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# Validation report for the international validation study on ROS (Reactive Oxygen Species) assay as a test evaluating phototoxic potential of chemicals (Atlas Suntest 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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Attachment 1 Protocol for Phase 1 study
INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING PHOTOTOXIC POTENTIAL OF CHEMICALS (VERSION 1.0)

Attachment 2 Protocol for Phase 2 study
INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING PHOTOTOXIC POTENTIAL OF CHEMICALS (ATLAS VERSION 3.02)

## 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 general conceptual framework [Hartung et al., 2004; OECD, 2005] was used for documenting the entire validation of a test method, which is called a modular approach to validation. 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.

## 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 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 1 hour. After agitation on a plate shaker, UV absorbance at 440 nm was measured. Superoxide was measured by irradiating samples containing the test chemical and nitroblue tetrazolium (NBT) with simulated sunlight for 1 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

An Atlas Suntest CPS series (CPS plus or CPS; Atlas Material Technology LLC, Chicago, IL, USA) equipped with a 1500-W xenon arc lamp was used for solar simulator. The irradiation test was carried out at 25°C with an irradiance of ca. 2.0 mW/cm$^2$ as determined using a calibrated UVA detector (Dr Hönle 0037, München, Germany).

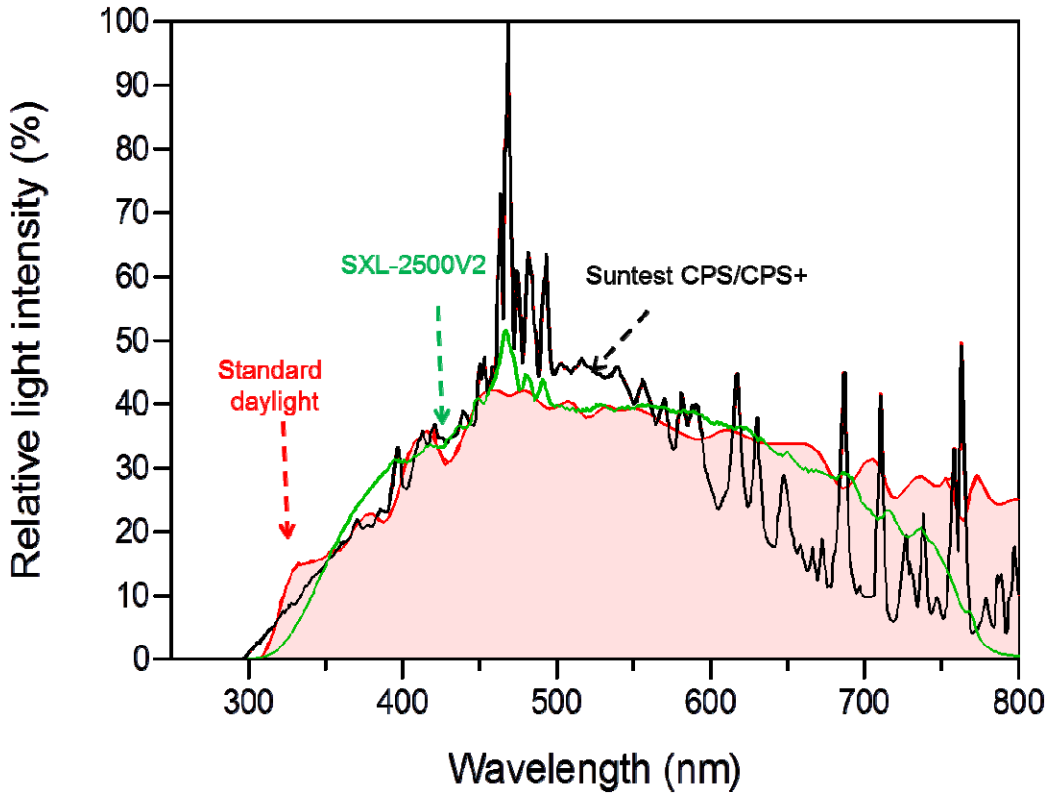


Figure 1 Spectral patterns of the light sources and standard daylight

## 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
Kazuichi Nakamura; Shionogi & Co., Ltd.
Tsuguto Toda; Shionogi & Co., Ltd.
Yasuhiro Matsumoto; ASKA Pharmaceutical Co., Ltd.
Manfred Liebsch; German Centre for the Documentation and Validation of Alternative Methods (ZEBET)
Hironori Takagi; Taisho Pharmaceutical Co., Ltd.
Naoto Osaki; Taisho Pharmaceutical Co., Ltd.
Satoru Kawakami; Asahi Kasei Pharma Co.
Valerie Zang; ECVAM
Warren Casey; ICCVAM

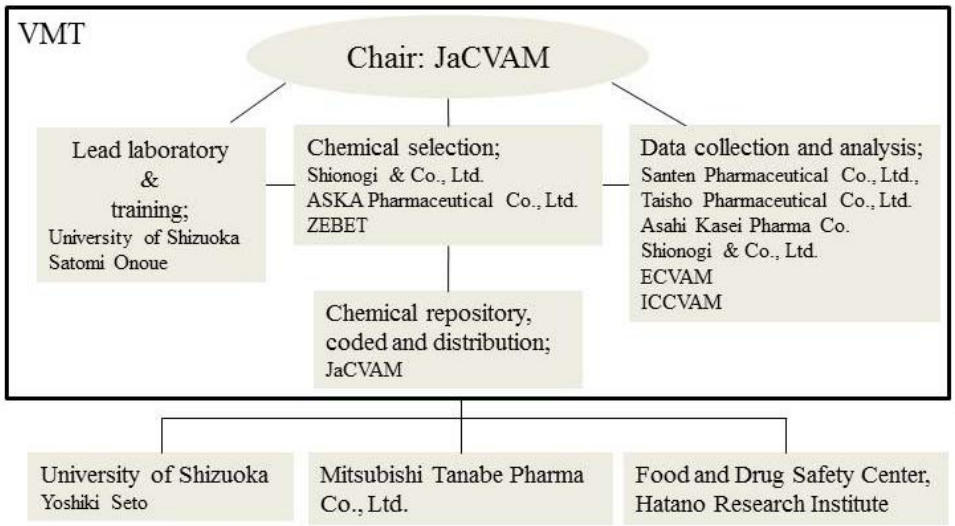


Figure 2 Management structure of the ROS assay validation study

## 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.
Naoto Osaki; Taisho Pharmaceutical Co., Ltd.
Satoru Kawakami; Asahi Kasei Pharma Co.
Kazuichi Nakamura; Shionogi & Co., Ltd.
Hironori Takagi; Taisho Pharmaceutical Co., Ltd.
Valerie Zang; ECVAM
Warren Casey; ICCVAM

### 4-4. Participating laboratory

The laboratories participating in the study were defined as shown in Figure 1.
The following three laboratories participated in the validation study for the evaluation of the ROS assays:
Laboratory 1: University of Shizuoka (Yoshiki Seto)
Laboratory 2: Food and Drug Safety Center, Hatano Research Institute (Shinobu Wakuri)
Laboratory 3: Mitsubishi Tanabe Pharma Co. (Toshinobu Yamamoto)

Although both the lead laboratory (Satomi Onoue) and Laboratory 1 (Yoshiki Seto) are located at the University of Shizuoka, Laboratory 1 participated in this validation study independently of the lead laboratory.
Each laboratory also was responsible for complying with GLP and specifying QA aspects.

## 5. Study Design

Before validation studies, a training course using Atlas Suntest CPS series 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, 8-methoxy psoralen, amiodarone, chlorpromazine, diclofenac, doxycycline, furosemide, ketoprofen, levofloxacin, norfloxacin, and omeprazole), the positive control, and the negative control (Table 1). These tests were conducted between April and May 2011 at three laboratories.
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 August and October 2011 at the three laboratories which had participated in both the training course and the Phase 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 it inappropriate to include rosiglitazone in the phototoxic chemical set, due to 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 these chemicals but could not find reliable human phototoxicity data.

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 11 October, 2011, 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 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 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 | Sulizobenzone 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 | *in vivo* | |
|---|---|---|---|---|---|---|---|---|
| | | | MEC (L/mol/cm) | λmax (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) λmax (nm) was a wavelength at which the UV/vis absorbance shows a peak between 290 and 700 nm. λmax (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].<br>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].<br>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].<br>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].<br>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 benzylindene 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 | Sulizobenzone 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) | λmax (nm) | | Animal | Human | 1 | 2 | 3 |
| **Phototoxic drugs** | | | | | | | | | | |
| II-1 | Acridine | 260-94-6 | 2773 | 354 | + [1)] | + [1)] | + [1)] | C-130 | B-090 | A-005 |
| II-2 | Acridine HCl | 17784-47-3 | 2635 | 354 | + [1)] | + [1)] | + [1)] | C-126 | B-086 | A-001 |
| II-3 | Amiodarone HCl | 19774-82-4 | 5400 | 290 [c)] | + [2)] | + [2)] | + [2)] | C-127 | B-087 | A-002 |
| II-4 | Chlorpromazine HCl | 69-09-0 | 1746 | 304 | + [2)] | + [2)] | + [2)] | C-106 | B-066 | A-026 |
| II-5 | Doxycycline HCl | 10592-13-9 | 3715 | 290 [c)] | + [2)] | + [2)] | + [2)] | C-116 | B-076 | A-036 |
| II-6 | Fenofibrate | 49562-28-9 | 3514 | 290 [c)] | + [1)] | NA | + [1)] | C-139 | B-054 | A-014 |
| II-7 | Furosemide | 54-31-9 | 2650 | 290 [c)] | +/- [1,3,4)] | NA | + [1)] | C-141 | B-056 | A-016 |
| II-8 | Ketoprofen | 22071-15-4 | 2092 | 290 [c)] | + [1)] | - [1)] | + [1)] | C-128 | B-088 | A-003 |
| II-9 | 6-Methylcoumarine | 92-48-8 | 3219 | 290 [c)] | + [1)] | + [1)] | + [1)] | C-113 | B-073 | A-033 |
| II-10 | 8-MOP | 298-81-7 | 3631 | 300 | + [2)] | + [2)] | + [2)] | C-131 | B-091 | A-006 |
| II-11 | Nalidixic acid | 389-08-2 | 3192 | 331 | + [1)] | + [1)] | + [1)] | C-137 | B-052 | A-012 |
| II-12 | Nalidixic acid (Na salt) | 3374-05-8 | 3019 | 333 | + [1)] | + [1)] | + [1)] | C-134 | B-094 | A-009 |
| II-13 | Norfloxacin | 70458-96-7 | 3562 | 323 | + [3)] | + [1)] | + [1)] | C-110 | B-070 | A-030 |
| II-14 | Ofloxacin | 82419-36-1 | 8443 | 290 [c)] | + [1)] | + [1)] | + [1)] | C-112 | B-072 | A-032 |
| II-15 | Piroxicam | 36322-90-4 | 3304 | 352 | - [2)] | - [2)] | + [2)] | C-135 | B-095 | A-010 |
| II-16 | Promethazine HCl | 58-33-3 | 1558 | 297 | + [2)] | NA | + [2)] | C-101 | B-061 | A-021 |
| II-17 | Rosiglitazone | 122320-73-4 | 1765 | 311 | + [d)] | NA | NA | C-117 | B-077 | A-037 |
| II-18 | Tetracycline | 60-54-8 | 3842 | 290 [c)] | + [2)] | + [2)] | + [2)] | C-102 | B-062 | A-022 |
| **Phototoxic non-drug chemicals** | | | | | | | | | | |
| II-19 | Anthracene | 120-12-7 | 2315 | 355 | + [1)] | + [1)] | + [1)] | C-121 | B-081 | A-041 |
| II-20 | Avobenzone | 70356-09-1 | 7686 | 354 | + [5)] | - [6)] | + [7)] | C-109 | B-069 | A-029 |
| II-21 | Bithionol | 97-18-7 | 2462 | 321 | + [2)] | + [2)] | + [2)] | C-115 | B-075 | A-035 |
| II-22 | Hexachlorophene | 70-30-4 | 2431 | 300 | - [1)] | - [1)] | + [1,8)] | C-107 | B-067 | A-027 |
| II-23 | Rose bengal | 632-69-9 | 19269 | 549 | + [1)] | - [1)] | + [1)] | C-104 | B-064 | A-024 |
| **Non-phototoxic drugs** | | | | | | | | | | |
| II-24 | Aspirin | 50-78-2 | 80 | 290 [c)] | - [3)] | NA | NA | C-140 | B-055 | A-015 |
| II-25 | Benzocaine | 94-09-7 | 4273 | 290 [c)] | - [3)] | NA | NA | C-114 | B-074 | A-034 |
| II-26 | Erythromycin | 114-07-8 | 0 | 290 [c)] | - [3)] | NA | NA | C-119 | B-079 | A-039 |
| II-27 | Penicillin G | 113-98-4 | 0 | 290 [c)] | - [2)] | NA | NA | C-118 | B-078 | A-038 |
| II-28 | Phenytoin | 57-41-0 | 0 | 290 [c)] | - [3)] | NA | NA | C-145 | B-060 | A-020 |

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) | λmax (nm) | | Animal | Human | 1 | 2 | 3 |
| **Non-phototoxic non-drug chemicals** | | | | | | | | | | |
| II-29 | Bumetrizole | 3896-11-5 | 3873 | 306 | - [d)] | NA | NA | C-138 | B-053 | A-013 |
| II-30 | Camphor sulfonic acid | 3144-16-9 | 0 | 290 [c)] | - [d)] | NA | NA | C-132 | B-092 | A-007 |
| II-31 | Chlorhexidine | 55-56-1 | 1338 | 290 [c)] | - [1)] | NA | - [1)] | C-144 | B-059 | A-019 |
| II-32 | Cinnamic acid | 140-10-3 | 3373 | 290 [c)] | - [d)] | NA | NA | C-123 | B-083 | A-043 |
| II-33 | Drometrizole | 2440-22-4 | 3946 | 295 | - [d)] | NA | NA | C-129 | B-089 | A-004 |
| II-34 | L-Histidine | 71-00-1 | 0 | 290 [c)] | - [2)] | NA | NA | C-111 | B-071 | A-031 |
| II-35 | Methylbenzylidene camphor | 36861-47-9 | 9200 | 304 | - [9)] | - [9)] | - [9)] | C-136 | B-051 | A-011 |
| II-36 | Octrizole | 3147-75-9 | 3958 | 296 | - [d)] | NA | NA | C-133 | B-093 | A-008 |
| II-37 | Octyl methacrylate | 688-84-6 | 0 | 290 [c)] | - [d)] | NA | NA | C-105 | B-065 | A-025 |
| II-38 | Octyl methoxycinnamate | 5466-77-3 | 3000 | 290 [c)] | - [9)] | - [9)] | - [9)] | C-142 | B-057 | A-017 |
| II-39 | Octyl salicylate | 118-60-5 | 1500 | 290 [c)] | - [9)] | - [9)] | - [9)] | C-120 | B-080 | A-040 |
| II-40 | PABA | 150-13-0 | 2404 | 290 [c)] | - [2)] | - [1)] | NA | C-124 | B-084 | A-044 |
| II-41 | SDS | 151-21-3 | 0 | 290 [c)] | - [2)] | NA | NA | C-125 | B-085 | A-045 |
| II-42 | UV-571 | 125304-04-3 | 1900 | 290 [c)] | - [d)] | NA | NA | C-122 | B-082 | A-042 |
| **Positive/Negative controls** | | | | | | | | | | |
| PC | Quinine HCl | 6119-47-7 | 1938 | 330 | + [3)] | + [10)] | + [11)] | PC | PC | PC |
| NC | Sulisobenzone | 4065-45-6 | 3519 | 290 [c)] | - [3)] | NA | - [9)] | 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) λmax (nm) was a wavelength at which the UV/vis absorbance shows a peak between 290 and 700 nm. λmax (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 protocols used in this study is described in Attachment 1 and 2.

### 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:

Singlet oxygen + Imidazole → [Peroxide intermediate] → Oxidized imidazole

[Peroxide intermediate] + RNO → RNO + Products

The generation of superoxide 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 reduces NBT to $NBT^+$, whose formation can be monitored spectrophotometrically at 560 nm.

Superoxide + NBT → $O_2$ + $NBT^+$

## 7-2. Protocol of ROS assay

### 7-2-1. Apparatus

In the present validation study, an Atlas Suntest CPS series (CPS plus or CPS; Atlas Material Technology LLC, Chicago, IL, USA) equipped with a 1500-W xenon arc lamp was used for solar simulator. The irradiation tests were carried out at 25°C with an irradiance of ca. 2.0 $mW/cm^2$ as 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 0.1, 1, 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 9. 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 the storage condition.

### 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 (2–200 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 1 hour. After agitation on plate shaker, UV absorbance at 440 nm was measured. For the determination of superoxide, samples containing the test chemical (2–200 µM) and NBT (50 µM) in 20 mM NaPB were irradiated with the simulated sunlight for 1 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 study, 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. As the final concentrations, 200 µM of test chemical solutions were subjected to the

ROS assay. However, when precipitation could be observed at 200 μM under the optical microscope at 100× before light exposure, additional experiments were performed at 20 μM. Further experiments should be performed at 2 μM when precipitation was still observed at 20 μM. When precipitation was observed at 2 μM in the reaction mixture, further experimentation was not performed. 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 judgement

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

Every assay result was classified based on these judgement criteria. Final judgements about chemicals were performed on the following four draft criteria at each laboratory:

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

### 7-4. Quality assurance

Assays and quality assurance were carried out in the spirit of GLP. Two of three test facilities were GLP certified even though tests were not performed under GLP. 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 study

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

Results of within-laboratory variation, which comprise intra-day variation and inter-day variation of the positive and negative controls at Laboratories 1, 2, and 3 are shown in Table 3. Individual positive and negative control values are shown in Appendix 1. 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 an 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 CVs of the positive control at each laboratory were below 10%, and 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 three laboratories were 12.2% (singlet oxygen) and 26.6% (superoxide anion). Negative control was shown to be inactive in all assays.

#### 8-1-2. Results and judgements in the Phase 1 study

Results of the Phase 1 study were shown (Table 5 and Appendix 2). 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 ($\Delta A_{440\,nm}\times10^3$) of 25 or more and/or superoxide value ($\Delta A_{560\,nm}\times10^3$) of 20 or more or
ii. Negative with singlet oxygen value of less than 25 and superoxide value of less than 20.

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

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

Draft criteria A: Final judgement of positive when positive results were obtained in at least one of three assays (Table 6A).
Draft criteria B: Final judgement based on the mean value of three assays (Table 6B).
Draft criteria C: Final judgement based on the majority of three assay results (Table 6C).
Draft criteria D: Final judgement based on the first assay results (Table 6D).

As for the final judgements at 20 μM, there was no inconsistency between the three laboratories under draft criteria A or B, but there was one inconsistency (furosemide) under draft criteria C or D. In addition, 8-MOP showed negative results for all laboratories at 20 μM. However, there was no inconsistency in the final judgement between the three laboratories at 200 μM.

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

Inconsistencies of final judgements 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 83.3% under draft criteria A or B and 75.0% or 83.3% under the draft criteria C and D at each laboratory. 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 33.3% under criteria A or B and 25.0 or 33.3% under criteria C or D. Accuracies were 84.6% under criteria A or B and 76.9% or 84.6% under criteria C or 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).

## 8-2. Phase 2 study

### 8-2-1. Irradiance and temperature during the irradiation

The irradiances and temperatures at the beginning and the end of each irradiation are shown in Table 8 and Appendix 3. 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 (1.93 to 2.07 $mW/cm^2$) were within the specified range of values, and there was no significant inconsistency between facilities. There was no apparent variation in either irradiance or temperature between the facilities.

### 8-2-2. 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 1, 2 and, 3, are shown in Table 9 and Appendix 4. 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 CVs of the positive control at each laboratory were below 10%, and 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 10). The CVs of positive control in the three laboratories were 20.6% (singlet oxygen) and 20.1% (superoxide anion). Negative control was shown to be inactive in all assays.

### 8-2-3. Results and judgements 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 1, 2, and 3 (Table 11 and Appendix 5). 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 2 or 20 μM due to limited solubility in aqueous media. In particular, assays of amiodarone HCl (No. II-3), anthracene (No. II-19 ), avobenzone (II-20), Octrizole(No. II-36) and UV-571(No. II-42) were available only at 2 μM in most laboratories. For rose bengal (No. II-23), only singlet oxygen could be measured, since the intense UV absorption at 560 nm interfered directly with determination of superoxide anion. All three participating laboratories found that all phototoxins demonstrated potent ability to generate singlet oxygen, superoxide, or both under UV–vis exposure at concentrations of 20 and 200 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 μM in all assays, and the values for singlet oxygen and superoxide anion were similar in each laboratory. For some phototoxins, however, generation of ROS was negligible at 2 μM. Similar photochemical reactivity was also seen for test chemicals that were non-phototoxic drugs/compounds, although some exhibited potent photoreactivity in a few laboratories.

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

Positive: Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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 judgements were based on four draft criteria shown in Tables 12A to 12D. The draft criteria for the final judgement are followings:

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

Three chemicals at Laboratories 1 and 2 gave inconsistent results in the three independent repeat assays, as did one chemical at Laboratory 3.
Ten chemicals gave inconsistent final judgements between the laboratories for draft criteria A (chemical Nos. II-6, 19, 25, 26, 27, 31, 32, 36, 37, and 41), as did nine chemicals for draft criteria B and C (chemical Nos. 3, 6, 19, 27, 31, 32, 36, 37, and 41), and eight chemicals for draft criteria D (chemical Nos. 6, 19, 27, 31, 32, 36, 37, and 41).

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

Inconsistent final judgements were assessed using contingency tables in the Phase 2 study (Tables 13A to 13D).
Sensitivities and negative predictivities were 100% in each laboratory regardless of the four different criteria. Specificities were 33.3% to 81.8% under draft criteria A, 41.7% to 81.8% under draft criteria B and C, and 58.3% to 81.8% under draft criteria D. Positive predictivities were 74.2% to 91.7% under draft criteria A, 75.9% to 91.7% under draft criteria B and C, and 82.1% to 91.7% under draft criteria D. Accuracies were 77.1% to 93.9% under draft criteria A, 79.4% to 93.9% under draft criteria B and C, and 85.7% to 93.9% under draft criteria D. There were no false negatives. There were between two and seven false positives in 11 or 12 negative chemicals under draft criteria A, between two and eight false positives in 10 to 12 negative chemicals under draft criteria B or C, and between two and five false positives in 10 to 12 negative chemicals under draft criteria D.

## 8-3. Combined results of Phase 1 and Phase 2

### 8-3-1. Results and judgements for Phase 1 and 2 combined results

The results of four phototoxic chemicals (200 μM) evaluated only in the Phase 1 study were combined with the Phase 2 results (diclofenac, 5-FU, levofloxacin, omeprazole; Tables 14A to 14 D). The results and the final judgements of these four chemicals were consistent between the three laboratories.

### 8-3-2. Contingency tables for Phase 1 and 2 combined results

Inconsistencies of final judgements were assessed using contingency tables for the combined results (Tables 15A to 15D).

Sensitivities were 96.0% to 96.3% in each laboratory regardless of the four different criteria. Specificities were 33.3% to 81.8% under draft criteria A, 41.7% to 81.8% under draft criteria B and C, and 58.3% to 81.8% under draft criteria D. Variation in specificities was basically dependent on facility. Specificities were 33.3% to 58.3%, 36.4% to 60.0%, and 81.8% in Laboratories 1, 2 and 3, respectively. Positive predictivities were 76.5% to 92.6% under draft criteria A, 78.1% to 92.6% under draft criteria B and C, and 83.9% to 92.6% under the draft criteria D. Negative predictivities were 80.0% to 90.0% under draft criteria A, 83.3% to 90.0% under draft criteria B and C, and 85.7% to 90.0% under draft criteria D. Accuracies were 76.9% to 91.1% under draft criteria A, 79.0% to 91.9% under draft criteria B and C, and 84.6% to 91.9% under draft criteria D.

5-FU showed a false negative under all criteria, but no other chemicals showed false negatives under any criteria. There were between two and eight false positives in 11 or 12 negative chemicals under draft criteria A, between two and seven false positives in 10 to 12 negative chemicals under draft criteria B or C, and between two and five false positives in 10 to 12 negative chemicals under draft criteria D.

### 8-4. Contingency tables for integrated judgement results

Parameters in the contingency tables for the Phase 1 study (20 and 200 μM), the Phase 2 study, and the combined results are shown in Tables 16-1 to 16-4. Integrated final judgements were based on a majority of laboratory judgements. Parameters in the contingency tables of integrated judgement results for the Phase 1 study (200 μM), the Phase 2 study, and the combined results are shown in Table 17.

The parameters in Phase 1 at 200 μM were the same regardless of the draft criteria for judgement. For the Phase 2 and the combined results, whoever, 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 and slightly higher under draft criteria D.

### 8-5. 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 27$^{th}$ February to 2$^{nd}$ 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)

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 20μM did not cause any false negatives in validation studies with Atlas Suntest CPS/CPS+ or Seric SXL2500-V2. Therefore the negative results at 20μM were included in the secondary data 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 judgement criteria were established for the proposed protocol as follows:

Judgement criteria for the secondary data analysis
Each test chemical is to be classified as follows:

| Judgement[1)] | Concentration | SO (mean of 3 wells) | | SA (mean of 3 wells) |
|---|---|---|---|---|
| Photoreactive | 20 and/or 200 μM[2)] | ≥25 | and | ≥70 |
| | | <25 and/or P[3)] | and | ≥70 |
| | | ≥25 | and | <70 and/or P |
| Weakly photoreactive | 20 and 200 μM[2)] | <25 | and | ≥20, <70 |
| Non-photoreactive | 20 and 200 μM[2)] | <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 judgement 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.

### 8-5-1. Secondary data analysis based on the criteria for the proposed protocol: Results and judgements of Phase 2 study

The results of the Phase 2 study were classified based on the criteria for secondary data analysis, and final judgements were based on four draft criteria as shown in Tables 18A to 18D. The draft criteria for the final judgement are as follows:

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

For phototoxic chemicals which were evaluated at 20μM and 200μM, all of the judgements, the final judgements, and the integrated judgements were photoreactive. For the non-phototoxic chemicals, two of three assay results for one non-drug chemical (No. II-37, Octyl methacrylate) were classified as photoreactive at Laboratory 1. Except for this sole case, all other non-phototoxic chemicals were classified as non-photoreactive or weakly photoreactive.

### 8-5-2. Secondary data analysis based on the criteria for the proposed protocol: Contingency tables of Phase 2 study results

Contingency tables are shown in Tables 19A to 19D. One to four phototoxic chemicals and one to five non-phototoxic chemicals were considered incompatible due to precipitation at 20μM and 200μM. 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 20. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of the integrated judgements based on the original criteria and the criteria for the proposed protocol are summarized in Table 21.

## 8-6. Third data analysis after reconsidering the negative results at 20μM

After issuing a revised validation report with secondary data analysis, the VMT updated the validation studies and the peer review process at the ICH S10 Expert Working Group (EWG) during the ICH Brussels meeting, held on 3rd June, 2013. After introduction of updated results stated in the Section 8-5 above, discussion at the EWG focused on the predictivity of the negative results at 20 μM. Some EWG members also noticed that differences in precipitation between laboratories might lead to different test concentrations, which could result in false negatives without appropriate predictivity at 20 μM. 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. The results of this new series of assays at 20 μM and the Phase 2 assay results at 200 μM are presented in Table 22. 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. Therefore, we concluded that follow-up studies will be needed whenever negative results are seen only at 20μM.

Based on these results, there are at least two options for classifying negative results at 20μM. One option is to establish a new judgement, 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, judgement criteria for the proposed protocol would be as follows.

Judgement criteria for the third data analysis

Each test chemical will be classified as follows;

| Judgement[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[2)] | <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 judgement 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.

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

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

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

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

For phototoxic chemicals, the results of the third data analysis matched those of the second data analysis, and there were no differences in integrated judgement as shown in Tables 23A to 23D. For non-phototoxic chemicals, the integrated judgements were same in each table except penicillin G, chlorhexidine and cinnamic acid. Penicillin G was classified as weakly photoreactive according to Tables 23A, B, and C but as non-photoreactive according to Table 23D. Chlorhexidine was classified as weakly photoreactive according to Table 23A but as non-photoreactive according to Tables 23B, C, and D. Cinnamic acid was classified

as inconclusive according to Tables 23A but as weakly photoreactive according to Table 23B, C, and D.

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

Contingency tables are shown in Tables 24A to 24D. One to four phototoxic chemicals and eight to nine non-phototoxic chemicals were considered incompatible due to precipitation at 20μM and 200μM 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 25. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of the integrated judgements based on the original criteria and the criteria for the proposed protocol are summarized in Table 26.

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

Negative results at 20μM were discussed during the peer review meeting held on 21 and 22 August, 2013, in Japan. As stated in Section 8-6, 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 uM 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 200uM and 20uM 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 20uM be performed when the assay at 200uM 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 judgement 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 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.

Criteria for judgment

Each test chemical is to be judged as follows:

| **Judgment**[1, 2] | **Concentration**[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 judging results, because the ROS assay shows good intra- and inter-laboratory reproducibility in the validation studies.
2. If precipitation, coloration, or other interference is observed at both 20 and 200 μM, the chemical is considered incompatible with the ROS assay and judged as inconclusive.
3. 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.
4. Interference such as precipitation or coloration.

Judgement as photoreactive, weakly photoreactive, or inconclusive would be a flag for follow-up assessment. Judgement 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 judgements of Phase 2 study

The results of the Phase 2 study were classified based on the criteria in the proposed protocol, and final judgements were based on four draft criteria as shown in Tables 27A to 27D. The draft criteria for the final judgement are as follows:

A. The highest criteria among the three assay results was selected as the final judgement.
B. Final judgement based on the mean value of three assays.
C. Final judgement based on the majority of three assay results.
D. Final judgement 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 judgement as shown in Tables 27A to 27D. For non-phototoxic chemicals, the integrated judgements were the same in each table except penicillin G, chlorhexidine and cinnamic acid. Penicillin G was classified as weakly photoreactive according to Tables 27A, B, and C but as non-photoreactive by Table 27D. Chlorhexidine was classified as weakly photoreactive according to Table 27A but as non-photoreactive according to Tables 27B, C, and D. Cinnamic acid was classified as inconclusive according to Table 27A but as weakly photoreactive according to Table 27B, C, and D. In the third data analysis, eight (Table 23B, C and D) or nine (Tables 23A) out of 19 non-phototoxic chemicals were classified as

inconclusive, but in the fourth data analysis, only one 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 28A to 28D. One to four phototoxic chemicals and two to five 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 29. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of the integrated judgements based on the original criteria and the criteria for the proposed protocol are summarized in Table 30.

## 9. Discussion

### 9-1. Reliability

Variability was assessed using quinine for a positive control and sulisobenzone for a negative control in the Phase 1 study. Within-laboratory variations were sufficiently small to suggest high repeatability and reproducibility. In addition, preliminary findings show good between-laboratory transferability.

Between-laboratory transferability was assessed using 12 positive and 1 negative chemical at 20 and 200 μM. All chemicals showed the same final judgements in all criteria and facilities at 200 μM. One chemical, however, showed different results at Laboratory 3 depending on the draft criteria for final judgement at 20 μM, which suggests that weak ROS generative chemicals could show obscure result at 20 μM. In addition, sensitivities were lower at 20 μM than 200 μM for all criteria at all laboratories. 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 Feburary to 2 March, 2013, the peer review panel recommended the inclusion of negative results at 20 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 blind conditions. The secondary data analysis did not affect the reliability of the ROS assay. After the ICH Brussels meeting in 2013 and the peer review panel telephone conference on 17 June, 2013, Lab 1 performed a new series of ROS assays at 20 μM with the validation chemical set. 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. Therefore, we concluded that follow-up studies will be needed whenever negative results are seen only at 20μM. Based on these results, there are at least two options for classifying negative results at 20μM. One is to establish as new judgement, 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 8-5. 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.
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

The Phase 2 study was conducted with 42 coded test chemicals and 2 control chemicals, and the third data analysis was performed on the results of 41 chemicals, excluding rosiglitazone. As shown in Table 29, defining the weakly photoreactive chemicals as non-phototoxic chemicals resulted in a sensitivity of 100%, a specificity of 81.3% to 100%, a positive predictivity of 87.5% to 100%, a negative predictivity of 100%, and an accuracy of 91.9% to 100%, based on the criteria for the proposed protocol. In contrast, defining weakly photoreactive chemicals as phototoxic chemicals resulted in a sensitivity of 100%, a specificity of 56.3% to 88.2%, a positive predictivity of 75.0% to 90.9%, a negative predictivity of 100%, and an accuracy of 81.1% to 94.6%. Specificities exhibited some fluctuation between laboratories. Seven non-phototoxic drugs/compounds were judged to be negative in all criteria at Laboratory 3 but were each judged differently depending on criteria at Laboratories 1 and 2, resulting in high specificity at Laboratory 3 and lower specificity at Laboratories 1 and 2 in all decision criteria. Values for the positive control were somewhat higher at Laboratories 1 and 2 than at Laboratory 3, suggesting that conditions were more conducive to ROS generation at Laboratories 1 and 2. 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 25, 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 8-6. According to the original criteria, some of the false positives observed in the Phase 2 study, which included phenytoin [Chen Y. et al., 2009] (No. II-28), penicillin G [Ray R. S. et al., 1996] (No. II-27), chlorhexidine [Information from manufacture] (No. II-31), cinnamic acid [Marin M. L. et al., 2007] (No. II-32), L-histidine [Huvaere K. et al., 2009] (No. II-34), and octyl methacrylate [Information from manufacture] (No. II-37), had previously been reported to be photodegradable and photoreactive, the mechanisms of which included radical reactions and/or electron transfer. 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, and L-histidine were classified as weakly photoreactive. 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, bufexamac, and haloperidol—which could be related to clinical photosensitivity, would be classified as weakly photoreactive [Onoue et al., 2008a]. Test chemicals such as bumetrizole (II-29), drometrizole (II-33), methylbenzylidene camphor (II-35), octrizole (II-36), octyl methacrylate (II-37), octyl methoxycinnamate (II-38), 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 20μM 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 did satisfy 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 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 30, 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, although criteria D did show a slight advantage in specificity, positive predictivity and negative predictivity. Additionally, because only one assay is needed, draft criteria D also has an advantage in terms of throughput. Optimal criteria for final judgementjudgement will be determined in a comprehensive manner using results of another validation running parallel with this one but with a different solar simulator.

## 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 Atlas Suntest CPS/CPS+, predictivity and reproducibility in distinguishing phototoxic and non-phototoxic chemicals using the physico-chemical ROS assay to evaluate phototoxic potential. 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 judgement criteria based on ROS assay endpoints, which maximized applicability domain and assay performance with sensitivity of 100% (21chemicals/21 chemicals), specificity of 83.3% (15 chemicals/18 chemicals), positive predictivity of 87.5% (21 chemicals/24 chemicals), negative predictivity of 100% (15 chemicals/15 chemicals) and accuracy of 92.3% (36 chemicals/39 chemicals). These results support the routine use of the validated ROS assay protocol in preclinical drug screening for phototoxic potential.

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

| ROS assay Validation data (atlas) | | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | | N | Mean | SD | CV (%) | N | Mean | SD | CV (%) | N | Mean | SD | CV (%) |
| Intra-day | | | | | | | | | | | | | | |
| Positive Control | Quinine | SO | 6 | 462 | 14.4 | 3.1 | 4 | 402 | 14.8 | 3.7 | 4 | 359 | 6.9 | 1.9 |
| | | SA | 6 | 372 | 11.1 | 3.0 | 4 | 343 | 28.7 | 8.4 | 4 | 217 | 14.2 | 6.5 |
| Negative Control | Sulisobenzone | SO | 6 | 2 | 12.2 | - | 4 | 1 | 2.4 | - | 4 | -7 | 1.0 | - |
| | | SA | 6 | -12 | 2.3 | - | 4 | -7 | 2.4 | - | 4 | -2 | 0.5 | - |
| Inter-day | | | | | | | | | | | | | | |
| Positive Control | Quinine | SO | 3 | 455 | 4.5 | 1.0 | 3 | 401 | 18.6 | 4.6 | 3 | 359 | 7.6 | 2.1 |
| | | SA | 3 | 349 | 16.2 | 4.6 | 3 | 335 | 17.8 | 5.3 | 3 | 223 | 10.0 | 4.5 |
| Negative Control | Sulisobenzone | SO | 3 | 3 | 8.7 | - | 3 | -1 | 1.0 | - | 3 | -5 | 3.0 | - |
| | | SA | 3 | -14 | 6.4 | - | 3 | -7 | 4.6 | - | 3 | -2 | 1.0 | - |

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 based on the results of an assay of the day.

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

| ROS assay Validation data (atlas) | | | | | | |
|---|---|---|---|---|---|---|
| Chemicals | | | N | Mean | SD | CV (%) |
| Positive Control | Quinine | SO | 3 | 410 | 50.0 | 12.2 |
| | | SA | 3 | 312 | 82.9 | 26.6 |
| Negative Control | Sulisobenzone | SO | 3 | -2 | 2.0 | - |
| | | SA | 3 | -7 | 5.0 | - |

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 study

| ROS assay Validation data (atlas) | | | Lab 1 | | | | | | | Lab 2 | | | | | | | Lab 3 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | | Conc | Assay | | | Mean | SD | CV | Conc | Assay | | | Mean | SD | CV | Conc | Assay | | | Mean | SD | CV |
| No. | Name | | (μM) | 1st | 2nd | 3rd | | | (%) | (μM) | 1st | 2nd | 3rd | | | (%) | (μM) | 1st | 2nd | 3rd | | | (%) |
| I - 1 | 5-FU | SO | 20 | 2 | -1 | -1 | 0 | 1.7 | - | 20 | 4 | -1 | -9 | -2 | 6.6 | - | 20 | -4 | -4 | -1 | -3 | 1.7 | - |
| | | SA | 20 | -18 | -13 | -9 | -13 | 4.5 | - | 20 | -1 | -2 | -3 | -2 | 1 | - | 20 | 0 | 1 | 8 | 3 | 4.4 | - |
| I - 2 | 8-MOP | SO | 20 | 9 | 7 | 9 | 8 | 1.2 | - | 20 | 9 | 6 | 13 | 9 | 3.5 | - | 20 | 7 | 6 | 9 | 7 | 1.5 | - |
| | | SA | 20 | -34 | -32 | -37 | -34 | 2.5 | - | 20 | 7 | 6 | 1 | 5 | 3.2 | - | 20 | 3 | 4 | 2 | 3 | 1 | - |
| I - 3 | Amiodarone [a] | SO | 20 | 135 | 129 | 126 | 130 | 4.6 | 3.53 | 20 | 93 | 65 | 86 | 81 | 14.6 | 17.92 | 20 | 82 | 82 | 85 | 83 | 1.7 | 2.09 |
| | | SA | 20 | -27 | -18 | -16 | -20 | 5.9 | - | 20 | -4 | 3 | -5 | -2 | 4.4 | - | 20 | 5 | 8 | 7 | 7 | 1.5 | - |
| I - 4 | Chlorpromazine | SO | 20 | 94 | 89 | 89 | 91 | 2.9 | 3.18 | 20 | 75 | 75 | 60 | 70 | 8.7 | 12.37 | 20 | 92 | 100 | 101 | 98 | 4.9 | 5.05 |
| | | SA | 20 | -21 | -14 | -14 | -16 | 4 | - | 20 | 21 | 21 | 19 | 20 | 1.2 | 5.68 | 20 | 12 | 13 | 12 | 12 | 0.6 | - |
| I - 5 | Diclofenac | SO | 20 | 163 | 160 | 161 | 161 | 1.5 | 0.95 | 20 | 101 | 113 | 90 | 101 | 11.5 | 11.35 | 20 | 155 | 147 | 145 | 149 | 5.3 | 3.55 |
| | | SA | 20 | 8 | 14 | 14 | 12 | 3.5 | - | 20 | 9 | 8 | 5 | 7 | 2.1 | - | 20 | 8 | 10 | 9 | 9 | 1 | - |
| I - 6 | Doxycycline | SO | 20 | 85 | 67 | 94 | 82 | 13.7 | 16.77 | 20 | 79 | 57 | 66 | 67 | 11.1 | 16.43 | 20 | 50 | 51 | 56 | 52 | 3.2 | 6.14 |
| | | SA | 20 | 20 | 29 | 31 | 27 | 5.9 | 21.97 | 20 | 67 | 35 | 56 | 53 | 16.3 | 30.87 | 20 | 45 | 45 | 44 | 45 | 0.6 | 1.29 |
| I - 7 | Furosemide | SO | 20 | 35 | 35 | 42 | 37 | 4 | 10.83 | 20 | 30 | 31 | 19 | 27 | 6.7 | 24.97 | 20 | 24 | 24 | 31 | 26 | 4 | 15.35 |
| | | SA | 20 | -15 | -14 | -15 | -15 | 0.6 | - | 20 | 6 | 8 | -2 | 4 | 5.3 | - | 20 | 5 | 6 | 5 | 5 | 0.6 | - |
| I - 8 | Ketoprofen | SO | 20 | 41 | 30 | 48 | 40 | 9.1 | 22.88 | 20 | 30 | 31 | 33 | 31 | 1.5 | 4.88 | 20 | 41 | 40 | 44 | 42 | 2.1 | 5 |
| | | SA | 20 | -29 | -31 | -7 | -22 | 13.3 | - | 20 | 4 | 3 | 6 | 4 | 1.5 | - | 20 | 1 | 2 | 2 | 2 | 0.6 | - |
| I - 9 | Levofloxacin | SO | 20 | 33 | 46 | 36 | 38 | 6.8 | 17.76 | 20 | 28 | 30 | 20 | 26 | 5.3 | 20.35 | 20 | 35 | 34 | 39 | 36 | 2.6 | 7.35 |
| | | SA | 20 | 172 | 161 | 150 | 161 | 11 | 6.83 | 20 | 191 | 209 | 181 | 194 | 14.2 | 7.33 | 20 | 162 | 134 | 141 | 146 | 14.6 | 10 |
| I - 10 | Norfloxacin | SO | 20 | 82 | 77 | 122 | 94 | 24.7 | 26.33 | 20 | 60 | 59 | 55 | 58 | 2.6 | 4.56 | 20 | 58 | 53 | 56 | 56 | 2.5 | 4.52 |
| | | SA | 20 | 4 | 3 | 7 | 5 | 2.1 | - | 20 | 21 | 28 | 23 | 24 | 3.6 | 15.02 | 20 | 18 | 15 | 16 | 16 | 1.5 | - |
| I - 11 | Omeprazole | SO | 20 | 66 | 71 | 68 | 68 | 2.5 | 3.68 | 20 | 57 | 65 | 59 | 60 | 4.2 | 6.9 | 20 | 49 | 47 | 55 | 50 | 4.2 | 8.27 |
| | | SA | 20 | 12 | 8 | 8 | 9 | 2.3 | - | 20 | 36 | 35 | 36 | 36 | 0.6 | 1.62 | 20 | 22 | 14 | 19 | 18 | 4 | - |
| I - 12 | Quinine | SO | 20 | 124 | 121 | 113 | 119 | 5.7 | 4.77 | 20 | 117 | 115 | 109 | 114 | 4.2 | 3.66 | 20 | 74 | 81 | 84 | 80 | 5.1 | 6.44 |
| | | SA | 20 | 10 | 8 | 21 | 13 | 7 | - | 20 | 65 | 65 | 59 | 63 | 3.5 | 5.5 | 20 | 34 | 26 | 31 | 30 | 4 | 13.32 |
| I - 13 | Sulisobenzone | SO | 20 | -11 | -2 | -10 | -8 | 4.9 | - | 20 | -1 | 3 | -9 | -2 | 6.1 | - | 20 | -3 | -2 | 2 | -1 | 2.6 | - |
| | | SA | 20 | -6 | -7 | -22 | -12 | 9 | - | 20 | -3 | -2 | -4 | -3 | 1 | - | 20 | -2 | -1 | -1 | -1 | 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 20μM and 200μM in this study.

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

| ROS assay Validation data (atlas) | | | Lab 1 | | | | | | | Lab 2 | | | | | | | Lab 3 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | | Conc | Assay | | | Mean | SD | CV | Conc | Assay | | | Mean | SD | CV | Conc | Assay | | | Mean | SD | CV |
| No. | Name | | (μM) | 1st | 2nd | 3rd | | | (%) | (μM) | 1st | 2nd | 3rd | | | (%) | (μM) | 1st | 2nd | 3rd | | | (%) |
| I - 1 | 5-FU | SO | 200 | 0 | -2 | 1 | 0 | 1.5 | - | 200 | 1 | -2 | 0 | 0 | 1.5 | - | 200 | -6 | -2 | -1 | -3 | 2.6 | - |
| | | SA | 200 | -8 | -9 | -7 | -8 | 1 | - | 200 | -3 | 0 | -5 | -3 | 2.5 | - | 200 | 0 | 0 | 0 | 0 | 0 | - |
| I - 2 | 8-MOP | SO | 200 | 121 | 123 | 122 | 122 | 1 | 0.82 | 200 | 60 | 65 | 58 | 61 | 3.6 | 5.91 | 200 | 42 | 55 | 51 | 49 | 6.7 | 13.5 |
| | | SA | 200 | -2 | 5 | 4 | 2 | 3.8 | - | 200 | 64 | 76 | 50 | 63 | 13 | 20.55 | 200 | 15 | 18 | 15 | 16 | 1.7 | - |
| I - 3 | Amiodarone [a] | SO | 200 | 259 | 192 | 247 | 233 | 35.7 | 15.35 | 200 | 379 | 311 | 443 | 378 | 66 | 17.48 | 200 | 319 | 393 | 362 | 358 | 37.2 | 10.38 |
| | | SA | 200 | -230 | -196 | -232 | -219 | 20.2 | - | 200 | -59 | -194 | -159 | -137 | 70.1 | - | 200 | -114 | 20 | -82 | -59 | 70 | - |
| I - 4 | Chlorpromazine | SO | 200 | -3 | -12 | -7 | -7 | 4.5 | - | 200 | -36 | -35 | -37 | -36 | 1 | - | 200 | 16 | 19 | 9 | 15 | 5.1 | - |
| | | SA | 200 | 80 | 78 | 78 | 79 | 1.2 | 1.47 | 200 | 90 | 113 | 98 | 100 | 11.7 | 11.64 | 200 | 78 | 74 | 76 | 76 | 2 | 2.63 |
| I - 5 | Diclofenac | SO | 200 | 312 | 318 | 321 | 317 | 4.6 | 1.45 | 200 | 241 | 263 | 234 | 246 | 15.1 | 6.15 | 200 | 330 | 330 | 328 | 329 | 1.2 | 0.35 |
| | | SA | 200 | 364 | 362 | 365 | 364 | 1.5 | 0.42 | 200 | 263 | 305 | 302 | 290 | 23.4 | 8.08 | 200 | 303 | 286 | 300 | 296 | 9.1 | 3.06 |
| I - 6 | Doxycycline | SO | 200 | 113 | 111 | 117 | 114 | 3.1 | 2.69 | 200 | 198 | 227 | 186 | 204 | 21.1 | 10.35 | 200 | 175 | 161 | 170 | 169 | 7.1 | 4.21 |
| | | SA | 200 | 321 | 321 | 323 | 322 | 1.2 | 0.36 | 200 | 394 | 411 | 368 | 391 | 21.7 | 5.54 | 200 | 308 | 315 | 314 | 312 | 3.8 | 1.21 |
| I - 7 | Furosemide | SO | 200 | 190 | 196 | 198 | 195 | 4.2 | 2.14 | 200 | 129 | 138 | 131 | 133 | 4.7 | 3.56 | 200 | 110 | 117 | 117 | 115 | 4 | 3.52 |
| | | SA | 200 | 102 | 99 | 94 | 98 | 4 | 4.11 | 200 | 60 | 76 | 65 | 67 | 8.2 | 12.22 | 200 | 42 | 44 | 43 | 43 | 1 | 2.33 |
| I - 8 | Ketoprofen | SO | 200 | 252 | 248 | 255 | 252 | 3.5 | 1.4 | 200 | 210 | 202 | 242 | 218 | 21.2 | 9.71 | 200 | 204 | 197 | 209 | 203 | 6 | 2.96 |
| | | SA | 200 | 90 | 94 | 94 | 93 | 2.3 | 2.49 | 200 | 107 | 95 | 109 | 104 | 7.6 | 7.3 | 200 | 58 | 52 | 51 | 54 | 3.8 | 7.05 |
| I - 9 | Levofloxacin | SO | 200 | 204 | 188 | 191 | 194 | 8.5 | 4.38 | 200 | 125 | 127 | 134 | 129 | 4.7 | 3.67 | 200 | 116 | 118 | 125 | 120 | 4.7 | 3.95 |
| | | SA | 200 | 371 | 390 | 379 | 380 | 9.5 | 2.51 | 200 | 455 | 472 | 441 | 456 | 15.5 | 3.4 | 200 | 440 | 451 | 443 | 445 | 5.7 | 1.28 |
| I - 10 | Norfloxacin | SO | 200 | 211 | 208 | 213 | 211 | 2.5 | 1.19 | 200 | 154 | 148 | 159 | 154 | 5.5 | 3.58 | 200 | 155 | 143 | 155 | 151 | 6.9 | 4.59 |
| | | SA | 200 | 116 | 119 | 115 | 117 | 2.1 | 1.78 | 200 | 148 | 164 | 151 | 154 | 8.5 | 5.51 | 200 | 121 | 117 | 102 | 113 | 10 | 8.84 |
| I - 11 | Omeprazole | SO | 200 | -29 | -31 | -24 | -28 | 3.6 | - | 200 | -46 | -56 | -49 | -50 | 5.1 | - | 200 | -36 | -30 | -59 | -42 | 15.3 | - |
| | | SA | 200 | 118 | 109 | 116 | 114 | 4.7 | 4.13 | 200 | 141 | 147 | 149 | 146 | 4.2 | 2.86 | 200 | 141 | 131 | 144 | 139 | 6.8 | 4.91 |
| I - 12 | Quinine | SO | 200 | 433 | 432 | 456 | 440 | 13.6 | 3.08 | 200 | 406 | 405 | 415 | 409 | 5.5 | 1.35 | 200 | 346 | 337 | 345 | 343 | 4.9 | 1.44 |
| | | SA | 200 | 387 | 394 | 379 | 387 | 7.5 | 1.94 | 200 | 395 | 396 | 372 | 388 | 13.6 | 3.5 | 200 | 215 | 209 | 195 | 206 | 10.3 | 4.97 |
| I - 13 | Sulisobenzone | SO | 200 | 4 | 7 | 2 | 4 | 2.5 | - | 200 | -1 | -1 | 4 | 1 | 2.9 | - | 200 | -9 | -4 | -4 | -6 | 2.9 | - |
| | | SA | 200 | -13 | -9 | -16 | -13 | 3.5 | - | 200 | -6 | -6 | -6 | -6 | 0 | - | 200 | -1 | -3 | -3 | -2 | 1.2 | - |

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 20μM and 200μM in this study.

Table 6A Judgement from the Phase 1 results: Final judgement of positive when positive results were obtained in at least one of three assays

20 μM

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| I - 1 | 5-FU | - | - | - | - | - | - | - | - | - | - | - | - | - |
| I - 2 | 8-MOP | - | - | - | - | - | - | - | - | - | - | - | - | - |
| I - 3 | Amiodarone [a] | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 4 | Chlorpromazine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 5 | Diclofenac | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 6 | Doxycycline | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 7 | Furosemide | + | + | + | + | + | + | - | + | - | - | + | + | + |
| I - 8 | Ketoprofen | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 9 | Levofloxacin | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 10 | Norfloxacin | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 11 | Omeprazole | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 12 | Quinine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 13 | Sulisobenzone | - | - | - | - | - | - | - | - | - | - | - | - | - |

200 μM

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| I - 1 | 5-FU | - | - | - | - | - | - | - | - | - | - | - | - | - |
| I - 2 | 8-MOP | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 3 | Amiodarone [a] | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 4 | Chlorpromazine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 5 | Diclofenac | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 6 | Doxycycline | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 7 | Furosemide | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 8 | Ketoprofen | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 9 | Levofloxacin | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 10 | Norfloxacin | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 11 | Omeprazole | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 12 | Quinine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| I - 13 | Sulisobenzone | - | - | - | - | - | - | - | - | - | - | - | - | - |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20)
- : negative(Singlet oxygen results <25 and Superoxide 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 20μM and 200μM in this study.
b : Integrated judgements were made by the majority of each laboratory's final judgement.

# Table 6B Judgement from the Phase 1 results: Final judgement based on the mean value of three assays

## 20 μM

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

## 200 μM

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

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20)
- : negative(Singlet oxygen results <25 and Superoxide 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 20μM and 200μM in this study.
b : Integrated judgements were made by the majority of each laboratory's final judgement.

# Table 6C Judgement from the Phase 1 results: Final judgement based on the majority of three assay results

## 20 μM

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

## 200 μM

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

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20)
- : negative(Singlet oxygen results <25 and Superoxide 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 20μM and 200μM in this study.
b : Integrated judgements were made by the majority of each laboratory's final judgement.

# Table 6D Judgement from the Phase 1 results: Final judgement based on the first assay results

## 20 μM

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

## 200 μM

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

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20)
- : negative(Singlet oxygen results <25 and Superoxide 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 20μM and 200μM in this study.
b : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 7A Contingency table for the Phase 1 results at 20 μM: Final judgement 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 μM**

| Lab 1 | | 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 2 | | 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 3 | | 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 Judgement | | 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 7B Contingency table for the Phase 1 results at 20 μM: Final judgement based on the mean value of three assays

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

**Concentration: 20 μM**

| Lab 1 | | 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 2 | | 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 3 | | 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 Judgement | | 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 7C Contingency table for the Phase 1 results at 20 μM: Final judgement based on the majority of three assay results

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

**Concentration: 20 μM**

| Lab 1 | | 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 2 | | 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 3 | | 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 Judgement | | 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 7D Contingency table for the Phase 1 results at 20 μM: Final judgement based on the first assay results

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

**Concentration: 20 μM**

| Lab 1 | | 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 2 | | 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 3 | | 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 Judgement | | 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 7E Contingency table for the Phase 1 results at 200 μM: The final judgements were the same in all of the analysis methods

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

**Concentration: 200 μM**

| Lab 1 | | 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 2 | | 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 3 | | 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 Judgement | | 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 Irradiance and temperature during the irradiation in the Phase 2 study

| | | | Lab 1 | | | | | Lab 2 | | | | | Lab 3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mean | SD | CV (%) | Max | Min | Mean | SD | CV (%) | Max | Min | Mean | SD | CV (%) | Max | Min |
| Irradiance ($mW/cm^2$) | Beginning of Irradiation | A | 1.8 | 0 | - | 1.8 | 1.8 | 1.4 | 0.01 | 0.7 | 1.4 | 1.4 | 1.2 | 0.06 | 5.0 | 1.2 | 1.0 |
| | | B | 2.1 | 0 | - | 2.1 | 2.1 | 1.9 | 0.05 | 2.6 | 2.0 | 1.8 | 2.1 | 0.12 | 5.7 | 2.2 | 1.8 |
| | End of Irradiation | A | 1.8 | 0 | - | 1.8 | 1.8 | 1.4 | 0.01 | 0.7 | 1.5 | 1.4 | 1.2 | 0.05 | 4.2 | 1.2 | 1.1 |
| | | B | 2.1 | 0 | - | 2.1 | 2.1 | 1.9 | 0.06 | 3.2 | 2.0 | 1.8 | 2.1 | 0.10 | 4.8 | 2.2 | 2.0 |
| Temperature (ºC) | Beginning of Irradiation | | 26.3 | 1.46 | 5.6 | 29 | 24 | 24.5 | 0.55 | 2.2 | 25.4 | 22.8 | 24.6 | 1.21 | 4.9 | 27.6 | 21.9 |
| | End of Irradiation | | 26.1 | 1.58 | 6.1 | 29 | 24 | 25.0 | 0.12 | 0.5 | 25.2 | 24.8 | 25.1 | 1.43 | 5.7 | 28.3 | 23.0 |

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. Hőnle 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 9 Within-laboratory variation of Phase 2 study

| ROS assay Validation data (atlas) | | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | | N | Mean | SD | CV (%) | N | Mean | SD | CV (%) | N | Mean | SD | CV (%) |
| Intra-day | | | | | | | | | | | | | | |
| Positive Control | Quinine | SO | 7 | 553 | 14.4 | 2.6 | 6 | 438 | 10.7 | 2.4 | 3 | 366 | 5.0 | 1.4 |
| | | SA | 7 | 424 | 30.8 | 7.3 | 6 | 305 | 11.7 | 3.8 | 3 | 306 | 26.7 | 8.7 |
| Negative Control | Sulisobenzone | SO | 7 | 5 | 9.6 | - | 6 | 0 | 3.2 | - | 3 | -2 | 1.2 | - |
| | | SA | 7 | -13 | 6.6 | - | 6 | -12 | 3.0 | - | 3 | -5 | 0 | - |
| Inter-day | | | | | | | | | | | | | | |
| Positive Control | Quinine | SO | 5 | 532 | 11.9 | 2.2 | 8 | 430 | 6.1 | 1.4 | 11 | 359 | 9.8 | 2.7 |
| | | SA | 5 | 408 | 8.4 | 2.1 | 8 | 276 | 24.2 | 8.8 | 11 | 295 | 15.9 | 5.4 |
| Negative Control | Sulisobenzone | SO | 5 | 2 | 4.8 | - | 8 | 1 | 3.3 | - | 11 | 0 | 1.7 | - |
| | | SA | 5 | -14 | 7.8 | - | 8 | -11 | 3.3 | - | 11 | -6 | 1.2 | - |

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 based on using the assay results of the first assay of the each assay day.

# Table 10 Between-laboratory variation of Phase 2 study

| ROS assay Validation data (atlas) | | | | | | |
|---|---|---|---|---|---|---|
| Chemicals | | | N | Mean | SD | CV (%) |
| Positive Control | Quinine | SO | 3 | 445 | 91.8 | 20.6 |
| | | SA | 3 | 323 | 64.9 | 20.1 |
| Negative Control | Sulisobenzone | SO | 3 | 2 | 2.6 | - |
| | | SA | 3 | -11 | 5.3 | - |

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

# Table 11 Results of the ROS assay multi-center validation Phase 2 study

| ROS assay Validation data (atlas) | | | Lab 1 | | | | | | | Lab 2 | | | | | | | Lab 3 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | | Conc (μM) | Assay | | | Mean | SD | CV (%) | Conc (μM) | Assay | | | Mean | SD | CV (%) | Conc (μM) | Assay | | | Mean | SD | CV (%) |
| N0. | Name | | | 1st | 2nd | 3rd | | | | | 1st | 2nd | 3rd | | | | | 1st | 2nd | 3rd | | | |
| II - 1 | Acridine | SO | 200 | 225 | 233 | 222 | 227 | 5.7 | 2.51 | 200 | 229 | 218 | 214 | 220 | 7.8 | 3.53 | 200 | 223 | 221 | 223 | 222 | 1.2 | 0.52 |
| | | SA | 200 | 228 | 231 | 215 | 225 | 8.5 | 3.79 | 200 | 191 | 209 | 190 | 197 | 10.7 | 5.44 | 200 | 192 | 172 | 168 | 177 | 12.9 | 7.25 |
| II - 2 | Acridine HCl | SO | 200 | 224 | 214 | 223 | 220 | 5.5 | 2.5 | 200 | 226 | 216 | 224 | 222 | 5.3 | 2.38 | 200 | 215 | 215 | 218 | 216 | 1.7 | 0.8 |
| | | SA | 200 | 211 | 215 | 215 | 214 | 2.3 | 1.08 | 200 | 198 | 212 | 181 | 197 | 15.5 | 7.88 | 200 | 176 | 181 | 164 | 174 | 8.7 | 5.03 |
| II - 3 | Amiodarone HCl | SO | 2 | 33 | 9 | 22 | 21 | 12 | - | 2 | 25 | 38 | 28 | 30 | 6.8 | 22.44 | 2 | 36 | 51 | 48 | 45 | 7.9 | 17.64 |
| | | SA | 2 | -16 | -13 | -5 | -11 | 5.7 | - | 2 | 3 | 5 | -5 | 1 | 5.3 | - | 2 | 0 | 2 | 0 | 1 | 1.2 | - |
| II - 4 | Chlorpromazine HCl | SO | 200 | -18 | -41 | -15 | -25 | 14.2 | - | 200 | -2 | -13 | -6 | -7 | 5.6 | - | 200 | -22 | -32 | -23 | -26 | 5.5 | - |
| | | SA | 200 | 84 | 106 | 97 | 96 | 11.1 | 11.56 | 200 | 97 | 99 | 109 | 102 | 6.4 | 6.32 | 200 | 87 | 84 | 78 | 83 | 4.6 | 5.52 |
| II - 5 | Doxycycline HCl | SO | 200 | 271 | 269 | 262 | 267 | 4.7 | 1.77 | 200 | 222 | 234 | 222 | 226 | 6.9 | 3.07 | 200 | 166 | 160 | 160 | 162 | 3.5 | 2.14 |
| | | SA | 200 | 353 | 486 | 342 | 394 | 80.2 | 20.36 | 200 | 413 | 437 | 411 | 420 | 14.5 | 3.44 | 200 | 247 | 249 | 261 | 252 | 7.6 | 3 |
| II - 6 | Fenofibrate | SO | 20 | 124 | 202 | 173 | 166 | 39.4 | 23.7 | 2 | 5 | 18 | 11 | 11 | 6.5 | - | 20 | 161 | 161 | 161 | 161 | 0 | 0 |
| | | SA | 20 | 0 | -39 | -31 | -23 | 20.6 | - | 2 | -6 | 17 | -9 | 1 | 14.2 | - | 20 | -9 | -12 | -12 | -11 | 1.7 | - |
| II - 7 | Furosemide | SO | 200 | 227 | 238 | 224 | 230 | 7.4 | 3.21 | 200 | 138 | 131 | 133 | 134 | 3.6 | 2.69 | 200 | 145 | 146 | 144 | 145 | 1 | 0.69 |
| | | SA | 200 | 115 | 121 | 102 | 113 | 9.7 | 8.62 | 200 | 62 | 67 | 67 | 65 | 2.9 | 4.42 | 200 | 52 | 50 | 54 | 52 | 2 | 3.85 |
| II - 8 | Ketoprofen | SO | 200 | 358 | 362 | 368 | 363 | 5 | 1.39 | 200 | 245 | 259 | 240 | 248 | 9.8 | 3.97 | 200 | 224 | 220 | 206 | 217 | 9.5 | 4.36 |
| | | SA | 200 | 130 | 122 | 137 | 130 | 7.5 | 5.79 | 200 | 107 | 117 | 109 | 111 | 5.3 | 4.77 | 200 | 80 | 88 | 87 | 85 | 4.4 | 5.13 |
| II - 9 | 6-methylcoumarine | SO | 200 | 114 | 111 | 120 | 115 | 4.6 | 3.98 | 200 | 103 | 122 | 142 | 122 | 19.5 | 15.94 | 200 | 106 | 96 | 99 | 100 | 5.1 | 5.11 |
| | | SA | 200 | 109 | 128 | 130 | 122 | 11.6 | 9.47 | 200 | 87 | 110 | 100 | 99 | 11.5 | 11.65 | 200 | 62 | 70 | 67 | 66 | 4 | 6.09 |
| II - 10 | 8-MOP | SO | 200 | 83 | 101 | 78 | 87 | 12.1 | 13.85 | 200 | 81 | 79 | 60 | 73 | 11.6 | 15.8 | 200 | 65 | 77 | 70 | 71 | 6 | 8.53 |
| | | SA | 200 | 76 | 138 | 113 | 109 | 31.2 | 28.62 | 200 | 87 | 103 | 92 | 94 | 8.2 | 8.71 | 200 | 23 | 30 | 31 | 28 | 4.4 | 15.57 |
| II - 11 | Nalidixic acid | SO | 200 | 348 | 185 | 182 | 238 | 95 | 39.85 | 200 | 147 | 144 | 145 | 145 | 1.5 | 1.05 | 200 | 134 | 130 | 119 | 128 | 7.8 | 6.08 |
| | | SA | 200 | 355 | 271 | 264 | 297 | 50.6 | 17.07 | 200 | 254 | 206 | 252 | 237 | 27.2 | 11.44 | 200 | 294 | 356 | 314 | 321 | 31.6 | 9.85 |
| II - 12 | Nalidixic acid (Na salt) | SO | 200 | 183 | 165 | 183 | 177 | 10.4 | 5.87 | 200 | 144 | 140 | 149 | 144 | 4.5 | 3.12 | 200 | 125 | 124 | 119 | 123 | 3.2 | 2.62 |
| | | SA | 200 | 287 | 233 | 203 | 241 | 42.6 | 17.66 | 200 | 218 | 192 | 211 | 207 | 13.5 | 6.5 | 200 | 299 | 341 | 320 | 320 | 21 | 6.56 |
| II - 13 | Norfloxacin | SO | 200 | 215 | 219 | 214 | 216 | 2.6 | 1.22 | 200 | 188 | 222 | 200 | 203 | 17.2 | 8.48 | 200 | 164 | 171 | 169 | 168 | 3.6 | 2.15 |
| | | SA | 200 | 145 | 139 | 113 | 132 | 17 | 12.85 | 200 | 132 | 120 | 119 | 124 | 7.2 | 5.85 | 200 | 149 | 149 | 139 | 146 | 5.8 | 3.96 |
| II - 14 | Ofloxacin | SO | 200 | 193 | 203 | 192 | 196 | 6.1 | 3.1 | 200 | 132 | 137 | 149 | 139 | 8.7 | 6.27 | 200 | 126 | 125 | 117 | 123 | 4.9 | 4.02 |
| | | SA | 200 | 351 | 228 | 274 | 284 | 62.1 | 21.86 | 200 | 288 | 292 | 292 | 291 | 2.3 | 0.79 | 200 | 420 | 439 | 446 | 435 | 13.5 | 3.09 |
| II - 15 | Piroxicam | SO | 200 | 191 | 210 | 232 | 211 | 20.5 | 9.72 | 200 | 207 | 230 | 221 | 219 | 11.6 | 5.28 | 200 | 168 | 160 | 153 | 160 | 7.5 | 4.68 |
| | | SA | 20 | 28 | 38 | 26 | 31 | 6.4 | 20.96 | 20 | 56 | 47 | 43 | 49 | 6.7 | 13.68 | 20 | 4 | 3 | 7 | 5 | 2.1 | - |
| II - 16 | Promethazine HCl | SO | 200 | 70 | 62 | 84 | 72 | 11.1 | 15.47 | 200 | 91 | 89 | 103 | 94 | 7.6 | 8.03 | 200 | 43 | 46 | 39 | 43 | 3.5 | 8.23 |
| | | SA | 200 | 67 | 86 | 86 | 80 | 11 | 13.77 | 200 | 43 | 51 | 48 | 47 | 4 | 8.54 | 200 | 35 | 33 | 36 | 35 | 1.5 | 4.41 |
| II - 17 | Rosiglitazone | SO | 200 | 118 | 104 | 131 | 118 | 13.5 | 11.48 | 200 | 90 | 87 | 93 | 90 | 3 | 3.33 | 200 | 57 | 54 | 54 | 55 | 1.7 | 3.15 |
| | | SA | 20 | 41 | 36 | 29 | 35 | 6 | 17.06 | 20 | 27 | 29 | 35 | 30 | 4.2 | 13.73 | 20 | 15 | 17 | 15 | 16 | 1.2 | - |
| II - 18 | Tetracycline | SO | 200 | 200 | 194 | 200 | 198 | 3.5 | 1.75 | 200 | 167 | 160 | 166 | 164 | 3.8 | 2.3 | 200 | 134 | 129 | 129 | 131 | 2.9 | 2.21 |
| | | SA | 200 | 197 | 216 | 218 | 210 | 11.6 | 5.51 | 200 | 240 | 255 | 239 | 245 | 9 | 3.66 | 200 | 146 | 101 | 123 | 123 | 22.5 | 18.24 |

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.

# Table 11 Results of the ROS assay multi-center validation Phase 2 study (continued)

| ROS assay Validation data (atlas) | | | Lab 1 | | | | | | | Lab 2 | | | | | | | Lab 3 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | | Conc (μM) | Assay | | | Mean | SD | CV (%) | Conc (μM) | Assay | | | Mean | SD | CV (%) | Conc (μM) | Assay | | | Mean | SD | CV (%) |
| N0. | Name | | | 1st | 2nd | 3rd | | | | | 1st | 2nd | 3rd | | | | | 1st | 2nd | 3rd | | | |
| II - 19 | Anthracene | SO | 20 | 261 | 340 | 272 | 291 | 42.8 | 14.7 | 2 | -2 | 7 | 6 | 4 | 4.9 | - | 2 | 5 | 3 | 4 | 4 | 1 | - |
| | | SA | 2 | 39 | 48 | 1 | 29 | 24.9 | 85.05 | 2 | 3 | 8 | -3 | 3 | 5.5 | - | 2 | 4 | 4 | 4 | 4 | 0 | - |
| II - 20 | Avobenzone | SO | 20 | 142 | 121 | 117 | 127 | 13.4 | 10.6 | 2 | 0 | 12 | 10 | 7 | 6.4 | - | 20 | 58 | 60 | 65 | 61 | 3.6 | 5.91 |
| | | SA | 2 | 52 | 19 | 32 | 34 | 16.6 | 48.42 | 2 | 29 | 36 | 25 | 30 | 5.6 | 18.56 | 20 | 13 | 9 | 18 | 13 | 4.5 | - |
| II - 21 | Bithionol | SO | 200 | 81 | 113 | 114 | 103 | 18.8 | 18.28 | 200 | 137 | 143 | 140 | 140 | 3 | 2.14 | 200 | 81 | 68 | 72 | 74 | 6.7 | 9.04 |
| | | SA | 20 | 21 | 28 | 24 | 24 | 3.5 | 14.43 | 2 | 13 | 15 | 15 | 14 | 1.2 | - | 200 | 34 | 30 | 33 | 32 | 2.1 | 6.44 |
| II - 22 | Hexachlorophene | SO | 200 | 318 | 333 | 355 | 335 | 18.6 | 5.55 | 200 | 246 | 255 | 258 | 253 | 6.2 | 2.47 | 200 | 227 | 226 | 217 | 223 | 5.5 | 2.47 |
| | | SA | 200 | 6 | 22 | 7 | 12 | 9 | - | 2 | -6 | 15 | -3 | 2 | 11.4 | - | 200 | 10 | 8 | 6 | 8 | 2 | - |
| II - 23 | Rose bengal | SO | 200 | 682 | 685 | 679 | 682 | 3 | 0.44 | 200 | 631 | 634 | 667 | 644 | 20 | 3.1 | 200 | 608 | 589 | 607 | 601 | 10.7 | 1.78 |
| | | SA | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| II - 24 | Aspirin | SO | 200 | 1 | 2 | 4 | 2 | 1.5 | - | 200 | 1 | -1 | 1 | 0 | 1.2 | - | 200 | -2 | 3 | 1 | 1 | 2.5 | - |
| | | SA | 200 | -12 | -15 | 10 | -6 | 13.7 | - | 200 | 0 | -6 | -4 | -3 | 3.1 | - | 200 | -1 | -1 | 0 | -1 | 0.6 | - |
| II - 25 | Benzocaine | SO | 200 | 6 | 3 | -2 | 2 | 4 | - | 200 | 9 | 1 | -12 | -1 | 10.6 | - | 200 | 0 | 4 | 3 | 2 | 2.1 | - |
| | | SA | 200 | 9 | 20 | 0 | 10 | 10 | - | 200 | -4 | 12 | 6 | 5 | 8.1 | - | 200 | 0 | 1 | 0 | 0 | 0.6 | - |
| II - 26 | Erythromycin | SO | 200 | -16 | 5 | 8 | -1 | 13.1 | - | 200 | 6 | -1 | 13 | 6 | 7 | - | 200 | 0 | -4 | -3 | -2 | 2.1 | - |
| | | SA | 200 | 4 | 8 | 6 | 6 | 2 | - | 200 | 14 | 4 | 35 | 18 | 15.8 | - | 200 | 1 | 4 | 2 | 2 | 1.5 | - |
| II – 27 | Penicillin G | SO | 200 | 17 | 6 | 9 | 11 | 5.7 | - | 200 | 0 | -1 | 4 | 1 | 2.6 | - | 200 | 0 | 5 | 4 | 3 | 2.6 | - |
| | | SA | 200 | 11 | 26 | 40 | 26 | 14.5 | 56.5 | 200 | 37 | 36 | 34 | 36 | 1.5 | 4.28 | 200 | 4 | 11 | 16 | 10 | 6 | - |
| II - 28 | Phenytoin | SO | 200 | 1 | 19 | 3 | 8 | 9.9 | - | 200 | 0 | -4 | 2 | -1 | 3.1 | - | 200 | 2 | 6 | 4 | 4 | 2 | - |
| | | SA | 200 | 63 | 55 | 32 | 50 | 16.1 | 32.19 | 200 | 52 | 53 | 48 | 51 | 2.6 | 5.19 | 200 | 35 | 17 | 26 | 26 | 9 | 34.62 |
| II - 29 | Bumetrizole | SO | 20 | -7 | -11 | -11 | -10 | 2.3 | - | 2 | -5 | 10 | 0 | 2 | 7.6 | - | 20 | -6 | -16 | -19 | -14 | 6.8 | - |
| | | SA | 2 | -8 | 9 | -6 | -2 | 9.3 | - | 2 | -7 | 0 | -7 | -5 | 4 | - | 20 | -2 | 2 | 9 | 3 | 5.6 | - |
| II - 30 | Camphor sulfonic acid | SO | 200 | 7 | -2 | 8 | 4 | 5.5 | - | 200 | -1 | 3 | 2 | 1 | 2.1 | - | 200 | 3 | 1 | 4 | 3 | 1.5 | - |
| | | SA | 200 | -4 | -12 | -22 | -13 | 9 | - | 200 | -4 | 5 | -2 | 0 | 4.7 | - | 200 | -4 | -1 | 0 | -2 | 2.1 | - |
| II - 31 | Chlorhexidine | SO | 200 | -5 | 8 | -12 | -3 | 10.1 | - | 200 | 11 | -9 | 8 | 3 | 10.8 | - | 200 | 23 | 22 | 22 | 22 | 0.6 | - |
| | | SA | 200 | 28 | 35 | 6 | 23 | 15.1 | 65.79 | 200 | 13 | 21 | 16 | 17 | 4 | - | 200 | 13 | 7 | 10 | 10 | 3 | - |
| II - 32 | Cinnamic acid | SO | 200 | 6 | 0 | 0 | 2 | 3.5 | - | 200 | 5 | 0 | -8 | -1 | 6.6 | - | 200 | 0 | -1 | 2 | 0 | 1.5 | - |
| | | SA | 200 | 61 | 70 | 36 | 56 | 17.6 | 31.65 | 200 | 52 | 36 | 37 | 42 | 9 | 21.51 | 200 | 9 | 10 | 7 | 9 | 1.5 | - |
| II - 33 | Drometrizole | SO | 20 | 7 | -4 | -7 | -1 | 7.4 | - | 20 | 2 | 6 | 8 | 5 | 3.1 | - | 2 | 8 | 2 | 4 | 5 | 3.1 | - |
| | | SA | 20 | -11 | 13 | -8 | -2 | 13.1 | - | 20 | 8 | 3 | 2 | 4 | 3.2 | - | 20 | 6 | 7 | 7 | 7 | 0.6 | - |
| II - 34 | L-Histidine | SO | 200 | 12 | 15 | 7 | 11 | 4 | - | 200 | 4 | 9 | -3 | 3 | 6 | - | 200 | 4 | 3 | 4 | 4 | 0.6 | - |
| | | SA | 200 | 61 | 73 | 51 | 62 | 11 | 17.86 | 200 | 55 | -4 | 68 | 40 | 38.4 | 96.73 | 200 | 51 | 48 | 48 | 49 | 1.7 | 3.53 |
| II - 35 | Methylbenzylidene camphor | SO | 20 | -7 | -3 | -4 | -5 | 2.1 | - | 20 | -3 | -1 | -1 | -2 | 1.2 | - | 20 | -4 | -3 | -8 | -5 | 2.6 | - |
| | | SA | 20 | -6 | 5 | -4 | -2 | 5.9 | - | 20 | 4 | 7 | -5 | 2 | 6.2 | - | 20 | -2 | -3 | -1 | -2 | 1 | - |
| II - 36 | Octrizole | SO | 2 | 4 | -6 | 1 | 0 | 5.1 | - | 2 | -3 | 3 | -3 | -1 | 3.5 | - | 20 | -13 | -11 | -17 | -14 | 3.1 | - |
| | | SA | 2 | 56 | 47 | 22 | 42 | 17.6 | 42.28 | 2 | 2 | 20 | 6 | 9 | 9.5 | - | 20 | 4 | 6 | 11 | 7 | 3.6 | - |
| II - 37 | Octyl methacrylate | SO | 200 | 17 | 26 | 52 | 32 | 18.2 | 57.39 | 20 | 1 | -1 | 4 | 1 | 2.5 | - | 20 | 10 | 4 | 6 | 7 | 3.1 | - |
| | | SA | 200 | -3 | -25 | -38 | -22 | 17.7 | - | 20 | -6 | -1 | -7 | -5 | 3.2 | - | 20 | 0 | 0 | -1 | 0 | 0.6 | - |
| II - 38 | Octyl methoxycinnamate | SO | 20 | 6 | 2 | 5 | 4 | 2.1 | - | 2 | -4 | 6 | 2 | 1 | 5 | - | 20 | 3 | 2 | 4 | 3 | 1 | - |
| | | SA | 20 | -17 | -15 | -11 | -14 | 3.1 | - | 2 | -5 | 2 | 1 | -1 | 3.8 | - | 20 | -5 | -5 | -4 | -5 | 0.6 | - |
| II - 39 | Octyl salicylate | SO | 20 | 0 | 14 | 2 | 5 | 7.6 | - | 20 | 1 | 7 | 7 | 5 | 3.5 | - | 20 | -3 | -3 | -3 | -3 | 0 | - |
| | | SA | 20 | 12 | 2 | 1 | 5 | 6.1 | - | 20 | 7 | 2 | -4 | 2 | 5.5 | - | 20 | 0 | 0 | 0 | 0 | 0 | - |
| II - 40 | PABA | SO | 200 | 18 | -8 | 5 | 5 | 13 | - | 200 | 2 | 2 | 7 | 4 | 2.9 | - | 200 | -5 | -3 | 3 | -2 | 4.2 | - |
| | | SA | 200 | 10 | -5 | 8 | 4 | 8.1 | - | 200 | -9 | -2 | -1 | -4 | 4.4 | - | 200 | -1 | 0 | -1 | -1 | 0.6 | - |
| II - 41 | SDS | SO | 200 | 18 | 17 | 15 | 17 | 1.5 | - | 200 | 5 | 8 | 12 | 8 | 3.5 | - | 200 | 5 | 5 | 8 | 6 | 1.7 | - |
| | | SA | 20 | 16 | 5 | 3 | 8 | 7 | - | 2 | 1 | -4 | 1 | -1 | 2.9 | - | 200 | 5 | 6 | 14 | 8 | 4.9 | - |
| II - 42 | UV-571 | SO | 20 | -16 | -19 | -15 | -17 | 2.1 | - | 2 | -10 | 7 | -4 | -2 | 8.6 | - | 2 | -5 | -4 | -4 | -4 | 0.6 | - |
| | | SA | 2 | 10 | 4 | 15 | 10 | 5.5 | - | 2 | 2 | 8 | 0 | 3 | 4.2 | - | 2 | 1 | 2 | 1 | 1 | 0.6 | - |

SO : Singlet oxygen SA : Superoxide anion ND : no data

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

Table 12A Judgement from the Phase 2 results: Final judgement of positive when positive results were obtained in at least one of three assays

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | + | 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 |
| II - 20 | Avobenzone | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 12A Judgement from the Phase 2 results: Final judgement of positive when positive results were obtained in at least one of three assays (continued)

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | + | + | - | + | - | + | - | + | - | - | - | - | + |
| II - 32 | Cinnamic acid | + | + | + | + | + | + | + | + | - | - | - | - | + |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | + | + | + | + | I | + | I | + | I | I | I | 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 |
| 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 |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

# Table 12B Judgement from the Phase 2 results: Final judgement based on the mean value of three assays

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | + | 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 |
| II - 20 | Avobenzone | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 12B Judgement from the Phase 2 results: Final judgement based on the mean value of three assays (continued)

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | + | + | - | + | - | + | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | + | + | + | + | + | + | + | + | - | - | - | - | + |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | + | + | + | + | I | + | I | I | I | I | I | I | 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 |
| 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 |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 12C Judgement from the Phase 2 results: Final judgement based on the majority of three assay results

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | + | 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 |
| II - 20 | Avobenzone | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 12C Judgement from the Phase 2 results: Final judgement based on the majority of three assay results (continued)

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | + | + | - | + | - | + | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | + | + | + | + | + | + | + | + | - | - | - | - | + |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | + | + | + | + | I | + | I | I | I | I | I | I | 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 |
| 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 |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 12D Judgement from the Phase 2 results: Final judgement based on the first assay results

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | + | 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 |
| II - 20 | Avobenzone | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 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 judgements were made by the majority of each laboratory's final judgement.

Table 12D Judgement from the Phase 2 results: Final judgement based on the first assay results (continued)

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | + | + | - | + | - | + | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | + | + | + | + | + | + | + | + | - | - | - | - | + |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | + | + | + | + | I | + | I | I | I | I | I | I | 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 |
| 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 |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 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 judgements were made by the majority of each laboratory's final judgement.

