

Validation report for the international validation study on ROS
(Reactive Oxygen Species) assay as a test evaluating phototoxic
potential of chemicals
(Seric version)

Conducted by:
ROS assay Validation Management Team

This version of the validation report was prepared for peer review.

Revisions made after a peer review panel meeting held from 27 February to 2 March are shown in blue.

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INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING PHOTOTOXIC POTENTIAL OF CHEMICALS (SERIC VERSION 1.2)

1. Background

The use of a reactive oxygen species (ROS) assay to detect chemicals with phototoxicity potential is expected to conform to the ICH framework.

The aim of this study was to validate the ROS assay method for between-laboratory variability and transferability in order to incorporate this assay for photoreactivity testing of drug candidates into an ICH framework. The ROS assay multi-study validation trials were undertaken in accordance with:

- i. the principles and criteria documented in the OECD No. 34 guidance document on the validation and international acceptance of new or updated test methods for hazard assessment [OECD, 2005],
- ii. the modular approach to validation [Hartung et al., 2004], and
- iii. discussions on multi-study validation trials with participation of good laboratory practice (GLP) test facilities [Cooper-Hannan et al., 1999] where the whole concept of multi-study validation trials was described in the context of GLP.

The studies part of multi-study trials should ideally be performed in accordance with GLP and should include but not necessarily be limited to the use of standard operating procedures (SOP) as well as adequate data recording, reporting, and record keeping.

A “modular approach” to validation is a general conceptual framework [Hartung et al., 2004; OECD, 2005] used for documenting the entire validation of a test method. In this approach, the information needed to support the validity of the method is organized into modules that provide the following information:

Module 1: Test definition

Module 2: Within-laboratory repeatability and reproducibility

Module 3: Between-laboratory transferability

Module 4: Between-laboratory reproducibility

Module 5: Predictive capacity

Module 6: Applicability domain

Module 7: Performance standards

The modular approach, as introduced by Hartung et al., allows the use of datasets from various sources and studies, and we took advantage of this approach in assessing the scientific validity of the ROS assay.

2. Objective of the study

The multi-study validation trial assessed the reliability (reproducibility within and between laboratories) and relevance (predictive capacity) of the ROS assay with a challenging set of test chemicals for which high quality in vivo data are available. In addition, to verify the applicability of other light sources for the ROS assay, a Seric SXL-2500V2 (SERIC LTD., Tokyo, Japan) was employed as a solar simulator in the present validation study.

3. Test Method

3-1. ROS assay

The ROS assay was developed by Onoue et al. [2008a] and is a high-throughput and high-performance system for predicting the phototoxic potential of pharmaceutical substances. This assay is a multiwell plate-based study using a quartz reaction container, the advantages of which include reduced sample volumes, improved assay

productivity, and highly-uniform irradiation.

In this study, the generation of ROS, including superoxide anion and singlet oxygen was detected by spectrophotometric measurement. Singlet oxygen was measured by monitoring the bleaching of p-nitrosodimethyl aniline (RNO) at 440 nm using imidazole as a selective acceptor of singlet oxygen. Two hundred microliters of samples containing the test chemical, RNO, and imidazole were transferred to the wells of a plastic 96-well plate before light exposure. The plate was subjected to measurement of absorbance at 440 nm using a microplate spectrophotometer. The plate was then fixed in the quartz reaction container with a quartz cover and irradiated with simulated sunlight for one hour. After agitation on a plate shaker, UV absorbance at 440 nm was measured. Superoxide anion was measured by irradiating samples containing the test chemical and nitroblue tetrazolium (NBT) with simulated sunlight for one hour, and then measuring the reduction in NBT by observing the increase in absorbance at 560 nm in the same manner as the singlet oxygen determination.

3-2. Sunlight simulator

A Seric SXL-2500V2 equipped with a 1500-W xenon arc lamp was used as a solar simulator.

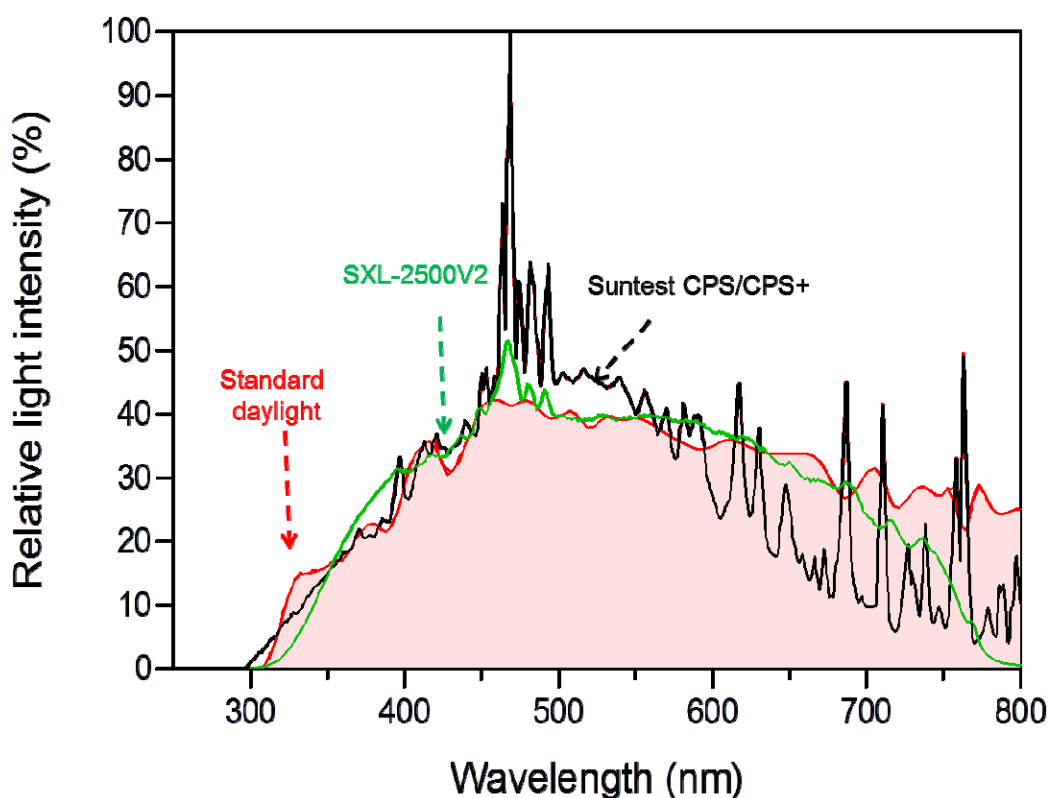


Figure 1 Spectral patterns of the light sources and standard daylight

4. Validation Management Structure

This validation study was performed by the Japanese Center for the Validation of

Alternative Methods (JaCVAM). The management structure is shown in Figure 1.

4-1. Validation Management Team

The validation management team (VMT) comprised individuals with the collective expertise in the underlying science to perform the scientific design, management, and evaluation of this study. The VMT played a central role in overseeing the validation study, including:

- 1) Goal statement
- 2) Project plan including objective
- 3) Study protocol/amendments
- 4) Outcome of QC audits
- 5) Test chemicals
- 6) Data management procedures
- 7) Timeline/study progression
- 8) Study interpretation and conclusions
- 9) Reports and publication

Final determination of which laboratories would participate in the validation study was the responsibility of the VMT.

Members:

Hajime Kojima; JaCVAM, VMT Chairperson

Kazuhiro Hosoi; Santen Pharmaceutical Co., Ltd., VMT Co-chair

Satomi Onoue; University of Shizuoka, lead laboratory

Tsuguto Toda; Shionogi & Co., Ltd.

Yasuhiro Matsumoto; ASKA Pharmaceutical Co., Ltd.

Manfred Liebsch; German Centre for the Documentation and Validation of Alternative Methods (ZEBET).

Yumiko Iwase; Mitsubishi Tanabe Pharma Corp.

Toshinobu Yamamoto; Mitsubishi Tanabe Pharma Corp.

Valerie Zang; ECVAM

Warren Casey; ICCVAM

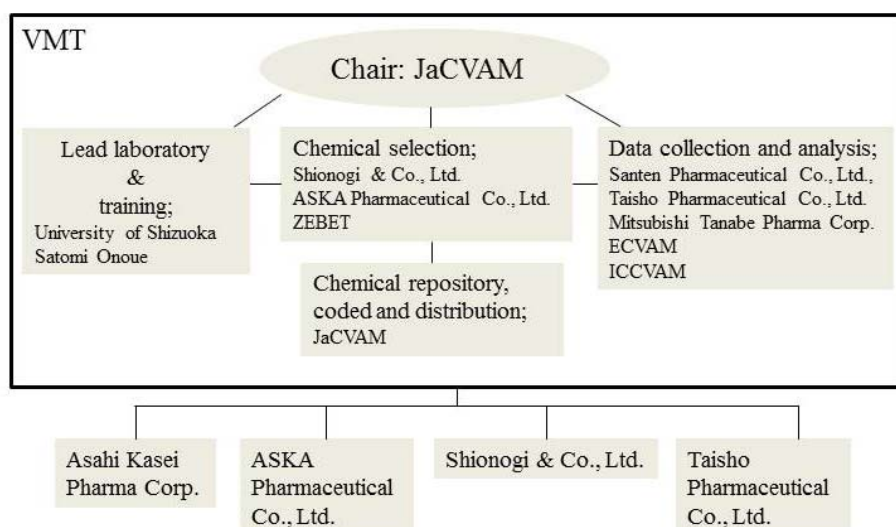


Figure 2 Management structure of the ROS assay validation study

4-2. Chemical selection, acquisition, coding and distribution

1) Definition of selection criteria

2) Chemical selection

Members:

Tsuguto Toda; Shionogi & Co., Ltd.

Yasuhiro Matsumoto; ASKA Pharmaceutical Co., Ltd.

Manfred Liebsch; ZEBET

(1) Liaise with suppliers

(2) Final check of chemicals provided

(3) Acquisition

(4) Coding

(5) Distribution

Member:

Hajime Kojima; JaCVAM

4-3. Independent biostatistician

1) Approve spreadsheets

2) Data collection

3) Data analysis

Members:

Kazuhiro Hosoi; Santen Pharmaceutical Co., Ltd.

Yumiko Iwase; Mitsubishi Tanabe Pharma Corp.

Toshinobu Yamamoto; Mitsubishi Tanabe Pharma Corp.

Valerie Zang; ECVAM

Warren Casey; ICCVAM

4-4. Participating laboratory

The following four laboratories participated in the validation study for the evaluation of the ROS assays, as shown in Figure 1.:

- Laboratory 4 - Asahi Kasei Pharma Corp. (Satoru Kawakami)
- Laboratory 5 - ASKA Pharmaceutical Co., Ltd. (Yasuhiro Matsumoto)
- Laboratory 6 - Shionogi & Co., Ltd. (Tsuguto Toda)
- Laboratory 7 - Taisho Pharmaceutical Co., Ltd. (Naoto Osaki)

Each laboratory also was responsible for complying with GLP-like principles and specifying QA aspects.

5. Study Design

Before this validation study, a training course using a Seric SXL-2500V2 was performed by the lead laboratory in March 2011. All technicians at each laboratory participated in this training course, which used quinine as a positive control and sulisobenzone as a negative control. After the training course, the two phases of the validation study were performed.

In the Phase 1 study, within-laboratory repeatability and reproducibility were assessed using 11 new chemicals (5-fluorouracil [5-FU], 8-methoxy psoralen, amiodarone,

chlorpromazine, diclofenac, doxycycline, furosemide, ketoprofen, levofloxacin, norfloxacin, and omeprazole), the positive control, and the negative control (Table 1). Phase 1-1 study was conducted with an irradiance of ca. 2.3 mW/cm² at four laboratories. And then, Phase 1-2 study was performed with an irradiance of ca. 3.0 mW/cm² or more except for laboratory 4. These studies were conducted between May and October 2011.

In the Phase 2 study, between-laboratory reproducibility and predictivity were assessed using 42 coded chemicals, the positive control, and the negative control (Table 2). This study was conducted between January and May 2012 at four laboratories which had participated in both the training course and the Phase 1-1 study.

6. Test Chemicals

6-1. Chemical selection

6-1-1. Chemicals for the Phase 1 study

Chemicals selected for the Phase 1 study are listed in Table 1-1 and 1-2. Twelve phototoxic chemicals and one non-phototoxic chemical were used. Chemicals for the Phase 1 study were not coded.

Quinine (No. I-12) was selected as the positive control and sulisobenzone (No. I-13) was selected as the negative control in the ROS assay according to the method of Onoue et al. [2008a]. Quinine HCl was classified as a phototoxic chemical for human per an article by Ljunggren et al [1986]. Sulisobenzone was classified as a non-phototoxic chemical, because human data on this chemical was described as negative per an article on the in vitro 3T3 neutral red uptake phototoxicity test (3T3NRU-PT) validation study [Spielmann et al, 1998b].

5-fluorouracil (5-FU, No. I-1) was reportedly a phototoxic chemical in humans [Dillaha et al., 1983], but it was negative in the 3T3NRU-PT [Kleinman et al., 2010 and Onoue et al., 2010]. Reported causes of human phototoxicity in 5-FU include photocytotoxicity induced by UV-B alone [Kirkup M.E. et al., 2003 and Andersen K.E. et al., 1984] and/or ROS generation derived from UV-B induced photodegradation [Miolo G. et al., 2011]. 5-FU absorbs mainly UV-B (290–320 nm) within the range of natural sunlight (Appendix 7), UV-B irradiation might be essential for photochemical activation of 5-FU. Therefore it was unknown whether 5-FU was a phototoxic chemical, and high quality human data was not available. 5-FU was selected in order to provide information on the limits of the ROS assay.

The remaining 10 chemicals (Nos. I-2, I-3, I-4, I-5, I-6, I-7, I-8, I-9, I-10 and I-11) were selected from typical phototoxic chemicals.

8-MOP (No. I-2), amiodarone HCl (No. I-3), chlorpromazine (No. I-4), doxycycline HCl (No. I-6), furosemide (No. I-7), ketoprofen (No. I-8) and norfloxacin (No. I-10) were selected from the list of phototoxic chemicals used in the 3T3NRU-PT validation study. Human data describing these chemicals as positive were given in the article on the 3T3NRU-PT validation study [Spielmann et al, 1994a and 1998a].

Diclofenac (No. I-5), levofloxacin (No. I-9) and omeprazole (No. I-11) were selected as phototoxic chemicals in humans per an article of Przybilla et al [1987], Boccumini et al [2000] and Dam et al [2008], respectively.

6-1-2. Chemicals for the Phase 2 study

Chemicals selected for the Phase 2 study are listed in Table 2-1 and 2-2. An equal

number of phototoxic and non-phototoxic chemicals were selected (approximately 1:1). Chemicals for the Phase 2 study were coded.

1) Phototoxic chemicals

Twenty-three positive chemicals (18 drugs and 5 non-drug chemicals) were selected based on the results in human and 3T3 NRU-PT.

Twenty-one chemicals (Nos. II-1, II-2, II-3, II-4, II-5, II-6, II-7, II-8, II-9, II-10, II-11, II-12, II-13, II-14, II-15, II-16, II-18, II-19, II-21, II-22 and II-23) of 23 positive chemicals were selected from the list of positive chemicals used in the 3T3NRU-PT validation study. Human data describing these chemicals as phototoxic were given in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a and 1998a].

Acridine (No. II-1) and acridine HCl (No. II-2) or nalidixic acid (II-11) and nalidixic acid (Na salt) (II-12) were tested both as a free form and a salt respectively in order to test whether the aqueous solubility of chemicals might limit the predictive power of the ROS assay.

Rosiglitazone (No. II-17) was selected because 3T3 NRU-PT was positive, although high quality human data regarding phototoxicity was not available. After the secondary data analysis on the Phase 2 study results, the VMT decided to exclude rosiglitazone from the third data analysis. The VMT considered that it is inappropriate to include rosiglitazone in the “phototoxic” chemical set because of lack of high quality human data regarding its phototoxicity.

Avobenzone was reported as negative for photoallergy by a photopatch test in the article by Szczurko C et al. [1994] and Trevisi P et al. [1994], but was reported to induce photoallergic reactions in the article by Schauder, S. et al. [1997]. Therefore, avobenzone was classified as a phototoxic chemical, because we were not able to completely judge that avobenzone was a non-phototoxic chemical.

2) Non-phototoxic chemicals

Nineteen negative chemicals (5 drugs and 14 non-drug chemicals) were selected mainly based on the negative results of 3T3 NRU-PT, because clinical information was not available for many chemicals.

We searched for human data for of these chemicals but and could not find reliable human phototoxicity data in human.

Five chemicals (Nos. II-27, II-31, II-34, II-40 and II-41) were selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a and 1998a]. Negative results in human and animals were described for chlorhexidine (No. II-31) and PABA (II-40) respectively in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a].

Seven chemicals (Nos. II-29, II-33, II-35, II-36, II-38, II-39 and II-42) were selected from UV absorbers. Three (Nos. II-35, II-38 and II-39) of them were in the list of negative chemicals in the 3T3 NRU-PT validation study and human data of these chemicals were described as negative in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998b]. We thought that human data for four UV absorbers (Nos. II-29, II-33, II-36 and II-42) would be provided. Human data, however, were not available for these chemicals. Therefore after confirming negative results in 3T3 NRU-PT, these chemicals were classified as non-phototoxic chemicals.

Four chemicals (Nos. II-24, II-25, II-26 and II-28) were selected based on negative

results in the 3T3 NRU-PT per the article by Onoue et al. [2010].

We originally intended to use cinnamic aldehyde, an aromatic ingredient used in cinnamon, as Chemical No. II-32, because this chemical used in the 3T3 NRU-PT validation study. However, we inadvertently added cinnamic acid instead of cinnamic aldehyde to the list of chemicals. Cinnamic acid is known to form a dimer by light irradiation when in the solid state. The difference between cinnamic aldehyde and cinnamic acid is that the former has a side chain of aldehyde and the later one of carboxyl. In addition, we carried out 3T3NRU-PT for cinnamic acid and the result was negative, just like cinnamic aldehyde. Therefore, we concluded that either chemical was suitable for the ROS assay validation study, because cinnamic acid resembles cinnamic aldehyde structurally, and the result of 3T3 NRU-PT testing was the same.

Chemical No. II-30 and II-37 were registered at first as benzylindene camphor sulphonic acid and octyl methoxycinnamate, respectively. These were UV absorbers used in the 3T3 NRU-PT validation study. However, we carried out the Phase 2 study without noticing a transcription error in the CAS number on the final chemical list. Because we did not notice this mistake until after the Phase 2 study, these chemicals were reclassified as non-phototoxic chemicals after confirming negative results in 3T3 NRU-PT and low molar extinction coefficient (MEC).

6-2. Chemical coding, distribution and disclosure of code

Coding and distribution of chemicals were performed by JaCVAM. The coded chemicals were sent to the safety officer, who is not involved in the experiments, together with a sealed envelope containing the material safety data sheets (MSDS). Since the chemicals were coded, the laboratories did not know their identity and therefore all chemicals were treated as hazardous chemicals. The disclosure of codes was performed at a VMT meeting on 8 May, 2012, after the data had been finalized per QC confirmation.

Table 1-1 List of reasons for chemical selection

NO.	Compound	Reasons for chemical selection
I-1	5-Fluorouracil (5-FU)	5-FU was reportedly a phototoxic chemical in human [Dillaha et al., 1983], but it was negative in the 3T3NRU-PT [Kleinman et al., 2010 and Onoue et al., 2010]. Reported causes of human phototoxicity in 5-FU include photocytotoxicity induced by UV-B alone [Kirkup M.E. et al., 2003 and Andersen K.E. et al., 1984] and/or ROS generation derived from UV-B induced photodegradation [Miolo G. et al., 2011]. 5-FU absorbs mainly UV-B (290–320 nm) within the range of natural sunlight (Appendix 6), UV-B irradiation might be essential for photochemical activation of 5-FU. Therefore it was unknown whether 5-FU was a phototoxic chemical, and high quality human data was not available. 5-FU was selected in order to provide information on the limits of the ROS assay. Photosensitivity was mentioned on both the US and the JPN label.
I-2	8-Methoxy psoralen (8-MOP)	8-MOP was selected from the list of phototoxic chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
I-3	Amiodarone HCl	Amiodarone HCl was selected from the list of phototoxic chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
I-4	Chlorpromazine HCl	Chlorpromazine HCl was selected from the list of phototoxic chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
I-5	Diclofenac	Diclofenac was selected as a phototoxic chemical for human per the article by Przybilla et al [1987]. Photosensitivity was mentioned on both the US and the JPN label.
I-6	Doxycycline HCl	Doxycycline HCl was selected from the list of phototoxic chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
I-7	Furosemide	Furosemide was selected from the list of phototoxic chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Although the 2011 US label did mention photosensitivity, 2012 US label did not. Photosensitivity was mentioned on the JPN label.
I-8	Ketoprofen	Ketoprofen was selected from the list of phototoxic chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Photosensitivity was mentioned on both the US and the JPN label.
I-9	Levofloxacin	Levofloxacin was selected as a phototoxicity positive chemical for human per the article by Boccumini et al [2000]. Photosensitivity was mentioned on both the US and the JPN label.
I-10	Norfloxacin	Norfloxacin was selected from the list of phototoxic chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Photosensitivity was mentioned on both the US and the JPN label.
I-11	Omeprazole	Omeprazole was selected as a phototoxic chemical for human per to the article by Dam et al [2008]. Photosensitivity was mentioned on both the US and the JPN label.
I-12	Quinine HCl	Quinine HCl was used as positive control according to the method of Onoue et al. [2008a]. Quinine HCl was classified as a phototoxic chemical for human per the article by Ljunggren et al [1986]. There was a mention of the photosensitivity in the US label.
I-13	Sulisobenzone	Sulisobenzone was used as negative control according to the method of Onoue et al. [2008a]. Sulisobenzone was classified as a non-phototoxic chemical, because Human data for this chemical was described as negative in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998b].

Table 1-2 Test chemicals for the Phase 1 study

No.	Chemical name	CAS No. ^{a)}	UV/vis absorption ^{b)}		ROS assay	3T3 NRU	<i>in vivo</i>	
			MEC (L/mol/cm)	λ_{\max} (nm)			Animal	Human
I-1	5-FU	51-21-8	1800 ^{c)}	290 ^{d)}	- ¹⁾	- ^{2,3)}	NA	+? ⁴⁾
I-2	8-MOP	298-81-7	3631	300	+ ¹⁾	+ ⁵⁾	+ ⁵⁾	+ ⁵⁾
I-3	Amiodarone HCl	19774-82-4	5400	290 ^{d)}	+ ³⁾	+ ⁵⁾	+ ⁵⁾	+ ⁵⁾
I-4	Chlorpromazine HCl	69-09-0	1746	304	+ ¹⁾	+ ⁵⁾	+ ⁵⁾	+ ⁵⁾
I-5	Diclofenac	15307-79-6	7800 ^{c)}	290 ^{d)}	+ ¹⁾	+ ³⁾	+ ⁶⁾	+ ⁷⁾
I-6	Doxycycline HCl	10592-13-9	3715	290 ^{d)}	+ ¹⁾	+ ⁵⁾	+ ⁵⁾	+ ⁵⁾
I-7	Furosemide	54-31-9	2650	290 ^{d)}	+ ¹⁾	+/- ^{3,8,9)}	NA	+ ⁸⁾
I-8	Ketoprofen	22071-15-4	2092	290 ^{d)}	+ ¹⁾	+ ⁸⁾	- ⁸⁾	+ ⁸⁾
I-9	Levofloxacin	100986-85-4	13000 ^{c)}	333	+ ¹⁰⁾	+ ¹⁰⁾	+ ¹¹⁾	+ ¹²⁾
I-10	Norfloxacin	70458-96-7	3562	323	+ ¹⁾	+ ³⁾	+ ⁸⁾	+ ⁸⁾
I-11	Omeprazole	73590-58-6	15000 ^{c)}	301	+ ¹⁾	+/- ³⁾	NA	+ ¹³⁾
I-12	Quinine HCl	6119-47-7	1938	330	+ ¹⁾	+ ³⁾	+ ⁶⁾	+ ¹⁴⁾
I-13	Sulisobenzone	4065-45-6	3519	290 ^{d)}	- ¹⁾	- ³⁾	NA	- ¹⁵⁾

5-FU: 5-fluorouracil, 8-MOP: 8-methoxy psoralen

+ : Positive, - : Negative, +/- : Equivocal, NA : Not available, ? : unclear

a) CAS No.: Chemical abstracts service registry number, b) The UV/vis absorbance (290-700 nm) of chemicals was measured in 20 mM phosphate buffer (pH 7.4). Test chemicals were dissolved in dimethylsulfoxide (DMSO) at 10 mM and diluted with 20 mM phosphate buffer (pH 7.4). Final concentration of DMSO was unified to 0.5%. c) Molar extinction coefficient (MEC) of 5-FU, diclofenac, levofloxacin, and omeprazole were extracted from the articles of Onoue et al. (2008a) and Seto et al. (2011).

d) λ_{\max} (nm) was a wavelength at which the UV/vis absorbance shows a peak between 290 and 700 nm. λ_{\max} (nm) was indicated as 290 nm in the case where the peak absorption is located below 290 nm and the maximum absorption is at 290 nm.

1) Onoue et al., 2008a, 2) Kleinman et al., 2010, 3) Onoue et al., 2010, 4) Dillaha et al., 1983, 5) Spielmann et al., 1994a, 6) Spielmann et al., 1994b, 7) Przybilla et al., 1987, 8) Spielmann et al., 1998a, 9) Peters et al., 2002, 10) Seto et al., 2011, 11) Wagai et al., 1992, 12) Boccumini et al., 2000, 13) Dam et al., 2008, 14) Ljunggren et al., 1986, 15) Spielmann et al., 1998b

Table 2-1 List of reasons for chemical selection

NO.	Chemical name	Reasons for chemical selection
Phototoxic drugs		
II-1	Acridine	Acridine was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Acridine (No. II-1) and acridine HCl (No. II-2) were tested both as a free form and a salt in order to test whether the aqueous solubility of chemicals might limit the predictive power of the ROS assay.
II-2	Acridine HCl	Acridine HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Acridine (No. II-1) and acridine HCl (No. II-2) were tested both as a free form and a salt in order to test whether the aqueous solubility of chemicals might limit the predictive power of the ROS assay.
II-3	Amiodarone HCl	Amiodarone HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
II-4	Chlorpromazine HCl	Chlorpromazine HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
II-5	Doxycycline HCl	Doxycycline HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
II-6	Fenofibrate	Fenofibrate was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Although the 2011 US label did mention photosensitivity, 2012 US label did not. Photosensitivity was mentioned on the JPN label.
II-7	Furosemide	Furosemide was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Although the 2011 US label did mention photosensitivity, 2012 US label did not. Photosensitivity was mentioned on the JPN label.
II-8	Ketoprofen	Ketoprofen was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Photosensitivity was mentioned on both the US and the JPN label.
II-9	6-Methylcoumarine	6-Methylcoumarine was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-10	8-MOP	8-MOP was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
II-11	Nalidixic acid	Nalidixic acid was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Nalidixic acid (II-11) and nalidixic acid (Na salt) (II-12) were tested both as a free form and a salt in order to test whether the aqueous solubility of chemicals might limit the predictive power of the ROS assay. Photosensitivity was mentioned on both the US and the JPN label.

II-12	Nalidixic acid (Na salt)	Nalidixic acid (Na salt) was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Nalidixic acid (II-11) and nalidixic acid (Na salt) (II-12) were tested both as a free form and a salt in order to test whether the aqueous solubility of chemicals might limit the predictive power of the ROS assay. Photosensitivity was mentioned on both the US and the JPN label.
II-13	Norfloxacin	Norfloxacin was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Photosensitivity was mentioned on both the US and the JPN label.
II-14	Ofloxacin	Ofloxacin was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Photosensitivity was mentioned on both the US and the JPN label.
II-15	Piroxicam	Piroxicam was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
II-16	Promethazine HCl	Promethazine HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
II-17	Rosiglitazone	Rosiglitazone was selected because 3T3 NRU-PT was positive, but high quality human data regarding phototoxicity was not available.
II-18	Tetracycline	Tetracycline was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was mentioned on both the US and the JPN label.
Phototoxic non-drug chemicals		
II-19	Anthracene	Anthracene was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-20	Avobenzone	Avobenzone was reported with photoallergy negative by a photopatch test in the article by Szczurko C et al. [1994] and Trevisi P et al. [1994], but was reported to induce photoallergic reactions in the article by Schauder, S. et al. [1997]. Therefore, avobenzone was classified as a phototoxic chemical because we were not able to completely judge that avobenzone was a non-phototoxic chemical.
II-21	Bithionol	Bithionol was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-22	Hexachlorophene	Hexachlorophene was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Masuda et al., 1971 and Spielmann et al, 1998a].
II-23	Rose bengal	Rose bengal was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as positive in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
Non-phototoxic drugs		
II-24	Aspirin	Aspirin was selected based on negative results of the 3T3 NRU-PT per the article by Onoue et al. [2010]. Photosensitivity was not mentioned on either the US or the JPN label.
II-25	Benzocaine	Benzocaine was selected based on negative results of the 3T3 NRU-PT per the article by Onoue et al. [2010]. Photosensitivity was not mentioned on either the US or the JPN label.
II-26	Erythromycin	Erythromycin was selected based on negative results of the 3T3 NRU-PT per the article by Onoue et al. [2010]. Photosensitivity was not mentioned on either the US or the JPN label.
II-27	Penicillin G	Penicillin G was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a]. Photosensitivity was not mentioned on

		either the US or the JPN label.
II-28	Phenytoin	Phenytoin was selected based on negative results of the 3T3 NRU-PT per the article by Onoue et al. [2010]. Photosensitivity was not mentioned on either the US or the JPN label.

Non-phototoxic non-drug chemicals		
II-29	Bumetrizole	Bumetrizole was selected from UV absorbers. We thought that human data of this UV absorber would be provided. Human data for this chemical, however, was not available. Therefore after confirming negative result in 3T3 NRU-PT, this chemical was classified as a non-phototoxic chemical.
II-30	Camphor sulfonic acid	Chemical No. II-30 was registered at first as benzylidene camphor sulphonic acid. This was a UV absorber used in the 3T3 NRU-PT validation study. However, we carried out the Phase 2 study without noticing a transcription error of the CAS number on the final chemical list. Because we did not notice this mistake until after the Phase 2 study, this chemical was reclassified as a non-phototoxic chemical after confirming negative results in 3T3 NRU-PT and low molar extinction coefficient (MEC).
II-31	Chlorhexidine	Chlorhexidine was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a and 1998a]. Negative result in human was described in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-32	Cinnamic acid	We originally intended to use cinnamic aldehyde, an aromatic ingredient used in cinnamon, as Chemical No. II-32, because this chemical used in the 3T3 NRU-PT validation study. However, we inadvertently added cinnamic acid instead of cinnamic aldehyde to the list of chemicals. Cinnamic acid is known to form a dimer by light irradiation when in the solid state. The difference between cinnamic aldehyde and cinnamic acid is that the former has a side chain of aldehyde and the later one of carboxyl. In addition, we carried out 3T3NRU-PT for cinnamic acid and the result was negative, just like cinnamic aldehyde. Therefore, we concluded that either chemical was suitable for the ROS assay validation study, because cinnamic acid resembles cinnamic aldehyde structurally, and the result of 3T3 NRU-PT testing was the same.
II-33	Drometrizole	Drometrizole was selected from UV absorbers. We thought that human data of this UV absorber would be provided. Human data for this chemical, however, was not available. Therefore after confirming negative result in 3T3 NRU-PT, this chemical was classified as a non-phototoxic chemical.
II-34	L-Histidine	L-Histidine was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-35	Methylbenzylidene camphor	Methylbenzylidene camphor was selected from UV absorbers. This was in the list of negative chemicals in the 3T3 NRU-PT validation study and Human data for this chemical was described as negative in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998b].
II-36	Octrizole	Octrizole was selected from UV absorbers. We thought that human data of this UV absorber would be provided. Human data for this chemical, however, was not available. Therefore after confirming negative result in 3T3 NRU-PT, this chemical was classified as a non-phototoxic chemical.
II-37	Octyl methacrylate	Chemical No. II-37 was registered at first as octyl methoxycinnamate. This was UV absorber used in the 3T3 NRU-PT validation study. However, we have carried out the Phase 2 study without noticing a transcribing error of the CAS number when we made the final chemical list. Because we noticed to take it wrong after the Phase 2 study, this chemical was classified as non-phototoxic chemical again after confirming negative result in 3T3 NRU-PT and low molar extinction coefficient (MEC).
II-38	Octyl methoxycinnamate	Octyl methoxycinnamate was selected from UV absorbers. This was in the list of negative chemicals in the 3T3 NRU-PT validation study and Human data for this chemical was described as negative in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998b].
II-39	Octyl salicylate	Octyl salicylate was selected from UV absorbers. This was in the list of negative chemicals in the 3T3 NRU-PT validation study and Human data for this chemical was described as negative in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998b].
II-40	PABA	PABA was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a and 1998a]. Negative result in animals was described in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-41	SDS	SDS was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a].

II-42	UV-571	UV-571 was selected from UV absorbers. We thought that human data of this UV absorber would be provided. Human data for this chemical, however, was not available. Therefore after confirming negative result in 3T3 NRU-PT, this chemical was classified as a non-phototoxic chemical.
Positive/Negative controls		
PC	Quinine HCl	Quinine HCl was used as positive control according to the method of Onoue et al. [2008a]. Quinine HCl was classified as a phototoxic chemical for human per the article by Ljunggren et al [1986]. Photosensitivity was mentioned on the US label.
NC	Sulisobenzone	Sulisobenzone was used as negative control according to the method of Onoue et al. [2008a]. Sulisobenzone was classified as a non-phototoxic chemical, because Human data for this chemical was described as negative in the article on the 3T3 NRU-PT validation study [Spielmann et al, 1998b].

Table 2-2 Test chemicals for Phase 2 study and code list

NO.	Chemical name	CAS No. ^{a)}	UV/vis absorption ^{b)}		3T3 NRU	in vivo		Laboratory			
			MEC (L/mol/cm)	λ_{max} (nm)		animal	human	4	5	6	7
Phototoxic drugs											
II-1	Acridine	260-94-6	2773	354	+ ¹⁾	+ ¹⁾	+ ¹⁾	G-330	F-270	E-215	H-390
II-2	Acridine HCl	17784-47-3	2635	354	+ ¹⁾	+ ¹⁾	+ ¹⁾	G-326	F-266	E-211	H-386
II-3	Amiodarone HCl	19774-82-4	5400	290 ^{e)}	+ ²⁾	+ ²⁾	+ ²⁾	G-327	F-267	E-212	H-387
II-4	Chlorpromazine HCl	69-09-0	1746	304	+ ²⁾	+ ²⁾	+ ²⁾	G-306	F-291	E-236	H-366
II-5	Doxycycline HCl	10592-13-9	3715	290 ^{e)}	+ ²⁾	+ ²⁾	+ ²⁾	G-316	F-256	E-201	H-376
II-6	Fenofibrate	49562-28-9	3514	290 ^{e)}	+ ¹⁾	NA	+ ¹⁾	G-339	F-279	E-224	H-354
II-7	Furosemide	54-31-9	2650	290 ^{e)}	+/- ^{1,3,4)}	NA	+ ¹⁾	G-341	F-281	E-226	H-356
II-8	Ketoprofen	22071-15-4	2092	290 ^{e)}	+ ¹⁾	- ¹⁾	+ ¹⁾	G-328	F-268	E-213	H-388
II-9	6-Methylcoumarine	92-48-8	3219	290 ^{e)}	+ ¹⁾	+ ¹⁾	+ ¹⁾	G-313	F-253	E-243	H-373
II-10	8-MOP	298-81-7	3631	300	+ ²⁾	+ ²⁾	+ ²⁾	G-331	F-271	E-216	H-391
II-11	Nalidixic acid	389-08-2	3192	331	+ ¹⁾	+ ¹⁾	+ ¹⁾	G-337	F-277	E-222	H-352
II-12	Nalidixic acid (Na salt)	3374-05-8	3019	333	+ ¹⁾	+ ¹⁾	+ ¹⁾	G-334	F-274	E-219	H-394
II-13	Norfloxacin	70458-96-7	3562	323	+ ³⁾	+ ¹⁾	+ ¹⁾	G-310	F-295	E-240	H-370
II-14	Ofloxacin	82419-36-1	8443	290 ^{e)}	+ ¹⁾	+ ¹⁾	+ ¹⁾	G-312	F-252	E-242	H-372
II-15	Piroxicam	36322-90-4	3304	352	- ²⁾	- ²⁾	+ ²⁾	G-335	F-275	E-220	H-395
II-16	Promethazine HCl	58-33-3	1558	297	+ ²⁾	NA	+ ²⁾	G-301	F-286	E-231	H-361
II-17	Rosiglitazone	122320-73-4	1765	311	+ ^{d)}	NA	NA	G-317	F-257	E-202	H-377
II-18	Tetracycline	60-54-8	3842	290 ^{e)}	+ ²⁾	+ ²⁾	+ ²⁾	G-302	F-287	E-232	H-362
Phototoxic compounds											
II-19	Anthracene	120-12-7	2315	355	+ ¹⁾	+ ¹⁾	+ ¹⁾	G-321	F-261	E-206	H-381
II-20	Avobenzene	70356-09-1	7686	354	+ ⁵⁾	- ⁶⁾	+ ⁷⁾	G-309	F-294	E-239	H-369
II-21	Bithionol	97-18-7	2462	321	+ ²⁾	+ ²⁾	+ ²⁾	G-315	F-255	E-245	H-375
II-22	Hexachlorophene	70-30-4	2431	300	- ¹⁾	- ¹⁾	+ ^{1,8)}	G-307	F-292	E-237	H-367
II-23	Rose bengal	632-69-9	19269	549	+ ¹⁾	- ¹⁾	+ ¹⁾	G-304	F-289	E-234	H-364
Non-phototoxic drugs											
II-24	Aspirin	50-78-2	80	290 ^{e)}	- ³⁾	NA	NA	G-340	F-280	E-225	H-355
II-25	Benzocaine	94-09-7	4273	290 ^{e)}	- ³⁾	NA	NA	G-314	F-254	E-244	H-374
II-26	Erythromycin	114-07-8	0	290 ^{e)}	- ³⁾	NA	NA	G-319	F-259	E-204	H-379
II-27	Penicillin G	113-98-4	0	290 ^{e)}	- ²⁾	NA	NA	G-318	F-258	E-203	H-378
II-28	Phenytoin	57-41-0	0	290 ^{e)}	- ³⁾	NA	NA	G-345	F-285	E-230	H-360

Table 2-2 Test chemicals for Phase 2 study and code list (continued)

NO.	Chemical name	CAS No. ^{a)}	UV/vis absorption ^{b)}		3T3 NRU	in vivo		Laboratory			
			MEC (L/mol/cm)	λ_{max} (nm)		animal	human	4	5	6	7
Non-phototoxic compounds											
II-29	Bumetrizole	3896-11-5	3873	306	- ^{d)}	NA	NA	G-338	F-278	E-223	H-353
II-30	Camphor sulfonic acid	3144-16-9	0	290 ^{c)}	- ^{d)}	NA	NA	G-332	F-272	E-217	H-392
II-31	Chlorhexidine	55-56-1	1338	290 ^{c)}	- ¹⁾	NA	- ¹⁾	G-344	F-284	E-229	H-359
II-32	Cinnamic acid	140-10-3	3373	290 ^{c)}	- ^{d)}	NA	NA	G-323	F-263	E-208	H-383
II-33	Drometrizole	2440-22-4	3946	295	- ^{d)}	NA	NA	G-329	F-269	E-214	H-389
II-34	L-Histidine	71-00-1	0	290 ^{c)}	- ²⁾	NA	NA	G-311	F-251	E-241	H-371
II-35	Methylbenzylidene camphor	36861-47-9	9200	304	- ⁹⁾	- ⁹⁾	- ⁹⁾	G-336	F-276	E-221	H-351
II-36	Octrizole	3147-75-9	3958	296	- ^{d)}	NA	NA	G-333	F-273	E-218	H-393
II-37	Octyl methacrylate	688-84-6	0	290 ^{c)}	- ^{d)}	NA	NA	G-305	F-290	E-235	H-365
II-38	Octyl methoxycinnamate	5466-77-3	3000	290 ^{c)}	- ⁹⁾	- ⁹⁾	- ⁹⁾	G-342	F-282	E-227	H-357
II-39	Octyl salicylate	118-60-5	1500	290 ^{c)}	- ⁹⁾	- ⁹⁾	- ⁹⁾	G-320	F-260	E-205	H-380
II-40	PABA	150-13-0	2404	290 ^{c)}	- ²⁾	- ¹⁾	NA	G-324	F-264	E-209	H-384
II-41	SDS	151-21-3	0	290 ^{c)}	- ²⁾	NA	NA	G-325	F-265	E-210	H-385
II-42	UV-571	125304-04-3	1900	290 ^{c)}	- ^{d)}	NA	NA	G-322	F-262	E-207	H-382
Positive/Negative controls											
PC	Quinine HCl	6119-47-7	1938	330	+ ³⁾	+ ¹⁰⁾	+ ¹¹⁾	PC	PC	PC	PC
NC	Sulizobenzone	4065-45-6	3519	290 ^{c)}	- ³⁾	NA	- ⁹⁾	NC	NC	NC	NC

8-MOP: 8-methoxy psoralen, PABA: *p*-aminobenzoic acid, SDS: sodium dodecyl sulfate

+ : Positive, - : Negative, +/- : Equivocal, NA : Not available, PC : Positive control, NC : Negative control

a) CAS No.: Chemical abstracts service registry number, b) The UV/vis absorbance (290-700 nm) of most chemicals was measured in 20 mM phosphate buffer (pH 7.4). However, the UV/vis absorbance of chemical Nos. II-19, II-20, II-29, II-33 and II-36 were measured in methanol, because these chemicals were not solved in 20 mM phosphate buffer (pH 7.4). In the each case, test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM phosphate buffer (pH 7.4) or methanol. Final concentration of DMSO was unified to 0.5%. c) λ_{max} (nm) was a wavelength at which the UV/vis absorbance shows a peak between 290 and 700 nm. λ_{max} (nm) was indicated as 290 nm in the case where the peak absorption is located below 290 nm and the maximum absorption is at 290 nm. d) In vitro phototoxicity was assessed by the 3T3 NRU PT in the participating laboratories, according to the OECD 432 guideline.

1) Spielmann et al., 1998a, 2) Spielmann et al., 1994a, 3) Onoue et al., 2010, 4) Peters et al., 2002, 5) Gaspar et al., 2012, 6) ZEBET in house data, 7) Schauder et al., 1997, 8) Masuda et al., 1971, 9) Spielmann et al., 1998b, 10) Spielmann et al., 1994b, 11) Ljunggren et al., 1986

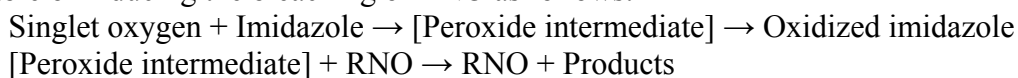
7. Protocols

The detailed test protocol used in this study is described in Attachments 1, 2 and 3.

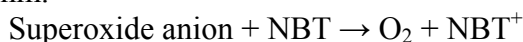
7-1. Prediction model of photoreactivity

In the ROS assay, generation of singlet oxygen is detected by spectrophotometric measurement of *p*-nitrosodimethyl aniline (RNO) bleaching, followed by decreased absorbance of RNO at 440 nm. Although singlet oxygen does not react chemically with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the

imidazole ring, resulting in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO as follows:



The generation of superoxide anion is detected by the reduction of nitroblue tetrazolium (NBT) by superoxide anion via a one-electron transfer reaction, yielding partially reduced ($2 e^-$) monoformazan (NBT^+) as a stable intermediate. Thus, superoxide anion reduces NBT to NBT^+ , whose formation can be monitored spectrophotometrically at 560 nm.



7-2. Protocol of ROS assay

7-2-1. Apparatus

In the present validation study, a Seric SXL-2500V2 (SERIC LTD., Tokyo, Japan) equipped with a xenon arc lamp, UV cut filter (<300 nm) and a fan, or their equivalents was used for solar simulator.

The irradiation tests were carried out at 20–29°C with an irradiance of ca. 2.3 mW/cm² (Phase 1-1 study) determined using the UVA detector (UD-36, UD-360 or UD-T36, TOPCON CORPORATION, Tokyo, Japan) or 3.0 or more mW/cm² (Phase 1-2 and 2 studies) determined using the calibrated UVA detector (Dr Hönle 0037, München, Germany) provided by the VMT. Quartz reaction containers for the ROS assay were manufactured by Ozawa Science (Aichi, Japan) and provided by the VMT.

7-2-2. Preparation of test chemicals and controls

The stock solutions were thawed just before use and used within the day. The coded test chemicals were dissolved in dimethylsulfoxide (DMSO) or 20 mM sodium phosphate buffer (NaPB, pH7.4) at concentrations of 1, 2.5, 5 or 10 mM just before use under UV-cut illumination or shade. All preparations were protected from light. [Detailed information on preparation of test chemicals is shown in Appendix 11.](#) The stock solutions of quinine for positive control and sulisobenzone for negative control were prepared at 10 mM in DMSO and kept frozen in tubes for up to 1 month. According to a chromatographic analysis, these stock solutions were stable for at least 1 month under these storage conditions.

7-2-3. ROS assay procedure

Singlet oxygen was measured in an aqueous solution by spectrophotometrically monitoring the bleaching of RNO at 440 nm using imidazole as a selective acceptor of singlet oxygen. Samples containing the tested chemical (20–200 μM), RNO (50 μM) and imidazole (50 μM) in 20 mM NaPB were mixed in a tube. Two hundred microliters of the sample were transferred to a well in a plastic 96-well plate (clear, non-treated, flat-bottom). The plate was subjected to measurement of absorbance at 440 nm using a microplate spectrophotometer. The plate was fixed in the quartz reaction container with a quartz cover, and then irradiated with simulated sunlight for one hour. After agitation on plate shaker, UV absorbance at 440 nm was measured. For the determination of superoxide anion, samples containing the test chemical (20–200 μM) and NBT (50 μM) in 20 mM NaPB were irradiated with the simulated

sunlight for one hour, and the reduction in NBT was measured by the increase in absorbance at 560 nm in the same manner as the singlet oxygen determination.

7-3. Data collection, handling, and criteria

7-3-1. Data collection

In the Phase 1-1 and 1-2 studies, experiments were performed in triplicate wells in three independent runs. As the final concentrations, 20 μM and 200 μM of test chemical solutions were subjected to the ROS assay. When questionable data (*e.g.* technical error) were obtained, each testing facility could perform an additional experiment using the questionable chemical(s) and the positive/negative control chemicals.

In the Phase 2 study, experiments were performed in triplicate wells in three independent runs.

Generally, the final concentration of test chemical solutions was 200 μM . However, when precipitation could be observed at 200 μM under the optical microscope at 100 \times before light exposure, additional experiments were performed at 100 μM . Further experiments were performed at 50 μM when precipitation was observed at 100 μM . When precipitation was observed at 50 μM in the reaction mixture, further experiment was performed at 20 μM . When precipitation was observed at 20 μM in the reaction mixture, further experimentation was no longer needed. When questionable data (*e.g.* technical error) were obtained, each testing facility could perform an additional experiment using the questionable chemical(s) and the positive/negative chemicals.

7-3-2. Data handling

The study report and all raw data from this study were retained according to the protocol in each testing facility. All raw data and the results were submitted to the VMT for review.

7-3-3. Criteria for data acceptance and judgment

The acceptance criteria for a valid assay were:

- i. No precipitation of the test chemical in the reaction mixture before light exposure,
- ii. No missing data for the positive control, negative control, blanks, or chemicals; and
- iii. Net absorbance of 0.02–1.5 in the controls and the chemicals.
- iv. Positive control value at 200 μM (mean of 3 wells)
Singlet oxygen: 150 or more
Superoxide anion: 200 or more
- v. Negative control value at 200 μM (mean of 3 wells)
Singlet oxygen: less than 25
Superoxide anion: less than 20

According to the results (mean of triplicate determinations) of the ROS assay, the photoreactivity on each test chemical was judged to be:

- i. Positive with singlet oxygen value ($\Delta A_{440 \text{ nm}} \times 10^3$) of 25 or more and/or superoxide anion value ($\Delta A_{560 \text{ nm}} \times 10^3$) of 20 or more; or
- ii. Negative with singlet oxygen value of less than 25 and superoxide anion value of less than 20.

Every assay result was classified based on these classification criteria. Final

judgments about chemicals were performed on the following four draft criteria at each laboratory:

- A. Final judgment of positive when positive results were obtained in at least one of three assays.
- B. Final judgment based on the mean value of three assays.
- C. Final judgment based on the majority of three assay results.
- D. Final judgment based on the first assay results.

7-4. Quality assurance

Assays and quality assurance were carried out in the spirit of GLP, although not all the participating laboratories routinely worked under GLP certification. The participating laboratories conducted the experiments in accordance with the protocol provided by the VMT. All raw data and data analysis sheets were pre-checked for quality in each laboratory and then reviewed by the VMT quality assurance group. The results accurately reflect the raw data.

8. Results

8-1. Phase 1-1 study

8-1-1. Within- and between-laboratory variation assessments in the Phase 1-1 study

Results of within-laboratory variation, which comprise intra-day variation and inter-day variation of the positive and negative controls at Laboratories 4, 5, 6, and 7 are shown in Table 3. Individual positive and negative control values are shown in Appendix 3. Parameters for intra-day variations were calculated based on results from the date on which the most assays were performed at each laboratory. Parameters for inter-day variations were calculated based on the results of the first assay of the day. Intra-day variation and inter-day variation were evaluated using the coefficient of variation (CV) of the positive control as well as values for mean and standard deviation of the positive and negative controls at each laboratory. The intra-day CVs for singlet oxygen and superoxide anion generated from the positive control were 2.5–8.6% and 4.8–12.8%, respectively. In addition, the inter-day CVs for the positive control were 2.6–18.7% for singlet oxygen and 2.8–16.4% for superoxide anion. Variations of each control value were sufficiently small to suggest good within-laboratory reproducibility.

Parameters for between-laboratory variations were calculated from the average values from all of the assay results for the positive and negative controls of each laboratory (Table 4). The CVs of positive control in the four laboratories were 14.5% (singlet oxygen) and 26.7% (superoxide anion).

In all the laboratories, the positive control generated both singlet oxygen and superoxide anion under light exposure, whereas ROS generation from the negative control was negligible in all assays. The assays for positive control had superoxide anion values in the range of 103 to 250, many of which failed to achieve the criteria for data acceptance (positive control value at 200 μ M, superoxide anion: 200 or more) even though other criteria for data acceptance were achieved.

8-1-2. Results and judgments in the Phase 1-1 study

Results of the Phase 1-1 study were shown (Table 5 and Appendix 4). According to the results (mean values of triplicate determinations) of the ROS assay, the

photoreactivity on each test chemical was judged to be

- i. Positive with singlet oxygen value ($\Delta A_{440\text{ nm}} \times 10^3$) of 25 or more and/or superoxide anion value ($\Delta A_{560\text{ nm}} \times 10^3$) of 20 or more or
- ii. Negative with singlet oxygen value of less than 25 and superoxide anion value of less than 20.

Although data for precipitation were not recorded, precipitations were observed for amiodarone.

In order to select criteria for final judgment in a recommendation protocol for the ROS assay, four different draft criteria for final judgments were used in this validation study.

Draft criteria A: Final judgment of positive when positive results were obtained in at least one of three assays (Table 6A).

Draft criteria B: Final judgment based on the mean value of three assays (Table 6B).

Draft criteria C: Final judgment based on the majority of three assay results (Table 6C).

Draft criteria D: Final judgment on the first assay results (Table 6D).

As for the final judgments at 20 μM , one chemical at Laboratories 4 and 7 gave inconsistent result in the three independent repeat assays, as did two chemicals at Laboratory 5, and three chemicals at Laboratory 6. Five chemicals gave inconsistent final judgments between the laboratories for draft criteria A (chemical Nos. I-2, I-4, I-5, I-7, and I-11), as did six chemicals for draft criteria B (chemical Nos. I-2, I-3, I-4, I-5, I-10, and I-11), five chemicals for draft criteria C (chemical Nos. I-3, I-4, I-5, I-10, and I-11), and six chemicals for draft criteria D (chemical Nos. I-3, I-4, I-5, I-7, I-10, and I-11). However, there was no inconsistency in the final judgment between the four laboratories at 200 μM .

8-1-3. Contingency tables in the Phase 1-1 study

Inconsistencies of final judgments were assessed using contingency tables at 20 μM (Tables 7A to 7D) and at 200 μM (Table 7E).

At a concentration of 20 μM , sensitivities were 50.0–75.0% under draft criteria A, 25.0–66.7% under draft criteria B and C, and 33.3–66.7% under the draft criteria D. Although there was only one non-phototoxic chemical, specificities were 100% under all criteria at all laboratories. Positive predictivities were also 100% under all criteria at all laboratories. Negative predictivities were 14.3–25.0% under draft criteria A, 10.0–20.0% under draft criteria B and C, and 11.1–20.0% under the draft criteria D. Accuracies were 53.8–76.9% under draft criteria A, 30.8–69.2% under draft criteria B and C, and 38.5–69.2% under the draft criteria D.

At a concentration of 200 μM , sensitivities were 91.7%, specificities and positive predictivities were 100%, negative predictivities were 50.0%, and accuracies were 92.3% at all laboratories regardless of criteria. A false negative result was obtained for one of 12 phototoxic chemicals (5-FU). It should be noted that the transferability of the ROS assay could be achieved among all laboratories, in which satisfactory consistency for the thirteen test chemicals was observed at a concentration of 200 μM .

8-2. Phase 1-2 study

8-2-1. Within- and between-laboratory variation assessments in the Phase

1-2 study

For the Phase 1-1 study, many assays failed to achieve the criteria for data acceptance (positive control value at 200 μM , superoxide anion: 200 or more). Therefore, in the Phase 1-2 study, the ROS assay was carried out under irradiance conditions that could achieve the criteria for data acceptance. The satisfactory transferability of the ROS assay had been already demonstrated among all laboratories by the Phase 1-1 study. Herein, in the Phase 1-2 study, the ROS assay for the thirteen test chemicals for the Phase 1-1 study was carried out in two independent runs at Laboratories 5, 6, and 7, with the aim of verifying the reproducibility of the ROS assay under the different irradiance condition from the Phase 1-1 study.

Results of within-laboratory variation, which comprise intra-day variation and inter-day variation of the positive and negative controls at Laboratories 5, 6, and 7 are shown in Table 8. Individual positive and negative control values are shown in Appendix 5. Parameters for intra-day variations were calculated based on results from the date on which the most assays were performed at each laboratory. Parameters for inter-day variations were calculated based on the results of the first assay of the day. Intra-day variation and inter-day variation were evaluated using the CV of the positive control as well as values for mean and standard deviation of the positive and negative controls at each laboratory. The intra-day CVs for singlet oxygen and superoxide anion generated from irradiated quinine were 3.3–5.8% and 5.2–10.0%, respectively. In addition, the inter-day CVs for quinine were 0.2–4.3% for singlet oxygen and 0.9–12.9% for superoxide anion. Variations of each control value were sufficiently small to suggest good within-laboratory reproducibility.

Parameters for between-laboratory variations were calculated from the average values from all of the assay results for the positive and negative controls of each laboratory (Table 9). The CVs of positive control in the four laboratories were 5.9% (singlet oxygen) and 10.4% (superoxide anion). Negative control was shown to be inactive in all assays.

8-2-2. Results and judgments in the Phase 1-2 study

Results of the Phase 1-2 study were shown (Table 10 and Appendix 6). According to the results (mean values of triplicate determinations) of the ROS assay, the photoreactivity on each test chemical was judged to be

- i. Positive with singlet oxygen value ($\Delta A_{440 \text{ nm}} \times 10^3$) of 25 or more and/or superoxide anion value ($\Delta A_{560 \text{ nm}} \times 10^3$) of 20 or more or
- ii. Negative with singlet oxygen value of less than 25 and superoxide anion value of less than 20.

Every ROS assay was classified based on these criteria and final judgments were based on four draft criteria shown in Tables 11A to 11D. The draft criteria for the final judgment are followings:

- A. Final judgment of positive when positive results were obtained in at least one of two assays.
- B. Final judgment based on the mean value of two assays.
- C. Final judgment based on the majority of two assay results.
- D. Final judgment based on the first assay results.

Under the draft criteria C, final judgment was judged to be inconclusive when the

number of positive equaled that of negative.

Although data for precipitation were not recorded, precipitations were observed for amiodarone.

As for the final judgments at 20 μM , one chemical at Laboratories 5 and 6 gave inconsistent result in the two independent repeat assays, as did two chemicals at Laboratory 7.

Three chemicals gave inconsistent final judgments between the laboratories for draft criteria A and D (chemical Nos. I-2, I-7, and I-8), as did two chemicals for draft criteria B (chemical Nos. I-7 and I-8), and four chemicals for draft criteria C (chemical Nos. I-2, I-5, I-7, and I-8). However, there was no inconsistency in the final judgment between the three laboratories at 200 μM .

8-2-3. Contingency tables in the Phase 1-2 study

Inconsistencies of final judgments were assessed using contingency tables at 20 μM (Tables 12A to 12D) and at 200 μM (Table 12E).

At a concentration of 20 μM , sensitivities were 75.0–83.3% under draft criteria A and B, 72.7–81.8% under draft criteria C, and 66.7–83.3% under the draft criteria D. Although there was only one non-phototoxic chemical, specificities were 100% under all criteria at all laboratories. Positive predictivities were also 100% under all criteria at all laboratories. Negative predictivities were 25.0–33.3% under draft criteria A, B, and C and 20.0–33.3% under the draft criteria D. Accuracies were 76.9–84.6% under draft criteria A and B, 75.0–83.3% under draft criteria C, and 69.2–84.6% under the draft criteria D.

At a concentration of 200 μM , sensitivities were 91.7%, specificities and positive predictivities were 100%, negative predictivities were 50.0%, and accuracies were 92.3% at all laboratories regardless of criteria. A false negative result was obtained for one of 12 phototoxic chemicals (5-FU).

8-3. Phase 2 study

8-3-1. Irradiance and temperature during the irradiation

The irradiances and temperatures at the beginning and the end of each irradiation are shown in Table 13 and Appendix 7. Irradiance A was measured by each laboratory with its own UVA detector. Irradiance B was a standardized irradiance calculated using values obtained using the calibrated UVA detector (Dr. Hönle), which was transported to each laboratory and conversion factors for standardized irradiance were prepared. Irradiance in each laboratory (3.9 to 4.0 mW/cm^2 at Laboratory 4, 5.0 mW/cm^2 at Laboratory 5, 3.5 mW/cm^2 at Laboratory 6, and 3.0 to 3.2 mW/cm^2 at Laboratory 7) were within the specified range of values. There was no apparent variation in either irradiance or temperature within the facilities.

8-3-2. Within- and between-laboratory variation for Phase 2 study

Results of within-laboratory variation, which comprise intra-day variation and inter-day variation of the positive and negative controls at Laboratories 4, 5, 6, and 7, are shown in Table 14 and Appendix 8. Parameters for intra-day variations were calculated based on results from the date on which the most assays were performed at each laboratory. Parameters for inter-day variations were calculated based on the results of the first assay

of the day. Intra-day variation and inter-day variation were evaluated using the CV of the positive control as well as values for mean and standard deviation of the positive and negative controls of each laboratory. The intra-day CVs for singlet oxygen and superoxide anion generated from the positive control were 1.9–3.8% and 0.6–12.0%, respectively. In addition, the inter-day CVs for the positive control were 2.4–4.6% for singlet oxygen and 4.4–8.3% for superoxide anion. Variations of each control value were sufficiently small to suggest good within-laboratory reproducibility.

Parameters for between-laboratory variations were calculated from the average value from all of the assay results for the positive and negative controls of each facility (Table 15). The CVs of positive control in the three laboratories were 14.4% (singlet oxygen) and 6.0% (superoxide anion). Negative control was shown to be inactive in all assays.

8-3-3. Results and judgments in the Phase 2 study

The photochemical reactivities of 42 coded chemicals, comprising 23 known phototoxins and 19 non-phototoxic drugs/compounds, were assessed using the ROS assay at Laboratories 4, 5, 6, and 7 (Table 16 and Appendix 9). Assessment of between 25 and 28 chemicals (60–67% of total) were made at a concentration of 200 μM , and that of between 14 and 17 chemicals had to be diluted to a final concentration of 20, 50, 100 μM due to limited solubility in aqueous media. In particular, assays of amiodarone HCl (No. II-3), anthracene (No. II-19), avobenzone (No. II-20), drometrizole (No. II-33), octrizole (No. II-36), and UV-571 (No. II-42) couldn't be carried out in Laboratories 4, 5, 6, and/ or 7 because precipitations were observed at 20 μM . [Nalidixic acid \(No. II-11\) and its sodium salt \(No. II-12\) were selected to evaluate the influence of the free form and Na salt form which may affect the solubility profile. The results of nalidixic acid and its Na salt were positive at 200 \$\mu\text{M}\$ in all assays, and the values for singlet oxygen and superoxide anion were similar in each laboratory.](#) All four participating laboratories found that all evaluable phototoxins demonstrated a potent ability to generate singlet oxygen, superoxide anion, or both under UV–vis exposure at concentrations of 20, 50, 100, and 200 μM . Similar photochemical reactivity was also seen for test chemicals that were non-phototoxic drugs/compounds, although some exhibited a potent photoreactivity in a few laboratories.

Classification criteria for positive, negative and inconclusive ROS assays are:

Positive: Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM

Negative: Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM

Inconclusive: The results do not meet the positive or negative criteria

Every ROS assay was classified based on these criteria, and final judgments were based on four draft criteria shown in Tables 17A to 17D. The draft criteria for the final judgment are followings:

- A. Final judgment of positive when positive results were obtained in at least one of three assays.
- B. Final judgment based on the mean value of three assays.
- C. Final judgment based on the majority of three assay results.
- D. Final judgment based on the first assay results.

One chemical at Laboratories 4 and 7 gave inconsistent result in the three independent repeat assays.

Thirteen chemicals gave inconsistent final judgments between the laboratories for draft criteria A (chemical Nos. II-6, II-19, II-20, II-27, II-31, II-32, II-35, II-36, II-37, II-38, II-39, II-41, and II-42), as did eleven chemicals for draft criteria B, C, and D (chemical Nos. II-6, II-19, II-20, II-27, II-31, II-32, II-35, II-36, II-38, II-39, and II-41).

8-3-4. Contingency tables in the Phase 2 study

Inconsistent final judgments were assessed using contingency tables in the Phase 2 study (Tables 18A to 18D).

Sensitivities and negative predictivities were 100% in each laboratory regardless of the four different criteria. Specificities were 40.0–63.6% under draft criteria A, 46.2–63.6% under draft criteria B, and 42.9–63.6% under draft criteria C and D. Positive predictivities were 70.0–84.0% under draft criteria A, 75.0–84.0% under draft criteria B, and 72.4–84.0% under draft criteria C and D. Accuracies were 75.0–87.5% under draft criteria A, 79.4–87.5% under draft criteria B, and 77.1–87.5% under draft criteria C and D. There were no false negatives. There were between four and nine false positives in 10 to 15 negative chemicals under draft criteria A, between four and seven false positives in 10 to 13 negative chemicals under draft criteria B, and between four and eight false positives in 10 to 14 negative chemicals under draft criteria C and D.

8-4. Combined results of Phase 1-2 and Phase 2

8-4-1. Results and judgments for Phase 1-2 and 2 combined results

The results of four phototoxic chemicals (200 μ M) evaluated in the Phase 1-2 study were combined with the Phase 2 results (diclofenac, 5-FU, levofloxacin, omeprazole; Tables 19A to 19 D). The results and the final judgments of these four chemicals were consistent between the four laboratories.

8-4-2. Contingency tables for Phase 1-2 and 2 combined results

Inconsistencies of final judgments were assessed using contingency tables for the combined results (Tables 20A to 20D).

Sensitivities were 95.8–100% in each laboratory regardless of the four different criteria. Specificities were 40.0–63.6% under draft criteria A, 46.2–63.6% under draft criteria B, and 42.9–63.6% under draft criteria C and D. Positive predictivities were 72.4–85.7% under draft criteria A, 77.4–85.7% under draft criteria B, and 75.0–85.7% under the draft criteria C and D. Negative predictivities were 85.7–100% under draft criteria A, B, C, and D. Accuracies were 75.0–86.1% under draft criteria A, 79.4–86.1% under draft criteria B, and 76.9–86.1% under draft criteria C and D.

5-FU showed a false negative under all criteria, but no other chemicals showed false negatives under any criteria. There were between four and nine false positives in 10 to 15 negative chemicals under draft criteria A, between four and seven false positives in 10 to 13 negative chemicals under draft criteria B, and between four and eight false positives in 10 to 14 negative chemicals under draft criteria C and D.

8-5. Contingency tables for integrated judgment results

Parameters in the contingency tables for the Phase 1-1 study (20 and 200 μ M), the

Phase 1-2 study (20 and 200 μM), the Phase 2 study, and the combined results are shown in Tables 21-1 to 21-6. Integrated final judgments were based on a majority of laboratory judgments. Integrated final judgments were considered inconclusive when the number of positive equaled that of negative or inconclusive. Parameters in the contingency tables of integrated judgment results for the Phase 1-2 study (200 μM), the Phase 2 study, and the combined results are shown in Table 22.

The parameters in Phase 1-1 and 1-2 at 200 μM were the same regardless of the draft criteria for judgment. For the Phase 2 and the combined results, however, specificities were lower than the other parameters, especially under draft criteria A. The other parameters showed no apparent inconsistency regardless of draft criteria, although some were slightly lower under draft criteria A.

8-6. Secondary data analysis after receiving the comments from the peer review committee

After issuing the validation report, the peer reviewers reviewed the report in a meeting held from 27th February to 2nd March, 2013. Major comments from the peer reviewers are as follows:

- 1) It might have been better to limit data to just blind Phase 2 study. The basis for overall evaluation of sensitivity, specificity, positive and negative predictivity, and overall accuracy (performance criteria) should focus on this data. In this data set, 100% negative predictivity is highly encouraging, even though refers to chemicals that provided conclusive data. Re-examine whether the human data for 5-FU phototoxicity could be regarded as “high quality human data” or not.
- 2) The database could be enlarged by taking solubility into account and accepting negative results at 20 μM .
- 3) Low specificity of data is problematic. If possible, widen the borderline for the acceptance criteria based on validation study data. (from 20 to x for superoxide anion)

According to comment No. 1, the data re-analysis conducted below is focused on the Phase 2 study, which was conducted under blind conditions. The VMT members re-examined the information on 5-FU phototoxicity in human. Upon re-examination, we concluded that it is unknown whether 5-FU was phototoxic or not, and high quality human data was not available (See Table 1-1.).

Regarding comment No. 2, accepting negative results at 20 μM did not cause any false negatives in validation studies with Atlas Suntest CPS/CPS+ or Seric SXL2500-V2. Therefore the negative results at 20 μM were included in this data re-analysis.

Regarding comment No. 3, no false negatives were found after changing the borderline for superoxide anion from 20 to 70 in the validation study results using Atlas Suntest CPS/CPS+ or Seric SXL2500-V2. If we use a borderline of 25 for singlet oxygen and 70 for superoxide anion, then amlodipine, amoxapine, bufexamac, and haloperidol would all be below the borderline [Onoue et al., 2008a].

Chemicals with singlet oxygen values of less than 25 and superoxide anion values of between 20 and 70 are considered to be weakly photoreactive. New judgment criteria

were established for the proposed protocol as follows:

Judgment criteria the secondary data analysis

Each test chemical will be judged as follows;

Judgment	Concentration judged	SO (mean of 3 wells)	SA (mean of 3 wells)
Photoreactive	20, 50, 100, and/or 200 μM ¹⁾	≥ 25	and ≥ 70
		< 25 and/or P ³⁾	and ≥ 70
		≥ 25	and < 70 and/or P
Weakly photoreactive	20, 50, 100, and 200 μM ¹⁾	< 25	and $\geq 20, < 70$
Non-photoreactive	20, 50, 100, and 200 μM ¹⁾	< 25	and < 20
Inconclusive	The results do not meet the above-mentioned criterion. ³⁾		

Notes

- 1) A single experiment is sufficient for classifying results, because the ROS assay shows good reproducibility in the validation studies.
- 2) Twenty μM can be used for classification when precipitation or coloration is observed at 200 μM .
- 3) Precipitation before irradiation.
- 4) If precipitation, coloration, or other interference before irradiation is observed at both at 20 and 200 μM , the chemical is considered incompatible with the ROS assay.

As described in the following sections, the Phase 2 study results were re-analyzed based the above criteria. Since the ROS assay is intended for screening photoreactivity during initial photosafety evaluation, it is preferable to minimize false negatives even at the cost of increased false positives. Although all chemicals that were classified as weakly photoreactive in the validation studies were non-phototoxic drugs or non-phototoxic non-drug chemicals, there are some drugs—such as amlodipine, amoxapine, bufexamac and haloperidol—which might show clinical photosensitivity despite being classified as weakly photoreactive [Onoue et al., 2008a]. Follow-up tests for non-clinical and/or clinical photosafety should be considered if a drug candidate is classified as weakly photoreactive. Therefore, in the secondary data analysis, we evaluated weakly photoreactive chemicals when defined as both non-phototoxic and phototoxic

8-6-1. Secondary data analysis based on the criteria for the proposed protocol: Results and judgments of Phase 2 study

The results of the Phase 2 study were classified based on the criteria in the proposed protocol, and final judgments were based on four draft criteria as shown in Tables 23A to 23D. The draft criteria for the final judgment are as follows:

- A. Final judgment of positive when positive results were obtained in at least one of three assays.
- B. Final judgment based on the mean value of three assays.

C. Final judgment based on the majority of three assay results.

D. Final judgment based on the first assay results.

For phototoxic chemicals which were evaluated at 20, 50, 100, and 200 μM , all of the judgments, the final judgments and the integrated judgments were “photoreactive”. For the non-phototoxic chemicals, four chemicals (Nos. II-28, II-34, II-35, II-37) were classified as “photoreactive” in at least one of three assay results at Laboratory 4, 5, 6, and/or 7. Except for these cases, all other non-phototoxic chemicals were classified as “non-photoreactive” or “weakly photoreactive”.

8-6-2. Secondary data analysis based on the criteria for the proposed protocol: Contingency tables of Phase 2 study results

Contingency tables are shown in Tables 24A to 24D. Two to four phototoxic chemicals and two to six non-phototoxic chemicals were considered incompatible due to precipitation at 20, 50, 100, and 200 μM . Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of each laboratory based on the original criteria and the criteria for the proposed protocol are summarized in Table 25. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of the integrated judgments based on the original criteria and the criteria for the proposed protocol are summarized in Table 26.

8-7. Third data analysis after reconsidering the negative results at 20 μM

After issuing a revised validation report with secondary data analysis, the VMT members updated the validation studies and the peer review process at the ICH S10 Expert Working Group (EWG) during the ICH Brussels meeting on 3rd June, 2013. After introduction of updated results which stated in Section 8-5 above, discussion at the EWG was focused on the predictivity of the negative results at 20 μM . Some EWG members also noticed that differences in precipitation between laboratories might lead the test concentration differences between Labs which could result in false negatives if the predictivity at 20 μM is not appropriate. The VMT promised to obtain assay results for the test chemicals at 20 μM .

After the ICH Brussels meeting, Lab 1 performed a new series of ROS assays at 20 μM using the validation chemical set with the Atlas CPS+. The results of this new series of assays at 20 μM and the Phase 2 assay results at 200 μM were presented in the validation report for the ROS assay with the Atlas CPS/CPS+. As the results, all of phototoxic chemicals were judged as “Photoreactive” even at 20 μM . The intensities of ROS generation, however, were generally decreased at 20 μM in comparison with the results at 200 μM in the validation study. Therefore, we concluded that follow-up studies will be needed whenever negative results are seen only at 20 μM .

Based on these results, there are at least two options for classifying negative results at 20 μM . One option is to establish a new classification, such as “probably non-photoreactive.” Another is to classify them as inconclusive. If “probably non-photoreactive” were to be adopted, the VMT could follow the suggestions from the peer review committee given in Section 8-5. An ICH S10 EWG member suggested that since negative ROS would not require any follow up in the draft ICH S10 guideline, and the term “probably non-photoreactive” would suggest somewhat negative results, its adoption would require additional explanation in the S10 guideline, which might

cause confusion after implementation. On the other hand, classifying negative results at 20µM as inconclusive would reduce the nominal applicability domain of ROS assay, even though the number of chemicals needing follow up after ROS assay would not change. After discussing these options, the VMT proposed that negative results at 20µM be classified as inconclusive. Therefore, classification criteria for the proposed protocol would be as follows.

Judgment criteria for the proposed protocol

Each test chemical will be judged as follows;

Judgment ¹⁾	Concentration	SO (mean of 3 wells)	SA (mean of 3 wells)
Photoreactive	20 and/or 200 µM ²⁾	≥25	and/or ≥70
Weakly photoreactive	20 and 200 µM ¹⁾	<25	and ≥20, <70
Non-photoreactive	20 and 200 µM ³⁾	<25	and <20
Inconclusive	The results do not meet the above-mentioned criterion. ⁴⁾		

Notes

- 1) A single experiment is sufficient for classifying results, because the ROS assay shows good reproducibility in the validation studies.
- 2) Twenty µM can be used for classification when precipitation or coloration is observed at 200 µM.
- 3) Two concentration levels without precipitation would be needed to classify a chemical as non-photoreactive. If precipitation is observed at 200 µM, the chemical is classified as inconclusive.
- 4) If precipitation, coloration, or other interference before irradiation is observed at both 20 and 200 µM, the chemical is considered incompatible with the ROS assay.

Classification as either photoreactive or weakly photoreactive would be a flag for follow-up assessment. Classification as non-photoreactive would indicate a very low probability of phototoxicity.

8-7-1. Third data analysis based on the criteria for the proposed protocol: Results and judgments of Phase 2 study

The results of the Phase 2 study were classified based on the criteria in the proposed protocol, and final judgments were based on four draft criteria as shown in Tables 27A to 27D. Since the ROS assay will be conducted at 20 µM and 200 µM in the proposed protocol, the validation study results with 50 µM and 100 µM were used as reference data, and were not used for data analysis. The draft criteria for the final judgment are as follows:

- A. The highest criteria among the three assay results were selected as the final judgment.
- B. Final judgment based on the mean value of three assays.
- C. Final judgment based on the majority of three assay results.
- D. Final judgment based on the first assay result.

For the phototoxic chemicals, the results of the third data analysis matched those of the second data analysis except the results at 50 μM and 100 μM which were not used in the third data analysis. There were no differences in integrated judgment as shown in Tables 27A to 27D. For the non-phototoxic chemicals, the integrated judgements were same in each table except phenytoin and chlorhexidine. Phenytoin was classified as photoreactive in Table 27A, and was classified as weakly photoreactive by three criteria (Tables 27B, C and D). Chlorhexidine was classified as weakly photoreactive by one criteria (Tables 27A), and was classified as non-photoreactive by three criteria (Tables 27B, C and D).

8-7-2. Third data analysis based on the criteria for the proposed protocol: Contingency tables of Phase 2 study results

Contingency tables are shown in Tables 28A to 28D. Three to four phototoxic chemicals and four to seven non-phototoxic chemicals were considered incompatible due to precipitation at 20 μM , 50 μM , 100 μM and 200 μM . Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of each laboratory based on the original criteria and the criteria for the proposed protocol are summarized in Table 29. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of the integrated judgments based on the original criteria and the criteria for the proposed protocol are summarized in Table 30.

8-7. Fourth data analysis after reconsidering negative results at 20 μM

Negative results at 20 μM were discussed during the peer review meeting held on 21 and 22 August, 2013, in Japan. As stated in Section 8-7, false negatives were not observed at 20 μM in results obtained by Dr. Onoue after the Brussels meeting. The VMT, however, proposed that negative results at 20 μM be classified as inconclusive in order to avoid the risk of false negatives. On the other hand, in Phase 1 of the validation study, which is included in the validation reports, the 11 phototoxicants tested at 200 μM and 20 μM were all positive at 200 μM and of these, only 8-MOP was negative at 20 μM . There were no differences between test facilities in these results. The peer review panel recommended that assays at 20 μM be performed when the assay at 200 μM did not provide results due to precipitation, coloration or other interference. Since implementation of this sequential protocol would reduce the risk of false negatives, the VMT decided to follow this recommendation. So far, we have not found any chemicals that are phototoxic in humans but inconclusive at 200 μM and negative at 20 μM in the ROS assay.

Regarding the criteria for classification as weakly photoreactive, of 18 chemicals that are non-phototoxic in vivo, three were weakly photoreactive in the ROS assay. Also, there are four other chemicals—amlodipine, amoxapine, bufexamac, and haloperidol—that could be considered weakly photoreactive based on existing ROS assay literature [Onoue et al., 2008a]. Photosensitivity is mentioned on amlodipine labels in Japan but not in the US. Photosensitivity is mentioned on amoxapine labels in the US not in Japan. Incidence of photosensitivity under clinical conditions was less than 0.1% for amlodipine according to a Japanese interview form and less than 1% for amoxapine according to

<http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=261006c8-3fd0-491b-b322-42beff6f9880>. In terms of UV A and UV B absorption of bufexamac and haloperidol, their absorption

peak wavelengths were shorter than the lower limit of UVB (290 nm). Their MEC values were 130 and 180 L/mol/cm at 290 nm[Onoue et al., 2008a]. In the ICH S10 photosafety draft guideline (Step 2 version, dated 15 November 2012), it is stated that absorption with a MEC less than 1000 L/mol/cm is not considered to result in a photosafety concern. These two compounds would not be subject to photosafety evaluation including ROS assay. Therefore, phototoxicity of these four drugs is not very noticeable, and it seems that the adverse events which are possibly related to the phototoxic potential of these drugs are not common events.

Classification criteria for the proposed protocol

Each test chemical will be judged as follows;

Classification ^{1,2)}	Concentration judged ³⁾	SO (mean of 3 wells)	SA (mean of 3 wells)
Photoreactive	200 µM	≥25	and ≥70
		<25 and/or I ⁴⁾	and ≥70
		≥25	and <70 and/or I ⁴⁾
Weakly photoreactive	200 µM	<25	and ≥ 20, <70
Non-photoreactive	200 µM	<25	and <20
Inconclusive	The results do not meet the above-mentioned criterion.		

Notes

- 1) A single experiment is sufficient for classifying results, because the ROS assay shows good reproducibility in the validation studies.
- 2) If precipitation, coloration, or other interference before irradiation is observed at both at 20 and 200 µM, the chemical is considered incompatible with the ROS assay.
- 3) Twenty µM can be used for classification when precipitation or coloration is observed at 200 µM.
- 4) Precipitation, coloration or other interference.

Classification as photoreactive, weakly photoreactive, or inconclusive would be a flag for follow-up assessment. Classification as non-photoreactive would indicate a very low probability of phototoxicity, with no further testing recommended.

8-7-1. Fourth data analysis based on the criteria for the proposed protocol: Results and classifications of Phase 2 study

The results of the Phase 2 study were classified based on the criteria in the proposed protocol, and final classifications were based on four draft criteria as shown in Tables 31A to 31D. The draft criteria for the final classification are as follows:

- A. The highest criteria among the three assay results was selected as the final classification.
- B. Final classification based on the mean value of three assays.
- C. Final classification based on the majority of three assay results.
- D. Final classification based on one of three assay results.
(For draft criteria D, the result of the first assay was used as one of three assay results.)

For phototoxic chemicals, the results of the fourth data analysis matched those of the second and third data analysis, and there were no differences in the integrated classification as shown in Tables 31A to 31D. For non-phototoxic chemicals, the integrated classifications were the same in each table except phenytoin, chlorhexidine and octyl methoxycinnamate. Phenytoin was classified as photoreactive according to Tables 31A but as weakly photoreactive by Table 31B, C and D. Chlorhexidine was classified as weakly photoreactive according to Table 31A but as non-photoreactive according to Tables 31B, C, and D. Octyl methoxycinnamate was classified as inconclusive according to Table 31A and D but as non- photoreactive according to Table 27B and C. In the third data analysis, eight (Tables 27A, B, C and D) out of 19 non-phototoxic chemicals were classified as inconclusive, but in the fourth data analysis, three (Tables 31A and D) or two (Tables 31B and C) out of 19 non-phototoxic chemicals was classified as inconclusive.

8-7-2. Fourth data analysis based on the criteria for the proposed protocol: Contingency tables of Phase 2 study results

Contingency tables are shown in Tables 32A to 32D. Three to four phototoxic chemicals and three to seven non-phototoxic chemicals were considered incompatible due to precipitation at 20 μM and 200 μM or classified as inconclusive. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of each laboratory based on the original criteria and the criteria for the proposed protocol are summarized in Table 33. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of the integrated classifications based on the original criteria and the criteria for the proposed protocol are summarized in Table 34.

9. Discussion

9-1. Reliability

Variability was assessed using quinine for a positive control and sulisobenzone for a negative control in the Phase 1-1 study. Whereas within-laboratory variations were sufficiently small in the Phase 1-1 study, superoxide anion values for the positive control did not achieve the criteria for data acceptance in many assays. In the Phase 1-1 study, the solar simulator (SXL-2500V2) produced a UVA intensity on the plate position (2.3 mW/cm^2) equivalent to the level of the Atlas Suntest CPS series used in the other validation study for the same aim [Kojima et al., 2013]. Since the Seric SXL-2500V2 was originally developed for in vitro phototoxicity assays, it includes a UV filter system that largely removes the cytotoxic UVB-region. Thus, the Seric SXL-2500V2 emitted less light from the UVB-region than the Atlas Suntest CPS series. These differences in spectral pattern of emitted light provide a partial explanation of why superoxide anion values for the positive control did not achieve criteria for data acceptance in the Phase 1-1 study. In the Phase 1-2 study, the assays were carried out under irradiance conditions that could achieve the data acceptance criteria for positive and negative control values. High repeatability and reproducibility were also confirmed in the Phase 1-2 study.

Between-laboratory transferability was assessed using 12 positive and 1 negative chemical at 20 and 200 μM in the Phase 1-1 study and Phase 1-2 study. All chemicals showed the same final judgments in all criteria and facilities at 200 μM in both studies.

On the other hand, some phototoxic chemicals showed negative or inconclusive results at 20 μM even in the Phase 1-2 study. There were no criteria that canceled these discrepancies. Thus, we decided to accept negative results only at 200 μM in the Phase 2 study, although we did accept positive results at any concentration. After the peer review panel meeting held from 27 February to 2 March, 2013, the peer review panel recommended the inclusion of negative results at lower concentrations (20, 50, and 100 μM) and widening of the borderline, in order to reduce the number of inconclusive and false positives in the validation study results. Secondary data analysis was conducted for the results of the Phase 2 study, which was conducted under masked condition. The secondary data analysis did not affect the reliability of the ROS assay. After the ICH Brussels meeting, Lab 1 performed a new series of ROS assays at 20 μM with the validation chemical set with the Atlas CPS+. The newly conducted assay results at 20 μM and the Phase 2 assay results at 200 μM were presented in the validation report for the ROS assay with the Atlas CPS/CPS+. All phototoxic chemicals were classified photoreactive even at 20 μM . The intensities of ROS generation, however, generally decreased at 20 μM compared with the results at 200 μM in the validation study. From this, VMT concluded that follow-up studies would be necessary when negative results are seen only at 20 μM . Based on these results, there are at least two options for classifying negative results at 20 μM . One is to establish as new classification, such as “probably non-photoreactive.” Another is to classify them as inconclusive. If “probably non-photoreactive” were to be adopted, the VMT could follow the suggestions the peer review committee given in Section 8-6. An ICH S10 EWG member suggested that since negative ROS would not require any follow up in the draft ICH S10 guideline, and the term “probably non-photoreactive” would suggest somewhat negative results, its adoption would require additional explanation in the S10 guideline, which might cause confusion after implementation. On the other hand, classifying negative results at 20 μM as inconclusive would reduce the nominal applicability domain of ROS assay, even though the number of chemicals needing follow up after ROS assay would not change. After discussing these options, the VMT proposed that negative results at 20 μM be classified as inconclusive. The results of the third data analysis reduced the potential risk for false positives from the secondary data analysis even though the number of inconclusives was not improved from the original data analysis. Therefore, the third data analysis did not affect the reliability of the ROS assay.

9-2. Between-laboratory reproducibility

In the Phase 2 study conducted with 42 coded test chemicals and 2 control chemicals, and the third data analysis was performed on the results with 41 chemicals except rosiglitazone. As shown in Table 29, the sensitivities, specificities, positive predictivities, negative predictivities and accuracies from each laboratory, based on the criteria for the proposed protocol, were 100%, 83.3% to 100%, 90.5% to 100%, 100%, and 93.5% to 100% when the weakly positive chemicals were defined as “non-phototoxic” chemicals; 100%, 50.0% to 63.6%, 76.0% to 82.6%, 100%, and 80.6% to 86.7% when the weakly positive chemicals were defined as “phototoxic” chemicals. Sensitivities and negative predictivities showed perfect results but specificities exhibited relative fluctuation per laboratory. This ROS assay protocol is intended for use in screening phototoxicity potential and therefore requires high

sensitivity. High sensitivity is more important than specificity in acquiring reliable photoreactivity assessments without false negatives, so these variations in specificity are acceptable.

9-3. Predictivity

In the Phase 2 results, as shown in Table 33, the sensitivity of each laboratory was 100%. In theory, the ROS assay is designed to capture all photochemically active substances that can be detected as type I and/or II photochemical reactions induced by irradiated chemicals. These photochemical reactions were observed at a very early stage of chemical-induced phototoxic cascades, so that the ROS assay had been thought effective for photosafety evaluation of pharmaceuticals. There is, however, a good probability that some photolabile substances would also be recognized as phototoxic by the ROS assay because of significant ROS generation during the photodegradation processes. Based on the validation study results, the criteria for the ROS assay results was revised in the proposed protocol as stated in Section 8-7. According to the original criteria, thirteen chemicals gave inconsistent final judgments between the laboratories for draft criteria A, as did 11 chemicals for draft criteria B, C, and D. Three phototoxic chemicals (Nos. II-6, II-19, and II-20) showed inconclusive final judgments in some laboratories because of precipitation. No laboratories, however, showed false negatives, and high sensitivity of the ROS assay was confirmed. Ten non-phototoxic chemicals (Nos. II-27, II-31, II-32, II-35, II-36, II-37, II-38, II-39, II-41, and II-42) showed false positives at least in one laboratory. In addition, the other 2 chemicals (Nos. II-28 and II-34) showed false positives in all laboratories. Within these 12 chemicals, 5 chemicals (Nos. II-27, II-28, II-31, II-32, and II-34) showed high solubility and assays were performed at 200 μM . There is a good probability that some photolabile substances would also be recognized as phototoxic by the ROS assay because of significant ROS generation during the photodegradation processes. These five chemicals had previously been reported to be photodegradable and photoreactive, the mechanisms of which included radical reactions and/or electron transfer (penicillin G [Ray R. S. et al., 1996] (No. II-27), phenytoin [Chen Y. et al., 2009] (No. II-28), chlorhexidine [Information from manufacture] (No. II-31), cinnamic acid [Marin M. L. et al., 2007] (No. II-32), and L-histidine [Huvaere K. et al., 2009] (No. II-34)). This could explain in part the data discrepancy observed between the ROS assay and in vitro/in vivo phototoxicity, and a better understanding of this limitation would be of great help in avoiding overestimation or misleading conclusions. After the criteria for the proposed protocol was adopted, penicillin G, phenytoin, chlorhexidine, cinnamic acid, L-histidine, octrizole, and SDS were classified as “weakly photoreactive” at least in one laboratory. Since the ROS assay is intended for screening photoreactivity during initial photosafety evaluation, it is preferable to minimize false negatives even at the cost of increased false positives. Although all chemicals that, were classified as weakly photoreactive in the validation studies, were non-phototoxic drugs or non-phototoxic non-drug chemicals, some drugs—such as amlodipine, amoxapine, bupropion, and haloperidol—which could be related to clinical photosensitivity would be classified as weakly photoreactive [Onoue et al., 2008a]. Test chemicals such as methylbenzylidene camphor (II-35), octyl methacrylate (II-37), octyl salicylate (II-39), and SDS (II-41), which could not be evaluated at 200 μM due to low solubility and were other than positive at 20 μM , were classified as inconclusive under the

original criteria but were classified as non-photoreactive when the criteria for the proposed protocol was applied. The VMT propose that negative results at 20 μ M not be accepted in order to avoid potential confusion after implementation of ICH S10 guideline. Drug candidates that are classified as weakly photoreactive or photoreactive in the ROS assay should be considered for follow-up non-clinical and/or clinical photosafety studies.

As observed in the present validation study, poorly water-soluble chemicals satisfied neither positive nor negative criteria, leading to inconclusive results, and it appears that photosafety assessment of poorly water-soluble chemicals will require some modification of the protocols. Although analysis throughput would be decreased, the use of micellar solution systems could be effective for poorly soluble chemicals [Onoue S. et al., 2008b]. In order to overcome limitations of poorly water-soluble chemicals, a modified ROS assay system has been developed using bovine serum albumin in the lead laboratory. Careful elucidation of predictivity will of course be made for modified protocols, if such protocols are intended to use in regulatory decisions. In this validation study, volatile substances were not included as the test chemicals. It was found that water droplets attached to the inside of the quartz plate and sealed by the quartz plate, but were not dispersed from the wells during the ROS assay; chemicals with volatility to some extent can be applied to the ROS assay and may not interfere other assay results in the same reaction container.

As shown in Table 34, sensitivities were the same under all criteria but specificities, positive predictivities, negative predictivities, and accuracies were lowest under draft criteria A. These parameters were relatively consistent for criteria B, C and D. Additionally, because only one assay is needed, draft criteria D also has an advantage in terms of throughput. Optimal criteria for final classification will be determined in a comprehensive manner using results of another validation running parallel with this one but with a different solar simulator.

As shown in Table 29, sensitivities and negative predictivities were the same under all criteria but specificities, positive predictivities, and accuracies were lowest under draft criteria A. There was no apparent inconsistency of parameters among draft criteria B, C, and D, although draft criteria D has an advantage in terms of throughput. A standard protocol with optimal criteria for final judgment will be proposed based on the present study (Seric SXL-2500V2) and parallel running study (Atlas Suntest CPS series).

10. Performance standards

Performance standards have not been established for the ROS assays based on the present validation study, however a list of chemicals for testing the adequacy of solar simulators other than the Atlas Suntest CPS/CPS+ and Seric SXL2500v2 evaluated in the two present studies has been provided in the recommended protocol.

11. Conclusion

The present validation study assessed, on the basis of the standardized protocol utilizing Seric solar simulator, robustness of the ROS assay was assessed using 27 phototoxic and 19 non-phototoxic chemicals at four facilities. High within- and between-laboratory reproducibility and transferability of methods were demonstrated at

three facilities. Assessment demonstrated the capacity to classify a balanced set of 41 test chemicals with a high degree of accuracy and no false negatives. Significant effort went into establishing well-defined classification criteria based on ROS assay endpoints, which maximized applicability domain and assay performance with sensitivity of 100% (20 chemicals/20 chemicals), specificity of 80.0% (12 chemicals/15 chemicals), positive predictivity of 86.4% (19 chemicals/22 chemicals), negative predictivity of 100% (12 chemicals/12 chemicals) and accuracy of 91.2% (31 chemicals/34 chemicals). These results support the routine use of the validated ROS assay protocol in preclinical drug screening for phototoxic potential. We propose a standard ROS assay protocol with defined data analytical methods based on the validation studies in which the two different solar simulators were used.

11. References

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Table 3 Within-laboratory variation of Phase 1-1 study

ROS assay Validation data (seric)			Lab 4				Lab 5				Lab 6				Lab 7			
			CV				CV				CV				CV			
Chemicals			N	Mean	SD	(%)	N	Mean	SD	(%)	N	Mean	SD	(%)	N	Mean	SD	(%)
Intra-day																		
Positive	Quinine HCl	SO	6	242	5.5	2.5	4	288	24.7	8.6	4	200	13.2	6.6	6	335	28.1	8.4
Control		SA	6	110	7.0	6.4	4	153	19.6	12.8	4	209	16.1	7.7	6	189	9.0	4.8
Negative	Sulisobenzone	SO	6	-3	3.5	/	4	1	1.3	/	4	1	4.7	/	6	3	3.7	/
Control		SA	6	-22	0.9	/	4	-7	6.7	/	4	-8	1.5	/	6	-2	1.3	/
Inter-day																		
Positive	Quinine HCl	SO	3	251	6.5	2.6	3	240	12.8	5.3	3	214	22.5	10.5	4	277	51.9	18.7
Control		SA	3	123	3.5	2.8	3	132	14.8	11.2	3	222	28.5	12.8	4	171	28.1	16.4
Negative	Sulisobenzone	SO	3	-1	2.6	/	3	-2	2.0	/	3	2	5.5	/	4	1	2.6	/
Control		SA	3	-22	0.6	/	3	-4	10.5	/	3	-8	4.6	/	4	-1	1.3	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Intra-day variations were calculated by selecting the date on which a number of assay results were obtained most.

Inter-day variations were calculated using the assay results of the first time of the each assay day.

Table 4 Between-laboratory variation of Phase 1-1 study

ROS assay Validation data (seric)

Chemicals			N	Mean	SD	CV
						(%)
Positive	Quinine HCl	SO	4	251	36.4	14.5
Control		SA	4	162	43.2	26.7
Negative	Sulisobenzone	SO	4	0	1.7	/
Control		SA	4	-9	9.7	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values. Between-laboratory variations were calculated from the average value of the results of each facility.

Table 5 Results of the ROS assay multi-center variation Phase 1-1 study
20 µM

ROS assay Validation data (seric)																										
		ab 4							Lab 5					Lab 6					Lab 7							
No.	Chemicals Name		Assay					CV (%)	Assay					CV (%)	Assay					CV (%)	Assay					CV (%)
			1st	2nd	3rd	Mean	SD		1st	2nd	3rd	Mean	SD		1st	2nd	3rd	Mean	SD		1st	2nd	3rd	Mean	SD	
I-1	5-FU	SO	-2	1	2	0	2.1	/	1	3	2	2	1	/	1	-4	2	0	3.2	/	-4	-3	1	-2	2.6	/
		SA	-3	-1	-5	-3	2	/	-3	-2	0	-2	1.5	/	-1	0	1	0	1	/	-1	-2	-2	-2	0.6	/
I-2	8-MOP	SO	3	8	6	6	2.5	/	7	8	5	7	1.5	/	2	0	2	1	1.2	/	18	7	6	10	6.7	/
		SA	9	10	9	9	0.6	/	-6	5	95	31	55.4	178.7	5	2	3	3	1.5	/	3	2	1	2	1	/
I-3	Amiodarone HCl ^a	SO	97	106	102	102	4.5	4.4	85	69	72	75	8.5	11.3	17	5	27	16	11	/	78	88	86	84	5.3	6.3
		SA	-20	-18	-19	-19	1	/	21	11	17	16	5	/	12	8	13	11	2.6	/	12	-10	-3	0	11.2	/
I-4	Chlorpromazine HCl	SO	80	86	79	82	3.8	4.6	113	91	80	95	16.8	17.7	-6	-8	1	-4	4.7	/	60	108	108	92	27.7	30.1
		SA	-10	-9	-9	-9	0.6	/	14	7	13	11	3.8	/	3	9	8	7	3.2	/	7	8	7	7	0.6	/
I-5	Diclofenac	SO	24	32	25	27	4.4	16.3	25	19	15	20	5	/	4	4	10	6	3.5	/	15	22	24	20	4.7	/
		SA	-6	-4	-4	-5	1.2	/	-16	5	3	-3	11.6	/	8	4	6	6	2	/	3	1	0	1	1.5	/
I-6	Doxycycline HCl	SO	45	57	47	50	6.4	12.8	67	56	53	59	7.4	12.5	55	33	43	44	11	25	49	61	56	55	6	10.9
		SA	12	13	14	13	1	/	42	40	44	42	2	4.8	43	52	47	47	4.5	9.6	39	39	37	38	1.2	3.2
I-7	Furosemide	SO	7	12	12	10	2.9	/	8	5	5	6	1.7	/	25	-1	10	11	13.1	/	4	11	8	8	3.5	/
		SA	-15	-12	-13	-13	1.5	/	17	-40	-44	-22	34.1	/	0	2	2	1	1.2	/	3	1	1	2	1.2	/
I-8	Ketoprofen	SO	17	20	20	19	1.7	/	16	16	16	16	0	/	3	13	20	12	8.5	/	8	19	14	14	5.5	/
		SA	5	6	8	6	1.5	/	6	-3	7	3	5.5	/	6	5	4	5	1	/	3	2	1	2	1	/
I-9	Levofloxacin	SO	18	21	22	20	2.1	/	58	21	16	32	22.9	71.6	8	19	28	18	10	/	9	23	16	16	7	/
		SA	181	174	182	179	4.4	2.5	174	162	172	169	6.4	3.8	170	129	125	141	24.9	17.7	95	118	106	106	11.5	10.8
I-10	Norfloxacin	SO	34	35	38	36	2.1	5.8	55	28	30	38	15	39.5	12	23	35	23	11.5	/	46	40	49	45	4.6	10.2
		SA	7	6	7	7	0.6	/	10	10	9	10	0.6	/	13	16	15	15	1.5	/	16	12	12	13	2.3	/
I-11	Omeprazole	SO	62	62	55	60	4	6.7	44	23	18	28	13.8	49.3	-3	5	22	8	12.8	/	17	33	4	18	14.5	/
		SA	1	0	3	1	1.5	/	30	37	28	32	4.7	14.7	-10	18	18	9	16.2	/	9	22	17	16	6.6	/
I-12	Quinine HCl	SO	61	57	62	60	2.6	4.3	76	42	43	54	19.3	35.7	36	39	56	44	10.8	24.5	43	63	57	54	10.3	19.1
		SA	23	19	26	23	3.5	15.2	48	32	28	36	10.6	29.4	43	33	34	37	5.5	14.9	23	30	26	26	3.5	13.5
I-13	Sulisobenzone	SO	-2	2	2	1	2.3	/	3	2	1	2	1	/	-4	-1	4	0	4	/	-5	4	0	0	4.5	/
		SA	-5	-4	-5	-5	0.6	/	-3	-7	-5	-5	2	/	-2	4	-1	0	3.2	/	-1	-2	-2	-2	0.6	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 µM and 200 µM in this study.

Table 5 Results of the ROS assay multi-center variation Phase 1-1 study (continued)

200 μ M

ROS assay Validation data (seric)			Lab 4							Lab 5					Lab 6					Lab 7						
No.	Chemicals Name		Assay			CV			Assay			CV			Assay			CV			Assay			CV		
			1st	2nd	3rd	Mean	SD	(%)	1st	2nd	3rd	Mean	SD	(%)	1st	2nd	3rd	Mean	SD	(%)	1st	2nd	3rd	Mean	SD	(%)
I-1	5-FU	SO	-3	-4	-2	-3	1	/	-3	-1	-4	-3	1.5	/	8	4	0	4	4	/	6	-5	1	1	5.5	/
		SA	11	5	4	7	3.8	/	-5	8	2	2	6.5	/	4	0	4	3	2.3	/	0	3	1	1	1.5	/
I-2	8-MOP	SO	41	34	46	40	6	15	49	52	47	49	2.5	5.1	19	24	21	21	2.5	/	40	42	55	46	8.1	17.6
		SA	31	26	26	28	2.9	10.4	13	36	20	23	11.8	51.3	52	32	67	50	17.6	35.2	25	18	27	23	4.7	20.4
I-3	Amiodarone HCl ^a	SO	221	190	199	203	15.9	7.8	358	573	526	486	113	23.3	216	111	134	154	55.2	35.8	131	128	61	107	39.6	37
		SA	-196	-193	-199	-196	3	/	307	171	141	206	88.5	43	-178	-100	-146	-141	39.2	/	-39	-80	-19	-46	31.1	/
I-4	Chlorpromazine HCl	SO	26	19	30	25	5.6	22.4	52	37	32	40	10.4	26	-64	-36	-28	-43	18.9	/	-37	47	35	15	45.4	/
		SA	39	42	43	41	2.1	5.1	54	75	62	64	10.6	16.6	54	59	55	56	2.6	4.6	65	52	63	60	7	11.7
I-5	Diclofenac	SO	104	106	101	104	2.5	2.4	96	67	70	78	15.9	20.4	41	62	57	53	11	20.8	94	65	82	80	14.6	18.3
		SA	39	43	39	40	2.3	5.8	71	108	95	91	18.8	20.7	86	127	120	111	21.9	19.7	108	81	97	95	13.6	14.3
I-6	Doxycycline HCl	SO	131	145	136	137	7.1	5.2	244	256	254	251	6.4	2.5	161	231	236	209	41.9	20	139	181	166	162	21.3	13.1
		SA	222	209	219	217	6.8	3.1	275	316	295	295	20.5	6.9	353	332	365	350	16.7	4.8	329	274	311	305	28	9.2
I-7	Furosemide	SO	44	41	43	43	1.5	3.5	40	32	29	34	5.7	16.8	40	49	35	41	7.1	17.3	37	22	37	32	8.7	27.2
		SA	7	5	4	5	1.5	/	-3	23	10	10	13	/	23	22	29	25	3.8	15.2	22	16	26	21	5	23.8
I-8	Ketoprofen	SO	152	157	153	154	2.6	1.7	119	124	118	120	3.2	2.7	91	122	123	112	18.2	16.3	106	109	139	118	18.2	15.4
		SA	70	70	77	72	4	5.6	105	107	98	103	4.7	4.6	88	100	99	96	6.7	7	75	71	72	73	2.1	2.9
I-9	Levofloxacin	SO	87	85	89	87	2	2.3	88	88	85	87	1.7	2	64	91	75	77	13.6	17.7	59	68	103	77	23.2	30.1
		SA	264	290	260	271	16.3	6	271	279	294	281	11.7	4.2	333	369	309	337	30.2	9	411	287	305	334	67	20.1
I-10	Norfloxacin	SO	127	119	118	121	4.9	4	128	145	164	146	18	12.3	61	95	78	78	17	21.8	111	156	198	155	43.5	28.1
		SA	74	73	69	72	2.6	3.6	87	87	74	83	7.5	9	90	94	95	93	2.6	2.8	97	80	89	89	8.5	9.6
I-11	Omeprazole	SO	55	51	62	56	5.6	10	38	22	4	21	17	/	-22	11	-28	-13	21	/	-16	54	28	22	35.4	/
		SA	7	10	5	7	2.5	/	33	40	36	36	3.5	9.7	36	42	38	39	3.1	7.9	36	24	45	35	10.5	30
I-12	Quinine HCl	SO	264	234	255	251	15.4	6.1	279	244	201	241	39.1	16.2	181	231	214	209	25.4	12.2	251	269	363	294	60.1	20.4
		SA	132	104	118	118	14	11.9	158	146	123	142	17.8	12.5	200	219	216	212	10.2	4.8	163	149	190	167	20.8	12.5
I-13	Sulisobenzone	SO	3	2	3	3	0.6	/	0	3	3	2	1.7	/	-4	3	-12	-4	7.5	/	-5	1	-1	-2	3.1	/
		SA	-24	-20	-24	-23	2.3	/	-11	-4	-2	-6	4.7	/	-9	-4	-13	-9	4.5	/	-1	-2	-3	-2	1	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

Table 6A Judgment from the Phase 1-1 results: Final judgment of positive when positive results were obtained in at least one of three assays.

20 μ M

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Judgment ^b
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
I-5	Diclofenac	-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	I
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
I-8	Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacine	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

I : inconclusive (the number of positive equals that of negative in integrated judgment)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 6A Judgment from the Phase 1-1 results: Final judgment of positive when positive results were obtained in at least one of three assays (continued).

200 μ M

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Judgment ^b
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacina	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 6B Judgment from the Phase 1-1 results: Final judgment based on the mean value of three assays.

20 µM

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^b
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
I-5	Diclofenac	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
I-8	Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	I
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥25 or Superoxide anion results ≥20)

- : negative(Singlet oxygen results <25 and Superoxide anion results <20)

I : inconclusive (the number of positive equals that of negative in integrated judgment)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 µM and 200 µM in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 6B Judgment from the Phase 1-1 results: Final judgment based on the mean value of three assays (continued).
200 µM

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^b
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 µM and 200 µM in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 6C Judgment from the Phase 1-1 results: Final judgment based on the majority of three assay results.

20 µM

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^b
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
I-5	Diclofenac	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
I-8	Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	I
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥25 or Superoxide anion results ≥20)

- : negative(Singlet oxygen results <25 and Superoxide anion results <20)

I : inconclusive (the number of positive equals that of negative in integrated judgment)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 µM and 200 µM in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 6C Judgment from the Phase 1-1 results: Final judgment based on the majority of three assay results (continued).
200 µM

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^b
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 µM and 200 µM in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 6D Judgment from the Phase 1-1 results: Final judgment on the first assay results.

20 µM

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^b
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+
I-5	Diclofenac	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
I-8	Ketoprofen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	I
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥25 or Superoxide anion results ≥20)

- : negative(Singlet oxygen results <25 and Superoxide anion results <20)

I : inconclusive (the number of positive equals that of negative in integrated judgment)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 µM and 200 µM in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 6D Judgment from the Phase 1-1 results: Final judgment on the first assay results (continued).

200 µM

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^b
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥25 or Superoxide anion results ≥20)

- : negative(Singlet oxygen results <25 and Superoxide anion results <20)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 µM and 200 µM in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 7A Contingency table for the Phase 1-1 results: Final judgment of positive when positive results were obtained in at least one of three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 4		ROS		Total
		+	-	
Phototoxic	+	8	4	12
	-	0	1	1
Total		8	5	13

Sensitivity : 66.7% (8/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 20.0% (1/5)
 Accuracy : 69.2% (9/13)

Lab 5		ROS		Total
		+	-	
Phototoxic	+	9	3	12
	-	0	1	1
Total		9	4	13

Sensitivity : 75.0% (9/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (9/9)
 Negative predictivity : 25.0% (1/4)
 Accuracy : 76.9% (10/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	6	6	12
	-	0	1	1
Total		6	7	13

Sensitivity : 50.0% (6/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (6/6)
 Negative predictivity : 14.3% (1/7)
 Accuracy : 53.8% (7/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	7	5	12
	-	0	1	1
Total		7	6	13

Sensitivity : 58.3% (7/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (7/7)
 Negative predictivity : 16.7% (1/6)
 Accuracy : 61.5% (8/13)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	7	4	(1)	11(12)
	-	0	1	(0)	1(1)
Total		7	5	(1)	12(13)

Sensitivity : 63.6% (7/11)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (7/7)
 Negative predictivity : 20.0% (1/5)
 Accuracy : 66.7% (8/12)

Table 7B Contingency table for the Phase 1-1 results: Final judgment based on the mean value of three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 4		ROS		Total
		+	-	
Phototoxic	+	8	4	12
	-	0	1	1
Total		8	5	13

Sensitivity : 66.7% (8/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 20.0% (1/5)
 Accuracy : 69.2% (9/13)

Lab 5		ROS		Total
		+	-	
Phototoxic	+	8	4	12
	-	0	1	1
Total		8	5	13

Sensitivity : 66.7% (8/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 20.0% (1/5)
 Accuracy : 69.2% (9/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	3	9	12
	-	0	1	1
Total		3	10	13

Sensitivity : 25.0% (3/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (3/3)
 Negative predictivity : 10.0% (1/10)
 Accuracy : 30.8% (4/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	6	6	12
	-	0	1	1
Total		6	7	13

Sensitivity : 50.0% (6/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (6/6)
 Negative predictivity : 14.3% (1/7)
 Accuracy : 53.8% (7/13)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	6	5	(1)	11(12)
	-	0	1	(0)	1(1)
Total		6	6	(1)	12(13)

Sensitivity : 54.5% (6/11)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (6/6)
 Negative predictivity : 16.7% (1/6)
 Accuracy : 58.3% (7/12)

Table 7C Contingency table for the Phase 1-1 results: Final judgment based on the majority of three assay results.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 4		ROS		Total
		+	-	
Phototoxic	+	8	4	12
	-	0	1	1
Total		8	5	13

Sensitivity : 66.7% (8/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 20.0% (1/5)
 Accuracy : 69.2% (9/13)

Lab 5		ROS		Total
		+	-	
Phototoxic	+	7	5	12
	-	0	1	1
Total		7	6	13

Sensitivity : 58.3% (8/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (7/7)
 Negative predictivity : 16.7% (1/6)
 Accuracy : 61.5% (8/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	3	9	12
	-	0	1	1
Total		3	10	13

Sensitivity : 25.0% (3/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (3/3)
 Negative predictivity : 10.0% (1/10)
 Accuracy : 30.8% (4/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	6	6	12
	-	0	1	1
Total		6	7	13

Sensitivity : 50.0% (6/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (6/6)
 Negative predictivity : 14.3% (1/7)
 Accuracy : 53.8% (7/13)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	6	5	(1)	11(12)
	-	0	1	(0)	1(1)
Total		6	6	(1)	12(13)

Sensitivity : 54.5% (6/11)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (6/6)
 Negative predictivity : 16.7% (1/6)
 Accuracy : 58.3% (7/12)

Table 7D Contingency table for the Phase 1-1 results: Final judgment on the first assay results.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 4		ROS		Total
		+	-	
Phototoxic	+	7	5	12
	-	0	1	1
Total		7	6	13

Sensitivity : 58.3% (7/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (7/7)
 Negative predictivity : 16.7% (1/6)
 Accuracy : 61.5% (8/13)

Lab 5		ROS		Total
		+	-	
Phototoxic	+	8	4	12
	-	0	1	1
Total		8	5	13

Sensitivity : 66.7% (8/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 20.0% (1/5)
 Accuracy : 69.2% (9/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	4	8	12
	-	0	1	1
Total		4	9	13

Sensitivity : 33.3% (4/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (4/4)
 Negative predictivity : 11.1% (1/9)
 Accuracy : 38.5% (5/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	6	6	12
	-	0	1	1
Total		6	7	13

Sensitivity : 50.0% (6/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (6/6)
 Negative predictivity : 14.3% (1/7)
 Accuracy : 53.8% (7/13)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	6	5	(1)	11(12)
	-	0	1	(0)	1(1)
Total		6	6	(1)	12(13)

Sensitivity : 54.5% (6/11)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (6/6)
 Negative predictivity : 16.7% (1/6)
 Accuracy : 58.3% (7/13)

Table 7E Contingency table for the Phase 1-1 results: The final judgments were the same in all of the analysis methods.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 200 μ M

Lab 4		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Lab 5		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Table 8 Within-laboratory variation of Phase 1-2 study

ROS assay Validation data (seric)			Lab 5				Lab 6				Lab 7			
Chemicals			N	Mean	SD	CV (%)	N	Mean	SD	CV (%)	N	Mean	SD	CV (%)
Intra-day														
Positive	Quinine HCl	SO	4	360	21.0	5.8	4	377	12.4	3.3	4	401	15.0	3.7
Control		SA	4	235	23.6	10.0	4	271	21.0	7.7	4	268	14.0	5.2
Negative	Sulisobenzone	SO	4	0	2.3	/	4	2	2.2	/	4	-1	1.0	/
Control		SA	4	-15	13.5	/	4	-3	0.5	/	4	-11	4.0	/
Inter-day														
Positive	Quinine HCl	SO	2	370	8.5	2.2	2	359	0.7	0.2	3	410	17.7	4.3
Control		SA	2	235	13.4	5.7	2	246	2.1	0.9	3	290	37.3	12.9
Negative	Sulisobenzone	SO	2	3	0.7	/	2	-4	5.7	/	3	-5	4	/
Control		SA	2	-6	2.8	/	2	-1	2.8	/	3	-10	6.5	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Intra-day variations were calculated by selecting the date on which a number of assay results were obtained most.

Inter-day variations were calculated using the assay results of the first time of the each assay day.

Table 9 Between-laboratory variation of Phase 1-2 study

ROS assay Validation data (seric)			N	Mean	SD	CV (%)
Positive	Quinine HCl	SO	3	382	22.7	5.9
Control		SA	3	262	27.2	10.4
Negative	Sulisobenzone	SO	3	-1	1.7	/
Control		SA	3	-7	4.7	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Between-laboratory variations were calculated from the average value of the results of each facility.

Table 10 Results of the ROS assay multi-center variation Phase 1-2 study

20 μM

ROS assay Validation data (seric)			Lab 5					Lab 6					Lab 7				
No.	Chemicals		Assay		Mean	SD	CV (%)	Assay		Mean	SD	CV (%)	Assay		Mean	SD	CV (%)
	Name		1st	2nd				1st	2nd				1st	2nd			
I-1	5-FU	SO	1	1	1	0	/	0	2	1	1.4	/	3	-7	-2	7.1	/
		SA	4	5	5	0.7	/	-1	-1	-1	0	/	-6	-7	-7	0.7	/
I-2	8-MOP	SO	8	10	9	1.4	/	9	11	10	1.4	/	11	5	8	4.2	/
		SA	25	7	16	12.7	/	2	1	2	0.7	/	8	3	6	3.5	/
I-3	Amiodarone HCl ^a	SO	86	43	65	30.4	46.8	80	90	85	7.1	8.4	113	85	99	19.8	20
		SA	30	16	23	9.9	43	3	2	3	0.7	/	-16	-16	-16	0	/
I-4	Chlorpromazine HCl	SO	87	92	90	3.5	3.9	50	66	58	11.3	19.5	109	102	106	4.9	4.6
		SA	30	27	29	2.1	7.2	15	13	14	1.4	/	-1	0	-1	0.7	/
I-5	Diclofenac	SO	29	27	28	1.4	5	31	31	31	0	0	39	11	25	19.8	79.2
		SA	15	17	16	1.4	/	9	6	8	2.1	/	7	2	5	3.5	/
I-6	Doxycycline HCl	SO	78	94	86	11.3	13.1	67	76	72	6.4	8.9	60	52	56	5.7	10.2
		SA	68	60	64	5.7	8.9	59	48	54	7.8	14.4	42	40	41	1.4	3.4
I-7	Furosemide	SO	17	17	17	0	/	21	32	27	7.8	28.9	31	18	25	9.2	36.8
		SA	-6	-1	-4	3.5	/	4	3	4	0.7	/	-4	-5	-5	0.7	/
I-8	Ketoprofen	SO	30	33	32	2.1	6.6	23	23	23	0	/	23	26	25	2.1	8.4
		SA	11	12	12	0.7	/	4	7	6	2.1	/	0	-1	-1	0.7	/
I-9	Levofloxacin	SO	28	42	35	9.9	28.3	31	36	34	3.5	10.3	31	35	33	2.8	8.5
		SA	209	223	216	9.9	4.6	158	154	156	2.8	1.8	207	188	198	13.4	6.8
I-10	Norfloxacin	SO	51	51	51	0	0	38	36	37	1.4	3.8	42	44	43	1.4	3.3
		SA	22	24	23	1.4	6.1	15	12	14	2.1	/	10	9	10	0.7	/
I-11	Omeprazole	SO	37	41	39	2.8	7.2	44	42	43	1.4	3.3	31	28	30	2.1	7
		SA	57	47	52	7.1	13.7	28	28	28	0	0	24	23	24	0.7	2.9
I-12	Quinine HCl	SO	81	72	77	6.4	8.3	73	71	72	1.4	1.9	75	85	80	7.1	8.9
		SA	64	55	60	6.4	10.7	50	50	50	0	0	54	55	55	0.7	1.3
I-13	Sulisobenzone	SO	3	2	3	0.7	/	-1	-3	-2	1.4	/	4	6	5	1.4	/
		SA	-14	-3	-9	7.8	/	0	-1	-1	0.7	/	-8	-9	-9	0.7	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

a : Since the precipitation was observed at a concentration of 20 μM of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μM and 200 μM in this study.