Table 13A Contingency table for Phase 2 results: Final judgement 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 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 23 | 0 | (0) | 23(23) |
| | - | 8 | 4 | (7) | 12(19) |
| Total | | 31 | 4 | (7) | 35(42) |

Sensitivity : 100% (23/23)
Specificity : 33.3% (4/12)
Positive predictivity : 74.2% (23/31)
Negative predictivity : 100% (4/4)
Accuracy : 77.1% (27/35)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 21 | 0 | (2) | 21(23) |
| | - | 7 | 4 | (8) | 11(19) |
| Total | | 28 | 4 | (10) | 32(42) |

Sensitivity : 100% (21/21)
Specificity : 36.4% (4/11)
Positive predictivity : 75.0% (21/28)
Negative predictivity : 100% (4/4)
Accuracy : 78.1% (25/32)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 2 | 9 | (8) | 11(19) |
| Total | | 24 | 9 | (9) | 33(42) |

Sensitivity : 100% (22/22)
Specificity : 81.8% (9/11)
Positive predictivity : 91.7% (22/24)
Negative predictivity : 100% (9/9)
Accuracy : 93.9% (31/33)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 6 | 5 | (8) | 11(19) |
| Total | | 28 | 5 | (9) | 33(42) |

Sensitivity : 100% (22/22)
Specificity : 45.5% (5/11)
Positive predictivity : 78.6% (22/28)
Negative predictivity : 100% (5/5)
Accuracy : 81.8% (27/33)

Table 13B Contingency table for Phase 2 results: Final judgement based on the mean value of three assays

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 7 | 5 | (7) | 12(19) |
| Total | | 29 | 5 | (8) | 34(42) |

Sensitivity : 100% (22/22)
Specificity : 41.7% (5/12)
Positive predictivity : 75.9% (22/29)
Negative predictivity : 100% (5/5)
Accuracy : 79.4% (27/34)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 21 | 0 | (2) | 21(23) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 25 | 6 | (11) | 31(42) |

Sensitivity : 100% (21/21)
Specificity : 60.0% (6/10)
Positive predictivity : 84.0% (21/25)
Negative predictivity : 100% (6/6)
Accuracy : 87.1% (27/31)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 2 | 9 | (8) | 11(19) |
| Total | | 24 | 9 | (9) | 33(42) |

Sensitivity : 100% (22/22)
Specificity : 81.8% (9/11)
Positive predictivity : 91.7% (22/24)
Negative predictivity : 100% (9/9)
Accuracy : 93.9% (31/33)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 26 | 6 | (10) | 32(42) |

Sensitivity : 100% (22/22)
Specificity : 60.0% (6/10)
Positive predictivity : 84.6% (22/26)
Negative predictivity : 100% (6/6)
Accuracy : 87.5% (28/32)

Table 13C Contingency table for Phase 2 results: Final judgement based on the majority of three assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 7 | 5 | (7) | 12(19) |
| Total | | 29 | 5 | (8) | 34(42) |

Sensitivity : 100% (22/22)
Specificity : 41.7% (5/12)
Positive predictivity : 75.9% (22/29)
Negative predictivity : 100% (5/5)
Accuracy : 79.4% (27/34)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 21 | 0 | (2) | 21(23) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 25 | 6 | (11) | 31(42) |

Sensitivity : 100% (21/21)
Specificity : 60.0% (6/10)
Positive predictivity : 84.0% (21/25)
Negative predictivity : 100% (6/6)
Accuracy : 87.1% (27/31)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 2 | 9 | (8) | 11(19) |
| Total | | 24 | 9 | (9) | 33(42) |

Sensitivity : 100% (22/22)
Specificity : 81.8% (9/11)
Positive predictivity : 91.7% (22/24)
Negative predictivity : 100% (9/9)
Accuracy : 93.9% (31/33)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 26 | 6 | (10) | 32(42) |

Sensitivity : 100% (22/22)
Specificity : 60.0% (6/10)
Positive predictivity : 84.6% (22/26)
Negative predictivity : 100% (6/6)
Accuracy : 87.5% (28/32)

Table 13D Contingency table for Phase 2 results: Final judgement based on the first assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 23 | 0 | (0) | 23(23) |
| | - | 5 | 7 | (7) | 12(19) |
| Total | | 28 | 7 | (7) | 35(42) |

Sensitivity : 100% (23/23)
Specificity : 58.3% (7/12)
Positive predictivity : 82.1% (23/28)
Negative predictivity : 100% (7/7)
Accuracy : 85.7% (30/35)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 21 | 0 | (2) | 21(23) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 25 | 6 | (11) | 31(42) |

Sensitivity : 100% (21/21)
Specificity : 60.0% (6/10)
Positive predictivity : 84.0% (21/25)
Negative predictivity : 100% (6/6)
Accuracy : 87.1% (27/31)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 2 | 9 | (8) | 11(19) |
| Total | | 24 | 9 | (9) | 33(42) |

Sensitivity : 100% (22/22)
Specificity : 81.8% (9/11)
Positive predictivity : 91.7% (22/24)
Negative predictivity : 100% (9/9)
Accuracy : 93.9% (31/33)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 22 | 0 | (1) | 22(23) |
| | - | 3 | 7 | (9) | 10(19) |
| Total | | 25 | 7 | (10) | 32(42) |

Sensitivity : 100% (22/22)
Specificity : 70.0% (7/10)
Positive predictivity : 88.0% (22/25)
Negative predictivity : 100% (7/7)
Accuracy : 90.6% (29/32)

Table 14A Judgement from the Phase 1 and 2 results: Final judgement of positive when positive results were obtained in at least one of three assays

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | + | 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 | Ketopofen | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 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 |
| II - 20 | Avobenzone | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 14A Judgement from the Phase 1 and 2 results: Final judgement of positive when positive results were obtained in at least one of three assays (continued)

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | + | + | - | + | - | + | - | + | - | - | - | - | + |
| II - 32 | Cinnamic acid | + | + | + | + | + | + | + | + | - | - | - | - | + |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | + | + | + | + | I | + | I | + | I | I | I | 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 |
| 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 |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 14B Judgement from the Phase 1 and 2 results: Final judgement based on the mean value of three assays

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | + | 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 | Ketopofen | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 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 |
| II - 20 | Avobenzone | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 14B Judgement from the Phase 1 and 2 results: Final judgement based on the mean value of three assays (continued)

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | + | + | - | + | - | + | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | + | + | + | + | + | + | + | + | - | - | - | - | + |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | + | + | + | + | I | + | I | I | I | I | I | I | 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 |
| 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 |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

# Table 14C Judgement from the Phase 1 and 2 results: Final judgement based on the majority of three assay results

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | + | 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 | Ketopofen | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 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 |
| II - 20 | Avobenzone | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 14C Judgement from the Phase 1 and 2 results: Final judgement based on the majority of three assay results (continued)

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | + | + | - | + | - | + | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | + | + | + | + | + | + | + | + | - | - | - | - | + |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | + | + | + | + | I | + | I | I | I | I | I | I | 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 |
| 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 |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 μM)
- : negative(Singlet oxygen results <25 and Superoxide results <20 at 200 μM)
I : inconclusive(The results does not meet the positive or negative criterion)
a : Integrated judgements were made by the majority of each laboratory's final judgement.
Compound Numbers from the phase1 study were shown in the brackets.

# Table 14D Judgement from the Phase 1 and 2 results: Final judgement based on the first assay results

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | + | 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 | Ketopofen | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 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 |
| II - 20 | Avobenzone | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 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 judgements were made by the majority of each laboratory's final judgement.
Compound Numbers from the phase1 study were shown in the brackets.

Table 14D Judgement from the Phase 1 and 2 results: Final judgement based on the first assay results (continued)

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | + | + | - | + | - | + | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | + | + | + | + | + | + | + | + | - | - | - | - | + |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | + | + | + | + | I | + | I | I | I | I | I | I | 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 |
| 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 |

+ : positive(Singlet oxygen results ≥25 or Superoxide results ≥20 at 200, 20 or 2 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 judgements were made by the majority of each laboratory's final judgement.
Compound Numbers from the phase1 study were shown in the brackets.

Table 15A Contingency table for Phase 1 and 2 results: Final judgement 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 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 26 | 1 | (0) | 27(27) |
| | - | 8 | 4 | (7) | 12(19) |
| Total | | 34 | 5 | (7) | 39(46) |

Sensitivity : 96.3% (26/27)
Specificity : 33.3% (4/12)
Positive predictivity : 76.5% (26/34)
Negative predictivity : 80.0% (4/5)
Accuracy : 76.9% (30/39)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 24 | 1 | (2) | 25(27) |
| | - | 7 | 4 | (8) | 11(19) |
| Total | | 31 | 5 | (10) | 36(46) |

Sensitivity : 96.0% (24/25)
Specificity : 36.4% (4/11)
Positive predictivity : 77.4% (24/31)
Negative predictivity : 80.0% (4/5)
Accuracy : 77.8% (28/36)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 2 | 9 | (8) | 11(19) |
| Total | | 27 | 10 | (9) | 37(46) |

Sensitivity : 96.2% (25/26)
Specificity : 81.8% (9/11)
Positive predictivity : 92.6% (25/27)
Negative predictivity : 90.0% (9/10)
Accuracy : 91.9% (34/37)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 6 | 5 | (8) | 11(19) |
| Total | | 31 | 6 | (9) | 37(46) |

Sensitivity : 96.2% (25/26)
Specificity : 45.5% (5/11)
Positive predictivity : 80.7% (25/31)
Negative predictivity : 83.3% (5/6)
Accuracy : 81.1% (30/37)

Table 15B Contingency table for Phase 1 and 2 results: Final judgement based on the mean value of three assays

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 7 | 5 | (7) | 12(19) |
| Total | | 32 | 6 | (8) | 38(46) |

Sensitivity : 96.2% (25/26)
Specificity : 41.7% (5/12)
Positive predictivity : 78.1% (25/32)
Negative predictivity : 83.3% (5/6)
Accuracy : 79.0% (30/38)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 24 | 1 | (2) | 25(27) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 28 | 7 | (11) | 35(46) |

Sensitivity : 96.0% (24/25)
Specificity : 60.0% (6/10)
Positive predictivity : 85.7% (24/28)
Negative predictivity : 85.7% (6/7)
Accuracy : 85.7% (30/35)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 2 | 9 | (8) | 11(19) |
| Total | | 27 | 10 | (9) | 37(46) |

Sensitivity : 96.2% (25/26)
Specificity : 81.8% (9/11)
Positive predictivity : 92.6% (25/27)
Negative predictivity : 90.0% (9/10)
Accuracy : 91.9% (34/37)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 29 | 7 | (10) | 36(46) |

Sensitivity : 96.2% (25/26)
Specificity : 60.0% (6/10)
Positive predictivity : 86.2% (25/29)
Negative predictivity : 85.7% (6/7)
Accuracy : 86.1% (31/36)

Table 15C Contingency table for Phase 1 and 2 results: Final judgement based on the majority of three assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 7 | 5 | (7) | 12(19) |
| Total | | 32 | 6 | (8) | 38(46) |

Sensitivity : 96.2% (25/26)
Specificity : 41.7% (5/12)
Positive predictivity : 78.1% (25/32)
Negative predictivity : 83.3% (5/6)
Accuracy : 79.0% (30/38)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 24 | 1 | (2) | 25(27) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 28 | 7 | (11) | 35(46) |

Sensitivity : 96.0% (24/25)
Specificity : 60.0% (6/10)
Positive predictivity : 85.7% (24/28)
Negative predictivity : 85.7% (6/7)
Accuracy : 85.7% (30/35)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 2 | 9 | (8) | 11(19) |
| Total | | 27 | 10 | (9) | 37(46) |

Sensitivity : 96.2% (25/26)
Specificity : 81.8% (9/11)
Positive predictivity : 92.6% (25/27)
Negative predictivity : 90.0% (9/10)
Accuracy : 91.9% (34/37)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 29 | 7 | (10) | 36(46) |

Sensitivity : 96.2% (25/26)
Specificity : 60.0% (6/10)
Positive predictivity : 86.2% (25/29)
Negative predictivity : 85.7% (6/7)
Accuracy : 86.1% (31/36)

Table 15D Contingency table for Phase 1 and 2 results: Final judgement based on the first assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 26 | 1 | (0) | 27(27) |
| | - | 5 | 7 | (7) | 12(19) |
| Total | | 31 | 8 | (7) | 39(46) |

Sensitivity : 96.3% (26/27)
Specificity : 58.3% (7/12)
Positive predictivity : 83.9% (26/31)
Negative predictivity : 87.5% (7/8)
Accuracy : 84.6% (33/39)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 24 | 1 | (2) | 25(27) |
| | - | 4 | 6 | (9) | 10(19) |
| Total | | 28 | 7 | (11) | 35(46) |

Sensitivity : 96.0% (24/25)
Specificity : 60.0% (6/10)
Positive predictivity : 85.7% (24/28)
Negative predictivity : 85.7% (6/7)
Accuracy : 85.7% (30/35)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 2 | 9 | (8) | 11(19) |
| Total | | 27 | 10 | (9) | 37(46) |

Sensitivity : 96.2% (25/26)
Specificity : 81.8% (9/11)
Positive predictivity : 92.6% (25/27)
Negative predictivity : 90.0% (9/10)
Accuracy : 91.9% (34/37)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | - | I | |
| Phototoxic | + | 25 | 1 | (1) | 26(27) |
| | - | 3 | 7 | (9) | 10(19) |
| Total | | 28 | 8 | (10) | 36(46) |

Sensitivity : 96.2% (25/26)
Specificity : 70.0% (7/10)
Positive predictivity : 89.3% (25/28)
Negative predictivity : 87.5% (7/8)
Accuracy : 88.9% (32/36)

# Table 16-1 Contingency table for Phase 1 results at 20 μM

**Concentration: 20 μM**

| Lab 1 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 83.3% (10/12) | 83.3% (10/12) | 83.3% (10/12) | 83.3% (10/12) |
| Specificity | 100% (1/1) | 100% (1/1) | 100% (1/1) | 100% (1/1) |
| Positive predictivity | 100% (10/10) | 100% (10/10) | 100% (10/10) | 100% (10/10) |
| Negative predictivity | 33.3% (1/3) | 33.3% (1/3) | 33.3% (1/3) | 33.3% (1/3) |
| Accuracy | 84.6% (11/13) | 84.6% (11/13) | 84.6% (11/13) | 84.6% (11/13) |

| Lab 2 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 83.3% (10/12) | 83.3% (10/12) | 83.3% (10/12) | 83.3% (10/12) |
| Specificity | 100% (1/1) | 100% (1/1) | 100% (1/1) | 100% (1/1) |
| Positive predictivity | 100% (10/10) | 100% (10/10) | 100% (10/10) | 100% (10/10) |
| Negative predictivity | 33.3% (1/3) | 33.3% (1/3) | 33.3% (1/3) | 33.3% (1/3) |
| Accuracy | 84.6% (11/13) | 84.6% (11/13) | 84.6% (11/13) | 84.6% (11/13) |

| Lab 3 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 83.3% (10/12) | 83.3% (10/12) | 75.0% (9/12) | 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% (9/9) | 100% (9/9) |
| Negative predictivity | 33.3% (1/3) | 33.3% (1/3) | 25.0% (1/4) | 25.0% (1/4) |
| Accuracy | 84.6% (11/13) | 84.6% (11/13) | 76.9% (10/13) | 76.9% (10/13) |

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

# Table 16-2 Contingency table for Phase 1 results at 200 μM

**Concentration: 200 μM**

| Lab 1 | Draft criteria for the final judgement [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 2 | Draft criteria for the final judgement [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 3 | Draft criteria for the final judgement [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 : Final judgement of positive when positive results were obtained in at least one of three assays.
B : Final judgement based on the mean value of three assays.
C : Final judgement based on the majority of three assay results
D : Final judgement based on the first assay results

Table 16-3 Contingency table for Phase 2 results

| Lab 1 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 100% (23/23) | 100% (22/22) | 100% (22/22) | 100% (23/23) |
| Specificity | 33.3% (4/12) | 41.7% (5/12) | 41.7% (5/12) | 58.3% (7/12) |
| Positive predictivity | 74.2% (23/31) | 75.9% (22/29) | 75.9% (22/29) | 82.1% (23/28) |
| Negative predictivity | 100% (4/4) | 100% (5/5) | 100% (5/5) | 100% (7/7) |
| Accuracy | 77.1% (27/35) | 79.4% (27/34) | 79.4% (27/34) | 85.7% (30/35) |

| Lab 2 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 100% (21/21) | 100% (21/21) | 100% (21/21) | 100% (21/21) |
| Specificity | 36.4% (4/11) | 60.0% (6/10) | 60.0% (6/10) | 60.0% (6/10) |
| Positive predictivity | 75.0% (21/28) | 84.0% (21/25) | 84.0% (21/25) | 84.0% (21/25) |
| Negative predictivity | 100% (4/4) | 100% (6/6) | 100% (6/6) | 100% (6/6) |
| Accuracy | 78.1% (25/32) | 87.1% (27/31) | 87.1% (27/31) | 87.1% (27/31) |

| Lab 3 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) |
| Specificity | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) |
| Positive predictivity | 91.7% (22/24) | 91.7% (22/24) | 91.7% (22/24) | 91.7% (22/24) |
| Negative predictivity | 100% (9/9) | 100% (9/9) | 100% (9/9) | 100% (9/9) |
| Accuracy | 93.9% (31/33) | 93.9% (31/33) | 93.9% (31/33) | 93.9% (31/33) |

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

Table 16-4 Contingency table for Phase 1 and 2 results

| Lab 1 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 96.3% (26/27) | 96.2% (25/26) | 96.2% (25/26) | 96.3% (26/27) |
| Specificity | 33.3% (4/12) | 41.7% (5/12) | 41.7% (5/12) | 58.3% (7/12) |
| Positive predictivity | 76.5% (26/34) | 78.1% (25/32) | 78.1% (25/32) | 83.9% (26/31) |
| Negative predictivity | 80.0% (4/5) | 83.3% (5/6) | 83.3% (5/6) | 87.5% (7/8) |
| Accuracy | 76.9% (30/39) | 79.0% (30/38) | 79.0% (30/38) | 84.6% (33/39) |

| Lab 2 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 96.0% (24/25) | 96.0% (24/25) | 96.0% (24/25) | 96.0% (24/25) |
| Specificity | 36.4% (4/11) | 60.0% (6/10) | 60.0% (6/10) | 60.0% (6/10) |
| Positive predictivity | 77.4% (24/31) | 85.7% (24/28) | 85.7% (24/28) | 85.7% (24/28) |
| Negative predictivity | 80.0% (4/5) | 85.7% (6/7) | 85.7% (6/7) | 85.7% (6/7) |
| Accuracy | 77.8% (28/36) | 85.7% (30/35) | 85.7% (30/35) | 85.7% (30/35) |

| Lab 3 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Sensitivity | 96.2% (25/26) | 96.2% (25/26) | 96.2% (25/26) | 96.2% (25/26) |
| Specificity | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) |
| Positive predictivity | 92.6% (25/27) | 92.6% (25/27) | 92.6% (25/27) | 92.6% (25/27) |
| Negative predictivity | 90.0% (9/10) | 90.0% (9/10) | 90.0% (9/10) | 90.0% (9/10) |
| Accuracy | 91.9% (34/37) | 91.9% (34/37) | 91.9% (34/37) | 91.9% (34/37) |

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

# Table 17 Contingency table for integrated judgement results

| Phase 1 (200 μM) | Draft criteria for the final judgement [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 2 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A [c] | B | C | D |
| Sensitivity | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) |
| Specificity | 45.5% (5/11) | 60.0% (6/10) | 60.0% (6/10) | 70.0% (7/10) |
| Positive predictivity | 78.6% (22/28) | 84.6% (22/26) | 84.6% (22/26) | 88.0% (22/25) |
| Negative predictivity | 100% (5/5) | 100% (6/6) | 100% (6/6) | 100% (7/7) |
| Accuracy | 81.8% (27/33) | 87.5% (28/32) | 87.5% (28/32) | 90.6% (29/32) |

| Phase 1 and 2 | Draft criteria for the final judgement [a] | | | |
|---|---|---|---|---|
| | A [d] | B | C | D |
| Sensitivity | 96.2% (25/26) | 96.2% (25/26) | 96.2% (25/26) | 96.2% (25/26) |
| Specificity | 45.5% (5/11) | 60.0% (6/10) | 60.0% (6/10) | 70.0% (7/10) |
| Positive predictivity | 80.7% (25/31) | 86.2% (25/29) | 86.2% (25/29) | 89.3% (25/28) |
| Negative predictivity | 83.3% (5/6) | 85.7% (6/7) | 85.7% (6/7) | 87.5% (7/8) |
| Accuracy | 81.1% (30/37) | 86.1% (31/36) | 86.1% (31/36) | 88.9% (32/36) |

Integrated judgements were made by the majority of each laboratory's final judgement.

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

b : See, tables 6A to 6D

c : See, tables 12A to 12D

d : See, tables 14A to 14D

Table 18A Secondary data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgement

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : Photoreactive (Singlet oxygen results ≥25 or Superoxide results ≥70 at 200 or 20 μM)
±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70 at 200 or 20 μM)
- : Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 or 20 μM)
(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I : inconclusive(The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 18A Secondary data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgement (continued)

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | ± | - | - | - | - | ± |
| II - 32 | Cinnamic acid | ± | + | ± | + | ± | ± | ± | ± | - | - | - | - | I |
| II - 33 | Drometrizole | - | - | - | - | - | - | - | - | (-) | (-) | (-) | (-) | - |
| II - 34 | L-Histidine | ± | + | ± | + | ± | - | ± | ± | ± | ± | ± | ± | ± |
| II - 35 | Methylbenzylidene camphor | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (±) | - | - | - | - | - |
| II - 37 | Octyl methacrylate | - | + | + | + | - | - | - | - | - | - | - | - | - |
| II - 38 | Octyl methoxycinnamate | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 39 | Octyl salicylate | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 40 | PABA | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 41 | SDS | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+ : Photoreactive (Singlet oxygen results ≥25 or Superoxide results ≥70 at 200 or 20 μM)
±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70 at 200 or 20 μM)
- : Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 or 20 μM)
(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I : inconclusive(The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 18B Secondary data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the mean value of three assays

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : Photoreactive (Singlet oxygen results ≥25 or Superoxide results ≥70 at 200 or 20 μM)
±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70 at 200 or 20 μM)
- : Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 or 20 μM)
(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I : inconclusive(The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 18B Secondary data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the mean value of three assays (continued)

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | - | - | - | - | - | - | - | - | (-) | (-) | (-) | (-) | - |
| II - 34 | L-Histidine | ± | + | ± | ± | ± | - | ± | ± | ± | ± | ± | ± | ± |
| II - 35 | Methylbenzylidene camphor | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | - | - | - | - | - |
| II - 37 | Octyl methacrylate | - | + | + | + | - | - | - | - | - | - | - | - | - |
| II - 38 | Octyl methoxycinnamate | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 39 | Octyl salicylate | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 40 | PABA | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 41 | SDS | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+ : Photoreactive (Singlet oxygen results ≥25 or Superoxide results ≥70 at 200 or 20 μM)
±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70 at 200 or 20 μM)
- : Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 or 20 μM)
(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I : inconclusive(The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 18C Secondary data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the majority of three assay results

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : Photoreactive (Singlet oxygen results ≥25 or Superoxide results ≥70 at 200 or 20 μM)
±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70 at 200 or 20 μM)
- : Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 or 20 μM)
(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I : inconclusive(The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 18C Secondary data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the majority of three assay results (continued)

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | - | - | - | - | - | - | - | - | (-) | (-) | (-) | (-) | - |
| II - 34 | L-Histidine | ± | + | ± | ± | ± | - | ± | ± | ± | ± | ± | ± | ± |
| II - 35 | Methylbenzylidene camphor | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | - | - | - | - | - |
| II - 37 | Octyl methacrylate | - | + | + | + | - | - | - | - | - | - | - | - | - |
| II - 38 | Octyl methoxycinnamate | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 39 | Octyl salicylate | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 40 | PABA | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 41 | SDS | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+ : Photoreactive (Singlet oxygen results ≥25 or Superoxide results ≥70 at 200 or 20 μM)
±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70 at 200 or 20 μM)
- : Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 or 20 μM)
(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I : inconclusive(The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 18D Secondary data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the first assay results

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| II - 21 | Bithionol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 22 | Hexachlorophene | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 23 | Rose bengal | + | + | + | + | + | + | + | + | + | + | + | + | + |

+ : Photoreactive (Singlet oxygen results ≥25 or Superoxide results ≥70 at 200 or 20 μM)
±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70 at 200 or 20 μM)
- : Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 or 20 μM)
(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I : inconclusive(The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 18D Secondary data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the first assay results (continued)

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | - | - | - | - | - | - | - | - | (-) | (-) | (-) | (-) | - |
| II - 34 | L-Histidine | ± | + | ± | ± | ± | - | ± | ± | ± | ± | ± | ± | ± |
| II - 35 | Methylbenzylidene camphor | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | - | - | - | - | - |
| II - 37 | Octyl methacrylate | - | + | + | - | - | - | - | - | - | - | - | - | - |
| II - 38 | Octyl methoxycinnamate | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 39 | Octyl salicylate | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 40 | PABA | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 41 | SDS | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+ : Photoreactive (Singlet oxygen results ≥25 or Superoxide results ≥70 at 200 or 20 μM)
±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70 at 200 or 20 μM)
- : Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 or 20 μM)
(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I : inconclusive(The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 19A Secondary 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 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 22 | 0 | 0 | 22[a] |
| | - | 3 | 4 | 9 | 16[b] |
| Total | | 25 | 4 | 9 | 38 |

a: One of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl).
b: 3 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 81.3% (13/16)
Positive predictivity : 88.0% (22/25) Negative predictivity : 100% (13/13)
Accuracy : 92.1% (35/38)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 56.3% (9/16)
Positive predictivity : 75.9%(22/29) Negative predictivity : 100% (9/9)
Accuracy : 81.6%(31/38)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 19 | 0 | 0 | 19[c] |
| | - | 0 | 6 | 8 | 14[d] |
| Total | | 19 | 6 | 8 | 33 |

c: 4 of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: 5 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (14/14)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (14/14)
Accuracy : 100% (33/33)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 57.1%(8/14)
Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (8/8)
Accuracy : 81.8%(27/33)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[e] |
| | - | 0 | 2 | 15 | 17[f] |
| Total | | 21 | 2 | 15 | 38 |

e: 2 of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Anthracene)
f: 2 of 19 non-phototoxic chemicals were not evaluated at20 or 200μM due to precipitation (Drometrizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (17/17)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (17/17)
Accuracy : 100% (38/38)

When the ±: Weakly photoreactive chemicals were defined as 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)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 22 | 0 | 0 | 22[g] |
| | - | 0 | 4 | 13 | 17[h] |
| Total | | 22 | 4 | 13 | 39 |

g: One of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl) .
h: 2 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Cinnamic acid, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 100% (17/17)
Positive predictivity : 100% (22/22) Negative predictivity : 100% (17/17)
Accuracy : 100% (39/39)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 76.5%(13/17)
Positive predictivity : 84.6%(22/26) Negative predictivity : 100% (13/13)
Accuracy : 89.7%(35/39)

Table 19B Secondary data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the mean value of three assays

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 22 | 0 | 0 | 22[a] |
| | - | 1 | 5 | 10 | 16[b] |
| Total | | 23 | 5 | 10 | 38 |

a: One of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl).
b: 3 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 93.8% (15/16)
Positive predictivity : 95.7% (22/23) Negative predictivity : 100% (15/15)
Accuracy : 97.4% (37/38)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 62.5% (10/16)
Positive predictivity : 78.6%(22/28) Negative predictivity : 100% (10/10)
Accuracy : 84.2%(32/38)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 19 | 0 | 0 | 19[c] |
| | - | 0 | 4 | 10 | 14[d] |
| Total | | 19 | 4 | 10 | 33 |

c: 4 of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: 5 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (14/14)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (14/14)
Accuracy : 100% (33/33)

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 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[e] |
| | - | 0 | 2 | 15 | 17[f] |
| Total | | 21 | 2 | 15 | 38 |

e: 2 of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Anthracene)
f: 2 of 19 non-phototoxic chemicals were not evaluated at20 or 200μM due to precipitation (Drometrizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (17/17)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (17/17)
Accuracy : 100% (38/38)

When the ±: Weakly photoreactive chemicals were defined as 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)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 22 | 0 | 0 | 22[g] |
| | - | 0 | 4 | 14 | 18[h] |
| Total | | 22 | 4 | 14 | 40 |

g: One of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl) .
h: One of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 100% (18/18)
Positive predictivity : 100% (22/22) Negative predictivity : 100% (18/18)
Accuracy : 100% (40/40)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 77.8%(14/18)
Positive predictivity : 84.6%(22/26) Negative predictivity : 100% (14/14)
Accuracy : 90.0%(36/40)

Table 19C Secondary data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the majority of three assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 22 | 0 | 0 | 22[a] |
| | - | 1 | 5 | 10 | 16[b] |
| Total | | 23 | 5 | 10 | 38 |

a: One of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl).
b: 3 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 93.8% (15/16)
Positive predictivity : 95.7% (22/23) Negative predictivity : 100% (15/15)
Accuracy : 97.4% (37/38)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 62.5% (10/16)
Positive predictivity : 78.6%(22/28) Negative predictivity : 100% (10/10)
Accuracy : 84.2%(32/38)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 19 | 0 | 0 | 19[c] |
| | - | 0 | 4 | 10 | 14[d] |
| Total | | 19 | 4 | 10 | 33 |

c: 4 of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: 5 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (14/14)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (14/14)
Accuracy : 100% (33/33)

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 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[e] |
| | - | 0 | 2 | 15 | 17[f] |
| Total | | 21 | 2 | 15 | 38 |

e: 2 of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Anthracene)
f: 2 of 19 non-phototoxic chemicals were not evaluated at20 or 200μM due to precipitation (Drometrizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (17/17)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (17/17)
Accuracy : 100% (38/38)

When the ±: Weakly photoreactive chemicals were defined as 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)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 22 | 0 | 0 | 22[g] |
| | - | 0 | 4 | 14 | 18[h] |
| Total | | 22 | 4 | 14 | 40 |

g: One of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl) .
h: One of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 100% (18/18)
Positive predictivity : 100% (22/22) Negative predictivity : 100% (18/18)
Accuracy : 100% (40/40)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 77.8%(14/18)
Positive predictivity : 84.6%(22/26) Negative predictivity : 100% (14/14)
Accuracy : 90.0%(36/40)

Table 19D Secondary data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the first assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 22 | 0 | 0 | 22[a] |
| | - | 0 | 4 | 12 | 16[b] |
| Total | | 22 | 4 | 12 | 38 |

a: One of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl).
b: 3 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 100% (16/16)
Positive predictivity : 100% (22/22) Negative predictivity : 100% (16/16)
Accuracy : 100% (38/38)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 75.0% (12/16)
Positive predictivity : 84.6%(22/26) Negative predictivity : 100% (12/12)
Accuracy : 89.5%(34/38)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 19 | 0 | 0 | 19[c] |
| | - | 0 | 4 | 10 | 14[d] |
| Total | | 19 | 4 | 10 | 33 |

c: 4 of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: 5 of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (14/14)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (14/14)
Accuracy : 100% (33/33)

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 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[e] |
| | - | 0 | 2 | 15 | 17[f] |
| Total | | 21 | 2 | 15 | 38 |

e: 2 of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Anthracene)
f: 2 of 19 non-phototoxic chemicals were not evaluated at20 or 200μM due to precipitation (Drometrizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (17/17)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (17/17)
Accuracy : 100% (38/38)

When the ±: Weakly photoreactive chemicals were defined as 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)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 22 | 0 | 0 | 22[g] |
| | - | 0 | 3 | 15 | 18[h] |
| Total | | 22 | 3 | 15 | 40 |

g: One of 23 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl) .
h: One of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 100% (18/18)
Positive predictivity : 100% (22/22) Negative predictivity : 100% (18/18)
Accuracy : 100% (40/40)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (22/22) Specificity : 83.3%(15/18)
Positive predictivity : 88.0%(22/25) Negative predictivity : 100% (15/15)
Accuracy : 92.5%(37/40)

Table 20 Secondary data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results

| Lab 1 | Draft criteria for the final judgement [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Original | | | | Data analysis based on the criteria for the secondary data analysis | | | | | | | |
| | | | | | 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% (23/23) | 100% (22/22) | 100% (22/22) | 100% (23/23) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) |
| Specificity | 33.3% (4/12) | 41.7% (5/12) | 41.7% (5/12) | 58.3% (7/12) | 81.3% (13/16) | 93.8% (15/16) | 93.8% (15/16) | 100% (16/16) | 56.3% (9/16) | 62.5% (10/16) | 62.5% (10/16) | 75.0% (12/16) |
| Positive predictivity | 74.2% (23/31) | 75.9% (22/29) | 75.9% (22/29) | 82.1% (23/28) | 88.0% (22/25) | 95.7% (22/23) | 95.7% (22/23) | 100% (22/22) | 75.9% (22/29) | 78.6% (22/28) | 78.6% (22/28) | 84.6% (22/26) |
| Negative predictivity | 100% (4/4) | 100% (5/5) | 100% (5/5) | 100% (7/7) | 100% (13/13) | 100% (15/15) | 100% (15/15) | 100% (16/16) | 100% (9/9) | 100% (10/10) | 100% (10/10) | 100% (12/12) |
| Accuracy | 77.1% (27/35) | 79.4% (27/34) | 79.4% (27/34) | 85.7% (30/35) | 92.1% (35/38) | 97.4% (37/38) | 97.4% (37/38) | 100% (38/38) | 81.6% (31/38) | 84.2% (32/38) | 84.2% (32/38) | 89.5% (34/38) |

| Lab 2 | Draft criteria for the final judgement [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Original | | | | Data analysis based on the criteria for the secondary data analysis | | | | | | | |
| | | | | | 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% (21/21) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 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 | 36.4% (4/11) | 60.0% (6/10) | 60.0% (6/10) | 60.0% (6/10) | 100% (14/14) | 100% (14/14) | 100% (14/14) | 100% (14/14) | 57.1% (8/14) | 71.4% (10/14) | 71.4% (10/14) | 71.4% (10/14) |
| Positive predictivity | 75.0% (21/28) | 84.0% (21/25) | 84.0% (21/25) | 84.0% (21/25) | 100% (19/19) | 100% (19/19) | 100% (19/19) | 100% (19/19) | 76.0% (19/25) | 82.6% (19/23) | 82.6% (19/23) | 82.6% (19/23) |
| Negative predictivity | 100% (4/4) | 100% (6/6) | 100% (6/6) | 100% (6/6) | 100% (14/14) | 100% (14/14) | 100% (14/14) | 100% (14/14) | 100% (8/8) | 100% (10/10) | 100% (10/10) | 100% (10/10) |
| Accuracy | 78.1% (25/32) | 87.1% (27/31) | 87.1% (27/31) | 87.1% (27/31) | 100% (33/33) | 100% (33/33) | 100% (33/33) | 100% (33/33) | 81.8% (27/33) | 87.9% (29/33) | 87.9% (29/33) | 87.9% (29/33) |

a : A : Original : Final judgement of positive when positive results were obtained in at least one of three assays

Data analysis based on the criteria for the secondary data analysis: Final judgement of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

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

C : Final judgement based on the majority of three assay results

D : Final judgement based on the first assay results

Table 20 Secondary data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results (continued).

| Lab 3 | Draft criteria for the final judgement [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Original | | | | Data analysis based on the criteria for the secondary data analysis | | | | | | | |
| | | | | | 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% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 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 | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 100% (17/17) | 100% (17/17) | 100% (17/17) | 100% (17/17) | 88.2% (15/17) | 88.2% (15/17) | 88.2% (15/17) | 88.2% (15/17) |
| Positive predictivity | 91.7% (22/24) | 91.7% (22/24) | 91.7% (22/24) | 91.7% (22/24) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 91.3% (21/23) | 91.3% (21/23) | 91.3% (21/23) | 91.3% (21/23) |
| Negative predictivity | 100% (9/9) | 100% (9/9) | 100% (9/9) | 100% (9/9) | 100% (17/17) | 100% (17/17) | 100% (17/17) | 100% (17/17) | 100% (15/15) | 100% (15/15) | 100% (15/15) | 100% (15/15) |
| Accuracy | 93.9% (31/33) | 93.9% (31/33) | 93.9% (31/33) | 93.9% (31/33) | 100% (38/38) | 100% (38/38) | 100% (38/38) | 100% (38/38) | 94.7% (36/38) | 94.7% (36/38) | 94.7% (36/38) | 94.7% (36/38) |

a : A : Original : Final judgement of positive when positive results were obtained in at least one of three assays

Data analysis based on the criteria for the secondary data analysis: Final judgement of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

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

C : Final judgement based on the majority of three assay results

D : Final judgement based on the first assay results

Table 21 Secondary data analysis based on the criteria for the proposed protocol: Contingency table for integrated judgement results.

| Phase 2 | Draft criteria for the final judgement [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Original | | | | Data analysis based on the criteria for the secondary data analysis | | | | | | | |
| | | | | | When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals | | | | When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals | | | |
| | A [c] | B | C | D | A [b] | B | C | D | A [b] | B | C | D |
| Sensitivity | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) |
| Specificity | 45.5% (5/11) | 60.0% (6/10) | 60.0% (6/10) | 70.0% (7/10) | 100% (17/17) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 76.5% (13/17) | 77.8% (14/18) | 77.8% (14/18) | 83.3% (15/18) |
| Positive predictivity | 78.6% (22/28) | 84.6% (22/26) | 84.6% (22/26) | 88.0% (22/25) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 100% (22/22) | 84.6% (22/26) | 84.6% (22/26) | 84.6% (22/26) | 88.0% (22/25) |
| Negative predictivity | 100% (5/5) | 100% (6/6) | 100% (6/6) | 100% (7/7) | 100% (17/17) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (13/13) | 100% (14/14) | 100% (14/14) | 100% (15/15) |
| Accuracy | 81.8% (27/33) | 87.5% (28/32) | 87.5% (28/32) | 90.6% (29/32) | 100% (39/39) | 100% (40/40) | 100% (40/40) | 100% (40/40) | 89.7% (35/39) | 90.0% (36/40) | 90.0% (36/40) | 92.5% (37/40) |

Integrated judgements were made by the majority of each laboratory's final judgement.

a : A : Original : Final judgement of positive when positive results were obtained in at least one of three assays

Data analysis based on the criteria for the secondary data analysis: Final judgement of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

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

C : Final judgement based on the majority of three assay results

D : Final judgement based on the first assay results

b : See, tables 18A to 18D

c : See, tables 12A to 12D

Table 22 Results of newly conducted ROS assay at 20 μM and the Phase 2 results of Lab 1

| ROS assay Validation data (atlas) | | | Lab 1 | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Phase 2 results | | | Additional data at 20 μM | | |
| Chemicals N0. | Name | | Conc (μM) | Mean | SD | Conc (μM) | Mean | SD |
| II - 1 | Acridine | SO | 200 | 227 | 5.7 | 20 | 73 | 13 |
| | | SA | 200 | 225 | 8.5 | 20 | 26 | 11 |
| II - 2 | Acridine HCl | SO | 200 | 220 | 5.5 | 20 | 78 | 7 |
| | | SA | 200 | 214 | 2.3 | 20 | 46 | 8 |
| II - 3 | Amiodarone HCl | SO | 2 | 21 | 12 | 20 | Precipitation | |
| | | SA | 2 | -11 | 5.7 | 20 | Precipitation | |
| II - 4 | Chlorpromazine HCl | SO | 200 | -25 | 14.2 | 20 | 91 | 5 |
| | | SA | 200 | 96 | 11.1 | 20 | Not detected | |
| II - 5 | Doxycycline HCl | SO | 200 | 267 | 4.7 | 20 | 100 | 12 |
| | | SA | 200 | 394 | 80.2 | 20 | 39 | 17 |
| II - 6 | Fenofibrate | SO | 20 | 166 | 39.4 | 20 | 202 | 1 |
| | | SA | 20 | -23 | 20.6 | 20 | Not detected | |
| II - 7 | Furosemide | SO | 200 | 230 | 7.4 | 20 | 60 | 3 |
| | | SA | 200 | 113 | 9.7 | 20 | 27 | 2 |
| II - 8 | Ketoprofen | SO | 200 | 363 | 5 | 20 | 50 | 10 |
| | | SA | 200 | 130 | 7.5 | 20 | 18 | 6 |
| II - 9 | 6-methylcoumarine | SO | 200 | 115 | 4.6 | 20 | 13 | 2 |
| | | SA | 200 | 122 | 11.6 | 20 | 26 | 14 |
| II - 10 | 8-MOP | SO | 200 | 87 | 12.1 | 20 | 21 | 3 |
| | | SA | 200 | 109 | 31.2 | 20 | 43 | 4 |
| II - 11 | Nalidixic acid | SO | 200 | 238 | 95 | 20 | 58 | 3 |
| | | SA | 200 | 297 | 50.6 | 20 | 292 | 6 |
| II - 12 | Nalidixic acid (Na salt) | SO | 200 | 177 | 10.4 | 20 | 32 | 2 |
| | | SA | 200 | 241 | 42.6 | 20 | 128 | 5 |
| II - 13 | Norfloxacin | SO | 200 | 216 | 2.6 | 20 | 61 | 7 |
| | | SA | 200 | 132 | 17 | 20 | 31 | 5 |
| II - 14 | Ofloxacin | SO | 200 | 196 | 6.1 | 20 | 14 | 6 |
| | | SA | 200 | 284 | 62.1 | 20 | 79 | 17 |
| II - 15 | Piroxicam | SO | 200 | 211 | 20.5 | 20 | 63 | 3 |
| | | SA | 20 | 31 | 6.4 | 20 | 51 | 7 |
| II - 16 | Promethazine HCl | SO | 200 | 72 | 11.1 | 20 | 94 | 6 |
| | | SA | 200 | 80 | 11 | 20 | Not detected | |
| II - 17 | Rosiglitazone | SO | 200 | 118 | 13.5 | 20 | Not detected | |
| | | SA | 20 | 35 | 6 | 20 | 30 | 4 |
| II - 18 | Tetracycline | SO | 200 | 198 | 3.5 | 20 | 87 | 7 |
| | | SA | 200 | 210 | 11.6 | 20 | 70 | 16 |

SO : Singlet oxygen SA : Superoxide anion ND : no data.

Table 22 Results of newly conducted ROS assay at 20 μM and the Phase 2 results of Lab 1 (continued)

| ROS assay Validation data (atlas) | | | Lab 1 | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Phase 2 results | | | Additional data at 20 μM | | |
| Chemicals | | | Conc (μM) | Mean | SD | Conc (μM) | Mean | SD |
| N0. | Name | | | | | | | |
| II - 19 | Anthracene | SO | 20 | 291 | 42.8 | 20 | 131 | 11 |
| | | SA | 2 | 29 | 24.9 | 20 | 106 | 5 |
| II - 20 | Avobenzone | SO | 20 | 127 | 13.4 | 20 | 117 | 7 |
| | | SA | 2 | 34 | 16.6 | 20 | Precipitation | |
| II - 21 | Bithionol | SO | 200 | 103 | 18.8 | 20 | 103 | 10 |
| | | SA | 20 | 24 | 3.5 | 20 | 1 | 14 |
| II - 22 | Hexachlorophene | SO | 200 | 335 | 18.6 | 20 | 71 | 11 |
| | | SA | 200 | 12 | 9 | 20 | Not detected | |
| II - 23 | Rose bengal | SO | 200 | 682 | 3 | 20 | 463 | 25 |
| | | SA | ND | ND | ND | 20 | Not detected | |
| II - 24 | Aspirin | SO | 200 | 2 | 1.5 | 20 | 2 | 6 |
| | | SA | 200 | -6 | 13.7 | 20 | Not detected | |
| II - 25 | Benzocaine | SO | 200 | 2 | 4 | 20 | 17 | 3 |
| | | SA | 200 | 10 | 10 | 20 | 22 | 11 |
| II - 26 | Erythromycin | SO | 200 | -1 | 13.1 | 20 | 6 | 1 |
| | | SA | 200 | 6 | 2 | 20 | Not detected | |
| II – 27 | Penicillin G | SO | 200 | 11 | 5.7 | 20 | Not detected | |
| | | SA | 200 | 26 | 14.5 | 20 | Not detected | |
| II - 28 | Phenytoin | SO | 200 | 8 | 9.9 | 20 | Not detected | |
| | | SA | 200 | 50 | 16.1 | 20 | 11 | 3 |
| II - 29 | Bumetrizole | SO | 20 | -10 | 2.3 | 20 | Not detected | |
| | | SA | 2 | -2 | 9.3 | 20 | Precipitation | |
| II - 30 | Camphor sulfonic acid | SO | 200 | 4 | 5.5 | 20 | Not detected | |
| | | SA | 200 | -13 | 9 | 20 | Not detected | |
| II - 31 | Chlorhexidine | SO | 200 | -3 | 10.1 | 20 | 33 | 3 |
| | | SA | 200 | 23 | 15.1 | 20 | 28 | 9 |
| II - 32 | Cinnamic acid | SO | 200 | 2 | 3.5 | 20 | Not detected | |
| | | SA | 200 | 56 | 17.6 | 20 | Not detected | |
| II - 33 | Drometrizole | SO | 20 | -1 | 7.4 | 20 | 7 | 7 |
| | | SA | 20 | -2 | 13.1 | 20 | Not detected | |
| II - 34 | L-Histidine | SO | 200 | 11 | 4 | 20 | Not detected | |
| | | SA | 200 | 62 | 11 | 20 | 18 | 3 |
| II - 35 | Methylbenzylidene camphor | SO | 20 | -5 | 2.1 | 20 | 3 | 1 |
| | | SA | 20 | -2 | 5.9 | 20 | 5 | 7 |
| II - 36 | Octrizole | SO | 2 | 0 | 5.1 | 20 | Precipitation | |
| | | SA | 2 | 42 | 17.6 | 20 | Precipitation | |
| II - 37 | Octyl methacrylate | SO | 200 | 32 | 18.2 | 20 | Not detected | |
| | | SA | 200 | -22 | 17.7 | 20 | Not detected | |
| II - 38 | Octyl methoxycinnamate | SO | 20 | 4 | 2.1 | 20 | 6 | 2 |
| | | SA | 20 | -14 | 3.1 | 20 | Not detected | |
| II - 39 | Octyl salicylate | SO | 20 | 5 | 7.6 | 20 | 2 | 3 |
| | | SA | 20 | 5 | 6.1 | 20 | 1 | 2 |
| II - 40 | PABA | SO | 200 | 5 | 13 | 20 | Not detected | |
| | | SA | 200 | 4 | 8.1 | 20 | Not detected | |
| II - 41 | SDS | SO | 200 | 17 | 1.5 | 20 | Not detected | |
| | | SA | 20 | 8 | 7 | 20 | 5 | 4 |
| II - 42 | UV-571 | SO | 20 | -17 | 2.1 | 20 | Not detected | |
| | | SA | 2 | 10 | 5.5 | 20 | Precipitation | |

SO : Singlet oxygen SA : Superoxide anion ND : no data.

Table 23A Third data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgement

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| 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 judgement of Non-photoreactive, when precipitation is observed at 200 μM, the compound should be judged as Inconclusive)
(+): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I: inconclusive (The results do not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a: Integrated judgements were made by the majority of each laboratory's final judgement.

Table 23A Third data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgement (continued)

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | ± | - | - | - | - | ± |
| II - 32 | Cinnamic acid | ± | + | ± | + | ± | ± | ± | ± | - | - | - | - | I |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (±) | I | I | I | I | 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 |
| 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 |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+: 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 judgement of Non-photoreactive, when precipitation is observed at 200 μM, the compound should be judged as Inconclusive)
(+): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I: inconclusive (The results do not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a: Integrated judgements were made by the majority of each laboratory's final judgement.

Table 23B Third data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the mean value of three assays

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| 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 judgement of Non-photoreactive, when precipitation is observed at 200 μM, the compound should be judged as Inconclusive)
(+): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I: inconclusive (The results do not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a: Integrated judgements were made by the majority of each laboratory's final judgement.

Table 23B Third data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the mean value of three assays (continued)

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | I | I | I | I | 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 |
| 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 |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+: 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 judgement of Non-photoreactive, when precipitation is observed at 200 μM, the compound should be judged as Inconclusive)
(+): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I: inconclusive (The results do not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a: Integrated judgements were made by the majority of each laboratory's final judgement.
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Table 23C Third data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the majority of three assay results

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| 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 judgement of Non-photoreactive, when precipitation is observed at 200 μM, the compound should be judged as Inconclusive)
(+): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I: inconclusive (The results do not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a: Integrated judgements were made by the majority of each laboratory's final judgement.

Table 23C Third data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the majority of three assay results (continued)

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | I | I | I | I | 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 |
| 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 |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+: 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 judgement of Non-photoreactive, when precipitation is observed at 200 μM, the compound should be judged as Inconclusive)
(+): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I: inconclusive (The results do not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a: Integrated judgements were made by the majority of each laboratory's final judgement.

Table 23D Third data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the first assay results

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| 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 judgement of Non-photoreactive, when precipitation is observed at 200 μM, the compound should be judged as Inconclusive)
(+): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I: inconclusive (The results do not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a: Integrated judgements were made by the majority of each laboratory's final judgement.

Table 23D Third data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the first assay results (continued)

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | 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 | I | I |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | I | I | I | I | 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 |
| 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 |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+: 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 judgement of Non-photoreactive, when precipitation is observed at 200 μM, the compound should be judged as Inconclusive)
(+): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 or Superoxide results ≥70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(±): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
(-): ROS assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.
I: inconclusive (The results do not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)
ND: not determined due to precipitation
a: Integrated judgements were made by the majority of each laboratory's final judgement.
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Table 24A 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 judgement

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[a] |
| | - | 3 | 4 | 4 | 11[b] |
| Total | | 24 | 4 | 4 | 32 |

a: One of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl).
b: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-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)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 36.4% (4/11)
Positive predictivity : 75.0%(21/28) Negative predictivity : 100% (4/4)
Accuracy : 78.1%(25/32)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 18 | 0 | 0 | 18[c] |
| | - | 0 | 6 | 4 | 10[d] |
| Total | | 18 | 6 | 4 | 28 |

c: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, 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 : 100% (10/10)
Positive predictivity : 100% (18/18) Negative predictivity : 100% (10/10)
Accuracy : 100% (28/28)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 40.0%(4/10)
Positive predictivity : 75.0%(18/24) Negative predictivity : 100% (4/4)
Accuracy : 78.6%(22/28)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 20 | 0 | 0 | 20[e] |
| | - | 0 | 2 | 9 | 11[f] |
| Total | | 20 | 2 | 9 | 31 |

e: Two of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl, Anthracene)
f: Eight of 19 non-phototoxic chemicals were not evaluated at20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (20/20) Specificity : 100% (11/11)
Positive predictivity : 100% (20/20) Negative predictivity : 100% (11/11)
Accuracy : 100% (31/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (20/20) Specificity : 81.8%(9/11)
Positive predictivity : 90.9%(20/22) Negative predictivity : 100% (9/9)
Accuracy : 93.5%(29/31)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[g] |
| | - | 0 | 4 | 5 | 9[h] |
| Total | | 21 | 4 | 5 | 30 |

g: One of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl) .
h: Ten of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation or the results does not meet the photoreactive, weakly photoreactive or non-photoreactive criterion. (Bumetrizole, Cinnamic acid, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (9/9)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (9/9)
Accuracy : 100% (30/30)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
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 24B Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the mean value of three assays

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[a] |
| | - | 1 | 5 | 5 | 11[b] |
| Total | | 22 | 5 | 5 | 32 |

a: One of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl).
b: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, Octyl salicylate, SDS, 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 : 45.5% (5/11)
Positive predictivity : 77.8%(21/27) Negative predictivity : 100% (5/5)
Accuracy : 81.3%(26/32)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 18 | 0 | 0 | 18[c] |
| | - | 0 | 4 | 6 | 10[d] |
| Total | | 18 | 4 | 6 | 28 |

c: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, 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 : 100% (10/10)
Positive predictivity : 100% (18/18) Negative predictivity : 100% (10/10)
Accuracy : 100% (28/28)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 60.0%(6/10)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (6/6)
Accuracy : 85.7%(24/28)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 20 | 0 | 0 | 20[e] |
| | - | 0 | 2 | 9 | 11[f] |
| Total | | 20 | 2 | 9 | 31 |

e: Two of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl, Anthracene)
f: Eight of 19 non-phototoxic chemicals were not evaluated at20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (20/20) Specificity : 100% (11/11)
Positive predictivity : 100% (20/20) Negative predictivity : 100% (11/11)
Accuracy : 100% (31/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (20/20) Specificity : 81.8%(9/11)
Positive predictivity : 90.9%(20/22) Negative predictivity : 100% (9/9)
Accuracy : 93.5%(29/31)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[g] |
| | - | 0 | 4 | 6 | 10[h] |
| Total | | 21 | 4 | 6 | 31 |

g: One of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl) .
h: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation or the results does not meet the photoreactive, weakly photoreactive or non-photoreactive criterion. (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (10/10)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (10/10)
Accuracy : 100% (31/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 60.0%(6/10)
Positive predictivity : 84.0%(21/25) Negative predictivity : 100% (6/6)
Accuracy : 87.1%(27/31)

Table 24C Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the majority of three assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[a] |
| | - | 1 | 5 | 5 | 11[b] |
| Total | | 22 | 5 | 5 | 32 |

a: One of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl).
b: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, Octyl salicylate, SDS, 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 : 45.5% (5/11)
Positive predictivity : 77.8%(21/27) Negative predictivity : 100% (5/5)
Accuracy : 81.3%(26/32)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 18 | 0 | 0 | 18[c] |
| | - | 0 | 4 | 6 | 10[d] |
| Total | | 18 | 4 | 6 | 28 |

c: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, 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 : 100% (10/10)
Positive predictivity : 100% (18/18) Negative predictivity : 100% (10/10)
Accuracy : 100% (28/28)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 60.0%(6/10)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (6/6)
Accuracy : 85.7%(24/28)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 20 | 0 | 0 | 20[e] |
| | - | 0 | 2 | 9 | 11[f] |
| Total | | 20 | 2 | 9 | 31 |

e: Two of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl, Anthracene)
f: Eight of 19 non-phototoxic chemicals were not evaluated at20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (20/20) Specificity : 100% (11/11)
Positive predictivity : 100% (20/20) Negative predictivity : 100% (11/11)
Accuracy : 100% (31/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (20/20) Specificity : 81.8%(9/11)
Positive predictivity : 90.9%(20/22) Negative predictivity : 100% (9/9)
Accuracy : 93.5%(29/31)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[g] |
| | - | 0 | 4 | 6 | 10[h] |
| Total | | 21 | 4 | 6 | 31 |

g: One of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl) .
h: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation or the results does not meet the photoreactive, weakly photoreactive or non-photoreactive criterion. (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (10/10)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (10/10)
Accuracy : 100% (31/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 60.0%(6/10)
Positive predictivity : 84.0%(21/25) Negative predictivity : 100% (6/6)
Accuracy : 87.1%(27/31)

Table 24D Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the first assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[a] |
| | - | 0 | 4 | 7 | 11[b] |
| Total | | 21 | 4 | 7 | 32 |

a: One of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl).
b: Eight of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (11/11)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (11/11)
Accuracy : 100% (32/32)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
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)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 18 | 0 | 0 | 18[c] |
| | - | 0 | 4 | 6 | 10[d] |
| Total | | 18 | 4 | 6 | 28 |

c: Four of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, 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 : 100% (10/10)
Positive predictivity : 100% (18/18) Negative predictivity : 100% (10/10)
Accuracy : 100% (28/28)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 60.0%(6/10)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (6/6)
Accuracy : 85.7%(24/28)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 20 | 0 | 0 | 20[e] |
| | - | 0 | 2 | 9 | 11[f] |
| Total | | 20 | 2 | 9 | 31 |

e: Two of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl, Anthracene)
f: Eight of 19 non-phototoxic chemicals were not evaluated at20 or 200 μM due to precipitation (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (20/20) Specificity : 100% (11/11)
Positive predictivity : 100% (20/20) Negative predictivity : 100% (11/11)
Accuracy : 100% (31/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (20/20) Specificity : 81.8%(9/11)
Positive predictivity : 90.9%(20/22) Negative predictivity : 100% (9/9)
Accuracy : 93.5%(29/31)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[g] |
| | - | 0 | 3 | 7 | 10[h] |
| Total | | 21 | 3 | 7 | 31 |

g: One of 22 phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation (Amiodarone HCl) .
h: Nine of 19 non-phototoxic chemicals were not evaluated at 20 or 200 μM due to precipitation or the results does not meet the photoreactive, weakly photoreactive or non-photoreactive criterion. (Bumetrizole, Drometrizole, Methylbenzylidene camphor, Octizole, Octyl methacrylate, Octyl methoxycinnamate, Octyl salicylate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (10/10)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (10/10)
Accuracy : 100% (31/31)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 70.0%(7/10)
Positive predictivity : 87.5%(21/24) Negative predictivity : 100% (7/7)
Accuracy : 90.3%(28/31)

Table 25 Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results

| Lab 1 | Draft criteria for the final judgement [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% (22/22) | 100% (21/21) | 100% (21/21) | 100% (22/22) | 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 | 33.3% (4/12) | 41.7% (5/12) | 41.7% (5/12) | 58.3% (7/12) | 72.7% (8/11) | 90.9% (10/11) | 90.9% (10/11) | 100% (11/11) | 36.4% (4/11) | 45.5% (5/11) | 45.5% (5/11) | 63.6% (7/11) |
| Positive predictivity | 73.3% (22/30) | 75.0% (21/28) | 75.0% (21/28) | 81.5% (22/27) | 87.5% (21/24) | 95.5% (21/22) | 95.5% (21/22) | 100% (21/21) | 75.0% (21/28) | 77.8% (21/27) | 77.8% (21/27) | 84.0% (21/25) |
| Negative predictivity | 100% (4/4) | 100% (5/5) | 100% (5/5) | 100% (7/7) | 100% (8/8) | 100% (10/10) | 100% (10/10) | 100% (11/11) | 100% (4/4) | 100% (5/5) | 100% (5/5) | 100% (7/7) |
| Accuracy | 76.5% (26/34) | 78.8% (26/33) | 78.8% (26/33) | 85.3% (29/34) | 90.6% (29/32) | 96.9% (31/32) | 96.9% (31/32) | 100% (32/32) | 78.1% (25/32) | 81.3% (26/32) | 81.3% (26/32) | 87.5% (28/32) |

| Lab 2 | Draft criteria for the final judgement [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% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) |
| Specificity | 36.4% (4/11) | 60.0% (6/10) | 60.0% (6/10) | 60.0% (6/10) | 100% (10/10) | 100% (10/10) | 100% (10/10) | 100% (10/10) | 40.0% (4/10) | 60.0% (6/10) | 60.0% (6/10) | 60.0% (6/10) |
| Positive predictivity | 74.1% (20/27) | 83.3% (20/24) | 83.3% (20/24) | 83.3% (20/24) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 75.0% (18/24) | 81.8% (18/22) | 81.8% (18/22) | 81.8% (18/22) |
| Negative predictivity | 100% (4/4) | 100% (6/6) | 100% (6/6) | 100% (6/6) | 100% (10/10) | 100% (10/10) | 100% (10/10) | 100% (10/10) | 100% (4/4) | 100% (6/6) | 100% (6/6) | 100% (6/6) |
| Accuracy | 77.4% (24/31) | 86.7% (26/30) | 86.7% (26/30) | 86.7% (26/30) | 100% (28/28) | 100% (28/28) | 100% (28/28) | 100% (28/28) | 78.6% (22/28) | 85.7% (24/28) | 85.7% (24/28) | 85.7% (24/28) |

a : A : Original : Final judgement 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 judgement
B : Final judgement based on the mean value of three assays.
C : Final judgement based on the majority of three assay results
D : Final judgement based on the first assay results

Table 25 Third data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results (continued).

| Lab 3 | Draft criteria for the final judgement [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% (21/21) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 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 | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 100% (11/11) | 100% (11/11) | 100% (11/11) | 100% (11/11) | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) |
| Positive predictivity | 91.3% (21/23) | 91.3% (21/23) | 91.3% (21/23) | 91.3% (21/23) | 100% (20/20) | 100% (20/20) | 100% (20/20) | 100% (20/20) | 90.9% (20/22) | 90.9% (20/22) | 90.9% (20/22) | 90.9% (20/22) |
| Negative predictivity | 100% (9/9) | 100% (9/9) | 100% (9/9) | 100% (9/9) | 100% (11/11) | 100% (11/11) | 100% (11/11) | 100% (11/11) | 100% (9/9) | 100% (9/9) | 100% (9/9) | 100% (9/9) |
| Accuracy | 93.8% (30/32) | 93.8% (30/32) | 93.8% (30/32) | 93.8% (30/32) | 100% (31/31) | 100% (31/31) | 100% (31/31) | 100% (31/31) | 93.5% (29/31) | 93.5% (29/31) | 93.5% (29/31) | 93.5% (29/31) |

a : A : Original : Final judgement 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 judgement
B : Final judgement based on the mean value of three assays.
C : Final judgement based on the majority of three assay results
D : Final judgement based on the first assay results

Table 26 Third data analysis based on the criteria for the proposed protocol: Contingency table for integrated judgement results.

| Phase 2 | Draft criteria for the final judgement [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% (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 | 45.5% (5/11) | 60.0% (6/10) | 60.0% (6/10) | 70.0% (7/10) | 100% (9/9) | 100% (10/10) | 100% (10/10) | 100% (10/10) | 55.6% (5/9) | 60.0% (6/10) | 60.0% (6/10) | 70.0% (7/10) |
| Positive predictivity | 77.8% (21/27) | 84.0% (21/25) | 84.0% (21/25) | 87.5% (21/24) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 84.0% (21/25) | 84.0% (21/25) | 84.0% (21/25) | 87.5% (21/24) |
| Negative predictivity | 100% (5/5) | 100% (6/6) | 100% (6/6) | 100% (7/7) | 100% (9/9) | 100% (10/10) | 100% (10/10) | 100% (10/10) | 100% (5/5) | 100% (6/6) | 100% (6/6) | 100% (7/7) |
| Accuracy | 81.3% (26/32) | 87.1% (27/31) | 87.1% (27/31) | 90.3% (28/31) | 100% (30/30) | 100% (31/31) | 100% (31/31) | 100% (31/31) | 86.7% (26/30) | 87.1% (27/31) | 87.1% (27/31) | 90.3% (28/31) |

Integrated judgements were made by the majority of each laboratory's final judgement.

a : A : Original : Final judgement 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 judgement
B : Final judgement based on the mean value of three assays.
C : Final judgement based on the majority of three assay results
D : Final judgement based on the first assay results

b : See, tables 12A to 12D

c : See, tables 23A to 23D

Table 27A Fourth data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgement

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| 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: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

-: Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

ND: not determined due to precipitation

a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 27A Fourth data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: The highest criteria among the three assay results was selected as the final judgement (continued)

| ROS assay Validation data (atlas) pattern A | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | ± | - | - | - | - | ± |
| II - 32 | Cinnamic acid | ± | + | ± | + | ± | ± | ± | ± | - | - | - | - | I |
| II - 33 | Drometrizole | - | - | - | - | - | - | - | - | (-) | (-) | (-) | (-) | - |
| II - 34 | L-Histidine | ± | + | ± | + | ± | - | ± | ± | ± | ± | ± | ± | ± |
| II - 35 | Methylbenzylidene camphor | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (±) | - | - | - | - | - |
| II - 37 | Octyl methacrylate | - | + | + | + | - | - | - | - | - | - | - | - | - |
| II - 38 | Octyl methoxycinnamate | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 39 | Octyl salicylate | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 40 | PABA | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 41 | SDS | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+: 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: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

-: Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

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

ND: not determined due to precipitation

a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 27B Fourth data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the mean value of three assays

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| 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: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

-: Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

ND: not determined due to precipitation

a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 27B Fourth data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the mean value of three assays (continued)

| ROS assay Validation data (atlas) pattern B | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | - | - | - | - | - | - | - | - | (-) | (-) | (-) | (-) | - |
| II - 34 | L-Histidine | ± | + | ± | ± | ± | - | ± | ± | ± | ± | ± | ± | ± |
| II - 35 | Methylbenzylidene camphor | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | - | - | - | - | - |
| II - 37 | Octyl methacrylate | - | + | + | + | - | - | - | - | - | - | - | - | - |
| II - 38 | Octyl methoxycinnamate | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 39 | Octyl salicylate | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 40 | PABA | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 41 | SDS | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+: 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: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

-: Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

(+):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(-):Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

ND: not determined due to precipitation

a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 27C Fourth data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the majority of three assay results

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| 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: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

-: Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

ND: not determined due to precipitation

a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 27C Fourth data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the majority of three assay results (continued)

| ROS assay Validation data (atlas) pattern C | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | - | - | - | - | - | - | - | - | (-) | (-) | (-) | (-) | - |
| II - 34 | L-Histidine | ± | + | ± | ± | ± | - | ± | ± | ± | ± | ± | ± | ± |
| II - 35 | Methylbenzylidene camphor | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | - | - | - | - | - |
| II - 37 | Octyl methacrylate | - | + | + | + | - | - | - | - | - | - | - | - | - |
| II - 38 | Octyl methoxycinnamate | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 39 | Octyl salicylate | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 40 | PABA | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 41 | SDS | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+: 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: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

-: Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

ND: not determined due to precipitation

a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 27D Fourth data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the single assay results

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | 1st | 2nd | 3rd | | |
| II - 1 | Acridine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 2 | Acridine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 3 | Amiodarone HCl | (+) | (-) | (-) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | (+) | ND |
| II - 4 | Chlorpromazine HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 5 | Doxycycline HCl | + | + | + | + | + | + | + | + | + | + | + | + | + |
| II - 6 | Fenofibrate | + | + | + | + | (-) | (-) | (-) | (-) | + | + | + | + | + |
| 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 | + | + | + | + | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | + |
| II - 20 | Avobenzone | + | + | + | + | (±) | (±) | (±) | (±) | + | + | + | + | + |
| 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: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

-: Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

ND: not determined due to precipitation

a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 27D Fourth data analysis based on the criteria for the proposed protocol: Judgement from the Phase 2 results: Final judgement based on the single assay results (continued)

| ROS assay Validation data (atlas) pattern D | | Lab 1 | | | | Lab 2 | | | | Lab 3 | | | | Integrated Judgement [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chemicals | | Assay | | | Final judg-ment | Assay | | | Final judg-ment | Assay | | | Final judg-ment | |
| No. | Name | 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 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 30 | Camphor sulfonic acid | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 31 | Chlorhexidine | ± | ± | - | ± | - | ± | - | - | - | - | - | - | - |
| II - 32 | Cinnamic acid | ± | + | ± | ± | ± | ± | ± | ± | - | - | - | - | ± |
| II - 33 | Drometrizole | - | - | - | - | - | - | - | - | (-) | (-) | (-) | (-) | - |
| II - 34 | L-Histidine | ± | + | ± | ± | ± | - | ± | ± | ± | ± | ± | ± | ± |
| II - 35 | Methylbenzylidene camphor | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 36 | Octrizole | (±) | (±) | (±) | (±) | (-) | (±) | (-) | (-) | - | - | - | - | - |
| II - 37 | Octyl methacrylate | - | + | + | - | - | - | - | - | - | - | - | - | - |
| II - 38 | Octyl methoxycinnamate | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 39 | Octyl salicylate | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 40 | PABA | - | - | - | - | - | - | - | - | - | - | - | - | - |
| II - 41 | SDS | - | - | - | - | (-) | (-) | (-) | (-) | - | - | - | - | - |
| II - 42 | UV-571 | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | (-) | ND |

+: 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: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

±: Weakly photoreactive (Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

-: Non-photoreactive (Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgement only when precipitation or coloration is observed at 200 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgement of integrated results or data analyses.

ND: not determined due to precipitation

a : Integrated judgements were made by the majority of each laboratory's final judgement.

Table 28A 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 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[a] |
| | - | 3 | 4 | 9 | 16[b] |
| Total | | 24 | 4 | 9 | 37 |

a: One of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl).
b: Three of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 81.3% (13/16)
Positive predictivity : 87.5% (21/24) Negative predictivity : 100% (13/13)
Accuracy : 91.9% (34/37)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 56.3% (9/16)
Positive predictivity : 75.0%(21/28) Negative predictivity : 100% (9/9)
Accuracy : 81.1%(30/37)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 18 | 0 | 0 | 18[c] |
| | - | 0 | 6 | 8 | 14[d] |
| Total | | 18 | 6 | 8 | 32 |

c: Four of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: Five of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 100% (14/14)
Positive predictivity : 100% (18/18) Negative predictivity : 100% (14/14)
Accuracy : 100% (32/32)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 57.1%(8/14)
Positive predictivity : 75.0%(18/24) Negative predictivity : 100% (8/8)
Accuracy : 81.3%(26/32)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 20 | 0 | 0 | 20[e] |
| | - | 0 | 2 | 15 | 17[f] |
| Total | | 20 | 2 | 15 | 37 |

e: Two of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Anthracene)
f: Two of 19 non-phototoxic chemicals were not evaluated at20 or 200μM due to precipitation (Drometrizole, 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 : 88.2%(15/17)
Positive predictivity : 90.9%(20/22) Negative predictivity : 100% (15/15)
Accuracy : 94.6%(35/37)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[g] |
| | - | 0 | 4 | 13 | 17[h] |
| Total | | 21 | 4 | 13 | 38 |

g: One of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl) .
h: Two of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation or the results does not meet the photoreactive, weakly photoreactive or non-photoreactive criterion (Cinnamic acid, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (17/17)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (17/17)
Accuracy : 100% (38/38)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 76.5%(13/17)
Positive predictivity : 84.0%(21/25) Negative predictivity : 100% (13/13)
Accuracy : 89.5%(34/38)

Table 28B Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the mean value of three assays

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[a] |
| | - | 1 | 5 | 10 | 16[b] |
| Total | | 22 | 5 | 10 | 37 |

a: One of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl).
b: Three of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 93.8% (15/16)
Positive predictivity : 95.5% (21/22) Negative predictivity : 100% (15/15)
Accuracy : 97.3% (36/37)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 62.5% (10/16)
Positive predictivity : 77.8%(21/27) Negative predictivity : 100% (10/10)
Accuracy : 83.8%(31/37)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 18 | 0 | 0 | 18[c] |
| | - | 0 | 4 | 10 | 14[d] |
| Total | | 18 | 4 | 10 | 32 |

c: Four of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: Five of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 100% (14/14)
Positive predictivity : 100% (18/18) Negative predictivity : 100% (14/14)
Accuracy : 100% (32/32)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 71.4%(10/14)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (10/10)
Accuracy : 87.5%(28/32)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 20 | 0 | 0 | 20[e] |
| | - | 0 | 2 | 15 | 17[f] |
| Total | | 20 | 2 | 15 | 37 |

e: Two of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Anthracene)
f: Two of 19 non-phototoxic chemicals were not evaluated at20 or 200μM due to precipitation (Drometrizole, 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 : 88.2%(15/17)
Positive predictivity : 90.9%(20/22) Negative predictivity : 100% (15/15)
Accuracy : 94.6%(35/37)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[g] |
| | - | 0 | 4 | 14 | 18[h] |
| Total | | 21 | 4 | 14 | 39 |

g: One of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl) .
h: One of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (18/18)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (18/18)
Accuracy : 100% (39/39)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 77.8%(14/18)
Positive predictivity : 84.0%(21/25) Negative predictivity : 100% (14/14)
Accuracy : 89.7%(35/39)

Table 28C Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the majority of three assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[a] |
| | - | 1 | 5 | 10 | 16[b] |
| Total | | 22 | 5 | 10 | 37 |

a: One of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl).
b: Three of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 93.8% (15/16)
Positive predictivity : 95.5% (21/22) Negative predictivity : 100% (15/15)
Accuracy : 97.3% (36/37)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 62.5% (10/16)
Positive predictivity : 77.8%(21/27) Negative predictivity : 100% (10/10)
Accuracy : 83.8%(31/37)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 18 | 0 | 0 | 18[c] |
| | - | 0 | 4 | 10 | 14[d] |
| Total | | 18 | 4 | 10 | 32 |

c: Four of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: Five of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 100% (14/14)
Positive predictivity : 100% (18/18) Negative predictivity : 100% (14/14)
Accuracy : 100% (32/32)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 71.4%(10/14)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (10/10)
Accuracy : 87.5%(28/32)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 20 | 0 | 0 | 20[e] |
| | - | 0 | 2 | 15 | 17[f] |
| Total | | 20 | 2 | 15 | 37 |

e: Two of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Anthracene)
f: Two of 19 non-phototoxic chemicals were not evaluated at20 or 200μM due to precipitation (Drometrizole, 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 : 88.2%(15/17)
Positive predictivity : 90.9%(20/22) Negative predictivity : 100% (15/15)
Accuracy : 94.6%(35/37)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[g] |
| | - | 0 | 4 | 14 | 18[h] |
| Total | | 21 | 4 | 14 | 39 |

g: One of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl) .
h: One of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (18/18)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (18/18)
Accuracy : 100% (39/39)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 77.8%(14/18)
Positive predictivity : 84.0%(21/25) Negative predictivity : 100% (14/14)
Accuracy : 89.7%(35/39)

Table 28D Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results: Final judgement based on the Final judgement based on the single assay results

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

| Lab 1 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[a] |
| | - | 0 | 4 | 12 | 16[b] |
| Total | | 21 | 4 | 12 | 37 |

a: One of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl).
b: Three of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octizole, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (16/16)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (16/16)
Accuracy : 100% (37/37)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 75.0% (12/16)
Positive predictivity : 84.0%(21/25) Negative predictivity : 100% (12/12)
Accuracy : 89.2%(33/37)

| Lab 2 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 18 | 0 | 0 | 18[c] |
| | - | 0 | 4 | 10 | 14[d] |
| Total | | 18 | 4 | 10 | 32 |

c: Four of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: Five of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 100% (14/14)
Positive predictivity : 100% (18/18) Negative predictivity : 100% (14/14)
Accuracy : 100% (32/32)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (18/18) Specificity : 71.4%(10/14)
Positive predictivity : 81.8%(18/22) Negative predictivity : 100% (10/10)
Accuracy : 87.5%(28/32)

| Lab 3 | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 20 | 0 | 0 | 20[e] |
| | - | 0 | 2 | 15 | 17[f] |
| Total | | 20 | 2 | 15 | 37 |

e: Two of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl, Anthracene)
f: Two of 19 non-phototoxic chemicals were not evaluated at20 or 200μM due to precipitation (Drometrizole, 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 : 88.2%(15/17)
Positive predictivity : 90.9%(20/22) Negative predictivity : 100% (15/15)
Accuracy : 94.6%(35/37)

| Integrated Judgement | | ROS | | | Total |
|---|---|---|---|---|---|
| | | + | ± | - | |
| Phototoxic | + | 21 | 0 | 0 | 21[g] |
| | - | 0 | 3 | 15 | 18[h] |
| Total | | 21 | 3 | 15 | 39 |

g: One of 22 phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (Amiodarone HCl) .
h: One of 19 non-phototoxic chemicals were not evaluated at 20 or 200μM due to precipitation (UV-571).

When the ±: Weakly photoreactive chemicals were defined as non-phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (18/18)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (18/18)
Accuracy : 100% (39/39)

When the ±: Weakly photoreactive chemicals were defined as phototoxic chemicals;
Sensitivity : 100% (21/21) Specificity : 83.3%(15/18)
Positive predictivity : 87.5%(21/24) Negative predictivity : 100% (15/15)
Accuracy : 92.3%(36/39)

Table 29 Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results

| Lab 1 | Draft criteria for the final judgement [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Original | | | | Data analysis based on the criteria for the fourth data analysis | | | | | | | |
| | | | | | 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% (22/22) | 100% (21/21) | 100% (21/21) | 100% (22/22) | 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 | 33.3% (4/12) | 41.7% (5/12) | 41.7% (5/12) | 58.3% (7/12) | 81.3% (13/16) | 93.8% (15/16) | 93.8% (15/16) | 100% (16/16) | 56.3% (9/16) | 62.5% (10/16) | 62.5% (10/16) | 75.0% (12/16) |
| Positive predictivity | 73.3% (22/30) | 75.0% (21/28) | 75.0% (21/28) | 81.5% (22/27) | 87.5% (21/24) | 95.5% (21/22) | 95.5% (21/22) | 100% (21/21) | 75.0% (21/28) | 77.8% (21/27) | 77.8% (21/27) | 84.0% (21/25) |
| Negative predictivity | 100% (4/4) | 100% (5/5) | 100% (5/5) | 100% (7/7) | 100% (13/13) | 100% (15/15) | 100% (15/15) | 100% (16/16) | 100% (9/9) | 100% (10/10) | 100% (10/10) | 100% (12/12) |
| Accuracy | 76.5% (26/34) | 78.8% (26/33) | 78.8% (26/33) | 85.3% (29/34) | 91.9% (34/37) | 97.3% (36/37) | 97.3% (36/37) | 100% (37/37) | 81.1% (30/37) | 83.8% (31/37) | 83.8% (31/37) | 89.2% (33/37) |

| Lab 2 | Draft criteria for the final judgement [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Original | | | | Data analysis based on the criteria for the fourth data analysis | | | | | | | |
| | | | | | 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% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) |
| Specificity | 36.4% (4/11) | 60.0% (6/10) | 60.0% (6/10) | 60.0% (6/10) | 100% (14/14) | 100% (14/14) | 100% (14/14) | 100% (14/14) | 57.1% (8/14) | 71.4% (10/14) | 71.4% (10/14) | 71.4% (10/14) |
| Positive predictivity | 74.1% (20/27) | 83.3% (20/24) | 83.3% (20/24) | 83.3% (20/24) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 75.0% (18/24) | 81.8% (18/22) | 81.8% (18/22) | 81.8% (18/22) |
| Negative predictivity | 100% (4/4) | 100% (6/6) | 100% (6/6) | 100% (6/6) | 100% (14/14) | 100% (14/14) | 100% (14/14) | 100% (14/14) | 100% (8/8) | 100% (10/10) | 100% (10/10) | 100% (10/10) |
| Accuracy | 77.4% (24/31) | 86.7% (26/30) | 86.7% (26/30) | 86.7% (26/30) | 100% (32/32) | 100% (32/32) | 100% (32/32) | 100% (32/32) | 81.3% (26/32) | 87.5% (28/32) | 87.5% (28/32) | 87.5% (28/32) |

a : A : Original : Final judgement of positive when positive results were obtained in at least one of three assays

Data analysis based on the criteria for the secondary data analysis: Final judgement of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

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

C : Final judgement based on the majority of three assay results

D : Final judgement based on the single assay results

Table 29 Fourth data analysis based on the criteria for the proposed protocol: Contingency table for Phase 2 results (continued).

| Lab 3 | Draft criteria for the final judgement [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Original | | | | Data analysis based on the criteria for the fourth data analysis | | | | | | | |
| | | | | | 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% (21/21) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 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 | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 81.8% (9/11) | 100% (17/17) | 100% (17/17) | 100% (17/17) | 100% (17/17) | 88.2% (15/17) | 88.2% (15/17) | 88.2% (15/17) | 88.2% (15/17) |
| Positive predictivity | 91.3% (21/23) | 91.3% (21/23) | 91.3% (21/23) | 91.3% (21/23) | 100% (20/20) | 100% (20/20) | 100% (20/20) | 100% (20/20) | 90.9% (20/22) | 90.9% (20/22) | 90.9% (20/22) | 90.9% (20/22) |
| Negative predictivity | 100% (9/9) | 100% (9/9) | 100% (9/9) | 100% (9/9) | 100% (17/17) | 100% (17/17) | 100% (17/17) | 100% (17/17) | 100% (15/15) | 100% (15/15) | 100% (15/15) | 100% (15/15) |
| Accuracy | 93.8% (30/32) | 93.8% (30/32) | 93.8% (30/32) | 93.8% (30/32) | 100% (37/37) | 100% (37/37) | 100% (37/37) | 100% (37/37) | 94.6% (35/37) | 94.6% (35/37) | 94.6% (35/37) | 94.6% (35/37) |

a : A : Original : Final judgement of positive when positive results were obtained in at least one of three assays

Data analysis based on the criteria for the secondary data analysis: Final judgement of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

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

C : Final judgement based on the majority of three assay results

D : Final judgement based on the single assay results

Table 30 Fourth data analysis based on the criteria for the proposed protocol: Contingency table for integrated judgement results.

| Phase 2 | Draft criteria for the final judgement [a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Original [b] | | | | Data analysis based on the criteria for the fourth data analysis [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% (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 | 45.5% (5/11) | 60.0% (6/10) | 60.0% (6/10) | 70.0% (7/10) | 100% (17/17) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 76.5% (13/17) | 77.8% (14/18) | 77.8% (14/18) | 83.3% (15/18) |
| Positive predictivity | 77.8% (21/27) | 84.0% (21/25) | 84.0% (21/25) | 87.5% (21/24) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 100% (21/21) | 84.0% (21/25) | 84.0% (21/25) | 84.0% (21/25) | 87.5% (21/24) |
| Negative predictivity | 100% (5/5) | 100% (6/6) | 100% (6/6) | 100% (7/7) | 100% (17/17) | 100% (18/18) | 100% (18/18) | 100% (18/18) | 100% (13/13) | 100% (14/14) | 100% (14/14) | 100% (15/15) |
| Accuracy | 81.3% (26/32) | 87.1% (27/31) | 87.1% (27/31) | 90.3% (28/31) | 100% (38/38) | 100% (39/39) | 100% (39/39) | 100% (39/39) | 89.5% (34/38) | 89.7% (35/39) | 89.7% (35/39) | 92.3% (36/39) |

Integrated judgements were made by the majority of each laboratory's final judgement.

a : A : Original : Final judgement of positive when positive results were obtained in at least one of three assays

Data analysis based on the criteria for the secondary data analysis: Final judgement of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

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

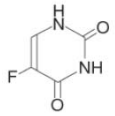
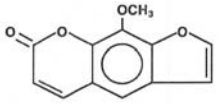
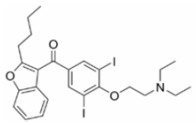
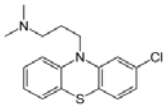
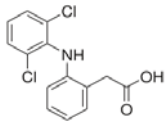
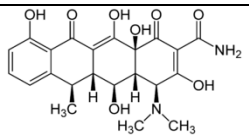
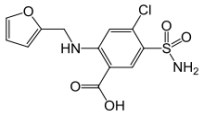
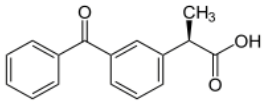
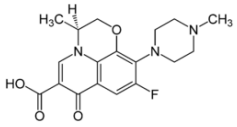
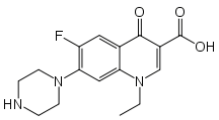
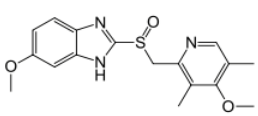
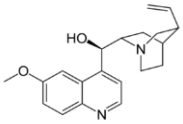
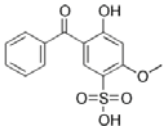
C : Final judgement based on the majority of three assay results

D : Final judgement based on the single assay results

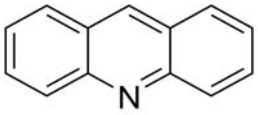
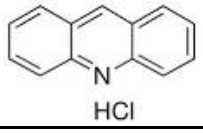
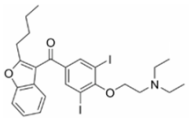
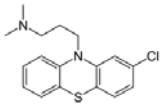
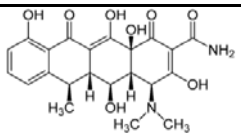
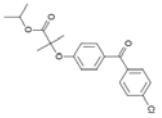
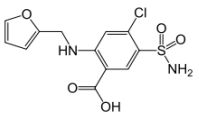
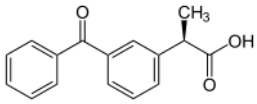
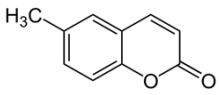
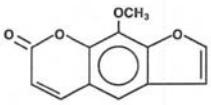
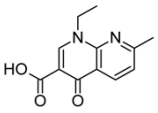
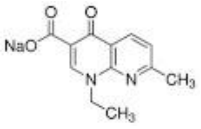
b : See, tables 12A to 12D

c : See, tables 27A to 27D

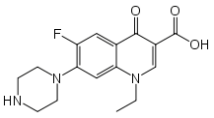
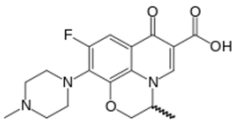
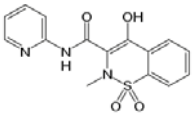
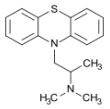
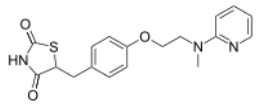
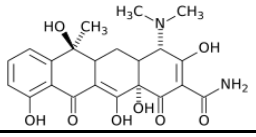
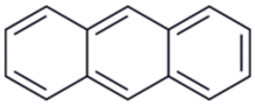
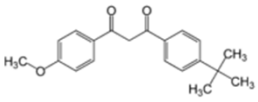
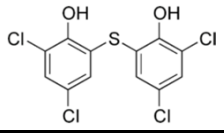
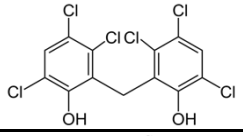
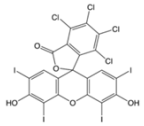
Appendix 1 Chemical structures of the test chemicals for the Phase 1 study

| 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 |  |

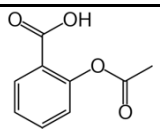
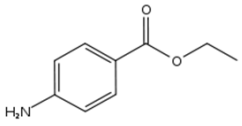
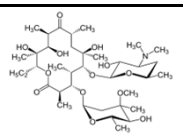
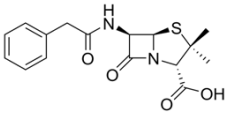
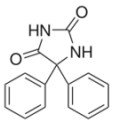
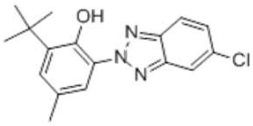
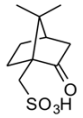
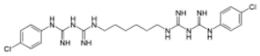
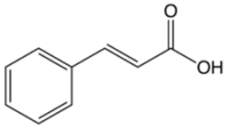
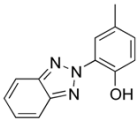
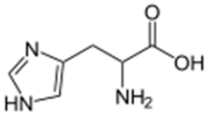
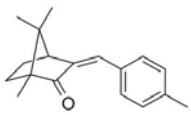
Appendix 2　Chemical structures of the test chemicals for the Phase 2 study

| 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 |  |

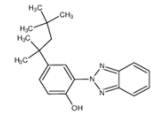
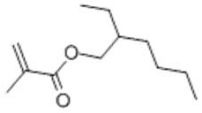
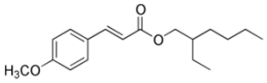
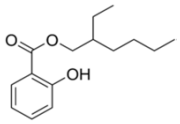
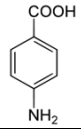
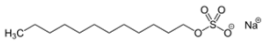
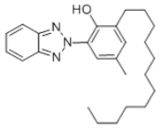
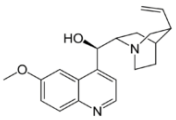
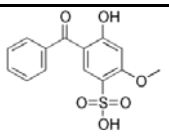
Appendix 2　Chemical structures of the test chemicals for the Phase 2 study

| NO. | Chemical name | CAS No.[a)] | Molecular weight | Chemical structure |
|---|---|---|---|---|
| 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 |  |

Appendix 2 Chemical structures of the test chemicals for the Phase 2 study

| NO. | Chemical name | CAS No.[a)] | Molecular weight | Chemical structure |
|---|---|---|---|---|
| **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 |  |

Appendix 2　Chemical structures of the test chemicals for the Phase 2 study

| NO. | Chemical name | CAS No.[a)] | Molecular weight | Chemical structure |
|---|---|---|---|---|
| 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 study

Laboratory : 1
Chemical Name : Qunine 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 | 0.869 | 0.873 | 0.394 | 0.867 | 469 | 1 | 1 | 0.036 | 0.032 | 0.394 | 0.036 | 350 |
| | 2 | 0.875 | 0.880 | 0.411 | 0.873 | 458 | | 2 | 0.034 | 0.047 | 0.406 | 0.040 | 363 |
| | 3 | 0.871 | 0.882 | 0.416 | 0.875 | 449 | | 3 | 0.045 | 0.030 | 0.411 | 0.061 | 357 |
| | Mean | 0.872 | 0.878 | 0.407 | 0.872 | 459 | | Mean | 0.038 | 0.036 | 0.404 | 0.045 | 357 |
| 2 | 1 | 0.867 | 0.880 | 0.407 | 0.869 | 451 | 2 | 1 | 0.037 | 0.033 | 0.396 | 0.036 | 348 |
| | 2 | 0.878 | 0.889 | 0.418 | 0.882 | 451 | | 2 | 0.035 | 0.052 | 0.399 | 0.050 | 353 |
| | 3 | 0.877 | 0.879 | 0.420 | 0.871 | 447 | | 3 | 0.041 | 0.033 | 0.415 | 0.066 | 362 |
| | Mean | 0.874 | 0.883 | 0.415 | 0.874 | 450 | | Mean | 0.038 | 0.039 | 0.403 | 0.051 | 354 |
| 3 | 1 | 0.890 | 0.886 | 0.395 | 0.877 | 486 | 3 | 1 | 0.035 | 0.033 | 0.415 | 0.039 | 371 |
| | 2 | 0.884 | 0.891 | 0.408 | 0.885 | 469 | | 2 | 0.035 | 0.048 | 0.412 | 0.044 | 367 |
| | 3 | 0.879 | 0.892 | 0.412 | 0.884 | 459 | | 3 | 0.042 | 0.032 | 0.425 | 0.057 | 373 |
| | Mean | 0.884 | 0.890 | 0.405 | 0.882 | 471 | | Mean | 0.037 | 0.037 | 0.417 | 0.047 | 370 |
| 4 | 1 | 0.860 | 0.871 | 0.390 | 0.864 | 463 | 4 | 1 | 0.036 | 0.033 | 0.387 | 0.058 | 332 |
| | 2 | 0.860 | 0.870 | 0.405 | 0.864 | 449 | | 2 | 0.034 | 0.035 | 0.383 | 0.041 | 329 |
| | 3 | 0.873 | 0.875 | 0.413 | 0.867 | 453 | | 3 | 0.041 | 0.030 | 0.389 | 0.058 | 328 |
| | Mean | 0.864 | 0.872 | 0.403 | 0.865 | 455 | | Mean | 0.037 | 0.033 | 0.386 | 0.053 | 330 |
| 5 | 1 | 0.859 | 0.859 | 0.399 | 0.850 | 452 | 5 | 1 | 0.041 | 0.030 | 0.394 | 0.038 | 342 |
| | 2 | 0.852 | 0.876 | 0.406 | 0.868 | 438 | | 2 | 0.035 | 0.058 | 0.386 | 0.056 | 340 |
| | 3 | 0.866 | 0.871 | 0.415 | 0.863 | 441 | | 3 | 0.041 | 0.031 | 0.406 | 0.057 | 354 |
| | Mean | 0.859 | 0.869 | 0.407 | 0.860 | 444 | | Mean | 0.039 | 0.039 | 0.395 | 0.050 | 345 |
| 6 | 1 | 0.853 | 0.868 | 0.382 | 0.861 | 464 | 6 | 1 | 0.040 | 0.029 | 0.393 | 0.036 | 344 |
| | 2 | 0.850 | 0.865 | 0.398 | 0.858 | 445 | | 2 | 0.035 | 0.045 | 0.403 | 0.042 | 359 |
| | 3 | 0.864 | 0.865 | 0.401 | 0.857 | 455 | | 3 | 0.043 | 0.031 | 0.414 | 0.055 | 362 |
| | Mean | 0.856 | 0.866 | 0.394 | 0.858 | 455 | | Mean | 0.039 | 0.035 | 0.403 | 0.044 | 355 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

The optical absorbance values were rounded to the third decimal place. If the calculated "results" are different from the numerical results in the raw data sheets by the number of significant digits in the data sheet and the appendices are different, the data in the raw data sheet is used in the appendices.