Table 10 Results of the ROS assay multi-center variation Phase 1-2 study (continued)

200 μ M

ROS assay Validation data (seric)			Lab 5					Lab 6					Lab 7				
No.	Chemicals Name		Assay		Mean	SD	CV (%)	Assay		Mean	SD	CV (%)	Assay		Mean	SD	CV (%)
			1st	2nd				1st	2nd				1st	2nd			
I-1	5-FU	SO	1	2	2	0.7	/	0	3	2	2.1	/	-4	0	-2	2.8	/
		SA	5	5	5	0.0	/	1	2	2	0.7	/	3	4	4	0.7	/
I-2	8-MOP	SO	89	76	83	9.2	11.1	53	76	65	16.3	25.1	48	52	50	2.8	5.6
		SA	69	73	71	2.8	3.9	29	29	29	0.0	0.0	60	75	68	10.6	15.6
I-3	Amiodarone HCl ^a	SO	400	372	386	19.8	5.1	350	417	384	47.4	12.3	291	205	248	60.8	24.5
		SA	192	253	223	43.1	19.3	116	-66	25	128.7	514.8	-212	-216	-214	2.8	/
I-4	Chlorpromazine HCl	SO	31	28	30	2.1	7.0	12	-11	1	16.3	/	24	24	24	0.0	/
		SA	89	84	87	3.5	4.0	72	75	74	2.1	2.8	80	73	77	4.9	6.4
I-5	Diclofenac	SO	118	122	120	2.8	2.3	97	76	87	14.8	17.0	141	147	144	4.2	2.9
		SA	140	135	138	3.5	2.5	127	126	127	0.7	0.6	127	120	124	4.9	4.0
I-6	Doxycycline HCl	SO	297	300	299	2.1	0.7	278	237	258	29.0	11.2	280	293	287	9.2	3.2
		SA	357	370	364	9.2	2.5	329	321	325	5.7	1.8	359	342	351	12.0	3.4
I-7	Furosemide	SO	76	70	73	4.2	5.8	70	34	52	25.5	49.0	82	84	83	1.4	1.7
		SA	31	34	33	2.1	6.4	30	30	30	0.0	0.0	38	32	35	4.2	12.0
I-8	Ketoprofen	SO	179	174	177	3.5	2.0	166	169	168	2.1	1.3	163	165	164	1.4	0.9
		SA	81	113	97	22.6	23.3	104	92	98	8.5	8.7	108	99	104	6.4	6.2
I-9	Levofloxacin	SO	143	162	153	13.4	8.8	128	131	130	2.1	1.6	141	129	135	8.5	6.3
		SA	380	351	366	20.5	5.6	430	437	434	4.9	1.1	418	414	416	2.8	0.7
I-10	Norfloxacin	SO	181	192	187	7.8	4.2	136	136	136	0.0	0.0	190	182	186	5.7	3.1
		SA	92	120	106	19.8	18.7	101	105	103	2.8	2.7	123	109	116	9.9	8.5
I-11	Omeprazole	SO	-34	0	-17	24.0	/	-249	-260	-255	7.8	/	24	0	12	17.0	/
		SA	28	74	51	32.5	63.7	201	204	203	2.1	1.0	77	70	74	4.9	6.6
I-12	Quinine HCl	SO	359	346	353	9.2	2.6	381	353	367	19.8	5.4	448	389	419	41.7	10.0
		SA	203	252	228	34.6	15.2	249	241	245	5.7	2.3	341	290	316	36.1	11.4
I-13	Sulisobenzone	SO	0	2	1	1.4	/	1	0	1	0.7	/	5	0	3	3.5	/
		SA	-38	-7	-23	21.9	/	-4	-3	-4	0.7	/	-8	-9	-9	0.7	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

Table 11A Judgment from the Phase 1-2 results: Final judgment of positive when positive results were obtained in at least one of two assays.

20 μ M

ROS assay Validation data (seric) pattern A		Lab 5			Lab 6			Lab 7			Integrated
No.	Chemicals Name	Assay		Final Judgment	Assay		Final Judgment	Assay		Final Judgment	Judgment ^b
		1st	2nd		1st	2nd		1st	2nd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	-	+	-	-	-	-	-	-	-
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	-	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	-	-	-	-	+	+	+	-	+	+
I-8	Ketoprofen	+	+	+	-	-	-	-	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacine	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 11A Judgment from the Phase 1-2 results: Final judgment of positive when positive results were obtained in at least one of two assays (continued).

200 μ M

ROS assay Validation data (seric) pattern A		Lab 5			Lab 6			Lab 7			Integrated
No.	Chemicals Name	Assay		Final Judgment	Assay		Final Judgment	Assay		Final Judgment	Judgment ^b
		1st	2nd		1st	2nd		1st	2nd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacine	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 11B Judgment from the Phase 1-2 results: Final judgment based on the mean value of two assays.
20 μ M

ROS assay Validation data (seric) pattern B		Lab 5			Lab 6			Lab 7			Integrated
No.	Chemicals Name	Assay		Final Judgment	Assay		Final Judgment	Assay		Final Judgment	Judgment ^b
		1st	2nd		1st	2nd		1st	2nd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	-	-	-	-	-	-	-	-	-
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	-	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	-	-	-	-	+	+	+	-	+	+
I-8	Ketoprofen	+	+	+	-	-	-	-	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacine	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 11B Judgment from the Phase 1-2 results: Final judgment based on the mean value of two assays (continued).
200 μ M

ROS assay Validation data (seric) pattern B		Lab 5			Lab 6			Lab 7			Integrated
No.	Chemicals Name	Assay		Final Judg-	Assay		Final Judg-	Assay		Final Judg-	Judgment ^b
		1st	2nd	ment	1st	2nd	ment	1st	2nd	ment	
I-1	5-FU	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacine	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 11C Judgment from the Phase 1-2 results: Final judgment based on the majority of two assay results.
20 μ M

ROS assay Validation data (seric) pattern C		Lab 5			Lab 6			Lab 7			Integrated
No.	Chemicals Name	Assay		Final Judgment	Assay		Final Judgment	Assay		Final Judgment	Judgment ^b
		1st	2nd		1st	2nd		1st	2nd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	-	I	-	-	-	-	-	-	-
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	-	I	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	-	-	-	-	+	I	+	-	I	I
I-8	Ketoprofen	+	+	+	-	-	-	-	+	I	I
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacine	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

I : inconclusive (the number of positive equals that of negative in final judgment or the number of positive equals that of negative and inconclusive in integrated judgment)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 11C Judgment from the Phase 1-2 results: Final judgment based on the majority of two assay results (continued).
200 μ M

ROS assay Validation data (seric) pattern C		Lab 5			Lab 6			Lab 7			Integrated
No.	Chemicals Name	Assay		Final Judg-	Assay		Final Judg-	Assay		Final Judg-	Judgment ^b
		1st	2nd	ment	1st	2nd	ment	1st	2nd	ment	
I-1	5-FU	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacine	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 11D Judgment from the Phase 1-2 results: Final judgment on the first assay results.
20 μ M

ROS assay Validation data (seric) pattern D		Lab 5			Lab 6			Lab 7			Integrated
No.	Chemicals Name	Assay		Final Judg-	Assay		Final Judg-	Assay		Final Judg-	Judgment ^b
		1st	2nd	ment	1st	2nd	ment	1st	2nd	ment	
I-1	5-FU	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	-	+	-	-	-	-	-	-	-
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	-	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	-	-	-	-	+	-	+	-	+	-
I-8	Ketoprofen	+	+	+	-	-	-	-	+	-	-
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacine	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 11D Judgment from the Phase 1-2 results: Final judgment on the first assay results (continued).
200 μ M

ROS assay Validation data (seric) pattern D		Lab 5			Lab 6			Lab 7			Integrated
No.	Chemicals Name	Assay		Final Judg-	Assay		Final Judg-	Assay		Final Judg-	Judgment ^b
		1st	2nd	ment	1st	2nd	ment	1st	2nd	ment	
I-1	5-FU	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone HCl ^a	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+
I-10	Norfloracin	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+
I-12	Quinine HCl	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the Phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 12A Contingency table for the Phase 1-2 results at 20 μ M: Final judgment of positive when positive results were obtained in at least one of two assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 5		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	9	3	12
	-	0	1	1
Total		9	4	13

Sensitivity : 75.0% (9/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (9/9)
 Negative predictivity : 25.0% (1/4)
 Accuracy : 76.9% (10/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Table 12B Contingency table for the Phase 1-2 results at 20 μ M: Final judgment based on the mean value of two assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 5		ROS		Total
		+	-	
Phototoxic	+	9	3	12
	-	0	1	1
Total		9	4	13

Sensitivity : 75.0% (9/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (9/9)
 Negative predictivity : 25.0% (1/4)
 Accuracy : 76.9% (10/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	9	3	12
	-	0	1	1
Total		9	4	13

Sensitivity : 75.0% (9/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (9/9)
 Negative predictivity : 25.0% (1/4)
 Accuracy : 76.9% (10/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Table 12C Contingency table for the Phase 1-2 results at 20 μ M: Final judgment based on the majority of two assay results.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	9	2	(1)	11(12)
	-	0	1	(0)	1(1)
Total		9	3	(1)	12(13)

Sensitivity : 81.8% (9/11)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (9/9)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 83.3% (10/12)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	8	3	(1)	11(12)
	-	0	1	(0)	1(1)
Total		8	4	(1)	12(13)

Sensitivity : 72.7% (8/11)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 25.0% (1/4)
 Accuracy : 75.0% (9/12)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	7	2	(3)	9(12)
	-	0	1	(0)	1(1)
Total		7	3	(3)	10(13)

Sensitivity : 77.8% (7/9)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (7/7)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 80.0% (8/10)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	8	2	(2)	10(12)
	-	0	1	(0)	1(1)
Total		8	3	(2)	11(13)

Sensitivity : 80.0% (8/10)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 81.8% (9/11)

Table 12D Contingency table for the Phase 1 results at 20 μ M: Final judgment on the first assay results.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 5		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	8	4	12
	-	0	1	1
Total		8	5	13

Sensitivity : 66.7% (8/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 20.0% (1/5)
 Accuracy : 69.2% (9/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	9	3	12
	-	0	1	1
Total		9	4	13

Sensitivity : 75.0% (9/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (9/9)
 Negative predictivity : 25.0% (1/4)
 Accuracy : 76.9% (10/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	8	4	12
	-	0	1	1
Total		8	5	13

Sensitivity : 66.7% (8/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (8/8)
 Negative predictivity : 20.0% (1/5)
 Accuracy : 69.2% (9/13)

Table 12E Contingency table for the Phase 1 results at 200 μM : The final judgments were the same in all of the analysis methods.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 200 μM

Lab 5		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Lab 6		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Lab 7		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Table 13 Irradiance and Temperature during the irradiation in the Phase 2 study

			Lab 4					Lab 5					Lab 6					Lab 7				
			Mean	SD	CV (%)	Max	Min	Mean	SD	CV (%)	Max	Min	Mean	SD	CV (%)	Max	Min	Mean	SD	CV (%)	Max	Min
Irradiance (mW/cm ²)	Beginning of Irradiation	A	4.8	0.02	0.4	4.9	4.8	4.7	0	/	4.7	4.7	3.3	0	/	3.3	3.3	3.6	0.09	2.5	3.7	3.5
		B	3.9	0.04	1.0	4.0	3.9	5	0	/	5.0	5.0	3.5	0	/	3.5	3.5	3.1	0.09	2.9	3.2	3.0
	End of Irradiation	A	4.8	0.04	0.8	4.9	4.8	4.7	0	/	4.7	4.7	3.3	0	/	3.3	3.3	3.6	0.08	2.2	3.7	3.5
		B	3.9	0.05	1.3	4.0	3.9	5	0	/	5.0	5.0	3.5	0	/	3.5	3.5	3.1	0.08	2.6	3.2	3.0
Temperature (°C)	Beginning of Irradiation		26.9	1.14	4.2	28.6	24.7	25.4	0.5	2.0	26.0	25.0	25.3	0.46	1.8	26.0	24.5	27.6	0.62	2.2	29.0	26.7
	End of Irradiation		26.7	0.8	3.0	27.8	25.1	25.2	0.59	2.3	26.0	24.0	25.8	0.52	2	27.0	25.0	27.5	0.54	2.0	28.2	26.5

A : Irradiances which were measured with each test facility's UVA detector.

B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr. Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the values from the UVA detectors.

CV : Coefficient of variation

Table 14 Within-laboratory variation of Phase 2 study

ROS assay Validation data (seric)			Lab 4				Lab 5				Lab 6				Lab 7			
						CV				CV				CV				CV
Chemicals			N	Mean	SD	(%)	N	Mean	SD	(%)	N	Mean	SD	(%)	N	Mean	SD	(%)
Intra-day																		
Positive	Quinine HCl	SO	3	478	9.2	1.9	5	548	16.9	3.1	5	397	14.9	3.8	6	443	8.3	1.9
Control		SA	3	259	1.5	0.6	4	270	32.4	12.0	5	274	9	3.3	6	291	20.9	7.2
Negative	Sulisobenzone	SO	3	0	2.6	/	5	0	5.8	/	5	2	0.5	/	6	1	0.9	/
Control		SA	3	-6	1.0	/	4	-8	0.8	/	5	-8	1.3	/	6	-2	0.4	/
Inter-day																		
Positive	Quinine HCl	SO	18	484	18.0	3.7	6	542	24.8	4.6	9	384	15.8	4.1	4	440	10.5	2.4
Control		SA	17	248	13.2	5.3	6	270	22.4	8.3	9	283	14.9	5.3	5	289	12.8	4.4
Negative	Sulisobenzone	SO	18	-1	2.4	/	6	1	4.7	/	9	0	1.6	/	4	0	2.3	/
Control		SA	17	-9	4.2	/	6	-6	1.5	/	9	-7	0.8	/	5	-3	1.8	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Intra-day variations were calculated by selecting the date on which a number of assay results were obtained most.

Inter-day variations were calculated using the assay results of the first time of the each assay day.

Table 15 Between-laboratory variation of Phase 2 study

ROS assay Validation data (seric)						CV
Chemicals			N	Mean	SD	(%)
Positive	Quinine HCl	SO	4	462	66.4	14.4
Control		SA	4	268	16.0	6.0
Negative	Sulisobenzone	SO	4	0	1	/
Control		SA	4	-7	2.1	/

SO : Singlet oxygen, SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Between-laboratory variations were calculated from the average value of the results of each facility.

Table 16 Results of the ROS assay multi-center validation phase 2 study (continued).

ROS assay Validation data (serie)		Lab 4							Lab 5					Lab 6					Lab 7														
N0.	Chemicals Name	Conc (µM)	Assay			Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)				
			1st	2nd	3rd					1st	2nd	3rd					1st	2nd	3rd					1st	2nd	3rd				1st	2nd	3rd	
II-24	Aspirin	SO	200	1	0	0	0	0.6	200	-1	1	-2	-1	1.5	200	4	2	3	3	1.0	200	-1	-2	1	-1	1.5	200	-1	-2	1	-1	1.5	
		SA	200	-2	-1	-2	-2	0.6	200	-3	-8	-2	-4	3.2	200	0	0	-1	0	0.6	200	-1	0	-1	-1	0.6	200	-1	0	-1	-1	0.6	
II-25	Benzocaine	SO	200	1	1	1	1	0	200	1	-2	-1	-1	1.5	200	0	0	7	2	4.0	200	-1	0	5	1	3.2	200	-1	0	5	1	3.2	
		SA	200	7	4	5	5	1.5	200	9	7	10	9	1.5	200	5	6	4	5	1.0	200	1	-7	1	-2	4.6	200	1	-7	1	-2	4.6	
II-26	Erythromycin	SO	200	-2	-10	-1	-4	4.9	200	-7	-4	2	-3	4.6	200	-3	-2	-3	-3	0.6	200	3	-10	-7	-5	6.8	200	3	-10	-7	-5	6.8	
		SA	200	9	2	1	4	4.4	200	3	2	4	3	1.0	200	5	4	6	5	1.0	200	-1	3	1	1	2.0	200	-1	3	1	1	2.0	
II-27	Penicillin G	SO	200	-1	2	1	1	1.5	200	1	8	0	3	4.4	200	0	3	2	2	1.5	200	1	2	-1	1	1.5	200	1	2	-1	1	1.5	
		SA	200	39	39	33	37	3.5	9.5	200	49	43	47	46	3.1	6.7	200	37	37	44	39	4.0	10.3	200	13	9	5	9	4.0	200	13	9	5
II-28	Phenytoin	SO	200	0	2	0	1	1.2	200	0	6	-1	2	3.8	200	-3	-2	-2	-2	0.6	200	0	-7	1	-2	4.4	200	0	-7	1	-2	4.4	
		SA	200	63	62	70	65	4.4	6.8	200	93	82	101	92	9.5	10.3	200	75	65	57	66	9.0	13.6	200	53	37	12	34	20.7	60.9			
II-29	Bumetrizole	SO	20	-10	-18	-36	-21	13.3	20	-13	-18	-8	-13	5.0	20	-4	-1	1	-1	2.5	u	u	u	u	u	u	u	u	u	u	u		
		SA	20	8	7	13	9	3.2	u	u	u	u	u	u	u	20	16	13	18	16	2.5	20	6	8	10	8	2.0	20	6	8	10	8	2.0
II-30	Camphor sulfonic acid	SO	200	0	0	-1	0	0.6	200	0	1	-2	0	1.5	200	5	0	-1	1	3.2	200	3	1	0	1	1.5	200	3	1	0	1	1.5	
		SA	200	-2	0	-1	-1	1.0	200	-1	-6	-2	-3	2.6	200	-2	-3	0	-2	1.5	200	-1	-1	4	1	2.9	200	-1	-1	4	1	2.9	
II-31	Chlorhexidine	SO	200	5	4	7	5	1.5	200	-1	2	4	2	2.5	200	8	-6	18	7	12.1	200	4	1	-1	1	2.5	200	4	1	-1	1	2.5	
		SA	200	13	20	16	16	3.5	200	20	28	27	25	4.4	17.6	200	16	17	14	16	1.5	200	25	24	28	26	2.1	8.1					
II-32	Cinnamic acid	SO	200	0	0	0	0	0	200	-1	9	0	3	5.5	200	0	2	-1	0	1.5	200	2	0	1	1	1.0	200	2	0	1	1	1.0	
		SA	200	10	13	7	10	3.0	200	33	45	40	39	6.0	15.4	200	43	36	32	37	5.6	15.1	200	10	3	2	5	4.4					
II-33	Drometrizole	SO	u	u	u	u	u	u	u	u	u	u	u	u	50	5	1	3	3	2.0	u	u	u	u	u	u	u	u	u	u			
		SA	u	u	u	u	u	u	u	u	u	u	u	u	u	50	3	6	2	4	2.1	u	u	u	u	u	u	u	u	u	u		
II-34	L-Histidine	SO	200	-1	1	0	0	1.0	200	-2	-2	-1	-2	0.6	200	1	0	-1	0	1.0	200	-1	-4	1	-1	2.5	200	-1	-4	1	-1	2.5	
		SA	200	78	74	57	70	11.2	16	200	112	101	110	108	5.9	5.5	200	42	31	28	34	7.4	21.8	200	91	85	85	87	3.5	4			
II-35	Methylbenzylidene camphor	SO	50	36	25	44	35	9.5	27.1	50	30	11	25	22	9.8	20	-1	-3	-1	-2	1.2	20	-2	1	0	0	1.5	20	-2	1	0	0	1.5
		SA	50	-17	-24	-26	-22	4.7	20	-10	-12	-20	-14	5.3	20	2	3	1	2	1.0	20	-5	-2	0	-2	2.5							
II-36	Ocotrizole	SO	20	-6	-11	-18	-12	6.0	20	-14	-24	-28	-22	7.2	u	u	u	u	u	u	20	-21	-23	-34	-26	7.0	20	-21	-23	-34	-26	7.0	
		SA	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	20	28	27	25	27	1.5	5.6					
II-37	Octyl methacrylate	SO	20	4	0	3	2	2.1	100	18	25	18	20	4.0	50	4	7	4	5	1.7	u	u	u	u	u	u	u	u	u	u			
		SA	20	-1	-2	-2	-2	0.6	100	7	4	3	5	2.1	50	-3	-3	-1	-2	1.2	20	-15	3	-1	-4	9.5							
II-38	Octyl methoxycinnamate	SO	20	12	10	11	11	1.0	20	2	11	7	7	4.5	20	20	14	17	17	3.0	u	u	u	u	u	u	u	u	u	u			
		SA	50	23	26	16	22	5.1	23.2	20	35	21	17	24	9.5	39.6	20	10	17	17	15	4.0	20	-2	-4	0	-2	2.0					
II-39	Octyl salicylate	SO	20	4	6	4	5	1.2	100	7	18	5	10	7.0	20	1	4	3	3	1.5	20	7	3	4	5	2.1	20	7	3	4	5	2.1	
		SA	20	16	12	14	14	2.0	50	42	30	33	35	6.2	17.7	20	17	14	11	14	3.0	20	3	-1	-2	0	2.6						
II-40	PABA	SO	200	-1	1	-1	0	1.2	200	0	9	1	3	4.9	200	0	2	1	1	1.0	200	3	-1	1	1	2.0	200	3	-1	1	1	2.0	
		SA	200	0	0	0	0	0	200	-3	-5	-5	-4	1.2	200	-6	-8	-5	-6	1.5	200	-7	0	-3	-3	3.5							
II-41	SDS	SO	200	5	10	9	8	2.6	200	5	15	8	9	5.1	200	7	9	6	7	1.5	200	3	1	-3	0	3.1	200	3	1	-3	0	3.1	
		SA	200	31	22	25	26	4.6	17.7	200	13	19	18	17	3.2	20	8	10	7	8	1.5	u	u	u	u	u	u	u	u	u	u		
II-42	UV-571	SO	50	12	3	5	7	4.7	20	-10	-3	-3	-5	4.0	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u	u		
		SA	20	15	18	23	19	4.0	u	u	u	u	u	u	u	u	u	u	u	u	u	20	12	17	13	14	2.6						

SO : Singlet oxygen, SA : Superoxide anion

u : undetermined due to precipitation

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged photoreactive based on the mean value

Table 17A Judgment from the Phase 2 results: Final judgment of positive when positive results were obtained in at least one of three assays.

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-20	Avobenzene	+	+	+	+	+	+	+	+	I	I	I	I	+	+	+	+	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 17A Judgment from the Phase 2 results: Final judgment of positive when positive results were obtained in at least one of three assays (continued).

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated	
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^a	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd			
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+
II-28	Phenytoin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
II-29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	+	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
II-32	Cinnamic acid	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	I
II-33	Drometizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-34	L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	I	+	+	I	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	+	I
II-37	Octyl methacrylate	I	I	I	I	I	+	I	+	I	I	I	I	I	I	I	I	I	I
II-38	Octyl methoxycinnamate	+	+	I	+	+	+	I	+	I	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	+	+	+	+	I	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	+	+	+	+	-	-	-	-	I	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	+	+	I	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative or inconclusive in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 17B Judgment from the Phase 2 results: Final judgment based on the mean value of three assays.

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-20	Avobenzone	+	+	+	+	+	+	+	+	I	I	I	I	+	+	+	+	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 17B Judgment from the Phase 2 results: Final judgment based on the mean value of three assays (continued).

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
II-28	Phenytoin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
II-29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	+	-	-	+	+	+	+	-	-	-	-	+	+	+	+	I
II-32	Cinnamic acid	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	I
II-33	Drometizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-34	L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	I	+	I	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-37	Octyl methacrylate	I	I	I	I	I	+	I	I	I	I	I	I	I	I	I	I	I
II-38	Octyl methoxycinnamate	+	+	I	+	+	+	I	+	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	+	+	+	+	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	+	+	+	+	-	-	-	-	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	+	I	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative or inconclusive in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 17C Judgment from the Phase 2 results: Final judgment based on the majority of three assay results.

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-20	Avobenzene	+	+	+	+	+	+	+	+	I	I	I	I	+	+	+	+	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 17C Judgment from the Phase 2 results: Final judgment based on the majority of three assay results (continued).

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
II-28	Phenytoin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
II-29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	+	-	-	+	+	+	+	-	-	-	-	+	+	+	+	I
II-32	Cinnamic acid	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	I
II-33	Drometizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-34	L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	I	+	+	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-37	Octyl methacrylate	I	I	I	I	I	+	I	I	I	I	I	I	I	I	I	I	I
II-38	Octyl methoxycinnamate	+	+	I	+	+	+	I	+	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	+	+	+	+	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	+	+	+	+	-	-	-	-	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	+	I	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative or inconclusive in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 17D Judgment from the Phase 2 results: Final judgment on the first assay results.

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-20	Avobenzene	+	+	+	+	+	+	+	+	I	I	I	I	+	+	+	+	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 17D Judgment from the Phase 2 results: Final judgment on the first assay results (continued).

ROS assay Validation data (seric) pattern D		Lab 4			Lab 5				Lab 6				Lab 7				Integrated	
No.	Chemicals Name	Assay			Final Judge- ment	Assay			Final Judge- ment	Assay			Final Judge- ment	Assay			Final Judge- ment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
II-28	Phenytoin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
II-29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	+	-	-	+	+	+	+	-	-	-	-	+	+	+	+	I
II-32	Cinnamic acid	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	I
II-33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-34	L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	I	+	+	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-37	Octyl methacrylate	I	I	I	I	I	+	I	I	I	I	I	I	I	I	I	I	I
II-38	Octyl methoxycinnamate	+	+	I	+	+	+	I	+	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	+	+	+	+	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	+	+	+	+	-	-	-	-	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	+	I	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative or inconclusive in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 18A Contingency table for Phase 2 results: Final judgment of positive when positive results were obtained in at least one of three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	8	6	(5)	14(19)
Total		29	6	(7)	35(42)

Sensitivity : 100% (21/21)
 Specificity : 42.9% (6/14)
 Positive predictivity : 72.4% (21/29)
 Negative predictivity : 100% (6/6)
 Accuracy : 77.1% (27/35)

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	9	6	(4)	15(19)
Total		30	6	(6)	36(42)

Sensitivity : 100% (21/21)
 Specificity : 40.0% (6/15)
 Positive predictivity : 70.0% (21/30)
 Negative predictivity : 100% (6/6)
 Accuracy : 75.0% (27/36)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	20	0	(3)	20(23)
	-	4	6	(9)	10(19)
Total		24	6	(12)	30(42)

Sensitivity : 100% (20/20)
 Specificity : 60.0% (6/10)
 Positive predictivity : 83.3% (20/24)
 Negative predictivity : 100% (6/6)
 Accuracy : 86.7% (26/30)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	4	7	(8)	11(19)
Total		25	7	(10)	32(42)

Sensitivity : 100% (21/21)
 Specificity : 63.6% (7/11)
 Positive predictivity : 84.0% (21/25)
 Negative predictivity : 100% (7/7)
 Accuracy : 87.5% (28/32)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	4	5	(10)	9(19)
Total		25	5	(12)	30(42)

Sensitivity : 100% (21/21)
 Specificity : 55.6% (5/9)
 Positive predictivity : 84.0% (21/25)
 Negative predictivity : 100% (5/5)
 Accuracy : 86.7% (26/30)

Table 18B Contingency table for Phase 2 results: Final judgment based on the mean value of three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	6	7	(6)	13(19)
Total		27	7	(8)	34(42)

Sensitivity : 100% (21/21)
 Specificity : 53.8% (7/13)
 Positive predictivity : 77.8% (21/27)
 Negative predictivity : 100% (7/7)
 Accuracy : 82.4% (28/34)

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	7	6	(6)	13(19)
Total		28	6	(8)	34(42)

Sensitivity : 100% (21/21)
 Specificity : 46.2% (6/13)
 Positive predictivity : 75.0% (21/28)
 Negative predictivity : 100% (6/6)
 Accuracy : 79.4% (27/34)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	20	0	(3)	20(23)
	-	4	6	(9)	10(19)
Total		24	6	(12)	30(42)

Sensitivity : 100% (20/20)
 Specificity : 60.0% (6/10)
 Positive predictivity : 83.3% (20/24)
 Negative predictivity : 100% (6/6)
 Accuracy : 86.7% (26/30)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	4	7	(8)	11(19)
Total		25	7	(10)	32(42)

Sensitivity : 100% (21/21)
 Specificity : 63.6% (7/11)
 Positive predictivity : 84.0% (21/25)
 Negative predictivity : 100% (7/7)
 Accuracy : 87.5% (28/32)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	3	5	(11)	8(19)
Total		24	5	(13)	29(42)

Sensitivity : 100% (21/21)
 Specificity : 62.5% (5/8)
 Positive predictivity : 87.5% (21/24)
 Negative predictivity : 100% (5/5)
 Accuracy : 89.7% (26/29)

Table 18C Contingency table for Phase 2 results: Final judgment based on the majority of three assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	6	7	(6)	13(19)
Total		27	7	(8)	34(42)

Sensitivity : 100% (21/21)
 Specificity : 53.8% (7/13)
 Positive predictivity : 77.8% (21/27)
 Negative predictivity : 100% (7/7)
 Accuracy : 82.4% (28/34)

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	8	6	(5)	14(19)
Total		29	6	(7)	35(42)

Sensitivity : 100% (21/21)
 Specificity : 42.9% (6/14)
 Positive predictivity : 72.4% (21/29)
 Negative predictivity : 100% (6/6)
 Accuracy : 77.1% (27/35)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	20	0	(3)	20(23)
	-	4	6	(9)	10(19)
Total		24	6	(12)	30(42)

Sensitivity : 100% (20/20)
 Specificity : 60.0% (6/10)
 Positive predictivity : 83.3% (20/24)
 Negative predictivity : 100% (6/6)
 Accuracy : 86.7% (26/30)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	4	7	(8)	11(19)
Total		25	7	(10)	32(42)

Sensitivity : 100% (21/21)
 Specificity : 63.6% (7/11)
 Positive predictivity : 84.0% (21/25)
 Negative predictivity : 100% (7/7)
 Accuracy : 87.5% (28/32)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	3	5	(11)	8(19)
Total		24	5	(13)	29(42)

Sensitivity : 100% (21/21)
 Specificity : 62.5% (5/8)
 Positive predictivity : 87.5% (21/24)
 Negative predictivity : 100% (5/5)
 Accuracy : 89.7% (26/29)

Table 18D Contingency table for Phase 2 results: Final judgment on the first assay results.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	6	7	(6)	13(19)
Total		27	7	(8)	34(42)

Sensitivity : 100% (21/21)
 Specificity : 53.8% (7/13)
 Positive predictivity : 77.8% (21/27)
 Negative predictivity : 100% (7/7)
 Accuracy : 82.4% (28/34)

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	8	6	(5)	14(19)
Total		29	6	(7)	35(42)

Sensitivity : 100% (21/21)
 Specificity : 42.9% (6/14)
 Positive predictivity : 72.4% (21/29)
 Negative predictivity : 100% (6/6)
 Accuracy : 77.1% (27/35)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	20	0	(3)	20(23)
	-	4	6	(9)	10(19)
Total		24	6	(12)	30(42)

Sensitivity : 100% (20/20)
 Specificity : 60.0% (6/10)
 Positive predictivity : 83.3% (20/24)
 Negative predictivity : 100% (6/6)
 Accuracy : 86.7% (26/30)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	4	7	(8)	11(19)
Total		25	7	(10)	32(42)

Sensitivity : 100% (21/21)
 Specificity : 63.6% (7/11)
 Positive predictivity : 84.0% (21/25)
 Negative predictivity : 100% (7/7)
 Accuracy : 87.5% (28/32)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	3	5	(11)	8(19)
Total		24	5	(13)	29(42)

Sensitivity : 100% (21/21)
 Specificity : 62.5% (5/8)
 Positive predictivity : 87.5% (21/24)
 Negative predictivity : 100% (5/5)
 Accuracy : 89.7% (26/29)

Table 19A Judgment from the Phase 1-2 and 2 results: Final judgment of positive when positive results were obtained in at least one of two or three assays.

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	/	/	/	/	+	+	/	+	+	+	+	+	+	+	/	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
I-1	5-FU	/	/	/	/	-	-	/	-	-	/	-	-	/	-	-	/	-
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	/	/	/	/	+	+	/	+	+	/	+	+	/	+	+	/	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	/	/	/	/	+	+	/	+	+	/	+	+	/	+	+	/	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-20	Avobenzene	+	+	+	+	+	+	+	+	I	I	I	I	+	+	+	+	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 19A Judgment from the Phase 1-2 and 2 results: Final judgment of positive when positive results were obtained in at least one of two or three assays (continued).

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated	
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^a	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd			
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+
II-28	Phenytoin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
II-29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	+	-	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
II-32	Cinnamic acid	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	I
II-33	Drometizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-34	L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	I	+	+	I	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	+	I
II-37	Octyl methacrylate	I	I	I	I	I	+	I	+	I	I	I	I	I	I	I	I	I	I
II-38	Octyl methoxycinnamate	+	+	I	+	+	+	I	+	I	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	+	+	+	+	I	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	+	+	+	+	-	-	-	-	I	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	+	+	I	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative or inconclusive in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 19B Judgment from the Phase 1-2 and 2 results: Final judgment based on the mean value of three assays.

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
Chemicals		Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	Judgment ^a
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
I-1	5-FU	/	/	/	/	-	-	/	-	-	-	/	-	-	-	/	-	-
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-20	Avobenzene	+	+	+	+	+	+	+	+	I	I	I	I	+	+	+	+	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 19B Judgment from the Phase 1-2 and 2 results: Final judgment based on the mean value of three assays (continued).

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
II-28	Phenytoin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
II-29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	+	-	-	+	+	+	+	-	-	-	-	+	+	+	+	I
II-32	Cinnamic acid	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	I
II-33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-34	L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	I	+	I	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-37	Octyl methacrylate	I	I	I	I	I	+	I	I	I	I	I	I	I	I	I	I	I
II-38	Octyl methoxycinnamate	+	+	I	+	+	+	I	+	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	+	+	+	+	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	+	+	+	+	-	-	-	-	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	+	I	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative or inconclusive in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 19C Judgment from the Phase 1-2 and 2 results: Final judgment based on the majority of three assay results.

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Judgment ^a
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
I-1	5-FU	/	/	/	/	-	-	/	-	-	/	-	-	/	-	-	/	-
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-20	Avobenzene	+	+	+	+	+	+	+	+	I	I	I	I	+	+	+	+	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 19C Judgment from the Phase 1-2 and 2 results: Final judgment based on the majority of three assay results (continued).

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	Judgment ^a
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
II-28	Phenytoin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
II-29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	+	-	-	+	+	+	+	-	-	-	-	+	+	+	+	I
II-32	Cinnamic acid	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	I
II-33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-34	L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	I	+	+	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-37	Octyl methacrylate	I	I	I	I	I	+	I	I	I	I	I	I	I	I	I	I	I
II-38	Octyl methoxycinnamate	+	+	I	+	+	+	I	+	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	+	+	+	+	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	+	+	+	+	-	-	-	-	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	+	I	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative or inconclusive in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 19D Judgment from the Phase 1-2 and 2 results: Final judgment on the first assay results.

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Judgment ^a
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
I-1	5-FU	/	/	/	/	-	-	/	-	-	-	/	-	-	-	/	-	-
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	/	/	/	/	+	+	/	+	+	+	/	+	+	+	/	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-20	Avobenzene	+	+	+	+	+	+	+	+	I	I	I	I	+	+	+	+	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 19D Judgment from the Phase 1-2 and 2 results: Final judgment on the first assay results (continued).

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Judgment ^a
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+
II-28	Phenytoin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
II-29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	+	-	-	+	+	+	+	-	-	-	-	+	+	+	+	I
II-32	Cinnamic acid	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	I
II-33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-34	L-Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	I	+	+	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	I	I	I	I	+	+	+	+	I
II-37	Octyl methacrylate	I	I	I	I	I	+	I	I	I	I	I	I	I	I	I	I	I
II-38	Octyl methoxycinnamate	+	+	I	+	+	+	I	+	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	+	+	+	+	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	+	+	+	+	-	-	-	-	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	+	I	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide anion anion results ≥ 20 at 200, 100, 50, or 20 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion or the number of positive equals that of negative or inconclusive in integrated judgment)

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 20A Contingency table for Phase 1-2 and 2 results: Final judgment of positive when positive results were obtained in at least one of two or three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	8	6	(5)	14(19)
Total		29	6	(7)	35(42)

Sensitivity : 100% (21/21)
 Specificity : 42.9% (6/14)
 Positive predictivity : 72.4% (21/29)
 Negative predictivity : 100% (6/6)
 Accuracy : 77.1% (27/35)

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	9	6	(4)	15(19)
Total		33	7	(6)	40(46)

Sensitivity : 96.0% (24/25)
 Specificity : 40.0% (6/15)
 Positive predictivity : 72.7% (24/33)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 75.0% (30/40)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	23	1	(3)	24(27)
	-	4	6	(9)	10(19)
Total		27	7	(12)	34(46)

Sensitivity : 95.8% (23/24)
 Specificity : 60.0% (6/10)
 Positive predictivity : 85.2% (23/27)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 85.3% (29/34)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	4	7	(8)	11(19)
Total		28	8	(10)	36(46)

Sensitivity : 96.0% (24/25)
 Specificity : 63.6% (7/11)
 Positive predictivity : 85.7% (24/28)
 Negative predictivity : 87.5% (7/8)
 Accuracy : 86.1% (31/36)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	4	5	(10)	9(19)
Total		28	6	(12)	34(46)

Sensitivity : 96.0% (24/25)
 Specificity : 55.6% (5/9)
 Positive predictivity : 85.7% (24/28)
 Negative predictivity : 83.3% (5/6)
 Accuracy : 85.3% (29/34)

Table 20B Contingency table for Phase 1-2 and 2 results: Final judgment based on the mean value of two or three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	6	7	(6)	13(19)
Total		27	7	(8)	34(42)

Sensitivity : 100% (21/21)
 Specificity : 53.8% (7/13)
 Positive predictivity : 77.8% (21/27)
 Negative predictivity : 100% (7/7)
 Accuracy : 82.4% (28/34)

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	7	6	(6)	13(19)
Total		31	7	(8)	38(46)

Sensitivity : 96.0% (24/25)
 Specificity : 46.2% (6/13)
 Positive predictivity : 77.4% (24/31)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 78.9% (30/38)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	23	1	(3)	24(27)
	-	4	6	(9)	10(19)
Total		27	7	(12)	34(46)

Sensitivity : 95.8% (23/24)
 Specificity : 60.0% (6/10)
 Positive predictivity : 85.2% (23/27)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 85.3% (29/34)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	4	7	(8)	11(19)
Total		28	8	(10)	36(46)

Sensitivity : 96.0% (24/25)
 Specificity : 63.6% (7/11)
 Positive predictivity : 85.7% (24/28)
 Negative predictivity : 87.5% (7/8)
 Accuracy : 86.1% (31/36)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	3	5	(11)	8(19)
Total		27	6	(13)	33(46)

Sensitivity : 96.0% (24/25)
 Specificity : 62.5% (5/8)
 Positive predictivity : 88.9% (24/27)
 Negative predictivity : 83.3% (5/6)
 Accuracy : 87.9% (29/33)

Table 20C Contingency table for Phase 1-2 and 2 results: Final judgment based on the majority of two or three assay results.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	6	7	(6)	13(19)
Total		27	7	(8)	34(42)

Sensitivity : 100% (21/21)
 Specificity : 53.8% (7/13)
 Positive predictivity : 77.8% (21/27)
 Negative predictivity : 100% (7/7)
 Accuracy : 82.4% (28/34)

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	8	6	(5)	14(19)
Total		32	7	(7)	39(46)

Sensitivity : 96.0% (24/25)
 Specificity : 42.9% (6/14)
 Positive predictivity : 75.0% (24/32)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 76.9% (30/39)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	23	1	(3)	24(27)
	-	4	6	(9)	10(19)
Total		27	7	(12)	34(46)

Sensitivity : 95.8% (23/24)
 Specificity : 60.0% (6/10)
 Positive predictivity : 85.2% (23/27)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 85.3% (29/34)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	4	7	(8)	11(19)
Total		28	8	(10)	36(46)

Sensitivity : 96.0% (24/25)
 Specificity : 63.6% (7/11)
 Positive predictivity : 85.7% (24/28)
 Negative predictivity : 87.5% (7/8)
 Accuracy : 86.1% (31/36)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	3	5	(11)	8(19)
Total		27	6	(13)	33(46)

Sensitivity : 96.0% (24/25)
 Specificity : 62.5% (5/8)
 Positive predictivity : 88.9% (24/27)
 Negative predictivity : 83.3% (5/6)
 Accuracy : 87.9% (29/33)

Table 20D Contingency table for Phase 1-2 and 2 results: Final judgment on the first assay results.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	6	7	(6)	13(19)
Total		27	7	(8)	34(42)

Sensitivity : 100% (21/21)
 Specificity : 53.8% (7/13)
 Positive predictivity : 77.8% (21/27)
 Negative predictivity : 100% (7/7)
 Accuracy : 82.4% (28/34)

Lab 5		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	8	6	(5)	14(19)
Total		32	7	(7)	39(46)

Sensitivity : 96.0% (24/25)
 Specificity : 42.9% (6/14)
 Positive predictivity : 75.0% (24/32)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 76.9% (30/39)

Lab 6		ROS			Total
		+	-	I	
Phototoxic	+	23	1	(3)	24(27)
	-	4	6	(9)	10(19)
Total		27	7	(12)	34(46)

Sensitivity : 95.8% (23/24)
 Specificity : 60.0% (6/10)
 Positive predictivity : 85.2% (23/27)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 85.3% (29/34)

Lab 7		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	4	7	(8)	11(19)
Total		28	8	(10)	36(46)

Sensitivity : 96.0% (24/25)
 Specificity : 63.6% (7/11)
 Positive predictivity : 85.7% (24/28)
 Negative predictivity : 87.5% (7/8)
 Accuracy : 86.1% (31/36)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	3	5	(11)	8(19)
Total		27	6	(13)	33(46)

Sensitivity : 96.0% (24/25)
 Specificity : 62.5% (5/8)
 Positive predictivity : 88.9% (24/27)
 Negative predictivity : 83.3%(5/6)
 Accuracy : 87.9% (29/33)

Table 21-1 Contingency table for Phase 1-1 results at 20 µM.

Concentration: 20 µM

Lab 4	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	66.7% (8/12)	66.7% (8/12)	66.7% (8/12)	58.3% (7/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (8/8)	100% (8/8)	100% (8/8)	100% (7/7)
Negative predictivity	20.0% (1/5)	20.0% (1/5)	20.0% (1/5)	16.7% (1/6)
Accuracy	69.2% (9/13)	69.2% (9/13)	69.2% (9/13)	61.5% (8/13)

Lab 5	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	75.0% (9/12)	66.7% (8/12)	58.3% (7/12)	66.7% (8/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (9/9)	100% (8/8)	100% (7/7)	100% (8/8)
Negative predictivity	25.0% (1/4)	20.0% (1/5)	16.7% (1/6)	20.0% (1/5)
Accuracy	76.9% (10/13)	69.2% (9/13)	61.5% (8/13)	69.2% (9/13)

Lab 6	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	50.0% (6/12)	25.0% (3/12)	25.0% (3/12)	33.3% (4/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (6/6)	100% (3/3)	100% (3/3)	100% (4/4)
Negative predictivity	14.3% (1/7)	10.0% (1/10)	10.0% (1/10)	11.1% (1/9)
Accuracy	53.8% (7/13)	30.8% (4/13)	30.8% (4/13)	38.5% (5/13)

Lab 7	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	58.3% (7/12)	50.0% (6/12)	50.0% (6/12)	50.0% (6/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (7/7)	100% (6/6)	100% (6/6)	100% (6/6)
Negative predictivity	16.7% (1/6)	14.3% (1/7)	14.3% (1/7)	14.3% (1/7)
Accuracy	61.5% (8/13)	53.8% (7/13)	53.8% (7/13)	53.8% (7/13)

a : A : The final judgments were determined as positive when positive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results.

D : Final judgment on the first assay results.

Table 21-2 Contingency table for Phase 1-1 results at 200 µM.

Concentration: 200 µM

Lab 4	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Lab 5	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Lab 6	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Lab 7	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

a : A : The final judgments were determined as positive when positive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results.

D : Final judgment on the first assay results

Table 21-3 Contingency table for Phase 1-2 results at 20 μ M.

Concentration: 20 μ M

Lab 5	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	83.3% (10/12)	75.0% (9/12)	81.8% (9/11)	83.3% (10/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (10/10)	100% (9/9)	100% (9/9)	100% (10/10)
Negative predictivity	33.3% (1/3)	25.0% (1/4)	33.3% (1/3)	33.3% (1/3)
Accuracy	84.6% (11/13)	76.9% (10/13)	83.3% (10/12)	84.6% (11/13)

Lab 6	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	75.0% (9/12)	75.0% (9/12)	72.7% (8/11)	66.7% (8/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (9/9)	100% (9/9)	100% (8/8)	100% (9/9)
Negative predictivity	25.0% (1/4)	25.0% (1/4)	25.0% (1/4)	20.0% (1/5)
Accuracy	76.9% (10/13)	76.9% (10/13)	75.0% (9/12)	69.2% (9/13)

Lab 7	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	83.3% (10/12)	83.3% (10/12)	77.8% (7/9)	75.0% (9/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (10/10)	100% (10/10)	100% (7/7)	100% (9/9)
Negative predictivity	33.3% (1/3)	33.3% (1/3)	33.3% (1/3)	25.0% (1/4)
Accuracy	84.6% (11/13)	84.6% (11/13)	80.0% (8/10)	76.9% (10/13)

a : A : The final judgments were determined as positive when positive results were obtained in at least one of two assays.

B : Final judgment based on the mean value of two assays.

C : Final judgment based on the majority of two assay results.

D : Final judgment on the first assay results.

Table 21-4 Contingency table for Phase 1-2 results at 200 μ M.

Concentration: 200 μ M

Lab 5	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Lab 6	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Lab 7	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

a : A : The final judgments were determined as positive when positive results were obtained in at least one of two assays.

B : Final judgment based on the mean value of two assays.

C : Final judgment based on the majority of two assay results.

D : Final judgment on the first assay results

Table 21-5 Contingency table for Phase 2 results.

Lab 4	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	42.9% (6/14)	53.8% (7/13)	53.8% (7/13)	53.8% (7/13)
Positive predictivity	72.4% (21/29)	77.8% (21/27)	77.8% (21/27)	77.8% (21/27)
Negative predictivity	100% (6/6)	100% (7/7)	100% (7/7)	100% (7/7)
Accuracy	77.1% (27/35)	82.4% (28/34)	82.4% (28/34)	82.4% (28/34)

Lab 5	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	40.0% (6/15)	46.2% (6/13)	42.9% (6/14)	42.9% (6/14)
Positive predictivity	70.0% (21/30)	75.0% (21/28)	72.4% (21/29)	72.4% (21/29)
Negative predictivity	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)
Accuracy	75.0% (27/36)	79.4% (27/34)	77.1% (27/35)	77.1% (27/35)

Lab 6	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
Specificity	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)
Positive predictivity	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)
Negative predictivity	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)
Accuracy	86.7% (26/30)	86.7% (26/30)	86.7% (26/30)	86.7% (26/30)

Lab 7	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)
Positive predictivity	84.0% (21/25)	84.0% (21/25)	84.0% (21/25)	84.0% (21/25)
Negative predictivity	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)
Accuracy	87.5% (28/32)	87.5% (28/32)	87.5% (28/32)	87.5% (28/32)

a : A : The final judgments were determined as positive when positive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results.

D : Final judgment on the first assay results.

Table 21-6 Contingency table for Phase 1-2 and 2 results.

Lab 4	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	42.9% (6/14)	53.8% (7/13)	53.8% (7/13)	53.8% (7/13)
Positive predictivity	72.4% (21/29)	77.8% (21/27)	77.8% (21/27)	77.8% (21/27)
Negative predictivity	100% (6/6)	100% (7/7)	100% (7/7)	100% (7/7)
Accuracy	77.1% (27/35)	82.4% (28/34)	82.4% (28/34)	82.4% (28/34)

Lab 5	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	96.0% (24/25)	96.0% (24/25)	96.0% (24/25)	96.0% (24/25)
Specificity	40.0% (6/15)	46.2% (6/13)	42.9% (6/14)	42.9% (6/14)
Positive predictivity	72.7% (24/33)	77.4% (24/31)	75.0% (24/32)	75.0% (24/32)
Negative predictivity	85.7% (6/7)	85.7% (6/7)	85.7% (6/7)	85.7% (6/7)
Accuracy	75.0% (30/40)	78.9% (30/38)	76.9% (30/39)	76.9% (30/39)

Lab 6	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	95.8% (23/24)	95.8% (23/24)	95.8% (23/24)	95.8% (23/24)
Specificity	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)
Positive predictivity	85.2% (23/27)	85.2% (23/27)	85.2% (23/27)	85.2% (23/27)
Negative predictivity	85.7% (6/7)	85.7% (6/7)	85.7% (6/7)	85.7% (6/7)
Accuracy	85.3% (29/34)	85.3% (29/34)	85.3% (29/34)	85.3% (29/34)

Lab 7	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	96.0% (24/25)	96.0% (24/25)	96.0% (24/25)	96.0% (24/25)
Specificity	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)
Positive predictivity	85.7% (24/28)	85.7% (24/28)	85.7% (24/28)	85.7% (24/28)
Negative predictivity	87.5% (7/8)	87.5% (7/8)	87.5% (7/8)	87.5% (7/8)
Accuracy	86.1% (31/36)	86.1% (31/36)	86.1% (31/36)	86.1% (31/36)

a : A : The final judgments were determined as positive when positive results were obtained in at least one of two or three assays.

B : Final judgment based on the mean value of two or three assays.

C : Final judgment based on the majority of two or three assay results.

D : Final judgment on the first assay results.

Table 22 Contingency table for integrated judgment results.

Phase 1-1 (200 µM)	Draft criteria for the final judgment ^a			
	A ^b	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Phase 1-2 (200 µM)	Draft criteria for the final judgment ^a			
	A ^c	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Phase 2	Draft criteria for the final judgment ^a			
	A ^d	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	55.6% (5/9)	62.5% (5/8)	62.5% (5/8)	62.5% (5/8)
Positive predictivity	84.0% (21/25)	87.5% (21/24)	87.5% (21/24)	87.5% (21/24)
Negative predictivity	100% (5/5)	100% (5/5)	100% (5/5)	100% (5/5)
Accuracy	86.7% (26/30)	89.7% (26/29)	89.7% (26/29)	89.7% (26/29)

Phase 1-2 and 2	Draft criteria for the final judgment ^a			
	A ^e	B	C	D
Sensitivity	96.0% (24/25)	96.0% (24/25)	96.0% (24/25)	96.0% (24/25)
Specificity	55.6% (5/9)	62.5% (5/8)	62.5% (5/8)	62.5% (5/8)
Positive predictivity	85.7% (24/28)	88.9% (24/27)	88.9% (24/27)	88.9% (24/27)
Negative predictivity	83.3% (5/6)	83.3% (5/6)	83.3% (5/6)	83.3% (5/6)
Accuracy	85.3% (29/34)	87.9% (29/33)	87.9% (29/33)	87.9% (29/33)

a : A : The final judgments were determined as positive when positive results were obtained in at least one of two or three assays.

B : Final judgment based on the mean value of two or three assays.

C : Final judgment based on the majority of two or three assay results.

D : Final judgment on the first assay results.

b : See, tables 6A to 6D

c : See, tables 11A to 11D

d : See, tables 17A to 17D

e : See, tables 19A to 19D

Table 23A Secondary data analysis based on the criteria for the proposed protocol: The highest criteria among the three assay results was selected as the final judgment

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	+	
II-20	Avobenzene	+	+	+	+	+	+	+	+	u	u	u	u	I	I	I	I	
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

+ : photoreactive (Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200, 100, 50, or 20 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200, 100, 50, or 20 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide anion results < 20 at 200, 100, 50, or 20 μM)

I : inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 23A Secondary data analysis based on the criteria for the proposed protocol: The highest criteria among the three assay results was selected as the final judgment (continued).

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	+ ^b	+	+	+	+	+	±	±	+ ^b	±	±	-	±	+
II-29	Bumetizole	-	-	-	-	I	I	I	I	-	-	-	-	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	±	±	±	±	±	-	-	-	-	±	±	±	±	±
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	I
II-33	Drometrizole	u	u	u	u	u	u	u	u	-	-	-	-	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	I
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	-	-	-	-	-	+	-	+	-	-	-	-	I	I	I	I	-
II-38	Octyl methoxycinnamate	±	±	-	±	±	±	-	±	-	-	-	-	I	I	I	I	±
II-39	Octyl salicylate	-	-	-	-	±	±	±	±	-	-	-	-	-	-	-	-	-
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	-	-	-	-	I	I	I	I	-
II-42	UV-571	-	-	±	±	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : photoreactive (Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200, 100, 50, or 20 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200, 100, 50, or 20 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide anion results < 20 at 200, 100, 50, or 20 μM)

I : inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion or the number of non-photoreactive equals that of photoreactive, weakly photoreactive, or inconclusive in integrated judgment)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

b : Final judgment of photoreactive when photoreactive results were obtained in at least one of three assays

Table 23B Secondary data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the mean value of three assays.

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzon	+	+	+	+	+	+	+	+	u	u	u	u	I	I	I	I	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : photoreactive (Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200, 100, 50, or 20 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200, 100, 50, or 20 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide anion results < 20 at 200, 100, 50, or 20 μM)

I : inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 23B Secondary data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the mean value of three assays (continued).

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	±	±	±	-	±	±
II-29	Bumetizole	-	-	-	-	I	I	I	I	-	-	-	-	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	I
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	I
II-33	Drometrizole	u	u	u	u	u	u	u	u	-	-	-	-	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	I
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	-	-	-	-	-	+	-	-	-	-	-	-	I	I	I	I	-
II-38	Octyl methoxycinnamate	±	±	-	±	±	±	-	±	-	-	-	-	I	I	I	I	±
II-39	Octyl salicylate	-	-	-	-	±	±	±	±	-	-	-	-	-	-	-	-	-
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	-	-	-	-	I	I	I	I	-
II-42	UV-571	-	-	±	-	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : photoreactive (Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200, 100, 50, or 20 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200, 100, 50, or 20 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide anion results < 20 at 200, 100, 50, or 20 μM)

I : inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion or the number of non-photoreactive equals that of photoreactive, weakly photoreactive, or inconclusive in integrated judgment)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 23C Secondary data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the majority of three assay results.

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzon	+	+	+	+	+	+	+	+	u	u	u	u	I	I	I	I	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : photoreactive (Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200, 100, 50, or 20 μM)
 \pm : weakly photoreactive (Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200, 100, 50, or 20 μM)
- : non-photoreactive (Singlet oxygen results < 25 and Superoxide anion results < 20 at 200, 100, 50, or 20 μM)
I : inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)
u : undetermined due to precipitation
a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 23C Secondary data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the majority of three assay results (continued).

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	±	±	±	-	±	±
II-29	Bumetrizole	-	-	-	-	I	I	I	I	-	-	-	-	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	I
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	I
II-33	Drometrizole	u	u	u	u	u	u	u	u	-	-	-	-	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	I
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	-	-	-	-	-	+	-	-	-	-	-	-	I	I	I	I	-
II-38	Octyl methoxycinnamate	±	±	-	±	±	±	-	±	-	-	-	-	I	I	I	I	±
II-39	Octyl salicylate	-	-	-	-	±	±	±	±	-	-	-	-	-	-	-	-	-
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	-	-	-	-	I	I	I	I	-
II-42	UV-571	-	-	±	-	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : photoreactive (Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200, 100, 50, or 20 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200, 100, 50, or 20 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide anion results < 20 at 200, 100, 50, or 20 μM)

I : inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion or the number of non-photoreactive equals that of photoreactive, weakly photoreactive, or inconclusive in integrated judgment)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 23D Secondary data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment on the first assay results.

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	+	+	+	+	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzene	+	+	+	+	+	+	+	+	u	u	u	u	I	I	I	I	+
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : photoreactive (Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200, 100, 50, or 20 μM)
 \pm : weakly photoreactive (Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200, 100, 50, or 20 μM)
- : non-photoreactive (Singlet oxygen results < 25 and Superoxide anion results < 20 at 200, 100, 50, or 20 μM)
I : inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)
u : undetermined due to precipitation
a : Integrated final judgments were made by the majority of each laboratory's judgment.

Table 23D Secondary data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment on the first assay results (continued).

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	+	±	±	-	±	I
II-29	Bumetrizole	-	-	-	-	I	I	I	I	-	-	-	-	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	I
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	I
II-33	Drometrizole	u	u	u	u	u	u	u	u	-	-	-	-	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	+	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	I
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	-	-	-	-	-	+	-	-	-	-	-	-	I	I	I	I	-
II-38	Octyl methoxycinnamate	±	±	-	±	±	±	-	±	-	-	-	-	I	I	I	I	±
II-39	Octyl salicylate	-	-	-	-	±	±	±	±	-	-	-	-	-	-	-	-	-
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	-	-	-	-	I	I	I	I	-
II-42	UV-571	-	-	±	-	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : photoreactive (Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200, 100, 50, or 20 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200, 100, 50, or 20 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide anion results < 20 at 200, 100, 50, or 20 μM)

I : inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion, the number of non-photoreactive equals that of photoreactive, weakly photoreactive, or inconclusive in integrated judgment or the number of photoreactive equals that of weakly photoreactive in integrated judgment)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

**Table 24A Secondary data analysis based on the criteria for the proposed protocol:
The highest criteria among the three assay results was selected as the final judgment
Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)**

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^a
	-	3	5	9	17 ^b
Total		24	5	9	38

a: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).
b: Two of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Drometizole, Octizole).
When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 82.4% (14/17)
Positive predictivity : 87.5% (21/24) Negative predictivity : 100% (14/14)
Accuracy : 92.1% (35/38)
When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 52.9% (9/17)
Positive predictivity : 72.4%(21/29) Negative predictivity : 100% (9/9)
Accuracy : 78.9%(30/38)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^c
	-	4	5	6	15 ^d
Total		25	5	6	36

c: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).
d: Four of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Bumetizole, Drometizole, Octizole, UV-571).
When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 73.3% (11/15)
Positive predictivity : 84.0% (21/25) Negative predictivity : 100% (11/11)
Accuracy : 88.9% (32/36)
When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 40.0%(6/15)
Positive predictivity : 70.0%(21/30) Negative predictivity : 100% (6/6)
Accuracy : 75.0%(27/36)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	20	0	0	20 ^e
	-	1	3	13	17 ^f
Total		21	3	13	37

e: Three of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
f: Two of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Octizole, UV-571).
When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (20/20) Specificity : 94.1% (16/17)
Positive predictivity : 95.2% (20/21) Negative predictivity : 100% (16/16)
Accuracy : 97.3% (36/37)
When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (20/20) Specificity : 76.5%(13/17)
Positive predictivity : 83.3%(20/24) Negative predictivity : 100% (13/13)
Accuracy : 89.2%(33/37)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	20	0	0	20 ^g
	-	1	3	9	13 ^h
Total		21	3	9	33

g: Three of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Avobenzone).
h: Six of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Bumetizole, Drometizole, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).
When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (20/20) Specificity : 92.3% (12/13)
Positive predictivity : 95.2% (20/21) Negative predictivity : 100% (12/12)
Accuracy : 97.0% (32/33)
When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (20/20) Specificity : 69.2%(9/13)
Positive predictivity : 83.3%(20/24) Negative predictivity : 100% (9/9)
Accuracy : 87.9%(29/33)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ⁱ
	-	2	3	8	13 ^j
Total		23	3	8	34

i: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).
j: Six of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM since precipitation was observed in the reaction mixture or the number of non-photoreactive equals that of photoreactive, weakly photoreactive, or inconclusive in integrated judgment. (Bumetizole, Cinnamic acid, Drometizole, Methylbenzylidene camphor, Octizole, UV-571).
When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 84.6% (11/13)
Positive predictivity : 91.3% (21/23) Negative predictivity : 100% (11/11)
Accuracy : 94.1% (32/34)
When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 61.5%(8/13)
Positive predictivity : 80.8%(21/26) Negative predictivity : 100% (8/8)
Accuracy : 85.3%(29/34)

Table 24B Secondary data analysis based on the criteria for secondary data analysis: Contingency table for Phase 2 results: Final judgment based on the mean value of three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^a
	-	2	4	11	17 ^b
Total		23	4	11	38

a: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).

b: Two of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Drometrizole, Octizole).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 88.2% (15/17)
Positive predictivity : 91.3% (21/23) Negative predictivity : 100% (15/15)
Accuracy : 94.7% (36/38)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 64.7% (11/17)
Positive predictivity : 77.8%(21/27) Negative predictivity : 100% (11/11)
Accuracy : 84.2%(32/38)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^c
	-	3	5	7	15 ^d
Total		24	5	7	36

c: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).

d: Four of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 80.0% (12/15)
Positive predictivity : 87.5% (21/24) Negative predictivity : 100% (12/12)
Accuracy : 91.7% (33/36)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 46.7%(7/15)
Positive predictivity : 72.4%(21/29) Negative predictivity : 100% (7/7)
Accuracy : 77.8%(28/36)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	20	0	0	20 ^e
	-	0	4	13	17 ^f
Total		20	4	13	37

e: Three of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzon).

f: Two of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (20/20) Specificity : 100% (17/17)
Positive predictivity : 100% (20/20) Negative predictivity : 100% (17/17)
Accuracy : 100% (37/37)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (20/20) Specificity : 76.5%(13/17)
Positive predictivity : 83.3%(20/24) Negative predictivity : 100% (13/13)
Accuracy : 89.2%(33/37)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^g
	-	1	3	9	13 ^h
Total		20	3	9	32

g: Four of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzon).

h: Six of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 92.3% (12/13)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (12/12)
Accuracy : 96.9% (31/32)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 69.2%(9/13)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (9/9)
Accuracy : 87.5%(28/32)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ⁱ
	-	1	3	8	12 ^j
Total		22	3	8	33

i: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).

j: Seven of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM since precipitation was observed in the reaction mixture or the number of non-photoreactive equals that of photoreactive, weakly photoreactive, or inconclusive in integrated judgment (Bumetrizole, Chlorhexidine, Cinnamic acid, Drometrizole, Methylbenzylidene camphor, Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 91.7% (11/12)
Positive predictivity : 95.5% (21/22) Negative predictivity : 100% (11/11)
Accuracy : 97.0% (32/33)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 66.7%(8/12)
Positive predictivity : 84.0%(21/25) Negative predictivity : 100% (8/8)
Accuracy : 87.9%(29/33)

Table 24C Secondary data analysis based on the criteria for secondary data analysis: Contingency table for Phase 2 results: Final judgment based on the majority of three assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^a
	-	2	4	11	17 ^b
Total		23	4	11	38

a: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).

b: Two of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Drometrizole, Octizole).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 88.2% (15/17)
Positive predictivity : 91.3% (21/23) Negative predictivity : 100% (15/15)
Accuracy : 94.7% (36/38)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 64.7% (11/17)
Positive predictivity : 77.8% (21/27) Negative predictivity : 100% (11/11)
Accuracy : 84.2% (32/38)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^c
	-	3	5	7	15 ^d
Total		24	5	7	36

c: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).

d: Four of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 80.0% (12/15)
Positive predictivity : 87.5% (21/24) Negative predictivity : 100% (12/12)
Accuracy : 91.7% (33/36)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 46.7% (7/15)
Positive predictivity : 72.4% (21/29) Negative predictivity : 100% (7/7)
Accuracy : 77.8% (28/36)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	20	0	0	20 ^e
	-	0	4	13	17 ^f
Total		20	4	13	37

e: Three of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzonone).

f: Two of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (20/20) Specificity : 100% (17/17)
Positive predictivity : 100% (20/20) Negative predictivity : 100% (17/17)
Accuracy : 100% (37/37)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (20/20) Specificity : 76.5% (13/17)
Positive predictivity : 83.3% (20/24) Negative predictivity : 100% (13/13)
Accuracy : 89.2% (33/37)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^g
	-	1	3	9	13 ^h
Total		20	3	9	32

g: Four of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzonone).

h: Six of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 92.3% (12/13)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (12/12)
Accuracy : 96.9% (31/32)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 69.2% (9/13)
Positive predictivity : 82.6% (19/23) Negative predictivity : 100% (9/9)
Accuracy : 87.5% (28/32)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ⁱ
	-	1	3	8	12 ^j
Total		22	3	8	33

i: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).

j: Seven of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM since precipitation was observed in the reaction mixture or the number of non-photoreactive equals that of photoreactive, weakly photoreactive, or inconclusive in integrated judgment (Bumetrizole, Chlorhexidine, Cinnamic acid, Drometrizole, Methylbenzylidene camphor, Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 91.7% (11/12)
Positive predictivity : 95.5% (21/22) Negative predictivity : 100% (11/11)
Accuracy : 97.0% (32/33)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 66.7% (8/12)
Positive predictivity : 84.0% (21/25) Negative predictivity : 100% (8/8)
Accuracy : 87.9% (29/33)

**Table 24D Secondary data analysis based on the criteria for secondary data analysis:
Contingency table for Phase 2 results: Final judgment on the first assay results.
Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)**

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^a
	-	2	4	11	17 ^b
Total		23	4	11	38

a: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).
b: Two of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Drometizole, Octizole).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 88.2% (15/17)
Positive predictivity : 91.3% (21/23) Negative predictivity : 100% (15/15)
Accuracy : 94.7% (36/38)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 64.7% (11/17)
Positive predictivity : 77.8% (21/27) Negative predictivity : 100% (11/11)
Accuracy : 84.2% (32/38)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^c
	-	3	5	7	15 ^d
Total		24	5	7	36

c: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).
d: Four of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Bumetizole, Drometizole, Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 80.0% (12/15)
Positive predictivity : 87.5% (21/24) Negative predictivity : 100% (12/12)
Accuracy : 91.7% (33/36)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 46.7% (7/15)
Positive predictivity : 72.4% (21/29) Negative predictivity : 100% (7/7)
Accuracy : 77.8% (28/36)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	20	0	0	20 ^e
	-	1	3	13	17 ^f
Total		21	3	13	37

e: Three of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
f: Two of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (20/20) Specificity : 94.1% (16/17)
Positive predictivity : 95.2% (20/21) Negative predictivity : 100% (16/16)
Accuracy : 97.3% (36/37)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (20/20) Specificity : 76.5% (13/17)
Positive predictivity : 83.3% (20/24) Negative predictivity : 100% (13/13)
Accuracy : 89.2% (33/37)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^g
	-	1	3	9	13 ^h
Total		20	3	9	32

g: Four of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
h: Six of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Bumetizole, Drometizole, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 92.3% (12/13)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (12/12)
Accuracy : 96.9% (31/32)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 69.2% (9/13)
Positive predictivity : 82.6% (19/23) Negative predictivity : 100% (9/9)
Accuracy : 87.5% (28/32)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ⁱ
	-	1	2	8	11 ^j
Total		22	2	8	32

i: Two of 23 phototoxic compounds were not evaluated at 20, 50 100, or 200 µM due to precipitation (Amiodarone HCl, Anthracene).
j: Eight of 19 non-phototoxic compounds were not evaluated at 20, 50 100, or 200 µM since precipitation was observed in the reaction mixture, the number of non-photoreactive equals that of photoreactive, weakly photoreactive, or inconclusive in integrated judgment or the number of photoreactive equals that of weakly photoreactive (Phenytoin, Bumetizole, Chlorhexidine, Cinnamic acid, Drometizole, Methylbenzylidene camphor, Octizole, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 90.9% (10/11)
Positive predictivity : 95.5% (21/22) Negative predictivity : 100% (10/10)
Accuracy : 96.9% (31/32)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (21/21) Specificity : 72.7% (8/11)
Positive predictivity : 87.5% (21/24) Negative predictivity : 100% (8/8)
Accuracy : 90.6% (29/32)

Table 25 Secondary data analysis based on the criteria for secondary data analysis: Contingency table for Phase 2 results.

Lab 4	Draft criteria for the final judgment ^a											
	Original				When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	42.9% (6/14)	53.8% (7/13)	53.8% (7/13)	53.8% (7/13)	82.4% (14/17)	88.2% (15/17)	88.2% (15/17)	88.2% (15/17)	52.9% (9/17)	64.7% (11/17)	64.7% (11/17)	64.7% (11/17)
Positive predictivity	72.4% (21/29)	77.8% (21/27)	77.8% (21/27)	77.8% (21/27)	87.5% (21/24)	91.3% (21/23)	91.3% (21/23)	91.3% (21/23)	72.4% (21/29)	77.8% (21/27)	77.8% (21/27)	77.8% (21/27)
Negative predictivity	100% (6/6)	100% (7/7)	100% (7/7)	100% (7/7)	100% (14/14)	100% (15/15)	100% (15/15)	100% (15/15)	100% (9/9)	100% (11/11)	100% (11/11)	100% (11/11)
Accuracy	77.1% (27/35)	82.4% (28/34)	82.4% (28/34)	82.4% (28/34)	92.1% (35/38)	94.7% (36/38)	94.7% (36/38)	94.7% (36/38)	78.9% (30/38)	84.2% (32/38)	84.2% (32/38)	84.2% (32/38)

Lab 5	Draft criteria for the final judgment ^a											
	Original				When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	40.0% (6/15)	46.2% (6/13)	42.9% (6/14)	42.9% (6/14)	73.3% (11/15)	80.0% (12/15)	80.0% (12/15)	80.0% (12/15)	40.0% (6/15)	46.7% (7/15)	46.7% (7/15)	46.7% (7/15)
Positive predictivity	70.0% (21/30)	75.0% (21/28)	72.4% (21/29)	72.4% (21/29)	84.0% (21/25)	87.5% (21/24)	87.5% (21/24)	87.5% (21/24)	70.0% (21/30)	72.4% (21/29)	72.4% (21/29)	72.4% (21/29)
Negative predictivity	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)	100% (11/11)	100% (12/12)	100% (12/12)	100% (12/12)	100% (6/6)	100% (7/7)	100% (7/7)	100% (7/7)
Accuracy	75.0% (27/36)	79.4% (27/34)	77.1% (27/35)	77.1% (27/35)	88.9% (32/36)	91.7% (33/36)	91.7% (33/36)	91.7% (33/36)	75.0% (27/36)	77.8% (28/36)	77.8% (28/36)	77.8% (28/36)

^a : A : Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays.
 B : Final judgment based on the mean value of three assays.
 C : Final judgment based on the majority of three assay results.
 D : Final judgment on the first assay results.

Table 25 Secondary data analysis based on the criteria for secondary data analysis: Contingency table for Phase 2 results (continued).

Lab 6	Draft criteria for the final judgment ^a											
	Original				When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
Specificity	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	94.1% (16/17)	100% (17/17)	100% (17/17)	94.1% (16/17)	76.5% (13/17)	76.5% (13/17)	76.5% (13/17)	76.5% (13/17)
Positive predictivity	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	95.2% (20/21)	100% (20/20)	100% (20/20)	95.2% (20/21)	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)
Negative predictivity	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)	100% (16/16)	100% (17/17)	100% (17/17)	100% (16/16)	100% (13/13)	100% (13/13)	100% (13/13)	100% (13/13)
Accuracy	86.7% (26/30)	86.7% (26/30)	86.7% (26/30)	86.7% (26/30)	97.3% (36/37)	100% (37/37)	100% (37/37)	97.3% (36/37)	89.2% (33/37)	89.2% (33/37)	89.2% (33/37)	89.2% (33/37)

Lab 7	Draft criteria for the final judgment ^a											
	Original				When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (20/20)	100% (19/19)	100% (19/19)	100% (19/19)	100% (20/20)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	69.2% (9/13)	69.2% (9/13)	69.2% (9/13)	69.2% (9/13)
Positive predictivity	84.0% (21/25)	84.0% (21/25)	84.0% (21/25)	84.0% (21/25)	95.2% (20/21)	95.0% (19/20)	95.0% (19/20)	95.0% (19/20)	83.3% (20/24)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)
Negative predictivity	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	100% (12/12)	100% (12/12)	100% (12/12)	100% (12/12)	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)
Accuracy	87.5% (28/32)	87.5% (28/32)	87.5% (28/32)	87.5% (28/32)	97.0% (32/33)	96.9% (31/32)	96.9% (31/32)	96.9% (31/32)	87.9% (29/33)	87.5% (28/32)	87.5% (28/32)	87.5% (28/32)

a : A : Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays.
 B : Final judgment based on the mean value of three assays.
 C : Final judgment based on the majority of three assay results.
 D : Final judgment on the first assay results.

Table 26 Secondary data analysis based on the criteria for secondary data analysis: Contingency table for integrated judgment results.

Phase 2	Draft criteria for the final judgment ^a											
	Original				When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
	A ^b	B	C	D	A ^c	B	C	D	A ^c	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	84.6% (11/13)	91.7% (11/12)	91.7% (11/12)	90.9% (10/11)	61.5% (8/13)	66.7% (8/12)	66.7% (8/12)	72.7% (8/11)
Positive predictivity	84.0% (21/25)	84.0% (21/25)	84.0% (21/25)	84.0% (21/25)	91.3% (21/23)	95.5% (21/22)	95.5% (21/22)	95.5% (21/22)	80.8% (21/26)	84.0% (21/25)	84.0% (21/25)	87.5% (21/24)
Negative predictivity	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	100% (11/11)	100% (11/11)	100% (11/11)	100% (10/10)	100% (8/8)	100% (8/8)	100% (8/8)	100% (8/8)
Accuracy	87.5% (28/32)	87.5% (28/32)	87.5% (28/32)	87.5% (28/32)	94.1% (32/34)	97.0% (32/33)	97.0% (32/33)	96.9% (31/32)	85.3% (29/34)	87.9% (29/33)	87.9% (29/33)	90.6% (29/32)

Integrated judgments were made by the majority of each laboratory’s final judgment.

a : A : Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results.

D : Final judgment on the first assay results.

b : See, tables 17A to 17D

c : See, tables 23A to 23D

Table 27A Third data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgment.

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	I	I	I	I ¹⁾	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	+	u
II-20	Avobenzone	(+)	(+)	(+)	(+)	+	+	+	+	u	u	u	u	I	I	I	I	I
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : photoreactive (Singlet oxygen results ≥ 25 and/or Superoxide results ≥ 70 at 20 and/or 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)
 \pm : weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 20 and 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)
- : non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 20 and 200 μM , two concentration levels without precipitation would be needed for judgment of "Non-photoreactive", when precipitation is observed at 200 μM , the compound should be judged as "Inconclusive")
(+), (\pm), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.
I : inconclusive (The results do not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)
u : undetermined due to precipitation
a : Integrated final judgments were made by the majority of each laboratory's judgment.
Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.
If there are 2 "photoreactive" results and 2 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.
If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.
1): The chemical was judged as "Inconclusive" since the SO result was "Photoreactive" at 50 μM and the SA result was < 20 at 20 μM .

Table 27A Third data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgment (continued).

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	
II-28	Phenytoin	±	±	+	+ ^b	+	+	+	+	+	±	±	+ ^b	±	±	-	±	
II-29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-31	Chlorhexidine	-	±	-	±	±	±	±	±	-	-	-	-	±	±	±	±	
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	
II-33	Drometrizole	u	u	u	u	u	u	u	u	(-)	(-)	(-)	(-)	u	u	u	u	
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	
II-35	Methylbenzylidene camphor	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(+)	I	I	I	I	I	I	I	I	
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	
II-37	Octyl methacrylate	I	I	I	I	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	I	I	I	I	
II-38	Octyl methoxycinnamate	I	I	I	I	±	±	I	±	I	I	I	I	I	I	I	I	
II-39	Octyl salicylate	I	I	I	I	(±)	(±)	(±)	(±)	I	I	I	I	I	I	I	I	
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-41	SDS	±	±	±	±	-	-	-	-	I	I	I	I	I	I	I	I	
II-42	UV-571	I	I	± ¹⁾	±	I	I	I	I	u	u	u	u	I	I	I	I	

+ : photoreactive (Singlet oxygen results ≥ 25 and/or Superoxide results ≥ 70 at 20 and/or 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

± : weakly photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 20 , < 70 at 20 and 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 and 200 μM , two concentration levels without precipitation would be needed for judgment of "Non-photoreactive", when precipitation is observed at 200 μM , the compound should be judged as "Inconclusive")

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive (The results do not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

b : Final judgment of photoreactive when photoreactive results were obtained in at least one of three assays

1): The chemical was judged as "Weakly photoreactive" since the SO result was "Non-photoreactive" at 50 μM and the SA result was "Weakly photoreactive" at 20 μM .

Table 27B Third data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the mean value of three assays.

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzon	(+)	(+)	(+)	(+)	+	+	+	+	u	u	u	u	I	I	I	I	I
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : photoreactive (Singlet oxygen results ≥ 25 and/or Superoxide results ≥ 70 at 20 and/or 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)
 \pm : weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 20 and 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)
- : non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 20 and 200 μM , two concentration levels without precipitation would be needed for judgment of "Non-photoreactive", when precipitation is observed at 200 μM , the compound should be judged as "Inconclusive")
(+), (\pm), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.
I : inconclusive (The results do not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)
u : undetermined due to precipitation
a : Integrated final judgments were made by the majority of each laboratory's judgment.
Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.
If there are 2 "photoreactive" results and 2 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.
If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 27B Third data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the mean value of three assays (continued).

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	±	±	±	-	±	
II-29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	
II-33	Drometizole	u	u	u	u	u	u	u	u	(-)	(-)	(-)	(-)	u	u	u	u	
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	
II-35	Methylbenzylidene camphor	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(-)	I	I	I	I	I	I	I	I	
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	
II-37	Octyl methacrylate	I	I	I	I	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	I	I	I	I	
II-38	Octyl methoxycinnamate	I	I	I	I	±	±	I	±	I	I	I	I	I	I	I	I	
II-39	Octyl salicylate	I	I	I	I	(±)	(±)	(±)	(±)	I	I	I	I	I	I	I	I	
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
II-41	SDS	±	±	±	±	-	-	-	-	I	I	I	I	I	I	I	I	
II-42	UV-571	I	I	±	I	I	I	I	I	u	u	u	u	I	I	I	I	

+ : photoreactive (Singlet oxygen results ≥ 25 and/or Superoxide results ≥ 70 at 20 and/or 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 20 and 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 20 and 200 μM , two concentration levels without precipitation would be needed for judgment of "Non-photoreactive", when precipitation is observed at 200 μM , the compound should be judged as "Inconclusive")

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive (The results do not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 27C Third data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the majority of three assay results.

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzene	(+)	(+)	(+)	(+)	+	+	+	+	u	u	u	u	I	I	I	I	I
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : photoreactive (Singlet oxygen results ≥ 25 and/or Superoxide results ≥ 70 at 20 and/or 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 20 and 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 20 and 200 μM , two concentration levels without precipitation would be needed for judgment of "Non-photoreactive", when precipitation is observed at 200 μM , the compound should be judged as "Inconclusive")

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive (The results do not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 27C Third data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the majority of three assay results (continued).

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	±	±	±	±	-	±
II-29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	-
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	-
II-33	Drometrizole	u	u	u	u	u	u	u	u	(-)	(-)	(-)	(-)	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(+)	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	I	I	I	I	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	I	I	I	I	I
II-38	Octyl methoxycinnamate	I	I	I	I	±	±	I	±	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	(±)	(±)	(±)	(±)	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	±	I	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : photoreactive (Singlet oxygen results ≥ 25 and/or Superoxide results ≥ 70 at 20 and/or 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 20 and 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 20 and 200 μM , two concentration levels without precipitation would be needed for judgment of "Non-photoreactive", when precipitation is observed at 200 μM , the compound should be judged as "Inconclusive")

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive (The results do not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 27D Third data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment on the first assay results.

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
No.	Name	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzene	(+)	(+)	(+)	(+)	+	+	+	+	u	u	u	u	I	I	I	I	I
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : photoreactive (Singlet oxygen results ≥ 25 and/or Superoxide results ≥ 70 at 20 and/or 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 20 and 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 20 and 200 μM , two concentration levels without precipitation would be needed for judgment of "Non-photoreactive", when precipitation is observed at 200 μM , the compound should be judged as "Inconclusive")

(+), (+), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive (The results do not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 27D Third data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment on the first assay results (continued).

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	+	±	±	-	±	±
II-29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	-
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	-
II-33	Drometizole	u	u	u	u	u	u	u	u	(-)	(-)	(-)	(-)	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(+)	I	I	I	I	I	I	I	I	I
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	I	I	I	I	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	I	I	I	I	I
II-38	Octyl methoxycinnamate	I	I	I	I	±	±	I	±	I	I	I	I	I	I	I	I	I
II-39	Octyl salicylate	I	I	I	I	(±)	(±)	(±)	(±)	I	I	I	I	I	I	I	I	I
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	I	I	I	I	I	I	I	I	I
II-42	UV-571	I	I	±	I	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : photoreactive (Singlet oxygen results ≥ 25 and/or Superoxide results ≥ 70 at 20 and/or 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

± : weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 20 and 200 μM , it would be judged at 20 μM only when precipitation is observed at 200 μM)

- : non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 20 and 200 μM , two concentration levels without precipitation would be needed for judgment of "Non-photoreactive", when precipitation is observed at 200 μM , the compound should be judged as "Inconclusive")

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive (The results do not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated final judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 28A Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: The highest criteria among the three assay results was selected as the final judgment.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^a
	-	2	4	6	12 ^b
Total		21	4	6	31

a: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene) or the results of 50 µM (Avobenzone).
 b: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate) or the results of 50 µM (Methylbenzylidene camphor).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
 Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
 Accuracy : 93.5% (29/31)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 50.0% (6/12)
 Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (6/6)
 Accuracy : 80.6%(25/31)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	2	4	6	12 ^d
Total		21	4	6	31

c: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene) or the results of 50 µM (Fenofibrate).
 d: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Octizole, UV-571) or the results of 50 and/or 100 µM (Methylbenzylidene camphor, Octyl methacrylate, Octyl salicylate).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
 Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
 Accuracy : 93.5% (29/31)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 50.0%(6/12)
 Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (6/6)
 Accuracy : 80.6%(25/31)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^e
	-	1	3	6	10 ^f
Total		20	3	6	29

e: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
 f: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Methylbenzylidene camphor, Octyl methoxycinnamate, Octyl salicylate, Octizole, SDS, UV-571) or the results of 50 µM (Drometizole, Octyl methacrylate).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 90.0% (9/10)
 Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (9/9)
 Accuracy : 96.6% (28/29)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 60.0%(6/10)
 Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (6/6)
 Accuracy : 86.2%(25/29)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^g
	-	1	3	7	11 ^h
Total		20	3	7	30

g: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Avobenzone).
 h: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Methylbenzylidene camphor, Octyl salicylate, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 90.9% (10/11)
 Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (10/10)
 Accuracy : 96.7% (29/30)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 63.6%(7/11)
 Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (7/7)
 Accuracy : 86.7%(26/30)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ⁱ
	-	2	2	6	10 ^j
Total		21	2	6	29

i: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
 j: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Methylbenzylidene camphor, Octizole, Octyl salicylate, UV-571), or could not be determined the majority of results (Octyl methacrylate, Octyl methoxycinnamate, SDS).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 80.0% (8/10)
 Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (8/8)
 Accuracy : 93.1% (27/29)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 60.0%(6/10)
 Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (6/6)
 Accuracy : 86.2%(25/29)

Table 28B Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgment based on the mean value of three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^a
	-	1	3	7	11 ^b
Total		20	3	7	30

a: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene) or the results of 50 µM (Avobenzone).
b: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, UV-571) or the results of 50 µM (Methylbenzylidene camphor).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 90.9% (10/11)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (10/10)
Accuracy : 96.7% (29/30)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 63.6% (7/11)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (7/7)
Accuracy : 86.7%(26/30)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	2	4	6	12 ^d
Total		21	4	6	31

c: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene) or the results of 50 µM (Fenofibrate).
d: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation mixtures (Bumetizole, Drometizole, Octizole, UV-571) or the results of 50 and/or 100 µM (Methyl benzylidene camphor, Octyl methacrylate, Octyl salicylate).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
Accuracy : 93.5% (29/31)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 50.0%(6/12)
Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (6/6)
Accuracy : 80.6%(25/31)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^e
	-	0	4	6	10 ^f
Total		19	4	6	29

e: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
f: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571) or the results of 50 µM (Drometizole, Octyl methacrylate).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (10/10)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (10/10)
Accuracy : 100% (29/29)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 60.0%(6/10)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (6/6)
Accuracy : 86.2%(25/29)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	18	0	0	18 ^g
	-	1	3	7	11 ^h
Total		19	3	7	29

g: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
h: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Methylbenzylidene camphor, Octyl salicylate, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (18/18) Specificity : 90.9% (10/11)
Positive predictivity : 94.7% (18/19) Negative predictivity : 100% (10/10)
Accuracy : 96.6% (28/29)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (18/18) Specificity : 63.6%(7/11)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (7/7)
Accuracy : 86.2%(25/29)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ⁱ
	-	1	2	7	10 ^j
Total		20	2	7	29

i: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
j: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Methylbenzylidene camphor, Octizole, Octyl salicylate, UV-571), or could not be determined the majority of results (Octyl methacrylate, Octyl methoxycinnamate, SDS).

When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 90.0% (9/10)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (9/9)
Accuracy : 96.6% (28/29)

When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
Sensitivity : 100% (19/19) Specificity : 70.0%(7/10)
Positive predictivity : 86.4%(19/22) Negative predictivity : 100% (7/7)
Accuracy : 89.7%(26/29)

Table 28C Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgment based on the majority of three assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^a
	-	1	3	7	11 ^b
Total		20	3	7	30

a: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene) or the results of 50 µM (Avobenzone).
 b: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, UV-571) or the results of 50 µM (Methylbenzylidene camphor).
 When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 90.9% (10/11)
 Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (10/10)
 Accuracy : 96.7% (29/30)
 When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 63.6% (7/11)
 Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (7/7)
 Accuracy : 86.7%(26/30)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	2	4	6	12 ^d
Total		21	4	6	31

c: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene) or the results of 50 µM (Fenofibrate).
 d: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Octizole, UV-571) or the results of 50 and/or 100 µM (Methyl benzylidene camphor, Octyl methacrylate, Octyl salicylate).
 When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
 Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
 Accuracy : 93.5% (29/31)
 When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 50.0%(6/12)
 Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (6/6)
 Accuracy : 80.6%(25/31)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^e
	-	0	4	6	10 ^f
Total		19	4	6	29

e: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
 f: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571) or the results of 50 µM (Drometizole, Octyl methacrylate).
 When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 100% (10/10)
 Positive predictivity : 100% (19/19) Negative predictivity : 100% (10/10)
 Accuracy : 100% (29/29)
 When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 60.0%(6/10)
 Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (6/6)
 Accuracy : 86.2%(25/29)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	18	0	0	18 ^g
	-	1	3	7	11 ^h
Total		19	3	7	29

g: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
 h: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Methylbenzylidene camphor, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571).
 When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (18/18) Specificity : 90.9% (10/11)
 Positive predictivity : 94.7% (18/19) Negative predictivity : 100% (10/10)
 Accuracy : 96.6% (28/29)
 When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (18/18) Specificity : 63.6%(7/11)
 Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (7/7)
 Accuracy : 86.2%(25/29)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ⁱ
	-	1	2	7	10 ^j
Total		20	2	7	29

i: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
 j: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetizole, Drometizole, Methylbenzylidene camphor, Octizole, UV-571), or could not be determined the majority of results (Octyl salicylate, Octyl metacrylate, Octyl methoxycinnamate, SDS).
 When the "±: weakly photoreactive" chemicals were defined as "non-phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 90.0% (9/10)
 Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (9/9)
 Accuracy : 96.6% (28/29)
 When the "±: weakly photoreactive" chemicals were defined as "phototoxic" chemicals;
 Sensitivity : 100% (19/19) Specificity : 70.0%(7/10)
 Positive predictivity : 86.4%(19/22) Negative predictivity : 100% (7/7)
 Accuracy : 89.7%(26/29)

Table 28D Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgment on the first assay results. Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^a
	-	1	3	7	11 ^b
Total		20	3	7	30

a: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene) or the results of 50 µM (Avobenzone).
b: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, UV-571) or the results of 50 µM (Methylbenzylidene camphor).

When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 90.9% (10/11)
Positive predictivity : 95.0%(19/20) Negative predictivity : 100% (10/10)
Accuracy : 96.7% (29/30)

When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;
Sensitivity : 100% (19/19) Specificity : 63.6% (7/11)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (7/7)
Accuracy : 86.7%(26/30)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	2	4	6	12 ^d
Total		21	4	6	31

c: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene) or the results of 50 µM (Fenofibrate).
d: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octizole, UV-571) or the results of 50 and/or 100 µM (Methyl benzylidene camphor, Octyl methacrylate, Octyl salicylate).

When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
Accuracy : 93.5% (29/31)

When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 50.0%(6/12)
Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (6/6)
Accuracy : 80.6%(25/31)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^e
	-	1	3	6	10 ^f
Total		20	3	6	29

e: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
f: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571) or the results of 50 µM (Drometrizole, Octyl methacrylate).

When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 90.0% (9/10)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (9/9)
Accuracy : 96.6% (28/29)

When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 60.0%(6/10)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (6/6)
Accuracy : 86.2%(25/29)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	18	0	0	18 ^g
	-	1	3	7	11 ^h
Total		19	3	7	29

g: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
h: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate SDS, UV-571).

When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (18/18) Specificity : 90.9% (10/11)
Positive predictivity : 94.7% (18/19) Negative predictivity : 100% (10/10)
Accuracy : 96.6% (28/29)

When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (18/18) Specificity : 63.6%(7/11)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (7/7)
Accuracy : 86.2%(25/29)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ⁱ
	-	1	2	7	10 ^j
Total		20	2	7	29

i: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
j: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl salicylate, UV-571), or could not be determined the majority of results (Octyl metacrylate, Octyl methoxycinnamate, SDS).

When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 90.0% (9/10)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (9/9)
Accuracy : 96.6% (28/29)

When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 70.0%(7/10)
Positive predictivity : 86.4%(19/22) Negative predictivity : 100% (7/7)
Accuracy : 89.7%(26/29)

Table 29 Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results.

Lab 4	Draft criteria for the final judgment ^a											
	Original				Third data analysis based on the criteria for the proposed protocol							
					When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	42.9% (6/14)	53.8% (7/13)	53.8% (7/13)	53.8% (7/13)	83.3% (10/12)	90.9% (10/11)	90.9% (10/11)	90.9% (10/11)	50.0% (6/12)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)
Positive predictivity	71.4% (20/28)	76.9% (20/26)	76.9% (20/26)	76.9% (20/26)	90.5% (19/21)	95.0% (19/20)	95.0% (19/20)	95.0% (19/20)	76.0% (19/25)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)
Negative predictivity	100% (6/6)	100% (7/7)	100% (7/7)	100% (7/7)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (6/6)	100% (7/7)	100% (7/7)	100% (7/7)
Accuracy	76.5% (26/34)	81.8% (27/33)	81.8% (27/33)	81.8% (27/33)	93.5% (29/31)	96.7% (29/30)	96.7% (29/30)	96.7% (29/30)	80.6% (25/31)	86.7% (26/30)	86.7% (26/30)	86.7% (26/30)

Lab 5	Draft criteria for the final judgment ^a											
	Original				Third data analysis based on the criteria for the proposed protocol							
					When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	40.0% (6/15)	46.2% (6/13)	42.9% (6/14)	42.9% (6/14)	83.3% (10/12)	83.3% (10/12)	83.3% (10/12)	83.3% (10/12)	50.0% (6/12)	50.0% (6/12)	50.0% (6/12)	50.0% (6/12)
Positive predictivity	69.0% (20/29)	74.1% (20/27)	71.4% (20/28)	71.4% (20/28)	90.5% (19/21)	90.5% (19/21)	90.5% (19/21)	90.5% (19/21)	76.0% (19/25)	76.0% (19/25)	76.0% (19/25)	76.0% (19/25)
Negative predictivity	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)
Accuracy	74.3% (26/35)	78.8% (26/33)	76.5% (26/34)	76.5% (26/34)	93.5% (29/31)	93.5% (29/31)	93.5% (29/31)	93.5% (29/31)	80.6% (25/31)	80.6% (25/31)	80.6% (25/31)	80.6% (25/31)

a : A : Original : Final judgment of positive when positive results were obtained in at least one of three assays
 Third data analysis based on the criteria for the proposed protocol: The highest criteria among the three assay results was selected as the final judgment
 B : Final judgment based on the mean value of three assays.
 C : Final judgment based on the majority of three assay results.
 D : Final judgment on the first assay results.

Table 29 Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results (continued).

Lab 6	Draft criteria for the final judgment ^a											
	Original	Third data analysis based on the criteria for the proposed protocol										
		When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals						
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	90.0% (9/10)	100% (10/10)	100% (10/10)	90.0% (9/10)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)
Positive predictivity	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)	95.0% (19/20)	100% (19/19)	100% (19/19)	95.0% (19/20)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)
Negative predictivity	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)	100% (9/9)	100% (10/10)	100% (10/10)	100% (9/9)	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)
Accuracy	86.2% (25/29)	86.2% (25/29)	86.2% (25/29)	86.2% (25/29)	96.6% (28/29)	100% (29/29)	100% (29/29)	96.6% (28/29)	86.2% (25/29)	86.2% (25/29)	86.2% (25/29)	86.2% (25/29)

Lab 7	Draft criteria for the final judgment ^a											
	Original	Third data analysis based on the criteria for the proposed protocol										
		When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals						
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (19/19)	100% (18/18)	100% (18/18)	100% (18/18)	100% (19/19)	100% (18/18)	100% (18/18)	100% (18/18)
Specificity	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	90.9% (10/11)	90.9% (10/11)	90.9% (10/11)	90.9% (10/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)
Positive predictivity	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	95.0% (19/20)	94.7% (18/19)	94.7% (18/19)	94.7% (18/19)	82.6% (19/23)	81.8% (18/22)	81.8% (18/22)	81.8% (18/22)
Negative predictivity	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)
Accuracy	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	96.7% (29/30)	96.6% (28/29)	96.6% (28/29)	96.6% (28/29)	86.7% (26/30)	86.2% (25/29)	86.2% (25/29)	86.2% (25/29)

a : A : Original : Final judgment of positive when positive results were obtained in at least one of three assays

Third data analysis based on the criteria for the proposed protocol: The highest criteria among the three assay results was selected as the final judgment

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results.

D : Final judgment on the first assay results.

Table 30 Third data analysis based on the criteria for the proposed protocol: Contingency table for integrated judgment results.

Phase 2	Draft criteria for the final judgment ^a											
	Original ^b				Third data analysis based on the criteria for the proposed protocol ^c							
					When the “±: weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: weakly photoreactive” chemicals were defined as “phototoxic” chemicals			
A	B	C	D	A	B	C	D	A	B	C	D	
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	80.0% (8/10)	90.0% (9/10)	90.0% (9/10)	90.0% (9/10)	60.0% (6/10)	70.0% (7/10)	70.0% (7/10)	70.0% (7/10)
Positive predictivity	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	90.5% (19/21)	95.0% (19/20)	95.0% (19/20)	95.0% (19/20)	82.6% (19/23)	86.4% (19/22)	86.4% (19/22)	86.4% (19/22)
Negative predictivity	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	100% (8/8)	100% (9/9)	100% (9/9)	100% (9/9)	100% (6/6)	100% (7/7)	100% (7/7)	100% (7/7)
Accuracy	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	93.1% (27/29)	96.6% (28/29)	96.6% (28/29)	96.6% (28/29)	86.2% (25/29)	89.7% (26/29)	89.7% (26/29)	89.7% (26/29)

Integrated judgments were made by the majority of each laboratory's final judgment.

a : A : Original : Final judgment of positive when positive results were obtained in at least one of three assays

Third data analysis based on the criteria for the proposed protocol: The highest criteria among the three assay results was selected as the final judgment

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results.

D : Final judgment on the first assay results.

b : See, tables 17A to 17D

c : See, tables 27A to 27D

Table 31A Fourth data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgement.

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	I	I	I	I ¹⁾	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	+	u
II-20	Avobenzone	(+)	(+)	(+)	(+)	+	+	+	+	u	u	u	u	I	I	I	I	I
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

-: Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM . For regulatory purposes, the stability of the test chemical in the reaction mixture both before and after light exposure is to be confirmed when results at 20 μM are used for judgment as a non-photoreactive chemical for which no further phototoxicity testing is necessary.)

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive(The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 or 1-1 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results or 1 "photoreactive" results and 1 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results or 1 "weakly photoreactive" results and 1 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

1): The chemical was judged as "Inconclusive" since the SO result was "Photoreactive" at 50 μM and the SA result was < 20 at 20 μM

Table 31A Fourth data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgement (continued).

ROS assay Validation data (seric) pattern A		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	+ ^b	+	+	+	+	+	±	±	+ ^b	±	±	-	±	+
II-29	Bumetizole	-	-	-	-	I	I	I	I	-	-	-	-	I	I	I	I	-
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	±	±	±	±	±	-	-	-	-	±	±	±	±	±
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	-
II-33	Drometizole	u	u	u	u	u	u	u	u	(-)	(-)	(-)	(-)	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(+)	-	-	-	-	-	-	-	-	-
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	-	-	-	-	(-)	(+)	(-)	(+)	(-)	(-)	(-)	(-)	I	I	I	I	-
II-38	Octyl methoxycinnamate	I	I	I	I	±	-	-	±	-	-	-	-	I	I	I	I	I
II-39	Octyl salicylate	-	-	-	-	(±)	(±)	(±)	(±)	-	-	-	-	-	-	-	-	-
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	-	-	-	-	I	I	I	I	-
II-42	UV-571	I	I	I	I	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : Photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

± : Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM . For regulatory purposes, the stability of the test chemical in the reaction mixture both before and after light exposure is to be confirmed when results at 20 μM are used for judgment as a non-photoreactive chemical for which no further phototoxicity testing is necessary.)

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive(The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 or 1-1-split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results or 1 "photoreactive" results and 1 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results or 1 "weakly photoreactive" results and 1 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

b : Final judgment of photoreactive when photoreactive results were obtained in at least one of three assays.

Table 31B Fourth data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the mean value of three assays.

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	I	I	I	I ¹⁾	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzone	(+)	(+)	(+)	(+)	+	+	+	+	u	u	u	u	I	I	I	I	I
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

-: Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM . For regulatory purposes, the stability of the test chemical in the reaction mixture both before and after light exposure is to be confirmed when results at 20 μM are used for judgment as a non-photoreactive chemical for which no further phototoxicity testing is necessary.)

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I: inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u: undetermined due to precipitation

a: Integrated judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 or 1-1 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results or 1 "photoreactive" results and 1 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results or 1 "weakly photoreactive" results and 1 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

1): The chemical was judged as "Inconclusive" since the SO result was "Photoreactive" at 50 μM and the SA result was < 20 at 20 μM

Table 31B Fourth data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the mean value of three assays (continued).

ROS assay Validation data (seric) pattern B		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	±	±	±	-	±	±
II-29	Bumetrizole	-	-	-	-	I	I	I	I	-	-	-	-	I	I	I	I	-
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	-
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	-
II-33	Drometrizole	u	u	u	u	u	u	u	u	(-)	(-)	(-)	(-)	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(-)	-	-	-	-	-	-	-	-	-
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	-	-	-	-	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	I	I	I	I	-
II-38	Octyl methoxycinnamate	I	I	I	I	±	-	-	-	-	-	-	-	I	I	I	I	-
II-39	Octyl salicylate	-	-	-	-	(±)	(±)	(±)	(±)	-	-	-	-	-	-	-	-	-
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	-	-	-	-	I	I	I	I	-
II-42	UV-571	I	I	I	I	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : Photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

± : Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM . For regulatory purposes, the stability of the test chemical in the reaction mixture both before and after light exposure is to be confirmed when results at 20 μM are used for judgment as a non-photoreactive chemical for which no further phototoxicity testing is necessary.)

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive(The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 or 1-1 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results or 1 "photoreactive" results and 1 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results or 1 "weakly photoreactive" results and 1 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 31C Fourth data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the majority of three assay results.

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	I	I	I	I ¹⁾	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzone	(+)	(+)	(+)	(+)	+	+	+	+	u	u	u	u	I	I	I	I	I
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

-: Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM . For regulatory purposes, the stability of the test chemical in the reaction mixture both before and after light exposure is to be confirmed when results at 20 μM are used for judgment as a non-photoreactive chemical for which no further phototoxicity testing is necessary.)

(+), (+), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I: inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u: undetermined due to precipitation

a: Integrated judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 or 1-1 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results or 1 "photoreactive" results and 1 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results or 1 "weakly photoreactive" results and 1 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

1): The chemical was judged as "Inconclusive" since the SO result was "Photoreactive" at 50 μM and the SA result was < 20 at 20 μM

Table 31C Fourth data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the majority of three assay results (continued).

ROS assay Validation data (seric) pattern C		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	Assay			Final Judge-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	±	±	±	±	-	±
II-29	Bumetizole	-	-	-	-	I	I	I	I	-	-	-	-	I	I	I	I	-
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	-
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	-
II-33	Drometizole	u	u	u	u	u	u	u	u	(-)	(-)	(-)	(-)	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(+)	-	-	-	-	-	-	-	-	-
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	-	-	-	-	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	I	I	I	I	-
II-38	Octyl methoxycinnamate	I	I	I	I	±	-	-	-	-	-	-	-	I	I	I	I	-
II-39	Octyl salicylate	-	-	-	-	(±)	(±)	(±)	(±)	-	-	-	-	-	-	-	-	-
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	-	-	-	-	I	I	I	I	-
II-42	UV-571	I	I	I	I	I	I	I	I	u	u	u	u	I	I	I	I	I

+: Photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

-: Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM . For regulatory purposes, the stability of the test chemical in the reaction mixture both before and after light exposure is to be confirmed when results at 20 μM are used for judgment as a non-photoreactive chemical for which no further phototoxicity testing is necessary.)

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive(The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 or 1-1 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results or 1 "photoreactive" results and 1 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results or 1 "weakly photoreactive" results and 1 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 31D Fourth data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the single assay results.

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	Assay			Final Judg-	
No.	Name	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	1st	2nd	3rd	ment	
II-1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-3	Amiodarone HCl	u	u	u	u	u	u	u	u	u	u	u	u	I	I	I	I	u
II-4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-6	Fenofibrate	+	+	+	+	I	I	I	I ¹⁾	+	+	+	+	I	I	I	I	+
II-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-9	6-Methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-19	Anthracene	u	u	u	u	u	u	u	u	u	u	u	u	I	+	I	I	u
II-20	Avobenzone	(+)	(+)	(+)	(+)	+	+	+	+	u	u	u	u	I	I	I	I	I
II-21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II-23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: Photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

-: Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM . For regulatory purposes, the stability of the test chemical in the reaction mixture both before and after light exposure is to be confirmed when results at 20 μM are used for judgment as a non-photoreactive chemical for which no further phototoxicity testing is necessary.)

(+), (+), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I: inconclusive (The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u: undetermined due to precipitation

a: Integrated judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 or 1-1 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results or 1 "photoreactive" results and 1 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results or 1 "weakly photoreactive" results and 1 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

1): The chemical was judged as "Inconclusive" since the SO result was "Photoreactive" at 50 μM and the SA result was < 20 at 20 μM

Table 31D Fourth data analysis based on the criteria for the proposed protocol: Judgment from the Phase 2 results: Final judgment based on the single assay results (continued).

ROS assay Validation data (seric) pattern D		Lab 4				Lab 5				Lab 6				Lab 7				Integrated Judgment ^a
Chemicals		Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
No.	Name	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II-24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-25	Benzocaine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-26	Erythromycin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-27	Penicillin G	±	±	±	±	±	±	±	±	±	±	±	±	-	-	-	-	±
II-28	Phenytoin	±	±	+	±	+	+	+	+	+	±	±	+	±	±	-	±	±
II-29	Bumetizole	-	-	-	-	I	I	I	I	-	-	-	-	I	I	I	I	-
II-30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-31	Chlorhexidine	-	±	-	-	±	±	±	±	-	-	-	-	±	±	±	±	-
II-32	Cinnamic acid	-	-	-	-	±	±	±	±	±	±	±	±	-	-	-	-	-
II-33	Drometizole	u	u	u	u	u	u	u	u	(-)	(-)	(-)	(-)	u	u	u	u	u
II-34	L-Histidine	+	+	±	+	+	+	+	+	±	±	±	±	+	+	+	+	+
II-35	Methylbenzylidene camphor	(+)	(+)	(+)	(+)	(+)	(-)	(+)	(+)	-	-	-	-	-	-	-	-	-
II-36	Octrizole	I	I	I	I	I	I	I	I	u	u	u	u	±	±	±	±	I
II-37	Octyl methacrylate	-	-	-	-	(-)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	I	I	I	I	-
II-38	Octyl methoxycinnamate	I	I	I	I	±	-	-	±	-	-	-	-	I	I	I	I	I
II-39	Octyl salicylate	-	-	-	-	(±)	(±)	(±)	(±)	-	-	-	-	-	-	-	-	-
II-40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II-41	SDS	±	±	±	±	-	-	-	-	-	-	-	-	I	I	I	I	-
II-42	UV-571	I	I	I	I	I	I	I	I	u	u	u	u	I	I	I	I	I

+ : Photoreactive (Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM .)

-: Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM : Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM . For regulatory purposes, the stability of the test chemical in the reaction mixture both before and after light exposure is to be confirmed when results at 20 μM are used for judgment as a non-photoreactive chemical for which no further phototoxicity testing is necessary.)

(+), (±), (-): The results shown in the parenthesis are the results at 50 μM or 100 μM which are not used for the integrated judgment.

I : inconclusive(The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion)

u : undetermined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's judgment.

Integrated judgments were followed by the following criteria when final judgments by the four laboratories were reached on a 2-2 or 1-1 split, modest results other than "Inconclusive" were chosen as an Integrated judgment.

If there are 2 "photoreactive" results and 2 "weakly photoreactive" results or 1 "photoreactive" results and 1 "weakly photoreactive" results, "weakly photoreactive" was chosen as an Integrated judgment.

If there are 2 "weakly photoreactive" results and 2 "non-photoreactive" results or 1 "weakly photoreactive" results and 1 "non-photoreactive" results, "non-photoreactive" was chosen as an Integrated judgment.

Table 32A Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: The highest criteria among the three assay results was selected as the final judgement.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^a
	-	2	3	9	14 ^b
Total		21	3	9	33

a: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
b: Five of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 85.7% (12/14)
Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (12/12)
Accuracy : 93.9% (31/33)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 64.3% (9/14)
Positive predictivity : 79.2%(19/24) Negative predictivity : 100% (9/9)
Accuracy : 84.8%(28/33)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	2	4	6	12 ^d
Total		21	4	6	31

c: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene).
d: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl salicylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
Accuracy : 93.5% (29/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 50.0%(6/12)
Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (6/6)
Accuracy : 80.6%(25/31)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^e
	-	1	3	11	15 ^f
Total		20	3	11	34

e: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
f: Four of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Drometrizole, Octizole, Octyl methacrylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 93.3% (14/15)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (14/14)
Accuracy : 97.1% (33/34)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 73.3%(11/15)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (11/11)
Accuracy : 88.2%(30/34)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^g
	-	1	3	9	13 ^h
Total		20	3	9	32

g: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Avobenzone).
h: Six of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 92.3% (12/13)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (12/12)
Accuracy : 96.9% (31/32)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 69.2%(9/13)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (9/9)
Accuracy : 87.5%(28/32)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ⁱ
	-	2	2	11	15 ^j
Total		21	2	11	34

i: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation, or could not be determined the majority of results. (Amiodarone HCl, Anthracene, Avobenzone).
j: Four of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation, or could not be determined the majority of results. (Drometrizole, Octizole, Octyl methoxycinnamate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 86.7% (13/15)
Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (13/13)
Accuracy : 94.1% (32/34)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 73.3% (11/15)
Positive predictivity : 82.6% (19/23) Negative predictivity : 100% (11/11)
Accuracy : 88.2% (30/34)

Table 32B Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgment based on the mean value of three assays.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^a
	-	1	3	10	14 ^b
Total		20	3	10	33

a: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
b: Five of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 92.9% (13/14)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (13/13)
Accuracy : 97.0% (32/33)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 71.4% (10/14)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (10/10)
Accuracy : 87.9%(29/33)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	2	3	7	12 ^d
Total		21	3	7	31

c: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene).
d: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl salicylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
Accuracy : 93.5% (29/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 58.3%(7/12)
Positive predictivity : 79.2%(19/24) Negative predictivity : 100% (7/7)
Accuracy : 83.9%(26/31)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^e
	-	0	4	11	15 ^f
Total		19	4	11	34

e: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
f: Four of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Drometrizole, Octizole, Octyl methacrylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (15/15)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (15/15)
Accuracy : 100% (34/34)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 73.3%(11/15)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (11/11)
Accuracy : 88.2%(30/34)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	18	0	0	18 ^g
	-	1	3	9	13 ^h
Total		19	3	9	31

g: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
h: Six of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 92.3% (12/13)
Positive predictivity : 94.7% (18/19) Negative predictivity : 100% (12/12)
Accuracy : 96.8% (30/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 69.2%(9/13)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (9/9)
Accuracy : 87.1%(27/31)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ⁱ
	-	1	2	13	16 ^j
Total		20	2	13	35

i: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation, or could not be determined the majority of results. (Amiodarone HCl, Anthracene, Avobenzone).
j: Three of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation, or could not be determined the majority of results. (Drometrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 93.8% (15/16)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (15/15)
Accuracy : 97.1% (34/35)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 81.3% (13/16)
Positive predictivity : 86.4% (19/22) Negative predictivity : 100% (13/13)
Accuracy : 91.4% (32/35)

Table 32C Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgment based on the majority of three assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^a
	-	1	3	10	14 ^b
Total		20	3	10	33

a: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
b: Five of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 92.9% (13/14)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (13/13)
Accuracy : 97.0% (32/33)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 71.4% (10/14)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (10/10)
Accuracy : 87.9%(29/33)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	2	3	7	12 ^d
Total		21	3	7	31

c: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene).
d: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl salicylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
Accuracy : 93.5% (29/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 58.3%(7/12)
Positive predictivity : 79.2%(19/24) Negative predictivity : 100% (7/7)
Accuracy : 83.9%(26/31)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^e
	-	0	4	11	15 ^f
Total		19	4	11	34

e: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
f: Four of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Drometrizole, Octizole, Octyl methacrylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (15/15)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (15/15)
Accuracy : 100% (34/34)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 73.3%(11/15)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (11/11)
Accuracy : 88.2%(30/34)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	18	0	0	18 ^g
	-	1	3	9	13 ^h
Total		19	3	9	31

g: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
h: Six of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 92.3% (12/13)
Positive predictivity : 94.7% (18/19) Negative predictivity : 100% (12/12)
Accuracy : 96.8% (30/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 69.2%(9/13)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (9/9)
Accuracy : 87.1%(27/31)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ⁱ
	-	1	2	13	16 ^j
Total		20	2	13	35

i: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation, or could not be determined the majority of results. (Amiodarone HCl, Anthracene, Avobenzone).
j: Three of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation, or could not be determined the majority of results. (Drometrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 93.8% (15/16)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (15/15)
Accuracy : 97.1% (34/35)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 81.3% (13/16)
Positive predictivity : 86.4% (19/22) Negative predictivity : 100% (13/13)
Accuracy : 91.4% (32/35)

Table 32D Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgment based on the single assay results.

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 4		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^a
	-	1	3	10	14 ^b
Total		20	3	10	33

a: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
b: Five of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 92.9% (13/14)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (13/13)
Accuracy : 97.0% (32/33)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 71.4% (10/14)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (10/10)
Accuracy : 87.9%(29/33)

Lab 5		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	2	4	6	12 ^d
Total		21	4	6	31

c: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene).
d: Seven of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl salicylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 83.3% (10/12)
Positive predictivity : 90.5% (19/21) Negative predictivity : 100% (10/10)
Accuracy : 93.5% (29/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 50.0%(6/12)
Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (6/6)
Accuracy : 80.6%(25/31)

Lab 6		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^e
	-	1	3	11	15 ^f
Total		20	3	11	34

e: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Avobenzone).
f: Four of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Drometrizole, Octizole, Octyl methacrylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 93.3% (14/15)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (14/14)
Accuracy : 97.1% (33/34)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 73.3%(11/15)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (11/11)
Accuracy : 88.2%(30/34)

Lab 7		ROS			Total
		+	±	-	
Phototoxic	+	18	0	0	18 ^g
	-	1	3	9	13 ^h
Total		19	3	9	31

g: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Amiodarone HCl, Anthracene, Fenofibrate, Avobenzone).
h: Six of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation (Bumetrizole, Drometrizole, Octyl methacrylate, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 92.3% (12/13)
Positive predictivity : 94.7% (18/19) Negative predictivity : 100% (12/12)
Accuracy : 96.8% (30/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 69.2%(9/13)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (9/9)
Accuracy : 87.1%(27/31)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ⁱ
	-	1	2	12	15 ^j
Total		20	2	12	34

i: Three of 22 phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation, or could not be determined the majority of results. (Amiodarone HCl, Anthracene, Avobenzone).
j: Four of 19 non-phototoxic chemicals were not evaluated at 20 or 200 µM due to precipitation, or could not be determined the majority of results. (Drometrizole, Octizole, Octyl methoxycinnamate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 93.3% (14/15)
Positive predictivity : 95.0% (19/20) Negative predictivity : 100% (14/14)
Accuracy : 97.1% (33/34)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 80.0% (12/15)
Positive predictivity : 86.4% (19/22) Negative predictivity : 100% (12/12)
Accuracy : 91.2% (31/34)

Table 33 Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results.

Lab 4	Draft criteria for the final judgment ^a											
	Original				Data analysis based on the criteria for the fourth data analysis							
					When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
A	B	C	D	A	B	C	D	A	B	C	D	
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	42.9% (6/14)	53.8% (7/13)	53.8% (7/13)	53.8% (7/13)	85.7% (12/14)	92.9% (13/14)	92.9% (13/14)	92.9% (13/14)	64.3% (9/14)	71.4% (10/14)	71.4% (10/14)	71.4% (10/14)
Positive predictivity	71.4% (20/28)	76.9% (20/26)	76.9% (20/26)	76.9% (20/26)	90.5% (19/21)	95.0% (19/20)	95.0% (19/20)	95.0% (19/20)	79.2% (19/24)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)
Negative predictivity	100% (6/6)	100% (7/7)	100% (7/7)	100% (7/7)	100% (12/12)	100% (13/13)	100% (13/13)	100% (13/13)	100% (9/9)	100% (10/10)	100% (10/10)	100% (10/10)
Accuracy	76.5% (26/34)	81.8% (27/33)	81.8% (27/33)	81.8% (27/33)	93.9% (31/33)	97.0% (32/33)	97.0% (32/33)	97.0% (32/33)	84.8% (28/33)	87.9% (29/33)	87.9% (29/33)	87.9% (29/33)

Lab 5	Draft criteria for the final judgment ^a											
	Original				Data analysis based on the criteria for the fourth data analysis							
					When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
A	B	C	D	A	B	C	D	A	B	C	D	
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	40.0% (6/15)	46.2% (6/13)	42.9% (6/14)	42.9% (6/14)	83.3% (10/12)	83.3% (10/12)	83.3% (10/12)	83.3% (10/12)	50.0% (6/12)	58.3% (7/12)	58.3% (7/12)	50.0% (6/12)
Positive predictivity	69.0% (20/29)	74.1% (20/27)	71.4% (20/28)	71.4% (20/28)	90.5% (19/21)	90.5% (19/21)	90.5% (19/21)	90.5% (19/21)	76.0% (19/25)	79.2% (19/24)	79.2% (19/24)	76.0% (19/25)
Negative predictivity	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)	100% (6/6)	100% (7/7)	100% (7/7)	100% (6/6)
Accuracy	74.3% (26/35)	78.8% (26/33)	76.5% (26/34)	76.5% (26/34)	93.5% (29/31)	93.5% (29/31)	93.5% (29/31)	93.5% (29/31)	80.6% (25/31)	83.9% (26/31)	83.9% (26/31)	80.6% (25/31)

a : A : Original: Final judgement of positive when positive results were obtained in at least one of three assays
 Fourth data analysis based on the criteria for the proposed protocol: The highest criteria among the three assay results was selected as the final judgement
 B : Final judgment based on the mean value of three assays.
 C : Final judgment based on the majority of three assay results.
 D : Final judgment based on the single assay results

Table 33 Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results (continued).

Lab 6	Draft criteria for the final judgment ^a											
	Original				Data analysis based on the criteria for the fourth data analysis							
					When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	93.3% (14/15)	100% (15/15)	100% (15/15)	93.3% (14/15)	73.3% (11/15)	73.3% (11/15)	73.3% (11/15)	73.3% (11/15)
Positive predictivity	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)	95.0% (19/20)	100% (19/19)	100% (19/19)	95.0% (19/20)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)
Negative predictivity	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)	100% (14/14)	100% (15/15)	100% (15/15)	100% (14/14)	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Accuracy	86.2% (25/29)	86.2% (25/29)	86.2% (25/29)	86.2% (25/29)	97.1% (33/34)	100% (34/34)	100% (34/34)	97.1% (33/34)	88.2% (30/34)	88.2% (30/34)	88.2% (30/34)	88.2% (30/34)

Lab 7	Draft criteria for the final judgment ^a											
	Original				Data analysis based on the criteria for the fourth data analysis							
					When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (19/19)	100% (18/18)	100% (18/18)	100% (18/18)	100% (19/19)	100% (18/18)	100% (18/18)	100% (18/18)
Specificity	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	69.2% (9/13)	69.2% (9/13)	69.2% (9/13)	69.2% (9/13)
Positive predictivity	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	95.0% (19/20)	94.7% (18/19)	94.7% (18/19)	94.7% (18/19)	82.6% (19/23)	81.8% (18/22)	81.8% (18/22)	81.8% (18/22)
Negative predictivity	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	100% (12/12)	100% (12/12)	100% (12/12)	100% (12/12)	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)
Accuracy	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	96.9% (31/32)	96.8% (30/31)	96.8% (30/31)	96.8% (30/31)	87.5% (28/32)	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)

a : A : Original: Final judgement of positive when positive results were obtained in at least one of three assays
 Fourth data analysis based on the criteria for the proposed protocol: The highest criteria among the three assay results was selected as the final judgement
 B : Final judgment based on the mean value of three assays.
 C : Final judgment based on the majority of three assay results.
 D : Final judgment based on the single assay results

Table 34 Fourth data analysis based on the criteria for the proposed protocol: Contingency table for integrated judgment results.