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 1

Chemical Name : Qunine 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 | 0.846 | 0.871 | 0.380 | 0.862 | 457 | 7 | 1 | 0.037 | 0.028 | 0.402 | 0.037 | 353 |
| | 2 | 0.851 | 0.877 | 0.395 | 0.868 | 447 | | 2 | 0.035 | 0.046 | 0.404 | 0.042 | 359 |
| | 3 | 0.857 | 0.880 | 0.401 | 0.871 | 447 | | 3 | 0.040 | 0.031 | 0.415 | 0.061 | 364 |
| | Mean | 0.851 | 0.876 | 0.392 | 0.867 | 450 | | Mean | 0.037 | 0.035 | 0.407 | 0.046 | 359 |
| 8 | 1 | 0.824 | 0.853 | 0.372 | 0.849 | 444 | 8 | 1 | 0.038 | 0.033 | 0.425 | 0.037 | 375 |
| | 2 | 0.840 | 0.859 | 0.391 | 0.852 | 442 | | 2 | 0.036 | 0.048 | 0.423 | 0.039 | 375 |
| | 3 | 0.845 | 0.861 | 0.398 | 0.853 | 440 | | 3 | 0.040 | 0.032 | 0.427 | 0.074 | 375 |
| | Mean | 0.836 | 0.858 | 0.387 | 0.851 | 442 | | Mean | 0.038 | 0.038 | 0.425 | 0.050 | 375 |
| 9 | 1 | 0.865 | 0.884 | 0.386 | 0.878 | 473 | 9 | 1 | 0.043 | 0.032 | 0.396 | 0.037 | 343 |
| | 2 | 0.848 | 0.886 | 0.398 | 0.879 | 444 | | 2 | 0.034 | 0.043 | 0.408 | 0.037 | 364 |
| | 3 | 0.867 | 0.880 | 0.407 | 0.873 | 455 | | 3 | 0.040 | 0.030 | 0.424 | 0.061 | 374 |
| | Mean | 0.860 | 0.883 | 0.397 | 0.877 | 457 | | Mean | 0.039 | 0.035 | 0.409 | 0.045 | 360 |
| 10 | 1 | 0.870 | 0.865 | 0.379 | 0.856 | 483 | 10 | 1 | 0.054 | 0.033 | 0.434 | 0.036 | 370 |
| | 2 | 0.855 | 0.880 | 0.389 | 0.874 | 458 | | 2 | 0.045 | 0.033 | 0.438 | 0.039 | 384 |
| | 3 | 0.880 | 0.873 | 0.400 | 0.865 | 472 | | 3 | 0.061 | 0.029 | 0.451 | 0.050 | 381 |
| | Mean | 0.868 | 0.873 | 0.389 | 0.865 | 471 | | Mean | 0.053 | 0.032 | 0.441 | 0.042 | 378 |
| 11 | 1 | 0.862 | 0.863 | 0.373 | 0.853 | 480 | 11 | 1 | 0.062 | 0.029 | 0.435 | 0.035 | 359 |
| | 2 | 0.859 | 0.873 | 0.392 | 0.865 | 458 | | 2 | 0.040 | 0.030 | 0.444 | 0.038 | 390 |
| | 3 | 0.886 | 0.875 | 0.406 | 0.867 | 472 | | 3 | 0.063 | 0.029 | 0.446 | 0.054 | 369 |
| | Mean | 0.869 | 0.870 | 0.390 | 0.861 | 470 | | Mean | 0.055 | 0.029 | 0.442 | 0.043 | 373 |
| 12 | 1 | 0.873 | 0.844 | 0.372 | 0.836 | 496 | 12 | 1 | 0.039 | 0.030 | 0.435 | 0.037 | 383 |
| | 2 | 0.857 | 0.844 | 0.386 | 0.842 | 466 | | 2 | 0.035 | 0.052 | 0.434 | 0.040 | 386 |
| | 3 | 0.879 | 0.851 | 0.395 | 0.845 | 479 | | 3 | 0.042 | 0.032 | 0.449 | 0.075 | 394 |
| | Mean | 0.870 | 0.846 | 0.384 | 0.841 | 480 | | Mean | 0.039 | 0.038 | 0.439 | 0.051 | 388 |
| Mean for all assays | | - | - | - | - | 459 | Mean for all assays | | - | - | - | - | 362 |
| SD for all assays | | - | - | - | - | 12 | SD for all assays | | - | - | - | - | 16 |
| CV for all assays | | - | - | - | - | 2.6 | CV for all assays | | - | - | - | - | 4.4 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 1
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 | 0.888 | 0.873 | 0.886 | 0.867 | -4 | 1 | 1 | 0.041 | 0.032 | 0.041 | 0.036 | -9 |
| | 2 | 0.884 | 0.880 | 0.882 | 0.873 | -4 | | 2 | 0.051 | 0.047 | 0.046 | 0.040 | -14 |
| | 3 | 0.892 | 0.882 | 0.889 | 0.875 | -4 | | 3 | 0.040 | 0.030 | 0.045 | 0.061 | -4 |
| | Mean | 0.888 | 0.878 | 0.886 | 0.872 | -4 | | Mean | 0.044 | 0.036 | 0.044 | 0.045 | -9 |
| 2 | 1 | 0.895 | 0.880 | 0.893 | 0.869 | -8 | 2 | 1 | 0.042 | 0.033 | 0.039 | 0.036 | -15 |
| | 2 | 0.903 | 0.889 | 0.897 | 0.882 | -4 | | 2 | 0.042 | 0.052 | 0.040 | 0.050 | -14 |
| | 3 | 0.900 | 0.879 | 0.897 | 0.871 | -6 | | 3 | 0.039 | 0.033 | 0.041 | 0.066 | -10 |
| | Mean | 0.899 | 0.883 | 0.896 | 0.874 | -6 | | Mean | 0.041 | 0.039 | 0.040 | 0.051 | -13 |
| 3 | 1 | 0.888 | 0.886 | 0.886 | 0.877 | -6 | 3 | 1 | 0.039 | 0.033 | 0.041 | 0.039 | -8 |
| | 2 | 0.892 | 0.891 | 0.885 | 0.885 | -1 | | 2 | 0.049 | 0.048 | 0.048 | 0.044 | -10 |
| | 3 | 0.891 | 0.892 | 0.892 | 0.884 | -9 | | 3 | 0.040 | 0.032 | 0.040 | 0.057 | -10 |
| | Mean | 0.890 | 0.890 | 0.888 | 0.882 | -5 | | Mean | 0.043 | 0.037 | 0.043 | 0.047 | -9 |
| 4 | 1 | 0.892 | 0.871 | 0.886 | 0.864 | -1 | 4 | 1 | 0.043 | 0.033 | 0.041 | 0.058 | -21 |
| | 2 | 0.886 | 0.870 | 0.877 | 0.864 | 2 | | 2 | 0.050 | 0.035 | 0.048 | 0.041 | -22 |
| | 3 | 0.901 | 0.875 | 0.893 | 0.867 | 1 | | 3 | 0.040 | 0.030 | 0.040 | 0.058 | -20 |
| | Mean | 0.893 | 0.872 | 0.885 | 0.865 | 1 | | Mean | 0.044 | 0.033 | 0.043 | 0.053 | -21 |
| 5 | 1 | 0.894 | 0.859 | 0.888 | 0.850 | -4 | 5 | 1 | 0.042 | 0.030 | 0.040 | 0.038 | -13 |
| | 2 | 0.886 | 0.876 | 0.874 | 0.868 | 3 | | 2 | 0.046 | 0.058 | 0.042 | 0.056 | -15 |
| | 3 | 0.886 | 0.871 | 0.879 | 0.863 | -2 | | 3 | 0.039 | 0.031 | 0.039 | 0.057 | -11 |
| | Mean | 0.889 | 0.869 | 0.880 | 0.860 | -1 | | Mean | 0.042 | 0.039 | 0.040 | 0.050 | -13 |
| 6 | 1 | 0.881 | 0.868 | 0.874 | 0.861 | -1 | 6 | 1 | 0.040 | 0.029 | 0.039 | 0.036 | -10 |
| | 2 | 0.872 | 0.865 | 0.864 | 0.858 | 0 | | 2 | 0.045 | 0.045 | 0.040 | 0.042 | -14 |
| | 3 | 0.884 | 0.865 | 0.875 | 0.857 | 0 | | 3 | 0.039 | 0.031 | 0.039 | 0.055 | -8 |
| | Mean | 0.879 | 0.866 | 0.871 | 0.858 | 0 | | Mean | 0.041 | 0.035 | 0.039 | 0.044 | -11 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 1

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 | 0.882 | 0.871 | 0.857 | 0.862 | 16 | 7 | 1 | 0.040 | 0.028 | 0.042 | 0.037 | -9 |
| | 2 | 0.883 | 0.877 | 0.865 | 0.868 | 8 | | 2 | 0.046 | 0.046 | 0.043 | 0.042 | -14 |
| | 3 | 0.896 | 0.880 | 0.871 | 0.871 | 16 | | 3 | 0.038 | 0.031 | 0.040 | 0.061 | -10 |
| | Mean | 0.887 | 0.876 | 0.864 | 0.867 | 13 | | Mean | 0.041 | 0.035 | 0.042 | 0.046 | -11 |
| 8 | 1 | 0.874 | 0.853 | 0.849 | 0.849 | 18 | 8 | 1 | 0.039 | 0.033 | 0.040 | 0.037 | -11 |
| | 2 | 0.873 | 0.859 | 0.854 | 0.852 | 12 | | 2 | 0.045 | 0.048 | 0.042 | 0.039 | -16 |
| | 3 | 0.876 | 0.861 | 0.852 | 0.853 | 17 | | 3 | 0.039 | 0.032 | 0.040 | 0.074 | -11 |
| | Mean | 0.874 | 0.858 | 0.852 | 0.851 | 16 | | Mean | 0.041 | 0.038 | 0.041 | 0.050 | -13 |
| 9 | 1 | 0.869 | 0.884 | 0.859 | 0.878 | 4 | 9 | 1 | 0.038 | 0.032 | 0.039 | 0.037 | -9 |
| | 2 | 0.868 | 0.886 | 0.857 | 0.879 | 5 | | 2 | 0.044 | 0.043 | 0.041 | 0.037 | -13 |
| | 3 | 0.876 | 0.880 | 0.866 | 0.873 | 4 | | 3 | 0.039 | 0.030 | 0.039 | 0.061 | -11 |
| | Mean | 0.871 | 0.883 | 0.861 | 0.877 | 4 | | Mean | 0.040 | 0.035 | 0.040 | 0.045 | -11 |
| 10 | 1 | 0.862 | 0.865 | 0.871 | 0.856 | -17 | 10 | 1 | 0.039 | 0.033 | 0.041 | 0.036 | -7 |
| | 2 | 0.877 | 0.880 | 0.875 | 0.874 | -6 | | 2 | 0.039 | 0.033 | 0.041 | 0.039 | -9 |
| | 3 | 0.876 | 0.873 | 0.887 | 0.865 | -19 | | 3 | 0.037 | 0.029 | 0.038 | 0.050 | -9 |
| | Mean | 0.872 | 0.873 | 0.878 | 0.865 | -14 | | Mean | 0.038 | 0.032 | 0.040 | 0.042 | -8 |
| 11 | 1 | 0.868 | 0.863 | 0.857 | 0.853 | 2 | 11 | 1 | 0.039 | 0.029 | 0.039 | 0.035 | -14 |
| | 2 | 0.875 | 0.873 | 0.863 | 0.865 | 3 | | 2 | 0.039 | 0.030 | 0.040 | 0.038 | -13 |
| | 3 | 0.873 | 0.875 | 0.862 | 0.867 | 2 | | 3 | 0.039 | 0.029 | 0.038 | 0.054 | -15 |
| | Mean | 0.872 | 0.870 | 0.861 | 0.861 | 2 | | Mean | 0.039 | 0.029 | 0.039 | 0.043 | -14 |
| 12 | 1 | 0.863 | 0.844 | 0.872 | 0.836 | -14 | 12 | 1 | 0.039 | 0.030 | 0.038 | 0.037 | -14 |
| | 2 | 0.866 | 0.844 | 0.864 | 0.842 | -3 | | 2 | 0.041 | 0.052 | 0.039 | 0.040 | -15 |
| | 3 | 0.867 | 0.851 | 0.878 | 0.845 | -16 | | 3 | 0.038 | 0.032 | 0.038 | 0.075 | -13 |
| | Mean | 0.865 | 0.846 | 0.871 | 0.841 | -11 | | Mean | 0.039 | 0.038 | 0.038 | 0.051 | -14 |
| Mean for all assays | | - | - | - | - | 0 | Mean for all assays | | - | - | - | - | -12 |
| SD for all assays | | - | - | - | - | 9 | SD for all assays | | - | - | - | - | 3 |
| CV for all assays | | - | - | - | - | - | CV for all assays | | - | - | - | - | - |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 2

Chemical Name : Qunine 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 | 0.996 | 1.003 | 0.597 | 0.992 | 390 | 1 | 1 | 0.034 | 0.038 | 0.380 | 0.042 | 335 |
| | 2 | 0.993 | 1.002 | 0.609 | 0.994 | 375 | | 2 | 0.032 | 0.036 | 0.374 | 0.059 | 331 |
| | 3 | 0.990 | 1.005 | 0.606 | 0.995 | 375 | | 3 | 0.037 | 0.034 | 0.376 | 0.040 | 328 |
| | Mean | 0.993 | 1.003 | 0.604 | 0.994 | 380 | | Mean | 0.034 | 0.036 | 0.377 | 0.047 | 331 |
| 3 | 1 | 1.000 | 1.005 | 0.573 | 1.004 | 421 | 2 | 1 | 0.032 | 0.033 | 0.341 | 0.037 | 304 |
| | 2 | 0.995 | 1.005 | 0.582 | 0.998 | 407 | | 2 | 0.034 | 0.035 | 0.339 | 0.040 | 300 |
| | 3 | 0.993 | 1.009 | 0.584 | 0.998 | 403 | | 3 | 0.033 | 0.034 | 0.357 | 0.039 | 319 |
| | Mean | 0.996 | 1.006 | 0.580 | 1.000 | 410 | | Mean | 0.033 | 0.034 | 0.346 | 0.039 | 308 |
| 2 | 1 | 0.994 | 1.007 | 0.570 | 0.999 | 412 | 3 | 1 | 0.033 | 0.032 | 0.437 | 0.041 | 396 |
| | 2 | 1.001 | 1.013 | 0.586 | 0.997 | 403 | | 2 | 0.032 | 0.032 | 0.413 | 0.039 | 373 |
| | 3 | 1.001 | 1.009 | 0.589 | 0.998 | 400 | | 3 | 0.032 | 0.034 | 0.384 | 0.042 | 344 |
| | Mean | 0.999 | 1.010 | 0.582 | 0.998 | 405 | | Mean | 0.032 | 0.033 | 0.411 | 0.041 | 371 |
| 4 | 1 | 0.985 | 1.001 | 0.559 | 0.996 | 417 | 4 | 1 | 0.033 | 0.033 | 0.412 | 0.041 | 371 |
| | 2 | 1.000 | 1.003 | 0.581 | 0.993 | 410 | | 2 | 0.033 | 0.033 | 0.404 | 0.041 | 363 |
| | 3 | 0.994 | 1.004 | 0.575 | 0.992 | 410 | | 3 | 0.033 | 0.033 | 0.389 | 0.042 | 348 |
| | Mean | 0.993 | 1.003 | 0.572 | 0.994 | 412 | | Mean | 0.033 | 0.033 | 0.402 | 0.041 | 361 |
| 5 | 1 | 0.996 | 1.000 | 0.558 | 0.991 | 428 | 5 | 1 | 0.032 | 0.032 | 0.358 | 0.038 | 319 |
| | 2 | 0.990 | 0.995 | 0.572 | 0.988 | 408 | | 2 | 0.032 | 0.033 | 0.352 | 0.040 | 313 |
| | 3 | 0.992 | 1.011 | 0.575 | 0.996 | 407 | | 3 | 0.032 | 0.033 | 0.365 | 0.042 | 326 |
| | Mean | 0.993 | 1.002 | 0.568 | 0.992 | 414 | | Mean | 0.032 | 0.033 | 0.358 | 0.040 | 319 |
| 6 | 1 | 1.001 | 1.004 | 0.570 | 1.000 | 424 | 6 | 1 | 0.032 | 0.033 | 0.345 | 0.038 | 307 |
| | 2 | 0.991 | 1.000 | 0.584 | 0.994 | 400 | | 2 | 0.032 | 0.032 | 0.344 | 0.038 | 306 |
| | 3 | 0.992 | 1.008 | 0.586 | 0.998 | 399 | | 3 | 0.032 | 0.032 | 0.352 | 0.038 | 314 |
| | Mean | 0.995 | 1.004 | 0.580 | 0.997 | 408 | | Mean | 0.032 | 0.032 | 0.347 | 0.038 | 309 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 2

Chemical Name : Qunine 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 | 0.988 | 1.002 | 0.548 | 0.986 | 428 | 7 | 1 | 0.032 | 0.032 | 0.443 | 0.038 | 404 |
| | 2 | 0.990 | 1.002 | 0.570 | 0.993 | 408 | | 2 | 0.032 | 0.032 | 0.444 | 0.040 | 405 |
| | 3 | 0.997 | 1.004 | 0.578 | 0.995 | 407 | | 3 | 0.032 | 0.033 | 0.464 | 0.040 | 425 |
| | Mean | 0.992 | 1.003 | 0.565 | 0.991 | 414 | | Mean | 0.032 | 0.032 | 0.450 | 0.039 | 411 |
| 8 | 1 | 0.994 | 1.000 | 0.554 | 0.991 | 432 | 8 | 1 | 0.032 | 0.032 | 0.489 | 0.038 | 449 |
| | 2 | 0.989 | 0.996 | 0.567 | 0.990 | 414 | | 2 | 0.032 | 0.033 | 0.475 | 0.045 | 435 |
| | 3 | 0.991 | 1.003 | 0.570 | 0.995 | 413 | | 3 | 0.032 | 0.034 | 0.438 | 0.040 | 398 |
| | Mean | 0.991 | 1.000 | 0.564 | 0.992 | 420 | | Mean | 0.032 | 0.033 | 0.467 | 0.041 | 427 |
| 9 | 1 | 0.987 | 0.995 | 0.554 | 0.986 | 424 | 9 | 1 | 0.033 | 0.032 | 0.401 | 0.038 | 357 |
| | 2 | 0.983 | 0.994 | 0.575 | 0.986 | 399 | | 2 | 0.033 | 0.034 | 0.377 | 0.051 | 333 |
| | 3 | 0.994 | 1.001 | 0.579 | 0.993 | 406 | | 3 | 0.033 | 0.032 | 0.415 | 0.044 | 371 |
| | Mean | 0.988 | 0.997 | 0.569 | 0.988 | 410 | | Mean | 0.033 | 0.033 | 0.398 | 0.044 | 354 |
| 11 | 1 | 0.988 | 0.994 | 0.545 | 0.965 | 427 | 10 | 1 | 0.034 | 0.033 | 0.423 | 0.038 | 381 |
| | 2 | 0.988 | 0.997 | 0.561 | 0.987 | 411 | | 2 | 0.032 | 0.032 | 0.405 | 0.045 | 365 |
| | 3 | 0.994 | 0.997 | 0.569 | 0.989 | 409 | | 3 | 0.033 | 0.033 | 0.398 | 0.040 | 357 |
| | Mean | 0.990 | 0.996 | 0.558 | 0.980 | 416 | | Mean | 0.033 | 0.033 | 0.409 | 0.041 | 368 |
| 10 | 1 | 0.993 | 1.002 | 0.545 | 0.990 | 438 | 11 | 1 | 0.032 | 0.033 | 0.395 | 0.038 | 357 |
| | 2 | 0.995 | 1.004 | 0.561 | 0.992 | 424 | | 2 | 0.036 | 0.033 | 0.382 | 0.038 | 340 |
| | 3 | 0.991 | 1.008 | 0.571 | 1.003 | 410 | | 3 | 0.032 | 0.033 | 0.417 | 0.040 | 379 |
| | Mean | 0.993 | 1.005 | 0.559 | 0.995 | 424 | | Mean | 0.033 | 0.033 | 0.398 | 0.039 | 359 |
| 12 | 1 | 0.996 | 1.009 | 0.540 | 0.999 | 439 | 12 | 1 | 0.033 | 0.035 | 0.395 | 0.040 | 356 |
| | 2 | 1.003 | 1.007 | 0.560 | 0.977 | 426 | | 2 | 0.032 | 0.034 | 0.397 | 0.041 | 359 |
| | 3 | 1.000 | 1.010 | 0.566 | 1.000 | 417 | | 3 | 0.032 | 0.033 | 0.416 | 0.039 | 378 |
| | Mean | 1.000 | 1.009 | 0.555 | 0.992 | 427 | | Mean | 0.032 | 0.034 | 0.403 | 0.040 | 364 |
| Mean for all assays | | - | - | - | - | 412 | Mean for all assays | | - | - | - | - | 357 |
| SD for all assays | | - | - | - | - | 12 | SD for all assays | | - | - | - | - | 37 |
| CV for all assays | | - | - | - | - | 2.9 | CV for all assays | | - | - | - | - | 10.4 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 2
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 | 0.996 | 0.992 | -1 | 1 | 1 | 0.038 | 0.038 | 0.039 | 0.042 | -10 |
| | 2 | 1.009 | 1.002 | 1.003 | 0.994 | -3 | | 2 | 0.037 | 0.036 | 0.038 | 0.059 | -10 |
| | 3 | 1.008 | 1.005 | 0.999 | 0.995 | 0 | | 3 | 0.037 | 0.034 | 0.038 | 0.040 | -10 |
| | Mean | 1.007 | 1.003 | 0.999 | 0.994 | -1 | | Mean | 0.037 | 0.036 | 0.038 | 0.047 | -10 |
| 2 | 1 | 1.006 | 1.005 | 0.997 | 1.004 | 3 | 2 | 1 | 0.038 | 0.033 | 0.039 | 0.037 | -4 |
| | 2 | 1.011 | 1.005 | 1.003 | 0.998 | 2 | | 2 | 0.039 | 0.035 | 0.040 | 0.040 | -4 |
| | 3 | 1.013 | 1.009 | 1.001 | 0.998 | 6 | | 3 | 0.039 | 0.034 | 0.041 | 0.039 | -3 |
| | Mean | 1.010 | 1.006 | 1.000 | 1.000 | 4 | | Mean | 0.039 | 0.034 | 0.040 | 0.039 | -4 |
| 3 | 1 | 1.007 | 1.007 | 0.999 | 0.999 | -4 | 3 | 1 | 0.038 | 0.032 | 0.038 | 0.041 | -8 |
| | 2 | 1.012 | 1.013 | 0.999 | 0.997 | 1 | | 2 | 0.037 | 0.032 | 0.039 | 0.039 | -6 |
| | 3 | 1.011 | 1.009 | 0.999 | 0.998 | 0 | | 3 | 0.037 | 0.034 | 0.038 | 0.042 | -7 |
| | Mean | 1.010 | 1.010 | 0.999 | 0.998 | -1 | | Mean | 0.037 | 0.033 | 0.038 | 0.041 | -7 |
| 4 | 1 | 1.002 | 1.001 | 0.997 | 0.996 | -4 | 4 | 1 | 0.038 | 0.033 | 0.039 | 0.041 | -7 |
| | 2 | 0.990 | 1.003 | 0.978 | 0.993 | 3 | | 2 | 0.037 | 0.033 | 0.039 | 0.041 | -6 |
| | 3 | 1.009 | 1.004 | 0.998 | 0.992 | 2 | | 3 | 0.039 | 0.033 | 0.040 | 0.042 | -7 |
| | Mean | 1.000 | 1.003 | 0.991 | 0.994 | 0 | | Mean | 0.038 | 0.033 | 0.039 | 0.041 | -7 |
| 5 | 1 | 1.002 | 1.000 | 0.991 | 0.991 | 1 | 5 | 1 | 0.038 | 0.032 | 0.038 | 0.038 | -7 |
| | 2 | 1.002 | 0.995 | 0.993 | 0.988 | -1 | | 2 | 0.038 | 0.033 | 0.051 | 0.040 | 6 |
| | 3 | 1.008 | 1.011 | 0.999 | 0.996 | -1 | | 3 | 0.042 | 0.033 | 0.045 | 0.042 | -4 |
| | Mean | 1.004 | 1.002 | 0.994 | 0.992 | 0 | | Mean | 0.039 | 0.033 | 0.045 | 0.040 | -2 |
| 6 | 1 | 1.004 | 1.004 | 1.002 | 1.000 | -5 | 6 | 1 | 0.038 | 0.033 | 0.039 | 0.038 | -5 |
| | 2 | 1.005 | 1.000 | 0.997 | 0.994 | 1 | | 2 | 0.037 | 0.032 | 0.039 | 0.038 | -4 |
| | 3 | 1.007 | 1.008 | 1.001 | 0.998 | -1 | | 3 | 0.037 | 0.032 | 0.038 | 0.038 | -5 |
| | Mean | 1.005 | 1.004 | 1.000 | 0.997 | -2 | | Mean | 0.037 | 0.032 | 0.039 | 0.038 | -5 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 2

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 | 0.998 | 1.002 | 0.989 | 0.986 | -3 | 7 | 1 | 0.037 | 0.032 | 0.039 | 0.038 | -5 |
| | 2 | 1.005 | 1.002 | 0.995 | 0.993 | -2 | | 2 | 0.038 | 0.032 | 0.038 | 0.040 | -7 |
| | 3 | 1.007 | 1.004 | 0.999 | 0.995 | -4 | | 3 | 0.037 | 0.033 | 0.040 | 0.040 | -4 |
| | Mean | 1.003 | 1.003 | 0.994 | 0.991 | -3 | | Mean | 0.037 | 0.032 | 0.039 | 0.039 | -5 |
| 8 | 1 | 1.000 | 1.000 | 0.987 | 0.991 | 5 | 8 | 1 | 0.037 | 0.032 | 0.037 | 0.038 | -8 |
| | 2 | 1.003 | 0.996 | 0.998 | 0.990 | -3 | | 2 | 0.037 | 0.033 | 0.038 | 0.045 | -7 |
| | 3 | 1.007 | 1.003 | 1.000 | 0.995 | -1 | | 3 | 0.039 | 0.034 | 0.039 | 0.040 | -8 |
| | Mean | 1.003 | 1.000 | 0.995 | 0.992 | 0 | | Mean | 0.038 | 0.033 | 0.038 | 0.041 | -8 |
| 9 | 1 | 0.995 | 0.995 | 0.990 | 0.986 | -4 | 9 | 1 | 0.037 | 0.032 | 0.038 | 0.038 | -10 |
| | 2 | 1.001 | 0.994 | 0.992 | 0.986 | 0 | | 2 | 0.037 | 0.034 | 0.038 | 0.051 | -10 |
| | 3 | 1.004 | 1.001 | 0.998 | 0.993 | -3 | | 3 | 0.037 | 0.032 | 0.039 | 0.044 | -9 |
| | Mean | 1.000 | 0.997 | 0.993 | 0.988 | -2 | | Mean | 0.037 | 0.033 | 0.038 | 0.044 | -10 |
| 10 | 1 | 1.000 | 0.994 | 0.992 | 0.965 | -8 | 10 | 1 | 0.037 | 0.033 | 0.039 | 0.038 | -6 |
| | 2 | 1.000 | 0.997 | 0.993 | 0.987 | -9 | | 2 | 0.037 | 0.032 | 0.038 | 0.045 | -7 |
| | 3 | 1.003 | 0.997 | 0.995 | 0.989 | -8 | | 3 | 0.038 | 0.033 | 0.038 | 0.040 | -8 |
| | Mean | 1.001 | 0.996 | 0.993 | 0.980 | -8 | | Mean | 0.037 | 0.033 | 0.038 | 0.041 | -7 |
| 11 | 1 | 1.000 | 1.002 | 0.990 | 0.990 | 0 | 11 | 1 | 0.040 | 0.033 | 0.039 | 0.038 | -7 |
| | 2 | 1.007 | 1.004 | 0.999 | 0.992 | -2 | | 2 | 0.038 | 0.033 | 0.038 | 0.038 | -6 |
| | 3 | 1.007 | 1.008 | 1.004 | 1.003 | -7 | | 3 | 0.037 | 0.033 | 0.038 | 0.040 | -5 |
| | Mean | 1.005 | 1.005 | 0.998 | 0.995 | -3 | | Mean | 0.038 | 0.033 | 0.038 | 0.039 | -6 |
| 12 | 1 | 1.008 | 1.009 | 0.998 | 0.999 | -7 | 12 | 1 | 0.038 | 0.035 | 0.039 | 0.040 | -5 |
| | 2 | 1.008 | 1.007 | 0.990 | 0.977 | 1 | | 2 | 0.038 | 0.034 | 0.039 | 0.041 | -5 |
| | 3 | 1.013 | 1.010 | 1.008 | 1.000 | -12 | | 3 | 0.038 | 0.033 | 0.040 | 0.039 | -4 |
| | Mean | 1.010 | 1.009 | 0.999 | 0.992 | -6 | | Mean | 0.038 | 0.034 | 0.039 | 0.040 | -5 |
| Mean for all assays | | - | - | - | - | -2 | Mean for all assays | | - | - | - | - | -6 |
| SD for all assays | | - | - | - | - | 3 | SD for all assays | | - | - | - | - | 2 |
| CV for all assays | | - | - | - | - | - | CV for all assays | | - | - | - | - | - |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 3

Chemical Name : Qunine 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 | 0.988 | 0.996 | 0.606 | 0.982 | 366 | 1 | 1 | 0.037 | 0.038 | 0.245 | 0.040 | 205 |
| | 2 | 0.991 | 1.001 | 0.614 | 0.987 | 361 | | 2 | 0.039 | 0.039 | 0.248 | 0.041 | 206 |
| | 3 | 0.984 | 1.000 | 0.614 | 0.983 | 355 | | 3 | 0.038 | 0.038 | 0.269 | 0.040 | 228 |
| | Mean | 0.987 | 0.999 | 0.612 | 0.984 | 361 | | Mean | 0.038 | 0.038 | 0.254 | 0.041 | 213 |
| 2 | 1 | 0.978 | 0.980 | 0.593 | 0.965 | 371 | 2 | 1 | 0.036 | 0.037 | 0.261 | 0.040 | 223 |
| | 2 | 0.975 | 0.989 | 0.602 | 0.977 | 359 | | 2 | 0.038 | 0.039 | 0.254 | 0.041 | 213 |
| | 3 | 0.974 | 0.988 | 0.598 | 0.974 | 363 | | 3 | 0.038 | 0.037 | 0.287 | 0.040 | 247 |
| | Mean | 0.976 | 0.986 | 0.598 | 0.972 | 364 | | Mean | 0.038 | 0.038 | 0.267 | 0.040 | 228 |
| 3 | 1 | 0.967 | 0.982 | 0.598 | 0.967 | 357 | 3 | 1 | 0.037 | 0.038 | 0.236 | 0.040 | 197 |
| | 2 | 0.966 | 0.980 | 0.606 | 0.967 | 347 | | 2 | 0.039 | 0.039 | 0.229 | 0.041 | 188 |
| | 3 | 0.962 | 0.976 | 0.608 | 0.964 | 342 | | 3 | 0.038 | 0.038 | 0.254 | 0.040 | 213 |
| | Mean | 0.965 | 0.979 | 0.604 | 0.966 | 349 | | Mean | 0.038 | 0.038 | 0.240 | 0.040 | 199 |
| 4 | 1 | 0.984 | 0.990 | 0.605 | 0.979 | 368 | 4 | 1 | 0.037 | 0.039 | 0.268 | 0.041 | 228 |
| | 2 | 0.986 | 0.997 | 0.618 | 0.985 | 357 | | 2 | 0.039 | 0.039 | 0.262 | 0.041 | 220 |
| | 3 | 0.982 | 0.995 | 0.607 | 0.985 | 364 | | 3 | 0.039 | 0.038 | 0.280 | 0.040 | 238 |
| | Mean | 0.984 | 0.994 | 0.610 | 0.983 | 363 | | Mean | 0.038 | 0.038 | 0.270 | 0.041 | 229 |
| 5 | 1 | 0.979 | 0.988 | 0.610 | 0.975 | 356 | 5 | 1 | 0.037 | 0.038 | 0.258 | 0.041 | 218 |
| | 2 | 0.980 | 0.993 | 0.621 | 0.981 | 346 | | 2 | 0.039 | 0.039 | 0.259 | 0.042 | 218 |
| | 3 | 0.974 | 0.992 | 0.609 | 0.979 | 352 | | 3 | 0.038 | 0.038 | 0.270 | 0.040 | 229 |
| | Mean | 0.978 | 0.991 | 0.613 | 0.978 | 351 | | Mean | 0.038 | 0.038 | 0.262 | 0.041 | 222 |
| 6 | 1 | 0.968 | 0.987 | 0.590 | 0.976 | 368 | 6 | 1 | 0.037 | 0.038 | 0.248 | 0.040 | 209 |
| | 2 | 0.966 | 0.981 | 0.594 | 0.971 | 362 | | 2 | 0.039 | 0.039 | 0.250 | 0.041 | 209 |
| | 3 | 0.960 | 0.978 | 0.594 | 0.968 | 356 | | 3 | 0.039 | 0.038 | 0.267 | 0.040 | 226 |
| | Mean | 0.964 | 0.982 | 0.593 | 0.972 | 362 | | Mean | 0.038 | 0.038 | 0.255 | 0.040 | 215 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 3

Chemical Name : Qunine 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 | 0.969 | 0.984 | 0.593 | 0.972 | 365 | 7 | 1 | 0.037 | 0.038 | 0.233 | 0.041 | 194 |
| | 2 | 0.966 | 0.984 | 0.602 | 0.974 | 353 | | 2 | 0.039 | 0.040 | 0.262 | 0.041 | 221 |
| | 3 | 0.963 | 0.982 | 0.600 | 0.970 | 351 | | 3 | 0.038 | 0.038 | 0.280 | 0.040 | 240 |
| | Mean | 0.966 | 0.984 | 0.598 | 0.972 | 356 | | Mean | 0.038 | 0.039 | 0.258 | 0.041 | 218 |
| 8 | 1 | 0.957 | 0.994 | 0.584 | 0.985 | 364 | 8 | 1 | 0.037 | 0.038 | 0.239 | 0.040 | 200 |
| | 2 | 0.954 | 0.978 | 0.593 | 0.969 | 352 | | 2 | 0.039 | 0.039 | 0.235 | 0.041 | 194 |
| | 3 | 0.949 | 0.965 | 0.596 | 0.957 | 344 | | 3 | 0.039 | 0.038 | 0.247 | 0.040 | 207 |
| | Mean | 0.954 | 0.979 | 0.591 | 0.970 | 353 | | Mean | 0.038 | 0.038 | 0.240 | 0.040 | 200 |
| 9 | 1 | 0.970 | 0.982 | 0.589 | 0.970 | 371 | 9 | 1 | 0.037 | 0.038 | 0.274 | 0.041 | 235 |
| | 2 | 0.971 | 0.987 | 0.595 | 0.980 | 366 | | 2 | 0.038 | 0.039 | 0.271 | 0.041 | 230 |
| | 3 | 0.968 | 0.994 | 0.596 | 0.984 | 362 | | 3 | 0.042 | 0.037 | 0.278 | 0.040 | 233 |
| | Mean | 0.970 | 0.988 | 0.593 | 0.978 | 366 | | Mean | 0.039 | 0.038 | 0.275 | 0.041 | 233 |
| 10 | 1 | 0.962 | 0.987 | 0.594 | 0.978 | 359 | 10 | 1 | 0.037 | 0.038 | 0.255 | 0.040 | 216 |
| | 2 | 0.965 | 0.976 | 0.598 | 0.965 | 357 | | 2 | 0.038 | 0.039 | 0.247 | 0.041 | 207 |
| | 3 | 0.953 | 0.974 | 0.590 | 0.966 | 354 | | 3 | 0.038 | 0.039 | 0.260 | 0.041 | 221 |
| | Mean | 0.960 | 0.979 | 0.594 | 0.970 | 357 | | Mean | 0.038 | 0.039 | 0.254 | 0.041 | 215 |
| 11 | 1 | 0.962 | 0.986 | 0.583 | 0.978 | 372 | 11 | 1 | 0.037 | 0.038 | 0.255 | 0.040 | 216 |
| | 2 | 0.964 | 0.976 | 0.593 | 0.968 | 364 | | 2 | 0.038 | 0.039 | 0.253 | 0.041 | 213 |
| | 3 | 0.950 | 0.973 | 0.588 | 0.967 | 355 | | 3 | 0.038 | 0.039 | 0.258 | 0.041 | 218 |
| | Mean | 0.958 | 0.978 | 0.588 | 0.971 | 364 | | Mean | 0.038 | 0.039 | 0.255 | 0.041 | 216 |
| 12 | 1 | 0.981 | 1.000 | 0.604 | 0.991 | 367 | 12 | 1 | 0.037 | 0.038 | 0.246 | 0.041 | 206 |
| | 2 | 0.969 | 0.976 | 0.597 | 0.965 | 362 | | 2 | 0.038 | 0.040 | 0.246 | 0.041 | 206 |
| | 3 | 0.954 | 0.969 | 0.592 | 0.959 | 352 | | 3 | 0.038 | 0.039 | 0.252 | 0.041 | 212 |
| | Mean | 0.968 | 0.982 | 0.597 | 0.972 | 360 | | Mean | 0.038 | 0.039 | 0.248 | 0.041 | 208 |
| Mean for all assays | | - | - | - | - | 359 | Mean for all assays | | - | - | - | - | 216 |
| SD for all assays | | - | - | - | - | 6 | SD for all assays | | - | - | - | - | 11 |
| CV for all assays | | - | - | - | - | 1.7 | CV for all assays | | - | - | - | - | 5.1 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 3
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 | 0.996 | 0.996 | 0.989 | 0.982 | -8 | 1 | 1 | 0.039 | 0.038 | 0.040 | 0.040 | -3 |
| | 2 | 0.997 | 1.001 | 0.992 | 0.987 | -9 | | 2 | 0.040 | 0.039 | 0.041 | 0.041 | -2 |
| | 3 | 1.001 | 1.000 | 0.993 | 0.983 | -7 | | 3 | 0.039 | 0.038 | 0.040 | 0.040 | -2 |
| | Mean | 0.998 | 0.999 | 0.991 | 0.984 | -8 | | Mean | 0.039 | 0.038 | 0.040 | 0.041 | -2 |
| 2 | 1 | 0.983 | 0.980 | 0.978 | 0.965 | -8 | 2 | 1 | 0.039 | 0.038 | 0.039 | 0.040 | -2 |
| | 2 | 0.990 | 0.989 | 0.981 | 0.977 | -6 | | 2 | 0.040 | 0.039 | 0.040 | 0.041 | -2 |
| | 3 | 0.989 | 0.988 | 0.982 | 0.974 | -7 | | 3 | 0.039 | 0.037 | 0.039 | 0.040 | -2 |
| | Mean | 0.987 | 0.986 | 0.980 | 0.972 | -7 | | Mean | 0.039 | 0.038 | 0.039 | 0.040 | -2 |
| 3 | 1 | 0.987 | 0.982 | 0.981 | 0.967 | -7 | 3 | 1 | 0.040 | 0.038 | 0.040 | 0.040 | -2 |
| | 2 | 0.979 | 0.980 | 0.975 | 0.967 | -9 | | 2 | 0.041 | 0.039 | 0.041 | 0.041 | -2 |
| | 3 | 0.979 | 0.976 | 0.974 | 0.964 | -8 | | 3 | 0.039 | 0.038 | 0.040 | 0.040 | -1 |
| | Mean | 0.982 | 0.979 | 0.976 | 0.966 | -8 | | Mean | 0.040 | 0.038 | 0.040 | 0.040 | -2 |
| 4 | 1 | 0.994 | 0.990 | 0.989 | 0.979 | -6 | 4 | 1 | 0.039 | 0.039 | 0.039 | 0.041 | -3 |
| | 2 | 0.993 | 0.997 | 0.989 | 0.985 | -7 | | 2 | 0.040 | 0.039 | 0.040 | 0.041 | -3 |
| | 3 | 0.997 | 0.995 | 0.993 | 0.985 | -6 | | 3 | 0.039 | 0.038 | 0.040 | 0.040 | -2 |
| | Mean | 0.995 | 0.994 | 0.990 | 0.983 | -6 | | Mean | 0.039 | 0.038 | 0.040 | 0.041 | -3 |
| 5 | 1 | 0.988 | 0.988 | 0.983 | 0.975 | -7 | 5 | 1 | 0.039 | 0.038 | 0.039 | 0.041 | -3 |
| | 2 | 0.989 | 0.993 | 0.980 | 0.981 | -4 | | 2 | 0.043 | 0.039 | 0.042 | 0.042 | -3 |
| | 3 | 0.992 | 0.992 | 0.982 | 0.979 | -3 | | 3 | 0.039 | 0.038 | 0.039 | 0.040 | -3 |
| | Mean | 0.990 | 0.991 | 0.982 | 0.978 | -5 | | Mean | 0.040 | 0.038 | 0.040 | 0.041 | -3 |
| 6 | 1 | 0.989 | 0.987 | 0.982 | 0.976 | -2 | 6 | 1 | 0.039 | 0.038 | 0.039 | 0.040 | -2 |
| | 2 | 0.982 | 0.981 | 0.973 | 0.971 | -1 | | 2 | 0.040 | 0.039 | 0.040 | 0.041 | -2 |
| | 3 | 0.978 | 0.978 | 0.970 | 0.968 | -2 | | 3 | 0.039 | 0.038 | 0.039 | 0.040 | -2 |
| | Mean | 0.983 | 0.982 | 0.975 | 0.972 | -2 | | Mean | 0.039 | 0.038 | 0.039 | 0.040 | -2 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 3 Positive control and negative control data of Phase 1 study

Laboratory : 3

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 | 0.984 | 0.985 | 0.972 | -4 | 7 | 1 | 0.039 | 0.038 | 0.040 | 0.041 | -1 |
| | 2 | 0.984 | 0.984 | 0.975 | 0.974 | -4 | | 2 | 0.041 | 0.040 | 0.041 | 0.041 | -1 |
| | 3 | 0.982 | 0.982 | 0.976 | 0.970 | -5 | | 3 | 0.039 | 0.038 | 0.041 | 0.040 | -1 |
| | Mean | 0.986 | 0.984 | 0.979 | 0.972 | -4 | | Mean | 0.040 | 0.039 | 0.041 | 0.041 | -1 |
| 8 | 1 | 0.995 | 0.994 | 0.988 | 0.985 | -2 | 8 | 1 | 0.039 | 0.038 | 0.039 | 0.040 | -2 |
| | 2 | 0.975 | 0.978 | 0.968 | 0.969 | -2 | | 2 | 0.040 | 0.039 | 0.040 | 0.041 | -2 |
| | 3 | 0.969 | 0.965 | 0.961 | 0.957 | -1 | | 3 | 0.039 | 0.038 | 0.040 | 0.040 | -2 |
| | Mean | 0.980 | 0.979 | 0.972 | 0.970 | -2 | | Mean | 0.039 | 0.038 | 0.040 | 0.040 | -2 |
| 9 | 1 | 0.984 | 0.982 | 0.976 | 0.970 | -2 | 9 | 1 | 0.039 | 0.038 | 0.039 | 0.041 | -3 |
| | 2 | 0.986 | 0.987 | 0.977 | 0.980 | -1 | | 2 | 0.040 | 0.039 | 0.040 | 0.041 | -2 |
| | 3 | 0.987 | 0.994 | 0.979 | 0.984 | -2 | | 3 | 0.038 | 0.037 | 0.039 | 0.040 | -2 |
| | Mean | 0.985 | 0.988 | 0.977 | 0.978 | -2 | | Mean | 0.039 | 0.038 | 0.040 | 0.041 | -2 |
| 10 | 1 | 0.980 | 0.987 | 0.974 | 0.978 | -2 | 10 | 1 | 0.040 | 0.038 | 0.040 | 0.040 | -2 |
| | 2 | 0.982 | 0.976 | 0.975 | 0.965 | -2 | | 2 | 0.039 | 0.039 | 0.039 | 0.041 | -2 |
| | 3 | 0.976 | 0.974 | 0.969 | 0.966 | -2 | | 3 | 0.039 | 0.039 | 0.039 | 0.041 | -1 |
| | Mean | 0.980 | 0.979 | 0.973 | 0.970 | -2 | | Mean | 0.039 | 0.039 | 0.039 | 0.041 | -2 |
| 11 | 1 | 0.981 | 0.986 | 0.973 | 0.978 | 1 | 11 | 1 | 0.039 | 0.038 | 0.040 | 0.040 | -2 |
| | 2 | 0.981 | 0.976 | 0.974 | 0.968 | 1 | | 2 | 0.039 | 0.039 | 0.039 | 0.041 | -2 |
| | 3 | 0.976 | 0.973 | 0.970 | 0.967 | -1 | | 3 | 0.039 | 0.039 | 0.040 | 0.041 | -1 |
| | Mean | 0.979 | 0.978 | 0.972 | 0.971 | 0 | | Mean | 0.039 | 0.039 | 0.040 | 0.041 | -2 |
| 12 | 1 | 0.993 | 1.000 | 0.987 | 0.991 | -4 | 12 | 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 |
| Mean for all assays | | - | - | - | - | -4 | Mean for all assays | | - | - | - | - | -2 |
| SD for all assays | | - | - | - | - | 3 | SD for all assays | | - | - | - | - | 1 |
| CV for all assays | | - | - | - | - | - | CV for all assays | | - | - | - | - | - |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

# Appendix 4 Individual data of Phase 1 study

Laboratory : 1
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 | 0.888 | 0.871 | 0.879 | 0.864 | 3 | 1 | 1 | 0.045 | 0.033 | 0.042 | 0.058 | -24 | |
| | 2 | 0.887 | 0.870 | 0.878 | 0.864 | 2 | | 2 | 0.031 | 0.035 | 0.040 | 0.041 | -11 | |
| | 3 | 0.852 | 0.875 | 0.844 | 0.867 | 1 | | 3 | 0.039 | 0.030 | 0.040 | 0.058 | -19 | |
| | Mean | 0.876 | 0.872 | 0.867 | 0.865 | 2 | | Mean | 0.038 | 0.033 | 0.041 | 0.053 | -18 | Negative |
| 2 | 1 | 0.874 | 0.859 | 0.866 | 0.850 | -1 | 2 | 1 | 0.045 | 0.030 | 0.044 | 0.038 | -12 | |
| | 2 | 0.873 | 0.876 | 0.865 | 0.868 | 0 | | 2 | 0.033 | 0.058 | 0.039 | 0.056 | -5 | |
| | 3 | 0.872 | 0.871 | 0.864 | 0.863 | -1 | | 3 | 0.051 | 0.031 | 0.039 | 0.057 | -23 | |
| | Mean | 0.873 | 0.869 | 0.865 | 0.860 | -1 | | Mean | 0.043 | 0.039 | 0.041 | 0.050 | -13 | Negative |
| 3 | 1 | 0.868 | 0.868 | 0.859 | 0.861 | 1 | 3 | 1 | 0.048 | 0.029 | 0.044 | 0.036 | -13 | |
| | 2 | 0.869 | 0.865 | 0.863 | 0.858 | -3 | | 2 | 0.032 | 0.045 | 0.046 | 0.042 | 5 | |
| | 3 | 0.869 | 0.865 | 0.861 | 0.857 | 0 | | 3 | 0.052 | 0.031 | 0.042 | 0.055 | -20 | |
| | Mean | 0.869 | 0.866 | 0.861 | 0.858 | -1 | | Mean | 0.044 | 0.035 | 0.044 | 0.044 | -9 | Negative |
| Mean for 3 assays | | - | - | - | - | 0 | Mean for 3 assays | | - | - | - | - | -13 | Negative [*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 | 0.879 | 0.873 | 0.872 | 0.867 | 1 | 1 | 1 | 0.035 | 0.032 | 0.046 | 0.036 | 2 | |
| | 2 | 0.889 | 0.880 | 0.882 | 0.873 | 0 | | 2 | 0.030 | 0.047 | 0.038 | 0.040 | 0 | |
| | 3 | 0.887 | 0.882 | 0.881 | 0.875 | 0 | | 3 | 0.059 | 0.030 | 0.042 | 0.061 | -26 | |
| | Mean | 0.885 | 0.878 | 0.878 | 0.872 | 0 | | Mean | 0.041 | 0.036 | 0.042 | 0.045 | -8 | Negative |
| 2 | 1 | 0.882 | 0.880 | 0.875 | 0.869 | -2 | 2 | 1 | 0.041 | 0.033 | 0.048 | 0.036 | -6 | |
| | 2 | 0.888 | 0.889 | 0.881 | 0.882 | -2 | | 2 | 0.031 | 0.052 | 0.040 | 0.050 | -3 | |
| | 3 | 0.893 | 0.879 | 0.885 | 0.871 | -1 | | 3 | 0.049 | 0.033 | 0.045 | 0.066 | -17 | |
| | Mean | 0.888 | 0.883 | 0.880 | 0.874 | -2 | | Mean | 0.040 | 0.039 | 0.044 | 0.051 | -9 | Negative |
| 3 | 1 | 0.881 | 0.886 | 0.873 | 0.877 | 0 | 3 | 1 | 0.039 | 0.033 | 0.040 | 0.039 | -10 | |
| | 2 | 0.890 | 0.891 | 0.881 | 0.885 | 2 | | 2 | 0.032 | 0.048 | 0.039 | 0.044 | -3 | |
| | 3 | 0.890 | 0.892 | 0.883 | 0.884 | 0 | | 3 | 0.041 | 0.032 | 0.044 | 0.057 | -7 | |
| | Mean | 0.887 | 0.890 | 0.879 | 0.882 | 1 | | Mean | 0.037 | 0.037 | 0.041 | 0.047 | -7 | Negative |
| Mean for 3 assays | | - | - | - | - | 0 | Mean for 3 assays | | - | - | - | - | -8 | Negative [*3] |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
Negative : Singlet oxygen results <25 and Superoxide anion results <25

The optical absorbance values were rounded to the third decimal place. If the calculated "results" are different from the numerical results in the raw data sheets by the number of significant digits in the data sheet and the appendices are different, the data in the raw data sheet is used in the appendices.

# Appendix 4 Individual data of Phase 1 study

Laboratory : 1
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 | 0.903 | 0.871 | 0.887 | 0.864 | 8 | 1 | 1 | 0.065 | 0.033 | 0.048 | 0.058 | -37 | |
| | 2 | 0.882 | 0.870 | 0.863 | 0.864 | 12 | | 2 | 0.051 | 0.035 | 0.057 | 0.041 | -14 | |
| | 3 | 0.898 | 0.875 | 0.885 | 0.867 | 6 | | 3 | 0.077 | 0.030 | 0.047 | 0.058 | -50 | |
| | Mean | 0.894 | 0.872 | 0.878 | 0.865 | 9 | | Mean | 0.064 | 0.033 | 0.051 | 0.053 | -34 | Negative |
| 2 | 1 | 0.879 | 0.859 | 0.866 | 0.850 | 4 | 2 | 1 | 0.070 | 0.030 | 0.047 | 0.038 | -34 | |
| | 2 | 0.870 | 0.876 | 0.852 | 0.868 | 9 | | 2 | 0.057 | 0.058 | 0.047 | 0.056 | -21 | |
| | 3 | 0.881 | 0.871 | 0.865 | 0.863 | 7 | | 3 | 0.077 | 0.031 | 0.048 | 0.057 | -40 | |
| | Mean | 0.877 | 0.869 | 0.861 | 0.860 | 7 | | Mean | 0.068 | 0.039 | 0.047 | 0.050 | -32 | Negative |
| 3 | 1 | 0.878 | 0.868 | 0.862 | 0.861 | 8 | 3 | 1 | 0.080 | 0.029 | 0.048 | 0.036 | -41 | |
| | 2 | 0.882 | 0.865 | 0.861 | 0.858 | 13 | | 2 | 0.050 | 0.045 | 0.044 | 0.042 | -15 | |
| | 3 | 0.885 | 0.865 | 0.870 | 0.857 | 7 | | 3 | 0.091 | 0.031 | 0.046 | 0.055 | -54 | |
| | Mean | 0.882 | 0.866 | 0.864 | 0.858 | 9 | | Mean | 0.074 | 0.035 | 0.046 | 0.044 | -37 | Negative |
| Mean for 3 assays | | - | - | - | - | 8 | Mean for 3 assays | | - | - | - | - | -34 | Negative *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 | 0.887 | 0.873 | 0.758 | 0.867 | 124 | 1 | 1 | 0.073 | 0.032 | 0.075 | 0.036 | -7 | |
| | 2 | 0.875 | 0.880 | 0.758 | 0.873 | 111 | | 2 | 0.056 | 0.047 | 0.072 | 0.040 | 8 | |
| | 3 | 0.894 | 0.882 | 0.760 | 0.875 | 128 | | 3 | 0.073 | 0.030 | 0.074 | 0.061 | -7 | |
| | Mean | 0.885 | 0.878 | 0.759 | 0.872 | 121 | | Mean | 0.067 | 0.036 | 0.074 | 0.045 | -2 | Positive |
| 2 | 1 | 0.884 | 0.880 | 0.745 | 0.869 | 130 | 2 | 1 | 0.068 | 0.033 | 0.072 | 0.036 | -8 | |
| | 2 | 0.879 | 0.889 | 0.755 | 0.882 | 115 | | 2 | 0.045 | 0.052 | 0.071 | 0.050 | 15 | |
| | 3 | 0.891 | 0.879 | 0.759 | 0.871 | 123 | | 3 | 0.054 | 0.033 | 0.072 | 0.066 | 7 | |
| | Mean | 0.885 | 0.883 | 0.753 | 0.874 | 123 | | Mean | 0.056 | 0.039 | 0.072 | 0.051 | 5 | Positive |
| 3 | 1 | 0.889 | 0.886 | 0.755 | 0.877 | 126 | 3 | 1 | 0.074 | 0.033 | 0.073 | 0.039 | -11 | |
| | 2 | 0.880 | 0.891 | 0.758 | 0.885 | 114 | | 2 | 0.043 | 0.048 | 0.070 | 0.044 | 17 | |
| | 3 | 0.895 | 0.892 | 0.760 | 0.884 | 127 | | 3 | 0.056 | 0.032 | 0.073 | 0.057 | 7 | |
| | Mean | 0.888 | 0.890 | 0.758 | 0.882 | 122 | | Mean | 0.058 | 0.037 | 0.072 | 0.047 | 4 | Positive |
| Mean for 3 assays | | - | - | - | - | 122 | Mean for 3 assays | | - | - | - | - | 2 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
Chemical Name : Amiodarone

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 | 0.946 | 0.871 | 0.801 | 0.864 | 138 | 1 | 1 | 0.096 | 0.033 | 0.089 | 0.058 | -27 | |
| | 2 | 0.941 | 0.870 | 0.802 | 0.864 | 132 | | 2 | 0.093 | 0.035 | 0.087 | 0.041 | -27 | |
| | 3 | 0.955 | 0.875 | 0.813 | 0.867 | 135 | | 3 | 0.095 | 0.030 | 0.088 | 0.058 | -27 | |
| | Mean | 0.947 | 0.872 | 0.805 | 0.865 | 135 | | Mean | 0.095 | 0.033 | 0.088 | 0.053 | -27 | Positive |
| 2 | 1 | 0.951 | 0.859 | 0.812 | 0.850 | 131 | 2 | 1 | 0.088 | 0.030 | 0.084 | 0.038 | -15 | |
| | 2 | 0.934 | 0.876 | 0.802 | 0.868 | 123 | | 2 | 0.086 | 0.058 | 0.083 | 0.056 | -14 | |
| | 3 | 0.946 | 0.871 | 0.804 | 0.863 | 133 | | 3 | 0.097 | 0.031 | 0.084 | 0.057 | -24 | |
| | Mean | 0.944 | 0.869 | 0.806 | 0.860 | 129 | | Mean | 0.090 | 0.039 | 0.084 | 0.050 | -18 | Positive |
| 3 | 1 | 0.941 | 0.868 | 0.812 | 0.861 | 122 | 3 | 1 | 0.091 | 0.029 | 0.083 | 0.036 | -17 | |
| | 2 | 0.935 | 0.865 | 0.804 | 0.858 | 124 | | 2 | 0.089 | 0.045 | 0.083 | 0.042 | -15 | |
| | 3 | 0.943 | 0.865 | 0.804 | 0.857 | 131 | | 3 | 0.092 | 0.031 | 0.084 | 0.055 | -17 | |
| | Mean | 0.940 | 0.866 | 0.807 | 0.858 | 126 | | Mean | 0.091 | 0.035 | 0.083 | 0.044 | -16 | Positive |
| Mean for 3 assays | | - | - | - | - | 130 | Mean for 3 assays | | - | - | - | - | -20 | Positive *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 | 1.579 | 0.873 | 1.296 | 0.867 | 278 | 1 | 1 | 0.733 | 0.032 | 0.502 | 0.036 | -240 | |
| | 2 | 1.566 | 0.880 | 1.303 | 0.873 | 256 | | 2 | 0.733 | 0.047 | 0.526 | 0.040 | -216 | |
| | 3 | 1.582 | 0.882 | 1.332 | 0.875 | 243 | | 3 | 0.732 | 0.030 | 0.506 | 0.061 | -234 | |
| | Mean | 1.576 | 0.878 | 1.310 | 0.872 | 259 | | Mean | 0.733 | 0.036 | 0.511 | 0.045 | -230 | Positive |
| 2 | 1 | 1.515 | 0.880 | 1.295 | 0.869 | 211 | 2 | 1 | 0.681 | 0.033 | 0.491 | 0.036 | -203 | |
| | 2 | 1.496 | 0.889 | 1.304 | 0.882 | 183 | | 2 | 0.684 | 0.052 | 0.505 | 0.050 | -191 | |
| | 3 | 1.495 | 0.879 | 1.304 | 0.871 | 182 | | 3 | 0.687 | 0.033 | 0.505 | 0.066 | -194 | |
| | Mean | 1.502 | 0.883 | 1.301 | 0.874 | 192 | | Mean | 0.684 | 0.039 | 0.500 | 0.051 | -196 | Positive |
| 3 | 1 | 1.553 | 0.886 | 1.285 | 0.877 | 260 | 3 | 1 | 0.721 | 0.033 | 0.492 | 0.039 | -239 | |
| | 2 | 1.535 | 0.891 | 1.294 | 0.885 | 234 | | 2 | 0.717 | 0.048 | 0.503 | 0.044 | -224 | |
| | 3 | 1.555 | 0.892 | 1.298 | 0.884 | 248 | | 3 | 0.719 | 0.032 | 0.496 | 0.057 | -233 | |
| | Mean | 1.548 | 0.890 | 1.292 | 0.882 | 247 | | Mean | 0.719 | 0.037 | 0.497 | 0.047 | -232 | Positive |
| Mean for 3 assays | | - | - | - | - | 233 | Mean for 3 assays | | - | - | - | - | -219 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
Chemical Name : Chlorpromazine

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 | 0.893 | 0.871 | 0.785 | 0.864 | 101 | 1 | 1 | 0.067 | 0.033 | 0.057 | 0.058 | -30 | |
| | 2 | 0.870 | 0.870 | 0.787 | 0.864 | 75 | | 2 | 0.037 | 0.035 | 0.056 | 0.041 | -1 | |
| | 3 | 0.902 | 0.875 | 0.789 | 0.867 | 106 | | 3 | 0.068 | 0.030 | 0.056 | 0.058 | -32 | |
| | Mean | 0.888 | 0.872 | 0.787 | 0.865 | 94 | | Mean | 0.057 | 0.033 | 0.056 | 0.053 | -21 | Positive |
| 2 | 1 | 0.884 | 0.859 | 0.777 | 0.850 | 98 | 2 | 1 | 0.062 | 0.030 | 0.059 | 0.038 | -14 | |
| | 2 | 0.848 | 0.876 | 0.766 | 0.868 | 73 | | 2 | 0.052 | 0.058 | 0.056 | 0.056 | -7 | |
| | 3 | 0.901 | 0.871 | 0.796 | 0.863 | 96 | | 3 | 0.066 | 0.031 | 0.055 | 0.057 | -22 | |
| | Mean | 0.878 | 0.869 | 0.780 | 0.860 | 89 | | Mean | 0.060 | 0.039 | 0.057 | 0.050 | -14 | Positive |
| 3 | 1 | 0.896 | 0.868 | 0.786 | 0.861 | 102 | 3 | 1 | 0.076 | 0.029 | 0.056 | 0.036 | -30 | |
| | 2 | 0.860 | 0.865 | 0.788 | 0.858 | 65 | | 2 | 0.040 | 0.045 | 0.057 | 0.042 | 9 | |
| | 3 | 0.897 | 0.865 | 0.789 | 0.857 | 100 | | 3 | 0.067 | 0.031 | 0.056 | 0.055 | -20 | |
| | Mean | 0.884 | 0.866 | 0.788 | 0.858 | 89 | | Mean | 0.061 | 0.035 | 0.056 | 0.044 | -14 | Positive |
| Mean for 3 assays | | - | - | - | - | 91 | Mean for 3 assays | | - | - | - | - | -16 | Positive *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 | 0.867 | 0.873 | 0.862 | 0.867 | -1 | 1 | 1 | 0.047 | 0.032 | 0.133 | 0.036 | 76 | |
| | 2 | 0.861 | 0.880 | 0.865 | 0.873 | -10 | | 2 | 0.038 | 0.047 | 0.134 | 0.040 | 87 | |
| | 3 | 0.877 | 0.882 | 0.870 | 0.875 | 1 | | 3 | 0.050 | 0.030 | 0.135 | 0.061 | 76 | |
| | Mean | 0.868 | 0.878 | 0.866 | 0.872 | -3 | | Mean | 0.045 | 0.036 | 0.134 | 0.045 | 80 | Positive |
| 2 | 1 | 0.846 | 0.880 | 0.852 | 0.869 | -16 | 2 | 1 | 0.043 | 0.033 | 0.130 | 0.036 | 75 | |
| | 2 | 0.860 | 0.889 | 0.867 | 0.882 | -16 | | 2 | 0.038 | 0.052 | 0.133 | 0.050 | 82 | |
| | 3 | 0.870 | 0.879 | 0.865 | 0.871 | -4 | | 3 | 0.045 | 0.033 | 0.133 | 0.066 | 76 | |
| | Mean | 0.859 | 0.883 | 0.861 | 0.874 | -12 | | Mean | 0.042 | 0.039 | 0.132 | 0.051 | 78 | Positive |
| 3 | 1 | 0.862 | 0.886 | 0.856 | 0.877 | -2 | 3 | 1 | 0.045 | 0.033 | 0.130 | 0.039 | 75 | |
| | 2 | 0.861 | 0.891 | 0.871 | 0.885 | -18 | | 2 | 0.037 | 0.048 | 0.132 | 0.044 | 85 | |
| | 3 | 0.882 | 0.892 | 0.873 | 0.884 | 0 | | 3 | 0.047 | 0.032 | 0.132 | 0.057 | 75 | |
| | Mean | 0.868 | 0.890 | 0.867 | 0.882 | -7 | | Mean | 0.043 | 0.037 | 0.131 | 0.047 | 78 | Positive |
| Mean for 3 assays | | - | - | - | - | -7 | Mean for 3 assays | | - | - | - | - | 79 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
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 | 0.873 | 0.871 | 0.704 | 0.864 | 162 | 1 | 1 | 0.043 | 0.033 | 0.072 | 0.058 | 9 | |
| | 2 | 0.871 | 0.870 | 0.700 | 0.864 | 164 | | 2 | 0.046 | 0.035 | 0.069 | 0.041 | 3 | |
| | 3 | 0.874 | 0.875 | 0.704 | 0.867 | 162 | | 3 | 0.040 | 0.030 | 0.072 | 0.058 | 12 | |
| | Mean | 0.873 | 0.872 | 0.703 | 0.865 | 163 | | Mean | 0.043 | 0.033 | 0.071 | 0.053 | 8 | Positive |
| 2 | 1 | 0.880 | 0.859 | 0.710 | 0.850 | 161 | 2 | 1 | 0.039 | 0.030 | 0.068 | 0.038 | 18 | |
| | 2 | 0.880 | 0.876 | 0.712 | 0.868 | 159 | | 2 | 0.052 | 0.058 | 0.072 | 0.056 | 9 | |
| | 3 | 0.885 | 0.871 | 0.715 | 0.863 | 161 | | 3 | 0.045 | 0.031 | 0.070 | 0.057 | 14 | |
| | Mean | 0.882 | 0.869 | 0.712 | 0.860 | 160 | | Mean | 0.045 | 0.039 | 0.070 | 0.050 | 14 | Positive |
| 3 | 1 | 0.859 | 0.868 | 0.690 | 0.861 | 162 | 3 | 1 | 0.044 | 0.029 | 0.069 | 0.036 | 16 | |
| | 2 | 0.873 | 0.865 | 0.703 | 0.858 | 162 | | 2 | 0.046 | 0.045 | 0.067 | 0.042 | 11 | |
| | 3 | 0.873 | 0.865 | 0.705 | 0.857 | 160 | | 3 | 0.043 | 0.031 | 0.068 | 0.055 | 16 | |
| | Mean | 0.868 | 0.866 | 0.699 | 0.858 | 161 | | Mean | 0.044 | 0.035 | 0.068 | 0.044 | 14 | Positive |
| Mean for 3 assays | | - | - | - | - | 161 | Mean for 3 assays | | - | - | - | - | 12 | Positive *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 | 0.866 | 0.873 | 0.548 | 0.867 | 312 | 1 | 1 | 0.050 | 0.032 | 0.410 | 0.036 | 351 | |
| | 2 | 0.887 | 0.880 | 0.570 | 0.873 | 311 | | 2 | 0.045 | 0.047 | 0.427 | 0.040 | 372 | |
| | 3 | 0.886 | 0.882 | 0.567 | 0.875 | 313 | | 3 | 0.050 | 0.030 | 0.428 | 0.061 | 369 | |
| | Mean | 0.880 | 0.878 | 0.562 | 0.872 | 312 | | Mean | 0.048 | 0.036 | 0.422 | 0.045 | 364 | Positive |
| 2 | 1 | 0.880 | 0.880 | 0.551 | 0.869 | 319 | 2 | 1 | 0.047 | 0.033 | 0.411 | 0.036 | 352 | |
| | 2 | 0.890 | 0.889 | 0.565 | 0.882 | 316 | | 2 | 0.043 | 0.052 | 0.431 | 0.050 | 376 | |
| | 3 | 0.888 | 0.879 | 0.562 | 0.871 | 318 | | 3 | 0.050 | 0.033 | 0.421 | 0.066 | 359 | |
| | Mean | 0.886 | 0.883 | 0.559 | 0.874 | 318 | | Mean | 0.047 | 0.039 | 0.421 | 0.051 | 362 | Positive |
| 3 | 1 | 0.899 | 0.886 | 0.566 | 0.877 | 325 | 3 | 1 | 0.048 | 0.033 | 0.411 | 0.039 | 353 | |
| | 2 | 0.894 | 0.891 | 0.566 | 0.885 | 321 | | 2 | 0.048 | 0.048 | 0.430 | 0.044 | 372 | |
| | 3 | 0.886 | 0.892 | 0.559 | 0.884 | 318 | | 3 | 0.050 | 0.032 | 0.431 | 0.057 | 371 | |
| | Mean | 0.893 | 0.890 | 0.564 | 0.882 | 321 | | Mean | 0.049 | 0.037 | 0.424 | 0.047 | 365 | Positive |
| Mean for 3 assays | | - | - | - | - | 317 | Mean for 3 assays | | - | - | - | - | 364 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
Chemical Name : Doxycycline

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 | 0.869 | 0.871 | 0.770 | 0.864 | 92 | 1 | 1 | 0.056 | 0.033 | 0.102 | 0.058 | 26 | |
| | 2 | 0.876 | 0.870 | 0.791 | 0.864 | 78 | | 2 | 0.071 | 0.035 | 0.105 | 0.041 | 14 | |
| | 3 | 0.881 | 0.875 | 0.788 | 0.867 | 86 | | 3 | 0.060 | 0.030 | 0.100 | 0.058 | 20 | |
| | Mean | 0.875 | 0.872 | 0.783 | 0.865 | 85 | | Mean | 0.062 | 0.033 | 0.102 | 0.053 | 20 | Positive |
| 2 | 1 | 0.874 | 0.859 | 0.790 | 0.850 | 75 | 2 | 1 | 0.053 | 0.030 | 0.092 | 0.038 | 28 | |
| | 2 | 0.840 | 0.876 | 0.776 | 0.868 | 55 | | 2 | 0.051 | 0.058 | 0.095 | 0.056 | 32 | |
| | 3 | 0.863 | 0.871 | 0.783 | 0.863 | 72 | | 3 | 0.055 | 0.031 | 0.092 | 0.057 | 26 | |
| | Mean | 0.859 | 0.869 | 0.783 | 0.860 | 67 | | Mean | 0.053 | 0.039 | 0.093 | 0.050 | 29 | Positive |
| 3 | 1 | 0.876 | 0.868 | 0.757 | 0.861 | 110 | 3 | 1 | 0.054 | 0.029 | 0.094 | 0.036 | 30 | |
| | 2 | 0.837 | 0.865 | 0.757 | 0.858 | 72 | | 2 | 0.054 | 0.045 | 0.097 | 0.042 | 33 | |
| | 3 | 0.882 | 0.865 | 0.775 | 0.857 | 100 | | 3 | 0.057 | 0.031 | 0.095 | 0.055 | 29 | |
| | Mean | 0.865 | 0.866 | 0.763 | 0.858 | 94 | | Mean | 0.055 | 0.035 | 0.095 | 0.044 | 31 | Positive |
| Mean for 3 assays | | - | - | - | - | 82 | Mean for 3 assays | | - | - | - | - | 27 | Positive *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 | 0.801 | 0.873 | 0.670 | 0.867 | 125 | 1 | 1 | 0.048 | 0.032 | 0.373 | 0.036 | 316 | |
| | 2 | 0.810 | 0.880 | 0.699 | 0.873 | 105 | | 2 | 0.055 | 0.047 | 0.384 | 0.040 | 319 | |
| | 3 | 0.814 | 0.882 | 0.700 | 0.875 | 108 | | 3 | 0.050 | 0.030 | 0.388 | 0.061 | 329 | |
| | Mean | 0.808 | 0.878 | 0.690 | 0.872 | 113 | | Mean | 0.051 | 0.036 | 0.382 | 0.045 | 321 | Positive |
| 2 | 1 | 0.810 | 0.880 | 0.674 | 0.869 | 127 | 2 | 1 | 0.047 | 0.033 | 0.371 | 0.036 | 312 | |
| | 2 | 0.813 | 0.889 | 0.703 | 0.882 | 100 | | 2 | 0.052 | 0.052 | 0.387 | 0.050 | 323 | |
| | 3 | 0.826 | 0.879 | 0.712 | 0.871 | 106 | | 3 | 0.048 | 0.033 | 0.388 | 0.066 | 328 | |
| | Mean | 0.816 | 0.883 | 0.696 | 0.874 | 111 | | Mean | 0.049 | 0.039 | 0.382 | 0.051 | 321 | Positive |
| 3 | 1 | 0.811 | 0.886 | 0.672 | 0.877 | 131 | 3 | 1 | 0.049 | 0.033 | 0.373 | 0.039 | 315 | |
| | 2 | 0.814 | 0.891 | 0.698 | 0.885 | 107 | | 2 | 0.047 | 0.048 | 0.384 | 0.044 | 327 | |
| | 3 | 0.834 | 0.892 | 0.713 | 0.884 | 113 | | 3 | 0.052 | 0.032 | 0.389 | 0.057 | 327 | |
| | Mean | 0.820 | 0.890 | 0.694 | 0.882 | 117 | | Mean | 0.049 | 0.037 | 0.382 | 0.047 | 323 | Positive |
| Mean for 3 assays | | - | - | - | - | 114 | Mean for 3 assays | | - | - | - | - | 322 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
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 | 0.878 | 0.871 | 0.843 | 0.864 | 28 | 1 | 1 | 0.048 | 0.033 | 0.067 | 0.058 | -1 | |
| | 2 | 0.878 | 0.870 | 0.823 | 0.864 | 48 | | 2 | 0.093 | 0.035 | 0.076 | 0.041 | -38 | |
| | 3 | 0.884 | 0.875 | 0.849 | 0.867 | 28 | | 3 | 0.051 | 0.030 | 0.064 | 0.058 | -7 | |
| | Mean | 0.880 | 0.872 | 0.838 | 0.865 | 35 | | Mean | 0.064 | 0.033 | 0.069 | 0.053 | -15 | Positive |
| 2 | 1 | 0.887 | 0.859 | 0.850 | 0.850 | 28 | 2 | 1 | 0.061 | 0.030 | 0.063 | 0.038 | -9 | |
| | 2 | 0.881 | 0.876 | 0.826 | 0.868 | 46 | | 2 | 0.087 | 0.058 | 0.075 | 0.056 | -22 | |
| | 3 | 0.898 | 0.871 | 0.859 | 0.863 | 30 | | 3 | 0.066 | 0.031 | 0.065 | 0.057 | -12 | |
| | Mean | 0.889 | 0.869 | 0.845 | 0.860 | 35 | | Mean | 0.071 | 0.039 | 0.068 | 0.050 | -14 | Positive |
| 3 | 1 | 0.883 | 0.868 | 0.835 | 0.861 | 41 | 3 | 1 | 0.073 | 0.029 | 0.071 | 0.036 | -11 | |
| | 2 | 0.879 | 0.865 | 0.822 | 0.858 | 50 | | 2 | 0.085 | 0.045 | 0.079 | 0.042 | -15 | |
| | 3 | 0.890 | 0.865 | 0.847 | 0.857 | 35 | | 3 | 0.077 | 0.031 | 0.067 | 0.055 | -19 | |
| | Mean | 0.884 | 0.866 | 0.835 | 0.858 | 42 | | Mean | 0.078 | 0.035 | 0.072 | 0.044 | -15 | Positive |
| Mean for 3 assays | | - | - | - | - | 37 | Mean for 3 assays | | - | - | - | - | -15 | Positive *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 | 0.894 | 0.873 | 0.696 | 0.867 | 192 | 1 | 1 | 0.047 | 0.032 | 0.153 | 0.036 | 97 | |
| | 2 | 0.915 | 0.880 | 0.748 | 0.873 | 161 | | 2 | 0.058 | 0.047 | 0.169 | 0.040 | 102 | |
| | 3 | 0.903 | 0.882 | 0.681 | 0.875 | 216 | | 3 | 0.047 | 0.030 | 0.164 | 0.061 | 107 | |
| | Mean | 0.904 | 0.878 | 0.708 | 0.872 | 190 | | Mean | 0.051 | 0.036 | 0.162 | 0.045 | 102 | Positive |
| 2 | 1 | 0.893 | 0.880 | 0.669 | 0.869 | 215 | 2 | 1 | 0.042 | 0.033 | 0.149 | 0.036 | 95 | |
| | 2 | 0.886 | 0.889 | 0.706 | 0.882 | 171 | | 2 | 0.052 | 0.052 | 0.164 | 0.050 | 100 | |
| | 3 | 0.903 | 0.879 | 0.692 | 0.871 | 202 | | 3 | 0.048 | 0.033 | 0.163 | 0.066 | 103 | |
| | Mean | 0.894 | 0.883 | 0.689 | 0.874 | 196 | | Mean | 0.047 | 0.039 | 0.159 | 0.051 | 99 | Positive |
| 3 | 1 | 0.892 | 0.886 | 0.677 | 0.877 | 207 | 3 | 1 | 0.054 | 0.033 | 0.151 | 0.039 | 87 | |
| | 2 | 0.896 | 0.891 | 0.706 | 0.885 | 182 | | 2 | 0.050 | 0.048 | 0.158 | 0.044 | 98 | |
| | 3 | 0.909 | 0.892 | 0.695 | 0.884 | 205 | | 3 | 0.049 | 0.032 | 0.155 | 0.057 | 96 | |
| | Mean | 0.899 | 0.890 | 0.693 | 0.882 | 198 | | Mean | 0.051 | 0.037 | 0.155 | 0.047 | 94 | Positive |
| Mean for 3 assays | | - | - | - | - | 195 | Mean for 3 assays | | - | - | - | - | 98 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
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 | 0.864 | 0.865 | 0.822 | 0.856 | 34 | 1 | 1 | 0.064 | 0.033 | 0.049 | 0.036 | -25 | |
| | 2 | 0.869 | 0.880 | 0.807 | 0.874 | 53 | | 2 | 0.059 | 0.033 | 0.042 | 0.039 | -27 | |
| | 3 | 0.874 | 0.873 | 0.829 | 0.865 | 37 | | 3 | 0.073 | 0.029 | 0.047 | 0.050 | -36 | |
| | Mean | 0.869 | 0.873 | 0.819 | 0.865 | 41 | | Mean | 0.065 | 0.032 | 0.046 | 0.042 | -29 | Positive |
| 2 | 1 | 0.877 | 0.863 | 0.843 | 0.853 | 25 | 2 | 1 | 0.058 | 0.029 | 0.047 | 0.035 | -25 | |
| | 2 | 0.876 | 0.873 | 0.823 | 0.865 | 43 | | 2 | 0.057 | 0.030 | 0.045 | 0.038 | -26 | |
| | 3 | 0.876 | 0.875 | 0.846 | 0.867 | 21 | | 3 | 0.079 | 0.029 | 0.051 | 0.054 | -42 | |
| | Mean | 0.876 | 0.870 | 0.837 | 0.861 | 30 | | Mean | 0.065 | 0.029 | 0.048 | 0.043 | -31 | Positive |
| 3 | 1 | 0.864 | 0.844 | 0.816 | 0.836 | 42 | 3 | 1 | 0.047 | 0.030 | 0.050 | 0.037 | -9 | |
| | 2 | 0.864 | 0.844 | 0.799 | 0.842 | 60 | | 2 | 0.033 | 0.052 | 0.044 | 0.040 | -3 | |
| | 3 | 0.862 | 0.851 | 0.814 | 0.845 | 42 | | 3 | 0.046 | 0.032 | 0.051 | 0.075 | -8 | |
| | Mean | 0.863 | 0.846 | 0.810 | 0.841 | 48 | | Mean | 0.042 | 0.038 | 0.048 | 0.051 | -7 | Positive |
| Mean for 3 assays | | - | - | - | - | 40 | Mean for 3 assays | | - | - | - | - | -22 | Positive *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 | 0.878 | 0.871 | 0.612 | 0.862 | 258 | 1 | 1 | 0.034 | 0.028 | 0.132 | 0.037 | 87 | |
| | 2 | 0.878 | 0.877 | 0.617 | 0.868 | 252 | | 2 | 0.033 | 0.046 | 0.131 | 0.042 | 87 | |
| | 3 | 0.882 | 0.880 | 0.626 | 0.871 | 247 | | 3 | 0.034 | 0.031 | 0.141 | 0.061 | 96 | |
| | Mean | 0.879 | 0.876 | 0.618 | 0.867 | 252 | | Mean | 0.034 | 0.035 | 0.135 | 0.046 | 90 | Positive |
| 2 | 1 | 0.857 | 0.853 | 0.601 | 0.849 | 249 | 2 | 1 | 0.034 | 0.033 | 0.136 | 0.037 | 91 | |
| | 2 | 0.863 | 0.859 | 0.606 | 0.852 | 249 | | 2 | 0.032 | 0.048 | 0.137 | 0.039 | 93 | |
| | 3 | 0.866 | 0.861 | 0.613 | 0.853 | 247 | | 3 | 0.035 | 0.032 | 0.145 | 0.074 | 98 | |
| | Mean | 0.862 | 0.858 | 0.607 | 0.851 | 248 | | Mean | 0.034 | 0.038 | 0.139 | 0.050 | 94 | Positive |
| 3 | 1 | 0.868 | 0.884 | 0.605 | 0.878 | 256 | 3 | 1 | 0.034 | 0.032 | 0.142 | 0.037 | 98 | |
| | 2 | 0.872 | 0.886 | 0.607 | 0.879 | 259 | | 2 | 0.033 | 0.043 | 0.132 | 0.037 | 89 | |
| | 3 | 0.873 | 0.880 | 0.618 | 0.873 | 249 | | 3 | 0.034 | 0.030 | 0.140 | 0.061 | 95 | |
| | Mean | 0.871 | 0.883 | 0.610 | 0.877 | 255 | | Mean | 0.034 | 0.035 | 0.138 | 0.045 | 94 | Positive |
| Mean for 3 assays | | - | - | - | - | 252 | Mean for 3 assays | | - | - | - | - | 93 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
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 | 0.866 | 0.865 | 0.829 | 0.856 | 29 | 1 | 1 | 0.037 | 0.033 | 0.224 | 0.036 | 177 | |
| | 2 | 0.875 | 0.880 | 0.825 | 0.874 | 42 | | 2 | 0.030 | 0.033 | 0.208 | 0.039 | 168 | |
| | 3 | 0.872 | 0.873 | 0.838 | 0.865 | 27 | | 3 | 0.033 | 0.029 | 0.214 | 0.050 | 171 | |
| | Mean | 0.871 | 0.873 | 0.831 | 0.865 | 33 | | Mean | 0.033 | 0.032 | 0.215 | 0.042 | 172 | Positive |
| 2 | 1 | 0.868 | 0.863 | 0.812 | 0.853 | 47 | 2 | 1 | 0.030 | 0.029 | 0.211 | 0.035 | 166 | |
| | 2 | 0.876 | 0.873 | 0.822 | 0.865 | 46 | | 2 | 0.044 | 0.030 | 0.209 | 0.038 | 152 | |
| | 3 | 0.876 | 0.875 | 0.823 | 0.867 | 44 | | 3 | 0.033 | 0.029 | 0.213 | 0.054 | 166 | |
| | Mean | 0.873 | 0.870 | 0.819 | 0.861 | 46 | | Mean | 0.036 | 0.029 | 0.211 | 0.043 | 161 | Positive |
| 3 | 1 | 0.861 | 0.844 | 0.822 | 0.836 | 34 | 3 | 1 | 0.060 | 0.030 | 0.217 | 0.037 | 144 | |
| | 2 | 0.864 | 0.844 | 0.814 | 0.842 | 45 | | 2 | 0.051 | 0.052 | 0.227 | 0.040 | 163 | |
| | 3 | 0.865 | 0.851 | 0.832 | 0.845 | 28 | | 3 | 0.067 | 0.032 | 0.224 | 0.075 | 144 | |
| | Mean | 0.863 | 0.846 | 0.823 | 0.841 | 36 | | Mean | 0.059 | 0.038 | 0.223 | 0.051 | 150 | Positive |
| Mean for 3 assays | | - | - | - | - | 38 | Mean for 3 assays | | - | - | - | - | 161 | Positive *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 | 0.866 | 0.871 | 0.632 | 0.862 | 225 | 1 | 1 | 0.076 | 0.028 | 0.449 | 0.037 | 362 | |
| | 2 | 0.872 | 0.877 | 0.675 | 0.868 | 188 | | 2 | 0.054 | 0.046 | 0.456 | 0.042 | 391 | |
| | 3 | 0.887 | 0.880 | 0.679 | 0.871 | 199 | | 3 | 0.082 | 0.031 | 0.453 | 0.061 | 360 | |
| | Mean | 0.875 | 0.876 | 0.662 | 0.867 | 204 | | Mean | 0.071 | 0.035 | 0.453 | 0.046 | 371 | Positive |
| 2 | 1 | 0.874 | 0.853 | 0.656 | 0.849 | 211 | 2 | 1 | 0.069 | 0.033 | 0.455 | 0.037 | 374 | |
| | 2 | 0.863 | 0.859 | 0.684 | 0.852 | 173 | | 2 | 0.046 | 0.048 | 0.479 | 0.039 | 422 | |
| | 3 | 0.875 | 0.861 | 0.689 | 0.853 | 179 | | 3 | 0.064 | 0.032 | 0.450 | 0.074 | 374 | |
| | Mean | 0.871 | 0.858 | 0.676 | 0.851 | 188 | | Mean | 0.060 | 0.038 | 0.461 | 0.050 | 390 | Positive |
| 3 | 1 | 0.865 | 0.884 | 0.668 | 0.878 | 191 | 3 | 1 | 0.074 | 0.032 | 0.447 | 0.037 | 363 | |
| | 2 | 0.873 | 0.886 | 0.681 | 0.879 | 186 | | 2 | 0.048 | 0.043 | 0.458 | 0.037 | 400 | |
| | 3 | 0.905 | 0.880 | 0.701 | 0.873 | 197 | | 3 | 0.073 | 0.030 | 0.457 | 0.061 | 375 | |
| | Mean | 0.881 | 0.883 | 0.683 | 0.877 | 191 | | Mean | 0.065 | 0.035 | 0.454 | 0.045 | 379 | Positive |
| Mean for 3 assays | | - | - | - | - | 194 | Mean for 3 assays | | - | - | - | - | 380 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
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 | 0.873 | 0.865 | 0.786 | 0.856 | 79 | 1 | 1 | 0.053 | 0.033 | 0.061 | 0.036 | -2 | |
| | 2 | 0.870 | 0.880 | 0.795 | 0.874 | 66 | | 2 | 0.036 | 0.033 | 0.063 | 0.039 | 17 | |
| | 3 | 0.875 | 0.873 | 0.765 | 0.865 | 102 | | 3 | 0.054 | 0.029 | 0.061 | 0.050 | -3 | |
| | Mean | 0.873 | 0.873 | 0.782 | 0.865 | 82 | | Mean | 0.048 | 0.032 | 0.062 | 0.042 | 4 | Positive |
| 2 | 1 | 0.867 | 0.863 | 0.779 | 0.853 | 79 | 2 | 1 | 0.051 | 0.029 | 0.062 | 0.035 | -3 | |
| | 2 | 0.869 | 0.873 | 0.795 | 0.865 | 65 | | 2 | 0.035 | 0.030 | 0.061 | 0.038 | 13 | |
| | 3 | 0.876 | 0.875 | 0.781 | 0.867 | 86 | | 3 | 0.049 | 0.029 | 0.062 | 0.054 | -2 | |
| | Mean | 0.871 | 0.870 | 0.785 | 0.861 | 77 | | Mean | 0.045 | 0.029 | 0.062 | 0.043 | 3 | Positive |
| 3 | 1 | 0.866 | 0.844 | 0.750 | 0.836 | 111 | 3 | 1 | 0.044 | 0.030 | 0.060 | 0.037 | 2 | |
| | 2 | 0.865 | 0.844 | 0.733 | 0.842 | 127 | | 2 | 0.033 | 0.052 | 0.060 | 0.040 | 14 | |
| | 3 | 0.867 | 0.851 | 0.734 | 0.845 | 129 | | 3 | 0.044 | 0.032 | 0.061 | 0.075 | 4 | |
| | Mean | 0.866 | 0.846 | 0.739 | 0.841 | 122 | | Mean | 0.040 | 0.038 | 0.060 | 0.051 | 7 | Positive |
| Mean for 3 assays | | - | - | - | - | 94 | Mean for 3 assays | | - | - | - | - | 5 | Positive *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 | 0.870 | 0.871 | 0.643 | 0.862 | 218 | 1 | 1 | 0.037 | 0.028 | 0.162 | 0.037 | 114 | |
| | 2 | 0.870 | 0.877 | 0.657 | 0.868 | 205 | | 2 | 0.035 | 0.046 | 0.165 | 0.042 | 120 | |
| | 3 | 0.871 | 0.880 | 0.654 | 0.871 | 209 | | 3 | 0.043 | 0.031 | 0.168 | 0.061 | 114 | |
| | Mean | 0.870 | 0.876 | 0.651 | 0.867 | 211 | | Mean | 0.038 | 0.035 | 0.165 | 0.046 | 116 | Positive |
| 2 | 1 | 0.861 | 0.853 | 0.641 | 0.849 | 214 | 2 | 1 | 0.036 | 0.033 | 0.165 | 0.037 | 117 | |
| | 2 | 0.862 | 0.859 | 0.650 | 0.852 | 205 | | 2 | 0.036 | 0.048 | 0.168 | 0.039 | 120 | |
| | 3 | 0.862 | 0.861 | 0.649 | 0.853 | 206 | | 3 | 0.040 | 0.032 | 0.172 | 0.074 | 120 | |
| | Mean | 0.862 | 0.858 | 0.647 | 0.851 | 208 | | Mean | 0.037 | 0.038 | 0.168 | 0.050 | 119 | Positive |
| 3 | 1 | 0.859 | 0.884 | 0.633 | 0.878 | 219 | 3 | 1 | 0.037 | 0.032 | 0.159 | 0.037 | 112 | |
| | 2 | 0.857 | 0.886 | 0.642 | 0.879 | 208 | | 2 | 0.036 | 0.043 | 0.162 | 0.037 | 116 | |
| | 3 | 0.870 | 0.880 | 0.650 | 0.873 | 213 | | 3 | 0.039 | 0.030 | 0.167 | 0.061 | 118 | |
| | Mean | 0.862 | 0.883 | 0.642 | 0.877 | 213 | | Mean | 0.037 | 0.035 | 0.163 | 0.045 | 115 | Positive |
| Mean for 3 assays | | - | - | - | - | 211 | Mean for 3 assays | | - | - | - | - | 117 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
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 | 0.879 | 0.865 | 0.802 | 0.856 | 68 | 1 | 1 | 0.044 | 0.033 | 0.068 | 0.036 | 15 | |
| | 2 | 0.885 | 0.880 | 0.812 | 0.874 | 65 | | 2 | 0.039 | 0.033 | 0.066 | 0.039 | 17 | |
| | 3 | 0.877 | 0.873 | 0.804 | 0.865 | 65 | | 3 | 0.052 | 0.029 | 0.065 | 0.050 | 3 | |
| | Mean | 0.880 | 0.873 | 0.806 | 0.865 | 66 | | Mean | 0.045 | 0.032 | 0.066 | 0.042 | 12 | Positive |
| 2 | 1 | 0.865 | 0.863 | 0.785 | 0.853 | 72 | 2 | 1 | 0.041 | 0.029 | 0.063 | 0.035 | 8 | |
| | 2 | 0.872 | 0.873 | 0.796 | 0.865 | 67 | | 2 | 0.036 | 0.030 | 0.062 | 0.038 | 12 | |
| | 3 | 0.878 | 0.875 | 0.795 | 0.867 | 74 | | 3 | 0.045 | 0.029 | 0.062 | 0.054 | 3 | |
| | Mean | 0.872 | 0.870 | 0.792 | 0.861 | 71 | | Mean | 0.041 | 0.029 | 0.062 | 0.043 | 8 | Positive |
| 3 | 1 | 0.876 | 0.844 | 0.803 | 0.836 | 68 | 3 | 1 | 0.045 | 0.030 | 0.064 | 0.037 | 6 | |
| | 2 | 0.875 | 0.844 | 0.808 | 0.842 | 62 | | 2 | 0.041 | 0.052 | 0.066 | 0.040 | 12 | |
| | 3 | 0.874 | 0.851 | 0.795 | 0.845 | 74 | | 3 | 0.045 | 0.032 | 0.064 | 0.075 | 6 | |
| | Mean | 0.875 | 0.846 | 0.802 | 0.841 | 68 | | Mean | 0.044 | 0.038 | 0.065 | 0.051 | 8 | Positive |
| Mean for 3 assays | | - | - | - | - | 68 | Mean for 3 assays | | - | - | - | - | 9 | Positive *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 | 0.845 | 0.871 | 0.861 | 0.862 | -24 | 1 | 1 | 0.045 | 0.028 | 0.173 | 0.037 | 117 | |
| | 2 | 0.829 | 0.877 | 0.857 | 0.868 | -37 | | 2 | 0.046 | 0.046 | 0.179 | 0.042 | 122 | |
| | 3 | 0.849 | 0.880 | 0.867 | 0.871 | -27 | | 3 | 0.045 | 0.031 | 0.171 | 0.061 | 115 | |
| | Mean | 0.841 | 0.876 | 0.862 | 0.867 | -29 | | Mean | 0.045 | 0.035 | 0.174 | 0.046 | 118 | Positive |
| 2 | 1 | 0.836 | 0.853 | 0.859 | 0.849 | -29 | 2 | 1 | 0.057 | 0.033 | 0.175 | 0.037 | 107 | |
| | 2 | 0.829 | 0.859 | 0.856 | 0.852 | -35 | | 2 | 0.047 | 0.048 | 0.174 | 0.039 | 116 | |
| | 3 | 0.830 | 0.861 | 0.853 | 0.853 | -30 | | 3 | 0.055 | 0.032 | 0.172 | 0.074 | 105 | |
| | Mean | 0.832 | 0.858 | 0.856 | 0.851 | -31 | | Mean | 0.053 | 0.038 | 0.174 | 0.050 | 109 | Positive |
| 3 | 1 | 0.838 | 0.884 | 0.844 | 0.878 | -12 | 3 | 1 | 0.044 | 0.032 | 0.169 | 0.037 | 116 | |
| | 2 | 0.833 | 0.886 | 0.854 | 0.879 | -27 | | 2 | 0.045 | 0.043 | 0.173 | 0.037 | 117 | |
| | 3 | 0.839 | 0.880 | 0.867 | 0.873 | -33 | | 3 | 0.047 | 0.030 | 0.171 | 0.061 | 114 | |
| | Mean | 0.837 | 0.883 | 0.855 | 0.877 | -24 | | Mean | 0.045 | 0.035 | 0.171 | 0.045 | 116 | Positive |
| Mean for 3 assays | | - | - | - | - | -28 | Mean for 3 assays | | - | - | - | - | 114 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
Chemical Name : Quinine

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 | 0.867 | 0.865 | 0.733 | 0.856 | 126 | 1 | 1 | 0.064 | 0.033 | 0.081 | 0.036 | 8 | |
| | 2 | 0.866 | 0.880 | 0.741 | 0.874 | 116 | | 2 | 0.048 | 0.033 | 0.078 | 0.039 | 20 | |
| | 3 | 0.873 | 0.873 | 0.735 | 0.865 | 131 | | 3 | 0.067 | 0.029 | 0.080 | 0.050 | 3 | |
| | Mean | 0.869 | 0.873 | 0.736 | 0.865 | 124 | | Mean | 0.060 | 0.032 | 0.080 | 0.042 | 10 | Positive |
| 2 | 1 | 0.868 | 0.863 | 0.741 | 0.853 | 118 | 2 | 1 | 0.055 | 0.029 | 0.075 | 0.035 | 6 | |
| | 2 | 0.865 | 0.873 | 0.737 | 0.865 | 119 | | 2 | 0.044 | 0.030 | 0.079 | 0.038 | 21 | |
| | 3 | 0.876 | 0.875 | 0.741 | 0.867 | 126 | | 3 | 0.062 | 0.029 | 0.074 | 0.054 | -2 | |
| | Mean | 0.870 | 0.870 | 0.740 | 0.861 | 121 | | Mean | 0.054 | 0.029 | 0.076 | 0.043 | 8 | Positive |
| 3 | 1 | 0.863 | 0.844 | 0.778 | 0.836 | 80 | 3 | 1 | 0.049 | 0.030 | 0.081 | 0.037 | 19 | |
| | 2 | 0.867 | 0.844 | 0.736 | 0.842 | 126 | | 2 | 0.033 | 0.052 | 0.079 | 0.040 | 33 | |
| | 3 | 0.870 | 0.851 | 0.730 | 0.845 | 134 | | 3 | 0.054 | 0.032 | 0.079 | 0.075 | 12 | |
| | Mean | 0.867 | 0.846 | 0.748 | 0.841 | 113 | | Mean | 0.045 | 0.038 | 0.080 | 0.051 | 21 | Positive |
| Mean for 3 assays | | - | - | - | - | 119 | Mean for 3 assays | | - | - | - | - | 13 | Positive *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 | 0.853 | 0.871 | 0.396 | 0.862 | 448 | 1 | 1 | 0.034 | 0.028 | 0.429 | 0.037 | 384 | |
| | 2 | 0.854 | 0.877 | 0.419 | 0.868 | 427 | | 2 | 0.034 | 0.046 | 0.424 | 0.042 | 380 | |
| | 3 | 0.863 | 0.880 | 0.430 | 0.871 | 424 | | 3 | 0.037 | 0.031 | 0.446 | 0.061 | 398 | |
| | Mean | 0.857 | 0.876 | 0.415 | 0.867 | 433 | | Mean | 0.035 | 0.035 | 0.433 | 0.046 | 387 | Positive |
| 2 | 1 | 0.855 | 0.853 | 0.400 | 0.849 | 448 | 2 | 1 | 0.034 | 0.033 | 0.434 | 0.037 | 389 | |
| | 2 | 0.844 | 0.859 | 0.417 | 0.852 | 420 | | 2 | 0.035 | 0.048 | 0.438 | 0.039 | 391 | |
| | 3 | 0.856 | 0.861 | 0.421 | 0.853 | 428 | | 3 | 0.035 | 0.032 | 0.448 | 0.074 | 401 | |
| | Mean | 0.852 | 0.858 | 0.413 | 0.851 | 432 | | Mean | 0.035 | 0.038 | 0.440 | 0.050 | 394 | Positive |
| 3 | 1 | 0.883 | 0.884 | 0.398 | 0.878 | 479 | 3 | 1 | 0.060 | 0.032 | 0.424 | 0.037 | 354 | |
| | 2 | 0.861 | 0.886 | 0.421 | 0.879 | 434 | | 2 | 0.035 | 0.043 | 0.433 | 0.037 | 388 | |
| | 3 | 0.885 | 0.880 | 0.425 | 0.873 | 455 | | 3 | 0.035 | 0.030 | 0.441 | 0.061 | 396 | |
| | Mean | 0.876 | 0.883 | 0.415 | 0.877 | 456 | | Mean | 0.043 | 0.035 | 0.433 | 0.045 | 379 | Positive |
| Mean for 3 assays | | - | - | - | - | 440 | Mean for 3 assays | | - | - | - | - | 387 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 1
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 | 0.865 | 0.865 | 0.869 | 0.856 | -11 | 1 | 1 | 0.040 | 0.033 | 0.047 | 0.036 | -3 | |
| | 2 | 0.877 | 0.880 | 0.875 | 0.874 | -6 | | 2 | 0.039 | 0.033 | 0.042 | 0.039 | -7 | |
| | 3 | 0.885 | 0.873 | 0.893 | 0.865 | -16 | | 3 | 0.044 | 0.029 | 0.047 | 0.050 | -8 | |
| | Mean | 0.876 | 0.873 | 0.879 | 0.865 | -11 | | Mean | 0.041 | 0.032 | 0.045 | 0.042 | -6 | Negative |
| 2 | 1 | 0.862 | 0.863 | 0.854 | 0.853 | -1 | 2 | 1 | 0.043 | 0.029 | 0.051 | 0.035 | -6 | |
| | 2 | 0.864 | 0.873 | 0.857 | 0.865 | -2 | | 2 | 0.038 | 0.030 | 0.042 | 0.038 | -10 | |
| | 3 | 0.870 | 0.875 | 0.864 | 0.867 | -3 | | 3 | 0.039 | 0.029 | 0.050 | 0.054 | -4 | |
| | Mean | 0.865 | 0.870 | 0.858 | 0.861 | -2 | | Mean | 0.040 | 0.029 | 0.048 | 0.043 | -7 | Negative |
| 3 | 1 | 0.851 | 0.844 | 0.862 | 0.836 | -16 | 3 | 1 | 0.055 | 0.030 | 0.047 | 0.037 | -21 | |
| | 2 | 0.854 | 0.844 | 0.852 | 0.842 | -3 | | 2 | 0.045 | 0.052 | 0.040 | 0.040 | -18 | |
| | 3 | 0.860 | 0.851 | 0.867 | 0.845 | -12 | | 3 | 0.054 | 0.032 | 0.040 | 0.075 | -27 | |
| | Mean | 0.855 | 0.846 | 0.860 | 0.841 | -10 | | Mean | 0.051 | 0.038 | 0.042 | 0.051 | -22 | Negative |
| Mean for 3 assays | | - | - | - | - | -8 | Mean for 3 assays | | - | - | - | - | -12 | Negative *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 | 0.879 | 0.871 | 0.864 | 0.862 | 6 | 1 | 1 | 0.048 | 0.028 | 0.045 | 0.037 | -13 | |
| | 2 | 0.876 | 0.877 | 0.871 | 0.868 | -4 | | 2 | 0.048 | 0.046 | 0.046 | 0.042 | -13 | |
| | 3 | 0.887 | 0.880 | 0.867 | 0.871 | 11 | | 3 | 0.047 | 0.031 | 0.047 | 0.061 | -12 | |
| | Mean | 0.881 | 0.876 | 0.867 | 0.867 | 4 | | Mean | 0.048 | 0.035 | 0.046 | 0.046 | -13 | Negative |
| 2 | 1 | 0.876 | 0.853 | 0.860 | 0.849 | 9 | 2 | 1 | 0.043 | 0.033 | 0.048 | 0.037 | -7 | |
| | 2 | 0.868 | 0.859 | 0.859 | 0.852 | 2 | | 2 | 0.047 | 0.048 | 0.050 | 0.039 | -10 | |
| | 3 | 0.880 | 0.861 | 0.862 | 0.853 | 11 | | 3 | 0.046 | 0.032 | 0.050 | 0.074 | -9 | |
| | Mean | 0.875 | 0.858 | 0.860 | 0.851 | 7 | | Mean | 0.045 | 0.038 | 0.049 | 0.050 | -9 | Negative |
| 3 | 1 | 0.880 | 0.884 | 0.872 | 0.878 | 2 | 3 | 1 | 0.046 | 0.032 | 0.041 | 0.037 | -15 | |
| | 2 | 0.882 | 0.886 | 0.873 | 0.879 | 3 | | 2 | 0.047 | 0.043 | 0.041 | 0.037 | -16 | |
| | 3 | 0.884 | 0.880 | 0.876 | 0.873 | 2 | | 3 | 0.048 | 0.030 | 0.042 | 0.061 | -16 | |
| | Mean | 0.882 | 0.883 | 0.874 | 0.877 | 2 | | Mean | 0.047 | 0.035 | 0.041 | 0.045 | -16 | Negative |
| Mean for 3 assays | | - | - | - | - | 4 | Mean for 3 assays | | - | - | - | - | -13 | Negative *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 1.005 | 1.005 | 0.998 | 1.004 | 1 | 1 | 1 | 0.034 | 0.033 | 0.037 | 0.037 | -2 | |
| | 2 | 1.007 | 1.005 | 0.999 | 0.998 | 2 | | 2 | 0.034 | 0.035 | 0.038 | 0.040 | -1 | |
| | 3 | 1.015 | 1.009 | 1.000 | 0.998 | 9 | | 3 | 0.033 | 0.034 | 0.038 | 0.039 | 0 | |
| | Mean | 1.009 | 1.006 | 0.999 | 1.000 | 4 | | Mean | 0.034 | 0.034 | 0.038 | 0.039 | -1 | Negative |
| 2 | 1 | 0.999 | 1.004 | 0.995 | 1.000 | -3 | 2 | 1 | 0.033 | 0.033 | 0.038 | 0.038 | -1 | |
| | 2 | 1.001 | 1.000 | 0.993 | 0.994 | 1 | | 2 | 0.032 | 0.032 | 0.036 | 0.038 | -2 | |
| | 3 | 1.003 | 1.008 | 0.997 | 0.998 | -1 | | 3 | 0.034 | 0.032 | 0.038 | 0.038 | -2 | |
| | Mean | 1.001 | 1.004 | 0.995 | 0.997 | -1 | | Mean | 0.033 | 0.032 | 0.037 | 0.038 | -2 | Negative |
| 3 | 1 | 0.991 | 0.994 | 0.985 | 0.965 | -10 | 3 | 1 | 0.032 | 0.033 | 0.037 | 0.038 | -3 | |
| | 2 | 0.998 | 0.997 | 0.992 | 0.987 | -10 | | 2 | 0.032 | 0.032 | 0.038 | 0.045 | -2 | |
| | 3 | 0.999 | 0.997 | 0.990 | 0.989 | -7 | | 3 | 0.032 | 0.033 | 0.037 | 0.040 | -3 | |
| | Mean | 0.996 | 0.996 | 0.989 | 0.980 | -9 | | Mean | 0.032 | 0.033 | 0.037 | 0.041 | -3 | Negative |
| Mean for 3 assays | | - | - | - | - | -2 | Mean for 3 assays | | - | - | - | - | -2 | Negative *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 | 1.001 | 1.003 | 0.991 | 0.992 | 1 | 1 | 1 | 0.032 | 0.038 | 0.039 | 0.042 | -4 | |
| | 2 | 1.003 | 1.002 | 0.994 | 0.994 | 0 | | 2 | 0.032 | 0.036 | 0.041 | 0.059 | -2 | |
| | 3 | 1.005 | 1.005 | 0.995 | 0.995 | 1 | | 3 | 0.034 | 0.034 | 0.041 | 0.040 | -4 | |
| | Mean | 1.003 | 1.003 | 0.993 | 0.994 | 1 | | Mean | 0.033 | 0.036 | 0.040 | 0.047 | -3 | Negative |
| 2 | 1 | 1.002 | 1.000 | 0.993 | 0.991 | -1 | 2 | 1 | 0.031 | 0.032 | 0.038 | 0.038 | 0 | |
| | 2 | 0.997 | 0.995 | 0.990 | 0.988 | -3 | | 2 | 0.032 | 0.033 | 0.040 | 0.040 | 1 | |
| | 3 | 1.007 | 1.011 | 0.998 | 0.996 | -1 | | 3 | 0.034 | 0.033 | 0.041 | 0.042 | 0 | |
| | Mean | 1.002 | 1.002 | 0.994 | 0.992 | -2 | | Mean | 0.032 | 0.033 | 0.040 | 0.040 | 0 | Negative |
| 3 | 1 | 0.993 | 0.995 | 0.983 | 0.986 | 1 | 3 | 1 | 0.031 | 0.032 | 0.038 | 0.038 | -4 | |
| | 2 | 0.996 | 0.994 | 0.987 | 0.986 | 0 | | 2 | 0.033 | 0.034 | 0.039 | 0.051 | -5 | |
| | 3 | 1.001 | 1.001 | 0.992 | 0.993 | 0 | | 3 | 0.033 | 0.032 | 0.039 | 0.044 | -5 | |
| | Mean | 0.997 | 0.997 | 0.987 | 0.988 | 0 | | Mean | 0.032 | 0.033 | 0.039 | 0.044 | -5 | Negative |
| Mean for 3 assays | | - | - | - | - | 0 | Mean for 3 assays | | - | - | - | - | -3 | Negative *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 1.004 | 1.005 | 0.985 | 1.004 | 13 | 1 | 1 | 0.032 | 0.033 | 0.044 | 0.037 | 7 | |
| | 2 | 1.006 | 1.005 | 0.996 | 0.998 | 4 | | 2 | 0.035 | 0.035 | 0.046 | 0.040 | 6 | |
| | 3 | 1.006 | 1.009 | 0.990 | 0.998 | 10 | | 3 | 0.032 | 0.034 | 0.044 | 0.039 | 7 | |
| | Mean | 1.005 | 1.006 | 0.990 | 1.000 | 9 | | Mean | 0.033 | 0.034 | 0.045 | 0.039 | 7 | Negative |
| 2 | 1 | 1.001 | 1.004 | 0.987 | 1.000 | 7 | 2 | 1 | 0.032 | 0.033 | 0.048 | 0.038 | 10 | |
| | 2 | 1.001 | 1.000 | 0.985 | 0.994 | 9 | | 2 | 0.031 | 0.032 | 0.042 | 0.038 | 5 | |
| | 3 | 0.995 | 1.008 | 0.986 | 0.998 | 2 | | 3 | 0.033 | 0.032 | 0.042 | 0.038 | 3 | |
| | Mean | 0.999 | 1.004 | 0.986 | 0.997 | 6 | | Mean | 0.032 | 0.032 | 0.044 | 0.038 | 6 | Negative |
| 3 | 1 | 0.994 | 0.994 | 0.960 | 0.965 | 18 | 3 | 1 | 0.033 | 0.033 | 0.042 | 0.038 | 1 | |
| | 2 | 1.000 | 0.997 | 0.982 | 0.987 | 2 | | 2 | 0.032 | 0.032 | 0.042 | 0.045 | 2 | |
| | 3 | 1.003 | 0.997 | 0.968 | 0.989 | 19 | | 3 | 0.033 | 0.033 | 0.042 | 0.040 | 1 | |
| | Mean | 0.999 | 0.996 | 0.970 | 0.980 | 13 | | Mean | 0.033 | 0.033 | 0.042 | 0.041 | 1 | Negative |
| Mean for 3 assays | | - | - | - | - | 9 | Mean for 3 assays | | - | - | - | - | 5 | Negative *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 | 0.993 | 1.003 | 0.922 | 0.992 | 62 | 1 | 1 | 0.032 | 0.038 | 0.107 | 0.042 | 64 | |
| | 2 | 0.993 | 1.002 | 0.924 | 0.994 | 60 | | 2 | 0.035 | 0.036 | 0.106 | 0.059 | 60 | |
| | 3 | 0.993 | 1.005 | 0.927 | 0.995 | 57 | | 3 | 0.033 | 0.034 | 0.112 | 0.040 | 68 | |
| | Mean | 0.993 | 1.003 | 0.924 | 0.994 | 60 | | Mean | 0.033 | 0.036 | 0.108 | 0.047 | 64 | Positive |
| 2 | 1 | 0.993 | 1.000 | 0.916 | 0.991 | 67 | 2 | 1 | 0.031 | 0.032 | 0.113 | 0.038 | 75 | |
| | 2 | 0.988 | 0.995 | 0.914 | 0.988 | 64 | | 2 | 0.032 | 0.033 | 0.112 | 0.040 | 73 | |
| | 3 | 0.996 | 1.011 | 0.922 | 0.996 | 64 | | 3 | 0.032 | 0.033 | 0.119 | 0.042 | 80 | |
| | Mean | 0.992 | 1.002 | 0.917 | 0.992 | 65 | | Mean | 0.032 | 0.033 | 0.115 | 0.040 | 76 | Positive |
| 3 | 1 | 0.987 | 0.995 | 0.917 | 0.986 | 61 | 3 | 1 | 0.032 | 0.032 | 0.093 | 0.038 | 50 | |
| | 2 | 0.992 | 0.994 | 0.931 | 0.986 | 52 | | 2 | 0.033 | 0.034 | 0.091 | 0.051 | 47 | |
| | 3 | 0.991 | 1.001 | 0.920 | 0.993 | 62 | | 3 | 0.033 | 0.032 | 0.097 | 0.044 | 53 | |
| | Mean | 0.990 | 0.997 | 0.923 | 0.988 | 58 | | Mean | 0.033 | 0.033 | 0.094 | 0.044 | 50 | Positive |
| Mean for 3 assays | | - | - | - | - | 61 | Mean for 3 assays | | - | - | - | - | 63 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
Chemical Name : Amiodarone

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 | 1.096 | 1.005 | 0.994 | 1.004 | 96 | 1 | 1 | 0.102 | 0.033 | 0.103 | 0.037 | -4 | |
| | 2 | 1.103 | 1.005 | 1.007 | 0.998 | 90 | | 2 | 0.104 | 0.035 | 0.107 | 0.040 | -2 | |
| | 3 | 1.099 | 1.009 | 1.000 | 0.998 | 93 | | 3 | 0.102 | 0.034 | 0.100 | 0.039 | -7 | |
| | Mean | 1.099 | 1.006 | 1.000 | 1.000 | 93 | | Mean | 0.103 | 0.034 | 0.103 | 0.039 | -4 | Positive |
| 2 | 1 | 1.019 | 1.004 | 0.937 | 1.000 | 75 | 2 | 1 | 0.050 | 0.033 | 0.060 | 0.038 | 4 | |
| | 2 | 1.021 | 1.000 | 0.950 | 0.994 | 64 | | 2 | 0.049 | 0.032 | 0.058 | 0.038 | 3 | |
| | 3 | 1.018 | 1.008 | 0.955 | 0.998 | 56 | | 3 | 0.049 | 0.032 | 0.057 | 0.038 | 2 | |
| | Mean | 1.019 | 1.004 | 0.947 | 0.997 | 65 | | Mean | 0.049 | 0.032 | 0.058 | 0.038 | 3 | Positive |
| 3 | 1 | 1.102 | 0.994 | 0.995 | 0.965 | 91 | 3 | 1 | 0.091 | 0.033 | 0.093 | 0.038 | -6 | |
| | 2 | 1.095 | 0.997 | 0.994 | 0.987 | 85 | | 2 | 0.090 | 0.032 | 0.094 | 0.045 | -4 | |
| | 3 | 1.097 | 0.997 | 1.000 | 0.989 | 81 | | 3 | 0.089 | 0.033 | 0.092 | 0.040 | -5 | |
| | Mean | 1.098 | 0.996 | 0.996 | 0.980 | 86 | | Mean | 0.090 | 0.033 | 0.093 | 0.041 | -5 | Positive |
| Mean for 3 assays | | - | - | - | - | 81 | Mean for 3 assays | | - | - | - | - | -2 | Positive *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 | 1.904 | 1.003 | 1.514 | 0.992 | 381 | 1 | 1 | 0.847 | 0.038 | 0.822 | 0.042 | -36 | |
| | 2 | 1.907 | 1.002 | 1.528 | 0.994 | 370 | | 2 | 0.849 | 0.036 | 0.793 | 0.059 | -67 | |
| | 3 | 1.894 | 1.005 | 1.498 | 0.995 | 387 | | 3 | 0.838 | 0.034 | 0.775 | 0.040 | -74 | |
| | Mean | 1.902 | 1.003 | 1.513 | 0.994 | 379 | | Mean | 0.845 | 0.036 | 0.797 | 0.047 | -59 | Positive |
| 2 | 1 | 1.797 | 1.000 | 1.485 | 0.991 | 302 | 2 | 1 | 0.813 | 0.032 | 0.641 | 0.038 | -179 | |
| | 2 | 1.804 | 0.995 | 1.467 | 0.988 | 327 | | 2 | 0.813 | 0.033 | 0.633 | 0.040 | -187 | |
| | 3 | 1.793 | 1.011 | 1.478 | 0.996 | 305 | | 3 | 0.806 | 0.033 | 0.597 | 0.042 | -216 | |
| | Mean | 1.798 | 1.002 | 1.477 | 0.992 | 311 | | Mean | 0.811 | 0.033 | 0.624 | 0.040 | -194 | Positive |
| 3 | 1 | 1.955 | 0.995 | 1.485 | 0.986 | 461 | 3 | 1 | 0.760 | 0.032 | 0.621 | 0.038 | -150 | |
| | 2 | 1.950 | 0.994 | 1.516 | 0.986 | 425 | | 2 | 0.765 | 0.034 | 0.622 | 0.051 | -154 | |
| | 3 | 1.940 | 1.001 | 1.489 | 0.993 | 442 | | 3 | 0.758 | 0.032 | 0.597 | 0.044 | -172 | |
| | Mean | 1.948 | 0.997 | 1.497 | 0.988 | 443 | | Mean | 0.761 | 0.033 | 0.613 | 0.044 | -159 | Positive |
| Mean for 3 assays | | - | - | - | - | 378 | Mean for 3 assays | | - | - | - | - | -137 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
Chemical Name : Chlorpromazine

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 | 0.994 | 1.005 | 0.919 | 1.004 | 69 | 1 | 1 | 0.033 | 0.033 | 0.059 | 0.037 | 21 | |
| | 2 | 1.002 | 1.005 | 0.918 | 0.998 | 78 | | 2 | 0.035 | 0.035 | 0.060 | 0.040 | 20 | |
| | 3 | 1.001 | 1.009 | 0.916 | 0.998 | 79 | | 3 | 0.034 | 0.034 | 0.060 | 0.039 | 21 | |
| | Mean | 0.999 | 1.006 | 0.918 | 1.000 | 75 | | Mean | 0.034 | 0.034 | 0.060 | 0.039 | 21 | Positive |
| 2 | 1 | 0.994 | 1.004 | 0.897 | 1.000 | 90 | 2 | 1 | 0.033 | 0.033 | 0.059 | 0.038 | 20 | |
| | 2 | 0.999 | 1.000 | 0.910 | 0.994 | 82 | | 2 | 0.033 | 0.032 | 0.061 | 0.038 | 22 | |
| | 3 | 1.000 | 1.008 | 0.939 | 0.998 | 54 | | 3 | 0.035 | 0.032 | 0.061 | 0.038 | 20 | |
| | Mean | 0.998 | 1.004 | 0.915 | 0.997 | 75 | | Mean | 0.034 | 0.032 | 0.060 | 0.038 | 21 | Positive |
| 3 | 1 | 0.999 | 0.994 | 0.929 | 0.965 | 54 | 3 | 1 | 0.034 | 0.033 | 0.060 | 0.038 | 18 | |
| | 2 | 0.993 | 0.997 | 0.910 | 0.987 | 67 | | 2 | 0.033 | 0.032 | 0.061 | 0.045 | 20 | |
| | 3 | 1.001 | 0.997 | 0.925 | 0.989 | 60 | | 3 | 0.033 | 0.033 | 0.059 | 0.040 | 18 | |
| | Mean | 0.998 | 0.996 | 0.921 | 0.980 | 60 | | Mean | 0.033 | 0.033 | 0.060 | 0.041 | 19 | Positive |
| Mean for 3 assays | | - | - | - | - | 70 | Mean for 3 assays | | - | - | - | - | 20 | Positive *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 | 0.953 | 1.003 | 0.980 | 0.992 | -36 | 1 | 1 | 0.035 | 0.038 | 0.137 | 0.042 | 91 | |
| | 2 | 0.983 | 1.002 | 1.012 | 0.994 | -38 | | 2 | 0.042 | 0.036 | 0.137 | 0.059 | 84 | |
| | 3 | 0.990 | 1.005 | 1.014 | 0.995 | -33 | | 3 | 0.035 | 0.034 | 0.140 | 0.040 | 94 | |
| | Mean | 0.975 | 1.003 | 1.002 | 0.994 | -36 | | Mean | 0.037 | 0.036 | 0.138 | 0.047 | 90 | Positive |
| 2 | 1 | 0.967 | 1.000 | 0.995 | 0.991 | -38 | 2 | 1 | 0.042 | 0.032 | 0.155 | 0.038 | 106 | |
| | 2 | 1.003 | 0.995 | 1.025 | 0.988 | -32 | | 2 | 0.036 | 0.033 | 0.159 | 0.040 | 116 | |
| | 3 | 0.982 | 1.011 | 1.006 | 0.996 | -34 | | 3 | 0.035 | 0.033 | 0.160 | 0.042 | 118 | |
| | Mean | 0.984 | 1.002 | 1.009 | 0.992 | -35 | | Mean | 0.038 | 0.033 | 0.158 | 0.040 | 113 | Positive |
| 3 | 1 | 0.972 | 0.995 | 0.996 | 0.986 | -33 | 3 | 1 | 0.034 | 0.032 | 0.142 | 0.038 | 97 | |
| | 2 | 0.999 | 0.994 | 1.029 | 0.986 | -39 | | 2 | 0.035 | 0.034 | 0.142 | 0.051 | 96 | |
| | 3 | 0.994 | 1.001 | 1.024 | 0.993 | -39 | | 3 | 0.036 | 0.032 | 0.148 | 0.044 | 101 | |
| | Mean | 0.988 | 0.997 | 1.016 | 0.988 | -37 | | Mean | 0.035 | 0.033 | 0.144 | 0.044 | 98 | Positive |
| Mean for 3 assays | | - | - | - | - | -36 | Mean for 3 assays | | - | - | - | - | 100 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 1.006 | 1.005 | 0.887 | 1.004 | 113 | 1 | 1 | 0.041 | 0.033 | 0.055 | 0.037 | 9 | |
| | 2 | 1.006 | 1.005 | 0.906 | 0.998 | 94 | | 2 | 0.039 | 0.035 | 0.053 | 0.040 | 9 | |
| | 3 | 1.006 | 1.009 | 0.903 | 0.998 | 97 | | 3 | 0.039 | 0.034 | 0.054 | 0.039 | 10 | |
| | Mean | 1.006 | 1.006 | 0.899 | 1.000 | 101 | | Mean | 0.040 | 0.034 | 0.054 | 0.039 | 9 | Positive |
| 2 | 1 | 1.004 | 1.004 | 0.870 | 1.000 | 127 | 2 | 1 | 0.038 | 0.033 | 0.054 | 0.038 | 10 | |
| | 2 | 1.000 | 1.000 | 0.884 | 0.994 | 109 | | 2 | 0.039 | 0.032 | 0.051 | 0.038 | 6 | |
| | 3 | 1.003 | 1.008 | 0.892 | 0.998 | 104 | | 3 | 0.037 | 0.032 | 0.052 | 0.038 | 9 | |
| | Mean | 1.002 | 1.004 | 0.882 | 0.997 | 113 | | Mean | 0.038 | 0.032 | 0.052 | 0.038 | 8 | Positive |
| 3 | 1 | 1.000 | 0.994 | 0.884 | 0.965 | 100 | 3 | 1 | 0.046 | 0.033 | 0.055 | 0.038 | 1 | |
| | 2 | 1.006 | 0.997 | 0.911 | 0.987 | 79 | | 2 | 0.039 | 0.032 | 0.053 | 0.045 | 6 | |
| | 3 | 1.008 | 0.997 | 0.901 | 0.989 | 91 | | 3 | 0.039 | 0.033 | 0.054 | 0.040 | 7 | |
| | Mean | 1.005 | 0.996 | 0.899 | 0.980 | 90 | | Mean | 0.041 | 0.033 | 0.054 | 0.041 | 5 | Positive |
| Mean for 3 assays | | - | - | - | - | 101 | Mean for 3 assays | | - | - | - | - | 7 | Positive *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 | 0.996 | 1.003 | 0.740 | 0.992 | 247 | 1 | 1 | 0.040 | 0.038 | 0.310 | 0.042 | 259 | |
| | 2 | 0.997 | 1.002 | 0.751 | 0.994 | 237 | | 2 | 0.038 | 0.036 | 0.312 | 0.059 | 263 | |
| | 3 | 1.001 | 1.005 | 0.752 | 0.995 | 240 | | 3 | 0.039 | 0.034 | 0.317 | 0.040 | 267 | |
| | Mean | 0.998 | 1.003 | 0.748 | 0.994 | 241 | | Mean | 0.039 | 0.036 | 0.313 | 0.047 | 263 | Positive |
| 2 | 1 | 1.005 | 1.000 | 0.721 | 0.991 | 274 | 2 | 1 | 0.040 | 0.032 | 0.347 | 0.038 | 300 | |
| | 2 | 0.996 | 0.995 | 0.729 | 0.988 | 257 | | 2 | 0.038 | 0.033 | 0.354 | 0.040 | 309 | |
| | 3 | 1.001 | 1.011 | 0.733 | 0.996 | 258 | | 3 | 0.038 | 0.033 | 0.352 | 0.042 | 307 | |
| | Mean | 1.001 | 1.002 | 0.728 | 0.992 | 263 | | Mean | 0.039 | 0.033 | 0.351 | 0.040 | 305 | Positive |
| 3 | 1 | 0.998 | 0.995 | 0.748 | 0.986 | 241 | 3 | 1 | 0.041 | 0.032 | 0.344 | 0.038 | 292 | |
| | 2 | 0.998 | 0.994 | 0.766 | 0.986 | 223 | | 2 | 0.037 | 0.034 | 0.349 | 0.051 | 301 | |
| | 3 | 1.000 | 1.001 | 0.752 | 0.993 | 239 | | 3 | 0.037 | 0.032 | 0.360 | 0.044 | 312 | |
| | Mean | 0.999 | 0.997 | 0.755 | 0.988 | 234 | | Mean | 0.038 | 0.033 | 0.351 | 0.044 | 302 | Positive |
| Mean for 3 assays | | - | - | - | - | 246 | Mean for 3 assays | | - | - | - | - | 290 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
Chemical Name : Doxycycline

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 | 1.006 | 1.005 | 0.913 | 1.004 | 87 | 1 | 1 | 0.035 | 0.033 | 0.109 | 0.037 | 69 | |
| | 2 | 1.009 | 1.005 | 0.927 | 0.998 | 76 | | 2 | 0.035 | 0.035 | 0.105 | 0.040 | 65 | |
| | 3 | 1.006 | 1.009 | 0.927 | 0.998 | 73 | | 3 | 0.035 | 0.034 | 0.106 | 0.039 | 66 | |
| | Mean | 1.007 | 1.006 | 0.922 | 1.000 | 79 | | Mean | 0.035 | 0.034 | 0.107 | 0.039 | 67 | Positive |
| 2 | 1 | 1.007 | 1.004 | 0.935 | 1.000 | 65 | 2 | 1 | 0.034 | 0.033 | 0.075 | 0.038 | 35 | |
| | 2 | 1.008 | 1.000 | 0.944 | 0.994 | 57 | | 2 | 0.035 | 0.032 | 0.076 | 0.038 | 35 | |
| | 3 | 1.003 | 1.008 | 0.946 | 0.998 | 50 | | 3 | 0.033 | 0.032 | 0.075 | 0.038 | 36 | |
| | Mean | 1.006 | 1.004 | 0.942 | 0.997 | 57 | | Mean | 0.034 | 0.032 | 0.075 | 0.038 | 35 | Positive |
| 3 | 1 | 1.004 | 0.994 | 0.925 | 0.965 | 63 | 3 | 1 | 0.034 | 0.033 | 0.095 | 0.038 | 53 | |
| | 2 | 1.006 | 0.997 | 0.922 | 0.987 | 68 | | 2 | 0.035 | 0.032 | 0.098 | 0.045 | 55 | |
| | 3 | 0.998 | 0.997 | 0.916 | 0.989 | 66 | | 3 | 0.035 | 0.033 | 0.104 | 0.040 | 61 | |
| | Mean | 1.003 | 0.996 | 0.921 | 0.980 | 66 | | Mean | 0.035 | 0.033 | 0.099 | 0.041 | 56 | Positive |
| Mean for 3 assays | | - | - | - | - | 67 | Mean for 3 assays | | - | - | - | - | 53 | Positive *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 | 0.974 | 1.003 | 0.762 | 0.992 | 203 | 1 | 1 | 0.041 | 0.038 | 0.453 | 0.042 | 401 | |
| | 2 | 0.990 | 1.002 | 0.788 | 0.994 | 193 | | 2 | 0.039 | 0.036 | 0.450 | 0.059 | 400 | |
| | 3 | 0.989 | 1.005 | 0.782 | 0.995 | 198 | | 3 | 0.040 | 0.034 | 0.433 | 0.040 | 382 | |
| | Mean | 0.984 | 1.003 | 0.777 | 0.994 | 198 | | Mean | 0.040 | 0.036 | 0.445 | 0.047 | 394 | Positive |
| 2 | 1 | 0.985 | 1.000 | 0.733 | 0.991 | 242 | 2 | 1 | 0.040 | 0.032 | 0.458 | 0.038 | 411 | |
| | 2 | 0.992 | 0.995 | 0.759 | 0.988 | 223 | | 2 | 0.040 | 0.033 | 0.461 | 0.040 | 414 | |
| | 3 | 0.992 | 1.011 | 0.766 | 0.996 | 216 | | 3 | 0.040 | 0.033 | 0.455 | 0.042 | 408 | |
| | Mean | 0.990 | 1.002 | 0.753 | 0.992 | 227 | | Mean | 0.040 | 0.033 | 0.458 | 0.040 | 411 | Positive |
| 3 | 1 | 0.981 | 0.995 | 0.774 | 0.986 | 198 | 3 | 1 | 0.039 | 0.032 | 0.426 | 0.038 | 376 | |
| | 2 | 0.987 | 0.994 | 0.810 | 0.986 | 168 | | 2 | 0.040 | 0.034 | 0.419 | 0.051 | 368 | |
| | 3 | 0.992 | 1.001 | 0.792 | 0.993 | 191 | | 3 | 0.039 | 0.032 | 0.411 | 0.044 | 361 | |
| | Mean | 0.987 | 0.997 | 0.792 | 0.988 | 186 | | Mean | 0.039 | 0.033 | 0.419 | 0.044 | 368 | Positive |
| Mean for 3 assays | | - | - | - | - | 204 | Mean for 3 assays | | - | - | - | - | 391 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 1.008 | 1.005 | 0.973 | 1.004 | 29 | 1 | 1 | 0.037 | 0.033 | 0.053 | 0.037 | 11 | |
| | 2 | 1.015 | 1.005 | 0.982 | 0.998 | 27 | | 2 | 0.045 | 0.035 | 0.049 | 0.040 | -1 | |
| | 3 | 1.010 | 1.009 | 0.971 | 0.998 | 33 | | 3 | 0.038 | 0.034 | 0.051 | 0.039 | 8 | |
| | Mean | 1.011 | 1.006 | 0.975 | 1.000 | 30 | | Mean | 0.040 | 0.034 | 0.051 | 0.039 | 6 | Positive |
| 2 | 1 | 1.004 | 1.004 | 0.963 | 1.000 | 34 | 2 | 1 | 0.038 | 0.033 | 0.056 | 0.038 | 12 | |
| | 2 | 1.009 | 1.000 | 0.970 | 0.994 | 32 | | 2 | 0.044 | 0.032 | 0.052 | 0.038 | 2 | |
| | 3 | 1.006 | 1.008 | 0.971 | 0.998 | 28 | | 3 | 0.036 | 0.032 | 0.051 | 0.038 | 9 | |
| | Mean | 1.006 | 1.004 | 0.968 | 0.997 | 31 | | Mean | 0.039 | 0.032 | 0.053 | 0.038 | 8 | Positive |
| 3 | 1 | 1.003 | 0.994 | 0.967 | 0.965 | 20 | 3 | 1 | 0.046 | 0.033 | 0.050 | 0.038 | -4 | |
| | 2 | 1.006 | 0.997 | 0.970 | 0.987 | 20 | | 2 | 0.045 | 0.032 | 0.050 | 0.045 | -3 | |
| | 3 | 1.004 | 0.997 | 0.971 | 0.989 | 17 | | 3 | 0.042 | 0.033 | 0.050 | 0.040 | 0 | |
| | Mean | 1.004 | 0.996 | 0.969 | 0.980 | 19 | | Mean | 0.044 | 0.033 | 0.050 | 0.041 | -2 | Negative |
| Mean for 3 assays | | - | - | - | - | 27 | Mean for 3 assays | | - | - | - | - | 4 | Positive *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 | 1.003 | 1.003 | 0.862 | 0.992 | 132 | 1 | 1 | 0.038 | 0.038 | 0.106 | 0.042 | 57 | |
| | 2 | 1.005 | 1.002 | 0.863 | 0.994 | 133 | | 2 | 0.041 | 0.036 | 0.110 | 0.059 | 58 | |
| | 3 | 1.000 | 1.005 | 0.870 | 0.995 | 121 | | 3 | 0.038 | 0.034 | 0.113 | 0.040 | 64 | |
| | Mean | 1.003 | 1.003 | 0.865 | 0.994 | 129 | | Mean | 0.039 | 0.036 | 0.110 | 0.047 | 60 | Positive |
| 2 | 1 | 1.000 | 1.000 | 0.848 | 0.991 | 142 | 2 | 1 | 0.039 | 0.032 | 0.118 | 0.038 | 72 | |
| | 2 | 1.005 | 0.995 | 0.847 | 0.988 | 148 | | 2 | 0.041 | 0.033 | 0.126 | 0.040 | 78 | |
| | 3 | 1.003 | 1.011 | 0.868 | 0.996 | 125 | | 3 | 0.042 | 0.033 | 0.127 | 0.042 | 78 | |
| | Mean | 1.003 | 1.002 | 0.854 | 0.992 | 138 | | Mean | 0.041 | 0.033 | 0.124 | 0.040 | 76 | Positive |
| 3 | 1 | 0.999 | 0.995 | 0.856 | 0.986 | 134 | 3 | 1 | 0.038 | 0.032 | 0.114 | 0.038 | 65 | |
| | 2 | 1.009 | 0.994 | 0.866 | 0.986 | 134 | | 2 | 0.037 | 0.034 | 0.112 | 0.051 | 64 | |
| | 3 | 1.001 | 1.001 | 0.867 | 0.993 | 125 | | 3 | 0.038 | 0.032 | 0.115 | 0.044 | 66 | |
| | Mean | 1.003 | 0.997 | 0.863 | 0.988 | 131 | | Mean | 0.038 | 0.033 | 0.114 | 0.044 | 65 | Positive |
| Mean for 3 assays | | - | - | - | - | 133 | Mean for 3 assays | | - | - | - | - | 67 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 0.994 | 1.001 | 0.956 | 0.996 | 29 | 1 | 1 | 0.034 | 0.033 | 0.045 | 0.041 | 3 | |
| | 2 | 1.000 | 1.003 | 0.961 | 0.993 | 30 | | 2 | 0.034 | 0.033 | 0.046 | 0.041 | 4 | |
| | 3 | 1.003 | 1.004 | 0.963 | 0.992 | 31 | | 3 | 0.033 | 0.033 | 0.047 | 0.042 | 6 | |
| | Mean | 0.999 | 1.003 | 0.960 | 0.994 | 30 | | Mean | 0.034 | 0.033 | 0.046 | 0.041 | 4 | Positive |
| 2 | 1 | 0.998 | 1.000 | 0.952 | 0.991 | 38 | 2 | 1 | 0.032 | 0.032 | 0.043 | 0.038 | 3 | |
| | 2 | 0.996 | 0.996 | 0.960 | 0.990 | 28 | | 2 | 0.032 | 0.033 | 0.044 | 0.045 | 4 | |
| | 3 | 1.000 | 1.003 | 0.965 | 0.995 | 27 | | 3 | 0.032 | 0.034 | 0.043 | 0.040 | 3 | |
| | Mean | 0.998 | 1.000 | 0.959 | 0.992 | 31 | | Mean | 0.032 | 0.033 | 0.043 | 0.041 | 3 | Positive |
| 3 | 1 | 1.013 | 1.009 | 0.968 | 0.999 | 28 | 3 | 1 | 0.032 | 0.035 | 0.042 | 0.040 | 4 | |
| | 2 | 1.011 | 1.007 | 0.945 | 0.977 | 49 | | 2 | 0.033 | 0.034 | 0.047 | 0.041 | 8 | |
| | 3 | 1.010 | 1.010 | 0.971 | 1.000 | 22 | | 3 | 0.036 | 0.033 | 0.047 | 0.039 | 5 | |
| | Mean | 1.011 | 1.009 | 0.961 | 0.992 | 33 | | Mean | 0.034 | 0.034 | 0.045 | 0.040 | 6 | Positive |
| Mean for 3 assays | | - | - | - | - | 31 | Mean for 3 assays | | - | - | - | - | 4 | Positive *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 | 0.999 | 1.007 | 0.774 | 0.999 | 213 | 1 | 1 | 0.033 | 0.032 | 0.150 | 0.041 | 109 | |
| | 2 | 1.007 | 1.013 | 0.787 | 0.997 | 208 | | 2 | 0.033 | 0.032 | 0.151 | 0.039 | 110 | |
| | 3 | 1.005 | 1.009 | 0.785 | 0.998 | 208 | | 3 | 0.033 | 0.034 | 0.142 | 0.042 | 101 | |
| | Mean | 1.004 | 1.010 | 0.782 | 0.998 | 210 | | Mean | 0.033 | 0.033 | 0.148 | 0.041 | 107 | Positive |
| 2 | 1 | 0.992 | 1.002 | 0.771 | 0.986 | 209 | 2 | 1 | 0.033 | 0.032 | 0.134 | 0.038 | 94 | |
| | 2 | 0.999 | 1.002 | 0.787 | 0.993 | 200 | | 2 | 0.032 | 0.032 | 0.133 | 0.040 | 94 | |
| | 3 | 1.000 | 1.004 | 0.792 | 0.995 | 196 | | 3 | 0.033 | 0.033 | 0.137 | 0.040 | 97 | |
| | Mean | 0.997 | 1.003 | 0.783 | 0.991 | 202 | | Mean | 0.033 | 0.032 | 0.135 | 0.039 | 95 | Positive |
| 3 | 1 | 1.001 | 1.002 | 0.742 | 0.990 | 249 | 3 | 1 | 0.032 | 0.033 | 0.160 | 0.038 | 122 | |
| | 2 | 1.004 | 1.004 | 0.756 | 0.992 | 238 | | 2 | 0.035 | 0.033 | 0.142 | 0.038 | 101 | |
| | 3 | 1.006 | 1.008 | 0.757 | 1.003 | 239 | | 3 | 0.034 | 0.033 | 0.144 | 0.040 | 104 | |
| | Mean | 1.004 | 1.005 | 0.752 | 0.995 | 242 | | Mean | 0.034 | 0.033 | 0.149 | 0.039 | 109 | Positive |
| Mean for 3 assays | | - | - | - | - | 218 | Mean for 3 assays | | - | - | - | - | 104 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 1.000 | 1.001 | 0.961 | 0.996 | 30 | 1 | 1 | 0.033 | 0.033 | 0.228 | 0.041 | 187 | |
| | 2 | 1.005 | 1.003 | 0.969 | 0.993 | 27 | | 2 | 0.033 | 0.033 | 0.231 | 0.041 | 190 | |
| | 3 | 1.008 | 1.004 | 0.971 | 0.992 | 28 | | 3 | 0.034 | 0.033 | 0.237 | 0.042 | 195 | |
| | Mean | 1.004 | 1.003 | 0.967 | 0.994 | 28 | | Mean | 0.033 | 0.033 | 0.232 | 0.041 | 191 | Positive |
| 2 | 1 | 1.000 | 1.000 | 0.957 | 0.991 | 35 | 2 | 1 | 0.031 | 0.032 | 0.255 | 0.038 | 216 | |
| | 2 | 1.001 | 0.996 | 0.965 | 0.990 | 28 | | 2 | 0.031 | 0.033 | 0.252 | 0.045 | 213 | |
| | 3 | 1.003 | 1.003 | 0.968 | 0.995 | 27 | | 3 | 0.032 | 0.034 | 0.239 | 0.040 | 199 | |
| | Mean | 1.001 | 1.000 | 0.963 | 0.992 | 30 | | Mean | 0.031 | 0.033 | 0.249 | 0.041 | 209 | Positive |
| 3 | 1 | 1.004 | 1.009 | 0.965 | 0.999 | 22 | 3 | 1 | 0.033 | 0.035 | 0.219 | 0.040 | 180 | |
| | 2 | 1.009 | 1.007 | 0.970 | 0.977 | 22 | | 2 | 0.032 | 0.034 | 0.217 | 0.041 | 179 | |
| | 3 | 1.005 | 1.010 | 0.971 | 1.000 | 17 | | 3 | 0.033 | 0.033 | 0.222 | 0.039 | 183 | |
| | Mean | 1.006 | 1.009 | 0.969 | 0.992 | 20 | | Mean | 0.033 | 0.034 | 0.219 | 0.040 | 181 | Positive |
| Mean for 3 assays | | - | - | - | - | 26 | Mean for 3 assays | | - | - | - | - | 194 | Positive *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 | 0.998 | 1.007 | 0.859 | 0.999 | 127 | 1 | 1 | 0.033 | 0.032 | 0.487 | 0.041 | 446 | |
| | 2 | 1.005 | 1.013 | 0.867 | 0.997 | 126 | | 2 | 0.032 | 0.032 | 0.496 | 0.039 | 456 | |
| | 3 | 1.003 | 1.009 | 0.869 | 0.998 | 122 | | 3 | 0.034 | 0.034 | 0.505 | 0.042 | 463 | |
| | Mean | 1.002 | 1.010 | 0.865 | 0.998 | 125 | | Mean | 0.033 | 0.033 | 0.496 | 0.041 | 455 | Positive |
| 2 | 1 | 0.994 | 1.002 | 0.850 | 0.986 | 132 | 2 | 1 | 0.032 | 0.032 | 0.507 | 0.038 | 468 | |
| | 2 | 1.000 | 1.002 | 0.861 | 0.993 | 127 | | 2 | 0.032 | 0.032 | 0.511 | 0.040 | 472 | |
| | 3 | 1.000 | 1.004 | 0.867 | 0.995 | 121 | | 3 | 0.032 | 0.033 | 0.515 | 0.040 | 476 | |
| | Mean | 0.998 | 1.003 | 0.859 | 0.991 | 127 | | Mean | 0.032 | 0.032 | 0.511 | 0.039 | 472 | Positive |
| 3 | 1 | 0.996 | 1.002 | 0.846 | 0.990 | 140 | 3 | 1 | 0.036 | 0.033 | 0.477 | 0.038 | 435 | |
| | 2 | 1.003 | 1.004 | 0.845 | 0.992 | 148 | | 2 | 0.033 | 0.033 | 0.482 | 0.038 | 443 | |
| | 3 | 1.003 | 1.008 | 0.878 | 1.003 | 115 | | 3 | 0.032 | 0.033 | 0.484 | 0.040 | 446 | |
| | Mean | 1.001 | 1.005 | 0.856 | 0.995 | 134 | | Mean | 0.034 | 0.033 | 0.481 | 0.039 | 441 | Positive |
| Mean for 3 assays | | - | - | - | - | 129 | Mean for 3 assays | | - | - | - | - | 456 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 0.996 | 1.001 | 0.918 | 0.996 | 69 | 1 | 1 | 0.032 | 0.033 | 0.061 | 0.041 | 21 | |
| | 2 | 0.996 | 1.003 | 0.930 | 0.993 | 57 | | 2 | 0.032 | 0.033 | 0.061 | 0.041 | 21 | |
| | 3 | 1.001 | 1.004 | 0.937 | 0.992 | 55 | | 3 | 0.032 | 0.033 | 0.062 | 0.042 | 22 | |
| | Mean | 0.998 | 1.003 | 0.928 | 0.994 | 60 | | Mean | 0.032 | 0.033 | 0.061 | 0.041 | 21 | Positive |
| 2 | 1 | 0.993 | 1.000 | 0.920 | 0.991 | 65 | 2 | 1 | 0.031 | 0.032 | 0.066 | 0.038 | 27 | |
| | 2 | 0.995 | 0.996 | 0.933 | 0.990 | 54 | | 2 | 0.031 | 0.033 | 0.067 | 0.045 | 28 | |
| | 3 | 0.993 | 1.003 | 0.928 | 0.995 | 57 | | 3 | 0.030 | 0.034 | 0.068 | 0.040 | 30 | |
| | Mean | 0.994 | 1.000 | 0.927 | 0.992 | 59 | | Mean | 0.031 | 0.033 | 0.067 | 0.041 | 28 | Positive |
| 3 | 1 | 1.001 | 1.009 | 0.928 | 0.999 | 56 | 3 | 1 | 0.031 | 0.035 | 0.061 | 0.040 | 24 | |
| | 2 | 1.007 | 1.007 | 0.930 | 0.977 | 60 | | 2 | 0.030 | 0.034 | 0.059 | 0.041 | 23 | |
| | 3 | 1.002 | 1.010 | 0.936 | 1.000 | 49 | | 3 | 0.032 | 0.033 | 0.061 | 0.039 | 23 | |
| | Mean | 1.003 | 1.009 | 0.931 | 0.992 | 55 | | Mean | 0.031 | 0.034 | 0.060 | 0.040 | 23 | Positive |
| Mean for 3 assays | | - | - | - | - | 58 | Mean for 3 assays | | - | - | - | - | 24 | Positive *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 | 0.995 | 1.007 | 0.821 | 0.999 | 162 | 1 | 1 | 0.031 | 0.032 | 0.190 | 0.041 | 151 | |
| | 2 | 0.998 | 1.013 | 0.834 | 0.997 | 152 | | 2 | 0.031 | 0.032 | 0.185 | 0.039 | 146 | |
| | 3 | 0.998 | 1.009 | 0.837 | 0.998 | 149 | | 3 | 0.031 | 0.034 | 0.186 | 0.042 | 147 | |
| | Mean | 0.997 | 1.010 | 0.831 | 0.998 | 154 | | Mean | 0.031 | 0.033 | 0.187 | 0.041 | 148 | Positive |
| 2 | 1 | 0.992 | 1.002 | 0.825 | 0.986 | 155 | 2 | 1 | 0.031 | 0.032 | 0.207 | 0.038 | 169 | |
| | 2 | 0.994 | 1.002 | 0.838 | 0.993 | 144 | | 2 | 0.031 | 0.032 | 0.200 | 0.040 | 162 | |
| | 3 | 0.996 | 1.004 | 0.838 | 0.995 | 146 | | 3 | 0.032 | 0.033 | 0.201 | 0.040 | 162 | |
| | Mean | 0.994 | 1.003 | 0.834 | 0.991 | 148 | | Mean | 0.031 | 0.032 | 0.203 | 0.039 | 164 | Positive |
| 3 | 1 | 0.989 | 1.002 | 0.818 | 0.990 | 161 | 3 | 1 | 0.031 | 0.033 | 0.188 | 0.038 | 151 | |
| | 2 | 1.000 | 1.004 | 0.827 | 0.992 | 163 | | 2 | 0.032 | 0.033 | 0.185 | 0.038 | 147 | |
| | 3 | 0.999 | 1.008 | 0.837 | 1.003 | 152 | | 3 | 0.031 | 0.033 | 0.193 | 0.040 | 156 | |
| | Mean | 0.996 | 1.005 | 0.827 | 0.995 | 159 | | Mean | 0.031 | 0.033 | 0.189 | 0.039 | 151 | Positive |
| Mean for 3 assays | | - | - | - | - | 154 | Mean for 3 assays | | - | - | - | - | 154 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 0.997 | 1.001 | 0.930 | 0.996 | 58 | 1 | 1 | 0.034 | 0.033 | 0.078 | 0.041 | 36 | |
| | 2 | 1.003 | 1.003 | 0.940 | 0.993 | 54 | | 2 | 0.034 | 0.033 | 0.078 | 0.041 | 36 | |
| | 3 | 1.013 | 1.004 | 0.946 | 0.992 | 58 | | 3 | 0.034 | 0.033 | 0.079 | 0.042 | 37 | |
| | Mean | 1.004 | 1.003 | 0.939 | 0.994 | 57 | | Mean | 0.034 | 0.033 | 0.078 | 0.041 | 36 | Positive |
| 2 | 1 | 1.001 | 1.000 | 0.913 | 0.991 | 80 | 2 | 1 | 0.032 | 0.032 | 0.076 | 0.038 | 36 | |
| | 2 | 1.006 | 0.996 | 0.943 | 0.990 | 55 | | 2 | 0.033 | 0.033 | 0.075 | 0.045 | 34 | |
| | 3 | 1.006 | 1.003 | 0.938 | 0.995 | 60 | | 3 | 0.033 | 0.034 | 0.075 | 0.040 | 34 | |
| | Mean | 1.004 | 1.000 | 0.931 | 0.992 | 65 | | Mean | 0.033 | 0.033 | 0.075 | 0.041 | 35 | Positive |
| 3 | 1 | 1.008 | 1.009 | 0.930 | 0.999 | 61 | 3 | 1 | 0.037 | 0.035 | 0.078 | 0.040 | 35 | |
| | 2 | 1.016 | 1.007 | 0.927 | 0.977 | 72 | | 2 | 0.033 | 0.034 | 0.074 | 0.041 | 35 | |
| | 3 | 1.012 | 1.010 | 0.952 | 1.000 | 43 | | 3 | 0.037 | 0.033 | 0.080 | 0.039 | 37 | |
| | Mean | 1.012 | 1.009 | 0.936 | 0.992 | 59 | | Mean | 0.036 | 0.034 | 0.077 | 0.040 | 36 | Positive |
| Mean for 3 assays | | - | - | - | - | 60 | Mean for 3 assays | | - | - | - | - | 36 | Positive *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 | 0.993 | 1.007 | 1.036 | 0.999 | -55 | 1 | 1 | 0.036 | 0.032 | 0.187 | 0.041 | 143 | |
| | 2 | 0.998 | 1.013 | 1.033 | 0.997 | -47 | | 2 | 0.037 | 0.032 | 0.187 | 0.039 | 142 | |
| | 3 | 1.003 | 1.009 | 1.027 | 0.998 | -36 | | 3 | 0.035 | 0.034 | 0.180 | 0.042 | 137 | |
| | Mean | 0.998 | 1.010 | 1.032 | 0.998 | -46 | | Mean | 0.036 | 0.033 | 0.185 | 0.041 | 141 | Positive |
| 2 | 1 | 0.990 | 1.002 | 1.041 | 0.986 | -63 | 2 | 1 | 0.035 | 0.032 | 0.187 | 0.038 | 145 | |
| | 2 | 0.993 | 1.002 | 1.042 | 0.993 | -61 | | 2 | 0.036 | 0.032 | 0.190 | 0.040 | 147 | |
| | 3 | 1.002 | 1.004 | 1.035 | 0.995 | -45 | | 3 | 0.036 | 0.033 | 0.192 | 0.040 | 149 | |
| | Mean | 0.995 | 1.003 | 1.039 | 0.991 | -56 | | Mean | 0.036 | 0.032 | 0.190 | 0.039 | 147 | Positive |
| 3 | 1 | 0.990 | 1.002 | 1.033 | 0.990 | -53 | 3 | 1 | 0.036 | 0.033 | 0.190 | 0.038 | 148 | |
| | 2 | 1.006 | 1.004 | 1.041 | 0.992 | -45 | | 2 | 0.041 | 0.033 | 0.191 | 0.038 | 144 | |
| | 3 | 0.998 | 1.008 | 1.037 | 1.003 | -49 | | 3 | 0.037 | 0.033 | 0.197 | 0.040 | 154 | |
| | Mean | 0.998 | 1.005 | 1.037 | 0.995 | -49 | | Mean | 0.038 | 0.033 | 0.193 | 0.039 | 149 | Positive |
| Mean for 3 assays | | - | - | - | - | -50 | Mean for 3 assays | | - | - | - | - | 146 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
Chemical Name : Quinine

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 | 0.989 | 1.001 | 0.861 | 0.996 | 119 | 1 | 1 | 0.035 | 0.033 | 0.109 | 0.041 | 66 | |
| | 2 | 1.003 | 1.003 | 0.881 | 0.993 | 113 | | 2 | 0.033 | 0.033 | 0.105 | 0.041 | 64 | |
| | 3 | 1.007 | 1.004 | 0.880 | 0.992 | 118 | | 3 | 0.034 | 0.033 | 0.107 | 0.042 | 65 | |
| | Mean | 1.000 | 1.003 | 0.874 | 0.994 | 117 | | Mean | 0.034 | 0.033 | 0.107 | 0.041 | 65 | Positive |
| 2 | 1 | 0.997 | 1.000 | 0.860 | 0.991 | 129 | 2 | 1 | 0.033 | 0.032 | 0.107 | 0.038 | 66 | |
| | 2 | 0.998 | 0.996 | 0.881 | 0.990 | 109 | | 2 | 0.032 | 0.033 | 0.102 | 0.045 | 62 | |
| | 3 | 1.001 | 1.003 | 0.885 | 0.995 | 108 | | 3 | 0.034 | 0.034 | 0.108 | 0.040 | 66 | |
| | Mean | 0.999 | 1.000 | 0.875 | 0.992 | 115 | | Mean | 0.033 | 0.033 | 0.106 | 0.041 | 65 | Positive |
| 3 | 1 | 1.003 | 1.009 | 0.873 | 0.999 | 113 | 3 | 1 | 0.033 | 0.035 | 0.099 | 0.040 | 60 | |
| | 2 | 1.005 | 1.007 | 0.877 | 0.977 | 111 | | 2 | 0.033 | 0.034 | 0.096 | 0.041 | 57 | |
| | 3 | 1.006 | 1.010 | 0.885 | 1.000 | 104 | | 3 | 0.034 | 0.033 | 0.099 | 0.039 | 59 | |
| | Mean | 1.005 | 1.009 | 0.878 | 0.992 | 109 | | Mean | 0.033 | 0.034 | 0.098 | 0.040 | 59 | Positive |
| Mean for 3 assays | | - | - | - | - | 114 | Mean for 3 assays | | - | - | - | - | 63 | Positive *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 | 0.993 | 1.007 | 0.568 | 0.999 | 413 | 1 | 1 | 0.033 | 0.032 | 0.443 | 0.041 | 402 | |
| | 2 | 1.000 | 1.013 | 0.583 | 0.997 | 405 | | 2 | 0.033 | 0.032 | 0.436 | 0.039 | 395 | |
| | 3 | 0.999 | 1.009 | 0.587 | 0.998 | 400 | | 3 | 0.033 | 0.034 | 0.428 | 0.042 | 387 | |
| | Mean | 0.997 | 1.010 | 0.579 | 0.998 | 406 | | Mean | 0.033 | 0.033 | 0.436 | 0.041 | 395 | Positive |
| 2 | 1 | 0.991 | 1.002 | 0.557 | 0.986 | 422 | 2 | 1 | 0.033 | 0.032 | 0.430 | 0.038 | 390 | |
| | 2 | 0.994 | 1.002 | 0.583 | 0.993 | 399 | | 2 | 0.032 | 0.032 | 0.427 | 0.040 | 388 | |
| | 3 | 0.999 | 1.004 | 0.592 | 0.995 | 395 | | 3 | 0.033 | 0.033 | 0.449 | 0.040 | 409 | |
| | Mean | 0.995 | 1.003 | 0.577 | 0.991 | 405 | | Mean | 0.033 | 0.032 | 0.435 | 0.039 | 396 | Positive |
| 3 | 1 | 0.996 | 1.002 | 0.556 | 0.990 | 430 | 3 | 1 | 0.034 | 0.033 | 0.408 | 0.038 | 368 | |
| | 2 | 0.998 | 1.004 | 0.575 | 0.992 | 413 | | 2 | 0.033 | 0.033 | 0.400 | 0.038 | 361 | |
| | 3 | 0.999 | 1.008 | 0.588 | 1.003 | 401 | | 3 | 0.033 | 0.033 | 0.427 | 0.040 | 388 | |
| | Mean | 0.998 | 1.005 | 0.573 | 0.995 | 415 | | Mean | 0.033 | 0.033 | 0.412 | 0.039 | 372 | Positive |
| Mean for 3 assays | | - | - | - | - | 409 | Mean for 3 assays | | - | - | - | - | 388 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 2
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 | 1.006 | 1.001 | 0.998 | 0.996 | -1 | 1 | 1 | 0.038 | 0.033 | 0.042 | 0.041 | -4 | |
| | 2 | 1.012 | 1.003 | 1.003 | 0.993 | 0 | | 2 | 0.038 | 0.033 | 0.041 | 0.041 | -5 | |
| | 3 | 1.010 | 1.004 | 1.003 | 0.992 | -2 | | 3 | 0.036 | 0.033 | 0.044 | 0.042 | 0 | |
| | Mean | 1.009 | 1.003 | 1.001 | 0.994 | -1 | | Mean | 0.037 | 0.033 | 0.042 | 0.041 | -3 | Negative |
| 2 | 1 | 1.008 | 1.000 | 0.993 | 0.991 | 7 | 2 | 1 | 0.035 | 0.032 | 0.040 | 0.038 | -3 | |
| | 2 | 1.011 | 0.996 | 1.002 | 0.990 | 1 | | 2 | 0.036 | 0.033 | 0.046 | 0.045 | 2 | |
| | 3 | 1.007 | 1.003 | 0.999 | 0.995 | 0 | | 3 | 0.038 | 0.034 | 0.041 | 0.040 | -5 | |
| | Mean | 1.009 | 1.000 | 0.998 | 0.992 | 3 | | Mean | 0.036 | 0.033 | 0.041 | 0.041 | -2 | Negative |
| 3 | 1 | 1.011 | 1.009 | 1.002 | 0.999 | -8 | 3 | 1 | 0.039 | 0.035 | 0.042 | 0.040 | -3 | |
| | 2 | 1.016 | 1.007 | 1.006 | 0.977 | -7 | | 2 | 0.040 | 0.034 | 0.040 | 0.041 | -6 | |
| | 3 | 1.010 | 1.010 | 1.004 | 1.000 | -11 | | 3 | 0.038 | 0.033 | 0.042 | 0.039 | -2 | |
| | Mean | 1.012 | 1.009 | 1.004 | 0.992 | -9 | | Mean | 0.039 | 0.034 | 0.041 | 0.040 | -4 | Negative |
| Mean for 3 assays | | - | - | - | - | -2 | Mean for 3 assays | | - | - | - | - | -3 | Negative *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 | 1.005 | 1.007 | 0.994 | 0.999 | -1 | 1 | 1 | 0.037 | 0.032 | 0.039 | 0.041 | -6 | |
| | 2 | 1.013 | 1.013 | 1.000 | 0.997 | 1 | | 2 | 0.037 | 0.032 | 0.040 | 0.039 | -5 | |
| | 3 | 1.006 | 1.009 | 0.996 | 0.998 | -2 | | 3 | 0.039 | 0.034 | 0.041 | 0.042 | -6 | |
| | Mean | 1.008 | 1.010 | 0.997 | 0.998 | -1 | | Mean | 0.038 | 0.033 | 0.040 | 0.041 | -6 | Negative |
| 2 | 1 | 1.002 | 1.002 | 0.989 | 0.986 | 1 | 2 | 1 | 0.040 | 0.032 | 0.040 | 0.038 | -7 | |
| | 2 | 1.008 | 1.002 | 0.997 | 0.993 | -1 | | 2 | 0.038 | 0.032 | 0.040 | 0.040 | -5 | |
| | 3 | 1.006 | 1.004 | 0.997 | 0.995 | -3 | | 3 | 0.038 | 0.033 | 0.040 | 0.040 | -5 | |
| | Mean | 1.005 | 1.003 | 0.994 | 0.991 | -1 | | Mean | 0.039 | 0.032 | 0.040 | 0.039 | -6 | Negative |
| 3 | 1 | 1.006 | 1.002 | 0.995 | 0.990 | 1 | 3 | 1 | 0.041 | 0.033 | 0.041 | 0.038 | -6 | |
| | 2 | 1.016 | 1.004 | 0.988 | 0.992 | 18 | | 2 | 0.040 | 0.033 | 0.039 | 0.038 | -7 | |
| | 3 | 1.009 | 1.008 | 1.006 | 1.003 | -7 | | 3 | 0.041 | 0.033 | 0.042 | 0.040 | -5 | |
| | Mean | 1.010 | 1.005 | 0.996 | 0.995 | 4 | | Mean | 0.041 | 0.033 | 0.041 | 0.039 | -6 | Negative |
| Mean for 3 assays | | - | - | - | - | 1 | Mean for 3 assays | | - | - | - | - | -6 | Negative *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.984 | 0.982 | 0.975 | 0.967 | -4 | 1 | 1 | 0.037 | 0.038 | 0.039 | 0.040 | 0 | |
| | 2 | 0.980 | 0.980 | 0.969 | 0.967 | -3 | | 2 | 0.039 | 0.039 | 0.041 | 0.041 | 0 | |
| | 3 | 0.983 | 0.976 | 0.974 | 0.964 | -4 | | 3 | 0.038 | 0.038 | 0.040 | 0.040 | 0 | |
| | Mean | 0.982 | 0.979 | 0.973 | 0.966 | -4 | | Mean | 0.038 | 0.038 | 0.040 | 0.040 | 0 | Negative |
| 2 | 1 | 0.989 | 0.984 | 0.982 | 0.972 | -4 | 2 | 1 | 0.037 | 0.038 | 0.040 | 0.041 | 1 | |
| | 2 | 0.983 | 0.984 | 0.975 | 0.974 | -4 | | 2 | 0.038 | 0.040 | 0.041 | 0.041 | 0 | |
| | 3 | 0.989 | 0.982 | 0.980 | 0.970 | -3 | | 3 | 0.038 | 0.038 | 0.041 | 0.040 | 1 | |
| | Mean | 0.987 | 0.984 | 0.979 | 0.972 | -4 | | Mean | 0.038 | 0.039 | 0.040 | 0.041 | 1 | Negative |
| 3 | 1 | 0.982 | 0.987 | 0.974 | 0.978 | -1 | 3 | 1 | 0.038 | 0.038 | 0.040 | 0.040 | 0 | |
| | 2 | 0.982 | 0.976 | 0.972 | 0.965 | 1 | | 2 | 0.037 | 0.039 | 0.039 | 0.041 | 0 | |
| | 3 | 0.980 | 0.974 | 0.974 | 0.966 | -3 | | 3 | 0.038 | 0.039 | 0.065 | 0.041 | 25 | |
| | Mean | 0.981 | 0.979 | 0.974 | 0.970 | -1 | | Mean | 0.038 | 0.039 | 0.048 | 0.041 | 8 | Negative |
| Mean for 3 assays | | - | - | - | - | -3 | Mean for 3 assays | | - | - | - | - | 3 | Negative *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 | 0.996 | 0.996 | 0.987 | 0.982 | -7 | 1 | 1 | 0.037 | 0.038 | 0.040 | 0.040 | 0 | |
| | 2 | 0.999 | 1.001 | 0.991 | 0.987 | -6 | | 2 | 0.038 | 0.039 | 0.041 | 0.041 | 0 | |
| | 3 | 1.004 | 1.000 | 0.993 | 0.983 | -5 | | 3 | 0.037 | 0.038 | 0.040 | 0.040 | 0 | |
| | Mean | 1.000 | 0.999 | 0.991 | 0.984 | -6 | | Mean | 0.037 | 0.038 | 0.040 | 0.041 | 0 | Negative |
| 2 | 1 | 0.989 | 0.987 | 0.981 | 0.976 | -2 | 2 | 1 | 0.037 | 0.038 | 0.039 | 0.040 | 0 | |
| | 2 | 0.982 | 0.981 | 0.973 | 0.971 | -1 | | 2 | 0.038 | 0.039 | 0.040 | 0.041 | 0 | |
| | 3 | 0.985 | 0.978 | 0.977 | 0.968 | -2 | | 3 | 0.038 | 0.038 | 0.040 | 0.040 | 1 | |
| | Mean | 0.985 | 0.982 | 0.977 | 0.972 | -2 | | Mean | 0.038 | 0.038 | 0.040 | 0.040 | 0 | Negative |
| 3 | 1 | 0.982 | 0.982 | 0.973 | 0.970 | -1 | 3 | 1 | 0.037 | 0.038 | 0.039 | 0.041 | 0 | |
| | 2 | 0.984 | 0.987 | 0.975 | 0.980 | -1 | | 2 | 0.038 | 0.039 | 0.041 | 0.041 | 0 | |
| | 3 | 0.991 | 0.994 | 0.982 | 0.984 | -1 | | 3 | 0.037 | 0.037 | 0.040 | 0.040 | 0 | |
| | Mean | 0.986 | 0.988 | 0.977 | 0.978 | -1 | | Mean | 0.037 | 0.038 | 0.040 | 0.041 | 0 | Negative |
| Mean for 3 assays | | - | - | - | - | -3 | Mean for 3 assays | | - | - | - | - | 0 | Negative *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.982 | 0.982 | 0.965 | 0.967 | 5 | 1 | 1 | 0.037 | 0.038 | 0.042 | 0.040 | 3 | |
| | 2 | 0.985 | 0.980 | 0.964 | 0.967 | 8 | | 2 | 0.038 | 0.039 | 0.043 | 0.041 | 3 | |
| | 3 | 0.977 | 0.976 | 0.957 | 0.964 | 8 | | 3 | 0.038 | 0.038 | 0.042 | 0.040 | 2 | |
| | Mean | 0.981 | 0.979 | 0.962 | 0.966 | 7 | | Mean | 0.038 | 0.038 | 0.042 | 0.040 | 3 | Negative |
| 2 | 1 | 0.989 | 0.984 | 0.973 | 0.972 | 4 | 2 | 1 | 0.037 | 0.038 | 0.042 | 0.041 | 4 | |
| | 2 | 0.991 | 0.984 | 0.973 | 0.974 | 6 | | 2 | 0.038 | 0.040 | 0.044 | 0.041 | 4 | |
| | 3 | 0.983 | 0.982 | 0.965 | 0.970 | 7 | | 3 | 0.038 | 0.038 | 0.043 | 0.040 | 3 | |
| | Mean | 0.988 | 0.984 | 0.970 | 0.972 | 6 | | Mean | 0.038 | 0.039 | 0.043 | 0.041 | 4 | Negative |
| 3 | 1 | 0.988 | 0.987 | 0.969 | 0.978 | 10 | 3 | 1 | 0.037 | 0.038 | 0.043 | 0.040 | 3 | |
| | 2 | 0.981 | 0.976 | 0.962 | 0.965 | 9 | | 2 | 0.038 | 0.039 | 0.042 | 0.041 | 1 | |
| | 3 | 0.978 | 0.974 | 0.960 | 0.966 | 9 | | 3 | 0.038 | 0.039 | 0.043 | 0.041 | 3 | |
| | Mean | 0.982 | 0.979 | 0.964 | 0.970 | 9 | | Mean | 0.038 | 0.039 | 0.042 | 0.041 | 2 | Negative |
| Mean for 3 assays | | - | - | - | - | 7 | Mean for 3 assays | | - | - | - | - | 3 | Negative *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 | 0.980 | 0.996 | 0.925 | 0.982 | 40 | 1 | 1 | 0.037 | 0.038 | 0.055 | 0.040 | 15 | |
| | 2 | 0.989 | 1.001 | 0.930 | 0.987 | 43 | | 2 | 0.039 | 0.039 | 0.057 | 0.041 | 15 | |
| | 3 | 0.988 | 1.000 | 0.931 | 0.983 | 42 | | 3 | 0.038 | 0.038 | 0.056 | 0.040 | 15 | |
| | Mean | 0.985 | 0.999 | 0.929 | 0.984 | 42 | | Mean | 0.038 | 0.038 | 0.056 | 0.041 | 15 | Positive |
| 2 | 1 | 0.975 | 0.987 | 0.911 | 0.976 | 54 | 2 | 1 | 0.037 | 0.038 | 0.055 | 0.040 | 16 | |
| | 2 | 0.977 | 0.981 | 0.911 | 0.971 | 56 | | 2 | 0.039 | 0.039 | 0.058 | 0.041 | 17 | |
| | 3 | 0.970 | 0.978 | 0.905 | 0.968 | 55 | | 3 | 0.038 | 0.038 | 0.060 | 0.040 | 20 | |
| | Mean | 0.974 | 0.982 | 0.909 | 0.972 | 55 | | Mean | 0.038 | 0.038 | 0.057 | 0.040 | 18 | Positive |
| 3 | 1 | 0.955 | 0.982 | 0.894 | 0.970 | 50 | 3 | 1 | 0.037 | 0.038 | 0.055 | 0.041 | 15 | |
| | 2 | 0.980 | 0.987 | 0.918 | 0.980 | 52 | | 2 | 0.039 | 0.039 | 0.056 | 0.041 | 14 | |
| | 3 | 0.979 | 0.994 | 0.916 | 0.984 | 52 | | 3 | 0.038 | 0.037 | 0.056 | 0.040 | 16 | |
| | Mean | 0.971 | 0.988 | 0.909 | 0.978 | 51 | | Mean | 0.038 | 0.038 | 0.056 | 0.041 | 15 | Positive |
| Mean for 3 assays | | - | - | - | - | 49 | Mean for 3 assays | | - | - | - | - | 16 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
Chemical Name : Amiodarone