Phase 2	Draft criteria for the final judgment ^a											
	Original				Data analysis based on the criteria for the fourth data analysis							
					When the results judged “±: weakly photoreactive” were regarded as the “non-photoreactive” results;				When the results judged “±: weakly photoreactive” were regarded as the “photoreactive” results;			
	A ^b	B	C	D	A ^c	B	C	D	A ^c	B	C	D
Sensitivity	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	63.6% (7/11)	86.7% (13/15)	93.8% (15/16)	93.8% (15/16)	93.3% (14/15)	73.3% (11/15)	81.3% (13/16)	81.3% (13/16)	80.0% (12/15)
Positive predictivity	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	83.3% (20/24)	90.5% (19/21)	95.0% (19/20)	95.0% (19/20)	95.0% (19/20)	82.6% (19/23)	86.4% (19/22)	86.4% (19/22)	86.4% (19/22)
Negative predictivity	100% (7/7)	100% (7/7)	100% (7/7)	100% (7/7)	100% (13/13)	100% (15/15)	100% (15/15)	100% (14/14)	100% (11/11)	100% (13/13)	100% (13/13)	100% (12/12)
Accuracy	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	94.1% (32/34)	97.1% (34/35)	97.1% (34/35)	97.1% (33/34)	88.6% (30/34)	91.4% (32/35)	91.4% (32/35)	91.2% (31/34)

Integrated judgments were made by the majority of each laboratory's final judgment.

a : A :Original: Final judgement of positive when positive results were obtained in at least one of three assays

Fourth data analysis based on the criteria for the proposed protocol: The highest criteria among the three assay results was selected as the final judgement

B : Final judgment based on the mean value of three assays.

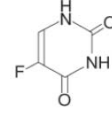
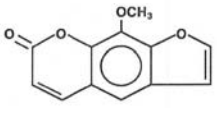
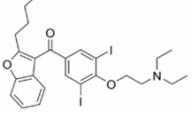
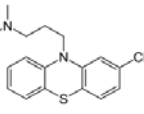
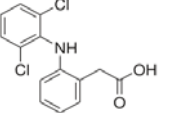
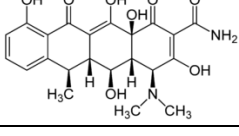
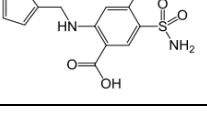
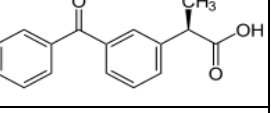
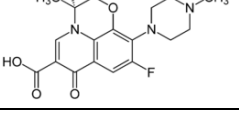
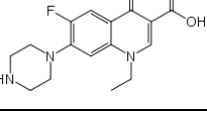
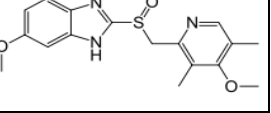
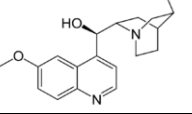
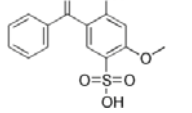
C : Final judgment based on the majority of three assay results.

D : Final judgment based on the single assay results.

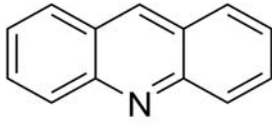
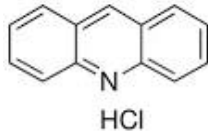
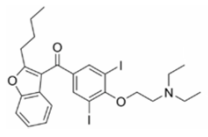
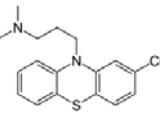
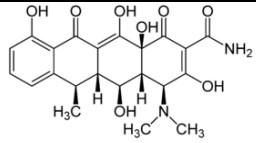
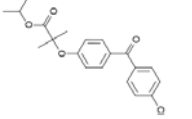
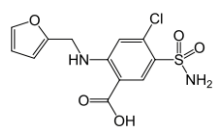
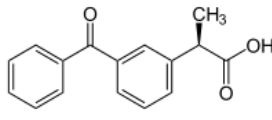
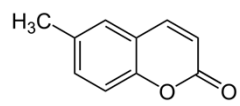
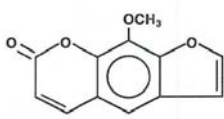
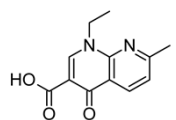
b : See, tables 17A to 17D

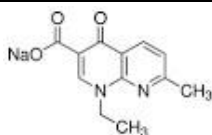
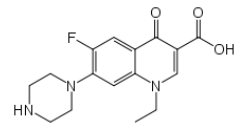
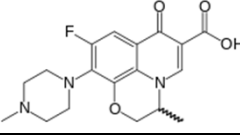
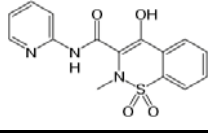
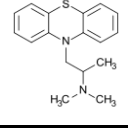
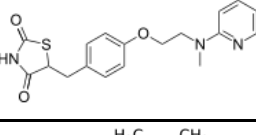
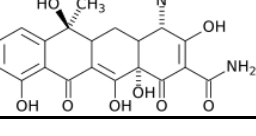
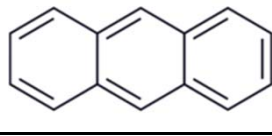
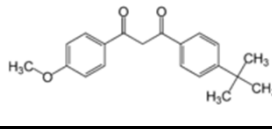
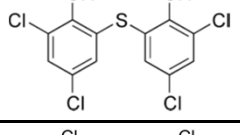
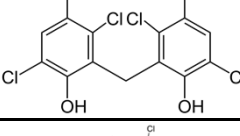
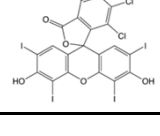
c : See, tables 31A to 31D

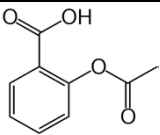
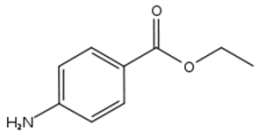
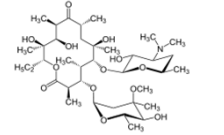
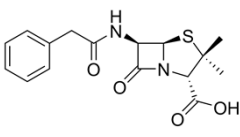
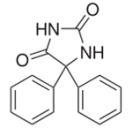
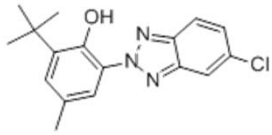
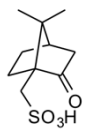
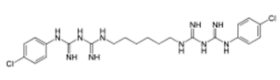
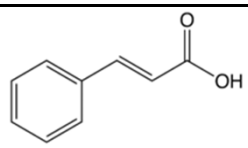
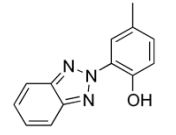
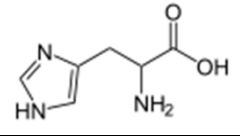
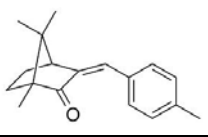
Appendix 1 Chemical structures of the test chemicals for the Phase 1 study

No.	Chemical name	CAS No.	Molecular weight	Chemical structure
I-1	5-FU	51-21-8	130.1	
I-2	8-MOP	298-81-7	216.2	
I-3	Amiodarone HCl	19774-82-4	681.8	
I-4	Chlorpromazine HCl	69-09-0	355.3	
I-5	Diclofenac	15307-79-6	318.1	
I-6	Doxycycline HCl	10592-13-9	512.9	
I-7	Furosemide	54-31-9	330.7	
I-8	Ketoprofen	22071-15-4	254.3	
I-9	Levofloxacin	100986-85-4	361.4	
I-10	Norfloxacin	70458-96-7	319.3	
I-11	Omeprazole	73590-58-6	345.4	
I-12	Quinine HCl	6119-47-7	396.9	
I-13	Sulisobenzone	4065-45-6	308.3	

Appendix 2 Chemical structures of the test chemicals for the Phase 2 study

NO.	Chemical name	CAS No. ^{a)}	Molecular weight	Chemical structure
Phototoxic drugs				
II-1	Acridine	260-94-6	179.2	
II-2	Acridine HCl	17784-47-3	215.7	
II-3	Amiodarone HCl	19774-82-4	681.8	
II-4	Chlorpromazine HCl	69-09-0	355.3	
II-5	Doxycycline HCl	10592-13-9	480.9	
II-6	Fenofibrate	49562-28-9	360.8	
II-7	Furosemide	54-31-9	330.7	
II-8	Ketoprofen	22071-15-4	254.3	
II-9	6-Methylcoumarine	92-48-8	160.2	
II-10	8-MOP	298-81-7	216.2	
II-11	Nalidixic acid	389-08-2	232.2	

II-12	Nalidixic acid (Na salt)	3374-05-8	254.2	
II-13	Norfloxacin	70458-96-7	319.3	
II-14	Ofloxacin	82419-36-1	361.4	
II-15	Piroxicam	36322-90-4	331.4	
II-16	Promethazine HCl	58-33-3	320.9	
II-17	Rosiglitazone	122320-73-4	357.4	
II-18	Tetracycline	60-54-8	444.4	
Phototoxic non-drug chemicals				
II-19	Anthracene	120-12-7	178.2	
II-20	Avobenzone	70356-09-1	310.39	
II-21	Bithionol	97-18-7	356.1	
II-22	Hexachlorophene	70-30-4	406.9	
II-23	Rose bengal	632-69-9	1017.6	
Non-phototoxic drugs				

II-24	Aspirin	50-78-2	180.2	
II-25	Benzocaine	94-09-7	165.2	
II-26	Erythromycin	114-07-8	733.9	
II-27	Penicillin G	113-98-4	372.5	
II-28	Phenytoin	57-41-0	252.3	
Non-phototoxic non-drug chemicals				
II-29	Bumetrizole	3896-11-5	315.8	
II-30	Camphor sulfonic acid	3144-16-9	232.3	
II-31	Chlorhexidine	55-56-1	505.5	
II-32	Cinnamic acid	140-10-3	148.2	
II-33	Drometrizole	2440-22-4	225.25	
II-34	L-Histidine	71-00-1	155.2	
II-35	Methylbenzylidene camphor	36861-47-9	254.4	

II-36	Octrizole	3147-75-9	323.43	
II-37	Octyl methacrylate	688-84-6	198.3	
II-38	Octyl methoxycinnamate	5466-77-3	290.4	
II-39	Octyl salicylate	118-60-5	250.3	
II-40	PABA	150-13-0	137.1	
II-41	SDS	151-21-3	288.4	
II-42	UV-571	125304-04-3	393.56	
Positive/Negative control				
PC	Quinine HCl	6119-47-7	396.9	
NC	Sulizobenzone	4065-45-6	308.3	

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.025	1.041	0.757	1.038	265	1	1	0.031	0.031	0.180	0.056	124
	2	1.020	1.040	0.761	1.036	256		2	0.031	0.030	0.180	0.055	124
	3	1.022	1.035	0.765	1.033	254		3	0.032	0.031	0.191	0.056	134
	Mean	1.022	1.039	0.761	1.036	258		Mean	0.031	0.031	0.184	0.056	127
2	1	1.021	1.041	0.761	1.034	252	2	1	0.031	0.032	0.193	0.057	138
	2	1.026	1.040	0.765	1.031	253		2	0.030	0.031	0.189	0.055	135
	3	1.024	1.044	0.765	1.036	251		3	0.031	0.032	0.190	0.057	135
	Mean	1.024	1.042	0.764	1.034	252		Mean	0.031	0.032	0.191	0.056	136
3	1	1.023	1.035	0.758	1.028	257	3	1	0.030	0.030	0.177	0.054	123
	2	1.015	1.030	0.760	1.019	247		2	0.031	0.030	0.175	0.054	120
	3	1.022	1.035	0.766	1.029	248		3	0.031	0.031	0.182	0.055	127
	Mean	1.020	1.033	0.761	1.025	251		Mean	0.031	0.030	0.178	0.054	123
4	1	1.024	1.039	0.772	1.031	244	4	1	0.030	0.030	0.166	0.053	113
	2	1.022	1.038	0.777	1.029	237		2	0.030	0.030	0.163	0.053	110
	3	1.029	1.039	0.790	1.034	231		3	0.031	0.031	0.169	0.054	115
	Mean	1.025	1.039	0.780	1.031	237		Mean	0.030	0.030	0.166	0.053	113
5	1	1.040	1.054	0.773	1.039	253	5	1	0.030	0.030	0.161	0.053	108
	2	1.037	1.055	0.784	1.038	239		2	0.030	0.030	0.157	0.052	104
	3	1.039	1.056	0.793	1.045	232		3	0.031	0.030	0.163	0.053	109
	Mean	1.039	1.055	0.783	1.041	241		Mean	0.030	0.030	0.160	0.053	107
6	1	1.032	1.040	0.787	1.040	244	6	1	0.030	0.032	0.158	0.053	107
	2	1.026	1.045	0.788	1.043	237		2	0.031	0.031	0.156	0.053	104
	3	1.031	1.040	0.798	1.040	232		3	0.031	0.032	0.162	0.054	110
	Mean	1.030	1.042	0.791	1.041	238		Mean	0.031	0.032	0.159	0.053	107

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.035	1.046	0.776	1.042	255	7	1	0.030	0.030	0.162	0.054	109
	2	1.030	1.040	0.782	1.038	244		2	0.030	0.031	0.159	0.055	106
	3	1.033	1.050	0.787	1.043	242		3	0.030	0.031	0.165	0.054	112
	Mean	1.033	1.045	0.782	1.041	247		Mean	0.030	0.031	0.162	0.054	109
8	1	1.035	1.054	0.784	1.047	245	8	1	0.031	0.031	0.159	0.053	106
	2	1.029	1.045	0.783	1.038	240		2	0.030	0.030	0.153	0.052	101
	3	1.032	1.043	0.792	1.038	234		3	0.031	0.032	0.154	0.055	101
	Mean	1.032	1.047	0.786	1.041	240		Mean	0.031	0.031	0.155	0.053	103
9	1	1.015	1.035	0.759	1.029	248	9	1	0.029	0.031	0.170	0.053	118
	2	1.019	1.033	0.767	1.027	244		2	0.030	0.030	0.169	0.053	116
	3	1.017	1.036	0.766	1.026	243		3	0.031	0.032	0.179	0.055	125
	Mean	1.017	1.035	0.764	1.027	245		Mean	0.030	0.031	0.173	0.054	120
10	1	1.034	1.065	0.766	1.063	265	10	1	0.031	0.030	0.175	0.055	119
	2	1.032	1.053	0.773	1.049	256		2	0.030	0.030	0.171	0.054	116
	3	1.034	1.058	0.781	1.056	250		3	0.030	0.031	0.175	0.056	120
	Mean	1.033	1.059	0.773	1.056	257		Mean	0.030	0.030	0.174	0.055	118
11	1	1.031	1.045	0.779	1.041	248	11	1	0.030	0.032	0.161	0.054	108
	2	1.032	1.039	0.789	1.034	239		2	0.030	0.032	0.159	0.056	106
	3	1.030	1.046	0.793	1.041	233		3	0.031	0.033	0.162	0.056	108
	Mean	1.031	1.043	0.787	1.039	240		Mean	0.030	0.032	0.161	0.055	107
12	1	1.030	1.057	0.769	1.053	256	12	1	0.029	0.031	0.177	0.054	124
	2	1.027	1.041	0.771	1.035	251		2	0.030	0.031	0.174	0.054	120
	3	1.030	1.044	0.777	1.037	248		3	0.031	0.031	0.182	0.056	127
	Mean	1.029	1.047	0.772	1.042	252		Mean	0.030	0.031	0.178	0.055	124
Mean for all assays		-	-	-	-	247	Mean for all assays		-	-	-	-	116
SD for all assays		-	-	-	-	7	SD for all assays		-	-	-	-	10
CV for all assays		-	-	-	-	2.8	CV for all assays		-	-	-	-	8.6

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.044	1.041	1.037	1.038	4	1	1	0.034	0.031	0.036	0.056	-23
	2	1.045	1.040	1.042	1.036	0		2	0.033	0.030	0.035	0.055	-23
	3	1.043	1.035	1.039	1.033	1		3	0.034	0.031	0.035	0.056	-24
	Mean	1.044	1.039	1.039	1.036	2		Mean	0.034	0.031	0.035	0.056	-23
2	1	1.048	1.041	1.045	1.034	-5	2	1	0.035	0.032	0.035	0.057	-24
	2	1.047	1.040	1.043	1.031	-4		2	0.033	0.031	0.035	0.055	-22
	3	1.044	1.044	1.040	1.036	-4		3	0.034	0.032	0.035	0.057	-23
	Mean	1.046	1.042	1.043	1.034	-4		Mean	0.034	0.032	0.035	0.056	-23
3	1	1.043	1.035	1.037	1.028	-2	3	1	0.034	0.030	0.034	0.054	-24
	2	1.043	1.030	1.039	1.019	-4		2	0.033	0.030	0.034	0.054	-23
	3	1.040	1.035	1.032	1.029	0		3	0.034	0.031	0.038	0.055	-20
	Mean	1.042	1.033	1.036	1.025	-2		Mean	0.034	0.030	0.035	0.054	-22
4	1	1.052	1.039	1.047	1.031	-3	4	1	0.034	0.030	0.034	0.053	-23
	2	1.048	1.038	1.042	1.029	-2		2	0.034	0.030	0.034	0.053	-23
	3	1.047	1.039	1.042	1.034	-3		3	0.035	0.031	0.035	0.054	-23
	Mean	1.049	1.039	1.044	1.031	-3		Mean	0.034	0.030	0.034	0.053	-23
5	1	1.057	1.054	1.052	1.039	-9	5	1	0.033	0.030	0.034	0.053	-22
	2	1.058	1.055	1.052	1.038	-8		2	0.033	0.030	0.034	0.052	-22
	3	1.055	1.056	1.050	1.045	-9		3	0.034	0.030	0.035	0.053	-22
	Mean	1.057	1.055	1.051	1.041	-9		Mean	0.033	0.030	0.034	0.053	-22
6	1	1.053	1.040	1.053	1.040	-1	6	1	0.033	0.032	0.034	0.053	-20
	2	1.054	1.045	1.054	1.043	-1		2	0.033	0.031	0.033	0.053	-21
	3	1.052	1.040	1.050	1.040	1		3	0.034	0.032	0.034	0.054	-21
	Mean	1.053	1.042	1.052	1.041	0		Mean	0.033	0.032	0.034	0.053	-21

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.051	1.046	1.047	1.042	0	7	1	0.034	0.030	0.034	0.054	-23
	2	1.047	1.040	1.042	1.038	1		2	0.034	0.031	0.034	0.055	-23
	3	1.042	1.050	1.037	1.043	1		3	0.034	0.031	0.034	0.054	-23
	Mean	1.047	1.045	1.042	1.041	1		Mean	0.034	0.031	0.034	0.054	-23
8	1	1.058	1.054	1.054	1.047	-2	8	1	0.035	0.031	0.035	0.053	-22
	2	1.049	1.045	1.046	1.038	-3		2	0.035	0.030	0.036	0.052	-21
	3	1.044	1.043	1.041	1.038	-3		3	0.034	0.032	0.035	0.055	-21
	Mean	1.050	1.047	1.047	1.041	-3		Mean	0.035	0.031	0.035	0.053	-21
9	1	1.043	1.035	1.036	1.029	-1	9	1	0.034	0.031	0.034	0.053	-23
	2	1.042	1.033	1.039	1.027	-5		2	0.033	0.030	0.034	0.053	-22
	3	1.040	1.036	1.035	1.026	-3		3	0.034	0.032	0.036	0.055	-21
	Mean	1.042	1.035	1.037	1.027	-3		Mean	0.034	0.031	0.035	0.054	-22
10	1	1.059	1.065	1.058	1.063	-2	10	1	0.038	0.030	0.039	0.055	-24
	2	1.056	1.053	1.056	1.049	-3		2	0.034	0.030	0.034	0.054	-25
	3	1.057	1.058	1.054	1.056	0		3	0.034	0.031	0.034	0.056	-25
	Mean	1.057	1.059	1.056	1.056	-2		Mean	0.035	0.030	0.036	0.055	-25
11	1	1.048	1.045	1.045	1.041	-1	11	1	0.033	0.032	0.034	0.054	-22
	2	1.051	1.039	1.047	1.034	0		2	0.033	0.032	0.034	0.056	-22
	3	1.049	1.046	1.046	1.041	-1		3	0.034	0.033	0.035	0.056	-22
	Mean	1.049	1.043	1.046	1.039	-1		Mean	0.033	0.032	0.034	0.055	-22
12	1	1.049	1.057	1.047	1.053	-3	12	1	0.033	0.031	0.034	0.054	-23
	2	1.048	1.041	1.046	1.035	-3		2	0.033	0.031	0.034	0.054	-23
	3	1.050	1.044	1.047	1.037	-2		3	0.033	0.031	0.035	0.056	-22
	Mean	1.049	1.047	1.047	1.042	-3		Mean	0.033	0.031	0.034	0.055	-23
Mean for all assays	-	-	-	-	-2	Mean for all assays	-	-	-	-	-	-23	
SD for all assays	-	-	-	-	3	SD for all assays	-	-	-	-	-	1	
CV for all assays	-	-	-	-	-	CV for all assays	-	-	-	-	-	-	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.046	1.058	0.791	1.059	256	1	1	0.034	0.037	0.167	0.064	115
	2	1.042	1.048	0.789	1.049	254		2	0.032	0.037	0.167	0.047	117
	3	1.017	1.023	0.774	1.023	244		3	0.031	0.035	0.191	0.050	142
	Mean	1.035	1.043	0.785	1.044	251		Mean	0.032	0.036	0.175	0.054	125
2	1	1.053	1.061	0.746	1.060	306	2	1	0.031	0.033	0.199	0.042	156
	2	1.055	1.054	0.755	1.053	299		2	0.033	0.032	0.200	0.040	155
	3	1.029	1.037	0.750	1.037	278		3	0.031	0.033	0.219	0.052	176
	Mean	1.046	1.051	0.750	1.050	294		Mean	0.032	0.033	0.206	0.045	162
3	1	1.051	1.058	0.738	1.058	315	3	1	0.032	0.035	0.191	0.039	152
	2	1.050	1.053	0.744	1.052	308		2	0.033	0.035	0.188	0.038	148
	3	1.023	1.038	0.738	1.046	287		3	0.032	0.033	0.200	0.046	161
	Mean	1.041	1.050	0.740	1.052	303		Mean	0.032	0.034	0.193	0.041	154
4	1	1.059	1.065	0.744	1.066	315	4	1	0.032	0.037	0.202	0.039	166
	2	1.052	1.052	0.746	1.052	306		2	0.032	0.035	0.200	0.037	164
	3	1.026	1.034	0.740	1.032	286		3	0.031	0.034	0.216	0.040	181
	Mean	1.046	1.050	0.743	1.050	302		Mean	0.032	0.035	0.206	0.039	170
5	1	1.012	1.019	0.754	1.016	255	5	1	0.033	0.036	0.173	0.036	142
	2	1.002	1.018	0.762	1.014	237		2	0.032	0.036	0.175	0.037	145
	3	0.983	0.999	0.743	0.996	237		3	0.031	0.041	0.189	0.036	160
	Mean	0.999	1.012	0.753	1.009	243		Mean	0.032	0.038	0.179	0.036	149
6	1	1.022	1.029	0.764	1.027	256	6	1	0.031	0.034	0.174	0.042	136
	2	1.017	1.026	0.769	1.025	246		2	0.032	0.033	0.174	0.038	135
	3	1.004	1.004	0.775	1.002	227		3	0.030	0.032	0.187	0.041	150
	Mean	1.014	1.020	0.769	1.018	243		Mean	0.031	0.033	0.178	0.040	140

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.025	1.024	0.767	1.022	256	7	1	0.031	0.036	0.171	0.041	132
	2	1.015	1.018	0.766	1.015	247		2	0.032	0.032	0.173	0.039	133
	3	0.993	0.998	0.759	0.995	232		3	0.031	0.031	0.184	0.044	145
	Mean	1.011	1.013	0.764	1.011	245		Mean	0.031	0.033	0.176	0.041	137
8	1	1.018	1.020	0.759	1.017	256	8	1	0.031	0.033	0.181	0.045	141
	2	1.014	1.016	0.762	1.013	249		2	0.032	0.032	0.184	0.039	143
	3	0.993	0.997	0.755	0.995	235		3	0.030	0.031	0.193	0.038	154
	Mean	1.008	1.011	0.759	1.008	247		Mean	0.031	0.032	0.186	0.041	146
9	1	1.010	1.016	0.770	1.011	235	9	1	0.032	0.032	0.162	0.038	122
	2	1.005	1.011	0.772	1.006	228		2	0.034	0.032	0.160	0.038	118
	3	0.982	0.987	0.763	0.983	214		3	0.039	0.031	0.172	0.044	125
	Mean	0.999	1.005	0.768	1.000	226		Mean	0.035	0.032	0.165	0.040	122
10	1	1.020	1.027	0.785	1.026	234	10	1	0.031	0.034	0.157	0.039	120
	2	1.013	1.018	0.787	1.017	225		2	0.033	0.033	0.159	0.040	120
	3	0.993	1.000	0.788	1.000	204		3	0.032	0.034	0.168	0.040	130
	Mean	1.009	1.015	0.787	1.014	221		Mean	0.032	0.034	0.161	0.040	123
11	1	1.004	1.014	0.760	1.012	242	11	1	0.031	0.034	0.163	0.038	127
	2	1.000	1.013	0.762	1.011	236		2	0.032	0.033	0.168	0.039	131
	3	0.987	0.995	0.757	0.992	228		3	0.030	0.034	0.174	0.039	139
	Mean	0.997	1.007	0.760	1.005	235		Mean	0.031	0.034	0.168	0.039	132
12	1	1.008	1.014	0.772	1.010	231	12	1	0.032	0.034	0.166	0.040	126
	2	1.003	1.007	0.777	1.002	221		2	0.034	0.033	0.166	0.039	124
	3	0.993	0.984	0.779	0.980	209		3	0.033	0.033	0.176	0.044	135
	Mean	1.001	1.002	0.776	0.997	220		Mean	0.033	0.033	0.169	0.041	128
Mean for all assays		-	-	-	-	253	Mean for all assays		-	-	-	-	141
SD for all assays		-	-	-	-	30	SD for all assays		-	-	-	-	16
CV for all assays		-	-	-	-	11.9	CV for all assays		-	-	-	-	11.3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.048	1.058	1.049	1.059	0	1	1	0.036	0.037	0.039	0.064	-15
	2	1.036	1.048	1.037	1.049	0		2	0.036	0.037	0.039	0.047	-15
	3	1.013	1.023	1.013	1.023	1		3	0.041	0.035	0.044	0.050	-15
	Mean	1.032	1.043	1.033	1.044	0		Mean	0.038	0.036	0.041	0.054	-15
2	1	1.070	1.061	1.068	1.060	1	2	1	0.035	0.033	0.037	0.042	-10
	2	1.063	1.054	1.061	1.053	1		2	0.035	0.032	0.038	0.040	-9
	3	1.041	1.037	1.038	1.037	2		3	0.038	0.033	0.042	0.052	-8
	Mean	1.058	1.051	1.056	1.050	1		Mean	0.036	0.033	0.039	0.045	-9
3	1	1.062	1.058	1.066	1.058	-2	3	1	0.035	0.035	0.037	0.039	-5
	2	1.058	1.053	1.057	1.052	3		2	0.037	0.035	0.037	0.038	-7
	3	1.033	1.038	1.034	1.046	1		3	0.037	0.033	0.041	0.046	-3
	Mean	1.051	1.050	1.052	1.052	1		Mean	0.036	0.034	0.038	0.041	-5
4	1	1.069	1.065	1.068	1.066	1	4	1	0.039	0.037	0.039	0.039	-4
	2	1.060	1.052	1.057	1.052	3		2	0.034	0.035	0.039	0.037	1
	3	1.041	1.034	1.037	1.032	4		3	0.034	0.034	0.043	0.040	5
	Mean	1.057	1.050	1.054	1.050	3		Mean	0.036	0.035	0.040	0.039	1
5	1	1.018	1.019	1.015	1.016	0	5	1	0.037	0.036	0.042	0.036	7
	2	1.004	1.018	1.007	1.014	-6		2	0.041	0.036	0.041	0.037	2
	3	0.993	0.999	0.990	0.996	0		3	0.054	0.041	0.062	0.036	10
	Mean	1.005	1.012	1.004	1.009	-2		Mean	0.044	0.038	0.048	0.036	6
6	1	1.032	1.029	1.029	1.027	1	6	1	0.038	0.034	0.042	0.042	-3
	2	1.028	1.026	1.027	1.025	-1		2	0.041	0.033	0.041	0.038	-7
	3	1.010	1.004	1.009	1.002	-1		3	0.043	0.032	0.045	0.041	-5
	Mean	1.023	1.020	1.022	1.018	0		Mean	0.041	0.033	0.043	0.040	-5

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.028	1.024	1.025	1.022	1	7	1	0.038	0.036	0.038	0.041	-8
	2	1.021	1.018	1.019	1.015	0		2	0.039	0.032	0.039	0.039	-8
	3	1.001	0.998	0.999	0.995	0		3	0.045	0.031	0.044	0.044	-9
	Mean	1.017	1.013	1.014	1.011	0		Mean	0.041	0.033	0.040	0.041	-8
8	1	1.022	1.020	1.019	1.017	0	8	1	0.037	0.033	0.038	0.045	-8
	2	1.021	1.016	1.017	1.013	1		2	0.037	0.032	0.040	0.039	-6
	3	1.000	0.997	0.997	0.995	0		3	0.038	0.031	0.044	0.038	-3
	Mean	1.014	1.011	1.011	1.008	0		Mean	0.037	0.032	0.041	0.041	-6
9	1	1.010	1.016	1.009	1.011	-4	9	1	0.035	0.032	0.038	0.038	-5
	2	1.009	1.011	1.007	1.006	-3		2	0.035	0.032	0.039	0.038	-4
	3	0.985	0.987	0.984	0.983	-4		3	0.037	0.031	0.044	0.044	-1
	Mean	1.001	1.005	1.000	1.000	-4		Mean	0.036	0.032	0.040	0.040	-3
10	1	1.022	1.027	1.019	1.026	2	10	1	0.034	0.034	0.037	0.039	-3
	2	1.025	1.018	1.020	1.017	4		2	0.034	0.033	0.039	0.040	-1
	3	1.004	1.000	1.000	1.000	3		3	0.033	0.034	0.045	0.040	6
	Mean	1.017	1.015	1.013	1.014	3		Mean	0.034	0.034	0.040	0.040	1
11	1	1.017	1.014	1.014	1.012	1	11	1	0.036	0.034	0.038	0.038	-3
	2	1.018	1.013	1.014	1.011	2		2	0.036	0.033	0.042	0.039	1
	3	0.994	0.995	0.992	0.992	0		3	0.037	0.034	0.045	0.039	3
	Mean	1.010	1.007	1.007	1.005	1		Mean	0.036	0.034	0.042	0.039	0
12	1	1.017	1.014	1.013	1.010	-1	12	1	0.038	0.034	0.039	0.040	-7
	2	1.012	1.007	0.998	1.002	9		2	0.038	0.033	0.040	0.039	-6
	3	0.999	0.984	0.996	0.980	-2		3	0.041	0.033	0.046	0.044	-3
	Mean	1.009	1.002	1.002	0.997	2		Mean	0.039	0.033	0.042	0.041	-5
Mean for all assays		-	-	-	-	0	Mean for all assays		-	-	-	-	-4
SD for all assays		-	-	-	-	2	SD for all assays		-	-	-	-	6
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.064	1.060	0.825	1.016	197	1	1	0.042	0.037	0.232	0.046	180
	2	1.065	1.050	0.830	1.010	193		2	0.037	0.037	0.234	0.047	187
	3	1.068	1.060	0.829	1.018	197		3	0.037	0.037	0.259	0.048	212
	Mean	1.066	1.057	0.828	1.015	196		Mean	0.039	0.037	0.242	0.047	193
2	1	1.056	1.051	0.818	1.014	196	2	1	0.037	0.036	0.246	0.047	198
	2	1.059	1.058	0.823	1.016	193		2	0.037	0.036	0.245	0.047	197
	3	1.059	1.065	0.820	1.015	197		3	0.037	0.037	0.273	0.049	224
	Mean	1.058	1.058	0.820	1.015	195		Mean	0.037	0.036	0.255	0.048	206
3	1	1.078	1.063	0.826	1.014	200	3	1	0.038	0.037	0.245	0.047	198
	2	1.071	1.060	0.832	1.009	188		2	0.037	0.037	0.243	0.047	196
	3	1.068	1.061	0.839	1.006	178		3	0.037	0.037	0.266	0.047	219
	Mean	1.072	1.061	0.833	1.010	189		Mean	0.037	0.037	0.252	0.047	204
4	1	1.081	1.076	0.818	1.039	224	4	1	0.037	0.038	0.272	0.050	222
	2	1.080	1.077	0.825	1.039	217		2	0.038	0.037	0.272	0.050	222
	3	1.079	1.087	0.826	1.049	215		3	0.037	0.037	0.299	0.050	249
	Mean	1.080	1.080	0.823	1.042	219		Mean	0.037	0.037	0.281	0.050	231
5	1	1.242	1.237	0.994	1.194	208	5	1	0.037	0.037	0.252	0.041	210
	2	1.241	1.228	0.998	1.188	203		2	0.041	0.039	0.261	0.044	215
	3	1.249	1.229	1.002	1.190	207		3	0.038	0.037	0.284	0.042	241
	Mean	1.244	1.231	0.998	1.191	206		Mean	0.039	0.037	0.265	0.042	222
6	1	1.235	1.223	0.983	1.190	219	6	1	0.039	0.038	0.263	0.043	219
	2	1.238	1.223	0.992	1.191	212		2	0.038	0.038	0.260	0.043	217
	3	1.232	1.225	0.987	1.192	213		3	0.038	0.038	0.295	0.043	252
	Mean	1.235	1.224	0.987	1.191	215		Mean	0.038	0.038	0.273	0.043	229

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.238	1.238	0.963	1.170	207	7	1	0.039	0.039	0.247	0.042	204
	2	1.230	1.238	0.956	1.173	206		2	0.038	0.038	0.246	0.042	203
	3	1.232	1.240	0.955	1.171	209		3	0.038	0.038	0.248	0.042	206
	Mean	1.234	1.239	0.958	1.171	207		Mean	0.038	0.038	0.247	0.042	204
8	1	1.215	1.202	0.969	1.172	215	8	1	0.037	0.037	0.252	0.041	211
	2	1.214	1.197	0.981	1.165	202		2	0.037	0.037	0.246	0.040	206
	3	1.204	1.206	0.976	1.174	197		3	0.037	0.037	0.268	0.040	228
	Mean	1.211	1.201	0.975	1.170	205		Mean	0.037	0.037	0.255	0.040	215
9	1	1.247	1.229	0.974	1.210	243	9	1	0.037	0.038	0.300	0.051	249
	2	1.248	1.246	0.981	1.207	237		2	0.038	0.037	0.291	0.051	239
	3	1.252	1.239	0.984	1.208	237		3	0.037	0.037	0.313	0.053	262
	Mean	1.249	1.238	0.980	1.208	239		Mean	0.037	0.037	0.301	0.051	250
10	1	1.255	1.248	0.957	1.183	230	10	1	0.038	0.037	0.263	0.053	210
	2	1.252	1.247	0.955	1.180	230		2	0.037	0.037	0.256	0.052	203
	3	1.262	1.262	0.975	1.192	220		3	0.038	0.037	0.275	0.054	221
	Mean	1.256	1.252	0.962	1.185	227		Mean	0.037	0.037	0.265	0.053	211
11	1	1.208	1.204	0.969	1.170	202	11	1	0.038	0.039	0.238	0.043	197
	2	1.203	1.198	0.965	1.161	201		2	0.038	0.037	0.238	0.041	196
	3	1.205	1.214	0.966	1.175	201		3	0.038	0.037	0.240	0.042	198
	Mean	1.205	1.206	0.967	1.169	201		Mean	0.038	0.038	0.239	0.042	197
12	1	1.210	1.197	0.978	1.169	202	12	1	0.048	0.042	0.263	0.045	211
	2	1.211	1.194	0.975	1.163	205		2	0.039	0.041	0.249	0.046	206
	3	1.227	1.203	1.001	1.171	195		3	0.041	0.038	0.272	0.042	227
	Mean	1.216	1.198	0.985	1.167	201		Mean	0.043	0.040	0.261	0.044	215
Mean for all assays		-	-	-	-	208	Mean for all assays		-	-	-	-	215
SD for all assays		-	-	-	-	14	SD for all assays		-	-	-	-	16
CV for all assays		-	-	-	-	6.7	CV for all assays		-	-	-	-	7.4

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.066	1.060	1.011	1.016	13	1	1	0.037	0.037	0.040	0.046	-7
	2	1.064	1.050	1.017	1.010	5		2	0.037	0.037	0.040	0.047	-7
	3	1.064	1.060	1.018	1.018	4		3	0.037	0.037	0.040	0.048	-7
	Mean	1.065	1.057	1.015	1.015	7		Mean	0.037	0.037	0.040	0.047	-7
2	1	1.054	1.051	1.016	1.014	-6	2	1	0.037	0.036	0.041	0.047	-8
	2	1.056	1.058	1.017	1.016	-4		2	0.037	0.036	0.039	0.047	-9
	3	1.052	1.065	1.009	1.015	0		3	0.037	0.037	0.040	0.049	-9
	Mean	1.054	1.058	1.014	1.015	-3		Mean	0.037	0.036	0.040	0.048	-9
3	1	1.065	1.063	1.014	1.014	0	3	1	0.037	0.037	0.040	0.047	-7
	2	1.063	1.060	1.010	1.009	2		2	0.038	0.037	0.039	0.047	-8
	3	1.065	1.061	1.011	1.006	2		3	0.037	0.037	0.039	0.047	-7
	Mean	1.064	1.061	1.012	1.010	1		Mean	0.037	0.037	0.040	0.047	-7
4	1	1.078	1.076	1.044	1.039	-4	4	1	0.037	0.038	0.041	0.050	-10
	2	1.082	1.077	1.047	1.039	-4		2	0.038	0.037	0.041	0.050	-10
	3	1.080	1.087	1.044	1.049	-2		3	0.038	0.037	0.040	0.050	-10
	Mean	1.080	1.080	1.045	1.042	-3		Mean	0.038	0.037	0.041	0.050	-10
5	1	1.238	1.237	1.196	1.194	2	5	1	0.047	0.037	0.048	0.041	-4
	2	1.244	1.228	1.201	1.188	2		2	0.042	0.039	0.043	0.044	-4
	3	1.243	1.229	1.200	1.190	3		3	0.037	0.037	0.038	0.042	-4
	Mean	1.241	1.231	1.199	1.191	2		Mean	0.042	0.037	0.043	0.042	-4
6	1	1.234	1.223	1.188	1.190	12	6	1	0.038	0.038	0.040	0.043	-4
	2	1.224	1.223	1.189	1.191	2		2	0.038	0.038	0.040	0.043	-4
	3	1.226	1.225	1.182	1.192	11		3	0.038	0.038	0.039	0.043	-5
	Mean	1.228	1.224	1.186	1.191	8		Mean	0.038	0.038	0.039	0.043	-4

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.242	1.238	1.173	1.170	0	7	1	0.038	0.039	0.041	0.042	-1
	2	1.230	1.238	1.164	1.173	-2		2	0.038	0.038	0.041	0.042	-1
	3	1.229	1.240	1.159	1.171	2		3	0.038	0.038	0.041	0.042	-1
	Mean	1.233	1.239	1.165	1.171	0		Mean	0.038	0.038	0.041	0.042	-1
8	1	1.210	1.202	1.174	1.172	5	8	1	0.039	0.037	0.041	0.041	-2
	2	1.206	1.197	1.170	1.165	5		2	0.037	0.037	0.038	0.040	-2
	3	1.211	1.206	1.179	1.174	2		3	0.037	0.037	0.038	0.040	-2
	Mean	1.209	1.201	1.174	1.170	4		Mean	0.038	0.037	0.039	0.040	-2
9	1	1.255	1.248	1.193	1.183	-6	9	1	0.038	0.037	0.041	0.053	-13
	2	1.252	1.247	1.189	1.180	-4		2	0.037	0.037	0.041	0.052	-12
	3	1.249	1.262	1.185	1.192	-2		3	0.037	0.037	0.040	0.054	-13
	Mean	1.252	1.252	1.189	1.185	-4		Mean	0.037	0.037	0.041	0.053	-13
10	1	0.993	1.000	0.987	0.991	-4	10	1	0.039	0.038	0.040	0.041	-2
	2	0.980	0.976	0.973	0.965	-3		2	0.039	0.040	0.039	0.041	-2
	3	0.973	0.969	0.966	0.959	-3		3	0.042	0.039	0.039	0.041	-4
	Mean	0.982	0.982	0.975	0.972	-3		Mean	0.040	0.039	0.039	0.041	-3
11	1	1.205	1.204	1.167	1.170	1	11	1	0.037	0.039	0.042	0.043	0
	2	1.207	1.198	1.169	1.161	1		2	0.038	0.037	0.041	0.041	-1
	3	1.202	1.214	1.161	1.175	4		3	0.038	0.037	0.041	0.042	0
	Mean	1.204	1.206	1.165	1.169	2		Mean	0.038	0.038	0.041	0.042	0
12	1	1.206	1.197	1.166	1.169	9	12	1	0.039	0.042	0.039	0.045	-4
	2	1.200	1.194	1.160	1.163	10		2	0.038	0.041	0.040	0.046	-2
	3	1.200	1.203	1.161	1.171	8		3	0.038	0.038	0.038	0.042	-4
	Mean	1.202	1.198	1.162	1.167	9		Mean	0.038	0.040	0.039	0.044	-3
Mean for all assays		-	-	-	-	2	Mean for all assays		-	-	-	-	-5
SD for all assays		-	-	-	-	5	SD for all assays		-	-	-	-	4
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.017	1.052	0.751	1.047	259	1	1	0.038	0.038	0.207	0.040	168
	2	1.029	1.047	0.756	1.041	266		2	0.038	0.041	0.201	0.042	162
	3	1.016	1.052	0.757	1.041	252		3	0.039	0.041	0.209	0.042	169
	Mean	1.021	1.050	0.755	1.043	259		Mean	0.038	0.040	0.206	0.041	166
2	1	1.016	1.042	0.710	1.027	291	2	1	0.039	0.038	0.227	0.043	186
	2	1.016	1.043	0.726	1.025	275		2	0.042	0.041	0.215	0.042	171
	3	1.010	1.039	0.746	1.027	249		3	0.041	0.041	0.225	0.042	182
	Mean	1.014	1.041	0.727	1.026	272		Mean	0.041	0.040	0.222	0.042	180
3	1	1.009	1.029	0.733	1.019	264	3	1	0.040	0.040	0.235	0.045	192
	2	1.022	1.042	0.743	1.026	267		2	0.040	0.041	0.223	0.042	180
	3	1.010	1.026	0.748	1.014	250		3	0.039	0.040	0.222	0.043	180
	Mean	1.014	1.032	0.741	1.020	260		Mean	0.040	0.040	0.227	0.043	184
4	1	1.009	1.026	0.739	1.014	257	4	1	0.041	0.040	0.228	0.044	185
	2	1.017	1.029	0.748	1.017	256		2	0.041	0.040	0.218	0.041	175
	3	1.003	1.025	0.749	1.011	241		3	0.039	0.043	0.230	0.045	189
	Mean	1.010	1.027	0.745	1.014	251		Mean	0.040	0.041	0.225	0.043	183
5	1	1.050	1.074	0.779	1.068	265	5	1	0.037	0.045	0.175	0.040	138
	2	1.058	1.067	0.843	1.062	209		2	0.037	0.036	0.167	0.038	130
	3	1.051	1.065	0.791	1.060	255		3	0.036	0.036	0.174	0.039	138
	Mean	1.053	1.069	0.804	1.063	243		Mean	0.037	0.039	0.172	0.039	135
6	1	1.037	1.060	0.751	1.053	280	6	1	0.037	0.037	0.182	0.039	142
	2	1.031	1.053	0.759	1.047	266		2	0.037	0.037	0.178	0.039	139
	3	1.035	1.052	0.760	1.046	269		3	0.037	0.039	0.187	0.041	148
	Mean	1.034	1.055	0.757	1.049	272		Mean	0.037	0.037	0.182	0.040	143

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.031	1.049	0.663	1.039	359	7	1	0.037	0.037	0.240	0.041	199
	2	1.027	1.043	0.665	1.034	354		2	0.038	0.037	0.232	0.041	189
	3	1.036	1.050	0.677	1.040	350		3	0.038	0.038	0.257	0.040	215
	Mean	1.031	1.047	0.668	1.038	354		Mean	0.038	0.037	0.243	0.041	201
8	1	1.030	1.047	0.678	1.039	344	8	1	0.038	0.038	0.231	0.042	190
	2	1.024	1.040	0.680	1.033	337		2	0.038	0.037	0.238	0.041	197
	3	1.028	1.049	0.687	1.041	333		3	0.038	0.038	0.226	0.041	185
	Mean	1.027	1.045	0.682	1.038	338		Mean	0.038	0.038	0.231	0.041	191
9	1	1.039	1.046	0.704	1.038	327	9	1	0.037	0.036	0.226	0.039	187
	2	1.035	1.044	0.709	1.037	318		2	0.037	0.037	0.221	0.039	182
	3	1.042	1.052	0.719	1.044	317		3	0.044	0.037	0.229	0.040	183
	Mean	1.038	1.047	0.711	1.040	321		Mean	0.039	0.037	0.225	0.039	184
10	1	1.011	1.019	0.616	1.008	384	10	1	0.037	0.037	0.234	0.041	193
	2	1.007	1.021	0.622	1.010	375		2	0.037	0.037	0.232	0.040	191
	3	1.023	1.018	0.638	1.008	374		3	0.037	0.039	0.242	0.042	202
	Mean	1.014	1.019	0.625	1.008	378		Mean	0.037	0.038	0.236	0.041	195
11	1	1.046	1.063	0.710	1.055	328	11	1	0.037	0.037	0.220	0.041	178
	2	1.045	1.066	0.716	1.057	321		2	0.038	0.040	0.211	0.044	169
	3	1.046	1.064	0.722	1.056	316		3	0.037	0.037	0.218	0.041	177
	Mean	1.046	1.064	0.716	1.056	322		Mean	0.037	0.038	0.216	0.042	175
12	1	1.045	1.055	0.735	1.047	303	12	1	0.038	0.037	0.231	0.041	189
	2	1.043	1.050	0.739	1.042	296		2	0.038	0.037	0.222	0.041	180
	3	1.049	1.058	0.745	1.050	296		3	0.038	0.038	0.234	0.041	192
	Mean	1.046	1.054	0.739	1.046	298		Mean	0.038	0.037	0.229	0.041	187
Mean for all assays		-	-	-	-	297	Mean for all assays		-	-	-	-	177
SD for all assays		-	-	-	-	45	SD for all assays		-	-	-	-	20
CV for all assays		-	-	-	-	15.2	CV for all assays		-	-	-	-	11.3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.051	1.052	1.041	1.047	3	1	1	0.039	0.038	0.039	0.040	-1
	2	1.049	1.047	1.036	1.041	6		2	0.039	0.041	0.040	0.042	0
	3	1.057	1.052	1.051	1.041	-1		3	0.039	0.041	0.039	0.042	-1
	Mean	1.052	1.050	1.043	1.043	3		Mean	0.039	0.040	0.039	0.041	-1
2	1	1.038	1.042	1.027	1.027	-4	2	1	0.040	0.038	0.040	0.043	-2
	2	1.043	1.043	1.037	1.025	-9		2	0.040	0.041	0.040	0.042	-2
	3	1.051	1.039	1.038	1.027	-2		3	0.039	0.041	0.040	0.042	-1
	Mean	1.044	1.041	1.034	1.026	-5		Mean	0.040	0.040	0.040	0.042	-2
3	1	1.043	1.029	1.029	1.019	2	3	1	0.040	0.040	0.040	0.045	-3
	2	1.050	1.042	1.040	1.026	-2		2	0.040	0.041	0.040	0.042	-3
	3	1.052	1.026	1.046	1.014	-6		3	0.039	0.040	0.040	0.043	-2
	Mean	1.048	1.032	1.038	1.020	-2		Mean	0.040	0.040	0.040	0.043	-3
4	1	1.030	1.026	1.019	1.014	-2	4	1	0.040	0.040	0.040	0.044	-2
	2	1.038	1.029	1.027	1.017	-2		2	0.040	0.040	0.041	0.041	-1
	3	1.043	1.025	1.033	1.011	-3		3	0.040	0.043	0.041	0.045	-1
	Mean	1.037	1.027	1.026	1.014	-2		Mean	0.040	0.041	0.041	0.043	-1
5	1	1.065	1.074	1.060	1.068	-1	5	1	0.037	0.045	0.038	0.040	0
	2	1.063	1.067	1.058	1.062	-1		2	0.038	0.036	0.039	0.038	1
	3	1.069	1.065	1.063	1.060	0		3	0.037	0.036	0.038	0.039	0
	Mean	1.066	1.069	1.060	1.063	-1		Mean	0.037	0.039	0.038	0.039	0
6	1	1.060	1.060	1.053	1.053	1	6	1	0.038	0.037	0.038	0.039	-2
	2	1.052	1.053	1.046	1.047	0		2	0.038	0.037	0.038	0.039	-3
	3	1.055	1.052	1.048	1.046	0		3	0.037	0.039	0.038	0.041	-2
	Mean	1.055	1.055	1.049	1.049	0		Mean	0.038	0.037	0.038	0.040	-2

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 3 Positive control and negative control data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results* ¹	Assay	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.045	1.049	1.033	1.039	3	7	1	0.039	0.037	0.040	0.041	-3
	2	1.042	1.043	1.031	1.034	3		2	0.040	0.037	0.041	0.041	-3
	3	1.048	1.050	1.036	1.040	3		3	0.039	0.038	0.040	0.040	-3
	Mean	1.045	1.047	1.033	1.038	3		Mean	0.039	0.037	0.040	0.041	-3
8	1	1.057	1.047	1.044	1.039	6	8	1	0.038	0.038	0.042	0.042	1
	2	1.043	1.040	1.030	1.033	6		2	0.039	0.037	0.041	0.041	-1
	3	1.050	1.049	1.036	1.041	7		3	0.039	0.038	0.041	0.041	-1
	Mean	1.050	1.045	1.036	1.038	6		Mean	0.039	0.038	0.041	0.041	0
9	1	1.055	1.046	1.048	1.038	1	9	1	0.038	0.036	0.038	0.039	-2
	2	1.052	1.044	1.045	1.037	0		2	0.038	0.037	0.039	0.039	-1
	3	1.058	1.052	1.050	1.044	0		3	0.041	0.037	0.042	0.040	-1
	Mean	1.055	1.047	1.048	1.040	0		Mean	0.039	0.037	0.040	0.039	-1
10	1	1.015	1.019	1.010	1.008	-6	10	1	0.038	0.037	0.038	0.041	-3
	2	1.041	1.021	1.032	1.010	-2		2	0.039	0.037	0.039	0.040	-3
	3	1.044	1.018	1.035	1.008	-2		3	0.038	0.039	0.039	0.042	-2
	Mean	1.033	1.019	1.026	1.008	-3		Mean	0.038	0.038	0.039	0.041	-3
11	1	1.056	1.063	1.043	1.055	6	11	1	0.038	0.037	0.040	0.041	-2
	2	1.058	1.066	1.044	1.057	6		2	0.038	0.040	0.041	0.044	-2
	3	1.065	1.064	1.052	1.056	5		3	0.038	0.037	0.040	0.041	-2
	Mean	1.060	1.064	1.046	1.056	6		Mean	0.038	0.038	0.040	0.042	-2
12	1	1.063	1.055	1.050	1.047	5	12	1	0.038	0.037	0.039	0.041	-3
	2	1.059	1.050	1.046	1.042	5		2	0.038	0.037	0.039	0.041	-3
	3	1.066	1.058	1.053	1.050	5		3	0.038	0.038	0.039	0.041	-4
	Mean	1.063	1.054	1.050	1.046	5		Mean	0.038	0.037	0.039	0.041	-3
Mean for all assays		-	-	-	-	1	Mean for all assays		-	-	-	-	-2
SD for all assays		-	-	-	-	4	SD for all assays		-	-	-	-	1
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank					Test Chemical	Blank	Test Chemical		Blank
1	1	1.048	1.035	1.043	1.028	-3	1	1	0.030	0.030	0.051	0.054	-3	Negative
	2	1.040	1.030	1.033	1.019	-1		2	0.030	0.030	0.051	0.054	-3	
	3	1.039	1.035	1.033	1.029	-2		3	0.031	0.031	0.053	0.055	-2	
	Mean	1.042	1.033	1.036	1.025	-2		Mean	0.030	0.030	0.052	0.054	-3	
2	1	1.053	1.046	1.046	1.042	3	2	1	0.030	0.030	0.051	0.054	-2	Negative
	2	1.052	1.040	1.044	1.038	4		2	0.030	0.031	0.052	0.055	-1	
	3	1.048	1.050	1.047	1.043	-3		3	0.030	0.031	0.053	0.054	0	
	Mean	1.051	1.045	1.046	1.041	1		Mean	0.030	0.031	0.052	0.054	-1	
3	1	1.047	1.045	1.041	1.041	2	3	1	0.031	0.032	0.048	0.054	-6	Negative
	2	1.046	1.039	1.039	1.034	3		2	0.031	0.032	0.049	0.056	-5	
	3	1.046	1.046	1.042	1.041	0		3	0.031	0.033	0.050	0.056	-4	
	Mean	1.046	1.043	1.041	1.039	2		Mean	0.031	0.032	0.049	0.055	-5	
Mean for 3 assays		-	-	-	-	0	Mean for 3 assays		-	-	-	-	-3	

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank					Test Chemical	Blank	Test Chemical		Blank
1	1	1.047	1.041	1.043	1.034	-4	1	1	0.031	0.032	0.066	0.057	11	Negative
	2	1.046	1.040	1.041	1.031	-3		2	0.030	0.031	0.066	0.055	12	
	3	1.046	1.044	1.041	1.036	-3		3	0.032	0.032	0.067	0.057	11	
	Mean	1.046	1.042	1.042	1.034	-3		Mean	0.031	0.032	0.066	0.056	11	
2	1	1.052	1.054	1.041	1.039	-3	2	1	0.030	0.030	0.057	0.053	4	Negative
	2	1.057	1.055	1.048	1.038	-5		2	0.030	0.030	0.058	0.052	5	
	3	1.057	1.056	1.048	1.045	-5		3	0.030	0.030	0.059	0.053	6	
	Mean	1.055	1.055	1.046	1.041	-4		Mean	0.030	0.030	0.058	0.053	5	
3	1	1.044	1.035	1.037	1.029	-1	3	1	0.030	0.031	0.057	0.053	4	Negative
	2	1.038	1.033	1.034	1.027	-4		2	0.030	0.030	0.056	0.053	3	
	3	1.044	1.036	1.038	1.026	-2		3	0.030	0.032	0.058	0.055	5	
	Mean	1.042	1.035	1.036	1.027	-2		Mean	0.030	0.031	0.057	0.054	4	
Mean for 3 assays		-	-	-	-	-3	Mean for 3 assays		-	-	-	-	7	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.041	1.035	1.030	1.028	3	1	1	0.029	0.030	0.062	0.054	9	Negative
	2	1.040	1.030	1.030	1.019	2		2	0.030	0.030	0.062	0.054	8	
	3	1.039	1.035	1.028	1.029	3		3	0.031	0.031	0.064	0.055	9	
	Mean	1.040	1.033	1.029	1.025	3		Mean	0.030	0.030	0.063	0.054	9	
2	1	1.048	1.046	1.036	1.042	8	2	1	0.030	0.030	0.061	0.054	8	Negative
	2	1.046	1.040	1.035	1.038	7		2	0.029	0.031	0.062	0.055	10	
	3	1.050	1.050	1.038	1.043	8		3	0.030	0.031	0.064	0.054	11	
	Mean	1.048	1.045	1.036	1.041	8		Mean	0.030	0.031	0.062	0.054	10	
3	1	1.048	1.045	1.038	1.041	6	3	1	0.030	0.032	0.061	0.054	8	Negative
	2	1.054	1.039	1.043	1.034	7		2	0.031	0.032	0.062	0.056	8	
	3	1.052	1.046	1.043	1.041	5		3	0.031	0.033	0.065	0.056	11	
	Mean	1.051	1.043	1.041	1.039	6		Mean	0.031	0.032	0.063	0.055	9	
Mean for 3 assays		-	-	-	-	6	Mean for 3 assays		-	-	-	-	9	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.039	1.041	0.985	1.034	46	1	1	0.031	0.032	0.086	0.057	31	Positive
	2	1.046	1.040	0.997	1.031	41		2	0.029	0.031	0.084	0.055	31	
	3	1.040	1.044	0.997	1.036	35		3	0.031	0.032	0.086	0.057	31	
	Mean	1.042	1.042	0.993	1.034	41		Mean	0.030	0.032	0.085	0.056	31	
2	1	1.051	1.054	1.004	1.039	33	2	1	0.029	0.030	0.078	0.053	26	Positive
	2	1.049	1.055	1.000	1.038	35		2	0.028	0.030	0.076	0.052	25	
	3	1.051	1.056	1.004	1.045	33		3	0.030	0.030	0.079	0.053	26	
	Mean	1.050	1.055	1.003	1.041	34		Mean	0.029	0.030	0.078	0.053	26	
3	1	1.036	1.035	0.982	1.029	46	3	1	0.029	0.031	0.077	0.053	25	Positive
	2	1.038	1.033	0.984	1.027	46		2	0.029	0.030	0.077	0.053	25	
	3	1.036	1.036	0.983	1.026	45		3	0.029	0.032	0.080	0.055	28	
	Mean	1.037	1.035	0.983	1.027	46		Mean	0.029	0.031	0.078	0.054	26	
Mean for 3 assays		-	-	-	-	40	Mean for 3 assays		-	-	-	-	28	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Amiodarone HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.084	1.035	0.980	1.028	96	1	1	0.073	0.030	0.077	0.054	-20	
	2	1.081	1.030	0.975	1.019	98		2	0.073	0.030	0.077	0.054	-20	
	3	1.084	1.035	0.978	1.029	98		3	0.073	0.031	0.077	0.055	-20	
	Mean	1.083	1.033	0.978	1.025	97		Mean	0.073	0.030	0.077	0.054	-20	
2	1	1.091	1.046	0.982	1.042	105	2	1	0.072	0.030	0.077	0.054	-18	
	2	1.094	1.040	0.982	1.038	108		2	0.071	0.031	0.077	0.055	-17	
	3	1.089	1.050	0.980	1.043	105		3	0.071	0.031	0.076	0.054	-18	
	Mean	1.091	1.045	0.981	1.041	106		Mean	0.071	0.031	0.077	0.054	-18	
3	1	1.086	1.045	0.979	1.041	103	3	1	0.073	0.032	0.077	0.054	-19	
	2	1.084	1.039	0.978	1.034	102		2	0.073	0.032	0.076	0.056	-20	
	3	1.088	1.046	0.982	1.041	102		3	0.073	0.033	0.077	0.056	-19	
	Mean	1.086	1.043	0.980	1.039	102		Mean	0.073	0.032	0.077	0.055	-19	
Mean for 3 assays		-	-	-	-	102	Mean for 3 assays		-	-	-	-	-19	

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.601	1.041	1.372	1.034	221	1	1	0.549	0.032	0.379	0.057	-194	
	2	1.588	1.040	1.366	1.031	214		2	0.532	0.031	0.363	0.055	-193	
	3	1.601	1.044	1.366	1.036	227		3	0.534	0.032	0.358	0.057	-200	
	Mean	1.597	1.042	1.368	1.034	221		Mean	0.538	0.032	0.367	0.056	-196	
2	1	1.583	1.054	1.390	1.039	179	2	1	0.542	0.030	0.367	0.053	-198	
	2	1.576	1.055	1.365	1.038	197		2	0.528	0.030	0.362	0.052	-189	
	3	1.576	1.056	1.368	1.045	194		3	0.529	0.030	0.360	0.053	-192	
	Mean	1.578	1.055	1.374	1.041	190		Mean	0.533	0.030	0.363	0.053	-193	
3	1	1.580	1.035	1.373	1.029	199	3	1	0.547	0.031	0.365	0.053	-205	
	2	1.573	1.033	1.367	1.027	198		2	0.532	0.030	0.358	0.053	-197	
	3	1.585	1.036	1.377	1.026	200		3	0.534	0.032	0.361	0.055	-196	
	Mean	1.579	1.035	1.372	1.027	199		Mean	0.538	0.031	0.361	0.054	-199	
Mean for 3 assays		-	-	-	-	203	Mean for 3 assays		-	-	-	-	-196	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Chlorpromazine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.032	1.035	0.945	1.028	79	1	1	0.028	0.030	0.044	0.054	-8	Positive
	2	1.027	1.030	0.939	1.019	80		2	0.029	0.030	0.042	0.054	-11	
	3	1.027	1.035	0.939	1.029	80		3	0.029	0.031	0.043	0.055	-10	
	Mean	1.029	1.033	0.941	1.025	80		Mean	0.029	0.030	0.043	0.054	-10	
2	1	1.042	1.046	0.952	1.042	86	2	1	0.028	0.030	0.042	0.054	-9	Positive
	2	1.039	1.040	0.947	1.038	88		2	0.028	0.031	0.042	0.055	-9	
	3	1.043	1.050	0.956	1.043	83		3	0.029	0.031	0.043	0.054	-9	
	Mean	1.041	1.045	0.952	1.041	86		Mean	0.028	0.031	0.042	0.054	-9	
3	1	1.042	1.045	0.958	1.041	80	3	1	0.029	0.032	0.042	0.054	-10	Positive
	2	1.039	1.039	0.955	1.034	80		2	0.028	0.032	0.042	0.056	-9	
	3	1.037	1.046	0.956	1.041	77		3	0.029	0.033	0.043	0.056	-9	
	Mean	1.039	1.043	0.956	1.039	79		Mean	0.029	0.032	0.042	0.055	-9	
Mean for 3 assays		-	-	-	-	82	Mean for 3 assays		-	-	-	-	-9	

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.020	1.041	0.988	1.034	24	1	1	0.030	0.032	0.091	0.057	37	Positive
	2	1.018	1.040	0.985	1.031	25		2	0.029	0.031	0.092	0.055	39	
	3	0.999	1.044	0.963	1.036	28		3	0.031	0.032	0.096	0.057	41	
	Mean	1.012	1.042	0.979	1.034	26		Mean	0.030	0.032	0.093	0.056	39	
2	1	1.037	1.054	1.005	1.039	18	2	1	0.029	0.030	0.093	0.053	41	Positive
	2	1.026	1.055	0.993	1.038	19		2	0.029	0.030	0.093	0.052	41	
	3	1.028	1.056	0.995	1.045	19		3	0.030	0.030	0.096	0.053	43	
	Mean	1.030	1.055	0.998	1.041	19		Mean	0.029	0.030	0.094	0.053	42	
3	1	1.015	1.035	0.977	1.029	30	3	1	0.029	0.031	0.093	0.053	41	Positive
	2	1.012	1.033	0.973	1.027	31		2	0.029	0.030	0.094	0.053	42	
	3	1.011	1.036	0.973	1.026	30		3	0.030	0.032	0.099	0.055	46	
	Mean	1.013	1.035	0.974	1.027	30		Mean	0.029	0.031	0.095	0.054	43	
Mean for 3 assays		-	-	-	-	25	Mean for 3 assays		-	-	-	-	41	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.039	1.035	1.007	1.028	24	1	1	0.033	0.030	0.051	0.054	-6	Negative
	2	1.034	1.030	1.001	1.019	25		2	0.033	0.030	0.051	0.054	-6	
	3	1.038	1.035	1.006	1.029	24		3	0.034	0.031	0.052	0.055	-6	
	Mean	1.037	1.033	1.005	1.025	24		Mean	0.033	0.030	0.051	0.054	-6	
2	1	1.055	1.046	1.018	1.042	33	2	1	0.033	0.030	0.052	0.054	-4	Positive
	2	1.053	1.040	1.016	1.038	33		2	0.033	0.031	0.052	0.055	-4	
	3	1.051	1.050	1.016	1.043	31		3	0.034	0.031	0.053	0.054	-4	
	Mean	1.053	1.045	1.017	1.041	32		Mean	0.033	0.031	0.052	0.054	-4	
3	1	1.049	1.045	1.019	1.041	26	3	1	0.033	0.032	0.051	0.054	-5	Positive
	2	1.051	1.039	1.022	1.034	25		2	0.033	0.032	0.051	0.056	-5	
	3	1.049	1.046	1.020	1.041	25		3	0.033	0.033	0.053	0.056	-3	
	Mean	1.050	1.043	1.020	1.039	25		Mean	0.033	0.032	0.052	0.055	-4	
Mean for 3 assays		-	-	-	-	27	Mean for 3 assays		-	-	-	-	-5	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.045	1.041	0.931	1.034	106	1	1	0.034	0.032	0.095	0.057	37	Positive
	2	1.044	1.040	0.930	1.031	106		2	0.033	0.031	0.095	0.055	38	
	3	1.037	1.044	0.928	1.036	101		3	0.034	0.032	0.101	0.057	43	
	Mean	1.042	1.042	0.930	1.034	104		Mean	0.034	0.032	0.097	0.056	39	
2	1	1.051	1.054	0.931	1.039	106	2	1	0.033	0.030	0.097	0.053	41	Positive
	2	1.050	1.055	0.932	1.038	104		2	0.033	0.030	0.098	0.052	42	
	3	1.055	1.056	0.934	1.045	107		3	0.034	0.030	0.102	0.053	45	
	Mean	1.052	1.055	0.932	1.041	106		Mean	0.033	0.030	0.099	0.053	43	
3	1	1.037	1.035	0.926	1.029	103	3	1	0.034	0.031	0.093	0.053	36	Positive
	2	1.034	1.033	0.925	1.027	101		2	0.033	0.030	0.095	0.053	39	
	3	1.030	1.036	0.922	1.026	100		3	0.034	0.032	0.100	0.055	43	
	Mean	1.034	1.035	0.924	1.027	101		Mean	0.034	0.031	0.096	0.054	39	
Mean for 3 assays		-	-	-	-	104	Mean for 3 assays		-	-	-	-	40	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Doxycycline HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.038	1.035	0.985	1.028	45	1	1	0.032	0.030	0.068	0.054	12	
	2	1.037	1.030	0.983	1.019	46		2	0.032	0.030	0.067	0.054	11	
	3	1.033	1.035	0.981	1.029	44		3	0.033	0.031	0.070	0.055	13	
	Mean	1.036	1.033	0.983	1.025	45		Mean	0.032	0.030	0.068	0.054	12	
2	1	1.045	1.046	0.982	1.042	59	2	1	0.032	0.030	0.066	0.054	11	
	2	1.046	1.040	0.984	1.038	58		2	0.031	0.031	0.068	0.055	14	
	3	1.048	1.050	0.990	1.043	54		3	0.032	0.031	0.069	0.054	14	
	Mean	1.046	1.045	0.985	1.041	57		Mean	0.032	0.031	0.068	0.054	13	
3	1	1.046	1.045	0.993	1.041	49	3	1	0.032	0.032	0.068	0.054	13	
	2	1.046	1.039	0.995	1.034	47		2	0.032	0.032	0.069	0.056	14	
	3	1.042	1.046	0.994	1.041	44		3	0.032	0.033	0.070	0.056	15	
	Mean	1.045	1.043	0.994	1.039	47		Mean	0.032	0.032	0.069	0.055	14	
Mean for 3 assays	-	-	-	-	50	Mean for 3 assays	-	-	-	-	13			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.026	1.041	0.884	1.034	134	1	1	0.035	0.032	0.278	0.057	219	
	2	1.025	1.040	0.889	1.031	128		2	0.035	0.031	0.280	0.055	221	
	3	1.033	1.044	0.893	1.036	132		3	0.036	0.032	0.285	0.057	225	
	Mean	1.028	1.042	0.889	1.034	131		Mean	0.035	0.032	0.281	0.056	222	
2	1	1.034	1.054	0.869	1.039	151	2	1	0.035	0.030	0.265	0.053	207	
	2	1.037	1.055	0.881	1.038	142		2	0.035	0.030	0.265	0.052	207	
	3	1.039	1.056	0.883	1.045	142		3	0.035	0.030	0.270	0.053	212	
	Mean	1.037	1.055	0.878	1.041	145		Mean	0.035	0.030	0.267	0.053	209	
3	1	1.021	1.035	0.872	1.029	141	3	1	0.035	0.031	0.271	0.053	213	
	2	1.021	1.033	0.881	1.027	132		2	0.035	0.030	0.276	0.053	218	
	3	1.024	1.036	0.881	1.026	135		3	0.035	0.032	0.285	0.055	227	
	Mean	1.022	1.035	0.878	1.027	136		Mean	0.035	0.031	0.277	0.054	219	
Mean for 3 assays	-	-	-	-	137	Mean for 3 assays	-	-	-	-	217			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.044	1.035	1.027	1.028	9	1	1	0.033	0.030	0.043	0.054	-14	
	2	1.036	1.030	1.023	1.019	5		2	0.033	0.030	0.042	0.054	-15	
	3	1.039	1.035	1.023	1.029	8		3	0.035	0.031	0.044	0.055	-15	
	Mean	1.040	1.033	1.024	1.025	7		Mean	0.034	0.030	0.043	0.054	-15	
2	1	1.050	1.046	1.034	1.042	12	2	1	0.033	0.030	0.043	0.054	-13	
	2	1.054	1.040	1.038	1.038	12		2	0.033	0.031	0.044	0.055	-12	
	3	1.051	1.050	1.035	1.043	12		3	0.033	0.031	0.044	0.054	-12	
	Mean	1.052	1.045	1.036	1.041	12		Mean	0.033	0.031	0.044	0.054	-12	
3	1	1.051	1.045	1.037	1.041	10	3	1	0.033	0.032	0.043	0.054	-13	
	2	1.054	1.039	1.037	1.034	13		2	0.033	0.032	0.042	0.056	-14	
	3	1.048	1.046	1.032	1.041	12		3	0.033	0.033	0.043	0.056	-13	
	Mean	1.051	1.043	1.035	1.039	12		Mean	0.033	0.032	0.043	0.055	-13	
Mean for 3 assays	-	-	-	-	10	Mean for 3 assays	-	-	-	-	-	-13		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.047	1.041	0.993	1.034	46	1	1	0.034	0.032	0.065	0.057	7	
	2	1.048	1.040	0.997	1.031	43		2	0.034	0.031	0.065	0.055	7	
	3	1.047	1.044	0.997	1.036	42		3	0.035	0.032	0.065	0.057	6	
	Mean	1.047	1.042	0.996	1.034	44		Mean	0.034	0.032	0.065	0.056	7	
2	1	1.055	1.054	0.997	1.039	44	2	1	0.034	0.030	0.062	0.053	5	
	2	1.057	1.055	1.002	1.038	41		2	0.034	0.030	0.061	0.052	4	
	3	1.053	1.056	1.000	1.045	39		3	0.034	0.030	0.062	0.053	5	
	Mean	1.055	1.055	1.000	1.041	41		Mean	0.034	0.030	0.062	0.053	5	
3	1	1.042	1.035	0.988	1.029	46	3	1	0.034	0.031	0.060	0.053	3	
	2	1.040	1.033	0.989	1.027	43		2	0.034	0.030	0.060	0.053	3	
	3	1.041	1.036	0.993	1.026	40		3	0.034	0.032	0.062	0.055	5	
	Mean	1.041	1.035	0.990	1.027	43		Mean	0.034	0.031	0.061	0.054	4	
Mean for 3 assays	-	-	-	-	43	Mean for 3 assays	-	-	-	-	-	5		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.042	1.039	1.017	1.031	17	1	1	0.031	0.030	0.059	0.053	5	
	2	1.052	1.038	1.027	1.029	17		2	0.030	0.030	0.059	0.053	6	
	3	1.051	1.039	1.026	1.034	17		3	0.034	0.031	0.062	0.054	5	
	Mean	1.048	1.039	1.023	1.031	17		Mean	0.032	0.030	0.060	0.053	5	
2	1	1.047	1.054	1.019	1.047	22	2	1	0.030	0.031	0.058	0.053	6	
	2	1.047	1.045	1.021	1.038	20		2	0.031	0.030	0.058	0.052	5	
	3	1.047	1.043	1.023	1.038	18		3	0.030	0.032	0.059	0.055	7	
	Mean	1.047	1.047	1.021	1.041	20		Mean	0.030	0.031	0.058	0.053	6	
3	1	1.051	1.057	1.026	1.053	20	3	1	0.029	0.031	0.060	0.054	7	
	2	1.050	1.041	1.023	1.035	22		2	0.029	0.031	0.060	0.054	7	
	3	1.050	1.044	1.028	1.037	17		3	0.030	0.031	0.063	0.056	9	
	Mean	1.050	1.047	1.026	1.042	20		Mean	0.029	0.031	0.061	0.055	8	
Mean for 3 assays	-	-	-	-	19	Mean for 3 assays	-	-	-	-	6	Negative		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.050	1.041	0.892	1.038	155	1	1	0.033	0.031	0.127	0.056	69	
	2	1.049	1.040	0.894	1.036	152		2	0.031	0.030	0.126	0.055	70	
	3	1.049	1.035	0.898	1.033	148		3	0.032	0.031	0.128	0.056	71	
	Mean	1.049	1.039	0.895	1.036	152		Mean	0.032	0.031	0.127	0.056	70	
2	1	1.054	1.040	0.894	1.040	159	2	1	0.032	0.032	0.123	0.053	70	
	2	1.052	1.045	0.893	1.043	158		2	0.032	0.031	0.122	0.053	69	
	3	1.052	1.040	0.897	1.040	154		3	0.033	0.032	0.125	0.054	71	
	Mean	1.053	1.042	0.895	1.041	157		Mean	0.032	0.032	0.123	0.053	70	
3	1	1.051	1.065	0.891	1.063	157	3	1	0.033	0.030	0.135	0.055	77	
	2	1.051	1.053	0.895	1.049	153		2	0.033	0.030	0.132	0.054	74	
	3	1.057	1.058	0.905	1.056	149		3	0.033	0.031	0.137	0.056	79	
	Mean	1.053	1.059	0.897	1.056	153		Mean	0.033	0.030	0.135	0.055	77	
Mean for 3 assays	-	-	-	-	154	Mean for 3 assays	-	-	-	-	72	Positive		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.050	1.039	1.022	1.031	20	1	1	0.030	0.030	0.236	0.053	183	
	2	1.046	1.038	1.021	1.029	17		2	0.030	0.030	0.233	0.053	180	
	3	1.048	1.039	1.022	1.034	18		3	0.031	0.031	0.234	0.054	180	
	Mean	1.048	1.039	1.022	1.031	18		Mean	0.030	0.030	0.234	0.053	181	
2	1	1.053	1.054	1.024	1.047	23	2	1	0.031	0.031	0.227	0.053	174	
	2	1.055	1.045	1.028	1.038	21		2	0.030	0.030	0.227	0.052	175	
	3	1.050	1.043	1.025	1.038	19		3	0.031	0.032	0.227	0.055	174	
	Mean	1.053	1.047	1.026	1.041	21		Mean	0.031	0.031	0.227	0.053	174	
3	1	1.045	1.057	1.019	1.053	21	3	1	0.029	0.031	0.237	0.054	184	
	2	1.047	1.041	1.018	1.035	24		2	0.030	0.031	0.233	0.054	179	
	3	1.055	1.044	1.029	1.037	21		3	0.030	0.031	0.237	0.056	183	
	Mean	1.049	1.047	1.022	1.042	22		Mean	0.030	0.031	0.236	0.055	182	
Mean for 3 assays		-	-	-	-	20	Mean for 3 assays		-	-	-	-	179	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.031	1.041	0.937	1.038	91	1	1	0.031	0.031	0.311	0.056	255	
	2	1.037	1.040	0.947	1.036	87		2	0.031	0.030	0.318	0.055	262	
	3	1.039	1.035	0.952	1.033	84		3	0.032	0.031	0.331	0.056	274	
	Mean	1.036	1.039	0.945	1.036	87		Mean	0.031	0.031	0.320	0.056	264	
2	1	1.047	1.040	0.956	1.040	90	2	1	0.032	0.032	0.372	0.053	319	
	2	1.041	1.045	0.957	1.043	83		2	0.031	0.031	0.322	0.053	270	
	3	1.042	1.040	0.959	1.040	82		3	0.032	0.032	0.333	0.054	280	
	Mean	1.043	1.042	0.957	1.041	85		Mean	0.032	0.032	0.342	0.053	290	
3	1	1.050	1.065	0.953	1.063	94	3	1	0.032	0.030	0.319	0.055	262	
	2	1.045	1.053	0.954	1.049	88		2	0.032	0.030	0.311	0.054	254	
	3	1.047	1.058	0.960	1.056	84		3	0.032	0.031	0.321	0.056	264	
	Mean	1.047	1.059	0.956	1.056	89		Mean	0.032	0.030	0.317	0.055	260	
Mean for 3 assays		-	-	-	-	87	Mean for 3 assays		-	-	-	-	271	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.047	1.039	1.003	1.031	36	1	1	0.030	0.030	0.060	0.053	7	
	2	1.039	1.038	0.998	1.029	33		2	0.030	0.030	0.060	0.053	7	
	3	1.039	1.039	0.997	1.034	34		3	0.031	0.031	0.062	0.054	8	
	Mean	1.042	1.039	0.999	1.031	34		Mean	0.030	0.030	0.061	0.053	7	
2	1	1.048	1.054	1.002	1.047	40	2	1	0.030	0.031	0.058	0.053	6	
	2	1.048	1.045	1.006	1.038	36		2	0.031	0.030	0.059	0.052	6	
	3	1.042	1.043	1.007	1.038	29		3	0.031	0.032	0.058	0.055	5	
	Mean	1.046	1.047	1.005	1.041	35		Mean	0.031	0.031	0.058	0.053	6	
3	1	1.052	1.057	1.010	1.053	37	3	1	0.029	0.031	0.060	0.054	7	
	2	1.057	1.041	1.012	1.035	40		2	0.029	0.031	0.059	0.054	6	
	3	1.054	1.044	1.012	1.037	37		3	0.030	0.031	0.061	0.056	7	
	Mean	1.054	1.047	1.011	1.042	38		Mean	0.029	0.031	0.060	0.055	7	
Mean for 3 assays	-	-	-	-	36	Mean for 3 assays	-	-	-	-	7	Positive		

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.045	1.041	0.912	1.038	130	1	1	0.032	0.031	0.130	0.056	73	
	2	1.039	1.040	0.909	1.036	127		2	0.031	0.030	0.130	0.055	74	
	3	1.039	1.035	0.913	1.033	123		3	0.032	0.031	0.133	0.056	76	
	Mean	1.041	1.039	0.911	1.036	127		Mean	0.032	0.031	0.131	0.056	74	
2	1	1.049	1.040	0.928	1.040	120	2	1	0.031	0.032	0.125	0.053	73	
	2	1.040	1.045	0.920	1.043	119		2	0.031	0.031	0.124	0.053	72	
	3	1.047	1.040	0.928	1.040	118		3	0.032	0.032	0.126	0.054	73	
	Mean	1.045	1.042	0.925	1.041	119		Mean	0.031	0.032	0.125	0.053	73	
3	1	1.052	1.065	0.931	1.063	118	3	1	0.031	0.030	0.126	0.055	70	
	2	1.051	1.053	0.928	1.049	120		2	0.031	0.030	0.124	0.054	68	
	3	1.044	1.058	0.926	1.056	115		3	0.031	0.031	0.125	0.056	69	
	Mean	1.049	1.059	0.928	1.056	118		Mean	0.031	0.030	0.125	0.055	69	
Mean for 3 assays	-	-	-	-	121	Mean for 3 assays	-	-	-	-	72	Positive		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.047	1.039	0.976	1.031	63	1	1	0.030	0.030	0.054	0.053	1	
	2	1.040	1.038	0.969	1.029	63		2	0.030	0.030	0.053	0.053	0	
	3	1.038	1.039	0.971	1.034	59		3	0.031	0.031	0.056	0.054	2	
	Mean	1.042	1.039	0.972	1.031	62		Mean	0.030	0.030	0.054	0.053	1	
2	1	1.049	1.054	0.979	1.047	64	2	1	0.030	0.031	0.053	0.053	1	
	2	1.041	1.045	0.972	1.038	63		2	0.030	0.030	0.052	0.052	0	
	3	1.042	1.043	0.976	1.038	60		3	0.031	0.032	0.052	0.055	-1	
	Mean	1.044	1.047	0.976	1.041	62		Mean	0.030	0.031	0.052	0.053	0	
3	1	1.050	1.057	0.989	1.053	56	3	1	0.029	0.031	0.057	0.054	4	
	2	1.048	1.041	0.984	1.035	59		2	0.029	0.031	0.056	0.054	3	
	3	1.053	1.044	0.997	1.037	51		3	0.031	0.031	0.058	0.056	3	
	Mean	1.050	1.047	0.990	1.042	55		Mean	0.030	0.031	0.057	0.055	3	
Mean for 3 assays	-	-	-	-	60	Mean for 3 assays	-	-	-	-	-	1		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.028	1.041	0.974	1.038	51	1	1	0.033	0.031	0.064	0.056	6	
	2	1.022	1.040	0.963	1.036	56		2	0.032	0.030	0.063	0.055	6	
	3	1.031	1.035	0.969	1.033	59		3	0.033	0.031	0.066	0.056	8	
	Mean	1.027	1.039	0.969	1.036	55		Mean	0.033	0.031	0.064	0.056	7	
2	1	1.040	1.040	0.993	1.040	46	2	1	0.032	0.032	0.062	0.053	9	
	2	1.034	1.045	0.981	1.043	52		2	0.032	0.031	0.062	0.053	9	
	3	1.034	1.040	0.979	1.040	54		3	0.033	0.032	0.066	0.054	12	
	Mean	1.036	1.042	0.984	1.041	51		Mean	0.032	0.032	0.063	0.053	10	
3	1	1.039	1.065	0.976	1.063	60	3	1	0.032	0.030	0.062	0.055	5	
	2	1.036	1.053	0.970	1.049	63		2	0.032	0.030	0.061	0.054	4	
	3	1.043	1.058	0.977	1.056	63		3	0.033	0.031	0.064	0.056	6	
	Mean	1.039	1.059	0.974	1.056	62		Mean	0.032	0.030	0.062	0.055	5	
Mean for 3 assays	-	-	-	-	56	Mean for 3 assays	-	-	-	-	-	7		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Quinine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.030	1.039	0.962	1.031	60	1	1	0.030	0.030	0.077	0.053	24	
	2	1.037	1.038	0.967	1.029	62		2	0.031	0.030	0.075	0.053	21	
	3	1.033	1.039	0.964	1.034	61		3	0.031	0.031	0.077	0.054	23	
	Mean	1.033	1.039	0.964	1.031	61		Mean	0.031	0.030	0.076	0.053	23	
2	1	1.036	1.054	0.972	1.047	58	2	1	0.031	0.031	0.074	0.053	21	
	2	1.036	1.045	0.971	1.038	59		2	0.031	0.030	0.072	0.052	19	
	3	1.035	1.043	0.975	1.038	54		3	0.031	0.032	0.071	0.055	18	
	Mean	1.036	1.047	0.973	1.041	57		Mean	0.031	0.031	0.072	0.053	19	
3	1	1.039	1.057	0.973	1.053	61	3	1	0.029	0.031	0.079	0.054	26	
	2	1.042	1.041	0.975	1.035	62		2	0.029	0.031	0.078	0.054	25	
	3	1.045	1.044	0.977	1.037	63		3	0.030	0.031	0.080	0.056	26	
	Mean	1.042	1.047	0.975	1.042	62		Mean	0.029	0.031	0.079	0.055	26	
Mean for 3 assays	-	-	-	-	60	Mean for 3 assays	-	-	-	-	23			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.030	1.041	0.760	1.038	267	1	1	0.031	0.031	0.185	0.056	129	
	2	1.031	1.040	0.763	1.036	265		2	0.031	0.030	0.184	0.055	128	
	3	1.032	1.035	0.769	1.033	260		3	0.032	0.031	0.195	0.056	138	
	Mean	1.031	1.039	0.764	1.036	264		Mean	0.031	0.031	0.188	0.056	132	
2	1	1.029	1.040	0.788	1.040	240	2	1	0.031	0.032	0.155	0.053	103	
	2	1.026	1.045	0.794	1.043	231		2	0.031	0.031	0.154	0.053	102	
	3	1.030	1.040	0.799	1.040	230		3	0.032	0.032	0.159	0.054	106	
	Mean	1.028	1.042	0.794	1.041	234		Mean	0.031	0.032	0.156	0.053	104	
3	1	1.024	1.065	0.763	1.063	258	3	1	0.031	0.030	0.175	0.055	119	
	2	1.025	1.053	0.766	1.049	256		2	0.033	0.030	0.174	0.054	116	
	3	1.027	1.058	0.774	1.056	250		3	0.032	0.031	0.175	0.056	118	
	Mean	1.025	1.059	0.768	1.056	255		Mean	0.032	0.030	0.175	0.055	118	
Mean for 3 assays	-	-	-	-	251	Mean for 3 assays	-	-	-	-	118			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 4
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.046	1.039	1.040	1.031	-2	1	1	0.033	0.030	0.051	0.053	-5	Negative
	2	1.049	1.038	1.043	1.029	-2		2	0.033	0.030	0.051	0.053	-5	
	3	1.049	1.039	1.044	1.034	-3		3	0.034	0.031	0.053	0.054	-4	
	Mean	1.048	1.039	1.042	1.031	-2		Mean	0.033	0.030	0.052	0.053	-5	
2	1	1.048	1.054	1.041	1.047	1	2	1	0.033	0.031	0.051	0.053	-4	Negative
	2	1.048	1.045	1.040	1.038	2		2	0.033	0.030	0.051	0.052	-4	
	3	1.046	1.043	1.036	1.038	4		3	0.034	0.032	0.052	0.055	-4	
	Mean	1.047	1.047	1.039	1.041	2		Mean	0.033	0.031	0.051	0.053	-4	
3	1	1.051	1.057	1.045	1.053	1	3	1	0.032	0.031	0.052	0.054	-4	Negative
	2	1.055	1.041	1.047	1.035	3		2	0.033	0.031	0.051	0.054	-6	
	3	1.059	1.044	1.051	1.037	3		3	0.034	0.031	0.053	0.056	-5	
	Mean	1.055	1.047	1.048	1.042	2		Mean	0.033	0.031	0.052	0.055	-5	
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	-5	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.040	1.041	1.033	1.038	4	1	1	0.034	0.031	0.035	0.056	-24	Negative
	2	1.046	1.040	1.040	1.036	3		2	0.033	0.030	0.035	0.055	-23	
	3	1.047	1.035	1.041	1.033	3		3	0.034	0.031	0.035	0.056	-24	
	Mean	1.044	1.039	1.038	1.036	3		Mean	0.034	0.031	0.035	0.056	-24	
2	1	1.058	1.040	1.056	1.040	1	2	1	0.032	0.032	0.033	0.053	-20	Negative
	2	1.056	1.045	1.054	1.043	1		2	0.033	0.031	0.033	0.053	-21	
	3	1.053	1.040	1.049	1.040	3		3	0.033	0.032	0.034	0.054	-20	
	Mean	1.056	1.042	1.053	1.041	2		Mean	0.033	0.032	0.033	0.053	-20	
3	1	1.054	1.065	1.047	1.063	4	3	1	0.034	0.030	0.035	0.055	-24	Negative
	2	1.053	1.053	1.046	1.049	4		2	0.033	0.030	0.034	0.054	-24	
	3	1.054	1.058	1.050	1.056	1		3	0.034	0.031	0.035	0.056	-24	
	Mean	1.054	1.059	1.048	1.056	3		Mean	0.034	0.030	0.035	0.055	-24	
Mean for 3 assays		-	-	-	-	3	Mean for 3 assays		-	-	-	-	-23	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.056	1.058	1.059	1.058	-1	1	1	0.033	0.035	0.038	0.039	-2	Negative
	2	1.052	1.053	1.052	1.052	2		2	0.033	0.035	0.039	0.038	-1	
	3	1.031	1.038	1.031	1.046	2		3	0.033	0.033	0.033	0.046	-7	
	Mean	1.046	1.050	1.047	1.052	1		Mean	0.033	0.034	0.037	0.041	-3	
2	1	1.032	1.024	1.028	1.022	2	2	1	0.032	0.036	0.035	0.041	-5	Negative
	2	1.020	1.018	1.014	1.015	4		2	0.032	0.032	0.037	0.039	-3	
	3	1.005	0.998	0.999	0.995	4		3	0.030	0.031	0.040	0.044	2	
	Mean	1.019	1.013	1.014	1.011	3		Mean	0.031	0.033	0.037	0.041	-2	
3	1	1.019	1.014	1.016	1.012	1	3	1	0.032	0.034	0.034	0.038	-3	Negative
	2	1.018	1.013	1.014	1.011	2		2	0.032	0.033	0.036	0.039	-1	
	3	0.990	0.995	0.986	0.992	2		3	0.031	0.034	0.039	0.039	3	
	Mean	1.009	1.007	1.005	1.005	2		Mean	0.032	0.034	0.036	0.039	0	
Mean for 3 assays		-	-	-	-	2	Mean for 3 assays		-	-	-	-	-2	

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.053	1.058	1.054	1.059	0	1	1	0.035	0.037	0.041	0.064	-12	Negative
	2	1.043	1.048	1.047	1.049	-3		2	0.032	0.037	0.041	0.047	-9	
	3	1.020	1.023	1.026	1.023	-5		3	0.032	0.035	0.057	0.050	7	
	Mean	1.039	1.043	1.042	1.044	-3		Mean	0.033	0.036	0.046	0.054	-5	
2	1	1.018	1.019	1.016	1.016	-1	2	1	0.033	0.036	0.039	0.036	8	Negative
	2	1.004	1.018	1.002	1.014	-1		2	0.033	0.036	0.036	0.037	5	
	3	0.990	0.999	0.987	0.996	0		3	0.032	0.041	0.041	0.036	11	
	Mean	1.004	1.012	1.002	1.009	-1		Mean	0.033	0.038	0.039	0.036	8	
3	1	1.017	1.016	1.015	1.011	-3	3	1	0.032	0.032	0.040	0.038	0	Negative
	2	1.015	1.011	1.014	1.006	-4		2	0.033	0.032	0.038	0.038	-3	
	3	0.992	0.987	0.992	0.983	-5		3	0.033	0.031	0.049	0.044	8	
	Mean	1.008	1.005	1.007	1.000	-4		Mean	0.033	0.032	0.042	0.040	2	
Mean for 3 assays		-	-	-	-	-3	Mean for 3 assays		-	-	-	-	2	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.056	1.058	1.054	1.058	4	1	1	0.033	0.035	0.036	0.039	-4	Negative
	2	1.056	1.053	1.051	1.052	7		2	0.036	0.035	0.037	0.038	-6	
	3	1.030	1.038	1.023	1.046	9		3	0.035	0.033	0.035	0.046	-7	
	Mean	1.047	1.050	1.043	1.052	7		Mean	0.035	0.034	0.036	0.041	-6	
2	1	1.028	1.024	1.019	1.022	7	2	1	0.031	0.036	0.042	0.041	3	Negative
	2	1.023	1.018	1.013	1.015	8		2	0.033	0.032	0.049	0.039	8	
	3	1.004	0.998	0.994	0.995	8		3	0.032	0.031	0.044	0.044	4	
	Mean	1.018	1.013	1.009	1.011	8		Mean	0.032	0.033	0.045	0.041	5	
3	1	1.010	1.014	1.002	1.012	6	3	1	0.033	0.034	0.119	0.038	81	Positive
	2	1.006	1.013	0.999	1.011	5		2	0.035	0.033	0.052	0.039	12	
	3	0.988	0.995	0.981	0.992	5		3	0.033	0.034	0.229	0.039	191	
	Mean	1.001	1.007	0.994	1.005	5		Mean	0.034	0.034	0.133	0.039	95	
Mean for 3 assays		-	-	-	-	7	Mean for 3 assays		-	-	-	-	31	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.042	1.058	0.935	1.059	108	1	1	0.033	0.037	0.056	0.064	5	Positive
	2	1.034	1.048	1.056	1.049	-21		2	0.033	0.037	0.062	0.047	11	
	3	1.012	1.023	0.954	1.023	59		3	0.031	0.035	0.071	0.050	22	
	Mean	1.029	1.043	0.982	1.044	49		Mean	0.032	0.036	0.063	0.054	13	
2	1	1.012	1.019	0.953	1.016	56	2	1	0.031	0.036	0.061	0.036	32	Positive
	2	1.000	1.018	0.945	1.014	52		2	0.032	0.036	0.066	0.037	36	
	3	0.980	0.999	0.928	0.996	49		3	0.030	0.041	0.069	0.036	41	
	Mean	0.997	1.012	0.942	1.009	52		Mean	0.031	0.038	0.065	0.036	36	
3	1	1.007	1.016	0.950	1.011	52	3	1	0.032	0.032	0.054	0.038	14	Positive
	2	1.000	1.011	0.950	1.006	45		2	0.034	0.032	0.063	0.038	21	
	3	0.989	0.987	0.941	0.983	43		3	0.032	0.031	0.064	0.044	24	
	Mean	0.999	1.005	0.947	1.000	47		Mean	0.033	0.032	0.060	0.040	20	
Mean for 3 assays		-	-	-	-	49	Mean for 3 assays		-	-	-	-	23	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Amiodarone HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.144	1.058	1.048	1.058	98	1	1	0.052	0.035	0.082	0.039	23	Positive
	2	1.135	1.053	1.052	1.052	85		2	0.053	0.035	0.079	0.038	19	
	3	1.112	1.038	1.041	1.046	73		3	0.062	0.033	0.091	0.046	22	
	Mean	1.130	1.050	1.047	1.052	85		Mean	0.056	0.034	0.084	0.041	21	
2	1	1.101	1.024	1.036	1.022	63	2	1	0.077	0.036	0.095	0.041	10	Positive
	2	1.097	1.018	1.014	1.015	81		2	0.078	0.032	0.097	0.039	11	
	3	1.074	0.998	1.008	0.995	64		3	0.077	0.031	0.098	0.044	13	
	Mean	1.091	1.013	1.019	1.011	69		Mean	0.077	0.033	0.097	0.041	11	
3	1	1.078	1.014	1.002	1.012	74	3	1	0.070	0.034	0.091	0.038	16	Positive
	2	1.069	1.013	0.989	1.011	78		2	0.071	0.033	0.090	0.039	14	
	3	1.048	0.995	0.982	0.992	64		3	0.072	0.034	0.099	0.039	22	
	Mean	1.065	1.007	0.991	1.005	72		Mean	0.071	0.034	0.093	0.039	17	
Mean for 3 assays	-	-	-	-	75	Mean for 3 assays	-	-	-	-	16			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.742	1.058	1.339	1.059	404	1	1	0.305	0.037	0.623	0.064	300	Positive
	2	1.735	1.048	1.380	1.049	356		2	0.304	0.037	0.622	0.047	300	
	3	1.684	1.023	1.372	1.023	313		3	0.306	0.035	0.646	0.050	322	
	Mean	1.720	1.043	1.364	1.044	358		Mean	0.305	0.036	0.630	0.054	307	
2	1	1.843	1.019	1.251	1.016	589	2	1	0.475	0.036	0.635	0.036	162	Positive
	2	1.853	1.018	1.254	1.014	596		2	0.476	0.036	0.641	0.037	167	
	3	1.802	0.999	1.265	0.996	534		3	0.477	0.041	0.660	0.036	185	
	Mean	1.833	1.012	1.257	1.009	573		Mean	0.476	0.038	0.645	0.036	171	
3	1	1.835	1.016	1.263	1.011	567	3	1	0.479	0.032	0.614	0.038	127	Positive
	2	1.837	1.011	1.314	1.006	518		2	0.479	0.032	0.635	0.038	148	
	3	1.807	0.987	1.310	0.983	492		3	0.481	0.031	0.637	0.044	148	
	Mean	1.826	1.005	1.296	1.000	526		Mean	0.480	0.032	0.629	0.040	141	
Mean for 3 assays	-	-	-	-	486	Mean for 3 assays	-	-	-	-	206			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Chlorpromazine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.054	1.058	0.938	1.058	118	1	1	0.032	0.035	0.050	0.039	11	
	2	1.050	1.053	0.934	1.052	118		2	0.032	0.035	0.052	0.038	13	
	3	1.027	1.038	0.925	1.046	104		3	0.031	0.033	0.057	0.046	19	
	Mean	1.044	1.050	0.932	1.052	113		Mean	0.032	0.034	0.053	0.041	14	
2	1	1.017	1.024	0.918	1.022	97	2	1	0.033	0.036	0.049	0.041	8	
	2	1.009	1.018	0.918	1.015	89		2	0.037	0.032	0.051	0.039	6	
	3	0.983	0.998	0.895	0.995	86		3	0.037	0.031	0.053	0.044	8	
	Mean	1.003	1.013	0.910	1.011	91		Mean	0.036	0.033	0.051	0.041	7	
3	1	1.002	1.014	0.915	1.012	85	3	1	0.032	0.034	0.048	0.038	11	
	2	0.989	1.013	0.908	1.011	79		2	0.035	0.033	0.053	0.039	13	
	3	0.972	0.995	0.894	0.992	76		3	0.034	0.034	0.055	0.039	16	
	Mean	0.988	1.007	0.906	1.005	80		Mean	0.034	0.034	0.052	0.039	13	
Mean for 3 assays	-	-	-	-	95	Mean for 3 assays	-	-	-	-	11			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.032	1.058	0.974	1.059	59	1	1	0.035	0.037	0.101	0.064	48	
	2	1.026	1.048	0.974	1.049	53		2	0.035	0.037	0.105	0.047	52	
	3	1.004	1.023	0.962	1.023	43		3	0.037	0.035	0.116	0.050	61	
	Mean	1.021	1.043	0.970	1.044	52		Mean	0.036	0.036	0.107	0.054	54	
2	1	0.981	1.019	0.936	1.016	42	2	1	0.034	0.036	0.102	0.036	70	
	2	0.990	1.018	0.951	1.014	36		2	0.034	0.036	0.109	0.037	77	
	3	0.970	0.999	0.933	0.996	34		3	0.033	0.041	0.110	0.036	79	
	Mean	0.980	1.012	0.940	1.009	37		Mean	0.034	0.038	0.107	0.036	75	
3	1	0.986	1.016	0.946	1.011	35	3	1	0.033	0.032	0.098	0.038	57	
	2	0.986	1.011	0.949	1.006	32		2	0.037	0.032	0.106	0.038	61	
	3	0.970	0.987	0.936	0.983	29		3	0.033	0.031	0.109	0.044	68	
	Mean	0.981	1.005	0.944	1.000	32		Mean	0.034	0.032	0.104	0.040	62	
Mean for 3 assays	-	-	-	-	40	Mean for 3 assays	-	-	-	-	64			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.061	1.058	1.038	1.058	25	1	1	0.047	0.035	0.045	0.039	-9	Positive
	2	1.060	1.053	1.035	1.052	27		2	0.043	0.035	0.046	0.038	-4	
	3	1.033	1.038	1.012	1.046	23		3	0.079	0.033	0.051	0.046	-35	
	Mean	1.051	1.050	1.028	1.052	25		Mean	0.056	0.034	0.047	0.041	-16	
2	1	1.030	1.024	1.009	1.022	19	2	1	0.042	0.036	0.045	0.041	-5	Negative
	2	1.024	1.018	1.003	1.015	19		2	0.043	0.032	0.084	0.039	33	
	3	1.003	0.998	0.983	0.995	18		3	0.056	0.031	0.050	0.044	-14	
	Mean	1.019	1.013	0.998	1.011	19		Mean	0.047	0.033	0.060	0.041	5	
3	1	1.013	1.014	0.995	1.012	16	3	1	0.035	0.034	0.044	0.038	4	Negative
	2	1.009	1.013	0.991	1.011	16		2	0.040	0.033	0.046	0.039	1	
	3	0.991	0.995	0.975	0.992	14		3	0.041	0.034	0.050	0.039	4	
	Mean	1.004	1.007	0.987	1.005	15		Mean	0.039	0.034	0.047	0.039	3	
Mean for 3 assays		-	-	-	-	20	Mean for 3 assays		-	-	-	-	-3	

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.049	1.058	0.939	1.059	111	1	1	0.040	0.037	0.126	0.064	68	Positive
	2	1.043	1.048	0.947	1.049	97		2	0.037	0.037	0.125	0.047	70	
	3	1.022	1.023	0.942	1.023	81		3	0.044	0.035	0.136	0.050	74	
	Mean	1.038	1.043	0.943	1.044	96		Mean	0.040	0.036	0.129	0.054	71	
2	1	1.008	1.019	0.933	1.016	72	2	1	0.034	0.036	0.136	0.036	104	Positive
	2	1.006	1.018	0.936	1.014	67		2	0.036	0.036	0.140	0.037	106	
	3	0.986	0.999	0.922	0.996	61		3	0.036	0.041	0.147	0.036	113	
	Mean	1.000	1.012	0.930	1.009	67		Mean	0.035	0.038	0.141	0.036	108	
3	1	1.005	1.016	0.928	1.011	72	3	1	0.036	0.032	0.135	0.038	91	Positive
	2	1.007	1.011	0.929	1.006	73		2	0.037	0.032	0.138	0.038	93	
	3	0.998	0.987	0.927	0.983	66		3	0.038	0.031	0.148	0.044	102	
	Mean	1.003	1.005	0.928	1.000	70		Mean	0.037	0.032	0.140	0.040	95	
Mean for 3 assays		-	-	-	-	78	Mean for 3 assays		-	-	-	-	91	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Doxycycline HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.066	1.058	0.999	1.058	69	1	1	0.035	0.035	0.081	0.039	39	Positive
	2	1.063	1.053	0.997	1.052	68		2	0.038	0.035	0.081	0.038	36	
	3	1.041	1.038	0.978	1.046	65		3	0.038	0.033	0.095	0.046	50	
	Mean	1.057	1.050	0.991	1.052	67		Mean	0.037	0.034	0.086	0.041	42	
2	1	1.023	1.024	0.958	1.022	63	2	1	0.035	0.036	0.081	0.041	38	Positive
	2	1.008	1.018	0.954	1.015	52		2	0.035	0.032	0.083	0.039	40	
	3	0.994	0.998	0.940	0.995	52		3	0.037	0.031	0.086	0.044	41	
	Mean	1.008	1.013	0.951	1.011	56		Mean	0.036	0.033	0.083	0.041	40	
3	1	1.015	1.014	0.957	1.012	56	3	1	0.036	0.034	0.084	0.038	43	Positive
	2	1.018	1.013	0.962	1.011	54		2	0.035	0.033	0.085	0.039	45	
	3	0.993	0.995	0.942	0.992	49		3	0.039	0.034	0.089	0.039	45	
	Mean	1.009	1.007	0.954	1.005	53		Mean	0.037	0.034	0.086	0.039	44	
Mean for 3 assays		-	-	-	-	59	Mean for 3 assays		-	-	-	-	42	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.024	1.058	0.761	1.059	264	1	1	0.038	0.037	0.318	0.064	262	Positive
	2	1.012	1.048	0.775	1.049	238		2	0.035	0.037	0.326	0.047	273	
	3	0.993	1.023	0.765	1.023	229		3	0.036	0.035	0.344	0.050	290	
	Mean	1.010	1.043	0.767	1.044	244		Mean	0.036	0.036	0.329	0.054	275	
2	1	1.003	1.019	0.730	1.016	270	2	1	0.039	0.036	0.349	0.036	312	Positive
	2	0.994	1.018	0.738	1.014	253		2	0.041	0.036	0.355	0.037	316	
	3	0.981	0.999	0.732	0.996	246		3	0.042	0.041	0.361	0.036	321	
	Mean	0.993	1.012	0.733	1.009	256		Mean	0.041	0.038	0.355	0.036	316	
3	1	1.002	1.016	0.727	1.011	270	3	1	0.050	0.032	0.344	0.038	286	Positive
	2	0.999	1.011	0.741	1.006	253		2	0.049	0.032	0.348	0.038	291	
	3	0.981	0.987	0.737	0.983	239		3	0.039	0.031	0.355	0.044	308	
	Mean	0.994	1.005	0.735	1.000	254		Mean	0.046	0.032	0.349	0.040	295	
Mean for 3 assays		-	-	-	-	251	Mean for 3 assays		-	-	-	-	295	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.056	1.058	1.050	1.058	8	1	1	0.036	0.035	0.042	0.039	-1	
	2	1.054	1.053	1.048	1.052	8		2	0.040	0.035	0.048	0.038	1	
	3	1.031	1.038	1.024	1.046	9		3	0.040	0.033	0.098	0.046	51	
	Mean	1.047	1.050	1.041	1.052	8		Mean	0.039	0.034	0.063	0.041	17	
2	1	1.027	1.024	1.018	1.022	7	2	1	0.063	0.036	0.050	0.041	-21	
	2	1.022	1.018	1.014	1.015	6		2	0.120	0.032	0.050	0.039	-78	
	3	0.998	0.998	0.993	0.995	3		3	0.066	0.031	0.052	0.044	-22	
	Mean	1.016	1.013	1.008	1.011	5		Mean	0.083	0.033	0.051	0.041	-40	
3	1	0.996	1.014	0.990	1.012	4	3	1	0.084	0.034	0.044	0.038	-45	
	2	0.996	1.013	0.990	1.011	4		2	0.076	0.033	0.053	0.039	-28	
	3	0.981	0.995	0.973	0.992	6		3	0.104	0.034	0.050	0.039	-59	
	Mean	0.991	1.007	0.984	1.005	5		Mean	0.088	0.034	0.049	0.039	-44	
Mean for 3 assays	-	-	-	-	6	Mean for 3 assays	-	-	-	-	-22	Negative		

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.053	1.058	1.009	1.059	45	1	1	0.040	0.037	0.054	0.064	-4	
	2	1.050	1.048	1.010	1.049	41		2	0.042	0.037	0.060	0.047	0	
	3	1.026	1.023	0.993	1.023	34		3	0.051	0.035	0.064	0.050	-5	
	Mean	1.043	1.043	1.004	1.044	40		Mean	0.044	0.036	0.059	0.054	-3	
2	1	1.016	1.019	0.982	1.016	31	2	1	0.038	0.036	0.058	0.036	22	
	2	1.021	1.018	0.983	1.014	35		2	0.043	0.036	0.064	0.037	23	
	3	0.998	0.999	0.966	0.996	29		3	0.041	0.041	0.062	0.036	23	
	Mean	1.012	1.012	0.977	1.009	32		Mean	0.041	0.038	0.061	0.036	23	
3	1	1.012	1.016	0.977	1.011	30	3	1	0.041	0.032	0.056	0.038	7	
	2	1.014	1.011	0.980	1.006	29		2	0.042	0.032	0.063	0.038	13	
	3	0.999	0.987	0.967	0.983	27		3	0.044	0.031	0.063	0.044	11	
	Mean	1.008	1.005	0.975	1.000	29		Mean	0.042	0.032	0.061	0.040	10	
Mean for 3 assays	-	-	-	-	34	Mean for 3 assays	-	-	-	-	10	Positive		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.067	1.065	1.049	1.066	18	1	1	0.036	0.037	0.047	0.039	7	Negative
	2	1.058	1.052	1.043	1.052	15		2	0.033	0.035	0.042	0.037	5	
	3	1.035	1.034	1.020	1.032	15		3	0.033	0.034	0.044	0.040	7	
	Mean	1.053	1.050	1.037	1.050	16		Mean	0.034	0.035	0.044	0.039	6	
2	1	1.024	1.020	1.003	1.017	18	2	1	0.032	0.033	0.038	0.045	-3	Negative
	2	1.018	1.016	0.998	1.013	17		2	0.033	0.032	0.039	0.039	-3	
	3	0.998	0.997	0.982	0.995	13		3	0.033	0.031	0.040	0.038	-2	
	Mean	1.013	1.011	0.994	1.008	16		Mean	0.033	0.032	0.039	0.041	-3	
3	1	1.020	1.014	0.999	1.010	16	3	1	0.031	0.034	0.044	0.040	5	Negative
	2	1.008	1.007	0.986	1.002	17		2	0.031	0.033	0.045	0.039	6	
	3	0.997	0.984	0.976	0.980	16		3	0.031	0.033	0.048	0.044	9	
	Mean	1.008	1.002	0.987	0.997	16		Mean	0.031	0.033	0.046	0.041	7	
Mean for 3 assays		-	-	-	-	16	Mean for 3 assays		-	-	-	-	3	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.063	1.061	0.939	1.060	123	1	1	0.032	0.033	0.147	0.042	103	Positive
	2	1.056	1.054	0.935	1.053	120		2	0.032	0.032	0.148	0.040	104	
	3	1.037	1.037	0.923	1.037	113		3	0.032	0.033	0.151	0.052	107	
	Mean	1.052	1.051	0.932	1.050	119		Mean	0.032	0.033	0.149	0.045	105	
2	1	1.036	1.029	0.905	1.027	129	2	1	0.032	0.034	0.144	0.042	105	Positive
	2	1.027	1.026	0.900	1.025	125		2	0.032	0.033	0.145	0.038	106	
	3	1.004	1.004	0.885	1.002	117		3	0.031	0.032	0.147	0.041	109	
	Mean	1.022	1.020	0.897	1.018	124		Mean	0.032	0.033	0.145	0.040	107	
3	1	1.028	1.027	0.904	1.026	123	3	1	0.032	0.034	0.135	0.039	97	Positive
	2	1.018	1.018	0.898	1.017	119		2	0.033	0.033	0.135	0.040	96	
	3	1.002	1.000	0.889	1.000	112		3	0.031	0.034	0.139	0.040	102	
	Mean	1.016	1.015	0.897	1.014	118		Mean	0.032	0.034	0.136	0.040	98	
Mean for 3 assays		-	-	-	-	120	Mean for 3 assays		-	-	-	-	103	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.980	1.065	0.924	1.066	56	1	1	0.034	0.037	0.206	0.039	168	
	2	0.985	1.052	0.922	1.052	63		2	0.036	0.035	0.213	0.037	173	
	3	0.960	1.034	0.906	1.032	54		3	0.035	0.034	0.220	0.040	181	
	Mean	0.975	1.050	0.917	1.050	58		Mean	0.035	0.035	0.213	0.039	174	
2	1	1.022	1.020	0.997	1.017	22	2	1	0.033	0.033	0.206	0.045	164	
	2	1.019	1.016	0.995	1.013	21		2	0.036	0.032	0.210	0.039	165	
	3	0.999	0.997	0.975	0.995	21		3	0.031	0.031	0.197	0.038	157	
	Mean	1.013	1.011	0.989	1.008	21		Mean	0.033	0.032	0.204	0.041	162	
3	1	1.017	1.014	0.994	1.010	18	3	1	0.033	0.034	0.208	0.040	167	
	2	1.014	1.007	0.995	1.002	14		2	0.036	0.033	0.219	0.039	175	
	3	0.997	0.984	0.976	0.980	16		3	0.034	0.033	0.217	0.044	175	
	Mean	1.009	1.002	0.988	0.997	16		Mean	0.034	0.033	0.215	0.041	172	
Mean for 3 assays		-	-	-	-	32	Mean for 3 assays		-	-	-	-	169	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.062	1.061	0.965	1.060	96	1	1	0.034	0.033	0.300	0.042	254	
	2	1.059	1.054	0.973	1.053	85		2	0.039	0.032	0.312	0.040	261	
	3	1.032	1.037	0.948	1.037	83		3	0.036	0.033	0.346	0.052	298	
	Mean	1.051	1.051	0.962	1.050	88		Mean	0.036	0.033	0.319	0.045	271	
2	1	1.018	1.029	0.921	1.027	95	2	1	0.033	0.034	0.296	0.042	256	
	2	1.015	1.026	0.926	1.025	87		2	0.036	0.033	0.303	0.038	260	
	3	1.001	1.004	0.916	1.002	83		3	0.034	0.032	0.361	0.041	320	
	Mean	1.011	1.020	0.921	1.018	88		Mean	0.034	0.033	0.320	0.040	279	
3	1	1.019	1.027	0.927	1.026	91	3	1	0.032	0.034	0.323	0.039	285	
	2	1.012	1.018	0.926	1.017	85		2	0.035	0.033	0.323	0.040	282	
	3	0.997	1.000	0.916	1.000	80		3	0.033	0.034	0.353	0.040	314	
	Mean	1.009	1.015	0.923	1.014	85		Mean	0.033	0.034	0.333	0.040	294	
Mean for 3 assays		-	-	-	-	87	Mean for 3 assays		-	-	-	-	281	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.073	1.065	1.018	1.066	55	1	1	0.038	0.037	0.052	0.039	10	
	2	1.067	1.052	1.011	1.052	56		2	0.038	0.035	0.052	0.037	10	
	3	1.039	1.034	0.986	1.032	53		3	0.044	0.034	0.057	0.040	9	
	Mean	1.060	1.050	1.005	1.050	55		Mean	0.040	0.035	0.054	0.039	10	
2	1	1.022	1.020	0.990	1.017	29	2	1	0.033	0.033	0.053	0.045	11	
	2	1.015	1.016	0.983	1.013	29		2	0.033	0.032	0.052	0.039	10	
	3	0.994	0.997	0.965	0.995	26		3	0.035	0.031	0.053	0.038	9	
	Mean	1.010	1.011	0.979	1.008	28		Mean	0.034	0.032	0.053	0.041	10	
3	1	1.015	1.014	0.978	1.010	32	3	1	0.033	0.034	0.050	0.040	9	
	2	1.007	1.007	0.971	1.002	31		2	0.033	0.033	0.050	0.039	9	
	3	0.994	0.984	0.961	0.980	28		3	0.037	0.033	0.053	0.044	8	
	Mean	1.005	1.002	0.970	0.997	30		Mean	0.034	0.033	0.051	0.041	9	
Mean for 3 assays	-	-	-	-	38	Mean for 3 assays	-	-	-	-	10			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.061	1.061	0.926	1.060	134	1	1	0.033	0.033	0.129	0.042	84	
	2	1.054	1.054	0.921	1.053	132		2	0.034	0.032	0.129	0.040	83	
	3	1.032	1.037	0.912	1.037	119		3	0.038	0.033	0.145	0.052	95	
	Mean	1.049	1.051	0.920	1.050	128		Mean	0.035	0.033	0.134	0.045	87	
2	1	1.036	1.029	0.867	1.027	167	2	1	0.031	0.034	0.124	0.042	86	
	2	1.023	1.026	0.868	1.025	153		2	0.032	0.033	0.125	0.038	86	
	3	1.004	1.004	0.887	1.002	115		3	0.038	0.032	0.134	0.041	89	
	Mean	1.021	1.020	0.874	1.018	145		Mean	0.034	0.033	0.128	0.040	87	
3	1	1.023	1.027	0.848	1.026	174	3	1	0.038	0.034	0.117	0.039	73	
	2	1.021	1.018	0.855	1.017	165		2	0.037	0.033	0.119	0.040	76	
	3	0.997	1.000	0.843	1.000	153		3	0.049	0.034	0.128	0.040	73	
	Mean	1.014	1.015	0.849	1.014	164		Mean	0.041	0.034	0.121	0.040	74	
Mean for 3 assays	-	-	-	-	146	Mean for 3 assays	-	-	-	-	83			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.057	1.065	1.001	1.066	56	1	1	0.035	0.037	0.069	0.039	30	Positive
	2	1.047	1.052	1.004	1.052	43		2	0.037	0.035	0.073	0.037	32	
	3	1.026	1.034	0.992	1.032	34		3	0.037	0.034	0.068	0.040	27	
	Mean	1.043	1.050	0.999	1.050	44		Mean	0.036	0.035	0.070	0.039	30	
2	1	1.018	1.020	0.991	1.017	24	2	1	0.033	0.033	0.069	0.045	27	Positive
	2	1.015	1.016	0.987	1.013	25		2	0.034	0.032	0.071	0.039	28	
	3	0.992	0.997	0.969	0.995	20		3	0.034	0.031	0.100	0.038	57	
	Mean	1.008	1.011	0.982	1.008	23		Mean	0.034	0.032	0.080	0.041	37	
3	1	1.013	1.014	0.981	1.010	27	3	1	0.032	0.034	0.066	0.040	26	Positive
	2	1.016	1.007	0.996	1.002	15		2	0.032	0.033	0.072	0.039	32	
	3	0.989	0.984	0.973	0.980	11		3	0.031	0.033	0.065	0.044	26	
	Mean	1.006	1.002	0.983	0.997	18		Mean	0.032	0.033	0.068	0.041	28	
Mean for 3 assays		-	-	-	-	28	Mean for 3 assays		-	-	-	-	32	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.053	1.061	1.014	1.060	38	1	1	0.036	0.033	0.078	0.042	30	Positive
	2	1.050	1.054	1.009	1.053	40		2	0.037	0.032	0.081	0.040	32	
	3	1.027	1.037	0.989	1.037	37		3	0.039	0.033	0.089	0.052	38	
	Mean	1.043	1.051	1.004	1.050	38		Mean	0.037	0.033	0.083	0.045	33	
2	1	1.020	1.029	1.001	1.027	17	2	1	0.035	0.034	0.080	0.042	38	Positive
	2	1.013	1.026	0.984	1.025	27		2	0.040	0.033	0.083	0.038	36	
	3	0.994	1.004	0.969	1.002	23		3	0.036	0.032	0.088	0.041	45	
	Mean	1.009	1.020	0.985	1.018	22		Mean	0.037	0.033	0.084	0.040	40	
3	1	1.003	1.027	1.002	1.026	0	3	1	0.042	0.034	0.085	0.039	37	Positive
	2	1.001	1.018	0.993	1.017	7		2	0.043	0.033	0.082	0.040	33	
	3	0.981	1.000	0.975	1.000	5		3	0.038	0.034	0.083	0.040	39	
	Mean	0.995	1.015	0.990	1.014	4		Mean	0.041	0.034	0.083	0.040	36	
Mean for 3 assays		-	-	-	-	21	Mean for 3 assays		-	-	-	-	36	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Quinine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.045	1.065	0.964	1.066	81	1	1	0.036	0.037	0.087	0.039	47	Positive
	2	1.038	1.052	0.958	1.052	80		2	0.035	0.035	0.084	0.037	45	
	3	1.021	1.034	0.955	1.032	66		3	0.036	0.034	0.091	0.040	51	
	Mean	1.035	1.050	0.959	1.050	76		Mean	0.036	0.035	0.087	0.039	48	
2	1	1.016	1.020	0.971	1.017	42	2	1	0.034	0.033	0.074	0.045	31	Positive
	2	1.011	1.016	0.967	1.013	41		2	0.034	0.032	0.075	0.039	32	
	3	0.994	0.997	0.949	0.995	42		3	0.035	0.031	0.078	0.038	34	
	Mean	1.007	1.011	0.962	1.008	42		Mean	0.034	0.032	0.076	0.041	32	
3	1	1.011	1.014	0.960	1.010	46	3	1	0.033	0.034	0.069	0.040	28	Positive
	2	1.013	1.007	0.964	1.002	44		2	0.034	0.033	0.069	0.039	27	
	3	0.994	0.984	0.949	0.980	40		3	0.033	0.033	0.070	0.044	29	
	Mean	1.006	1.002	0.958	0.997	43		Mean	0.033	0.033	0.069	0.041	28	
Mean for 3 assays		-	-	-	-	54	Mean for 3 assays		-	-	-	-	36	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.052	1.061	0.764	1.060	287	1	1	0.035	0.033	0.196	0.042	149	Positive
	2	1.044	1.054	0.762	1.053	281		2	0.036	0.032	0.202	0.040	154	
	3	1.021	1.037	0.751	1.037	269		3	0.035	0.033	0.218	0.052	171	
	Mean	1.039	1.051	0.759	1.050	279		Mean	0.035	0.033	0.205	0.045	158	
2	1	1.027	1.029	0.771	1.027	254	2	1	0.033	0.034	0.181	0.042	141	Positive
	2	1.021	1.026	0.774	1.025	245		2	0.035	0.033	0.183	0.038	141	
	3	1.007	1.004	0.772	1.002	233		3	0.034	0.032	0.198	0.041	157	
	Mean	1.018	1.020	0.772	1.018	244		Mean	0.034	0.033	0.187	0.040	146	
3	1	1.006	1.027	0.797	1.026	208	3	1	0.034	0.034	0.163	0.039	123	Positive
	2	1.000	1.018	0.798	1.017	201		2	0.036	0.033	0.160	0.040	118	
	3	0.989	1.000	0.794	1.000	194		3	0.038	0.034	0.171	0.040	127	
	Mean	0.998	1.015	0.796	1.014	201		Mean	0.036	0.034	0.165	0.040	123	
Mean for 3 assays		-	-	-	-	241	Mean for 3 assays		-	-	-	-	142	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.062	1.065	1.058	1.066	4	1	1	0.034	0.037	0.036	0.039	-2	Negative
	2	1.054	1.052	1.051	1.052	3		2	0.036	0.035	0.037	0.037	-3	
	3	1.030	1.034	1.027	1.032	3		3	0.037	0.034	0.038	0.040	-3	
	Mean	1.049	1.050	1.045	1.050	3		Mean	0.036	0.035	0.037	0.039	-3	
2	1	1.024	1.020	1.020	1.017	1	2	1	0.034	0.033	0.036	0.045	-7	Negative
	2	1.015	1.016	1.009	1.013	3		2	0.035	0.032	0.037	0.039	-7	
	3	0.997	0.997	0.992	0.995	2		3	0.036	0.031	0.038	0.038	-7	
	Mean	1.012	1.011	1.007	1.008	2		Mean	0.035	0.032	0.037	0.041	-7	
3	1	1.014	1.014	1.009	1.010	0	3	1	0.035	0.034	0.036	0.040	-7	Negative
	2	1.006	1.007	1.000	1.002	1		2	0.036	0.033	0.041	0.039	-3	
	3	0.997	0.984	0.990	0.980	2		3	0.031	0.033	0.033	0.044	-6	
	Mean	1.006	1.002	1.000	0.997	1		Mean	0.034	0.033	0.037	0.041	-5	
Mean for 3 assays		-	-	-	-	2	Mean for 3 assays		-	-	-	-	-5	