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 | 0.982 | 0.966 | 0.967 | 82 | 1 | 1 | 0.089 | 0.038 | 0.095 | 0.040 | 4 | |
| | 2 | 1.059 | 0.980 | 0.962 | 0.967 | 84 | | 2 | 0.091 | 0.039 | 0.097 | 0.041 | 4 | |
| | 3 | 1.058 | 0.976 | 0.966 | 0.964 | 79 | | 3 | 0.089 | 0.038 | 0.098 | 0.040 | 7 | |
| | Mean | 1.060 | 0.979 | 0.965 | 0.966 | 82 | | Mean | 0.090 | 0.038 | 0.097 | 0.040 | 5 | Positive |
| 2 | 1 | 1.058 | 0.984 | 0.967 | 0.972 | 79 | 2 | 1 | 0.079 | 0.038 | 0.088 | 0.041 | 7 | |
| | 2 | 1.057 | 0.984 | 0.957 | 0.974 | 88 | | 2 | 0.084 | 0.040 | 0.095 | 0.041 | 8 | |
| | 3 | 1.062 | 0.982 | 0.972 | 0.970 | 78 | | 3 | 0.085 | 0.038 | 0.097 | 0.040 | 10 | |
| | Mean | 1.059 | 0.984 | 0.965 | 0.972 | 82 | | Mean | 0.083 | 0.039 | 0.093 | 0.041 | 8 | Positive |
| 3 | 1 | 1.062 | 0.987 | 0.968 | 0.978 | 85 | 3 | 1 | 0.080 | 0.038 | 0.088 | 0.040 | 6 | |
| | 2 | 1.053 | 0.976 | 0.957 | 0.965 | 87 | | 2 | 0.084 | 0.039 | 0.093 | 0.041 | 7 | |
| | 3 | 1.055 | 0.974 | 0.964 | 0.966 | 82 | | 3 | 0.087 | 0.039 | 0.096 | 0.041 | 7 | |
| | Mean | 1.057 | 0.979 | 0.963 | 0.970 | 85 | | Mean | 0.084 | 0.039 | 0.092 | 0.041 | 7 | Positive |
| Mean for 3 assays | | - | - | - | - | 83 | Mean for 3 assays | | - | - | - | - | 7 | Positive [*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 | 1.772 | 0.996 | 1.416 | 0.982 | 341 | 1 | 1 | 0.660 | 0.038 | 0.543 | 0.040 | -119 | |
| | 2 | 1.778 | 1.001 | 1.456 | 0.987 | 307 | | 2 | 0.664 | 0.039 | 0.548 | 0.041 | -119 | |
| | 3 | 1.784 | 1.000 | 1.459 | 0.983 | 310 | | 3 | 0.661 | 0.038 | 0.559 | 0.040 | -105 | |
| | Mean | 1.778 | 0.999 | 1.444 | 0.984 | 319 | | Mean | 0.662 | 0.038 | 0.550 | 0.041 | -114 | Positive |
| 2 | 1 | 1.830 | 0.987 | 1.403 | 0.976 | 416 | 2 | 1 | 0.504 | 0.038 | 0.523 | 0.040 | 17 | |
| | 2 | 1.818 | 0.981 | 1.417 | 0.971 | 391 | | 2 | 0.513 | 0.039 | 0.534 | 0.041 | 19 | |
| | 3 | 1.820 | 0.978 | 1.439 | 0.968 | 371 | | 3 | 0.534 | 0.038 | 0.559 | 0.040 | 24 | |
| | Mean | 1.823 | 0.982 | 1.420 | 0.972 | 393 | | Mean | 0.517 | 0.038 | 0.539 | 0.040 | 20 | Positive |
| 3 | 1 | 1.806 | 0.982 | 1.410 | 0.970 | 386 | 3 | 1 | 0.641 | 0.038 | 0.566 | 0.041 | -79 | |
| | 2 | 1.809 | 0.987 | 1.442 | 0.980 | 357 | | 2 | 0.653 | 0.039 | 0.580 | 0.041 | -76 | |
| | 3 | 1.823 | 0.994 | 1.469 | 0.984 | 344 | | 3 | 0.644 | 0.037 | 0.557 | 0.040 | -90 | |
| | Mean | 1.813 | 0.988 | 1.440 | 0.978 | 362 | | Mean | 0.646 | 0.038 | 0.567 | 0.041 | -82 | Positive |
| Mean for 3 assays | | - | - | - | - | 358 | Mean for 3 assays | | - | - | - | - | -59 | Positive [*3] |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
Chemical Name : Chlorpromazine

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 | 0.982 | 0.862 | 0.967 | 96 | 1 | 1 | 0.038 | 0.038 | 0.052 | 0.040 | 12 | |
| | 2 | 0.968 | 0.980 | 0.873 | 0.967 | 82 | | 2 | 0.038 | 0.039 | 0.051 | 0.041 | 11 | |
| | 3 | 0.965 | 0.976 | 0.855 | 0.964 | 97 | | 3 | 0.037 | 0.038 | 0.052 | 0.040 | 12 | |
| | Mean | 0.968 | 0.979 | 0.863 | 0.966 | 92 | | Mean | 0.038 | 0.038 | 0.051 | 0.040 | 12 | Positive |
| 2 | 1 | 0.980 | 0.984 | 0.867 | 0.972 | 101 | 2 | 1 | 0.038 | 0.038 | 0.052 | 0.041 | 12 | |
| | 2 | 0.972 | 0.984 | 0.859 | 0.974 | 102 | | 2 | 0.038 | 0.040 | 0.054 | 0.041 | 13 | |
| | 3 | 0.969 | 0.982 | 0.860 | 0.970 | 97 | | 3 | 0.038 | 0.038 | 0.053 | 0.040 | 13 | |
| | Mean | 0.974 | 0.984 | 0.862 | 0.972 | 100 | | Mean | 0.038 | 0.039 | 0.053 | 0.041 | 13 | Positive |
| 3 | 1 | 0.976 | 0.987 | 0.861 | 0.978 | 105 | 3 | 1 | 0.038 | 0.038 | 0.051 | 0.040 | 11 | |
| | 2 | 0.968 | 0.976 | 0.857 | 0.965 | 102 | | 2 | 0.037 | 0.039 | 0.051 | 0.041 | 12 | |
| | 3 | 0.966 | 0.974 | 0.860 | 0.966 | 96 | | 3 | 0.038 | 0.039 | 0.053 | 0.041 | 13 | |
| | Mean | 0.970 | 0.979 | 0.860 | 0.970 | 101 | | Mean | 0.037 | 0.039 | 0.052 | 0.041 | 12 | Positive |
| Mean for 3 assays | | - | - | - | - | 98 | Mean for 3 assays | | - | - | - | - | 12 | Positive *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 | 0.963 | 0.996 | 0.937 | 0.982 | 11 | 1 | 1 | 0.038 | 0.038 | 0.117 | 0.040 | 76 | |
| | 2 | 0.992 | 1.001 | 0.945 | 0.987 | 32 | | 2 | 0.039 | 0.039 | 0.120 | 0.041 | 78 | |
| | 3 | 0.963 | 1.000 | 0.943 | 0.983 | 5 | | 3 | 0.037 | 0.038 | 0.119 | 0.040 | 79 | |
| | Mean | 0.973 | 0.999 | 0.941 | 0.984 | 16 | | Mean | 0.038 | 0.038 | 0.119 | 0.041 | 78 | Positive |
| 2 | 1 | 0.960 | 0.987 | 0.926 | 0.976 | 24 | 2 | 1 | 0.038 | 0.038 | 0.112 | 0.040 | 72 | |
| | 2 | 0.956 | 0.981 | 0.926 | 0.971 | 21 | | 2 | 0.038 | 0.039 | 0.109 | 0.041 | 69 | |
| | 3 | 0.951 | 0.978 | 0.930 | 0.968 | 12 | | 3 | 0.037 | 0.038 | 0.120 | 0.040 | 81 | |
| | Mean | 0.956 | 0.982 | 0.927 | 0.972 | 19 | | Mean | 0.038 | 0.038 | 0.114 | 0.040 | 74 | Positive |
| 3 | 1 | 0.955 | 0.982 | 0.929 | 0.970 | 16 | 3 | 1 | 0.038 | 0.038 | 0.114 | 0.041 | 73 | |
| | 2 | 0.956 | 0.987 | 0.935 | 0.980 | 11 | | 2 | 0.040 | 0.039 | 0.117 | 0.041 | 75 | |
| | 3 | 0.951 | 0.994 | 0.941 | 0.984 | 0 | | 3 | 0.037 | 0.037 | 0.121 | 0.040 | 80 | |
| | Mean | 0.954 | 0.988 | 0.935 | 0.978 | 9 | | Mean | 0.038 | 0.038 | 0.117 | 0.041 | 76 | Positive |
| Mean for 3 assays | | - | - | - | - | 15 | Mean for 3 assays | | - | - | - | - | 76 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.982 | 0.982 | 0.808 | 0.967 | 160 | 1 | 1 | 0.039 | 0.038 | 0.049 | 0.040 | 8 | |
| | 2 | 0.987 | 0.980 | 0.815 | 0.967 | 159 | | 2 | 0.041 | 0.039 | 0.051 | 0.041 | 8 | |
| | 3 | 0.978 | 0.976 | 0.818 | 0.964 | 146 | | 3 | 0.040 | 0.038 | 0.051 | 0.040 | 9 | |
| | Mean | 0.982 | 0.979 | 0.814 | 0.966 | 155 | | Mean | 0.040 | 0.038 | 0.050 | 0.040 | 8 | Positive |
| 2 | 1 | 0.986 | 0.984 | 0.832 | 0.972 | 142 | 2 | 1 | 0.038 | 0.038 | 0.049 | 0.041 | 9 | |
| | 2 | 0.995 | 0.984 | 0.829 | 0.974 | 154 | | 2 | 0.040 | 0.040 | 0.051 | 0.041 | 9 | |
| | 3 | 0.982 | 0.982 | 0.825 | 0.970 | 145 | | 3 | 0.039 | 0.038 | 0.052 | 0.040 | 11 | |
| | Mean | 0.988 | 0.984 | 0.829 | 0.972 | 147 | | Mean | 0.039 | 0.039 | 0.050 | 0.041 | 10 | Positive |
| 3 | 1 | 0.989 | 0.987 | 0.837 | 0.978 | 143 | 3 | 1 | 0.039 | 0.038 | 0.049 | 0.040 | 8 | |
| | 2 | 0.977 | 0.976 | 0.823 | 0.965 | 145 | | 2 | 0.039 | 0.039 | 0.049 | 0.041 | 8 | |
| | 3 | 0.980 | 0.974 | 0.824 | 0.966 | 147 | | 3 | 0.039 | 0.039 | 0.051 | 0.041 | 10 | |
| | Mean | 0.982 | 0.979 | 0.828 | 0.970 | 145 | | Mean | 0.039 | 0.039 | 0.050 | 0.041 | 9 | Positive |
| Mean for 3 assays | | - | - | - | - | 149 | Mean for 3 assays | | - | - | - | - | 9 | Positive *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 | 0.990 | 0.996 | 0.644 | 0.982 | 331 | 1 | 1 | 0.039 | 0.038 | 0.329 | 0.040 | 287 | |
| | 2 | 1.002 | 1.001 | 0.659 | 0.987 | 328 | | 2 | 0.040 | 0.039 | 0.346 | 0.041 | 303 | |
| | 3 | 0.998 | 1.000 | 0.653 | 0.983 | 330 | | 3 | 0.039 | 0.038 | 0.361 | 0.040 | 319 | |
| | Mean | 0.996 | 0.999 | 0.652 | 0.984 | 330 | | Mean | 0.039 | 0.038 | 0.345 | 0.041 | 303 | Positive |
| 2 | 1 | 0.985 | 0.987 | 0.640 | 0.976 | 335 | 2 | 1 | 0.039 | 0.038 | 0.303 | 0.040 | 263 | |
| | 2 | 0.988 | 0.981 | 0.651 | 0.971 | 327 | | 2 | 0.040 | 0.039 | 0.331 | 0.041 | 289 | |
| | 3 | 0.978 | 0.978 | 0.641 | 0.968 | 327 | | 3 | 0.039 | 0.038 | 0.346 | 0.040 | 306 | |
| | Mean | 0.984 | 0.982 | 0.644 | 0.972 | 330 | | Mean | 0.039 | 0.038 | 0.327 | 0.040 | 286 | Positive |
| 3 | 1 | 0.980 | 0.982 | 0.638 | 0.970 | 331 | 3 | 1 | 0.038 | 0.038 | 0.321 | 0.041 | 280 | |
| | 2 | 0.992 | 0.987 | 0.652 | 0.980 | 330 | | 2 | 0.040 | 0.039 | 0.341 | 0.041 | 299 | |
| | 3 | 0.983 | 0.994 | 0.651 | 0.984 | 322 | | 3 | 0.039 | 0.037 | 0.362 | 0.040 | 320 | |
| | Mean | 0.985 | 0.988 | 0.647 | 0.978 | 328 | | Mean | 0.039 | 0.038 | 0.341 | 0.041 | 300 | Positive |
| Mean for 3 assays | | - | - | - | - | 329 | Mean for 3 assays | | - | - | - | - | 296 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
Chemical Name : Doxycycline

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 | 0.973 | 0.982 | 0.913 | 0.967 | 47 | 1 | 1 | 0.039 | 0.038 | 0.084 | 0.040 | 43 | |
| | 2 | 0.970 | 0.980 | 0.906 | 0.967 | 51 | | 2 | 0.039 | 0.039 | 0.086 | 0.041 | 45 | |
| | 3 | 0.969 | 0.976 | 0.904 | 0.964 | 52 | | 3 | 0.039 | 0.038 | 0.087 | 0.040 | 46 | |
| | Mean | 0.971 | 0.979 | 0.908 | 0.966 | 50 | | Mean | 0.039 | 0.038 | 0.085 | 0.040 | 45 | Positive |
| 2 | 1 | 0.978 | 0.984 | 0.918 | 0.972 | 49 | 2 | 1 | 0.038 | 0.038 | 0.082 | 0.041 | 42 | |
| | 2 | 0.977 | 0.984 | 0.913 | 0.974 | 52 | | 2 | 0.039 | 0.040 | 0.086 | 0.041 | 46 | |
| | 3 | 0.971 | 0.982 | 0.908 | 0.970 | 52 | | 3 | 0.038 | 0.038 | 0.086 | 0.040 | 46 | |
| | Mean | 0.976 | 0.984 | 0.913 | 0.972 | 51 | | Mean | 0.038 | 0.039 | 0.085 | 0.041 | 45 | Positive |
| 3 | 1 | 0.981 | 0.987 | 0.917 | 0.978 | 55 | 3 | 1 | 0.038 | 0.038 | 0.082 | 0.040 | 42 | |
| | 2 | 0.975 | 0.976 | 0.908 | 0.965 | 58 | | 2 | 0.038 | 0.039 | 0.084 | 0.041 | 44 | |
| | 3 | 0.970 | 0.974 | 0.906 | 0.966 | 55 | | 3 | 0.039 | 0.039 | 0.087 | 0.041 | 46 | |
| | Mean | 0.975 | 0.979 | 0.910 | 0.970 | 56 | | Mean | 0.038 | 0.039 | 0.084 | 0.041 | 44 | Positive |
| Mean for 3 assays | | - | - | - | - | 52 | Mean for 3 assays | | - | - | - | - | 45 | Positive *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 | 0.959 | 0.996 | 0.767 | 0.982 | 177 | 1 | 1 | 0.041 | 0.038 | 0.348 | 0.040 | 304 | |
| | 2 | 0.965 | 1.001 | 0.777 | 0.987 | 173 | | 2 | 0.041 | 0.039 | 0.348 | 0.041 | 304 | |
| | 3 | 0.965 | 1.000 | 0.775 | 0.983 | 175 | | 3 | 0.041 | 0.038 | 0.359 | 0.040 | 315 | |
| | Mean | 0.963 | 0.999 | 0.773 | 0.984 | 175 | | Mean | 0.041 | 0.038 | 0.352 | 0.041 | 308 | Positive |
| 2 | 1 | 0.955 | 0.987 | 0.779 | 0.976 | 166 | 2 | 1 | 0.040 | 0.038 | 0.345 | 0.040 | 303 | |
| | 2 | 0.949 | 0.981 | 0.784 | 0.971 | 156 | | 2 | 0.041 | 0.039 | 0.355 | 0.041 | 312 | |
| | 3 | 0.948 | 0.978 | 0.778 | 0.968 | 160 | | 3 | 0.040 | 0.038 | 0.373 | 0.040 | 331 | |
| | Mean | 0.951 | 0.982 | 0.780 | 0.972 | 161 | | Mean | 0.040 | 0.038 | 0.357 | 0.040 | 315 | Positive |
| 3 | 1 | 0.950 | 0.982 | 0.766 | 0.970 | 174 | 3 | 1 | 0.040 | 0.038 | 0.348 | 0.041 | 305 | |
| | 2 | 0.949 | 0.987 | 0.771 | 0.980 | 168 | | 2 | 0.041 | 0.039 | 0.359 | 0.041 | 315 | |
| | 3 | 0.953 | 0.994 | 0.775 | 0.984 | 167 | | 3 | 0.040 | 0.037 | 0.364 | 0.040 | 321 | |
| | Mean | 0.950 | 0.988 | 0.771 | 0.978 | 170 | | Mean | 0.040 | 0.038 | 0.357 | 0.041 | 314 | Positive |
| Mean for 3 assays | | - | - | - | - | 169 | Mean for 3 assays | | - | - | - | - | 312 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.983 | 0.982 | 0.946 | 0.967 | 23 | 1 | 1 | 0.040 | 0.038 | 0.047 | 0.040 | 5 | |
| | 2 | 0.980 | 0.980 | 0.943 | 0.967 | 23 | | 2 | 0.041 | 0.039 | 0.047 | 0.041 | 5 | |
| | 3 | 0.973 | 0.976 | 0.936 | 0.964 | 25 | | 3 | 0.041 | 0.038 | 0.048 | 0.040 | 5 | |
| | Mean | 0.979 | 0.979 | 0.942 | 0.966 | 24 | | Mean | 0.040 | 0.038 | 0.047 | 0.040 | 5 | Negative |
| 2 | 1 | 0.993 | 0.984 | 0.956 | 0.972 | 25 | 2 | 1 | 0.039 | 0.038 | 0.047 | 0.041 | 6 | |
| | 2 | 0.985 | 0.984 | 0.949 | 0.974 | 24 | | 2 | 0.040 | 0.040 | 0.048 | 0.041 | 6 | |
| | 3 | 0.982 | 0.982 | 0.946 | 0.970 | 24 | | 3 | 0.040 | 0.038 | 0.047 | 0.040 | 5 | |
| | Mean | 0.987 | 0.984 | 0.950 | 0.972 | 24 | | Mean | 0.040 | 0.039 | 0.047 | 0.041 | 6 | Negative |
| 3 | 1 | 0.982 | 0.987 | 0.942 | 0.978 | 31 | 3 | 1 | 0.039 | 0.038 | 0.046 | 0.040 | 5 | |
| | 2 | 0.979 | 0.976 | 0.939 | 0.965 | 31 | | 2 | 0.039 | 0.039 | 0.046 | 0.041 | 5 | |
| | 3 | 0.981 | 0.974 | 0.942 | 0.966 | 30 | | 3 | 0.040 | 0.039 | 0.047 | 0.041 | 5 | |
| | Mean | 0.981 | 0.979 | 0.941 | 0.970 | 31 | | Mean | 0.039 | 0.039 | 0.046 | 0.041 | 5 | Positive |
| Mean for 3 assays | | - | - | - | - | 26 | Mean for 3 assays | | - | - | - | - | 5 | Positive *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 | 0.992 | 0.996 | 0.864 | 0.982 | 113 | 1 | 1 | 0.040 | 0.038 | 0.083 | 0.040 | 40 | |
| | 2 | 0.993 | 1.001 | 0.869 | 0.987 | 109 | | 2 | 0.041 | 0.039 | 0.085 | 0.041 | 41 | |
| | 3 | 0.992 | 1.000 | 0.869 | 0.983 | 108 | | 3 | 0.040 | 0.038 | 0.087 | 0.040 | 44 | |
| | Mean | 0.992 | 0.999 | 0.867 | 0.984 | 110 | | Mean | 0.040 | 0.038 | 0.085 | 0.041 | 42 | Positive |
| 2 | 1 | 0.984 | 0.987 | 0.854 | 0.976 | 120 | 2 | 1 | 0.040 | 0.038 | 0.084 | 0.040 | 42 | |
| | 2 | 0.980 | 0.981 | 0.853 | 0.971 | 117 | | 2 | 0.041 | 0.039 | 0.086 | 0.041 | 43 | |
| | 3 | 0.974 | 0.978 | 0.850 | 0.968 | 114 | | 3 | 0.040 | 0.038 | 0.088 | 0.040 | 47 | |
| | Mean | 0.979 | 0.982 | 0.852 | 0.972 | 117 | | Mean | 0.040 | 0.038 | 0.086 | 0.040 | 44 | Positive |
| 3 | 1 | 0.980 | 0.982 | 0.850 | 0.970 | 120 | 3 | 1 | 0.039 | 0.038 | 0.084 | 0.041 | 42 | |
| | 2 | 0.982 | 0.987 | 0.856 | 0.980 | 116 | | 2 | 0.040 | 0.039 | 0.085 | 0.041 | 42 | |
| | 3 | 0.980 | 0.994 | 0.855 | 0.984 | 115 | | 3 | 0.040 | 0.037 | 0.087 | 0.040 | 44 | |
| | Mean | 0.981 | 0.988 | 0.854 | 0.978 | 117 | | Mean | 0.040 | 0.038 | 0.085 | 0.041 | 43 | Positive |
| Mean for 3 assays | | - | - | - | - | 115 | Mean for 3 assays | | - | - | - | - | 43 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.990 | 0.990 | 0.936 | 0.979 | 43 | 1 | 1 | 0.037 | 0.039 | 0.042 | 0.041 | 1 | |
| | 2 | 0.996 | 0.997 | 0.946 | 0.985 | 40 | | 2 | 0.039 | 0.039 | 0.043 | 0.041 | 1 | |
| | 3 | 1.001 | 0.995 | 0.950 | 0.985 | 40 | | 3 | 0.038 | 0.038 | 0.043 | 0.040 | 2 | |
| | Mean | 0.996 | 0.994 | 0.944 | 0.983 | 41 | | Mean | 0.038 | 0.038 | 0.042 | 0.041 | 1 | Positive |
| 2 | 1 | 0.993 | 0.994 | 0.942 | 0.985 | 42 | 2 | 1 | 0.037 | 0.038 | 0.041 | 0.040 | 2 | |
| | 2 | 0.976 | 0.978 | 0.926 | 0.969 | 41 | | 2 | 0.038 | 0.039 | 0.043 | 0.041 | 2 | |
| | 3 | 0.976 | 0.965 | 0.930 | 0.957 | 37 | | 3 | 0.038 | 0.038 | 0.042 | 0.040 | 2 | |
| | Mean | 0.982 | 0.979 | 0.933 | 0.970 | 40 | | Mean | 0.038 | 0.038 | 0.042 | 0.040 | 2 | Positive |
| 3 | 1 | 0.981 | 0.986 | 0.928 | 0.978 | 46 | 3 | 1 | 0.037 | 0.038 | 0.042 | 0.040 | 3 | |
| | 2 | 0.980 | 0.976 | 0.930 | 0.968 | 44 | | 2 | 0.038 | 0.039 | 0.041 | 0.041 | 2 | |
| | 3 | 0.980 | 0.973 | 0.930 | 0.967 | 43 | | 3 | 0.038 | 0.039 | 0.042 | 0.041 | 2 | |
| | Mean | 0.980 | 0.978 | 0.929 | 0.971 | 44 | | Mean | 0.038 | 0.039 | 0.042 | 0.041 | 2 | Positive |
| Mean for 3 assays | | - | - | - | - | 42 | Mean for 3 assays | | - | - | - | - | 2 | Positive *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 | 0.984 | 0.980 | 0.764 | 0.965 | 207 | 1 | 1 | 0.037 | 0.038 | 0.098 | 0.040 | 59 | |
| | 2 | 0.991 | 0.989 | 0.770 | 0.977 | 207 | | 2 | 0.038 | 0.039 | 0.094 | 0.041 | 54 | |
| | 3 | 0.996 | 0.988 | 0.785 | 0.974 | 197 | | 3 | 0.038 | 0.037 | 0.101 | 0.040 | 61 | |
| | Mean | 0.991 | 0.986 | 0.773 | 0.972 | 204 | | Mean | 0.038 | 0.038 | 0.098 | 0.040 | 58 | Positive |
| 2 | 1 | 0.984 | 0.988 | 0.770 | 0.975 | 201 | 2 | 1 | 0.037 | 0.038 | 0.090 | 0.041 | 50 | |
| | 2 | 0.989 | 0.993 | 0.780 | 0.981 | 196 | | 2 | 0.039 | 0.039 | 0.094 | 0.042 | 52 | |
| | 3 | 0.994 | 0.992 | 0.787 | 0.979 | 194 | | 3 | 0.038 | 0.038 | 0.096 | 0.040 | 55 | |
| | Mean | 0.989 | 0.991 | 0.779 | 0.978 | 197 | | Mean | 0.038 | 0.038 | 0.093 | 0.041 | 52 | Positive |
| 3 | 1 | 0.994 | 1.000 | 0.770 | 0.991 | 214 | 3 | 1 | 0.038 | 0.038 | 0.089 | 0.041 | 50 | |
| | 2 | 0.980 | 0.976 | 0.764 | 0.965 | 206 | | 2 | 0.038 | 0.040 | 0.091 | 0.041 | 51 | |
| | 3 | 0.979 | 0.969 | 0.763 | 0.959 | 206 | | 3 | 0.038 | 0.039 | 0.093 | 0.041 | 53 | |
| | Mean | 0.984 | 0.982 | 0.766 | 0.972 | 209 | | Mean | 0.038 | 0.039 | 0.091 | 0.041 | 51 | Positive |
| Mean for 3 assays | | - | - | - | - | 203 | Mean for 3 assays | | - | - | - | - | 54 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.989 | 0.990 | 0.943 | 0.979 | 35 | 1 | 1 | 0.037 | 0.039 | 0.202 | 0.041 | 162 | |
| | 2 | 0.998 | 0.997 | 0.951 | 0.985 | 36 | | 2 | 0.038 | 0.039 | 0.203 | 0.041 | 162 | |
| | 3 | 0.997 | 0.995 | 0.953 | 0.985 | 33 | | 3 | 0.038 | 0.038 | 0.204 | 0.040 | 163 | |
| | Mean | 0.995 | 0.994 | 0.949 | 0.983 | 35 | | Mean | 0.038 | 0.038 | 0.203 | 0.041 | 162 | Positive |
| 2 | 1 | 0.992 | 0.994 | 0.948 | 0.985 | 35 | 2 | 1 | 0.037 | 0.038 | 0.179 | 0.040 | 140 | |
| | 2 | 0.979 | 0.978 | 0.936 | 0.969 | 34 | | 2 | 0.039 | 0.039 | 0.169 | 0.041 | 129 | |
| | 3 | 0.970 | 0.965 | 0.928 | 0.957 | 33 | | 3 | 0.038 | 0.038 | 0.174 | 0.040 | 134 | |
| | Mean | 0.980 | 0.979 | 0.938 | 0.970 | 34 | | Mean | 0.038 | 0.038 | 0.174 | 0.040 | 134 | Positive |
| 3 | 1 | 0.987 | 0.986 | 0.941 | 0.978 | 39 | 3 | 1 | 0.037 | 0.038 | 0.178 | 0.040 | 139 | |
| | 2 | 0.979 | 0.976 | 0.934 | 0.968 | 39 | | 2 | 0.037 | 0.039 | 0.178 | 0.041 | 139 | |
| | 3 | 0.979 | 0.973 | 0.935 | 0.967 | 38 | | 3 | 0.038 | 0.039 | 0.186 | 0.041 | 146 | |
| | Mean | 0.982 | 0.978 | 0.936 | 0.971 | 39 | | Mean | 0.037 | 0.039 | 0.181 | 0.041 | 141 | Positive |
| Mean for 3 assays | | - | - | - | - | 36 | Mean for 3 assays | | - | - | - | - | 146 | Positive *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 | 0.979 | 0.980 | 0.855 | 0.965 | 109 | 1 | 1 | 0.037 | 0.038 | 0.483 | 0.040 | 444 | |
| | 2 | 0.989 | 0.989 | 0.853 | 0.977 | 123 | | 2 | 0.038 | 0.039 | 0.485 | 0.041 | 445 | |
| | 3 | 0.986 | 0.988 | 0.855 | 0.974 | 117 | | 3 | 0.038 | 0.037 | 0.470 | 0.040 | 430 | |
| | Mean | 0.985 | 0.986 | 0.854 | 0.972 | 116 | | Mean | 0.037 | 0.038 | 0.479 | 0.040 | 440 | Positive |
| 2 | 1 | 0.980 | 0.988 | 0.846 | 0.975 | 121 | 2 | 1 | 0.037 | 0.038 | 0.485 | 0.041 | 445 | |
| | 2 | 0.988 | 0.993 | 0.860 | 0.981 | 115 | | 2 | 0.040 | 0.039 | 0.501 | 0.042 | 458 | |
| | 3 | 0.986 | 0.992 | 0.856 | 0.979 | 117 | | 3 | 0.038 | 0.038 | 0.492 | 0.040 | 451 | |
| | Mean | 0.985 | 0.991 | 0.854 | 0.978 | 118 | | Mean | 0.038 | 0.038 | 0.492 | 0.041 | 451 | Positive |
| 3 | 1 | 0.994 | 1.000 | 0.854 | 0.991 | 129 | 3 | 1 | 0.038 | 0.038 | 0.484 | 0.041 | 444 | |
| | 2 | 0.975 | 0.976 | 0.842 | 0.965 | 123 | | 2 | 0.037 | 0.040 | 0.494 | 0.041 | 455 | |
| | 3 | 0.969 | 0.969 | 0.838 | 0.959 | 122 | | 3 | 0.039 | 0.039 | 0.472 | 0.041 | 431 | |
| | Mean | 0.979 | 0.982 | 0.844 | 0.972 | 125 | | Mean | 0.038 | 0.039 | 0.483 | 0.041 | 443 | Positive |
| Mean for 3 assays | | - | - | - | - | 120 | Mean for 3 assays | | - | - | - | - | 445 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.991 | 0.990 | 0.922 | 0.979 | 57 | 1 | 1 | 0.037 | 0.039 | 0.058 | 0.041 | 18 | |
| | 2 | 0.996 | 0.997 | 0.926 | 0.985 | 59 | | 2 | 0.039 | 0.039 | 0.060 | 0.041 | 18 | |
| | 3 | 0.996 | 0.995 | 0.927 | 0.985 | 58 | | 3 | 0.038 | 0.038 | 0.059 | 0.040 | 18 | |
| | Mean | 0.994 | 0.994 | 0.925 | 0.983 | 58 | | Mean | 0.038 | 0.038 | 0.059 | 0.041 | 18 | Positive |
| 2 | 1 | 0.994 | 0.994 | 0.932 | 0.985 | 53 | 2 | 1 | 0.037 | 0.038 | 0.054 | 0.040 | 15 | |
| | 2 | 0.976 | 0.978 | 0.913 | 0.969 | 54 | | 2 | 0.039 | 0.039 | 0.055 | 0.041 | 15 | |
| | 3 | 0.974 | 0.965 | 0.912 | 0.957 | 52 | | 3 | 0.038 | 0.038 | 0.055 | 0.040 | 15 | |
| | Mean | 0.981 | 0.979 | 0.919 | 0.970 | 53 | | Mean | 0.038 | 0.038 | 0.055 | 0.040 | 15 | Positive |
| 3 | 1 | 0.982 | 0.986 | 0.920 | 0.978 | 55 | 3 | 1 | 0.037 | 0.038 | 0.055 | 0.040 | 16 | |
| | 2 | 0.977 | 0.976 | 0.912 | 0.968 | 57 | | 2 | 0.037 | 0.039 | 0.055 | 0.041 | 16 | |
| | 3 | 0.974 | 0.973 | 0.912 | 0.967 | 55 | | 3 | 0.038 | 0.039 | 0.057 | 0.041 | 16 | |
| | Mean | 0.978 | 0.978 | 0.915 | 0.971 | 56 | | Mean | 0.038 | 0.039 | 0.056 | 0.041 | 16 | Positive |
| Mean for 3 assays | | - | - | - | - | 56 | Mean for 3 assays | | - | - | - | - | 16 | Positive *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 | 0.982 | 0.980 | 0.807 | 0.965 | 161 | 1 | 1 | 0.039 | 0.038 | 0.158 | 0.040 | 117 | |
| | 2 | 0.985 | 0.989 | 0.817 | 0.977 | 154 | | 2 | 0.038 | 0.039 | 0.161 | 0.041 | 120 | |
| | 3 | 0.988 | 0.988 | 0.823 | 0.974 | 150 | | 3 | 0.037 | 0.037 | 0.164 | 0.040 | 125 | |
| | Mean | 0.985 | 0.986 | 0.816 | 0.972 | 155 | | Mean | 0.038 | 0.038 | 0.161 | 0.040 | 121 | Positive |
| 2 | 1 | 0.980 | 0.988 | 0.818 | 0.975 | 149 | 2 | 1 | 0.037 | 0.038 | 0.154 | 0.041 | 114 | |
| | 2 | 0.983 | 0.993 | 0.829 | 0.981 | 141 | | 2 | 0.039 | 0.039 | 0.158 | 0.042 | 117 | |
| | 3 | 0.987 | 0.992 | 0.836 | 0.979 | 138 | | 3 | 0.038 | 0.038 | 0.161 | 0.040 | 120 | |
| | Mean | 0.983 | 0.991 | 0.828 | 0.978 | 143 | | Mean | 0.038 | 0.038 | 0.158 | 0.041 | 117 | Positive |
| 3 | 1 | 0.991 | 1.000 | 0.818 | 0.991 | 163 | 3 | 1 | 0.038 | 0.038 | 0.140 | 0.041 | 101 | |
| | 2 | 0.973 | 0.976 | 0.809 | 0.965 | 154 | | 2 | 0.037 | 0.040 | 0.140 | 0.041 | 100 | |
| | 3 | 0.969 | 0.969 | 0.809 | 0.959 | 149 | | 3 | 0.039 | 0.039 | 0.146 | 0.041 | 105 | |
| | Mean | 0.978 | 0.982 | 0.812 | 0.972 | 155 | | Mean | 0.038 | 0.039 | 0.142 | 0.041 | 102 | Positive |
| Mean for 3 assays | | - | - | - | - | 151 | Mean for 3 assays | | - | - | - | - | 113 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.989 | 0.990 | 0.930 | 0.979 | 48 | 1 | 1 | 0.038 | 0.039 | 0.064 | 0.041 | 22 | |
| | 2 | 0.982 | 0.997 | 0.924 | 0.985 | 48 | | 2 | 0.039 | 0.039 | 0.063 | 0.041 | 21 | |
| | 3 | 0.987 | 0.995 | 0.924 | 0.985 | 52 | | 3 | 0.037 | 0.038 | 0.063 | 0.040 | 23 | |
| | Mean | 0.986 | 0.994 | 0.926 | 0.983 | 49 | | Mean | 0.038 | 0.038 | 0.063 | 0.041 | 22 | Positive |
| 2 | 1 | 0.976 | 0.994 | 0.918 | 0.985 | 49 | 2 | 1 | 0.038 | 0.038 | 0.056 | 0.040 | 16 | |
| | 2 | 0.966 | 0.978 | 0.909 | 0.969 | 48 | | 2 | 0.038 | 0.039 | 0.054 | 0.041 | 14 | |
| | 3 | 0.962 | 0.965 | 0.908 | 0.957 | 45 | | 3 | 0.038 | 0.038 | 0.053 | 0.040 | 13 | |
| | Mean | 0.968 | 0.979 | 0.912 | 0.970 | 47 | | Mean | 0.038 | 0.038 | 0.054 | 0.040 | 14 | Positive |
| 3 | 1 | 0.981 | 0.986 | 0.916 | 0.978 | 58 | 3 | 1 | 0.038 | 0.038 | 0.059 | 0.040 | 20 | |
| | 2 | 0.974 | 0.976 | 0.912 | 0.968 | 55 | | 2 | 0.038 | 0.039 | 0.058 | 0.041 | 18 | |
| | 3 | 0.972 | 0.973 | 0.913 | 0.967 | 52 | | 3 | 0.038 | 0.039 | 0.059 | 0.041 | 19 | |
| | Mean | 0.975 | 0.978 | 0.914 | 0.971 | 55 | | Mean | 0.038 | 0.039 | 0.059 | 0.041 | 19 | Positive |
| Mean for 3 assays | | - | - | - | - | 50 | Mean for 3 assays | | - | - | - | - | 18 | Positive *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 | 0.966 | 0.980 | 0.990 | 0.965 | -38 | 1 | 1 | 0.038 | 0.038 | 0.178 | 0.040 | 138 | |
| | 2 | 0.969 | 0.989 | 0.989 | 0.977 | -34 | | 2 | 0.038 | 0.039 | 0.181 | 0.041 | 140 | |
| | 3 | 0.965 | 0.988 | 0.988 | 0.974 | -37 | | 3 | 0.038 | 0.037 | 0.185 | 0.040 | 145 | |
| | Mean | 0.967 | 0.986 | 0.989 | 0.972 | -36 | | Mean | 0.038 | 0.038 | 0.181 | 0.040 | 141 | Positive |
| 2 | 1 | 0.968 | 0.988 | 0.979 | 0.975 | -24 | 2 | 1 | 0.039 | 0.038 | 0.170 | 0.041 | 128 | |
| | 2 | 0.969 | 0.993 | 0.988 | 0.981 | -33 | | 2 | 0.041 | 0.039 | 0.174 | 0.042 | 130 | |
| | 3 | 0.966 | 0.992 | 0.987 | 0.979 | -34 | | 3 | 0.038 | 0.038 | 0.177 | 0.040 | 136 | |
| | Mean | 0.967 | 0.991 | 0.985 | 0.978 | -30 | | Mean | 0.039 | 0.038 | 0.174 | 0.041 | 131 | Positive |
| 3 | 1 | 0.975 | 1.000 | 1.025 | 0.991 | -60 | 3 | 1 | 0.039 | 0.038 | 0.183 | 0.041 | 143 | |
| | 2 | 0.958 | 0.976 | 1.005 | 0.965 | -58 | | 2 | 0.039 | 0.040 | 0.184 | 0.041 | 144 | |
| | 3 | 0.954 | 0.969 | 1.003 | 0.959 | -60 | | 3 | 0.039 | 0.039 | 0.185 | 0.041 | 144 | |
| | Mean | 0.962 | 0.982 | 1.011 | 0.972 | -59 | | Mean | 0.039 | 0.039 | 0.184 | 0.041 | 144 | Positive |
| Mean for 3 assays | | - | - | - | - | -42 | Mean for 3 assays | | - | - | - | - | 139 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
Chemical Name : Quinine

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 | 0.990 | 0.904 | 0.979 | 72 | 1 | 1 | 0.038 | 0.039 | 0.075 | 0.041 | 34 | |
| | 2 | 0.999 | 0.997 | 0.910 | 0.985 | 78 | | 2 | 0.038 | 0.039 | 0.075 | 0.041 | 34 | |
| | 3 | 0.993 | 0.995 | 0.909 | 0.985 | 73 | | 3 | 0.038 | 0.038 | 0.075 | 0.040 | 34 | |
| | Mean | 0.993 | 0.994 | 0.908 | 0.983 | 74 | | Mean | 0.038 | 0.038 | 0.075 | 0.041 | 34 | Positive |
| 2 | 1 | 0.985 | 0.994 | 0.890 | 0.985 | 86 | 2 | 1 | 0.037 | 0.038 | 0.067 | 0.040 | 28 | |
| | 2 | 0.976 | 0.978 | 0.882 | 0.969 | 84 | | 2 | 0.039 | 0.039 | 0.066 | 0.041 | 26 | |
| | 3 | 0.965 | 0.965 | 0.883 | 0.957 | 74 | | 3 | 0.038 | 0.038 | 0.065 | 0.040 | 25 | |
| | Mean | 0.975 | 0.979 | 0.885 | 0.970 | 81 | | Mean | 0.038 | 0.038 | 0.066 | 0.040 | 26 | Positive |
| 3 | 1 | 0.977 | 0.986 | 0.885 | 0.978 | 85 | 3 | 1 | 0.037 | 0.038 | 0.071 | 0.040 | 32 | |
| | 2 | 0.968 | 0.976 | 0.878 | 0.968 | 84 | | 2 | 0.037 | 0.039 | 0.070 | 0.041 | 31 | |
| | 3 | 0.971 | 0.973 | 0.882 | 0.967 | 82 | | 3 | 0.038 | 0.039 | 0.071 | 0.041 | 31 | |
| | Mean | 0.972 | 0.978 | 0.881 | 0.971 | 84 | | Mean | 0.038 | 0.039 | 0.071 | 0.041 | 31 | Positive |
| Mean for 3 assays | | - | - | - | - | 80 | Mean for 3 assays | | - | - | - | - | 30 | Positive *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 | 0.968 | 0.980 | 0.605 | 0.965 | 349 | 1 | 1 | 0.037 | 0.038 | 0.247 | 0.040 | 209 | |
| | 2 | 0.982 | 0.989 | 0.621 | 0.977 | 347 | | 2 | 0.038 | 0.039 | 0.253 | 0.041 | 213 | |
| | 3 | 0.976 | 0.988 | 0.619 | 0.974 | 343 | | 3 | 0.037 | 0.037 | 0.262 | 0.040 | 223 | |
| | Mean | 0.975 | 0.986 | 0.615 | 0.972 | 346 | | Mean | 0.037 | 0.038 | 0.254 | 0.040 | 215 | Positive |
| 2 | 1 | 0.976 | 0.988 | 0.632 | 0.975 | 331 | 2 | 1 | 0.037 | 0.038 | 0.246 | 0.041 | 206 | |
| | 2 | 0.985 | 0.993 | 0.638 | 0.981 | 334 | | 2 | 0.039 | 0.039 | 0.250 | 0.042 | 208 | |
| | 3 | 0.975 | 0.992 | 0.616 | 0.979 | 346 | | 3 | 0.038 | 0.038 | 0.255 | 0.040 | 214 | |
| | Mean | 0.978 | 0.991 | 0.628 | 0.978 | 337 | | Mean | 0.038 | 0.038 | 0.250 | 0.041 | 209 | Positive |
| 3 | 1 | 0.982 | 1.000 | 0.616 | 0.991 | 355 | 3 | 1 | 0.037 | 0.038 | 0.232 | 0.041 | 192 | |
| | 2 | 0.967 | 0.976 | 0.612 | 0.965 | 345 | | 2 | 0.038 | 0.040 | 0.232 | 0.041 | 192 | |
| | 3 | 0.965 | 0.969 | 0.620 | 0.959 | 335 | | 3 | 0.038 | 0.039 | 0.242 | 0.041 | 202 | |
| | Mean | 0.971 | 0.982 | 0.616 | 0.972 | 345 | | Mean | 0.038 | 0.039 | 0.235 | 0.041 | 195 | Positive |
| Mean for 3 assays | | - | - | - | - | 343 | Mean for 3 assays | | - | - | - | - | 206 | Positive *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

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 study

Laboratory : 3
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 | 0.990 | 0.990 | 0.984 | 0.979 | -5 | 1 | 1 | 0.039 | 0.039 | 0.041 | 0.041 | -2 | |
| | 2 | 0.995 | 0.997 | 0.984 | 0.985 | 0 | | 2 | 0.039 | 0.039 | 0.041 | 0.041 | -2 | |
| | 3 | 0.993 | 0.995 | 0.985 | 0.985 | -3 | | 3 | 0.039 | 0.038 | 0.040 | 0.040 | -2 | |
| | Mean | 0.993 | 0.994 | 0.984 | 0.983 | -3 | | Mean | 0.039 | 0.038 | 0.040 | 0.041 | -2 | Negative |
| 2 | 1 | 0.991 | 0.994 | 0.985 | 0.985 | -3 | 2 | 1 | 0.039 | 0.038 | 0.040 | 0.040 | -1 | |
| | 2 | 0.978 | 0.978 | 0.971 | 0.969 | -1 | | 2 | 0.040 | 0.039 | 0.040 | 0.041 | -1 | |
| | 3 | 0.969 | 0.965 | 0.960 | 0.957 | -1 | | 3 | 0.039 | 0.038 | 0.040 | 0.040 | -1 | |
| | Mean | 0.979 | 0.979 | 0.972 | 0.970 | -2 | | Mean | 0.039 | 0.038 | 0.040 | 0.040 | -1 | Negative |
| 3 | 1 | 0.988 | 0.986 | 0.979 | 0.978 | 2 | 3 | 1 | 0.038 | 0.038 | 0.040 | 0.040 | -1 | |
| | 2 | 0.983 | 0.976 | 0.974 | 0.968 | 2 | | 2 | 0.038 | 0.039 | 0.040 | 0.041 | -1 | |
| | 3 | 0.976 | 0.973 | 0.966 | 0.967 | 3 | | 3 | 0.040 | 0.039 | 0.042 | 0.041 | 0 | |
| | Mean | 0.982 | 0.978 | 0.973 | 0.971 | 2 | | Mean | 0.039 | 0.039 | 0.040 | 0.041 | -1 | Negative |
| Mean for 3 assays | | - | - | - | - | -1 | Mean for 3 assays | | - | - | - | - | -1 | Negative *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 | 0.982 | 0.980 | 0.977 | 0.965 | -9 | 1 | 1 | 0.040 | 0.038 | 0.040 | 0.040 | -1 | |
| | 2 | 0.984 | 0.989 | 0.979 | 0.977 | -9 | | 2 | 0.040 | 0.039 | 0.041 | 0.041 | -1 | |
| | 3 | 0.988 | 0.988 | 0.983 | 0.974 | -9 | | 3 | 0.039 | 0.037 | 0.040 | 0.040 | -1 | |
| | Mean | 0.985 | 0.986 | 0.980 | 0.972 | -9 | | Mean | 0.039 | 0.038 | 0.040 | 0.040 | -1 | Negative |
| 2 | 1 | 0.983 | 0.988 | 0.974 | 0.975 | -4 | 2 | 1 | 0.040 | 0.038 | 0.040 | 0.041 | -3 | |
| | 2 | 0.988 | 0.993 | 0.978 | 0.981 | -3 | | 2 | 0.040 | 0.039 | 0.040 | 0.042 | -3 | |
| | 3 | 0.986 | 0.992 | 0.978 | 0.979 | -4 | | 3 | 0.040 | 0.038 | 0.040 | 0.040 | -3 | |
| | Mean | 0.986 | 0.991 | 0.977 | 0.978 | -4 | | Mean | 0.040 | 0.038 | 0.040 | 0.041 | -3 | Negative |
| 3 | 1 | 1.000 | 1.000 | 0.995 | 0.991 | -4 | 3 | 1 | 0.039 | 0.038 | 0.039 | 0.041 | -2 | |
| | 2 | 0.985 | 0.976 | 0.979 | 0.965 | -4 | | 2 | 0.041 | 0.040 | 0.039 | 0.041 | -4 | |
| | 3 | 0.976 | 0.969 | 0.969 | 0.959 | -4 | | 3 | 0.041 | 0.039 | 0.040 | 0.041 | -2 | |
| | Mean | 0.987 | 0.982 | 0.981 | 0.972 | -4 | | Mean | 0.040 | 0.039 | 0.040 | 0.041 | -3 | Negative |
| Mean for 3 assays | | - | - | - | - | -6 | Mean for 3 assays | | - | - | - | - | -2 | Negative *3 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20
Negative : Singlet oxygen results <25 and Superoxide anion results <25

## Appendix 5 Irradiance and temperature during the irradiation in the Phase 2 study

Laboratory 1

| Experimental No. (US-) | | | 001 | 002 | 003 | 004 | 005 | 006 | 007 | 023 | 024 | 025 | 026 | 027 | 028 | 029 | 030 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UVA intensity ($mW/cm^2$) | Beginning of Irradiation | A | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| | | B | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 |
| | End of Irradiation | A | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| | | B | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 |
| Temperature (°C) | Beginning of Irradiation | | 29 | 27 | 28 | 29 | 27 | 27 | 27 | 24 | 26 | 26 | 27 | 27 | 26 | 27 | 25 |
| | End of Irradiation | | 27 | 28 | 28 | 28 | 28 | 29 | 28 | 25 | 26 | 26 | 25 | 26 | 26 | 26 | 25 |

| Experimental No. (US-) | | | 031 | 032 | 033 | 034 | 035 | 036 |
|---|---|---|---|---|---|---|---|---|
| UVA intensity ($mW/cm^2$) | Beginning of Irradiation | A | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| | | B | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 |
| | End of Irradiation | A | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| | | B | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 |
| Temperature (°C) | Beginning of Irradiation | | 25 | 25 | 27 | 24 | 24 | 26 |
| | End of Irradiation | | 24 | 25 | 26 | 24 | 24 | 24 |

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.Hőnle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the value from the UVA detectors.
B=A × f f : correlation factor on the values of the UVA detectors (= 1.18)
Thermometer : Suntest CPS+ (Atlas)

Appendix 5 Irradiance and temperature during the irradiation in the Phase 2 study

Laboratory 2

| Experimental No. (FDSC-) | | | 001 | 002 | 003 | 004 | 005 | 006 | 007 | 008 | 009 | 010 | 011 | 012 | 013 | 014 | 015 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UVA intensity ($mW/cm^2$) | Beginning of Irradiation | A | 1.435 | 1.439 | 1.437 | 1.439 | 1.437 | 1.437 | 1.436 | 1.441 | 1.442 | 1.437 | 1.424 | 1.428 | 1.420 | 1.422 | 1.426 |
| | | B | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | 2.0 | 2.0 | 1.9 | 1.8 | 1.9 | 1.8 | 1.8 | 1.9 |
| | End of Irradiation | A | 1.437 | 1.427 | 1.431 | 1.450 | 1.440 | 1.431 | 1.423 | 1.434 | 1.436 | 1.434 | 1.429 | 1.433 | 1.433 | 1.426 | 1.424 |
| | | B | 1.9 | 1.9 | 1.9 | 2.0 | 1.9 | 1.9 | 1.8 | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | 1.9 | 1.8 |
| Temperature (°C) | Beginning of Irradiation | | 22.8 | 24.9 | 24.1 | 24.1 | 24.8 | 24.6 | 23.6 | 24.7 | 23.7 | 24.4 | 24.6 | 24.0 | 25.0 | 24.9 | 24.9 |
| | End of Irradiation | | 25.0 | 25.0 | 24.9 | 24.9 | 25.0 | 25.2 | 24.9 | 24.9 | 25.0 | 24.9 | 25.2 | 25.1 | 25.1 | 25.0 | 25.2 |

| Experimental No. (FDSC-) | | | 016 | 017 | 018 | 019 | 020 | 021 | 022 | 023 | 024 | 025 | 026 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UVA intensity ($mW/cm^2$) | Beginning of Irradiation | A | 1.426 | 1.422 | 1.434 | 1.432 | 1.435 | 1.419 | 1.424 | 1.426 | 1.439 | 1.434 | 1.440 |
| | | B | 1.9 | 1.8 | 1.9 | 1.9 | 1.9 | 1.8 | 1.8 | 1.9 | 1.9 | 1.9 | 1.9 |
| | End of Irradiation | A | 1.423 | 1.424 | 1.446 | 1.437 | 1.438 | 1.426 | 1.436 | 1.424 | 1.423 | 1.436 | 1.442 |
| | | B | 1.8 | 1.8 | 2.0 | 1.9 | 1.9 | 1.9 | 1.9 | 1.8 | 1.8 | 1.9 | 2.0 |
| Temperature (°C) | Beginning of Irradiation | | 24.9 | 25.4 | 24.4 | 24.8 | 24.9 | 24.5 | 24.8 | 24.9 | 24.8 | 24.8 | 24.3 |
| | End of Irradiation | | 25.0 | 25.2 | 24.8 | 24.8 | 24.9 | 24.9 | 24.9 | 25.1 | 25.1 | 25.0 | 24.9 |

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 value from the UVA detectors.
B=A × f - 7.3943 f : correlation factor on the values of the UVA detectors (=6.4855)
Thermometer : Thermo Recorder RTR-52 (TANDD)

## Appendix 5 Irradiance and temperature during the irradiation in the Phase 2 study

Laboratory 3

| Experimental No. (MT-) | | | 001 | 002 | 003 | 004 | 005 | 006 | 007 | 008 | 009 | 010 | 011 | 012 | 013 | 014 | 015 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UVA intensity ($mW/cm^2$) | Beginning of Irradiation | A | 1.0 | 1.1 | 1.1 | 1.2 | 1.1 | 1.2 | 1.1 | 1.2 | 1.1 | 1.1 | 1.2 | 1.2 | 1.2 | 1.1 | 1.2 |
| | | B | 1.8 | 2.0 | 2.0 | 2.2 | 2.0 | 2.2 | 2.0 | 2.2 | 2.0 | 2.0 | 2.2 | 2.2 | 2.2 | 2.0 | 2.2 |
| | End of Irradiation | A | 1.2 | 1.1 | 1.1 | 1.1 | 1.2 | 1.2 | 1.2 | 1.1 | 1.1 | 1.1 | 1.2 | 1.2 | 1.2 | 1.2 | 1.1 |
| | | B | 2.2 | 2.0 | 2.0 | 2.0 | 2.2 | 2.2 | 2.2 | 2.0 | 2.0 | 2.0 | 2.2 | 2.2 | 2.2 | 2.2 | 2.0 |
| Temperature (°C) | Beginning of Irradiation | | 25.4 | 24.6 | 23.8 | 25.3 | 24.6 | 24.0 | 25.5 | 27.6 | 21.9 | 24.7 | 23.3 | 23.5 | 23.0 | 24.5 | 23.6 |
| | End of Irradiation | | 28,3 | 23.8 | 23,7 | 24.6 | 24.0 | 24.2 | 28.3 | 26.4 | 26.6 | 23.6 | 23.5 | 23.0 | 23.3 | 23.8 | 24.6 |

| Experimental No. (MT-) | | | 016 | 018 | 019 | 020 | 021 | 022 | 023 |
|---|---|---|---|---|---|---|---|---|---|
| UVA intensity ($mW/cm^2$) | Beginning of Irradiation | A | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.1 | 1.1 |
| | | B | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.0 | 2.0 |
| | End of Irradiation | A | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.1 | 1.1 |
| | | B | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.0 | 2.0 |
| Temperature (°C) | Beginning of Irradiation | | 25.0 | 25.5 | 24.3 | 25.1 | 26.5 | 24.4 | 24.6 |
| | End of Irradiation | | 26.2 | 26.3 | 25.1 | 26.5 | 25.9 | 26.0 | 25.8 |

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.Hőnle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the value from the UVA detectors.
$B=A \times f$ f : correlation factor on the values of the UVA detectors (= 1.8)
Thermometer : ondotori Jr. TR-51i (TANDD)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 1

Chemical Name : Qunine 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 | |
| US-001 | 1 | 1.023 | 1.014 | 0.481 | 1.006 | 531 | US-001 | 1 | 0.049 | 0.033 | 0.463 | 0.046 | 400 |
| | 2 | 1.029 | 1.028 | 0.506 | 1.014 | 512 | | 2 | 0.042 | 0.046 | 0.461 | 0.053 | 405 |
| | 3 | 1.036 | 1.029 | 0.525 | 1.020 | 501 | | 3 | 0.047 | 0.034 | 0.465 | 0.056 | 405 |
| | Mean | 1.029 | 1.024 | 0.504 | 1.013 | 515 | | Mean | 0.046 | 0.038 | 0.463 | 0.052 | 403 |
| US-002 | 1 | 1.013 | 1.017 | 0.470 | 1.007 | 534 | US-002 | 1 | 0.114 | 0.031 | 0.457 | 0.038 | 336 |
| | 2 | 1.040 | 1.028 | 0.504 | 1.021 | 527 | | 2 | 0.110 | 0.043 | 0.453 | 0.041 | 336 |
| | 3 | 1.037 | 1.031 | 0.516 | 1.020 | 512 | | 3 | 0.115 | 0.030 | 0.457 | 0.048 | 335 |
| | Mean | 1.030 | 1.025 | 0.497 | 1.016 | 524 | | Mean | 0.113 | 0.035 | 0.455 | 0.042 | 336 |
| US-003 | 1 | 1.018 | 1.011 | 0.459 | 1.001 | 549 | US-003 | 1 | 0.046 | 0.029 | 0.454 | 0.049 | 385 |
| | 2 | 1.032 | 1.028 | 0.487 | 1.017 | 535 | | 2 | 0.042 | 0.044 | 0.448 | 0.052 | 383 |
| | 3 | 1.035 | 1.024 | 0.493 | 1.016 | 532 | | 3 | 0.046 | 0.030 | 0.471 | 0.070 | 402 |
| | Mean | 1.028 | 1.021 | 0.480 | 1.011 | 539 | | Mean | 0.044 | 0.034 | 0.457 | 0.057 | 390 |
| US-004 | 1 | 0.996 | 0.998 | 0.441 | 0.987 | 545 | US-004 | 1 | 0.052 | 0.029 | 0.370 | 0.046 | 293 |
| | 2 | 1.028 | 1.027 | 0.481 | 1.019 | 537 | | 2 | 0.045 | 0.029 | 0.381 | 0.059 | 313 |
| | 3 | 1.029 | 1.021 | 0.491 | 1.010 | 528 | | 3 | 0.066 | 0.029 | 0.378 | 0.054 | 288 |
| | Mean | 1.018 | 1.015 | 0.471 | 1.005 | 537 | | Mean | 0.054 | 0.029 | 0.376 | 0.053 | 298 |
| US-005 | 1 | 1.006 | 1.015 | 0.454 | 1.004 | 541 | US-005 | 1 | 0.040 | 0.029 | 0.470 | 0.043 | 419 |
| | 2 | 1.011 | 1.012 | 0.477 | 1.002 | 523 | | 2 | 0.036 | 0.043 | 0.470 | 0.044 | 423 |
| | 3 | 1.015 | 1.016 | 0.492 | 1.007 | 511 | | 3 | 0.041 | 0.029 | 0.464 | 0.046 | 412 |
| | Mean | 1.011 | 1.015 | 0.474 | 1.004 | 525 | | Mean | 0.039 | 0.033 | 0.468 | 0.044 | 418 |
| US-006 | 1 | 1.002 | 1.020 | 0.438 | 1.010 | 553 | US-006 | 1 | 0.063 | 0.031 | 0.462 | 0.044 | 376 |
| | 2 | 1.016 | 1.026 | 0.465 | 1.011 | 540 | | 2 | 0.047 | 0.031 | 0.466 | 0.048 | 396 |
| | 3 | 1.017 | 1.017 | 0.477 | 1.008 | 529 | | 3 | 0.066 | 0.031 | 0.461 | 0.069 | 373 |
| | Mean | 1.012 | 1.021 | 0.460 | 1.010 | 541 | | Mean | 0.058 | 0.031 | 0.463 | 0.054 | 382 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 1

Chemical Name : Qunine 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 | |
| US-007 | 1 | 1.017 | 1.006 | 0.439 | 0.991 | 567 | US-007 | 1 | 0.062 | 0.035 | 0.467 | 0.046 | 390 |
| | 2 | 1.015 | 1.015 | 0.462 | 1.005 | 541 | | 2 | 0.040 | 0.034 | 0.458 | 0.054 | 403 |
| | 3 | 1.031 | 1.010 | 0.478 | 1.002 | 542 | | 3 | 0.062 | 0.033 | 0.455 | 0.049 | 378 |
| | Mean | 1.021 | 1.010 | 0.460 | 0.999 | 550 | | Mean | 0.055 | 0.034 | 0.460 | 0.049 | 390 |
| US-023 | 1 | 0.998 | 1.008 | 0.430 | 0.991 | 554 | US-023 | 1 | 0.037 | 0.033 | 0.458 | 0.039 | 414 |
| | 2 | 1.005 | 1.024 | 0.458 | 1.013 | 533 | | 2 | 0.033 | 0.049 | 0.451 | 0.044 | 411 |
| | 3 | 1.003 | 1.022 | 0.472 | 1.008 | 517 | | 3 | 0.039 | 0.029 | 0.463 | 0.051 | 418 |
| | Mean | 1.002 | 1.018 | 0.453 | 1.004 | 535 | | Mean | 0.036 | 0.037 | 0.457 | 0.044 | 414 |
| US-024 | 1 | 1.001 | 1.014 | 0.411 | 1.003 | 576 | US-024 | 1 | 0.038 | 0.030 | 0.464 | 0.045 | 410 |
| | 2 | 1.011 | 1.023 | 0.439 | 1.009 | 558 | | 2 | 0.033 | 0.040 | 0.477 | 0.052 | 428 |
| | 3 | 1.013 | 1.023 | 0.452 | 1.006 | 546 | | 3 | 0.038 | 0.029 | 0.466 | 0.051 | 412 |
| | Mean | 1.008 | 1.020 | 0.434 | 1.006 | 560 | | Mean | 0.036 | 0.033 | 0.469 | 0.049 | 417 |
| US-025 | 1 | 0.992 | 0.990 | 0.411 | 0.969 | 559 | US-025 | 1 | 0.039 | 0.029 | 0.469 | 0.046 | 409 |
| | 2 | 1.012 | 1.013 | 0.439 | 0.988 | 551 | | 2 | 0.034 | 0.047 | 0.457 | 0.047 | 402 |
| | 3 | 1.006 | 1.011 | 0.455 | 0.993 | 530 | | 3 | 0.037 | 0.030 | 0.473 | 0.073 | 415 |
| | Mean | 1.003 | 1.005 | 0.435 | 0.983 | 547 | | Mean | 0.037 | 0.035 | 0.466 | 0.056 | 409 |
| US-026 | 1 | 0.988 | 0.973 | 0.410 | 0.965 | 570 | US-026 | 1 | 0.036 | 0.032 | 0.470 | 0.040 | 422 |
| | 2 | 0.987 | 0.982 | 0.433 | 0.977 | 546 | | 2 | 0.033 | 0.038 | 0.473 | 0.049 | 428 |
| | 3 | 0.994 | 0.986 | 0.440 | 0.976 | 546 | | 3 | 0.039 | 0.029 | 0.630 | 0.046 | 579 |
| | Mean | 0.990 | 0.981 | 0.427 | 0.973 | 554 | | Mean | 0.036 | 0.033 | 0.524 | 0.045 | 476 |
| US-027 | 1 | 0.967 | 0.970 | 0.375 | 0.965 | 584 | US-027 | 1 | 0.058 | 0.033 | 0.466 | 0.040 | 395 |
| | 2 | 0.997 | 0.979 | 0.417 | 0.970 | 572 | | 2 | 0.041 | 0.030 | 0.468 | 0.047 | 413 |
| | 3 | 1.008 | 0.978 | 0.431 | 0.969 | 569 | | 3 | 0.061 | 0.029 | 0.465 | 0.046 | 391 |
| | Mean | 0.991 | 0.976 | 0.408 | 0.968 | 575 | | Mean | 0.053 | 0.030 | 0.466 | 0.044 | 400 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 1

Chemical Name : Qunine 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 | |
| US-028 | 1 | 0.962 | 0.980 | 0.397 | 0.971 | 554 | US-028 | 1 | 0.052 | 0.029 | 0.469 | 0.051 | 391 |
| | 2 | 0.977 | 0.988 | 0.428 | 0.977 | 538 | | 2 | 0.041 | 0.030 | 0.465 | 0.053 | 399 |
| | 3 | 0.974 | 0.995 | 0.448 | 0.983 | 515 | | 3 | 0.063 | 0.028 | 0.477 | 0.061 | 389 |
| | Mean | 0.971 | 0.988 | 0.424 | 0.977 | 536 | | Mean | 0.052 | 0.029 | 0.471 | 0.055 | 393 |
| US-029 | 1 | 0.947 | 0.973 | 0.370 | 0.960 | 567 | US-029 | 1 | 0.040 | 0.030 | 0.497 | 0.038 | 449 |
| | 2 | 0.988 | 0.986 | 0.413 | 0.979 | 565 | | 2 | 0.036 | 0.044 | 0.506 | 0.044 | 461 |
| | 3 | 0.990 | 0.987 | 0.429 | 0.978 | 551 | | 3 | 0.041 | 0.029 | 0.511 | 0.047 | 462 |
| | Mean | 0.975 | 0.982 | 0.404 | 0.972 | 561 | | Mean | 0.039 | 0.034 | 0.505 | 0.043 | 457 |
| US-030 | 1 | 0.978 | 0.997 | 0.422 | 1.002 | 556 | US-030 | 1 | 0.045 | 0.029 | 0.474 | 0.047 | 408 |
| | 2 | 0.985 | 1.003 | 0.444 | 0.996 | 542 | | 2 | 0.034 | 0.043 | 0.462 | 0.051 | 407 |
| | 3 | 0.982 | 1.001 | 0.455 | 1.005 | 528 | | 3 | 0.039 | 0.030 | 0.463 | 0.066 | 403 |
| | Mean | 0.982 | 1.000 | 0.441 | 1.001 | 542 | | Mean | 0.039 | 0.034 | 0.466 | 0.055 | 406 |
| US-031 | 1 | 0.976 | 0.986 | 0.416 | 0.973 | 551 | US-031 | 1 | 0.042 | 0.029 | 0.449 | 0.042 | 394 |
| | 2 | 0.987 | 0.985 | 0.442 | 0.979 | 535 | | 2 | 0.035 | 0.042 | 0.462 | 0.046 | 414 |
| | 3 | 0.991 | 0.995 | 0.455 | 0.985 | 525 | | 3 | 0.041 | 0.029 | 0.459 | 0.049 | 405 |
| | Mean | 0.984 | 0.989 | 0.438 | 0.979 | 537 | | Mean | 0.039 | 0.033 | 0.457 | 0.046 | 404 |
| US-032 | 1 | 0.964 | 0.987 | 0.413 | 0.976 | 539 | US-032 | 1 | 0.037 | 0.028 | 0.468 | 0.045 | 411 |
| | 2 | 0.987 | 0.988 | 0.442 | 0.975 | 533 | | 2 | 0.035 | 0.045 | 0.465 | 0.045 | 410 |
| | 3 | 0.994 | 1.007 | 0.462 | 0.995 | 520 | | 3 | 0.038 | 0.028 | 0.467 | 0.072 | 409 |
| | Mean | 0.982 | 0.994 | 0.439 | 0.982 | 531 | | Mean | 0.037 | 0.034 | 0.467 | 0.054 | 410 |
| US-033 | 1 | 0.971 | 0.982 | 0.423 | 0.972 | 536 | US-033 | 1 | 0.061 | 0.033 | 0.459 | 0.041 | 379 |
| | 2 | 0.979 | 0.989 | 0.447 | 0.975 | 520 | | 2 | 0.041 | 0.034 | 0.466 | 0.051 | 405 |
| | 3 | 0.993 | 0.987 | 0.457 | 0.975 | 524 | | 3 | 0.064 | 0.028 | 0.482 | 0.064 | 398 |
| | Mean | 0.981 | 0.986 | 0.442 | 0.974 | 527 | | Mean | 0.055 | 0.032 | 0.469 | 0.052 | 394 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 1

Chemical Name : Qunine 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 | |
| US-034 | 1 | 0.964 | 0.974 | 0.399 | 0.965 | 556 | US-034 | 1 | 0.037 | 0.036 | 0.468 | 0.049 | 407 |
| | 2 | 0.979 | 1.001 | 0.422 | 0.992 | 548 | | 2 | 0.033 | 0.042 | 0.449 | 0.045 | 392 |
| | 3 | 0.985 | 0.994 | 0.451 | 0.985 | 525 | | 3 | 0.038 | 0.029 | 0.454 | 0.085 | 392 |
| | Mean | 0.976 | 0.990 | 0.424 | 0.981 | 543 | | Mean | 0.036 | 0.036 | 0.457 | 0.060 | 397 |
| US-035 | 1 | 0.984 | 0.982 | 0.424 | 0.975 | 552 | US-035 | 1 | 0.058 | 0.034 | 0.461 | 0.045 | 380 |
| | 2 | 0.985 | 0.985 | 0.449 | 0.974 | 527 | | 2 | 0.039 | 0.030 | 0.485 | 0.048 | 424 |
| | 3 | 0.993 | 0.985 | 0.459 | 0.976 | 525 | | 3 | 0.059 | 0.028 | 0.459 | 0.066 | 378 |
| | Mean | 0.987 | 0.984 | 0.444 | 0.975 | 535 | | Mean | 0.052 | 0.031 | 0.468 | 0.053 | 394 |
| US-036 | 1 | 0.984 | 0.977 | 0.414 | 0.965 | 560 | US-036 | 1 | 0.058 | 0.028 | 0.436 | 0.043 | 354 |
| | 2 | 0.978 | 0.990 | 0.441 | 0.984 | 527 | | 2 | 0.038 | 0.032 | 0.460 | 0.048 | 398 |
| | 3 | 0.986 | 0.988 | 0.450 | 0.977 | 526 | | 3 | 0.069 | 0.029 | 0.479 | 0.072 | 386 |
| | Mean | 0.983 | 0.985 | 0.435 | 0.975 | 538 | | Mean | 0.055 | 0.030 | 0.459 | 0.054 | 379 |
| Mean for all assays | | - | - | - | - | 541 | Mean for all assays | | - | - | - | - | 398 |
| SD for all assays | | - | - | - | - | 14 | SD for all assays | | - | - | - | - | 36 |
| CV for all assays | | - | - | - | - | 2.6 | CV for all assays | | - | - | - | - | 9.0 |

*1 : decrease of A440 $x10^3$ = (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 exposure)

*2 : increase of A560 $x10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 1
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 | |
| US-001 | 1 | 1.015 | 1.014 | 1.006 | 1.006 | -2 | US-001 | 1 | 0.044 | 0.033 | 0.044 | 0.046 | -14 |
| | 2 | 1.020 | 1.028 | 1.014 | 1.014 | -5 | | 2 | 0.043 | 0.046 | 0.045 | 0.053 | -12 |
| | 3 | 1.009 | 1.029 | 1.002 | 1.020 | -4 | | 3 | 0.041 | 0.034 | 0.044 | 0.056 | -12 |
| | Mean | 1.015 | 1.024 | 1.007 | 1.013 | -4 | | Mean | 0.043 | 0.038 | 0.044 | 0.052 | -13 |
| US-002 | 1 | 1.069 | 1.017 | 1.058 | 1.007 | 2 | US-002 | 1 | 0.044 | 0.031 | 0.045 | 0.038 | -6 |
| | 2 | 1.051 | 1.028 | 1.037 | 1.021 | 5 | | 2 | 0.042 | 0.043 | 0.045 | 0.041 | -4 |
| | 3 | 1.062 | 1.031 | 1.048 | 1.020 | 5 | | 3 | 0.044 | 0.030 | 0.045 | 0.048 | -6 |
| | Mean | 1.061 | 1.025 | 1.048 | 1.016 | 4 | | Mean | 0.043 | 0.035 | 0.045 | 0.042 | -5 |
| US-003 | 1 | 1.037 | 1.011 | 1.012 | 1.001 | 15 | US-003 | 1 | 0.045 | 0.029 | 0.044 | 0.049 | -24 |
| | 2 | 1.044 | 1.028 | 1.021 | 1.017 | 14 | | 2 | 0.042 | 0.044 | 0.046 | 0.052 | -19 |
| | 3 | 1.049 | 1.024 | 1.021 | 1.016 | 18 | | 3 | 0.046 | 0.030 | 0.045 | 0.070 | -24 |
| | Mean | 1.043 | 1.021 | 1.018 | 1.011 | 16 | | Mean | 0.044 | 0.034 | 0.045 | 0.057 | -22 |
| US-004 | 1 | 1.016 | 0.998 | 1.005 | 0.987 | 1 | US-004 | 1 | 0.038 | 0.029 | 0.056 | 0.046 | -5 |
| | 2 | 1.042 | 1.027 | 1.027 | 1.019 | 5 | | 2 | 0.039 | 0.029 | 0.059 | 0.059 | -4 |
| | 3 | 1.050 | 1.021 | 1.036 | 1.010 | 4 | | 3 | 0.039 | 0.029 | 0.058 | 0.054 | -5 |
| | Mean | 1.036 | 1.015 | 1.023 | 1.005 | 3 | | Mean | 0.039 | 0.029 | 0.058 | 0.053 | -5 |
| US-005 | 1 | 1.029 | 1.015 | 1.015 | 1.004 | 3 | US-005 | 1 | 0.048 | 0.029 | 0.047 | 0.043 | -11 |
| | 2 | 1.026 | 1.012 | 1.012 | 1.002 | 3 | | 2 | 0.042 | 0.043 | 0.044 | 0.044 | -8 |
| | 3 | 1.041 | 1.016 | 1.028 | 1.007 | 2 | | 3 | 0.045 | 0.029 | 0.046 | 0.046 | -10 |
| | Mean | 1.032 | 1.015 | 1.018 | 1.004 | 3 | | Mean | 0.045 | 0.033 | 0.046 | 0.044 | -10 |
| US-006 | 1 | 1.027 | 1.020 | 1.001 | 1.010 | 15 | US-006 | 1 | 0.042 | 0.031 | 0.043 | 0.044 | -22 |
| | 2 | 1.035 | 1.026 | 1.013 | 1.011 | 11 | | 2 | 0.041 | 0.031 | 0.044 | 0.048 | -21 |
| | 3 | 1.042 | 1.017 | 1.015 | 1.008 | 16 | | 3 | 0.043 | 0.031 | 0.044 | 0.069 | -22 |
| | Mean | 1.035 | 1.021 | 1.010 | 1.010 | 14 | | Mean | 0.042 | 0.031 | 0.043 | 0.054 | -22 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 1

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 | |
| US-007 | 1 | 1.039 | 1.006 | 1.026 | 0.991 | 2 | US-007 | 1 | 0.041 | 0.035 | 0.045 | 0.046 | -11 |
| | 2 | 1.027 | 1.015 | 1.013 | 1.005 | 3 | | 2 | 0.040 | 0.034 | 0.046 | 0.054 | -10 |
| | 3 | 1.030 | 1.010 | 1.017 | 1.002 | 2 | | 3 | 0.041 | 0.033 | 0.045 | 0.049 | -11 |
| | Mean | 1.032 | 1.010 | 1.019 | 0.999 | 2 | | Mean | 0.041 | 0.034 | 0.045 | 0.049 | -11 |
| US-023 | 1 | 1.021 | 1.008 | 1.003 | 0.991 | 4 | US-023 | 1 | 0.042 | 0.033 | 0.048 | 0.039 | -1 |
| | 2 | 1.028 | 1.024 | 1.012 | 1.013 | 2 | | 2 | 0.041 | 0.049 | 0.043 | 0.044 | -5 |
| | 3 | 1.030 | 1.022 | 1.012 | 1.008 | 4 | | 3 | 0.041 | 0.029 | 0.043 | 0.051 | -6 |
| | Mean | 1.026 | 1.018 | 1.009 | 1.004 | 3 | | Mean | 0.041 | 0.037 | 0.045 | 0.044 | -4 |
| US-024 | 1 | 1.024 | 1.014 | 1.003 | 1.003 | 8 | US-024 | 1 | 0.045 | 0.030 | 0.046 | 0.045 | -15 |
| | 2 | 1.024 | 1.023 | 1.008 | 1.009 | 2 | | 2 | 0.042 | 0.040 | 0.046 | 0.052 | -12 |
| | 3 | 1.038 | 1.023 | 1.004 | 1.006 | 21 | | 3 | 0.047 | 0.029 | 0.047 | 0.051 | -15 |
| | Mean | 1.029 | 1.020 | 1.005 | 1.006 | 10 | | Mean | 0.044 | 0.033 | 0.046 | 0.049 | -14 |
| US-025 | 1 | 1.018 | 0.990 | 0.987 | 0.969 | 9 | US-025 | 1 | 0.041 | 0.029 | 0.042 | 0.046 | -20 |
| | 2 | 1.022 | 1.013 | 0.996 | 0.988 | 4 | | 2 | 0.040 | 0.047 | 0.043 | 0.047 | -18 |
| | 3 | 1.022 | 1.011 | 0.993 | 0.993 | 6 | | 3 | 0.043 | 0.030 | 0.043 | 0.073 | -21 |
| | Mean | 1.020 | 1.005 | 0.992 | 0.983 | 6 | | Mean | 0.041 | 0.035 | 0.043 | 0.056 | -20 |
| US-026 | 1 | 0.988 | 0.973 | 0.998 | 0.965 | -18 | US-026 | 1 | 0.041 | 0.032 | 0.042 | 0.040 | -11 |
| | 2 | 1.003 | 0.982 | 1.001 | 0.977 | -6 | | 2 | 0.041 | 0.038 | 0.043 | 0.049 | -10 |
| | 3 | 1.005 | 0.986 | 1.018 | 0.976 | -20 | | 3 | 0.044 | 0.029 | 0.041 | 0.046 | -15 |
| | Mean | 0.999 | 0.981 | 1.005 | 0.973 | -15 | | Mean | 0.042 | 0.033 | 0.042 | 0.045 | -12 |
| US-027 | 1 | 0.973 | 0.970 | 0.963 | 0.965 | 3 | US-027 | 1 | 0.037 | 0.033 | 0.044 | 0.040 | -7 |
| | 2 | 0.992 | 0.979 | 0.973 | 0.970 | 11 | | 2 | 0.040 | 0.030 | 0.043 | 0.047 | -10 |
| | 3 | 0.996 | 0.978 | 0.985 | 0.969 | 3 | | 3 | 0.038 | 0.029 | 0.044 | 0.046 | -8 |
| | Mean | 0.987 | 0.976 | 0.973 | 0.968 | 6 | | Mean | 0.038 | 0.030 | 0.044 | 0.044 | -8 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 1