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.060	1.061	1.059	1.060	0	1	1	0.035	0.033	0.036	0.042	-11	Negative
	2	1.056	1.054	1.055	1.053	0		2	0.036	0.032	0.036	0.040	-12	
	3	1.036	1.037	1.035	1.037	0		3	0.037	0.033	0.040	0.052	-9	
	Mean	1.051	1.051	1.050	1.050	0		Mean	0.036	0.033	0.037	0.045	-11	
2	1	1.035	1.029	1.029	1.027	4	2	1	0.036	0.034	0.038	0.042	-5	Negative
	2	1.031	1.026	1.026	1.025	3		2	0.036	0.033	0.039	0.038	-4	
	3	1.008	1.004	1.003	1.002	3		3	0.038	0.032	0.043	0.041	-2	
	Mean	1.025	1.020	1.019	1.018	3		Mean	0.037	0.033	0.040	0.040	-4	
3	1	1.024	1.027	1.019	1.026	4	3	1	0.037	0.034	0.038	0.039	-5	Negative
	2	1.019	1.018	1.015	1.017	3		2	0.034	0.033	0.039	0.040	-1	
	3	1.008	1.000	1.005	1.000	2		3	0.033	0.034	0.040	0.040	1	
	Mean	1.017	1.015	1.013	1.014	3		Mean	0.035	0.034	0.039	0.040	-2	
Mean for 3 assays		-	-	-	-	2	Mean for 3 assays		-	-	-	-	-6	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.063	1.063	1.012	1.014	-1	1	1	0.042	0.037	0.051	0.047	-1	
	2	1.063	1.060	1.011	1.009	1		2	0.040	0.037	0.049	0.047	-1	
	3	1.063	1.061	1.010	1.006	2		3	0.038	0.037	0.048	0.047	0	
	Mean	1.063	1.061	1.011	1.010	1		Mean	0.040	0.037	0.049	0.047	-1	
2	1	1.226	1.238	1.161	1.170	-4	2	1	0.038	0.039	0.042	0.042	0	
	2	1.231	1.238	1.168	1.173	-4		2	0.038	0.038	0.042	0.042	0	
	3	1.223	1.240	1.157	1.171	-3		3	0.038	0.038	0.042	0.042	0	
	Mean	1.226	1.239	1.162	1.171	-4		Mean	0.038	0.038	0.042	0.042	0	
3	1	1.206	1.204	1.168	1.170	1	3	1	0.038	0.039	0.043	0.043	1	
	2	1.207	1.198	1.168	1.161	2		2	0.038	0.037	0.042	0.041	1	
	3	1.202	1.214	1.161	1.175	4		3	0.037	0.037	0.042	0.042	1	
	Mean	1.205	1.206	1.166	1.169	2		Mean	0.037	0.038	0.042	0.042	1	
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	0			

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.063	1.060	1.011	1.016	10	1	1	0.037	0.037	0.050	0.046	3	
	2	1.068	1.050	1.019	1.010	7		2	0.037	0.037	0.051	0.047	4	
	3	1.064	1.060	1.015	1.018	8		3	0.037	0.037	0.052	0.048	5	
	Mean	1.065	1.057	1.015	1.015	8		Mean	0.037	0.037	0.051	0.047	4	
2	1	1.247	1.237	1.202	1.194	4	2	1	0.037	0.037	0.042	0.041	0	
	2	1.242	1.228	1.198	1.188	5		2	0.037	0.039	0.042	0.044	0	
	3	1.239	1.229	1.194	1.190	4		3	0.037	0.037	0.043	0.042	1	
	Mean	1.242	1.231	1.198	1.191	4		Mean	0.037	0.037	0.043	0.042	0	
3	1	1.240	1.229	1.211	1.210	-1	3	1	0.038	0.038	0.054	0.051	3	
	2	1.237	1.246	1.211	1.207	-4		2	0.037	0.037	0.054	0.051	3	
	3	1.251	1.239	1.216	1.208	5		3	0.037	0.037	0.057	0.053	6	
	Mean	1.243	1.238	1.212	1.208	0		Mean	0.037	0.037	0.055	0.051	4	
Mean for 3 assays	-	-	-	-	4	Mean for 3 assays	-	-	-	-	3			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.072	1.063	1.021	1.014	0	1	1	0.042	0.037	0.057	0.047	5	
	2	1.077	1.060	1.026	1.009	1		2	0.039	0.037	0.053	0.047	4	
	3	1.095	1.061	1.039	1.006	4		3	0.038	0.037	0.054	0.047	6	
	Mean	1.081	1.061	1.029	1.010	2		Mean	0.040	0.037	0.054	0.047	5	
2	1	1.228	1.238	1.161	1.170	-1	2	1	0.038	0.039	0.044	0.042	2	
	2	1.235	1.238	1.167	1.173	0		2	0.038	0.038	0.044	0.042	2	
	3	1.231	1.240	1.163	1.171	0		3	0.038	0.038	0.045	0.042	3	
	Mean	1.231	1.239	1.163	1.171	0		Mean	0.038	0.038	0.044	0.042	2	
3	1	1.200	1.204	1.160	1.170	4	3	1	0.037	0.039	0.044	0.043	3	
	2	1.200	1.198	1.162	1.161	1		2	0.037	0.037	0.045	0.041	3	
	3	1.202	1.214	1.164	1.175	1		3	0.037	0.037	0.044	0.042	3	
	Mean	1.201	1.206	1.162	1.169	2		Mean	0.037	0.038	0.044	0.042	3	
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	3			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.057	1.060	0.996	1.016	19	1	1	0.037	0.037	0.096	0.046	48	
	2	1.061	1.050	1.000	1.010	19		2	0.037	0.037	0.097	0.047	50	
	3	1.061	1.060	0.999	1.018	20		3	0.037	0.037	0.106	0.048	59	
	Mean	1.060	1.057	0.998	1.015	19		Mean	0.037	0.037	0.100	0.047	52	
2	1	1.239	1.237	1.175	1.194	24	2	1	0.039	0.037	0.072	0.041	29	
	2	1.247	1.228	1.185	1.188	22		2	0.037	0.039	0.073	0.044	31	
	3	1.247	1.229	1.182	1.190	25		3	0.039	0.037	0.079	0.042	35	
	Mean	1.244	1.231	1.181	1.191	24		Mean	0.038	0.037	0.075	0.042	32	
3	1	1.240	1.229	1.194	1.210	16	3	1	0.037	0.038	0.114	0.051	63	
	2	1.244	1.246	1.194	1.207	21		2	0.037	0.037	0.116	0.051	65	
	3	1.255	1.239	1.198	1.208	27		3	0.037	0.037	0.124	0.053	73	
	Mean	1.246	1.238	1.195	1.208	21		Mean	0.037	0.037	0.118	0.051	67	
Mean for 3 assays	-	-	-	-	21	Mean for 3 assays	-	-	-	-	50			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Amiodarone HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.162	1.063	1.101	1.014	10	1	1	0.081	0.037	0.104	0.047	13	
	2	1.172	1.060	1.110	1.009	11		2	0.081	0.037	0.105	0.047	14	
	3	1.153	1.061	1.071	1.006	31		3	0.080	0.037	0.100	0.047	10	
	Mean	1.162	1.061	1.094	1.010	17		Mean	0.081	0.037	0.103	0.047	12	
2	1	1.321	1.238	1.249	1.170	4	2	1	0.088	0.039	0.102	0.042	10	
	2	1.332	1.238	1.259	1.173	5		2	0.088	0.038	0.099	0.042	7	
	3	1.324	1.240	1.250	1.171	6		3	0.088	0.038	0.099	0.042	6	
	Mean	1.326	1.239	1.252	1.171	5		Mean	0.088	0.038	0.100	0.042	8	
3	1	1.305	1.204	1.240	1.170	29	3	1	0.088	0.039	0.105	0.043	13	
	2	1.303	1.198	1.252	1.161	14		2	0.087	0.037	0.106	0.041	15	
	3	1.306	1.214	1.230	1.175	39		3	0.087	0.037	0.103	0.042	12	
	Mean	1.305	1.206	1.241	1.169	27		Mean	0.088	0.038	0.105	0.042	13	
Mean for 3 assays	-	-	-	-	16	Mean for 3 assays	-	-	-	-	11			

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.918	1.060	1.657	1.016	219	1	1	0.664	0.037	0.521	0.046	-153	
	2	1.913	1.050	1.643	1.010	228		2	0.667	0.037	0.511	0.047	-167	
	3	1.854	1.060	1.611	1.018	201		3	0.723	0.037	0.518	0.048	-215	
	Mean	1.895	1.057	1.637	1.015	216		Mean	0.685	0.037	0.517	0.047	-178	
2	1	2.059	1.237	1.878	1.194	141	2	1	0.599	0.037	0.541	0.041	-64	
	2	2.022	1.228	1.891	1.188	91		2	0.623	0.039	0.533	0.044	-95	
	3	1.935	1.229	1.793	1.190	102		3	0.642	0.037	0.506	0.042	-141	
	Mean	2.005	1.231	1.854	1.191	111		Mean	0.621	0.037	0.527	0.042	-100	
3	1	2.104	1.229	1.900	1.210	174	3	1	0.656	0.038	0.575	0.051	-95	
	2	2.100	1.246	1.936	1.207	134		2	0.665	0.037	0.558	0.051	-121	
	3	2.007	1.239	1.882	1.208	94		3	0.707	0.037	0.499	0.053	-222	
	Mean	2.070	1.238	1.906	1.208	134		Mean	0.676	0.037	0.544	0.051	-146	
Mean for 3 assays	-	-	-	-	154	Mean for 3 assays	-	-	-	-	-141			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Chlorpromazine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.056	1.063	1.011	1.014	-6	1	1	0.048	0.037	0.062	0.047	4	
	2	1.057	1.060	1.014	1.009	-7		2	0.037	0.037	0.048	0.047	2	
	3	1.063	1.061	1.018	1.006	-6		3	0.037	0.037	0.050	0.047	3	
	Mean	1.059	1.061	1.014	1.010	-6		Mean	0.040	0.037	0.053	0.047	3	
2	1	1.227	1.238	1.167	1.170	-8	2	1	0.038	0.039	0.051	0.042	9	
	2	1.226	1.238	1.167	1.173	-9		2	0.038	0.038	0.051	0.042	9	
	3	1.223	1.240	1.163	1.171	-8		3	0.038	0.038	0.052	0.042	9	
	Mean	1.225	1.239	1.165	1.171	-8		Mean	0.038	0.038	0.051	0.042	9	
3	1	1.198	1.204	1.160	1.170	1	3	1	0.037	0.039	0.049	0.043	8	
	2	1.206	1.198	1.167	1.161	2		2	0.037	0.037	0.049	0.041	8	
	3	1.196	1.214	1.159	1.175	1		3	0.037	0.037	0.049	0.042	8	
	Mean	1.200	1.206	1.162	1.169	1		Mean	0.037	0.038	0.049	0.042	8	
Mean for 3 assays	-	-	-	-	-4	Mean for 3 assays	-	-	-	-	7			

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.050	1.060	1.052	1.016	-45	1	1	0.037	0.037	0.097	0.046	49	
	2	1.070	1.050	1.131	1.010	-103		2	0.037	0.037	0.101	0.047	53	
	3	1.073	1.060	1.074	1.018	-43		3	0.037	0.037	0.106	0.048	59	
	Mean	1.064	1.057	1.086	1.015	-64		Mean	0.037	0.037	0.101	0.047	54	
2	1	1.240	1.237	1.235	1.194	-35	2	1	0.037	0.037	0.100	0.041	57	
	2	1.261	1.228	1.258	1.188	-37		2	0.037	0.039	0.100	0.044	58	
	3	1.257	1.229	1.253	1.190	-36		3	0.037	0.037	0.105	0.042	63	
	Mean	1.253	1.231	1.249	1.191	-36		Mean	0.037	0.037	0.101	0.042	59	
3	1	1.232	1.229	1.220	1.210	-19	3	1	0.037	0.038	0.104	0.051	53	
	2	1.268	1.246	1.272	1.207	-34		2	0.038	0.037	0.106	0.051	55	
	3	1.274	1.239	1.275	1.208	-31		3	0.037	0.037	0.109	0.053	58	
	Mean	1.258	1.238	1.256	1.208	-28		Mean	0.037	0.037	0.107	0.051	55	
Mean for 3 assays	-	-	-	-	-43	Mean for 3 assays	-	-	-	-	56			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.059	1.063	1.005	1.014	3	1	1	0.037	0.037	0.054	0.047	7	Negative
	2	1.055	1.060	1.001	1.009	3		2	0.041	0.037	0.059	0.047	8	
	3	1.059	1.061	1.003	1.006	5		3	0.052	0.037	0.070	0.047	8	
	Mean	1.058	1.061	1.003	1.010	4		Mean	0.043	0.037	0.061	0.047	8	
2	1	1.221	1.238	1.154	1.170	0	2	1	0.038	0.039	0.047	0.042	4	Negative
	2	1.234	1.238	1.158	1.173	8		2	0.051	0.038	0.059	0.042	4	
	3	1.216	1.240	1.145	1.171	3		3	0.038	0.038	0.047	0.042	5	
	Mean	1.224	1.239	1.152	1.171	4		Mean	0.042	0.038	0.051	0.042	4	
3	1	1.198	1.204	1.152	1.170	10	3	1	0.038	0.039	0.052	0.043	10	Negative
	2	1.199	1.198	1.152	1.161	10		2	0.037	0.037	0.046	0.041	4	
	3	1.199	1.214	1.151	1.175	11		3	0.037	0.037	0.046	0.042	5	
	Mean	1.199	1.206	1.152	1.169	10		Mean	0.037	0.038	0.048	0.042	6	
Mean for 3 assays		-	-	-	-	6	Mean for 3 assays		-	-	-	-	6	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.060	1.060	0.977	1.016	41	1	1	0.037	0.037	0.127	0.046	80	Positive
	2	1.059	1.050	0.977	1.010	40		2	0.038	0.037	0.134	0.047	86	
	3	1.059	1.060	0.974	1.018	43		3	0.037	0.037	0.139	0.048	92	
	Mean	1.059	1.057	0.976	1.015	41		Mean	0.037	0.037	0.134	0.047	86	
2	1	1.245	1.237	1.145	1.194	61	2	1	0.037	0.037	0.163	0.041	121	Positive
	2	1.246	1.228	1.145	1.188	60		2	0.037	0.039	0.166	0.044	124	
	3	1.239	1.229	1.133	1.190	66		3	0.037	0.037	0.177	0.042	136	
	Mean	1.243	1.231	1.141	1.191	62		Mean	0.037	0.037	0.169	0.042	127	
3	1	1.239	1.229	1.152	1.210	57	3	1	0.038	0.038	0.164	0.051	113	Positive
	2	1.241	1.246	1.158	1.207	53		2	0.037	0.037	0.169	0.051	118	
	3	1.271	1.239	1.181	1.208	60		3	0.037	0.037	0.182	0.053	130	
	Mean	1.250	1.238	1.164	1.208	57		Mean	0.037	0.037	0.172	0.051	120	
Mean for 3 assays		-	-	-	-	53	Mean for 3 assays		-	-	-	-	111	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Doxycycline HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.058	1.063	0.951	1.014	56	1	1	0.037	0.037	0.091	0.047	44	
	2	1.059	1.060	0.954	1.009	55		2	0.037	0.037	0.091	0.047	44	
	3	1.059	1.061	0.954	1.006	55		3	0.043	0.037	0.093	0.047	40	
	Mean	1.059	1.061	0.953	1.010	55		Mean	0.039	0.037	0.091	0.047	43	
2	1	1.224	1.238	1.123	1.170	33	2	1	0.039	0.039	0.094	0.042	51	
	2	1.223	1.238	1.123	1.173	32		2	0.038	0.038	0.094	0.042	51	
	3	1.228	1.240	1.127	1.171	33		3	0.038	0.038	0.097	0.042	55	
	Mean	1.225	1.239	1.124	1.171	33		Mean	0.038	0.038	0.095	0.042	52	
3	1	1.206	1.204	1.126	1.170	43	3	1	0.038	0.039	0.088	0.043	46	
	2	1.203	1.198	1.124	1.161	42		2	0.038	0.037	0.088	0.041	46	
	3	1.207	1.214	1.126	1.175	43		3	0.038	0.037	0.089	0.042	48	
	Mean	1.205	1.206	1.126	1.169	43		Mean	0.038	0.038	0.088	0.042	47	
Mean for 3 assays		-	-	-	-	44	Mean for 3 assays		-	-	-	-	47	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.072	1.060	0.862	1.016	168	1	1	0.038	0.037	0.386	0.046	338	
	2	1.075	1.050	0.876	1.010	157		2	0.038	0.037	0.402	0.047	354	
	3	1.078	1.060	0.877	1.018	159		3	0.038	0.037	0.416	0.048	367	
	Mean	1.075	1.057	0.872	1.015	161		Mean	0.038	0.037	0.401	0.047	353	
2	1	1.254	1.237	0.972	1.194	242	2	1	0.038	0.037	0.367	0.041	324	
	2	1.254	1.228	0.989	1.188	224		2	0.038	0.039	0.373	0.044	330	
	3	1.246	1.229	0.979	1.190	227		3	0.037	0.037	0.383	0.042	341	
	Mean	1.251	1.231	0.980	1.191	231		Mean	0.038	0.037	0.374	0.042	332	
3	1	1.240	1.229	0.962	1.210	248	3	1	0.039	0.038	0.407	0.051	354	
	2	1.250	1.246	0.992	1.207	229		2	0.038	0.037	0.417	0.051	365	
	3	1.254	1.239	0.993	1.208	231		3	0.039	0.037	0.427	0.053	375	
	Mean	1.248	1.238	0.982	1.208	236		Mean	0.039	0.037	0.417	0.051	365	
Mean for 3 assays		-	-	-	-	209	Mean for 3 assays		-	-	-	-	350	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.051	1.063	0.977	1.014	24	1	1	0.037	0.037	0.047	0.047	0	
	2	1.054	1.060	0.979	1.009	25		2	0.037	0.037	0.047	0.047	0	
	3	1.053	1.061	0.976	1.006	26		3	0.037	0.037	0.047	0.047	0	
	Mean	1.053	1.061	0.977	1.010	25		Mean	0.037	0.037	0.047	0.047	0	
2	1	1.224	1.238	1.158	1.170	-2	2	1	0.038	0.039	0.044	0.042	2	
	2	1.217	1.238	1.151	1.173	-3		2	0.038	0.038	0.044	0.042	1	
	3	1.233	1.240	1.163	1.171	1		3	0.038	0.038	0.044	0.042	2	
	Mean	1.225	1.239	1.158	1.171	-1		Mean	0.038	0.038	0.044	0.042	2	
3	1	1.202	1.204	1.155	1.170	11	3	1	0.037	0.039	0.044	0.043	2	
	2	1.201	1.198	1.156	1.161	8		2	0.037	0.037	0.043	0.041	2	
	3	1.197	1.214	1.150	1.175	11		3	0.037	0.037	0.043	0.042	2	
	Mean	1.200	1.206	1.153	1.169	10		Mean	0.037	0.038	0.043	0.042	2	
Mean for 3 assays		-	-	-	-	11	Mean for 3 assays		-	-	-	-	1	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.064	1.060	0.961	1.016	61	1	1	0.037	0.037	0.069	0.046	22	
	2	1.064	1.050	0.993	1.010	29		2	0.037	0.037	0.069	0.047	22	
	3	1.066	1.060	0.994	1.018	30		3	0.037	0.037	0.071	0.048	24	
	Mean	1.065	1.057	0.982	1.015	40		Mean	0.037	0.037	0.070	0.047	23	
2	1	1.239	1.237	1.150	1.194	49	2	1	0.038	0.037	0.062	0.041	20	
	2	1.246	1.228	1.158	1.188	49		2	0.037	0.039	0.063	0.044	22	
	3	1.240	1.229	1.151	1.190	49		3	0.037	0.037	0.066	0.042	23	
	Mean	1.242	1.231	1.153	1.191	49		Mean	0.037	0.037	0.064	0.042	22	
3	1	1.236	1.229	1.167	1.210	39	3	1	0.040	0.038	0.085	0.051	31	
	2	1.242	1.246	1.179	1.207	33		2	0.037	0.037	0.076	0.051	25	
	3	1.239	1.239	1.176	1.208	33		3	0.037	0.037	0.083	0.053	32	
	Mean	1.239	1.238	1.174	1.208	35		Mean	0.038	0.037	0.081	0.051	29	
Mean for 3 assays		-	-	-	-	41	Mean for 3 assays		-	-	-	-	25	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.072	1.076	1.033	1.039	1	1	1	0.038	0.038	0.056	0.050	5	
	2	1.077	1.077	1.035	1.039	4		2	0.037	0.037	0.056	0.050	5	
	3	1.075	1.087	1.033	1.049	5		3	0.037	0.037	0.057	0.050	7	
	Mean	1.075	1.080	1.034	1.042	3		Mean	0.037	0.037	0.056	0.050	6	
2	1	1.211	1.202	1.165	1.172	15	2	1	0.037	0.037	0.045	0.041	5	
	2	1.199	1.197	1.155	1.165	13		2	0.037	0.037	0.045	0.040	5	
	3	1.203	1.206	1.160	1.174	12		3	0.037	0.037	0.045	0.040	6	
	Mean	1.204	1.201	1.160	1.170	13		Mean	0.037	0.037	0.045	0.040	5	
3	1	1.209	1.197	1.159	1.169	19	3	1	0.038	0.042	0.046	0.045	4	
	2	1.202	1.194	1.152	1.163	20		2	0.038	0.041	0.046	0.046	4	
	3	1.202	1.203	1.149	1.171	22		3	0.037	0.038	0.046	0.042	4	
	Mean	1.204	1.198	1.153	1.167	20		Mean	0.038	0.040	0.046	0.044	4	
Mean for 3 assays	-	-	-	-	12	Mean for 3 assays	-	-	-	-	5			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.047	1.051	0.913	1.014	91	1	1	0.038	0.036	0.134	0.047	85	
	2	1.051	1.058	0.916	1.016	91		2	0.037	0.036	0.139	0.047	90	
	3	1.044	1.065	0.910	1.015	91		3	0.037	0.037	0.137	0.049	89	
	Mean	1.047	1.058	0.913	1.015	91		Mean	0.037	0.036	0.137	0.048	88	
2	1	1.229	1.223	1.076	1.190	120	2	1	0.039	0.038	0.143	0.043	99	
	2	1.226	1.223	1.076	1.191	118		2	0.039	0.038	0.145	0.043	102	
	3	1.233	1.225	1.071	1.192	129		3	0.038	0.038	0.143	0.043	99	
	Mean	1.229	1.224	1.074	1.191	122		Mean	0.038	0.038	0.144	0.043	100	
3	1	1.242	1.248	1.055	1.183	120	3	1	0.038	0.037	0.147	0.053	94	
	2	1.253	1.247	1.063	1.180	123		2	0.037	0.037	0.153	0.052	100	
	3	1.246	1.262	1.053	1.192	126		3	0.038	0.037	0.157	0.054	103	
	Mean	1.247	1.252	1.057	1.185	123		Mean	0.037	0.037	0.152	0.053	99	
Mean for 3 assays	-	-	-	-	112	Mean for 3 assays	-	-	-	-	96			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.066	1.076	1.021	1.039	7	1	1	0.038	0.038	0.222	0.050	171	
	2	1.072	1.077	1.027	1.039	7		2	0.037	0.037	0.219	0.050	169	
	3	1.059	1.087	1.011	1.049	11		3	0.037	0.037	0.220	0.050	170	
	Mean	1.066	1.080	1.020	1.042	8		Mean	0.037	0.037	0.220	0.050	170	
2	1	1.215	1.202	1.164	1.172	21	2	1	0.037	0.037	0.167	0.041	127	
	2	1.205	1.197	1.156	1.165	19		2	0.037	0.037	0.166	0.040	127	
	3	1.199	1.206	1.150	1.174	18		3	0.037	0.037	0.173	0.040	133	
	Mean	1.206	1.201	1.156	1.170	19		Mean	0.037	0.037	0.169	0.040	129	
3	1	1.215	1.197	1.158	1.169	26	3	1	0.038	0.042	0.163	0.045	121	
	2	1.207	1.194	1.148	1.163	28		2	0.039	0.041	0.166	0.046	123	
	3	1.208	1.203	1.147	1.171	30		3	0.037	0.038	0.171	0.042	130	
	Mean	1.210	1.198	1.151	1.167	28		Mean	0.038	0.040	0.167	0.044	125	
Mean for 3 assays	-	-	-	-	18	Mean for 3 assays	-	-	-	-	141			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.044	1.051	0.939	1.014	63	1	1	0.037	0.036	0.370	0.047	321	
	2	1.045	1.058	0.940	1.016	62		2	0.037	0.036	0.395	0.047	346	
	3	1.047	1.065	0.938	1.015	66		3	0.037	0.037	0.381	0.049	332	
	Mean	1.045	1.058	0.939	1.015	64		Mean	0.037	0.036	0.382	0.048	333	
2	1	1.241	1.223	1.115	1.190	93	2	1	0.038	0.038	0.402	0.043	359	
	2	1.230	1.223	1.109	1.191	88		2	0.038	0.038	0.415	0.043	372	
	3	1.227	1.225	1.102	1.192	92		3	0.038	0.038	0.419	0.043	376	
	Mean	1.232	1.224	1.108	1.191	91		Mean	0.038	0.038	0.412	0.043	369	
3	1	1.240	1.248	1.100	1.183	73	3	1	0.037	0.037	0.349	0.053	296	
	2	1.244	1.247	1.103	1.180	74		2	0.037	0.037	0.365	0.052	312	
	3	1.243	1.262	1.099	1.192	77		3	0.037	0.037	0.373	0.054	320	
	Mean	1.242	1.252	1.101	1.185	75		Mean	0.037	0.037	0.362	0.053	309	
Mean for 3 assays	-	-	-	-	77	Mean for 3 assays	-	-	-	-	337			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.065	1.076	1.015	1.039	11	1	1	0.038	0.038	0.064	0.050	13	Negative
	2	1.068	1.077	1.018	1.039	12		2	0.037	0.037	0.063	0.050	13	
	3	1.068	1.087	1.016	1.049	14		3	0.037	0.037	0.063	0.050	13	
	Mean	1.067	1.080	1.016	1.042	12		Mean	0.037	0.037	0.063	0.050	13	
2	1	1.209	1.202	1.153	1.172	25	2	1	0.037	0.037	0.057	0.041	16	Negative
	2	1.206	1.197	1.153	1.165	22		2	0.037	0.037	0.056	0.040	16	
	3	1.199	1.206	1.147	1.174	21		3	0.037	0.037	0.056	0.040	16	
	Mean	1.205	1.201	1.151	1.170	23		Mean	0.037	0.037	0.056	0.040	16	
3	1	1.216	1.197	1.149	1.169	36	3	1	0.038	0.042	0.057	0.045	15	Positive
	2	1.215	1.194	1.148	1.163	35		2	0.038	0.041	0.056	0.046	15	
	3	1.204	1.203	1.138	1.171	34		3	0.037	0.038	0.056	0.042	15	
	Mean	1.212	1.198	1.145	1.167	35		Mean	0.038	0.040	0.056	0.044	15	
Mean for 3 assays		-	-	-	-	23	Mean for 3 assays		-	-	-	-	15	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.045	1.051	0.940	1.014	62	1	1	0.037	0.036	0.138	0.047	89	Positive
	2	1.038	1.058	0.937	1.016	58		2	0.037	0.036	0.139	0.047	90	
	3	1.041	1.065	0.934	1.015	64		3	0.036	0.037	0.141	0.049	92	
	Mean	1.041	1.058	0.937	1.015	61		Mean	0.037	0.036	0.139	0.048	90	
2	1	1.233	1.223	1.105	1.190	95	2	1	0.038	0.038	0.135	0.043	92	Positive
	2	1.229	1.223	1.103	1.191	94		2	0.038	0.038	0.136	0.043	93	
	3	1.222	1.225	1.094	1.192	95		3	0.038	0.038	0.140	0.043	97	
	Mean	1.228	1.224	1.101	1.191	95		Mean	0.038	0.038	0.137	0.043	94	
3	1	1.239	1.248	1.096	1.183	77	3	1	0.037	0.037	0.145	0.053	92	Positive
	2	1.245	1.247	1.101	1.180	76		2	0.037	0.037	0.147	0.052	94	
	3	1.242	1.262	1.096	1.192	80		3	0.037	0.037	0.152	0.054	99	
	Mean	1.242	1.252	1.098	1.185	78		Mean	0.037	0.037	0.148	0.053	95	
Mean for 3 assays		-	-	-	-	78	Mean for 3 assays		-	-	-	-	93	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.066	1.076	1.031	1.039	-3	1	1	0.037	0.038	0.041	0.050	-10	Negative
	2	1.070	1.077	1.037	1.039	-4		2	0.037	0.037	0.041	0.050	-10	
	3	1.068	1.087	1.034	1.049	-3		3	0.037	0.037	0.041	0.050	-10	
	Mean	1.068	1.080	1.034	1.042	-3		Mean	0.037	0.037	0.041	0.050	-10	
2	1	1.205	1.202	1.167	1.172	8	2	1	0.037	0.037	0.057	0.041	17	Negative
	2	1.199	1.197	1.163	1.165	4		2	0.037	0.037	0.057	0.040	17	
	3	1.203	1.206	1.168	1.174	4		3	0.037	0.037	0.059	0.040	19	
	Mean	1.202	1.201	1.166	1.170	5		Mean	0.037	0.037	0.058	0.040	18	
3	1	1.213	1.197	1.161	1.169	21	3	1	0.038	0.042	0.058	0.045	17	Negative
	2	1.211	1.194	1.157	1.163	23		2	0.037	0.041	0.059	0.046	17	
	3	1.216	1.203	1.161	1.171	23		3	0.038	0.038	0.061	0.042	20	
	Mean	1.213	1.198	1.160	1.167	22		Mean	0.038	0.040	0.059	0.044	18	
Mean for 3 assays		-	-	-	-	8	Mean for 3 assays		-	-	-	-	9	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.042	1.051	1.018	1.014	-20	1	1	0.037	0.036	0.081	0.047	33	Positive
	2	1.048	1.058	1.028	1.016	-23		2	0.037	0.036	0.083	0.047	35	
	3	1.051	1.065	1.031	1.015	-23		3	0.037	0.037	0.088	0.049	39	
	Mean	1.047	1.058	1.026	1.015	-22		Mean	0.037	0.036	0.084	0.048	36	
2	1	1.234	1.223	1.187	1.190	15	2	1	0.038	0.038	0.081	0.043	38	Positive
	2	1.231	1.223	1.189	1.191	9		2	0.038	0.038	0.084	0.043	41	
	3	1.237	1.225	1.196	1.192	8		3	0.038	0.038	0.089	0.043	46	
	Mean	1.234	1.224	1.191	1.191	11		Mean	0.038	0.038	0.085	0.043	42	
3	1	1.230	1.248	1.187	1.183	-24	3	1	0.037	0.037	0.087	0.053	33	Positive
	2	1.229	1.247	1.191	1.180	-29		2	0.037	0.037	0.090	0.052	37	
	3	1.235	1.262	1.200	1.192	-32		3	0.037	0.037	0.096	0.054	43	
	Mean	1.231	1.252	1.193	1.185	-28		Mean	0.037	0.037	0.091	0.053	38	
Mean for 3 assays		-	-	-	-	-13	Mean for 3 assays		-	-	-	-	39	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Quinine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.067	1.076	0.993	1.039	36	1	1	0.038	0.038	0.093	0.050	43	Positive
	2	1.069	1.077	0.995	1.039	36		2	0.037	0.037	0.092	0.050	42	
	3	1.068	1.087	0.995	1.049	35		3	0.037	0.037	0.094	0.050	44	
	Mean	1.068	1.080	0.994	1.042	36		Mean	0.037	0.037	0.093	0.050	43	
2	1	1.208	1.202	1.135	1.172	41	2	1	0.037	0.037	0.073	0.041	33	Positive
	2	1.200	1.197	1.131	1.165	38		2	0.037	0.037	0.072	0.040	32	
	3	1.202	1.206	1.133	1.174	37		3	0.038	0.037	0.075	0.040	34	
	Mean	1.203	1.201	1.133	1.170	39		Mean	0.037	0.037	0.073	0.040	33	
3	1	1.214	1.197	1.129	1.169	54	3	1	0.038	0.042	0.075	0.045	33	Positive
	2	1.208	1.194	1.120	1.163	58		2	0.038	0.041	0.076	0.046	34	
	3	1.210	1.203	1.122	1.171	56		3	0.038	0.038	0.078	0.042	36	
	Mean	1.211	1.198	1.124	1.167	56		Mean	0.038	0.040	0.076	0.044	34	
Mean for 3 assays		-	-	-	-	44	Mean for 3 assays		-	-	-	-	37	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.046	1.051	0.819	1.014	183	1	1	0.037	0.036	0.244	0.047	195	Positive
	2	1.048	1.058	0.825	1.016	180		2	0.036	0.036	0.241	0.047	193	
	3	1.043	1.065	0.820	1.015	179		3	0.036	0.037	0.259	0.049	211	
	Mean	1.045	1.058	0.821	1.015	181		Mean	0.036	0.036	0.248	0.048	200	
2	1	1.245	1.223	0.962	1.190	250	2	1	0.038	0.038	0.254	0.043	211	Positive
	2	1.233	1.223	0.979	1.191	221		2	0.038	0.038	0.260	0.043	217	
	3	1.231	1.225	0.977	1.192	221		3	0.038	0.038	0.271	0.043	228	
	Mean	1.236	1.224	0.972	1.191	231		Mean	0.038	0.038	0.262	0.043	219	
3	1	1.242	1.248	0.959	1.183	217	3	1	0.037	0.037	0.263	0.053	210	Positive
	2	1.242	1.247	0.965	1.180	210		2	0.037	0.037	0.259	0.052	207	
	3	1.239	1.262	0.958	1.192	214		3	0.038	0.037	0.286	0.054	232	
	Mean	1.241	1.252	0.960	1.185	214		Mean	0.037	0.037	0.270	0.053	216	
Mean for 3 assays		-	-	-	-	209	Mean for 3 assays		-	-	-	-	212	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.071	1.076	1.036	1.039	-4	1	1	0.038	0.038	0.049	0.050	-2	Negative
	2	1.068	1.077	1.036	1.039	-5		2	0.037	0.037	0.048	0.050	-2	
	3	1.065	1.087	1.031	1.049	-3		3	0.038	0.037	0.050	0.050	-1	
	Mean	1.068	1.080	1.034	1.042	-4		Mean	0.037	0.037	0.049	0.050	-2	
2	1	1.202	1.202	1.166	1.172	6	2	1	0.037	0.037	0.040	0.041	0	Negative
	2	1.193	1.197	1.165	1.165	-3		2	0.037	0.037	0.040	0.040	0	
	3	1.200	1.206	1.174	1.174	-5		3	0.038	0.037	0.054	0.040	13	
	Mean	1.198	1.201	1.168	1.170	-1		Mean	0.037	0.037	0.045	0.040	4	
3	1	1.211	1.197	1.174	1.169	6	3	1	0.038	0.042	0.041	0.045	-1	Negative
	2	1.205	1.194	1.174	1.163	1		2	0.038	0.041	0.040	0.046	-1	
	3	1.209	1.203	1.172	1.171	6		3	0.038	0.038	0.040	0.042	-2	
	Mean	1.208	1.198	1.173	1.167	4		Mean	0.038	0.040	0.040	0.044	-1	
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	0			

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.044	1.051	1.004	1.014	-3	1	1	0.037	0.036	0.040	0.047	-9	Negative
	2	1.042	1.058	1.003	1.016	-4		2	0.037	0.036	0.040	0.047	-9	
	3	1.041	1.065	1.001	1.015	-4		3	0.036	0.037	0.040	0.049	-9	
	Mean	1.042	1.058	1.003	1.015	-4		Mean	0.036	0.036	0.040	0.048	-9	
2	1	1.234	1.223	1.194	1.190	8	2	1	0.038	0.038	0.039	0.043	-4	Negative
	2	1.229	1.223	1.194	1.191	2		2	0.038	0.038	0.039	0.043	-4	
	3	1.235	1.225	1.202	1.192	0		3	0.038	0.038	0.039	0.043	-4	
	Mean	1.232	1.224	1.196	1.191	3		Mean	0.038	0.038	0.039	0.043	-4	
3	1	1.227	1.248	1.172	1.183	-12	3	1	0.041	0.037	0.044	0.053	-13	Negative
	2	1.233	1.247	1.178	1.180	-12		2	0.037	0.037	0.040	0.052	-13	
	3	1.235	1.262	1.179	1.192	-11		3	0.037	0.037	0.040	0.054	-13	
	Mean	1.232	1.252	1.177	1.185	-12		Mean	0.038	0.037	0.042	0.053	-13	
Mean for 3 assays	-	-	-	-	-4	Mean for 3 assays	-	-	-	-	-9			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : 5-FU

Test concentration 20 μM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.034	1.029	1.027	1.019	-5	1	1	0.039	0.040	0.043	0.045	1	
	2	1.044	1.042	1.036	1.026	-4		2	0.039	0.041	0.041	0.042	-1	
	3	1.044	1.026	1.034	1.014	-2		3	0.040	0.040	0.040	0.043	-3	
	Mean	1.041	1.032	1.032	1.020	-4		Mean	0.039	0.040	0.041	0.043	-1	
2	1	1.044	1.049	1.034	1.039	1	2	1	0.038	0.037	0.039	0.041	-2	
	2	1.035	1.043	1.036	1.034	-10		2	0.038	0.037	0.040	0.041	-2	
	3	1.046	1.050	1.036	1.040	1		3	0.037	0.038	0.039	0.040	-2	
	Mean	1.042	1.047	1.035	1.038	-3		Mean	0.037	0.037	0.039	0.041	-2	
3	1	1.057	1.063	1.047	1.055	1	3	1	0.036	0.037	0.039	0.041	-2	
	2	1.054	1.066	1.044	1.057	1		2	0.037	0.040	0.039	0.044	-2	
	3	1.062	1.064	1.053	1.056	0		3	0.037	0.037	0.039	0.041	-2	
	Mean	1.057	1.064	1.048	1.056	1		Mean	0.037	0.038	0.039	0.042	-2	
Mean for 3 assays		-	-	-	-	-2	Mean for 3 assays		-	-	-	-	-2	

Test concentration 200 μM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.057	1.052	1.043	1.047	7	1	1	0.038	0.039	0.040	0.043	-1	
	2	1.050	1.047	1.041	1.041	2		2	0.038	0.038	0.041	0.042	-1	
	3	1.047	1.052	1.031	1.041	9		3	0.038	0.039	0.042	0.042	1	
	Mean	1.051	1.050	1.038	1.043	6		Mean	0.038	0.039	0.041	0.042	0	
2	1	1.066	1.074	1.069	1.068	-9	2	1	0.037	0.045	0.039	0.040	3	
	2	1.039	1.067	1.037	1.062	-4		2	0.037	0.036	0.040	0.038	3	
	3	1.061	1.065	1.056	1.060	-1		3	0.038	0.036	0.042	0.039	4	
	Mean	1.055	1.069	1.054	1.063	-5		Mean	0.037	0.039	0.040	0.039	3	
3	1	1.049	1.046	1.042	1.038	1	3	1	0.037	0.036	0.040	0.039	1	
	2	1.046	1.044	1.037	1.037	2		2	0.037	0.037	0.040	0.039	1	
	3	1.054	1.052	1.046	1.044	1		3	0.037	0.037	0.040	0.040	1	
	Mean	1.050	1.047	1.042	1.040	1		Mean	0.037	0.037	0.040	0.039	1	
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	1	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.994	1.029	0.934	1.019	48	1	1	0.042	0.040	0.045	0.045	0	
	2	0.987	1.042	0.959	1.026	16		2	0.037	0.041	0.046	0.042	6	
	3	0.946	1.026	0.943	1.014	-9		3	0.038	0.040	0.045	0.043	4	
	Mean	0.976	1.032	0.945	1.020	18		Mean	0.039	0.040	0.045	0.043	3	
2	1	1.041	1.049	1.025	1.039	7	2	1	0.037	0.037	0.043	0.041	2	
	2	1.035	1.043	1.018	1.034	8		2	0.040	0.037	0.045	0.041	1	
	3	1.041	1.050	1.026	1.040	6		3	0.038	0.038	0.045	0.040	3	
	Mean	1.039	1.047	1.023	1.038	7		Mean	0.038	0.037	0.044	0.041	2	
3	1	1.059	1.063	1.045	1.055	5	3	1	0.037	0.037	0.042	0.041	1	
	2	1.051	1.066	1.037	1.057	6		2	0.037	0.040	0.042	0.044	1	
	3	1.059	1.064	1.045	1.056	6		3	0.037	0.037	0.042	0.041	1	
	Mean	1.056	1.064	1.043	1.056	6		Mean	0.037	0.038	0.042	0.042	1	
Mean for 3 assays		-	-	-	-	10	Mean for 3 assays		-	-	-	-	2	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.034	1.052	0.971	1.047	56	1	1	0.039	0.038	0.062	0.040	22	
	2	1.036	1.047	0.994	1.041	35		2	0.037	0.041	0.062	0.042	24	
	3	1.029	1.052	0.992	1.041	30		3	0.041	0.041	0.071	0.042	29	
	Mean	1.033	1.050	0.986	1.043	40		Mean	0.039	0.040	0.065	0.041	25	
2	1	1.058	1.074	1.009	1.068	43	2	1	0.043	0.045	0.059	0.040	17	
	2	1.052	1.067	1.005	1.062	42		2	0.037	0.036	0.054	0.038	17	
	3	1.056	1.065	1.008	1.060	41		3	0.039	0.036	0.059	0.039	20	
	Mean	1.055	1.069	1.007	1.063	42		Mean	0.039	0.039	0.057	0.039	18	
3	1	1.042	1.046	0.980	1.038	55	3	1	0.037	0.036	0.064	0.039	25	
	2	1.039	1.044	0.975	1.037	57		2	0.037	0.037	0.066	0.039	28	
	3	1.045	1.052	0.984	1.044	54		3	0.037	0.037	0.066	0.040	27	
	Mean	1.042	1.047	0.980	1.040	55		Mean	0.037	0.037	0.065	0.039	27	
Mean for 3 assays		-	-	-	-	46	Mean for 3 assays		-	-	-	-	23	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Amiodarone HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.077	1.029	1.063	1.019	2	1	1	0.103	0.040	0.119	0.045	13	
	2	1.107	1.042	1.025	1.026	70		2	0.104	0.041	0.118	0.042	11	
	3	1.235	1.026	1.060	1.014	163		3	0.103	0.040	0.118	0.043	12	
	Mean	1.140	1.032	1.049	1.020	78		Mean	0.103	0.040	0.118	0.043	12	
2	1	1.098	1.049	1.000	1.039	88	2	1	0.085	0.037	0.078	0.041	-11	
	2	1.101	1.043	1.003	1.034	89		2	0.085	0.037	0.079	0.041	-9	
	3	1.096	1.050	1.000	1.040	87		3	0.085	0.038	0.079	0.040	-10	
	Mean	1.098	1.047	1.001	1.038	88		Mean	0.085	0.037	0.079	0.041	-10	
3	1	1.132	1.063	1.032	1.055	92	3	1	0.091	0.037	0.092	0.041	-3	
	2	1.120	1.066	1.024	1.057	88		2	0.091	0.040	0.094	0.044	-2	
	3	1.126	1.064	1.039	1.056	79		3	0.092	0.037	0.093	0.041	-3	
	Mean	1.126	1.064	1.032	1.056	86		Mean	0.091	0.038	0.093	0.042	-3	
Mean for 3 assays		-	-	-	-	84	Mean for 3 assays		-	-	-	-	0	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.450	1.052	1.306	1.047	137	1	1	0.579	0.038	0.542	0.040	-38	
	2	1.398	1.047	1.335	1.041	56		2	0.552	0.041	0.509	0.042	-44	
	3	1.539	1.052	1.331	1.041	201		3	0.546	0.041	0.513	0.042	-34	
	Mean	1.462	1.050	1.324	1.043	131		Mean	0.559	0.040	0.521	0.041	-39	
2	1	1.372	1.074	1.204	1.068	162	2	1	0.415	0.045	0.325	0.040	-90	
	2	1.355	1.067	1.215	1.062	135		2	0.422	0.036	0.354	0.038	-69	
	3	1.347	1.065	1.253	1.060	88		3	0.416	0.036	0.334	0.039	-82	
	Mean	1.358	1.069	1.224	1.063	128		Mean	0.418	0.039	0.337	0.039	-80	
3	1	1.254	1.046	1.168	1.038	79	3	1	0.306	0.036	0.290	0.039	-19	
	2	1.248	1.044	1.189	1.037	52		2	0.304	0.037	0.287	0.039	-20	
	3	1.250	1.052	1.191	1.044	53		3	0.304	0.037	0.290	0.040	-17	
	Mean	1.251	1.047	1.183	1.040	61		Mean	0.305	0.037	0.289	0.039	-19	
Mean for 3 assays		-	-	-	-	107	Mean for 3 assays		-	-	-	-	-46	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Chlorpromazine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.022	1.029	0.953	1.019	57	1	1	0.039	0.040	0.051	0.045	9	Positive
	2	1.035	1.042	0.969	1.026	54		2	0.045	0.041	0.052	0.042	4	
	3	1.034	1.026	0.952	1.014	70		3	0.039	0.040	0.051	0.043	9	
	Mean	1.030	1.032	0.958	1.020	60		Mean	0.041	0.040	0.051	0.043	7	
2	1	1.019	1.049	0.902	1.039	108	2	1	0.037	0.037	0.049	0.041	8	Positive
	2	1.020	1.043	0.904	1.034	107		2	0.038	0.037	0.049	0.041	7	
	3	1.026	1.050	0.908	1.040	109		3	0.037	0.038	0.050	0.040	9	
	Mean	1.022	1.047	0.905	1.038	108		Mean	0.037	0.037	0.049	0.041	8	
3	1	1.038	1.063	0.924	1.055	107	3	1	0.038	0.037	0.049	0.041	7	Positive
	2	1.039	1.066	0.923	1.057	108		2	0.037	0.040	0.048	0.044	7	
	3	1.042	1.064	0.926	1.056	108		3	0.037	0.037	0.048	0.041	7	
	Mean	1.040	1.064	0.924	1.056	108		Mean	0.037	0.038	0.048	0.042	7	
Mean for 3 assays		-	-	-	-	92	Mean for 3 assays		-	-	-	-	7	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.010	1.052	1.054	1.047	-51	1	1	0.039	0.038	0.104	0.040	64	Positive
	2	0.996	1.047	1.024	1.041	-35		2	0.039	0.041	0.104	0.042	64	
	3	1.017	1.052	1.034	1.041	-24		3	0.039	0.041	0.108	0.042	68	
	Mean	1.008	1.050	1.037	1.043	-37		Mean	0.039	0.040	0.105	0.041	65	
2	1	1.017	1.074	0.965	1.068	46	2	1	0.040	0.045	0.089	0.040	49	Positive
	2	1.032	1.067	0.979	1.062	46		2	0.037	0.036	0.089	0.038	52	
	3	1.043	1.065	0.987	1.060	50		3	0.037	0.036	0.091	0.039	54	
	Mean	1.030	1.069	0.977	1.063	47		Mean	0.038	0.039	0.089	0.039	52	
3	1	1.003	1.046	0.962	1.038	34	3	1	0.037	0.036	0.099	0.039	60	Positive
	2	1.017	1.044	0.976	1.037	34		2	0.038	0.037	0.103	0.039	63	
	3	1.013	1.052	0.969	1.044	37		3	0.037	0.037	0.104	0.040	65	
	Mean	1.011	1.047	0.969	1.040	35		Mean	0.037	0.037	0.102	0.039	63	
Mean for 3 assays		-	-	-	-	15	Mean for 3 assays		-	-	-	-	60	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.035	1.029	1.006	1.019	17	1	1	0.040	0.040	0.047	0.045	4	Negative
	2	1.044	1.042	1.022	1.026	10		2	0.039	0.041	0.045	0.042	3	
	3	1.042	1.026	1.011	1.014	19		3	0.039	0.040	0.045	0.043	3	
	Mean	1.040	1.032	1.013	1.020	15		Mean	0.039	0.040	0.046	0.043	3	
2	1	1.033	1.049	1.002	1.039	23	2	1	0.038	0.037	0.042	0.041	1	Negative
	2	1.033	1.043	1.001	1.034	23		2	0.038	0.037	0.042	0.041	1	
	3	1.036	1.050	1.007	1.040	20		3	0.038	0.038	0.043	0.040	1	
	Mean	1.034	1.047	1.003	1.038	22		Mean	0.038	0.037	0.042	0.041	1	
3	1	1.057	1.063	1.025	1.055	24	3	1	0.037	0.037	0.042	0.041	0	Negative
	2	1.051	1.066	1.019	1.057	24		2	0.037	0.040	0.042	0.044	0	
	3	1.062	1.064	1.030	1.056	24		3	0.037	0.037	0.042	0.041	1	
	Mean	1.057	1.064	1.025	1.056	24		Mean	0.037	0.038	0.042	0.042	0	
Mean for 3 assays		-	-	-	-	20	Mean for 3 assays		-	-	-	-	1	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.039	1.052	0.910	1.047	122	1	1	0.038	0.038	0.146	0.040	107	Positive
	2	1.042	1.047	0.957	1.041	78		2	0.038	0.041	0.138	0.042	99	
	3	1.041	1.052	0.952	1.041	82		3	0.038	0.041	0.157	0.042	118	
	Mean	1.041	1.050	0.940	1.043	94		Mean	0.038	0.040	0.147	0.041	108	
2	1	1.059	1.074	0.987	1.068	66	2	1	0.039	0.045	0.116	0.040	76	Positive
	2	1.058	1.067	0.988	1.062	64		2	0.038	0.036	0.118	0.038	81	
	3	1.060	1.065	0.989	1.060	65		3	0.037	0.036	0.123	0.039	86	
	Mean	1.059	1.069	0.988	1.063	65		Mean	0.038	0.039	0.119	0.039	81	
3	1	1.048	1.046	0.957	1.038	84	3	1	0.038	0.036	0.132	0.039	92	Positive
	2	1.048	1.044	0.963	1.037	77		2	0.038	0.037	0.136	0.039	96	
	3	1.047	1.052	0.956	1.044	85		3	0.038	0.037	0.141	0.040	102	
	Mean	1.048	1.047	0.959	1.040	82		Mean	0.038	0.037	0.136	0.039	97	
Mean for 3 assays		-	-	-	-	80	Mean for 3 assays		-	-	-	-	95	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Doxycycline HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.033	1.029	0.972	1.019	49	1	1	0.039	0.039	0.082	0.043	40	
	2	1.025	1.042	0.957	1.026	56		2	0.040	0.038	0.081	0.042	38	
	3	1.028	1.026	0.975	1.014	41		3	0.039	0.039	0.080	0.042	39	
	Mean	1.029	1.032	0.968	1.020	49		Mean	0.039	0.039	0.081	0.042	39	
2	1	1.032	1.049	0.958	1.039	65	2	1	0.038	0.037	0.080	0.041	38	
	2	1.029	1.043	0.958	1.034	62		2	0.038	0.037	0.081	0.041	39	
	3	1.036	1.050	0.970	1.040	57		3	0.038	0.038	0.081	0.040	39	
	Mean	1.032	1.047	0.962	1.038	61		Mean	0.038	0.037	0.081	0.041	39	
3	1	1.051	1.063	0.985	1.055	58	3	1	0.037	0.037	0.078	0.041	37	
	2	1.045	1.066	0.982	1.057	56		2	0.039	0.040	0.078	0.044	36	
	3	1.052	1.064	0.989	1.056	55		3	0.038	0.037	0.079	0.041	37	
	Mean	1.049	1.064	0.985	1.056	56		Mean	0.038	0.038	0.078	0.042	37	
Mean for 3 assays		-	-	-	-	55	Mean for 3 assays		-	-	-	-	38	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.003	1.052	0.843	1.047	153	1	1	0.041	0.038	0.364	0.040	322	
	2	1.011	1.047	0.875	1.041	129		2	0.041	0.041	0.374	0.042	332	
	3	0.996	1.052	0.855	1.041	134		3	0.042	0.041	0.376	0.042	333	
	Mean	1.003	1.050	0.858	1.043	139		Mean	0.041	0.040	0.371	0.041	329	
2	1	1.025	1.074	0.837	1.068	182	2	1	0.041	0.045	0.309	0.040	269	
	2	1.025	1.067	0.843	1.062	176		2	0.040	0.036	0.314	0.038	274	
	3	1.036	1.065	0.844	1.060	186		3	0.040	0.036	0.318	0.039	278	
	Mean	1.029	1.069	0.841	1.063	181		Mean	0.040	0.039	0.314	0.039	274	
3	1	0.979	1.046	0.802	1.038	170	3	1	0.041	0.036	0.345	0.039	302	
	2	0.973	1.044	0.808	1.037	158		2	0.041	0.037	0.352	0.039	309	
	3	0.985	1.052	0.808	1.044	170		3	0.042	0.037	0.366	0.040	322	
	Mean	0.979	1.047	0.806	1.040	166		Mean	0.041	0.037	0.354	0.039	311	
Mean for 3 assays		-	-	-	-	162	Mean for 3 assays		-	-	-	-	305	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.047	1.029	1.029	1.019	6	1	1	0.040	0.040	0.046	0.045	3	
	2	1.039	1.042	1.023	1.026	4		2	0.039	0.041	0.046	0.042	4	
	3	1.049	1.026	1.034	1.014	3		3	0.039	0.040	0.045	0.043	3	
	Mean	1.045	1.032	1.029	1.020	4		Mean	0.039	0.040	0.046	0.043	3	
2	1	1.034	1.049	1.016	1.039	9	2	1	0.038	0.037	0.043	0.041	2	
	2	1.029	1.043	1.008	1.034	12		2	0.038	0.037	0.043	0.041	1	
	3	1.036	1.050	1.015	1.040	12		3	0.038	0.038	0.043	0.040	1	
	Mean	1.033	1.047	1.013	1.038	11		Mean	0.038	0.037	0.043	0.041	1	
3	1	1.059	1.054	1.044	1.046	8	3	1	0.037	0.037	0.041	0.040	1	
	2	1.052	1.055	1.036	1.048	9		2	0.037	0.037	0.042	0.040	1	
	3	1.050	1.057	1.036	1.049	7		3	0.037	0.037	0.041	0.040	2	
	Mean	1.054	1.055	1.039	1.048	8		Mean	0.037	0.037	0.041	0.040	1	
Mean for 3 assays	-	-	-	-	8	Mean for 3 assays	-	-	-	-	-	2		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.043	1.052	0.998	1.047	38	1	1	0.038	0.038	0.062	0.040	23	
	2	1.041	1.047	0.995	1.041	39		2	0.039	0.041	0.061	0.042	21	
	3	1.051	1.052	1.009	1.041	35		3	0.038	0.041	0.062	0.042	23	
	Mean	1.045	1.050	1.001	1.043	37		Mean	0.038	0.040	0.062	0.041	22	
2	1	1.063	1.074	1.036	1.068	21	2	1	0.037	0.045	0.053	0.040	16	
	2	1.058	1.067	1.027	1.062	24		2	0.038	0.036	0.054	0.038	16	
	3	1.062	1.065	1.036	1.060	20		3	0.037	0.036	0.055	0.039	17	
	Mean	1.061	1.069	1.033	1.063	22		Mean	0.038	0.039	0.054	0.039	16	
3	1	1.049	1.046	1.003	1.038	39	3	1	0.038	0.036	0.076	0.039	36	
	2	1.046	1.044	1.004	1.037	35		2	0.038	0.037	0.060	0.039	20	
	3	1.051	1.052	1.008	1.044	37		3	0.038	0.037	0.061	0.040	21	
	Mean	1.049	1.047	1.005	1.040	37		Mean	0.038	0.037	0.065	0.039	26	
Mean for 3 assays	-	-	-	-	32	Mean for 3 assays	-	-	-	-	-	21		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.033	1.026	1.011	1.014	9	1	1	0.039	0.040	0.044	0.044	3	Negative
	2	1.038	1.029	1.023	1.017	2		2	0.039	0.040	0.044	0.041	3	
	3	1.028	1.025	1.001	1.011	14		3	0.040	0.043	0.044	0.045	2	
	Mean	1.033	1.027	1.012	1.014	8		Mean	0.039	0.041	0.044	0.043	3	
2	1	1.051	1.047	1.024	1.039	20	2	1	0.037	0.038	0.043	0.042	2	Negative
	2	1.036	1.040	1.010	1.033	19		2	0.038	0.037	0.043	0.041	2	
	3	1.047	1.049	1.021	1.041	19		3	0.037	0.038	0.042	0.041	2	
	Mean	1.045	1.045	1.018	1.038	19		Mean	0.037	0.038	0.043	0.041	2	
3	1	1.059	1.055	1.036	1.047	15	3	1	0.037	0.037	0.042	0.041	1	Negative
	2	1.055	1.050	1.033	1.042	14		2	0.038	0.037	0.043	0.041	1	
	3	1.065	1.058	1.043	1.050	14		3	0.037	0.038	0.042	0.041	1	
	Mean	1.060	1.054	1.037	1.046	14		Mean	0.037	0.037	0.043	0.041	1	
Mean for 3 assays		-	-	-	-	14	Mean for 3 assays		-	-	-	-	2	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.032	1.042	0.904	1.027	113	1	1	0.043	0.038	0.115	0.043	70	Positive
	2	1.047	1.043	0.921	1.025	111		2	0.039	0.041	0.120	0.042	79	
	3	1.037	1.039	0.928	1.027	94		3	0.038	0.041	0.116	0.042	76	
	Mean	1.039	1.041	0.918	1.026	106		Mean	0.040	0.040	0.117	0.042	75	
2	1	1.057	1.060	0.939	1.053	113	2	1	0.047	0.037	0.113	0.039	63	Positive
	2	1.052	1.053	0.939	1.047	107		2	0.037	0.037	0.115	0.039	75	
	3	1.055	1.052	0.942	1.046	107		3	0.037	0.039	0.115	0.041	75	
	Mean	1.055	1.055	0.940	1.049	109		Mean	0.040	0.037	0.114	0.040	71	
3	1	1.020	1.019	0.867	1.008	142	3	1	0.037	0.037	0.108	0.041	69	Positive
	2	1.035	1.021	0.886	1.010	138		2	0.039	0.037	0.114	0.040	72	
	3	1.023	1.018	0.876	1.008	136		3	0.037	0.039	0.116	0.042	75	
	Mean	1.026	1.019	0.876	1.008	139		Mean	0.038	0.038	0.113	0.041	72	
Mean for 3 assays		-	-	-	-	118	Mean for 3 assays		-	-	-	-	73	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.032	1.026	1.008	1.014	11	1	1	0.037	0.040	0.136	0.044	97	
	2	0.948	1.029	0.932	1.017	3		2	0.038	0.040	0.131	0.041	91	
	3	1.035	1.025	1.010	1.011	12		3	0.038	0.043	0.136	0.045	96	
	Mean	1.005	1.027	0.983	1.014	9		Mean	0.038	0.041	0.134	0.043	95	
2	1	1.041	1.047	1.014	1.039	20	2	1	0.037	0.038	0.157	0.042	117	
	2	1.039	1.040	1.002	1.033	29		2	0.037	0.037	0.158	0.041	117	
	3	1.040	1.049	1.012	1.041	20		3	0.037	0.038	0.160	0.041	120	
	Mean	1.040	1.045	1.010	1.038	23		Mean	0.037	0.038	0.158	0.041	118	
3	1	1.057	1.055	1.033	1.047	16	3	1	0.037	0.037	0.147	0.041	106	
	2	1.056	1.050	1.033	1.042	15		2	0.037	0.037	0.147	0.041	106	
	3	1.060	1.058	1.036	1.050	17		3	0.038	0.038	0.148	0.041	106	
	Mean	1.058	1.054	1.034	1.046	16		Mean	0.037	0.037	0.147	0.041	106	
Mean for 3 assays		-	-	-	-	16	Mean for 3 assays		-	-	-	-	106	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.026	1.042	0.947	1.027	64	1	1	0.039	0.038	0.447	0.043	406	
	2	1.025	1.043	0.952	1.025	58		2	0.037	0.041	0.456	0.042	417	
	3	1.031	1.039	0.962	1.027	54		3	0.039	0.041	0.452	0.042	411	
	Mean	1.027	1.041	0.954	1.026	59		Mean	0.038	0.040	0.452	0.042	411	
2	1	1.052	1.060	0.976	1.053	70	2	1	0.037	0.037	0.321	0.039	281	
	2	1.041	1.053	0.969	1.047	67		2	0.037	0.037	0.330	0.039	290	
	3	1.045	1.052	0.972	1.046	68		3	0.037	0.039	0.329	0.041	289	
	Mean	1.046	1.055	0.972	1.049	68		Mean	0.037	0.037	0.326	0.040	287	
3	1	1.026	1.019	0.908	1.008	107	3	1	0.037	0.037	0.350	0.041	310	
	2	1.022	1.021	0.912	1.010	100		2	0.037	0.037	0.327	0.040	287	
	3	1.047	1.018	0.934	1.008	102		3	0.042	0.039	0.364	0.042	319	
	Mean	1.032	1.019	0.918	1.008	103		Mean	0.039	0.038	0.347	0.041	305	
Mean for 3 assays		-	-	-	-	77	Mean for 3 assays		-	-	-	-	334	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.041	1.026	0.976	1.014	52	1	1	0.037	0.040	0.056	0.044	17	
	2	1.032	1.029	0.983	1.017	36		2	0.037	0.040	0.056	0.041	17	
	3	1.042	1.025	0.978	1.011	51		3	0.039	0.043	0.056	0.045	15	
	Mean	1.038	1.027	0.979	1.014	46		Mean	0.038	0.041	0.056	0.043	16	
2	1	1.039	1.047	0.988	1.039	44	2	1	0.037	0.038	0.052	0.042	12	
	2	1.037	1.040	0.992	1.033	38		2	0.037	0.037	0.052	0.041	12	
	3	1.039	1.049	0.994	1.041	38		3	0.037	0.038	0.052	0.041	12	
	Mean	1.038	1.045	0.991	1.038	40		Mean	0.037	0.038	0.052	0.041	12	
3	1	1.037	1.055	0.985	1.047	43	3	1	0.037	0.037	0.053	0.041	12	
	2	1.035	1.050	0.977	1.042	50		2	0.037	0.037	0.053	0.041	12	
	3	1.041	1.058	0.979	1.050	54		3	0.037	0.038	0.053	0.041	12	
	Mean	1.038	1.054	0.980	1.046	49		Mean	0.037	0.037	0.053	0.041	12	
Mean for 3 assays		-	-	-	-	45	Mean for 3 assays		-	-	-	-	13	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.078	1.042	0.956	1.027	107	1	1	0.038	0.038	0.143	0.043	103	
	2	1.001	1.043	0.884	1.025	102		2	0.056	0.041	0.142	0.042	84	
	3	1.042	1.039	0.904	1.027	123		3	0.039	0.041	0.146	0.042	105	
	Mean	1.040	1.041	0.915	1.026	111		Mean	0.044	0.040	0.144	0.042	97	
2	1	1.062	1.060	0.889	1.053	167	2	1	0.038	0.037	0.120	0.039	79	
	2	1.043	1.053	0.886	1.047	151		2	0.038	0.037	0.119	0.039	79	
	3	1.045	1.052	0.890	1.046	149		3	0.037	0.039	0.121	0.041	81	
	Mean	1.050	1.055	0.889	1.049	156		Mean	0.037	0.037	0.120	0.040	80	
3	1	1.035	1.019	0.823	1.008	201	3	1	0.037	0.037	0.128	0.041	88	
	2	1.039	1.021	0.832	1.010	196		2	0.037	0.037	0.129	0.040	88	
	3	1.044	1.018	0.836	1.008	197		3	0.037	0.039	0.131	0.042	91	
	Mean	1.039	1.019	0.830	1.008	198		Mean	0.037	0.038	0.129	0.041	89	
Mean for 3 assays		-	-	-	-	155	Mean for 3 assays		-	-	-	-	89	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.025	1.026	0.998	1.014	14	1	1	0.037	0.040	0.050	0.044	11	
	2	1.032	1.029	1.010	1.017	9		2	0.040	0.040	0.050	0.041	8	
	3	1.031	1.025	0.989	1.011	29		3	0.040	0.043	0.050	0.045	8	
	Mean	1.029	1.027	0.999	1.014	17		Mean	0.039	0.041	0.050	0.043	9	
2	1	1.043	1.047	1.000	1.039	36	2	1	0.037	0.038	0.062	0.042	22	
	2	1.044	1.040	1.005	1.033	32		2	0.037	0.037	0.061	0.041	20	
	3	1.047	1.049	1.007	1.041	32		3	0.039	0.038	0.066	0.041	23	
	Mean	1.044	1.045	1.004	1.038	33		Mean	0.038	0.038	0.063	0.041	22	
3	1	1.076	1.055	1.066	1.047	2	3	1	0.037	0.037	0.058	0.041	17	
	2	1.078	1.050	1.067	1.042	3		2	0.038	0.037	0.058	0.041	16	
	3	1.077	1.058	1.063	1.050	6		3	0.038	0.038	0.060	0.041	19	
	Mean	1.077	1.054	1.065	1.046	4		Mean	0.037	0.037	0.059	0.041	17	
Mean for 3 assays	-	-	-	-	18	Mean for 3 assays	-	-	-	-	-	16	Positive	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.010	1.042	1.008	1.027	-13	1	1	0.041	0.038	0.076	0.043	33	
	2	0.999	1.043	0.987	1.025	-3		2	0.039	0.041	0.076	0.042	35	
	3	0.992	1.039	1.009	1.027	-32		3	0.039	0.041	0.081	0.042	40	
	Mean	1.000	1.041	1.001	1.026	-16		Mean	0.040	0.040	0.078	0.042	36	
2	1	1.036	1.060	0.975	1.053	54	2	1	0.037	0.037	0.066	0.039	26	
	2	1.025	1.053	0.965	1.047	54		2	0.038	0.037	0.059	0.039	18	
	3	1.031	1.052	0.971	1.046	54		3	0.037	0.039	0.069	0.041	29	
	Mean	1.030	1.055	0.970	1.049	54		Mean	0.037	0.037	0.065	0.040	24	
3	1	0.995	1.019	0.951	1.008	33	3	1	0.038	0.037	0.084	0.041	43	
	2	0.987	1.021	0.946	1.010	30		2	0.039	0.037	0.085	0.040	44	
	3	1.001	1.018	0.968	1.008	22		3	0.038	0.039	0.090	0.042	49	
	Mean	0.995	1.019	0.955	1.008	28		Mean	0.038	0.038	0.086	0.041	45	
Mean for 3 assays	-	-	-	-	22	Mean for 3 assays	-	-	-	-	-	35	Positive	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Quinine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.022	1.026	0.970	1.014	39	1	1	0.040	0.040	0.067	0.044	25	
	2	1.032	1.029	0.976	1.017	43		2	0.038	0.040	0.064	0.041	24	
	3	1.029	1.025	0.968	1.011	48		3	0.041	0.043	0.064	0.045	21	
	Mean	1.028	1.027	0.971	1.014	43		Mean	0.040	0.041	0.065	0.043	23	
2	1	1.049	1.054	0.978	1.046	64	2	1	0.036	0.037	0.070	0.040	30	
	2	1.046	1.055	0.977	1.048	62		2	0.037	0.037	0.069	0.040	30	
	3	1.054	1.057	0.984	1.049	63		3	0.036	0.037	0.069	0.040	30	
	Mean	1.049	1.055	0.980	1.048	63		Mean	0.036	0.037	0.069	0.040	30	
3	1	1.048	1.055	0.985	1.047	56	3	1	0.037	0.037	0.068	0.041	27	
	2	1.048	1.050	0.985	1.042	55		2	0.037	0.037	0.067	0.041	25	
	3	1.053	1.058	0.984	1.050	61		3	0.038	0.038	0.067	0.041	26	
	Mean	1.050	1.054	0.984	1.046	57		Mean	0.037	0.037	0.067	0.041	26	
Mean for 3 assays		-	-	-	-	54	Mean for 3 assays		-	-	-	-	26	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.017	1.042	0.749	1.027	253	1	1	0.038	0.039	0.222	0.043	181	
	2	1.029	1.043	0.756	1.025	258		2	0.038	0.038	0.191	0.042	150	
	3	1.016	1.039	0.760	1.027	241		3	0.038	0.039	0.199	0.042	157	
	Mean	1.021	1.041	0.755	1.026	251		Mean	0.038	0.039	0.204	0.042	163	
2	1	1.030	1.060	0.748	1.053	276	2	1	0.037	0.037	0.192	0.039	152	
	2	1.023	1.053	0.749	1.047	267		2	0.038	0.037	0.183	0.039	142	
	3	1.026	1.052	0.757	1.046	263		3	0.037	0.039	0.193	0.041	154	
	Mean	1.026	1.055	0.751	1.049	269		Mean	0.037	0.037	0.189	0.040	149	
3	1	1.039	1.019	0.654	1.008	374	3	1	0.038	0.037	0.225	0.041	185	
	2	1.033	1.021	0.667	1.010	355		2	0.037	0.037	0.227	0.040	187	
	3	1.041	1.018	0.669	1.008	361		3	0.037	0.039	0.237	0.042	197	
	Mean	1.037	1.019	0.663	1.008	363		Mean	0.037	0.038	0.230	0.041	190	
Mean for 3 assays		-	-	-	-	294	Mean for 3 assays		-	-	-	-	167	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 4 Individual data of Phase 1-1 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.037	1.026	1.029	1.014	-5	1	1	0.039	0.039	0.040	0.043	-1	
	2	1.035	1.029	1.025	1.017	-3		2	0.039	0.038	0.041	0.042	-1	
	3	1.035	1.025	1.030	1.011	-8		3	0.039	0.039	0.041	0.042	0	
	Mean	1.036	1.027	1.028	1.014	-5		Mean	0.039	0.039	0.041	0.042	-1	
2	1	1.064	1.047	1.052	1.039	4	2	1	0.037	0.038	0.038	0.042	-2	
	2	1.057	1.040	1.046	1.033	4		2	0.038	0.037	0.039	0.041	-2	
	3	1.064	1.049	1.053	1.041	4		3	0.038	0.038	0.039	0.041	-2	
	Mean	1.061	1.045	1.050	1.038	4		Mean	0.038	0.038	0.038	0.041	-2	
3	1	1.054	1.055	1.046	1.047	0	3	1	0.038	0.037	0.039	0.041	-2	
	2	1.053	1.050	1.045	1.042	0		2	0.038	0.037	0.040	0.041	-2	
	3	1.061	1.058	1.052	1.050	1		3	0.038	0.038	0.039	0.041	-3	
	Mean	1.056	1.054	1.048	1.046	0		Mean	0.038	0.037	0.039	0.041	-2	
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	-	-2		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.042	1.042	1.033	1.027	-6	1	1	0.040	0.038	0.041	0.043	-1	
	2	1.033	1.043	1.023	1.025	-5		2	0.039	0.041	0.040	0.042	-1	
	3	1.051	1.039	1.040	1.027	-4		3	0.039	0.041	0.040	0.042	-1	
	Mean	1.042	1.041	1.032	1.026	-5		Mean	0.039	0.040	0.040	0.042	-1	
2	1	1.060	1.060	1.052	1.053	2	2	1	0.038	0.037	0.038	0.039	-2	
	2	1.053	1.053	1.046	1.047	1		2	0.038	0.037	0.039	0.039	-2	
	3	1.055	1.052	1.048	1.046	0		3	0.038	0.039	0.039	0.041	-2	
	Mean	1.056	1.055	1.049	1.049	1		Mean	0.038	0.037	0.039	0.040	-2	
3	1	1.051	1.019	1.041	1.008	-1	3	1	0.038	0.037	0.038	0.041	-3	
	2	1.055	1.021	1.045	1.010	-1		2	0.038	0.037	0.039	0.040	-2	
	3	1.056	1.018	1.047	1.008	-1		3	0.038	0.039	0.038	0.042	-3	
	Mean	1.054	1.019	1.044	1.008	-1		Mean	0.038	0.038	0.039	0.041	-3	
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	-	-2		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <20

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	0.998	1.003	0.613	0.998	384	1	1	0.031	0.032	0.275	0.040	234
	2	0.990	0.999	0.611	0.997	378		2	0.032	0.034	0.275	0.045	233
	3	0.987	1.003	0.620	1.007	366		3	0.032	0.032	0.306	0.045	264
	Mean	0.992	1.002	0.615	1.001	376		Mean	0.032	0.033	0.285	0.043	244
2	1	0.986	1.000	0.638	0.995	343	2	1	0.032	0.033	0.268	0.068	200
	2	0.987	1.000	0.647	0.997	335		2	0.032	0.031	0.267	0.064	199
	3	0.989	0.999	0.650	0.994	334		3	0.032	0.031	0.289	0.072	221
	Mean	0.987	1.000	0.645	0.995	337		Mean	0.032	0.032	0.275	0.068	207
3	1	1.004	0.997	0.605	0.991	395	3	1	0.033	0.031	0.256	0.041	213
	2	0.990	1.005	0.608	1.001	378		2	0.032	0.031	0.266	0.043	224
	3	0.989	1.004	0.619	1.001	366		3	0.033	0.031	0.284	0.039	241
	Mean	0.994	1.002	0.611	0.998	380		Mean	0.033	0.031	0.269	0.041	226
4	1	0.990	0.997	0.633	0.993	353	4	1	0.034	0.032	0.295	0.037	255
	2	0.984	0.998	0.632	0.994	348		2	0.033	0.031	0.291	0.039	252
	3	0.992	1.000	0.645	0.995	343		3	0.033	0.032	0.317	0.037	278
	Mean	0.989	0.998	0.637	0.994	348		Mean	0.033	0.032	0.301	0.038	262
5	1	0.984	0.998	0.612	0.996	369	5	1	0.037	0.033	0.268	0.041	222
	2	0.980	1.008	0.615	1.005	362		2	0.034	0.032	0.260	0.042	217
	3	0.982	1.006	0.619	1.001	360		3	0.032	0.032	0.278	0.039	237
	Mean	0.982	1.004	0.615	1.001	364		Mean	0.034	0.032	0.269	0.041	225
6	1	0.988	0.992	0.605	0.989	380	6	1	0.032	0.031	0.263	0.040	222
	2	0.983	1.000	0.607	0.996	373		2	0.032	0.031	0.261	0.040	220
	3	0.986	0.996	0.613	0.993	370		3	0.032	0.032	0.285	0.041	244
	Mean	0.986	0.996	0.608	0.993	374		Mean	0.032	0.031	0.270	0.040	229

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.990	1.000	0.610	0.996	377	7	1	0.033	0.032	0.272	0.040	229
	2	0.985	1.002	0.612	0.999	370		2	0.032	0.031	0.260	0.041	218
	3	0.989	1.004	0.616	1.001	370		3	0.033	0.032	0.290	0.044	247
	Mean	0.988	1.002	0.613	0.999	372		Mean	0.033	0.032	0.274	0.042	231
8	1	0.999	0.994	0.612	0.989	383	8	1	0.033	0.032	0.274	0.042	230
	2	0.987	0.998	0.611	0.993	372		2	0.033	0.031	0.274	0.042	230
	3	0.993	1.003	0.615	0.999	374		3	0.032	0.032	0.298	0.044	255
	Mean	0.993	0.998	0.613	0.994	376		Mean	0.033	0.032	0.282	0.043	238
Mean for all assays		-	-	-	-	366	Mean for all assays		-	-	-	-	233
SD for all assays		-	-	-	-	15	SD for all assays		-	-	-	-	16
CV for all assays		-	-	-	-	4.1	CV for all assays		-	-	-	-	6.9

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.004	1.003	0.999	0.998	4	1	1	0.044	0.032	0.052	0.040	-2
	2	1.003	0.999	1.000	0.997	2		2	0.045	0.034	0.044	0.045	-11
	3	1.003	1.003	0.998	1.007	4		3	0.046	0.032	0.056	0.045	0
	Mean	1.003	1.002	0.999	1.001	3		Mean	0.045	0.033	0.051	0.043	-4
2	1	1.001	1.000	0.997	0.995	-1	2	1	0.046	0.033	0.055	0.068	-27
	2	1.000	1.000	0.997	0.997	-2		2	0.047	0.031	0.045	0.064	-38
	3	1.002	0.999	0.999	0.994	-2		3	0.047	0.031	0.047	0.072	-36
	Mean	1.001	1.000	0.998	0.995	-2		Mean	0.047	0.032	0.049	0.068	-34
3	1	1.002	0.997	0.997	0.991	1	3	1	0.048	0.031	0.049	0.041	-9
	2	1.004	1.005	1.003	1.001	-3		2	0.043	0.031	0.044	0.043	-9
	3	1.002	1.004	0.998	1.001	0		3	0.064	0.031	0.048	0.039	-26
	Mean	1.003	1.002	0.999	0.998	-1		Mean	0.052	0.031	0.047	0.041	-15
4	1	0.999	0.997	0.996	0.993	-1	4	1	0.046	0.032	0.044	0.037	-8
	2	1.000	0.998	0.995	0.994	1		2	0.044	0.031	0.041	0.039	-9
	3	0.998	1.000	0.993	0.995	1		3	0.041	0.032	0.042	0.037	-5
	Mean	0.999	0.998	0.995	0.994	0		Mean	0.044	0.032	0.042	0.038	-7
5	1	1.000	0.998	0.994	0.996	3	5	1	0.051	0.033	0.053	0.041	-7
	2	0.997	1.008	0.993	1.005	1		2	0.047	0.032	0.044	0.042	-12
	3	0.996	1.006	0.991	1.001	2		3	0.051	0.032	0.055	0.039	-5
	Mean	0.998	1.004	0.993	1.001	2		Mean	0.050	0.032	0.051	0.041	-8
6	1	1.001	0.992	0.996	0.989	2	6	1	0.046	0.031	0.048	0.040	-7
	2	1.004	1.000	0.997	0.996	4		2	0.049	0.031	0.046	0.040	-12
	3	1.004	0.996	1.000	0.993	1		3	0.040	0.032	0.047	0.041	-2
	Mean	1.003	0.996	0.998	0.993	2		Mean	0.045	0.031	0.047	0.040	-7

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	1.001	1.000	0.999	0.996	-1	7	1	0.043	0.032	0.047	0.040	-6
	2	0.999	1.002	0.998	0.999	-2		2	0.045	0.031	0.049	0.041	-6
	3	1.003	1.004	0.999	1.001	1		3	0.040	0.032	0.045	0.044	-5
	Mean	1.001	1.002	0.999	0.999	-1		Mean	0.043	0.032	0.047	0.042	-6
8	1	1.001	0.994	0.998	0.989	-1	8	1	0.042	0.032	0.049	0.042	-4
	2	0.999	0.998	0.993	0.993	2		2	0.047	0.031	0.049	0.042	-9
	3	0.997	1.003	0.994	0.999	-1		3	0.039	0.032	0.051	0.044	1
	Mean	0.999	0.998	0.995	0.994	0		Mean	0.043	0.032	0.050	0.043	-4
Mean for all assays		-	-	-	-	0	Mean for all assays		-	-	-	-	-11
SD for all assays		-	-	-	-	2	SD for all assays		-	-	-	-	10
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.013	1.031	0.618	1.022	388	1	1	0.035	0.035	0.291	0.038	253
	2	1.019	1.028	0.656	1.021	356		2	0.035	0.035	0.283	0.038	245
	3	1.019	1.036	0.678	1.029	334		3	0.036	0.035	0.283	0.038	244
	Mean	1.017	1.031	0.651	1.024	359		Mean	0.035	0.035	0.286	0.038	247
2	1	0.999	1.014	0.587	1.008	409	2	1	0.035	0.035	0.315	0.039	276
	2	1.002	1.018	0.620	1.013	378		2	0.035	0.035	0.294	0.038	255
	3	1.002	1.014	0.641	1.011	357		3	0.036	0.035	0.297	0.038	257
	Mean	1.001	1.015	0.616	1.011	381		Mean	0.035	0.035	0.302	0.039	263
3	1	0.991	1.003	0.582	0.995	402	3	1	0.036	0.036	0.335	0.038	297
	2	0.991	1.003	0.604	0.996	380		2	0.036	0.037	0.317	0.039	278
	3	0.994	1.002	0.622	0.997	365		3	0.036	0.035	0.352	0.038	312
	Mean	0.992	1.003	0.603	0.996	382		Mean	0.036	0.036	0.335	0.039	296
4	1	0.995	1.008	0.577	1.001	411	4	1	0.036	0.036	0.317	0.038	279
	2	0.997	1.007	0.606	1.001	384		2	0.036	0.036	0.314	0.038	276
	3	0.999	1.012	0.627	1.005	366		3	0.037	0.036	0.318	0.038	280
	Mean	0.997	1.009	0.603	1.002	387		Mean	0.036	0.036	0.316	0.038	278
5	1	0.989	1.006	0.557	0.953	383	5	1	0.035	0.035	0.258	0.037	221
	2	0.991	1.006	0.583	0.950	358		2	0.048	0.035	0.331	0.038	281
	3	0.989	1.001	0.605	0.958	334		3	0.036	0.035	0.268	0.037	231
	Mean	0.990	1.004	0.582	0.954	358		Mean	0.040	0.035	0.286	0.037	244
6	1	0.977	0.998	0.571	0.993	400	6	1	0.035	0.035	0.304	0.037	266
	2	0.985	1.004	0.600	0.999	380		2	0.035	0.035	0.299	0.038	260
	3	0.984	0.999	0.614	0.994	364		3	0.036	0.035	0.303	0.038	264
	Mean	0.982	1.001	0.595	0.995	381		Mean	0.035	0.035	0.302	0.038	263

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.976	0.991	0.616	0.985	354	7	1	0.035	0.035	0.289	0.038	251
	2	0.977	0.992	0.638	0.986	334		2	0.035	0.036	0.283	0.038	245
	3	0.977	0.991	0.648	0.985	323		3	0.036	0.035	0.312	0.038	273
	Mean	0.977	0.991	0.634	0.985	337		Mean	0.035	0.035	0.294	0.038	256
8	1	0.994	0.996	0.593	0.985	392	8	1	0.035	0.035	0.316	0.037	278
	2	0.994	0.992	0.599	0.984	386		2	0.035	0.036	0.313	0.038	275
	3	0.994	0.991	0.600	0.985	386		3	0.036	0.035	0.316	0.038	277
	Mean	0.994	0.993	0.597	0.984	388		Mean	0.036	0.035	0.315	0.038	277
Mean for all assays		-	-	-	-	372	Mean for all assays		-	-	-	-	266
SD for all assays		-	-	-	-	18	SD for all assays		-	-	-	-	17
CV for all assays		-	-	-	-	4.8	CV for all assays		-	-	-	-	6.4

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.029	1.031	1.021	1.022	1	1	1	0.036	0.035	0.036	0.038	-3
	2	1.032	1.028	1.026	1.021	-1		2	0.035	0.035	0.036	0.038	-3
	3	1.033	1.036	1.027	1.029	-1		3	0.036	0.035	0.037	0.038	-3
	Mean	1.031	1.031	1.025	1.024	0		Mean	0.036	0.035	0.036	0.038	-3
2	1	1.017	1.014	1.012	1.008	1	2	1	0.036	0.035	0.036	0.039	-3
	2	1.019	1.018	1.013	1.013	1		2	0.035	0.035	0.036	0.038	-3
	3	1.017	1.014	1.012	1.011	0		3	0.037	0.035	0.037	0.038	-4
	Mean	1.017	1.015	1.012	1.011	1		Mean	0.036	0.035	0.036	0.039	-3
3	1	1.002	1.003	0.993	0.995	2	3	1	0.037	0.036	0.037	0.038	-3
	2	1.001	1.003	0.992	0.996	1		2	0.036	0.037	0.036	0.039	-3
	3	1.001	1.002	0.993	0.997	1		3	0.037	0.035	0.037	0.038	-3
	Mean	1.001	1.003	0.993	0.996	1		Mean	0.037	0.036	0.037	0.039	-3
4	1	1.010	1.008	1.003	1.001	0	4	1	0.037	0.036	0.037	0.038	-2
	2	1.013	1.007	0.999	1.001	7		2	0.037	0.036	0.037	0.038	-2
	3	1.012	1.012	0.998	1.005	7		3	0.037	0.036	0.038	0.038	-2
	Mean	1.012	1.009	1.000	1.002	5		Mean	0.037	0.036	0.037	0.038	-2
5	1	1.003	1.006	0.956	0.953	-3	5	1	0.036	0.035	0.037	0.037	-2
	2	1.011	1.006	0.972	0.950	-11		2	0.036	0.035	0.045	0.038	7
	3	1.005	1.001	0.964	0.958	-10		3	0.036	0.035	0.037	0.037	-2
	Mean	1.007	1.004	0.964	0.954	-8		Mean	0.036	0.035	0.039	0.037	1
6	1	0.997	0.998	0.991	0.993	-1	6	1	0.036	0.035	0.036	0.037	-3
	2	0.991	1.004	0.984	0.999	1		2	0.036	0.035	0.036	0.038	-3
	3	0.995	0.999	0.988	0.994	1		3	0.036	0.035	0.037	0.038	-3
	Mean	0.994	1.001	0.988	0.995	0		Mean	0.036	0.035	0.036	0.038	-3

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.992	0.991	0.982	0.985	4	7	1	0.036	0.035	0.036	0.038	-3
	2	0.986	0.992	0.979	0.986	1		2	0.035	0.036	0.036	0.038	-2
	3	0.987	0.991	0.980	0.985	1		3	0.036	0.035	0.037	0.038	-3
	Mean	0.988	0.991	0.980	0.985	2		Mean	0.036	0.035	0.036	0.038	-3
8	1	0.979	0.996	0.973	0.985	-3	8	1	0.035	0.035	0.037	0.037	-1
	2	0.959	0.992	0.951	0.984	-1		2	0.035	0.036	0.037	0.038	-1
	3	0.999	0.991	0.993	0.985	-3		3	0.036	0.035	0.038	0.038	0
	Mean	0.979	0.993	0.972	0.984	-2		Mean	0.035	0.035	0.037	0.038	-1
Mean for all assays		-	-	-	-	0	Mean for all assays		-	-	-	-	-2
SD for all assays		-	-	-	-	4	SD for all assays		-	-	-	-	1
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	0.968	0.972	0.527	0.959	429	1	1	0.034	0.034	0.366	0.038	328
	2	0.965	0.998	0.534	0.988	419		2	0.033	0.033	0.362	0.037	325
	3	0.969	0.979	0.540	0.967	417		3	0.050	0.032	0.397	0.036	343
	Mean	0.967	0.983	0.534	0.971	422		Mean	0.039	0.033	0.375	0.037	332
2	1	0.961	0.986	0.501	0.979	452	2	1	0.039	0.034	0.381	0.038	338
	2	0.962	0.998	0.509	0.990	445		2	0.033	0.033	0.381	0.037	344
	3	0.968	0.995	0.516	0.986	444		3	0.034	0.032	0.414	0.036	376
	Mean	0.964	0.993	0.509	0.985	447		Mean	0.035	0.033	0.392	0.037	353
3	1	0.968	0.981	0.532	0.973	428	3	1	0.034	0.034	0.320	0.049	271
	2	0.973	0.986	0.551	0.977	414		2	0.034	0.034	0.318	0.048	269
	3	0.975	0.988	0.552	0.980	415		3	0.034	0.032	0.347	0.048	298
	Mean	0.972	0.985	0.545	0.977	419		Mean	0.034	0.033	0.328	0.048	279
4	1	0.964	0.978	0.559	0.970	397	4	1	0.034	0.034	0.311	0.049	262
	2	0.967	0.979	0.569	0.972	390		2	0.033	0.033	0.294	0.047	246
	3	0.968	0.982	0.578	0.974	382		3	0.034	0.032	0.335	0.047	286
	Mean	0.966	0.980	0.569	0.972	390		Mean	0.034	0.033	0.313	0.048	265
5	1	0.970	0.972	0.546	0.964	415	5	1	0.034	0.035	0.317	0.047	272
	2	0.966	0.980	0.550	0.971	407		2	0.033	0.037	0.311	0.046	267
	3	0.967	0.984	0.559	0.975	399		3	0.035	0.033	0.344	0.046	298
	Mean	0.968	0.979	0.552	0.970	407		Mean	0.034	0.035	0.324	0.046	279
6	1	0.967	0.984	0.571	0.977	389	6	1	0.034	0.034	0.281	0.042	240
	2	0.964	0.978	0.569	0.972	388		2	0.032	0.034	0.285	0.041	246
	3	0.968	0.983	0.575	0.976	386		3	0.034	0.033	0.301	0.040	260
	Mean	0.966	0.982	0.572	0.975	388		Mean	0.033	0.034	0.289	0.041	249

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.971	0.982	0.558	0.974	398	7	1	0.038	0.034	0.301	0.048	249
	2	0.970	0.984	0.567	0.976	388		2	0.033	0.034	0.290	0.046	243
	3	0.973	0.967	0.575	0.938	383		3	0.034	0.032	0.335	0.047	287
	Mean	0.971	0.978	0.567	0.963	390		Mean	0.035	0.033	0.309	0.047	260
8	1	0.961	0.972	0.549	0.965	405	8	1	0.034	0.034	0.321	0.050	272
	2	0.966	0.977	0.560	0.970	399		2	0.033	0.034	0.316	0.049	268
	3	0.972	0.981	0.566	0.974	399		3	0.034	0.033	0.345	0.049	296
	Mean	0.966	0.977	0.558	0.970	401		Mean	0.034	0.034	0.327	0.049	279
Mean for all assays		-	-	-	-	408	Mean for all assays		-	-	-	-	287
SD for all assays		-	-	-	-	20	SD for all assays		-	-	-	-	36
CV for all assays		-	-	-	-	4.9	CV for all assays		-	-	-	-	12.5

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	0.983	0.972	0.975	0.959	-4	1	1	0.035	0.034	0.035	0.038	-4
	2	0.977	0.998	0.973	0.988	-8		2	0.033	0.033	0.034	0.037	-3
	3	0.981	0.979	0.976	0.967	-7		3	0.035	0.032	0.036	0.036	-3
	Mean	0.980	0.983	0.975	0.971	-6		Mean	0.034	0.033	0.035	0.037	-3
2	1	0.992	0.986	0.984	0.979	0	2	1	0.035	0.034	0.036	0.038	-3
	2	0.981	0.998	0.974	0.990	-1		2	0.034	0.033	0.035	0.037	-3
	3	0.999	0.995	0.991	0.986	0		3	0.036	0.032	0.039	0.036	-1
	Mean	0.991	0.993	0.983	0.985	0		Mean	0.035	0.033	0.037	0.037	-2
3	1	0.988	0.981	0.982	0.973	-2	3	1	0.035	0.034	0.037	0.049	-13
	2	0.984	0.986	0.978	0.977	-2		2	0.034	0.034	0.035	0.048	-14
	3	0.989	0.988	0.983	0.980	-2		3	0.035	0.032	0.036	0.048	-14
	Mean	0.987	0.985	0.981	0.977	-2		Mean	0.035	0.033	0.036	0.048	-14
4	1	0.988	0.978	0.981	0.970	-1	4	1	0.035	0.034	0.038	0.049	-12
	2	0.979	0.979	0.974	0.972	-3		2	0.033	0.033	0.035	0.047	-13
	3	0.981	0.982	0.975	0.974	-2		3	0.035	0.032	0.037	0.047	-13
	Mean	0.983	0.980	0.977	0.972	-2		Mean	0.034	0.033	0.037	0.048	-13
5	1	0.988	0.972	0.980	0.964	-1	5	1	0.036	0.035	0.036	0.047	-11
	2	0.979	0.980	0.972	0.971	-2		2	0.034	0.037	0.035	0.046	-10
	3	0.983	0.984	0.975	0.975	-1		3	0.035	0.033	0.036	0.046	-10
	Mean	0.983	0.979	0.976	0.970	-1		Mean	0.035	0.035	0.036	0.046	-10
6	1	0.983	0.984	0.976	0.977	0	6	1	0.035	0.034	0.036	0.042	-6
	2	0.983	0.978	0.975	0.972	1		2	0.034	0.034	0.035	0.041	-6
	3	0.983	0.983	0.976	0.976	0		3	0.035	0.033	0.036	0.040	-6
	Mean	0.983	0.982	0.976	0.975	0		Mean	0.035	0.034	0.036	0.041	-6

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 5 Positive control and negative control data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.993	0.982	0.985	0.974	-7	7	1	0.044	0.034	0.037	0.048	-21
	2	0.984	0.984	0.978	0.976	-9		2	0.036	0.034	0.035	0.046	-15
	3	0.987	0.967	0.982	0.938	-10		3	0.035	0.032	0.037	0.047	-12
	Mean	0.988	0.978	0.982	0.963	-9		Mean	0.038	0.033	0.036	0.047	-16
8	1	0.978	0.972	0.969	0.965	2	8	1	0.035	0.034	0.036	0.050	-14
	2	0.977	0.977	0.970	0.970	0		2	0.033	0.034	0.034	0.049	-14
	3	0.979	0.981	0.974	0.974	-2		3	0.052	0.033	0.065	0.049	-2
	Mean	0.978	0.977	0.971	0.970	0		Mean	0.040	0.034	0.045	0.049	-10
Mean for all assays		-	-	-	-	-3	Mean for all assays		-	-	-	-	-9
SD for all assays		-	-	-	-	3	SD for all assays		-	-	-	-	5
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.995	0.997	0.987	0.991	4	1	1	0.031	0.031	0.054	0.041	13	Negative
	2	0.997	1.005	0.995	1.001	-2		2	0.031	0.031	0.039	0.043	-2	
	3	0.999	1.004	0.993	1.001	2		3	0.031	0.031	0.042	0.039	1	
	Mean	0.997	1.002	0.992	0.998	1		Mean	0.031	0.031	0.045	0.041	4	
2	1	0.997	1.000	0.994	0.996	0	2	1	0.034	0.032	0.047	0.040	3	Negative
	2	0.997	1.002	0.993	0.999	1		2	0.035	0.031	0.043	0.041	-2	
	3	0.996	1.004	0.991	1.001	2		3	0.031	0.032	0.056	0.044	15	
	Mean	0.997	1.002	0.993	0.999	1		Mean	0.033	0.032	0.049	0.042	5	
3							3							
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	5	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.999	1.003	0.998	0.998	0	1	1	0.032	0.032	0.042	0.040	0	Negative
	2	1.002	0.999	0.998	0.997	3		2	0.032	0.034	0.046	0.045	4	
	3	0.999	1.003	0.997	1.007	1		3	0.032	0.032	0.053	0.045	11	
	Mean	1.000	1.002	0.998	1.001	1		Mean	0.032	0.033	0.047	0.043	5	
2	1	0.990	0.998	0.987	0.996	0	2	1	0.032	0.033	0.047	0.041	6	Negative
	2	0.988	1.008	0.982	1.005	3		2	0.031	0.032	0.045	0.042	5	
	3	1.003	1.006	0.997	1.001	3		3	0.032	0.032	0.046	0.039	5	
	Mean	0.994	1.004	0.989	1.001	2		Mean	0.032	0.032	0.046	0.041	5	
3							3							
Mean for 3 assays		-	-	-	-	2	Mean for 3 assays		-	-	-	-	5	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)		Results* ²	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	0.991	0.997	0.980	0.991	7	1	1	0.032	0.031	0.059	0.041	17	Positive
	2	1.001	1.005	0.990	1.001	7		2	0.031	0.031	0.062	0.043	21	
	3	0.999	1.004	0.984	1.001	11		3	0.035	0.031	0.082	0.039	37	
	Mean	0.997	1.002	0.985	0.998	8		Mean	0.033	0.031	0.068	0.041	25	
2	1	0.989	1.000	0.976	0.996	10	2	1	0.032	0.032	0.049	0.040	7	Negative
	2	0.992	1.002	0.978	0.999	11		2	0.031	0.031	0.048	0.041	7	
	3	0.991	1.004	0.980	1.001	8		3	0.032	0.032	0.049	0.044	7	
	Mean	0.991	1.002	0.978	0.999	10		Mean	0.032	0.032	0.049	0.042	7	
3							3							
Mean for 3 assays		-	-	-	-	9	Mean for 3 assays		-	-	-	-	16	

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)		Results* ²	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	0.985	1.003	0.889	0.998	95	1	1	0.032	0.032	0.094	0.040	52	Positive
	2	0.987	0.999	0.893	0.997	93		2	0.032	0.034	0.122	0.045	80	
	3	0.988	1.003	0.909	1.007	78		3	0.032	0.032	0.116	0.045	74	
	Mean	0.987	1.002	0.897	1.001	89		Mean	0.032	0.033	0.111	0.043	69	
2	1	0.980	0.998	0.884	0.996	93	2	1	0.032	0.033	0.103	0.041	62	Positive
	2	0.981	1.008	0.918	1.005	60		2	0.032	0.032	0.133	0.042	92	
	3	0.979	1.006	0.900	1.001	76		3	0.036	0.032	0.110	0.039	65	
	Mean	0.980	1.004	0.901	1.001	76		Mean	0.033	0.032	0.115	0.041	73	
3							3							
Mean for 3 assays		-	-	-	-	83	Mean for 3 assays		-	-	-	-	71	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Amiodarone HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)		Results* ²	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.115	0.997	0.982	0.991	129	1	1	0.065	0.031	0.108	0.041	33	Positive
	2	1.051	1.005	0.981	1.001	66		2	0.066	0.031	0.108	0.043	32	
	3	1.047	1.004	0.981	1.001	62		3	0.065	0.031	0.101	0.039	26	
	Mean	1.071	1.002	0.981	0.998	86		Mean	0.065	0.031	0.106	0.041	30	
2	1	1.048	1.000	1.031	0.996	14	2	1	0.067	0.032	0.097	0.040	20	Positive
	2	1.050	1.002	0.991	0.999	56		2	0.072	0.031	0.094	0.041	12	
	3	1.047	1.004	0.984	1.001	60		3	0.069	0.032	0.094	0.044	15	
	Mean	1.048	1.002	1.002	0.999	43		Mean	0.069	0.032	0.095	0.042	16	
3	1						3							Positive
	2							2						
	3							3						
	Mean							Mean						
Mean for 3 assays		-	-	-	-	65	Mean for 3 assays		-	-	-	-	23	

Test concentration 200 µM

Singlet oxygen							Superoxide anion					Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)		Results* ²	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank
1	1	1.882	1.003	1.420	0.998	461	1	1	0.513	0.032	0.732	0.040	209	Positive
	2	1.852	0.999	1.443	0.997	408		2	0.513	0.034	0.716	0.045	193	
	3	1.854	1.003	1.522	1.007	331		3	0.510	0.032	0.694	0.045	174	
	Mean	1.863	1.002	1.462	1.001	400		Mean	0.512	0.033	0.714	0.043	192	
2	1	1.756	0.998	1.306	0.996	447	2	1	0.358	0.033	0.630	0.041	263	Positive
	2	1.690	1.008	1.316	1.005	371		2	0.358	0.032	0.622	0.042	255	
	3	1.693	1.006	1.392	1.001	298		3	0.357	0.032	0.607	0.039	241	
	Mean	1.713	1.004	1.338	1.001	372		Mean	0.358	0.032	0.620	0.041	253	
3	1						3							Positive
	2							2						
	3							3						
	Mean							Mean						
Mean for 3 assays		-	-	-	-	386	Mean for 3 assays		-	-	-	-	223	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (B-A) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Chlorpromazine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results *1	Run	Run#	A560(-)		A560(+)			Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.989	0.997	0.891	0.991	94	1	1	0.033	0.031	0.068	0.041	25	
	2	0.985	1.005	0.892	1.001	89		2	0.032	0.031	0.081	0.043	39	
	3	0.986	1.004	0.904	1.001	78		3	0.034	0.031	0.070	0.039	26	
	Mean	0.987	1.002	0.896	0.998	87		Mean	0.033	0.031	0.073	0.041	30	
2	1	0.991	1.000	0.893	0.996	95	2	1	0.033	0.032	0.074	0.040	31	
	2	0.986	1.002	0.895	0.999	88		2	0.033	0.031	0.068	0.041	25	
	3	0.984	1.004	0.889	1.001	92		3	0.032	0.032	0.068	0.044	26	
	Mean	0.987	1.002	0.892	0.999	92		Mean	0.033	0.032	0.070	0.042	27	
3							3							
Mean for 3 assays		-	-	-	-	90	Mean for 3 assays		-	-	-	-	29	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results *1	Run	Run#	A560(-)		A560(+)			Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.976	1.003	0.946	0.998	29	1	1	0.037	0.032	0.132	0.040	85	
	2	0.969	0.999	0.938	0.997	30		2	0.036	0.034	0.136	0.045	90	
	3	0.973	1.003	0.939	1.007	33		3	0.037	0.032	0.138	0.045	91	
	Mean	0.973	1.002	0.941	1.001	31		Mean	0.037	0.033	0.135	0.043	89	
2	1	0.977	0.998	0.946	0.996	28	2	1	0.035	0.033	0.124	0.041	80	
	2	0.975	1.008	0.946	1.005	26		2	0.036	0.032	0.128	0.042	83	
	3	0.975	1.006	0.941	1.001	31		3	0.036	0.032	0.134	0.039	89	
	Mean	0.976	1.004	0.944	1.001	28		Mean	0.036	0.032	0.129	0.041	84	
3							3							
Mean for 3 assays		-	-	-	-	30	Mean for 3 assays		-	-	-	-	87	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results *1	Run	Run#	A560(-)		A560(+)			Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.000	0.997	0.974	0.991	22	1	1	0.049	0.031	0.075	0.041	16	
	2	0.996	1.005	0.960	1.001	32		2	0.046	0.031	0.073	0.043	17	
	3	0.997	1.004	0.961	1.001	32		3	0.046	0.031	0.067	0.039	11	
	Mean	0.998	1.002	0.965	0.998	29		Mean	0.047	0.031	0.072	0.041	15	
2	1	1.005	1.000	0.978	0.996	24	2	1	0.046	0.032	0.069	0.040	13	
	2	0.994	1.002	0.965	0.999	26		2	0.042	0.031	0.073	0.041	21	
	3	0.997	1.004	0.964	1.001	30		3	0.048	0.032	0.076	0.044	18	
	Mean	0.999	1.002	0.969	0.999	27		Mean	0.045	0.032	0.073	0.042	17	
3							3							
Mean for 3 assays		-	-	-	-	28	Mean for 3 assays		-	-	-	-	16	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results *1	Run	Run#	A560(-)		A560(+)			Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.995	1.003	0.883	0.998	111	1	1	0.044	0.032	0.193	0.040	139	
	2	0.994	0.999	0.875	0.997	118		2	0.044	0.034	0.196	0.045	142	
	3	0.993	1.003	0.868	1.007	124		3	0.052	0.032	0.202	0.045	140	
	Mean	0.994	1.002	0.875	1.001	118		Mean	0.047	0.033	0.197	0.043	140	
2	1	0.997	0.998	0.873	0.996	121	2	1	0.046	0.033	0.188	0.041	133	
	2	0.996	1.008	0.872	1.005	121		2	0.048	0.032	0.191	0.042	134	
	3	1.000	1.006	0.874	1.001	123		3	0.050	0.032	0.197	0.039	138	
	Mean	0.998	1.004	0.873	1.001	122		Mean	0.048	0.032	0.192	0.041	135	
3							3							
Mean for 3 assays		-	-	-	-	120	Mean for 3 assays		-	-	-	-	138	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Doxycycline HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.996	0.997	0.916	0.991	76	1	1	0.035	0.031	0.105	0.041	60	Positive
	2	0.990	1.005	0.903	1.001	83		2	0.036	0.031	0.122	0.043	76	
	3	0.999	1.004	0.920	1.001	75		3	0.040	0.031	0.118	0.039	68	
	Mean	0.995	1.002	0.913	0.998	78		Mean	0.037	0.031	0.115	0.041	68	
2	1	0.998	1.000	0.912	0.996	83	2	1	0.041	0.032	0.114	0.040	63	Positive
	2	0.994	1.002	0.895	0.999	96		2	0.038	0.031	0.103	0.041	55	
	3	0.991	1.004	0.884	1.001	104		3	0.040	0.032	0.112	0.044	62	
	Mean	0.994	1.002	0.897	0.999	94		Mean	0.040	0.032	0.110	0.042	60	
3							3							
Mean for 3 assays		-	-	-	-	86	Mean for 3 assays		-	-	-	-	64	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.971	1.003	0.672	0.998	298	1	1	0.064	0.032	0.431	0.040	357	Positive
	2	0.966	0.999	0.670	0.997	295		2	0.052	0.034	0.417	0.045	355	
	3	0.972	1.003	0.673	1.007	298		3	0.050	0.032	0.418	0.045	358	
	Mean	0.970	1.002	0.672	1.001	297		Mean	0.055	0.033	0.422	0.043	357	
2	1	0.985	0.998	0.688	0.996	294	2	1	0.049	0.033	0.426	0.041	368	Positive
	2	0.988	1.008	0.681	1.005	304		2	0.053	0.032	0.433	0.042	371	
	3	0.982	1.006	0.678	1.001	301		3	0.061	0.032	0.440	0.039	370	
	Mean	0.985	1.004	0.682	1.001	300		Mean	0.054	0.032	0.433	0.041	370	
3							3							
Mean for 3 assays		-	-	-	-	299	Mean for 3 assays		-	-	-	-	364	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.994	0.997	0.977	0.991	13	1	1	0.046	0.031	0.066	0.041	10	Negative
	2	0.999	1.005	0.975	1.001	20		2	0.060	0.031	0.061	0.043	-9	
	3	1.002	1.004	0.980	1.001	18		3	0.067	0.031	0.059	0.039	-18	
	Mean	0.998	1.002	0.977	0.998	17		Mean	0.058	0.031	0.062	0.041	-6	
2	1	0.995	1.000	0.978	0.996	14	2	1	0.040	0.032	0.063	0.040	13	Negative
	2	0.993	1.002	0.970	0.999	20		2	0.046	0.031	0.050	0.041	-6	
	3	0.997	1.004	0.978	1.001	16		3	0.052	0.032	0.052	0.044	-10	
	Mean	0.995	1.002	0.975	0.999	17		Mean	0.046	0.032	0.055	0.042	-1	
3							3							
Mean for 3 assays		-	-	-	-	17	Mean for 3 assays		-	-	-	-	-4	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.001	1.003	0.932	0.998	68	1	1	0.042	0.032	0.083	0.040	31	Positive
	2	0.992	0.999	0.911	0.997	80		2	0.045	0.034	0.096	0.045	41	
	3	0.993	1.003	0.912	1.007	80		3	0.060	0.032	0.092	0.045	22	
	Mean	0.995	1.002	0.918	1.001	76		Mean	0.049	0.033	0.090	0.043	31	
2	1	0.995	0.998	0.926	0.996	66	2	1	0.041	0.033	0.086	0.041	36	Positive
	2	1.001	1.008	0.926	1.005	72		2	0.046	0.032	0.085	0.042	30	
	3	1.001	1.006	0.926	1.001	72		3	0.044	0.032	0.089	0.039	36	
	Mean	0.999	1.004	0.926	1.001	70		Mean	0.044	0.032	0.087	0.041	34	
3							3							
Mean for 3 assays		-	-	-	-	73	Mean for 3 assays		-	-	-	-	33	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.999	0.997	0.965	0.993	30	1	1	0.032	0.032	0.045	0.037	7	Positive
	2	0.994	0.998	0.963	0.994	27		2	0.033	0.031	0.045	0.039	6	
	3	1.002	1.000	0.966	0.995	32		3	0.034	0.032	0.061	0.037	21	
	Mean	0.998	0.998	0.965	0.994	30		Mean	0.033	0.032	0.050	0.038	11	
2	1	0.994	0.994	0.959	0.989	31	2	1	0.033	0.032	0.064	0.042	20	Positive
	2	0.991	0.998	0.953	0.993	34		2	0.033	0.031	0.049	0.042	5	
	3	0.992	1.003	0.954	0.999	34		3	0.031	0.032	0.053	0.044	11	
	Mean	0.992	0.998	0.955	0.994	33		Mean	0.032	0.032	0.055	0.043	12	
3							3							
Mean for 3 assays		-	-	-	-	32	Mean for 3 assays		-	-	-	-	12	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.999	1.000	0.810	0.995	184	1	1	0.041	0.033	0.153	0.068	76	Positive
	2	0.997	1.000	0.814	0.997	178		2	0.038	0.031	0.154	0.064	80	
	3	0.997	0.999	0.816	0.994	176		3	0.038	0.031	0.161	0.072	87	
	Mean	0.998	1.000	0.813	0.995	179		Mean	0.039	0.032	0.156	0.068	81	
2	1	0.993	0.992	0.816	0.989	174	2	1	0.036	0.031	0.159	0.040	114	Positive
	2	0.992	1.000	0.816	0.996	173		2	0.043	0.031	0.159	0.040	107	
	3	0.994	0.996	0.816	0.993	175		3	0.037	0.032	0.164	0.041	118	
	Mean	0.993	0.996	0.816	0.993	174		Mean	0.039	0.031	0.161	0.040	113	
3							3							
Mean for 3 assays		-	-	-	-	177	Mean for 3 assays		-	-	-	-	97	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.996	0.997	0.963	0.993	29	1	1	0.032	0.032	0.249	0.037	211	Positive
	2	1.000	0.998	0.969	0.994	27		2	0.032	0.031	0.234	0.039	196	
	3	0.995	1.000	0.964	0.995	27		3	0.032	0.032	0.258	0.037	220	
	Mean	0.997	0.998	0.965	0.994	28		Mean	0.032	0.032	0.247	0.038	209	
2	1	0.996	0.994	0.949	0.989	43	2	1	0.032	0.032	0.267	0.042	224	Positive
	2	0.995	0.998	0.948	0.993	43		2	0.033	0.031	0.261	0.042	217	
	3	0.993	1.003	0.948	0.999	41		3	0.033	0.032	0.271	0.044	227	
	Mean	0.995	0.998	0.948	0.994	42		Mean	0.033	0.032	0.266	0.043	223	
3							3							
Mean for 3 assays		-	-	-	-	35	Mean for 3 assays		-	-	-	-	216	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.995	1.000	0.839	0.995	151	1	1	0.034	0.033	0.448	0.068	378	Positive
	2	0.992	1.000	0.844	0.997	143		2	0.035	0.031	0.459	0.064	388	
	3	0.999	0.999	0.859	0.994	135		3	0.032	0.031	0.442	0.072	374	
	Mean	0.995	1.000	0.847	0.995	143		Mean	0.034	0.032	0.450	0.068	380	
2	1	0.988	0.992	0.815	0.989	170	2	1	0.032	0.031	0.375	0.040	334	Positive
	2	0.988	1.000	0.829	0.996	156		2	0.032	0.031	0.399	0.040	358	
	3	0.985	0.996	0.821	0.993	161		3	0.032	0.032	0.401	0.041	360	
	Mean	0.987	0.996	0.822	0.993	162		Mean	0.032	0.031	0.392	0.040	351	
3							3							
Mean for 3 assays		-	-	-	-	153	Mean for 3 assays		-	-	-	-	366	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.001	0.997	0.944	0.993	53	1	1	0.033	0.032	0.066	0.037	27	Positive
	2	0.995	0.998	0.943	0.994	48		2	0.032	0.031	0.059	0.039	21	
	3	1.002	1.000	0.947	0.995	51		3	0.037	0.032	0.061	0.037	18	
	Mean	0.999	0.998	0.945	0.994	51		Mean	0.034	0.032	0.062	0.038	22	
2	1	0.999	0.994	0.944	0.989	51	2	1	0.033	0.032	0.067	0.042	23	Positive
	2	0.991	0.998	0.934	0.993	53		2	0.032	0.031	0.066	0.042	23	
	3	0.991	1.003	0.938	0.999	49		3	0.033	0.032	0.069	0.044	25	
	Mean	0.994	0.998	0.939	0.994	51		Mean	0.033	0.032	0.067	0.043	24	
3							3							
Mean for 3 assays		-	-	-	-	51	Mean for 3 assays		-	-	-	-	23	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.003	1.000	0.803	0.995	195	1	1	0.034	0.033	0.162	0.068	92	Positive
	2	0.996	1.000	0.803	0.997	188		2	0.033	0.031	0.157	0.064	88	
	3	1.000	0.999	0.836	0.994	159		3	0.032	0.031	0.164	0.072	96	
	Mean	1.000	1.000	0.814	0.995	181		Mean	0.033	0.032	0.161	0.068	92	
2	1	0.993	0.992	0.789	0.989	201	2	1	0.034	0.031	0.162	0.040	119	Positive
	2	0.988	1.000	0.791	0.996	194		2	0.033	0.031	0.160	0.040	118	
	3	0.987	0.996	0.803	0.993	181		3	0.032	0.032	0.165	0.041	124	
	Mean	0.989	0.996	0.794	0.993	192		Mean	0.033	0.031	0.162	0.040	120	
3							3							
Mean for 3 assays		-	-	-	-	187	Mean for 3 assays		-	-	-	-	106	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results *1	Run	Run#	A560(-)		A560(+)			Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.999	0.997	0.952	0.993	43	1	1	0.031	0.032	0.096	0.037	59	Positive
	2	0.998	0.998	0.957	0.994	37		2	0.032	0.031	0.093	0.039	55	
	3	0.995	1.000	0.961	0.995	30		3	0.034	0.032	0.097	0.037	57	
	Mean	0.997	0.998	0.957	0.994	37		Mean	0.032	0.032	0.095	0.038	57	
2	1	0.998	0.994	0.949	0.989	45	2	1	0.034	0.032	0.089	0.042	44	Positive
	2	0.995	0.998	0.949	0.993	42		2	0.033	0.031	0.090	0.042	46	
	3	0.992	1.003	0.953	0.999	35		3	0.035	0.032	0.096	0.044	50	
	Mean	0.995	0.998	0.950	0.994	41		Mean	0.034	0.032	0.092	0.043	47	
3							3							
Mean for 3 assays		-	-	-	-	39	Mean for 3 assays		-	-	-	-	52	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results *1	Run	Run#	A560(-)		A560(+)			Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.984	1.000	1.026	0.995	-47	1	1	0.046	0.033	0.112	0.068	30	Positive
	2	0.984	1.000	1.031	0.997	-52		2	0.049	0.031	0.104	0.064	19	
	3	0.985	0.999	0.983	0.994	-3		3	0.040	0.031	0.112	0.072	36	
	Mean	0.984	1.000	1.013	0.995	-34		Mean	0.045	0.032	0.109	0.068	28	
2	1	0.986	0.992	0.979	0.989	4	2	1	0.037	0.031	0.121	0.040	75	Positive
	2	0.981	1.000	0.979	0.996	-1		2	0.037	0.031	0.120	0.040	74	
	3	0.979	0.996	0.979	0.993	-3		3	0.044	0.032	0.125	0.041	72	
	Mean	0.982	0.996	0.979	0.993	0		Mean	0.039	0.031	0.122	0.040	74	
3							3							
Mean for 3 assays		-	-	-	-	-17	Mean for 3 assays		-	-	-	-	51	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Quinine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.007	0.997	0.921	0.993	82	1	1	0.033	0.032	0.104	0.037	65	Positive
	2	0.998	0.998	0.910	0.994	84		2	0.033	0.031	0.100	0.039	61	
	3	0.993	1.000	0.912	0.995	77		3	0.031	0.032	0.102	0.037	65	
	Mean	0.999	0.998	0.914	0.994	81		Mean	0.032	0.032	0.102	0.038	64	
2	1	0.995	0.994	0.913	0.989	78	2	1	0.032	0.032	0.097	0.042	54	Positive
	2	0.986	0.998	0.909	0.993	73		2	0.032	0.031	0.108	0.042	65	
	3	0.995	1.003	0.926	0.999	65		3	0.034	0.032	0.092	0.044	47	
	Mean	0.992	0.998	0.916	0.994	72		Mean	0.033	0.032	0.099	0.043	55	
3							3							
Mean for 3 assays		-	-	-	-	77	Mean for 3 assays		-	-	-	-	60	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results* ¹	Run	Run#	A560(-)		A560(+)			Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.985	1.000	0.615	0.995	365	1	1	0.035	0.033	0.276	0.068	205	Positive
	2	0.989	1.000	0.620	0.997	364		2	0.037	0.031	0.268	0.064	195	
	3	0.982	0.999	0.629	0.994	348		3	0.032	0.031	0.278	0.072	210	
	Mean	0.985	1.000	0.621	0.995	359		Mean	0.035	0.032	0.274	0.068	203	
2	1	0.987	0.992	0.629	0.989	355	2	1	0.033	0.031	0.292	0.040	250	Positive
	2	0.978	1.000	0.628	0.996	347		2	0.039	0.031	0.295	0.040	247	
	3	0.984	0.996	0.644	0.993	337		3	0.032	0.032	0.299	0.041	258	
	Mean	0.983	0.996	0.634	0.993	346		Mean	0.035	0.031	0.295	0.040	252	
3							3							
Mean for 3 assays		-	-	-	-	353	Mean for 3 assays		-	-	-	-	228	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.000	0.997	0.998	0.993	-2	1	1	0.051	0.032	0.051	0.037	-6	Negative
	2	1.012	0.998	0.996	0.994	12		2	0.057	0.031	0.039	0.039	-24	
	3	1.002	1.000	0.998	0.995	0		3	0.047	0.032	0.042	0.037	-11	
	Mean	1.005	0.998	0.997	0.994	3		Mean	0.052	0.032	0.044	0.038	-14	
2	1	0.997	0.994	0.991	0.989	2	2	1	0.040	0.032	0.058	0.042	7	Negative
	2	0.994	0.998	0.989	0.993	1		2	0.039	0.031	0.042	0.042	-8	
	3	0.992	1.003	0.986	0.999	2		3	0.041	0.032	0.045	0.044	-7	
	Mean	0.994	0.998	0.989	0.994	2		Mean	0.040	0.032	0.048	0.043	-3	
3							3							
Mean for 3 assays		-	-	-	-	3	Mean for 3 assays		-	-	-	-	-9	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.997	1.000	0.990	0.995	2	1	1	0.049	0.033	0.046	0.068	-39	Negative
	2	1.002	1.000	0.998	0.997	-1		2	0.058	0.031	0.052	0.064	-42	
	3	1.001	0.999	0.997	0.994	-1		3	0.040	0.031	0.042	0.072	-34	
	Mean	1.000	1.000	0.995	0.995	0		Mean	0.049	0.032	0.047	0.068	-38	
2	1	0.999	0.992	0.994	0.989	2	2	1	0.055	0.031	0.051	0.040	-13	Negative
	2	0.993	1.000	0.990	0.996	0		2	0.050	0.031	0.063	0.040	4	
	3	0.995	0.996	0.989	0.993	3		3	0.053	0.032	0.051	0.041	-11	
	Mean	0.996	0.996	0.991	0.993	2		Mean	0.053	0.031	0.055	0.040	-7	
3							3							
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	-23	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.004	1.003	0.997	0.995	0	1	1	0.036	0.036	0.038	0.038	0	Negative
	2	1.001	1.003	0.993	0.996	1		2	0.036	0.037	0.038	0.039	-1	
	3	1.003	1.002	0.996	0.997	0		3	0.036	0.035	0.038	0.038	-1	
	Mean	1.002	1.003	0.995	0.996	0		Mean	0.036	0.036	0.038	0.039	-1	
2	1	0.988	0.991	0.981	0.985	1	2	1	0.035	0.035	0.038	0.038	-1	Negative
	2	0.990	0.992	0.982	0.986	2		2	0.035	0.036	0.037	0.038	-1	
	3	0.989	0.991	0.981	0.985	2		3	0.036	0.035	0.038	0.038	-1	
	Mean	0.989	0.991	0.981	0.985	2		Mean	0.036	0.035	0.038	0.038	-1	
3							3							
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	-1	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.022	1.031	1.015	1.022	1	1	1	0.035	0.035	0.039	0.038	1	Negative
	2	1.028	1.028	1.021	1.021	0		2	0.035	0.035	0.039	0.038	0	
	3	1.024	1.036	1.017	1.029	0		3	0.036	0.035	0.040	0.038	1	
	Mean	1.025	1.031	1.018	1.024	0		Mean	0.035	0.035	0.039	0.038	1	
2	1	1.006	1.006	0.950	0.953	6	2	1	0.036	0.035	0.039	0.037	2	Negative
	2	1.008	1.006	0.955	0.950	3		2	0.036	0.035	0.039	0.038	2	
	3	1.008	1.001	0.960	0.958	-1		3	0.036	0.035	0.039	0.037	2	
	Mean	1.007	1.004	0.955	0.954	3		Mean	0.036	0.035	0.039	0.037	2	
3							3							
Mean for 3 assays		-	-	-	-	2	Mean for 3 assays		-	-	-	-	2	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.002	1.003	0.986	0.995	9	1	1	0.036	0.036	0.042	0.038	3	
	2	1.003	1.003	0.986	0.996	11		2	0.036	0.037	0.042	0.039	2	
	3	1.001	1.002	0.986	0.997	8		3	0.036	0.035	0.041	0.038	2	
	Mean	1.002	1.003	0.986	0.996	9		Mean	0.036	0.036	0.041	0.039	2	
2	1	0.988	0.991	0.970	0.985	12	2	1	0.036	0.035	0.040	0.038	2	
	2	0.992	0.992	0.974	0.986	11		2	0.036	0.036	0.040	0.038	1	
	3	0.988	0.991	0.972	0.985	10		3	0.036	0.035	0.040	0.038	1	
	Mean	0.989	0.991	0.972	0.985	11		Mean	0.036	0.035	0.040	0.038	1	
3							3							
Mean for 3 assays		-	-	-	-	10	Mean for 3 assays		-	-	-	-	2	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.018	1.031	0.948	1.022	63	1	1	0.035	0.035	0.067	0.038	29	
	2	1.017	1.028	0.970	1.021	39		2	0.036	0.035	0.067	0.038	28	
	3	1.022	1.036	0.956	1.029	58		3	0.036	0.035	0.070	0.038	31	
	Mean	1.019	1.031	0.958	1.024	53		Mean	0.036	0.035	0.068	0.038	29	
2	1	0.998	1.006	0.869	0.953	79	2	1	0.036	0.035	0.067	0.037	29	
	2	1.001	1.006	0.873	0.950	78		2	0.036	0.035	0.065	0.038	28	
	3	0.998	1.001	0.877	0.958	70		3	0.036	0.035	0.069	0.037	31	
	Mean	0.999	1.004	0.873	0.954	76		Mean	0.036	0.035	0.067	0.037	29	
3							3							
Mean for 3 assays		-	-	-	-	65	Mean for 3 assays		-	-	-	-	29	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Amiodarone HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.083	1.003	0.996	0.995	80	1	1	0.085	0.036	0.090	0.038	2	Positive
	2	1.086	1.003	0.998	0.996	81		2	0.085	0.037	0.091	0.039	3	
	3	1.087	1.002	0.999	0.997	80		3	0.085	0.035	0.092	0.038	4	
	Mean	1.085	1.003	0.998	0.996	80		Mean	0.085	0.036	0.091	0.039	3	
2	1	1.073	0.991	0.975	0.985	92	2	1	0.087	0.035	0.091	0.038	1	Positive
	2	1.074	0.992	0.982	0.986	87		2	0.087	0.036	0.092	0.038	2	
	3	1.074	0.991	0.979	0.985	90		3	0.086	0.035	0.091	0.038	2	
	Mean	1.074	0.991	0.978	0.985	90		Mean	0.087	0.035	0.091	0.038	2	
3							3							
Mean for 3 assays		-	-	-	-	85	Mean for 3 assays		-	-	-	-	3	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.891	1.031	1.531	1.022	353	1	1	0.506	0.035	0.629	0.038	120	Positive
	2	1.887	1.028	1.508	1.021	372		2	0.509	0.035	0.637	0.038	125	
	3	1.832	1.036	1.501	1.029	324		3	0.508	0.035	0.613	0.038	102	
	Mean	1.870	1.031	1.513	1.024	350		Mean	0.508	0.035	0.626	0.038	116	
2	1	1.886	1.006	1.458	0.953	378	2	1	0.583	0.035	0.508	0.037	-78	Positive
	2	1.881	1.006	1.378	0.950	452		2	0.581	0.035	0.521	0.038	-62	
	3	1.819	1.001	1.347	0.958	421		3	0.582	0.035	0.527	0.037	-58	
	Mean	1.862	1.004	1.395	0.954	417		Mean	0.582	0.035	0.518	0.037	-66	
3							3							
Mean for 3 assays		-	-	-	-	384	Mean for 3 assays		-	-	-	-	25	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Chlorpromazine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.991	1.003	0.929	0.995	55	1	1	0.036	0.036	0.052	0.038	14	Positive
	2	0.990	1.003	0.931	0.996	51		2	0.035	0.037	0.053	0.039	15	
	3	0.993	1.002	0.940	0.997	45		3	0.037	0.035	0.055	0.038	15	
	Mean	0.991	1.003	0.934	0.996	50		Mean	0.036	0.036	0.054	0.039	15	
2	1	0.974	0.991	0.898	0.985	70	2	1	0.036	0.035	0.052	0.038	13	Positive
	2	0.974	0.992	0.907	0.986	61		2	0.036	0.036	0.052	0.038	13	
	3	0.970	0.991	0.896	0.985	68		3	0.036	0.035	0.052	0.038	13	
	Mean	0.972	0.991	0.900	0.985	66		Mean	0.036	0.035	0.052	0.038	13	
3	/						3	/						
Mean for 3 assays		-	-	-	-	58	Mean for 3 assays		-	-	-	-	14	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.983	1.031	0.966	1.022	10	1	1	0.036	0.035	0.110	0.038	71	Positive
	2	0.996	1.028	0.975	1.021	14		2	0.036	0.035	0.111	0.038	72	
	3	1.012	1.036	0.994	1.029	11		3	0.036	0.035	0.114	0.038	74	
	Mean	0.997	1.031	0.978	1.024	12		Mean	0.036	0.035	0.112	0.038	72	
2	1	0.961	1.006	0.929	0.953	-17	2	1	0.036	0.035	0.111	0.037	73	Positive
	2	0.996	1.006	0.956	0.950	-10		2	0.036	0.035	0.112	0.038	74	
	3	0.983	1.001	0.939	0.958	-5		3	0.036	0.035	0.115	0.037	77	
	Mean	0.980	1.004	0.941	0.954	-11		Mean	0.036	0.035	0.113	0.037	75	
3	/						3	/						
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	74	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.002	1.003	0.967	0.995	29	1	1	0.037	0.036	0.049	0.038	9	
	2	1.003	1.003	0.962	0.996	34		2	0.037	0.037	0.049	0.039	9	
	3	1.000	1.002	0.963	0.997	30		3	0.037	0.035	0.050	0.038	10	
	Mean	1.002	1.003	0.964	0.996	31		Mean	0.037	0.036	0.049	0.039	9	
2	1	0.989	0.991	0.951	0.985	31	2	1	0.037	0.035	0.046	0.038	6	
	2	0.989	0.992	0.950	0.986	33		2	0.036	0.036	0.045	0.038	6	
	3	0.987	0.991	0.952	0.985	29		3	0.036	0.035	0.046	0.038	7	
	Mean	0.988	0.991	0.951	0.985	31		Mean	0.036	0.035	0.045	0.038	6	
3							3							
Mean for 3 assays		-	-	-	-	31	Mean for 3 assays		-	-	-	-	8	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.023	1.031	0.909	1.022	107	1	1	0.036	0.035	0.160	0.038	121	
	2	1.019	1.028	0.917	1.021	96		2	0.036	0.035	0.164	0.038	126	
	3	1.028	1.036	0.934	1.029	88		3	0.036	0.035	0.172	0.038	133	
	Mean	1.024	1.031	0.920	1.024	97		Mean	0.036	0.035	0.165	0.038	127	
2	1	0.999	1.006	0.864	0.953	85	2	1	0.038	0.035	0.154	0.037	114	
	2	0.999	1.006	0.877	0.950	72		2	0.036	0.035	0.167	0.038	129	
	3	1.000	1.001	0.879	0.958	71		3	0.036	0.035	0.173	0.037	135	
	Mean	0.999	1.004	0.874	0.954	76		Mean	0.037	0.035	0.165	0.037	126	
3							3							
Mean for 3 assays		-	-	-	-	87	Mean for 3 assays		-	-	-	-	127	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Doxycycline HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.004	1.003	0.928	0.995	69	1	1	0.037	0.036	0.097	0.038	57	Positive
	2	1.004	1.003	0.931	0.996	67		2	0.036	0.037	0.098	0.039	59	
	3	1.002	1.002	0.930	0.997	65		3	0.037	0.035	0.100	0.038	60	
	Mean	1.003	1.003	0.930	0.996	67		Mean	0.037	0.036	0.098	0.039	59	
2	1	0.986	0.991	0.901	0.985	79	2	1	0.036	0.035	0.087	0.038	48	Positive
	2	0.987	0.992	0.907	0.986	74		2	0.036	0.036	0.086	0.038	47	
	3	0.985	0.991	0.905	0.985	74		3	0.037	0.035	0.088	0.038	49	
	Mean	0.986	0.991	0.904	0.985	76		Mean	0.036	0.035	0.087	0.038	48	
3							3							
Mean for 3 assays		-	-	-	-	72	Mean for 3 assays		-	-	-	-	54	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.007	1.031	0.711	1.022	289	1	1	0.037	0.035	0.363	0.038	324	Positive
	2	1.010	1.028	0.728	1.021	275		2	0.036	0.035	0.369	0.038	330	
	3	1.009	1.036	0.731	1.029	271		3	0.037	0.035	0.374	0.038	334	
	Mean	1.009	1.031	0.723	1.024	278		Mean	0.037	0.035	0.369	0.038	329	
2	1	0.982	1.006	0.684	0.953	248	2	1	0.039	0.035	0.356	0.037	316	Positive
	2	0.983	1.006	0.704	0.950	229		2	0.036	0.035	0.356	0.038	318	
	3	0.983	1.001	0.700	0.958	233		3	0.037	0.035	0.368	0.037	329	
	Mean	0.983	1.004	0.696	0.954	237		Mean	0.037	0.035	0.360	0.037	321	
3							3							
Mean for 3 assays		-	-	-	-	258	Mean for 3 assays		-	-	-	-	325	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.003	1.003	0.975	0.995	22	1	1	0.037	0.036	0.044	0.038	4	Negative
	2	1.005	1.003	0.976	0.996	21		2	0.037	0.037	0.044	0.039	4	
	3	1.007	1.002	0.980	0.997	19		3	0.037	0.035	0.044	0.038	4	
	Mean	1.005	1.003	0.977	0.996	21		Mean	0.037	0.036	0.044	0.039	4	
2	1	0.989	0.991	0.931	0.985	52	2	1	0.036	0.035	0.043	0.038	4	Positive
	2	0.990	0.992	0.962	0.986	22		2	0.036	0.036	0.043	0.038	3	
	3	0.995	0.991	0.968	0.985	21		3	0.036	0.035	0.042	0.038	3	
	Mean	0.991	0.991	0.954	0.985	32		Mean	0.036	0.035	0.043	0.038	3	
3							3							
Mean for 3 assays		-	-	-	-	27	Mean for 3 assays		-	-	-	-	4	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.025	1.031	0.941	1.022	76	1	1	0.035	0.035	0.068	0.038	30	Positive
	2	1.031	1.028	0.954	1.021	69		2	0.035	0.035	0.067	0.038	29	
	3	1.024	1.036	0.951	1.029	66		3	0.035	0.035	0.068	0.038	30	
	Mean	1.026	1.031	0.949	1.024	70		Mean	0.035	0.035	0.068	0.038	30	
2	1	1.005	1.006	0.915	0.953	39	2	1	0.036	0.035	0.068	0.037	29	Positive
	2	1.006	1.006	0.922	0.950	33		2	0.036	0.035	0.067	0.038	29	
	3	1.006	1.001	0.925	0.958	31		3	0.037	0.035	0.071	0.037	32	
	Mean	1.005	1.004	0.921	0.954	34		Mean	0.037	0.035	0.069	0.037	30	
3							3							
Mean for 3 assays		-	-	-	-	52	Mean for 3 assays		-	-	-	-	30	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.012	1.008	0.981	1.001	24	1	1	0.036	0.036	0.042	0.038	4	
	2	1.014	1.007	0.983	1.001	24		2	0.036	0.036	0.042	0.038	4	
	3	1.010	1.012	0.981	1.005	22		3	0.037	0.036	0.043	0.038	5	
	Mean	1.012	1.009	0.982	1.002	23		Mean	0.036	0.036	0.042	0.038	4	
2	1	0.993	0.996	0.961	0.985	24	2	1	0.035	0.035	0.041	0.037	3	
	2	0.995	0.992	0.963	0.984	23		2	0.035	0.036	0.054	0.038	15	
	3	0.993	0.991	0.962	0.985	21		3	0.036	0.035	0.041	0.038	2	
	Mean	0.994	0.993	0.962	0.984	23		Mean	0.036	0.035	0.045	0.038	7	
3							3							
Mean for 3 assays		-	-	-	-	23	Mean for 3 assays		-	-	-	-	6	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.011	1.014	0.844	1.008	162	1	1	0.036	0.035	0.143	0.039	103	
	2	1.015	1.018	0.840	1.013	171		2	0.036	0.035	0.143	0.038	103	
	3	1.014	1.014	0.846	1.011	164		3	0.036	0.035	0.146	0.038	106	
	Mean	1.013	1.015	0.843	1.011	166		Mean	0.036	0.035	0.144	0.039	104	
2	1	0.991	0.998	0.807	0.993	177	2	1	0.036	0.035	0.128	0.037	89	
	2	0.990	1.004	0.816	0.999	168		2	0.035	0.035	0.132	0.038	93	
	3	0.987	0.999	0.818	0.994	163		3	0.036	0.035	0.134	0.038	95	
	Mean	0.989	1.001	0.814	0.995	169		Mean	0.036	0.035	0.131	0.038	92	
3							3							
Mean for 3 assays		-	-	-	-	168	Mean for 3 assays		-	-	-	-	98	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.016	1.008	0.976	1.001	33	1	1	0.035	0.036	0.198	0.038	161	
	2	1.017	1.007	0.978	1.001	31		2	0.036	0.036	0.192	0.038	154	
	3	1.016	1.012	0.979	1.005	29		3	0.036	0.036	0.197	0.038	159	
	Mean	1.016	1.009	0.978	1.002	31		Mean	0.036	0.036	0.196	0.038	158	
2	1	0.993	0.996	0.948	0.985	36	2	1	0.035	0.035	0.198	0.037	160	
	2	0.994	0.992	0.947	0.984	39		2	0.036	0.036	0.187	0.038	148	
	3	0.995	0.991	0.953	0.985	33		3	0.036	0.035	0.192	0.038	153	
	Mean	0.994	0.993	0.949	0.984	36		Mean	0.036	0.035	0.192	0.038	154	
3	/						3	/						
	/							/						
	/							/						
	/							/						
Mean for 3 assays		-	-	-	-	34	Mean for 3 assays		-	-	-	-	156	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.010	1.014	0.867	1.008	139	1	1	0.036	0.035	0.474	0.039	434	
	2	1.012	1.018	0.879	1.013	128		2	0.036	0.035	0.468	0.038	429	
	3	1.004	1.014	0.883	1.011	117		3	0.036	0.035	0.468	0.038	428	
	Mean	1.008	1.015	0.876	1.011	128		Mean	0.036	0.035	0.470	0.039	430	
2	1	0.992	0.998	0.848	0.993	138	2	1	0.035	0.035	0.485	0.037	447	
	2	0.992	1.004	0.857	0.999	129		2	0.036	0.035	0.469	0.038	431	
	3	0.992	0.999	0.860	0.994	126		3	0.036	0.035	0.473	0.038	434	
	Mean	0.992	1.001	0.855	0.995	131		Mean	0.036	0.035	0.476	0.038	437	
3	/						3	/						
	/							/						
	/							/						
	/							/						
Mean for 3 assays		-	-	-	-	130	Mean for 3 assays		-	-	-	-	434	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.012	1.008	0.967	1.001	38	1	1	0.036	0.036	0.054	0.038	16	
	2	1.013	1.007	0.966	1.001	40		2	0.036	0.036	0.052	0.038	14	
	3	1.013	1.012	0.969	1.005	37		3	0.037	0.036	0.053	0.038	14	
	Mean	1.013	1.009	0.968	1.002	38		Mean	0.036	0.036	0.053	0.038	15	
2	1	0.995	0.996	0.950	0.985	37	2	1	0.035	0.035	0.050	0.037	12	
	2	0.995	0.992	0.950	0.984	36		2	0.036	0.036	0.050	0.038	12	
	3	0.996	0.991	0.952	0.985	35		3	0.036	0.035	0.051	0.038	12	
	Mean	0.995	0.993	0.951	0.984	36		Mean	0.036	0.035	0.050	0.038	12	
3	/						3	/						
	/							/						
	/							/						
	/							/						
Mean for 3 assays		-	-	-	-	37	Mean for 3 assays		-	-	-	-	14	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.013	1.014	0.865	1.008	144	1	1	0.036	0.035	0.142	0.039	102	
	2	1.009	1.018	0.873	1.013	132		2	0.036	0.035	0.137	0.038	97	
	3	1.009	1.014	0.873	1.011	132		3	0.036	0.035	0.144	0.038	104	
	Mean	1.010	1.015	0.870	1.011	136		Mean	0.036	0.035	0.141	0.039	101	
2	1	0.994	0.998	0.848	0.993	140	2	1	0.036	0.035	0.144	0.037	105	
	2	0.991	1.004	0.850	0.999	135		2	0.036	0.035	0.145	0.038	106	
	3	0.996	0.999	0.856	0.994	134		3	0.036	0.035	0.143	0.038	104	
	Mean	0.994	1.001	0.851	0.995	136		Mean	0.036	0.035	0.144	0.038	105	
3	/						3	/						
	/							/						
	/							/						
	/							/						
Mean for 3 assays		-	-	-	-	136	Mean for 3 assays		-	-	-	-	103	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.004	1.008	0.956	1.001	41	1	1	0.036	0.036	0.066	0.038	28	Positive
	2	1.006	1.007	0.950	1.001	49		2	0.036	0.036	0.065	0.038	27	
	3	1.006	1.012	0.956	1.005	43		3	0.037	0.036	0.067	0.038	29	
	Mean	1.005	1.009	0.954	1.002	44		Mean	0.036	0.036	0.066	0.038	28	
2	1	1.004	0.996	0.956	0.985	39	2	1	0.035	0.035	0.066	0.037	28	Positive
	2	1.006	0.992	0.950	0.984	47		2	0.036	0.036	0.067	0.038	28	
	3	1.006	0.991	0.956	0.985	41		3	0.036	0.035	0.067	0.038	28	
	Mean	1.005	0.993	0.954	0.984	42		Mean	0.036	0.035	0.067	0.038	28	
3	/						3	/						
Mean for 3 assays		-	-	-	-	43	Mean for 3 assays		-	-	-	-	28	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.980	1.014	1.267	1.008	-291	1	1	0.036	0.035	0.237	0.039	197	Positive
	2	0.983	1.018	1.219	1.013	-240		2	0.036	0.035	0.235	0.038	195	
	3	0.985	1.014	1.198	1.011	-217		3	0.037	0.035	0.253	0.038	212	
	Mean	0.982	1.015	1.228	1.011	-249		Mean	0.037	0.035	0.242	0.039	201	
2	1	0.965	0.998	1.246	0.993	-288	2	1	0.036	0.035	0.239	0.037	199	Positive
	2	0.964	1.004	1.205	0.999	-247		2	0.037	0.035	0.243	0.038	203	
	3	0.964	0.999	1.202	0.994	-244		3	0.037	0.035	0.251	0.038	211	
	Mean	0.964	1.001	1.217	0.995	-260		Mean	0.037	0.035	0.244	0.038	204	
3	/						3	/						
Mean for 3 assays		-	-	-	-	-255	Mean for 3 assays		-	-	-	-	203	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.011	1.008	0.929	1.001	75	1	1	0.036	0.036	0.090	0.038	52	
	2	1.009	1.007	0.931	1.001	70		2	0.036	0.036	0.086	0.038	49	
	3	1.011	1.012	0.931	1.005	74		3	0.037	0.036	0.087	0.038	48	
	Mean	1.010	1.009	0.930	1.002	73		Mean	0.036	0.036	0.088	0.038	50	
2	1	1.011	0.996	0.929	0.985	73	2	1	0.036	0.035	0.091	0.037	52	
	2	1.009	0.992	0.931	0.984	68		2	0.036	0.036	0.086	0.038	48	
	3	1.011	0.991	0.931	0.985	72		3	0.037	0.035	0.089	0.038	49	
	Mean	1.010	0.993	0.930	0.984	71		Mean	0.036	0.035	0.089	0.038	50	
3	/						3	/						
Mean for 3 assays		-	-	-	-	72	Mean for 3 assays		-	-	-	-	50	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.002	1.014	0.595	1.008	403	1	1	0.036	0.035	0.287	0.039	247	
	2	1.000	1.018	0.618	1.013	378		2	0.036	0.035	0.286	0.038	246	
	3	1.001	1.014	0.635	1.011	363		3	0.036	0.035	0.295	0.038	254	
	Mean	1.001	1.015	0.616	1.011	381		Mean	0.036	0.035	0.289	0.039	249	
2	1	0.986	0.998	0.608	0.993	372	2	1	0.036	0.035	0.287	0.037	248	
	2	0.984	1.004	0.634	0.999	345		2	0.035	0.035	0.271	0.038	233	
	3	0.985	0.999	0.638	0.994	341		3	0.036	0.035	0.280	0.038	241	
	Mean	0.985	1.001	0.627	0.995	353		Mean	0.036	0.035	0.279	0.038	241	
3	/						3	/						
Mean for 3 assays		-	-	-	-	367	Mean for 3 assays		-	-	-	-	245	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.007	1.008	1.002	1.001	-2	1	1	0.037	0.036	0.039	0.038	0	
	2	1.011	1.007	1.005	1.001	-1		2	0.037	0.036	0.038	0.038	-1	
	3	1.011	1.012	1.005	1.005	-1		3	0.037	0.036	0.039	0.038	0	
	Mean	1.010	1.009	1.004	1.002	-1		Mean	0.037	0.036	0.038	0.038	0	
2	1	1.007	0.996	1.002	0.985	-4	2	1	0.037	0.035	0.038	0.037	-1	
	2	1.011	0.992	1.005	0.984	-3		2	0.037	0.036	0.039	0.038	-1	
	3	1.011	0.991	1.005	0.985	-3		3	0.037	0.035	0.039	0.038	-1	
	Mean	1.010	0.993	1.004	0.984	-3		Mean	0.037	0.035	0.038	0.038	-1	
3	3						3	3						
	Mean for 3 assays							Mean for 3 assays						
	-							-						
	-2							-1						

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.014	1.014	1.008	1.008	2	1	1	0.037	0.035	0.037	0.039	-4	
	2	1.017	1.018	1.012	1.013	1		2	0.036	0.035	0.037	0.038	-4	
	3	1.015	1.014	1.011	1.011	1		3	0.037	0.035	0.037	0.038	-4	
	Mean	1.015	1.015	1.010	1.011	1		Mean	0.037	0.035	0.037	0.039	-4	
2	1	0.995	0.998	0.988	0.993	0	2	1	0.037	0.035	0.037	0.037	-3	
	2	0.995	1.004	0.988	0.999	1		2	0.036	0.035	0.036	0.038	-3	
	3	0.995	0.999	0.989	0.994	0		3	0.037	0.035	0.037	0.038	-3	
	Mean	0.995	1.001	0.989	0.995	0		Mean	0.036	0.035	0.037	0.038	-3	
3	3						3	3						
	Mean for 3 assays							Mean for 3 assays						
	-							-						
	1							-4						

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.987	0.981	0.977	0.973	2	1	1	0.033	0.034	0.044	0.049	-4	Negative
	2	0.983	0.986	0.972	0.977	3		2	0.035	0.034	0.042	0.048	-8	
	3	0.986	0.988	0.975	0.980	3		3	0.035	0.032	0.044	0.048	-6	
	Mean	0.985	0.985	0.975	0.977	3		Mean	0.034	0.033	0.043	0.048	-6	
2	1	0.988	0.982	0.979	0.974	-6	2	1	0.033	0.034	0.039	0.048	-8	Negative
	2	0.981	0.984	0.973	0.976	-7		2	0.033	0.034	0.043	0.046	-4	
	3	0.982	0.967	0.974	0.938	-7		3	0.038	0.032	0.043	0.047	-9	
	Mean	0.984	0.978	0.975	0.963	-7		Mean	0.035	0.033	0.042	0.047	-7	
3							3							
Mean for 3 assays		-	-	-	-	-2	Mean for 3 assays		-	-	-	-	-7	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.985	0.972	0.976	0.959	-3	1	1	0.033	0.034	0.040	0.038	3	Negative
	2	0.980	0.998	0.973	0.988	-5		2	0.033	0.033	0.040	0.037	3	
	3	0.979	0.979	0.972	0.967	-5		3	0.034	0.032	0.042	0.036	4	
	Mean	0.981	0.983	0.974	0.971	-4		Mean	0.033	0.033	0.041	0.037	3	
2	1	0.982	0.972	0.974	0.964	-1	2	1	0.033	0.035	0.048	0.047	4	Negative
	2	0.980	0.980	0.970	0.971	1		2	0.033	0.037	0.047	0.046	3	
	3	0.979	0.984	0.971	0.975	-1		3	0.035	0.033	0.051	0.046	5	
	Mean	0.980	0.979	0.972	0.970	0		Mean	0.034	0.035	0.049	0.046	4	
3							3							
Mean for 3 assays		-	-	-	-	-2	Mean for 3 assays		-	-	-	-	4	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.985	0.981	0.965	0.973	12	1	1	0.034	0.034	0.056	0.049	7	
	2	0.983	0.986	0.965	0.977	10		2	0.035	0.034	0.058	0.048	8	
	3	0.986	0.988	0.968	0.980	10		3	0.034	0.032	0.058	0.048	9	
	Mean	0.985	0.985	0.966	0.977	11		Mean	0.034	0.033	0.057	0.048	8	
2	1	0.984	0.982	0.964	0.974	5	2	1	0.034	0.034	0.050	0.048	2	
	2	0.983	0.984	0.966	0.976	2		2	0.034	0.034	0.049	0.046	1	
	3	0.983	0.967	0.961	0.938	7		3	0.034	0.032	0.054	0.047	6	
	Mean	0.983	0.978	0.964	0.963	5		Mean	0.034	0.033	0.051	0.047	3	
3							3							
Mean for 3 assays		-	-	-	-	8	Mean for 3 assays		-	-	-	-	6	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.972	0.972	0.903	0.959	57	1	1	0.034	0.034	0.095	0.038	57	
	2	0.965	0.998	0.907	0.988	46		2	0.035	0.033	0.096	0.037	57	
	3	0.967	0.979	0.915	0.967	40		3	0.036	0.032	0.105	0.036	65	
	Mean	0.968	0.983	0.908	0.971	48		Mean	0.035	0.033	0.099	0.037	60	
2	1	0.968	0.972	0.906	0.964	53	2	1	0.034	0.035	0.119	0.047	74	
	2	0.969	0.980	0.908	0.971	52		2	0.035	0.037	0.117	0.046	71	
	3	0.972	0.984	0.913	0.975	50		3	0.034	0.033	0.125	0.046	80	
	Mean	0.970	0.979	0.909	0.970	52		Mean	0.034	0.035	0.120	0.046	75	
3							3							
Mean for 3 assays		-	-	-	-	50	Mean for 3 assays		-	-	-	-	68	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Amiodarone HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.071	0.981	0.944	0.973	119	1	1	0.085	0.034	0.088	0.049	-12	Positive
	2	1.066	0.986	0.943	0.977	115		2	0.099	0.034	0.087	0.048	-27	
	3	1.065	0.988	0.951	0.980	106		3	0.087	0.032	0.092	0.048	-10	
	Mean	1.067	0.985	0.946	0.977	113		Mean	0.090	0.033	0.089	0.048	-16	
2	1	1.064	0.982	0.952	0.974	97	2	1	0.089	0.034	0.086	0.048	-17	Positive
	2	1.051	0.984	0.961	0.976	75		2	0.088	0.034	0.086	0.046	-16	
	3	1.055	0.967	0.957	0.938	83		3	0.088	0.032	0.086	0.047	-16	
	Mean	1.057	0.978	0.957	0.963	85		Mean	0.088	0.033	0.086	0.047	-16	
3							3							
Mean for 3 assays		-	-	-	-	99	Mean for 3 assays		-	-	-	-	-16	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	1.631	0.972	1.340	0.959	279	1	1	0.618	0.034	0.408	0.038	-214	Positive
	2	1.597	0.998	1.277	0.988	308		2	0.621	0.033	0.423	0.037	-202	
	3	1.575	0.979	1.276	0.967	287		3	0.619	0.032	0.402	0.036	-221	
	Mean	1.601	0.983	1.298	0.971	291		Mean	0.619	0.033	0.411	0.037	-212	
2	1	1.469	0.972	1.275	0.964	185	2	1	0.658	0.035	0.451	0.047	-218	Positive
	2	1.465	0.980	1.222	0.971	234		2	0.666	0.037	0.473	0.046	-204	
	3	1.452	0.984	1.247	0.975	196		3	0.664	0.033	0.448	0.046	-227	
	Mean	1.462	0.979	1.248	0.970	205		Mean	0.663	0.035	0.457	0.046	-216	
3							3							
Mean for 3 assays		-	-	-	-	248	Mean for 3 assays		-	-	-	-	-214	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - (A560(-) - (B-A)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Chlorpromazine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.970	0.981	0.852	0.973	110	1	1	0.034	0.034	0.048	0.049	-1	
	2	0.969	0.986	0.852	0.977	109		2	0.033	0.034	0.047	0.048	-1	
	3	0.977	0.988	0.861	0.980	108		3	0.033	0.032	0.047	0.048	-1	
	Mean	0.972	0.985	0.855	0.977	109		Mean	0.033	0.033	0.047	0.048	-1	
2	1	0.973	0.982	0.855	0.974	103	2	1	0.034	0.034	0.048	0.048	0	
	2	0.968	0.984	0.852	0.976	101		2	0.033	0.034	0.046	0.046	-1	
	3	0.971	0.967	0.853	0.938	103		3	0.034	0.032	0.048	0.047	0	
	Mean	0.971	0.978	0.853	0.963	102		Mean	0.034	0.033	0.047	0.047	0	
3							3							
Mean for 3 assays		-	-	-	-	106	Mean for 3 assays		-	-	-	-	-1	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.936	0.972	0.902	0.959	22	1	1	0.036	0.034	0.114	0.038	74	
	2	0.941	0.998	0.904	0.988	25		2	0.037	0.033	0.127	0.037	86	
	3	0.949	0.979	0.911	0.967	26		3	0.035	0.032	0.120	0.036	81	
	Mean	0.942	0.983	0.906	0.971	24		Mean	0.036	0.033	0.120	0.037	80	
2	1	0.936	0.972	0.905	0.964	22	2	1	0.036	0.035	0.117	0.047	70	
	2	0.955	0.980	0.922	0.971	24		2	0.035	0.037	0.119	0.046	73	
	3	0.959	0.984	0.923	0.975	27		3	0.035	0.033	0.123	0.046	77	
	Mean	0.950	0.979	0.917	0.970	24		Mean	0.035	0.035	0.120	0.046	73	
3							3							
Mean for 3 assays		-	-	-	-	24	Mean for 3 assays		-	-	-	-	77	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.983	0.981	0.936	0.973	39	1	1	0.035	0.034	0.056	0.049	6	Positive
	2	0.981	0.986	0.934	0.977	39		2	0.034	0.034	0.054	0.048	5	
	3	0.985	0.988	0.938	0.980	39		3	0.035	0.032	0.060	0.048	10	
	Mean	0.983	0.985	0.936	0.977	39		Mean	0.035	0.033	0.057	0.048	7	
2	1	0.981	0.982	0.941	0.974	25	2	1	0.037	0.034	0.053	0.048	2	Negative
	2	0.981	0.984	0.987	0.976	-21		2	0.034	0.034	0.049	0.046	1	
	3	0.981	0.967	0.938	0.938	28		3	0.036	0.032	0.053	0.047	3	
	Mean	0.981	0.978	0.955	0.963	11		Mean	0.036	0.033	0.052	0.047	2	
3							3							
Mean for 3 assays		-	-	-	-	25	Mean for 3 assays		-	-	-	-	5	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.969	0.972	0.816	0.959	141	1	1	0.035	0.034	0.157	0.038	118	Positive
	2	0.965	0.998	0.816	0.988	137		2	0.034	0.033	0.163	0.037	125	
	3	0.968	0.979	0.810	0.967	146		3	0.035	0.032	0.177	0.036	138	
	Mean	0.967	0.983	0.814	0.971	141		Mean	0.035	0.033	0.166	0.037	127	
2	1	0.979	0.972	0.826	0.964	144	2	1	0.039	0.035	0.159	0.047	109	Positive
	2	0.979	0.980	0.822	0.971	148		2	0.035	0.037	0.164	0.046	118	
	3	0.981	0.984	0.823	0.975	149		3	0.035	0.033	0.178	0.046	132	
	Mean	0.980	0.979	0.824	0.970	147		Mean	0.036	0.035	0.167	0.046	120	
3							3							
Mean for 3 assays		-	-	-	-	144	Mean for 3 assays		-	-	-	-	124	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Doxycycline HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.971	0.981	0.900	0.973	63	1	1	0.035	0.034	0.092	0.049	42	Positive
	2	0.972	0.986	0.905	0.977	59		2	0.035	0.034	0.092	0.048	42	
	3	0.977	0.988	0.910	0.980	59		3	0.035	0.032	0.092	0.048	42	
	Mean	0.973	0.985	0.905	0.977	60		Mean	0.035	0.033	0.092	0.048	42	
2	1	0.977	0.982	0.909	0.974	53	2	1	0.039	0.034	0.094	0.048	41	Positive
	2	0.975	0.984	0.908	0.976	52		2	0.034	0.034	0.087	0.046	39	
	3	0.976	0.967	0.909	0.938	52		3	0.035	0.032	0.088	0.047	39	
	Mean	0.976	0.978	0.909	0.963	52		Mean	0.036	0.033	0.090	0.047	40	
3							3							
Mean for 3 assays		-	-	-	-	56	Mean for 3 assays		-	-	-	-	41	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.942	0.972	0.658	0.959	272	1	1	0.038	0.034	0.387	0.038	345	Positive
	2	0.958	0.998	0.662	0.988	284		2	0.037	0.033	0.403	0.037	362	
	3	0.956	0.979	0.661	0.967	283		3	0.037	0.032	0.411	0.036	370	
	Mean	0.952	0.983	0.660	0.971	280		Mean	0.037	0.033	0.400	0.037	359	
2	1	0.961	0.972	0.656	0.964	296	2	1	0.037	0.035	0.382	0.047	334	Positive
	2	0.957	0.980	0.662	0.971	286		2	0.037	0.037	0.387	0.046	339	
	3	0.970	0.984	0.664	0.975	297		3	0.037	0.033	0.402	0.046	354	
	Mean	0.963	0.979	0.661	0.970	293		Mean	0.037	0.035	0.390	0.046	342	
3							3							
Mean for 3 assays		-	-	-	-	287	Mean for 3 assays		-	-	-	-	351	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.988	0.981	0.948	0.973	32	1	1	0.035	0.034	0.046	0.049	-4	Positive
	2	0.981	0.986	0.942	0.977	31		2	0.036	0.034	0.047	0.048	-4	
	3	0.988	0.988	0.950	0.980	30		3	0.035	0.032	0.046	0.048	-4	
	Mean	0.986	0.985	0.947	0.977	31		Mean	0.035	0.033	0.046	0.048	-4	
2	1	0.989	0.982	0.955	0.974	19	2	1	0.035	0.034	0.045	0.048	-4	Negative
	2	0.984	0.984	0.952	0.976	17		2	0.047	0.034	0.059	0.046	-2	
	3	0.986	0.967	0.953	0.938	18		3	0.039	0.032	0.043	0.047	-10	
	Mean	0.986	0.978	0.953	0.963	18		Mean	0.040	0.033	0.049	0.047	-5	
3							3							
Mean for 3 assays		-	-	-	-	25	Mean for 3 assays		-	-	-	-	-5	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.971	0.972	0.875	0.959	84	1	1	0.035	0.034	0.073	0.038	34	Positive
	2	0.978	0.998	0.884	0.988	82		2	0.035	0.033	0.077	0.037	38	
	3	0.977	0.979	0.884	0.967	81		3	0.034	0.032	0.079	0.036	41	
	Mean	0.975	0.983	0.881	0.971	82		Mean	0.035	0.033	0.076	0.037	38	
2	1	0.983	0.972	0.890	0.964	84	2	1	0.035	0.035	0.076	0.047	30	Positive
	2	0.982	0.980	0.890	0.971	83		2	0.035	0.037	0.078	0.046	32	
	3	0.983	0.984	0.890	0.975	84		3	0.035	0.033	0.080	0.046	34	
	Mean	0.983	0.979	0.890	0.970	84		Mean	0.035	0.035	0.078	0.046	32	
3							3							
Mean for 3 assays		-	-	-	-	83	Mean for 3 assays		-	-	-	-	35	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.980	0.978	0.948	0.970	24	1	1	0.033	0.034	0.047	0.049	-1	Negative
	2	0.978	0.979	0.947	0.972	23		2	0.033	0.033	0.047	0.047	-1	
	3	0.983	0.982	0.952	0.974	23		3	0.033	0.032	0.050	0.047	2	
	Mean	0.980	0.980	0.949	0.972	23		Mean	0.033	0.033	0.048	0.048	0	
2	1	0.980	0.972	0.946	0.965	27	2	1	0.033	0.034	0.047	0.050	-1	Positive
	2	0.976	0.977	0.943	0.970	26		2	0.033	0.034	0.046	0.049	-2	
	3	0.980	0.981	0.949	0.974	24		3	0.033	0.033	0.048	0.049	0	
	Mean	0.979	0.977	0.946	0.970	26		Mean	0.033	0.034	0.047	0.049	-1	
3							3							
Mean for 3 assays		-	-	-	-	25	Mean for 3 assays		-	-	-	-	-1	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.993	0.986	0.819	0.979	166	1	1	0.033	0.034	0.139	0.038	102	Positive
	2	0.991	0.998	0.822	0.990	161		2	0.033	0.033	0.146	0.037	109	
	3	0.995	0.995	0.826	0.986	161		3	0.034	0.032	0.151	0.036	113	
	Mean	0.993	0.993	0.822	0.985	163		Mean	0.033	0.033	0.145	0.037	108	
2	1	0.977	0.984	0.805	0.977	165	2	1	0.034	0.034	0.138	0.042	97	Positive
	2	0.972	0.978	0.801	0.972	164		2	0.034	0.034	0.139	0.041	98	
	3	0.971	0.983	0.799	0.976	165		3	0.034	0.033	0.144	0.040	103	
	Mean	0.973	0.982	0.802	0.975	165		Mean	0.034	0.034	0.140	0.041	99	
3							3							
Mean for 3 assays		-	-	-	-	164	Mean for 3 assays		-	-	-	-	104	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.987	0.978	0.953	0.970	26	1	1	0.033	0.034	0.255	0.049	207	Positive
	2	0.981	0.979	0.941	0.972	32		2	0.034	0.033	0.254	0.047	205	
	3	0.981	0.982	0.939	0.974	34		3	0.034	0.032	0.259	0.047	210	
	Mean	0.983	0.980	0.944	0.972	31		Mean	0.034	0.033	0.256	0.048	207	
2	1	0.976	0.972	0.933	0.965	36	2	1	0.034	0.034	0.236	0.050	187	Positive
	2	0.974	0.977	0.932	0.970	35		2	0.034	0.034	0.237	0.049	188	
	3	0.975	0.981	0.933	0.974	35		3	0.034	0.033	0.237	0.049	188	
	Mean	0.975	0.977	0.933	0.970	35		Mean	0.034	0.034	0.237	0.049	188	
3							3							
Mean for 3 assays		-	-	-	-	33	Mean for 3 assays		-	-	-	-	198	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.987	0.986	0.833	0.979	146	1	1	0.034	0.034	0.450	0.038	412	Positive
	2	0.985	0.998	0.838	0.990	139		2	0.034	0.033	0.451	0.037	413	
	3	0.975	0.995	0.829	0.986	138		3	0.034	0.032	0.466	0.036	428	
	Mean	0.982	0.993	0.833	0.985	141		Mean	0.034	0.033	0.456	0.037	418	
2	1	0.980	0.984	0.842	0.977	131	2	1	0.034	0.034	0.448	0.042	407	Positive
	2	0.975	0.978	0.843	0.972	125		2	0.034	0.034	0.457	0.041	416	
	3	0.977	0.983	0.839	0.976	131		3	0.034	0.033	0.460	0.040	419	
	Mean	0.977	0.982	0.841	0.975	129		Mean	0.034	0.034	0.455	0.041	414	
3							3							
Mean for 3 assays		-	-	-	-	135	Mean for 3 assays		-	-	-	-	416	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.977	0.978	0.927	0.970	42	1	1	0.034	0.034	0.059	0.049	10	
	2	0.975	0.979	0.925	0.972	42		2	0.034	0.033	0.059	0.047	10	
	3	0.977	0.982	0.927	0.974	42		3	0.033	0.032	0.059	0.047	11	
	Mean	0.976	0.980	0.926	0.972	42		Mean	0.034	0.033	0.059	0.048	10	
2	1	0.978	0.972	0.924	0.965	47	2	1	0.034	0.034	0.058	0.050	9	
	2	0.973	0.977	0.923	0.970	43		2	0.033	0.034	0.057	0.049	9	
	3	0.976	0.981	0.926	0.974	43		3	0.034	0.033	0.058	0.049	9	
	Mean	0.976	0.977	0.924	0.970	44		Mean	0.034	0.034	0.058	0.049	9	
3	/						3	/						
Mean for 3 assays		-	-	-	-	43	Mean for 3 assays		-	-	-	-	10	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.981	0.986	0.790	0.979	183	1	1	0.035	0.034	0.158	0.038	119	
	2	0.981	0.998	0.762	0.990	211		2	0.033	0.033	0.162	0.037	125	
	3	0.984	0.995	0.799	0.986	177		3	0.034	0.032	0.164	0.036	126	
	Mean	0.982	0.993	0.784	0.985	190		Mean	0.034	0.033	0.161	0.037	123	
2	1	0.972	0.984	0.782	0.977	183	2	1	0.035	0.034	0.151	0.042	109	
	2	0.969	0.978	0.776	0.972	186		2	0.034	0.034	0.141	0.041	100	
	3	0.972	0.983	0.788	0.976	177		3	0.034	0.033	0.159	0.040	118	
	Mean	0.971	0.982	0.782	0.975	182		Mean	0.034	0.034	0.150	0.041	109	
3	/						3	/						
Mean for 3 assays		-	-	-	-	186	Mean for 3 assays		-	-	-	-	116	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20

Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.975	0.978	0.937	0.970	30	1	1	0.034	0.034	0.074	0.049	25	Positive
	2	0.971	0.979	0.932	0.972	31		2	0.032	0.033	0.070	0.047	23	
	3	0.975	0.982	0.934	0.974	33		3	0.036	0.032	0.075	0.047	24	
	Mean	0.974	0.980	0.934	0.972	31		Mean	0.034	0.033	0.073	0.048	24	
2	1	0.972	0.972	0.935	0.965	30	2	1	0.034	0.034	0.072	0.050	23	Positive
	2	0.970	0.977	0.937	0.970	26		2	0.033	0.034	0.069	0.049	21	
	3	0.974	0.981	0.938	0.974	29		3	0.034	0.033	0.073	0.049	24	
	Mean	0.972	0.977	0.937	0.970	28		Mean	0.034	0.034	0.071	0.049	23	
3							3							
Mean for 3 assays		-	-	-	-	30	Mean for 3 assays		-	-	-	-	24	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.974	0.986	0.945	0.979	21	1	1	0.035	0.034	0.112	0.038	73	Positive
	2	0.986	0.998	0.947	0.990	31		2	0.033	0.033	0.112	0.037	75	
	3	0.980	0.995	0.953	0.986	19		3	0.035	0.032	0.123	0.036	84	
	Mean	0.980	0.993	0.948	0.985	24		Mean	0.034	0.033	0.116	0.037	77	
2	1	0.964	0.984	0.958	0.977	-1	2	1	0.035	0.034	0.106	0.042	64	Positive
	2	0.961	0.978	0.956	0.972	-2		2	0.034	0.034	0.109	0.041	68	
	3	0.965	0.983	0.956	0.976	2		3	0.035	0.033	0.120	0.040	78	
	Mean	0.963	0.982	0.957	0.975	0		Mean	0.035	0.034	0.112	0.041	70	
3							3							
Mean for 3 assays		-	-	-	-	12	Mean for 3 assays		-	-	-	-	74	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Quinine HCl

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.975	0.978	0.892	0.970	75	1	1	0.034	0.034	0.103	0.049	54	
	2	0.973	0.979	0.890	0.972	75		2	0.033	0.033	0.102	0.047	54	
	3	0.976	0.982	0.893	0.974	75		3	0.034	0.032	0.102	0.047	53	
	Mean	0.975	0.980	0.892	0.972	75		Mean	0.034	0.033	0.102	0.048	54	
2	1	0.973	0.972	0.883	0.965	83	2	1	0.034	0.034	0.105	0.050	56	
	2	0.966	0.977	0.866	0.970	93		2	0.033	0.034	0.102	0.049	54	
	3	0.973	0.981	0.886	0.974	80		3	0.034	0.033	0.105	0.049	56	
	Mean	0.971	0.977	0.878	0.970	85		Mean	0.034	0.034	0.104	0.049	55	
3							3							
Mean for 3 assays		-	-	-	-	80	Mean for 3 assays		-	-	-	-	55	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.977	0.986	0.508	0.979	461	1	1	0.035	0.034	0.366	0.038	327	
	2	0.976	0.998	0.510	0.990	458		2	0.050	0.033	0.385	0.037	331	
	3	0.978	0.995	0.544	0.986	426		3	0.034	0.032	0.402	0.036	364	
	Mean	0.977	0.993	0.521	0.985	448		Mean	0.040	0.033	0.384	0.037	341	
2	1	0.967	0.984	0.550	0.977	410	2	1	0.035	0.034	0.323	0.042	281	
	2	0.965	0.978	0.578	0.972	380		2	0.034	0.034	0.326	0.041	285	
	3	0.968	0.983	0.584	0.976	377		3	0.034	0.033	0.346	0.040	305	
	Mean	0.967	0.982	0.571	0.975	389		Mean	0.034	0.034	0.332	0.041	290	
3							3							
Mean for 3 assays		-	-	-	-	419	Mean for 3 assays		-	-	-	-	316	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 6 Individual data of Phase 1-2 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.979	0.978	0.966	0.970	5	1	1	0.035	0.034	0.042	0.049	-8	Negative
	2	0.978	0.979	0.967	0.972	3		2	0.035	0.033	0.042	0.047	-8	
	3	0.979	0.982	0.968	0.974	3		3	0.034	0.032	0.042	0.047	-7	
	Mean	0.979	0.980	0.967	0.972	4		Mean	0.035	0.033	0.042	0.048	-8	
2	1	0.984	0.972	0.970	0.965	7	2	1	0.035	0.034	0.042	0.050	-8	Negative
	2	0.979	0.977	0.966	0.970	6		2	0.035	0.034	0.041	0.049	-9	
	3	0.980	0.981	0.969	0.974	4		3	0.035	0.033	0.041	0.049	-9	
	Mean	0.981	0.977	0.968	0.970	6		Mean	0.035	0.034	0.041	0.049	-9	
3							3							
Mean for 3 assays		-	-	-	-	5	Mean for 3 assays		-	-	-	-	-9	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
1	1	0.958	0.986	0.948	0.979	2	1	1	0.050	0.034	0.037	0.038	-17	Negative
	2	0.958	0.998	0.949	0.990	1		2	0.035	0.033	0.036	0.037	-3	
	3	0.971	0.995	0.950	0.986	13		3	0.035	0.032	0.036	0.036	-3	
	Mean	0.962	0.993	0.949	0.985	5		Mean	0.040	0.033	0.036	0.037	-8	
2	1	0.983	0.984	0.975	0.977	1	2	1	0.044	0.034	0.037	0.042	-14	Negative
	2	0.985	0.978	0.977	0.972	1		2	0.035	0.034	0.036	0.041	-6	
	3	0.987	0.983	0.981	0.976	-1		3	0.035	0.033	0.036	0.040	-6	
	Mean	0.985	0.982	0.978	0.975	0		Mean	0.038	0.034	0.036	0.041	-9	
3							3							
Mean for 3 assays		-	-	-	-	3	Mean for 3 assays		-	-	-	-	-9	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
 Negative : Singlet oxygen results <25 and Superoxide anion results <25

Appendix 7 Irradiance and temperature during the irradiation in the Phase 2 study

Laboratory 4

Experimental No. (AKP-)			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	4.9	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	
		B	4.0	3.9	3.9	4.0	3.9	4.0	3.9	4.0	3.9	4.0	3.9	4.0	3.9	4.0	3.9	
	End of Irradiation	A	4.9	4.9	4.9	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
		B	4.0	4.0	4.0	3.9	4.0	3.9	4.0	3.9	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.9
Temperature (°C)	Beginning of Irradiation		25	28	27	27	27	28	27	25	27	26	26	28	27	29	27	
	End of Irradiation		27	28	28	28	27	27	25	26	27	26	26	28	27	26	27	

Experimental No. (AKP-)			16	17	18	19	21	22	23	24	25	26	27	28	29	30
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
		B	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9
	End of Irradiation	A	4.8	4.8	4.8	4.8	4.9	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8	4.8
		B	3.9	3.9	3.9	3.9	4.0	3.9	3.9	3.9	3.9	3.9	3.9	3.9	4.0	3.9
Temperature (°C)	Beginning of Irradiation		26	28	26	28	28	25	28	27	28	26	28	27	28	25
	End of Irradiation		26	28	26	28	28	25	27	27	26	26	27	27	27	28

A : Irradiances which were measured with each test facility's UVA detector.

B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr.Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the values

$B=A \times f - 0.0807$ f : correlation factor on the values of the UVA detectors (= 0.8343)

Thermometer : RT-100 (AS ONE)

Laboratory 5

Experimental No. (AS-)			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
		B	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	End of Irradiation	A	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
		B	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Temperature (°C)	Beginning of Irradiation		25	26	25	25	25	25	26	26	26	26	25	26	26	26	26
	End of Irradiation		25	24	25	25	26	25	26	26	25	25	25	26	26	25	25

Experimental No. (AS-)			16	17	18	19	20	21	22
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	4.7	4.7	4.7	4.7	4.7	4.7	4.7
		B	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	End of Irradiation	A	4.7	4.7	4.7	4.7	4.7	4.7	4.7
		B	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Temperature (°C)	Beginning of Irradiation		25	25	25	25	25	25	
	End of Irradiation		24	25	26	25	25	25	

A : Irradiances which were measured with each test facility's UVA detector.

B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr.Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the values

$B=A \times f - 0.0105$ f : correlation factor on the values of the UVA detectors (=1.0621)

Thermometer : THERMOMETER NTS9888 (ASONE)

Appendix 7 Irradiance and temperature during the irradiation in the Phase 2 study

Laboratory 6

Experimental No. (SI-)			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16		
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	
		B	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
	End of Irradiation	A	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
		B	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Temperature (°C)	Beginning of Irradiation		25.0	24.5	24.5	25.5	25.0	25.0	25.0	25.0	25.5	25.0	26.0	26.0	26.0	25.0	25.0	25.0	25.0	
	End of Irradiation		26.0	25.0	26.0	26.5	26.0	25.5	25.5	25.5	26.5	25.5	26.5	27.0	26.5	25.5	25.5	25.5	25.5	

A : Irradiances which were measured with each test facility's UVA detector.
 B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr.Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the values
 $B=A \times f + 0.008$ f : correlation factor on the values of the UVA detectors (= 1.053)
 Thermometer : TOYAMA KANSHITSUKEI (ANDO KEIKI)

Laboratory 7

Experimental No. (TS-)			20	21	22	23	24	26	27	28	29	30	31	32	33	34
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	3.7	3.5	3.7	3.5	3.7	3.7	3.5	3.7	3.5	3.7	3.5	3.5	3.6	3.5
		B	3.2	3.0	3.2	3.0	3.2	3.2	3.0	3.2	3.0	3.2	3.0	3.0	3.1	3.0
	End of Irradiation	A	3.7	3.6	3.7	3.5	3.7	3.7	3.6	3.7	3.5	3.7	3.6	3.5	3.6	3.5
		B	3.2	3.1	3.2	3.0	3.2	3.2	3.1	3.2	3.0	3.2	3.1	3.0	3.1	3.0
Temperature (°C)	Beginning of Irradiation		27.7	27.7	27.2	27.2	26.8	26.7	26.7	27.8	27.8	27.4	27.4	27.4	27.4	27.1
	End of Irradiation		26.5	26.5	27.3	27.3	26.8	27.2	27.2	27.4	27.4	28.2	28.2	27.1	27.1	27.8

A : Irradiances which were measured with each test facility's UVA detector.
 B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr.Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the values
 $B=A \times f - 0.21$ f : correlation factor on the values of the UVA detectors (=0.91)
 Thermometer : RTR72 (T&D)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-01	1	0.938	0.954	0.447	0.951	488	AKP-01	1	0.028	0.027	0.276	0.033	242
	2	0.939	0.949	0.467	0.946	469		2	0.028	0.027	0.267	0.033	233
	3	0.940	0.959	0.478	0.956	459		3	0.028	0.027	0.273	0.034	239
	Mean	0.939	0.954	0.464	0.951	472		Mean	0.028	0.027	0.272	0.033	238
AKP-02	1	0.956	0.979	0.468	0.976	485	AKP-02	1	0.028	0.028	0.296	0.033	262
	2	0.961	0.986	0.495	0.982	463		2	0.028	0.027	0.287	0.033	253
	3	0.962	0.984	0.504	0.981	455		3	0.028	0.028	0.297	0.035	263
	Mean	0.960	0.983	0.489	0.980	468		Mean	0.028	0.028	0.293	0.034	259
AKP-03	1	0.978	0.985	0.478	0.983	497	AKP-03	1	0.028	0.027	0.297	0.034	262
	2	0.978	1.007	0.499	1.005	476		2	0.027	0.027	0.290	0.033	256
	3	0.978	1.005	0.509	1.001	466		3	0.028	0.028	0.299	0.035	264
	Mean	0.978	0.999	0.495	0.996	480		Mean	0.028	0.027	0.295	0.034	261
AKP-04	1	0.972	0.988	0.465	0.985	504	AKP-04	1	0.028	0.027	0.297	0.034	262
	2	0.975	1.001	0.489	0.998	483		2	0.027	0.026	0.287	0.033	253
	3	0.976	1.002	0.501	0.998	472		3	0.028	0.027	0.295	0.035	260
	Mean	0.974	0.997	0.485	0.994	486		Mean	0.028	0.027	0.293	0.034	258
AKP-05	1	0.961	0.977	0.450	0.974	508	AKP-05	1	0.027	0.027	0.279	0.038	244
	2	0.957	0.979	0.475	0.976	479		2	0.027	0.027	0.272	0.033	237
	3	0.966	0.988	0.494	0.984	469		3	0.028	0.027	0.275	0.034	239
	Mean	0.961	0.981	0.473	0.978	485		Mean	0.027	0.027	0.275	0.035	240
AKP-06	1	0.964	0.981	0.474	0.979	488	AKP-06	1	0.027	0.026	0.305	0.033	271
	2	0.967	0.985	0.496	0.982	469		2	0.030	0.026	0.297	0.033	260
	3	0.968	0.984	0.506	0.981	460		3	0.028	0.027	0.303	0.034	268
	Mean	0.966	0.983	0.492	0.981	472		Mean	0.028	0.026	0.302	0.033	266

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-07	1	0.971	0.992	0.482	0.984	483	AKP-07	1	0.029	0.028	0.321	0.033	269
	2	0.979	0.997	0.506	0.992	467		2	0.028	0.027	0.309	0.032	258
	3	0.982	0.995	0.516	0.991	460		3	0.030	0.028	0.320	0.089	267
	Mean	0.977	0.995	0.501	0.989	470		Mean	0.029	0.028	0.317	0.051	265
AKP-08	1	0.971	0.993	0.490	0.990	478	AKP-08	1	0.028	0.027	0.290	0.033	256
	2	0.979	0.989	0.516	0.985	460		2	0.027	0.027	0.283	0.033	250
	3	0.985	0.988	0.527	0.985	455		3	0.028	0.027	0.294	0.034	260
	Mean	0.978	0.990	0.511	0.987	464		Mean	0.028	0.027	0.289	0.033	255
AKP-09	1	0.974	0.987	0.464	0.982	506	AKP-09	1	0.028	0.028	0.298	0.033	265
	2	0.974	0.988	0.486	0.983	484		2	0.028	0.028	0.287	0.033	254
	3	0.975	0.987	0.496	0.983	475		3	0.029	0.028	0.304	0.034	270
	Mean	0.974	0.987	0.482	0.983	488		Mean	0.028	0.028	0.296	0.033	263
AKP-10	1	0.962	0.967	0.482	0.966	477	AKP-10	1	0.028	0.027	0.273	0.033	238
	2	0.966	0.969	0.506	0.966	457		2	0.027	0.026	0.270	0.033	236
	3	0.972	0.973	0.521	0.969	448		3	0.028	0.028	0.281	0.035	246
	Mean	0.967	0.970	0.503	0.967	461		Mean	0.028	0.027	0.275	0.034	240
AKP-11	1	0.973	0.970	0.572	0.966	398	AKP-11	1	0.028	0.027	0.259	0.032	226
	2	0.967	0.978	0.591	0.976	373		2	0.028	0.027	0.253	0.032	220
	3	0.973	0.977	0.606	0.973	364		3	0.029	0.028	0.261	0.033	227
	Mean	0.971	0.975	0.590	0.972	378		Mean	0.028	0.027	0.258	0.032	224
AKP-12	1	0.971	0.986	0.499	0.984	469	AKP-12	1	0.028	0.027	0.272	0.033	238
	2	0.968	0.983	0.520	0.977	445		2	0.027	0.027	0.263	0.032	230
	3	0.969	0.984	0.537	0.981	429		3	0.028	0.027	0.278	0.034	244
	Mean	0.969	0.984	0.519	0.981	448		Mean	0.028	0.027	0.271	0.033	237

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results* ¹	Experimental No.	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-13	1	0.973	0.985	0.500	0.980	469	AKP-13	1	0.029	0.027	0.275	0.033	240
	2	0.970	0.986	0.523	0.984	443		2	0.028	0.026	0.267	0.033	233
	3	0.970	0.992	0.537	0.989	429		3	0.028	0.027	0.283	0.034	249
	Mean	0.971	0.988	0.520	0.984	447		Mean	0.028	0.027	0.275	0.033	241
AKP-14	1	0.959	0.959	0.451	0.956	505	AKP-14	1	0.029	0.028	0.303	0.034	268
	2	0.963	0.973	0.481	0.971	479		2	0.029	0.027	0.289	0.033	254
	3	0.962	0.974	0.495	0.972	464		3	0.029	0.028	0.291	0.035	256
	Mean	0.961	0.969	0.476	0.966	483		Mean	0.029	0.028	0.294	0.034	259
AKP-15	1	0.951	0.971	0.449	0.970	499	AKP-15	1	0.028	0.027	0.295	0.034	260
	2	0.954	0.965	0.471	0.959	480		2	0.029	0.027	0.285	0.034	249
	3	0.957	0.965	0.484	0.962	470		3	0.029	0.027	0.296	0.035	260
	Mean	0.954	0.967	0.468	0.964	483		Mean	0.029	0.027	0.292	0.034	256
AKP-16	1	0.967	0.984	0.461	0.981	503	AKP-16	1	0.029	0.028	0.297	0.033	262
	2	0.968	0.985	0.489	0.981	476		2	0.028	0.027	0.285	0.033	251
	3	0.969	0.984	0.500	0.982	466		3	0.028	0.027	0.297	0.033	263
	Mean	0.968	0.984	0.483	0.981	482		Mean	0.028	0.027	0.293	0.033	259
AKP-17	1	0.989	1.009	0.454	1.004	531	AKP-17	1	0.029	0.027	0.311	0.044	265
	2	0.993	1.001	0.478	0.997	511		2	0.029	0.027	0.301	0.043	255
	3	0.993	1.009	0.489	1.005	500		3	0.029	0.028	0.318	0.046	272
	Mean	0.992	1.006	0.474	1.002	514		Mean	0.029	0.027	0.310	0.044	264
AKP-18	1	0.977	0.995	0.490	0.992	484	AKP-18	1	0.029	0.026	0.284	0.040	242
	2	0.976	0.995	0.512	0.991	461		2	0.028	0.027	0.273	0.039	232
	3	0.979	0.993	0.524	0.990	452		3	0.028	0.027	0.285	0.041	244
	Mean	0.977	0.994	0.509	0.991	466		Mean	0.028	0.027	0.281	0.040	239

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-19	1	0.959	0.967	0.419	0.965	536	AKP-19	1					
	2	0.968	0.986	0.444	0.982	520		2					
	3	0.968	0.986	0.455	0.982	509		3					
	Mean	0.965	0.980	0.439	0.976	522		Mean					
AKP-21	1	0.973	0.993	0.456	0.991	514	AKP-21	1	0.028	0.027	0.260	0.040	219
	2	0.969	0.993	0.479	0.988	487		2	0.028	0.027	0.258	0.039	217
	3	0.972	0.992	0.491	0.990	478		3	0.029	0.028	0.264	0.040	222
	Mean	0.971	0.993	0.475	0.990	493		Mean	0.028	0.027	0.261	0.040	219
AKP-22	1	0.987	0.987	0.491	0.968	487	AKP-22	1	0.028	0.027	0.277	0.039	237
	2	0.987	1.006	0.515	1.002	463		2	0.028	0.027	0.269	0.038	229
	3	0.990	1.008	0.527	1.003	454		3	0.029	0.028	0.281	0.041	240
	Mean	0.988	1.000	0.511	0.991	468		Mean	0.028	0.027	0.276	0.039	235
AKP-23	1	0.985	1.005	0.462	1.000	518	AKP-23	1	0.029	0.028	0.309	0.045	262
	2	0.987	1.007	0.486	1.003	496		2	0.028	0.027	0.300	0.045	254
	3	0.990	1.008	0.497	1.004	488		3	0.029	0.028	0.308	0.047	261
	Mean	0.987	1.007	0.482	1.002	501		Mean	0.029	0.028	0.306	0.046	259
AKP-24	1	0.974	1.001	0.464	0.996	505	AKP-24	1	0.030	0.028	0.300	0.042	257
	2	0.988	1.004	0.492	0.998	491		2	0.032	0.029	0.295	0.041	250
	3	0.992	1.004	0.502	0.999	485		3	0.036	0.032	0.312	0.045	263
	Mean	0.985	1.003	0.486	0.998	494		Mean	0.033	0.030	0.302	0.043	257

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-25	1	0.964	0.980	0.464	0.979	498	AKP-25	1	0.028	0.027	0.278	0.040	237
	2	0.974	0.987	0.490	0.985	482		2	0.027	0.027	0.274	0.039	234
	3	0.975	0.987	0.502	0.984	471		3	0.028	0.028	0.287	0.041	246
	Mean	0.971	0.985	0.485	0.983	484		Mean	0.028	0.027	0.280	0.040	239
AKP-026	1	0.976	0.991	0.469	0.989	505	AKP-026	1	0.028	0.028	0.267	0.040	228
	2	0.985	0.999	0.503	0.998	480		2	0.027	0.028	0.261	0.038	223
	3	0.988	0.999	0.515	0.995	471		3	0.030	0.027	0.274	0.039	233
	Mean	0.983	0.996	0.496	0.994	485		Mean	0.028	0.028	0.267	0.039	228
AKP-027	1	0.970	0.986	0.452	0.984	516	AKP-027	1	0.028	0.027	0.288	0.039	248
	2	0.970	0.988	0.476	0.985	492		2	0.027	0.027	0.283	0.038	244
	3	0.972	0.991	0.488	0.989	482		3	0.028	0.028	0.293	0.040	253
	Mean	0.971	0.988	0.472	0.986	497		Mean	0.028	0.027	0.288	0.039	248
AKP-028	1	0.964	0.974	0.463	0.972	498	AKP-028	1	0.030	0.028	0.300	0.040	258
	2	0.974	0.989	0.491	0.987	480		2	0.029	0.028	0.294	0.039	253
	3	0.980	0.988	0.503	0.984	474		3	0.029	0.027	0.303	0.041	262
	Mean	0.973	0.984	0.486	0.981	484		Mean	0.029	0.028	0.299	0.040	258
AKP-029	1	0.967	0.980	0.467	0.977	498	AKP-029	1	0.028	0.027	0.281	0.038	241
	2	0.970	0.989	0.492	0.987	476		2	0.028	0.026	0.279	0.038	239
	3	0.973	0.987	0.503	0.985	468		3	0.030	0.028	0.289	0.040	247
	Mean	0.970	0.985	0.487	0.983	481		Mean	0.029	0.027	0.283	0.039	242
AKP-030	1	0.961	0.978	0.421	0.975	537	AKP-030	1	/				
	2	0.979	0.996	0.442	0.993	534		2					
	3	0.979	0.994	0.421	0.990	555		3					
	Mean	0.973	0.989	0.428	0.986	542		Mean					
Mean for all assays	-	-	-	-	479	Mean for all assays	-	-	-	-	248		
SD for all assays	-	-	-	-	28	SD for all assays	-	-	-	-	13		
CV for all assays	-	-	-	-	5.8	CV for all assays	-	-	-	-	5.2		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank			
AKP-01	1	0.940	0.954	0.944	0.951	-7	AKP-01	1	0.033	0.027	0.033	0.033	-6
	2	0.957	0.949	0.955	0.946	-1		2	0.032	0.027	0.032	0.033	-6
	3	0.955	0.959	0.954	0.956	-2		3	0.033	0.027	0.034	0.034	-5
	Mean	0.951	0.954	0.951	0.951	-3		Mean	0.033	0.027	0.033	0.033	-6
AKP-02	1	0.981	0.979	0.980	0.976	-2	AKP-02	1	0.032	0.028	0.033	0.033	-5
	2	0.986	0.986	0.985	0.982	-2		2	0.032	0.027	0.032	0.033	-6
	3	0.984	0.984	0.982	0.981	-1		3	0.033	0.028	0.034	0.035	-5
	Mean	0.984	0.983	0.982	0.980	-2		Mean	0.032	0.028	0.033	0.034	-5
AKP-03	1	0.993	0.985	0.991	0.983	-1	AKP-03	1	0.033	0.027	0.033	0.034	-7
	2	1.001	1.007	1.000	1.005	-2		2	0.032	0.027	0.032	0.033	-7
	3	1.000	1.005	0.998	1.001	-1		3	0.033	0.028	0.034	0.035	-6
	Mean	0.998	0.999	0.996	0.996	-1		Mean	0.033	0.027	0.033	0.034	-7
AKP-04	1	0.992	0.988	0.978	0.985	11	AKP-04	1	0.032	0.027	0.033	0.034	-6
	2	0.998	1.001	0.996	0.998	-1		2	0.032	0.026	0.033	0.033	-6
	3	0.999	1.002	0.996	0.998	0		3	0.033	0.027	0.033	0.035	-7
	Mean	0.996	0.997	0.990	0.994	3		Mean	0.032	0.027	0.033	0.034	-6
AKP-05	1	0.985	0.977	0.983	0.974	-1	AKP-05	1	0.032	0.027	0.033	0.038	-7
	2	0.980	0.979	0.980	0.976	-3		2	0.033	0.027	0.033	0.033	-8
	3	0.987	0.988	0.985	0.984	-1		3	0.033	0.027	0.034	0.034	-7
	Mean	0.984	0.981	0.983	0.978	-2		Mean	0.033	0.027	0.033	0.035	-7
AKP-06	1	0.983	0.981	0.964	0.979	17	AKP-06	1	0.032	0.026	0.033	0.033	-6
	2	0.988	0.985	0.986	0.982	0		2	0.032	0.026	0.032	0.033	-7
	3	0.987	0.984	0.983	0.981	2		3	0.033	0.027	0.033	0.034	-7
	Mean	0.986	0.983	0.978	0.981	6		Mean	0.032	0.026	0.033	0.033	-7

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-07	1	0.989	0.992	0.987	0.984	-4	AKP-07	1	0.032	0.028	0.033	0.033	-22
	2	0.990	0.997	0.988	0.992	-4		2	0.033	0.027	0.033	0.032	-23
	3	0.988	0.995	0.985	0.991	-3		3	0.033	0.028	0.034	0.089	-22
	Mean	0.989	0.995	0.987	0.989	-4		Mean	0.033	0.028	0.033	0.051	-22
AKP-08	1	0.993	0.993	0.988	0.990	2	AKP-08	1	0.033	0.027	0.033	0.033	-6
	2	0.997	0.989	0.993	0.985	1		2	0.032	0.027	0.033	0.033	-5
	3	0.997	0.988	0.992	0.985	2		3	0.033	0.027	0.033	0.034	-6
	Mean	0.996	0.990	0.991	0.987	2		Mean	0.033	0.027	0.033	0.033	-6
AKP-09	1	0.982	0.987	0.980	0.982	-2	AKP-09	1	0.033	0.028	0.033	0.033	-5
	2	0.997	0.988	0.995	0.983	-2		2	0.032	0.028	0.033	0.033	-4
	3	0.996	0.987	0.994	0.983	-2		3	0.033	0.028	0.034	0.034	-4
	Mean	0.992	0.987	0.990	0.983	-2		Mean	0.033	0.028	0.033	0.033	-4
AKP-10	1	0.984	0.967	0.981	0.966	0	AKP-10	1	0.033	0.027	0.033	0.033	-7
	2	0.981	0.969	0.979	0.966	-1		2	0.032	0.026	0.032	0.033	-7
	3	0.980	0.973	0.978	0.969	-1		3	0.033	0.028	0.034	0.035	-6
	Mean	0.982	0.970	0.979	0.967	-1		Mean	0.033	0.027	0.033	0.034	-7
AKP-11	1	0.984	0.970	0.982	0.966	-1	AKP-11	1	0.033	0.027	0.033	0.032	-5
	2	0.983	0.978	0.982	0.976	-2		2	0.032	0.027	0.033	0.032	-4
	3	0.983	0.977	0.982	0.973	-2		3	0.034	0.028	0.033	0.033	-6
	Mean	0.983	0.975	0.982	0.972	-2		Mean	0.033	0.027	0.033	0.032	-5
AKP-12	1	0.988	0.986	0.986	0.984	-1	AKP-12	1	0.033	0.027	0.033	0.033	-6
	2	0.984	0.983	0.981	0.977	0		2	0.033	0.027	0.032	0.032	-7
	3	0.981	0.984	0.980	0.981	-2		3	0.033	0.027	0.034	0.034	-5
	Mean	0.984	0.984	0.982	0.981	-1		Mean	0.033	0.027	0.033	0.033	-6

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-13	1	0.997	0.985	0.994	0.980	-1	AKP-13	1	0.033	0.027	0.033	0.033	-6
	2	0.996	0.986	0.994	0.984	-2		2	0.032	0.026	0.034	0.033	-4
	3	0.997	0.992	0.995	0.989	-2		3	0.033	0.027	0.034	0.034	-5
	Mean	0.997	0.988	0.994	0.984	-2		Mean	0.033	0.027	0.034	0.033	-5
AKP-14	1	0.988	0.959	0.985	0.956	0	AKP-14	1	0.033	0.028	0.033	0.034	-6
	2	0.988	0.973	0.985	0.971	0		2	0.032	0.027	0.032	0.033	-6
	3	0.986	0.974	0.983	0.972	0		3	0.034	0.028	0.034	0.035	-6
	Mean	0.987	0.969	0.984	0.966	0		Mean	0.033	0.028	0.033	0.034	-6
AKP-15	1	1.000	0.971	0.998	0.970	-1	AKP-15	1	0.033	0.027	0.033	0.034	-7
	2	1.013	0.965	1.010	0.959	0		2	0.032	0.027	0.033	0.034	-6
	3	1.021	0.965	1.020	0.962	-2		3	0.034	0.027	0.033	0.035	-8
	Mean	1.011	0.967	1.009	0.964	-1		Mean	0.033	0.027	0.033	0.034	-7
AKP-16	1	0.988	0.984	0.985	0.981	0	AKP-16	1	0.032	0.028	0.033	0.033	-5
	2	0.985	0.985	0.983	0.981	-1		2	0.032	0.027	0.033	0.033	-5
	3	0.984	0.984	0.982	0.982	-1		3	0.033	0.027	0.033	0.033	-6
	Mean	0.986	0.984	0.983	0.981	-1		Mean	0.032	0.027	0.033	0.033	-5
AKP-17	1	1.016	1.009	1.013	1.004	-1	AKP-17	1	0.035	0.027	0.035	0.044	-17
	2	1.014	1.001	1.012	0.997	-2		2	0.033	0.027	0.033	0.043	-17
	3	1.014	1.009	1.012	1.005	-2		3	0.034	0.028	0.034	0.046	-17
	Mean	1.015	1.006	1.012	1.002	-2		Mean	0.034	0.027	0.034	0.044	-17
AKP-18	1	0.997	0.995	0.994	0.992	0	AKP-18	1	0.032	0.026	0.033	0.040	-12
	2	0.995	0.995	0.994	0.991	-2		2	0.033	0.027	0.033	0.039	-13
	3	0.988	0.993	0.986	0.990	-1		3	0.033	0.027	0.034	0.041	-12
	Mean	0.993	0.994	0.991	0.991	-1		Mean	0.033	0.027	0.033	0.040	-12

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-19	1	0.987	0.967	0.984	0.965	-1	AKP-19	1					
	2	0.983	0.986	0.979	0.982	0		2					
	3	0.980	0.986	0.978	0.982	-2		3					
	Mean	0.983	0.980	0.980	0.976	-1		Mean					
AKP-21	1	0.995	0.993	0.993	0.991	-1	AKP-21	1	0.032	0.027	0.033	0.040	-12
	2	0.993	0.993	0.991	0.988	-1		2	0.032	0.027	0.034	0.039	-11
	3	0.992	0.992	0.992	0.990	-3		3	0.033	0.028	0.033	0.040	-13
	Mean	0.993	0.993	0.992	0.990	-2		Mean	0.032	0.027	0.033	0.040	-12
AKP-22	1	1.008	0.987	1.003	0.968	-4	AKP-22	1	0.033	0.027	0.033	0.039	-12
	2	1.003	1.006	1.002	1.002	-8		2	0.033	0.027	0.033	0.038	-12
	3	1.003	1.008	1.001	1.003	-7		3	0.034	0.028	0.036	0.041	-10
	Mean	1.005	1.000	1.002	0.991	-6		Mean	0.033	0.027	0.034	0.039	-11
AKP-23	1	1.016	1.009	1.013	1.004	-1	AKP-23	1	0.033	0.028	0.033	0.045	-18
	2	1.014	1.001	1.012	0.997	-2		2	0.033	0.027	0.033	0.045	-18
	3	1.014	1.009	1.012	1.005	-2		3	0.033	0.028	0.034	0.047	-17
	Mean	1.015	1.006	1.012	1.002	-2		Mean	0.033	0.028	0.033	0.046	-18
AKP-24	1	1.000	1.001	0.997	0.996	-2	AKP-24	1	0.034	0.028	0.034	0.042	-13
	2	1.009	1.004	1.006	0.998	-2		2	0.033	0.029	0.033	0.041	-13
	3	1.009	1.004	1.005	0.999	-1		3	0.034	0.032	0.035	0.045	-12
	Mean	1.006	1.003	1.003	0.998	-2		Mean	0.034	0.030	0.034	0.043	-13

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 4
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AKP-25	1	0.978	0.980	0.976	0.979	0	AKP-25	1	0.033	0.027	0.033	0.040	-13
	2	0.993	0.987	0.991	0.985	0		2	0.032	0.027	0.033	0.039	-12
	3	0.989	0.987	0.987	0.984	0		3	0.034	0.028	0.034	0.041	-13
	Mean	0.987	0.985	0.985	0.983	0		Mean	0.033	0.027	0.033	0.040	-13
AKP-26	1	0.996	0.991	0.993	0.989	1	AKP-26	1	0.033	0.028	0.033	0.040	-11
	2	0.998	0.999	0.995	0.998	1		2	0.032	0.028	0.032	0.038	-11
	3	1.004	0.999	1.000	0.995	2		3	0.033	0.027	0.035	0.039	-9
	Mean	0.999	0.996	0.996	0.994	1		Mean	0.033	0.028	0.033	0.039	-10
AKP-27	1	0.989	0.986	0.987	0.984	0	AKP-27	1	0.033	0.027	0.033	0.039	-12
	2	0.992	0.988	0.991	0.985	-1		2	0.032	0.027	0.032	0.038	-12
	3	0.990	0.991	0.988	0.989	0		3	0.033	0.028	0.033	0.040	-12
	Mean	0.990	0.988	0.989	0.986	0		Mean	0.033	0.027	0.033	0.039	-12
AKP-28	1	0.993	0.974	0.990	0.972	0	AKP-28	1	0.037	0.028	0.038	0.040	-11
	2	0.987	0.989	0.985	0.987	-1		2	0.036	0.028	0.038	0.039	-10
	3	0.988	0.988	0.985	0.984	0		3	0.033	0.027	0.034	0.041	-11
	Mean	0.989	0.984	0.987	0.981	0		Mean	0.035	0.028	0.037	0.040	-11
AKP-29	1	0.987	0.980	0.985	0.977	0	AKP-29	1	0.032	0.027	0.033	0.038	-11
	2	0.991	0.989	0.989	0.987	0		2	0.033	0.026	0.032	0.038	-13
	3	0.989	0.987	0.986	0.985	1		3	0.034	0.028	0.034	0.040	-12
	Mean	0.989	0.985	0.987	0.983	0		Mean	0.033	0.027	0.033	0.039	-12
AKP-30	1	0.982	0.978	0.980	0.975	-1	AKP-30	1	/				
	2	0.993	0.996	0.990	0.993	0							
	3	0.991	0.994	0.988	0.990	0							
	Mean	0.989	0.989	0.986	0.986	0							
Mean for all assays		-	-	-	-	-1	Mean for all assays		-	-	-	-	-9
SD for all assays		-	-	-	-	2	SD for all assays		-	-	-	-	5
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 5
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AS-01	1	0.996	1.014	0.489	1.010	503	AS-01	1	0.034	0.034	0.295	0.038	256
	2	0.997	1.012	0.494	1.008	499		2	0.034	0.034	0.293	0.039	254
	3	0.993	1.009	0.486	1.005	503		3	0.034	0.034	0.314	0.040	275
	Mean	0.995	1.012	0.490	1.008	502		Mean	0.034	0.034	0.300	0.039	262
AS-02	1	1.012	1.025	0.533	1.023	474	AS-02	1	0.034	0.034	0.292	0.039	253
	2	1.013	1.026	0.533	1.022	476		2	0.034	0.034	0.284	0.039	245
	3	1.008	1.031	0.527	1.022	477		3	0.034	0.043	0.299	0.047	260
	Mean	1.011	1.027	0.531	1.022	476		Mean	0.034	0.037	0.292	0.042	253
AS-03	1	0.983	1.001	0.424	0.997	554	AS-03	1	0.034	0.034	0.301	0.038	263
	2	0.985	1.006	0.435	1.002	545		2	0.034	0.034	0.287	0.038	250
	3	0.983	1.009	0.437	1.005	541		3	0.033	0.034	0.303	0.039	266
	Mean	0.984	1.006	0.432	1.001	547		Mean	0.034	0.034	0.297	0.038	260
AS-04	1	1.000	1.018	0.437	1.014	560	AS-04	1	0.034	0.034	0.297	0.040	257
	2	0.999	1.019	0.442	1.014	552		2	0.034	0.034	0.294	0.041	254
	3	1.006	1.017	0.445	1.013	557		3	0.034	0.033	0.308	0.040	268
	Mean	1.002	1.018	0.441	1.014	556		Mean	0.034	0.034	0.300	0.040	260
AS-05	1	1.001	1.014	0.475	1.009	522	AS-05	1	0.034	0.034	0.281	0.038	243
	2	0.999	1.013	0.488	1.009	507		2	0.034	0.034	0.307	0.038	269
	3	0.995	1.018	0.473	1.014	518		3	0.033	0.034	0.320	0.039	283
	Mean	0.998	1.015	0.479	1.011	516		Mean	0.034	0.034	0.302	0.038	265
AS-06	1	1.005	1.008	0.483	1.005	517	AS-06	1	0.034	0.033	0.311	0.039	270
	2	1.001	1.012	0.480	1.007	517		2	0.033	0.033	0.290	0.039	249
	3	0.996	1.011	0.476	1.005	515		3	0.033	0.034	0.296	0.040	256
	Mean	1.000	1.010	0.480	1.006	516		Mean	0.033	0.033	0.299	0.040	258

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 5
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results* ¹	Experimental No.	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AS-07	1	0.996	1.011	0.432	1.007	560	AS-07	1	0.034	0.034	0.298	0.041	258
	2	0.995	1.016	0.436	1.011	554		2	0.033	0.034	0.299	0.040	259
	3	0.992	1.015	0.440	1.011	549		3	0.034	0.034	0.312	0.040	272
	Mean	0.994	1.014	0.436	1.010	554		Mean	0.034	0.034	0.303	0.040	263
AS-08	1	1.002	1.005	0.406	1.003	593	AS-08	1	0.034	0.034	0.302	0.040	262
	2	0.998	1.004	0.414	0.998	581		2	0.033	0.034	0.313	0.040	273
	3	0.998	1.013	0.425	1.010	570		3	0.034	0.033	0.320	0.040	281
	Mean	0.999	1.007	0.415	1.004	581		Mean	0.034	0.034	0.312	0.040	272
AS-09	1	0.987	1.006	0.382	0.999	598	AS-09	1	0.034	0.033	0.301	0.042	258
	2	0.989	1.006	0.392	0.999	589		2	0.034	0.034	0.316	0.042	274
	3	0.986	1.006	0.390	0.996	587		3	0.033	0.034	0.331	0.043	289
	Mean	0.987	1.006	0.388	0.998	591		Mean	0.033	0.033	0.316	0.042	274
AS-10	1	0.990	1.004	0.438	0.994	545	AS-10	1	0.034	0.034	0.351	0.041	309
	2	0.993	1.002	0.447	0.996	539		2	0.034	0.034	0.349	0.042	307
	3	0.988	1.008	0.446	1.003	535		3	0.033	0.034	0.371	0.042	330
	Mean	0.990	1.005	0.444	0.998	540		Mean	0.034	0.034	0.357	0.042	315
AS-11	1	0.985	0.991	0.426	0.989	563	AS-11	1	0.034	0.034	0.307	0.041	267
	2	0.985	0.998	0.430	0.995	559		2	0.033	0.034	0.305	0.042	265
	3	0.989	0.999	0.453	1.015	540		3	0.034	0.033	0.318	0.040	277
	Mean	0.986	0.996	0.436	1.000	554		Mean	0.034	0.034	0.310	0.041	270
AS-12	1	0.980	0.997	0.427	0.992	548	AS-12	1	0.034	0.034	0.279	0.040	235
	2	0.983	0.998	0.432	0.993	546		2	0.034	0.034	0.285	0.052	241
	3	0.987	0.997	0.467	0.991	514		3	0.033	0.034	0.298	0.040	255
	Mean	0.983	0.997	0.442	0.992	536		Mean	0.034	0.034	0.287	0.044	244

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 5
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AS-13	1	0.993	1.009	0.451	1.002	536	AS-13	1	0.034	0.034	0.300	0.047	257
	2	0.991	1.004	0.448	0.998	536		2	0.033	0.034	0.281	0.041	239
	3	0.997	1.014	0.459	1.008	532		3	0.034	0.033	0.295	0.040	252
	Mean	0.993	1.009	0.453	1.003	535		Mean	0.034	0.034	0.292	0.043	249
AS-14	1	1.002	1.011	0.431	1.009	567	AS-14	1					
	2	1.000	1.013	0.431	1.009	565		2					
	3	0.998	1.011	0.401	1.006	593		3					
	Mean	1.000	1.012	0.421	1.008	575		Mean					
AS-15	1	0.992	1.003	0.415	0.999	579	AS-15	1	0.034	0.034	0.299	0.041	259
	2	0.988	1.002	0.425	0.997	564		2	0.034	0.034	0.279	0.041	239
	3	0.986	1.003	0.415	1.016	573		3	0.034	0.033	0.307	0.041	267
	Mean	0.988	1.002	0.418	1.004	572		Mean	0.034	0.034	0.295	0.041	255
AS-16	1	0.965	1.003	0.414	1.001	548	AS-16	1	0.034	0.034	0.294	0.040	248
	2	0.965	1.011	0.417	1.008	545		2	0.034	0.034	0.298	0.058	252
	3	0.956	1.012	0.412	1.008	541		3	0.034	0.034	0.343	0.040	297
	Mean	0.962	1.009	0.414	1.006	545		Mean	0.034	0.034	0.311	0.046	266
AS-17	1	0.994	1.007	0.470	1.002	519	AS-17	1	0.034	0.033	0.285	0.038	246
	2	0.994	1.014	0.470	1.008	520		2	0.034	0.034	0.297	0.039	259
	3	0.991	1.013	0.465	1.008	521		3	0.033	0.034	0.315	0.039	277
	Mean	0.993	1.011	0.468	1.006	520		Mean	0.034	0.034	0.299	0.039	261
AS-18	1	0.987	1.003	0.415	0.998	567	AS-18	1	0.034	0.034	0.285	0.040	244
	2	0.996	1.007	0.424	1.003	567		2	0.034	0.034	0.297	0.041	256
	3	0.990	1.002	0.424	0.997	561		3	0.033	0.034	0.309	0.041	269
	Mean	0.991	1.004	0.421	0.999	565		Mean	0.034	0.034	0.297	0.041	256

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 5
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AS-19	1	0.999	1.004	0.455	0.998	538	AS-19	1	0.034	0.034	0.297	0.040	257
	2	0.997	1.004	0.462	0.998	528		2	0.034	0.034	0.288	0.040	249
	3	0.995	1.008	0.473	1.001	515		3	0.034	0.034	0.323	0.040	283
	Mean	0.997	1.006	0.463	0.999	527		Mean	0.034	0.034	0.303	0.040	263
AS-20	1	0.994	1.008	0.434	1.003	553	AS-20	1	0.034	0.034	0.292	0.039	254
	2	0.993	1.005	0.443	0.997	543		2	0.034	0.034	0.291	0.040	252
	3	0.995	1.004	0.450	0.998	539		3	0.034	0.033	0.309	0.039	270
	Mean	0.994	1.006	0.442	0.999	545		Mean	0.034	0.034	0.297	0.039	259
AS-21	1	0.995	1.004	0.444	1.000	547	AS-21	1	0.034	0.034	0.288	0.040	249
	2	0.994	1.003	0.452	0.999	538		2	0.034	0.034	0.290	0.040	250
	3	0.992	1.003	0.475	0.999	513		3	0.033	0.033	0.313	0.040	274
	Mean	0.994	1.003	0.457	0.999	533		Mean	0.034	0.034	0.297	0.040	258
AS-22	1	1.001	1.016	0.448	1.009	546	AS-22	1	0.034	0.034	0.283	0.040	243
	2	1.004	1.014	0.450	1.007	546		2	0.034	0.034	0.280	0.040	241
	3	1.000	1.014	0.458	1.008	535		3	0.033	0.033	0.301	0.040	262
	Mean	1.002	1.015	0.452	1.008	542		Mean	0.033	0.034	0.288	0.040	249
Mean for all assays	-	-	-	-	542	Mean for all assays	-	-	-	-	-	262	
SD for all assays	-	-	-	-	27	SD for all assays	-	-	-	-	-	14	
CV for all assays	-	-	-	-	5.0	CV for all assays	-	-	-	-	-	5.3	

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AS-01	1	1.013	1.014	1.009	1.010	0	AS-01	1	0.037	0.034	0.038	0.038	-4
	2	1.038	1.012	1.009	1.008	25		2	0.034	0.034	0.035	0.039	-4
	3	1.010	1.009	1.006	1.005	0		3	0.034	0.034	0.035	0.040	-5
	Mean	1.020	1.012	1.008	1.008	8		Mean	0.035	0.034	0.036	0.039	-4
AS-02	1	1.025	1.025	1.020	1.023	0	AS-02	1	0.035	0.034	0.050	0.039	10
	2	1.023	1.026	1.024	1.022	-6		2	0.034	0.034	0.035	0.039	-5
	3	1.027	1.031	1.022	1.022	0		3	0.035	0.043	0.035	0.047	-5
	Mean	1.025	1.027	1.022	1.022	-2		Mean	0.035	0.037	0.040	0.042	0
AS-03	1	1.005	1.001	1.000	0.997	0	AS-03	1	0.035	0.034	0.036	0.038	-4
	2	1.002	1.006	0.996	1.002	1		2	0.034	0.034	0.035	0.038	-4
	3	1.009	1.009	1.005	1.005	-1		3	0.034	0.034	0.035	0.039	-3
	Mean	1.005	1.006	1.000	1.001	0		Mean	0.035	0.034	0.035	0.038	-4
AS-04	1	1.013	1.018	1.008	1.014	1	AS-04	1	0.035	0.034	0.036	0.040	-6
	2	1.017	1.019	1.011	1.014	2		2	0.034	0.034	0.035	0.041	-5
	3	1.011	1.017	1.006	1.013	1		3	0.034	0.033	0.035	0.040	-5
	Mean	1.014	1.018	1.008	1.014	1		Mean	0.035	0.034	0.035	0.040	-5
AS-05	1	1.014	1.014	1.010	1.009	1	AS-05	1	0.035	0.034	0.036	0.038	-4
	2	1.015	1.013	1.011	1.009	1		2	0.034	0.034	0.034	0.038	-4
	3	1.009	1.018	1.003	1.014	2		3	0.034	0.034	0.035	0.039	-4
	Mean	1.013	1.015	1.008	1.011	1		Mean	0.034	0.034	0.035	0.038	-4
AS-06	1	1.016	1.008	1.011	1.005	1	AS-06	1	0.046	0.033	0.036	0.039	-17
	2	1.015	1.012	1.010	1.007	1		2	0.034	0.033	0.034	0.039	-6
	3	1.012	1.011	1.006	1.005	1		3	0.034	0.034	0.034	0.040	-7
	Mean	1.014	1.010	1.009	1.006	1		Mean	0.038	0.033	0.035	0.040	-10

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AS-07	1	1.010	1.011	1.007	1.007	-1	AS-07	1	0.035	0.034	0.035	0.041	-5
	2	1.006	1.016	1.004	1.011	-2		2	0.034	0.034	0.034	0.040	-6
	3	1.014	1.015	1.011	1.011	-1		3	0.034	0.034	0.035	0.040	-6
	Mean	1.010	1.014	1.007	1.010	-1		Mean	0.034	0.034	0.035	0.040	-6
AS-08	1	1.002	1.005	0.963	1.003	36	AS-08	1	0.035	0.034	0.036	0.040	-5
	2	1.004	1.004	1.002	0.998	0		2	0.034	0.034	0.035	0.040	-5
	3	1.004	1.013	1.001	1.010	0		3	0.034	0.033	0.035	0.040	-5
	Mean	1.003	1.007	0.989	1.004	12		Mean	0.034	0.034	0.035	0.040	-5
AS-09	1	1.011	1.006	1.005	0.999	-2	AS-09	1	0.035	0.033	0.036	0.042	-9
	2	1.008	1.006	1.002	0.999	-2		2	0.034	0.034	0.035	0.042	-9
	3	1.004	1.006	0.999	0.996	-3		3	0.034	0.034	0.035	0.043	-8
	Mean	1.008	1.006	1.002	0.998	-2		Mean	0.034	0.033	0.035	0.042	-9
AS-10	1	1.009	1.004	1.004	0.994	-2	AS-10	1	0.035	0.034	0.036	0.041	-8
	2	1.006	1.002	1.001	0.996	-2		2	0.034	0.034	0.034	0.042	-7
	3	1.008	1.008	1.003	1.003	-2		3	0.035	0.034	0.035	0.042	-8
	Mean	1.007	1.005	1.003	0.998	-2		Mean	0.035	0.034	0.035	0.042	-8
AS-11	1	0.999	0.991	0.994	0.989	9	AS-11	1	0.035	0.034	0.035	0.041	-7
	2	0.996	0.998	0.993	0.995	6		2	0.034	0.034	0.035	0.042	-7
	3	0.996	0.999	0.990	1.015	10		3	0.035	0.033	0.035	0.040	-7
	Mean	0.997	0.996	0.993	1.000	8		Mean	0.035	0.034	0.035	0.041	-7
AS-12	1	0.990	0.997	0.985	0.992	0	AS-12	1	0.035	0.034	0.036	0.040	-9
	2	0.989	0.998	0.985	0.993	-1		2	0.034	0.034	0.035	0.052	-9
	3	0.986	0.997	1.000	0.991	-19		3	0.034	0.034	0.035	0.040	-10
	Mean	0.988	0.997	0.990	0.992	-7		Mean	0.034	0.034	0.035	0.044	-9

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AS-13	1	1.013	1.009	1.006	1.002	1	AS-13	1	0.035	0.034	0.035	0.047	-8
	2	1.009	1.004	1.001	0.998	2		2	0.034	0.034	0.035	0.041	-8
	3	1.015	1.014	1.018	1.008	-9		3	0.034	0.033	0.035	0.040	-8
	Mean	1.012	1.009	1.008	1.003	-2		Mean	0.034	0.034	0.035	0.043	-8
AS-14	1	1.014	1.011	1.009	1.009	2	AS-14	1					
	2	1.014	1.013	1.010	1.009	0		2					
	3	1.019	1.011	1.004	1.006	11		3					
	Mean	1.015	1.012	1.007	1.008	4		Mean					
AS-15	1	1.006	1.003	1.001	0.999	6	AS-15	1	0.035	0.034	0.035	0.041	-7
	2	1.002	1.002	0.997	0.997	6		2	0.034	0.034	0.034	0.041	-7
	3	1.007	1.003	1.003	1.016	7		3	0.035	0.033	0.035	0.041	-7
	Mean	1.005	1.002	1.001	1.004	6		Mean	0.035	0.034	0.035	0.041	-7
AS-16	1	1.011	1.003	1.007	1.001	1	AS-16	1	0.035	0.034	0.036	0.040	-11
	2	1.009	1.011	1.005	1.008	1		2	0.034	0.034	0.035	0.058	-12
	3	1.007	1.012	1.002	1.008	2		3	0.034	0.034	0.035	0.040	-11
	Mean	1.009	1.009	1.005	1.006	1		Mean	0.035	0.034	0.035	0.046	-11
AS-17	1	1.009	1.007	1.004	1.002	0	AS-17	1	0.036	0.033	0.036	0.038	-5
	2	1.005	1.014	1.001	1.008	0		2	0.034	0.034	0.035	0.039	-5
	3	1.012	1.013	1.007	1.008	0		3	0.035	0.034	0.035	0.039	-5
	Mean	1.009	1.011	1.004	1.006	0		Mean	0.035	0.034	0.035	0.039	-5
AS-18	1	1.009	1.003	1.005	0.998	0	AS-18	1	0.035	0.034	0.036	0.040	-7
	2	1.005	1.007	1.018	1.003	-18		2	0.034	0.034	0.035	0.041	-7
	3	1.002	1.002	0.998	0.997	0		3	0.034	0.034	0.035	0.041	-6
	Mean	1.005	1.004	1.007	0.999	-6		Mean	0.035	0.034	0.035	0.041	-7

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 5
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
AS-19	1	1.009	1.004	1.003	0.998	-1	AS-19	1	0.035	0.034	0.036	0.040	-5
	2	1.003	1.004	0.996	0.998	0		2	0.034	0.034	0.035	0.040	-5
	3	1.005	1.008	1.007	1.001	-10		3	0.034	0.034	0.035	0.040	-6
	Mean	1.006	1.006	1.002	0.999	-4		Mean	0.034	0.034	0.035	0.040	-5
AS-20	1	1.005	1.008	0.999	1.003	-1	AS-20	1	0.035	0.034	0.035	0.039	-4
	2	1.004	1.005	0.998	0.997	-1		2	0.034	0.034	0.035	0.040	-5
	3	1.008	1.004	1.002	0.998	-1		3	0.034	0.033	0.035	0.039	-4
	Mean	1.006	1.006	1.000	0.999	-1		Mean	0.034	0.034	0.035	0.039	-4
AS-21	1	1.006	1.004	1.002	1.000	1	AS-21	1	0.035	0.034	0.035	0.040	-5
	2	1.005	1.003	1.001	0.999	1		2	0.034	0.034	0.052	0.040	12
	3	1.004	1.003	1.000	0.999	1		3	0.034	0.033	0.035	0.040	-5
	Mean	1.005	1.003	1.001	0.999	1		Mean	0.034	0.034	0.041	0.040	1
AS-22	1	1.014	1.016	1.002	1.009	6	AS-22	1	0.035	0.034	0.035	0.040	-5
	2	1.011	1.014	1.004	1.007	0		2	0.034	0.034	0.035	0.040	-5
	3	1.017	1.014	1.010	1.008	0		3	0.034	0.033	0.034	0.040	-6
	Mean	1.014	1.015	1.005	1.008	2		Mean	0.034	0.034	0.035	0.040	-5
Mean for all assays		-	-	-	-	1	Mean for all assays		-	-	-	-	-6
SD for all assays		-	-	-	-	5	SD for all assays		-	-	-	-	3
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-01	1	0.968	0.988	0.541	0.981	420	SI-01	1	0.035	0.035	0.328	0.044	284
	2	0.970	0.985	0.584	0.979	379		2	0.035	0.035	0.310	0.043	267
	3	0.971	0.984	0.600	0.978	365		3	0.036	0.035	0.319	0.044	275
	Mean	0.970	0.985	0.575	0.979	388		Mean	0.035	0.035	0.319	0.043	275
SI-02	1	0.975	0.989	0.564	0.977	400	SI-02	1	0.036	0.036	0.311	0.045	268
	2	0.977	0.998	0.590	0.986	375		2	0.035	0.036	0.315	0.044	272
	3	0.977	0.995	0.597	0.983	367		3	0.036	0.036	0.319	0.044	274
	Mean	0.976	0.994	0.584	0.982	381		Mean	0.036	0.036	0.315	0.044	271
SI-03	1	1.001	1.014	0.609	0.999	378	SI-03	1	0.035	0.035	0.277	0.050	227
	2	1.003	1.010	0.637	0.997	353		2	0.035	0.035	0.291	0.050	241
	3	0.999	1.010	0.644	0.994	341		3	0.036	0.035	0.298	0.050	247
	Mean	1.001	1.011	0.630	0.997	357		Mean	0.035	0.035	0.288	0.050	238
SI-04	1	0.960	0.976	0.533	0.970	421	SI-04	1	0.036	0.035	0.300	0.044	257
	2	0.962	0.976	0.562	0.971	394		2	0.035	0.036	0.302	0.044	259
	3	0.965	0.975	0.580	0.968	378		3	0.036	0.035	0.332	0.044	288
	Mean	0.962	0.976	0.559	0.970	398		Mean	0.036	0.036	0.311	0.044	268
SI-05	1	0.999	1.010	0.598	1.004	394	SI-05	1	0.035	0.035	0.322	0.043	279
	2	0.998	1.010	0.618	1.003	373		2	0.035	0.035	0.317	0.043	274
	3	0.995	1.010	0.631	1.004	357		3	0.036	0.035	0.351	0.044	307
	Mean	0.997	1.010	0.616	1.003	375		Mean	0.035	0.035	0.330	0.043	287
SI-06	1	0.971	0.985	0.569	0.978	395	SI-06	1	0.035	0.035	0.310	0.045	265
	2	0.974	0.987	0.616	0.980	351		2	0.035	0.036	0.313	0.045	268
	3	0.974	0.985	0.615	0.979	352		3	0.036	0.035	0.319	0.045	273
	Mean	0.973	0.986	0.600	0.979	366		Mean	0.035	0.035	0.314	0.045	269

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results* ¹	Experimental No.	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-07	1	0.963	0.982	0.546	0.975	411	SI-07	1	0.035	0.035	0.313	0.043	270
	2	0.965	0.979	0.578	0.974	380		2	0.035	0.035	0.307	0.043	264
	3	0.968	0.978	0.589	0.972	372		3	0.036	0.035	0.316	0.043	272
	Mean	0.966	0.980	0.571	0.973	388		Mean	0.035	0.035	0.312	0.043	269
SI-08	1	0.960	0.974	0.552	0.969	402	SI-08	1	0.036	0.036	0.304	0.044	261
	2	0.964	0.977	0.583	0.970	375		2	0.035	0.036	0.315	0.043	272
	3	0.962	0.976	0.586	0.970	370		3	0.036	0.036	0.321	0.044	277
	Mean	0.962	0.976	0.574	0.970	382		Mean	0.036	0.036	0.313	0.044	270
SI-09	1	0.955	0.966	0.501	0.954	445	SI-09	1	0.036	0.036	0.307	0.046	260
	2	0.953	0.964	0.533	0.955	412		2	0.036	0.036	0.314	0.047	267
	3	0.953	0.970	0.549	0.962	396		3	0.037	0.036	0.337	0.047	290
	Mean	0.954	0.966	0.527	0.957	418		Mean	0.036	0.036	0.319	0.047	272
SI-10	1	0.966	0.975	0.554	0.969	407	SI-10	1	0.036	0.036	0.314	0.044	270
	2	0.973	0.985	0.582	0.980	386		2	0.036	0.036	0.307	0.044	263
	3	0.973	0.984	0.587	0.979	381		3	0.036	0.036	0.318	0.045	274
	Mean	0.970	0.981	0.574	0.976	391		Mean	0.036	0.036	0.313	0.044	269
SI-11	1	0.976	0.990	0.543	0.983	425	SI-11	1	0.036	0.036	0.333	0.045	287
	2	0.983	0.987	0.571	0.980	405		2	0.035	0.036	0.324	0.045	279
	3	0.984	0.988	0.585	0.980	392		3	0.036	0.036	0.350	0.046	304
	Mean	0.981	0.988	0.567	0.981	407		Mean	0.036	0.036	0.335	0.046	290
SI-12	1	0.986	1.002	0.547	0.996	432	SI-12	1	0.035	0.035	0.342	0.045	296
	2	0.989	1.005	0.568	0.997	414		2	0.035	0.036	0.324	0.044	279
	3	0.989	1.003	0.580	0.996	402		3	0.036	0.035	0.338	0.045	292
	Mean	0.988	1.003	0.565	0.996	416		Mean	0.035	0.035	0.334	0.045	289

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results* ¹	Experimental No.	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-13	1	0.977	0.987	0.536	0.980	432	SI-13	1	0.036	0.035	0.344	0.046	298
	2	0.978	0.992	0.568	0.985	401		2	0.035	0.036	0.347	0.045	302
	3	0.978	0.989	0.573	0.980	396		3	0.036	0.036	0.369	0.046	323
	Mean	0.978	0.990	0.559	0.981	410		Mean	0.036	0.036	0.353	0.046	308
SI-14	1	0.978	0.998	0.571	0.992	401	SI-14	1	0.035	0.035	0.318	0.042	275
	2	0.982	0.996	0.598	0.990	378		2	0.035	0.035	0.309	0.042	266
	3	0.985	0.996	0.612	0.990	367		3	0.036	0.035	0.325	0.043	281
	Mean	0.982	0.997	0.594	0.991	382		Mean	0.035	0.035	0.317	0.043	274
SI-15	1	0.966	0.981	0.557	0.975	403	SI-15	1	0.036	0.036	0.294	0.043	251
	2	0.970	0.985	0.582	0.978	382		2	0.035	0.036	0.301	0.043	259
	3	0.967	0.985	0.592	0.977	369		3	0.036	0.036	0.329	0.043	286
	Mean	0.967	0.983	0.577	0.977	385		Mean	0.036	0.036	0.308	0.043	265
SI-16	1	0.965	0.977	0.558	0.972	402	SI-16	1	0.036	0.036	0.317	0.043	273
	2	0.963	0.975	0.582	0.969	376		2	0.035	0.036	0.313	0.043	269
	3	0.964	0.975	0.598	0.969	361		3	0.036	0.036	0.327	0.044	282
	Mean	0.964	0.975	0.579	0.970	380		Mean	0.036	0.036	0.319	0.044	275
SI-17	1	0.986	1.003	0.558	0.992	418	SI-17	1	0.036	0.036	0.309	0.044	267
	2	0.982	1.004	0.592	0.995	380		2	0.036	0.036	0.295	0.043	253
	3	0.993	1.002	0.609	0.992	374		3	0.036	0.036	0.298	0.043	255
	Mean	0.987	1.003	0.586	0.993	391		Mean	0.036	0.036	0.301	0.043	258
SI-18	1	0.971	0.981	0.556	0.972	404	SI-18	1	0.036	0.036	0.295	0.045	250
	2	0.972	0.980	0.579	0.968	382		2	0.035	0.036	0.293	0.044	248
	3	0.974	0.988	0.596	0.976	367		3	0.036	0.036	0.315	0.045	270
	Mean	0.972	0.983	0.577	0.972	384		Mean	0.036	0.036	0.301	0.045	256

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-19	1	0.975	0.997	0.581	0.985	385	SI-19	1	0.035	0.035	0.351	0.044	309
	2	0.977	0.991	0.601	0.984	367		2	0.035	0.036	0.329	0.043	287
	3	0.982	0.993	0.619	0.984	353		3	0.036	0.036	0.321	0.043	278
	Mean	0.978	0.993	0.600	0.984	368		Mean	0.035	0.036	0.334	0.043	291
SI-20	1	0.965	0.978	0.568	0.970	389	SI-20	1	0.035	0.035	0.307	0.043	265
	2	0.967	0.981	0.607	0.973	352		2	0.035	0.036	0.322	0.043	280
	3	0.967	0.978	0.622	0.971	337		3	0.036	0.036	0.295	0.043	253
	Mean	0.966	0.979	0.599	0.971	359		Mean	0.035	0.036	0.308	0.043	266
SI-21	1	0.979	0.999	0.585	0.992	388	SI-21	1	0.036	0.036	0.329	0.044	285
	2	0.983	0.996	0.616	0.989	361		2	0.035	0.036	0.315	0.043	272
	3	0.986	0.996	0.628	0.988	351		3	0.036	0.036	0.330	0.044	286
	Mean	0.983	0.997	0.609	0.990	367		Mean	0.036	0.036	0.325	0.044	281
SI-22	1	0.974	0.983	0.564	0.976	403	SI-22	1	0.036	0.036	0.312	0.043	269
	2	0.975	0.987	0.588	0.980	380		2	0.036	0.036	0.314	0.043	271
	3	0.974	0.988	0.597	0.980	370		3	0.037	0.036	0.338	0.043	295
	Mean	0.974	0.986	0.583	0.979	384		Mean	0.036	0.036	0.321	0.043	278
SI-23	1	0.981	1.004	0.574	0.997	400	SI-23	1	0.035	0.035	0.324	0.046	278
	2	0.985	0.999	0.600	0.992	378		2	0.035	0.036	0.324	0.045	279
	3	0.987	1.003	0.608	0.997	372		3	0.036	0.036	0.344	0.046	298
	Mean	0.984	1.002	0.594	0.995	383		Mean	0.035	0.036	0.330	0.046	285
SI-24	1	1.017	1.035	0.627	1.027	383	SI-24	1	0.035	0.035	0.322	0.043	279
	2	1.018	1.033	0.651	1.027	361		2	0.035	0.035	0.318	0.043	275
	3	1.017	1.033	0.662	1.026	349		3	0.036	0.035	0.345	0.044	302
	Mean	1.017	1.034	0.646	1.027	364		Mean	0.035	0.035	0.328	0.043	285

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results* ¹	Experimental No.	Run#	A560(-)		A560(+)		Results* ²
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-25	1	0.973	0.982	0.556	0.974	409	SI-25	1	0.037	0.036	0.368	0.047	321
	2	0.974	0.989	0.586	0.983	380		2	0.036	0.037	0.367	0.047	320
	3	0.976	0.987	0.599	0.978	369		3	0.037	0.036	0.393	0.047	345
	Mean	0.974	0.986	0.580	0.978	386		Mean	0.037	0.036	0.376	0.047	329
SI-26	1	0.975	0.991	0.573	0.985	393	SI-26	1	0.035	0.035	0.364	0.043	321
	2	0.974	0.993	0.594	0.984	372		2	0.035	0.035	0.347	0.043	304
	3	0.974	0.992	0.601	0.984	366		3	0.036	0.035	0.365	0.044	321
	Mean	0.974	0.992	0.589	0.984	377		Mean	0.035	0.035	0.358	0.043	315
SI-27	1	0.973	0.990	0.572	0.981	390	SI-27	1	0.037	0.036	0.295	0.045	250
	2	0.974	0.993	0.604	0.982	359		2	0.036	0.037	0.295	0.044	251
	3	0.979	0.992	0.624	0.981	344		3	0.037	0.037	0.313	0.045	268
	Mean	0.976	0.992	0.600	0.981	364		Mean	0.037	0.037	0.301	0.045	256
SI-28	1	1.007	1.016	0.605	1.006	392	SI-28	1	0.036	0.036	0.311	0.045	266
	2	1.010	1.020	0.602	1.011	397		2	0.035	0.036	0.306	0.044	262
	3	1.011	1.023	0.604	1.013	398		3	0.036	0.036	0.314	0.045	269
	Mean	1.009	1.020	0.604	1.010	396		Mean	0.036	0.036	0.310	0.045	266
SI-29	1	1.010	1.028	0.594	1.020	408	SI-29	1	0.036	0.035	0.329	0.045	284
	2	1.018	1.029	0.620	1.021	390		2	0.035	0.036	0.334	0.045	290
	3	1.018	1.033	0.633	1.025	377		3	0.036	0.036	0.343	0.046	298
	Mean	1.016	1.030	0.616	1.022	392		Mean	0.036	0.036	0.335	0.045	291
SI-30	1	0.997	1.017	0.576	1.012	413	SI-30	1	0.035	0.036	0.292	0.044	249
	2	0.994	1.019	0.604	1.009	382		2	0.035	0.036	0.297	0.044	254
	3	1.000	1.016	0.618	1.007	373		3	0.038	0.036	0.313	0.044	267
	Mean	0.997	1.017	0.600	1.009	389		Mean	0.036	0.036	0.301	0.044	257

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-31	1	0.999	1.017	0.571	1.007	413	SI-31	1	0.035	0.035	0.299	0.043	257
	2	1.000	1.019	0.592	1.004	393		2	0.035	0.036	0.295	0.043	253
	3	1.000	1.020	0.606	1.000	380		3	0.036	0.036	0.313	0.043	270
	Mean	1.000	1.019	0.590	1.004	395		Mean	0.036	0.036	0.302	0.043	260
SI-32	1	1.013	1.030	0.621	1.023	385	SI-32	1	0.036	0.036	0.320	0.044	276
	2	1.013	1.035	0.646	1.028	360		2	0.035	0.036	0.327	0.045	283
	3	1.017	1.035	0.655	1.027	355		3	0.036	0.036	0.349	0.046	304
	Mean	1.014	1.033	0.641	1.026	367		Mean	0.036	0.036	0.332	0.045	288
Mean for all assays		-	-	-	-	384	Mean for all assays		-	-	-	-	277
SD for all assays		-	-	-	-	16	SD for all assays		-	-	-	-	18
CV for all assays		-	-	-	-	4.2	CV for all assays		-	-	-	-	6.5