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 | |
| US-028 | 1 | 0.994 | 0.980 | 0.968 | 0.971 | 15 | US-028 | 1 | 0.040 | 0.029 | 0.042 | 0.051 | -24 |
| | 2 | 1.000 | 0.988 | 0.980 | 0.977 | 10 | | 2 | 0.040 | 0.030 | 0.042 | 0.053 | -24 |
| | 3 | 1.003 | 0.995 | 0.976 | 0.983 | 16 | | 3 | 0.037 | 0.028 | 0.042 | 0.061 | -22 |
| | Mean | 0.999 | 0.988 | 0.975 | 0.977 | 14 | | Mean | 0.039 | 0.029 | 0.042 | 0.055 | -23 |
| US-029 | 1 | 0.984 | 0.973 | 0.961 | 0.960 | 13 | US-029 | 1 | 0.044 | 0.030 | 0.040 | 0.038 | -12 |
| | 2 | 0.986 | 0.986 | 0.971 | 0.979 | 5 | | 2 | 0.043 | 0.044 | 0.042 | 0.044 | -9 |
| | 3 | 1.003 | 0.987 | 0.977 | 0.978 | 16 | | 3 | 0.043 | 0.029 | 0.041 | 0.047 | -11 |
| | Mean | 0.991 | 0.982 | 0.970 | 0.972 | 11 | | Mean | 0.043 | 0.034 | 0.041 | 0.043 | -11 |
| US-030 | 1 | 0.990 | 0.997 | 0.983 | 1.002 | 8 | US-030 | 1 | 0.039 | 0.029 | 0.042 | 0.047 | -18 |
| | 2 | 0.998 | 1.003 | 0.986 | 0.996 | 13 | | 2 | 0.040 | 0.043 | 0.043 | 0.051 | -18 |
| | 3 | 1.001 | 1.001 | 0.994 | 1.005 | 7 | | 3 | 0.042 | 0.030 | 0.043 | 0.066 | -20 |
| | Mean | 0.996 | 1.000 | 0.988 | 1.001 | 9 | | Mean | 0.040 | 0.034 | 0.043 | 0.055 | -19 |
| US-031 | 1 | 1.012 | 0.986 | 1.006 | 0.973 | -4 | US-031 | 1 | 0.045 | 0.029 | 0.042 | 0.042 | -16 |
| | 2 | 1.008 | 0.985 | 0.999 | 0.979 | -1 | | 2 | 0.039 | 0.042 | 0.043 | 0.046 | -9 |
| | 3 | 1.017 | 0.995 | 1.005 | 0.985 | 2 | | 3 | 0.045 | 0.029 | 0.044 | 0.049 | -14 |
| | Mean | 1.012 | 0.989 | 1.003 | 0.979 | -1 | | Mean | 0.043 | 0.033 | 0.043 | 0.046 | -13 |
| US-032 | 1 | 0.978 | 0.987 | 0.952 | 0.976 | 14 | US-032 | 1 | 0.041 | 0.028 | 0.043 | 0.045 | -17 |
| | 2 | 0.992 | 0.988 | 0.973 | 0.975 | 7 | | 2 | 0.040 | 0.045 | 0.043 | 0.045 | -17 |
| | 3 | 1.002 | 1.007 | 0.975 | 0.995 | 15 | | 3 | 0.042 | 0.028 | 0.044 | 0.072 | -18 |
| | Mean | 0.990 | 0.994 | 0.967 | 0.982 | 12 | | Mean | 0.041 | 0.034 | 0.043 | 0.054 | -17 |
| US-033 | 1 | 0.977 | 0.982 | 0.962 | 0.972 | 3 | US-033 | 1 | 0.040 | 0.033 | 0.041 | 0.041 | -18 |
| | 2 | 0.995 | 0.989 | 0.977 | 0.975 | 6 | | 2 | 0.039 | 0.034 | 0.041 | 0.051 | -18 |
| | 3 | 0.998 | 0.987 | 0.983 | 0.975 | 3 | | 3 | 0.041 | 0.028 | 0.041 | 0.064 | -20 |
| | Mean | 0.990 | 0.986 | 0.974 | 0.974 | 4 | | Mean | 0.040 | 0.032 | 0.041 | 0.052 | -19 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 1

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 | |
| US-034 | 1 | 0.991 | 0.974 | 0.982 | 0.965 | 0 | US-034 | 1 | 0.041 | 0.036 | 0.041 | 0.049 | -24 |
| | 2 | 1.001 | 1.001 | 0.992 | 0.992 | 1 | | 2 | 0.041 | 0.042 | 0.042 | 0.045 | -23 |
| | 3 | 1.001 | 0.994 | 0.993 | 0.985 | -2 | | 3 | 0.042 | 0.029 | 0.041 | 0.085 | -25 |
| | Mean | 0.998 | 0.990 | 0.989 | 0.981 | 0 | | Mean | 0.041 | 0.036 | 0.041 | 0.060 | -24 |
| US-035 | 1 | 0.982 | 0.982 | 0.974 | 0.975 | -1 | US-035 | 1 | 0.043 | 0.034 | 0.041 | 0.045 | -24 |
| | 2 | 0.988 | 0.985 | 0.979 | 0.974 | 0 | | 2 | 0.042 | 0.030 | 0.043 | 0.048 | -21 |
| | 3 | 0.996 | 0.985 | 0.985 | 0.976 | 2 | | 3 | 0.044 | 0.028 | 0.042 | 0.066 | -24 |
| | Mean | 0.989 | 0.984 | 0.979 | 0.975 | 0 | | Mean | 0.043 | 0.031 | 0.042 | 0.053 | -23 |
| US-036 | 1 | 0.984 | 0.977 | 0.975 | 0.965 | -1 | US-036 | 1 | 0.041 | 0.028 | 0.042 | 0.043 | -23 |
| | 2 | 0.995 | 0.990 | 0.987 | 0.984 | -2 | | 2 | 0.040 | 0.032 | 0.043 | 0.048 | -22 |
| | 3 | 0.997 | 0.988 | 0.989 | 0.977 | -3 | | 3 | 0.040 | 0.029 | 0.042 | 0.072 | -22 |
| | Mean | 0.992 | 0.985 | 0.984 | 0.975 | -2 | | Mean | 0.040 | 0.030 | 0.042 | 0.054 | -22 |
| Mean for all assays | | - | - | - | - | 5 | Mean for all assays | | - | - | - | - | -15 |
| SD for all assays | | - | - | - | - | 7 | SD for all assays | | - | - | - | - | 7 |
| CV for all assays | | - | - | - | - | - | CV for all assays | | - | - | - | - | - |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

Chemical Name : Qunine 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 | |
| FDSC-01 | 1 | 0.940 | 0.955 | 0.509 | 0.948 | 424 | FDSC-01 | 1 | 0.031 | 0.033 | 0.320 | 0.043 | 278 |
| | 2 | 0.959 | 0.963 | 0.530 | 0.956 | 422 | | 2 | 0.032 | 0.037 | 0.344 | 0.050 | 301 |
| | 3 | 0.963 | 0.967 | 0.539 | 0.961 | 417 | | 3 | 0.033 | 0.033 | 0.352 | 0.042 | 308 |
| | Mean | 0.954 | 0.962 | 0.526 | 0.955 | 421 | | Mean | 0.032 | 0.034 | 0.339 | 0.045 | 296 |
| FDSC-02 | 1 | 0.991 | 0.964 | 0.479 | 0.953 | 502 | FDSC-02 | 1 | 0.033 | 0.032 | 0.282 | 0.041 | 230 |
| | 2 | 0.996 | 0.971 | 0.507 | 0.964 | 479 | | 2 | 0.032 | 0.034 | 0.300 | 0.041 | 249 |
| | 3 | 0.999 | 0.978 | 0.510 | 0.965 | 479 | | 3 | 0.033 | 0.038 | 0.305 | 0.081 | 253 |
| | Mean | 0.995 | 0.971 | 0.499 | 0.961 | 487 | | Mean | 0.033 | 0.035 | 0.296 | 0.054 | 244 |
| FDSC-03 | 1 | 0.954 | 0.966 | 0.516 | 0.962 | 435 | FDSC-03 | 1 | 0.032 | 0.033 | 0.351 | 0.050 | 302 |
| | 2 | 0.954 | 0.970 | 0.527 | 0.968 | 424 | | 2 | 0.032 | 0.033 | 0.340 | 0.050 | 291 |
| | 3 | 0.965 | 0.974 | 0.537 | 0.970 | 425 | | 3 | 0.034 | 0.035 | 0.363 | 0.053 | 312 |
| | Mean | 0.958 | 0.970 | 0.527 | 0.967 | 428 | | Mean | 0.033 | 0.034 | 0.351 | 0.051 | 302 |
| FDSC-04 | 1 | 0.960 | 0.972 | 0.511 | 0.963 | 439 | FDSC-04 | 1 | 0.032 | 0.032 | 0.346 | 0.050 | 297 |
| | 2 | 0.970 | 0.970 | 0.526 | 0.959 | 434 | | 2 | 0.033 | 0.032 | 0.346 | 0.049 | 296 |
| | 3 | 0.961 | 0.975 | 0.526 | 0.963 | 425 | | 3 | 0.034 | 0.033 | 0.343 | 0.049 | 292 |
| | Mean | 0.964 | 0.972 | 0.521 | 0.962 | 433 | | Mean | 0.033 | 0.032 | 0.345 | 0.049 | 295 |
| FDSC-05 | 1 | 0.966 | 0.970 | 0.533 | 0.966 | 432 | FDSC-05 | 1 | 0.032 | 0.032 | 0.321 | 0.048 | 273 |
| | 2 | 0.963 | 0.966 | 0.538 | 0.973 | 424 | | 2 | 0.033 | 0.032 | 0.309 | 0.051 | 260 |
| | 3 | 0.961 | 0.975 | 0.539 | 0.968 | 421 | | 3 | 0.034 | 0.032 | 0.329 | 0.046 | 279 |
| | Mean | 0.963 | 0.970 | 0.537 | 0.969 | 426 | | Mean | 0.033 | 0.032 | 0.320 | 0.048 | 271 |
| FDSC-06 | 1 | 0.975 | 0.982 | 0.530 | 0.972 | 436 | FDSC-06 | 1 | 0.032 | 0.033 | 0.357 | 0.052 | 307 |
| | 2 | 0.981 | 0.977 | 0.544 | 0.965 | 428 | | 2 | 0.033 | 0.034 | 0.355 | 0.047 | 304 |
| | 3 | 0.975 | 0.982 | 0.547 | 0.976 | 419 | | 3 | 0.032 | 0.033 | 0.357 | 0.055 | 307 |
| | Mean | 0.977 | 0.980 | 0.540 | 0.971 | 428 | | Mean | 0.032 | 0.033 | 0.356 | 0.051 | 306 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

Chemical Name : Qunine 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 | |
| FDSC-07 | 1 | 0.963 | 0.973 | 0.520 | 0.964 | 438 | FDSC-07 | 1 | 0.032 | 0.032 | 0.296 | 0.048 | 248 |
| | 2 | 0.984 | 0.971 | 0.545 | 0.967 | 434 | | 2 | 0.032 | 0.032 | 0.283 | 0.049 | 235 |
| | 3 | 0.970 | 0.978 | 0.538 | 0.976 | 427 | | 3 | 0.032 | 0.033 | 0.295 | 0.047 | 247 |
| | Mean | 0.972 | 0.974 | 0.534 | 0.969 | 433 | | Mean | 0.032 | 0.032 | 0.291 | 0.048 | 243 |
| FDSC-08 | 1 | 0.977 | 0.987 | 0.517 | 0.980 | 453 | FDSC-08 | 1 | 0.031 | 0.032 | 0.358 | 0.046 | 314 |
| | 2 | 0.992 | 0.989 | 0.541 | 0.982 | 444 | | 2 | 0.032 | 0.032 | 0.325 | 0.048 | 280 |
| | 3 | 0.989 | 0.996 | 0.541 | 0.990 | 441 | | 3 | 0.033 | 0.033 | 0.333 | 0.042 | 287 |
| | Mean | 0.986 | 0.991 | 0.533 | 0.984 | 446 | | Mean | 0.032 | 0.032 | 0.339 | 0.045 | 294 |
| FDSC-09 | 1 | 0.981 | 0.984 | 0.532 | 0.975 | 443 | FDSC-09 | 1 | 0.032 | 0.032 | 0.329 | 0.052 | 274 |
| | 2 | 0.982 | 0.984 | 0.536 | 0.980 | 440 | | 2 | 0.032 | 0.032 | 0.318 | 0.057 | 263 |
| | 3 | 0.986 | 0.994 | 0.545 | 0.987 | 435 | | 3 | 0.032 | 0.032 | 0.342 | 0.055 | 287 |
| | Mean | 0.983 | 0.987 | 0.538 | 0.981 | 439 | | Mean | 0.032 | 0.032 | 0.330 | 0.055 | 275 |
| FDSC-10 | 1 | 0.976 | 0.982 | 0.519 | 0.976 | 448 | FDSC-10 | 1 | 0.032 | 0.032 | 0.334 | 0.047 | 283 |
| | 2 | 0.985 | 0.992 | 0.534 | 0.975 | 442 | | 2 | 0.032 | 0.032 | 0.329 | 0.053 | 278 |
| | 3 | 0.982 | 0.987 | 0.537 | 0.984 | 436 | | 3 | 0.032 | 0.034 | 0.351 | 0.055 | 300 |
| | Mean | 0.981 | 0.987 | 0.530 | 0.978 | 442 | | Mean | 0.032 | 0.033 | 0.338 | 0.052 | 287 |
| FDSC-11 | 1 | 0.976 | 0.977 | 0.516 | 0.965 | 452 | FDSC-11 | 1 | 0.037 | 0.031 | 0.312 | 0.049 | 256 |
| | 2 | 0.977 | 0.972 | 0.533 | 0.967 | 436 | | 2 | 0.032 | 0.032 | 0.313 | 0.055 | 262 |
| | 3 | 0.978 | 0.979 | 0.531 | 0.971 | 439 | | 3 | 0.033 | 0.034 | 0.325 | 0.050 | 273 |
| | Mean | 0.977 | 0.976 | 0.527 | 0.968 | 442 | | Mean | 0.034 | 0.032 | 0.317 | 0.051 | 264 |
| FDSC-12 | 1 | 0.959 | 0.967 | 0.513 | 0.961 | 439 | FDSC-12 | 1 | 0.033 | 0.033 | 0.340 | 0.049 | 292 |
| | 2 | 0.969 | 0.962 | 0.530 | 0.954 | 432 | | 2 | 0.033 | 0.035 | 0.337 | 0.050 | 289 |
| | 3 | 0.957 | 0.967 | 0.528 | 0.959 | 422 | | 3 | 0.034 | 0.033 | 0.338 | 0.047 | 289 |
| | Mean | 0.962 | 0.965 | 0.524 | 0.958 | 431 | | Mean | 0.033 | 0.034 | 0.338 | 0.049 | 290 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

Chemical Name : Qunine 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 | |
| FDSC-13 | 1 | 0.966 | 0.970 | 0.542 | 0.970 | 419 | FDSC-13 | 1 | 0.031 | 0.034 | 0.349 | 0.043 | 309 |
| | 2 | 0.975 | 0.970 | 0.539 | 0.962 | 431 | | 2 | 0.033 | 0.034 | 0.333 | 0.043 | 291 |
| | 3 | 0.963 | 0.972 | 0.529 | 0.965 | 429 | | 3 | 0.033 | 0.033 | 0.369 | 0.044 | 327 |
| | Mean | 0.968 | 0.971 | 0.537 | 0.966 | 426 | | Mean | 0.032 | 0.034 | 0.350 | 0.043 | 309 |
| FDSC-14 | 1 | 0.958 | 0.971 | 0.511 | 0.960 | 437 | FDSC-14 | 1 | 0.032 | 0.032 | 0.367 | 0.044 | 322 |
| | 2 | 0.967 | 0.968 | 0.526 | 0.959 | 431 | | 2 | 0.033 | 0.033 | 0.362 | 0.050 | 316 |
| | 3 | 0.969 | 0.974 | 0.530 | 0.963 | 429 | | 3 | 0.032 | 0.033 | 0.371 | 0.045 | 326 |
| | Mean | 0.965 | 0.971 | 0.522 | 0.961 | 432 | | Mean | 0.032 | 0.033 | 0.367 | 0.046 | 321 |
| FDSC-15 | 1 | 0.975 | 0.974 | 0.527 | 0.967 | 443 | FDSC-15 | 1 | 0.031 | 0.033 | 0.351 | 0.054 | 305 |
| | 2 | 0.979 | 0.975 | 0.541 | 0.971 | 433 | | 2 | 0.032 | 0.032 | 0.327 | 0.048 | 280 |
| | 3 | 0.981 | 0.982 | 0.543 | 0.977 | 433 | | 3 | 0.034 | 0.035 | 0.340 | 0.043 | 291 |
| | Mean | 0.978 | 0.977 | 0.537 | 0.972 | 436 | | Mean | 0.032 | 0.033 | 0.339 | 0.048 | 292 |
| FDSC-16 | 1 | 0.971 | 0.986 | 0.512 | 0.996 | 459 | FDSC-16 | 1 | 0.031 | 0.031 | 0.358 | 0.041 | 316 |
| | 2 | 0.978 | 0.982 | 0.530 | 0.980 | 448 | | 2 | 0.032 | 0.032 | 0.334 | 0.044 | 291 |
| | 3 | 0.984 | 0.993 | 0.539 | 0.986 | 445 | | 3 | 0.033 | 0.033 | 0.364 | 0.043 | 320 |
| | Mean | 0.978 | 0.987 | 0.527 | 0.987 | 451 | | Mean | 0.032 | 0.032 | 0.352 | 0.043 | 309 |
| FDSC-17 | 1 | 0.981 | 0.981 | 0.519 | 0.977 | 459 | FDSC-17 | 1 | 0.032 | 0.032 | 0.344 | 0.053 | 294 |
| | 2 | 0.990 | 0.989 | 0.538 | 0.984 | 449 | | 2 | 0.032 | 0.033 | 0.356 | 0.052 | 306 |
| | 3 | 0.990 | 0.995 | 0.541 | 0.993 | 446 | | 3 | 0.032 | 0.032 | 0.372 | 0.046 | 322 |
| | Mean | 0.987 | 0.988 | 0.533 | 0.985 | 451 | | Mean | 0.032 | 0.032 | 0.357 | 0.050 | 307 |
| FDSC-18 | 1 | 0.948 | 0.960 | 0.503 | 0.955 | 440 | FDSC-18 | 1 | 0.032 | 0.032 | 0.324 | 0.043 | 279 |
| | 2 | 0.953 | 0.955 | 0.520 | 0.951 | 428 | | 2 | 0.032 | 0.032 | 0.335 | 0.047 | 290 |
| | 3 | 0.953 | 0.966 | 0.520 | 0.958 | 428 | | 3 | 0.036 | 0.033 | 0.341 | 0.045 | 292 |
| | Mean | 0.951 | 0.960 | 0.514 | 0.955 | 432 | | Mean | 0.033 | 0.032 | 0.333 | 0.045 | 287 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

Chemical Name : Qunine 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 | |
| FDSC-19 | 1 | 0.979 | 0.986 | 0.527 | 0.990 | 448 | FDSC-19 | 1 | 0.031 | 0.033 | 0.301 | 0.049 | 252 |
| | 2 | 0.976 | 0.981 | 0.538 | 0.975 | 434 | | 2 | 0.032 | 0.032 | 0.293 | 0.054 | 243 |
| | 3 | 0.976 | 0.991 | 0.542 | 0.982 | 430 | | 3 | 0.033 | 0.033 | 0.319 | 0.050 | 268 |
| | Mean | 0.977 | 0.986 | 0.536 | 0.982 | 437 | | Mean | 0.032 | 0.033 | 0.304 | 0.051 | 254 |
| FDSC-20 | 1 | 0.965 | 0.974 | 0.515 | 0.949 | 431 | FDSC-20 | 1 | 0.032 | 0.032 | 0.343 | 0.046 | 299 |
| | 2 | 0.976 | 0.972 | 0.534 | 0.954 | 423 | | 2 | 0.032 | 0.032 | 0.348 | 0.045 | 304 |
| | 3 | 0.972 | 0.980 | 0.536 | 0.966 | 417 | | 3 | 0.034 | 0.034 | 0.354 | 0.045 | 308 |
| | Mean | 0.971 | 0.975 | 0.528 | 0.956 | 424 | | Mean | 0.033 | 0.033 | 0.348 | 0.045 | 304 |
| FDSC-21 | 1 | 0.955 | 0.959 | 0.509 | 0.967 | 444 | FDSC-21 | 1 | 0.031 | 0.031 | 0.279 | 0.043 | 239 |
| | 2 | 0.959 | 0.958 | 0.524 | 0.953 | 433 | | 2 | 0.033 | 0.032 | 0.267 | 0.041 | 225 |
| | 3 | 0.957 | 0.963 | 0.528 | 0.955 | 427 | | 3 | 0.032 | 0.035 | 0.286 | 0.042 | 245 |
| | Mean | 0.957 | 0.960 | 0.520 | 0.958 | 435 | | Mean | 0.032 | 0.033 | 0.277 | 0.042 | 236 |
| FDSC-22 | 1 | 0.968 | 0.980 | 0.519 | 0.974 | 443 | FDSC-22 | 1 | 0.032 | 0.031 | 0.314 | 0.040 | 268 |
| | 2 | 0.973 | 0.982 | 0.538 | 0.974 | 429 | | 2 | 0.034 | 0.033 | 0.275 | 0.057 | 227 |
| | 3 | 0.977 | 0.991 | 0.544 | 0.986 | 427 | | 3 | 0.033 | 0.032 | 0.307 | 0.042 | 260 |
| | Mean | 0.973 | 0.984 | 0.534 | 0.978 | 433 | | Mean | 0.033 | 0.032 | 0.299 | 0.046 | 252 |
| FDSC-23 | 1 | 0.988 | 0.957 | 0.536 | 0.946 | 442 | FDSC-23 | 1 | 0.031 | 0.032 | 0.317 | 0.059 | 268 |
| | 2 | 0.995 | 0.960 | 0.552 | 0.947 | 433 | | 2 | 0.032 | 0.032 | 0.312 | 0.045 | 262 |
| | 3 | 0.995 | 0.965 | 0.557 | 0.959 | 428 | | 3 | 0.033 | 0.033 | 0.344 | 0.045 | 293 |
| | Mean | 0.993 | 0.961 | 0.548 | 0.951 | 434 | | Mean | 0.032 | 0.032 | 0.324 | 0.050 | 274 |
| FDSC-24 | 1 | 0.967 | 0.972 | 0.518 | 0.963 | 442 | FDSC-24 | 1 | 0.032 | 0.031 | 0.258 | 0.045 | 212 |
| | 2 | 0.968 | 0.970 | 0.528 | 0.963 | 433 | | 2 | 0.034 | 0.032 | 0.258 | 0.048 | 210 |
| | 3 | 0.965 | 0.977 | 0.530 | 0.973 | 428 | | 3 | 0.033 | 0.032 | 0.270 | 0.046 | 223 |
| | Mean | 0.967 | 0.973 | 0.525 | 0.966 | 434 | | Mean | 0.033 | 0.032 | 0.262 | 0.046 | 215 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

Chemical Name : Qunine 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 | |
| FDSC-25 | 1 | 0.938 | 0.958 | 0.502 | 0.948 | 424 | FDSC-25 | 1 | 0.032 | 0.032 | 0.304 | 0.040 | 264 |
| | 2 | 0.945 | 0.952 | 0.514 | 0.949 | 419 | | 2 | 0.032 | 0.034 | 0.303 | 0.041 | 263 |
| | 3 | 0.943 | 0.951 | 0.517 | 0.928 | 414 | | 3 | 0.033 | 0.033 | 0.316 | 0.042 | 275 |
| | Mean | 0.942 | 0.954 | 0.511 | 0.942 | 419 | | Mean | 0.032 | 0.033 | 0.308 | 0.041 | 267 |
| FDSC-26 | 1 | 0.946 | 0.959 | 0.502 | 0.954 | 438 | FDSC-26 | 1 | 0.033 | 0.037 | 0.329 | 0.042 | 285 |
| | 2 | 0.960 | 0.955 | 0.519 | 0.949 | 435 | | 2 | 0.034 | 0.033 | 0.328 | 0.050 | 283 |
| | 3 | 0.955 | 0.964 | 0.523 | 0.956 | 426 | | 3 | 0.033 | 0.033 | 0.351 | 0.043 | 307 |
| | Mean | 0.954 | 0.959 | 0.515 | 0.953 | 433 | | Mean | 0.033 | 0.034 | 0.336 | 0.045 | 292 |
| Mean for all assays | | - | - | - | - | 436 | Mean for all assays | | - | - | - | - | 280 |
| SD for all assays | | - | - | - | - | 13 | SD for all assays | | - | - | - | - | 27 |
| CV for all assays | | - | - | - | - | 3.0 | CV for all assays | | - | - | - | - | 9.6 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2
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 | |
| FDSC-01 | 1 | 0.959 | 0.955 | 0.949 | 0.948 | 3 | FDSC-01 | 1 | 0.038 | 0.033 | 0.039 | 0.043 | -10 |
| | 2 | 0.974 | 0.963 | 0.966 | 0.956 | 1 | | 2 | 0.038 | 0.037 | 0.038 | 0.050 | -11 |
| | 3 | 0.976 | 0.967 | 0.971 | 0.961 | -2 | | 3 | 0.037 | 0.033 | 0.043 | 0.042 | -5 |
| | Mean | 0.970 | 0.962 | 0.962 | 0.955 | 1 | | Mean | 0.038 | 0.034 | 0.040 | 0.045 | -9 |
| FDSC-02 | 1 | 0.984 | 0.964 | 0.941 | 0.953 | 33 | FDSC-02 | 1 | 0.033 | 0.032 | 0.040 | 0.041 | -12 |
| | 2 | 0.990 | 0.971 | 0.963 | 0.964 | 17 | | 2 | 0.033 | 0.034 | 0.040 | 0.041 | -12 |
| | 3 | 0.998 | 0.978 | 0.966 | 0.965 | 22 | | 3 | 0.032 | 0.038 | 0.040 | 0.081 | -11 |
| | Mean | 0.991 | 0.971 | 0.957 | 0.961 | 24 | | Mean | 0.033 | 0.035 | 0.040 | 0.054 | -12 |
| FDSC-03 | 1 | 0.968 | 0.966 | 0.962 | 0.962 | 3 | FDSC-03 | 1 | 0.038 | 0.033 | 0.039 | 0.050 | -16 |
| | 2 | 0.977 | 0.970 | 0.976 | 0.968 | -2 | | 2 | 0.037 | 0.033 | 0.039 | 0.050 | -15 |
| | 3 | 0.976 | 0.974 | 0.972 | 0.970 | 1 | | 3 | 0.041 | 0.035 | 0.039 | 0.053 | -19 |
| | Mean | 0.974 | 0.970 | 0.970 | 0.967 | 1 | | Mean | 0.039 | 0.034 | 0.039 | 0.051 | -17 |
| FDSC-04 | 1 | 0.974 | 0.972 | 0.962 | 0.963 | 2 | FDSC-04 | 1 | 0.038 | 0.032 | 0.038 | 0.050 | -17 |
| | 2 | 0.976 | 0.970 | 0.960 | 0.959 | 6 | | 2 | 0.037 | 0.032 | 0.038 | 0.049 | -16 |
| | 3 | 0.975 | 0.975 | 0.963 | 0.963 | 2 | | 3 | 0.039 | 0.033 | 0.040 | 0.049 | -16 |
| | Mean | 0.975 | 0.972 | 0.962 | 0.962 | 3 | | Mean | 0.038 | 0.032 | 0.039 | 0.049 | -16 |
| FDSC-05 | 1 | 0.967 | 0.970 | 0.966 | 0.966 | 0 | FDSC-05 | 1 | 0.038 | 0.032 | 0.040 | 0.048 | -14 |
| | 2 | 0.969 | 0.966 | 0.968 | 0.973 | 0 | | 2 | 0.037 | 0.032 | 0.039 | 0.051 | -14 |
| | 3 | 0.968 | 0.975 | 0.963 | 0.968 | 4 | | 3 | 0.037 | 0.032 | 0.038 | 0.046 | -15 |
| | Mean | 0.968 | 0.970 | 0.966 | 0.969 | 1 | | Mean | 0.037 | 0.032 | 0.039 | 0.048 | -14 |
| FDSC-06 | 1 | 0.983 | 0.982 | 0.978 | 0.972 | -4 | FDSC-06 | 1 | 0.038 | 0.033 | 0.039 | 0.052 | -17 |
| | 2 | 0.982 | 0.977 | 0.972 | 0.965 | 1 | | 2 | 0.038 | 0.034 | 0.042 | 0.047 | -14 |
| | 3 | 0.987 | 0.982 | 0.981 | 0.976 | -3 | | 3 | 0.038 | 0.033 | 0.040 | 0.055 | -16 |
| | Mean | 0.984 | 0.980 | 0.977 | 0.971 | -2 | | Mean | 0.038 | 0.033 | 0.040 | 0.051 | -16 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

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 | |
| FDSC-07 | 1 | 0.974 | 0.973 | 0.974 | 0.964 | -5 | FDSC-07 | 1 | 0.038 | 0.032 | 0.039 | 0.048 | -15 |
| | 2 | 0.979 | 0.971 | 0.972 | 0.967 | 2 | | 2 | 0.038 | 0.032 | 0.043 | 0.049 | -11 |
| | 3 | 0.980 | 0.978 | 0.977 | 0.976 | -2 | | 3 | 0.038 | 0.033 | 0.041 | 0.047 | -13 |
| | Mean | 0.978 | 0.974 | 0.974 | 0.969 | -2 | | Mean | 0.038 | 0.032 | 0.041 | 0.048 | -13 |
| FDSC-08 | 1 | 0.991 | 0.987 | 0.985 | 0.980 | -1 | FDSC-08 | 1 | 0.037 | 0.032 | 0.043 | 0.046 | -7 |
| | 2 | 0.995 | 0.989 | 0.987 | 0.982 | 1 | | 2 | 0.038 | 0.032 | 0.041 | 0.048 | -10 |
| | 3 | 1.003 | 0.996 | 0.995 | 0.990 | 1 | | 3 | 0.037 | 0.033 | 0.040 | 0.042 | -10 |
| | Mean | 0.996 | 0.991 | 0.989 | 0.984 | 0 | | Mean | 0.037 | 0.032 | 0.041 | 0.045 | -9 |
| FDSC-09 | 1 | 0.994 | 0.984 | 0.982 | 0.975 | 6 | FDSC-09 | 1 | 0.038 | 0.032 | 0.039 | 0.052 | -22 |
| | 2 | 0.990 | 0.984 | 0.986 | 0.980 | -2 | | 2 | 0.038 | 0.032 | 0.040 | 0.057 | -21 |
| | 3 | 0.996 | 0.994 | 0.991 | 0.987 | -1 | | 3 | 0.038 | 0.032 | 0.038 | 0.055 | -23 |
| | Mean | 0.993 | 0.987 | 0.986 | 0.981 | 1 | | Mean | 0.038 | 0.032 | 0.039 | 0.055 | -22 |
| FDSC-10 | 1 | 0.988 | 0.982 | 0.982 | 0.976 | -3 | FDSC-10 | 1 | 0.039 | 0.032 | 0.039 | 0.047 | -19 |
| | 2 | 0.990 | 0.992 | 0.985 | 0.975 | -4 | | 2 | 0.037 | 0.032 | 0.039 | 0.053 | -17 |
| | 3 | 0.995 | 0.987 | 0.994 | 0.984 | -8 | | 3 | 0.039 | 0.034 | 0.040 | 0.055 | -18 |
| | Mean | 0.991 | 0.987 | 0.987 | 0.978 | -5 | | Mean | 0.038 | 0.033 | 0.039 | 0.052 | -18 |
| FDSC-11 | 1 | 0.984 | 0.977 | 0.977 | 0.965 | -1 | FDSC-11 | 1 | 0.037 | 0.031 | 0.039 | 0.049 | -17 |
| | 2 | 0.983 | 0.972 | 0.976 | 0.967 | -1 | | 2 | 0.037 | 0.032 | 0.038 | 0.055 | -18 |
| | 3 | 0.985 | 0.979 | 0.975 | 0.971 | 2 | | 3 | 0.037 | 0.034 | 0.039 | 0.050 | -17 |
| | Mean | 0.984 | 0.976 | 0.976 | 0.968 | 0 | | Mean | 0.037 | 0.032 | 0.039 | 0.051 | -17 |
| FDSC-12 | 1 | 0.977 | 0.967 | 0.964 | 0.961 | 6 | FDSC-12 | 1 | 0.037 | 0.033 | 0.039 | 0.049 | -13 |
| | 2 | 0.972 | 0.962 | 0.965 | 0.954 | 0 | | 2 | 0.037 | 0.035 | 0.039 | 0.050 | -13 |
| | 3 | 0.975 | 0.967 | 0.968 | 0.959 | 0 | | 3 | 0.037 | 0.033 | 0.041 | 0.047 | -11 |
| | Mean | 0.975 | 0.965 | 0.966 | 0.958 | 2 | | Mean | 0.037 | 0.034 | 0.040 | 0.049 | -12 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

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 | |
| FDSC-13 | 1 | 0.969 | 0.970 | 0.971 | 0.970 | -7 | FDSC-13 | 1 | 0.037 | 0.034 | 0.039 | 0.043 | -7 |
| | 2 | 0.970 | 0.970 | 0.972 | 0.962 | -7 | | 2 | 0.037 | 0.034 | 0.040 | 0.043 | -6 |
| | 3 | 0.968 | 0.972 | 0.961 | 0.965 | 2 | | 3 | 0.037 | 0.033 | 0.039 | 0.044 | -7 |
| | Mean | 0.969 | 0.971 | 0.968 | 0.966 | -4 | | Mean | 0.037 | 0.034 | 0.039 | 0.043 | -7 |
| FDSC-14 | 1 | 0.971 | 0.971 | 0.962 | 0.960 | -1 | FDSC-14 | 1 | 0.037 | 0.032 | 0.040 | 0.044 | -10 |
| | 2 | 0.974 | 0.968 | 0.969 | 0.959 | -5 | | 2 | 0.037 | 0.033 | 0.039 | 0.050 | -11 |
| | 3 | 0.982 | 0.974 | 0.973 | 0.963 | -1 | | 3 | 0.040 | 0.033 | 0.040 | 0.045 | -13 |
| | Mean | 0.976 | 0.971 | 0.968 | 0.961 | -2 | | Mean | 0.038 | 0.033 | 0.040 | 0.046 | -11 |
| FDSC-15 | 1 | 0.981 | 0.974 | 0.977 | 0.967 | -1 | FDSC-15 | 1 | 0.039 | 0.033 | 0.040 | 0.054 | -14 |
| | 2 | 0.985 | 0.975 | 0.981 | 0.971 | -1 | | 2 | 0.037 | 0.032 | 0.040 | 0.048 | -12 |
| | 3 | 0.987 | 0.982 | 0.983 | 0.977 | -1 | | 3 | 0.038 | 0.035 | 0.039 | 0.043 | -14 |
| | Mean | 0.984 | 0.977 | 0.980 | 0.972 | -1 | | Mean | 0.038 | 0.033 | 0.040 | 0.048 | -13 |
| FDSC-16 | 1 | 0.984 | 0.986 | 0.978 | 0.996 | 6 | FDSC-16 | 1 | 0.037 | 0.031 | 0.038 | 0.041 | -10 |
| | 2 | 0.985 | 0.982 | 0.981 | 0.980 | 4 | | 2 | 0.037 | 0.032 | 0.038 | 0.044 | -10 |
| | 3 | 0.989 | 0.993 | 0.983 | 0.986 | 6 | | 3 | 0.037 | 0.033 | 0.039 | 0.043 | -9 |
| | Mean | 0.986 | 0.987 | 0.981 | 0.987 | 5 | | Mean | 0.037 | 0.032 | 0.038 | 0.043 | -10 |
| FDSC-17 | 1 | 0.982 | 0.981 | 0.978 | 0.977 | 1 | FDSC-17 | 1 | 0.039 | 0.032 | 0.041 | 0.053 | -16 |
| | 2 | 0.991 | 0.989 | 0.988 | 0.984 | 0 | | 2 | 0.037 | 0.033 | 0.040 | 0.052 | -15 |
| | 3 | 0.995 | 0.995 | 0.989 | 0.993 | 3 | | 3 | 0.037 | 0.032 | 0.039 | 0.046 | -16 |
| | Mean | 0.989 | 0.988 | 0.985 | 0.985 | 1 | | Mean | 0.038 | 0.032 | 0.040 | 0.050 | -16 |
| FDSC-18 | 1 | 0.963 | 0.960 | 0.956 | 0.955 | 2 | FDSC-18 | 1 | 0.038 | 0.032 | 0.040 | 0.043 | -11 |
| | 2 | 0.964 | 0.955 | 0.959 | 0.951 | 0 | | 2 | 0.038 | 0.032 | 0.040 | 0.047 | -11 |
| | 3 | 0.968 | 0.966 | 0.961 | 0.958 | 2 | | 3 | 0.040 | 0.033 | 0.039 | 0.045 | -14 |
| | Mean | 0.965 | 0.960 | 0.959 | 0.955 | 1 | | Mean | 0.039 | 0.032 | 0.040 | 0.045 | -12 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

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 | |
| FDSC-19 | 1 | 0.985 | 0.986 | 0.976 | 0.990 | 5 | FDSC-19 | 1 | 0.038 | 0.033 | 0.039 | 0.049 | -17 |
| | 2 | 0.984 | 0.981 | 0.973 | 0.975 | 7 | | 2 | 0.038 | 0.032 | 0.039 | 0.054 | -17 |
| | 3 | 0.980 | 0.991 | 0.973 | 0.982 | 3 | | 3 | 0.039 | 0.033 | 0.038 | 0.050 | -19 |
| | Mean | 0.983 | 0.986 | 0.974 | 0.982 | 5 | | Mean | 0.038 | 0.033 | 0.039 | 0.051 | -18 |
| FDSC-20 | 1 | 0.978 | 0.974 | 0.962 | 0.949 | -3 | FDSC-20 | 1 | 0.038 | 0.032 | 0.040 | 0.046 | -10 |
| | 2 | 0.983 | 0.972 | 0.972 | 0.954 | -8 | | 2 | 0.037 | 0.032 | 0.040 | 0.045 | -9 |
| | 3 | 0.988 | 0.980 | 0.980 | 0.966 | -11 | | 3 | 0.039 | 0.034 | 0.038 | 0.045 | -13 |
| | Mean | 0.983 | 0.975 | 0.971 | 0.956 | -7 | | Mean | 0.038 | 0.033 | 0.039 | 0.045 | -11 |
| FDSC-21 | 1 | 0.967 | 0.959 | 0.957 | 0.967 | 8 | FDSC-21 | 1 | 0.038 | 0.031 | 0.039 | 0.043 | -8 |
| | 2 | 0.972 | 0.958 | 0.963 | 0.953 | 7 | | 2 | 0.037 | 0.032 | 0.039 | 0.041 | -7 |
| | 3 | 0.966 | 0.963 | 0.959 | 0.955 | 5 | | 3 | 0.037 | 0.035 | 0.039 | 0.042 | -7 |
| | Mean | 0.968 | 0.960 | 0.960 | 0.958 | 7 | | Mean | 0.037 | 0.033 | 0.039 | 0.042 | -7 |
| FDSC-22 | 1 | 0.979 | 0.980 | 0.972 | 0.974 | 1 | FDSC-22 | 1 | 0.038 | 0.031 | 0.039 | 0.040 | -13 |
| | 2 | 0.980 | 0.982 | 0.973 | 0.974 | 1 | | 2 | 0.037 | 0.033 | 0.039 | 0.057 | -12 |
| | 3 | 0.988 | 0.991 | 0.977 | 0.986 | 5 | | 3 | 0.037 | 0.032 | 0.039 | 0.042 | -12 |
| | Mean | 0.982 | 0.984 | 0.974 | 0.978 | 2 | | Mean | 0.037 | 0.032 | 0.039 | 0.046 | -12 |
| FDSC-23 | 1 | 0.964 | 0.957 | 0.954 | 0.946 | 0 | FDSC-23 | 1 | 0.038 | 0.032 | 0.040 | 0.059 | -16 |
| | 2 | 0.963 | 0.960 | 0.951 | 0.947 | 2 | | 2 | 0.038 | 0.032 | 0.039 | 0.045 | -17 |
| | 3 | 0.966 | 0.965 | 0.956 | 0.959 | 0 | | 3 | 0.038 | 0.033 | 0.043 | 0.045 | -13 |
| | Mean | 0.964 | 0.961 | 0.954 | 0.951 | 1 | | Mean | 0.038 | 0.032 | 0.041 | 0.050 | -15 |
| FDSC-24 | 1 | 0.975 | 0.972 | 0.965 | 0.963 | 3 | FDSC-24 | 1 | 0.038 | 0.031 | 0.039 | 0.045 | -13 |
| | 2 | 0.975 | 0.970 | 0.965 | 0.963 | 3 | | 2 | 0.038 | 0.032 | 0.040 | 0.048 | -12 |
| | 3 | 0.976 | 0.977 | 0.969 | 0.973 | 0 | | 3 | 0.039 | 0.032 | 0.043 | 0.046 | -10 |
| | Mean | 0.975 | 0.973 | 0.966 | 0.966 | 2 | | Mean | 0.038 | 0.032 | 0.041 | 0.046 | -12 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 2

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 | |
| FDSC-25 | 1 | 0.962 | 0.958 | 0.951 | 0.948 | -1 | FDSC-25 | 1 | 0.038 | 0.032 | 0.039 | 0.040 | -7 |
| | 2 | 0.959 | 0.952 | 0.952 | 0.949 | -5 | | 2 | 0.037 | 0.034 | 0.040 | 0.041 | -5 |
| | 3 | 0.963 | 0.951 | 0.956 | 0.928 | -5 | | 3 | 0.038 | 0.033 | 0.039 | 0.042 | -7 |
| | Mean | 0.961 | 0.954 | 0.953 | 0.942 | -4 | | Mean | 0.038 | 0.033 | 0.039 | 0.041 | -6 |
| FDSC-26 | 1 | 0.963 | 0.959 | 0.959 | 0.954 | -2 | FDSC-26 | 1 | 0.040 | 0.037 | 0.039 | 0.042 | -12 |
| | 2 | 0.964 | 0.955 | 0.954 | 0.949 | 4 | | 2 | 0.037 | 0.033 | 0.040 | 0.050 | -8 |
| | 3 | 0.966 | 0.964 | 0.961 | 0.956 | -1 | | 3 | 0.039 | 0.033 | 0.038 | 0.043 | -12 |
| | Mean | 0.964 | 0.959 | 0.958 | 0.953 | 0 | | Mean | 0.039 | 0.034 | 0.039 | 0.045 | -11 |
| Mean for all assays | | - | - | - | - | 1 | Mean for all assays | | - | - | - | - | -13 |
| SD for all assays | | - | - | - | - | 6 | SD for all assays | | - | - | - | - | 4 |
| CV for all assays | | - | - | - | - | - | CV for all assays | | - | - | - | - | - |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

## Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 3
Chemical Name : Qunine 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 | |
| MT-001 | 1 | 0.986 | 1.005 | 0.596 | 1.000 | 387 | MT-001 | 1 | 0.032 | 0.030 | 0.340 | 0.035 | 301 |
| | 2 | 0.990 | 1.009 | 0.612 | 1.005 | 376 | | 2 | 0.033 | 0.030 | 0.340 | 0.035 | 301 |
| | 3 | 0.987 | 1.010 | 0.624 | 1.012 | 361 | | 3 | 0.032 | 0.029 | 0.353 | 0.036 | 314 |
| | Mean | 0.988 | 1.008 | 0.611 | 1.006 | 375 | | Mean | 0.032 | 0.029 | 0.344 | 0.036 | 305 |
| MT-002 | 1 | 0.992 | 1.011 | 0.627 | 1.005 | 360 | MT-002 | 1 | 0.031 | 0.029 | 0.320 | 0.033 | 284 |
| | 2 | 0.995 | 1.016 | 0.633 | 1.011 | 357 | | 2 | 0.032 | 0.030 | 0.317 | 0.034 | 280 |
| | 3 | 0.989 | 1.018 | 0.639 | 1.013 | 345 | | 3 | 0.031 | 0.028 | 0.334 | 0.033 | 298 |
| | Mean | 0.992 | 1.015 | 0.633 | 1.010 | 354 | | Mean | 0.031 | 0.029 | 0.324 | 0.034 | 287 |
| MT-003 | 1 | | | | | | MT-003 | 1 | 0.031 | 0.029 | 0.337 | 0.036 | 300 |
| | 2 | | | | | | | 2 | 0.031 | 0.029 | 0.325 | 0.034 | 288 |
| | 3 | | | | | | | 3 | 0.031 | 0.028 | 0.343 | 0.034 | 307 |
| | Mean | | | | | | | Mean | 0.031 | 0.029 | 0.335 | 0.035 | 298 |
| MT-004 | 1 | 0.979 | 0.999 | 0.594 | 0.992 | 379 | MT-004 | 1 | 0.032 | 0.029 | 0.366 | 0.034 | 330 |
| | 2 | 0.982 | 1.003 | 0.605 | 0.997 | 370 | | 2 | 0.032 | 0.029 | 0.369 | 0.034 | 331 |
| | 3 | 0.979 | 1.001 | 0.608 | 0.996 | 364 | | 3 | 0.032 | 0.029 | 0.384 | 0.034 | 347 |
| | Mean | 0.980 | 1.001 | 0.602 | 0.995 | 371 | | Mean | 0.032 | 0.029 | 0.373 | 0.034 | 336 |
| MT-005 | 1 | 0.982 | 1.000 | 0.608 | 0.997 | 370 | MT-005 | 1 | 0.032 | 0.029 | 0.322 | 0.034 | 285 |
| | 2 | 0.981 | 1.003 | 0.617 | 1.000 | 360 | | 2 | 0.032 | 0.029 | 0.322 | 0.034 | 284 |
| | 3 | 0.977 | 1.002 | 0.621 | 0.998 | 352 | | 3 | 0.031 | 0.029 | 0.326 | 0.034 | 289 |
| | Mean | 0.980 | 1.002 | 0.615 | 0.998 | 361 | | Mean | 0.032 | 0.029 | 0.323 | 0.034 | 286 |
| MT-006 | 1 | 0.986 | 1.002 | 0.606 | 0.996 | 374 | MT-006 | 1 | 0.031 | 0.029 | 0.330 | 0.034 | 293 |
| | 2 | 0.987 | 1.006 | 0.616 | 0.999 | 365 | | 2 | 0.032 | 0.029 | 0.324 | 0.034 | 287 |
| | 3 | 0.982 | 1.007 | 0.617 | 1.001 | 360 | | 3 | 0.031 | 0.028 | 0.340 | 0.033 | 304 |
| | Mean | 0.985 | 1.005 | 0.613 | 0.999 | 366 | | Mean | 0.031 | 0.029 | 0.331 | 0.034 | 295 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

The optical absorbance values were rounded to the third decimal place. If the calculated "results" are different from the numerical results in the raw data sheets by the number of significant digits in the data sheet and the appendices are different, the data in the raw data sheet is used in the appendices.

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 3

Chemical Name : Qunine 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 | |
| MT-007 | 1 | 0.981 | 0.996 | 0.597 | 0.992 | 382 | MT-007 | 1 | 0.031 | 0.029 | 0.343 | 0.036 | 305 |
| | 2 | 0.984 | 1.002 | 0.607 | 0.998 | 374 | | 2 | 0.032 | 0.029 | 0.340 | 0.035 | 302 |
| | 3 | 0.978 | 1.000 | 0.606 | 0.997 | 369 | | 3 | 0.031 | 0.028 | 0.348 | 0.034 | 311 |
| | Mean | 0.981 | 0.999 | 0.603 | 0.996 | 375 | | Mean | 0.031 | 0.029 | 0.344 | 0.035 | 306 |
| MT-008 | 1 | 0.994 | 1.010 | 0.597 | 0.995 | 382 | MT-008 | 1 | 0.033 | 0.031 | 0.335 | 0.035 | 297 |
| | 2 | 0.993 | 1.012 | 0.605 | 0.997 | 372 | | 2 | 0.033 | 0.031 | 0.340 | 0.036 | 301 |
| | 3 | 0.988 | 1.011 | 0.610 | 0.997 | 362 | | 3 | 0.032 | 0.030 | 0.348 | 0.035 | 311 |
| | Mean | 0.992 | 1.011 | 0.604 | 0.996 | 372 | | Mean | 0.033 | 0.031 | 0.341 | 0.036 | 303 |
| MT-009 | 1 | 0.976 | 0.994 | 0.603 | 0.989 | 369 | MT-009 | 1 | 0.031 | 0.029 | 0.334 | 0.034 | 298 |
| | 2 | 0.978 | 0.997 | 0.618 | 0.994 | 357 | | 2 | 0.032 | 0.029 | 0.331 | 0.035 | 295 |
| | 3 | 0.975 | 0.999 | 0.619 | 0.995 | 352 | | 3 | 0.031 | 0.028 | 0.345 | 0.034 | 309 |
| | Mean | 0.976 | 0.997 | 0.613 | 0.993 | 359 | | Mean | 0.031 | 0.029 | 0.337 | 0.034 | 301 |
| MT-010 | 1 | 0.983 | 0.998 | 0.617 | 0.994 | 362 | MT-010 | 1 | 0.031 | 0.029 | 0.324 | 0.034 | 288 |
| | 2 | 0.983 | 1.002 | 0.622 | 0.997 | 357 | | 2 | 0.031 | 0.029 | 0.324 | 0.035 | 287 |
| | 3 | 0.978 | 1.001 | 0.623 | 0.996 | 351 | | 3 | 0.031 | 0.028 | 0.326 | 0.034 | 290 |
| | Mean | 0.981 | 1.000 | 0.621 | 0.996 | 357 | | Mean | 0.031 | 0.029 | 0.325 | 0.034 | 288 |
| MT-011 | 1 | 0.982 | 0.998 | 0.618 | 0.993 | 359 | MT-011 | 1 | 0.032 | 0.030 | 0.318 | 0.034 | 280 |
| | 2 | 0.985 | 1.005 | 0.627 | 0.999 | 353 | | 2 | 0.032 | 0.029 | 0.318 | 0.035 | 280 |
| | 3 | 0.980 | 1.003 | 0.629 | 1.000 | 347 | | 3 | 0.031 | 0.028 | 0.327 | 0.035 | 290 |
| | Mean | 0.982 | 1.002 | 0.625 | 0.997 | 353 | | Mean | 0.032 | 0.029 | 0.321 | 0.035 | 283 |
| MT-012 | 1 | 0.974 | 0.992 | 0.607 | 0.989 | 363 | MT-012 | 1 | 0.032 | 0.030 | 0.317 | 0.034 | 280 |
| | 2 | 0.979 | 0.999 | 0.618 | 0.994 | 357 | | 2 | 0.033 | 0.029 | 0.315 | 0.035 | 277 |
| | 3 | 0.975 | 0.999 | 0.620 | 0.996 | 351 | | 3 | 0.031 | 0.029 | 0.320 | 0.034 | 283 |
| | Mean | 0.976 | 0.997 | 0.615 | 0.993 | 357 | | Mean | 0.032 | 0.029 | 0.317 | 0.034 | 280 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 3

Chemical Name : Qunine 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 | |
| MT-013 | 1 | 0.970 | 0.994 | 0.611 | 0.988 | 356 | MT-013 | 1 | 0.031 | 0.029 | 0.317 | 0.034 | 280 |
| | 2 | 0.973 | 0.995 | 0.618 | 0.991 | 351 | | 2 | 0.032 | 0.029 | 0.309 | 0.035 | 272 |
| | 3 | 0.971 | 0.995 | 0.619 | 0.990 | 348 | | 3 | 0.031 | 0.028 | 0.319 | 0.034 | 283 |
| | Mean | 0.971 | 0.994 | 0.616 | 0.990 | 352 | | Mean | 0.031 | 0.029 | 0.315 | 0.034 | 278 |
| MT-014 | 1 | 0.988 | 1.007 | 0.622 | 1.003 | 362 | MT-014 | 1 | 0.032 | 0.029 | 0.317 | 0.035 | 279 |
| | 2 | 0.991 | 1.012 | 0.632 | 1.008 | 355 | | 2 | 0.033 | 0.029 | 0.315 | 0.035 | 276 |
| | 3 | 0.986 | 1.010 | 0.634 | 1.009 | 348 | | 3 | 0.032 | 0.029 | 0.318 | 0.037 | 280 |
| | Mean | 0.988 | 1.010 | 0.629 | 1.006 | 355 | | Mean | 0.032 | 0.029 | 0.317 | 0.035 | 278 |
| MT-015 | 1 | 0.983 | 1.002 | 0.617 | 0.997 | 359 | MT-015 | 1 | 0.031 | 0.030 | 0.326 | 0.035 | 290 |
| | 2 | 0.989 | 1.007 | 0.626 | 1.004 | 355 | | 2 | 0.032 | 0.030 | 0.322 | 0.035 | 286 |
| | 3 | 0.985 | 1.006 | 0.629 | 0.995 | 349 | | 3 | 0.031 | 0.029 | 0.326 | 0.034 | 291 |
| | Mean | 0.986 | 1.005 | 0.624 | 0.998 | 354 | | Mean | 0.031 | 0.030 | 0.325 | 0.034 | 289 |
| MT-016 | 1 | 0.975 | 0.996 | 0.609 | 0.992 | 362 | MT-016 | 1 | 0.032 | 0.029 | 0.329 | 0.034 | 293 |
| | 2 | 0.981 | 0.988 | 0.619 | 0.985 | 358 | | 2 | 0.032 | 0.029 | 0.326 | 0.033 | 290 |
| | 3 | 0.969 | 0.989 | 0.616 | 0.984 | 349 | | 3 | 0.031 | 0.029 | 0.330 | 0.033 | 295 |
| | Mean | 0.975 | 0.991 | 0.615 | 0.987 | 356 | | Mean | 0.032 | 0.029 | 0.328 | 0.033 | 293 |
| MT-018 | 1 | 0.969 | 0.997 | 0.594 | 0.993 | 371 | MT-018 | 1 | 0.032 | 0.029 | 0.335 | 0.033 | 298 |
| | 2 | 0.976 | 0.992 | 0.607 | 0.987 | 365 | | 2 | 0.032 | 0.028 | 0.332 | 0.033 | 295 |
| | 3 | 0.961 | 0.990 | 0.603 | 0.987 | 354 | | 3 | 0.031 | 0.028 | 0.340 | 0.033 | 304 |
| | Mean | 0.969 | 0.993 | 0.601 | 0.989 | 363 | | Mean | 0.032 | 0.028 | 0.336 | 0.033 | 299 |
| MT-019 | 1 | 0.985 | 1.009 | 0.626 | 1.001 | 350 | MT-019 | 1 | 0.033 | 0.030 | 0.325 | 0.035 | 287 |
| | 2 | 0.990 | 1.002 | 0.633 | 0.992 | 348 | | 2 | 0.033 | 0.030 | 0.327 | 0.035 | 289 |
| | 3 | 0.978 | 1.003 | 0.630 | 0.994 | 339 | | 3 | 0.032 | 0.030 | 0.335 | 0.035 | 298 |
| | Mean | 0.984 | 1.005 | 0.630 | 0.996 | 346 | | Mean | 0.033 | 0.030 | 0.329 | 0.035 | 291 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 3

Chemical Name : Qunine 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 | |
| MT-020 | 1 | 0.981 | 1.003 | 0.622 | 1.003 | 357 | MT-020 | 1 | 0.033 | 0.030 | 0.315 | 0.035 | 276 |
| | 2 | 0.986 | 1.000 | 0.638 | 0.998 | 346 | | 2 | 0.033 | 0.030 | 0.313 | 0.035 | 275 |
| | 3 | 0.974 | 0.999 | 0.633 | 0.997 | 339 | | 3 | 0.032 | 0.031 | 0.317 | 0.036 | 279 |
| | Mean | 0.980 | 1.001 | 0.631 | 0.999 | 347 | | Mean | 0.033 | 0.030 | 0.315 | 0.036 | 277 |
| MT-021 | 1 | 0.982 | 1.008 | 0.626 | 1.001 | 351 | MT-021 | 1 | 0.032 | 0.029 | 0.319 | 0.035 | 280 |
| | 2 | 0.989 | 1.004 | 0.638 | 1.000 | 346 | | 2 | 0.032 | 0.030 | 0.315 | 0.035 | 277 |
| | 3 | 0.979 | 1.004 | 0.633 | 1.001 | 340 | | 3 | 0.032 | 0.029 | 0.322 | 0.035 | 284 |
| | Mean | 0.983 | 1.005 | 0.632 | 1.000 | 346 | | Mean | 0.032 | 0.029 | 0.319 | 0.035 | 280 |
| MT-022 | 1 | 0.976 | 1.001 | 0.619 | 0.994 | 351 | MT-022 | 1 | 0.032 | 0.030 | 0.313 | 0.034 | 276 |
| | 2 | 0.981 | 1.000 | 0.627 | 0.992 | 348 | | 2 | 0.032 | 0.030 | 0.310 | 0.035 | 273 |
| | 3 | 0.971 | 0.996 | 0.624 | 0.993 | 341 | | 3 | 0.032 | 0.029 | 0.323 | 0.034 | 286 |
| | Mean | 0.976 | 0.999 | 0.623 | 0.993 | 347 | | Mean | 0.032 | 0.029 | 0.315 | 0.034 | 278 |
| MT-023 | 1 | 0.980 | 1.002 | 0.612 | 0.997 | 364 | MT-023 | 1 | 0.032 | 0.029 | 0.324 | 0.036 | 285 |
| | 2 | 0.985 | 0.998 | 0.626 | 0.993 | 355 | | 2 | 0.032 | 0.029 | 0.324 | 0.036 | 285 |
| | 3 | 0.973 | 0.998 | 0.620 | 0.995 | 349 | | 3 | 0.032 | 0.029 | 0.333 | 0.036 | 294 |
| | Mean | 0.979 | 0.999 | 0.619 | 0.995 | 356 | | Mean | 0.032 | 0.029 | 0.327 | 0.036 | 288 |
| Mean for all assays | | - | - | - | - | 358 | Mean for all assays | | - | - | - | - | 292 |
| SD for all assays | | - | - | - | - | 9 | SD for all assays | | - | - | - | - | 14 |
| CV for all assays | | - | - | - | - | 2.5 | CV for all assays | | - | - | - | - | 4.8 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 3
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 | |
| MT-001 | 1 | 1.008 | 1.005 | 1.005 | 1.000 | 1 | MT-001 | 1 | 0.034 | 0.030 | 0.034 | 0.035 | -7 |
| | 2 | 1.015 | 1.009 | 1.013 | 1.005 | 0 | | 2 | 0.036 | 0.030 | 0.036 | 0.035 | -8 |
| | 3 | 1.014 | 1.010 | 1.011 | 1.012 | 1 | | 3 | 0.034 | 0.029 | 0.034 | 0.036 | -7 |
| | Mean | 1.012 | 1.008 | 1.010 | 1.006 | 1 | | Mean | 0.035 | 0.029 | 0.035 | 0.036 | -7 |
| MT-002 | 1 | 1.011 | 1.011 | 1.008 | 1.005 | -2 | MT-002 | 1 | 0.033 | 0.029 | 0.033 | 0.033 | -6 |
| | 2 | 1.012 | 1.016 | 1.009 | 1.011 | -2 | | 2 | 0.034 | 0.030 | 0.033 | 0.034 | -6 |
| | 3 | 1.015 | 1.018 | 1.013 | 1.013 | -3 | | 3 | 0.034 | 0.028 | 0.032 | 0.033 | -6 |
| | Mean | 1.013 | 1.015 | 1.010 | 1.010 | -2 | | Mean | 0.034 | 0.029 | 0.033 | 0.034 | -6 |
| MT-003 | 1 | | | | | | MT-003 | 1 | 0.033 | 0.029 | 0.033 | 0.036 | -6 |
| | 2 | | | | | | | 2 | 0.034 | 0.029 | 0.034 | 0.034 | -6 |
| | 3 | | | | | | | 3 | 0.033 | 0.028 | 0.033 | 0.034 | -6 |
| | Mean | | | | | | | Mean | 0.033 | 0.029 | 0.033 | 0.035 | -6 |
| MT-004 | 1 | 1.001 | 0.999 | 0.998 | 0.992 | -4 | MT-004 | 1 | 0.033 | 0.029 | 0.033 | 0.034 | -5 |
| | 2 | 1.006 | 1.003 | 1.004 | 0.997 | -4 | | 2 | 0.033 | 0.029 | 0.033 | 0.034 | -5 |
| | 3 | 1.004 | 1.001 | 1.000 | 0.996 | -2 | | 3 | 0.033 | 0.029 | 0.033 | 0.034 | -6 |
| | Mean | 1.004 | 1.001 | 1.001 | 0.995 | -3 | | Mean | 0.033 | 0.029 | 0.033 | 0.034 | -5 |
| MT-005 | 1 | 1.002 | 1.000 | 1.000 | 0.997 | -1 | MT-005 | 1 | 0.033 | 0.029 | 0.033 | 0.034 | -5 |
| | 2 | 1.001 | 1.003 | 0.999 | 1.000 | -2 | | 2 | 0.034 | 0.029 | 0.034 | 0.034 | -5 |
| | 3 | 1.005 | 1.002 | 1.001 | 0.998 | 1 | | 3 | 0.033 | 0.029 | 0.033 | 0.034 | -6 |
| | Mean | 1.003 | 1.002 | 1.000 | 0.998 | -1 | | Mean | 0.033 | 0.029 | 0.033 | 0.034 | -5 |
| MT-006 | 1 | 1.003 | 1.002 | 1.001 | 0.996 | -3 | MT-006 | 1 | 0.033 | 0.029 | 0.033 | 0.034 | -5 |
| | 2 | 1.006 | 1.006 | 1.005 | 0.999 | -5 | | 2 | 0.034 | 0.029 | 0.034 | 0.034 | -5 |
| | 3 | 1.009 | 1.007 | 1.003 | 1.001 | 0 | | 3 | 0.033 | 0.028 | 0.033 | 0.033 | -6 |
| | Mean | 1.006 | 1.005 | 1.003 | 0.999 | -3 | | Mean | 0.033 | 0.029 | 0.033 | 0.034 | -5 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 3
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 | |
| MT-007 | 1 | 0.999 | 0.996 | 0.996 | 0.992 | 0 | MT-007 | 1 | 0.032 | 0.029 | 0.034 | 0.036 | -5 |
| | 2 | 1.001 | 1.002 | 0.999 | 0.998 | -1 | | 2 | 0.034 | 0.029 | 0.034 | 0.035 | -6 |
| | 3 | 1.004 | 1.000 | 1.002 | 0.997 | -1 | | 3 | 0.032 | 0.028 | 0.034 | 0.034 | -5 |
| | Mean | 1.001 | 0.999 | 0.999 | 0.996 | -1 | | Mean | 0.033 | 0.029 | 0.034 | 0.035 | -5 |
| MT-008 | 1 | 1.009 | 1.010 | 0.994 | 0.995 | 0 | MT-008 | 1 | 0.035 | 0.031 | 0.033 | 0.035 | -7 |
| | 2 | 1.010 | 1.012 | 0.996 | 0.997 | 0 | | 2 | 0.035 | 0.031 | 0.034 | 0.036 | -6 |
| | 3 | 1.011 | 1.011 | 0.998 | 0.997 | -2 | | 3 | 0.035 | 0.030 | 0.033 | 0.035 | -7 |
| | Mean | 1.010 | 1.011 | 0.996 | 0.996 | -1 | | Mean | 0.035 | 0.031 | 0.033 | 0.036 | -7 |
| MT-009 | 1 | 0.998 | 0.994 | 0.996 | 0.989 | -2 | MT-009 | 1 | 0.032 | 0.029 | 0.033 | 0.034 | -4 |
| | 2 | 0.998 | 0.997 | 0.995 | 0.994 | -1 | | 2 | 0.033 | 0.029 | 0.033 | 0.035 | -5 |
| | 3 | 1.001 | 0.999 | 0.995 | 0.995 | 2 | | 3 | 0.033 | 0.028 | 0.032 | 0.034 | -6 |
| | Mean | 0.999 | 0.997 | 0.995 | 0.993 | 0 | | Mean | 0.033 | 0.029 | 0.033 | 0.034 | -5 |
| MT-010 | 1 | 1.001 | 0.998 | 0.997 | 0.994 | 0 | MT-010 | 1 | 0.032 | 0.029 | 0.032 | 0.034 | -5 |
| | 2 | 1.001 | 1.002 | 0.996 | 0.997 | 1 | | 2 | 0.033 | 0.029 | 0.033 | 0.035 | -5 |
| | 3 | 1.004 | 1.001 | 0.999 | 0.996 | 1 | | 3 | 0.033 | 0.028 | 0.032 | 0.034 | -5 |
| | Mean | 1.002 | 1.000 | 0.997 | 0.996 | 1 | | Mean | 0.033 | 0.029 | 0.032 | 0.034 | -5 |
| MT-011 | 1 | 1.001 | 0.998 | 0.998 | 0.993 | -2 | MT-011 | 1 | 0.032 | 0.030 | 0.033 | 0.034 | -6 |
| | 2 | 1.001 | 1.005 | 0.997 | 0.999 | -1 | | 2 | 0.033 | 0.029 | 0.033 | 0.035 | -6 |
| | 3 | 1.005 | 1.003 | 1.000 | 1.000 | 1 | | 3 | 0.034 | 0.028 | 0.033 | 0.035 | -7 |
| | Mean | 1.002 | 1.002 | 0.998 | 0.997 | -1 | | Mean | 0.033 | 0.029 | 0.033 | 0.035 | -6 |
| MT-012 | 1 | 0.997 | 0.992 | 0.993 | 0.989 | 0 | MT-012 | 1 | 0.032 | 0.030 | 0.032 | 0.034 | -5 |
| | 2 | 0.997 | 0.999 | 0.992 | 0.994 | 1 | | 2 | 0.033 | 0.029 | 0.033 | 0.035 | -5 |
| | 3 | 0.998 | 0.999 | 0.992 | 0.996 | 2 | | 3 | 0.033 | 0.029 | 0.032 | 0.034 | -6 |
| | Mean | 0.997 | 0.997 | 0.992 | 0.993 | 1 | | Mean | 0.033 | 0.029 | 0.032 | 0.034 | -5 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 3
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 | |
| MT-013 | 1 | 0.994 | 0.994 | 0.991 | 0.988 | -1 | MT-013 | 1 | 0.032 | 0.029 | 0.032 | 0.034 | -5 |
| | 2 | 0.993 | 0.995 | 0.989 | 0.991 | 0 | | 2 | 0.033 | 0.029 | 0.033 | 0.035 | -5 |
| | 3 | 0.997 | 0.995 | 0.989 | 0.990 | 3 | | 3 | 0.033 | 0.028 | 0.032 | 0.034 | -5 |
| | Mean | 0.995 | 0.994 | 0.990 | 0.990 | 1 | | Mean | 0.033 | 0.029 | 0.032 | 0.034 | -5 |
| MT-014 | 1 | 1.010 | 1.007 | 1.006 | 1.003 | 0 | MT-014 | 1 | 0.033 | 0.029 | 0.033 | 0.035 | -6 |
| | 2 | 1.010 | 1.012 | 1.005 | 1.008 | 1 | | 2 | 0.034 | 0.029 | 0.033 | 0.035 | -7 |
| | 3 | 1.013 | 1.010 | 1.006 | 1.009 | 3 | | 3 | 0.034 | 0.029 | 0.032 | 0.037 | -7 |
| | Mean | 1.011 | 1.010 | 1.006 | 1.006 | 1 | | Mean | 0.034 | 0.029 | 0.033 | 0.035 | -7 |
| MT-015 | 1 | 1.008 | 1.002 | 1.003 | 0.997 | -3 | MT-015 | 1 | 0.033 | 0.030 | 0.032 | 0.035 | -4 |
| | 2 | 1.009 | 1.007 | 1.005 | 1.004 | -3 | | 2 | 0.034 | 0.030 | 0.033 | 0.035 | -4 |
| | 3 | 1.014 | 1.006 | 1.008 | 0.995 | -1 | | 3 | 0.033 | 0.029 | 0.032 | 0.034 | -5 |
| | Mean | 1.010 | 1.005 | 1.005 | 0.998 | -2 | | Mean | 0.033 | 0.030 | 0.032 | 0.034 | -4 |
| MT-016 | 1 | 0.989 | 0.996 | 0.984 | 0.992 | 1 | MT-016 | 1 | 0.034 | 0.029 | 0.033 | 0.034 | -4 |
| | 2 | 1.005 | 0.988 | 1.002 | 0.985 | -2 | | 2 | 0.034 | 0.029 | 0.033 | 0.033 | -5 |
| | 3 | 0.995 | 0.989 | 0.989 | 0.984 | 2 | | 3 | 0.032 | 0.029 | 0.032 | 0.033 | -4 |
| | Mean | 0.996 | 0.991 | 0.992 | 0.987 | 0 | | Mean | 0.033 | 0.029 | 0.033 | 0.033 | -4 |
| MT-018 | 1 | 0.989 | 0.997 | 0.984 | 0.993 | 0 | MT-018 | 1 | 0.033 | 0.029 | 0.033 | 0.033 | -5 |
| | 2 | 1.000 | 0.992 | 0.983 | 0.987 | 13 | | 2 | 0.033 | 0.028 | 0.033 | 0.033 | -5 |
| | 3 | 0.995 | 0.990 | 0.989 | 0.987 | 2 | | 3 | 0.032 | 0.028 | 0.033 | 0.033 | -5 |
| | Mean | 0.995 | 0.993 | 0.985 | 0.989 | 5 | | Mean | 0.033 | 0.028 | 0.033 | 0.033 | -5 |
| MT-019 | 1 | 1.001 | 1.009 | 0.995 | 1.001 | -3 | MT-019 | 1 | 0.033 | 0.030 | 0.033 | 0.035 | -5 |
| | 2 | 1.007 | 1.002 | 0.999 | 0.992 | 0 | | 2 | 0.033 | 0.030 | 0.033 | 0.035 | -5 |
| | 3 | 1.005 | 1.003 | 0.998 | 0.994 | -2 | | 3 | 0.033 | 0.030 | 0.033 | 0.035 | -5 |
| | Mean | 1.004 | 1.005 | 0.997 | 0.996 | -2 | | Mean | 0.033 | 0.030 | 0.033 | 0.035 | -5 |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

Appendix 6 Positive control and negative control data of Phase 2 study

Laboratory : 3
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 | |
| MT-020 | 1 | 1.000 | 1.003 | 0.996 | 1.003 | 2 | MT-020 | 1 | 0.034 | 0.030 | 0.034 | 0.035 | -6 |
| | 2 | 1.010 | 1.000 | 1.003 | 0.998 | 5 | | 2 | 0.033 | 0.030 | 0.033 | 0.035 | -6 |
| | 3 | 1.008 | 0.999 | 1.001 | 0.997 | 5 | | 3 | 0.032 | 0.031 | 0.032 | 0.036 | -5 |
| | Mean | 1.006 | 1.001 | 1.000 | 0.999 | 4 | | Mean | 0.033 | 0.030 | 0.033 | 0.036 | -6 |
| MT-021 | 1 | 1.000 | 1.008 | 0.993 | 1.001 | 1 | MT-021 | 1 | 0.033 | 0.029 | 0.034 | 0.035 | -6 |
| | 2 | 1.011 | 1.004 | 1.006 | 1.000 | 0 | | 2 | 0.032 | 0.030 | 0.033 | 0.035 | -5 |
| | 3 | 1.011 | 1.004 | 1.004 | 1.001 | 2 | | 3 | 0.031 | 0.029 | 0.033 | 0.035 | -5 |
| | Mean | 1.007 | 1.005 | 1.001 | 1.000 | 1 | | Mean | 0.032 | 0.029 | 0.033 | 0.035 | -5 |
| MT-022 | 1 | 0.993 | 1.001 | 0.988 | 0.994 | 0 | MT-022 | 1 | 0.033 | 0.030 | 0.034 | 0.034 | -4 |
| | 2 | 1.002 | 1.000 | 0.994 | 0.992 | 2 | | 2 | 0.032 | 0.030 | 0.033 | 0.035 | -4 |
| | 3 | 1.002 | 0.996 | 0.995 | 0.993 | 1 | | 3 | 0.033 | 0.029 | 0.033 | 0.034 | -5 |
| | Mean | 0.999 | 0.999 | 0.992 | 0.993 | 1 | | Mean | 0.033 | 0.029 | 0.033 | 0.034 | -4 |
| MT-023 | 1 | 0.996 | 1.002 | 0.990 | 0.997 | 2 | MT-023 | 1 | 0.034 | 0.029 | 0.033 | 0.036 | -8 |
| | 2 | 1.006 | 0.998 | 0.999 | 0.993 | 4 | | 2 | 0.035 | 0.029 | 0.033 | 0.036 | -8 |
| | 3 | 1.004 | 0.998 | 0.998 | 0.995 | 2 | | 3 | 0.033 | 0.029 | 0.033 | 0.036 | -7 |
| | Mean | 1.002 | 0.999 | 0.996 | 0.995 | 3 | | Mean | 0.034 | 0.029 | 0.033 | 0.036 | -8 |
| Mean for all assays | | - | - | - | - | 0 | Mean for all assays | | - | - | - | - | -5 |
| SD for all assays | | - | - | - | - | 2 | SD for all assays | | - | - | - | - | 1 |
| CV for all assays | | - | - | - | - | - | CV for all assays | | - | - | - | - | - |

*1 : decrease of A440 x$10^3$ = (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 exposure)

*2 : increase of A560 x$10^3$ = (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 exposure)

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Acridine

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-005 | 1 | 1.028 | 1.015 | 0.784 | 1.004 | 233 | US-005 | 1 | 0.055 | 0.029 | 0.283 | 0.043 | 217 | | |
| | 2 | 1.027 | 1.012 | 0.793 | 1.002 | 224 | | 2 | 0.033 | 0.043 | 0.293 | 0.044 | 249 | | |
| | 3 | 1.035 | 1.016 | 0.806 | 1.007 | 219 | | 3 | 0.053 | 0.029 | 0.282 | 0.046 | 218 | | |
| | Mean | 1.030 | 1.015 | 0.794 | 1.004 | 225 | | Mean | 0.047 | 0.033 | 0.286 | 0.044 | 228 | Positive | Photoreactive |
| US-025 | 1 | 0.980 | 0.990 | 0.728 | 0.969 | 231 | US-025 | 1 | 0.053 | 0.029 | 0.298 | 0.046 | 224 | | |
| | 2 | 1.007 | 1.013 | 0.759 | 0.988 | 226 | | 2 | 0.033 | 0.047 | 0.305 | 0.047 | 251 | | |
| | 3 | 1.019 | 1.011 | 0.755 | 0.993 | 242 | | 3 | 0.054 | 0.030 | 0.292 | 0.073 | 217 | | |
| | Mean | 1.002 | 1.005 | 0.747 | 0.983 | 233 | | Mean | 0.047 | 0.035 | 0.298 | 0.056 | 231 | Positive | Photoreactive |
| US-032 | 1 | 0.983 | 0.987 | 0.736 | 0.976 | 235 | US-032 | 1 | 0.053 | 0.028 | 0.286 | 0.045 | 214 | | |
| | 2 | 0.981 | 0.988 | 0.751 | 0.975 | 218 | | 2 | 0.042 | 0.045 | 0.279 | 0.045 | 216 | | |
| | 3 | 0.999 | 1.007 | 0.773 | 0.995 | 214 | | 3 | 0.053 | 0.028 | 0.287 | 0.072 | 215 | | |
| | Mean | 0.988 | 0.994 | 0.753 | 0.982 | 222 | | Mean | 0.049 | 0.034 | 0.284 | 0.054 | 215 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 227 | Mean for 3 assays | | - | - | - | - | 225 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Acridine HCl

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-005 | 1 | 1.012 | 1.015 | 0.764 | 1.004 | 236 | US-005 | 1 | 0.036 | 0.029 | 0.258 | 0.043 | 211 | | |
| | 2 | 1.015 | 1.012 | 0.779 | 1.002 | 224 | | 2 | 0.034 | 0.043 | 0.259 | 0.044 | 214 | | |
| | 3 | 1.019 | 1.016 | 0.796 | 1.007 | 212 | | 3 | 0.038 | 0.029 | 0.257 | 0.046 | 208 | | |
| | Mean | 1.015 | 1.015 | 0.780 | 1.004 | 224 | | Mean | 0.036 | 0.033 | 0.258 | 0.044 | 211 | Positive | Photoreactive |
| US-025 | 1 | 0.996 | 0.990 | 0.752 | 0.969 | 222 | US-025 | 1 | 0.034 | 0.029 | 0.272 | 0.046 | 217 | | |
| | 2 | 1.005 | 1.013 | 0.764 | 0.988 | 219 | | 2 | 0.031 | 0.047 | 0.266 | 0.047 | 214 | | |
| | 3 | 1.003 | 1.011 | 0.780 | 0.993 | 200 | | 3 | 0.036 | 0.030 | 0.271 | 0.073 | 214 | | |
| | Mean | 1.001 | 1.005 | 0.765 | 0.983 | 214 | | Mean | 0.034 | 0.035 | 0.269 | 0.056 | 215 | Positive | Photoreactive |
| US-031 | 1 | 0.998 | 0.986 | 0.758 | 0.973 | 229 | US-031 | 1 | 0.037 | 0.029 | 0.267 | 0.042 | 217 | | |
| | 2 | 1.003 | 0.985 | 0.769 | 0.979 | 224 | | 2 | 0.033 | 0.042 | 0.264 | 0.046 | 218 | | |
| | 3 | 0.998 | 0.995 | 0.773 | 0.985 | 215 | | 3 | 0.037 | 0.029 | 0.260 | 0.049 | 211 | | |
| | Mean | 0.999 | 0.989 | 0.767 | 0.979 | 223 | | Mean | 0.035 | 0.033 | 0.264 | 0.046 | 215 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 220 | Mean for 3 assays | | - | - | - | - | 214 | Positive [*3] | Photoreactive [*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Amiodarone HCl

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-025 | 1 | 1.028 | 0.990 | 0.968 | 0.969 | 38 | US-025 | 1 | 0.077 | 0.029 | 0.068 | 0.046 | -30 | | |
| | 2 | 1.024 | 1.013 | 0.972 | 0.988 | 30 | | 2 | 0.044 | 0.047 | 0.062 | 0.047 | -3 | | |
| | 3 | 1.035 | 1.011 | 0.982 | 0.993 | 31 | | 3 | 0.063 | 0.030 | 0.070 | 0.073 | -14 | | |
| | Mean | 1.029 | 1.005 | 0.974 | 0.983 | 33 | | Mean | 0.061 | 0.035 | 0.067 | 0.056 | -16 | Positive | (Photoreactive) |
| US-031 | 1 | 1.021 | 0.986 | 0.995 | 0.973 | 17 | US-031 | 1 | 0.074 | 0.029 | 0.062 | 0.042 | -26 | | |
| | 2 | 0.998 | 0.985 | 0.970 | 0.979 | 18 | | 2 | 0.042 | 0.042 | 0.062 | 0.046 | 7 | | |
| | 3 | 1.005 | 0.995 | 1.001 | 0.985 | -7 | | 3 | 0.077 | 0.029 | 0.071 | 0.049 | -19 | | |
| | Mean | 1.008 | 0.989 | 0.989 | 0.979 | 9 | | Mean | 0.065 | 0.033 | 0.065 | 0.046 | -13 | Inconclusive | (Non-photoreactive) |
| US-033 | 1 | 0.977 | 0.982 | 0.939 | 0.972 | 26 | US-033 | 1 | 0.036 | 0.033 | 0.047 | 0.041 | -9 | | |
| | 2 | 0.990 | 0.989 | 0.954 | 0.975 | 24 | | 2 | 0.034 | 0.034 | 0.054 | 0.051 | 0 | | |
| | 3 | 1.001 | 0.987 | 0.973 | 0.975 | 16 | | 3 | 0.038 | 0.028 | 0.052 | 0.064 | -6 | | |
| | Mean | 0.989 | 0.986 | 0.955 | 0.974 | 22 | | Mean | 0.036 | 0.032 | 0.051 | 0.052 | -5 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | 21 | Mean for 3 assays | | - | - | - | - | -11 | Inconclusive [*3] | (Non-photoreactive) [*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Chlorpromazine HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-001 | 1 | 1.020 | 1.014 | 1.028 | 1.006 | -18 | US-001 | 1 | 0.048 | 0.033 | 0.143 | 0.046 | 81 | | |
| | 2 | 1.002 | 1.028 | 1.009 | 1.014 | -17 | | 2 | 0.051 | 0.046 | 0.146 | 0.053 | 82 | | |
| | 3 | 1.017 | 1.029 | 1.024 | 1.020 | -18 | | 3 | 0.044 | 0.034 | 0.146 | 0.056 | 88 | | |
| | Mean | 1.013 | 1.024 | 1.020 | 1.013 | -18 | | Mean | 0.048 | 0.038 | 0.145 | 0.052 | 84 | Positive | Photoreactive |
| US-023 | 1 | 0.988 | 1.008 | 1.012 | 0.991 | -38 | US-023 | 1 | 0.042 | 0.033 | 0.149 | 0.039 | 101 | | |
| | 2 | 0.989 | 1.024 | 1.016 | 1.013 | -41 | | 2 | 0.038 | 0.049 | 0.154 | 0.044 | 109 | | |
| | 3 | 0.998 | 1.022 | 1.027 | 1.008 | -43 | | 3 | 0.040 | 0.029 | 0.154 | 0.051 | 107 | | |
| | Mean | 0.992 | 1.018 | 1.018 | 1.004 | -41 | | Mean | 0.040 | 0.037 | 0.152 | 0.044 | 106 | Positive | Photoreactive |
| US-029 | 1 | 0.956 | 0.973 | 0.960 | 0.960 | -13 | US-029 | 1 | 0.039 | 0.030 | 0.144 | 0.038 | 96 | | |
| | 2 | 0.968 | 0.986 | 0.974 | 0.979 | -16 | | 2 | 0.040 | 0.044 | 0.146 | 0.044 | 97 | | |
| | 3 | 0.976 | 0.987 | 0.981 | 0.978 | -15 | | 3 | 0.039 | 0.029 | 0.147 | 0.047 | 99 | | |
| | Mean | 0.967 | 0.982 | 0.971 | 0.972 | -15 | | Mean | 0.039 | 0.034 | 0.146 | 0.043 | 97 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | -25 | Mean for 3 assays | | - | - | - | - | 96 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Doxycycline HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-002 | 1 | 1.041 | 1.017 | 0.743 | 1.007 | 289 | US-002 | 1 | 0.062 | 0.031 | 0.412 | 0.038 | 344 | | |
| | 2 | 1.043 | 1.028 | 0.785 | 1.021 | 249 | | 2 | 0.067 | 0.043 | 0.436 | 0.041 | 362 | | |
| | 3 | 1.061 | 1.031 | 0.777 | 1.020 | 275 | | 3 | 0.063 | 0.030 | 0.424 | 0.048 | 354 | | |
| | Mean | 1.048 | 1.025 | 0.768 | 1.016 | 271 | | Mean | 0.064 | 0.035 | 0.424 | 0.042 | 353 | Positive | Photoreactive |
| US-004 | 1 | 1.039 | 0.998 | 0.734 | 0.987 | 295 | US-004 | 1 | 0.050 | 0.029 | 0.550 | 0.046 | 476 | | |
| | 2 | 1.016 | 1.027 | 0.764 | 1.019 | 243 | | 2 | 0.079 | 0.029 | 0.587 | 0.059 | 484 | | |
| | 3 | 1.049 | 1.021 | 0.769 | 1.010 | 270 | | 3 | 0.048 | 0.029 | 0.571 | 0.054 | 499 | | |
| | Mean | 1.035 | 1.015 | 0.756 | 1.005 | 269 | | Mean | 0.059 | 0.029 | 0.569 | 0.053 | 486 | Positive | Photoreactive |
| US-028 | 1 | 0.999 | 0.980 | 0.707 | 0.971 | 281 | US-028 | 1 | 0.054 | 0.029 | 0.415 | 0.051 | 335 | | |
| | 2 | 0.991 | 0.988 | 0.738 | 0.977 | 241 | | 2 | 0.063 | 0.030 | 0.433 | 0.053 | 344 | | |
| | 3 | 1.016 | 0.995 | 0.740 | 0.983 | 265 | | 3 | 0.054 | 0.028 | 0.428 | 0.061 | 347 | | |
| | Mean | 1.002 | 0.988 | 0.728 | 0.977 | 262 | | Mean | 0.057 | 0.029 | 0.425 | 0.055 | 342 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 267 | Mean for 3 assays | | - | - | - | - | 394 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Fenofibrate