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-01	1	0.985	0.988	0.977	0.981	2	SI-01	1	0.036	0.035	0.037	0.044	-7
	2	0.984	0.985	0.977	0.979	1		2	0.036	0.035	0.037	0.043	-7
	3	0.984	0.984	0.976	0.978	2		3	0.036	0.035	0.037	0.044	-7
	Mean	0.984	0.985	0.977	0.979	2		Mean	0.036	0.035	0.037	0.043	-7
SI-02	1	0.995	0.989	0.983	0.977	-1	SI-02	1	0.037	0.036	0.038	0.045	-8
	2	0.992	0.998	0.981	0.986	-1		2	0.037	0.036	0.038	0.044	-7
	3	0.993	0.995	0.982	0.983	-1		3	0.037	0.036	0.039	0.044	-7
	Mean	0.993	0.994	0.982	0.982	-1		Mean	0.037	0.036	0.038	0.044	-7
SI-03	1	1.019	1.014	1.003	0.999	2	SI-03	1	0.036	0.035	0.049	0.050	-2
	2	1.012	1.010	0.996	0.997	1		2	0.036	0.035	0.049	0.050	-1
	3	1.014	1.010	0.998	0.994	2		3	0.036	0.035	0.050	0.050	-1
	Mean	1.015	1.011	0.999	0.997	2		Mean	0.036	0.035	0.049	0.050	-1
SI-04	1	0.974	0.976	0.967	0.970	1	SI-04	1	0.036	0.035	0.037	0.044	-7
	2	0.975	0.976	0.967	0.971	2		2	0.036	0.036	0.037	0.044	-7
	3	0.976	0.975	0.969	0.968	1		3	0.036	0.035	0.037	0.044	-7
	Mean	0.975	0.976	0.967	0.970	1		Mean	0.036	0.036	0.037	0.044	-7
SI-05	1	1.012	1.010	1.001	1.004	3	SI-05	1	0.036	0.035	0.037	0.043	-7
	2	1.011	1.010	1.001	1.003	3		2	0.036	0.035	0.036	0.043	-7
	3	1.009	1.010	1.001	1.004	1		3	0.036	0.035	0.037	0.044	-7
	Mean	1.011	1.010	1.001	1.003	2		Mean	0.036	0.035	0.037	0.043	-7
SI-06	1	0.989	0.985	0.982	0.978	0	SI-06	1	0.036	0.035	0.038	0.045	-8
	2	0.987	0.987	0.979	0.980	1		2	0.035	0.036	0.036	0.045	-9
	3	0.986	0.985	0.979	0.979	0		3	0.036	0.035	0.037	0.045	-9
	Mean	0.987	0.986	0.980	0.979	0		Mean	0.036	0.035	0.037	0.045	-9

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-07	1	0.982	0.982	0.973	0.975	2	SI-07	1	0.036	0.035	0.037	0.043	-7
	2	0.980	0.979	0.972	0.974	2		2	0.036	0.035	0.037	0.043	-7
	3	0.980	0.978	0.972	0.972	1		3	0.036	0.035	0.037	0.043	-7
	Mean	0.981	0.980	0.972	0.973	2		Mean	0.036	0.035	0.037	0.043	-7
SI-08	1	0.979	0.974	0.973	0.969	0	SI-08	1	0.036	0.036	0.037	0.044	-7
	2	0.979	0.977	0.971	0.970	2		2	0.036	0.036	0.037	0.043	-8
	3	0.981	0.976	0.973	0.970	3		3	0.036	0.036	0.037	0.044	-8
	Mean	0.980	0.976	0.972	0.970	2		Mean	0.036	0.036	0.037	0.044	-8
SI-09	1	0.971	0.966	0.961	0.954	1	SI-09	1	0.037	0.036	0.037	0.046	-10
	2	0.967	0.964	0.958	0.955	1		2	0.036	0.036	0.037	0.047	-10
	3	0.967	0.970	0.955	0.962	3		3	0.037	0.036	0.038	0.047	-11
	Mean	0.968	0.966	0.958	0.957	2		Mean	0.037	0.036	0.037	0.047	-10
SI-10	1	0.980	0.975	0.975	0.969	0	SI-10	1	0.036	0.036	0.037	0.044	-7
	2	0.983	0.985	0.976	0.980	2		2	0.036	0.036	0.037	0.044	-7
	3	0.983	0.984	0.977	0.979	1		3	0.037	0.036	0.037	0.045	-8
	Mean	0.982	0.981	0.976	0.976	1		Mean	0.036	0.036	0.037	0.044	-7
SI-11	1	0.991	0.990	0.984	0.983	0	SI-11	1	0.036	0.036	0.037	0.045	-9
	2	0.993	0.987	0.984	0.980	1		2	0.036	0.036	0.037	0.045	-9
	3	0.999	0.988	0.990	0.980	2		3	0.036	0.036	0.037	0.046	-9
	Mean	0.994	0.988	0.986	0.981	1		Mean	0.036	0.036	0.037	0.046	-9
SI-12	1	1.007	1.002	1.000	0.996	0	SI-12	1	0.036	0.035	0.036	0.045	-9
	2	1.007	1.005	1.001	0.997	-1		2	0.035	0.036	0.036	0.044	-9
	3	1.006	1.003	0.998	0.996	0		3	0.036	0.035	0.037	0.045	-9
	Mean	1.007	1.003	1.000	0.996	0		Mean	0.036	0.035	0.036	0.045	-9

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank			
SI-13	1	0.992	0.987	0.985	0.980	-1	SI-13	1	0.037	0.035	0.037	0.046	-9
	2	0.992	0.992	0.983	0.985	0		2	0.037	0.036	0.038	0.045	-9
	3	0.993	0.989	0.982	0.980	2		3	0.036	0.036	0.037	0.046	-9
	Mean	0.992	0.990	0.983	0.981	0		Mean	0.036	0.036	0.037	0.046	-9
SI-14	1	0.997	0.998	0.992	0.992	-1	SI-14	1	0.036	0.035	0.037	0.042	-7
	2	0.997	0.996	0.990	0.990	1		2	0.036	0.035	0.036	0.042	-7
	3	0.998	0.996	0.992	0.990	0		3	0.036	0.035	0.037	0.043	-7
	Mean	0.997	0.997	0.991	0.991	0		Mean	0.036	0.035	0.037	0.043	-7
SI-15	1	0.984	0.981	0.979	0.975	-1	SI-15	1	0.036	0.036	0.037	0.043	-6
	2	0.983	0.985	0.976	0.978	1		2	0.036	0.036	0.037	0.043	-6
	3	0.985	0.985	0.979	0.977	0		3	0.036	0.036	0.037	0.043	-6
	Mean	0.984	0.983	0.978	0.977	0		Mean	0.036	0.036	0.037	0.043	-6
SI-16	1	0.979	0.977	0.973	0.972	1	SI-16	1	0.036	0.036	0.037	0.043	-8
	2	0.978	0.975	0.970	0.969	3		2	0.036	0.036	0.037	0.043	-8
	3	0.976	0.975	0.968	0.969	2		3	0.036	0.036	0.037	0.044	-7
	Mean	0.977	0.975	0.970	0.970	2		Mean	0.036	0.036	0.037	0.044	-8
SI-17	1	1.008	1.003	1.001	0.992	-3	SI-17	1	0.036	0.036	0.037	0.044	-7
	2	1.005	1.004	0.998	0.995	-3		2	0.036	0.036	0.037	0.043	-6
	3	1.006	1.002	0.999	0.992	-3		3	0.036	0.036	0.037	0.043	-6
	Mean	1.007	1.003	1.000	0.993	-3		Mean	0.036	0.036	0.037	0.043	-6
SI-18	1	0.976	0.981	0.953	0.972	13	SI-18	1	0.037	0.036	0.038	0.045	-8
	2	0.983	0.980	0.972	0.968	1		2	0.037	0.036	0.038	0.044	-8
	3	0.981	0.988	0.970	0.976	0		3	0.037	0.036	0.038	0.045	-8
	Mean	0.980	0.983	0.965	0.972	5		Mean	0.037	0.036	0.038	0.045	-8

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank			
SI-19	1	0.996	0.997	0.987	0.985	0	SI-19	1	0.036	0.035	0.037	0.044	-6
	2	0.994	0.991	0.987	0.984	-2		2	0.036	0.036	0.037	0.043	-6
	3	0.994	0.993	0.986	0.984	-1		3	0.036	0.036	0.037	0.043	-6
	Mean	0.995	0.993	0.986	0.984	-1		Mean	0.036	0.036	0.037	0.043	-6
SI-20	1	0.983	0.978	0.975	0.970	1	SI-20	1	0.036	0.035	0.036	0.043	-6
	2	0.983	0.981	0.975	0.973	0		2	0.036	0.036	0.037	0.043	-6
	3	0.984	0.978	0.976	0.971	0		3	0.036	0.036	0.037	0.043	-6
	Mean	0.983	0.979	0.975	0.971	0		Mean	0.036	0.036	0.037	0.043	-6
SI-21	1	0.998	0.999	0.989	0.992	2	SI-21	1	0.037	0.036	0.037	0.044	-7
	2	0.999	0.996	0.990	0.989	2		2	0.037	0.036	0.037	0.043	-8
	3	0.999	0.996	0.991	0.988	1		3	0.037	0.036	0.037	0.044	-7
	Mean	0.998	0.997	0.990	0.990	2		Mean	0.037	0.036	0.037	0.044	-7
SI-22	1	0.990	0.983	0.983	0.976	0	SI-22	1	0.037	0.036	0.038	0.043	-7
	2	0.991	0.987	0.981	0.980	3		2	0.037	0.036	0.037	0.043	-7
	3	0.999	0.988	0.990	0.980	2		3	0.037	0.036	0.037	0.043	-7
	Mean	0.993	0.986	0.984	0.979	2		Mean	0.037	0.036	0.037	0.043	-7
SI-23	1	1.004	1.004	0.994	0.997	3	SI-23	1	0.036	0.035	0.037	0.046	-9
	2	1.001	0.999	0.992	0.992	2		2	0.041	0.036	0.043	0.045	-8
	3	0.999	1.003	0.991	0.997	1		3	0.036	0.036	0.037	0.046	-9
	Mean	1.001	1.002	0.993	0.995	2		Mean	0.038	0.036	0.039	0.046	-9
SI-24	1	1.035	1.035	1.027	1.027	1	SI-24	1	0.036	0.035	0.036	0.043	-7
	2	1.034	1.033	1.027	1.027	0		2	0.035	0.035	0.036	0.043	-7
	3	1.033	1.033	1.025	1.026	0		3	0.036	0.035	0.037	0.044	-7
	Mean	1.034	1.034	1.026	1.027	0		Mean	0.035	0.035	0.036	0.043	-7

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-25	1	0.980	0.982	0.967	0.974	6	SI-25	1	0.038	0.036	0.038	0.047	-11
	2	0.985	0.989	0.976	0.983	1		2	0.037	0.037	0.038	0.047	-10
	3	0.987	0.987	0.978	0.978	0		3	0.037	0.036	0.039	0.047	-10
	Mean	0.984	0.986	0.974	0.978	2		Mean	0.037	0.036	0.038	0.047	-10
SI-26	1	0.993	0.991	0.986	0.985	-1	SI-26	1	0.035	0.035	0.036	0.043	-7
	2	0.990	0.993	0.984	0.984	-2		2	0.035	0.035	0.036	0.043	-7
	3	0.990	0.992	0.984	0.984	-2		3	0.035	0.035	0.036	0.044	-7
	Mean	0.991	0.992	0.985	0.984	-2		Mean	0.035	0.035	0.036	0.043	-7
SI-27	1	0.990	0.990	0.977	0.981	2	SI-27	1	0.038	0.036	0.038	0.045	-7
	2	0.989	0.993	0.978	0.982	0		2	0.037	0.037	0.038	0.044	-7
	3	0.988	0.992	0.977	0.981	0		3	0.038	0.037	0.038	0.045	-8
	Mean	0.989	0.992	0.977	0.981	1		Mean	0.038	0.037	0.038	0.045	-7
SI-28	1	1.014	1.016	1.003	1.006	1	SI-28	1	0.036	0.036	0.037	0.045	-8
	2	1.018	1.020	1.009	1.011	0		2	0.036	0.036	0.037	0.044	-8
	3	1.016	1.023	1.006	1.013	0		3	0.036	0.036	0.037	0.045	-8
	Mean	1.016	1.020	1.006	1.010	0		Mean	0.036	0.036	0.037	0.045	-8
SI-29	1	1.031	1.028	1.022	1.020	0	SI-29	1	0.036	0.035	0.037	0.045	-8
	2	1.030	1.029	1.022	1.021	0		2	0.036	0.036	0.037	0.045	-9
	3	1.038	1.033	1.028	1.025	2		3	0.037	0.036	0.037	0.046	-8
	Mean	1.033	1.030	1.024	1.022	1		Mean	0.036	0.036	0.037	0.045	-8
SI-30	1	1.017	1.017	1.009	1.012	0	SI-30	1	0.036	0.036	0.037	0.044	-7
	2	1.012	1.019	1.006	1.009	-2		2	0.036	0.036	0.037	0.044	-7
	3	1.014	1.016	1.006	1.007	0		3	0.036	0.036	0.037	0.044	-7
	Mean	1.014	1.017	1.007	1.009	-1		Mean	0.036	0.036	0.037	0.044	-7

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 6
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
SI-31	1	1.027	1.017	1.012	1.007	0	SI-31	1	0.036	0.035	0.037	0.043	-6
	2	1.018	1.019	1.006	1.004	-2		2	0.036	0.036	0.037	0.043	-6
	3	1.018	1.020	1.005	1.000	-2		3	0.036	0.036	0.037	0.043	-6
	Mean	1.021	1.019	1.008	1.004	-1		Mean	0.036	0.036	0.037	0.043	-6
SI-32	1	1.031	1.030	1.024	1.023	1	SI-32	1	0.036	0.036	0.037	0.044	-8
	2	1.032	1.035	1.025	1.028	0		2	0.036	0.036	0.037	0.045	-8
	3	1.025	1.035	1.018	1.027	0		3	0.036	0.036	0.037	0.046	-8
	Mean	1.030	1.033	1.022	1.026	0		Mean	0.036	0.036	0.037	0.045	-8
Mean for all assays		-	-	-	-	1	Mean for all assays		-	-	-	-	-7
SD for all assays		-	-	-	-	2	SD for all assays		-	-	-	-	2
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 7
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
TS-20	1	0.968	0.983	0.502	0.977	460	TS-20	1	0.035	0.035	0.335	0.038	297
	2	0.975	0.986	0.523	0.981	446		2	0.034	0.034	0.335	0.037	298
	3	0.979	0.991	0.528	0.985	445		3	0.035	0.034	0.360	0.037	322
	Mean	0.974	0.987	0.518	0.981	450		Mean	0.035	0.034	0.343	0.037	306
TS-21	1	0.991	0.983	0.510	0.976	475	TS-21	1	0.035	0.035	0.289	0.038	251
	2	0.969	0.983	0.526	0.977	437		2	0.034	0.035	0.293	0.038	256
	3	0.966	0.983	0.535	0.979	425		3	0.067	0.034	0.319	0.038	249
	Mean	0.975	0.983	0.524	0.977	446		Mean	0.045	0.035	0.300	0.038	252
TS-22	1	0.979	0.996	0.518	0.991	457	TS-22	1	0.035	0.034	0.319	0.039	275
	2	0.978	0.996	0.534	0.992	440		2	0.034	0.034	0.317	0.038	274
	3	0.986	1.005	0.540	1.001	442		3	0.035	0.038	0.352	0.055	308
	Mean	0.981	0.999	0.531	0.995	446		Mean	0.035	0.035	0.329	0.044	286
TS-23	1	0.967	0.983	0.499	0.979	463	TS-23	1	0.035	0.034	0.295	0.037	257
	2	0.963	0.978	0.520	0.972	438		2	0.034	0.034	0.294	0.037	257
	3	0.964	0.984	0.542	0.980	417		3	0.035	0.034	0.315	0.037	277
	Mean	0.965	0.982	0.520	0.977	439		Mean	0.035	0.034	0.301	0.037	264
TS-24	1	0.965	0.982	0.505	0.976	454	TS-24	1	0.034	0.034	0.305	0.038	268
	2	0.969	0.988	0.522	0.983	441		2	0.034	0.034	0.302	0.037	265
	3	0.978	0.994	0.539	0.988	433		3	0.034	0.034	0.322	0.037	285
	Mean	0.971	0.988	0.522	0.982	443		Mean	0.034	0.034	0.310	0.037	273
TS-26	1	0.986	1.000	0.536	0.994	445	TS-26	1	0.035	0.035	0.317	0.037	280
	2	0.989	1.013	0.549	1.009	435		2	0.034	0.034	0.309	0.036	273
	3	0.996	1.017	0.553	1.012	438		3	0.035	0.034	0.336	0.036	299
	Mean	0.990	1.010	0.546	1.005	439		Mean	0.035	0.034	0.321	0.036	284

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 7
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
TS-27	1	0.980	1.002	0.514	0.997	460	TS-27	1	0.034	0.035	0.310	0.037	275
	2	0.979	0.993	0.534	0.986	439		2	0.034	0.035	0.305	0.036	270
	3	0.982	0.999	0.545	0.993	431		3	0.035	0.035	0.318	0.036	282
	Mean	0.980	0.998	0.531	0.992	443		Mean	0.034	0.035	0.311	0.036	276
TS-28	1	0.985	0.999	0.533	0.994	448	TS-28	1	0.035	0.034	0.339	0.036	302
	2	0.987	1.002	0.552	0.999	431		2	0.034	0.034	0.337	0.036	301
	3	0.994	1.007	0.557	1.004	433		3	0.034	0.034	0.369	0.037	333
	Mean	0.989	1.003	0.547	0.999	437		Mean	0.034	0.034	0.348	0.036	312
TS-29	1	0.970	1.000	0.518	0.995	446	TS-29	1	0.035	0.035	0.298	0.037	260
	2	0.970	1.014	0.535	1.007	429		2	0.034	0.034	0.294	0.036	257
	3	0.972	1.007	0.546	1.000	420		3	0.035	0.034	0.313	0.037	275
	Mean	0.971	1.007	0.533	1.001	432		Mean	0.035	0.034	0.302	0.037	264
TS-30	1	0.988	1.010	0.515	0.997	464	TS-30	1	0.035	0.034	0.351	0.036	312
	2	0.990	1.001	0.535	0.995	446		2	0.034	0.035	0.345	0.042	307
	3	0.998	1.009	0.538	1.003	451		3	0.034	0.034	0.373	0.036	335
	Mean	0.992	1.007	0.529	0.998	454		Mean	0.034	0.034	0.356	0.038	318
TS-31	1	0.983	1.001	0.521	0.995	456	TS-31	1	0.034	0.035	0.327	0.037	291
	2	0.984	0.988	0.527	0.982	451		2	0.034	0.035	0.320	0.036	284
	3	0.991	1.015	0.543	1.009	442		3	0.035	0.034	0.339	0.037	302
	Mean	0.986	1.001	0.530	0.995	450		Mean	0.034	0.035	0.329	0.037	292
TS-32	1	0.981	0.991	0.548	0.986	431	TS-32	1	0.034	0.034	0.297	0.036	261
	2	0.980	0.995	0.554	0.990	424		2	0.035	0.034	0.300	0.036	263
	3	0.987	0.998	0.562	1.003	423		3	0.034	0.034	0.326	0.036	290
	Mean	0.983	0.995	0.555	0.993	426		Mean	0.034	0.034	0.308	0.036	271

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 7
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
TS-33	1	0.990	1.007	0.518	1.002	466	TS-33	1	0.035	0.034	0.301	0.037	263
	2	0.987	1.010	0.538	1.003	443		2	0.034	0.035	0.294	0.038	257
	3	0.992	1.012	0.549	1.007	437		3	0.035	0.034	0.320	0.036	282
	Mean	0.990	1.010	0.535	1.004	449		Mean	0.035	0.034	0.305	0.037	267
TS-34	1	0.968	0.994	0.521	0.988	442	TS-34	1	0.034	0.034	0.298	0.037	267
	2	0.975	0.991	0.529	0.986	441		2	0.034	0.034	0.294	0.036	263
	3	0.984	1.001	0.548	0.996	431		3	0.035	0.048	0.321	0.036	289
	Mean	0.976	0.995	0.533	0.990	438		Mean	0.034	0.039	0.304	0.036	273
TS-35	1	1.000	1.011	0.510	1.012	485	TS-35	1	0.034	0.036	0.328	0.038	289
	2	0.995	1.015	0.528	1.007	462		2	0.034	0.035	0.318	0.037	279
	3	0.997	1.015	0.536	1.009	456		3	0.034	0.034	0.341	0.046	302
	Mean	0.997	1.014	0.525	1.009	468		Mean	0.034	0.035	0.329	0.040	290
TS-39	1	0.971	0.990	0.513	0.985	452	TS-39	1	0.036	0.037	0.322	0.046	281
	2	0.975	0.994	0.527	0.984	442		2	0.034	0.034	0.323	0.037	284
	3	0.983	1.005	0.532	1.000	445		3	0.035	0.034	0.350	0.037	310
	Mean	0.976	0.996	0.524	0.990	446		Mean	0.035	0.035	0.332	0.040	292
TS-40	1	0.976	0.994	0.515	0.987	453	TS-40	1	0.034	0.034	0.320	0.036	266
	2	0.974	0.997	0.530	0.988	436		2	0.034	0.034	0.315	0.091	261
	3	0.979	1.002	0.542	0.996	429		3	0.034	0.034	0.336	0.036	282
	Mean	0.976	0.998	0.529	0.990	439		Mean	0.034	0.034	0.324	0.054	270
TS-41	1	0.950	0.961	0.487	0.956	458	TS-41	1	0.035	0.037	0.354	0.049	314
	2	0.940	0.961	0.483	0.955	452		2	0.034	0.034	0.363	0.036	324
	3	0.963	1.004	0.506	0.998	452		3	0.035	0.034	0.381	0.036	341
	Mean	0.951	0.975	0.492	0.970	454		Mean	0.035	0.035	0.366	0.040	326

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 7
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
TS-42	1	0.914	0.999	0.491	0.993	417	TS-42	1	0.035	0.035	0.341	0.037	303
	2	0.922	0.999	0.515	0.993	401		2	0.034	0.034	0.336	0.038	299
	3	0.931	1.001	0.530	0.995	395		3	0.034	0.034	0.351	0.036	314
	Mean	0.922	1.000	0.512	0.994	404		Mean	0.034	0.034	0.343	0.037	305
TS-43	1	0.895	0.904	0.445	0.900	445	TS-43	1	0.036	0.034	0.325	0.036	286
	2	0.889	0.910	0.455	0.904	429		2	0.034	0.034	0.327	0.036	290
	3	0.903	0.914	0.470	0.909	428		3	0.034	0.034	0.355	0.039	318
	Mean	0.896	0.909	0.457	0.904	434		Mean	0.035	0.034	0.336	0.037	298
TS-44	1	0.983	0.990	0.526	0.986	452	TS-44	1	0.034	0.034	0.278	0.037	241
	2	0.983	1.003	0.560	0.997	418		2	0.034	0.034	0.272	0.037	235
	3	0.988	1.010	0.552	1.004	431		3	0.035	0.034	0.283	0.037	245
	Mean	0.985	1.001	0.546	0.996	434		Mean	0.034	0.034	0.278	0.037	240
TS-45	1						TS-45	1	0.034	0.034	0.331	0.036	295
	2							2	0.034	0.034	0.316	0.036	280
	3							3	0.034	0.034	0.338	0.036	302
	Mean							Mean	0.034	0.034	0.328	0.036	292
Mean for all assays		-	-	-	-	441	Mean for all assays		-	-	-	-	284
SD for all assays		-	-	-	-	13	SD for all assays		-	-	-	-	22
CV for all assays		-	-	-	-	2.9	CV for all assays		-	-	-	-	7.7

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank			
TS-20	1	0.987	0.983	0.986	0.977	-5	TS-20	1	0.036	0.035	0.037	0.038	-2
	2	0.989	0.986	0.983	0.981	0		2	0.039	0.034	0.036	0.037	-6
	3	0.992	0.991	0.986	0.985	0		3	0.036	0.034	0.036	0.037	-3
	Mean	0.989	0.987	0.985	0.981	-2		Mean	0.037	0.034	0.036	0.037	-4
TS-21	1	0.986	0.983	0.975	0.976	5	TS-21	1	0.050	0.035	0.052	0.038	-1
	2	0.989	0.983	0.972	0.977	11		2	0.039	0.035	0.036	0.038	-6
	3	0.988	0.983	0.982	0.979	0		3	0.036	0.034	0.037	0.038	-2
	Mean	0.988	0.983	0.976	0.977	5		Mean	0.042	0.035	0.042	0.038	-3
TS-22	1	1.000	0.996	0.995	0.991	1	TS-22	1	0.036	0.034	0.036	0.039	-9
	2	0.997	0.996	0.993	0.992	0		2	0.035	0.034	0.036	0.038	-8
	3	1.004	1.005	0.998	1.001	2		3	0.036	0.038	0.036	0.055	-9
	Mean	1.000	0.999	0.995	0.995	1		Mean	0.036	0.035	0.036	0.044	-9
TS-23	1	0.985	0.983	0.978	0.979	2	TS-23	1	0.036	0.034	0.036	0.037	-3
	2	0.978	0.978	0.971	0.972	2		2	0.045	0.034	0.036	0.037	-12
	3	0.981	0.984	0.974	0.980	2		3	0.036	0.034	0.037	0.037	-2
	Mean	0.981	0.982	0.974	0.977	2		Mean	0.039	0.034	0.036	0.037	-6
TS-24	1	0.986	0.982	0.982	0.976	-2	TS-24	1	0.036	0.034	0.036	0.038	-3
	2	0.988	0.988	0.983	0.983	-1		2	0.036	0.034	0.036	0.037	-3
	3	0.995	0.994	0.990	0.988	-1		3	0.036	0.034	0.036	0.037	-3
	Mean	0.990	0.988	0.985	0.982	-1		Mean	0.036	0.034	0.036	0.037	-3
TS-26	1	1.010	1.000	1.005	0.994	0	TS-26	1	0.036	0.035	0.036	0.037	-2
	2	1.008	1.013	1.002	1.009	1		2	0.036	0.034	0.036	0.036	-2
	3	1.026	1.017	1.015	1.012	6		3	0.036	0.034	0.036	0.036	-2
	Mean	1.015	1.010	1.007	1.005	2		Mean	0.036	0.034	0.036	0.036	-2

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
TS-27	1	0.999	1.002	0.994	0.997	-1	TS-27	1	0.036	0.035	0.036	0.037	-1
	2	0.996	0.993	0.991	0.986	-1		2	0.036	0.035	0.036	0.036	-1
	3	0.996	0.999	0.989	0.993	1		3	0.039	0.035	0.036	0.036	-4
	Mean	0.997	0.998	0.991	0.992	0		Mean	0.037	0.035	0.036	0.036	-2
TS-28	1	1.007	0.999	1.004	0.994	0	TS-28	1	0.036	0.034	0.036	0.036	-2
	2	1.013	1.002	1.005	0.999	4		2	0.035	0.034	0.035	0.036	-2
	3	1.005	1.007	1.002	1.004	-1		3	0.036	0.034	0.036	0.037	-2
	Mean	1.008	1.003	1.004	0.999	1		Mean	0.036	0.034	0.036	0.036	-2
TS-29	1	1.004	1.000	0.998	0.995	0	TS-29	1	0.036	0.035	0.036	0.037	-3
	2	1.009	1.014	1.003	1.007	0		2	0.035	0.034	0.036	0.036	-2
	3	1.008	1.007	1.002	1.000	0		3	0.036	0.034	0.037	0.037	-2
	Mean	1.007	1.007	1.001	1.001	0		Mean	0.036	0.034	0.036	0.037	-2
TS-30	1	1.008	1.010	1.004	0.997	-5	TS-30	1	0.036	0.034	0.036	0.036	-4
	2	1.027	1.001	1.003	0.995	15		2	0.039	0.035	0.040	0.042	-3
	3	1.013	1.009	1.009	1.003	-5		3	0.035	0.034	0.036	0.036	-3
	Mean	1.016	1.007	1.005	0.998	2		Mean	0.037	0.034	0.037	0.038	-3
TS-31	1	1.004	1.001	0.999	0.995	-1	TS-31	1	0.036	0.035	0.036	0.037	-2
	2	1.003	0.988	0.993	0.982	4		2	0.037	0.035	0.036	0.036	-3
	3	0.999	1.015	0.994	1.009	-1		3	0.036	0.034	0.036	0.037	-2
	Mean	1.002	1.001	0.995	0.995	1		Mean	0.036	0.035	0.036	0.037	-2
TS-32	1	1.000	0.991	0.996	0.986	2	TS-32	1	0.035	0.034	0.036	0.036	-1
	2	0.996	0.995	0.992	0.990	2		2	0.035	0.034	0.036	0.036	-1
	3	0.999	0.998	0.995	1.003	2		3	0.036	0.034	0.036	0.036	-2
	Mean	0.998	0.995	0.994	0.993	2		Mean	0.035	0.034	0.036	0.036	-1

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
TS-33	1	1.009	1.007	1.003	1.002	0	TS-33	1	0.036	0.034	0.036	0.037	-3
	2	1.005	1.010	0.999	1.003	0		2	0.036	0.035	0.036	0.038	-3
	3	1.007	1.012	1.002	1.007	-1		3	0.036	0.034	0.036	0.036	-3
	Mean	1.007	1.010	1.001	1.004	0		Mean	0.036	0.034	0.036	0.037	-3
TS-34	1	0.992	0.994	0.988	0.988	-1	TS-34	1	0.036	0.034	0.036	0.037	3
	2	1.001	0.991	0.995	0.986	1		2	0.035	0.034	0.035	0.036	3
	3	1.003	1.001	0.997	0.996	1		3	0.036	0.048	0.036	0.036	3
	Mean	0.999	0.995	0.993	0.990	0		Mean	0.036	0.039	0.036	0.036	3
TS-35	1	1.016	1.011	1.019	1.012	-8	TS-35	1	0.036	0.036	0.037	0.038	-4
	2	1.015	1.015	1.008	1.007	2		2	0.035	0.035	0.036	0.037	-4
	3	1.015	1.015	1.014	1.009	-4		3	0.036	0.034	0.037	0.046	-4
	Mean	1.015	1.014	1.014	1.009	-3		Mean	0.036	0.035	0.037	0.040	-4
TS-39	1	0.996	0.990	0.989	0.985	1	TS-39	1	0.038	0.037	0.037	0.046	-6
	2	0.996	0.994	0.993	0.984	-3		2	0.035	0.034	0.035	0.037	-5
	3	1.004	1.005	1.001	1.000	-3		3	0.036	0.034	0.036	0.037	-5
	Mean	0.999	0.996	0.994	0.990	-2		Mean	0.036	0.035	0.036	0.040	-5
TS-40	1	1.006	0.994	0.999	0.987	-1	TS-40	1	0.036	0.034	0.036	0.036	-20
	2	0.996	0.997	0.990	0.988	-2		2	0.035	0.034	0.035	0.091	-20
	3	1.005	1.002	0.997	0.996	0		3	0.036	0.034	0.036	0.036	-20
	Mean	1.002	0.998	0.995	0.990	-1		Mean	0.036	0.034	0.036	0.054	-20
TS-41	1	0.971	0.961	0.967	0.956	-1	TS-41	1	0.036	0.037	0.036	0.049	-5
	2	0.974	0.961	0.967	0.955	2		2	0.035	0.034	0.035	0.036	-5
	3	0.979	1.004	0.976	0.998	-2		3	0.036	0.034	0.036	0.036	-5
	Mean	0.975	0.975	0.970	0.970	0		Mean	0.036	0.035	0.036	0.040	-5

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 8 Positive control and negative control data of Phase 2 study

Laboratory : 7
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
TS-42	1	0.999	0.999	0.993	0.993	0	TS-42	1	0.036	0.035	0.036	0.037	-3
	2	0.995	0.999	0.989	0.993	0		2	0.035	0.034	0.036	0.038	-2
	3	1.000	1.001	0.992	0.995	2		3	0.036	0.034	0.036	0.036	-3
	Mean	0.998	1.000	0.991	0.994	1		Mean	0.036	0.034	0.036	0.037	-3
TS-43	1	0.911	0.904	0.906	0.900	0	TS-43	1	0.036	0.034	0.036	0.036	-3
	2	0.906	0.910	0.901	0.904	0		2	0.035	0.034	0.035	0.036	-3
	3	0.912	0.914	0.907	0.909	0		3	0.036	0.034	0.036	0.039	-3
	Mean	0.910	0.909	0.905	0.904	0		Mean	0.036	0.034	0.036	0.037	-3
TS-44	1	1.000	0.990	0.994	0.986	1	TS-44	1	0.036	0.034	0.036	0.037	-3
	2	0.994	1.003	0.989	0.997	0		2	0.037	0.034	0.036	0.037	-4
	3	0.997	1.010	0.991	1.004	1		3	0.036	0.034	0.036	0.037	-3
	Mean	0.997	1.001	0.991	0.996	1		Mean	0.036	0.034	0.036	0.037	-3
TS-45	1						TS-45	1	0.036	0.034	0.036	0.036	-2
	2							2	0.035	0.034	0.036	0.036	-1
	3							3	0.036	0.034	0.037	0.036	-1
	Mean							Mean	0.036	0.034	0.036	0.036	-1
Mean for all assays		-	-	-	-	0	Mean for all assays		-	-	-	-	-4
SD for all assays		-	-	-	-	2	SD for all assays		-	-	-	-	4
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Acridine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-18	1	0.977	0.995	0.679	0.992	295	AKP-18	1	0.028	0.026	0.183	0.040	142	Positive	Photoreactive
	2	0.973	0.995	0.686	0.991	284		2	0.028	0.027	0.180	0.039	139		
	3	0.975	0.993	0.690	0.990	282		3	0.028	0.027	0.186	0.041	145		
	Mean	0.975	0.994	0.685	0.991	287		Mean	0.028	0.027	0.183	0.040	142		
AKP-26	1	0.972	0.991	0.667	0.989	303	AKP-26	1	0.030	0.028	0.188	0.040	147	Positive	Photoreactive
	2	0.981	0.999	0.679	0.998	300		2	0.027	0.028	0.187	0.038	149		
	3	0.982	0.999	0.684	0.995	296		3	0.028	0.027	0.186	0.039	147		
	Mean	0.978	0.996	0.677	0.994	300		Mean	0.028	0.028	0.187	0.039	148		
AKP-27	1	0.970	0.986	0.651	0.984	317	AKP-27	1	0.029	0.027	0.193	0.039	152	Positive	Photoreactive
	2	0.966	0.988	0.658	0.985	306		2	0.029	0.027	0.187	0.038	146		
	3	0.967	0.991	0.661	0.989	304		3	0.029	0.028	0.193	0.040	152		
	Mean	0.968	0.988	0.657	0.986	309		Mean	0.029	0.027	0.191	0.039	150		
Mean for 3 assays	-	-	-	-	299	Mean for 3 assays	-	-	-	-	147				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Acridine HCl

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-03	1	0.976	0.985	0.681	0.983	292	AKP-03	1	0.027	0.027	0.174	0.034	140	Positive	Photoreactive
	2	0.972	1.007	0.685	1.005	284		2	0.026	0.027	0.171	0.033	138		
	3	0.983	1.005	0.697	1.001	283		3	0.027	0.028	0.181	0.035	147		
	Mean	0.977	0.999	0.688	0.996	286		Mean	0.027	0.027	0.175	0.034	142		
AKP-09	1	0.975	0.987	0.655	0.982	316	AKP-09	1	0.027	0.028	0.177	0.033	145	Positive	Photoreactive
	2	0.978	0.988	0.666	0.983	308		2	0.027	0.028	0.173	0.033	141		
	3	0.977	0.987	0.673	0.983	300		3	0.027	0.028	0.176	0.034	144		
	Mean	0.977	0.987	0.665	0.983	308		Mean	0.027	0.028	0.175	0.033	143		
AKP-12	1	0.978	0.986	0.671	0.984	304	AKP-12	1	0.027	0.027	0.177	0.033	144	Positive	Photoreactive
	2	0.978	0.983	0.680	0.977	295		2	0.026	0.027	0.174	0.032	142		
	3	0.977	0.984	0.681	0.981	293		3	0.027	0.027	0.179	0.034	146		
	Mean	0.978	0.984	0.677	0.981	297		Mean	0.027	0.027	0.177	0.033	144		
Mean for 3 assays	-	-	-	-	297	Mean for 3 assays	-	-	-	-	143				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Amiodarone HCl

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Chlorpromazine HCl

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-16	1	0.956	0.984	0.925	0.981	28	AKP-16	1	0.029	0.028	0.120	0.033	85	Positive	Photoreactive
	2	0.952	0.985	0.920	0.981	29		2	0.029	0.027	0.119	0.033	84		
	3	0.955	0.984	0.921	0.982	31		3	0.029	0.027	0.123	0.033	88		
	Mean	0.954	0.984	0.922	0.981	29		Mean	0.029	0.027	0.121	0.033	86		
AKP-21	1	0.954	0.993	0.922	0.991	29	AKP-21	1	0.028	0.027	0.118	0.040	77	Positive	Photoreactive
	2	0.950	0.993	0.918	0.988	29		2	0.032	0.027	0.118	0.039	73		
	3	0.950	0.992	0.917	0.990	30		3	0.029	0.028	0.123	0.040	81		
	Mean	0.951	0.993	0.919	0.990	29		Mean	0.030	0.027	0.120	0.040	77		
AKP-24	1	0.977	1.001	0.922	0.996	50	AKP-24	1	0.031	0.028	0.115	0.042	71	Positive	Photoreactive
	2	0.964	1.004	0.918	0.998	41		2	0.030	0.029	0.116	0.041	73		
	3	0.971	1.004	0.920	0.999	46		3	0.030	0.032	0.119	0.045	76		
	Mean	0.971	1.003	0.920	0.998	46		Mean	0.030	0.030	0.117	0.043	73		
Mean for 3 assays	-	-	-	-	35	Mean for 3 assays	-	-	-	-	79				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Doxycycline HCl

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-17	1	0.983	1.009	0.656	1.004	323	AKP-17	1	0.034	0.027	0.368	0.044	317	Positive	Photoreactive
	2	0.978	1.001	0.669	0.997	305		2	0.034	0.027	0.364	0.043	313		
	3	0.982	1.009	0.676	1.005	302		3	0.035	0.028	0.375	0.046	323		
	Mean	0.981	1.006	0.667	1.002	310		Mean	0.034	0.027	0.369	0.044	318		
AKP-21	1	0.961	0.993	0.643	0.991	315	AKP-21	1	0.034	0.027	0.343	0.040	296	Positive	Photoreactive
	2	0.954	0.993	0.658	0.988	293		2	0.034	0.027	0.345	0.039	298		
	3	0.959	0.992	0.667	0.990	289		3	0.035	0.028	0.352	0.040	304		
	Mean	0.958	0.993	0.656	0.990	299		Mean	0.034	0.027	0.347	0.040	299		
AKP-23	1	0.979	1.005	0.669	1.000	305	AKP-23	1	0.036	0.028	0.346	0.045	292	Positive	Photoreactive
	2	0.973	1.007	0.687	1.003	281		2	0.035	0.027	0.347	0.045	294		
	3	0.975	1.008	0.688	1.004	282		3	0.035	0.028	0.363	0.047	310		
	Mean	0.976	1.007	0.681	1.002	289		Mean	0.035	0.028	0.352	0.046	299		
Mean for 3 assays	-	-	-	-	299	Mean for 3 assays	-	-	-	-	305				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Fenofibrate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-26	1	0.997	0.991	0.871	0.989	124	AKP-26	1	0.034	0.028	0.041	0.040	-4	Positive	Photoreactive
	2	1.005	0.999	0.877	0.998	126		2	0.034	0.028	0.040	0.038	-5		
	3	1.005	0.999	0.879	0.995	124		3	0.035	0.027	0.042	0.039	-4		
	Mean	1.002	0.996	0.876	0.994	125		Mean	0.034	0.028	0.041	0.039	-4		
AKP-28	1	0.988	0.974	0.861	0.972	124	AKP-28	1	0.037	0.028	0.044	0.040	-5	Positive	Photoreactive
	2	0.992	0.989	0.863	0.987	126		2	0.035	0.028	0.042	0.039	-5		
	3	0.990	0.988	0.862	0.984	125		3	0.034	0.027	0.043	0.041	-3		
	Mean	0.990	0.984	0.862	0.981	125		Mean	0.035	0.028	0.043	0.040	-4		
AKP-29	1	0.989	0.980	0.849	0.977	138	AKP-29	1	0.036	0.027	0.040	0.038	-8	Positive	Photoreactive
	2	0.994	0.989	0.851	0.987	141		2	0.036	0.026	0.040	0.038	-8		
	3	0.993	0.987	0.851	0.985	140		3	0.037	0.028	0.042	0.040	-7		
	Mean	0.992	0.985	0.850	0.983	140		Mean	0.036	0.027	0.041	0.039	-8		
Mean for 3 assays	-	-	-	-	130	Mean for 3 assays	-	-	-	-	-	-5			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Furosemide

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-05	1	0.977	0.977	0.864	0.974	110	AKP-05	1	0.033	0.027	0.062	0.038	21	Positive	Photoreactive
	2	0.982	0.979	0.870	0.976	109		2	0.033	0.027	0.063	0.033	22		
	3	0.981	0.988	0.874	0.984	104		3	0.034	0.027	0.067	0.034	25		
	Mean	0.980	0.981	0.869	0.978	108		Mean	0.033	0.027	0.064	0.035	23		
AKP-10	1	0.977	0.967	0.864	0.966	110	AKP-10	1	0.033	0.027	0.062	0.033	22	Positive	Photoreactive
	2	0.978	0.969	0.869	0.966	106		2	0.033	0.026	0.060	0.033	20		
	3	0.977	0.973	0.865	0.969	109		3	0.034	0.028	0.062	0.035	21		
	Mean	0.977	0.970	0.866	0.967	108		Mean	0.033	0.027	0.061	0.034	21		
AKP-15	1	0.970	0.971	0.844	0.970	123	AKP-15	1	0.033	0.027	0.072	0.034	32	Positive	Photoreactive
	2	0.973	0.965	0.851	0.959	119		2	0.032	0.027	0.069	0.034	30		
	3	0.972	0.965	0.858	0.962	111		3	0.034	0.027	0.073	0.035	32		
	Mean	0.972	0.967	0.851	0.964	118		Mean	0.033	0.027	0.071	0.034	31		
Mean for 3 assays	-	-	-	-	111	Mean for 3 assays	-	-	-	-	25				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Ketoprofen

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-03	1	0.993	0.985	0.762	0.983	228	AKP-03	1	0.031	0.027	0.146	0.034	108	Positive	Photoreactive
	2	0.999	1.007	0.778	1.005	218		2	0.030	0.027	0.146	0.033	109		
	3	0.998	1.005	0.784	1.001	211		3	0.032	0.028	0.151	0.035	112		
	Mean	0.997	0.999	0.775	0.996	219		Mean	0.031	0.027	0.148	0.034	110		
AKP-09	1	0.990	0.987	0.757	0.982	229	AKP-09	1	0.030	0.028	0.138	0.033	103	Positive	Photoreactive
	2	0.989	0.988	0.761	0.983	224		2	0.029	0.028	0.140	0.033	106		
	3	0.988	0.987	0.769	0.983	215		3	0.031	0.028	0.148	0.034	112		
	Mean	0.989	0.987	0.762	0.983	223		Mean	0.030	0.028	0.142	0.033	107		
AKP-13	1	0.990	0.985	0.773	0.980	213	AKP-13	1	0.030	0.027	0.134	0.033	98	Positive	Photoreactive
	2	0.992	0.986	0.782	0.984	206		2	0.030	0.026	0.132	0.033	96		
	3	0.989	0.992	0.792	0.989	193		3	0.031	0.027	0.137	0.034	100		
	Mean	0.990	0.988	0.782	0.984	204		Mean	0.030	0.027	0.134	0.033	98		
Mean for 3 assays	-	-	-	-	215	Mean for 3 assays	-	-	-	-	105				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : 6-methylcoumarine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-01	1	0.945	0.954	0.763	0.951	179	AKP-01	1	0.027	0.027	0.121	0.033	88	Positive	Photoreactive
	2	0.955	0.949	0.774	0.946	178		2	0.027	0.027	0.118	0.033	85		
	3	0.955	0.959	0.780	0.956	172		3	0.027	0.027	0.128	0.034	95		
	Mean	0.952	0.954	0.772	0.951	176		Mean	0.027	0.027	0.122	0.033	89		
AKP-08	1	0.994	0.993	0.822	0.990	169	AKP-08	1	0.027	0.027	0.112	0.033	79	Positive	Photoreactive
	2	0.996	0.989	0.831	0.985	162		2	0.027	0.027	0.109	0.033	76		
	3	0.995	0.988	0.840	0.985	152		3	0.028	0.027	0.117	0.034	83		
	Mean	0.995	0.990	0.831	0.987	161		Mean	0.027	0.027	0.113	0.033	79		
AKP-11	1	0.974	0.970	0.839	0.966	132	AKP-11	1	0.028	0.027	0.086	0.032	53	Positive	Photoreactive
	2	0.971	0.978	0.841	0.976	127		2	0.027	0.027	0.084	0.032	52		
	3	0.979	0.977	0.854	0.973	122		3	0.028	0.028	0.089	0.033	56		
	Mean	0.975	0.975	0.845	0.972	127		Mean	0.028	0.027	0.086	0.032	54		
Mean for 3 assays	-	-	-	-	155	Mean for 3 assays	-	-	-	-	74				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : 8-MOP

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-03	1	0.976	0.985	0.861	0.983	112	AKP-03	1	0.029	0.027	0.081	0.034	45	Positive	Photoreactive
	2	0.982	1.007	0.872	1.005	107		2	0.028	0.027	0.080	0.033	45		
	3	0.986	1.005	0.879	1.001	104		3	0.029	0.028	0.088	0.035	52		
	Mean	0.981	0.999	0.871	0.996	108		Mean	0.029	0.027	0.083	0.034	47		
AKP-09	1	0.976	0.987	0.843	0.982	129	AKP-09	1	0.028	0.028	0.085	0.033	52	Positive	Photoreactive
	2	0.974	0.988	0.854	0.983	116		2	0.028	0.028	0.083	0.033	50		
	3	0.977	0.987	0.863	0.983	110		3	0.029	0.028	0.092	0.034	58		
	Mean	0.976	0.987	0.853	0.983	118		Mean	0.028	0.028	0.087	0.033	53		
AKP-13	1	0.967	0.985	0.847	0.980	116	AKP-13	1	0.029	0.027	0.072	0.033	37	Positive	Photoreactive
	2	0.971	0.986	0.860	0.984	107		2	0.027	0.026	0.069	0.033	36		
	3	0.977	0.992	0.871	0.989	102		3	0.028	0.027	0.080	0.034	46		
	Mean	0.972	0.988	0.859	0.984	108		Mean	0.028	0.027	0.074	0.033	40		
Mean for 3 assays	-	-	-	-	111	Mean for 3 assays	-	-	-	-	47				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Nalidixic acid

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
	A440(-)			A440(+)			A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *2		
AKP-05	1	0.979	0.977	0.825	0.974	151	1	0.028	0.027	0.237	0.038	201	Positive	Photoreactive
	2	0.978	0.979	0.834	0.976	141	2	0.027	0.027	0.232	0.033	197		
	3	0.976	0.988	0.837	0.984	136	3	0.028	0.027	0.224	0.034	188		
	Mean	0.978	0.981	0.832	0.978	143	Mean	0.028	0.027	0.231	0.035	195		
AKP-10	1	0.982	0.967	0.832	0.966	147	1	0.028	0.027	0.257	0.033	222	Positive	Photoreactive
	2	0.981	0.969	0.837	0.966	141	2	0.027	0.026	0.233	0.033	199		
	3	0.977	0.973	0.839	0.969	135	3	0.028	0.028	0.249	0.035	214		
	Mean	0.980	0.970	0.836	0.967	141	Mean	0.028	0.027	0.246	0.034	212		
AKP-13	1	0.988	0.985	0.827	0.980	157	1	0.028	0.027	0.259	0.033	225	Positive	Photoreactive
	2	0.995	0.986	0.839	0.984	152	2	0.027	0.026	0.239	0.033	206		
	3	0.993	0.992	0.844	0.989	145	3	0.028	0.027	0.248	0.034	214		
	Mean	0.992	0.988	0.837	0.984	151	Mean	0.028	0.027	0.249	0.033	215		
Mean for 3 assays	-	-	-	-	145	Mean for 3 assays	-	-	-	-	207			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Nalidixic acid (Na salt)

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-18	1	0.985	0.995	0.815	0.992	167	AKP-18	1	0.028	0.026	0.231	0.040	190	Positive	Photoreactive
	2	0.986	0.995	0.818	0.991	165		2	0.027	0.027	0.224	0.039	184		
	3	0.988	0.993	0.828	0.990	157		3	0.028	0.027	0.227	0.041	186		
	Mean	0.986	0.994	0.820	0.991	163		Mean	0.028	0.027	0.227	0.040	187		
AKP-24	1	1.001	1.001	0.847	0.996	149	AKP-24	1	0.029	0.028	0.247	0.042	205	Positive	Photoreactive
	2	1.006	1.004	0.859	0.998	142		2	0.028	0.029	0.243	0.041	202		
	3	1.005	1.004	0.861	0.999	139		3	0.029	0.032	0.254	0.045	212		
	Mean	1.004	1.003	0.856	0.998	143		Mean	0.029	0.030	0.248	0.043	206		
AKP-26	1	0.995	0.991	0.843	0.989	150	AKP-26	1	0.027	0.028	0.246	0.040	208	Positive	Photoreactive
	2	1.002	0.999	0.859	0.998	141		2	0.028	0.028	0.243	0.038	204		
	3	1.002	0.999	0.862	0.995	138		3	0.027	0.027	0.238	0.039	200		
	Mean	1.000	0.996	0.855	0.994	143		Mean	0.027	0.028	0.242	0.039	204		
Mean for 3 assays	-	-	-	-	150	Mean for 3 assays	-	-	-	-	199				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Norfloxacin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-01	1	0.948	0.954	0.709	0.951	236	AKP-01	1	0.028	0.027	0.104	0.033	70	Positive	Photoreactive
	2	0.952	0.949	0.718	0.946	231		2	0.028	0.027	0.103	0.033	69		
	3	0.953	0.959	0.725	0.956	225		3	0.029	0.027	0.107	0.034	72		
	Mean	0.951	0.954	0.717	0.951	231		Mean	0.028	0.027	0.105	0.033	70		
AKP-07	1	0.995	0.992	0.753	0.984	236	AKP-11	1	0.028	0.027	0.104	0.032	71	Positive	Photoreactive
	2	0.994	0.997	0.765	0.992	223		2	0.028	0.027	0.104	0.032	71		
	3	0.993	0.995	0.772	0.991	215		3	0.028	0.028	0.108	0.033	75		
	Mean	0.994	0.995	0.763	0.989	225		Mean	0.028	0.027	0.105	0.032	72		
AKP-11	1	0.992	0.970	0.779	0.966	210	AKP-27	1	0.030	0.027	0.117	0.039	75	Positive	Photoreactive
	2	0.988	0.978	0.783	0.976	202		2	0.029	0.027	0.114	0.038	73		
	3	0.989	0.977	0.789	0.973	197		3	0.029	0.028	0.117	0.040	76		
	Mean	0.990	0.975	0.784	0.972	203		Mean	0.029	0.027	0.116	0.039	75		
Mean for 3 assays	-	-	-	-	220	Mean for 3 assays	-	-	-	-	72				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Ofloxacin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-16	1	0.990	0.984	0.807	0.981	180	AKP-16	1	0.030	0.028	0.350	0.033	314	Positive	Photoreactive
	2	0.988	0.985	0.813	0.981	172		2	0.029	0.027	0.354	0.033	319		
	3	0.992	0.984	0.821	0.982	168		3	0.029	0.027	0.362	0.033	327		
	Mean	0.990	0.984	0.814	0.981	173		Mean	0.029	0.027	0.355	0.033	320		
AKP-21	1	0.981	0.993	0.780	0.991	198	AKP-21	1	0.029	0.027	0.343	0.040	301	Positive	Photoreactive
	2	0.981	0.993	0.796	0.988	182		2	0.028	0.027	0.327	0.039	286		
	3	0.979	0.992	0.804	0.990	172		3	0.028	0.028	0.339	0.040	298		
	Mean	0.980	0.993	0.793	0.990	184		Mean	0.028	0.027	0.336	0.040	295		
AKP-23	1	0.994	1.005	0.799	1.000	190	AKP-23	1	0.030	0.028	0.340	0.045	292	Positive	Photoreactive
	2	0.995	1.007	0.818	1.003	172		2	0.029	0.027	0.331	0.045	284		
	3	0.996	1.008	0.822	1.004	169		3	0.030	0.028	0.345	0.047	297		
	Mean	0.995	1.007	0.813	1.002	177		Mean	0.030	0.028	0.339	0.046	291		
Mean for 3 assays	-	-	-	-	178	Mean for 3 assays	-	-	-	-	302				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Piroxicam

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 50 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-19	1	0.978	0.967	0.607	0.965	367	AKP-27	1	0.034	0.027	0.158	0.039	112	Positive	Photoreactive
	2	0.971	0.986	0.633	0.982	334		2	0.034	0.027	0.158	0.038	112		
	3	0.974	0.986	0.629	0.982	341		3	0.034	0.028	0.161	0.040	115		
	Mean	0.974	0.980	0.623	0.976	347		Mean	0.034	0.027	0.159	0.039	113		
AKP-23	1	0.996	1.005	0.662	1.000	329	AKP-28	1	0.036	0.028	0.149	0.040	101	Positive	Photoreactive
	2	0.995	1.007	0.695	1.003	295		2	0.034	0.028	0.148	0.039	102		
	3	1.001	1.008	0.703	1.004	293		3	0.033	0.027	0.157	0.041	112		
	Mean	0.997	1.007	0.687	1.002	306		Mean	0.034	0.028	0.151	0.040	105		
AKP-26	1	0.993	0.991	0.662	0.989	329	AKP-29	1	0.033	0.027	0.142	0.038	97	Positive	Photoreactive
	2	0.998	0.999	0.705	0.998	291		2	0.034	0.026	0.141	0.038	95		
	3	0.998	0.999	0.710	0.995	286		3	0.034	0.028	0.149	0.040	103		
	Mean	0.996	0.996	0.692	0.994	302		Mean	0.034	0.027	0.144	0.039	98		
Mean for 3 assays	-	-	-	-	318	Mean for 3 assays	-	-	-	-	105				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Promethazine HCl

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-16	1	0.982	0.984	0.856	0.981	123	AKP-16	1	0.027	0.028	0.061	0.033	28	Positive	Photoreactive
	2	0.977	0.985	0.855	0.981	119		2	0.028	0.027	0.060	0.033	26		
	3	0.977	0.984	0.853	0.982	121		3	0.028	0.027	0.064	0.033	30		
	Mean	0.979	0.984	0.855	0.981	121		Mean	0.028	0.027	0.062	0.033	28		
AKP-19	1	0.969	0.967	0.836	0.965	129	AKP-24	1	0.029	0.028	0.065	0.042	23	Positive	Photoreactive
	2	0.967	0.986	0.835	0.982	128		2	0.028	0.029	0.063	0.041	22		
	3	0.964	0.986	0.833	0.982	127		3	0.028	0.032	0.067	0.045	26		
	Mean	0.967	0.980	0.835	0.976	128		Mean	0.028	0.030	0.065	0.043	24		
AKP-24	1	0.981	1.001	0.846	0.996	130	AKP-25	1	0.027	0.027	0.063	0.040	23	Positive	Photoreactive
	2	0.978	1.004	0.848	0.998	125		2	0.027	0.027	0.059	0.039	19		
	3	0.992	1.004	0.862	0.999	125		3	0.028	0.028	0.064	0.041	23		
	Mean	0.984	1.003	0.852	0.998	127		Mean	0.027	0.027	0.062	0.040	22		
Mean for 3 assays	-	-	-	-	125	Mean for 3 assays	-	-	-	-	25				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Rosiglitazone

Rosiglitazone is excluded from the Fourth data analysis since the VMT considered that it is inappropriate to include rosiglitazone in the “phototoxic” chemical set because of lack of high quality human data regarding its phototoxicity.

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement				
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank					
AKP-17	1	0.963	1.009	0.890	1.004	69	AKP-21	1	0.032	0.027	0.076	0.040	31	Positive	/		
	2	0.960	1.001	0.892	0.997	64		2	0.032	0.027	0.077	0.039	32				
	3	0.954	1.009	0.888	1.005	62		3	0.033	0.028	0.082	0.040	36				
	Mean	0.959	1.006	0.890	1.002	65		Mean	0.032	0.027	0.078	0.040	33				
AKP-21	1	0.944	0.993	0.873	0.991	68	AKP-25	1	0.033	0.027	0.077	0.040	31	Positive		/	
	2	0.939	0.993	0.873	0.988	63		2	0.033	0.027	0.076	0.039	30				
	3	0.940	0.992	0.879	0.990	58		3	0.034	0.028	0.081	0.041	34				
	Mean	0.941	0.993	0.875	0.990	63		Mean	0.033	0.027	0.078	0.040	32				
AKP-25	1	0.941	0.980	0.863	0.979	76	AKP-27	1	0.034	0.027	0.080	0.039	34	Positive			/
	2	0.938	0.987	0.864	0.985	72		2	0.033	0.027	0.076	0.038	31				
	3	0.936	0.987	0.870	0.984	64		3	0.035	0.028	0.081	0.040	34				
	Mean	0.938	0.985	0.866	0.983	71		Mean	0.034	0.027	0.079	0.039	33				
Mean for 3 assays	-	-	-	-	66	Mean for 3 assays	-	-	-	-	33						

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Tetracycline

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol	
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *2			
AKP-16	1	0.992	0.984	0.769	0.981	220	AKP-16	1	0.035	0.028	0.204	0.033	163	Positive	Photoreactive
	2	0.986	0.985	0.781	0.981	202		2	0.033	0.027	0.198	0.033	159		
	3	0.993	0.984	0.787	0.982	203		3	0.034	0.027	0.197	0.033	157		
	Mean	0.990	0.984	0.779	0.981	208		Mean	0.034	0.027	0.200	0.033	160		
AKP-19	1	0.977	0.967	0.710	0.965	263	AKP-24	1	0.036	0.028	0.202	0.042	153	Positive	Photoreactive
	2	0.977	0.986	0.721	0.982	252		2	0.037	0.029	0.206	0.041	156		
	3	0.984	0.986	0.725	0.982	255		3	0.035	0.032	0.215	0.045	167		
	Mean	0.979	0.980	0.719	0.976	257		Mean	0.036	0.030	0.208	0.043	159		
AKP-24	1	0.991	1.001	0.745	0.996	241	AKP-25	1	0.033	0.027	0.197	0.040	151	Positive	Photoreactive
	2	0.995	1.004	0.772	0.998	218		2	0.033	0.027	0.192	0.039	146		
	3	0.998	1.004	0.774	0.999	219		3	0.034	0.028	0.203	0.041	156		
	Mean	0.995	1.003	0.764	0.998	226		Mean	0.033	0.027	0.197	0.040	151		
Mean for 3 assays	-	-	-	-	230	Mean for 3 assays	-	-	-	-	157				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Anthracene

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!	Undetermined	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Avobenzone

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Test concentration
 Singlet oxygen 50 µM
 Superoxide anion 50 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-06	1	1.165	0.981	0.977	0.979	186	AKP-06	1	0.128	0.026	0.136	0.033	1	Positive	(Photoreactive)
	2	1.176	0.985	0.971	0.982	203		2	0.126	0.026	0.134	0.033	1		
	3	1.170	0.984	0.983	0.981	185		3	0.127	0.027	0.136	0.034	2		
	Mean	1.170	0.983	0.977	0.981	191		Mean	0.127	0.026	0.135	0.033	1		
AKP-11	1	1.215	0.970	0.979	0.966	233	AKP-11	1	0.119	0.027	0.140	0.032	16	Positive	(Photoreactive)
	2	1.211	0.978	0.979	0.976	229		2	0.118	0.027	0.138	0.032	15		
	3	1.210	0.977	0.984	0.973	223		3	0.119	0.028	0.139	0.033	15		
	Mean	1.212	0.975	0.981	0.972	228		Mean	0.119	0.027	0.139	0.032	15		
AKP-12	1	1.182	0.986	0.939	0.984	240	AKP-12	1	0.132	0.027	0.126	0.033	-12	Positive	(Photoreactive)
	2	1.188	0.983	0.945	0.977	240		2	0.130	0.027	0.124	0.032	-12		
	3	1.184	0.984	0.947	0.981	234		3	0.131	0.027	0.125	0.034	-12		
	Mean	1.185	0.984	0.944	0.981	238		Mean	0.131	0.027	0.125	0.033	-12		
Mean for 3 assays	-	-	-	-	219	Mean for 3 assays	-	-	-	-	1				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Bithionol

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion Not tested

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-02	1	0.917	0.979	0.786	0.976	128		1							
	2	0.917	0.986	0.786	0.982	128		2							
	3	0.918	0.984	0.785	0.981	130		3							
	Mean	0.917	0.983	0.786	0.980	129		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Positive	Photoreactive
AKP-08	1	0.959	0.993	0.804	0.990	152		1							
	2	0.958	0.989	0.804	0.985	151		2							
	3	0.961	0.988	0.809	0.985	149		3							
	Mean	0.959	0.990	0.806	0.987	151		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Positive	Photoreactive
AKP-12	1	0.946	0.986	0.803	0.984	140		1							
	2	0.948	0.983	0.805	0.977	140		2							
	3	0.950	0.984	0.807	0.981	140		3							
	Mean	0.948	0.984	0.805	0.981	140		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Positive	Photoreactive
Mean for 3 assays	-	-	-	-	140		Mean for 3 assays	-	-	-	-	#DIV/0!			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Hexachlorophene

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results *1	Experimental No.	Run#	Superoxide anion				Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		A440(-)		A440(+)					A560(-)		A560(+)				
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-01	1	0.813	0.954	0.700	0.951	110	AKP-01	1	0.044	0.027	0.050	0.033	0	Positive	Photoreactive
	2	0.820	0.949	0.713	0.946	104		2	0.044	0.027	0.050	0.033	0		
	3	0.824	0.959	0.717	0.956	104		3	0.045	0.027	0.051	0.033	0		
	Mean	0.819	0.954	0.710	0.951	106		Mean	0.044	0.027	0.050	0.033	0		
AKP-11	1	0.869	0.970	0.766	0.966	100	AKP-11	1	0.046	0.027	0.050	0.032	-1	Positive	Photoreactive
	2	0.872	0.978	0.769	0.976	100		2	0.045	0.027	0.050	0.032	0		
	3	0.871	0.977	0.772	0.973	96		3	0.046	0.028	0.052	0.033	1		
	Mean	0.871	0.975	0.769	0.972	99		Mean	0.046	0.027	0.051	0.032	0		
AKP-15	1	0.875	0.971	0.730	0.970	142	AKP-15	1	0.046	0.027	0.052	0.034	-1	Positive	Photoreactive
	2	0.875	0.965	0.732	0.959	140		2	0.045	0.027	0.052	0.034	0		
	3	0.874	0.965	0.733	0.962	138		3	0.046	0.027	0.053	0.035	0		
	Mean	0.875	0.967	0.732	0.964	140		Mean	0.046	0.027	0.052	0.034	0		
Mean for 3 assays	-	-	-	-	115	Mean for 3 assays	-	-	-	-	0				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Rose bengal

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion Not tested

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-01	1	1.132	0.954	0.463	0.951	666	AKP-01	1	3.408	0.027	3.138	0.033	No data*3	Positive	Photoreactive
	2	1.138	0.949	0.478	0.946	657		2	3.397	0.027	3.134	0.033	No data*3		
	3	1.141	0.959	0.482	0.956	656		3	3.412	0.027	3.064	0.034	No data*3		
	Mean	1.137	0.954	0.474	0.951	660		Mean	3.406	0.027	3.112	0.033	#DIV/0!		
AKP-06	1	1.184	0.981	0.468	0.979	714	AKP-06	1					#DIV/0!	Positive	Photoreactive
	2	1.181	0.985	0.480	0.982	699		2					#DIV/0!		
	3	1.177	0.984	0.476	0.981	699		3					#DIV/0!		
	Mean	1.181	0.983	0.475	0.981	704		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
AKP-07	1	1.197	0.992	0.477	0.984	714	AKP-07	1					#DIV/0!	Positive	Photoreactive
	2	1.194	0.997	0.485	0.992	703		2					#DIV/0!		
	3	1.194	0.995	0.481	0.991	707		3					#DIV/0!		
	Mean	1.195	0.995	0.481	0.989	708		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
Mean for 3 assays		-	-	-	-	691	Mean for 3 assays		-	-	-	-	#DIV/0!		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*3 : over the OD criteria

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Aspirin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-05	1	0.980	0.977	0.976	0.974	1	AKP-05	1	0.027	0.027	0.032	0.038	-3	Negative	Non-photoreactive
	2	0.984	0.979	0.981	0.976	0		2	0.028	0.027	0.033	0.033	-3		
	3	0.985	0.988	0.981	0.984	1		3	0.027	0.027	0.034	0.034	-1		
	Mean	0.983	0.981	0.979	0.978	1		Mean	0.027	0.027	0.033	0.035	-2		
AKP-10	1	0.981	0.967	0.979	0.966	-1	AKP-10	1	0.027	0.027	0.032	0.033	-2	Negative	Non-photoreactive
	2	0.979	0.969	0.976	0.966	0		2	0.027	0.026	0.033	0.033	-1		
	3	0.979	0.973	0.976	0.969	0		3	0.027	0.028	0.034	0.035	0		
	Mean	0.980	0.970	0.977	0.967	0		Mean	0.027	0.027	0.033	0.034	-1		
AKP-15	1	0.968	0.971	0.965	0.970	0	AKP-15	1	0.028	0.027	0.033	0.034	-2	Negative	Non-photoreactive
	2	0.980	0.965	0.977	0.959	0		2	0.027	0.027	0.033	0.034	-1		
	3	0.980	0.965	0.976	0.962	1		3	0.028	0.027	0.033	0.035	-2		
	Mean	0.976	0.967	0.973	0.964	0		Mean	0.028	0.027	0.033	0.034	-2		
Mean for 3 assays		-	-	-	-	0	Mean for 3 assays		-	-	-	-	-2		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Benzocaine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-01	1	0.953	0.954	0.949	0.951	1	AKP-01	1	0.026	0.027	0.039	0.033	7	Negative	Non-photoreactive
	2	0.960	0.949	0.957	0.946	0		2	0.026	0.027	0.038	0.033	6		
	3	0.961	0.959	0.957	0.956	1		3	0.027	0.027	0.041	0.034	8		
	Mean	0.958	0.954	0.954	0.951	1		Mean	0.026	0.027	0.039	0.033	7		
AKP-08	1	0.999	0.993	0.995	0.990	1	AKP-08	1	0.026	0.027	0.036	0.033	4	Negative	Non-photoreactive
	2	0.998	0.989	0.994	0.985	1		2	0.027	0.027	0.036	0.033	3		
	3	0.998	0.988	0.994	0.985	1		3	0.027	0.027	0.038	0.034	5		
	Mean	0.998	0.990	0.994	0.987	1		Mean	0.027	0.027	0.037	0.033	4		
AKP-11	1	0.989	0.970	0.986	0.966	0	AKP-11	1	0.027	0.027	0.036	0.032	4	Negative	Non-photoreactive
	2	0.987	0.978	0.982	0.976	2		2	0.027	0.027	0.037	0.032	5		
	3	0.992	0.977	0.989	0.973	0		3	0.027	0.028	0.038	0.033	6		
	Mean	0.989	0.975	0.986	0.972	1		Mean	0.027	0.027	0.037	0.032	5		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	5				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Erythromycin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-17	1	0.962	1.009	0.962	1.004	-4	AKP-17	1	0.031	0.027	0.057	0.044	9	Negative	Non-photoreactive
	2	0.959	1.001	0.956	0.997	-1		2	0.030	0.027	0.055	0.043	8		
	3	0.953	1.009	0.949	1.005	0		3	0.032	0.028	0.059	0.046	10		
	Mean	0.958	1.006	0.956	1.002	-2		Mean	0.031	0.027	0.057	0.044	9		
AKP-22	1	0.938	0.987	0.938	0.968	-9	AKP-22	1	0.031	0.027	0.045	0.039	2	Negative	Non-photoreactive
	2	0.930	1.006	0.930	1.002	-9		2	0.029	0.027	0.043	0.038	2		
	3	0.942	1.008	0.944	1.003	-11		3	0.032	0.028	0.047	0.041	3		
	Mean	0.937	1.000	0.937	0.991	-10		Mean	0.031	0.027	0.045	0.039	2		
AKP-25	1	0.930	0.980	0.926	0.979	2	AKP-25	1	0.032	0.027	0.046	0.040	1	Negative	Non-photoreactive
	2	0.933	0.987	0.932	0.985	-1		2	0.031	0.027	0.044	0.039	0		
	3	0.934	0.987	0.935	0.984	-3		3	0.032	0.028	0.046	0.041	1		
	Mean	0.932	0.985	0.931	0.983	-1		Mean	0.032	0.027	0.045	0.040	1		
Mean for 3 assays	-	-	-	-	-4	Mean for 3 assays	-	-	-	-	4				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Penicillin G

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-17	1	1.006	1.009	1.002	1.004	0	AKP-17	1	0.030	0.027	0.085	0.044	38	Positive	Weakly photoreactive
	2	1.001	1.001	0.999	0.997	-2		2	0.029	0.027	0.084	0.043	38		
	3	1.008	1.009	1.004	1.005	0		3	0.030	0.028	0.089	0.046	42		
	Mean	1.005	1.006	1.002	1.002	-1		Mean	0.030	0.027	0.086	0.044	39		
AKP-21	1	0.987	0.993	0.982	0.991	2	AKP-21	1	0.029	0.027	0.081	0.040	39	Positive	Weakly photoreactive
	2	0.986	0.993	0.981	0.988	2		2	0.029	0.027	0.079	0.039	37		
	3	0.989	0.992	0.984	0.990	2		3	0.030	0.028	0.083	0.040	40		
	Mean	0.987	0.993	0.982	0.990	2		Mean	0.029	0.027	0.081	0.040	39		
AKP-25	1	0.982	0.980	0.979	0.979	1	AKP-25	1	0.030	0.027	0.075	0.040	32	Positive	Weakly photoreactive
	2	0.988	0.987	0.986	0.985	0		2	0.029	0.027	0.072	0.039	30		
	3	0.988	0.987	0.985	0.984	1		3	0.030	0.028	0.080	0.041	37		
	Mean	0.986	0.985	0.983	0.983	1		Mean	0.030	0.027	0.076	0.040	33		
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	37		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Phenytoin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-06	1	0.973	0.981	0.972	0.979	-1	AKP-06	1	0.031	0.026	0.101	0.033	63	Positive	Weakly photoreactive
	2	0.974	0.985	0.972	0.982	0		2	0.031	0.026	0.099	0.033	61		
	3	0.976	0.984	0.973	0.981	1		3	0.031	0.027	0.104	0.034	66		
	Mean	0.974	0.983	0.972	0.981	0		Mean	0.031	0.026	0.101	0.033	63		
AKP-10	1	0.975	0.967	0.971	0.966	1	AKP-10	1	0.031	0.027	0.098	0.033	60	Positive	Weakly photoreactive
	2	0.975	0.969	0.969	0.966	3		2	0.030	0.026	0.098	0.033	61		
	3	0.975	0.973	0.971	0.969	1		3	0.031	0.028	0.103	0.035	65		
	Mean	0.975	0.970	0.970	0.967	2		Mean	0.031	0.027	0.100	0.034	62		
AKP-15	1	0.965	0.971	0.962	0.970	0	AKP-15	1	0.031	0.027	0.106	0.034	68	Positive	Photoreactive
	2	0.974	0.965	0.971	0.959	0		2	0.031	0.027	0.107	0.034	69		
	3	0.972	0.965	0.970	0.962	-1		3	0.032	0.027	0.112	0.035	73		
	Mean	0.970	0.967	0.968	0.964	0		Mean	0.031	0.027	0.108	0.034	70		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	65				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Bumetrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-28	1	1.009	0.974	1.013	0.972	-7	AKP-26	1	0.038	0.028	0.057	0.040	8	Inconclusive	Non-photoreactive
	2	1.011	0.989	1.020	0.987	-12		2	0.038	0.028	0.056	0.038	7		
	3	1.005	0.988	1.013	0.984	-11		3	0.039	0.027	0.059	0.039	9		
	Mean	1.008	0.984	1.015	0.981	-10		Mean	0.038	0.028	0.057	0.039	8		
AKP-29	1	0.992	0.980	0.995	0.977	-5	AKP-28	1	0.051	0.028	0.070	0.040	7	Inconclusive	Non-photoreactive
	2	1.005	0.989	1.012	0.987	-9		2	0.052	0.028	0.070	0.039	6		
	3	1.002	0.987	1.039	0.985	-39		3	0.051	0.027	0.071	0.041	8		
	Mean	1.000	0.985	1.015	0.983	-18		Mean	0.051	0.028	0.070	0.040	7		
AKP-30	1	0.996	0.978	1.027	0.975	-34	AKP-29	1	0.037	0.027	0.062	0.038	13	Inconclusive	Non-photoreactive
	2	1.010	0.996	1.045	0.993	-38		2	0.037	0.026	0.062	0.038	13		
	3	1.003	0.994	1.035	0.990	-35		3	0.039	0.028	0.064	0.040	13		
	Mean	1.003	0.989	1.036	0.986	-36		Mean	0.038	0.027	0.063	0.039	13		
Mean for 3 assays	-	-	-	-	-21	Mean for 3 assays	-	-	-	-	9				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Camphor sulfonic acid

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-03	1	0.994	0.985	0.991	0.983	0	AKP-03	1	0.028	0.027	0.033	0.034	-2	Negative	Non-photoreactive
	2	1.002	1.007	0.999	1.005	0		2	0.027	0.027	0.032	0.033	-2		
	3	1.002	1.005	0.998	1.001	1		3	0.028	0.028	0.034	0.035	-1		
	Mean	0.999	0.999	0.996	0.996	0		Mean	0.028	0.027	0.033	0.034	-2		
AKP-09	1	0.993	0.987	0.990	0.982	-1	AKP-09	1	0.028	0.028	0.033	0.033	0	Negative	Non-photoreactive
	2	0.994	0.988	0.989	0.983	1		2	0.027	0.028	0.032	0.033	0		
	3	0.990	0.987	0.986	0.983	0		3	0.028	0.028	0.034	0.034	1		
	Mean	0.992	0.987	0.988	0.983	0		Mean	0.028	0.028	0.033	0.033	0		
AKP-13	1	0.989	0.985	0.986	0.980	-1	AKP-13	1	0.028	0.027	0.032	0.033	-2	Negative	Non-photoreactive
	2	0.992	0.986	0.989	0.984	-1		2	0.028	0.026	0.032	0.033	-2		
	3	0.992	0.992	0.990	0.989	-2		3	0.028	0.027	0.034	0.034	0		
	Mean	0.991	0.988	0.988	0.984	-1		Mean	0.028	0.027	0.033	0.033	-1		
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	-1				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Chlorhexidine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-19	1	0.889	0.967	0.879	0.965	6	AKP-23	1	0.034	0.028	0.064	0.045	12	Negative	Non-photoreactive
	2	0.880	0.986	0.872	0.982	4		2	0.035	0.027	0.067	0.045	14		
	3	0.883	0.986	0.875	0.982	4		3	0.033	0.028	0.064	0.047	13		
	Mean	0.884	0.980	0.875	0.976	5		Mean	0.034	0.028	0.065	0.046	13		
AKP-23	1	0.905	1.005	0.896	1.000	4	AKP-28	1	0.038	0.028	0.070	0.040	20	Positive	Weakly photoreactive
	2	0.905	1.007	0.897	1.003	3		2	0.037	0.028	0.068	0.039	19		
	3	0.906	1.008	0.897	1.004	4		3	0.035	0.027	0.069	0.041	22		
	Mean	0.905	1.007	0.897	1.002	4		Mean	0.037	0.028	0.069	0.040	20		
AKP-28	1	0.883	0.974	0.872	0.972	8	AKP-29	1	0.033	0.027	0.062	0.038	17	Negative	Non-photoreactive
	2	0.884	0.989	0.875	0.987	6		2	0.033	0.026	0.061	0.038	16		
	3	0.885	0.988	0.875	0.984	7		3	0.035	0.028	0.063	0.040	16		
	Mean	0.884	0.984	0.874	0.981	7		Mean	0.034	0.027	0.062	0.039	16		
Mean for 3 assays	-	-	-	-	5	Mean for 3 assays	-	-	-	-	16				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B)) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Cinnamic acid

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank				
AKP-02	1	0.983	0.979	0.979	0.976	1	AKP-02	1	0.027	0.028	0.042	0.033	9	Negative	Non-photoreactive	
	2	0.987	0.986	0.984	0.982	0		2	0.027	0.027	0.042	0.033				9
	3	0.988	0.984	0.985	0.981	0		3	0.027	0.028	0.046	0.035				13
	Mean	0.986	0.983	0.983	0.980	0		Mean	0.027	0.028	0.043	0.034				10
AKP-08	1	0.986	0.993	0.984	0.990	-1	AKP-08	1	0.027	0.027	0.045	0.033	12	Negative	Non-photoreactive	
	2	0.990	0.989	0.986	0.985	1		2	0.027	0.027	0.044	0.033				11
	3	0.990	0.988	0.987	0.985	0		3	0.027	0.027	0.048	0.034				15
	Mean	0.989	0.990	0.986	0.987	0		Mean	0.027	0.027	0.046	0.033				13
AKP-12	1	0.991	0.986	0.988	0.984	0	AKP-12	1	0.027	0.027	0.039	0.033	6	Negative	Non-photoreactive	
	2	0.988	0.983	0.986	0.977	-1		2	0.027	0.027	0.039	0.032				6
	3	0.990	0.984	0.987	0.981	0		3	0.027	0.027	0.041	0.034				8
	Mean	0.990	0.984	0.987	0.981	0		Mean	0.027	0.027	0.040	0.033				7
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	10					

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Drometrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Test concentration
 Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!	Undetermined	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : L-Histidine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-01	1	0.973	0.954	0.972	0.951	-2	AKP-01	1	0.027	0.027	0.110	0.033	77	Positive	Photoreactive
	2	0.979	0.949	0.976	0.946	0		2	0.027	0.027	0.108	0.033	75		
	3	0.978	0.959	0.975	0.956	0		3	0.028	0.027	0.115	0.034	81		
	Mean	0.977	0.954	0.974	0.951	-1		Mean	0.027	0.027	0.111	0.033	78		
AKP-08	1	0.985	0.993	0.981	0.990	1	AKP-08	1	0.027	0.027	0.106	0.033	73	Positive	Photoreactive
	2	0.994	0.989	0.989	0.985	2		2	0.027	0.027	0.106	0.033	73		
	3	0.991	0.988	0.987	0.985	1		3	0.028	0.027	0.111	0.034	77		
	Mean	0.990	0.990	0.986	0.987	1		Mean	0.027	0.027	0.108	0.033	74		
AKP-11	1	0.974	0.970	0.971	0.966	0	AKP-11	1	0.028	0.027	0.087	0.032	54	Positive	Weakly photoreactive
	2	0.981	0.978	0.978	0.976	0		2	0.027	0.027	0.087	0.032	55		
	3	0.981	0.977	0.978	0.973	0		3	0.028	0.028	0.095	0.033	62		
	Mean	0.979	0.975	0.976	0.972	0		Mean	0.028	0.027	0.090	0.032	57		
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	70				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Methylbenzylidene camphor

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Test concentration
 Singlet oxygen 50 µM
 Superoxide anion 50 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-07	1	1.014	0.992	0.972	0.984	36	AKP-09	1	0.046	0.028	0.034	0.033	-17	Positive	(Photoreactive)
	2	1.011	0.997	0.969	0.992	36		2	0.045	0.028	0.033	0.033	-17		
	3	1.017	0.995	0.974	0.991	37		3	0.047	0.028	0.035	0.034	-17		
	Mean	1.014	0.995	0.972	0.989	36		Mean	0.046	0.028	0.034	0.033	-17		
AKP-09	1	1.003	0.987	0.972	0.982	27	AKP-13	1	0.050	0.027	0.032	0.033	-24	Positive	(Photoreactive)
	2	0.998	0.988	0.969	0.983	25		2	0.050	0.026	0.031	0.033	-25		
	3	1.002	0.987	0.975	0.983	23		3	0.051	0.027	0.033	0.034	-24		
	Mean	1.001	0.987	0.972	0.983	25		Mean	0.050	0.027	0.032	0.033	-24		
AKP-13	1	1.022	0.985	0.974	0.980	44	AKP-15	1	0.052	0.027	0.033	0.034	-26	Positive	(Photoreactive)
	2	1.016	0.986	0.969	0.984	43		2	0.052	0.027	0.033	0.034	-26		
	3	1.019	0.992	0.971	0.989	44		3	0.053	0.027	0.033	0.035	-27		
	Mean	1.019	0.988	0.971	0.984	44		Mean	0.052	0.027	0.033	0.034	-26		
Mean for 3 assays	-	-	-	-	35	Mean for 3 assays	-	-	-	-	-22				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Octrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen 20 µM
 Superoxide anion Not tested

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-23	1	1.052	1.005	1.050	1.000	-3		1							
	2	1.049	1.007	1.052	1.003	-8		2							
	3	1.051	1.008	1.052	1.004	-6		3							
	Mean	1.051	1.007	1.051	1.002	-6		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	Inconclusive
AKP-26	1	1.038	0.991	1.047	0.989	-11		1							
	2	1.036	0.999	1.044	0.998	-10		2							
	3	1.035	0.999	1.046	0.995	-13		3							
	Mean	1.036	0.996	1.046	0.994	-11		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	Inconclusive
AKP-27	1	1.003	0.986	1.019	0.984	-18		1							
	2	1.010	0.988	1.025	0.985	-17		2							
	3	1.011	0.991	1.028	0.989	-19		3							
	Mean	1.008	0.988	1.024	0.986	-18		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	Inconclusive
Mean for 3 assays	-	-	-	-	-12		Mean for 3 assays	-	-	-	-	#DIV/0!			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Octyl methacrylate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-21	1	0.988	0.993	0.981	0.991	4	AKP-21	1	0.026	0.027	0.039	0.040	0	Inconclusive	Non-photoreactive
	2	0.986	0.993	0.979	0.988	4		2	0.027	0.027	0.038	0.039	-2		
	3	0.986	0.992	0.979	0.990	4		3	0.029	0.028	0.042	0.040	0		
	Mean	0.987	0.993	0.980	0.990	4		Mean	0.027	0.027	0.040	0.040	-1		
AKP-24	1	1.000	1.001	0.995	0.996	0	AKP-24	1	0.030	0.028	0.040	0.042	-3	Inconclusive	Non-photoreactive
	2	1.008	1.004	1.005	0.998	-2		2	0.027	0.029	0.039	0.041	-1		
	3	1.012	1.004	1.006	0.999	1		3	0.031	0.032	0.043	0.045	-1		
	Mean	1.007	1.003	1.002	0.998	0		Mean	0.029	0.030	0.041	0.043	-2		
AKP-25	1	0.992	0.980	0.988	0.979	2	AKP-25	1	0.028	0.027	0.039	0.040	-2	Inconclusive	Non-photoreactive
	2	0.991	0.987	0.986	0.985	3		2	0.027	0.027	0.037	0.039	-3		
	3	0.989	0.987	0.984	0.984	3		3	0.028	0.028	0.039	0.041	-2		
	Mean	0.991	0.985	0.986	0.983	3		Mean	0.028	0.027	0.038	0.040	-2		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	-2				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Octyl methoxycinnamate

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Test concentration
 Singlet oxygen 20 µM
 Superoxide anion 50 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-19	1	1.009	0.967	0.992	0.965	13	AKP-16	1	0.064	0.028	0.092	0.033	22	Positive	Inconclusive
	2	1.007	0.986	0.992	0.982	11		2	0.064	0.027	0.093	0.033	23		
	3	1.007	0.986	0.990	0.982	13		3	0.065	0.027	0.095	0.033	24		
	Mean	1.008	0.980	0.991	0.976	12		Mean	0.064	0.027	0.093	0.033	23		
AKP-24	1	1.023	1.001	1.008	0.996	10	AKP-17	1	0.059	0.027	0.106	0.044	30	Positive	Inconclusive
	2	1.030	1.004	1.016	0.998	9		2	0.059	0.027	0.105	0.043	29		
	3	1.025	1.004	1.009	0.999	11		3	0.060	0.028	0.097	0.046	20		
	Mean	1.026	1.003	1.011	0.998	10		Mean	0.059	0.027	0.103	0.044	26		
AKP-28	1	0.994	0.974	0.981	0.972	10	AKP-18	1	0.071	0.026	0.100	0.040	16	Inconclusive	Inconclusive
	2	0.995	0.989	0.982	0.987	10		2	0.070	0.027	0.098	0.039	15		
	3	1.008	0.988	0.993	0.984	12		3	0.071	0.027	0.101	0.041	17		
	Mean	0.999	0.984	0.985	0.981	11		Mean	0.071	0.027	0.100	0.040	16		
Mean for 3 assays	-	-	-	-	11	Mean for 3 assays	-	-	-	-	22				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : Octyl salicylate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-27	1	1.000	0.986	0.994	0.984	4	AKP-27	1	0.033	0.027	0.062	0.039	17	Inconclusive	Non-photoreactive
	2	0.992	0.988	0.987	0.985	3		2	0.035	0.027	0.062	0.038	15		
	3	0.996	0.991	0.990	0.989	4		3	0.034	0.028	0.063	0.040	17		
	Mean	0.996	0.988	0.990	0.986	4		Mean	0.034	0.027	0.062	0.039	16		
AKP-28	1	0.982	0.974	0.975	0.972	4	AKP-28	1	0.036	0.028	0.060	0.040	12	Inconclusive	Non-photoreactive
	2	1.007	0.989	0.997	0.987	7		2	0.033	0.028	0.055	0.039	10		
	3	0.999	0.988	0.990	0.984	6		3	0.032	0.027	0.057	0.041	13		
	Mean	0.996	0.984	0.987	0.981	6		Mean	0.034	0.028	0.057	0.040	12		
AKP-29	1	0.990	0.980	0.984	0.977	4	AKP-29	1	0.032	0.027	0.058	0.038	14	Inconclusive	Non-photoreactive
	2	0.992	0.989	0.985	0.987	5		2	0.032	0.026	0.057	0.038	13		
	3	0.999	0.987	0.993	0.985	4		3	0.032	0.028	0.059	0.040	15		
	Mean	0.994	0.985	0.987	0.983	4		Mean	0.032	0.027	0.058	0.039	14		
Mean for 3 assays	-	-	-	-	5	Mean for 3 assays	-	-	-	-	14				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : PABA

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-02	1	0.980	0.979	0.977	0.976	0	AKP-02	1	0.028	0.028	0.033	0.033	-1	Negative	Non-photoreactive
	2	0.979	0.986	0.977	0.982	-1		2	0.026	0.027	0.032	0.033	0		
	3	0.989	0.984	0.987	0.981	-1		3	0.027	0.028	0.034	0.035	1		
	Mean	0.983	0.983	0.980	0.980	-1		Mean	0.027	0.028	0.033	0.034	0		
AKP-08	1	0.994	0.993	0.989	0.990	2	AKP-08	1	0.027	0.027	0.033	0.033	0	Negative	Non-photoreactive
	2	0.989	0.989	0.985	0.985	1		2	0.027	0.027	0.033	0.033	0		
	3	0.993	0.988	0.989	0.985	1		3	0.027	0.027	0.034	0.034	1		
	Mean	0.992	0.990	0.988	0.987	1		Mean	0.027	0.027	0.033	0.033	0		
AKP-12	1	0.988	0.986	0.986	0.984	-1	AKP-12	1	0.027	0.027	0.033	0.033	0	Negative	Non-photoreactive
	2	0.991	0.983	0.989	0.977	-1		2	0.027	0.027	0.032	0.032	-1		
	3	0.991	0.984	0.989	0.981	-1		3	0.027	0.027	0.034	0.034	1		
	Mean	0.990	0.984	0.988	0.981	-1		Mean	0.027	0.027	0.033	0.033	0		
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	0				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : SDS

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-02	1	0.962	0.979	0.951	0.976	8	AKP-02	1	0.073	0.028	0.112	0.033	33	Positive	Weakly photoreactive
	2	0.963	0.986	0.961	0.982	-1		2	0.073	0.027	0.110	0.033	31		
	3	0.962	0.984	0.951	0.981	8		3	0.074	0.028	0.110	0.035	30		
	Mean	0.962	0.983	0.954	0.980	5		Mean	0.073	0.028	0.111	0.034	31		
AKP-09	1	0.972	0.987	0.959	0.982	9	AKP-09	1	0.075	0.028	0.103	0.033	23	Positive	Weakly photoreactive
	2	0.972	0.988	0.958	0.983	10		2	0.074	0.028	0.101	0.033	22		
	3	0.971	0.987	0.957	0.983	10		3	0.075	0.028	0.102	0.034	22		
	Mean	0.972	0.987	0.958	0.983	10		Mean	0.075	0.028	0.102	0.033	22		
AKP-12	1	0.957	0.986	0.945	0.984	9	AKP-12	1	0.074	0.027	0.106	0.033	26	Positive	Weakly photoreactive
	2	0.972	0.983	0.960	0.977	9		2	0.073	0.027	0.103	0.032	24		
	3	0.973	0.984	0.961	0.981	9		3	0.074	0.027	0.104	0.034	24		
	Mean	0.967	0.984	0.955	0.981	9		Mean	0.074	0.027	0.104	0.033	25		
Mean for 3 assays	-	-	-	-	8	Mean for 3 assays	-	-	-	-	26				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 4
 Chemical Name : UV-571

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen 50 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AKP-07	1	1.156	0.992	1.139	0.984	11	AKP-22	1	0.051	0.027	0.079	0.039	16	Inconclusive	Inconclusive
	2	1.163	0.997	1.144	0.992	13		2	0.050	0.027	0.076	0.038	14		
	3	1.154	0.995	1.136	0.991	12		3	0.052	0.028	0.080	0.041	16		
	Mean	1.158	0.995	1.140	0.989	12		Mean	0.051	0.027	0.078	0.039	15		
AKP-12	1	1.136	0.986	1.129	0.984	4	AKP-26	1	0.047	0.028	0.077	0.040	19	Inconclusive	Inconclusive
	2	1.134	0.983	1.131	0.977	0		2	0.047	0.028	0.074	0.038	16		
	3	1.135	0.984	1.128	0.981	4		3	0.047	0.027	0.076	0.039	18		
	Mean	1.135	0.984	1.129	0.981	3		Mean	0.047	0.028	0.076	0.039	18		
AKP-16	1	1.140	0.984	1.127	0.981	10	AKP-27	1	0.048	0.027	0.084	0.039	24	Positive	Inconclusive
	2	1.142	0.985	1.137	0.981	2		2	0.049	0.027	0.083	0.038	22		
	3	1.141	0.984	1.134	0.982	4		3	0.049	0.028	0.085	0.040	24		
	Mean	1.141	0.984	1.133	0.981	5		Mean	0.049	0.027	0.084	0.039	23		
Mean for 3 assays	-	-	-	-	7	Mean for 3 assays	-	-	-	-	19				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Acridine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-04	1	0.986	1.018	0.648	1.014	335	AS-04	1	0.033	0.034	0.196	0.040	157	Positive	Photoreactive
	2	0.981	1.019	0.658	1.014	319		2	0.033	0.034	0.190	0.041	151		
	3	0.983	1.017	0.657	1.013	322		3	0.034	0.033	0.202	0.040	162		
	Mean	0.983	1.018	0.654	1.014	325		Mean	0.034	0.034	0.196	0.040	157		
AS-12	1	0.976	0.997	0.654	0.992	317	AS-12	1	0.034	0.034	0.155	0.040	111	Positive	Photoreactive
	2	0.970	0.998	0.663	0.993	302		2	0.034	0.034	0.156	0.052	112		
	3	0.972	0.997	0.664	0.991	303		3	0.034	0.034	0.157	0.040	112		
	Mean	0.973	0.997	0.660	0.992	307		Mean	0.034	0.034	0.156	0.044	112		
AS-19	1	0.987	1.004	0.660	0.998	320	AS-19	1	0.034	0.034	0.200	0.040	159	Positive	Photoreactive
	2	0.984	1.004	0.670	0.998	307		2	0.034	0.034	0.209	0.040	168		
	3	0.985	1.008	0.670	1.001	307		3	0.034	0.034	0.201	0.040	160		
	Mean	0.985	1.006	0.667	0.999	311		Mean	0.034	0.034	0.203	0.040	162		
Mean for 3 assays	-	-	-	-	314	Mean for 3 assays	-	-	-	-	144				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Acridine HCl

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-04	1	0.999	1.018	0.672	1.014	323	AS-04	1	0.034	0.034	0.146	0.040	106	Positive	Photoreactive
	2	1.009	1.019	0.689	1.014	316		2	0.034	0.034	0.147	0.041	107		
	3	1.008	1.017	0.691	1.013	313		3	0.033	0.033	0.151	0.040	111		
	Mean	1.005	1.018	0.684	1.014	317		Mean	0.034	0.034	0.148	0.040	108		
AS-12	1	0.988	0.997	0.657	0.992	326	AS-12	1	0.034	0.034	0.151	0.040	106	Positive	Photoreactive
	2	0.979	0.998	0.661	0.993	313		2	0.033	0.034	0.172	0.052	128		
	3	0.982	0.997	0.664	0.991	313		3	0.033	0.034	0.156	0.040	113		
	Mean	0.983	0.997	0.661	0.992	317		Mean	0.034	0.034	0.160	0.044	116		
AS-19	1	0.998	1.004	0.665	0.998	326	AS-19	1	0.034	0.034	0.165	0.040	125	Positive	Photoreactive
	2	0.989	1.004	0.669	0.998	313		2	0.034	0.034	0.178	0.040	138		
	3	0.995	1.008	0.676	1.001	312		3	0.033	0.034	0.190	0.040	151		
	Mean	0.994	1.006	0.670	0.999	317		Mean	0.034	0.034	0.178	0.040	138		
Mean for 3 assays	-	-	-	-	317	Mean for 3 assays	-	-	-	-	121				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Amiodarone HCl

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Test concentration
 Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1							1							
	2							2							
	3							3							
	Mean							Mean					Inconclusive	Undetermined	
	1							1							
	2							2							
	3							3							
	Mean							Mean					Inconclusive	Undetermined	
	1							1							
	2							2							
	3							3							
	Mean							Mean					Inconclusive	Undetermined	
	Mean for 3 assays	-	-	-	-			Mean for 3 assays	-	-	-	-			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Chlorpromazine HCl

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-08	1	0.981	1.005	0.925	1.003	53	AS-08	1	0.034	0.034	0.133	0.040	92	Positive	Photoreactive
	2	0.979	1.004	0.924	0.998	52		2	0.034	0.034	0.134	0.040	94		
	3	0.979	1.013	0.925	1.010	51		3	0.043	0.033	0.137	0.040	88		
	Mean	0.980	1.007	0.925	1.004	52		Mean	0.037	0.034	0.134	0.040	91		
AS-16	1	0.976	1.003	0.899	1.001	74	AS-16	1	0.034	0.034	0.129	0.040	83	Positive	Photoreactive
	2	0.984	1.011	0.910	1.008	71		2	0.034	0.034	0.128	0.058	81		
	3	0.982	1.012	0.909	1.008	71		3	0.034	0.034	0.129	0.040	83		
	Mean	0.981	1.009	0.906	1.006	72		Mean	0.034	0.034	0.129	0.046	82		
AS-22	1	0.983	1.016	0.907	1.009	69	AS-22	1	0.034	0.034	0.131	0.040	91	Positive	Photoreactive
	2	0.983	1.014	0.909	1.007	67		2	0.034	0.034	0.132	0.040	92		
	3	0.984	1.014	0.909	1.008	68		3	0.034	0.033	0.134	0.040	94		
	Mean	0.983	1.015	0.908	1.008	68		Mean	0.034	0.034	0.132	0.040	92		
Mean for 3 assays	-	-	-	-	64	Mean for 3 assays	-	-	-	-	88				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Doxycycline HCl

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-01	1	0.988	1.014	0.544	1.010	440	AS-01	1	0.036	0.034	0.420	0.038	378	Positive	Photoreactive
	2	1.003	1.012	0.573	1.008	426		2	0.036	0.034	0.417	0.039	376		
	3	0.996	1.009	0.569	1.005	423		3	0.037	0.034	0.419	0.040	377		
	Mean	0.996	1.012	0.562	1.008	430		Mean	0.036	0.034	0.418	0.039	377		
AS-10	1	0.979	1.004	0.529	0.994	443	AS-10	1	0.035	0.034	0.417	0.041	375	Positive	Photoreactive
	2	0.980	1.002	0.546	0.996	427		2	0.034	0.034	0.409	0.042	368		
	3	0.976	1.008	0.560	1.003	409		3	0.037	0.034	0.426	0.042	381		
	Mean	0.978	1.005	0.545	0.998	426		Mean	0.035	0.034	0.418	0.042	375		
AS-17	1	0.979	1.007	0.535	1.002	440	AS-17	1	0.035	0.033	0.428	0.038	388	Positive	Photoreactive
	2	0.979	1.014	0.548	1.008	426		2	0.034	0.034	0.422	0.039	383		
	3	0.975	1.013	0.548	1.008	422		3	0.036	0.034	0.423	0.039	382		
	Mean	0.978	1.011	0.544	1.006	429		Mean	0.035	0.034	0.424	0.039	384		
Mean for 3 assays	-	-	-	-	428	Mean for 3 assays	-	-	-	-	379				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Fenofibrate

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen 50 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-06	1	1.042	1.008	0.825	1.005	214	AS-06	1	0.040	0.033	0.043	0.039	-3	Positive	Inconclusive
	2	1.043	1.012	0.831	1.007	208		2	0.039	0.033	0.043	0.039	-3		
	3	1.038	1.011	0.829	1.005	205		3	0.040	0.034	0.046	0.040	-1		
	Mean	1.041	1.010	0.828	1.006	209		Mean	0.040	0.033	0.044	0.040	-2		
AS-13	1	1.048	1.009	0.828	1.002	214	AS-13	1	0.044	0.034	0.042	0.047	-12	Positive	Inconclusive
	2	1.047	1.004	0.826	0.998	215		2	0.044	0.034	0.042	0.041	-11		
	3	1.054	1.014	0.836	1.008	212		3	0.045	0.033	0.044	0.040	-10		
	Mean	1.050	1.009	0.830	1.003	214		Mean	0.044	0.034	0.042	0.043	-11		
AS-20	1	1.055	1.008	0.830	1.003	218	AS-20	1	0.043	0.034	0.041	0.039	-7	Positive	Inconclusive
	2	1.055	1.005	0.831	0.997	217		2	0.042	0.034	0.040	0.040	-7		
	3	1.054	1.004	0.836	0.998	211		3	0.043	0.033	0.042	0.039	-6		
	Mean	1.055	1.006	0.832	0.999	215		Mean	0.043	0.034	0.041	0.039	-7		
Mean for 3 assays	-	-	-	-	213	Mean for 3 assays	-	-	-	-	-7				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Furosemide

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-07	1	1.002	1.011	0.884	1.007	114	AS-07	1	0.035	0.034	0.068	0.041	28	Positive	Photoreactive
	2	1.009	1.016	0.891	1.011	114		2	0.034	0.034	0.070	0.040	30		
	3	1.008	1.015	0.892	1.011	113		3	0.035	0.034	0.086	0.040	45		
	Mean	1.006	1.014	0.889	1.010	114		Mean	0.034	0.034	0.075	0.040	34		
AS-13	1	0.999	1.009	0.873	1.002	119	AS-13	1	0.035	0.034	0.073	0.047	29	Positive	Photoreactive
	2	0.994	1.004	0.874	0.998	114		2	0.035	0.034	0.076	0.041	33		
	3	0.995	1.014	0.872	1.008	117		3	0.034	0.033	0.079	0.040	36		
	Mean	0.996	1.009	0.873	1.003	117		Mean	0.034	0.034	0.076	0.043	33		
AS-20	1	1.002	1.008	0.866	1.003	128	AS-20	1	0.035	0.034	0.073	0.039	33	Positive	Photoreactive
	2	1.006	1.005	0.870	0.997	130		2	0.034	0.034	0.075	0.040	35		
	3	1.007	1.004	0.875	0.998	124		3	0.034	0.033	0.080	0.039	41		
	Mean	1.005	1.006	0.870	0.999	127		Mean	0.034	0.034	0.076	0.039	36		
Mean for 3 assays	-	-	-	-	119	Mean for 3 assays	-	-	-	-	34				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Ketoprofen

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-04	1	1.012	1.018	0.789	1.014	220	AS-04	1	0.034	0.034	0.179	0.040	139	Positive	Photoreactive
	2	1.024	1.019	0.798	1.014	222		2	0.034	0.034	0.180	0.041	140		
	3	1.025	1.017	0.803	1.013	218		3	0.034	0.033	0.182	0.040	141		
	Mean	1.020	1.018	0.796	1.014	220		Mean	0.034	0.034	0.180	0.040	140		
AS-12	1	0.989	0.997	0.771	0.992	213	AS-12	1	0.034	0.034	0.176	0.040	131	Positive	Photoreactive
	2	0.989	0.998	0.775	0.993	209		2	0.033	0.034	0.180	0.052	137		
	3	0.989	0.997	0.776	0.991	208		3	0.035	0.034	0.186	0.040	141		
	Mean	0.989	0.997	0.774	0.992	210		Mean	0.034	0.034	0.181	0.044	136		
AS-19	1	1.011	1.004	0.801	0.998	203	AS-19	1	0.034	0.034	0.174	0.040	134	Positive	Photoreactive
	2	1.009	1.004	0.805	0.998	197		2	0.033	0.034	0.172	0.040	133		
	3	1.007	1.008	0.803	1.001	197		3	0.034	0.034	0.172	0.040	132		
	Mean	1.009	1.006	0.803	0.999	199		Mean	0.034	0.034	0.173	0.040	133		
Mean for 3 assays	-	-	-	-	210	Mean for 3 assays	-	-	-	-	136				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : 6-methylcoumarine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-01	1	1.007	1.014	0.827	1.010	176	AS-01	1	0.034	0.034	0.145	0.038	106	Positive	Photoreactive
	2	1.018	1.012	0.839	1.008	175		2	0.033	0.034	0.144	0.039	106		
	3	1.017	1.009	0.841	1.005	172		3	0.034	0.034	0.152	0.040	113		
	Mean	1.014	1.012	0.836	1.008	174		Mean	0.034	0.034	0.147	0.039	108		
AS-10	1	0.995	1.004	0.847	0.994	141	AS-10	1	0.034	0.034	0.149	0.041	107	Positive	Photoreactive
	2	0.992	1.002	0.840	0.996	145		2	0.034	0.034	0.148	0.042	106		
	3	0.996	1.008	0.852	1.003	138		3	0.034	0.034	0.156	0.042	113		
	Mean	0.994	1.005	0.846	0.998	141		Mean	0.034	0.034	0.151	0.042	109		
AS-17	1	1.003	1.007	0.841	1.002	157	AS-17	1	0.033	0.033	0.136	0.038	97	Positive	Photoreactive
	2	1.007	1.014	0.845	1.008	157		2	0.034	0.034	0.133	0.039	94		
	3	1.008	1.013	0.868	1.008	135		3	0.034	0.034	0.138	0.039	99		
	Mean	1.006	1.011	0.851	1.006	150		Mean	0.034	0.034	0.136	0.039	97		
Mean for 3 assays	-	-	-	-	155	Mean for 3 assays	-	-	-	-	105				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : 8-MOP

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-04	1	0.993	1.018	0.841	1.014	148	AS-04	1	0.034	0.034	0.093	0.040	53	Positive	Photoreactive
	2	1.004	1.019	0.851	1.014	149		2	0.033	0.034	0.088	0.041	49		
	3	1.002	1.017	0.854	1.013	144		3	0.034	0.033	0.091	0.040	51		
	Mean	1.000	1.018	0.849	1.014	147		Mean	0.034	0.034	0.091	0.040	51		
AS-12	1	0.978	0.997	0.857	0.992	116	AS-12	1	0.033	0.034	0.089	0.040	46	Positive	Photoreactive
	2	0.974	0.998	0.860	0.993	110		2	0.033	0.034	0.086	0.052	43		
	3	0.975	0.997	0.862	0.991	108		3	0.034	0.034	0.109	0.040	65		
	Mean	0.976	0.997	0.860	0.992	111		Mean	0.033	0.034	0.095	0.044	51		
AS-19	1	0.990	1.004	0.853	0.998	130	AS-19	1	0.033	0.034	0.109	0.040	70	Positive	Photoreactive
	2	0.990	1.004	0.863	0.998	120		2	0.033	0.034	0.109	0.040	70		
	3	0.986	1.008	0.859	1.001	120		3	0.034	0.034	0.115	0.040	75		
	Mean	0.989	1.006	0.858	0.999	123		Mean	0.033	0.034	0.111	0.040	72		
Mean for 3 assays	-	-	-	-	127	Mean for 3 assays	-	-	-	-	58				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Nalidixic acid

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-05	1	0.993	1.014	0.840	1.009	149	AS-05	1	0.033	0.034	0.204	0.038	167	Positive	Photoreactive
	2	1.000	1.013	0.846	1.009	149		2	0.033	0.034	0.218	0.038	181		
	3	0.988	1.018	0.842	1.014	142		3	0.034	0.034	0.244	0.039	206		
	Mean	0.994	1.015	0.843	1.011	147		Mean	0.033	0.034	0.222	0.038	185		
AS-13	1	0.997	1.009	0.853	1.002	138	AS-13	1	0.034	0.034	0.225	0.047	182	Positive	Photoreactive
	2	0.993	1.004	0.839	0.998	147		2	0.034	0.034	0.290	0.041	247		
	3	0.996	1.014	0.839	1.008	152		3	0.034	0.033	0.292	0.040	249		
	Mean	0.995	1.009	0.844	1.003	146		Mean	0.034	0.034	0.269	0.043	226		
AS-20	1	1.012	1.008	0.844	1.003	162	AS-20	1	0.034	0.034	0.140	0.039	101	Positive	Photoreactive
	2	1.007	1.005	0.846	0.997	155		2	0.034	0.034	0.155	0.040	116		
	3	1.004	1.004	0.869	0.998	128		3	0.034	0.033	0.147	0.039	108		
	Mean	1.008	1.006	0.853	0.999	148		Mean	0.034	0.034	0.147	0.039	108		
Mean for 3 assays	-	-	-	-	147	Mean for 3 assays	-	-	-	-	173				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Nalidixic acid (Na salt)

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-05	1	1.005	1.014	0.858	1.009	143	AS-05	1	0.034	0.034	0.190	0.038	152	Positive	Photoreactive
	2	1.023	1.013	0.882	1.009	138		2	0.033	0.034	0.172	0.038	135		
	3	1.023	1.018	0.881	1.014	138		3	0.034	0.034	0.190	0.039	152		
	Mean	1.017	1.015	0.873	1.011	140		Mean	0.034	0.034	0.184	0.038	146		
AS-12	1	0.973	0.997	0.866	0.992	101	AS-12	1	0.034	0.034	0.218	0.040	175	Positive	Photoreactive
	2	0.992	0.998	0.830	0.993	157		2	0.034	0.034	0.248	0.052	204		
	3	0.989	0.997	0.829	0.991	156		3	0.034	0.034	0.248	0.040	204		
	Mean	0.985	0.997	0.842	0.992	138		Mean	0.034	0.034	0.238	0.044	194		
AS-19	1	1.004	1.004	0.841	0.998	156	AS-19	1	0.034	0.034	0.282	0.040	242	Positive	Photoreactive
	2	1.002	1.004	0.840	0.998	155		2	0.034	0.034	0.310	0.040	270		
	3	1.007	1.008	0.844	1.001	156		3	0.034	0.034	0.318	0.040	278		
	Mean	1.004	1.006	0.841	0.999	156		Mean	0.034	0.034	0.303	0.040	263		
Mean for 3 assays	-	-	-	-	145	Mean for 3 assays	-	-	-	-	201				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Norfloxacin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-08	1	1.007	1.005	0.745	1.003	259	AS-08	1	0.034	0.034	0.136	0.040	96	Positive	Photoreactive
	2	1.012	1.004	0.753	0.998	256		2	0.033	0.034	0.140	0.040	101		
	3	1.010	1.013	0.752	1.010	255		3	0.034	0.033	0.150	0.040	110		
	Mean	1.010	1.007	0.750	1.004	257		Mean	0.034	0.034	0.142	0.040	102		
AS-16	1	1.003	1.003	0.744	1.001	256	AS-16	1	0.033	0.034	0.113	0.040	68	Positive	Photoreactive
	2	1.002	1.011	0.759	1.008	240		2	0.033	0.034	0.129	0.058	84		
	3	1.001	1.012	0.767	1.008	231		3	0.034	0.034	0.144	0.040	98		
	Mean	1.002	1.009	0.756	1.006	242		Mean	0.033	0.034	0.129	0.046	83		
AS-22	1	1.009	1.016	0.742	1.009	260	AS-22	1	0.033	0.034	0.126	0.040	88	Positive	Photoreactive
	2	1.009	1.014	0.745	1.007	257		2	0.033	0.034	0.126	0.040	87		
	3	1.004	1.014	0.740	1.008	257		3	0.034	0.033	0.149	0.040	109		
	Mean	1.007	1.015	0.742	1.008	258		Mean	0.033	0.034	0.134	0.040	95		
Mean for 3 assays	-	-	-	-	252	Mean for 3 assays	-	-	-	-	93				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Ofloxacin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-01	1	1.001	1.014	0.788	1.010	208	AS-01	1	0.035	0.034	0.299	0.038	259	Positive	Photoreactive
	2	1.006	1.012	0.807	1.008	196		2	0.034	0.034	0.310	0.039	271		
	3	1.008	1.009	0.810	1.005	195		3	0.034	0.034	0.317	0.040	278		
	Mean	1.005	1.012	0.802	1.008	200		Mean	0.034	0.034	0.309	0.039	269		
AS-10	1	0.996	1.004	0.791	0.994	198	AS-10	1	0.034	0.034	0.330	0.041	288	Positive	Photoreactive
	2	0.990	1.002	0.781	0.996	202		2	0.033	0.034	0.370	0.042	329		
	3	0.989	1.008	0.804	1.003	178		3	0.034	0.034	0.404	0.042	362		
	Mean	0.991	1.005	0.792	0.998	193		Mean	0.034	0.034	0.368	0.042	326		
AS-17	1	0.997	1.007	0.797	1.002	195	AS-17	1	0.034	0.033	0.298	0.038	259	Positive	Photoreactive
	2	0.996	1.014	0.784	1.008	207		2	0.033	0.034	0.340	0.039	301		
	3	0.993	1.013	0.795	1.008	193		3	0.034	0.034	0.340	0.039	301		
	Mean	0.995	1.011	0.792	1.006	198		Mean	0.034	0.034	0.326	0.039	287		
Mean for 3 assays	-	-	-	-	197	Mean for 3 assays	-	-	-	-	294				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Piroxicam

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Solution 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 100 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-05	1	1.004	1.014	0.556	1.009	444	AS-06	1	0.041	0.033	0.166	0.039	117	Positive	Photoreactive
	2	1.019	1.013	0.583	1.009	432		2	0.041	0.033	0.210	0.039	162		
	3	1.016	1.018	0.591	1.014	421		3	0.042	0.034	0.186	0.040	137		
	Mean	1.013	1.015	0.577	1.011	432		Mean	0.041	0.033	0.187	0.040	139		
AS-13	1	0.996	1.009	0.553	1.002	437	AS-13	1	0.041	0.034	0.208	0.047	158	Positive	Photoreactive
	2	0.995	1.004	0.584	0.998	405		2	0.041	0.034	0.225	0.041	175		
	3	0.993	1.014	0.607	1.008	380		3	0.042	0.033	0.203	0.040	152		
	Mean	0.995	1.009	0.581	1.003	407		Mean	0.041	0.034	0.212	0.043	162		
AS-20	1	0.996	1.008	0.550	1.003	439	AS-20	1	0.039	0.034	0.173	0.039	129	Positive	Photoreactive
	2	0.997	1.005	0.593	0.997	397		2	0.039	0.034	0.170	0.040	126		
	3	0.995	1.004	0.590	0.998	398		3	0.038	0.033	0.171	0.039	128		
	Mean	0.996	1.006	0.578	0.999	411		Mean	0.039	0.034	0.171	0.039	128		
Mean for 3 assays	-	-	-	-	417	Mean for 3 assays	-	-	-	-	143				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Promethazine HCl

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-07	1	0.997	1.011	0.841	1.007	152	AS-07	1	0.034	0.034	0.067	0.041	27	Positive	Photoreactive
	2	0.995	1.016	0.842	1.011	149		2	0.033	0.034	0.065	0.040	26		
	3	1.010	1.015	0.858	1.011	147		3	0.034	0.034	0.070	0.040	30		
	Mean	1.001	1.014	0.847	1.010	149		Mean	0.034	0.034	0.067	0.040	28		
AS-15	1	0.976	1.003	0.825	0.999	153	AS-15	1	0.033	0.034	0.068	0.041	28	Positive	Photoreactive
	2	0.983	1.002	0.832	0.997	153		2	0.033	0.034	0.069	0.041	28		
	3	0.980	1.003	0.834	1.016	148		3	0.036	0.033	0.074	0.041	31		
	Mean	0.980	1.002	0.830	1.004	151		Mean	0.034	0.034	0.070	0.041	29		
AS-21	1	0.985	1.004	0.833	1.000	149	AS-21	1	0.033	0.034	0.067	0.040	28	Positive	Photoreactive
	2	0.993	1.003	0.841	0.999	148		2	0.033	0.034	0.067	0.040	28		
	3	0.987	1.003	0.837	0.999	145		3	0.066	0.033	0.071	0.040	-1		
	Mean	0.988	1.003	0.837	0.999	147		Mean	0.044	0.034	0.068	0.040	18		
Mean for 3 assays	-	-	-	-	149	Mean for 3 assays	-	-	-	-	25				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Laboratory : 5
 Chemical Name : Rosiglitazone

Rosiglitazone is excluded from the Fourth data analysis since the VMT considered that it is inappropriate to include rosiglitazone in the “phototoxic” chemical set because of lack of high quality human data regarding its phototoxicity.

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 50 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
	A440(-)		A440(+)		Results *1	A560(-)		A560(+)		Results *2				
	Run#	Test Chemical	Blank	Test Chemical		Blank	Run#	Test Chemical	Blank		Test Chemical	Blank		
AS-01	1	0.981	1.014	0.925	1.010	51	AS-03	1	0.037	0.034	0.115	0.038	75	
	2	0.983	1.012	0.930	1.008	49		2	0.036	0.034	0.128	0.038	88	
	3	0.978	1.009	0.928	1.005	46		3	0.036	0.034	0.112	0.039	72	
	Mean	0.981	1.012	0.928	1.008	49		Mean	0.036	0.034	0.118	0.038	78	
AS-10	1	0.971	1.004	0.913	0.994	51	AS-10	1	0.036	0.034	0.126	0.041	82	
	2	0.969	1.002	0.911	0.996	51		2	0.036	0.034	0.122	0.042	78	
	3	0.964	1.008	0.907	1.003	50		3	0.036	0.034	0.122	0.042	78	
	Mean	0.968	1.005	0.910	0.998	51		Mean	0.036	0.034	0.123	0.042	79	
AS-17	1	0.965	1.007	0.911	1.002	49	AS-17	1	0.036	0.033	0.115	0.038	74	
	2	0.961	1.014	0.907	1.008	50		2	0.036	0.034	0.111	0.039	70	
	3	0.973	1.013	0.924	1.008	45		3	0.036	0.034	0.111	0.039	70	
	Mean	0.967	1.011	0.914	1.006	48		Mean	0.036	0.034	0.112	0.039	71	
Mean for 3 assays	-	-	-	-	49	Mean for 3 assays	-	-	-	-	76			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Tetracycline

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-07	1	1.005	1.011	0.647	1.007	354	AS-07	1	0.036	0.034	0.272	0.041	230	Positive	Photoreactive
	2	1.011	1.016	0.662	1.011	345		2	0.035	0.034	0.259	0.040	217		
	3	1.009	1.015	0.666	1.011	339		3	0.035	0.034	0.254	0.040	213		
	Mean	1.008	1.014	0.658	1.010	346		Mean	0.036	0.034	0.261	0.040	220		
AS-15	1	0.990	1.003	0.648	0.999	344	AS-15	1	0.035	0.034	0.299	0.041	257	Positive	Photoreactive
	2	0.987	1.002	0.658	0.997	331		2	0.035	0.034	0.284	0.041	243		
	3	0.985	1.003	0.662	1.016	325		3	0.035	0.033	0.280	0.041	238		
	Mean	0.987	1.002	0.656	1.004	333		Mean	0.035	0.034	0.288	0.041	246		
AS-21	1	1.002	1.004	0.658	1.000	340	AS-21	1	0.035	0.034	0.281	0.040	240	Positive	Photoreactive
	2	0.996	1.003	0.668	0.999	325		2	0.034	0.034	0.271	0.040	231		
	3	0.996	1.003	0.673	0.999	318		3	0.035	0.033	0.274	0.040	233		
	Mean	0.998	1.003	0.666	0.999	328		Mean	0.035	0.034	0.275	0.040	235		
Mean for 3 assays	-	-	-	-	336	Mean for 3 assays	-	-	-	-	234				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Anthracene

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1							1					Inconclusive	Undetermined	
	2							2							
	3							3							
	Mean							Mean							
	1							1					Inconclusive	Undetermined	
	2							2							
	3							3							
	Mean							Mean							
	1							1					Inconclusive	Undetermined	
	2							2							
	3							3							
	Mean							Mean							
	Mean for 3 assays	-	-	-	-			Mean for 3 assays	-	-	-	-			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Avobenzone

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-09	1	1.021	1.006	0.925	0.999	89	AS-09	1	0.045	0.033	0.132	0.042	79	Positive	Photoreactive
	2	1.015	1.006	0.918	0.999	90		2	0.045	0.034	0.131	0.042	78		
	3	1.017	1.006	0.920	0.996	88		3	0.045	0.034	0.136	0.043	82		
	Mean	1.018	1.006	0.921	0.998	89		Mean	0.045	0.033	0.133	0.042	80		
AS-16	1	1.018	1.003	0.924	1.001	91	AS-16	1	0.052	0.034	0.113	0.040	50	Positive	Photoreactive
	2	1.018	1.011	0.924	1.008	91		2	0.052	0.034	0.111	0.058	47		
	3	1.016	1.012	0.923	1.008	90		3	0.052	0.034	0.116	0.040	52		
	Mean	1.017	1.009	0.924	1.006	91		Mean	0.052	0.034	0.113	0.046	50		
AS-22	1	1.027	1.016	0.950	1.009	69	AS-22	1	0.055	0.034	0.115	0.040	54	Positive	Photoreactive
	2	1.022	1.014	0.929	1.007	86		2	0.053	0.034	0.112	0.040	52		
	3	1.020	1.014	0.927	1.008	86		3	0.053	0.033	0.116	0.040	57		
	Mean	1.023	1.015	0.935	1.008	80		Mean	0.054	0.034	0.114	0.040	54		
Mean for 3 assays	-	-	-	-	87	Mean for 3 assays	-	-	-	-	61				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Bithionol

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-01	1	0.975	1.014	0.802	1.010	169	AS-01	1	0.059	0.034	0.084	0.038	20	Positive	Photoreactive
	2	0.973	1.012	0.805	1.008	164		2	0.059	0.034	0.084	0.039	20		
	3	0.985	1.009	0.815	1.005	166		3	0.061	0.034	0.087	0.040	22		
	Mean	0.978	1.012	0.807	1.008	166		Mean	0.059	0.034	0.085	0.039	21		
AS-10	1	0.967	1.004	0.842	0.994	118	AS-10	1	0.056	0.034	0.082	0.041	17	Positive	Photoreactive
	2	0.967	1.002	0.854	0.996	105		2	0.056	0.034	0.081	0.042	17		
	3	0.968	1.008	0.855	1.003	106		3	0.057	0.034	0.084	0.042	19		
	Mean	0.967	1.005	0.850	0.998	110		Mean	0.056	0.034	0.082	0.042	18		
As-17	1	0.963	1.007	0.787	1.002	171	As-17	1	0.056	0.033	0.081	0.038	20	Positive	Photoreactive
	2	0.964	1.014	0.791	1.008	168		2	0.055	0.034	0.084	0.039	24		
	3	0.958	1.013	0.790	1.008	163		3	0.057	0.034	0.083	0.039	22		
	Mean	0.962	1.011	0.789	1.006	167		Mean	0.056	0.034	0.083	0.039	22		
Mean for 3 assays	-	-	-	-	148	Mean for 3 assays	-	-	-	-	20				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Hexachlorophene

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-08	1	0.924	1.005	0.775	1.003	146	AS-08	1	0.046	0.034	0.051	0.040	-1	Positive	Photoreactive
	2	0.938	1.004	0.779	0.998	156		2	0.046	0.034	0.049	0.040	-3		
	3	0.928	1.013	0.776	1.010	148		3	0.047	0.033	0.050	0.040	-3		
	Mean	0.930	1.007	0.777	1.004	150		Mean	0.046	0.034	0.050	0.040	-2		
AS-16	1	0.908	1.003	0.758	1.001	147	AS-16	1	0.048	0.034	0.051	0.040	-9	Positive	Photoreactive
	2	0.913	1.011	0.760	1.008	150		2	0.047	0.034	0.050	0.058	-9		
	3	0.911	1.012	0.758	1.008	151		3	0.048	0.034	0.051	0.040	-8		
	Mean	0.911	1.009	0.758	1.006	149		Mean	0.047	0.034	0.051	0.046	-9		
AS-22	1	0.924	1.016	0.769	1.009	148	AS-22	1	0.049	0.034	0.052	0.040	-3	Positive	Photoreactive
	2	0.926	1.014	0.791	1.007	128		2	0.048	0.034	0.051	0.040	-3		
	3	0.917	1.014	0.761	1.008	149		3	0.049	0.033	0.052	0.040	-3		
	Mean	0.922	1.015	0.774	1.008	142		Mean	0.048	0.034	0.052	0.040	-3		
Mean for 3 assays	-	-	-	-	147	Mean for 3 assays	-	-	-	-	-	-5			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Rose bengal

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-08	1	1.217	1.005	0.381	1.003	833	AS-08	1	3.420	0.034	2.092	0.040	No data *3	Positive	Photoreactive
	2	1.215	1.004	0.400	0.998	811		2	3.477	0.034	2.150	0.040	No data *3		
	3	1.215	1.013	0.403	1.010	809		3	3.429	0.033	2.062	0.040	No data *3		
	Mean	1.216	1.007	0.395	1.004	818		Mean	3.442	0.034	2.101	0.040	No data *3		
AS-15	1	1.203	1.003	0.362	0.999	843	AS-15	1	3.428	0.034	1.983	0.041	No data *3	Positive	Photoreactive
	2	1.203	1.002	0.377	0.997	828		2	3.493	0.034	2.075	0.041	No data *3		
	3	1.222	1.003	0.386	1.016	838		3	3.471	0.033	2.038	0.041	No data *3		
	Mean	1.209	1.002	0.375	1.004	836		Mean	3.464	0.034	2.032	0.041	No data *3		
AS-21	1	1.216	1.004	0.379	1.000	833	AS-21	1	3.426	0.034	2.147	0.040	No data *3	Positive	Photoreactive
	2	1.218	1.003	0.403	0.999	811		2	3.475	0.034	2.195	0.040	No data *3		
	3	1.218	1.003	0.410	0.999	804		3	3.458	0.033	2.125	0.040	No data *3		
	Mean	1.217	1.003	0.397	0.999	816		Mean	3.453	0.034	2.156	0.040	No data *3		
Mean for 3 assays	-	-	-	-	823	Mean for 3 assays	-	-	-	-	-	No data *3			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Aspirin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-07	1	1.005	1.011	1.002	1.007	0	AS-07	1	0.034	0.034	0.037	0.041	-2	Negative	Non-photoreactive
	2	1.014	1.016	1.012	1.011	-2		2	0.034	0.034	0.037	0.040	-3		
	3	1.012	1.015	1.010	1.011	-2		3	0.034	0.034	0.038	0.040	-3		
	Mean	1.010	1.014	1.008	1.010	-1		Mean	0.034	0.034	0.037	0.040	-3		
AS-16	1	1.001	1.003	0.998	1.001	0	AS-16	1	0.034	0.034	0.038	0.040	-8	Negative	Non-photoreactive
	2	0.999	1.011	0.996	1.008	1		2	0.033	0.034	0.037	0.058	-8		
	3	1.009	1.012	1.004	1.008	1		3	0.034	0.034	0.038	0.040	-8		
	Mean	1.003	1.009	0.999	1.006	1		Mean	0.033	0.034	0.037	0.046	-8		
AS-20	1	1.010	1.008	1.005	1.003	-2	AS-20	1	0.034	0.034	0.037	0.039	-2	Negative	Non-photoreactive
	2	1.007	1.005	1.001	0.997	-1		2	0.033	0.034	0.036	0.040	-2		
	3	1.006	1.004	1.001	0.998	-2		3	0.034	0.033	0.037	0.039	-1		
	Mean	1.007	1.006	1.002	0.999	-2		Mean	0.033	0.034	0.037	0.039	-2		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	-4				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Benzocaine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol	
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *2			
AS-01	1	1.007	1.014	1.002	1.010	1	AS-01	1	0.034	0.034	0.047	0.038	8	Negative	Non-photoreactive
	2	1.010	1.012	1.004	1.008	1		2	0.036	0.034	0.050	0.039	9		
	3	1.014	1.009	1.007	1.005	2		3	0.034	0.034	0.050	0.040	10		
	Mean	1.010	1.012	1.004	1.008	1		Mean	0.035	0.034	0.049	0.039	9		
AS-10	1	0.993	1.004	0.989	0.994	-2	AS-10	1	0.033	0.034	0.046	0.041	5	Negative	Non-photoreactive
	2	0.996	1.002	0.990	0.996	-2		2	0.034	0.034	0.047	0.042	5		
	3	0.997	1.008	0.991	1.003	-2		3	0.034	0.034	0.053	0.042	10		
	Mean	0.995	1.005	0.990	0.998	-2		Mean	0.034	0.034	0.048	0.042	7		
AS-17	1	1.003	1.007	1.001	1.002	-3	AS-17	1	0.033	0.033	0.046	0.038	8	Negative	Non-photoreactive
	2	1.003	1.014	0.997	1.008	1		2	0.033	0.034	0.048	0.039	9		
	3	1.001	1.013	0.995	1.008	0		3	0.035	0.034	0.053	0.039	14		
	Mean	1.002	1.011	0.998	1.006	-1		Mean	0.034	0.034	0.049	0.039	10		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	9				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Erythromycin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-02	1	0.995	1.025	1.010	1.023	-20	AS-02	1	0.034	0.034	0.042	0.039	3	Negative	Non-photoreactive
	2	1.002	1.026	0.998	1.022	-1		2	0.034	0.034	0.042	0.039	2		
	3	1.017	1.031	1.012	1.022	-1		3	0.034	0.043	0.043	0.047	4		
	Mean	1.004	1.027	1.007	1.022	-7		Mean	0.034	0.037	0.042	0.042	3		
AS-11	1	0.963	0.991	0.974	0.989	-7	AS-11	1	0.034	0.034	0.044	0.041	2	Negative	Non-photoreactive
	2	0.958	0.998	0.970	0.995	-8		2	0.034	0.034	0.042	0.042	2		
	3	0.964	0.999	0.964	1.015	4		3	0.034	0.033	0.044	0.040	2		
	Mean	0.962	0.996	0.969	1.000	-4		Mean	0.034	0.034	0.043	0.041	2		
AS-18	1	0.985	1.003	0.976	0.998	4	AS-18	1	0.034	0.034	0.046	0.040	4	Negative	Non-photoreactive
	2	0.987	1.007	0.981	1.003	1		2	0.034	0.034	0.043	0.041	3		
	3	0.983	1.002	0.976	0.997	2		3	0.034	0.034	0.046	0.041	5		
	Mean	0.985	1.004	0.978	0.999	2		Mean	0.034	0.034	0.045	0.041	4		
Mean for 3 assays	-	-	-	-	-3	Mean for 3 assays	-	-	-	-	3				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Penicillin G

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-01	1	1.003	1.014	0.998	1.010	1	AS-01	1	0.034	0.034	0.090	0.038	51	Positive	Weakly photoreactive
	2	0.998	1.012	0.994	1.008	0		2	0.034	0.034	0.086	0.039	47		
	3	1.005	1.009	0.999	1.005	1		3	0.034	0.034	0.088	0.040	49		
	Mean	1.002	1.012	0.997	1.008	1		Mean	0.034	0.034	0.088	0.039	49		
AS-11	1	0.993	0.991	0.989	0.989	8	AS-11	1	0.034	0.034	0.085	0.041	44	Positive	Weakly photoreactive
	2	0.994	0.998	0.991	0.995	7		2	0.034	0.034	0.082	0.042	41		
	3	0.996	0.999	0.991	1.015	9		3	0.034	0.033	0.086	0.040	45		
	Mean	0.994	0.996	0.990	1.000	8		Mean	0.034	0.034	0.085	0.041	43		
AS-18	1	1.000	1.003	0.995	0.998	0	AS-18	1	0.035	0.034	0.089	0.040	48	Positive	Weakly photoreactive
	2	0.996	1.007	0.991	1.003	-1		2	0.034	0.034	0.085	0.041	44		
	3	1.006	1.002	1.000	0.997	1		3	0.034	0.034	0.089	0.041	49		
	Mean	1.000	1.004	0.995	0.999	0		Mean	0.034	0.034	0.088	0.041	47		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	46				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Phenytoin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-07	1	1.006	1.011	1.001	1.007	1	AS-07	1	0.034	0.034	0.136	0.041	96	Positive	Photoreactive
	2	1.009	1.016	1.006	1.011	0		2	0.033	0.034	0.130	0.040	91		
	3	1.008	1.015	1.004	1.011	0		3	0.037	0.034	0.136	0.040	93		
	Mean	1.008	1.014	1.004	1.010	0		Mean	0.035	0.034	0.134	0.040	93		
AS-15	1	0.998	1.003	0.994	0.999	7	AS-15	1	0.035	0.034	0.122	0.041	81	Positive	Photoreactive
	2	0.994	1.002	0.991	0.997	5		2	0.035	0.034	0.120	0.041	78		
	3	0.996	1.003	0.991	1.016	7		3	0.036	0.033	0.130	0.041	88		
	Mean	0.996	1.002	0.992	1.004	6		Mean	0.035	0.034	0.124	0.041	82		
AS-21	1	1.001	1.004	0.994	1.000	2	AS-21	1	0.035	0.034	0.140	0.040	99	Positive	Photoreactive
	2	0.999	1.003	0.994	0.999	2		2	0.034	0.034	0.138	0.040	98		
	3	0.996	1.003	0.999	0.999	-6		3	0.034	0.033	0.146	0.040	106		
	Mean	0.999	1.003	0.995	0.999	-1		Mean	0.034	0.034	0.141	0.040	101		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	92				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Bumetrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen 20 µM
 Superoxide anion Not tested

Experimental No.	Singlet oxygen						Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
	A440(-)			A440(+)			A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *2		
AS-06	1	1.021	1.008	1.026	1.005	-9	1						Inconclusive	Inconclusive
	2	1.024	1.012	1.029	1.007	-9	2							
	3	1.025	1.011	1.043	1.005	-22	3							
	Mean	1.023	1.010	1.033	1.006	-13	Mean							
AS-13	1	1.018	1.009	1.025	1.002	-13	1						Inconclusive	Inconclusive
	2	1.019	1.004	1.040	0.998	-28	2							
	3	1.025	1.014	1.030	1.008	-12	3							
	Mean	1.020	1.009	1.032	1.003	-18	Mean							
AS-20	1	1.014	1.008	1.015	1.003	-8	1						Inconclusive	Inconclusive
	2	1.017	1.005	1.019	0.997	-9	2							
	3	1.022	1.004	1.022	0.998	-7	3							
	Mean	1.018	1.006	1.019	0.999	-8	Mean							
Mean for 3 assays	-	-	-	-	-13	Mean for 3 assays	-	-	-	-	-			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Camphor sulfonic acid

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-04	1	1.004	1.018	1.001	1.014	-1	AS-04	1	0.034	0.034	0.039	0.040	-1	Negative	Non-photoreactive
	2	1.005	1.019	0.999	1.014	1		2	0.034	0.034	0.038	0.041	-2		
	3	1.003	1.017	0.998	1.013	0		3	0.033	0.033	0.038	0.040	-1		
	Mean	1.004	1.018	0.999	1.014	0		Mean	0.034	0.034	0.039	0.040	-1		
AS-12	1	0.997	0.997	0.990	0.992	2	AS-12	1	0.034	0.034	0.038	0.040	-6	Negative	Non-photoreactive
	2	0.998	0.998	0.993	0.993	0		2	0.033	0.034	0.036	0.052	-6		
	3	0.993	0.997	0.988	0.991	0		3	0.034	0.034	0.038	0.040	-6		
	Mean	0.996	0.997	0.990	0.992	1		Mean	0.033	0.034	0.037	0.044	-6		
AS-19	1	1.002	1.004	1.001	0.998	-6	AS-19	1	0.033	0.034	0.037	0.040	-2	Negative	Non-photoreactive
	2	1.000	1.004	0.993	0.998	-1		2	0.033	0.034	0.036	0.040	-3		
	3	1.002	1.008	0.996	1.001	0		3	0.034	0.034	0.038	0.040	-2		
	Mean	1.001	1.006	0.996	0.999	-2		Mean	0.033	0.034	0.037	0.040	-2		
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	-3				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Chlorhexidine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Results *1	Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
	A440(-)			A440(+)				A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Test Chemical		Blank	Run#	Test Chemical	Blank	Test Chemical	Blank		
AS-07	1	0.910	1.011	0.905	1.007	1	AS-07	1	0.033	0.034	0.063	0.041	24	Positive	Weakly photoreactive
	2	0.907	1.016	0.903	1.011	-1		2	0.034	0.034	0.059	0.040	19		
	3	0.903	1.015	0.901	1.011	-2		3	0.038	0.034	0.060	0.040	16		
	Mean	0.906	1.014	0.903	1.010	-1		Mean	0.035	0.034	0.061	0.040	20		
AS-15	1	0.900	1.003	0.899	0.999	4	AS-15	1	0.035	0.034	0.073	0.041	31	Positive	Weakly photoreactive
	2	0.895	1.002	0.895	0.997	2		2	0.034	0.034	0.067	0.041	26		
	3	0.894	1.003	0.895	1.016	1		3	0.035	0.033	0.068	0.041	26		
	Mean	0.897	1.002	0.896	1.004	2		Mean	0.035	0.034	0.069	0.041	28		
AS-21	1	0.910	1.004	0.900	1.000	6	AS-21	1	0.035	0.034	0.069	0.040	28	Positive	Weakly photoreactive
	2	0.908	1.003	0.899	0.999	5		2	0.034	0.034	0.065	0.040	26		
	3	0.907	1.003	0.902	0.999	1		3	0.035	0.033	0.066	0.040	26		
	Mean	0.908	1.003	0.900	0.999	4		Mean	0.034	0.034	0.067	0.040	27		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	25				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Cinnamic acid

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-02	1	1.019	1.025	1.016	1.023	-2	AS-02	1	0.034	0.034	0.069	0.039	30	Positive	Weakly photoreactive
	2	1.028	1.026	1.026	1.022	-2		2	0.034	0.034	0.067	0.039	29		
	3	1.028	1.031	1.022	1.022	1		3	0.034	0.043	0.080	0.047	41		
	Mean	1.025	1.027	1.021	1.022	-1		Mean	0.034	0.037	0.072	0.042	33		
AS-11	1	0.993	0.991	0.988	0.989	8	AS-11	1	0.033	0.034	0.081	0.041	40	Positive	Weakly photoreactive
	2	1.001	0.998	0.996	0.995	10		2	0.033	0.034	0.079	0.042	40		
	3	0.999	0.999	0.994	1.015	9		3	0.034	0.033	0.095	0.040	54		
	Mean	0.998	0.996	0.993	1.000	9		Mean	0.034	0.034	0.085	0.041	45		
AS-18	1	0.992	1.003	0.988	0.998	-1	AS-18	1	0.034	0.034	0.076	0.040	35	Positive	Weakly photoreactive
	2	1.001	1.007	0.996	1.003	0		2	0.033	0.034	0.077	0.041	37		
	3	0.995	1.002	0.989	0.997	1		3	0.034	0.034	0.090	0.041	49		
	Mean	0.996	1.004	0.991	0.999	0		Mean	0.034	0.034	0.081	0.041	40		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	39				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Drometrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
	1							1				Inconclusive	Undetermined	
	2							2						
	3							3						
	Mean							Mean						
	1							1				Inconclusive	Undetermined	
	2							2						
	3							3						
	Mean							Mean						
	1							1				Inconclusive	Undetermined	
	2							2						
	3							3						
	Mean							Mean						
	Mean for 3 assays	-	-	-	-			Mean for 3 assays	-	-	-			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : L-Histidine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-03	1	1.016	1.001	1.014	0.997	-3	AS-03	1	0.034	0.034	0.150	0.038	112	Positive	Photoreactive
	2	1.029	1.006	1.025	1.002	-2		2	0.033	0.034	0.147	0.038	110		
	3	1.031	1.009	1.027	1.005	-1		3	0.034	0.034	0.153	0.039	115		
	Mean	1.025	1.006	1.022	1.001	-2		Mean	0.034	0.034	0.150	0.038	112		
AS-10	1	1.006	1.004	1.001	0.994	-2	AS-10	1	0.035	0.034	0.142	0.041	100	Positive	Photoreactive
	2	1.002	1.002	0.998	0.996	-3		2	0.033	0.034	0.139	0.042	98		
	3	1.013	1.008	1.005	1.003	0		3	0.034	0.034	0.146	0.042	104		
	Mean	1.007	1.005	1.001	0.998	-2		Mean	0.034	0.034	0.142	0.042	101		
AS-17	1	1.006	1.007	1.003	1.002	-2	AS-17	1	0.034	0.033	0.154	0.038	115	Positive	Photoreactive
	2	1.004	1.014	0.999	1.008	0		2	0.033	0.034	0.152	0.039	114		
	3	1.016	1.013	1.012	1.008	-1		3	0.034	0.034	0.141	0.039	102		
	Mean	1.009	1.011	1.005	1.006	-1		Mean	0.034	0.034	0.149	0.039	110		
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	108				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Methylbenzylidene camphor

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Test concentration
 Singlet oxygen 50 µM
 Superoxide anion 50 µM

Experimental No.	Run#	Singlet oxygen					Results*1	Experimental No.	Run#	Superoxide anion					Results*2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		A440(-)		A440(+)		A560(-)				A560(+)							
		Test Chemical	Blank	Test Chemical	Blank	Test Chemical				Blank	Test Chemical	Blank					
AS-06	1	1.026	1.008	0.990	1.005	32	AS-06	1	0.053	0.033	0.048	0.039	-12	Positive	(Photoreactive)		
	2	1.024	1.012	0.991	1.007	29		2	0.054	0.033	0.051	0.039	-10				
	3	1.025	1.011	0.993	1.005	28		3	0.052	0.034	0.051	0.040	-9				
	Mean	1.025	1.010	0.992	1.006	30		Mean	0.053	0.033	0.050	0.040	-10				
AS-13	1	1.002	1.009	0.986	1.002	10	AS-13	1	0.048	0.034	0.047	0.047	-10	Inconclusive	(Non-photoreactive)		
	2	1.002	1.004	0.985	0.998	11		2	0.047	0.034	0.042	0.041	-15				
	3	1.001	1.014	0.983	1.008	11		3	0.048	0.033	0.045	0.040	-11				
	Mean	1.001	1.009	0.985	1.003	11		Mean	0.048	0.034	0.045	0.043	-12				
AS-20	1	1.021	1.008	0.989	1.003	25	AS-20	1	0.059	0.034	0.043	0.039	-21	Positive	(Photoreactive)		
	2	1.020	1.005	0.987	0.997	25		2	0.058	0.034	0.042	0.040	-21				
	3	1.015	1.004	0.984	0.998	24		3	0.058	0.033	0.045	0.039	-18				
	Mean	1.018	1.006	0.987	0.999	25		Mean	0.058	0.034	0.043	0.039	-20				
Mean for 3 assays	-	-	-	-	22	Mean for 3 assays	-	-	-	-	-14						

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Octrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen 20 µM
 Superoxide anion Not tested

Experimental No.	Singlet oxygen						Results *1	Superoxide anion						Judged by Original Criteria	Judgement
	A440(-)			A440(+)				A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Test Chemical		Blank	Run#	Test Chemical	Blank	Test Chemical	Blank		
AS-06	1	1.029	1.008	1.040	1.005	-15	1						Inconclusive	Inconclusive	
	2	1.029	1.012	1.041	1.007	-16	2								
	3	1.030	1.011	1.036	1.005	-10	3								
	Mean	1.029	1.010	1.039	1.006	-14	Mean								
AS-12	1	1.003	0.997	1.021	0.992	-23	1						Inconclusive	Inconclusive	
	2	1.004	0.998	1.022	0.993	-23	2								
	3	1.002	0.997	1.023	0.991	-26	3								
	Mean	1.003	0.997	1.022	0.992	-24	Mean								
AS-19	1	1.027	1.004	1.050	0.998	-30	1						Inconclusive	Inconclusive	
	2	1.025	1.004	1.047	0.998	-28	2								
	3	1.023	1.008	1.043	1.001	-27	3								
	Mean	1.025	1.006	1.047	0.999	-28	Mean								
Mean for 3 assays	-	-	-	-	-22	Mean for 3 assays	-	-	-	-	-				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Octyl methacrylate

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Solution 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Solution 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 100 µM
 Superoxide anion 100 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-09	1	1.025	1.006	0.999	0.999	17	AS-09	1	0.046	0.033	0.063	0.042	8	Inconclusive	(Non-photoreactive)
	2	1.022	1.006	0.996	0.999	18		2	0.045	0.034	0.059	0.042	5		
	3	1.025	1.006	0.999	0.996	18		3	0.046	0.034	0.064	0.043	8		
	Mean	1.024	1.006	0.998	0.998	18		Mean	0.046	0.033	0.062	0.042	7		
AS-15	1	1.017	1.003	0.994	0.999	26	AS-15	1	0.049	0.034	0.058	0.041	2	Positive	(Photoreactive)
	2	1.012	1.002	0.988	0.997	25		2	0.049	0.034	0.057	0.041	2		
	3	1.009	1.003	0.988	1.016	23		3	0.049	0.033	0.063	0.041	7		
	Mean	1.013	1.002	0.990	1.004	25		Mean	0.049	0.034	0.059	0.041	4		
AS-21	1	1.018	1.004	0.996	1.000	18	AS-21	1	0.048	0.034	0.055	0.040	2	Inconclusive	(Non-photoreactive)
	2	1.023	1.003	1.001	0.999	18		2	0.048	0.034	0.055	0.040	1		
	3	1.021	1.003	0.999	0.999	18		3	0.048	0.033	0.059	0.040	6		
	Mean	1.021	1.003	0.999	0.999	18		Mean	0.048	0.034	0.056	0.040	3		
Mean for 3 assays	-	-	-	-	20	Mean for 3 assays	-	-	-	-	-	5			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Octyl methoxycinnamate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank				
AS-09	1	1.014	1.006	1.003	0.999	2	AS-09	1	0.042	0.033	0.085	0.042	34	Positive	Weakly photoreactive	
	2	1.014	1.006	1.004	0.999	1		2	0.041	0.034	0.083	0.042				33
	3	1.015	1.006	1.005	0.996	2		3	0.041	0.034	0.089	0.043				
	Mean	1.014	1.006	1.004	0.998	2		Mean	0.041	0.033	0.085	0.042				35
AS-15	1	0.985	1.003	0.976	0.999	11	AS-15	1	0.043	0.034	0.070	0.041	20	Positive	Non-photoreactive	
	2	0.982	1.002	0.972	0.997	12		2	0.043	0.034	0.069	0.041				19
	3	0.982	1.003	0.973	1.016	11		3	0.043	0.033	0.073	0.041				
	Mean	0.983	1.002	0.974	1.004	11		Mean	0.043	0.034	0.071	0.041				21
AS-21	1	1.015	1.004	1.004	1.000	7	AS-21	1	0.046	0.034	0.068	0.040	16	Inconclusive	Non-photoreactive	
	2	1.015	1.003	1.004	0.999	7		2	0.046	0.034	0.067	0.040				15
	3	1.010	1.003	1.000	0.999	6		3	0.045	0.033	0.070	0.040				
	Mean	1.013	1.003	1.002	0.999	7		Mean	0.046	0.034	0.068	0.040				17
Mean for 3 assays	-	-	-	-	7	Mean for 3 assays	-	-	-	-	24					

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : Octyl salicylate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Solution 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined

Test concentration

Singlet oxygen 100 µM
 Superoxide anion 50 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-03	1	1.041	1.001	1.029	0.997	7	AS-03	1	0.045	0.034	0.090	0.038	41	Positive	(Weakly photoreactive)
	2	1.038	1.006	1.028	1.002	5		2	0.045	0.034	0.089	0.038	41		
	3	1.054	1.009	1.040	1.005	9		3	0.045	0.034	0.093	0.039	44		
	Mean	1.045	1.006	1.033	1.001	7		Mean	0.045	0.034	0.091	0.038	42		
AS-11	1	1.051	0.991	1.036	0.989	19	AS-11	1	0.053	0.034	0.089	0.041	29	Positive	(Weakly photoreactive)
	2	1.046	0.998	1.033	0.995	17		2	0.053	0.034	0.088	0.042	28		
	3	1.055	0.999	1.042	1.015	17		3	0.053	0.033	0.092	0.040	32		
	Mean	1.051	0.996	1.037	1.000	18		Mean	0.053	0.034	0.089	0.041	30		
AS-18	1	1.046	1.003	1.036	0.998	5	AS-18	1	0.049	0.034	0.088	0.040	32	Positive	(Weakly photoreactive)
	2	1.042	1.007	1.032	1.003	4		2	0.050	0.034	0.090	0.041	34		
	3	1.050	1.002	1.038	0.997	7		3	0.050	0.034	0.091	0.041	34		
	Mean	1.046	1.004	1.036	0.999	5		Mean	0.050	0.034	0.090	0.041	33		
Mean for 3 assays	-	-	-	-	10	Mean for 3 assays	-	-	-	-	35				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : PABA

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-02	1	1.027	1.025	1.022	1.023	0	AS-02	1	0.034	0.034	0.036	0.039	-3	Negative	Non-photoreactive
	2	1.031	1.026	1.025	1.022	0		2	0.033	0.034	0.035	0.039	-3		
	3	1.029	1.031	1.023	1.022	1		3	0.035	0.043	0.037	0.047	-3		
	Mean	1.029	1.027	1.023	1.022	0		Mean	0.034	0.037	0.036	0.042	-3		
AS-11	1	0.996	0.991	0.991	0.989	9	AS-11	1	0.034	0.034	0.036	0.041	-5	Negative	Non-photoreactive
	2	0.995	0.998	0.989	0.995	10		2	0.033	0.034	0.035	0.042	-5		
	3	1.003	0.999	0.998	1.015	9		3	0.034	0.033	0.037	0.040	-5		
	Mean	0.998	0.996	0.993	1.000	9		Mean	0.034	0.034	0.036	0.041	-5		
AS-18	1	1.003	1.003	0.997	0.998	1	AS-18	1	0.034	0.034	0.036	0.040	-5	Negative	Non-photoreactive
	2	1.003	1.007	0.998	1.003	1		2	0.033	0.034	0.035	0.041	-5		
	3	1.005	1.002	0.998	0.997	1		3	0.034	0.034	0.037	0.041	-5		
	Mean	1.004	1.004	0.998	0.999	1		Mean	0.034	0.034	0.036	0.041	-5		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	-4				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : SDS

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
AS-02	1	1.002	1.025	0.992	1.023	5	AS-02	1	0.079	0.034	0.098	0.039	14	Negative	Non-photoreactive
	2	1.004	1.026	0.994	1.022	5		2	0.080	0.034	0.098	0.039	12		
	3	0.999	1.031	0.990	1.022	4		3	0.077	0.043	0.093	0.047	12		
	Mean	1.002	1.027	0.992	1.022	5		Mean	0.078	0.037	0.096	0.042	13		
AS-11	1	0.983	0.991	0.973	0.989	14	AS-11	1	0.071	0.034	0.099	0.041	21	Negative	Non-photoreactive
	2	0.981	0.998	0.969	0.995	16		2	0.071	0.034	0.096	0.042	19		
	3	0.987	0.999	0.976	1.015	14		3	0.071	0.033	0.095	0.040	17		
	Mean	0.984	0.996	0.973	1.000	15		Mean	0.071	0.034	0.097	0.041	19		
AS-18	1	0.981	1.003	0.966	0.998	9	AS-18	1	0.070	0.034	0.097	0.040	20	Negative	Non-photoreactive
	2	0.979	1.007	0.967	1.003	7		2	0.070	0.034	0.095	0.041	17		
	3	0.978	1.002	0.966	0.997	7		3	0.070	0.034	0.093	0.041	16		
	Mean	0.979	1.004	0.966	0.999	8		Mean	0.070	0.034	0.095	0.041	18		
Mean for 3 assays	-	-	-	-	9	Mean for 3 assays	-	-	-	-	17				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 5
 Chemical Name : UV-571

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen 20 µM
 Superoxide anion Not tested

Experimental No.	Singlet oxygen						Results *1	Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
	A440(-)			A440(+)				A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Test Chemical		Blank	Run#	Test Chemical	Blank	Test Chemical	Blank		
AS-14	1	1.015	1.011	1.022	1.009	-11	1								
	2	1.014	1.013	1.020	1.009	-10	2								
	3	1.015	1.011	1.021	1.006	-10	3								
	Mean	1.015	1.012	1.021	1.008	-10	Mean							Inconclusive	
AS-18	1	1.012	1.003	1.011	0.998	-3	1								
	2	1.009	1.007	1.008	1.003	-3	2								
	3	1.018	1.002	1.016	0.997	-3	3								
	Mean	1.013	1.004	1.011	0.999	-3	Mean							Inconclusive	
AS-22	1	1.024	1.016	1.021	1.009	-4	1								
	2	1.024	1.014	1.021	1.007	-3	2								
	3	1.028	1.014	1.023	1.008	-2	3								
	Mean	1.025	1.015	1.022	1.008	-3	Mean							Inconclusive	
Mean for 3 assays	-	-	-	-	-5	Mean for 3 assays	-	-	-	-	-	-	Inconclusive		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Acridine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-03	1	1.003	1.014	0.744	0.999	245	SI-03	1	0.036	0.035	0.249	0.050	198	Positive	Photoreactive
	2	1.005	1.010	0.753	0.997	239		2	0.037	0.035	0.251	0.050	200		
	3	1.002	1.010	0.757	0.994	231		3	0.036	0.035	0.263	0.050	212		
	Mean	1.003	1.011	0.751	0.997	238		Mean	0.036	0.035	0.255	0.050	203		
SI-14	1	0.977	0.998	0.713	0.992	258	SI-14	1	0.036	0.035	0.244	0.042	201	Positive	Photoreactive
	2	0.978	0.996	0.727	0.990	246		2	0.036	0.035	0.253	0.042	209		
	3	0.973	0.996	0.729	0.990	238		3	0.036	0.035	0.258	0.043	213		
	Mean	0.976	0.997	0.723	0.991	247		Mean	0.036	0.035	0.252	0.043	208		
SI-20	1	0.964	0.978	0.705	0.970	251	SI-20	1	0.036	0.035	0.255	0.043	212	Positive	Photoreactive
	2	0.966	0.981	0.721	0.973	237		2	0.036	0.036	0.256	0.043	213		
	3	0.969	0.978	0.727	0.971	234		3	0.036	0.036	0.265	0.043	222		
	Mean	0.966	0.979	0.718	0.971	241		Mean	0.036	0.036	0.259	0.043	216		
Mean for 3 assays	-	-	-	-	242	Mean for 3 assays	-	-	-	-	209				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Acridine HCl

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-02	1	0.981	0.989	0.698	0.977	271	SI-02	1	0.036	0.036	0.230	0.045	186	Positive	Photoreactive
	2	0.979	0.998	0.708	0.986	259		2	0.036	0.036	0.229	0.044	185		
	3	0.978	0.995	0.708	0.983	258		3	0.036	0.036	0.246	0.044	201		
	Mean	0.979	0.994	0.705	0.982	263		Mean	0.036	0.036	0.235	0.044	191		
SI-13	1	1.202	0.987	0.929	0.980	264	SI-13	1	0.145	0.035	0.400	0.046	245	Positive	Photoreactive
	2	1.184	0.992	0.945	0.985	231		2	0.142	0.036	0.412	0.045	259		
	3	1.177	0.989	0.927	0.980	241		3	0.140	0.036	0.430	0.046	280		
	Mean	1.188	0.990	0.934	0.981	245		Mean	0.142	0.036	0.414	0.046	261		
SI-19	1	0.980	0.997	0.725	0.985	247	SI-19	1	0.036	0.035	0.238	0.044	195	Positive	Photoreactive
	2	0.978	0.991	0.731	0.984	238		2	0.036	0.036	0.219	0.043	176		
	3	0.981	0.993	0.736	0.984	236		3	0.036	0.036	0.225	0.043	182		
	Mean	0.980	0.993	0.731	0.984	240		Mean	0.036	0.036	0.227	0.043	184		
Mean for 3 assays	-	-	-	-	249	Mean for 3 assays	-	-	-	-	212				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Amiodarone HCl

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!	Undetermined	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Chlorpromazine HCl

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-05	1	0.961	1.010	0.952	1.004	2	SI-05	1	0.036	0.035	0.124	0.043	79	Positive	Photoreactive
	2	0.982	1.010	0.984	1.003	-9		2	0.036	0.035	0.120	0.043	76		
	3	0.979	1.010	0.988	1.004	-17		3	0.036	0.035	0.127	0.044	83		
	Mean	0.974	1.010	0.975	1.003	-8		Mean	0.036	0.035	0.123	0.043	79		
SI-16	1	0.930	0.977	0.916	0.972	9	SI-16	1	0.036	0.036	0.118	0.043	73	Positive	Photoreactive
	2	0.945	0.975	0.937	0.969	2		2	0.036	0.036	0.118	0.043	74		
	3	0.945	0.975	0.931	0.969	9		3	0.037	0.036	0.122	0.044	77		
	Mean	0.940	0.975	0.928	0.970	7		Mean	0.036	0.036	0.119	0.044	75		
SI-22	1	0.949	0.983	0.948	0.976	-6	SI-22	1	0.037	0.036	0.122	0.043	78	Positive	Photoreactive
	2	0.947	0.987	0.951	0.980	-11		2	0.037	0.036	0.122	0.043	78		
	3	0.948	0.988	0.951	0.980	-10		3	0.037	0.036	0.127	0.043	83		
	Mean	0.948	0.986	0.950	0.979	-9		Mean	0.037	0.036	0.123	0.043	80		
Mean for 3 assays	-	-	-	-	-3	Mean for 3 assays	-	-	-	-	-	78			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Doxycycline HCl

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-01	1	0.962	0.988	0.732	0.981	223	SI-01	1	0.037	0.035	0.358	0.044	313	Positive	Photoreactive
	2	0.964	0.985	0.758	0.979	200		2	0.037	0.035	0.370	0.043	325		
	3	0.963	0.984	0.761	0.978	196		3	0.037	0.035	0.379	0.044	335		
	Mean	0.963	0.985	0.750	0.979	206		Mean	0.037	0.035	0.369	0.043	324		
SI-12	1	0.985	1.002	0.746	0.996	232	SI-12	1	0.037	0.035	0.354	0.045	307	Positive	Photoreactive
	2	0.984	1.005	0.764	0.997	213		2	0.037	0.036	0.364	0.044	317		
	3	0.987	1.003	0.769	0.996	210		3	0.037	0.035	0.379	0.045	332		
	Mean	0.985	1.003	0.760	0.996	218		Mean	0.037	0.035	0.366	0.045	319		
SI-18	1	0.959	0.981	0.690	0.972	258	SI-18	1	0.038	0.036	0.405	0.045	358	Positive	Photoreactive
	2	0.966	0.980	0.689	0.968	266		2	0.038	0.036	0.413	0.044	367		
	3	0.962	0.988	0.692	0.976	258		3	0.038	0.036	0.417	0.045	369		
	Mean	0.962	0.983	0.690	0.972	261		Mean	0.038	0.036	0.411	0.045	365		
Mean for 3 assays	-	-	-	-	228	Mean for 3 assays	-	-	-	-	336				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Fenofibrate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-25	1	0.980	0.982	0.879	0.974	93	SI-25	1	0.046	0.036	0.046	0.047	-11	Positive	Photoreactive
	2	0.989	0.989	0.889	0.983	92		2	0.048	0.037	0.046	0.047	-13		
	3	1.007	0.987	0.905	0.978	94		3	0.047	0.036	0.048	0.047	-10		
	Mean	0.992	0.986	0.891	0.978	93		Mean	0.047	0.036	0.047	0.047	-11		
SI-27	1	0.994	0.990	0.880	0.981	103	SI-27	1	0.052	0.036	0.046	0.045	-15	Positive	Photoreactive
	2	0.986	0.993	0.877	0.982	99		2	0.051	0.037	0.046	0.044	-13		
	3	0.994	0.992	0.886	0.981	96		3	0.049	0.037	0.046	0.045	-11		
	Mean	0.991	0.992	0.881	0.981	99		Mean	0.051	0.037	0.046	0.045	-13		
SI-29	1	1.031	1.028	0.903	1.020	120	SI-29	1	0.050	0.035	0.044	0.045	-15	Positive	Photoreactive
	2	1.055	1.029	0.927	1.021	120		2	0.050	0.036	0.044	0.045	-14		
	3	1.054	1.033	0.929	1.025	117		3	0.050	0.036	0.046	0.046	-14		
	Mean	1.047	1.030	0.920	1.022	119		Mean	0.050	0.036	0.045	0.045	-14		
Mean for 3 assays	-	-	-	-	104	Mean for 3 assays	-	-	-	-	-	-13			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Furosemide

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-04	1	0.972	0.976	0.881	0.970	85	SI-04	1	0.038	0.035	0.079	0.044	34	Positive	Photoreactive
	2	0.965	0.976	0.882	0.971	77		2	0.037	0.036	0.078	0.044	33		
	3	0.971	0.975	0.890	0.968	74		3	0.038	0.035	0.082	0.044	37		
	Mean	0.969	0.976	0.885	0.970	79		Mean	0.037	0.036	0.080	0.044	35		
SI-15	1	0.978	0.981	0.891	0.975	81	SI-15	1	0.037	0.036	0.077	0.043	33	Positive	Photoreactive
	2	0.976	0.985	0.894	0.978	76		2	0.036	0.036	0.077	0.043	34		
	3	0.981	0.985	0.901	0.977	74		3	0.037	0.036	0.080	0.043	37		
	Mean	0.978	0.983	0.895	0.977	77		Mean	0.036	0.036	0.078	0.043	35		
SI-21	1	0.994	0.999	0.910	0.992	77	SI-21	1	0.037	0.036	0.077	0.044	32	Positive	Photoreactive
	2	0.993	0.996	0.917	0.989	70		2	0.037	0.036	0.075	0.043	30		
	3	0.994	0.996	0.922	0.988	65		3	0.038	0.036	0.079	0.044	33		
	Mean	0.994	0.997	0.916	0.990	71		Mean	0.037	0.036	0.077	0.044	32		
Mean for 3 assays	-	-	-	-	76	Mean for 3 assays	-	-	-	-	34				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Ketoprofen

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-02	1	0.988	0.989	0.766	0.977	210	SI-02	1	0.037	0.036	0.161	0.045	116	Positive	Photoreactive
	2	0.989	0.998	0.788	0.986	189		2	0.036	0.036	0.157	0.044	113		
	3	0.991	0.995	0.789	0.983	189		3	0.037	0.036	0.168	0.044	123		
	Mean	0.989	0.994	0.781	0.982	196		Mean	0.036	0.036	0.162	0.044	117		
SI-13	1	0.987	0.987	0.779	0.980	199	SI-13	1	0.036	0.035	0.176	0.046	130	Positive	Photoreactive
	2	0.989	0.992	0.785	0.985	195		2	0.036	0.036	0.179	0.045	133		
	3	0.991	0.989	0.810	0.980	172		3	0.037	0.036	0.188	0.046	141		
	Mean	0.989	0.990	0.792	0.981	189		Mean	0.036	0.036	0.181	0.046	135		
SI-19	1	0.980	0.997	0.751	0.985	220	SI-19	1	0.036	0.035	0.184	0.044	141	Positive	Photoreactive
	2	0.983	0.991	0.762	0.984	212		2	0.035	0.036	0.183	0.043	140		
	3	0.987	0.993	0.796	0.984	182		3	0.036	0.036	0.193	0.043	150		
	Mean	0.983	0.993	0.770	0.984	205		Mean	0.036	0.036	0.186	0.043	144		
Mean for 3 assays	-	-	-	-	197	Mean for 3 assays	-	-	-	-	132				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : 6-methylcoumarine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank				
SI-06	1	0.979	0.985	0.872	0.978	100	SI-06	1	0.036	0.035	0.145	0.045	99	Positive	Photoreactive	
	2	0.981	0.987	0.888	0.980	86		2	0.035	0.036	0.142	0.045				97
	3	0.982	0.985	0.891	0.979	85		3	0.036	0.035	0.157	0.045				111
	Mean	0.981	0.986	0.884	0.979	90		Mean	0.036	0.035	0.148	0.045				102
SI-17	1	0.999	1.003	0.894	0.992	96	SI-17	1	0.041	0.036	0.144	0.044	96	Positive	Photoreactive	
	2	0.997	1.004	0.899	0.995	88		2	0.036	0.036	0.137	0.043				94
	3	1.000	1.002	0.909	0.992	81		3	0.036	0.036	0.150	0.043				106
	Mean	0.999	1.003	0.901	0.993	88		Mean	0.038	0.036	0.144	0.043				99
SI-23	1	0.997	1.004	0.894	0.997	96	SI-23	1	0.036	0.035	0.151	0.046	105	Positive	Photoreactive	
	2	0.997	0.999	0.896	0.992	94		2	0.036	0.036	0.149	0.045				104
	3	0.997	1.003	0.902	0.997	89		3	0.036	0.036	0.160	0.046				113
	Mean	0.997	1.002	0.897	0.995	93		Mean	0.036	0.036	0.153	0.046				107
Mean for 3 assays	-	-	-	-	90	Mean for 3 assays	-	-	-	-	103					

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : 8-MOP

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-03	1	1.004	1.014	0.939	0.999	51	SI-03	1	0.035	0.035	0.126	0.050	75	Positive	Photoreactive
	2	1.005	1.010	0.944	0.997	48		2	0.036	0.035	0.125	0.050	74		
	3	1.006	1.010	0.942	0.994	50		3	0.036	0.035	0.128	0.050	77		
	Mean	1.005	1.011	0.942	0.997	50		Mean	0.036	0.035	0.126	0.050	75		
SI-14	1	0.981	0.998	0.928	0.992	47	SI-14	1	0.035	0.035	0.122	0.042	79	Positive	Photoreactive
	2	0.985	0.996	0.931	0.990	48		2	0.036	0.035	0.121	0.042	77		
	3	0.983	0.996	0.931	0.990	46		3	0.036	0.035	0.134	0.043	90		
	Mean	0.983	0.997	0.930	0.991	47		Mean	0.036	0.035	0.126	0.043	82		
SI-20	1	0.964	0.978	0.897	0.970	59	SI-20	1	0.035	0.035	0.120	0.043	78	Positive	Photoreactive
	2	0.971	0.981	0.904	0.973	59		2	0.036	0.036	0.118	0.043	75		
	3	0.975	0.978	0.907	0.971	60		3	0.036	0.036	0.126	0.043	83		
	Mean	0.970	0.979	0.903	0.971	59		Mean	0.036	0.036	0.121	0.043	79		
Mean for 3 assays	-	-	-	-	52	Mean for 3 assays	-	-	-	-	79				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Nalidixic acid

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-04	1	0.971	0.976	0.824	0.970	141	SI-04	1	0.036	0.035	0.574	0.044	530	Positive	Photoreactive
	2	0.971	0.976	0.835	0.971	130		2	0.036	0.036	0.457	0.044	414		
	3	0.973	0.975	0.838	0.968	129		3	0.036	0.035	0.538	0.044	494		
	Mean	0.972	0.976	0.832	0.970	133		Mean	0.036	0.036	0.523	0.044	479		
SI-15	1	0.982	0.981	0.848	0.975	128	SI-15	1	0.036	0.036	0.486	0.043	443	Positive	Photoreactive
	2	0.979	0.985	0.858	0.978	116		2	0.036	0.036	0.493	0.043	451		
	3	0.981	0.985	0.862	0.977	114		3	0.036	0.036	0.520	0.043	478		
	Mean	0.981	0.983	0.856	0.977	119		Mean	0.036	0.036	0.500	0.043	457		
SI-21	1	0.995	0.999	0.868	0.992	120	SI-21	1	0.036	0.036	0.539	0.044	495	Positive	Photoreactive
	2	0.997	0.996	0.880	0.989	110		2	0.036	0.036	0.539	0.043	496		
	3	0.995	0.996	0.881	0.988	107		3	0.037	0.036	0.480	0.044	436		
	Mean	0.996	0.997	0.876	0.990	112		Mean	0.036	0.036	0.520	0.044	476		
Mean for 3 assays	-	-	-	-	121	Mean for 3 assays	-	-	-	-	-	471			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Nalidixic acid (Na salt)

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-03	1	1.014	1.014	0.879	0.999	122	SI-03	1	0.036	0.035	0.336	0.050	285	Positive	Photoreactive
	2	1.016	1.010	0.892	0.997	110		2	0.035	0.035	0.383	0.050	333		
	3	1.013	1.010	0.892	0.994	107		3	0.036	0.035	0.381	0.050	330		
	Mean	1.014	1.011	0.888	0.997	113		Mean	0.036	0.035	0.367	0.050	316		
SI-14	1	0.997	0.998	0.873	0.992	118	SI-14	1	0.036	0.035	0.478	0.042	435	Positive	Photoreactive
	2	0.998	0.996	0.884	0.990	108		2	0.035	0.035	0.536	0.042	493		
	3	0.995	0.996	0.885	0.990	104		3	0.036	0.035	0.529	0.043	485		
	Mean	0.997	0.997	0.881	0.991	110		Mean	0.035	0.035	0.514	0.043	471		
SI-20	1	0.982	0.978	0.853	0.970	121	SI-20	1	0.036	0.035	0.494	0.043	451	Positive	Photoreactive
	2	0.979	0.981	0.863	0.973	108		2	0.036	0.036	0.455	0.043	412		
	3	0.981	0.978	0.869	0.971	104		3	0.037	0.036	0.486	0.043	442		
	Mean	0.981	0.979	0.862	0.971	111		Mean	0.036	0.036	0.478	0.043	435		
Mean for 3 assays	-	-	-	-	111	Mean for 3 assays	-	-	-	-	407				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Norfloxacin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol	
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results *2			
SI-06	1	0.984	0.985	0.831	0.978	147	SI-06	1	0.036	0.035	0.153	0.045	107	Positive	Photoreactive
	2	0.985	0.987	0.841	0.980	137		2	0.036	0.036	0.153	0.045	107		
	3	0.983	0.985	0.841	0.979	135		3	0.036	0.035	0.160	0.045	114		
	Mean	0.984	0.986	0.838	0.979	140		Mean	0.036	0.035	0.155	0.045	109		
SI-17	1	1.004	1.003	0.845	0.992	149	SI-17	1	0.036	0.036	0.150	0.044	107	Positive	Photoreactive
	2	1.004	1.004	0.858	0.995	137		2	0.036	0.036	0.150	0.043	106		
	3	1.001	1.002	0.859	0.992	132		3	0.036	0.036	0.159	0.043	116		
	Mean	1.003	1.003	0.854	0.993	139		Mean	0.036	0.036	0.153	0.043	110		
SI-23	1	0.998	1.004	0.841	0.997	150	SI-23	1	0.036	0.035	0.153	0.046	108	Positive	Photoreactive
	2	0.998	0.999	0.853	0.992	139		2	0.036	0.036	0.155	0.045	109		
	3	1.000	1.003	0.855	0.997	138		3	0.036	0.036	0.162	0.046	116		
	Mean	0.999	1.002	0.850	0.995	142		Mean	0.036	0.036	0.157	0.046	111		
Mean for 3 assays	-	-	-	-	140	Mean for 3 assays	-	-	-	-	110				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Ofloxacin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-06	1	0.982	0.985	0.809	0.978	166	SI-06	1	0.036	0.035	0.499	0.045	454	Positive	Photoreactive
	2	0.980	0.987	0.823	0.980	150		2	0.035	0.036	0.514	0.045	468		
	3	0.980	0.985	0.830	0.979	143		3	0.036	0.035	0.532	0.045	485		
	Mean	0.980	0.986	0.821	0.979	153		Mean	0.036	0.035	0.515	0.045	469		
SI-17	1	1.002	1.003	0.837	0.992	155	SI-17	1	0.036	0.036	0.490	0.044	447	Positive	Photoreactive
	2	1.002	1.004	0.852	0.995	139		2	0.036	0.036	0.503	0.043	460		
	3	1.001	1.002	0.857	0.992	135		3	0.036	0.036	0.511	0.043	468		
	Mean	1.002	1.003	0.849	0.993	143		Mean	0.036	0.036	0.502	0.043	458		
SI-23	1	0.995	1.004	0.837	0.997	152	SI-23	1	0.036	0.035	0.529	0.046	484	Positive	Photoreactive
	2	0.994	0.999	0.849	0.992	137		2	0.036	0.036	0.525	0.045	479		
	3	0.998	1.003	0.858	0.997	133		3	0.037	0.036	0.529	0.046	483		
	Mean	0.996	1.002	0.848	0.995	141		Mean	0.036	0.036	0.528	0.046	482		
Mean for 3 assays	-	-	-	-	146	Mean for 3 assays	-	-	-	-	470				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Piroxicam

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Solution 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 100 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-03	1	1.004	1.014	0.739	0.999	252	SI-08	1	0.052	0.036	0.298	0.044	238	Positive	Photoreactive
	2	1.010	1.010	0.768	0.997	228		2	0.051	0.036	0.304	0.043	245		
	3	1.005	1.010	0.773	0.994	218		3	0.052	0.036	0.315	0.044	255		
	Mean	1.007	1.011	0.760	0.997	233		Mean	0.052	0.036	0.306	0.044	246		
SI-14	1	0.985	0.998	0.700	0.992	280	SI-14	1	0.050	0.035	0.297	0.042	240	Positive	Photoreactive
	2	0.992	0.996	0.737	0.990	250		2	0.049	0.035	0.307	0.042	249		
	3	0.991	0.996	0.740	0.990	245		3	0.050	0.035	0.315	0.043	257		
	Mean	0.990	0.997	0.725	0.991	258		Mean	0.050	0.035	0.306	0.043	249		
SI-20	1	0.978	0.978	0.743	0.970	227	SI-20	1	0.052	0.035	0.304	0.043	245	Positive	Photoreactive
	2	0.976	0.981	0.760	0.973	209		2	0.050	0.036	0.305	0.043	248		
	3	0.976	0.978	0.770	0.971	198		3	0.049	0.036	0.305	0.043	249		
	Mean	0.977	0.979	0.757	0.971	211		Mean	0.050	0.036	0.304	0.043	247		
Mean for 3 assays	-	-	-	-	234	Mean for 3 assays	-	-	-	-	247				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Promethazine HCl

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-05	1	0.984	1.010	0.919	1.004	58	SI-05	1	0.035	0.035	0.058	0.043	15	Positive	Photoreactive
	2	0.994	1.010	0.930	1.003	56		2	0.036	0.035	0.062	0.043	18		
	3	0.991	1.010	0.948	1.004	36		3	0.036	0.035	0.066	0.044	22		
	Mean	0.989	1.010	0.932	1.003	50		Mean	0.036	0.035	0.062	0.043	18		
SI-16	1	0.957	0.977	0.906	0.972	46	SI-16	1	0.036	0.036	0.060	0.043	16	Positive	Photoreactive
	2	0.957	0.975	0.910	0.969	43		2	0.036	0.036	0.060	0.043	16		
	3	0.965	0.975	0.891	0.969	69		3	0.036	0.036	0.063	0.044	19		
	Mean	0.960	0.975	0.902	0.970	53		Mean	0.036	0.036	0.061	0.044	17		
SI-22	1	0.974	0.983	0.910	0.976	57	SI-22	1	0.036	0.036	0.061	0.043	18	Positive	Photoreactive
	2	0.978	0.987	0.898	0.980	73		2	0.037	0.036	0.061	0.043	17		
	3	0.978	0.988	0.914	0.980	57		3	0.037	0.036	0.065	0.043	21		
	Mean	0.976	0.986	0.907	0.979	62		Mean	0.037	0.036	0.062	0.043	19		
Mean for 3 assays	-	-	-	-	55	Mean for 3 assays	-	-	-	-	18				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Laboratory : 6
 Chemical Name : Rosiglitazone

Rosiglitazone is excluded from the Fourth data analysis since the VMT considered that it is inappropriate to include rosiglitazone in the “phototoxic” chemical set because of lack of high quality human data regarding its phototoxicity.

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 50 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol		
	A440(-)		A440(+)		Results *1	A560(-)		A560(+)		Results *2						
	Run#	Test Chemical	Blank	Test Chemical		Blank	Run#	Test Chemical	Blank		Test Chemical	Blank				
SI-01	1	0.926	0.988	0.881	0.981	39	SI-10	1	0.056	0.036	0.138	0.044	74	Positive		
	2	0.941	0.985	0.902	0.979	33		2	0.060	0.036	0.142	0.044	73			
	3	0.933	0.984	0.900	0.978	27		3	0.062	0.036	0.143	0.045	72			
	Mean	0.933	0.985	0.895	0.979	33		Mean	0.059	0.036	0.141	0.044	73			
SI-12	1	0.953	1.002	0.915	0.996	31	SI-12	1	0.065	0.035	0.165	0.045	90		Positive	
	2	0.952	1.005	0.921	0.997	25		2	0.064	0.036	0.166	0.044	92			
	3	0.966	1.003	0.936	0.996	23		3	0.063	0.035	0.166	0.045	93			
	Mean	0.957	1.003	0.924	0.996	26		Mean	0.064	0.035	0.166	0.045	92			
SI-18	1	0.950	0.981	0.894	0.972	46	SI-18	1	0.038	0.036	0.100	0.045	53			Positive
	2	0.945	0.980	0.894	0.968	40		2	0.041	0.036	0.104	0.044	54			
	3	0.954	0.988	0.904	0.976	39		3	0.039	0.036	0.111	0.045	62			
	Mean	0.950	0.983	0.897	0.972	42		Mean	0.039	0.036	0.105	0.045	56			
Mean for 3 assays	-	-	-	-	34	Mean for 3 assays	-	-	-	-	74					

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Tetracycline

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-05	1	1.014	1.010	0.835	1.004	172	SI-05	1	0.036	0.035	0.234	0.043	190	Positive	Photoreactive
	2	1.013	1.010	0.860	1.003	146		2	0.036	0.035	0.236	0.043	192		
	3	1.018	1.010	0.870	1.004	141		3	0.037	0.035	0.250	0.044	205		
	Mean	1.015	1.010	0.855	1.003	153		Mean	0.036	0.035	0.240	0.043	196		
SI-16	1	0.981	0.977	0.816	0.972	160	SI-16	1	0.038	0.036	0.234	0.043	189	Positive	Photoreactive
	2	0.980	0.975	0.835	0.969	140		2	0.038	0.036	0.240	0.043	194		
	3	0.981	0.975	0.838	0.969	138		3	0.038	0.036	0.249	0.044	203		
	Mean	0.981	0.975	0.830	0.970	146		Mean	0.038	0.036	0.241	0.044	195		
SI-22	1	1.001	0.983	0.845	0.976	149	SI-22	1	0.039	0.036	0.222	0.043	177	Positive	Photoreactive
	2	1.002	0.987	0.846	0.980	149		2	0.038	0.036	0.226	0.043	181		
	3	0.999	0.988	0.839	0.980	153		3	0.043	0.036	0.240	0.043	191		
	Mean	1.001	0.986	0.843	0.979	150		Mean	0.040	0.036	0.230	0.043	183		
Mean for 3 assays	-	-	-	-	150	Mean for 3 assays	-	-	-	-	191				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Anthracene

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!	Undetermined	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Avobenzone

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!	Undetermined	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Bithionol

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-06	1	0.927	0.985	0.850	0.978	70	SI-06	1	0.076	0.035	0.105	0.045	20	Positive	Photoreactive
	2	0.961	0.987	0.884	0.980	70		2	0.076	0.036	0.103	0.045	17		
	3	0.958	0.985	0.880	0.979	71		3	0.076	0.035	0.104	0.045	18		
	Mean	0.949	0.986	0.872	0.979	70		Mean	0.076	0.035	0.104	0.045	18		
SI-17	1	0.945	1.003	0.864	0.992	71	SI-17	1	0.070	0.036	0.101	0.044	24	Positive	Photoreactive
	2	0.978	1.004	0.899	0.995	69		2	0.070	0.036	0.100	0.043	24		
	3	0.982	1.002	0.903	0.992	69		3	0.070	0.036	0.099	0.043	22		
	Mean	0.968	1.003	0.888	0.993	70		Mean	0.070	0.036	0.100	0.043	23		
SI-23	1	0.954	1.004	0.864	0.997	83	SI-23	1	0.075	0.035	0.100	0.046	15	Positive	Photoreactive
	2	0.964	0.999	0.874	0.992	83		2	0.076	0.036	0.100	0.045	14		
	3	0.964	1.003	0.880	0.997	78		3	0.075	0.036	0.100	0.046	15		
	Mean	0.961	1.002	0.872	0.995	81		Mean	0.075	0.036	0.100	0.046	15		
Mean for 3 assays	-	-	-	-	74	Mean for 3 assays	-	-	-	-	19				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Hexachlorophene

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-05	1	0.895	1.010	0.761	1.004	126	SI-05	1	0.056	0.035	0.058	0.043	-5	Positive	Photoreactive
	2	0.930	1.010	0.806	1.003	117		2	0.056	0.035	0.058	0.043	-5		
	3	0.933	1.010	0.809	1.004	117		3	0.056	0.035	0.059	0.044	-6		
	Mean	0.919	1.010	0.792	1.003	120		Mean	0.056	0.035	0.058	0.043	-5		
SI-16	1	0.857	0.977	0.760	0.972	92	SI-16	1	0.054	0.036	0.056	0.043	-6	Positive	Photoreactive
	2	0.888	0.975	0.788	0.969	95		2	0.053	0.036	0.056	0.043	-5		
	3	0.882	0.975	0.784	0.969	94		3	0.053	0.036	0.056	0.044	-5		
	Mean	0.876	0.975	0.777	0.970	94		Mean	0.053	0.036	0.056	0.044	-5		
SI-22	1	0.953	0.983	0.840	0.976	106	SI-22	1	0.061	0.036	0.071	0.043	3	Positive	Photoreactive
	2	0.945	0.987	0.837	0.980	101		2	0.057	0.036	0.059	0.043	-5		
	3	0.949	0.988	0.846	0.980	96		3	0.056	0.036	0.059	0.043	-5		
	Mean	0.949	0.986	0.841	0.979	101		Mean	0.058	0.036	0.063	0.043	-2		
Mean for 3 assays	-	-	-	-	105	Mean for 3 assays	-	-	-	-	-	-4			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Rose bengal

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-05	1	1.217	1.010	0.522	1.004	689	SI-05	1	3.138	0.035	3.034	0.043	No data *3	Positive	Photoreactive
	2	1.231	1.010	0.529	1.003	696		2	3.178	0.035	3.070	0.043	No data *3		
	3	1.230	1.010	0.529	1.004	694		3	3.144	0.035	2.999	0.044	No data *3		
	Mean	1.226	1.010	0.526	1.003	693		Mean	3.153	0.035	3.034	0.043	No data *3		
SI-16	1	1.187	0.977	0.522	0.972	660	SI-16	1	3.123	0.036	3.049	0.043	No data *3	Positive	Photoreactive
	2	1.191	0.975	0.532	0.969	654		2	3.145	0.036	3.074	0.043	No data *3		
	3	1.212	0.975	0.543	0.969	664		3	3.115	0.036	3.012	0.044	No data *3		
	Mean	1.197	0.975	0.532	0.970	659		Mean	3.128	0.036	3.045	0.044	No data *3		
SI-22	1	1.179	0.983	0.540	0.976	631	SI-22	1	3.103	0.036	3.040	0.043	No data *3	Positive	Photoreactive
	2	1.215	0.987	0.554	0.980	654		2	3.138	0.036	3.074	0.043	No data *3		
	3	1.215	0.988	0.558	0.980	650		3	3.133	0.036	3.012	0.043	No data *3		
	Mean	1.203	0.986	0.551	0.979	645		Mean	3.125	0.036	3.042	0.043	No data *3		
Mean for 3 assays	-	-	-	-	666	Mean for 3 assays	-	-	-	-	-	No data *3			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Aspirin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-04	1	0.978	0.976	0.968	0.970	4	SI-04	1	0.036	0.035	0.043	0.044	0	Negative	Non-photoreactive
	2	0.976	0.976	0.965	0.971	4		2	0.036	0.036	0.043	0.044	0		
	3	0.976	0.975	0.966	0.968	4		3	0.036	0.035	0.044	0.044	0		
	Mean	0.976	0.976	0.966	0.970	4		Mean	0.036	0.036	0.044	0.044	0		
SI-15	1	0.981	0.981	0.974	0.975	1	SI-15	1	0.036	0.036	0.042	0.043	0	Negative	Non-photoreactive
	2	0.980	0.985	0.972	0.978	2		2	0.035	0.036	0.042	0.043	0		
	3	0.985	0.985	0.977	0.977	2		3	0.036	0.036	0.043	0.043	0		
	Mean	0.982	0.983	0.975	0.977	2		Mean	0.036	0.036	0.042	0.043	0		
SI-21	1	0.995	0.999	0.984	0.992	3	SI-21	1	0.036	0.036	0.043	0.044	-1	Negative	Non-photoreactive
	2	0.995	0.996	0.986	0.989	2		2	0.036	0.036	0.043	0.043	-1		
	3	0.995	0.996	0.985	0.988	3		3	0.036	0.036	0.044	0.044	0		
	Mean	0.995	0.997	0.985	0.990	3		Mean	0.036	0.036	0.043	0.044	-1		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	0				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Benzocaine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-06	1	0.984	0.985	0.977	0.978	0	SI-06	1	0.036	0.035	0.051	0.045	6	Negative	Non-photoreactive
	2	0.984	0.987	0.978	0.980	0		2	0.035	0.036	0.050	0.045	4		
	3	0.984	0.985	0.979	0.979	-1		3	0.036	0.035	0.051	0.045	5		
	Mean	0.984	0.986	0.978	0.979	0		Mean	0.036	0.035	0.050	0.045	5		
SI-17	1	1.001	1.003	0.992	0.992	-1	SI-17	1	0.036	0.036	0.048	0.044	5	Negative	Non-photoreactive
	2	1.001	1.004	0.991	0.995	0		2	0.036	0.036	0.048	0.043	6		
	3	1.003	1.002	0.993	0.992	0		3	0.036	0.036	0.050	0.043	7		
	Mean	1.002	1.003	0.992	0.993	0		Mean	0.036	0.036	0.049	0.043	6		
SI-23	1	0.993	1.004	0.986	0.997	1	SI-23	1	0.036	0.035	0.050	0.046	4	Negative	Non-photoreactive
	2	1.014	0.999	0.988	0.992	19		2	0.036	0.036	0.049	0.045	3		
	3	0.998	1.003	0.988	0.997	2		3	0.037	0.036	0.052	0.046	5		
	Mean	1.002	1.002	0.987	0.995	7		Mean	0.036	0.036	0.050	0.046	4		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	5				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Erythromycin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-01	1	0.938	0.988	0.934	0.981	-2	SI-01	1	0.036	0.035	0.049	0.044	5	Negative	Non-photoreactive
	2	0.953	0.985	0.951	0.979	-3		2	0.036	0.035	0.049	0.043	5		
	3	0.956	0.984	0.954	0.978	-5		3	0.036	0.035	0.050	0.044	6		
	Mean	0.949	0.985	0.946	0.979	-3		Mean	0.036	0.035	0.049	0.043	5		
SI-12	1	0.959	1.002	0.954	0.996	-2	SI-12	1	0.036	0.035	0.050	0.045	4	Negative	Non-photoreactive
	2	0.975	1.005	0.969	0.997	-1		2	0.036	0.036	0.048	0.044	3		
	3	0.976	1.003	0.971	0.996	-2		3	0.036	0.035	0.051	0.045	4		
	Mean	0.970	1.003	0.965	0.996	-2		Mean	0.036	0.035	0.050	0.045	4		
SI-18	1	0.942	0.981	0.934	0.972	-3	SI-18	1	0.037	0.036	0.052	0.045	6	Negative	Non-photoreactive
	2	0.959	0.980	0.952	0.968	-3		2	0.037	0.036	0.051	0.044	5		
	3	0.963	0.988	0.955	0.976	-4		3	0.037	0.036	0.053	0.045	7		
	Mean	0.955	0.983	0.947	0.972	-3		Mean	0.037	0.036	0.052	0.045	6		
Mean for 3 assays	-	-	-	-	-3	Mean for 3 assays	-	-	-	-	5				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Penicillin G

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-01	1	0.985	0.988	0.976	0.981	2	SI-01	1	0.036	0.035	0.080	0.044	36	Positive	Weakly photoreactive
	2	0.986	0.985	0.981	0.979	-1		2	0.035	0.035	0.079	0.043	36		
	3	0.986	0.984	0.981	0.978	0		3	0.036	0.035	0.084	0.044	40		
	Mean	0.986	0.985	0.979	0.979	0		Mean	0.036	0.035	0.081	0.043	37		
SI-12	1	1.004	1.002	0.995	0.996	2	SI-12	1	0.036	0.035	0.083	0.045	37	Positive	Weakly photoreactive
	2	1.001	1.005	0.992	0.997	3		2	0.036	0.036	0.081	0.044	35		
	3	1.007	1.003	0.996	0.996	3		3	0.036	0.035	0.087	0.045	40		
	Mean	1.004	1.003	0.994	0.996	3		Mean	0.036	0.035	0.083	0.045	37		
SI-18	1	0.980	0.981	0.966	0.972	3	SI-18	1	0.037	0.036	0.086	0.045	41	Positive	Weakly photoreactive
	2	0.980	0.980	0.968	0.968	1		2	0.036	0.036	0.085	0.044	40		
	3	0.981	0.988	0.969	0.976	1		3	0.037	0.036	0.096	0.045	50		
	Mean	0.980	0.983	0.968	0.972	2		Mean	0.036	0.036	0.089	0.045	44		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	39				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Phenytoin

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen					Results *1	Experimental No.	Run#	Superoxide anion					Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		A440(-)		A440(+)		A560(-)				A560(+)							
		Test Chemical	Blank	Test Chemical	Blank	Test Chemical				Blank	Test Chemical	Blank					
SI-05	1	0.994	1.010	0.992	1.004	-4	SI-05	1	0.036	0.035	0.118	0.043	74	Positive	Photoreactive		
	2	0.999	1.010	0.994	1.003	-2		2	0.036	0.035	0.117	0.043	73				
	3	0.997	1.010	0.993	1.004	-3		3	0.036	0.035	0.123	0.044	79				
	Mean	0.997	1.010	0.993	1.003	-3		Mean	0.036	0.035	0.119	0.043	75				
SI-16	1	0.966	0.977	0.963	0.972	-3	SI-16	1	0.036	0.036	0.104	0.043	60	Positive	Weakly photoreactive		
	2	0.969	0.975	0.966	0.969	-2		2	0.036	0.036	0.107	0.043	63				
	3	0.967	0.975	0.964	0.969	-2		3	0.036	0.036	0.116	0.044	71				
	Mean	0.967	0.975	0.965	0.970	-2		Mean	0.036	0.036	0.109	0.044	65				
SI-22	1	0.977	0.983	0.972	0.976	-2	SI-22	1	0.037	0.036	0.098	0.043	54	Positive	Weakly photoreactive		
	2	0.979	0.987	0.974	0.980	-2		2	0.037	0.036	0.099	0.043	55				
	3	0.981	0.988	0.977	0.980	-3		3	0.039	0.036	0.108	0.043	62				
	Mean	0.979	0.986	0.974	0.979	-2		Mean	0.037	0.036	0.101	0.043	57				
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	66						

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Bumetrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-24	1	1.087	1.035	1.084	1.027	-4	SI-24	1	0.048	0.035	0.073	0.043	17	Inconclusive	Non-photoreactive
	2	1.082	1.033	1.080	1.027	-5		2	0.048	0.035	0.072	0.043	16		
	3	1.075	1.033	1.071	1.026	-3		3	0.048	0.035	0.072	0.044	16		
	Mean	1.081	1.034	1.078	1.027	-4		Mean	0.048	0.035	0.072	0.043	16		
SI-26	1	1.063	0.991	1.056	0.985	0	SI-26	1	0.049	0.035	0.070	0.043	13	Inconclusive	Non-photoreactive
	2	1.063	0.993	1.058	0.984	-3		2	0.048	0.035	0.069	0.043	13		
	3	1.066	0.992	1.058	0.984	0		3	0.048	0.035	0.069	0.044	13		
	Mean	1.064	0.992	1.057	0.984	-1		Mean	0.048	0.035	0.069	0.043	13		
SI-28	1	1.078	1.016	1.066	1.006	1	SI-28	1	0.051	0.036	0.079	0.045	19	Inconclusive	Non-photoreactive
	2	1.076	1.020	1.065	1.011	1		2	0.050	0.036	0.078	0.044	19		
	3	1.085	1.023	1.074	1.013	1		3	0.051	0.036	0.077	0.045	17		
	Mean	1.080	1.020	1.068	1.010	1		Mean	0.051	0.036	0.078	0.045	18		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	-	16			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Camphor sulfonic acid

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-03	1	1.016	1.014	0.998	0.999	5	SI-03	1	0.036	0.035	0.049	0.050	-2	Negative	Non-photoreactive
	2	1.014	1.010	0.996	0.997	5		2	0.035	0.035	0.048	0.050	-2		
	3	1.015	1.010	0.996	0.994	5		3	0.036	0.035	0.049	0.050	-2		
	Mean	1.015	1.011	0.997	0.997	5		Mean	0.036	0.035	0.049	0.050	-2		
SI-14	1	0.994	0.998	0.988	0.992	0	SI-14	1	0.035	0.035	0.041	0.042	-3	Negative	Non-photoreactive
	2	0.992	0.996	0.986	0.990	0		2	0.035	0.035	0.040	0.042	-3		
	3	0.997	0.996	0.991	0.990	0		3	0.036	0.035	0.042	0.043	-2		
	Mean	0.994	0.997	0.988	0.991	0		Mean	0.036	0.035	0.041	0.043	-3		
SI-20	1	0.981	0.978	0.973	0.970	0	SI-20	1	0.042	0.035	0.051	0.043	3	Negative	Non-photoreactive
	2	0.977	0.981	0.971	0.973	-2		2	0.035	0.036	0.041	0.043	-2		
	3	0.980	0.978	0.972	0.971	0		3	0.036	0.036	0.042	0.043	-1		
	Mean	0.979	0.979	0.972	0.971	-1		Mean	0.038	0.036	0.045	0.043	0		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	-2				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Chlorhexidine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-04	1	0.908	0.976	0.895	0.970	7	SI-04	1	0.039	0.035	0.063	0.044	16	Negative	Non-photoreactive
	2	0.937	0.976	0.918	0.971	13		2	0.039	0.036	0.062	0.044	15		
	3	0.933	0.975	0.924	0.968	4		3	0.039	0.035	0.063	0.044	16		
	Mean	0.926	0.976	0.912	0.970	8		Mean	0.039	0.036	0.063	0.044	16		
SI-15	1	0.878	0.981	0.877	0.975	-5	SI-15	1	0.039	0.036	0.063	0.043	18	Negative	Non-photoreactive
	2	0.898	0.985	0.901	0.978	-9		2	0.038	0.036	0.063	0.043	17		
	3	0.900	0.985	0.899	0.977	-5		3	0.038	0.036	0.062	0.043	17		
	Mean	0.892	0.983	0.892	0.977	-6		Mean	0.038	0.036	0.063	0.043	17		
SI-21	1	0.917	0.999	0.894	0.992	16	SI-21	1	0.040	0.036	0.063	0.044	15	Negative	Non-photoreactive
	2	0.929	0.996	0.901	0.989	20		2	0.040	0.036	0.061	0.043	13		
	3	0.923	0.996	0.900	0.988	17		3	0.040	0.036	0.062	0.044	14		
	Mean	0.923	0.997	0.898	0.990	18		Mean	0.040	0.036	0.062	0.044	14		
Mean for 3 assays	-	-	-	-	7	Mean for 3 assays	-	-	-	-	16				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Cinnamic acid

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-02	1	0.993	0.989	0.981	0.977	0	SI-02	1	0.036	0.036	0.088	0.045	44	Positive	Weakly photoreactive
	2	0.993	0.998	0.981	0.986	0		2	0.036	0.036	0.087	0.044	43		
	3	0.995	0.995	0.982	0.983	1		3	0.036	0.036	0.087	0.044	43		
	Mean	0.994	0.994	0.981	0.982	0		Mean	0.036	0.036	0.088	0.044	43		
SI-13	1	0.989	0.987	0.980	0.980	0	SI-13	1	0.036	0.035	0.080	0.046	34	Positive	Weakly photoreactive
	2	0.991	0.992	0.980	0.985	2		2	0.036	0.036	0.079	0.045	33		
	3	0.993	0.989	0.981	0.980	3		3	0.036	0.036	0.087	0.046	41		
	Mean	0.991	0.990	0.980	0.981	2		Mean	0.036	0.036	0.082	0.046	36		
SI-19	1	0.991	0.997	0.983	0.985	-1	SI-19	1	0.035	0.035	0.072	0.044	29	Positive	Weakly photoreactive
	2	0.992	0.991	0.984	0.984	-1		2	0.035	0.036	0.071	0.043	29		
	3	0.990	0.993	0.982	0.984	-1		3	0.036	0.036	0.080	0.043	37		
	Mean	0.991	0.993	0.983	0.984	-1		Mean	0.035	0.036	0.074	0.043	32		
Mean for 3 assays		-	-	-	-	0	Mean for 3 assays		-	-	-	-	37		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Drometrizole

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Test concentration
 Singlet oxygen 50 µM
 Superoxide anion 50 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-10	1	0.986	0.975	0.978	0.969	3	SI-10	1	0.049	0.036	0.059	0.044	2	Inconclusive	(Non-photoreactive)
	2	0.988	0.985	0.974	0.980	9		2	0.048	0.036	0.059	0.044	3		
	3	0.989	0.984	0.980	0.979	4		3	0.049	0.036	0.061	0.045	4		
	Mean	0.988	0.981	0.977	0.976	5		Mean	0.049	0.036	0.060	0.044	3		
SI-13	1	0.991	0.987	0.983	0.980	-1	SI-13	1	0.048	0.035	0.064	0.046	6	Inconclusive	(Non-photoreactive)
	2	1.008	0.992	0.998	0.985	1		2	0.047	0.036	0.064	0.045	7		
	3	1.016	0.989	1.003	0.980	4		3	0.047	0.036	0.063	0.046	6		
	Mean	1.005	0.990	0.995	0.981	1		Mean	0.047	0.036	0.063	0.046	6		
SI-19	1	0.993	0.997	0.984	0.985	0	SI-19	1	0.049	0.035	0.057	0.044	1	Inconclusive	(Non-photoreactive)
	2	0.998	0.991	0.983	0.984	6		2	0.049	0.036	0.057	0.043	1		
	3	0.995	0.993	0.985	0.984	2		3	0.049	0.036	0.060	0.043	4		
	Mean	0.995	0.993	0.984	0.984	3		Mean	0.049	0.036	0.058	0.043	2		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	4				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : L-Histidine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-06	1	0.986	0.985	0.977	0.978	2	SI-06	1	0.035	0.035	0.087	0.045	41	Positive	Weakly photoreactive
	2	0.983	0.987	0.976	0.980	0		2	0.035	0.036	0.086	0.045	41		
	3	0.993	0.985	0.986	0.979	0		3	0.036	0.035	0.090	0.045	45		
	Mean	0.987	0.986	0.980	0.979	1		Mean	0.035	0.035	0.088	0.045	42		
SI-17	1	1.006	1.003	0.997	0.992	0	SI-17	1	0.036	0.036	0.073	0.044	30	Positive	Weakly photoreactive
	2	1.005	1.004	0.996	0.995	-1		2	0.036	0.036	0.073	0.043	30		
	3	1.006	1.002	0.996	0.992	0		3	0.036	0.036	0.075	0.043	32		
	Mean	1.006	1.003	0.996	0.993	0		Mean	0.036	0.036	0.073	0.043	31		
SI-23	1	1.002	1.004	0.997	0.997	-2	SI-23	1	0.036	0.035	0.073	0.046	27	Positive	Weakly photoreactive
	2	1.004	0.999	0.997	0.992	0		2	0.036	0.036	0.073	0.045	27		
	3	1.004	1.003	0.997	0.997	0		3	0.036	0.036	0.077	0.046	30		
	Mean	1.003	1.002	0.997	0.995	-1		Mean	0.036	0.036	0.074	0.046	28		
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	34				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Methylbenzylidene camphor

Solubility

Singlet oxygen 200 µM : Not determined 100 µM : Not determined 50 µM : Not determined 20 µM : Solution
 Superoxide anion 200 µM : Not determined 100 µM : Not determined 50 µM : Not determined 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-24	1	1.016	1.035	1.012	1.027	-2	SI-24	1	0.036	0.035	0.046	0.043	2	Inconclusive	Non-photoreactive
	2	1.022	1.033	1.015	1.027	1		2	0.035	0.035	0.044	0.043	1		
	3	1.027	1.033	1.022	1.026	-2		3	0.036	0.035	0.048	0.044	4		
	Mean	1.022	1.034	1.016	1.027	-1		Mean	0.036	0.035	0.046	0.043	2		
SI-26	1	0.982	0.991	0.976	0.985	-1	SI-26	1	0.036	0.035	0.046	0.043	3	Inconclusive	Non-photoreactive
	2	0.978	0.993	0.974	0.984	-4		2	0.035	0.035	0.045	0.043	2		
	3	0.978	0.992	0.975	0.984	-5		3	0.036	0.035	0.046	0.044	3		
	Mean	0.980	0.992	0.975	0.984	-3		Mean	0.036	0.035	0.046	0.043	3		
SI-28	1	1.011	1.016	1.003	1.006	-2	SI-28	1	0.037	0.036	0.047	0.045	1	Inconclusive	Non-photoreactive
	2	1.019	1.020	1.009	1.011	-1		2	0.036	0.036	0.046	0.044	1		
	3	1.019	1.023	1.010	1.013	-1		3	0.037	0.036	0.048	0.045	2		
	Mean	1.016	1.020	1.007	1.010	-1		Mean	0.037	0.036	0.047	0.045	1		
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	2				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Octrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!	Undetermined	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Octyl methacrylate

Solubility
 Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined
 Test concentration
 Singlet oxygen 50 µM
 Superoxide anion 50 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-11	1	0.990	0.990	0.978	0.983	5	SI-11	1	0.038	0.036	0.045	0.045	-3	Inconclusive	(Non-photoreactive)
	2	0.994	0.987	0.983	0.980	5		2	0.038	0.036	0.044	0.045	-4		
	3	0.998	0.988	0.988	0.980	3		3	0.039	0.036	0.046	0.046	-3		
	Mean	0.994	0.988	0.983	0.981	4		Mean	0.038	0.036	0.045	0.046	-3		
SI-16	1	0.930	0.977	0.916	0.972	9	SI-16	1	0.040	0.036	0.045	0.043	-3	Inconclusive	(Non-photoreactive)
	2	0.945	0.975	0.937	0.969	2		2	0.039	0.036	0.044	0.043	-4		
	3	0.945	0.975	0.931	0.969	9		3	0.040	0.036	0.046	0.044	-2		
	Mean	0.940	0.975	0.928	0.970	7		Mean	0.040	0.036	0.045	0.044	-3		
SI-22	1	0.994	0.983	0.983	0.976	4	SI-22	1	0.040	0.036	0.046	0.043	-1	Inconclusive	(Non-photoreactive)
	2	0.993	0.987	0.981	0.980	5		2	0.040	0.036	0.045	0.043	-1		
	3	0.994	0.988	0.983	0.980	4		3	0.040	0.036	0.046	0.043	0		
	Mean	0.994	0.986	0.982	0.979	4		Mean	0.040	0.036	0.046	0.043	-1		
Mean for 3 assays	-	-	-	-	5	Mean for 3 assays	-	-	-	-	-2				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Octyl methoxycinnamate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-25	1	1.013	0.982	0.985	0.974	19	SI-25	1	0.053	0.036	0.073	0.047	9	Inconclusive	Non-photoreactive
	2	1.028	0.989	1.000	0.983	20		2	0.053	0.037	0.073	0.047	10		
	3	1.026	0.987	0.997	0.978	20		3	0.053	0.036	0.074	0.047	11		
	Mean	1.022	0.986	0.994	0.978	20		Mean	0.053	0.036	0.073	0.047	10		
SI-27	1	1.001	0.990	0.979	0.981	11	SI-27	1	0.050	0.036	0.075	0.045	17	Inconclusive	Non-photoreactive
	2	1.007	0.993	0.985	0.982	11		2	0.050	0.037	0.075	0.044	17		
	3	1.022	0.992	0.991	0.981	21		3	0.050	0.037	0.075	0.045	17		
	Mean	1.010	0.992	0.985	0.981	14		Mean	0.050	0.037	0.075	0.045	17		
SI-29	1	1.050	1.028	1.025	1.020	18	SI-29	1	0.049	0.035	0.075	0.045	17	Inconclusive	Non-photoreactive
	2	1.066	1.029	1.042	1.021	17		2	0.050	0.036	0.075	0.045	16		
	3	1.066	1.033	1.044	1.025	15		3	0.050	0.036	0.077	0.046	19		
	Mean	1.061	1.030	1.037	1.022	17		Mean	0.049	0.036	0.076	0.045	17		
Mean for 3 assays	-	-	-	-	17	Mean for 3 assays	-	-	-	-	15				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : Octyl salicylate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-24	1	1.036	1.035	1.029	1.027	0	SI-24	1	0.042	0.035	0.067	0.043	17	Inconclusive	Non-photoreactive
	2	1.040	1.033	1.033	1.027	1		2	0.042	0.035	0.066	0.043	17		
	3	1.039	1.033	1.031	1.026	1		3	0.042	0.035	0.068	0.044	18		
	Mean	1.038	1.034	1.031	1.027	1		Mean	0.042	0.035	0.067	0.043	17		
SI-26	1	1.004	0.991	0.992	0.985	5	SI-26	1	0.041	0.035	0.063	0.043	14	Inconclusive	Non-photoreactive
	2	1.004	0.993	0.993	0.984	3		2	0.041	0.035	0.063	0.043	14		
	3	1.005	0.992	0.993	0.984	3		3	0.041	0.035	0.064	0.044	15		
	Mean	1.004	0.992	0.992	0.984	4		Mean	0.041	0.035	0.063	0.043	14		
SI-28	1	1.032	1.016	1.019	1.006	3	SI-28	1	0.042	0.036	0.062	0.045	11	Inconclusive	Non-photoreactive
	2	1.042	1.020	1.029	1.011	3		2	0.042	0.036	0.061	0.044	10		
	3	1.045	1.023	1.030	1.013	4		3	0.042	0.036	0.063	0.045	11		
	Mean	1.040	1.020	1.026	1.010	3		Mean	0.042	0.036	0.062	0.045	11		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	14				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : PABA

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-02	1	0.993	0.989	0.980	0.977	1	SI-02	1	0.036	0.036	0.038	0.045	-6	Negative	Non-photoreactive
	2	0.993	0.998	0.981	0.986	0		2	0.036	0.036	0.038	0.044	-6		
	3	0.991	0.995	0.980	0.983	-1		3	0.036	0.036	0.038	0.044	-6		
	Mean	0.992	0.994	0.981	0.982	0		Mean	0.036	0.036	0.038	0.044	-6		
SI-13	1	0.991	0.987	0.982	0.980	0	SI-13	1	0.036	0.035	0.038	0.046	-8	Negative	Non-photoreactive
	2	0.993	0.992	0.981	0.985	3		2	0.036	0.036	0.038	0.045	-8		
	3	0.994	0.989	0.983	0.980	2		3	0.037	0.036	0.039	0.046	-8		
	Mean	0.993	0.990	0.982	0.981	2		Mean	0.036	0.036	0.038	0.046	-8		
SI-19	1	0.996	0.997	0.985	0.985	1	SI-19	1	0.036	0.035	0.038	0.044	-5	Negative	Non-photoreactive
	2	0.995	0.991	0.984	0.984	1		2	0.036	0.036	0.038	0.043	-5		
	3	0.996	0.993	0.987	0.984	0		3	0.036	0.036	0.038	0.043	-5		
	Mean	0.995	0.993	0.985	0.984	1		Mean	0.036	0.036	0.038	0.043	-5		
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	-6		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : SDS

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
SI-02	1	0.970	0.989	0.953	0.977	4	SI-30	1	0.047	0.036	0.063	0.044	7	Inconclusive	Non-photoreactive
	2	0.971	0.998	0.953	0.986	6		2	0.047	0.036	0.063	0.044	8		
	3	0.970	0.995	0.948	0.983	10		3	0.048	0.036	0.064	0.044	9		
	Mean	0.970	0.994	0.951	0.982	7		Mean	0.047	0.036	0.063	0.044	8		
SI-13	1	0.968	0.987	0.950	0.980	9	SI-31	1	0.047	0.035	0.063	0.043	10	Inconclusive	Non-photoreactive
	2	0.967	0.992	0.950	0.985	9		2	0.046	0.036	0.063	0.043	10		
	3	0.969	0.989	0.950	0.980	10		3	0.047	0.036	0.065	0.043	11		
	Mean	0.968	0.990	0.950	0.981	9		Mean	0.047	0.036	0.064	0.043	10		
SI-19	1	0.971	0.997	0.957	0.985	5	SI-32	1	0.050	0.036	0.067	0.044	8	Inconclusive	Non-photoreactive
	2	0.978	0.991	0.964	0.984	5		2	0.050	0.036	0.065	0.045	5		
	3	0.977	0.993	0.961	0.984	7		3	0.051	0.036	0.067	0.046	7		
	Mean	0.975	0.993	0.961	0.984	6		Mean	0.051	0.036	0.066	0.045	7		
Mean for 3 assays	-	-	-	-	7	Mean for 3 assays	-	-	-	-	8				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 6
 Chemical Name : UV-571

Solubility

Singlet oxygen 200 μM : Precipitation 100 μM : Precipitation 50 μM : Precipitation 20 μM : Precipitation
 Superoxide anion 200 μM : Precipitation 100 μM : Precipitation 50 μM : Precipitation 20 μM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!	Undetermined	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 μM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 μM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 μM: Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 μM: Twenty μM can be used for judgment when precipitation or coloration is observed at 200 μM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 μM or 100 μM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Acridine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-23	1	0.956	0.983	0.702	0.979	249	TS-23	1	0.034	0.034	0.203	0.037	166	Positive	Photoreactive
	2	0.952	0.978	0.715	0.972	232		2	0.034	0.034	0.206	0.037	169		
	3	0.960	0.984	0.727	0.980	228		3	0.035	0.034	0.209	0.037	171		
	Mean	0.956	0.982	0.715	0.977	236		Mean	0.034	0.034	0.206	0.037	169		
TS-28	1	0.980	0.999	0.705	0.994	271	TS-28	1	0.036	0.034	0.216	0.036	178	Positive	Photoreactive
	2	0.983	1.002	0.716	0.999	263		2	0.035	0.034	0.211	0.036	174		
	3	0.989	1.007	0.716	1.004	269		3	0.036	0.034	0.221	0.037	183		
	Mean	0.984	1.003	0.712	0.999	268		Mean	0.036	0.034	0.216	0.036	178		
TS-33	1	0.988	1.007	0.703	1.002	279	TS-33	1	0.035	0.034	0.217	0.037	179	Positive	Photoreactive
	2	0.987	1.010	0.715	1.003	266		2	0.035	0.035	0.215	0.038	177		
	3	0.994	1.012	0.718	1.007	270		3	0.035	0.034	0.221	0.036	183		
	Mean	0.990	1.010	0.712	1.004	272		Mean	0.035	0.034	0.218	0.037	180		
Mean for 3 assays	-	-	-	-	259	Mean for 3 assays	-	-	-	-	176				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Acridine HCl

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-22	1	0.983	0.996	0.710	0.991	269	TS-22	1	0.035	0.034	0.197	0.039	153	Positive	Photoreactive
	2	0.984	0.996	0.720	0.992	260		2	0.035	0.034	0.193	0.038	149		
	3	0.988	1.005	0.721	1.001	263		3	0.035	0.038	0.214	0.055	170		
	Mean	0.985	0.999	0.717	0.995	264		Mean	0.035	0.035	0.201	0.044	157		
TS-28	1	0.995	0.999	0.716	0.994	275	TS-28	1	0.035	0.034	0.194	0.036	157	Positive	Photoreactive
	2	0.989	1.002	0.719	0.999	266		2	0.035	0.034	0.190	0.036	153		
	3	0.991	1.007	0.738	1.004	249		3	0.035	0.034	0.199	0.037	162		
	Mean	0.992	1.003	0.724	0.999	263		Mean	0.035	0.034	0.194	0.036	157		
TS-33	1	0.996	1.007	0.727	1.002	263	TS-33	1	0.035	0.034	0.197	0.037	159	Positive	Photoreactive
	2	0.995	1.010	0.734	1.003	255		2	0.035	0.035	0.194	0.038	156		
	3	0.996	1.012	0.740	1.007	250		3	0.035	0.034	0.203	0.036	165		
	Mean	0.996	1.010	0.734	1.004	256		Mean	0.035	0.034	0.198	0.037	160		
Mean for 3 assays	-	-	-	-	261	Mean for 3 assays	-	-	-	-	158				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Amiodarone HCl

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen Not tested
 Superoxide anion 20 µM

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1	0.066	0.034	0.072	0.036	4		
	2					#DIV/0!	TS-45	2	0.066	0.034	0.073	0.036	5		
	3					#DIV/0!		3	0.066	0.034	0.073	0.036	5		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.066	0.034	0.073	0.036	5	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.075	0.035	0.071	0.037	-7		
	2					#DIV/0!	TS-42	2	0.075	0.034	0.071	0.038	-7		
	3					#DIV/0!		3	0.075	0.034	0.072	0.036	-6		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.075	0.034	0.071	0.037	-7	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.073	0.034	0.069	0.037	-7		
	2					#DIV/0!	TS-44	2	0.073	0.034	0.070	0.037	-6		
	3					#DIV/0!		3	0.073	0.034	0.071	0.037	-5		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.073	0.034	0.070	0.037	-6	Inconclusive	Inconclusive
	Mean for 3 assays	-	-	-	-	#DIV/0!	Mean for 3 assays	-	-	-	-	-3			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Chlorpromazine HCl

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-21	1	0.914	0.983	0.893	0.976	15	TS-21	1	0.036	0.035	0.114	0.038	75	Positive	Photoreactive
	2	0.943	0.983	0.927	0.977	10		2	0.035	0.035	0.116	0.038	78		
	3	0.930	0.983	0.910	0.979	14		3	0.036	0.034	0.116	0.038	77		
	Mean	0.929	0.983	0.910	0.977	13		Mean	0.036	0.035	0.115	0.038	77		
TS-27	1	0.936	1.002	0.910	0.997	20	TS-27	1	0.035	0.035	0.114	0.037	78	Positive	Photoreactive
	2	0.952	0.993	0.936	0.986	10		2	0.035	0.035	0.116	0.036	80		
	3	0.954	0.999	0.935	0.993	13		3	0.036	0.035	0.118	0.036	81		
	Mean	0.947	0.998	0.927	0.992	14		Mean	0.035	0.035	0.116	0.036	80		
TS-32	1	0.939	0.991	0.928	0.986	9	TS-32	1	0.035	0.034	0.112	0.036	75	Positive	Photoreactive
	2	0.949	0.995	0.943	0.990	4		2	0.035	0.034	0.112	0.036	75		
	3	0.952	0.998	0.944	1.003	6		3	0.035	0.034	0.117	0.036	80		
	Mean	0.947	0.995	0.938	0.993	6		Mean	0.035	0.034	0.114	0.036	77		
Mean for 3 assays	-	-	-	-	11	Mean for 3 assays	-	-	-	-	78				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Doxycycline HCl

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-22	1	0.969	0.996	0.680	0.991	285	TS-22	1	0.042	0.034	0.387	0.039	336	Positive	Photoreactive
	2	0.972	0.996	0.696	0.992	272		2	0.039	0.034	0.389	0.038	341		
	3	0.978	1.005	0.693	1.001	281		3	0.039	0.038	0.395	0.055	347		
	Mean	0.973	0.999	0.690	0.995	279		Mean	0.040	0.035	0.390	0.044	341		
TS-27	1	0.975	1.002	0.654	0.997	315	TS-27	1	0.040	0.035	0.403	0.037	362	Positive	Photoreactive
	2	0.973	0.993	0.671	0.986	296		2	0.040	0.035	0.415	0.036	374		
	3	0.969	0.999	0.660	0.993	303		3	0.040	0.035	0.421	0.036	380		
	Mean	0.972	0.998	0.662	0.992	305		Mean	0.040	0.035	0.413	0.036	372		
TS-32	1	0.965	0.991	0.658	0.986	305	TS-32	1	0.039	0.034	0.386	0.036	345	Positive	Photoreactive
	2	0.969	0.995	0.692	0.990	275		2	0.039	0.034	0.388	0.036	347		
	3	0.971	0.998	0.675	1.003	294		3	0.040	0.034	0.403	0.036	361		
	Mean	0.968	0.995	0.675	0.993	291		Mean	0.039	0.034	0.392	0.036	351		
Mean for 3 assays	-	-	-	-	292	Mean for 3 assays	-	-	-	-	355				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Fenofibrate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen Not tested
 Superoxide anion 20 µM

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1	0.038	0.037	0.037	0.046	-6		
	2					#DIV/0!	TS-39	2	0.038	0.034	0.069	0.037	26		
	3					#DIV/0!	3	0.039	0.034	0.038	0.037	-6			
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Mean	0.038	0.035	0.048	0.040	5	Inconclusive	Inconclusive	
	1					#DIV/0!		1	0.034	0.037	0.039	0.049	0		
	2					#DIV/0!	TS-41	2	0.034	0.034	0.038	0.036	-1		
	3					#DIV/0!	3	0.034	0.034	0.038	0.036	-1			
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Mean	0.034	0.035	0.038	0.040	-1	Inconclusive	Inconclusive	
	1					#DIV/0!		1	0.034	0.034	0.038	0.036	1		
	2					#DIV/0!	TS-43	2	0.035	0.034	0.037	0.036	-1		
	3					#DIV/0!	3	0.037	0.034	0.039	0.039	-1			
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Mean	0.035	0.034	0.038	0.037	0	Inconclusive	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!	Mean for 3 assays	-	-	-	-	1			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Furosemide

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-20	1	0.976	0.983	0.882	0.977	88	TS-20	1	0.037	0.035	0.070	0.038	30	Positive	Photoreactive
	2	0.975	0.986	0.888	0.981	81		2	0.036	0.034	0.072	0.037	33		
	3	0.980	0.991	0.888	0.985	86		3	0.036	0.034	0.077	0.037	38		
	Mean	0.977	0.987	0.886	0.981	85		Mean	0.036	0.034	0.073	0.037	34		
TS-26	1	1.001	1.000	0.913	0.994	83	TS-26	1	0.036	0.035	0.070	0.037	32	Positive	Photoreactive
	2	1.002	1.013	0.919	1.009	78		2	0.036	0.034	0.070	0.036	32		
	3	1.008	1.017	0.924	1.012	79		3	0.036	0.034	0.074	0.036	36		
	Mean	1.004	1.010	0.919	1.005	80		Mean	0.036	0.034	0.071	0.036	33		
TS-31	1	0.997	1.001	0.911	0.995	80	TS-31	1	0.036	0.035	0.066	0.037	28	Positive	Photoreactive
	2	0.998	0.988	0.920	0.982	72		2	0.047	0.035	0.065	0.036	16		
	3	1.009	1.015	0.930	1.009	73		3	0.038	0.034	0.070	0.037	30		
	Mean	1.001	1.001	0.920	0.995	75		Mean	0.040	0.035	0.067	0.037	25		
Mean for 3 assays	-	-	-	-	80	Mean for 3 assays	-	-	-	-	31				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Ketoprofen

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-22	1	0.993	0.996	0.780	0.991	209	TS-22	1	0.036	0.034	0.149	0.039	104	Positive	Photoreactive
	2	0.992	0.996	0.784	0.992	204		2	0.036	0.034	0.154	0.038	109		
	3	0.998	1.005	0.786	1.001	208		3	0.036	0.038	0.158	0.055	113		
	Mean	0.994	0.999	0.783	0.995	207		Mean	0.036	0.035	0.154	0.044	109		
TS-28	1	0.992	0.999	0.811	0.994	177	TS-28	1	0.035	0.034	0.134	0.036	97	Positive	Photoreactive
	2	1.008	1.002	0.832	0.999	172		2	0.035	0.034	0.138	0.036	101		
	3	1.013	1.007	0.834	1.004	175		3	0.036	0.034	0.148	0.037	110		
	Mean	1.004	1.003	0.826	0.999	175		Mean	0.035	0.034	0.140	0.036	103		
TS-33	1	1.007	1.007	0.817	1.002	184	TS-33	1	0.036	0.034	0.127	0.037	88	Positive	Photoreactive
	2	1.003	1.010	0.831	1.003	166		2	0.036	0.035	0.129	0.038	90		
	3	1.008	1.012	0.833	1.007	169		3	0.036	0.034	0.138	0.036	99		
	Mean	1.006	1.010	0.827	1.004	173		Mean	0.036	0.034	0.131	0.037	92		
Mean for 3 assays	-	-	-	-	185	Mean for 3 assays	-	-	-	-	101				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : 6-methylcoumarine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results *1	Experimental No.	Run#	Superoxide anion				Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		A440(-)		A440(+)					A560(-)		A560(+)				
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-21	1	0.980	0.983	0.841	0.976	133	TS-21	1	0.036	0.035	0.099	0.038	60	Positive	Photoreactive
	2	0.993	0.983	0.845	0.977	142		2	0.035	0.035	0.096	0.038	58		
	3	0.990	0.983	0.850	0.979	134		3	0.036	0.034	0.103	0.038	64		
	Mean	0.988	0.983	0.845	0.977	136		Mean	0.036	0.035	0.099	0.038	61		
TS-27	1	1.002	1.002	0.883	0.997	113	TS-27	1	0.040	0.035	0.073	0.037	32	Positive	Photoreactive
	2	0.997	0.993	0.884	0.986	107		2	0.035	0.035	0.070	0.036	34		
	3	0.995	0.999	0.883	0.993	106		3	0.035	0.035	0.082	0.036	46		
	Mean	0.998	0.998	0.883	0.992	109		Mean	0.037	0.035	0.075	0.036	37		
TS-32	1	0.996	0.991	0.864	0.986	130	TS-32	1	0.035	0.034	0.098	0.036	61	Positive	Photoreactive
	2	0.990	0.995	0.866	0.990	122		2	0.035	0.034	0.096	0.036	59		
	3	1.002	0.998	0.873	1.003	127		3	0.035	0.034	0.108	0.036	71		
	Mean	0.996	0.995	0.868	0.993	126		Mean	0.035	0.034	0.101	0.036	64		
Mean for 3 assays	-	-	-	-	124	Mean for 3 assays	-	-	-	-	54				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : 8-MOP

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-23	1	0.964	0.983	0.892	0.979	67	TS-23	1	0.035	0.034	0.073	0.037	35	Positive	Photoreactive
	2	0.962	0.978	0.896	0.972	61		2	0.035	0.034	0.070	0.037	32		
	3	0.967	0.984	0.904	0.980	58		3	0.035	0.034	0.074	0.037	36		
	Mean	0.964	0.982	0.897	0.977	62		Mean	0.035	0.034	0.072	0.037	34		
TS-29	1	0.990	1.000	0.914	0.995	70	TS-29	1	0.034	0.035	0.060	0.037	23	Positive	Photoreactive
	2	0.980	1.014	0.914	1.007	60		2	0.034	0.034	0.059	0.036	22		
	3	0.983	1.007	0.920	1.000	57		3	0.035	0.034	0.062	0.037	24		
	Mean	0.984	1.007	0.916	1.001	62		Mean	0.034	0.034	0.060	0.037	23		
TS-34	1	0.980	0.994	0.899	0.988	76	TS-34	1	0.034	0.034	0.064	0.037	33	Positive	Photoreactive
	2	0.974	0.991	0.898	0.986	71		2	0.034	0.034	0.061	0.036	30		
	3	0.979	1.001	0.906	0.996	68		3	0.035	0.048	0.066	0.036	34		
	Mean	0.978	0.995	0.901	0.990	72		Mean	0.034	0.039	0.064	0.036	32		
Mean for 3 assays	-	-	-	-	65	Mean for 3 assays	-	-	-	-	30				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Nalidixic acid

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-20	1	0.979	0.983	0.839	0.977	134	TS-20	1	0.034	0.035	0.306	0.038	269	Positive	Photoreactive
	2	0.979	0.986	0.846	0.981	127		2	0.057	0.034	0.293	0.037	233		
	3	0.983	0.991	0.850	0.985	127		3	0.034	0.034	0.292	0.037	255		
	Mean	0.980	0.987	0.845	0.981	129		Mean	0.042	0.034	0.297	0.037	252		
TS-26	1	0.990	1.000	0.855	0.994	130	TS-26	1	0.034	0.035	0.278	0.037	242	Positive	Photoreactive
	2	1.001	1.013	0.868	1.009	128		2	0.035	0.034	0.278	0.036	241		
	3	1.005	1.017	0.874	1.012	126		3	0.034	0.034	0.279	0.036	243		
	Mean	0.999	1.010	0.866	1.005	128		Mean	0.034	0.034	0.278	0.036	242		
TS-31	1	0.988	1.001	0.856	0.995	126	TS-31	1	0.034	0.035	0.265	0.037	229	Positive	Photoreactive
	2	1.007	0.988	0.877	0.982	124		2	0.034	0.035	0.272	0.036	236		
	3	1.000	1.015	0.870	1.009	124		3	0.035	0.034	0.279	0.037	242		
	Mean	0.998	1.001	0.868	0.995	125		Mean	0.034	0.035	0.272	0.037	236		
Mean for 3 assays	-	-	-	-	127	Mean for 3 assays	-	-	-	-	243				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Nalidixic acid (Na salt)

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-23	1	0.975	0.983	0.843	0.979	127	TS-23	1	0.035	0.034	0.271	0.037	233	Positive	Photoreactive
	2	0.968	0.978	0.839	0.972	124		2	0.035	0.034	0.287	0.037	249		
	3	0.981	0.984	0.854	0.980	122		3	0.035	0.034	0.293	0.037	255		
	Mean	0.975	0.982	0.845	0.977	124		Mean	0.035	0.034	0.284	0.037	246		
TS-29	1	1.004	1.000	0.869	0.995	129	TS-29	1	0.035	0.035	0.276	0.037	238	Positive	Photoreactive
	2	0.999	1.014	0.881	1.007	112		2	0.035	0.034	0.298	0.036	260		
	3	1.005	1.007	0.885	1.000	114		3	0.035	0.034	0.298	0.037	260		
	Mean	1.003	1.007	0.878	1.001	118		Mean	0.035	0.034	0.291	0.037	253		
TS-34	1	0.987	0.994	0.853	0.988	129	TS-34	1	0.035	0.034	0.281	0.037	249	Positive	Photoreactive
	2	0.985	0.991	0.860	0.986	120		2	0.034	0.034	0.284	0.036	253		
	3	0.993	1.001	0.859	0.996	129		3	0.035	0.048	0.299	0.036	267		
	Mean	0.988	0.995	0.857	0.990	126		Mean	0.035	0.039	0.288	0.036	256		
Mean for 3 assays	-	-	-	-	123	Mean for 3 assays	-	-	-	-	252				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Norfloxacin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-21	1	0.979	0.983	0.749	0.976	224	TS-21	1	0.036	0.035	0.112	0.038	73	Positive	Photoreactive
	2	0.974	0.983	0.762	0.977	206		2	0.035	0.035	0.112	0.038	74		
	3	0.981	0.983	0.762	0.979	213		3	0.036	0.034	0.119	0.038	80		
	Mean	0.978	0.983	0.758	0.977	214		Mean	0.036	0.035	0.114	0.038	76		
TS-27	1	0.999	1.002	0.773	0.997	220	TS-27	1	0.037	0.035	0.127	0.037	89	Positive	Photoreactive
	2	0.983	0.993	0.784	0.986	193		2	0.035	0.035	0.127	0.036	91		
	3	0.999	0.999	0.805	0.993	188		3	0.035	0.035	0.133	0.036	97		
	Mean	0.994	0.998	0.787	0.992	200		Mean	0.036	0.035	0.129	0.036	92		
TS-32	1	0.986	0.991	0.758	0.986	226	TS-32	1	0.035	0.034	0.120	0.036	83	Positive	Photoreactive
	2	0.989	0.995	0.774	0.990	213		2	0.035	0.034	0.121	0.036	84		
	3	0.991	0.998	0.777	1.003	212		3	0.035	0.034	0.130	0.036	93		
	Mean	0.989	0.995	0.770	0.993	217		Mean	0.035	0.034	0.124	0.036	87		
Mean for 3 assays	-	-	-	-	210	Mean for 3 assays	-	-	-	-	85				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Ofloxacin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-21	1	0.972	0.983	0.830	0.976	136	TS-21	1	0.036	0.035	0.320	0.038	281	Positive	Photoreactive
	2	0.970	0.983	0.841	0.977	123		2	0.036	0.035	0.319	0.038	280		
	3	0.974	0.983	0.847	0.979	121		3	0.036	0.034	0.338	0.038	299		
	Mean	0.972	0.983	0.839	0.977	127		Mean	0.036	0.035	0.326	0.038	287		
TS-27	1	0.988	1.002	0.849	0.997	133	TS-27	1	0.035	0.035	0.428	0.037	392	Positive	Photoreactive
	2	0.994	0.993	0.866	0.986	122		2	0.035	0.035	0.428	0.036	392		
	3	0.993	0.999	0.871	0.993	116		3	0.037	0.035	0.437	0.036	399		
	Mean	0.992	0.998	0.862	0.992	124		Mean	0.036	0.035	0.431	0.036	394		
TS-32	1	0.989	0.991	0.847	0.986	140	TS-32	1	0.035	0.034	0.430	0.036	393	Positive	Photoreactive
	2	0.989	0.995	0.857	0.990	130		2	0.037	0.034	0.436	0.036	397		
	3	0.996	0.998	0.866	1.003	128		3	0.035	0.034	0.448	0.036	411		
	Mean	0.991	0.995	0.857	0.993	133		Mean	0.036	0.034	0.438	0.036	400		
Mean for 3 assays	-	-	-	-	128	Mean for 3 assays	-	-	-	-	360				

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Piroxicam

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 50 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-23	1	0.979	0.983	0.714	0.979	260	TS-23	1	0.036	0.034	0.104	0.037	65	Positive	Photoreactive
	2	0.965	0.978	0.723	0.972	237		2	0.036	0.034	0.109	0.037	70		
	3	0.970	0.984	0.722	0.980	243		3	0.037	0.034	0.116	0.037	76		
	Mean	0.971	0.982	0.720	0.977	247		Mean	0.036	0.034	0.110	0.037	70		
TS-29	1	1.002	1.000	0.739	0.995	257	TS-29	1	0.036	0.035	0.094	0.037	55	Positive	Photoreactive
	2	0.999	1.014	0.760	1.007	233		2	0.036	0.034	0.102	0.036	63		
	3	1.012	1.007	0.759	1.000	247		3	0.036	0.034	0.106	0.037	67		
	Mean	1.004	1.007	0.753	1.001	246		Mean	0.036	0.034	0.101	0.037	62		
TS-34	1	0.988	0.994	0.713	0.988	270	TS-34	1	0.036	0.034	0.098	0.037	65	Positive	Photoreactive
	2	0.989	0.991	0.741	0.986	243		2	0.036	0.034	0.103	0.036	70		
	3	0.992	1.001	0.725	0.996	262		3	0.036	0.048	0.111	0.036	78		
	Mean	0.990	0.995	0.726	0.990	258		Mean	0.036	0.039	0.104	0.036	71		
Mean for 3 assays	-	-	-	-	250	Mean for 3 assays	-	-	-	-	68				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Promethazine HCl

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-20	1	0.967	0.983	0.843	0.977	118	TS-20	1	0.036	0.035	0.065	0.038	26	Positive	Photoreactive
	2	0.959	0.986	0.846	0.981	107		2	0.036	0.034	0.066	0.037	27		
	3	0.987	0.991	0.863	0.985	118		3	0.037	0.034	0.070	0.037	30		
	Mean	0.971	0.987	0.851	0.981	114		Mean	0.036	0.034	0.067	0.037	28		
TS-26	1	0.990	1.000	0.857	0.994	128	TS-26	1	0.035	0.035	0.063	0.037	26	Positive	Photoreactive
	2	0.990	1.013	0.867	1.009	118		2	0.035	0.034	0.063	0.036	26		
	3	0.999	1.017	0.869	1.012	125		3	0.036	0.034	0.067	0.036	29		
	Mean	0.993	1.010	0.864	1.005	124		Mean	0.035	0.034	0.064	0.036	27		
TS-31	1	0.978	1.001	0.850	0.995	122	TS-31	1	0.035	0.035	0.061	0.037	24	Positive	Photoreactive
	2	0.986	0.988	0.870	0.982	110		2	0.035	0.035	0.066	0.036	29		
	3	0.984	1.015	0.858	1.009	120		3	0.035	0.034	0.064	0.037	27		
	Mean	0.983	1.001	0.859	0.995	117		Mean	0.035	0.035	0.064	0.037	27		
Mean for 3 assays	-	-	-	-	118	Mean for 3 assays	-	-	-	-	27				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Laboratory : 7
 Chemical Name : Rosiglitazone

Rosiglitazone is excluded from the Fourth data analysis since the VMT considered that it is inappropriate to include rosiglitazone in the “phototoxic” chemical set because of lack of high quality human data regarding its phototoxicity.