Solubility
Singlet oxygen 200 μM : Precipitation 20 μM : Solution 2 μM : Not determined
Superoxide anion 200 μM : Precipitation 20 μM : Solution 2 μM : Not determined
Test concentration
Singlet oxygen 20 μM
Superoxide anion 20 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-027 | 1 | 0.961 | 0.970 | 0.827 | 0.965 | 126 | US-027 | 1 | 0.042 | 0.033 | 0.058 | 0.040 | 2 | | |
| | 2 | 0.975 | 0.979 | 0.844 | 0.970 | 123 | | 2 | 0.041 | 0.030 | 0.058 | 0.047 | 3 | | |
| | 3 | 0.979 | 0.978 | 0.847 | 0.969 | 124 | | 3 | 0.043 | 0.029 | 0.053 | 0.046 | -4 | | |
| | Mean | 0.972 | 0.976 | 0.840 | 0.968 | 124 | | Mean | 0.042 | 0.030 | 0.057 | 0.044 | 0 | Positive | Photoreactive |
| US-034 | 1 | 1.021 | 0.974 | 0.810 | 0.965 | 202 | US-034 | 1 | 0.069 | 0.036 | 0.049 | 0.049 | -44 | | |
| | 2 | 1.014 | 1.001 | 0.802 | 0.992 | 203 | | 2 | 0.057 | 0.042 | 0.049 | 0.045 | -33 | | |
| | 3 | 1.029 | 0.994 | 0.819 | 0.985 | 201 | | 3 | 0.067 | 0.029 | 0.050 | 0.085 | -41 | | |
| | Mean | 1.022 | 0.990 | 0.810 | 0.981 | 202 | | Mean | 0.064 | 0.036 | 0.049 | 0.060 | -39 | Positive | Photoreactive |
| US-036 | 1 | 0.995 | 0.977 | 0.807 | 0.965 | 178 | US-036 | 1 | 0.066 | 0.028 | 0.059 | 0.043 | -31 | | |
| | 2 | 0.997 | 0.990 | 0.817 | 0.984 | 170 | | 2 | 0.063 | 0.032 | 0.062 | 0.048 | -25 | | |
| | 3 | 1.001 | 0.988 | 0.819 | 0.977 | 172 | | 3 | 0.066 | 0.029 | 0.053 | 0.072 | -38 | | |
| | Mean | 0.998 | 0.985 | 0.814 | 0.975 | 173 | | Mean | 0.065 | 0.030 | 0.058 | 0.054 | -31 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 166 | Mean for 3 assays | | - | - | - | - | -23 | Positive *3 | Photoreactive*3 |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1

Chemical Name : Furosemide

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-007 | 1 | 1.008 | 1.006 | 0.773 | 0.991 | 224 | US-007 | 1 | 0.042 | 0.035 | 0.166 | 0.046 | 109 | | |
| | 2 | 1.024 | 1.015 | 0.784 | 1.005 | 229 | | 2 | 0.043 | 0.034 | 0.173 | 0.054 | 116 | | |
| | 3 | 1.028 | 1.010 | 0.791 | 1.002 | 227 | | 3 | 0.044 | 0.033 | 0.179 | 0.049 | 120 | | |
| | Mean | 1.020 | 1.010 | 0.783 | 0.999 | 227 | | Mean | 0.043 | 0.034 | 0.173 | 0.049 | 115 | Positive | Photoreactive |
| US-027 | 1 | 0.980 | 0.970 | 0.728 | 0.965 | 244 | US-027 | 1 | 0.041 | 0.033 | 0.172 | 0.040 | 118 | | |
| | 2 | 0.988 | 0.979 | 0.742 | 0.970 | 238 | | 2 | 0.043 | 0.030 | 0.178 | 0.047 | 122 | | |
| | 3 | 0.998 | 0.978 | 0.759 | 0.969 | 231 | | 3 | 0.045 | 0.029 | 0.181 | 0.046 | 122 | | |
| | Mean | 0.989 | 0.976 | 0.743 | 0.968 | 238 | | Mean | 0.043 | 0.030 | 0.177 | 0.044 | 121 | Positive | Photoreactive |
| US-034 | 1 | 0.993 | 0.974 | 0.744 | 0.965 | 240 | US-034 | 1 | 0.044 | 0.036 | 0.164 | 0.049 | 97 | | |
| | 2 | 0.988 | 1.001 | 0.760 | 0.992 | 219 | | 2 | 0.044 | 0.042 | 0.169 | 0.045 | 101 | | |
| | 3 | 0.997 | 0.994 | 0.776 | 0.985 | 212 | | 3 | 0.044 | 0.029 | 0.175 | 0.085 | 107 | | |
| | Mean | 0.993 | 0.990 | 0.760 | 0.981 | 224 | | Mean | 0.044 | 0.036 | 0.169 | 0.060 | 102 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 230 | Mean for 3 assays | | - | - | - | - | 113 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Ketoprofen |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-005 | 1 | 1.012 | 1.015 | 0.640 | 1.004 | 361 | US-005 | 1 | 0.048 | 0.029 | 0.185 | 0.043 | 126 | | |
| | 2 | 1.024 | 1.012 | 0.652 | 1.002 | 362 | | 2 | 0.037 | 0.043 | 0.177 | 0.044 | 129 | | |
| | 3 | 1.027 | 1.016 | 0.664 | 1.007 | 351 | | 3 | 0.044 | 0.029 | 0.190 | 0.046 | 135 | | |
| | Mean | 1.021 | 1.015 | 0.652 | 1.004 | 358 | | Mean | 0.043 | 0.033 | 0.184 | 0.044 | 130 | Positive | Photoreactive |
| US-025 | 1 | 1.014 | 0.990 | 0.627 | 0.969 | 365 | US-025 | 1 | 0.047 | 0.029 | 0.184 | 0.046 | 116 | | |
| | 2 | 1.017 | 1.013 | 0.629 | 0.988 | 366 | | 2 | 0.036 | 0.047 | 0.183 | 0.047 | 126 | | |
| | 3 | 1.025 | 1.011 | 0.648 | 0.993 | 355 | | 3 | 0.043 | 0.030 | 0.190 | 0.073 | 125 | | |
| | Mean | 1.019 | 1.005 | 0.635 | 0.983 | 362 | | Mean | 0.042 | 0.035 | 0.185 | 0.056 | 122 | Positive | Photoreactive |
| US-031 | 1 | 1.004 | 0.986 | 0.624 | 0.973 | 371 | US-031 | 1 | 0.064 | 0.029 | 0.195 | 0.042 | 119 | | |
| | 2 | 1.005 | 0.985 | 0.623 | 0.979 | 372 | | 2 | 0.040 | 0.042 | 0.216 | 0.046 | 163 | | |
| | 3 | 1.008 | 0.995 | 0.638 | 0.985 | 360 | | 3 | 0.058 | 0.029 | 0.199 | 0.049 | 129 | | |
| | Mean | 1.006 | 0.989 | 0.628 | 0.979 | 368 | | Mean | 0.054 | 0.033 | 0.204 | 0.046 | 137 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 363 | Mean for 3 assays | | - | - | - | - | 130 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | 6-methylcoumarine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-002 | 1 | 1.018 | 1.017 | 0.894 | 1.007 | 115 | US-002 | 1 | 0.073 | 0.031 | 0.174 | 0.038 | 94 | | |
| | 2 | 1.036 | 1.028 | 0.918 | 1.021 | 109 | | 2 | 0.040 | 0.043 | 0.173 | 0.041 | 126 | | |
| | 3 | 1.048 | 1.031 | 0.919 | 1.020 | 119 | | 3 | 0.069 | 0.030 | 0.181 | 0.048 | 106 | | |
| | Mean | 1.034 | 1.025 | 0.910 | 1.016 | 114 | | Mean | 0.061 | 0.035 | 0.176 | 0.042 | 109 | Positive | Photoreactive |
| US-004 | 1 | 1.039 | 0.998 | 0.907 | 0.987 | 123 | US-004 | 1 | 0.037 | 0.029 | 0.187 | 0.046 | 126 | | |
| | 2 | 1.038 | 1.027 | 0.922 | 1.019 | 106 | | 2 | 0.033 | 0.029 | 0.186 | 0.059 | 129 | | |
| | 3 | 1.038 | 1.021 | 0.923 | 1.010 | 105 | | 3 | 0.038 | 0.029 | 0.190 | 0.054 | 128 | | |
| | Mean | 1.038 | 1.015 | 0.917 | 1.005 | 111 | | Mean | 0.036 | 0.029 | 0.188 | 0.053 | 128 | Positive | Photoreactive |
| US-028 | 1 | 1.000 | 0.980 | 0.859 | 0.971 | 130 | US-028 | 1 | 0.040 | 0.029 | 0.197 | 0.051 | 131 | | |
| | 2 | 1.001 | 0.988 | 0.874 | 0.977 | 116 | | 2 | 0.038 | 0.030 | 0.192 | 0.053 | 128 | | |
| | 3 | 1.009 | 0.995 | 0.885 | 0.983 | 114 | | 3 | 0.040 | 0.028 | 0.197 | 0.061 | 131 | | |
| | Mean | 1.003 | 0.988 | 0.872 | 0.977 | 120 | | Mean | 0.039 | 0.029 | 0.195 | 0.055 | 130 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 115 | Mean for 3 assays | | - | - | - | - | 122 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | 8-MOP |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-006 | 1 | 1.022 | 1.020 | 0.924 | 1.010 | 87 | US-006 | 1 | 0.073 | 0.031 | 0.166 | 0.044 | 69 | | |
| | 2 | 1.023 | 1.026 | 0.929 | 1.011 | 83 | | 2 | 0.047 | 0.031 | 0.160 | 0.048 | 90 | | |
| | 3 | 1.021 | 1.017 | 0.930 | 1.008 | 80 | | 3 | 0.077 | 0.031 | 0.168 | 0.069 | 68 | | |
| | Mean | 1.022 | 1.021 | 0.928 | 1.010 | 83 | | Mean | 0.066 | 0.031 | 0.164 | 0.054 | 76 | Positive | Photoreactive |
| US-026 | 1 | 0.978 | 0.973 | 0.869 | 0.965 | 101 | US-026 | 1 | 0.037 | 0.032 | 0.191 | 0.040 | 142 | | |
| | 2 | 0.986 | 0.982 | 0.879 | 0.977 | 98 | | 2 | 0.034 | 0.038 | 0.186 | 0.049 | 140 | | |
| | 3 | 0.991 | 0.986 | 0.880 | 0.976 | 103 | | 3 | 0.043 | 0.029 | 0.187 | 0.046 | 132 | | |
| | Mean | 0.985 | 0.981 | 0.876 | 0.973 | 101 | | Mean | 0.038 | 0.033 | 0.188 | 0.045 | 138 | Positive | Photoreactive |
| US-032 | 1 | 0.974 | 0.987 | 0.883 | 0.976 | 79 | US-032 | 1 | 0.041 | 0.028 | 0.174 | 0.045 | 113 | | |
| | 2 | 0.987 | 0.988 | 0.890 | 0.975 | 85 | | 2 | 0.034 | 0.045 | 0.167 | 0.045 | 113 | | |
| | 3 | 1.000 | 1.007 | 0.917 | 0.995 | 71 | | 3 | 0.043 | 0.028 | 0.175 | 0.072 | 112 | | |
| | Mean | 0.987 | 0.994 | 0.897 | 0.982 | 78 | | Mean | 0.040 | 0.034 | 0.172 | 0.054 | 113 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 87 | Mean for 3 assays | | - | - | - | - | 109 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Nalidixic acid |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-006 | 1 | 0.997 | 1.020 | 0.622 | 1.010 | 364 | US-006 | 1 | 0.047 | 0.031 | 0.376 | 0.044 | 306 | | |
| | 2 | 0.996 | 1.026 | 0.645 | 1.011 | 340 | | 2 | 0.041 | 0.031 | 0.383 | 0.048 | 319 | | |
| | 3 | 1.008 | 1.017 | 0.656 | 1.008 | 341 | | 3 | 0.046 | 0.031 | 0.510 | 0.069 | 441 | | |
| | Mean | 1.000 | 1.021 | 0.641 | 1.010 | 348 | | Mean | 0.045 | 0.031 | 0.423 | 0.054 | 355 | Positive | Photoreactive |
| US-026 | 1 | 0.992 | 0.973 | 0.799 | 0.965 | 185 | US-026 | 1 | 0.062 | 0.032 | 0.327 | 0.040 | 254 | | |
| | 2 | 0.992 | 0.982 | 0.791 | 0.977 | 193 | | 2 | 0.033 | 0.038 | 0.319 | 0.049 | 274 | | |
| | 3 | 0.999 | 0.986 | 0.814 | 0.976 | 177 | | 3 | 0.061 | 0.029 | 0.357 | 0.046 | 284 | | |
| | Mean | 0.994 | 0.981 | 0.801 | 0.973 | 185 | | Mean | 0.052 | 0.033 | 0.335 | 0.045 | 271 | Positive | Photoreactive |
| US-034 | 1 | 0.985 | 0.974 | 0.784 | 0.965 | 192 | US-034 | 1 | 0.054 | 0.036 | 0.360 | 0.049 | 282 | | |
| | 2 | 0.990 | 1.001 | 0.798 | 0.992 | 183 | | 2 | 0.044 | 0.042 | 0.351 | 0.045 | 283 | | |
| | 3 | 1.000 | 0.994 | 0.821 | 0.985 | 170 | | 3 | 0.058 | 0.029 | 0.309 | 0.085 | 227 | | |
| | Mean | 0.991 | 0.990 | 0.801 | 0.981 | 182 | | Mean | 0.052 | 0.036 | 0.340 | 0.060 | 264 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 238 | Mean for 3 assays | | - | - | - | - | 297 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Nalidixic acid (Na salt) |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-006 | 1 | 1.016 | 1.020 | 0.812 | 1.010 | 193 | US-006 | 1 | 0.047 | 0.031 | 0.319 | 0.044 | 249 | | |
| | 2 | 1.022 | 1.026 | 0.836 | 1.011 | 175 | | 2 | 0.035 | 0.031 | 0.309 | 0.048 | 251 | | |
| | 3 | 1.047 | 1.017 | 0.854 | 1.008 | 182 | | 3 | 0.043 | 0.031 | 0.427 | 0.069 | 361 | | |
| | Mean | 1.028 | 1.021 | 0.834 | 1.010 | 183 | | Mean | 0.042 | 0.031 | 0.352 | 0.054 | 287 | Positive | Photoreactive |
| US-026 | 1 | 0.993 | 0.973 | 0.823 | 0.965 | 162 | US-026 | 1 | 0.058 | 0.032 | 0.288 | 0.040 | 219 | | |
| | 2 | 1.005 | 0.982 | 0.823 | 0.977 | 175 | | 2 | 0.039 | 0.038 | 0.300 | 0.049 | 249 | | |
| | 3 | 1.009 | 0.986 | 0.843 | 0.976 | 159 | | 3 | 0.071 | 0.029 | 0.314 | 0.046 | 231 | | |
| | Mean | 1.003 | 0.981 | 0.829 | 0.973 | 165 | | Mean | 0.056 | 0.033 | 0.301 | 0.045 | 233 | Positive | Photoreactive |
| US-032 | 1 | 0.993 | 0.987 | 0.790 | 0.976 | 191 | US-032 | 1 | 0.068 | 0.028 | 0.284 | 0.045 | 196 | | |
| | 2 | 0.992 | 0.988 | 0.804 | 0.975 | 176 | | 2 | 0.040 | 0.045 | 0.266 | 0.045 | 206 | | |
| | 3 | 1.008 | 1.007 | 0.814 | 0.995 | 181 | | 3 | 0.068 | 0.028 | 0.296 | 0.072 | 208 | | |
| | Mean | 0.998 | 0.994 | 0.803 | 0.982 | 183 | | Mean | 0.058 | 0.034 | 0.282 | 0.054 | 203 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 177 | Mean for 3 assays | | - | - | - | - | 241 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Norfloxacin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-002 | 1 | 1.022 | 1.017 | 0.791 | 1.007 | 222 | US-002 | 1 | 0.042 | 0.031 | 0.197 | 0.038 | 147 | | |
| | 2 | 1.040 | 1.028 | 0.812 | 1.021 | 219 | | 2 | 0.037 | 0.043 | 0.192 | 0.041 | 148 | | |
| | 3 | 1.039 | 1.031 | 0.824 | 1.020 | 205 | | 3 | 0.042 | 0.030 | 0.189 | 0.048 | 140 | | |
| | Mean | 1.034 | 1.025 | 0.809 | 1.016 | 215 | | Mean | 0.040 | 0.035 | 0.192 | 0.042 | 145 | Positive | Photoreactive |
| US-004 | 1 | 1.000 | 0.998 | 0.766 | 0.987 | 224 | US-004 | 1 | 0.053 | 0.029 | 0.216 | 0.046 | 139 | | |
| | 2 | 1.036 | 1.027 | 0.807 | 1.019 | 220 | | 2 | 0.044 | 0.029 | 0.214 | 0.059 | 147 | | |
| | 3 | 1.038 | 1.021 | 0.815 | 1.010 | 214 | | 3 | 0.058 | 0.029 | 0.212 | 0.054 | 131 | | |
| | Mean | 1.025 | 1.015 | 0.796 | 1.005 | 219 | | Mean | 0.051 | 0.029 | 0.214 | 0.053 | 139 | Positive | Photoreactive |
| US-028 | 1 | 0.984 | 0.980 | 0.754 | 0.971 | 220 | US-028 | 1 | 0.056 | 0.029 | 0.199 | 0.051 | 117 | | |
| | 2 | 0.992 | 0.988 | 0.765 | 0.977 | 216 | | 2 | 0.045 | 0.030 | 0.192 | 0.053 | 120 | | |
| | 3 | 0.997 | 0.995 | 0.781 | 0.983 | 205 | | 3 | 0.060 | 0.028 | 0.188 | 0.061 | 102 | | |
| | Mean | 0.991 | 0.988 | 0.767 | 0.977 | 214 | | Mean | 0.054 | 0.029 | 0.193 | 0.055 | 113 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 216 | Mean for 3 assays | | - | - | - | - | 132 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Ofloxacin

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-002 | 1 | 1.026 | 1.017 | 0.813 | 1.007 | 204 | US-002 | 1 | 0.046 | 0.031 | 0.388 | 0.038 | 335 | | |
| | 2 | 1.015 | 1.028 | 0.814 | 1.021 | 192 | | 2 | 0.044 | 0.043 | 0.389 | 0.041 | 338 | | |
| | 3 | 1.043 | 1.031 | 0.852 | 1.020 | 182 | | 3 | 0.048 | 0.030 | 0.434 | 0.048 | 379 | | |
| | Mean | 1.028 | 1.025 | 0.826 | 1.016 | 193 | | Mean | 0.046 | 0.035 | 0.403 | 0.042 | 351 | Positive | Photoreactive |
| US-004 | 1 | 1.002 | 0.998 | 0.776 | 0.987 | 216 | US-004 | 1 | 0.060 | 0.029 | 0.296 | 0.046 | 212 | | |
| | 2 | 1.027 | 1.027 | 0.814 | 1.019 | 203 | | 2 | 0.041 | 0.029 | 0.303 | 0.059 | 238 | | |
| | 3 | 1.031 | 1.021 | 0.830 | 1.010 | 191 | | 3 | 0.059 | 0.029 | 0.316 | 0.054 | 233 | | |
| | Mean | 1.020 | 1.015 | 0.807 | 1.005 | 203 | | Mean | 0.053 | 0.029 | 0.305 | 0.053 | 228 | Positive | Photoreactive |
| US-028 | 1 | 0.986 | 0.980 | 0.769 | 0.971 | 206 | US-028 | 1 | 0.062 | 0.029 | 0.359 | 0.051 | 271 | | |
| | 2 | 0.989 | 0.988 | 0.783 | 0.977 | 194 | | 2 | 0.044 | 0.030 | 0.359 | 0.053 | 289 | | |
| | 3 | 0.993 | 0.995 | 0.805 | 0.983 | 177 | | 3 | 0.056 | 0.028 | 0.345 | 0.061 | 262 | | |
| | Mean | 0.989 | 0.988 | 0.786 | 0.977 | 192 | | Mean | 0.054 | 0.029 | 0.354 | 0.055 | 274 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 196 | Mean for 3 assays | | - | - | - | - | 284 | Positive *3 | Photoreactive*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Piroxicam |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-006 | 1 | 1.010 | 1.020 | 0.793 | 1.010 | 206 | US-0026 | 1 | 0.045 | 0.032 | 0.088 | 0.040 | 32 | | |
| | 2 | 1.027 | 1.026 | 0.824 | 1.011 | 191 | | 2 | 0.063 | 0.038 | 0.101 | 0.049 | 26 | | |
| | 3 | 1.033 | 1.017 | 0.846 | 1.008 | 177 | | 3 | 0.058 | 0.029 | 0.097 | 0.046 | 26 | | |
| | Mean | 1.023 | 1.021 | 0.821 | 1.010 | 191 | | Mean | 0.055 | 0.033 | 0.095 | 0.045 | 28 | Positive | Photoreactive |
| US-026 | 1 | 0.995 | 0.973 | 0.761 | 0.965 | 226 | US-0032 | 1 | 0.057 | 0.028 | 0.113 | 0.045 | 35 | | |
| | 2 | 0.999 | 0.982 | 0.792 | 0.977 | 200 | | 2 | 0.070 | 0.045 | 0.120 | 0.045 | 30 | | |
| | 3 | 1.004 | 0.986 | 0.793 | 0.976 | 203 | | 3 | 0.050 | 0.028 | 0.118 | 0.072 | 48 | | |
| | Mean | 0.999 | 0.981 | 0.782 | 0.973 | 210 | | Mean | 0.059 | 0.034 | 0.117 | 0.054 | 38 | Positive | Photoreactive |
| US-032 | 1 | 1.000 | 0.987 | 0.736 | 0.976 | 252 | US-033 | 1 | 0.039 | 0.033 | 0.088 | 0.041 | 29 | | |
| | 2 | 0.981 | 0.988 | 0.758 | 0.975 | 211 | | 2 | 0.051 | 0.034 | 0.092 | 0.051 | 21 | | |
| | 3 | 1.010 | 1.007 | 0.764 | 0.995 | 234 | | 3 | 0.047 | 0.028 | 0.094 | 0.064 | 27 | | |
| | Mean | 0.997 | 0.994 | 0.753 | 0.982 | 232 | | Mean | 0.045 | 0.032 | 0.091 | 0.052 | 26 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 211 | Mean for 3 assays | | - | - | - | - | 31 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Promethazine HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-001 | 1 | 1.002 | 1.014 | 0.911 | 1.006 | 80 | US-001 | 1 | 0.041 | 0.033 | 0.121 | 0.046 | 66 | | |
| | 2 | 1.017 | 1.028 | 0.934 | 1.014 | 72 | | 2 | 0.037 | 0.046 | 0.119 | 0.053 | 68 | | |
| | 3 | 1.008 | 1.029 | 0.939 | 1.020 | 59 | | 3 | 0.043 | 0.034 | 0.123 | 0.056 | 67 | | |
| | Mean | 1.009 | 1.024 | 0.928 | 1.013 | 70 | | Mean | 0.040 | 0.038 | 0.121 | 0.052 | 67 | Positive | Photoreactive |
| US-023 | 1 | 1.001 | 1.008 | 0.921 | 0.991 | 67 | US-023 | 1 | 0.040 | 0.033 | 0.129 | 0.039 | 82 | | |
| | 2 | 1.005 | 1.024 | 0.933 | 1.013 | 59 | | 2 | 0.034 | 0.049 | 0.127 | 0.044 | 86 | | |
| | 3 | 1.005 | 1.022 | 0.933 | 1.008 | 59 | | 3 | 0.041 | 0.029 | 0.136 | 0.051 | 89 | | |
| | Mean | 1.004 | 1.018 | 0.929 | 1.004 | 62 | | Mean | 0.038 | 0.037 | 0.131 | 0.044 | 86 | Positive | Photoreactive |
| US-029 | 1 | 0.971 | 0.973 | 0.872 | 0.960 | 89 | US-029 | 1 | 0.038 | 0.030 | 0.129 | 0.038 | 81 | | |
| | 2 | 0.984 | 0.986 | 0.894 | 0.979 | 80 | | 2 | 0.033 | 0.044 | 0.131 | 0.044 | 89 | | |
| | 3 | 0.994 | 0.987 | 0.900 | 0.978 | 84 | | 3 | 0.038 | 0.029 | 0.136 | 0.047 | 89 | | |
| | Mean | 0.983 | 0.982 | 0.889 | 0.972 | 84 | | Mean | 0.036 | 0.034 | 0.132 | 0.043 | 86 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 72 | Mean for 3 assays | | - | - | - | - | 80 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Rosiglitazone |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-003 | 1 | 1.000 | 1.011 | 0.861 | 1.001 | 129 | US-0024 | 1 | 0.043 | 0.030 | 0.099 | 0.045 | 40 | | |
| | 2 | 0.991 | 1.028 | 0.866 | 1.017 | 115 | | 2 | 0.037 | 0.040 | 0.098 | 0.052 | 45 | | |
| | 3 | 1.003 | 1.024 | 0.883 | 1.016 | 110 | | 3 | 0.044 | 0.029 | 0.096 | 0.051 | 37 | | |
| | Mean | 0.998 | 1.021 | 0.870 | 1.011 | 118 | | Mean | 0.041 | 0.033 | 0.098 | 0.049 | 41 | Positive | |
| US-024 | 1 | 0.966 | 1.014 | 0.846 | 1.003 | 107 | US-030 | 1 | 0.044 | 0.029 | 0.100 | 0.047 | 35 | | |
| | 2 | 0.995 | 1.023 | 0.876 | 1.009 | 105 | | 2 | 0.040 | 0.043 | 0.100 | 0.051 | 39 | | |
| | 3 | 0.994 | 1.023 | 0.880 | 1.006 | 100 | | 3 | 0.045 | 0.030 | 0.100 | 0.066 | 34 | | |
| | Mean | 0.985 | 1.020 | 0.868 | 1.006 | 104 | | Mean | 0.043 | 0.034 | 0.100 | 0.055 | 36 | Positive | |
| US-030 | 1 | 0.979 | 0.997 | 0.844 | 1.002 | 137 | US-035 | 1 | 0.041 | 0.034 | 0.092 | 0.045 | 29 | | |
| | 2 | 0.965 | 1.003 | 0.841 | 0.996 | 125 | | 2 | 0.041 | 0.030 | 0.092 | 0.048 | 29 | | |
| | 3 | 0.978 | 1.001 | 0.847 | 1.005 | 132 | | 3 | 0.042 | 0.028 | 0.092 | 0.066 | 28 | | |
| | Mean | 0.974 | 1.000 | 0.844 | 1.001 | 131 | | Mean | 0.041 | 0.031 | 0.092 | 0.053 | 29 | Positive | |
| Mean for 3 assays | | - | - | - | - | 118 | Mean for 3 assays | | - | - | - | - | 35 | Positive [*3] | |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Tetracycline |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-001 | 1 | 1.039 | 1.014 | 0.810 | 1.006 | 217 | US-001 | 1 | 0.060 | 0.033 | 0.271 | 0.046 | 197 | | |
| | 2 | 1.037 | 1.028 | 0.840 | 1.014 | 186 | | 2 | 0.058 | 0.046 | 0.266 | 0.053 | 194 | | |
| | 3 | 1.063 | 1.029 | 0.853 | 1.020 | 198 | | 3 | 0.056 | 0.034 | 0.269 | 0.056 | 200 | | |
| | Mean | 1.046 | 1.024 | 0.835 | 1.013 | 200 | | Mean | 0.058 | 0.038 | 0.269 | 0.052 | 197 | Positive | Photoreactive |
| US-023 | 1 | 1.026 | 1.008 | 0.807 | 0.991 | 205 | US-023 | 1 | 0.050 | 0.033 | 0.268 | 0.039 | 211 | | |
| | 2 | 1.011 | 1.024 | 0.812 | 1.013 | 185 | | 2 | 0.047 | 0.049 | 0.267 | 0.044 | 214 | | |
| | 3 | 1.039 | 1.022 | 0.833 | 1.008 | 192 | | 3 | 0.051 | 0.029 | 0.280 | 0.051 | 222 | | |
| | Mean | 1.025 | 1.018 | 0.817 | 1.004 | 194 | | Mean | 0.049 | 0.037 | 0.272 | 0.044 | 216 | Positive | Photoreactive |
| US-029 | 1 | 1.029 | 0.973 | 0.781 | 0.960 | 237 | US-029 | 1 | 0.054 | 0.030 | 0.274 | 0.038 | 212 | | |
| | 2 | 1.038 | 0.986 | 0.834 | 0.979 | 194 | | 2 | 0.048 | 0.044 | 0.280 | 0.044 | 224 | | |
| | 3 | 1.052 | 0.987 | 0.874 | 0.978 | 168 | | 3 | 0.052 | 0.029 | 0.279 | 0.047 | 218 | | |
| | Mean | 1.039 | 0.982 | 0.830 | 0.972 | 200 | | Mean | 0.051 | 0.034 | 0.278 | 0.043 | 218 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 198 | Mean for 3 assays | | - | - | - | - | 210 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Anthracene |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-024 | 1 | 1.088 | 1.014 | 0.790 | 1.003 | 284 | US-024 | 1 | 0.040 | 0.030 | 0.102 | 0.045 | 46 | | |
| | 2 | 1.094 | 1.023 | 0.819 | 1.009 | 261 | | 2 | 0.033 | 0.040 | 0.090 | 0.052 | 41 | | |
| | 3 | 1.099 | 1.023 | 0.849 | 1.006 | 237 | | 3 | 0.040 | 0.029 | 0.087 | 0.051 | 31 | | |
| | Mean | 1.094 | 1.020 | 0.819 | 1.006 | 261 | | Mean | 0.038 | 0.033 | 0.093 | 0.049 | 39 | Positive | Photoreactive |
| US-030 | 1 | 1.068 | 0.997 | 0.717 | 1.002 | 353 | US-030 | 1 | 0.059 | 0.029 | 0.106 | 0.047 | 26 | | |
| | 2 | 1.065 | 1.003 | 0.715 | 0.996 | 352 | | 2 | 0.033 | 0.043 | 0.146 | 0.051 | 92 | | |
| | 3 | 1.073 | 1.001 | 0.758 | 1.005 | 316 | | 3 | 0.061 | 0.030 | 0.107 | 0.066 | 25 | | |
| | Mean | 1.069 | 1.000 | 0.730 | 1.001 | 340 | | Mean | 0.051 | 0.034 | 0.120 | 0.055 | 48 | Positive | Photoreactive |
| US-033 | 1 | 1.056 | 0.982 | 0.755 | 0.972 | 289 | US-033 | 1 | 0.048 | 0.033 | 0.067 | 0.041 | 0 | | |
| | 2 | 1.078 | 0.989 | 0.786 | 0.975 | 280 | | 2 | 0.038 | 0.034 | 0.060 | 0.051 | 2 | | |
| | 3 | 1.074 | 0.987 | 0.814 | 0.975 | 248 | | 3 | 0.050 | 0.028 | 0.073 | 0.064 | 2 | | |
| | Mean | 1.069 | 0.986 | 0.785 | 0.974 | 272 | | Mean | 0.045 | 0.032 | 0.067 | 0.052 | 1 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 291 | Mean for 3 assays | | - | - | - | - | 29 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Avobenzone |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-029 | 1 | 1.079 | 0.973 | 0.923 | 0.960 | 146 | US-029 | 1 | 0.059 | 0.030 | 0.100 | 0.038 | 32 | | |
| | 2 | 1.091 | 0.986 | 0.943 | 0.979 | 138 | | 2 | 0.034 | 0.044 | 0.135 | 0.044 | 92 | | |
| | 3 | 1.076 | 0.987 | 0.925 | 0.978 | 142 | | 3 | 0.065 | 0.029 | 0.107 | 0.047 | 33 | | |
| | Mean | 1.082 | 0.982 | 0.930 | 0.972 | 142 | | Mean | 0.053 | 0.034 | 0.114 | 0.043 | 52 | Positive | Photoreactive |
| US-035 | 1 | 1.074 | 0.982 | 0.951 | 0.975 | 114 | US-035 | 1 | 0.063 | 0.034 | 0.101 | 0.045 | 17 | | |
| | 2 | 1.077 | 0.985 | 0.938 | 0.974 | 130 | | 2 | 0.039 | 0.030 | 0.101 | 0.048 | 40 | | |
| | 3 | 1.089 | 0.985 | 0.962 | 0.976 | 118 | | 3 | 0.075 | 0.028 | 0.098 | 0.066 | 1 | | |
| | Mean | 1.080 | 0.984 | 0.950 | 0.975 | 121 | | Mean | 0.059 | 0.031 | 0.100 | 0.053 | 19 | Positive | Photoreactive |
| US-036 | 1 | 1.068 | 0.977 | 0.933 | 0.965 | 125 | US-036 | 1 | 0.053 | 0.028 | 0.121 | 0.043 | 43 | | |
| | 2 | 1.063 | 0.990 | 0.938 | 0.984 | 115 | | 2 | 0.044 | 0.032 | 0.102 | 0.048 | 34 | | |
| | 3 | 1.072 | 0.988 | 0.951 | 0.977 | 111 | | 3 | 0.062 | 0.029 | 0.104 | 0.072 | 18 | | |
| | Mean | 1.067 | 0.985 | 0.941 | 0.975 | 117 | | Mean | 0.053 | 0.030 | 0.109 | 0.054 | 32 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 127 | Mean for 3 assays | | - | - | - | - | 34 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Bithionol |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-002 | 1 | 0.979 | 1.017 | 0.886 | 1.007 | 84 | US-028 | 1 | 0.061 | 0.029 | 0.106 | 0.051 | 19 | | |
| | 2 | 0.997 | 1.028 | 0.907 | 1.021 | 81 | | 2 | 0.058 | 0.030 | 0.109 | 0.053 | 25 | | |
| | 3 | 0.994 | 1.031 | 0.906 | 1.020 | 79 | | 3 | 0.063 | 0.028 | 0.107 | 0.061 | 18 | | |
| | Mean | 0.990 | 1.025 | 0.900 | 1.016 | 81 | | Mean | 0.060 | 0.029 | 0.107 | 0.055 | 21 | Positive | Photoreactive |
| US-004 | 1 | 0.989 | 0.998 | 0.855 | 0.987 | 125 | US-030 | 1 | 0.070 | 0.029 | 0.115 | 0.047 | 23 | | |
| | 2 | 0.996 | 1.027 | 0.880 | 1.019 | 106 | | 2 | 0.060 | 0.043 | 0.120 | 0.051 | 39 | | |
| | 3 | 1.003 | 1.021 | 0.884 | 1.010 | 109 | | 3 | 0.073 | 0.030 | 0.115 | 0.066 | 21 | | |
| | Mean | 0.996 | 1.015 | 0.873 | 1.005 | 113 | | Mean | 0.068 | 0.034 | 0.116 | 0.055 | 28 | Positive | Photoreactive |
| US-028 | 1 | 0.976 | 0.980 | 0.847 | 0.971 | 118 | US-035 | 1 | 0.074 | 0.034 | 0.114 | 0.045 | 18 | | |
| | 2 | 0.968 | 0.988 | 0.856 | 0.977 | 102 | | 2 | 0.063 | 0.030 | 0.114 | 0.048 | 29 | | |
| | 3 | 0.986 | 0.995 | 0.855 | 0.983 | 121 | | 3 | 0.064 | 0.028 | 0.112 | 0.066 | 26 | | |
| | Mean | 0.977 | 0.988 | 0.852 | 0.977 | 114 | | Mean | 0.067 | 0.031 | 0.113 | 0.053 | 24 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 103 | Mean for 3 assays | | - | - | - | - | 24 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Hexachlorophene

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-001 | 1 | 0.958 | 1.014 | 0.622 | 1.006 | 325 | US-001 | 1 | 0.085 | 0.033 | 0.095 | 0.046 | -3 | | |
| | 2 | 0.968 | 1.028 | 0.642 | 1.014 | 315 | | 2 | 0.063 | 0.046 | 0.096 | 0.053 | 19 | | |
| | 3 | 0.975 | 1.029 | 0.651 | 1.020 | 313 | | 3 | 0.084 | 0.034 | 0.099 | 0.056 | 1 | | |
| | Mean | 0.967 | 1.024 | 0.638 | 1.013 | 318 | | Mean | 0.077 | 0.038 | 0.097 | 0.052 | 6 | Positive | Photoreactive |
| US-023 | 1 | 0.973 | 1.008 | 0.615 | 0.991 | 344 | US-023 | 1 | 0.068 | 0.033 | 0.096 | 0.039 | 21 | | |
| | 2 | 0.955 | 1.024 | 0.619 | 1.013 | 322 | | 2 | 0.055 | 0.049 | 0.086 | 0.044 | 24 | | |
| | 3 | 0.963 | 1.022 | 0.616 | 1.008 | 332 | | 3 | 0.071 | 0.029 | 0.098 | 0.051 | 20 | | |
| | Mean | 0.964 | 1.018 | 0.617 | 1.004 | 333 | | Mean | 0.065 | 0.037 | 0.093 | 0.044 | 22 | Positive | Photoreactive |
| US-029 | 1 | 0.912 | 0.973 | 0.559 | 0.960 | 343 | US-029 | 1 | 0.069 | 0.030 | 0.074 | 0.038 | -3 | | |
| | 2 | 0.940 | 0.986 | 0.577 | 0.979 | 352 | | 2 | 0.045 | 0.044 | 0.078 | 0.044 | 24 | | |
| | 3 | 0.963 | 0.987 | 0.582 | 0.978 | 370 | | 3 | 0.068 | 0.029 | 0.077 | 0.047 | 0 | | |
| | Mean | 0.938 | 0.982 | 0.573 | 0.972 | 355 | | Mean | 0.061 | 0.034 | 0.077 | 0.043 | 7 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 335 | Mean for 3 assays | | - | - | - | - | 12 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Rose bengal |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-001 | 1 | 1.235 | 1.014 | 0.527 | 1.006 | 697 | US-001 | 1 | 3.648 | 0.033 | 3.165 | 0.046 | No data[*4] | | |
| | 2 | 1.227 | 1.028 | 0.550 | 1.014 | 666 | | 2 | 3.650 | 0.046 | 3.200 | 0.053 | No data[*4] | | |
| | 3 | 1.249 | 1.029 | 0.556 | 1.020 | 682 | | 3 | 3.634 | 0.034 | 3.160 | 0.056 | No data[*4] | | |
| | Mean | 1.237 | 1.024 | 0.544 | 1.013 | 682 | | Mean | 3.644 | 0.038 | 3.175 | 0.052 | No data[*4] | Positive | Photoreactive |
| US-023 | 1 | 1.255 | 1.008 | 0.544 | 0.991 | 697 | US-023 | 1 | 3.607 | 0.033 | 3.262 | 0.039 | No data[*4] | | |
| | 2 | 1.260 | 1.024 | 0.571 | 1.013 | 675 | | 2 | 3.630 | 0.049 | 3.287 | 0.044 | No data[*4] | | |
| | 3 | 1.270 | 1.022 | 0.574 | 1.008 | 682 | | 3 | 3.589 | 0.029 | 3.211 | 0.051 | No data[*4] | | |
| | Mean | 1.262 | 1.018 | 0.563 | 1.004 | 685 | | Mean | 3.609 | 0.037 | 3.253 | 0.044 | No data[*4] | Positive | Photoreactive |
| US-029 | 1 | 1.210 | 0.973 | 0.511 | 0.960 | 689 | US-029 | 1 | 3.667 | 0.030 | 3.171 | 0.038 | No data[*4] | | |
| | 2 | 1.219 | 0.986 | 0.547 | 0.979 | 662 | | 2 | 3.669 | 0.044 | 3.266 | 0.044 | No data[*4] | | |
| | 3 | 1.246 | 0.987 | 0.550 | 0.978 | 686 | | 3 | 3.645 | 0.029 | 3.172 | 0.047 | No data[*4] | | |
| | Mean | 1.225 | 0.982 | 0.536 | 0.972 | 679 | | Mean | 3.660 | 0.034 | 3.203 | 0.043 | No data[*4] | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 682 | Mean for 3 assays | | - | - | - | - | No data[*4] | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

*4 : Over the OD criteria

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Aspirin

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-007 | 1 | 1.022 | 1.006 | 1.012 | 0.991 | -1 | US-007 | 1 | 0.055 | 0.035 | 0.054 | 0.046 | -16 | | |
| | 2 | 1.023 | 1.015 | 1.010 | 1.005 | 3 | | 2 | 0.039 | 0.034 | 0.045 | 0.054 | -9 | | |
| | 3 | 1.031 | 1.010 | 1.020 | 1.002 | 0 | | 3 | 0.052 | 0.033 | 0.056 | 0.049 | -12 | | |
| | Mean | 1.026 | 1.010 | 1.014 | 0.999 | 1 | | Mean | 0.049 | 0.034 | 0.052 | 0.049 | -12 | Negative | Non-photoreactive |
| US-027 | 1 | 0.977 | 0.970 | 0.968 | 0.965 | 1 | US-027 | 1 | 0.051 | 0.033 | 0.047 | 0.040 | -18 | | |
| | 2 | 0.988 | 0.979 | 0.975 | 0.970 | 5 | | 2 | 0.040 | 0.030 | 0.044 | 0.047 | -9 | | |
| | 3 | 0.996 | 0.978 | 0.987 | 0.969 | 0 | | 3 | 0.053 | 0.029 | 0.050 | 0.046 | -17 | | |
| | Mean | 0.987 | 0.976 | 0.977 | 0.968 | 2 | | Mean | 0.048 | 0.030 | 0.047 | 0.044 | -15 | Negative | Non-photoreactive |
| US-034 | 1 | 0.975 | 0.974 | 0.962 | 0.965 | 4 | US-034 | 1 | 0.038 | 0.036 | 0.081 | 0.049 | 19 | | |
| | 2 | 0.996 | 1.001 | 0.982 | 0.992 | 5 | | 2 | 0.033 | 0.042 | 0.062 | 0.045 | 5 | | |
| | 3 | 0.995 | 0.994 | 0.983 | 0.985 | 3 | | 3 | 0.037 | 0.029 | 0.067 | 0.085 | 6 | | |
| | Mean | 0.989 | 0.990 | 0.976 | 0.981 | 4 | | Mean | 0.036 | 0.036 | 0.070 | 0.060 | 10 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 2 | Mean for 3 assays | | - | - | - | - | -6 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Benzocaine

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-002 | 1 | 1.029 | 1.017 | 1.016 | 1.007 | 4 | US-002 | 1 | 0.040 | 0.031 | 0.055 | 0.038 | 7 | | |
| | 2 | 1.039 | 1.028 | 1.020 | 1.021 | 10 | | 2 | 0.035 | 0.043 | 0.050 | 0.041 | 8 | | |
| | 3 | 1.040 | 1.031 | 1.025 | 1.020 | 5 | | 3 | 0.042 | 0.030 | 0.061 | 0.048 | 12 | | |
| | Mean | 1.036 | 1.025 | 1.020 | 1.016 | 6 | | Mean | 0.039 | 0.035 | 0.055 | 0.042 | 9 | Negative | Non-photoreactive |
| US-004 | 1 | 1.000 | 0.998 | 0.988 | 0.987 | 2 | US-004 | 1 | 0.053 | 0.029 | 0.095 | 0.046 | 18 | | |
| | 2 | 1.024 | 1.027 | 1.008 | 1.019 | 6 | | 2 | 0.041 | 0.029 | 0.091 | 0.059 | 26 | | |
| | 3 | 1.034 | 1.021 | 1.022 | 1.010 | 2 | | 3 | 0.056 | 0.029 | 0.096 | 0.054 | 15 | | |
| | Mean | 1.019 | 1.015 | 1.006 | 1.005 | 3 | | Mean | 0.050 | 0.029 | 0.094 | 0.053 | 20 | Positive | Weakly photoreactive |
| US-028 | 1 | 0.995 | 0.980 | 0.987 | 0.971 | -3 | US-028 | 1 | 0.053 | 0.029 | 0.076 | 0.051 | -3 | | |
| | 2 | 0.997 | 0.988 | 0.984 | 0.977 | 2 | | 2 | 0.041 | 0.030 | 0.067 | 0.053 | -1 | | |
| | 3 | 1.001 | 0.995 | 0.996 | 0.983 | -5 | | 3 | 0.052 | 0.028 | 0.081 | 0.061 | 3 | | |
| | Mean | 0.998 | 0.988 | 0.989 | 0.977 | -2 | | Mean | 0.049 | 0.029 | 0.075 | 0.055 | 0 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 2 | Mean for 3 assays | | - | - | - | - | 10 | Negative *3 | Non-photoreactive*3 |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Erythromycin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by criteria for Proposed Protocol |
| US-003 | 1 | 1.021 | 1.011 | 1.024 | 1.001 | -13 | US-003 | 1 | 0.058 | 0.029 | 0.085 | 0.049 | 4 | | |
| | 2 | 1.020 | 1.028 | 1.029 | 1.017 | -18 | | 2 | 0.051 | 0.044 | 0.078 | 0.052 | 4 | | |
| | 3 | 1.035 | 1.024 | 1.040 | 1.016 | -16 | | 3 | 0.059 | 0.030 | 0.085 | 0.070 | 4 | | |
| | Mean | 1.025 | 1.021 | 1.031 | 1.011 | -16 | | Mean | 0.056 | 0.034 | 0.082 | 0.057 | 4 | Negative | Non-photoreactive |
| US-024 | 1 | 0.996 | 1.014 | 0.976 | 1.003 | 6 | US-024 | 1 | 0.051 | 0.030 | 0.073 | 0.045 | 6 | | |
| | 2 | 0.999 | 1.023 | 0.980 | 1.009 | 5 | | 2 | 0.041 | 0.040 | 0.069 | 0.052 | 11 | | |
| | 3 | 1.002 | 1.023 | 0.984 | 1.006 | 4 | | 3 | 0.050 | 0.029 | 0.073 | 0.051 | 7 | | |
| | Mean | 0.999 | 1.020 | 0.980 | 1.006 | 5 | | Mean | 0.047 | 0.033 | 0.071 | 0.049 | 8 | Negative | Non-photoreactive |
| US-030 | 1 | 1.001 | 0.997 | 0.994 | 1.002 | 9 | US-030 | 1 | 0.046 | 0.029 | 0.072 | 0.047 | 5 | | |
| | 2 | 0.995 | 1.003 | 0.991 | 0.996 | 5 | | 2 | 0.041 | 0.043 | 0.071 | 0.051 | 9 | | |
| | 3 | 1.016 | 1.001 | 1.006 | 1.005 | 10 | | 3 | 0.049 | 0.030 | 0.075 | 0.066 | 5 | | |
| | Mean | 1.004 | 1.000 | 0.997 | 1.001 | 8 | | Mean | 0.045 | 0.034 | 0.073 | 0.055 | 6 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -1 | Mean for 3 assays | | - | - | - | - | 6 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Penicillin G |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-003 | 1 | 1.046 | 1.011 | 1.015 | 1.001 | 21 | US-003 | 1 | 0.064 | 0.029 | 0.097 | 0.049 | 10 | | |
| | 2 | 1.036 | 1.028 | 1.017 | 1.017 | 9 | | 2 | 0.049 | 0.044 | 0.096 | 0.052 | 24 | | |
| | 3 | 1.048 | 1.024 | 1.017 | 1.016 | 21 | | 3 | 0.076 | 0.030 | 0.097 | 0.070 | -2 | | |
| | Mean | 1.043 | 1.021 | 1.016 | 1.011 | 17 | | Mean | 0.063 | 0.034 | 0.097 | 0.057 | 11 | Negative | Non-photoreactive |
| US-024 | 1 | 1.007 | 1.014 | 0.987 | 1.003 | 6 | US-024 | 1 | 0.078 | 0.030 | 0.106 | 0.045 | 13 | | |
| | 2 | 1.016 | 1.023 | 0.994 | 1.009 | 8 | | 2 | 0.049 | 0.040 | 0.110 | 0.052 | 46 | | |
| | 3 | 1.033 | 1.023 | 1.015 | 1.006 | 4 | | 3 | 0.071 | 0.029 | 0.107 | 0.051 | 20 | | |
| | Mean | 1.019 | 1.020 | 0.999 | 1.006 | 6 | | Mean | 0.066 | 0.033 | 0.108 | 0.049 | 26 | Positive | Weakly photoreactive |
| US-030 | 1 | 1.012 | 0.997 | 1.006 | 1.002 | 6 | US-030 | 1 | 0.053 | 0.029 | 0.110 | 0.047 | 36 | | |
| | 2 | 1.008 | 1.003 | 0.996 | 0.996 | 13 | | 2 | 0.045 | 0.043 | 0.108 | 0.051 | 43 | | |
| | 3 | 1.014 | 1.001 | 1.008 | 1.005 | 7 | | 3 | 0.049 | 0.030 | 0.112 | 0.066 | 42 | | |
| | Mean | 1.011 | 1.000 | 1.003 | 1.001 | 9 | | Mean | 0.049 | 0.034 | 0.110 | 0.055 | 40 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 11 | Mean for 3 assays | | - | - | - | - | 26 | Positive [*3] | Weakly photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Phenytoin

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-007 | 1 | 1.019 | 1.006 | 1.006 | 0.991 | 2 | US-007 | 1 | 0.047 | 0.035 | 0.115 | 0.046 | 53 | | |
| | 2 | 1.016 | 1.015 | 1.006 | 1.005 | -1 | | 2 | 0.039 | 0.034 | 0.128 | 0.054 | 74 | | |
| | 3 | 1.025 | 1.010 | 1.013 | 1.002 | 1 | | 3 | 0.046 | 0.033 | 0.124 | 0.049 | 63 | | |
| | Mean | 1.020 | 1.010 | 1.008 | 0.999 | 1 | | Mean | 0.044 | 0.034 | 0.122 | 0.049 | 63 | Positive | Weakly photoreactive |
| US-027 | 1 | 0.999 | 0.970 | 0.970 | 0.965 | 21 | US-027 | 1 | 0.045 | 0.033 | 0.112 | 0.040 | 53 | | |
| | 2 | 1.006 | 0.979 | 0.982 | 0.970 | 17 | | 2 | 0.044 | 0.030 | 0.118 | 0.047 | 60 | | |
| | 3 | 1.011 | 0.978 | 0.982 | 0.969 | 20 | | 3 | 0.044 | 0.029 | 0.111 | 0.046 | 53 | | |
| | Mean | 1.005 | 0.976 | 0.978 | 0.968 | 19 | | Mean | 0.044 | 0.030 | 0.114 | 0.044 | 55 | Positive | Weakly photoreactive |
| US-035 | 1 | 0.985 | 0.982 | 0.975 | 0.975 | 1 | US-035 | 1 | 0.040 | 0.034 | 0.095 | 0.045 | 33 | | |
| | 2 | 0.996 | 0.985 | 0.982 | 0.974 | 5 | | 2 | 0.034 | 0.030 | 0.093 | 0.048 | 36 | | |
| | 3 | 1.001 | 0.985 | 0.989 | 0.976 | 2 | | 3 | 0.050 | 0.028 | 0.099 | 0.066 | 27 | | |
| | Mean | 0.994 | 0.984 | 0.982 | 0.975 | 3 | | Mean | 0.042 | 0.031 | 0.096 | 0.053 | 32 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 8 | Mean for 3 assays | | - | - | - | - | 50 | Positive *3 | Weakly photoreactive*3 |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Bumetrizole |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-027 | 1 | 1.041 | 0.970 | 1.041 | 0.965 | -8 | US-027 | 1 | 0.047 | 0.033 | 0.053 | 0.040 | -9 | | |
| | 2 | 1.081 | 0.979 | 1.079 | 0.970 | -5 | | 2 | 0.040 | 0.030 | 0.048 | 0.047 | -6 | | |
| | 3 | 1.086 | 0.978 | 1.085 | 0.969 | -7 | | 3 | 0.048 | 0.029 | 0.053 | 0.046 | -9 | | |
| | Mean | 1.069 | 0.976 | 1.068 | 0.968 | -7 | | Mean | 0.045 | 0.030 | 0.051 | 0.044 | -8 | Inconclusive | (Non-photoreactive) |
| US-034 | 1 | 1.031 | 0.974 | 1.031 | 0.965 | -9 | US-034 | 1 | 0.039 | 0.036 | 0.067 | 0.049 | 4 | | |
| | 2 | 1.038 | 1.001 | 1.040 | 0.992 | -11 | | 2 | 0.036 | 0.042 | 0.072 | 0.045 | 12 | | |
| | 3 | 1.038 | 0.994 | 1.041 | 0.985 | -12 | | 3 | 0.040 | 0.029 | 0.076 | 0.085 | 12 | | |
| | Mean | 1.036 | 0.990 | 1.037 | 0.981 | -11 | | Mean | 0.038 | 0.036 | 0.072 | 0.060 | 9 | Inconclusive | (Non-photoreactive) |
| US-036 | 1 | 1.041 | 0.977 | 1.040 | 0.965 | -8 | US-036 | 1 | 0.037 | 0.028 | 0.053 | 0.043 | -8 | | |
| | 2 | 1.050 | 0.990 | 1.054 | 0.984 | -14 | | 2 | 0.035 | 0.032 | 0.056 | 0.048 | -3 | | |
| | 3 | 1.051 | 0.988 | 1.052 | 0.977 | -11 | | 3 | 0.037 | 0.029 | 0.053 | 0.072 | -7 | | |
| | Mean | 1.048 | 0.985 | 1.049 | 0.975 | -11 | | Mean | 0.036 | 0.030 | 0.054 | 0.054 | -6 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | -10 | Mean for 3 assays | | - | - | - | - | -2 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Camphor sulfonic acid |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-006 | 1 | 1.038 | 1.020 | 1.017 | 1.010 | 10 | US-006 | 1 | 0.039 | 0.031 | 0.057 | 0.044 | -4 | | |
| | 2 | 1.039 | 1.026 | 1.030 | 1.011 | -2 | | 2 | 0.034 | 0.031 | 0.056 | 0.048 | -1 | | |
| | 3 | 1.054 | 1.017 | 1.031 | 1.008 | 12 | | 3 | 0.041 | 0.031 | 0.057 | 0.069 | -7 | | |
| | Mean | 1.044 | 1.021 | 1.026 | 1.010 | 7 | | Mean | 0.038 | 0.031 | 0.057 | 0.054 | -4 | Negative | Non-photoreactive |
| US-026 | 1 | 0.976 | 0.973 | 0.977 | 0.965 | -9 | US-026 | 1 | 0.060 | 0.032 | 0.050 | 0.040 | -22 | | |
| | 2 | 0.994 | 0.982 | 0.979 | 0.977 | 7 | | 2 | 0.043 | 0.038 | 0.051 | 0.049 | -4 | | |
| | 3 | 0.993 | 0.986 | 0.988 | 0.976 | -3 | | 3 | 0.061 | 0.029 | 0.062 | 0.046 | -11 | | |
| | Mean | 0.988 | 0.981 | 0.981 | 0.973 | -2 | | Mean | 0.055 | 0.033 | 0.054 | 0.045 | -12 | Negative | Non-photoreactive |
| US-032 | 1 | 1.001 | 0.987 | 0.977 | 0.976 | 12 | US-032 | 1 | 0.075 | 0.028 | 0.068 | 0.045 | -27 | | |
| | 2 | 1.004 | 0.988 | 0.990 | 0.975 | 2 | | 2 | 0.043 | 0.045 | 0.057 | 0.045 | -6 | | |
| | 3 | 1.014 | 1.007 | 0.993 | 0.995 | 9 | | 3 | 0.073 | 0.028 | 0.061 | 0.072 | -32 | | |
| | Mean | 1.006 | 0.994 | 0.987 | 0.982 | 8 | | Mean | 0.064 | 0.034 | 0.062 | 0.054 | -22 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 4 | Mean for 3 assays | | - | - | - | - | -13 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Chlorhexidine

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-007 | 1 | 0.911 | 1.006 | 0.906 | 0.991 | -6 | US-007 | 1 | 0.044 | 0.035 | 0.086 | 0.046 | 26 | | |
| | 2 | 0.964 | 1.015 | 0.958 | 1.005 | -5 | | 2 | 0.046 | 0.034 | 0.096 | 0.054 | 35 | | |
| | 3 | 0.983 | 1.010 | 0.975 | 1.002 | -3 | | 3 | 0.046 | 0.033 | 0.084 | 0.049 | 23 | | |
| | Mean | 0.953 | 1.010 | 0.947 | 0.999 | -5 | | Mean | 0.045 | 0.034 | 0.088 | 0.049 | 28 | Positive | Weakly photoreactive |
| US-027 | 1 | 0.880 | 0.970 | 0.865 | 0.965 | 7 | US-027 | 1 | 0.051 | 0.033 | 0.103 | 0.040 | 38 | | |
| | 2 | 0.925 | 0.979 | 0.909 | 0.970 | 8 | | 2 | 0.049 | 0.030 | 0.099 | 0.047 | 36 | | |
| | 3 | 0.937 | 0.978 | 0.921 | 0.969 | 8 | | 3 | 0.052 | 0.029 | 0.098 | 0.046 | 32 | | |
| | Mean | 0.914 | 0.976 | 0.898 | 0.968 | 8 | | Mean | 0.051 | 0.030 | 0.100 | 0.044 | 35 | Positive | Weakly photoreactive |
| US-035 | 1 | 0.882 | 0.982 | 0.882 | 0.975 | -9 | US-035 | 1 | 0.049 | 0.034 | 0.079 | 0.045 | 8 | | |
| | 2 | 0.917 | 0.985 | 0.922 | 0.974 | -13 | | 2 | 0.048 | 0.030 | 0.076 | 0.048 | 6 | | |
| | 3 | 0.932 | 0.985 | 0.937 | 0.976 | -14 | | 3 | 0.048 | 0.028 | 0.075 | 0.066 | 5 | | |
| | Mean | 0.910 | 0.984 | 0.913 | 0.975 | -12 | | Mean | 0.048 | 0.031 | 0.077 | 0.053 | 6 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -3 | Mean for 3 assays | | - | - | - | - | 23 | Positive [*3] | Weakly photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Cinnamic acid |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-003 | 1 | 1.022 | 1.011 | 1.001 | 1.001 | 11 | US-003 | 1 | 0.056 | 0.029 | 0.135 | 0.049 | 56 | | |
| | 2 | 1.030 | 1.028 | 1.025 | 1.017 | -5 | | 2 | 0.033 | 0.044 | 0.141 | 0.052 | 84 | | |
| | 3 | 1.054 | 1.024 | 1.033 | 1.016 | 11 | | 3 | 0.059 | 0.030 | 0.124 | 0.070 | 42 | | |
| | Mean | 1.036 | 1.021 | 1.020 | 1.011 | 6 | | Mean | 0.049 | 0.034 | 0.133 | 0.057 | 61 | Positive | Weakly photoreactive |
| US-024 | 1 | 1.034 | 1.014 | 1.020 | 1.003 | 0 | US-024 | 1 | 0.062 | 0.030 | 0.129 | 0.045 | 51 | | |
| | 2 | 1.026 | 1.023 | 1.013 | 1.009 | -1 | | 2 | 0.031 | 0.040 | 0.151 | 0.052 | 104 | | |
| | 3 | 1.039 | 1.023 | 1.025 | 1.006 | 0 | | 3 | 0.062 | 0.029 | 0.131 | 0.051 | 54 | | |
| | Mean | 1.033 | 1.020 | 1.020 | 1.006 | 0 | | Mean | 0.052 | 0.033 | 0.137 | 0.049 | 70 | Positive | Photoreactive |
| US-031 | 1 | 1.012 | 0.986 | 1.002 | 0.973 | 0 | US-031 | 1 | 0.062 | 0.029 | 0.102 | 0.042 | 27 | | |
| | 2 | 1.008 | 0.985 | 0.997 | 0.979 | 1 | | 2 | 0.040 | 0.042 | 0.098 | 0.046 | 46 | | |
| | 3 | 1.015 | 0.995 | 1.006 | 0.985 | -1 | | 3 | 0.058 | 0.029 | 0.105 | 0.049 | 34 | | |
| | Mean | 1.012 | 0.989 | 1.002 | 0.979 | 0 | | Mean | 0.053 | 0.033 | 0.102 | 0.046 | 36 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 2 | Mean for 3 assays | | - | - | - | - | 56 | Positive [*3] | Weakly photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Drometrizole

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-025 | 1 | 1.036 | 0.990 | 1.015 | 0.969 | 0 | US-025 | 1 | 0.064 | 0.029 | 0.071 | 0.046 | -14 | | |
| | 2 | 1.028 | 1.013 | 0.999 | 0.988 | 7 | | 2 | 0.045 | 0.047 | 0.057 | 0.047 | -9 | | |
| | 3 | 1.045 | 1.011 | 1.010 | 0.993 | 13 | | 3 | 0.065 | 0.030 | 0.078 | 0.073 | -9 | | |
| | Mean | 1.036 | 1.005 | 1.008 | 0.983 | 7 | | Mean | 0.058 | 0.035 | 0.069 | 0.056 | -11 | Inconclusive | Non-photoreactive |
| US-032 | 1 | 0.984 | 0.987 | 0.979 | 0.976 | -7 | US-032 | 1 | 0.037 | 0.028 | 0.068 | 0.045 | 11 | | |
| | 2 | 0.990 | 0.988 | 0.976 | 0.975 | 2 | | 2 | 0.034 | 0.045 | 0.066 | 0.045 | 12 | | |
| | 3 | 0.991 | 1.007 | 0.986 | 0.995 | -7 | | 3 | 0.038 | 0.028 | 0.074 | 0.072 | 15 | | |
| | Mean | 0.988 | 0.994 | 0.980 | 0.982 | -4 | | Mean | 0.037 | 0.034 | 0.069 | 0.054 | 13 | Inconclusive | Non-photoreactive |
| US-033 | 1 | 0.976 | 0.982 | 0.972 | 0.972 | -8 | US-033 | 1 | 0.055 | 0.033 | 0.073 | 0.041 | -2 | | |
| | 2 | 0.996 | 0.989 | 0.991 | 0.975 | -7 | | 2 | 0.047 | 0.034 | 0.056 | 0.051 | -10 | | |
| | 3 | 1.005 | 0.987 | 0.998 | 0.975 | -5 | | 3 | 0.058 | 0.028 | 0.068 | 0.064 | -11 | | |
| | Mean | 0.992 | 0.986 | 0.987 | 0.974 | -7 | | Mean | 0.053 | 0.032 | 0.066 | 0.052 | -8 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -1 | Mean for 3 assays | | - | - | - | - | -2 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | L-Histidine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-028 | 1 | 0.979 | 0.980 | 0.952 | 0.971 | 16 | US-028 | 1 | 0.031 | 0.029 | 0.116 | 0.051 | 59 | | |
| | 2 | 0.979 | 0.988 | 0.962 | 0.977 | 6 | | 2 | 0.030 | 0.030 | 0.118 | 0.053 | 62 | | |
| | 3 | 0.986 | 0.995 | 0.961 | 0.983 | 15 | | 3 | 0.032 | 0.028 | 0.119 | 0.061 | 61 | | |
| | Mean | 0.981 | 0.988 | 0.958 | 0.977 | 12 | | Mean | 0.031 | 0.029 | 0.117 | 0.055 | 61 | Positive | Weakly photoreactive |
| US-030 | 1 | 0.986 | 0.997 | 0.972 | 1.002 | 15 | US-030 | 1 | 0.033 | 0.029 | 0.129 | 0.047 | 76 | | |
| | 2 | 0.994 | 1.003 | 0.978 | 0.996 | 16 | | 2 | 0.031 | 0.043 | 0.119 | 0.051 | 67 | | |
| | 3 | 0.996 | 1.001 | 0.982 | 1.005 | 15 | | 3 | 0.034 | 0.030 | 0.131 | 0.066 | 76 | | |
| | Mean | 0.992 | 1.000 | 0.977 | 1.001 | 15 | | Mean | 0.032 | 0.034 | 0.126 | 0.055 | 73 | Positive | Photoreactive |
| US-035 | 1 | 0.985 | 0.982 | 0.969 | 0.975 | 7 | US-035 | 1 | 0.056 | 0.034 | 0.115 | 0.045 | 37 | | |
| | 2 | 0.993 | 0.985 | 0.977 | 0.974 | 7 | | 2 | 0.029 | 0.030 | 0.111 | 0.048 | 60 | | |
| | 3 | 0.997 | 0.985 | 0.983 | 0.976 | 6 | | 3 | 0.033 | 0.028 | 0.111 | 0.066 | 56 | | |
| | Mean | 0.992 | 0.984 | 0.976 | 0.975 | 7 | | Mean | 0.039 | 0.031 | 0.112 | 0.053 | 51 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 11 | Mean for 3 assays | | - | - | - | - | 62 | Positive [*3] | Weakly photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Methylbenzylidene camphor |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-026 | 1 | 0.985 | 0.973 | 0.988 | 0.965 | -11 | US-026 | 1 | 0.047 | 0.032 | 0.051 | 0.040 | -8 | | |
| | 2 | 0.994 | 0.982 | 0.987 | 0.977 | -1 | | 2 | 0.032 | 0.038 | 0.043 | 0.049 | -1 | | |
| | 3 | 0.995 | 0.986 | 0.997 | 0.976 | -9 | | 3 | 0.049 | 0.029 | 0.052 | 0.046 | -9 | | |
| | Mean | 0.991 | 0.981 | 0.991 | 0.973 | -7 | | Mean | 0.043 | 0.033 | 0.049 | 0.045 | -6 | Inconclusive | Non-photoreactive |
| US-034 | 1 | 0.968 | 0.974 | 0.961 | 0.965 | -2 | US-034 | 1 | 0.031 | 0.036 | 0.061 | 0.049 | 6 | | |
| | 2 | 0.981 | 1.001 | 0.975 | 0.992 | -3 | | 2 | 0.030 | 0.042 | 0.052 | 0.045 | -2 | | |
| | 3 | 0.978 | 0.994 | 0.972 | 0.985 | -3 | | 3 | 0.032 | 0.029 | 0.067 | 0.085 | 11 | | |
| | Mean | 0.976 | 0.990 | 0.969 | 0.981 | -3 | | Mean | 0.031 | 0.036 | 0.060 | 0.060 | 5 | Inconclusive | Non-photoreactive |
| US-036 | 1 | 0.971 | 0.977 | 0.965 | 0.965 | -4 | US-036 | 1 | 0.041 | 0.028 | 0.064 | 0.043 | -1 | | |
| | 2 | 0.981 | 0.990 | 0.973 | 0.984 | -2 | | 2 | 0.032 | 0.032 | 0.051 | 0.048 | -6 | | |
| | 3 | 0.982 | 0.988 | 0.978 | 0.977 | -6 | | 3 | 0.043 | 0.029 | 0.063 | 0.072 | -4 | | |
| | Mean | 0.978 | 0.985 | 0.972 | 0.975 | -4 | | Mean | 0.039 | 0.030 | 0.060 | 0.054 | -4 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -5 | Mean for 3 assays | | - | - | - | - | -2 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Octrizole |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-026 | 1 | 1.003 | 0.973 | 0.989 | 0.965 | 6 | US-026 | 1 | 0.039 | 0.032 | 0.105 | 0.040 | 54 | | |
| | 2 | 0.999 | 0.982 | 0.988 | 0.977 | 3 | | 2 | 0.036 | 0.038 | 0.094 | 0.049 | 46 | | |
| | 3 | 1.009 | 0.986 | 0.999 | 0.976 | 2 | | 3 | 0.041 | 0.029 | 0.121 | 0.046 | 67 | | |
| | Mean | 1.004 | 0.981 | 0.992 | 0.973 | 4 | | Mean | 0.039 | 0.033 | 0.106 | 0.045 | 56 | Positive | (Weakly photoreactive) |
| US-032 | 1 | 0.977 | 0.987 | 0.978 | 0.976 | -13 | US-032 | 1 | 0.041 | 0.028 | 0.113 | 0.045 | 52 | | |
| | 2 | 0.992 | 0.988 | 0.973 | 0.975 | 6 | | 2 | 0.035 | 0.045 | 0.089 | 0.045 | 34 | | |
| | 3 | 0.992 | 1.007 | 0.992 | 0.995 | -12 | | 3 | 0.038 | 0.028 | 0.114 | 0.072 | 56 | | |
| | Mean | 0.987 | 0.994 | 0.981 | 0.982 | -6 | | Mean | 0.038 | 0.034 | 0.106 | 0.054 | 47 | Positive | (Weakly photoreactive) |
| US-033 | 1 | 0.984 | 0.982 | 0.969 | 0.972 | 3 | US-033 | 1 | 0.037 | 0.033 | 0.082 | 0.041 | 25 | | |
| | 2 | 0.987 | 0.989 | 0.971 | 0.975 | 4 | | 2 | 0.035 | 0.034 | 0.071 | 0.051 | 16 | | |
| | 3 | 0.992 | 0.987 | 0.984 | 0.975 | -4 | | 3 | 0.040 | 0.028 | 0.085 | 0.064 | 25 | | |
| | Mean | 0.987 | 0.986 | 0.975 | 0.974 | 1 | | Mean | 0.037 | 0.032 | 0.079 | 0.052 | 22 | Positive | (Weakly photoreactive) |
| Mean for 3 assays | | - | - | - | - | 0 | Mean for 3 assays | | - | - | - | - | 42 | Positive [*3] | (Weakly photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Octyl methacrylate |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-001 | 1 | 1.028 | 1.014 | 0.994 | 1.006 | 24 | US-001 | 1 | 0.067 | 0.033 | 0.070 | 0.046 | -10 | | |
| | 2 | 1.034 | 1.028 | 1.011 | 1.014 | 12 | | 2 | 0.044 | 0.046 | 0.059 | 0.053 | 1 | | |
| | 3 | 1.049 | 1.029 | 1.022 | 1.020 | 16 | | 3 | 0.062 | 0.034 | 0.077 | 0.056 | 1 | | |
| | Mean | 1.037 | 1.024 | 1.009 | 1.013 | 17 | | Mean | 0.058 | 0.038 | 0.069 | 0.052 | -3 | Negative | Non-photoreactive |
| US-023 | 1 | 1.053 | 1.008 | 1.014 | 0.991 | 25 | US-023 | 1 | 0.086 | 0.033 | 0.066 | 0.039 | -27 | | |
| | 2 | 1.047 | 1.024 | 1.008 | 1.013 | 26 | | 2 | 0.071 | 0.049 | 0.052 | 0.044 | -27 | | |
| | 3 | 1.053 | 1.022 | 1.012 | 1.008 | 27 | | 3 | 0.083 | 0.029 | 0.070 | 0.051 | -20 | | |
| | Mean | 1.051 | 1.018 | 1.011 | 1.004 | 26 | | Mean | 0.080 | 0.037 | 0.063 | 0.044 | -25 | Positive | Photoreactive |
| US-029 | 1 | 1.031 | 0.973 | 0.967 | 0.960 | 54 | US-029 | 1 | 0.091 | 0.030 | 0.055 | 0.038 | -45 | | |
| | 2 | 1.036 | 0.986 | 0.975 | 0.979 | 51 | | 2 | 0.073 | 0.044 | 0.050 | 0.044 | -32 | | |
| | 3 | 1.044 | 0.987 | 0.983 | 0.978 | 51 | | 3 | 0.090 | 0.029 | 0.061 | 0.047 | -38 | | |
| | Mean | 1.037 | 0.982 | 0.975 | 0.972 | 52 | | Mean | 0.085 | 0.034 | 0.055 | 0.043 | -38 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 32 | Mean for 3 assays | | - | - | - | - | -22 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 1
Chemical Name : Octyl methoxycinnamate