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Solution 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 50 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
	Run#	A440(-)		A440(+)		Results *1	Run#	A560(-)		A560(+)		Results *2		
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
TS-23	1	0.909	0.983	0.864	0.979	40	TS-23	1	0.037	0.034	0.090	0.037	50	Positive
	2	0.918	0.978	0.878	0.972	35		2	0.040	0.034	0.096	0.037	53	
	3	0.912	0.984	0.869	0.980	38		3	0.037	0.034	0.097	0.037	57	
	Mean	0.913	0.982	0.870	0.977	38		Mean	0.038	0.034	0.094	0.037	53	
TS-29	1	0.959	1.000	0.916	0.995	37	TS-29	1	0.039	0.035	0.104	0.037	62	Positive
	2	0.958	1.014	0.921	1.007	31		2	0.039	0.034	0.111	0.036	69	
	3	0.951	1.007	0.912	1.000	33		3	0.039	0.034	0.110	0.037	68	
	Mean	0.956	1.007	0.916	1.001	34		Mean	0.039	0.034	0.108	0.037	66	
TS-34	1	0.942	0.994	0.888	0.988	49	TS-34	1	0.038	0.034	0.098	0.037	63	Positive
	2	0.943	0.991	0.897	0.986	41		2	0.038	0.034	0.096	0.036	61	
	3	0.957	1.001	0.904	0.996	48		3	0.038	0.048	0.105	0.036	70	
	Mean	0.947	0.995	0.896	0.990	46		Mean	0.038	0.039	0.100	0.036	65	
Mean for 3 assays	-	-	-	-	39	Mean for 3 assays	-	-	-	-	61			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Tetracycline

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-20	1	0.974	0.983	0.739	0.977	229	TS-20	1	0.041	0.035	0.256	0.038	212	Positive	Photoreactive
	2	0.975	0.986	0.751	0.981	218		2	0.040	0.034	0.252	0.037	209		
	3	0.985	0.991	0.755	0.985	224		3	0.040	0.034	0.255	0.037	212		
	Mean	0.978	0.987	0.748	0.981	224		Mean	0.040	0.034	0.254	0.037	211		
TS-26	1	0.997	1.000	0.748	0.994	244	TS-26	1	0.038	0.035	0.265	0.037	225	Positive	Photoreactive
	2	0.999	1.013	0.767	1.009	227		2	0.038	0.034	0.254	0.036	214		
	3	1.006	1.017	0.769	1.012	232		3	0.039	0.034	0.272	0.036	231		
	Mean	1.001	1.010	0.761	1.005	234		Mean	0.038	0.034	0.264	0.036	223		
TS-31	1	0.993	1.001	0.747	0.995	240	TS-31	1	0.040	0.035	0.291	0.037	249	Positive	Photoreactive
	2	0.998	0.988	0.769	0.982	223		2	0.040	0.035	0.300	0.036	258		
	3	1.000	1.015	0.764	1.009	230		3	0.040	0.034	0.299	0.037	257		
	Mean	0.997	1.001	0.760	0.995	231		Mean	0.040	0.035	0.297	0.037	255		
Mean for 3 assays	-	-	-	-	230	Mean for 3 assays	-	-	-	-	230				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Anthracene

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen Not tested
 Superoxide anion 20 µM

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1	0.046	0.034	0.119	0.036	71		
	2					#DIV/0!	TS-45	2	0.047	0.034	0.109	0.036	60		
	3					#DIV/0!		3	0.047	0.034	0.124	0.036	75		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.047	0.034	0.117	0.036	69	Positive	Inconclusive
	1					#DIV/0!		1	0.054	0.035	0.156	0.037	99		
	2					#DIV/0!	TS-42	2	0.055	0.034	0.131	0.038	73		
	3					#DIV/0!		3	0.055	0.034	0.143	0.036	85		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.055	0.034	0.143	0.037	86	Positive	Photoreactive
	1					#DIV/0!		1	0.052	0.034	0.096	0.037	41		
	2					#DIV/0!	TS-44	2	0.052	0.034	0.086	0.037	31		
	3					#DIV/0!		3	0.052	0.034	0.090	0.037	35		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.052	0.034	0.091	0.037	36	Positive	Inconclusive
	Mean for 3 assays	-	-	-	-	#DIV/0!	Mean for 3 assays	-	-	-	-	-	64		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Avobenzone

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen Not tested
 Superoxide anion 20 µM

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1	0.063	0.037	0.105	0.046	37		
	2					#DIV/0!	TS-39	2	0.064	0.034	0.095	0.037	26		
	3					#DIV/0!		3	0.064	0.034	0.100	0.037	31		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.064	0.035	0.100	0.040	31	Positive	Inconclusive
	1					#DIV/0!		1	0.066	0.037	0.106	0.049	35		
	2					#DIV/0!	TS-41	2	0.066	0.034	0.102	0.036	31		
	3					#DIV/0!		3	0.066	0.034	0.106	0.036	35		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.066	0.035	0.105	0.040	34	Positive	Inconclusive
	1					#DIV/0!		1	0.058	0.034	0.105	0.036	44		
	2					#DIV/0!	TS-43	2	0.058	0.034	0.101	0.036	40		
	3					#DIV/0!		3	0.065	0.034	0.105	0.039	37		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.060	0.034	0.104	0.037	40	Positive	Inconclusive
	Mean for 3 assays	-	-	-	-	#DIV/0!	Mean for 3 assays	-	-	-	-	-	35		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Bithionol

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen 200 µM
 Superoxide anion Not tested

Singlet oxygen							Superoxide anion					Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-24	1	0.901	0.982	0.768	0.976	127	1					#DIV/0!	Positive	Photoreactive	
	2	0.925	0.988	0.794	0.983	125	2					#DIV/0!			
	3	0.940	0.994	0.803	0.988	131	3					#DIV/0!			
	Mean	0.922	0.988	0.788	0.982	128	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!			
TS-29	1	0.935	1.000	0.779	0.995	150	1					#DIV/0!	Positive	Photoreactive	
	2	0.966	1.014	0.817	1.007	143	2					#DIV/0!			
	3	0.953	1.007	0.796	1.000	151	3					#DIV/0!			
	Mean	0.951	1.007	0.797	1.001	148	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!			
TS-34	1	0.939	0.994	0.778	0.988	156	1					#DIV/0!	Positive	Photoreactive	
	2	0.934	0.991	0.782	0.986	147	2					#DIV/0!			
	3	0.987	1.001	0.819	0.996	163	3					#DIV/0!			
	Mean	0.953	0.995	0.793	0.990	155	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!			
Mean for 3 assays	-	-	-	-	144	Mean for 3 assays	-	-	-	-	-	#DIV/0!			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Hexachlorophene

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion Not tested

Singlet oxygen							Superoxide anion					Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank		
TS-23	1	0.834	0.983	0.695	0.979	134	1					#DIV/0!	Positive	Photoreactive
	2	0.860	0.978	0.725	0.972	130	2					#DIV/0!		
	3	0.880	0.984	0.737	0.980	138	3					#DIV/0!		
	Mean	0.858	0.982	0.719	0.977	134	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
TS-29	1	0.857	1.000	0.718	0.995	133	1					#DIV/0!	Positive	Photoreactive
	2	0.891	1.014	0.756	1.007	129	2					#DIV/0!		
	3	0.911	1.007	0.769	1.000	136	3					#DIV/0!		
	Mean	0.886	1.007	0.748	1.001	133	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
TS-34	1	0.853	0.994	0.709	0.988	139	1					#DIV/0!	Positive	Photoreactive
	2	0.900	0.991	0.760	0.986	135	2					#DIV/0!		
	3	0.910	1.001	0.757	0.996	148	3					#DIV/0!		
	Mean	0.888	0.995	0.742	0.990	141	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		
Mean for 3 assays	-	-	-	-	136	Mean for 3 assays	-	-	-	-	-	#DIV/0!		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Rose bengal

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-21	1	1.155	0.983	0.490	0.976	659	TS-21	1	3.404	0.035	3.289	0.038	No data *3	Positive	Photoreactive
	2	1.111	0.983	0.502	0.977	603		2	3.363	0.035	3.265	0.038	No data *3		
	3	1.137	0.983	0.503	0.979	628		3	3.319	0.034	3.188	0.038	No data *3		
	Mean	1.134	0.983	0.498	0.977	630		Mean	3.362	0.035	3.247	0.038	No data *3		
TS-27	1	1.128	1.002	0.459	0.997	663	TS-27	1	3.407	0.035	3.258	0.037	No data *3	Positive	Photoreactive
	2	1.176	0.993	0.504	0.986	666		2	3.380	0.035	3.247	0.036	No data *3		
	3	1.151	0.999	0.490	0.993	655		3	3.350	0.035	3.193	0.036	No data *3		
	Mean	1.152	0.998	0.484	0.992	661		Mean	3.379	0.035	3.233	0.036	No data *3		
TS-32	1	1.171	0.991	0.490	0.986	679	TS-32	1	3.403	0.034	3.258	0.036	No data *3	Positive	Photoreactive
	2	1.168	0.995	0.507	0.990	659		2	3.378	0.034	3.266	0.036	No data *3		
	3	1.176	0.998	0.502	1.003	672		3	3.341	0.034	3.208	0.036	No data *3		
	Mean	1.172	0.995	0.500	0.993	670		Mean	3.374	0.034	3.244	0.036	No data *3		
Mean for 3 assays	-	-	-	-	654	Mean for 3 assays	-	-	-	-	-	No data *3			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Aspirin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-20	1	0.976	0.983	0.970	0.977	0	TS-20	1	0.034	0.035	0.037	0.038	0	Negative	Non-photoreactive
	2	0.991	0.986	0.987	0.981	-2		2	0.034	0.034	0.037	0.037	0		
	3	0.989	0.991	0.985	0.985	-2		3	0.037	0.034	0.037	0.037	-3		
	Mean	0.985	0.987	0.981	0.981	-1		Mean	0.035	0.034	0.037	0.037	-1		
TS-26	1	1.005	1.000	1.000	0.994	0	TS-26	1	0.034	0.035	0.037	0.037	1	Negative	Non-photoreactive
	2	1.005	1.013	1.004	1.009	-4		2	0.034	0.034	0.036	0.036	0		
	3	1.008	1.017	1.006	1.012	-3		3	0.035	0.034	0.036	0.036	-1		
	Mean	1.006	1.010	1.003	1.005	-2		Mean	0.034	0.034	0.036	0.036	0		
TS-31	1	1.003	1.001	0.993	0.995	4	TS-31	1	0.036	0.035	0.037	0.037	-1	Negative	Non-photoreactive
	2	0.995	0.988	0.990	0.982	-1		2	0.034	0.035	0.036	0.036	0		
	3	1.005	1.015	0.998	1.009	1		3	0.037	0.034	0.038	0.037	-1		
	Mean	1.001	1.001	0.994	0.995	1		Mean	0.036	0.035	0.037	0.037	-1		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	-1				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Benzocaine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-21	1	0.973	0.983	0.969	0.976	-2	TS-21	1	0.037	0.035	0.042	0.038	2	Negative	Non-photoreactive
	2	0.978	0.983	0.971	0.977	1		2	0.039	0.035	0.041	0.038	-1		
	3	0.984	0.983	0.979	0.979	-1		3	0.035	0.034	0.041	0.038	3		
	Mean	0.978	0.983	0.973	0.977	-1		Mean	0.037	0.035	0.041	0.038	1		
TS-27	1	0.998	1.002	0.992	0.997	0	TS-27	1	0.051	0.035	0.038	0.037	-14	Negative	Non-photoreactive
	2	0.995	0.993	0.989	0.986	0		2	0.036	0.035	0.038	0.036	1		
	3	1.003	0.999	0.996	0.993	1		3	0.044	0.035	0.037	0.036	-8		
	Mean	0.999	0.998	0.992	0.992	0		Mean	0.044	0.035	0.038	0.036	-7		
TS-32	1	0.989	0.991	0.981	0.986	6	TS-32	1	0.035	0.034	0.037	0.036	0	Negative	Non-photoreactive
	2	0.995	0.995	0.989	0.990	4		2	0.035	0.034	0.037	0.036	0		
	3	1.000	0.998	0.994	1.003	4		3	0.035	0.034	0.039	0.036	2		
	Mean	0.995	0.995	0.988	0.993	5		Mean	0.035	0.034	0.038	0.036	1		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	-	-2			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Erythromycin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-22	1	0.919	0.996	0.915	0.991	0	TS-22	1	0.035	0.034	0.044	0.039	0	Negative	Non-photoreactive
	2	0.945	0.996	0.935	0.992	6		2	0.035	0.034	0.043	0.038	-1		
	3	0.944	1.005	0.937	1.001	3		3	0.036	0.038	0.044	0.055	-1		
	Mean	0.936	0.999	0.929	0.995	3		Mean	0.035	0.035	0.044	0.044	-1		
TS-28	1	0.925	0.999	0.930	0.994	-9	TS-28	1	0.034	0.034	0.039	0.036	3	Negative	Non-photoreactive
	2	0.960	1.002	0.966	0.999	-10		2	0.035	0.034	0.039	0.036	2		
	3	0.960	1.007	0.967	1.004	-11		3	0.035	0.034	0.040	0.037	3		
	Mean	0.948	1.003	0.954	0.999	-10		Mean	0.035	0.034	0.039	0.036	3		
TS-33	1	0.924	1.007	0.925	1.002	-7	TS-33	1	0.035	0.034	0.039	0.037	1	Negative	Non-photoreactive
	2	0.949	1.010	0.950	1.003	-7		2	0.035	0.035	0.039	0.038	1		
	3	0.951	1.012	0.951	1.007	-6		3	0.036	0.034	0.040	0.036	1		
	Mean	0.941	1.010	0.942	1.004	-7		Mean	0.035	0.034	0.039	0.037	1		
Mean for 3 assays	-	-	-	-	-5	Mean for 3 assays	-	-	-	-	1				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Penicillin G

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-22	1	0.995	0.996	0.988	0.991	3	TS-22	1	0.034	0.034	0.055	0.039	12	Negative	Non-photoreactive
	2	0.996	0.996	0.993	0.992	-1		2	0.035	0.034	0.055	0.038	11		
	3	1.001	1.005	0.995	1.001	2		3	0.035	0.038	0.059	0.055	15		
	Mean	0.997	0.999	0.992	0.995	1		Mean	0.035	0.035	0.056	0.044	13		
TS-28	1	0.988	0.999	0.982	0.994	2	TS-28	1	0.034	0.034	0.044	0.036	8	Negative	Non-photoreactive
	2	1.020	1.002	1.012	0.999	4		2	0.034	0.034	0.044	0.036	8		
	3	1.005	1.007	1.002	1.004	-1		3	0.034	0.034	0.047	0.037	11		
	Mean	1.004	1.003	0.999	0.999	2		Mean	0.034	0.034	0.043	0.036	9		
TS-33	1	1.006	1.007	1.001	1.002	-1	TS-33	1	0.034	0.034	0.043	0.037	6	Negative	Non-photoreactive
	2	1.004	1.010	0.998	1.003	0		2	0.034	0.035	0.042	0.038	5		
	3	1.006	1.012	1.001	1.007	-1		3	0.035	0.034	0.043	0.036	5		
	Mean	1.005	1.010	1.000	1.004	-1		Mean	0.034	0.034	0.043	0.037	5		
Mean for 3 assays		-	-	-	-	1	Mean for 3 assays		-	-	-	-	9		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Phenytoin

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank				
TS-20	1	0.975	0.983	0.968	0.977	1	TS-20	1	0.036	0.035	0.088	0.038	49	Positive	Weakly photoreactive	
	2	0.970	0.986	0.966	0.981	-2		2	0.036	0.034	0.090	0.037				51
	3	0.979	0.991	0.973	0.985	0		3	0.036	0.034	0.097	0.037				58
	Mean	0.975	0.987	0.969	0.981	0		Mean	0.036	0.034	0.092	0.037				53
TS-26	1	0.996	1.000	0.991	0.994	0	TS-26	1	0.036	0.035	0.071	0.037	33	Positive	Weakly photoreactive	
	2	0.995	1.013	0.991	1.009	-1		2	0.036	0.034	0.070	0.036				32
	3	1.001	1.017	1.015	1.012	-19		3	0.036	0.034	0.083	0.036				45
	Mean	0.997	1.010	0.999	1.005	-7		Mean	0.036	0.034	0.075	0.036				37
TS-31	1	0.981	1.001	0.974	0.995	1	TS-31	1	0.036	0.035	0.054	0.037	16	Negative	Non-photoreactive	
	2	1.000	0.988	0.993	0.982	1		2	0.036	0.035	0.054	0.036				16
	3	0.998	1.015	0.991	1.009	1		3	0.036	0.034	0.042	0.037				4
	Mean	0.993	1.001	0.986	0.995	1		Mean	0.036	0.035	0.050	0.037				12
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	-	34				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Bumetrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen Not tested
 Superoxide anion 20 µM

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1	0.061	0.037	0.072	0.046	6		
	2					#DIV/0!	TS-39	2	0.060	0.034	0.071	0.037	6		
	3					#DIV/0!		3	0.062	0.034	0.074	0.037	7		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.061	0.035	0.072	0.040	6	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.062	0.037	0.075	0.049	8		
	2					#DIV/0!	TS-41	2	0.062	0.034	0.076	0.036	9		
	3					#DIV/0!		3	0.062	0.034	0.074	0.036	7		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.062	0.035	0.075	0.040	8	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.051	0.034	0.065	0.036	11		
	2					#DIV/0!	TS-43	2	0.051	0.034	0.064	0.036	10		
	3					#DIV/0!		3	0.058	0.034	0.071	0.039	10		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.053	0.034	0.067	0.037	10	Inconclusive	Inconclusive
	Mean for 3 assays	-	-	-	-	#DIV/0!	Mean for 3 assays	-	-	-	-	8			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Camphor sulfonic acid

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-23	1	0.976	0.983	0.968	0.979	3	TS-23	1	0.035	0.034	0.037	0.037	-1	Negative	Non-photoreactive
	2	0.977	0.978	0.969	0.972	3		2	0.035	0.034	0.037	0.037	-1		
	3	0.985	0.984	0.978	0.980	2		3	0.035	0.034	0.038	0.037	0		
	Mean	0.979	0.982	0.972	0.977	3		Mean	0.035	0.034	0.037	0.037	-1		
TS-29	1	1.009	1.000	1.002	0.995	1	TS-29	1	0.035	0.035	0.036	0.037	-2	Negative	Non-photoreactive
	2	0.999	1.014	0.993	1.007	0		2	0.035	0.034	0.037	0.036	-1		
	3	1.011	1.007	1.002	1.000	3		3	0.035	0.034	0.037	0.037	-1		
	Mean	1.006	1.007	0.999	1.001	1		Mean	0.035	0.034	0.037	0.037	-1		
TS-34	1	0.993	0.994	0.987	0.988	1	TS-34	1	0.035	0.034	0.036	0.037	4	Negative	Non-photoreactive
	2	0.990	0.991	0.986	0.986	-1		2	0.035	0.034	0.036	0.036	4		
	3	0.998	1.001	0.993	0.996	0		3	0.035	0.048	0.036	0.036	4		
	Mean	0.994	0.995	0.989	0.990	0		Mean	0.035	0.039	0.036	0.036	4		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	1				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - (A560(-)) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Chlorhexidine

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-20	1	0.860	0.983	0.848	0.977	6	TS-20	1	0.036	0.035	0.064	0.038	25	Positive	Weakly photoreactive
	2	0.869	0.986	0.863	0.981	0		2	0.036	0.034	0.066	0.037	27		
	3	0.884	0.991	0.871	0.985	7		3	0.038	0.034	0.065	0.037	24		
	Mean	0.871	0.987	0.861	0.981	4		Mean	0.037	0.034	0.065	0.037	25		
TS-26	1	0.867	1.000	0.861	0.994	1	TS-26	1	0.036	0.035	0.063	0.037	25	Positive	Weakly photoreactive
	2	0.909	1.013	0.901	1.009	3		2	0.036	0.034	0.061	0.036	23		
	3	0.909	1.017	0.905	1.012	-1		3	0.036	0.034	0.063	0.036	25		
	Mean	0.895	1.010	0.889	1.005	1		Mean	0.036	0.034	0.062	0.036	24		
TS-31	1	0.865	1.001	0.860	0.995	-1	TS-31	1	0.036	0.035	0.066	0.037	28	Positive	Weakly photoreactive
	2	0.905	0.988	0.902	0.982	-3		2	0.036	0.035	0.065	0.036	27		
	3	0.909	1.015	0.903	1.009	0		3	0.036	0.034	0.067	0.037	29		
	Mean	0.893	1.001	0.888	0.995	-1		Mean	0.036	0.035	0.066	0.037	28		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	26				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Cinnamic acid

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-22	1	0.996	0.996	0.990	0.991	2	TS-22	1	0.034	0.034	0.049	0.039	6	Negative	Non-photoreactive
	2	0.997	0.996	0.992	0.992	1		2	0.035	0.034	0.048	0.038	4		
	3	1.005	1.005	0.997	1.001	4		3	0.035	0.038	0.064	0.055	20		
	Mean	0.999	0.999	0.993	0.995	2		Mean	0.035	0.035	0.054	0.044	10		
TS-28	1	1.002	0.999	0.997	0.994	1	TS-28	1	0.036	0.034	0.041	0.036	3	Negative	Non-photoreactive
	2	0.996	1.002	0.993	0.999	-1		2	0.034	0.034	0.039	0.036	3		
	3	1.007	1.007	1.002	1.004	1		3	0.035	0.034	0.041	0.037	4		
	Mean	1.002	1.003	0.997	0.999	0		Mean	0.035	0.034	0.040	0.036	3		
TS-33	1	1.008	1.007	1.002	1.002	0	TS-33	1	0.036	0.034	0.040	0.037	1	Negative	Non-photoreactive
	2	1.004	1.010	0.995	1.003	3		2	0.035	0.035	0.039	0.038	1		
	3	1.006	1.012	0.999	1.007	1		3	0.037	0.034	0.043	0.036	3		
	Mean	1.006	1.010	0.999	1.004	1		Mean	0.036	0.034	0.041	0.037	2		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	5				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Drometrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation

Test concentration

Singlet oxygen Not tested
 Superoxide anion Not tested

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	1					#DIV/0!		1							
	2					#DIV/0!		2							
	3					#DIV/0!		3							
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	Inconclusive	
	Mean for 3 assays	-	-	-	-	#DIV/0!		Mean for 3 assays	-	-	-	-	#DIV/0!	Undetermined	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : L-Histidine

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-24	1	0.991	0.982	0.987	0.976	-2	TS-24	1	0.035	0.034	0.128	0.038	90	Positive	Photoreactive
	2	0.981	0.988	0.976	0.983	-1		2	0.035	0.034	0.128	0.037	90		
	3	0.998	0.994	0.992	0.988	0		3	0.035	0.034	0.130	0.037	92		
	Mean	0.990	0.988	0.985	0.982	-1		Mean	0.035	0.034	0.129	0.037	91		
TS-30	1	1.006	1.010	1.002	0.997	-5	TS-30	1	0.034	0.034	0.122	0.036	84	Positive	Photoreactive
	2	1.005	1.001	1.000	0.995	-4		2	0.034	0.035	0.122	0.042	84		
	3	1.013	1.009	1.007	1.003	-3		3	0.034	0.034	0.125	0.036	87		
	Mean	1.008	1.007	1.003	0.998	-4		Mean	0.034	0.034	0.123	0.038	85		
TS-35	1	1.006	1.011	1.000	1.012	1	TS-35	1	0.034	0.036	0.123	0.038	84	Positive	Photoreactive
	2	0.991	1.015	0.985	1.007	1		2	0.034	0.035	0.126	0.037	87		
	3	1.021	1.015	1.014	1.009	2		3	0.034	0.034	0.123	0.046	84		
	Mean	1.006	1.014	1.000	1.009	1		Mean	0.034	0.035	0.124	0.040	85		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	-	87			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Methylbenzylidene camphor

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-39	1	0.985	0.990	0.981	0.985	-2	TS-39	1	0.034	0.037	0.037	0.046	-2	Inconclusive	Non-photoreactive
	2	0.988	0.994	0.984	0.984	-2		2	0.034	0.034	0.037	0.037	-2		
	3	0.985	1.005	0.982	1.000	-3		3	0.044	0.034	0.039	0.037	-10		
	Mean	0.986	0.996	0.982	0.990	-2		Mean	0.037	0.035	0.038	0.040	-5		
TS-41	1	0.967	0.961	0.962	0.956	0	TS-41	1	0.038	0.037	0.041	0.049	-2	Inconclusive	Non-photoreactive
	2	0.973	0.961	0.963	0.955	5		2	0.034	0.034	0.037	0.036	-2		
	3	0.971	1.004	0.968	0.998	-2		3	0.034	0.034	0.038	0.036	-1		
	Mean	0.970	0.975	0.964	0.970	1		Mean	0.035	0.035	0.039	0.040	-2		
TS-43	1	0.900	0.904	0.896	0.900	-1	TS-43	1	0.034	0.034	0.038	0.036	1	Inconclusive	Non-photoreactive
	2	0.891	0.910	0.886	0.904	0		2	0.034	0.034	0.036	0.036	-1		
	3	0.910	0.914	0.905	0.909	0		3	0.034	0.034	0.037	0.039	0		
	Mean	0.900	0.909	0.896	0.904	0		Mean	0.034	0.034	0.037	0.037	0		
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	-2				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Octrizole

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-40	1	1.034	0.994	1.047	0.987	-21	TS-45	1	0.052	0.034	0.082	0.036	28	Positive	Weakly photoreactive
	2	1.030	0.997	1.043	0.988	-21		2	0.052	0.034	0.082	0.036	28		
	3	1.033	1.002	1.045	0.996	-20		3	0.052	0.034	0.083	0.036	29		
	Mean	1.032	0.998	1.045	0.990	-21		Mean	0.052	0.034	0.082	0.036	28		
TS-42	1	1.014	0.999	1.031	0.993	-23	TS-42	1	0.063	0.035	0.107	0.037	41	Positive	Weakly photoreactive
	2	1.015	0.999	1.031	0.993	-22		2	0.063	0.034	0.085	0.038	19		
	3	1.007	1.001	1.025	0.995	-24		3	0.063	0.034	0.088	0.036	22		
	Mean	1.012	1.000	1.029	0.994	-23		Mean	0.063	0.034	0.093	0.037	27		
TS-44	1	1.017	0.990	1.048	0.986	-36	TS-44	1	0.060	0.034	0.086	0.037	23	Positive	Weakly photoreactive
	2	1.006	1.003	1.032	0.997	-31		2	0.060	0.034	0.091	0.037	28		
	3	1.017	1.010	1.047	1.004	-35		3	0.060	0.034	0.087	0.037	24		
	Mean	1.013	1.001	1.042	0.996	-34		Mean	0.060	0.034	0.088	0.037	25		
Mean for 3 assays	-	-	-	-	-26	Mean for 3 assays	-	-	-	-	-	27			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Octyl methacrylate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen Not tested
 Superoxide anion 20 µM

Singlet oxygen						Superoxide anion					Judgement				
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1	0.045	0.037	0.045	0.046	-5		
	2					#DIV/0!	TS-39	2	0.045	0.034	0.045	0.037	-5		
	3					#DIV/0!		3	0.075	0.034	0.046	0.037	-34		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.055	0.035	0.045	0.040	-15	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.034	0.037	0.039	0.049	0		
	2					#DIV/0!	TS-41	2	0.034	0.034	0.050	0.036	11		
	3					#DIV/0!		3	0.035	0.034	0.037	0.036	-3		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.034	0.035	0.042	0.040	3	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.035	0.034	0.037	0.036	-1		
	2					#DIV/0!	TS-43	2	0.034	0.034	0.039	0.036	2		
	3					#DIV/0!		3	0.039	0.034	0.037	0.039	-5		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.036	0.034	0.038	0.037	-1	Inconclusive	Inconclusive
	Mean for 3 assays	-	-	-	-	#DIV/0!	Mean for 3 assays	-	-	-	-	-4			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Octyl methoxycinnamate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen Not tested
 Superoxide anion 20 µM

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1	0.034	0.037	0.037	0.046	-2		
	2					#DIV/0!	TS-39	2	0.034	0.034	0.037	0.037	-2		
	3					#DIV/0!		3	0.035	0.034	0.037	0.037	-3		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.034	0.035	0.037	0.040	-2	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.047	0.037	0.048	0.049	-4		
	2					#DIV/0!	TS-41	2	0.047	0.034	0.048	0.036	-4		
	3					#DIV/0!		3	0.048	0.034	0.049	0.036	-4		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.047	0.035	0.048	0.040	-4	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.043	0.034	0.046	0.036	0		
	2					#DIV/0!	TS-43	2	0.043	0.034	0.045	0.036	-1		
	3					#DIV/0!		3	0.043	0.034	0.046	0.039	0		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.043	0.034	0.046	0.037	0	Inconclusive	Inconclusive
	Mean for 3 assays	-	-	-	-	#DIV/0!	Mean for 3 assays	-	-	-	-	-2			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and/or interference such as precipitation or coloration, and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results <25 and Superoxide results <20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : Octyl salicylate

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-40	1	1.000	0.994	0.981	0.987	11	TS-45	1	0.040	0.034	0.045	0.036	3	Inconclusive	Non-photoreactive
	2	1.005	0.997	0.993	0.988	4		2	0.040	0.034	0.045	0.036	3		
	3	1.008	1.002	0.995	0.996	5		3	0.040	0.034	0.046	0.036	4		
	Mean	1.004	0.998	0.990	0.990	7		Mean	0.040	0.034	0.045	0.036	3		
TS-42	1	1.005	0.999	0.996	0.993	3	TS-42	1	0.039	0.035	0.041	0.037	-1	Inconclusive	Non-photoreactive
	2	1.002	0.999	0.992	0.993	4		2	0.040	0.034	0.042	0.038	-1		
	3	1.006	1.001	0.997	0.995	3		3	0.039	0.034	0.041	0.036	-1		
	Mean	1.004	1.000	0.995	0.994	3		Mean	0.039	0.034	0.041	0.037	-1		
TS-44	1	1.003	0.990	0.994	0.986	4	TS-44	1	0.039	0.034	0.040	0.037	-2	Inconclusive	Non-photoreactive
	2	0.999	1.003	0.989	0.997	5		2	0.039	0.034	0.041	0.037	-1		
	3	1.000	1.010	0.992	1.004	3		3	0.040	0.034	0.041	0.037	-2		
	Mean	1.001	1.001	0.992	0.996	4		Mean	0.039	0.034	0.041	0.037	-2		
Mean for 3 assays		-	-	-	-	5	Mean for 3 assays		-	-	-	-	0		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : PABA

Solubility
 Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Singlet oxygen							Superoxide anion							Judgement	
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-22	1	0.998	0.996	0.992	0.991	2	TS-22	1	0.035	0.034	0.037	0.039	-7	Negative	Non-photoreactive
	2	0.997	0.996	0.991	0.992	2		2	0.035	0.034	0.037	0.038	-7		
	3	1.007	1.005	0.997	1.001	6		3	0.035	0.038	0.038	0.055	-6		
	Mean	1.001	0.999	0.993	0.995	3		Mean	0.035	0.035	0.037	0.044	-7		
TS-28	1	0.995	0.999	0.992	0.994	-1	TS-28	1	0.035	0.034	0.037	0.036	0	Negative	Non-photoreactive
	2	1.002	1.002	0.999	0.999	-1		2	0.035	0.034	0.037	0.036	0		
	3	1.009	1.007	1.006	1.004	-1		3	0.035	0.034	0.037	0.037	0		
	Mean	1.002	1.003	0.999	0.999	-1		Mean	0.035	0.034	0.037	0.036	0		
TS-33	1	1.011	1.007	1.004	1.002	1	TS-33	1	0.041	0.034	0.037	0.037	-7	Negative	Non-photoreactive
	2	1.005	1.010	0.999	1.003	0		2	0.034	0.035	0.036	0.038	-1		
	3	1.011	1.012	1.003	1.007	2		3	0.037	0.034	0.038	0.036	-2		
	Mean	1.009	1.010	1.002	1.004	1		Mean	0.037	0.034	0.037	0.037	-3		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	-3				

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : SDS

Solubility

Singlet oxygen 200 µM : Solution 100 µM : Not determined 50 µM : Not determined 20 µM : Not determined
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion Not tested

Singlet oxygen							Superoxide anion						Judgement		
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
TS-24	1	0.961	0.982	0.952	0.976	3	1					#DIV/0!	Inconclusive	Inconclusive	
	2	0.964	0.988	0.955	0.983	3	2					#DIV/0!			
	3	0.969	0.994	0.959	0.988	4	3					#DIV/0!			
	Mean	0.965	0.988	0.955	0.982	3	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!			
TS-30	1	0.982	1.010	0.970	0.997	3	1					#DIV/0!	Inconclusive	Inconclusive	
	2	0.978	1.001	0.967	0.995	2	2					#DIV/0!			
	3	0.986	1.009	0.979	1.003	-2	3					#DIV/0!			
	Mean	0.982	1.007	0.972	0.998	1	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!			
TS-35	1	0.997	1.011	0.985	1.012	7	1					#DIV/0!	Inconclusive	Inconclusive	
	2	1.006	1.015	1.026	1.007	-25	2					#DIV/0!			
	3	0.999	1.015	0.985	1.009	9	3					#DIV/0!			
	Mean	1.001	1.014	0.999	1.009	-3	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!			
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	-	#DIV/0!			

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM
 Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM
 Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 9 Individual data of Phase 2 study

Laboratory : 7
 Chemical Name : UV-571

Solubility

Singlet oxygen 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Precipitation
 Superoxide anion 200 µM : Precipitation 100 µM : Precipitation 50 µM : Precipitation 20 µM : Solution
 Test concentration
 Singlet oxygen Not tested
 Superoxide anion 20 µM

Singlet oxygen						Superoxide anion						Judgement			
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2	Judged by Original Criteria	Fourth data analysis based on the criteria for the proposed protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
	1					#DIV/0!		1	0.050	0.034	0.064	0.036	12		
	2					#DIV/0!	TS-45	2	0.050	0.034	0.064	0.036	12		
	3					#DIV/0!		3	0.050	0.034	0.064	0.036	12		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.050	0.034	0.064	0.036	12	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.056	0.035	0.076	0.037	17		
	2					#DIV/0!	TS-42	2	0.056	0.034	0.075	0.038	16		
	3					#DIV/0!		3	0.055	0.034	0.076	0.036	18		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.056	0.034	0.076	0.037	17	Inconclusive	Inconclusive
	1					#DIV/0!		1	0.055	0.034	0.071	0.037	13		
	2					#DIV/0!	TS-44	2	0.055	0.034	0.071	0.037	13		
	3					#DIV/0!		3	0.055	0.034	0.071	0.037	13		
	Mean	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!		Mean	0.055	0.034	0.071	0.037	13	Inconclusive	Inconclusive
	Mean for 3 assays	-	-	-	-	#DIV/0!	Mean for 3 assays	-	-	-	-	-	14		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 100, 50 or 20 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Fourth data analysis based on the criteria for the proposed protocol:

Photoreactive: Singlet oxygen results ≥ 25 and Superoxide results ≥ 70 , Singlet oxygen results < 25 and/or interference such as precipitation or coloration, and Superoxide results ≥ 70 , Singlet oxygen results ≥ 25 and Superoxide results < 70 and/or interference such as precipitation or coloration, at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

Weakly photoreactive: Singlet oxygen results < 25 and Superoxide results ≥ 20 , < 70 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

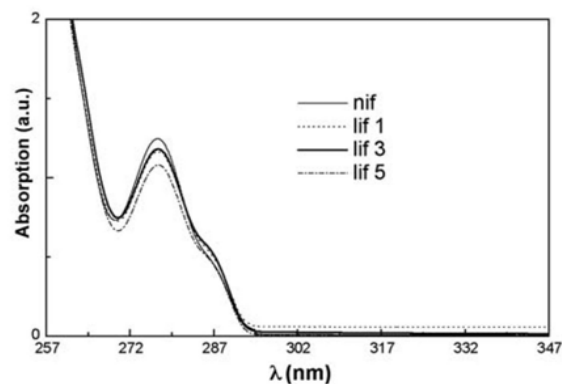
Non-photoreactive: Singlet oxygen results < 25 and Superoxide results < 20 at 200 µM: Twenty µM can be used for judgment when precipitation or coloration is observed at 200 µM.

(Photoreactive), (Weakly photoreactive), (Non-photoreactive) : The results shown in the parenthesis are the results at 50 µM or 100 µM.

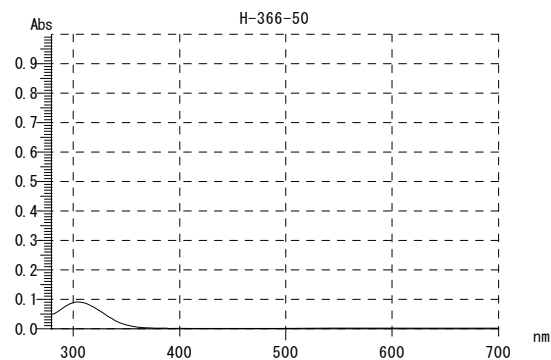
Inconclusive : The results does not meet the photoreactive, weakly photoreactive, or non-photoreactive criterion. Undetermined : Undetermined due to precipitation.

Appendix 10 UV spectrum of test chemicals

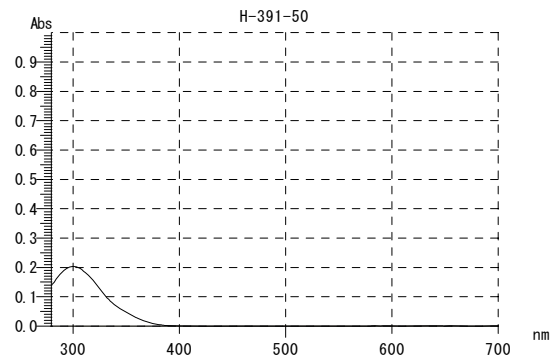
Chemical Name : 5-FU¹⁾
 Solvent : Saline(adjusted to pH8.4)
 Test concentration : 100 μ M



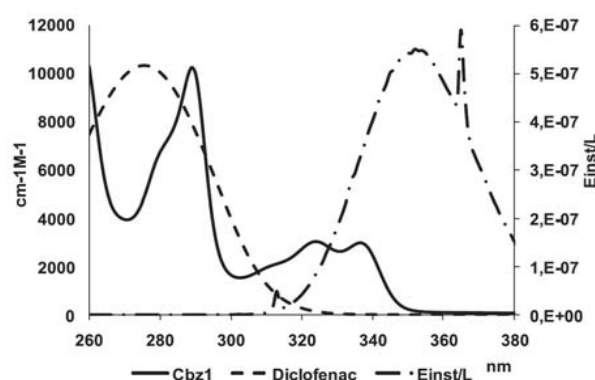
Chemical Name : Chlorpromazine HCl
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



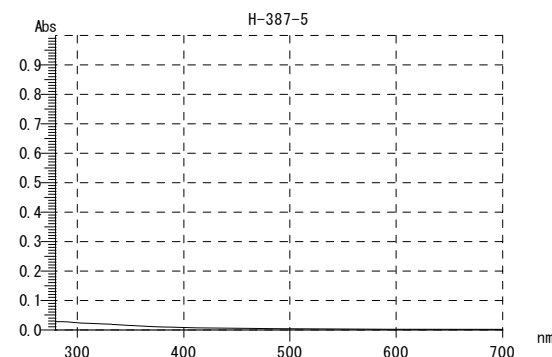
Chemical Name : 8-MOP
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



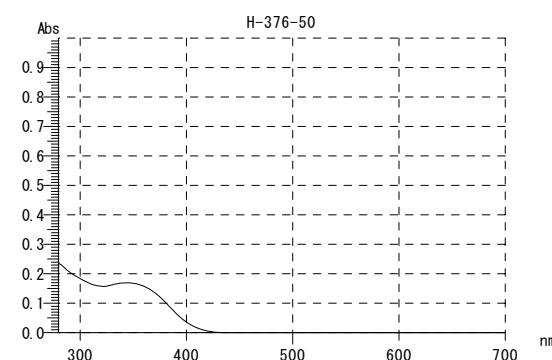
Chemical Name : Diclofenac²⁾
 Solvent : Phosphate buffer(pH8.5)
 Sample Concentration : <5 μ M



Chemical Name : Amiodarone HCl
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 5 μ M



Chemical Name : Doxycycline HCl
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M

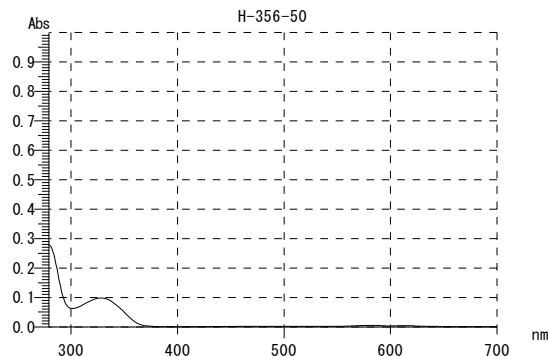


Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

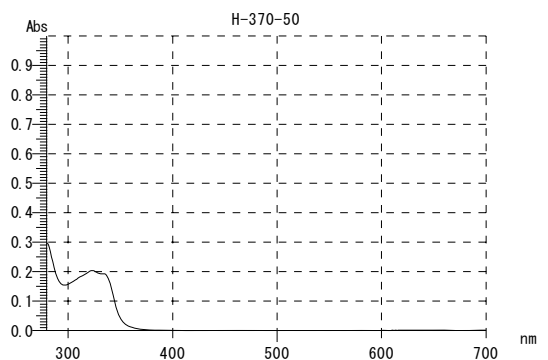
1)UV spectrum of 5-FU(normal line (nif)) was extracted from the article of M. L. Pascu, M. Brezeanu, L. Voicu, A. Staicu, B. Carstocea, R. A. Pascu (2005) *in vivo* , 19, 215-220
 2)UV spectrum of Diclofenac (dashed line) was extracted from the article of J. Eriksson, J. Svanfelt, L. Kronberg (2010) *Photochemistry and Photobiology* , 86, 528-532

Appendix 10 UV spectrum of test chemicals

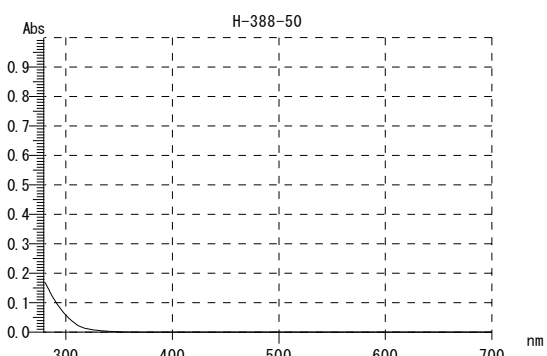
Chemical Name : Furoseimide
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



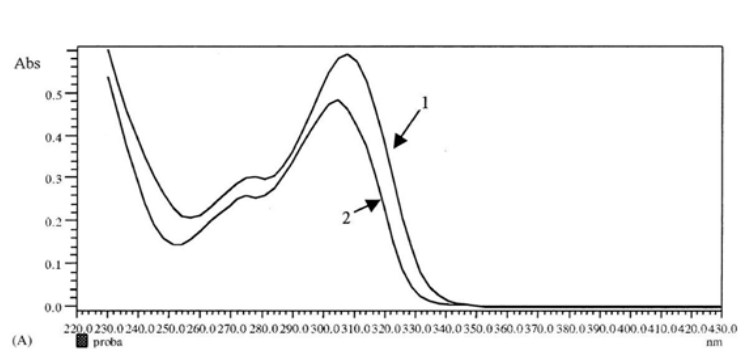
Chemical Name : Norfloxacin
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



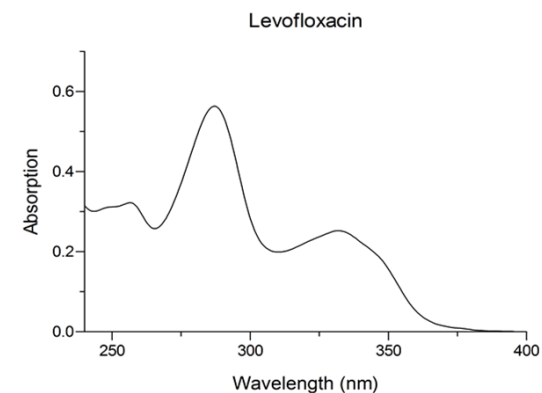
Chemical Name : Ketoprofen
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



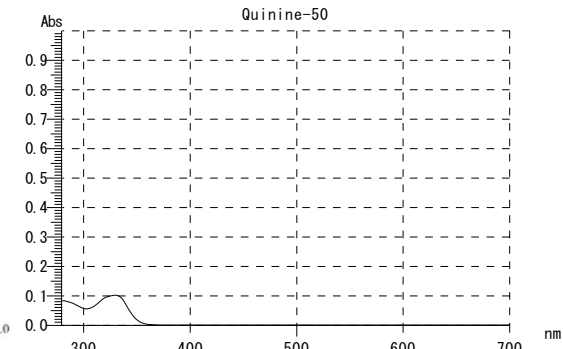
Chemical Name : Omeprazole²⁾
 Solvent : Methanol /NH₃ 4.0% v/v (pH* 9.0)
 Sample Concentration : 8.64 μ g/mL(23.5 μ M)



Chemical Name : Levofloxacin¹⁾
 Solvent : 20 mM NaPB(pH7.4)
 Test concentration : 20 μ M



Chemical Name : Quinine HCl
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



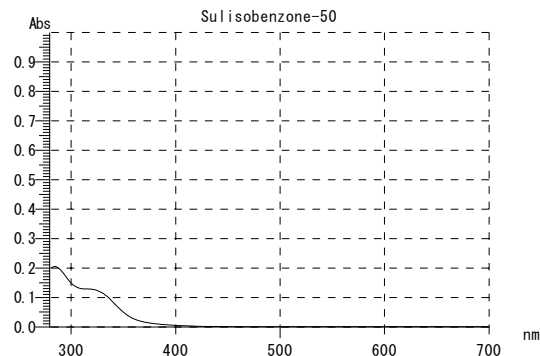
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

1) Test chemical was dissolved in 20 mM NaPB(pH7.4). UV/vis absorption spectra was recorded with a HITACHI U-2010 spectrophotometer (HITACHI, Tokyo, Japan).

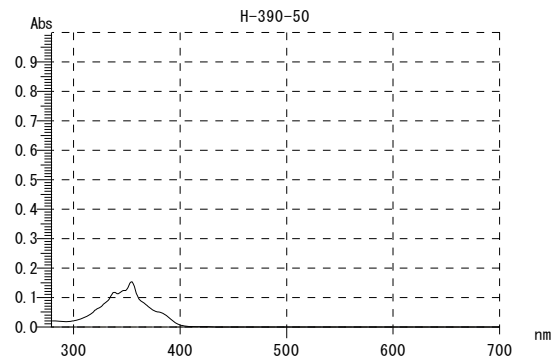
2) Omeprazole (curve 1) was extracted from the article of K. K. Rajic, D. Novovic, V. Mrinkovic, D. Agbaba (2003) *Journal of Pharmaceutical and Biomedical Analysis*, 32, 1019-1027

Appendix 10 UV spectrum of test chemicals

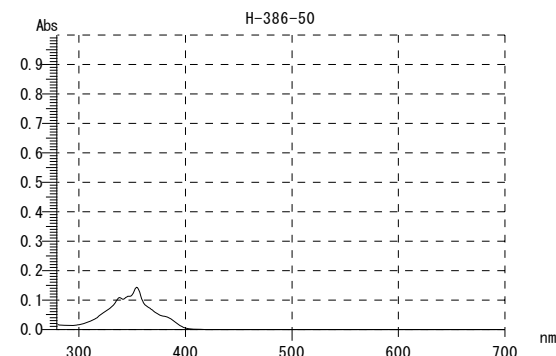
Chemical Name : Sulisobenzone
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



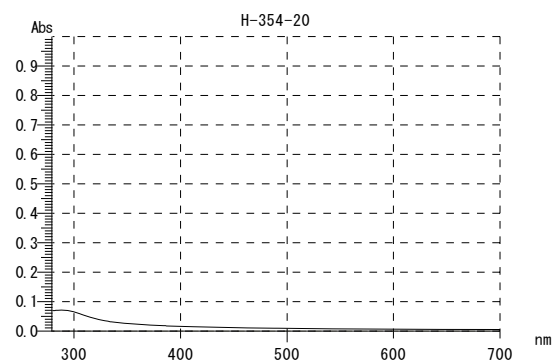
Chemical Name : Acridine
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



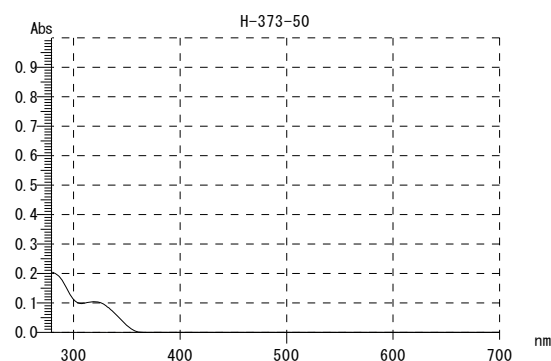
Chemical Name : Acridine HC
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



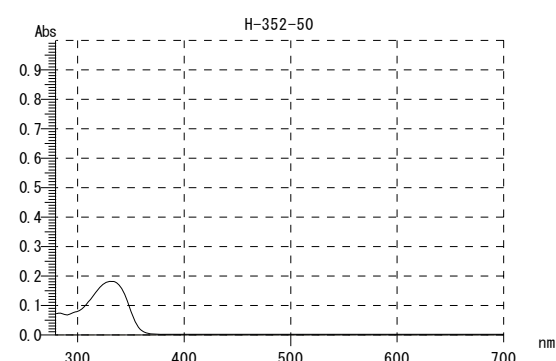
Chemical Name : Fenofibrate
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 20 μ M



Chemical Name : 6-methylcoumarin
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



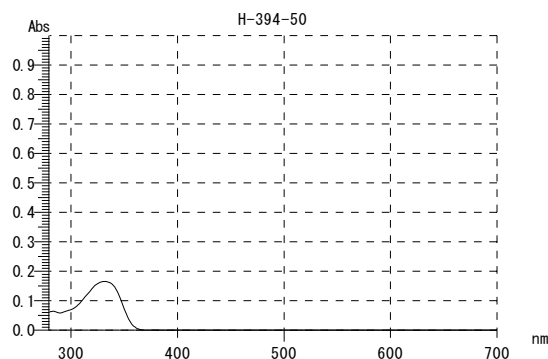
Chemical Name : Nalidixic acid free-acid
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



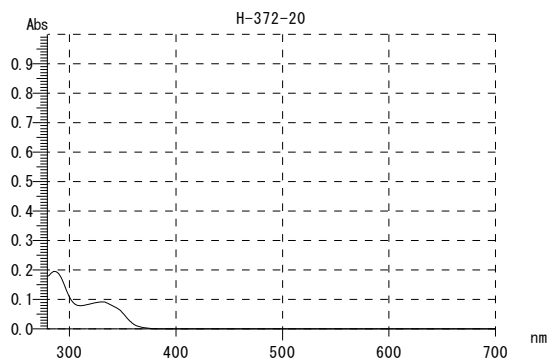
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 10 UV spectrum of test chemicals

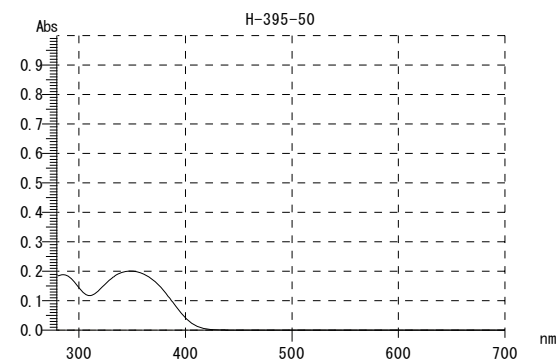
Chemical Name : Nalidixic acid Na
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



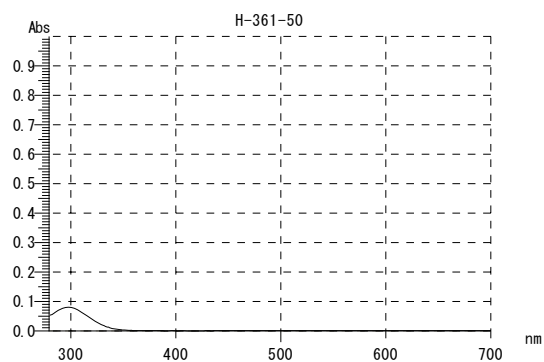
Chemical Name : Ofloxacin
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 20 μ M



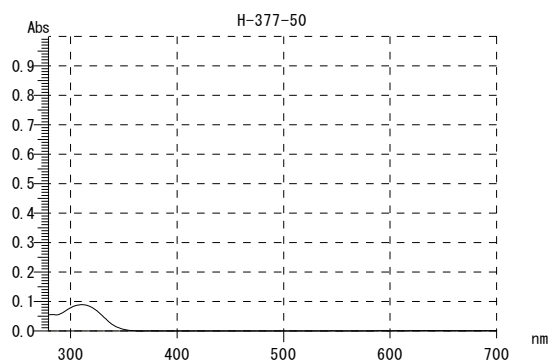
Chemical Name : Piroxicam
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



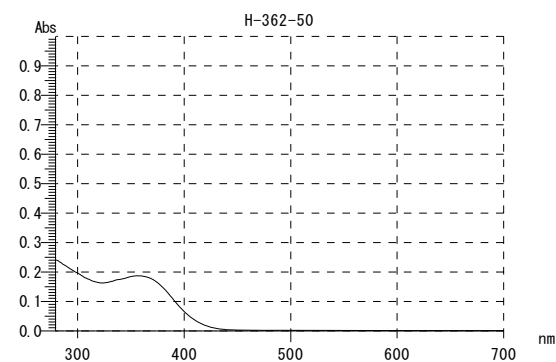
Chemical Name : Promethazine HCl
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



Chemical Name : Rosiglitazone
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



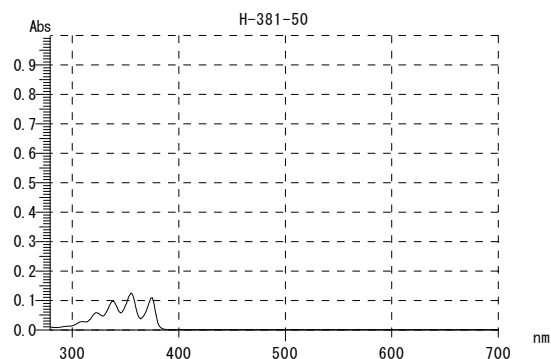
Chemical Name : Tetracycline
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



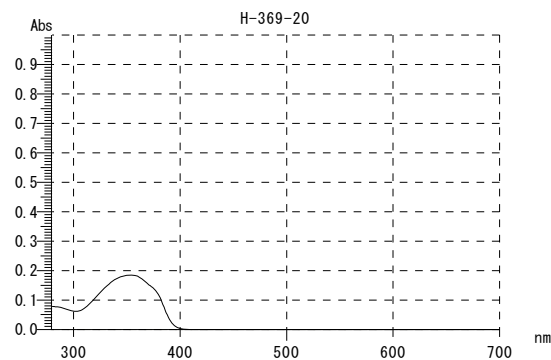
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 10 UV spectrum of test chemicals

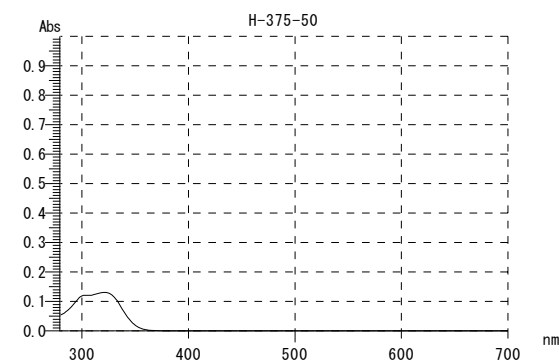
Chemical Name	: Anthracene
Solvent	: Methanol, 0.5% DMSO
Test concentration	: 50 μ M



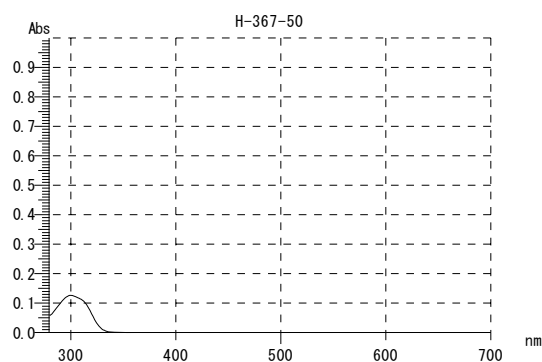
Chemical Name	: Butyl Methoxydibenzoylmethane (Avobenzone)
Solvent	: Methanol, 0.5% DMSO
Test concentration	: 20 μ M



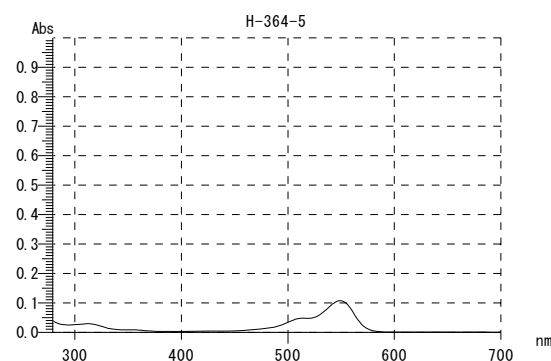
Chemical Name	: Bithionol
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 50 μ M



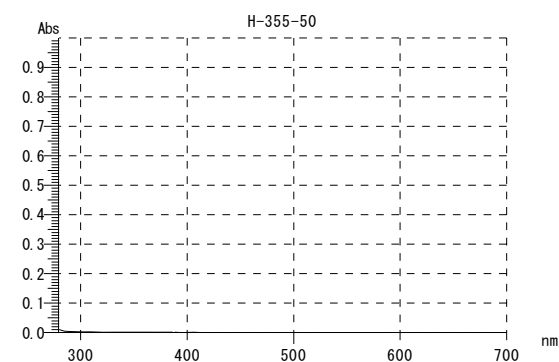
Chemical Name	: Hexachlorophene
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



Chemical Name	: Rose Bengal
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 5 μ M



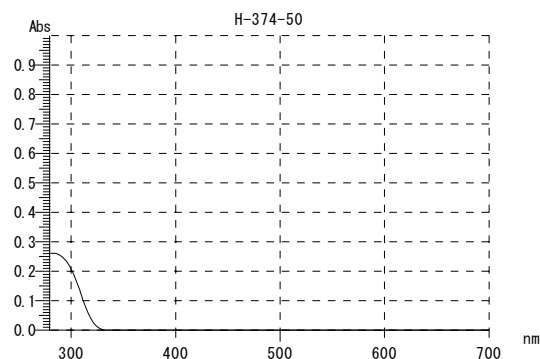
Chemical Name	: Aspirin
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



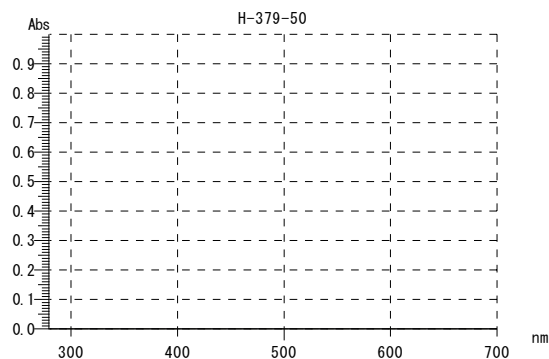
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 10 UV spectrum of test chemicals

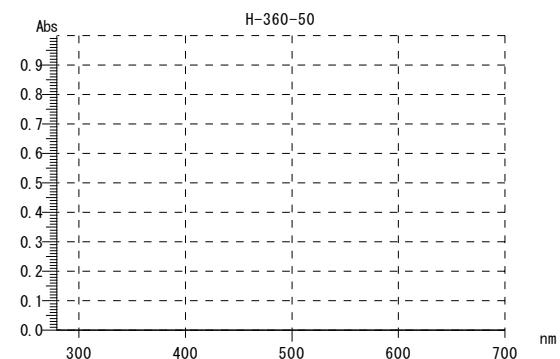
Chemical Name : Benzocaine
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



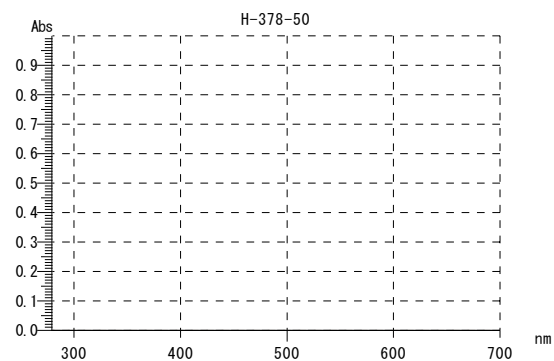
Chemical Name : Erythromycin
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



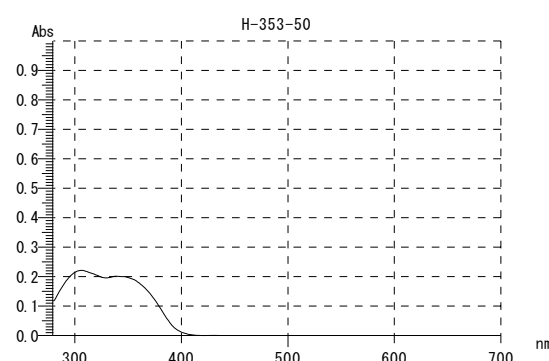
Chemical Name : Phenytoin
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



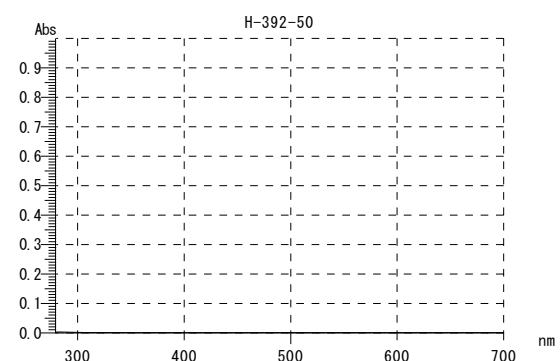
Chemical Name : Penicilin G
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



Chemical Name : 2-tert-Butyl-6-(5-chloro-2H-benzotriazol-2-yl)-4-methylphenol
 Solvent : Methanol, 0.5% DMSO
 Sample Concentration : 50 μ M



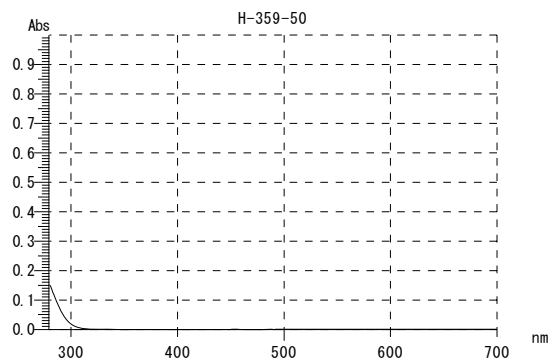
Chemical Name : D(+)-10-Camphorsulfonic acid
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



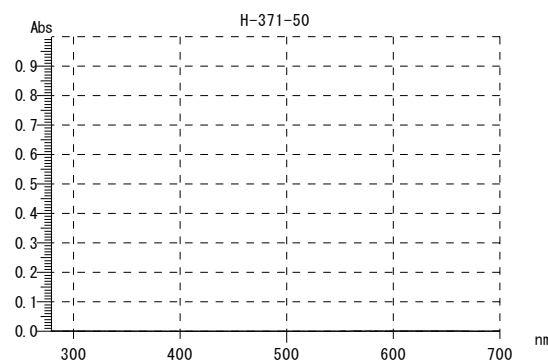
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 10 UV spectrum of test chemicals

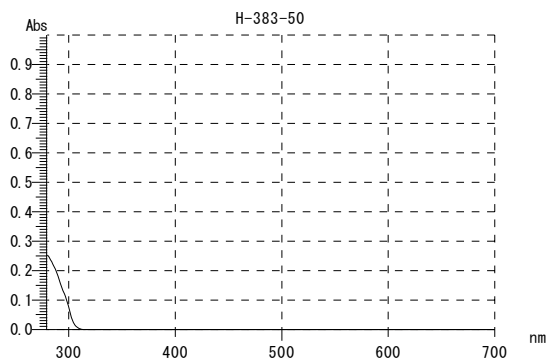
Chemical Name : Chlorhexidine
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



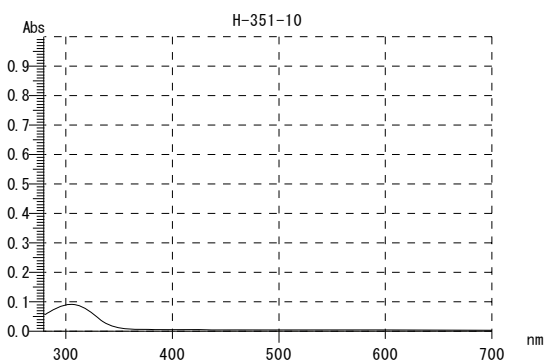
Chemical Name : L-histidine
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



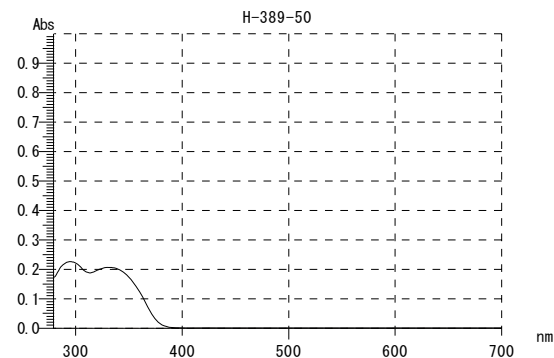
Chemical Name : Cinnamic acid
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



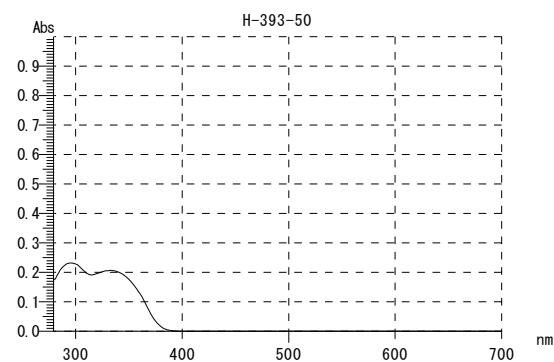
Chemical Name : 4-methyl benzylidene camphor
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 10 μ M



Chemical Name : 2-(2-Hydroxy-5-methylphenyl)benzotriazole
 Solvent : Methanol, 0.5% DMSO
 Test concentration : 50 μ M



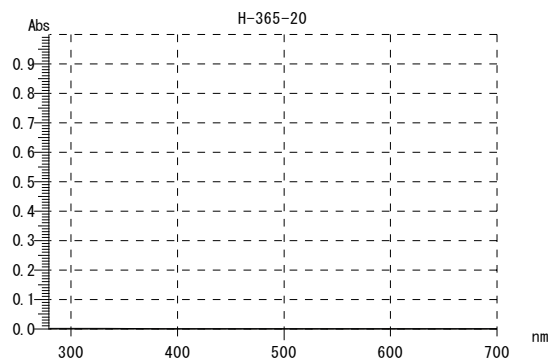
Chemical Name : 2-(2H-Benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol
 Solvent : Methanol, 0.5% DMSO
 Sample Concentration : 50 μ M



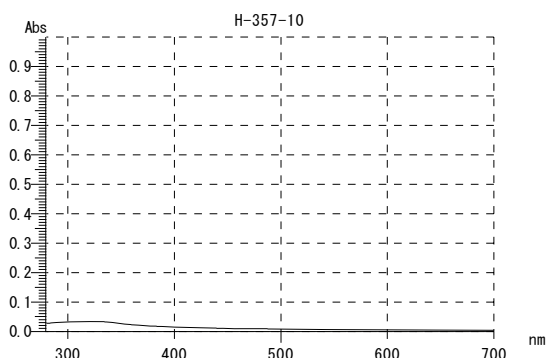
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 10 UV spectrum of test chemicals

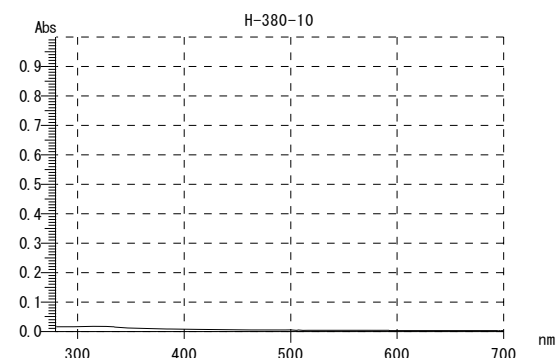
Chemical Name : 2-Ethylhexyl methacrylate
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 20 μ M



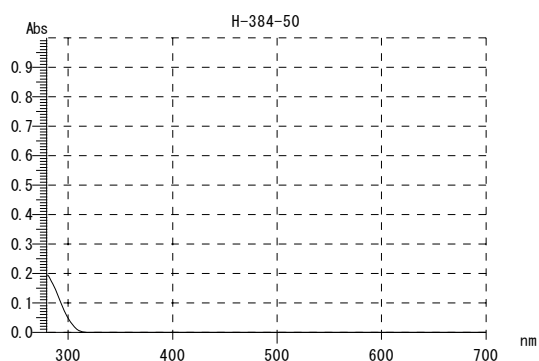
Chemical Name : Octyl 4-methoxycinnamate
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 10 μ M



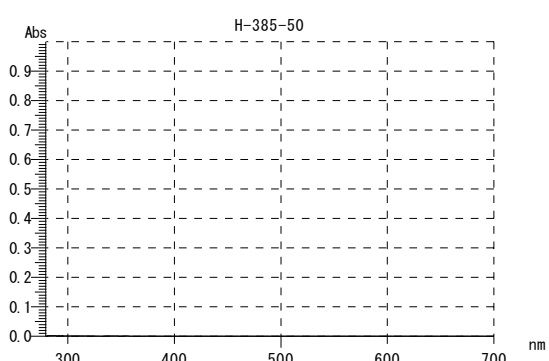
Chemical Name : Octyl salicylate
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 10 μ M



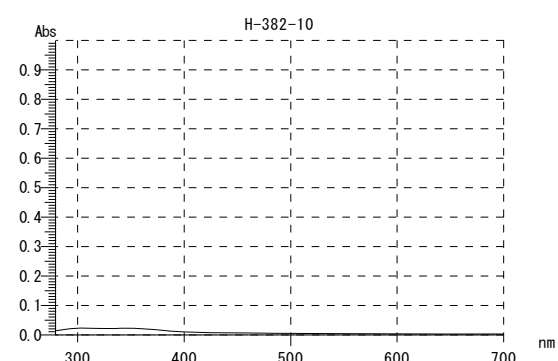
Chemical Name : PABA
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



Chemical Name : SDS
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



Chemical Name : 2-(2H-Benzotriazol-2-yl)-6-dodecyl-4-methylphenol
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 10 μ M



Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 11 Preparation information and appearance of the stock solutions and the reaction mixtures

N0.	Chemicals Name	Lab 4										Lab 5									
		Stock solution					Reaction mixture					Stock solution					Reaction mixture				
		Ist Solvent	Concentration				Concentration ¹⁾				Ist Solvent	Concentration				Concentration ¹⁾					
	10 mM	5 mM	2.5mM	1 mM	200 µM	100 µM	50 µM	20 µM		10 mM	5 mM	2.5mM	1 mM	200 µM	100 µM	50 µM	20 µM				
II - 1	Acridine	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 2	Acridine HCl	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 3	Amiodarone HCl	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.
							SA	Sus.	Sus.	Sus.	Sus.							SA	Sus.	Sus.	Sus.
II - 4	Chlorpromazine HCl	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 5	Doxycycline HCl	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 6	Fenofibrate	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	-
							SA	Sus.	Sus.	Sus.	Sol.							SA	Sus.	Sus.	Sol.
II - 7	Furosemide	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 8	Ketoprofen	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 9	6-methylcoumarine	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 10	8-MOP	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 11	Nalidixic acid	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 12	Nalidixic acid (Na salt)	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 13	Norfloxacin	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 14	Ofloxacin	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 15	Piroxicam	DMSO	Sol.	Sol.	Sol.	-	SO	Sol.	-	-	-	DMSO	Sol.	Sol.	-	-	SO	Sol.	-	-	-
							SA	Sus.	Sus.	Sol.	-							SA	Sus.	Sol.	-
II - 16	Promethazine HCl	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 17	Rosiglitazone	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sol.	-	-	-	DMSO	Sol.	Sol.	Sol.	-	SO	Sol.	-	-	-
							SA	Sus.	Sus.	Sus.	Sol.							SA	Sus.	Sus.	Sol.
II - 18	Tetracycline	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 19	Anthracene	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.
							SA	Sus.	Sus.	Sus.	Sus.							SA	Sus.	Sus.	Sus.
II - 20	Avobenzon	DMSO	Sol.	Sol.	Sol.	-	SO	Sus.	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.
							SA	Sus.	Sus.	Sol.	-							SA	Sus.	Sus.	Sus.
II - 21	Bithionol	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sus.	Sus.	Sus.	Sus.							SA	Sol.	-	-
II - 22	Hexachlorophene	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 23	Rose bengal	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	Sol.	Sol.	Sol.							SA	Sol.	-	-

Appearance; Sol. : Solution, Sus. : Suspension - : Not prepared

SO : Singlet oxygen SA : Superoxide anion DMSO : Dimethyl sulfoxide NaPB : 2% DMSO / 20 mM sodium phosphate buffer

a) For each test chemical, 20 µL of stock solution was added to the reaction mixtures whose final volume was 1000 µL. The stock solution whose concentration was 10 mM were used to prepare 200 µM reaction mixtures.

The stock solutions whose concentrations were 5, 2.5 and 1 mM were used to prepare 100, 50 and 20 µM.

Appendix 11 Preparation information and appearance of the stock solutions and the reaction mixtures (continued)

Chemicals N0. Name		Lab 4										Lab 5																			
		Stock solution					Reaction mixture					Stock solution					Reaction mixture														
		Ist Solvent	Concentration				Concentration ^{a)}					Ist Solvent	Concentration				Concentration ^{a)}														
	10 mM	5 mM	2.5mM	1 mM	200 µM	100 µM	50 µM	20 µM	200 µM	100 µM	50 µM	20 µM	Ist Solvent	10 mM	5 mM	2.5mM	1 mM	200 µM	100 µM	50 µM	20 µM										
II - 24 Aspirin	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 25 Benzocaine	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 26 Erythromycin	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 27 Penicillin G	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 28 Phenytoin	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 29 Bumetrizole	DMSO	Sus.	Sol.	Sol.	Sol.	SO	-	Sus.	Sus.	Sol.	SA	-	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	
						SO	-	-	-	-	SA	-	-	-	-	NaPB	-	-	-	-	SO	-	-	-	-	SA	-	-	-	-	
		NaPB	Sus.	-	-	-	SO	-	-	-	-	SA	-	-	-	-						SO	-	-	-	-	SA	-	-	-	-
II - 30 Camphor sulfonic acid	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 31 Chlorhexidine	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 32 Cinnamic acid	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 33 Drometrizole	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	SA	Sus.	Sus.	Sus.	Sus.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	SA	Sus.	Sus.	Sus.	Sus.	
II - 34 L-Histidine	DMSO	Sus.	Sus.	Sus.	Sus.	SO	-	-	-	-	SA	-	-	-	-	DMSO	Sus.	-	-	-	SO	-	-	-	-	SA	-	-	-	-	
						SO	-	-	-	-	SA	-	-	-	-	NaPB	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	-
		NaPB	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-						SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 35 Methylbenzylidene camphor	DMSO	Sol.	Sol.	Sol.	-	SO	Sus.	Sus.	Sol.	-	SA	Sus.	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	-	SO	Sus.	Sus.	Sol.	-	SA	Sus.	Sus.	Sol.	-	
II - 36 Octrizole	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	
II - 37 Octyl methacrylate	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	-	-	SO	Sus.	Sol.	-	-	SA	Sus.	Sol.	-	-	
II - 38 Octyl methoxycinnamate	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	-	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	
II - 39 Octyl salicylate	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	-	SO	Sus.	Sol.	-	-	SA	Sus.	Sus.	Sol.	-	
II - 40 PABA	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 41 SDS	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	
II - 42 UV-571	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	-	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	

Appearance; Sol. : Solution, Sus. : Suspension - : Not prepared

SO : Singlet oxygen SA : Superoxide anion DMSO : Dimethyl sulfoxide NaPB : 2% DMSO / 20 mM sodium phosphate buffer

a): For each test chemical, 20 µL of stock solution was added to the reaction mixtures whose final volume was 1000 µL. The stock solution whose concentration was 10 mM were used to prepare 200 µM reaction mixtures.

The stock solutions whose concentrations were 5, 2.5 and 1 mM were used to prepare 100, 50 and 20 µM.

Appendix 11 Preparation information and appearance of the stock solutions and the reaction mixtures (continued)

		Lab 6										Lab 7									
N0.	Chemicals Name	Stock solution				Reaction mixture					Stock solution					Reaction mixture					
		1st Solvent	Concentration			Concentration ^{a)}					1st Solvent	Concentration				Concentration ^{a)}					
		10 mM	5 mM	2.5mM	1 mM	200 µM	100 µM	50 µM	20 µM		10 mM	5 mM	2.5mM	1 mM	200 µM	100 µM	50 µM	20 µM			
II - 1	Acridine	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 2	Acridine HCl	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 3	Amiodarone HCl	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.
							SA	Sus.	Sus.	Sus.	Sus.							SA	Sus.	Sus.	Sus.
II - 4	Chlorpromazine HCl	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 5	Doxycycline HCl	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 6	Fenofibrate	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.
							SA	Sus.	Sus.	Sus.	Sol.							SA	Sus.	Sus.	Sus.
II - 7	Furosemide	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 8	Ketoprofen	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 9	6-methylcoumarine	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 10	8-MOP	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 11	Nalidixic acid	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 12	Nalidixic acid (Na salt)	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 13	Norfloxacin	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 14	Ofloxacin	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 15	Piroxicam	DMSO	Sol.	Sol.	-	-	SO	Sol.	-	-	-	DMSO	Sol.	Sol.	Sol.	-	SO	Sol.	-	-	-
							SA	Sus.	Sol.	-	-							SA	Sus.	Sus.	Sol.
II - 16	Promethazine HCl	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 17	Rosiglitazone	DMSO	Sol.	Sol.	Sol.	-	SO	Sol.	-	-	-	DMSO	Sol.	Sol.	Sol.	-	SO	Sol.	-	-	-
							SA	Sus.	Sus.	Sol.	-							SA	Sus.	Sus.	Sol.
II - 18	Tetracycline	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-
II - 19	Anthracene	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.
							SA	Sus.	Sus.	Sus.	Sus.							SA	Sus.	Sus.	Sus.
II - 20	Avobenzene	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.
							SA	Sus.	Sus.	Sus.	Sus.							SA	Sus.	Sus.	Sus.
II - 21	Bithionol	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sus.	Sus.	Sus.
II - 22	Hexachlorophene	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sus.	Sus.	Sus.
II - 23	Rose bengal	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-
							SA	Sol.	-	-	-							SA	Sol.	-	-

Appearance; Sol. : Solution, Sus. : Suspension - : Not prepared

SO : Singlet oxygen SA : Superoxide anion DMSO : Dimethyl sulfoxide NaPB : 2% DMSO / 20 mM sodium phosphate buffer

a) For each test chemical, 20 µL of stock solution was added to the reaction mixtures whose final volume was 1000 µL. The stock solution whose concentration was 10 mM were used to prepare 200 µM reaction mixtures.

The stock solutions whose concentrations were 5, 2.5 and 1 mM were used to prepare 100, 50 and 20 µM.

Appendix 11 Preparation information and appearance of the stock solutions and the reaction mixtures (continued)

Chemicals N0.	Name	Lab 6										Lab 7																			
		Stock solution					Reaction mixture					Stock solution					Reaction mixture														
		Ist Solvent	Concentration			1 mM	Concentration ^{a)}				Ist Solvent	Concentration			1 mM	Concentration ^{a)}															
10 mM	5 mM	2.5mM	1 mM	200 µM	100 µM	50 µM	20 µM	200 µM	100 µM	50 µM	20 µM	Ist Solvent	10 mM	5 mM	2.5mM	1 mM	200 µM	100 µM	50 µM	20 µM											
II - 24 Aspirin		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 25 Benzocaine		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 26 Erythromycin		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 27 Penicillin G		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 28 Phenytoin		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 29 Bumetrizole		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	SA	Sus.	Sus.	Sus.	Sol.
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 30 Camphor sulfonic acid		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 31 Chlorhexidine		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 32 Cinnamic acid		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 33 Drometrizole		DMSO	Sol.	Sol.	Sol.	-	SO	Sus.	Sus.	Sol.	-	SA	Sus.	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	SA	Sus.	Sus.	Sus.	Sus.
		DMSO	Sus.	-	-	-	SO	-	-	-	-	SA	-	-	-	-	DMSO	Sus.	-	-	-	SO	-	-	-	-	SA	-	-	-	-
		NaPB	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	NaPB	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 34 L-Histidine		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		NaPB	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	NaPB	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 35 Methylbenzylidene camphor		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
II - 36 Octrizole		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
II - 37 Octyl methacrylate		DMSO	Sol.	Sol.	Sol.	-	SO	Sus.	Sus.	Sol.	-	SA	Sus.	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sus.	SA	Sus.	Sus.	Sus.	Sol.
		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
II - 38 Octyl methoxycinnamate		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
II - 39 Octyl salicylate		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 40 PABA		DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	-	-	-	SO	Sol.	-	-	-	SA	Sol.	-	-	-
		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sol.	-	-	-	SA	Sol.	-	-	-	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sol.	-	-	-	SA	Sol.	-	-	-
II - 41 SDS		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
II - 42 UV-571		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.
		DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sus.	Sol.	SA	Sus.	Sus.	Sus.	Sol.

Appearance; Sol. : Solution, Sus. : Suspension - : Not prepared

SO : Singlet oxygen SA : Superoxide anion DMSO : Dimethyl sulfoxide NaPB : 2% DMSO / 20 mM sodium phosphate buffer

a): For each test chemical, 20 µL of stock solution was added to the reaction mixtures whose final volume was 1000 µL. The stock solution whose concentration was 10 mM were used to prepare 200 µM reaction mixtures.

The stock solutions whose concentrations were 5, 2.5 and 1 mM were used to prepare 100, 50 and 20 µM.

**INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE
OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING
PHOTOTOXIC POTENTIAL OF CHEMICALS
(Seric Phase 1, VERSION 1.0)**

Issued by: the Validation Management Team (VMT)

Date: 26 May 2011.

1. Purpose of this document

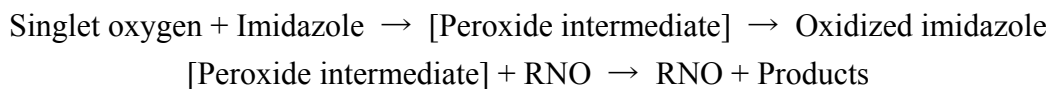
This document is provided to clarify the conduct of an international validation study to evaluate the ability of ROS (reactive oxidative species) assay as a photochemical property study to examine necessity of phototoxicity tests. This document represents the first study protocol developed as a result of the collaboration efforts of the participating testing facilities and the VMT. Each testing facility will develop a study protocol based on the information provided in this document.

2. Background of ROS assay

Drug-induced photoirritation can be defined as an inflammatory reaction of the skin after topical or systemic administration of pharmaceutical substances. Several classes of drugs including antibacterials, thiazide diuretics, non-steroidal anti-inflammatory drugs, quinolones, and tricyclic antidepressants, even though nontoxic by themselves, may become reactive under exposure to environmental light, leading to undesired side effects. The primary event in any photosensitization process is the absorption of photons of the appropriate wavelength, which allows chromophore to reach an excited state. The excitation energy is often transferred to oxygen molecules, followed by generation of ROS: superoxide through type I reaction and singlet oxygen through type II reaction by photo-excited drug molecules. These appear to be the principal intermediate species in the phototoxic response. From the standpoint of risk assessment, previous study demonstrated that determination of ROS from pharmaceutical substances irradiated with UVA/B and Vis would be of help in recognizing their phototoxic potential.

In the ROS assay, generation of singlet oxygen was detected by spectrophotometric measurement of *p*-nitrosodimethyl aniline (RNO) bleaching, followed by decreased absorbance of RNO at 440 nm. Although singlet oxygen does not react chemically

with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the imidazole ring, resulting in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO as follows;



The generation of superoxide could be determined by the reduction of nitroblue tetrazolium (NBT) as indicated below; NBT can be reduced by superoxide anion via a one-electron transfer reaction, yielding partially reduced ($2 e^-$) monoformazan (NBT^+) as a stable intermediate. Thus, superoxide can reduce NBT to NBT^+ , whose formation can be monitored spectrophotometrically at 560 nm.



3. Materials

3.1. Control compounds

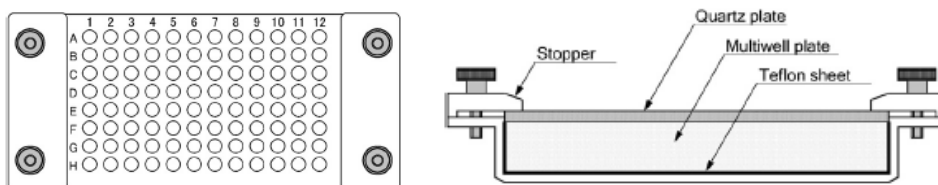
Name	CAS No.	Manufacture	Molecular weight
Quinine monohydrochloride dihydrate (Positive control)	6119-47-7	Sigma (Catalog No. 145920)	396.91
Sulisobenzone (Negative control)	4065-45-6	Tokyo Chemical Industry (Catalog No. H0466)	308.31

3.2. Solvent

Dimethylsulfoxide (DMSO): analytical grade

3.3. Instruments

Instrument	Model
Solar simulator	SXL-2500V2 (SERIC LTD., Tokyo, Japan) equipped with a xenon arc lamp, UV cut filter (<300 nm) and a fan, or their equivalents
UVA detector	UD-36, UD-360 or UD-T36, TOPCON CORPORATION, Tokyo, Japan
Microplate spectrophotometer, equipped 440 and 560 nm filters	Spectra Max M2 (Molecular Devices), Tecan Safire (Tecan), or their equivalents
Quartz reaction container	Made-to-order (Ozawa Sciences, Onoue et al. 2008, see below Figure)



4. Preparations

4.1. Reagents

All reagents should be sonicated and used within 1 month after preparation.

4.1.1. 20 mM Sodium phosphate buffer (NaPB), pH 7.4

Transfer 593 mg of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 5.8 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ to a 1L flask, add 900 mL of purified water, adjust with HCl to a pH of 7.4, dilute with purified water to volume, and mix.

Stored at refrigerator or room temperature.

4.1.2. p-Nitrosodimethylaniline (RNO)

Dissolve 3 mg of RNO in 100 mL of 20 mM NaPB at the concentration of 2×10^{-4} M.

Stored at refrigerator and keep to protect from light

4.1.3. Imidazole

Dissolve 13.6 mg of Imidazole in 10 mL of 20 mM NaPB at the concentration of 2×10^{-2} M

Dilute the 2×10^{-2} M Imidazole solution 100 times with 20 mM NaPB.

Stored at refrigerator and keep to protect from light

4.1.4. Nitroblue tetrazolium chloride (NBT)

Dissolve 32.7 mg of NBT in 100 mL of 20 mM NaPB at the concentration of 4×10^{-4} M.

Stored at refrigerator and keep to protect from light

4.2. Test compounds

Name	Concentration of preparation	Final concentration
Test compounds	1 and 10 mM ¹⁾	20 and 200 μM
Quinine (positive control)	10 mM	200 μM
Sulisobenzone (negative control)	10 mM ²⁾	200 μM
DMSO (blank)		

Notes

- 1) Compound will be weighed in a tube, and added DMSO at the concentration 10 mM as a stock solution. The tube will be mixed with vortex mixer and sonicated for 5 to 10 min under UV-cut illumination or shade. The solutions will be divided to 3 or more new tubes at volume of >50 μL each, kept to protect from light, and stored at freezer (below -20°C). Just before use, the stock solution will be thawed and diluted in DMSO at 1 mM. All preparations should be checked solubility (solution or suspension) with the naked eye.
- 2) Sulisobenzone solution will be prepared at 3.825 mg/mL (net weight) in DMSO because of dried material of 19.4% (lot Z61BE).

5. Methods

A tube (e.g. 1.5 mL of eppendorf tube) and a plastic clear flat bottomed 96-well microplate will be used. The reaction mixture should be prepared under UV-cut illumination or shade.

Experiments will be performed in triplicate wells in 3 independent runs.

【Singlet oxygen】		【Superoxide anion】	
20 mM NaPB	480 μL	20 mM NaPB	855 μL
Imidazole	250 μL	NBT	125 μL
RNO	250 μL	Compound	20 μL
Compound	20 μL		
↓		↓	
Mix (Vortex and Sonication for 5-10 min)		Mix (Vortex and sonication for 5-10 min)	
↓		↓	
Check solubility ¹⁾		Check solubility ¹⁾	
↓		↓	
Add 200 μL of mixture to each well (n=3)		Add 200 μL of mixture to each well (n=3)	
↓		↓	
Pre-read Abs at 440 nm after shaking for 5 sec		Pre-read Abs at 560 nm after shaking for 5 sec	
↓		↓	
Light exposure (ca. 2.3 mW/cm ² for 1 hr at 20-29°C) ²⁾		Light exposure (ca. 2.3 mW/cm ² for 1 hr at 20-29°C) ²⁾	
↓		↓	
Read Abs at 440 nm after shaking for 5 sec		Read Abs 560 nm after shaking for 5 sec	
↓		↓	
Check solubility ¹⁾		Check solubility ¹⁾	

Notes

- 1) The reaction mixture will be checked solubility (solution or suspension) with naked eye before or after light exposure.
- 2) The 96-well plate will be placed in to the Quartz reaction container. The container will be set quarts cover and fasten with bolts. After the final experiment of the day, UVA intensity will be measured using a UVA detector.. An example of 96-well plate configuration is shown as follows;

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
C		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
D		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
E		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
F		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
G		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
H												

B :Blank (DMSO)

P :Positive control (Quinine), 200 µM

N :Negative control (Sulisobenzone), 200 µM

SP1-SP7: test compound No. 1-7, 200 µM or 20 µM

6. Data analysis

Individual data will be presented in a fixed tabular form (excel file) provided form the VMT.

6.1. Singlet oxygen

$$\text{decrease of } A_{440} \times 10^3 = (A_{440}(-) - A_{440}(+) - (A-B)) \times 1000$$

A₄₄₀(-) : Absorbance before light exposure at 440 nm

A₄₄₀(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

6.2. Superoxide anion

$$\text{increase of A560} \times 10^3 = (\text{A560}(+) - \text{A560}(-) - (\text{B}-\text{A})) \times 1000$$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

7. Criteria for data acceptance

The following criteria should be satisfied in each experiment at the present. The final criteria will be decided after validation study.

7-1. Data

Without data lack in positive control, negative control, blank, and test compound.

7-2. OD values

Each net OD value of positive control, negative control, and test compound: 0.02 to 1.1

7-3. Calculation values

Positive control value at 200 μM (mean of 3 wells)

Singlet oxygen: 150 or more

Superoxide anion: 200 or more

Negative control value at 200 μM (mean of 3 wells)

Singlet oxygen: less than 25

Superoxide anion: less than 20

8. Criteria for judgment

The test compound will be judged as a positive response when mean value of 3 wells at 20 and/or 200 μM is over 25 for singlet oxygen and over 20 for superoxide anion. The final criteria will be decided after validation study.

9. Archives and review

The study report and all raw data from this study will be retained according to the SOP in each testing facility. All raw data will be submitted to the VMT for review if required.

10. Reference

S. Onoue, N. Igarashi, S. Yamada, Y. Tsuda, *Journal of Pharmaceutical and Biomedical Analysis*, 46 (2008) 187-193.

**INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE
OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING
PHOTOTOXIC POTENTIAL OF CHEMICALS
(Seric Phase 1-2, VERSION 1.1)**

Issued by: the Validation Management Team (VMT)

Date: 18 October 2011.

1. Purpose of this document

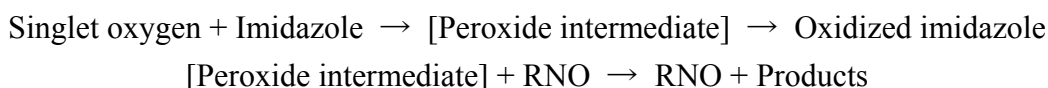
This document is provided to clarify the conduct of an international validation study to evaluate the ability of ROS (reactive oxidative species) assay as a photochemical property study to examine necessity of phototoxicity tests. This document represents the first study protocol developed as a result of the collaboration efforts of the participating testing facilities and the VMT. Each testing facility will develop a study protocol based on the information provided in this document. In the interim results of the phase 1 study, the criteria for data acceptance weren't exceeded in many assays. Therefore, the study conducted under the protocol issued on 26 May, 2011 is defined as phase 1-1 study, additionally phase 1-2 study will be carried out under the irradiance condition that could achieve the criteria for data acceptance. Phase 1-1 and 1-2 study will be stated in this protocol,

2. Background of ROS assay

Drug-induced photoirritation can be defined as an inflammatory reaction of the skin after topical or systemic administration of pharmaceutical substances. Several classes of drugs including antibacterials, thiazide diuretics, non-steroidal anti-inflammatory drugs, quinolones, and tricyclic antidepressants, even though nontoxic by themselves, may become reactive under exposure to environmental light, leading to undesired side effects. The primary event in any photosensitization process is the absorption of photons of the appropriate wavelength, which allows chromophore to reach an excited state. The excitation energy is often transferred to oxygen molecules, followed by generation of ROS: superoxide through type I reaction and singlet oxygen through type II reaction by photo-excited drug molecules. These appear to be the principal intermediate species in the phototoxic response. From the standpoint of risk assessment, previous study demonstrated that determination of ROS from pharmaceutical

substances irradiated with UVA/B and Vis would be of help in recognizing their phototoxic potential.

In the ROS assay, generation of singlet oxygen was detected by spectrophotometric measurement of *p*-nitrosodimethyl aniline (RNO) bleaching, followed by decreased absorbance of RNO at 440 nm. Although singlet oxygen does not react chemically with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the imidazole ring, resulting in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO as follows;



The generation of superoxide could be determined by the reduction of nitroblue tetrazolium (NBT) as indicated below; NBT can be reduced by superoxide anion via a one-electron transfer reaction, yielding partially reduced ($2 e^-$) monoformazan (NBT^+) as a stable intermediate. Thus, superoxide can reduce NBT to NBT^+ , whose formation can be monitored spectrophotometrically at 560 nm.



3. Materials

3.1. Control compounds

Name	CAS No.	Manufacture	Molecular weight
Quinine monohydrochloride dihydrate (Positive control)	6119-47-7	Sigma (Catalog No. 145920)	396.91
Sulisobenzone (Negative control)	4065-45-6	Tokyo Chemical Industry (Catalog No. H0466)	308.31

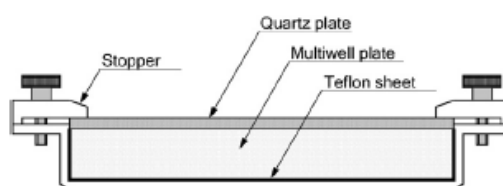
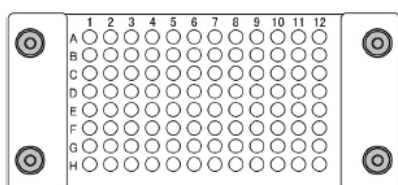
3.2. Solvent

Dimethylsulfoxide (DMSO): analytical grade

3.3. Instruments

Instrument	Model
Solar simulator	SXL-2500V2 (SERIC LTD., Tokyo, Japan) equipped with a xenon arc lamp, UV cut filter (<300 nm) and a fan, or their equivalents
UVA detector	Phase 1-1:

	UD-36, UD-360 or UD-T36, TOPCON CORPORATION, Tokyo, Japan Phase 1-2: the calibrated UVA detector (Dr Hönle 0037, München, Germany)
Microplate spectrophotometer, equipped 440 and 560 nm filters	Spectra Max M2 (Molecular Devices), Tecan Safire (Tecan), or their equivalents
Quartz reaction container	Made-to-order (Ozawa Sciences, Onoue et al. 2008, see below Figure)



4. Preparations

4.1. Reagents

All reagents should be sonicated and used within 1 month after preparation.

4.1.1. 20 mM Sodium phosphate buffer (NaPB), pH 7.4

Transfer 593 mg of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 5.8 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ to a 1L flask, add 900 mL of purified water, adjust with HCl to a pH of 7.4, dilute with purified water to volume, and mix.

Stored at refrigerator or room temperature.

4.1.2. p-Nitrosodimethylaniline (RNO)

Dissolve 3 mg of RNO in 100 mL of 20 mM NaPB at the concentration of 2×10^{-4} M.

Stored at refrigerator and keep to protect from light

4.1.3. Imidazole

Dissolve 13.6 mg of Imidazole in 10 mL of 20 mM NaPB at the concentration of 2×10^{-2} M

Dilute the 2×10^{-2} M Imidazole solution 100 times with 20 mM NaPB.

Stored at refrigerator and keep to protect from light

4.1.4. Nitroblue tetrazolium chloride (NBT)

Dissolve 32.7 mg of NBT in 100 mL of 20 mM NaPB at the concentration of 4×10^{-4} M.

Stored at refrigerator and keep to protect from light

4.2. Test compounds

Name	Concentration of preparation	Final concentration
Test compounds	1 and 10 mM ¹⁾	20 and 200 µM
Quinine (positive control)	10 mM	200 µM
Sulisobenzone (negative control)	10 mM ²⁾	200 µM
DMSO (blank)		

Notes

- 1) Compound will be weighed in a tube, and added DMSO at the concentration 10 mM as a stock solution. The tube will be mixed with vortex mixer and sonicated for 5 to 10 min under UV-cut illumination or shade. The solutions will be divided to 3 or more new tubes at volume of >50 µL each, kept to protect from light, and stored at freezer (below -20°C). Just before use, the stock solution will be thawed and diluted in DMSO at 1 mM. All preparations should be checked solubility (solution or suspension) with the naked eye.
- 2) Sulisobenzone solution will be prepared at 3.825 mg/mL (net weight) in DMSO because of dried material of 19.4% (lot Z6IBE).

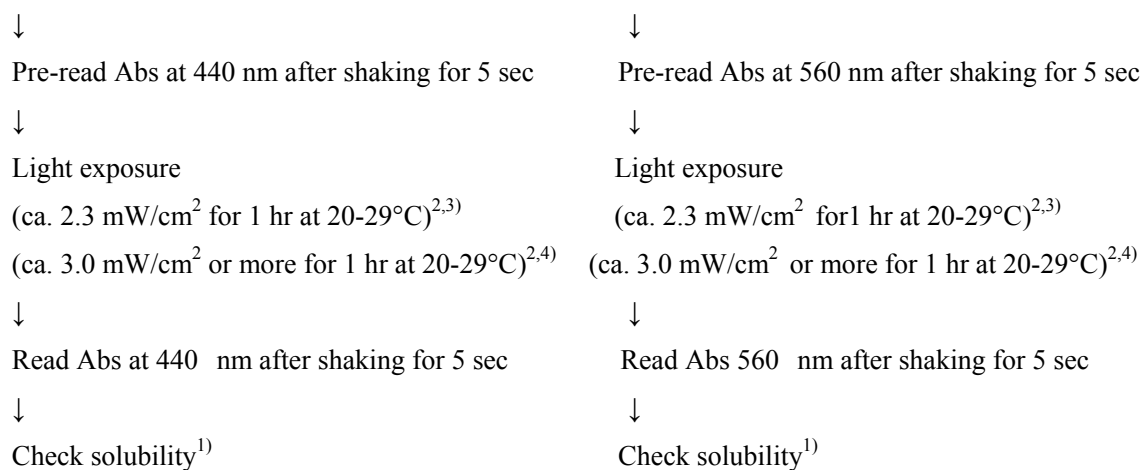
5. Methods

A tube (e.g. 1.5 mL of eppendorf tube) and a plastic clear flat bottomed 96-well microplate will be used. The reaction mixture should be prepared under UV-cut illumination or shade.

Experiments will be performed in triplicate wells in 3 independent runs.

【Singlet oxygen】		【Superoxide anion】	
20 mM NaPB	480 µL	20 mM NaPB	855 µL
Imidazole	250 µL	NBT	125 µL
RNO	250 µL	Compound	20 µL
Compound	20 µL		
↓		↓	
Mix (Vortex and Sonication for 5 – 10 min)		Mix (Vortex and sonication for 5-10 min)	
↓		↓	
Check solubility ¹⁾		Check solubility ¹⁾	
↓		↓	
Add 200 µL of mixture to each well (n=3)		Add 200 µL of mixture to each well (n=3)	

International Validation Study on ROS Assay as a Test Evaluating Phototoxic Potential of Chemicals
Version 1.1



Notes

- 1) The reaction mixture will be checked solubility (solution or suspension) with naked eye before or after light exposure.
- 2) The 96-well plate will be placed in to the Quartz reaction container. The container will be set quarts cover and fasten with bolts. After the final experiment of the day, UVA intensity will be measured using a UVA detector. An example of 96-well plate configuration is shown as follows;

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
C		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
D		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
E		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
F		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
G		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
H												

B :Blank (DMSO)

P :Positive control (Quinine), 200 µM

N :Negative control (Sulisobenzone), 200 µM

SP1-SP7: test compound No. 1-7, 200 µM or 20 µM

- 3) Irradiance condition at phase 1-1 study
- 4) Irradiance condition at phase 1-2 study

6. Data analysis

Individual data will be presented in a fixed tabular form (excel file) provided form the VMT.

6.1. Singlet oxygen

$$\text{decrease of } A_{440} \times 10^3 = (A_{440(-)} - A_{440(+)} - (A - B)) \times 1000$$

A₄₄₀₍₋₎ : Absorbance before light exposure at 440 nm

A₄₄₀₍₊₎ : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

6.2. Superoxide anion

$$\text{increase of A560} \times 10^3 = (\text{A560}(+) - \text{A560}(-) - (\text{B}-\text{A})) \times 1000$$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

7. Criteria for data acceptance

The following criteria should be satisfied in each experiment at the present. The final criteria will be decided after validation study.

7-1. Data

Without data lack in positive control, negative control, blank, and test compound.

7-2. OD values

Each net OD value of positive control, negative control, and test compound: 0.02 to 1.1

7-3. Calculation values

Positive control value at 200 μM (mean of 3 wells)

Singlet oxygen: 150 or more

Superoxide anion: 200 or more

Negative control value at 200 μM (mean of 3 wells)

Singlet oxygen: less than 25

Superoxide anion: less than 20

8. Criteria for judgment

The test compound will be judged as a positive response when mean value of 3 wells at 20 and/or 200 μM is over 25 for singlet oxygen and over 20 for superoxide anion. The final criteria will be decided after validation study.

9. Archives and review

The study report and all raw data from this study will be retained according to the SOP in each testing facility. All raw data will be submitted to the VMT for review if required.

10. Reference

S. Onoue, N. Igarashi, S. Yamada, Y. Tsuda, *Journal of Pharmaceutical and Biomedical Analysis*, 46 (2008) 187-193.

**INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE
OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING
PHOTOTOXIC POTENTIAL OF CHEMICALS
(SERIC VERSION 1.2)**

Issued by: the Validation Management Team (VMT)

Date: 21 May 2012

1. Purpose of this document

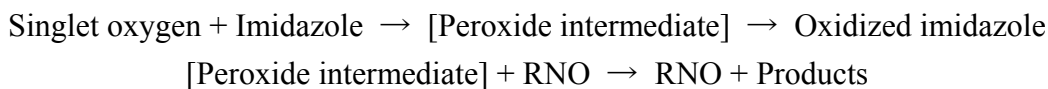
This document is provided to clarify the conduct of an international validation study to evaluate the ability of ROS (reactive oxidative species) assay as a photochemical property study to examine necessity of phototoxicity tests. This document represents the last study protocol developed as a result of the collaboration efforts of the participating testing facilities and the VMT. Each testing facility will develop a study protocol based on the information provided in this document.

2. Background of ROS assay

Drug-induced photoirritation can be defined as an inflammatory reaction of the skin after topical or systemic administration of pharmaceutical substances. Several classes of drugs including antibacterials, thiazide diuretics, non-steroidal anti-inflammatory drugs, quinolones, and tricyclic antidepressants, even though nontoxic by themselves, may become reactive under exposure to environmental light, leading to undesired side effects. The primary event in any photosensitization process is the absorption of photons of the appropriate wavelength, which allows chromophore to reach an excited state. The excitation energy is often transferred to oxygen molecules, followed by generation of ROS: superoxide through type I reaction and singlet oxygen through type II reaction by photo-excited drug molecules. These appear to be the principal intermediate species in the phototoxic response. From the standpoint of risk assessment, previous study demonstrated that determination of ROS from pharmaceutical substances irradiated with UVA/B and visible light would be of help in recognizing their phototoxic potential.

In the ROS assay, generation of singlet oxygen was detected by spectrophotometric measurement of *p*-nitrosodimethyl aniline (RNO) bleaching, followed by decreased absorbance of RNO at 440 nm. Although singlet oxygen does not react chemically

with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the imidazole ring, resulting in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO as follows;



The generation of superoxide could be determined by the reduction of nitroblue tetrazolium (NBT) as indicated below; NBT can be reduced by superoxide anion via a one-electron transfer reaction, yielding partially reduced ($2 e^-$) monoformazan (NBT^+) as a stable intermediate. Thus, superoxide can reduce NBT to NBT^+ , whose formation can be monitored spectrophotometrically at 560 nm.



3. Materials

3.1. Test chemicals

Coded test chemicals and essential information about the test substances (physical state, weight or volume of the test chemicals, specific density for liquids, storage instructions, molecular weight, and conversion factor) will be supplied to each testing facility by the VMT. Safety information of the test chemicals will be provided to an appropriate individual within the organization who is not involved in the study. The test chemicals should be stored according to the VMT instructions until termination of the study. Study personnel can confirm the safety information in the case of emergency. If the safety information is opened, appropriate documentation and justification will need to be provided to the VMT.

3.2. Control chemicals

Positive and negative control chemicals will be supplied to each testing facility by the VMT. Both chemicals will be stored in an air-tight container in a refrigerator and protected from light.

Name	CAS No.	Molecular weight
Quinine monohydrochloride dihydrate (Quinine, positive control)	6119-47-7	396.91
Sulisobenzone (Negative control)	4065-45-6	308.31

3.3. Solvent

Dimethylsulfoxide (DMSO, analytical grade) should be used at first. DMSO should be stored at room temperature. In the case of DMSO-insoluble chemical,

20 mM sodium phosphate buffer (NaPB, see Section 4.1.1.) should be used as a solvent. If a test chemical is insoluble in either DMSO or 20 mM NaPB, it is impossible for the chemical to evaluate in this assay.

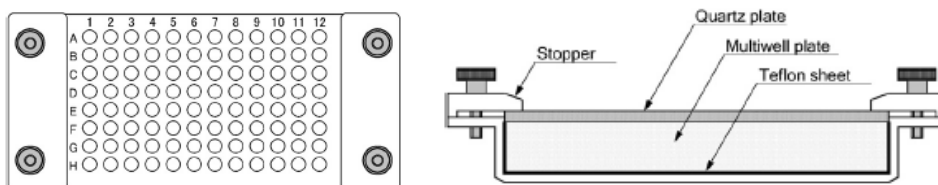
3.4. Reagents

The following reagents will be used and stored according to the instructions of manufacturers.

- $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$: e.g. Wako, catalog No. 192-02815
- $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$: e.g. Wako, catalog No. 194-02831
- p-Nitrosodimethylaniline (RNO): e.g. APOLLO, catalog No. OR30877
- Imidazole: e.g. Wako, catalog No. 099-00013
- Nitroblue tetrazolium chloride (NBT): e.g. Wako, catalog No. 144-01993
- Purified water

3.5. Technical equipments

- Solar simulator: SXL-2500V2 (Seric), equipped with a xenon arc lamp, UV cut filter(<300 nm) and a fan, or their equivalents
- UVA detector (as a calibrator, Dr. Hönle #0037)
- UVA detector for regular use: e.g. Topcon or Dr. Hönle
- Thermometer
- Microplate spectrophotometer, equipped 440 and 560 nm filters: Spectra Max M2 (Molecular Devices), Tecan Safire (Tecan), or equivalents
- Quartz reaction container: Made-to-order (Ozawa Sciences, Onoue et al. 2008, see below Figure), supplied from the VMT.
- Microscope
- Voltex mixer
- Plate shaker
- Sonicator
- Pipetting aid
- Pipettes, 8-channel-pipettes
- Polypropylene tubes
- Plastic 96-well plates (clear, non-treat flat-bottom)
- Plastic- and glassware



4. Preparations

4.1. Reagents

All reagents should be sonicated and used within 1 month after preparation. Representative preparation methods are shown as follows;

4.1.1. 20 mM sodium phosphate buffer (NaPB), pH 7.4

Weigh 593 mg of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 5.8 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, add 900 mL of purified water, adjust with HCl to a pH of 7.4, dilute with purified water up to 1 L, and mix.

Stored at refrigerator or room temperature.

4.1.2. p-Nitrosodimethylaniline (RNO)

Dissolve 3 mg of RNO in 100 mL of 20 mM NaPB at the concentration of 2×10^{-4} M.

Stored at refrigerator and keep to protect from light.

4.1.3. Imidazole

Dissolve 13.6 mg of imidazole in 10 mL of 20 mM NaPB at the concentration of 2×10^{-2} M.

Dilute the 2×10^{-2} M imidazole solution 100 times with 20 mM NaPB.

Stored at refrigerator and keep to protect from light.

4.1.4. Nitroblue tetrazolium chloride (NBT)

Dissolve 32.7 mg of NBT in 100 mL of 20 mM NaPB at the concentration of 4×10^{-4} M.

Stored at refrigerator and keep to protect from light.

4.2. Test chemicals

The test chemicals will be prepared using DMSO just before use.

Each test chemical will be weighed in a tube, and added DMSO at the concentration 10 mM at first. The tube will be mixed with vortex mixer and sonicated for 5 to 10 min under UV-cut illumination or shade. All preparations should be kept to protect from light. The final concentration in a reaction mixture (see section 5.3.) will be set at 200, 100, 50, or 20 μM . When precipitation is observed at 200 μM in the

reaction mixture under a microscope, 5 mM of the test chemical solution should be prepared using DMSO. Furthermore, 2.5 mM of the test chemical solution should be prepared using DMSO when precipitation is observed at 100 μ M in the reaction mixture. One mM of the test chemical solution should be prepared when precipitation is observed at 50 μ M in the reaction mixture.

In the case of DMSO-insoluble chemical, the final concentration in the reaction mixture including 20 μ L of DMSO (see Section 5.3.) will be used at the maximum concentration without precipitation (20, 50, 100, or 200 μ M).

4.3. Positive and negative control chemicals

Stock solutions of quinine and sulisobenzone will be prepared at 10 mM each in DMSO (the final concentration of 200 μ M) according to the procedure of section 4.2., divided into some tubes, and stored in a freezer (generally below -20°C) for up to 1 month. The stock solution will be thawed just before the experiment and used within the day.

5. Methods

5.1. Calibration of the UVA irradiance

The UVA irradiance should be calibrated as described below prior to the study.

UVA intensity should be set at an appropriate value to satisfy the criteria for data acceptance (see Section 7.). The solar simulator and the fan (or its equivalent) will be turned on the power supply. UVA intensity on the plate position will be measured under a stable condition using a UVA detector of the testing facility and the calibrated UVA detector (Dr. Hönle #0037) delivered by the VMT. The measurement will be performed at some different intensity using the two UVA detectors. Correlation curve on the values of the UVA detectors will be confirmed.

5.2. Calibration of the solar simulator

On the day of assays, an appropriate value (3.0 mW/cm^2 or more) of UVA intensity for the calibrated UVA detector will be set. Acceptable temperature range after light exposure is 20-29 $^{\circ}\text{C}$.

5.3. Test procedure

A tube (e.g. 1.5 mL of eppendorf tube) and a plastic clear flat bottomed 96-well microplate will be used. The reaction mixture should be prepared under UV-cut illumination or shade. DMSO will be used in a blank.

Experiments will be performed in triplicate wells in three independent runs. As the final concentrations, 200 μM of test chemical solutions will be used generally. When precipitation is observed at 200 μM , additional experiments should be performed at 100 μM . Further experiments should be performed at 50 μM when precipitation is observed at 100 μM . When precipitation is observed at 50 μM in the reaction mixture, further experiment should be performed at 20 μM . When precipitation is observed at 20 μM in the reaction mixture, further experiment is not needed. When questionable data (e.g. technical error) is obtained, each testing facility can perform an additional experiment using the questionable chemical(s) and the positive/negative chemicals. If the values of the positive/negative chemicals would not be met the criteria (see section 7), the additional experiment using the all chemicals of the plate should be needed. The reason of the additional experiment should be described in the raw data. The adoption of the triplicate data will be decided by the VMT.

【Singlet oxygen】		【Superoxide anion】	
20 mM NaPB	480 μL	20 mM NaPB	855 μL
Imidazole	250 μL	NBT	125 μL
RNO	250 μL	Chemical	20 μL
Chemical	20 μL		
↓		↓	
Mix (Vortex and Sonication for 5 – 10 min)		Mix (Vortex and sonication for 5-10 min)	
↓		↓	
Add 200 μL of mixture to each well (n=3) ¹⁾		Add 200 μL of mixture to each well (n=3) ¹⁾	
↓		↓	
Check solubility ²⁾		Check solubility ²⁾	
↓		↓	
Pre-read Abs at 440 nm after shaking for 5 sec		Pre-read Abs at 560 nm after shaking for 5 sec	
↓		↓	
Light exposure for 1 hr ³⁾		Light exposure for 1 hr ³⁾	
↓		↓	
Read Abs at 440 nm after shaking for 1 min		Read Abs 560 nm after shaking for 1 min	

Notes

1) An example of 96-well plate configuration is shown as follows;

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
C		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
D		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
E		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
F		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
G		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
H												

B: Blank (DMSO)

P: Positive control (Quinine), 200 µM

N: Negative control (Sulisobenzone), 200 µM

SP1-SP7: test chemical No. 1-7

B2-B11, C2-C11, and D2-D11 wells: singlet oxygen

E2-E11, F2-F11, and G2-G11 wells: superoxide anion

- The reaction mixture will be checked solubility (solution or suspension) using a microscope (×100) before light exposure.
- The 96-well plate will be placed in to the Quartz reaction container. The container will be set quarts cover and fasten with bolts. The solar simulator and the temperature control unit (or its equivalent) will be use under a stable condition. After the experiment, UVA intensity and temperature on the plate position will be measured using the UVA detector of the testing facility and thermometer. Acceptable ranges of temperature and UVA intensity after light exposure are shown in section 5.1.

6. Data analysis

Individual data will be presented in a fixed tabular form (excel file) provided by the VMT. The data of 3 wells in each chemical will be calculated as mean and standard division in each experiment.

6.1. Singlet oxygen

$$\text{decrease of A440} \times 10^3 = (\text{A440(-)} - \text{A440(+)} - (\text{A-B})) \times 1000$$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

6.2. Superoxide anion

$$\text{increase of A560} \times 10^3 = (\text{A560}(+) - \text{A560}(-) - (\text{B}-\text{A})) \times 1000$$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

7. Criteria for data acceptance

The following criteria should be satisfied in each experiment at the present. The criteria will be decided after validation study.

7.1. Precipitation

Without precipitation of test chemical in the reaction mixture before light exposure.

7.2. Data

Without data lack in positive control, negative control, blank, and test chemical.

7.3. OD values

Each net OD value of positive control, negative control, and test chemical: 0.02 to 1.5.

7.4. Calculation values

Positive control value at 200 μM (mean of 3 wells)

Singlet oxygen: 150 or more

Superoxide anion: 200 or more

Negative control value at 200 μM (mean of 3 wells)

Singlet oxygen: less than 25

Superoxide anion: less than 20

8. Criteria for judgment

The final criteria including obligatory endpoint(s) for ROS assay, singlet oxygen and/or superoxide, will be decided after validation study. At present, each test chemical will be judged in each experiment as follows;

Positive (mean of 3 wells)

Singlet oxygen: 25 or more at 200, 100, 50 or 20 μM , or

Superoxide anion: 20 or more at 200, 100, 50 or 20 μM

Negative (mean of 3 wells)

Singlet oxygen: less than 25 at 200 μM and

Superoxide anion: less than 20 at 200 μM

Inconclusive (mean of 3 wells)

The results does not meet the positive or negative criterion.

The final judgment will be estimated on the results of triplicate experiments in each testing facility as follows;

Positive (mean of 3 assays)

Singlet oxygen: 25 or more at 200, 100, 50 or 20 μM , or

Superoxide anion: 20 or more at 200, 100, 50 or 20 μM

Negative (mean of 3 assays)

Singlet oxygen: less than 25 at 200 μM and

Superoxide anion: less than 20 at 200 μM and

Inconclusive (mean of 3 wells)

The results does not meet the positive or negative criterion.

9. Archives and review

The study report and all raw data from this study will be retained according to the SOP in each testing facility. All raw data (pdf files) and the results (excel files) will be submitted to the VMT for review.

10. Reference

S. Onoue, N. Igarashi, S. Yamada, Y. Tsuda, *Journal of Pharmaceutical and Biomedical Analysis*, 46 (2008) 187-193.

Appendix 1: Amendment of Protocol

1. Version 1.1

Date: 28 February 2012

Reason for amendment

To evaluate some chemicals which were precipitated into the reaction mixture at 50 μM , the VMT decided to add a concentration (20 μM) in accordance with the protocol of Atlas version 3.01.

Version 1.0

Items: 4.2. Test chemicals (Page 4-5)

The final concentration in a reaction mixture (see section 5.3.) will be set at 200, 100, or 50 μM . When precipitation is observed at 200 μM in the reaction mixture under a microscope, 5 mM of the test chemical solution should be prepared using DMSO. Furthermore, 2.5 mM of the test chemical solution should be prepared using DMSO when precipitation is observed at 100 μM in the reaction mixture.

In the case of DMSO-insoluble chemical, the final concentration in the reaction mixture including 20 μL of DMSO (see Section 5.3.) will be used at the maximum concentration without precipitation (50, 100, or 200 μM).

Items: 5.3. Test procedure (Page 6)

When precipitation is observed at 50 μM in the reaction mixture, further experiment is not needed.

Version 1.1

Items: 4.2. Test chemicals (Page 4-5)

The final concentration in a reaction mixture (see section 5.3.) will be set at 200, 100, 50, or 20 μM . When precipitation is observed at 200 μM in the reaction mixture under a microscope, 5 mM of the test chemical solution should be prepared using DMSO. Furthermore, 2.5 mM of the test chemical solution should be prepared using DMSO when precipitation is observed at 100 μM in the reaction mixture. One mM of the test chemical solution should be prepared when precipitation is observed at 50 μM in the reaction mixture.

In the case of DMSO-insoluble chemical, the final concentration in the reaction mixture including 20 μL of DMSO (see Section 5.3.) will be used at

the maximum concentration without precipitation (20, 50, 100, or 200 μ M).

Items: 5.3. Test procedure (Page 6)

When precipitation is observed at 50 μ M in the reaction mixture, further experiment should be performed at 20 μ M. When precipitation is observed at 20 μ M in the reaction mixture, further experiment is not needed.

Version 1.2

Items: 8. Criteria for judgment (Page 8-9)

Positive (mean of 3 wells)

Singlet oxygen: 25 or more at 200, 100, 50 or 20 μ M, or

Superoxide anion: 20 or more at 200, 100, 50 or 20 μ M

Negative (mean of 3 wells)

Singlet oxygen: less than 25 at 200 μ M and

Superoxide anion: less than 20 at 200 μ M

Inconclusive (mean of 3 wells)

The results does not meet the positive or negative criterion.

The final judgment will be estimated on the results of triplicate experiments in each testing facility as follows;

Positive (mean of 3 assays)

Singlet oxygen: 25 or more at 200, 100, 50 or 20 μ M, or

Superoxide anion: 20 or more at 200, 100, 50 or 20 μ M

Negative (mean of 3 assays)

Singlet oxygen: less than 25 at 200 μ M and

Superoxide anion: less than 20 at 200 μ M and

Inconclusive (mean of 3 wells)

The results does not meet the positive or negative criterion.

Reason: Information on the test concentration was added to the positive and negative criterion. The negative results should be judged from the results at 200 μ M only. The positive results can be judged from the results at any concentration tested. And, when the test chemical is not judged either "Positive" or "Negative" from the assay results, the assay results should be judged as "Inconclusive". For the final judgment, the same criterion for the 3 assay results should be adopted.