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-027 | 1 | 0.990 | 0.970 | 0.977 | 0.965 | 5 | US-027 | 1 | 0.072 | 0.033 | 0.068 | 0.040 | -18 | | |
| | 2 | 1.001 | 0.979 | 0.989 | 0.970 | 5 | | 2 | 0.067 | 0.030 | 0.067 | 0.047 | -14 | | |
| | 3 | 1.007 | 0.978 | 0.991 | 0.969 | 8 | | 3 | 0.073 | 0.029 | 0.069 | 0.046 | -18 | | |
| | Mean | 0.999 | 0.976 | 0.985 | 0.968 | 6 | | Mean | 0.070 | 0.030 | 0.068 | 0.044 | -17 | Inconclusive | Non-photoreactive |
| US-034 | 1 | 1.008 | 0.974 | 0.998 | 0.965 | 1 | US-034 | 1 | 0.081 | 0.036 | 0.079 | 0.049 | -27 | | |
| | 2 | 1.021 | 1.001 | 1.010 | 0.992 | 3 | | 2 | 0.063 | 0.042 | 0.099 | 0.045 | 12 | | |
| | 3 | 1.032 | 0.994 | 1.021 | 0.985 | 3 | | 3 | 0.082 | 0.029 | 0.076 | 0.085 | -30 | | |
| | Mean | 1.021 | 0.990 | 1.010 | 0.981 | 2 | | Mean | 0.075 | 0.036 | 0.085 | 0.060 | -15 | Inconclusive | Non-photoreactive |
| US-036 | 1 | 1.012 | 0.977 | 0.993 | 0.965 | 9 | US-036 | 1 | 0.061 | 0.028 | 0.073 | 0.043 | -12 | | |
| | 2 | 1.017 | 0.990 | 0.999 | 0.984 | 8 | | 2 | 0.058 | 0.032 | 0.070 | 0.048 | -12 | | |
| | 3 | 1.018 | 0.988 | 1.010 | 0.977 | -1 | | 3 | 0.059 | 0.029 | 0.074 | 0.072 | -9 | | |
| | Mean | 1.016 | 0.985 | 1.000 | 0.975 | 5 | | Mean | 0.059 | 0.030 | 0.072 | 0.054 | -11 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 4 | Mean for 3 assays | | - | - | - | - | -14 | Inconclusive *3 | Non-photoreactive*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | Octyl salicylate |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-024 | 1 | 1.026 | 1.014 | 1.013 | 1.003 | -1 | US-024 | 1 | 0.054 | 0.030 | 0.081 | 0.045 | 11 | | |
| | 2 | 1.027 | 1.023 | 1.011 | 1.009 | 3 | | 2 | 0.041 | 0.040 | 0.067 | 0.052 | 10 | | |
| | 3 | 1.040 | 1.023 | 1.026 | 1.006 | -1 | | 3 | 0.054 | 0.029 | 0.084 | 0.051 | 14 | | |
| | Mean | 1.031 | 1.020 | 1.017 | 1.006 | 0 | | Mean | 0.050 | 0.033 | 0.077 | 0.049 | 12 | Inconclusive | Non-photoreactive |
| US-030 | 1 | 1.019 | 0.997 | 1.005 | 1.002 | 15 | US-030 | 1 | 0.061 | 0.029 | 0.086 | 0.047 | 4 | | |
| | 2 | 1.014 | 1.003 | 1.003 | 0.996 | 13 | | 2 | 0.048 | 0.043 | 0.065 | 0.051 | -4 | | |
| | 3 | 1.022 | 1.001 | 1.010 | 1.005 | 13 | | 3 | 0.058 | 0.030 | 0.086 | 0.066 | 7 | | |
| | Mean | 1.018 | 1.000 | 1.006 | 1.001 | 14 | | Mean | 0.056 | 0.034 | 0.079 | 0.055 | 2 | Inconclusive | Non-photoreactive |
| US-035 | 1 | 0.996 | 0.982 | 0.985 | 0.975 | 3 | US-035 | 1 | 0.042 | 0.034 | 0.067 | 0.045 | 2 | | |
| | 2 | 0.990 | 0.985 | 0.982 | 0.974 | -1 | | 2 | 0.042 | 0.030 | 0.063 | 0.048 | -1 | | |
| | 3 | 1.016 | 0.985 | 1.003 | 0.976 | 5 | | 3 | 0.042 | 0.028 | 0.068 | 0.066 | 3 | | |
| | Mean | 1.001 | 0.984 | 0.990 | 0.975 | 2 | | Mean | 0.042 | 0.031 | 0.066 | 0.053 | 1 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 5 | Mean for 3 assays | | - | - | - | - | 5 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | PABA |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-005 | 1 | 1.021 | 1.015 | 0.994 | 1.004 | 17 | US-005 | 1 | 0.038 | 0.029 | 0.057 | 0.043 | 8 | | |
| | 2 | 1.045 | 1.012 | 1.014 | 1.002 | 20 | | 2 | 0.032 | 0.043 | 0.051 | 0.044 | 9 | | |
| | 3 | 1.050 | 1.016 | 1.023 | 1.007 | 16 | | 3 | 0.036 | 0.029 | 0.059 | 0.046 | 12 | | |
| | Mean | 1.039 | 1.015 | 1.010 | 1.004 | 18 | | Mean | 0.035 | 0.033 | 0.056 | 0.044 | 10 | Negative | Non-photoreactive |
| US-025 | 1 | 0.997 | 0.990 | 0.985 | 0.969 | -10 | US-025 | 1 | 0.036 | 0.029 | 0.052 | 0.046 | -4 | | |
| | 2 | 1.003 | 1.013 | 0.982 | 0.988 | -1 | | 2 | 0.031 | 0.047 | 0.048 | 0.047 | -4 | | |
| | 3 | 1.004 | 1.011 | 0.995 | 0.993 | -13 | | 3 | 0.039 | 0.030 | 0.052 | 0.073 | -8 | | |
| | Mean | 1.001 | 1.005 | 0.987 | 0.983 | -8 | | Mean | 0.035 | 0.035 | 0.051 | 0.056 | -5 | Negative | Non-photoreactive |
| US-031 | 1 | 0.998 | 0.986 | 0.985 | 0.973 | 3 | US-031 | 1 | 0.038 | 0.029 | 0.057 | 0.042 | 6 | | |
| | 2 | 1.006 | 0.985 | 0.990 | 0.979 | 6 | | 2 | 0.033 | 0.042 | 0.055 | 0.046 | 9 | | |
| | 3 | 1.008 | 0.995 | 0.993 | 0.985 | 5 | | 3 | 0.038 | 0.029 | 0.059 | 0.049 | 8 | | |
| | Mean | 1.004 | 0.989 | 0.989 | 0.979 | 5 | | Mean | 0.036 | 0.033 | 0.057 | 0.046 | 8 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 5 | Mean for 3 assays | | - | - | - | - | 4 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | SDS |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-005 | 1 | 0.975 | 1.015 | 0.950 | 1.004 | 13 | US-031 | 1 | 0.057 | 0.029 | 0.089 | 0.042 | 19 | | |
| | 2 | 1.004 | 1.012 | 0.975 | 1.002 | 19 | | 2 | 0.056 | 0.042 | 0.088 | 0.046 | 19 | | |
| | 3 | 1.020 | 1.016 | 0.988 | 1.007 | 21 | | 3 | 0.059 | 0.029 | 0.083 | 0.049 | 11 | | |
| | Mean | 0.999 | 1.015 | 0.971 | 1.004 | 18 | | Mean | 0.057 | 0.033 | 0.087 | 0.046 | 16 | Inconclusive | Non-photoreactive |
| US-025 | 1 | 0.994 | 0.990 | 0.953 | 0.969 | 20 | US-033 | 1 | 0.066 | 0.033 | 0.088 | 0.041 | 2 | | |
| | 2 | 0.999 | 1.013 | 0.963 | 0.988 | 14 | | 2 | 0.064 | 0.034 | 0.088 | 0.051 | 4 | | |
| | 3 | 1.004 | 1.011 | 0.965 | 0.993 | 17 | | 3 | 0.066 | 0.028 | 0.096 | 0.064 | 10 | | |
| | Mean | 0.999 | 1.005 | 0.960 | 0.983 | 17 | | Mean | 0.065 | 0.032 | 0.091 | 0.052 | 5 | Inconclusive | Non-photoreactive |
| US-031 | 1 | 0.969 | 0.986 | 0.944 | 0.973 | 15 | US-036 | 1 | 0.063 | 0.028 | 0.091 | 0.043 | 3 | | |
| | 2 | 0.973 | 0.985 | 0.944 | 0.979 | 19 | | 2 | 0.066 | 0.032 | 0.098 | 0.048 | 8 | | |
| | 3 | 0.982 | 0.995 | 0.960 | 0.985 | 12 | | 3 | 0.064 | 0.029 | 0.086 | 0.072 | -2 | | |
| | Mean | 0.975 | 0.989 | 0.949 | 0.979 | 15 | | Mean | 0.064 | 0.030 | 0.092 | 0.054 | 3 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 17 | Mean for 3 assays | | - | - | - | - | 8 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 1 |
| Chemical Name | : | UV-571 |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| US-024 | 1 | 1.064 | 1.014 | 1.069 | 1.003 | -19 | US-024 | 1 | 0.057 | 0.030 | 0.084 | 0.045 | 11 | | |
| | 2 | 1.062 | 1.023 | 1.058 | 1.009 | -10 | | 2 | 0.040 | 0.040 | 0.070 | 0.052 | 14 | | |
| | 3 | 1.077 | 1.023 | 1.080 | 1.006 | -18 | | 3 | 0.066 | 0.029 | 0.087 | 0.051 | 5 | | |
| | Mean | 1.068 | 1.020 | 1.069 | 1.006 | -16 | | Mean | 0.054 | 0.033 | 0.080 | 0.049 | 10 | Inconclusive | (Non-photoreactive) |
| US-033 | 1 | 1.032 | 0.982 | 1.042 | 0.972 | -22 | US-033 | 1 | 0.037 | 0.033 | 0.065 | 0.041 | 8 | | |
| | 2 | 1.058 | 0.989 | 1.062 | 0.975 | -16 | | 2 | 0.036 | 0.034 | 0.056 | 0.051 | 0 | | |
| | 3 | 1.049 | 0.987 | 1.055 | 0.975 | -18 | | 3 | 0.039 | 0.028 | 0.064 | 0.064 | 5 | | |
| | Mean | 1.046 | 0.986 | 1.053 | 0.974 | -19 | | Mean | 0.037 | 0.032 | 0.062 | 0.052 | 4 | Inconclusive | (Non-photoreactive) |
| US-036 | 1 | 1.061 | 0.977 | 1.068 | 0.965 | -17 | US-036 | 1 | 0.038 | 0.028 | 0.082 | 0.043 | 19 | | |
| | 2 | 1.053 | 0.990 | 1.057 | 0.984 | -14 | | 2 | 0.033 | 0.032 | 0.060 | 0.048 | 3 | | |
| | 3 | 1.052 | 0.988 | 1.057 | 0.977 | -15 | | 3 | 0.039 | 0.029 | 0.086 | 0.072 | 23 | | |
| | Mean | 1.055 | 0.985 | 1.061 | 0.975 | -15 | | Mean | 0.037 | 0.030 | 0.076 | 0.054 | 15 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | -17 | Mean for 3 assays | | - | - | - | - | 10 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Acridine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-06 | 1 | 0.959 | 0.982 | 0.719 | 0.972 | 231 | FDSC-06 | 1 | 0.032 | 0.033 | 0.250 | 0.052 | 200 | | |
| | 2 | 0.965 | 0.977 | 0.732 | 0.965 | 224 | | 2 | 0.033 | 0.034 | 0.240 | 0.047 | 189 | | |
| | 3 | 0.964 | 0.982 | 0.723 | 0.976 | 232 | | 3 | 0.032 | 0.033 | 0.233 | 0.055 | 183 | | |
| | Mean | 0.963 | 0.980 | 0.725 | 0.971 | 229 | | Mean | 0.032 | 0.033 | 0.241 | 0.051 | 191 | Positive | Photoreactive |
| FDSC-15 | 1 | 0.955 | 0.974 | 0.724 | 0.967 | 226 | FDSC-15 | 1 | 0.032 | 0.033 | 0.256 | 0.054 | 209 | | |
| | 2 | 0.962 | 0.975 | 0.748 | 0.971 | 209 | | 2 | 0.032 | 0.032 | 0.255 | 0.048 | 208 | | |
| | 3 | 0.971 | 0.982 | 0.747 | 0.977 | 219 | | 3 | 0.033 | 0.035 | 0.259 | 0.043 | 211 | | |
| | Mean | 0.963 | 0.977 | 0.740 | 0.972 | 218 | | Mean | 0.032 | 0.033 | 0.257 | 0.048 | 209 | Positive | Photoreactive |
| FDSC-22 | 1 | 0.948 | 0.980 | 0.714 | 0.974 | 228 | FDSC-22 | 1 | 0.034 | 0.031 | 0.234 | 0.040 | 186 | | |
| | 2 | 0.956 | 0.982 | 0.755 | 0.974 | 195 | | 2 | 0.035 | 0.033 | 0.242 | 0.057 | 193 | | |
| | 3 | 0.956 | 0.991 | 0.732 | 0.986 | 218 | | 3 | 0.033 | 0.032 | 0.239 | 0.042 | 192 | | |
| | Mean | 0.953 | 0.984 | 0.734 | 0.978 | 214 | | Mean | 0.034 | 0.032 | 0.238 | 0.046 | 190 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 220 | Mean for 3 assays | | - | - | - | - | 197 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Acridine HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-04 | 1 | 0.951 | 0.972 | 0.707 | 0.963 | 234 | FDSC-04 | 1 | 0.030 | 0.032 | 0.233 | 0.050 | 186 | | |
| | 2 | 0.958 | 0.970 | 0.728 | 0.959 | 220 | | 2 | 0.034 | 0.032 | 0.256 | 0.049 | 205 | | |
| | 3 | 0.951 | 0.975 | 0.717 | 0.963 | 224 | | 3 | 0.031 | 0.033 | 0.250 | 0.049 | 202 | | |
| | Mean | 0.953 | 0.972 | 0.717 | 0.962 | 226 | | Mean | 0.032 | 0.032 | 0.246 | 0.049 | 198 | Positive | Photoreactive |
| FDSC-13 | 1 | 0.946 | 0.970 | 0.722 | 0.970 | 219 | FDSC-13 | 1 | 0.032 | 0.034 | 0.259 | 0.043 | 218 | | |
| | 2 | 0.964 | 0.970 | 0.743 | 0.962 | 216 | | 2 | 0.034 | 0.034 | 0.249 | 0.043 | 206 | | |
| | 3 | 0.955 | 0.972 | 0.736 | 0.965 | 214 | | 3 | 0.032 | 0.033 | 0.252 | 0.044 | 211 | | |
| | Mean | 0.955 | 0.971 | 0.734 | 0.966 | 216 | | Mean | 0.033 | 0.034 | 0.253 | 0.043 | 212 | Positive | Photoreactive |
| FDSC-20 | 1 | 0.959 | 0.974 | 0.684 | 0.949 | 256 | FDSC-20 | 1 | 0.032 | 0.032 | 0.204 | 0.046 | 160 | | |
| | 2 | 0.972 | 0.972 | 0.745 | 0.954 | 208 | | 2 | 0.032 | 0.032 | 0.218 | 0.045 | 174 | | |
| | 3 | 0.971 | 0.980 | 0.744 | 0.966 | 208 | | 3 | 0.033 | 0.034 | 0.254 | 0.045 | 209 | | |
| | Mean | 0.967 | 0.975 | 0.724 | 0.956 | 224 | | Mean | 0.032 | 0.033 | 0.225 | 0.045 | 181 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 222 | Mean for 3 assays | | - | - | - | - | 197 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Amiodarone HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-10 | 1 | 0.985 | 0.982 | 0.950 | 0.976 | 26 | FDSC-10 | 1 | 0.034 | 0.032 | 0.055 | 0.047 | 2 | | |
| | 2 | 0.988 | 0.992 | 0.951 | 0.975 | 28 | | 2 | 0.033 | 0.032 | 0.056 | 0.053 | 4 | | |
| | 3 | 0.990 | 0.987 | 0.961 | 0.984 | 20 | | 3 | 0.033 | 0.034 | 0.055 | 0.055 | 3 | | |
| | Mean | 0.988 | 0.987 | 0.954 | 0.978 | 25 | | Mean | 0.033 | 0.033 | 0.055 | 0.052 | 3 | Positive | (Photoreactive) |
| FDSC-16 | 1 | 0.985 | 0.986 | 0.939 | 0.996 | 46 | FDSC-16 | 1 | 0.033 | 0.031 | 0.049 | 0.041 | 5 | | |
| | 2 | 0.988 | 0.982 | 0.953 | 0.980 | 35 | | 2 | 0.034 | 0.032 | 0.051 | 0.044 | 6 | | |
| | 3 | 0.989 | 0.993 | 0.957 | 0.986 | 32 | | 3 | 0.035 | 0.033 | 0.049 | 0.043 | 3 | | |
| | Mean | 0.987 | 0.987 | 0.950 | 0.987 | 38 | | Mean | 0.034 | 0.032 | 0.050 | 0.043 | 5 | Positive | (Photoreactive) |
| FDSC-23 | 1 | 0.965 | 0.957 | 0.928 | 0.946 | 27 | FDSC-23 | 1 | 0.033 | 0.032 | 0.046 | 0.059 | -5 | | |
| | 2 | 0.977 | 0.960 | 0.937 | 0.947 | 30 | | 2 | 0.035 | 0.032 | 0.050 | 0.045 | -3 | | |
| | 3 | 0.975 | 0.965 | 0.938 | 0.959 | 27 | | 3 | 0.034 | 0.033 | 0.045 | 0.045 | -7 | | |
| | Mean | 0.972 | 0.961 | 0.934 | 0.951 | 28 | | Mean | 0.034 | 0.032 | 0.047 | 0.050 | -5 | Positive | (Photoreactive) |
| Mean for 3 assays | | - | - | - | - | 30 | Mean for 3 assays | | - | - | - | - | 1 | Positive [*3] | (Photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Chlorpromazine HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-05 | 1 | 0.954 | 0.970 | 0.961 | 0.966 | -8 | FDSC-05 | 1 | 0.038 | 0.032 | 0.147 | 0.048 | 93 | | |
| | 2 | 0.972 | 0.966 | 0.969 | 0.973 | 2 | | 2 | 0.033 | 0.032 | 0.147 | 0.051 | 98 | | |
| | 3 | 0.962 | 0.975 | 0.961 | 0.968 | 0 | | 3 | 0.034 | 0.032 | 0.150 | 0.046 | 100 | | |
| | Mean | 0.963 | 0.970 | 0.964 | 0.969 | -2 | | Mean | 0.035 | 0.032 | 0.148 | 0.048 | 97 | Positive | Photoreactive |
| FDSC-14 | 1 | 0.953 | 0.971 | 0.958 | 0.960 | -15 | FDSC-14 | 1 | 0.031 | 0.032 | 0.143 | 0.044 | 99 | | |
| | 2 | 0.960 | 0.968 | 0.965 | 0.959 | -15 | | 2 | 0.033 | 0.033 | 0.143 | 0.050 | 97 | | |
| | 3 | 0.960 | 0.974 | 0.960 | 0.963 | -10 | | 3 | 0.036 | 0.033 | 0.150 | 0.045 | 101 | | |
| | Mean | 0.958 | 0.971 | 0.961 | 0.961 | -13 | | Mean | 0.033 | 0.033 | 0.145 | 0.046 | 99 | Positive | Photoreactive |
| FDSC-21 | 1 | 0.939 | 0.959 | 0.944 | 0.967 | -7 | FDSC-21 | 1 | 0.032 | 0.031 | 0.147 | 0.043 | 106 | | |
| | 2 | 0.956 | 0.958 | 0.960 | 0.953 | -6 | | 2 | 0.032 | 0.032 | 0.145 | 0.041 | 104 | | |
| | 3 | 0.946 | 0.963 | 0.950 | 0.955 | -6 | | 3 | 0.032 | 0.035 | 0.158 | 0.042 | 117 | | |
| | Mean | 0.947 | 0.960 | 0.951 | 0.958 | -6 | | Mean | 0.032 | 0.033 | 0.150 | 0.042 | 109 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | -7 | Mean for 3 assays | | - | - | - | - | 102 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Doxycycline HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-06 | 1 | 0.952 | 0.982 | 0.717 | 0.972 | 226 | FDSC-06 | 1 | 0.038 | 0.033 | 0.463 | 0.052 | 407 | | |
| | 2 | 0.964 | 0.977 | 0.733 | 0.965 | 222 | | 2 | 0.038 | 0.034 | 0.466 | 0.047 | 410 | | |
| | 3 | 0.964 | 0.982 | 0.738 | 0.976 | 217 | | 3 | 0.039 | 0.033 | 0.480 | 0.055 | 423 | | |
| | Mean | 0.960 | 0.980 | 0.729 | 0.971 | 222 | | Mean | 0.038 | 0.033 | 0.470 | 0.051 | 413 | Positive | Photoreactive |
| FDSC-14 | 1 | 0.940 | 0.971 | 0.688 | 0.960 | 242 | FDSC-14 | 1 | 0.041 | 0.032 | 0.485 | 0.044 | 431 | | |
| | 2 | 0.950 | 0.968 | 0.711 | 0.959 | 229 | | 2 | 0.040 | 0.033 | 0.488 | 0.050 | 435 | | |
| | 3 | 0.956 | 0.974 | 0.716 | 0.963 | 230 | | 3 | 0.041 | 0.033 | 0.500 | 0.045 | 446 | | |
| | Mean | 0.949 | 0.971 | 0.705 | 0.961 | 234 | | Mean | 0.041 | 0.033 | 0.491 | 0.046 | 437 | Positive | Photoreactive |
| FDSC-21 | 1 | 0.934 | 0.959 | 0.705 | 0.967 | 227 | FDSC-21 | 1 | 0.040 | 0.031 | 0.453 | 0.043 | 404 | | |
| | 2 | 0.951 | 0.958 | 0.731 | 0.953 | 218 | | 2 | 0.040 | 0.032 | 0.459 | 0.041 | 410 | | |
| | 3 | 0.947 | 0.963 | 0.725 | 0.955 | 220 | | 3 | 0.041 | 0.035 | 0.468 | 0.042 | 418 | | |
| | Mean | 0.944 | 0.960 | 0.720 | 0.958 | 222 | | Mean | 0.040 | 0.033 | 0.460 | 0.042 | 411 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 226 | Mean for 3 assays | | - | - | - | - | 420 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Fenofibrate |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-10 | 1 | 0.975 | 0.982 | 0.960 | 0.976 | 6 | FDSC-10 | 1 | 0.033 | 0.032 | 0.044 | 0.047 | -8 | | |
| | 2 | 0.989 | 0.992 | 0.971 | 0.975 | 9 | | 2 | 0.032 | 0.032 | 0.046 | 0.053 | -5 | | |
| | 3 | 0.985 | 0.987 | 0.977 | 0.984 | -1 | | 3 | 0.032 | 0.034 | 0.045 | 0.055 | -6 | | |
| | Mean | 0.983 | 0.987 | 0.969 | 0.978 | 5 | | Mean | 0.032 | 0.033 | 0.045 | 0.052 | -6 | Inconclusive | (Non-photoreactive) |
| FDSC-16 | 1 | 0.972 | 0.986 | 0.954 | 0.996 | 18 | FDSC-16 | 1 | 0.032 | 0.031 | 0.046 | 0.041 | 3 | | |
| | 2 | 0.976 | 0.982 | 0.957 | 0.980 | 19 | | 2 | 0.031 | 0.032 | 0.059 | 0.044 | 17 | | |
| | 3 | 0.981 | 0.993 | 0.963 | 0.986 | 18 | | 3 | 0.032 | 0.033 | 0.075 | 0.043 | 32 | | |
| | Mean | 0.976 | 0.987 | 0.958 | 0.987 | 18 | | Mean | 0.032 | 0.032 | 0.060 | 0.043 | 17 | Inconclusive | (Non-photoreactive) |
| FDSC-23 | 1 | 0.958 | 0.957 | 0.938 | 0.946 | 10 | FDSC-23 | 1 | 0.031 | 0.032 | 0.040 | 0.059 | -9 | | |
| | 2 | 0.965 | 0.960 | 0.940 | 0.947 | 15 | | 2 | 0.031 | 0.032 | 0.041 | 0.045 | -8 | | |
| | 3 | 0.969 | 0.965 | 0.950 | 0.959 | 9 | | 3 | 0.032 | 0.033 | 0.041 | 0.045 | -9 | | |
| | Mean | 0.964 | 0.961 | 0.943 | 0.951 | 11 | | Mean | 0.031 | 0.032 | 0.041 | 0.050 | -9 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | 11 | Mean for 3 assays | | - | - | - | - | 1 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Furosemide |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-01 | 1 | 0.958 | 0.955 | 0.803 | 0.948 | 148 | FDSC-01 | 1 | 0.037 | 0.033 | 0.108 | 0.043 | 60 | | |
| | 2 | 0.978 | 0.963 | 0.830 | 0.956 | 141 | | 2 | 0.038 | 0.037 | 0.112 | 0.050 | 63 | | |
| | 3 | 0.972 | 0.967 | 0.839 | 0.961 | 126 | | 3 | 0.043 | 0.033 | 0.117 | 0.042 | 63 | | |
| | Mean | 0.969 | 0.962 | 0.824 | 0.955 | 138 | | Mean | 0.039 | 0.034 | 0.112 | 0.045 | 62 | Positive | Photoreactive |
| FDSC-12 | 1 | 0.962 | 0.967 | 0.815 | 0.961 | 140 | FDSC-12 | 1 | 0.038 | 0.033 | 0.119 | 0.049 | 66 | | |
| | 2 | 0.965 | 0.962 | 0.829 | 0.954 | 129 | | 2 | 0.036 | 0.035 | 0.113 | 0.050 | 62 | | |
| | 3 | 0.963 | 0.967 | 0.832 | 0.959 | 124 | | 3 | 0.040 | 0.033 | 0.127 | 0.047 | 72 | | |
| | Mean | 0.963 | 0.965 | 0.825 | 0.958 | 131 | | Mean | 0.038 | 0.034 | 0.120 | 0.049 | 67 | Positive | Photoreactive |
| FDSC-19 | 1 | 0.972 | 0.986 | 0.828 | 0.990 | 140 | FDSC-19 | 1 | 0.036 | 0.033 | 0.123 | 0.049 | 69 | | |
| | 2 | 0.970 | 0.981 | 0.832 | 0.975 | 134 | | 2 | 0.034 | 0.032 | 0.118 | 0.054 | 66 | | |
| | 3 | 0.969 | 0.991 | 0.841 | 0.982 | 124 | | 3 | 0.035 | 0.033 | 0.118 | 0.050 | 65 | | |
| | Mean | 0.970 | 0.986 | 0.834 | 0.982 | 133 | | Mean | 0.035 | 0.033 | 0.120 | 0.051 | 67 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 134 | Mean for 3 assays | | - | - | - | - | 65 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Ketoprofen |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-04 | 1 | 0.976 | 0.972 | 0.714 | 0.963 | 252 | FDSC-04 | 1 | 0.036 | 0.032 | 0.161 | 0.050 | 108 | | |
| | 2 | 0.982 | 0.970 | 0.728 | 0.959 | 244 | | 2 | 0.034 | 0.032 | 0.158 | 0.049 | 107 | | |
| | 3 | 0.975 | 0.975 | 0.725 | 0.963 | 240 | | 3 | 0.035 | 0.033 | 0.159 | 0.049 | 107 | | |
| | Mean | 0.978 | 0.972 | 0.722 | 0.962 | 245 | | Mean | 0.035 | 0.032 | 0.159 | 0.049 | 107 | Positive | Photoreactive |
| FDSC-13 | 1 | 0.972 | 0.970 | 0.696 | 0.970 | 271 | FDSC-13 | 1 | 0.035 | 0.034 | 0.160 | 0.043 | 116 | | |
| | 2 | 0.969 | 0.970 | 0.702 | 0.962 | 262 | | 2 | 0.035 | 0.034 | 0.159 | 0.043 | 115 | | |
| | 3 | 0.965 | 0.972 | 0.716 | 0.965 | 244 | | 3 | 0.034 | 0.033 | 0.162 | 0.044 | 119 | | |
| | Mean | 0.969 | 0.971 | 0.705 | 0.966 | 259 | | Mean | 0.035 | 0.034 | 0.160 | 0.043 | 117 | Positive | Photoreactive |
| FDSC-20 | 1 | 0.926 | 0.974 | 0.663 | 0.949 | 244 | FDSC-20 | 1 | 0.034 | 0.032 | 0.151 | 0.046 | 105 | | |
| | 2 | 0.932 | 0.972 | 0.677 | 0.954 | 236 | | 2 | 0.034 | 0.032 | 0.158 | 0.045 | 112 | | |
| | 3 | 0.936 | 0.980 | 0.677 | 0.966 | 240 | | 3 | 0.036 | 0.034 | 0.159 | 0.045 | 111 | | |
| | Mean | 0.931 | 0.975 | 0.672 | 0.956 | 240 | | Mean | 0.035 | 0.033 | 0.156 | 0.045 | 109 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 248 | Mean for 3 assays | | - | - | - | - | 111 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | 6-methylcoumarine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-03 | 1 | 0.961 | 0.966 | 0.835 | 0.962 | 123 | FDSC-03 | 1 | 0.032 | 0.033 | 0.136 | 0.050 | 87 | | |
| | 2 | 0.963 | 0.970 | 0.877 | 0.968 | 83 | | 2 | 0.032 | 0.033 | 0.133 | 0.050 | 84 | | |
| | 3 | 0.968 | 0.974 | 0.862 | 0.970 | 103 | | 3 | 0.034 | 0.035 | 0.141 | 0.053 | 90 | | |
| | Mean | 0.964 | 0.970 | 0.858 | 0.967 | 103 | | Mean | 0.033 | 0.034 | 0.137 | 0.051 | 87 | Positive | Photoreactive |
| FDSC-12 | 1 | 0.969 | 0.967 | 0.851 | 0.961 | 111 | FDSC-12 | 1 | 0.033 | 0.033 | 0.157 | 0.049 | 109 | | |
| | 2 | 0.975 | 0.962 | 0.842 | 0.954 | 126 | | 2 | 0.032 | 0.035 | 0.157 | 0.050 | 110 | | |
| | 3 | 0.974 | 0.967 | 0.839 | 0.959 | 128 | | 3 | 0.033 | 0.033 | 0.160 | 0.047 | 112 | | |
| | Mean | 0.973 | 0.965 | 0.844 | 0.958 | 122 | | Mean | 0.033 | 0.034 | 0.158 | 0.049 | 110 | Positive | Photoreactive |
| FDSC-19 | 1 | 0.967 | 0.986 | 0.818 | 0.990 | 145 | FDSC-19 | 1 | 0.032 | 0.033 | 0.146 | 0.049 | 96 | | |
| | 2 | 0.978 | 0.981 | 0.833 | 0.975 | 141 | | 2 | 0.031 | 0.032 | 0.149 | 0.054 | 100 | | |
| | 3 | 0.978 | 0.991 | 0.834 | 0.982 | 140 | | 3 | 0.031 | 0.033 | 0.152 | 0.050 | 103 | | |
| | Mean | 0.974 | 0.986 | 0.828 | 0.982 | 142 | | Mean | 0.031 | 0.033 | 0.149 | 0.051 | 100 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 122 | Mean for 3 assays | | - | - | - | - | 99 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | 8-MOP |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-04 | 1 | 0.963 | 0.972 | 0.868 | 0.963 | 85 | FDSC-04 | 1 | 0.032 | 0.032 | 0.135 | 0.050 | 86 | | |
| | 2 | 0.971 | 0.970 | 0.887 | 0.959 | 74 | | 2 | 0.033 | 0.032 | 0.135 | 0.049 | 85 | | |
| | 3 | 0.968 | 0.975 | 0.874 | 0.963 | 84 | | 3 | 0.033 | 0.033 | 0.141 | 0.049 | 91 | | |
| | Mean | 0.967 | 0.972 | 0.876 | 0.962 | 81 | | Mean | 0.033 | 0.032 | 0.137 | 0.049 | 87 | Positive | Photoreactive |
| FDSC-13 | 1 | 0.966 | 0.970 | 0.877 | 0.970 | 84 | FDSC-13 | 1 | 0.033 | 0.034 | 0.140 | 0.043 | 98 | | |
| | 2 | 0.969 | 0.970 | 0.885 | 0.962 | 79 | | 2 | 0.033 | 0.034 | 0.146 | 0.043 | 104 | | |
| | 3 | 0.963 | 0.972 | 0.885 | 0.965 | 73 | | 3 | 0.033 | 0.033 | 0.149 | 0.044 | 107 | | |
| | Mean | 0.966 | 0.971 | 0.882 | 0.966 | 79 | | Mean | 0.033 | 0.034 | 0.145 | 0.043 | 103 | Positive | Photoreactive |
| FDSC-20 | 1 | 0.939 | 0.974 | 0.843 | 0.949 | 77 | FDSC-20 | 1 | 0.033 | 0.032 | 0.136 | 0.046 | 91 | | |
| | 2 | 0.951 | 0.972 | 0.902 | 0.954 | 30 | | 2 | 0.032 | 0.032 | 0.130 | 0.045 | 86 | | |
| | 3 | 0.954 | 0.980 | 0.863 | 0.966 | 72 | | 3 | 0.033 | 0.034 | 0.145 | 0.045 | 100 | | |
| | Mean | 0.948 | 0.975 | 0.869 | 0.956 | 60 | | Mean | 0.033 | 0.033 | 0.137 | 0.045 | 92 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 73 | Mean for 3 assays | | - | - | - | - | 94 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Nalidixic acid |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-01 | 1 | 0.947 | 0.955 | 0.790 | 0.948 | 150 | FDSC-01 | 1 | 0.032 | 0.033 | 0.323 | 0.043 | 280 | | |
| | 2 | 0.966 | 0.963 | 0.812 | 0.956 | 147 | | 2 | 0.032 | 0.037 | 0.262 | 0.050 | 219 | | |
| | 3 | 0.970 | 0.967 | 0.818 | 0.961 | 145 | | 3 | 0.033 | 0.033 | 0.308 | 0.042 | 264 | | |
| | Mean | 0.961 | 0.962 | 0.807 | 0.955 | 147 | | Mean | 0.032 | 0.034 | 0.298 | 0.045 | 254 | Positive | Photoreactive |
| FDSC-12 | 1 | 0.961 | 0.967 | 0.804 | 0.961 | 150 | FDSC-12 | 1 | 0.033 | 0.033 | 0.265 | 0.049 | 217 | | |
| | 2 | 0.963 | 0.962 | 0.814 | 0.954 | 142 | | 2 | 0.034 | 0.035 | 0.230 | 0.050 | 181 | | |
| | 3 | 0.966 | 0.967 | 0.819 | 0.959 | 140 | | 3 | 0.034 | 0.033 | 0.270 | 0.047 | 221 | | |
| | Mean | 0.963 | 0.965 | 0.812 | 0.958 | 144 | | Mean | 0.034 | 0.034 | 0.255 | 0.049 | 206 | Positive | Photoreactive |
| FDSC-19 | 1 | 0.971 | 0.986 | 0.818 | 0.990 | 149 | FDSC-19 | 1 | 0.032 | 0.033 | 0.317 | 0.049 | 267 | | |
| | 2 | 0.973 | 0.981 | 0.824 | 0.975 | 145 | | 2 | 0.032 | 0.032 | 0.285 | 0.054 | 235 | | |
| | 3 | 0.977 | 0.991 | 0.832 | 0.982 | 141 | | 3 | 0.033 | 0.033 | 0.304 | 0.050 | 253 | | |
| | Mean | 0.974 | 0.986 | 0.825 | 0.982 | 145 | | Mean | 0.032 | 0.033 | 0.302 | 0.051 | 252 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 145 | Mean for 3 assays | | - | - | - | - | 237 | Positive *3 | Photoreactive*3 |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Nalidixic acid (Na salt) |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-15 | 1 | 0.972 | 0.974 | 0.814 | 0.967 | 153 | FDSC-15 | 1 | 0.032 | 0.033 | 0.252 | 0.054 | 205 | | |
| | 2 | 0.976 | 0.975 | 0.829 | 0.971 | 142 | | 2 | 0.031 | 0.032 | 0.282 | 0.048 | 236 | | |
| | 3 | 0.979 | 0.982 | 0.836 | 0.977 | 138 | | 3 | 0.032 | 0.035 | 0.261 | 0.043 | 214 | | |
| | Mean | 0.976 | 0.977 | 0.826 | 0.972 | 144 | | Mean | 0.032 | 0.033 | 0.265 | 0.048 | 218 | Positive | Photoreactive |
| FDSC-22 | 1 | 0.958 | 0.980 | 0.809 | 0.974 | 143 | FDSC-22 | 1 | 0.031 | 0.031 | 0.214 | 0.040 | 169 | | |
| | 2 | 0.958 | 0.982 | 0.813 | 0.974 | 139 | | 2 | 0.033 | 0.033 | 0.234 | 0.057 | 187 | | |
| | 3 | 0.966 | 0.991 | 0.822 | 0.986 | 138 | | 3 | 0.031 | 0.032 | 0.266 | 0.042 | 221 | | |
| | Mean | 0.961 | 0.984 | 0.815 | 0.978 | 140 | | Mean | 0.032 | 0.032 | 0.238 | 0.046 | 192 | Positive | Photoreactive |
| FDSC-26 | 1 | 0.962 | 0.959 | 0.800 | 0.954 | 156 | FDSC-26 | 1 | 0.033 | 0.037 | 0.270 | 0.042 | 226 | | |
| | 2 | 0.959 | 0.955 | 0.805 | 0.949 | 148 | | 2 | 0.033 | 0.033 | 0.253 | 0.050 | 209 | | |
| | 3 | 0.962 | 0.964 | 0.812 | 0.956 | 144 | | 3 | 0.034 | 0.033 | 0.242 | 0.043 | 197 | | |
| | Mean | 0.961 | 0.959 | 0.806 | 0.953 | 149 | | Mean | 0.033 | 0.034 | 0.255 | 0.045 | 211 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 144 | Mean for 3 assays | | - | - | - | - | 207 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Norfloxacin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-12 | 1 | 0.985 | 0.967 | 0.755 | 0.961 | 223 | FDSC-12 | 1 | 0.033 | 0.033 | 0.169 | 0.049 | 121 | | |
| | 2 | 0.986 | 0.962 | 0.804 | 0.954 | 175 | | 2 | 0.033 | 0.035 | 0.182 | 0.050 | 134 | | |
| | 3 | 0.977 | 0.967 | 0.803 | 0.959 | 167 | | 3 | 0.034 | 0.033 | 0.190 | 0.047 | 141 | | |
| | Mean | 0.983 | 0.965 | 0.787 | 0.958 | 188 | | Mean | 0.033 | 0.034 | 0.180 | 0.049 | 132 | Positive | Photoreactive |
| FDSC-19 | 1 | 0.976 | 0.986 | 0.745 | 0.990 | 227 | FDSC-19 | 1 | 0.032 | 0.033 | 0.153 | 0.049 | 103 | | |
| | 2 | 0.977 | 0.981 | 0.756 | 0.975 | 217 | | 2 | 0.032 | 0.032 | 0.172 | 0.054 | 122 | | |
| | 3 | 0.980 | 0.991 | 0.755 | 0.982 | 221 | | 3 | 0.033 | 0.033 | 0.185 | 0.050 | 134 | | |
| | Mean | 0.978 | 0.986 | 0.752 | 0.982 | 222 | | Mean | 0.032 | 0.033 | 0.170 | 0.051 | 120 | Positive | Photoreactive |
| FDSC-26 | 1 | 0.956 | 0.959 | 0.751 | 0.954 | 199 | FDSC-26 | 1 | 0.033 | 0.037 | 0.159 | 0.042 | 115 | | |
| | 2 | 0.956 | 0.955 | 0.747 | 0.949 | 203 | | 2 | 0.032 | 0.033 | 0.162 | 0.050 | 119 | | |
| | 3 | 0.963 | 0.964 | 0.759 | 0.956 | 198 | | 3 | 0.033 | 0.033 | 0.166 | 0.043 | 122 | | |
| | Mean | 0.958 | 0.959 | 0.752 | 0.953 | 200 | | Mean | 0.033 | 0.034 | 0.162 | 0.045 | 119 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 203 | Mean for 3 assays | | - | - | - | - | 124 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Ofloxacin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-06 | 1 | 0.973 | 0.982 | 0.826 | 0.972 | 138 | FDSC-06 | 1 | 0.032 | 0.033 | 0.338 | 0.052 | 288 | | |
| | 2 | 0.975 | 0.977 | 0.839 | 0.965 | 127 | | 2 | 0.034 | 0.034 | 0.322 | 0.047 | 270 | | |
| | 3 | 0.979 | 0.982 | 0.839 | 0.976 | 131 | | 3 | 0.032 | 0.033 | 0.356 | 0.055 | 306 | | |
| | Mean | 0.976 | 0.980 | 0.835 | 0.971 | 132 | | Mean | 0.033 | 0.033 | 0.339 | 0.051 | 288 | Positive | Photoreactive |
| FDSC-14 | 1 | 0.967 | 0.971 | 0.813 | 0.960 | 144 | FDSC-14 | 1 | 0.032 | 0.032 | 0.327 | 0.044 | 282 | | |
| | 2 | 0.974 | 0.968 | 0.830 | 0.959 | 134 | | 2 | 0.035 | 0.033 | 0.329 | 0.050 | 281 | | |
| | 3 | 0.976 | 0.974 | 0.832 | 0.963 | 134 | | 3 | 0.033 | 0.033 | 0.359 | 0.045 | 313 | | |
| | Mean | 0.972 | 0.971 | 0.825 | 0.961 | 137 | | Mean | 0.033 | 0.033 | 0.338 | 0.046 | 292 | Positive | Photoreactive |
| FDSC-21 | 1 | 0.963 | 0.959 | 0.784 | 0.967 | 177 | FDSC-21 | 1 | 0.033 | 0.031 | 0.368 | 0.043 | 326 | | |
| | 2 | 0.977 | 0.958 | 0.837 | 0.953 | 138 | | 2 | 0.034 | 0.032 | 0.272 | 0.041 | 229 | | |
| | 3 | 0.970 | 0.963 | 0.836 | 0.955 | 132 | | 3 | 0.033 | 0.035 | 0.363 | 0.042 | 321 | | |
| | Mean | 0.970 | 0.960 | 0.819 | 0.958 | 149 | | Mean | 0.033 | 0.033 | 0.334 | 0.042 | 292 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 139 | Mean for 3 assays | | - | - | - | - | 291 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Piroxicam |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-05 | 1 | 0.964 | 0.970 | 0.745 | 0.966 | 218 | FDSC-09 | 1 | 0.039 | 0.032 | 0.114 | 0.052 | 52 | | |
| | 2 | 0.966 | 0.966 | 0.764 | 0.973 | 201 | | 2 | 0.038 | 0.032 | 0.118 | 0.057 | 57 | | |
| | 3 | 0.965 | 0.975 | 0.762 | 0.968 | 202 | | 3 | 0.038 | 0.032 | 0.121 | 0.055 | 60 | | |
| | Mean | 0.965 | 0.970 | 0.757 | 0.969 | 207 | | Mean | 0.038 | 0.032 | 0.118 | 0.055 | 56 | Positive | Photoreactive |
| FDSC-09 | 1 | 0.998 | 0.984 | 0.751 | 0.975 | 241 | FDSC-17 | 1 | 0.038 | 0.032 | 0.096 | 0.053 | 40 | | |
| | 2 | 0.992 | 0.984 | 0.762 | 0.980 | 224 | | 2 | 0.037 | 0.033 | 0.102 | 0.052 | 47 | | |
| | 3 | 0.995 | 0.994 | 0.763 | 0.987 | 226 | | 3 | 0.037 | 0.032 | 0.108 | 0.046 | 53 | | |
| | Mean | 0.995 | 0.987 | 0.759 | 0.981 | 230 | | Mean | 0.037 | 0.032 | 0.102 | 0.050 | 47 | Positive | Photoreactive |
| FDSC-17 | 1 | 0.989 | 0.981 | 0.752 | 0.977 | 234 | FDSC-18 | 1 | 0.040 | 0.032 | 0.094 | 0.043 | 41 | | |
| | 2 | 0.995 | 0.989 | 0.777 | 0.984 | 215 | | 2 | 0.038 | 0.032 | 0.094 | 0.047 | 43 | | |
| | 3 | 0.988 | 0.995 | 0.771 | 0.993 | 214 | | 3 | 0.039 | 0.033 | 0.096 | 0.045 | 44 | | |
| | Mean | 0.991 | 0.988 | 0.767 | 0.985 | 221 | | Mean | 0.039 | 0.032 | 0.095 | 0.045 | 43 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 219 | Mean for 3 assays | | - | - | - | - | 49 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Promethazine HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-05 | 1 | 0.959 | 0.970 | 0.869 | 0.966 | 89 | FDSC-05 | 1 | 0.033 | 0.032 | 0.092 | 0.048 | 43 | | |
| | 2 | 0.966 | 0.966 | 0.873 | 0.973 | 92 | | 2 | 0.033 | 0.032 | 0.090 | 0.051 | 41 | | |
| | 3 | 0.966 | 0.975 | 0.874 | 0.968 | 91 | | 3 | 0.035 | 0.032 | 0.095 | 0.046 | 44 | | |
| | Mean | 0.964 | 0.970 | 0.872 | 0.969 | 91 | | Mean | 0.034 | 0.032 | 0.092 | 0.048 | 43 | Positive | Photoreactive |
| FDSC-14 | 1 | 0.950 | 0.971 | 0.851 | 0.960 | 89 | FDSC-14 | 1 | 0.031 | 0.032 | 0.094 | 0.044 | 50 | | |
| | 2 | 0.974 | 0.968 | 0.878 | 0.959 | 86 | | 2 | 0.034 | 0.033 | 0.094 | 0.050 | 47 | | |
| | 3 | 0.970 | 0.974 | 0.869 | 0.963 | 91 | | 3 | 0.032 | 0.033 | 0.100 | 0.045 | 55 | | |
| | Mean | 0.965 | 0.971 | 0.866 | 0.961 | 89 | | Mean | 0.032 | 0.033 | 0.096 | 0.046 | 51 | Positive | Photoreactive |
| FDSC-21 | 1 | 0.948 | 0.959 | 0.845 | 0.967 | 101 | FDSC-21 | 1 | 0.031 | 0.031 | 0.085 | 0.043 | 45 | | |
| | 2 | 0.964 | 0.958 | 0.858 | 0.953 | 104 | | 2 | 0.032 | 0.032 | 0.088 | 0.041 | 47 | | |
| | 3 | 0.961 | 0.963 | 0.856 | 0.955 | 103 | | 3 | 0.032 | 0.035 | 0.092 | 0.042 | 51 | | |
| | Mean | 0.958 | 0.960 | 0.853 | 0.958 | 103 | | Mean | 0.032 | 0.033 | 0.088 | 0.042 | 48 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 94 | Mean for 3 assays | | - | - | - | - | 47 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Rosiglitazone |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-06 | 1 | 0.955 | 0.982 | 0.860 | 0.972 | 86 | FDSC-09 | 1 | 0.036 | 0.032 | 0.080 | 0.052 | 21 | | |
| | 2 | 0.936 | 0.977 | 0.837 | 0.965 | 90 | | 2 | 0.036 | 0.032 | 0.096 | 0.057 | 37 | | |
| | 3 | 0.943 | 0.982 | 0.840 | 0.976 | 94 | | 3 | 0.037 | 0.032 | 0.083 | 0.055 | 23 | | |
| | Mean | 0.945 | 0.980 | 0.846 | 0.971 | 90 | | Mean | 0.036 | 0.032 | 0.086 | 0.055 | 27 | Positive | |
| FDSC-09 | 1 | 0.967 | 0.984 | 0.879 | 0.975 | 82 | FDSC-17 | 1 | 0.037 | 0.032 | 0.081 | 0.053 | 26 | | |
| | 2 | 0.967 | 0.984 | 0.874 | 0.980 | 87 | | 2 | 0.036 | 0.033 | 0.083 | 0.052 | 29 | | |
| | 3 | 0.963 | 0.994 | 0.864 | 0.987 | 93 | | 3 | 0.037 | 0.032 | 0.088 | 0.046 | 33 | | |
| | Mean | 0.966 | 0.987 | 0.872 | 0.981 | 87 | | Mean | 0.037 | 0.032 | 0.084 | 0.050 | 29 | Positive | |
| FDSC-17 | 1 | 0.946 | 0.981 | 0.862 | 0.977 | 81 | FDSC-18 | 1 | 0.037 | 0.032 | 0.086 | 0.043 | 36 | | |
| | 2 | 0.973 | 0.989 | 0.871 | 0.984 | 99 | | 2 | 0.037 | 0.032 | 0.082 | 0.047 | 32 | | |
| | 3 | 0.965 | 0.995 | 0.863 | 0.993 | 99 | | 3 | 0.036 | 0.033 | 0.085 | 0.045 | 36 | | |
| | Mean | 0.961 | 0.988 | 0.865 | 0.985 | 93 | | Mean | 0.037 | 0.032 | 0.084 | 0.045 | 35 | Positive | |
| Mean for 3 assays | | - | - | - | - | 90 | Mean for 3 assays | | - | - | - | - | 30 | Positive | [*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Tetracycline |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-05 | 1 | 0.968 | 0.970 | 0.792 | 0.966 | 175 | FDSC-05 | 1 | 0.039 | 0.032 | 0.289 | 0.048 | 234 | | |
| | 2 | 0.995 | 0.966 | 0.829 | 0.973 | 165 | | 2 | 0.042 | 0.032 | 0.299 | 0.051 | 241 | | |
| | 3 | 0.992 | 0.975 | 0.829 | 0.968 | 162 | | 3 | 0.040 | 0.032 | 0.301 | 0.046 | 245 | | |
| | Mean | 0.985 | 0.970 | 0.817 | 0.969 | 167 | | Mean | 0.040 | 0.032 | 0.296 | 0.048 | 240 | Positive | Photoreactive |
| FDSC-15 | 1 | 0.964 | 0.974 | 0.793 | 0.967 | 166 | FDSC-15 | 1 | 0.040 | 0.033 | 0.305 | 0.054 | 250 | | |
| | 2 | 0.971 | 0.975 | 0.809 | 0.971 | 157 | | 2 | 0.042 | 0.032 | 0.314 | 0.048 | 257 | | |
| | 3 | 0.986 | 0.982 | 0.823 | 0.977 | 158 | | 3 | 0.043 | 0.035 | 0.316 | 0.043 | 258 | | |
| | Mean | 0.974 | 0.977 | 0.808 | 0.972 | 160 | | Mean | 0.042 | 0.033 | 0.312 | 0.048 | 255 | Positive | Photoreactive |
| FDSC-22 | 1 | 0.961 | 0.980 | 0.783 | 0.974 | 172 | FDSC-22 | 1 | 0.040 | 0.031 | 0.291 | 0.040 | 237 | | |
| | 2 | 0.973 | 0.982 | 0.806 | 0.974 | 161 | | 2 | 0.046 | 0.033 | 0.297 | 0.057 | 237 | | |
| | 3 | 0.981 | 0.991 | 0.809 | 0.986 | 166 | | 3 | 0.038 | 0.032 | 0.296 | 0.042 | 244 | | |
| | Mean | 0.972 | 0.984 | 0.799 | 0.978 | 166 | | Mean | 0.041 | 0.032 | 0.295 | 0.046 | 239 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 164 | Mean for 3 assays | | - | - | - | - | 245 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Anthracene |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-10 | 1 | 0.982 | 0.982 | 0.977 | 0.976 | -4 | FDSC-10 | 1 | 0.033 | 0.032 | 0.054 | 0.047 | 2 | | |
| | 2 | 0.989 | 0.992 | 0.979 | 0.975 | 1 | | 2 | 0.036 | 0.032 | 0.059 | 0.053 | 4 | | |
| | 3 | 0.994 | 0.987 | 0.987 | 0.984 | -2 | | 3 | 0.033 | 0.034 | 0.055 | 0.055 | 3 | | |
| | Mean | 0.988 | 0.987 | 0.981 | 0.978 | -2 | | Mean | 0.034 | 0.033 | 0.056 | 0.052 | 3 | Inconclusive | (Non-photoreactive) |
| FDSC-16 | 1 | 0.981 | 0.986 | 0.976 | 0.996 | 5 | FDSC-16 | 1 | 0.032 | 0.031 | 0.050 | 0.041 | 7 | | |
| | 2 | 0.986 | 0.982 | 0.978 | 0.980 | 8 | | 2 | 0.032 | 0.032 | 0.053 | 0.044 | 10 | | |
| | 3 | 0.992 | 0.993 | 0.985 | 0.986 | 7 | | 3 | 0.033 | 0.033 | 0.052 | 0.043 | 8 | | |
| | Mean | 0.986 | 0.987 | 0.980 | 0.987 | 7 | | Mean | 0.032 | 0.032 | 0.052 | 0.043 | 8 | Inconclusive | (Non-photoreactive) |
| FDSC-23 | 1 | 0.968 | 0.957 | 0.953 | 0.946 | 5 | FDSC-23 | 1 | 0.033 | 0.032 | 0.049 | 0.059 | -2 | | |
| | 2 | 0.972 | 0.960 | 0.956 | 0.947 | 6 | | 2 | 0.032 | 0.032 | 0.048 | 0.045 | -2 | | |
| | 3 | 0.978 | 0.965 | 0.962 | 0.959 | 6 | | 3 | 0.033 | 0.033 | 0.047 | 0.045 | -4 | | |
| | Mean | 0.973 | 0.961 | 0.957 | 0.951 | 6 | | Mean | 0.033 | 0.032 | 0.048 | 0.050 | -3 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | 4 | Mean for 3 assays | | - | - | - | - | 3 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Avobenzone |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-10 | 1 | 0.986 | 0.982 | 0.975 | 0.976 | 2 | FDSC-10 | 1 | 0.033 | 0.032 | 0.080 | 0.047 | 28 | | |
| | 2 | 0.992 | 0.992 | 0.979 | 0.975 | 4 | | 2 | 0.034 | 0.032 | 0.084 | 0.053 | 31 | | |
| | 3 | 0.989 | 0.987 | 0.985 | 0.984 | -5 | | 3 | 0.033 | 0.034 | 0.081 | 0.055 | 29 | | |
| | Mean | 0.989 | 0.987 | 0.980 | 0.978 | 0 | | Mean | 0.033 | 0.033 | 0.082 | 0.052 | 29 | Positive | (Weakly photoreactive) |
| FDSC-16 | 1 | 0.979 | 0.986 | 0.966 | 0.996 | 13 | FDSC-16 | 1 | 0.033 | 0.031 | 0.079 | 0.041 | 35 | | |
| | 2 | 0.980 | 0.982 | 0.972 | 0.980 | 8 | | 2 | 0.033 | 0.032 | 0.081 | 0.044 | 37 | | |
| | 3 | 0.989 | 0.993 | 0.973 | 0.986 | 16 | | 3 | 0.034 | 0.033 | 0.081 | 0.043 | 36 | | |
| | Mean | 0.983 | 0.987 | 0.970 | 0.987 | 12 | | Mean | 0.033 | 0.032 | 0.080 | 0.043 | 36 | Positive | (Weakly photoreactive) |
| FDSC-23 | 1 | 0.970 | 0.957 | 0.951 | 0.946 | 9 | FDSC-23 | 1 | 0.034 | 0.032 | 0.080 | 0.059 | 28 | | |
| | 2 | 0.983 | 0.960 | 0.958 | 0.947 | 15 | | 2 | 0.035 | 0.032 | 0.082 | 0.045 | 29 | | |
| | 3 | 0.983 | 0.965 | 0.966 | 0.959 | 7 | | 3 | 0.045 | 0.033 | 0.080 | 0.045 | 17 | | |
| | Mean | 0.979 | 0.961 | 0.958 | 0.951 | 10 | | Mean | 0.038 | 0.032 | 0.081 | 0.050 | 25 | Positive | (Weakly photoreactive) |
| Mean for 3 assays | | - | - | - | - | 7 | Mean for 3 assays | | - | - | - | - | 30 | Positive [*3] | (Weakly photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Bithionol |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-03 | 1 | 0.935 | 0.966 | 0.795 | 0.962 | 137 | FDSC-11 | 1 | 0.034 | 0.031 | 0.064 | 0.049 | 11 | | |
| | 2 | 0.948 | 0.970 | 0.809 | 0.968 | 136 | | 2 | 0.033 | 0.032 | 0.067 | 0.055 | 15 | | |
| | 3 | 0.944 | 0.974 | 0.802 | 0.970 | 139 | | 3 | 0.034 | 0.034 | 0.067 | 0.050 | 14 | | |
| | Mean | 0.942 | 0.970 | 0.802 | 0.967 | 137 | | Mean | 0.034 | 0.032 | 0.066 | 0.051 | 13 | Positive | Photoreactive |
| FDSC-09 | 1 | 0.954 | 0.984 | 0.806 | 0.975 | 142 | FDSC-17 | 1 | 0.035 | 0.032 | 0.068 | 0.053 | 15 | | |
| | 2 | 0.946 | 0.984 | 0.798 | 0.980 | 142 | | 2 | 0.035 | 0.033 | 0.068 | 0.052 | 15 | | |
| | 3 | 0.966 | 0.994 | 0.815 | 0.987 | 145 | | 3 | 0.035 | 0.032 | 0.067 | 0.046 | 14 | | |
| | Mean | 0.955 | 0.987 | 0.806 | 0.981 | 143 | | Mean | 0.035 | 0.032 | 0.068 | 0.050 | 15 | Positive | Photoreactive |
| FDSC-11 | 1 | 0.950 | 0.977 | 0.803 | 0.965 | 139 | FDSC-18 | 1 | 0.034 | 0.032 | 0.068 | 0.043 | 21 | | |
| | 2 | 0.965 | 0.972 | 0.817 | 0.967 | 140 | | 2 | 0.034 | 0.032 | 0.059 | 0.047 | 12 | | |
| | 3 | 0.952 | 0.979 | 0.802 | 0.971 | 142 | | 3 | 0.034 | 0.033 | 0.060 | 0.045 | 13 | | |
| | Mean | 0.956 | 0.976 | 0.807 | 0.968 | 140 | | Mean | 0.034 | 0.032 | 0.062 | 0.045 | 15 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 140 | Mean for 3 assays | | - | - | - | - | 14 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Hexachlorophene |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-02 | 1 | 0.921 | 0.964 | 0.659 | 0.953 | 252 | FDSC-11 | 1 | 0.033 | 0.031 | 0.046 | 0.049 | -6 | | |
| | 2 | 0.928 | 0.971 | 0.671 | 0.964 | 247 | | 2 | 0.034 | 0.032 | 0.047 | 0.055 | -6 | | |
| | 3 | 0.918 | 0.978 | 0.668 | 0.965 | 240 | | 3 | 0.035 | 0.034 | 0.048 | 0.050 | -6 | | |
| | Mean | 0.922 | 0.971 | 0.666 | 0.961 | 246 | | Mean | 0.034 | 0.032 | 0.047 | 0.051 | -6 | Positive | Photoreactive |
| FDSC-09 | 1 | 0.929 | 0.984 | 0.669 | 0.975 | 254 | FDSC-17 | 1 | 0.035 | 0.032 | 0.069 | 0.053 | 16 | | |
| | 2 | 0.941 | 0.984 | 0.678 | 0.980 | 257 | | 2 | 0.035 | 0.033 | 0.066 | 0.052 | 13 | | |
| | 3 | 0.944 | 0.994 | 0.683 | 0.987 | 255 | | 3 | 0.035 | 0.032 | 0.068 | 0.046 | 15 | | |
| | Mean | 0.938 | 0.987 | 0.677 | 0.981 | 255 | | Mean | 0.035 | 0.032 | 0.068 | 0.050 | 15 | Positive | Photoreactive |
| FDSC-11 | 1 | 0.921 | 0.977 | 0.656 | 0.965 | 257 | FDSC-18 | 1 | 0.034 | 0.032 | 0.044 | 0.043 | -3 | | |
| | 2 | 0.924 | 0.972 | 0.658 | 0.967 | 258 | | 2 | 0.033 | 0.032 | 0.043 | 0.047 | -3 | | |
| | 3 | 0.932 | 0.979 | 0.665 | 0.971 | 259 | | 3 | 0.034 | 0.033 | 0.043 | 0.045 | -4 | | |
| | Mean | 0.926 | 0.976 | 0.660 | 0.968 | 258 | | Mean | 0.034 | 0.032 | 0.043 | 0.045 | -3 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 253 | Mean for 3 assays | | - | - | - | - | 2 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Rose bengal |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-02 | 1 | 1.247 | 0.964 | 0.607 | 0.953 | 630 | FDSC-02 | 1 | 3.498 | 0.032 | 3.334 | 0.041 | No data[*4] | | |
| | 2 | 1.259 | 0.971 | 0.618 | 0.964 | 631 | | 2 | 3.460 | 0.034 | 3.338 | 0.041 | No data[*4] | | |
| | 3 | 1.258 | 0.978 | 0.617 | 0.965 | 631 | | 3 | 3.566 | 0.038 | 3.374 | 0.081 | No data[*4] | | |
| | Mean | 1.255 | 0.971 | 0.614 | 0.961 | 631 | | Mean | 3.508 | 0.035 | 3.349 | 0.054 | No data[*4] | Positive | Photoreactive |
| FDSC-12 | 1 | 1.241 | 0.967 | 0.607 | 0.961 | 627 | FDSC-12 | 1 | OVER | 0.033 | 3.747 | 0.049 | No data[*4] | | |
| | 2 | 1.267 | 0.962 | 0.626 | 0.954 | 634 | | 2 | OVER | 0.035 | OVER | 0.050 | No data[*4] | | |
| | 3 | 1.276 | 0.967 | 0.629 | 0.959 | 640 | | 3 | OVER | 0.033 | 3.943 | 0.047 | No data[*4] | | |
| | Mean | 1.261 | 0.965 | 0.621 | 0.958 | 634 | | Mean | #DIV/0! | 0.034 | 3.845 | 0.049 | No data[*4] | Positive | Photoreactive |
| FDSC-19 | 1 | 1.254 | 0.986 | 0.576 | 0.990 | 674 | FDSC-19 | 1 | OVER | 0.033 | 3.513 | 0.049 | No data[*4] | | |
| | 2 | 1.250 | 0.981 | 0.583 | 0.975 | 663 | | 2 | OVER | 0.032 | 3.467 | 0.054 | No data[*4] | | |
| | 3 | 1.253 | 0.991 | 0.585 | 0.982 | 664 | | 3 | OVER | 0.033 | 3.424 | 0.050 | No data[*4] | | |
| | Mean | 1.252 | 0.986 | 0.581 | 0.982 | 667 | | Mean | #DIV/0! | 0.033 | 3.468 | 0.051 | No data[*4] | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 644 | Mean for 3 assays | | - | - | - | - | No data[*4] | Positive [*3] | Photoreactive[*3] |

OVER: Sptical dencity was not measurable because it was more thant 4.0.

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

*4 : Over the OD criteria

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 2
Chemical Name : Aspirin

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-01 | 1 | 0.957 | 0.955 | 0.949 | 0.948 | 1 | FDSC-01 | 1 | 0.034 | 0.033 | 0.049 | 0.043 | 4 | | |
| | 2 | 0.979 | 0.963 | 0.969 | 0.956 | 3 | | 2 | 0.035 | 0.037 | 0.045 | 0.050 | -1 | | |
| | 3 | 0.979 | 0.967 | 0.973 | 0.961 | -1 | | 3 | 0.035 | 0.033 | 0.043 | 0.042 | -3 | | |
| | Mean | 0.972 | 0.962 | 0.964 | 0.955 | 1 | | Mean | 0.035 | 0.034 | 0.046 | 0.045 | 0 | Negative | Non-photoreactive |
| FDSC-12 | 1 | 0.962 | 0.967 | 0.957 | 0.961 | -2 | FDSC-12 | 1 | 0.033 | 0.033 | 0.041 | 0.049 | -7 | | |
| | 2 | 0.972 | 0.962 | 0.965 | 0.954 | 0 | | 2 | 0.033 | 0.035 | 0.043 | 0.050 | -5 | | |
| | 3 | 0.967 | 0.967 | 0.961 | 0.959 | -1 | | 3 | 0.032 | 0.033 | 0.042 | 0.047 | -5 | | |
| | Mean | 0.967 | 0.965 | 0.961 | 0.958 | -1 | | Mean | 0.033 | 0.034 | 0.042 | 0.049 | -6 | Negative | Non-photoreactive |
| FDSC-19 | 1 | 0.970 | 0.986 | 0.965 | 0.990 | 1 | FDSC-19 | 1 | 0.031 | 0.033 | 0.045 | 0.049 | -4 | | |
| | 2 | 0.973 | 0.981 | 0.968 | 0.975 | 1 | | 2 | 0.032 | 0.032 | 0.046 | 0.054 | -4 | | |
| | 3 | 0.979 | 0.991 | 0.974 | 0.982 | 1 | | 3 | 0.032 | 0.033 | 0.046 | 0.050 | -4 | | |
| | Mean | 0.974 | 0.986 | 0.969 | 0.982 | 1 | | Mean | 0.032 | 0.033 | 0.046 | 0.051 | -4 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 0 | Mean for 3 assays | | - | - | - | - | -3 | Negative *3 | Non-photoreactive*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Benzocaine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-03 | 1 | 0.961 | 0.966 | 0.952 | 0.962 | 6 | FDSC-03 | 1 | 0.033 | 0.033 | 0.045 | 0.050 | -5 | | |
| | 2 | 0.970 | 0.970 | 0.967 | 0.968 | 0 | | 2 | 0.031 | 0.033 | 0.044 | 0.050 | -4 | | |
| | 3 | 0.973 | 0.974 | 0.950 | 0.970 | 20 | | 3 | 0.033 | 0.035 | 0.046 | 0.053 | -4 | | |
| | Mean | 0.968 | 0.970 | 0.956 | 0.967 | 9 | | Mean | 0.032 | 0.034 | 0.045 | 0.051 | -4 | Negative | Non-photoreactive |
| FDSC-13 | 1 | 0.963 | 0.970 | 0.956 | 0.970 | 2 | FDSC-13 | 1 | 0.031 | 0.034 | 0.054 | 0.043 | 14 | | |
| | 2 | 0.965 | 0.970 | 0.960 | 0.962 | 0 | | 2 | 0.034 | 0.034 | 0.054 | 0.043 | 11 | | |
| | 3 | 0.962 | 0.972 | 0.955 | 0.965 | 2 | | 3 | 0.032 | 0.033 | 0.051 | 0.044 | 10 | | |
| | Mean | 0.963 | 0.971 | 0.957 | 0.966 | 1 | | Mean | 0.032 | 0.034 | 0.053 | 0.043 | 12 | Negative | Non-photoreactive |
| FDSC-20 | 1 | 0.966 | 0.974 | 0.955 | 0.949 | -8 | FDSC-20 | 1 | 0.031 | 0.032 | 0.053 | 0.046 | 10 | | |
| | 2 | 0.973 | 0.972 | 0.967 | 0.954 | -13 | | 2 | 0.031 | 0.032 | 0.051 | 0.045 | 8 | | |
| | 3 | 0.982 | 0.980 | 0.977 | 0.966 | -14 | | 3 | 0.033 | 0.034 | 0.045 | 0.045 | 0 | | |
| | Mean | 0.974 | 0.975 | 0.966 | 0.956 | -12 | | Mean | 0.032 | 0.033 | 0.050 | 0.045 | 6 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -1 | Mean for 3 assays | | - | - | - | - | 5 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Erythromycin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-06 | 1 | 0.980 | 0.982 | 0.967 | 0.972 | 4 | FDSC-06 | 1 | 0.039 | 0.033 | 0.064 | 0.052 | 7 | | |
| | 2 | 0.958 | 0.977 | 0.946 | 0.965 | 3 | | 2 | 0.037 | 0.034 | 0.069 | 0.047 | 14 | | |
| | 3 | 0.975 | 0.982 | 0.956 | 0.976 | 10 | | 3 | 0.035 | 0.033 | 0.073 | 0.055 | 20 | | |
| | Mean | 0.971 | 0.980 | 0.956 | 0.971 | 6 | | Mean | 0.037 | 0.033 | 0.069 | 0.051 | 14 | Negative | Non-photoreactive |
| FDSC-14 | 1 | 0.960 | 0.971 | 0.950 | 0.960 | 0 | FDSC-14 | 1 | 0.034 | 0.032 | 0.052 | 0.044 | 5 | | |
| | 2 | 0.977 | 0.968 | 0.968 | 0.959 | -1 | | 2 | 0.035 | 0.033 | 0.052 | 0.050 | 4 | | |
| | 3 | 0.974 | 0.974 | 0.966 | 0.963 | -2 | | 3 | 0.036 | 0.033 | 0.053 | 0.045 | 4 | | |
| | Mean | 0.970 | 0.971 | 0.961 | 0.961 | -1 | | Mean | 0.035 | 0.033 | 0.052 | 0.046 | 4 | Negative | Non-photoreactive |
| FDSC-21 | 1 | 0.948 | 0.959 | 0.929 | 0.967 | 17 | FDSC-21 | 1 | 0.037 | 0.031 | 0.083 | 0.043 | 37 | | |
| | 2 | 0.963 | 0.958 | 0.950 | 0.953 | 11 | | 2 | 0.036 | 0.032 | 0.073 | 0.041 | 28 | | |
| | 3 | 0.967 | 0.963 | 0.954 | 0.955 | 11 | | 3 | 0.036 | 0.035 | 0.085 | 0.042 | 40 | | |
| | Mean | 0.959 | 0.960 | 0.944 | 0.958 | 13 | | Mean | 0.036 | 0.033 | 0.080 | 0.042 | 35 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 6 | Mean for 3 assays | | - | - | - | - | 18 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Penicillin G |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-06 | 1 | 0.979 | 0.982 | 0.969 | 0.972 | 1 | FDSC-06 | 1 | 0.034 | 0.033 | 0.090 | 0.052 | 38 | | |
| | 2 | 0.981 | 0.977 | 0.970 | 0.965 | 2 | | 2 | 0.035 | 0.034 | 0.091 | 0.047 | 38 | | |
| | 3 | 0.978 | 0.982 | 0.971 | 0.976 | -2 | | 3 | 0.041 | 0.033 | 0.094 | 0.055 | 35 | | |
| | Mean | 0.979 | 0.980 | 0.970 | 0.971 | 0 | | Mean | 0.037 | 0.033 | 0.092 | 0.051 | 37 | Positive | Weakly photoreactive |
| FDSC-14 | 1 | 0.975 | 0.971 | 0.967 | 0.960 | -2 | FDSC-14 | 1 | 0.034 | 0.032 | 0.082 | 0.044 | 35 | | |
| | 2 | 0.979 | 0.968 | 0.972 | 0.959 | -3 | | 2 | 0.034 | 0.033 | 0.081 | 0.050 | 34 | | |
| | 3 | 0.985 | 0.974 | 0.974 | 0.963 | 1 | | 3 | 0.034 | 0.033 | 0.087 | 0.045 | 40 | | |
| | Mean | 0.980 | 0.971 | 0.971 | 0.961 | -1 | | Mean | 0.034 | 0.033 | 0.083 | 0.046 | 36 | Positive | Weakly photoreactive |
| FDSC-21 | 1 | 0.970 | 0.959 | 0.964 | 0.967 | 4 | FDSC-21 | 1 | 0.034 | 0.031 | 0.077 | 0.043 | 34 | | |
| | 2 | 0.977 | 0.958 | 0.973 | 0.953 | 2 | | 2 | 0.036 | 0.032 | 0.075 | 0.041 | 30 | | |
| | 3 | 0.976 | 0.963 | 0.969 | 0.955 | 5 | | 3 | 0.034 | 0.035 | 0.081 | 0.042 | 38 | | |
| | Mean | 0.974 | 0.960 | 0.969 | 0.958 | 4 | | Mean | 0.035 | 0.033 | 0.078 | 0.042 | 34 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 1 | Mean for 3 assays | | - | - | - | - | 36 | Positive [*3] | Weakly photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 2
Chemical Name : Phenytoin

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-02 | 1 | 0.955 | 0.964 | 0.945 | 0.953 | 0 | | 1 | 0.033 | 0.032 | 0.102 | 0.041 | 50 | | |
| | 2 | 0.962 | 0.971 | 0.952 | 0.964 | 0 | | 2 | 0.036 | 0.034 | 0.104 | 0.041 | 49 | | |
| | 3 | 0.969 | 0.978 | 0.958 | 0.965 | 1 | | 3 | 0.034 | 0.038 | 0.109 | 0.081 | 56 | | |
| | Mean | 0.962 | 0.971 | 0.952 | 0.961 | 0 | | Mean | 0.034 | 0.035 | 0.105 | 0.054 | 52 | Positive | Weakly photoreactive |
| FDSC-12 | 1 | 0.970 | 0.967 | 0.967 | 0.961 | -4 | | 1 | 0.037 | 0.033 | 0.105 | 0.049 | 53 | | |
| | 2 | 0.971 | 0.962 | 0.967 | 0.954 | -3 | | 2 | 0.037 | 0.035 | 0.104 | 0.050 | 52 | | |
| | 3 | 0.966 | 0.967 | 0.963 | 0.959 | -4 | | 3 | 0.040 | 0.033 | 0.109 | 0.047 | 54 | | |
| | Mean | 0.969 | 0.965 | 0.966 | 0.958 | -4 | | Mean | 0.038 | 0.034 | 0.106 | 0.049 | 53 | Positive | Weakly photoreactive |
| FDSC-19 | 1 | 0.970 | 0.986 | 0.964 | 0.990 | 2 | | 1 | 0.033 | 0.033 | 0.099 | 0.049 | 48 | | |
| | 2 | 0.979 | 0.981 | 0.973 | 0.975 | 2 | | 2 | 0.035 | 0.032 | 0.100 | 0.054 | 47 | | |
| | 3 | 0.977 | 0.991 | 0.970 | 0.982 | 3 | | 3 | 0.038 | 0.033 | 0.104 | 0.050 | 48 | | |
| | Mean | 0.975 | 0.986 | 0.969 | 0.982 | 2 | | Mean | 0.035 | 0.033 | 0.101 | 0.051 | 48 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | -1 | Mean for 3 assays | | - | - | - | - | 51 | Positive [*3] | Weakly photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Bumetrizole |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-10 | 1 | 0.985 | 0.982 | 0.978 | 0.976 | -2 | FDSC-10 | 1 | 0.033 | 0.032 | 0.044 | 0.047 | -8 | | |
| | 2 | 0.990 | 0.992 | 0.985 | 0.975 | -4 | | 2 | 0.035 | 0.032 | 0.049 | 0.053 | -5 | | |
| | 3 | 0.997 | 0.987 | 0.996 | 0.984 | -8 | | 3 | 0.034 | 0.034 | 0.046 | 0.055 | -7 | | |
| | Mean | 0.991 | 0.987 | 0.986 | 0.978 | -5 | | Mean | 0.034 | 0.033 | 0.046 | 0.052 | -7 | Inconclusive | (Non-photoreactive) |
| FDSC-16 | 1 | 0.976 | 0.986 | 0.954 | 0.996 | 22 | FDSC-16 | 1 | 0.032 | 0.031 | 0.045 | 0.041 | 2 | | |
| | 2 | 0.983 | 0.982 | 0.979 | 0.980 | 4 | | 2 | 0.033 | 0.032 | 0.044 | 0.044 | 0 | | |
| | 3 | 0.983 | 0.993 | 0.979 | 0.986 | 4 | | 3 | 0.033 | 0.033 | 0.043 | 0.043 | -1 | | |
| | Mean | 0.981 | 0.987 | 0.971 | 0.987 | 10 | | Mean | 0.033 | 0.032 | 0.044 | 0.043 | 0 | Inconclusive | (Non-photoreactive) |
| FDSC-23 | 1 | 0.960 | 0.957 | 0.948 | 0.946 | 2 | FDSC-23 | 1 | 0.033 | 0.032 | 0.043 | 0.059 | -8 | | |
| | 2 | 0.966 | 0.960 | 0.954 | 0.947 | 2 | | 2 | 0.033 | 0.032 | 0.045 | 0.045 | -6 | | |
| | 3 | 0.968 | 0.965 | 0.961 | 0.959 | -3 | | 3 | 0.034 | 0.033 | 0.046 | 0.045 | -6 | | |
| | Mean | 0.965 | 0.961 | 0.954 | 0.951 | 0 | | Mean | 0.033 | 0.032 | 0.045 | 0.050 | -7 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | 2 | Mean for 3 assays | | - | - | - | - | -5 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 2
Chemical Name : Camphor sulfonic acid

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-04 | 1 | 0.969 | 0.972 | 0.956 | 0.963 | 3 | FDSC-04 | 1 | 0.032 | 0.032 | 0.045 | 0.050 | -4 | | |
| | 2 | 0.975 | 0.970 | 0.966 | 0.959 | -1 | | 2 | 0.033 | 0.032 | 0.046 | 0.049 | -4 | | |
| | 3 | 0.969 | 0.975 | 0.963 | 0.963 | -4 | | 3 | 0.034 | 0.033 | 0.046 | 0.049 | -5 | | |
| | Mean | 0.971 | 0.972 | 0.962 | 0.962 | -1 | | Mean | 0.033 | 0.032 | 0.046 | 0.049 | -4 | Negative | Non-photoreactive |
| FDSC-13 | 1 | 0.967 | 0.970 | 0.960 | 0.970 | 2 | FDSC-13 | 1 | 0.032 | 0.034 | 0.046 | 0.043 | 5 | | |
| | 2 | 0.974 | 0.970 | 0.965 | 0.962 | 4 | | 2 | 0.033 | 0.034 | 0.047 | 0.043 | 5 | | |
| | 3 | 0.970 | 0.972 | 0.963 | 0.965 | 2 | | 3 | 0.032 | 0.033 | 0.046 | 0.044 | 5 | | |
| | Mean | 0.970 | 0.971 | 0.963 | 0.966 | 3 | | Mean | 0.032 | 0.034 | 0.046 | 0.043 | 5 | Negative | Non-photoreactive |
| FDSC-26 | 1 | 0.963 | 0.959 | 0.954 | 0.954 | 3 | FDSC-26 | 1 | 0.033 | 0.037 | 0.042 | 0.042 | -2 | | |
| | 2 | 0.968 | 0.955 | 0.958 | 0.949 | 4 | | 2 | 0.033 | 0.033 | 0.044 | 0.050 | 0 | | |
| | 3 | 0.969 | 0.964 | 0.963 | 0.956 | 0 | | 3 | 0.033 | 0.033 | 0.041 | 0.043 | -3 | | |
| | Mean | 0.967 | 0.959 | 0.958 | 0.953 | 2 | | Mean | 0.033 | 0.034 | 0.042 | 0.045 | -2 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 1 | Mean for 3 assays | | - | - | - | - | 0 | Negative *3 | Non-photoreactive*3 |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Chlorhexidine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-05 | 1 | 0.916 | 0.970 | 0.902 | 0.966 | 13 | FDSC-05 | 1 | 0.039 | 0.032 | 0.068 | 0.048 | 13 | | |
| | 2 | 0.933 | 0.966 | 0.920 | 0.973 | 12 | | 2 | 0.038 | 0.032 | 0.064 | 0.051 | 10 | | |
| | 3 | 0.918 | 0.975 | 0.910 | 0.968 | 7 | | 3 | 0.038 | 0.032 | 0.069 | 0.046 | 15 | | |
| | Mean | 0.922 | 0.970 | 0.911 | 0.969 | 11 | | Mean | 0.038 | 0.032 | 0.067 | 0.048 | 13 | Negative | Non-photoreactive |
| FDSC-14 | 1 | 0.904 | 0.971 | 0.902 | 0.960 | -8 | FDSC-14 | 1 | 0.036 | 0.032 | 0.067 | 0.044 | 18 | | |
| | 2 | 0.904 | 0.968 | 0.901 | 0.959 | -7 | | 2 | 0.037 | 0.033 | 0.077 | 0.050 | 27 | | |
| | 3 | 0.905 | 0.974 | 0.907 | 0.963 | -12 | | 3 | 0.038 | 0.033 | 0.068 | 0.045 | 17 | | |
| | Mean | 0.904 | 0.971 | 0.903 | 0.961 | -9 | | Mean | 0.037 | 0.033 | 0.071 | 0.046 | 21 | Positive | Weakly photoreactive |
| FDSC-21 | 1 | 0.892 | 0.959 | 0.880 | 0.967 | 10 | FDSC-21 | 1 | 0.037 | 0.031 | 0.062 | 0.043 | 16 | | |
| | 2 | 0.901 | 0.958 | 0.892 | 0.953 | 7 | | 2 | 0.037 | 0.032 | 0.062 | 0.041 | 16 | | |
| | 3 | 0.889 | 0.963 | 0.880 | 0.955 | 7 | | 3 | 0.037 | 0.035 | 0.062 | 0.042 | 16 | | |
| | Mean | 0.894 | 0.960 | 0.884 | 0.958 | 8 | | Mean | 0.037 | 0.033 | 0.062 | 0.042 | 16 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 3 | Mean for 3 assays | | - | - | - | - | 17 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Cinnamic acid |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-03 | 1 | 0.977 | 0.966 | 0.967 | 0.962 | 7 | FDSC-03 | 1 | 0.035 | 0.033 | 0.100 | 0.050 | 48 | | |
| | 2 | 0.978 | 0.970 | 0.972 | 0.968 | 3 | | 2 | 0.032 | 0.033 | 0.102 | 0.050 | 53 | | |
| | 3 | 0.969 | 0.974 | 0.962 | 0.970 | 4 | | 3 | 0.033 | 0.035 | 0.104 | 0.053 | 54 | | |
| | Mean | 0.975 | 0.970 | 0.967 | 0.967 | 5 | | Mean | 0.033 | 0.034 | 0.102 | 0.051 | 52 | Positive | Weakly photoreactive |
| FDSC-13 | 1 | 0.962 | 0.970 | 0.956 | 0.970 | 1 | FDSC-13 | 1 | 0.032 | 0.034 | 0.075 | 0.043 | 34 | | |
| | 2 | 0.963 | 0.970 | 0.958 | 0.962 | 0 | | 2 | 0.033 | 0.034 | 0.077 | 0.043 | 35 | | |
| | 3 | 0.964 | 0.972 | 0.959 | 0.965 | 0 | | 3 | 0.032 | 0.033 | 0.081 | 0.044 | 40 | | |
| | Mean | 0.963 | 0.971 | 0.958 | 0.966 | 0 | | Mean | 0.032 | 0.034 | 0.078 | 0.043 | 36 | Positive | Weakly photoreactive |
| FDSC-20 | 1 | 0.966 | 0.974 | 0.951 | 0.949 | -4 | FDSC-20 | 1 | 0.031 | 0.032 | 0.080 | 0.046 | 37 | | |
| | 2 | 0.978 | 0.972 | 0.968 | 0.954 | -9 | | 2 | 0.031 | 0.032 | 0.077 | 0.045 | 34 | | |
| | 3 | 0.979 | 0.980 | 0.972 | 0.966 | -12 | | 3 | 0.033 | 0.034 | 0.084 | 0.045 | 39 | | |
| | Mean | 0.974 | 0.975 | 0.964 | 0.956 | -8 | | Mean | 0.032 | 0.033 | 0.080 | 0.045 | 37 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | -1 | Mean for 3 assays | | - | - | - | - | 42 | Positive [*3] | Weakly hotoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Drometrizole |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-15 | 1 | 0.975 | 0.974 | 0.966 | 0.967 | 4 | FDSC-15 | 1 | 0.035 | 0.033 | 0.058 | 0.054 | 8 | | |
| | 2 | 0.988 | 0.975 | 0.981 | 0.971 | 2 | | 2 | 0.034 | 0.032 | 0.058 | 0.048 | 9 | | |
| | 3 | 0.990 | 0.982 | 0.984 | 0.977 | 1 | | 3 | 0.037 | 0.035 | 0.059 | 0.043 | 7 | | |
| | Mean | 0.984 | 0.977 | 0.977 | 0.972 | 2 | | Mean | 0.035 | 0.033 | 0.058 | 0.048 | 8 | Inconclusive | Non-photoreactive |
| FDSC-22 | 1 | 0.967 | 0.980 | 0.957 | 0.974 | 4 | FDSC-22 | 1 | 0.034 | 0.031 | 0.050 | 0.040 | 2 | | |
| | 2 | 0.977 | 0.982 | 0.964 | 0.974 | 7 | | 2 | 0.034 | 0.033 | 0.052 | 0.057 | 4 | | |
| | 3 | 0.977 | 0.991 | 0.965 | 0.986 | 6 | | 3 | 0.033 | 0.032 | 0.050 | 0.042 | 3 | | |
| | Mean | 0.974 | 0.984 | 0.962 | 0.978 | 6 | | Mean | 0.034 | 0.032 | 0.051 | 0.046 | 3 | Inconclusive | Non-photoreactive |
| FDSC-26 | 1 | 0.969 | 0.959 | 0.955 | 0.954 | 8 | FDSC-26 | 1 | 0.035 | 0.037 | 0.047 | 0.042 | 1 | | |
| | 2 | 0.971 | 0.955 | 0.955 | 0.949 | 10 | | 2 | 0.036 | 0.033 | 0.050 | 0.050 | 3 | | |
| | 3 | 0.970 | 0.964 | 0.958 | 0.956 | 6 | | 3 | 0.035 | 0.033 | 0.049 | 0.043 | 3 | | |
| | Mean | 0.970 | 0.959 | 0.956 | 0.953 | 8 | | Mean | 0.035 | 0.034 | 0.049 | 0.045 | 2 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 5 | Mean for 3 assays | | - | - | - | - | 4 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | L-Histidine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-18 | 1 | 0.939 | 0.960 | 0.930 | 0.955 | 4 | FDSC-18 | 1 | 0.031 | 0.032 | 0.099 | 0.043 | 55 | | |
| | 2 | 0.940 | 0.955 | 0.933 | 0.951 | 2 | | 2 | 0.032 | 0.032 | 0.098 | 0.047 | 53 | | |
| | 3 | 0.947 | 0.966 | 0.937 | 0.958 | 5 | | 3 | 0.033 | 0.033 | 0.102 | 0.045 | 56 | | |
| | Mean | 0.942 | 0.960 | 0.933 | 0.955 | 4 | | Mean | 0.032 | 0.032 | 0.100 | 0.045 | 55 | Positive | Weakly photoreactive |
| FDSC-24 | 1 | 0.975 | 0.972 | 0.959 | 0.963 | 9 | FDSC-24 | 1 | 0.031 | 0.031 | 0.042 | 0.045 | -3 | | |
| | 2 | 0.980 | 0.970 | 0.955 | 0.963 | 18 | | 2 | 0.034 | 0.032 | 0.042 | 0.048 | -6 | | |
| | 3 | 0.981 | 0.977 | 0.974 | 0.973 | 0 | | 3 | 0.032 | 0.032 | 0.043 | 0.046 | -3 | | |
| | Mean | 0.979 | 0.973 | 0.963 | 0.966 | 9 | | Mean | 0.032 | 0.032 | 0.042 | 0.046 | -4 | Negative | Non-photoreactive |
| FDSC-25 | 1 | 0.940 | 0.958 | 0.928 | 0.948 | 0 | FDSC-25 | 1 | 0.031 | 0.032 | 0.109 | 0.040 | 70 | | |
| | 2 | 0.939 | 0.952 | 0.932 | 0.949 | -5 | | 2 | 0.032 | 0.034 | 0.108 | 0.041 | 68 | | |
| | 3 | 0.943 | 0.951 | 0.935 | 0.928 | -4 | | 3 | 0.033 | 0.033 | 0.106 | 0.042 | 65 | | |
| | Mean | 0.941 | 0.954 | 0.932 | 0.942 | -3 | | Mean | 0.032 | 0.033 | 0.108 | 0.041 | 68 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 3 | Mean for 3 assays | | - | - | - | - | 40 | Positive [*3] | Weakly photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Methylbenzylidene camphor |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-07 | 1 | 0.957 | 0.973 | 0.956 | 0.964 | -4 | FDSC-07 | 1 | 0.031 | 0.032 | 0.050 | 0.048 | 3 | | |
| | 2 | 0.967 | 0.971 | 0.963 | 0.967 | -1 | | 2 | 0.033 | 0.032 | 0.053 | 0.049 | 4 | | |
| | 3 | 0.968 | 0.978 | 0.966 | 0.976 | -3 | | 3 | 0.035 | 0.033 | 0.055 | 0.047 | 4 | | |
| | Mean | 0.964 | 0.974 | 0.962 | 0.969 | -3 | | Mean | 0.033 | 0.032 | 0.053 | 0.048 | 4 | Inconclusive | Non-photoreactive |
| FDSC-15 | 1 | 0.964 | 0.974 | 0.960 | 0.967 | -1 | FDSC-15 | 1 | 0.033 | 0.033 | 0.061 | 0.054 | 13 | | |
| | 2 | 0.982 | 0.975 | 0.979 | 0.971 | -2 | | 2 | 0.034 | 0.032 | 0.052 | 0.048 | 3 | | |
| | 3 | 0.980 | 0.982 | 0.976 | 0.977 | -1 | | 3 | 0.032 | 0.035 | 0.051 | 0.043 | 4 | | |
| | Mean | 0.975 | 0.977 | 0.972 | 0.972 | -1 | | Mean | 0.033 | 0.033 | 0.055 | 0.048 | 7 | Inconclusive | Non-photoreactive |
| FDSC-22 | 1 | 0.962 | 0.980 | 0.956 | 0.974 | 0 | FDSC-22 | 1 | 0.033 | 0.031 | 0.043 | 0.040 | -4 | | |
| | 2 | 0.966 | 0.982 | 0.962 | 0.974 | -2 | | 2 | 0.034 | 0.033 | 0.043 | 0.057 | -5 | | |
| | 3 | 0.965 | 0.991 | 0.959 | 0.986 | 0 | | 3 | 0.034 | 0.032 | 0.042 | 0.042 | -6 | | |
| | Mean | 0.964 | 0.984 | 0.959 | 0.978 | -1 | | Mean | 0.034 | 0.032 | 0.043 | 0.046 | -5 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -2 | Mean for 3 assays | | - | - | - | - | 2 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 2
Chemical Name : Octrizole

Solubility
Singlet oxygen 200 μM : Precipitation 20 μM : Precipitation 2 μM : Solution
Superoxide anion 200 μM : Precipitation 20 μM : Precipitation 2 μM : Solution
Test concentration
Singlet oxygen 2 μM
Superoxide anion 2 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-11 | 1 | 0.973 | 0.977 | 0.969 | 0.965 | -4 | FDSC-11 | 1 | 0.034 | 0.031 | 0.055 | 0.049 | 2 | | |
| | 2 | 0.979 | 0.972 | 0.973 | 0.967 | -2 | | 2 | 0.035 | 0.032 | 0.057 | 0.055 | 3 | | |
| | 3 | 0.983 | 0.979 | 0.977 | 0.971 | -2 | | 3 | 0.035 | 0.034 | 0.056 | 0.050 | 2 | | |
| | Mean | 0.978 | 0.976 | 0.973 | 0.968 | -3 | | Mean | 0.035 | 0.032 | 0.056 | 0.051 | 2 | Inconclusive | (Non-photoreactive) |
| FDSC-17 | 1 | 0.982 | 0.981 | 0.975 | 0.977 | 4 | FDSC-17 | 1 | 0.033 | 0.032 | 0.068 | 0.053 | 17 | | |
| | 2 | 0.997 | 0.989 | 0.991 | 0.984 | 3 | | 2 | 0.034 | 0.033 | 0.076 | 0.052 | 24 | | |
| | 3 | 0.997 | 0.995 | 0.993 | 0.993 | 1 | | 3 | 0.033 | 0.032 | 0.069 | 0.046 | 18 | | |
| | Mean | 0.992 | 0.988 | 0.986 | 0.985 | 3 | | Mean | 0.033 | 0.032 | 0.071 | 0.050 | 20 | Positive | (Weakly photoreactive) |
| FDSC-18 | 1 | 0.958 | 0.960 | 0.952 | 0.955 | 1 | FDSC-18 | 1 | 0.034 | 0.032 | 0.053 | 0.043 | 6 | | |
| | 2 | 0.967 | 0.955 | 0.964 | 0.951 | -2 | | 2 | 0.033 | 0.032 | 0.053 | 0.047 | 7 | | |
| | 3 | 0.967 | 0.966 | 0.970 | 0.958 | -8 | | 3 | 0.035 | 0.033 | 0.053 | 0.045 | 5 | | |
| | Mean | 0.964 | 0.960 | 0.962 | 0.955 | -3 | | Mean | 0.034 | 0.032 | 0.053 | 0.045 | 6 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | -1 | Mean for 3 assays | | - | - | - | - | 9 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Octyl methacrylate |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-07 | 1 | 0.975 | 0.973 | 0.973 | 0.964 | -3 | FDSC-07 | 1 | 0.034 | 0.032 | 0.044 | 0.048 | -6 | | |
| | 2 | 0.982 | 0.971 | 0.973 | 0.967 | 4 | | 2 | 0.033 | 0.032 | 0.043 | 0.049 | -6 | | |
| | 3 | 0.984 | 0.978 | 0.977 | 0.976 | 2 | | 3 | 0.034 | 0.033 | 0.044 | 0.047 | -6 | | |
| | Mean | 0.980 | 0.974 | 0.974 | 0.969 | 1 | | Mean | 0.034 | 0.032 | 0.044 | 0.048 | -6 | Inconclusive | Non-photoreactive |
| FDSC-15 | 1 | 0.981 | 0.974 | 0.975 | 0.967 | 1 | FDSC-15 | 1 | 0.032 | 0.033 | 0.046 | 0.054 | -1 | | |
| | 2 | 0.981 | 0.975 | 0.979 | 0.971 | -3 | | 2 | 0.032 | 0.032 | 0.047 | 0.048 | 0 | | |
| | 3 | 0.987 | 0.982 | 0.984 | 0.977 | -2 | | 3 | 0.033 | 0.035 | 0.046 | 0.043 | -2 | | |
| | Mean | 0.983 | 0.977 | 0.979 | 0.972 | -1 | | Mean | 0.032 | 0.033 | 0.046 | 0.048 | -1 | Inconclusive | Non-photoreactive |
| FDSC-22 | 1 | 0.963 | 0.980 | 0.952 | 0.974 | 5 | FDSC-22 | 1 | 0.033 | 0.031 | 0.040 | 0.040 | -7 | | |
| | 2 | 0.965 | 0.982 | 0.956 | 0.974 | 3 | | 2 | 0.036 | 0.033 | 0.042 | 0.057 | -8 | | |
| | 3 | 0.970 | 0.991 | 0.959 | 0.986 | 5 | | 3 | 0.033 | 0.032 | 0.040 | 0.042 | -7 | | |
| | Mean | 0.966 | 0.984 | 0.956 | 0.978 | 4 | | Mean | 0.034 | 0.032 | 0.041 | 0.046 | -7 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 1 | Mean for 3 assays | | - | - | - | - | -5 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | Octyl methoxycinnamate |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-10 | 1 | 0.977 | 0.982 | 0.971 | 0.976 | -3 | FDSC-10 | 1 | 0.032 | 0.032 | 0.045 | 0.047 | -6 | | |
| | 2 | 0.982 | 0.992 | 0.977 | 0.975 | -4 | | 2 | 0.031 | 0.032 | 0.046 | 0.053 | -4 | | |
| | 3 | 0.985 | 0.987 | 0.982 | 0.984 | -6 | | 3 | 0.032 | 0.034 | 0.046 | 0.055 | -5 | | |
| | Mean | 0.981 | 0.987 | 0.977 | 0.978 | -4 | | Mean | 0.032 | 0.033 | 0.046 | 0.052 | -5 | Inconclusive | (Non-photoreactive) |
| FDSC-16 | 1 | 0.977 | 0.986 | 0.972 | 0.996 | 5 | FDSC-16 | 1 | 0.031 | 0.031 | 0.042 | 0.041 | 0 | | |
| | 2 | 0.979 | 0.982 | 0.974 | 0.980 | 5 | | 2 | 0.030 | 0.032 | 0.044 | 0.044 | 3 | | |
| | 3 | 0.980 | 0.993 | 0.973 | 0.986 | 7 | | 3 | 0.031 | 0.033 | 0.044 | 0.043 | 2 | | |
| | Mean | 0.979 | 0.987 | 0.973 | 0.987 | 6 | | Mean | 0.031 | 0.032 | 0.043 | 0.043 | 2 | Inconclusive | (Non-photoreactive) |
| FDSC-23 | 1 | 0.962 | 0.957 | 0.950 | 0.946 | 2 | FDSC-23 | 1 | 0.030 | 0.032 | 0.046 | 0.059 | -2 | | |
| | 2 | 0.969 | 0.960 | 0.955 | 0.947 | 4 | | 2 | 0.031 | 0.032 | 0.050 | 0.045 | 1 | | |
| | 3 | 0.965 | 0.965 | 0.955 | 0.959 | 0 | | 3 | 0.032 | 0.033 | 0.055 | 0.045 | 5 | | |
| | Mean | 0.965 | 0.961 | 0.953 | 0.951 | 2 | | Mean | 0.031 | 0.032 | 0.050 | 0.050 | 1 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | 1 | Mean for 3 assays | | - | - | - | - | -1 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 2
Chemical Name : Octyl salicylate

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-07 | 1 | 0.983 | 0.973 | 0.979 | 0.964 | -1 | FDSC-07 | 1 | 0.041 | 0.032 | 0.062 | 0.048 | 5 | | |
| | 2 | 0.992 | 0.971 | 0.985 | 0.967 | 2 | | 2 | 0.042 | 0.032 | 0.068 | 0.049 | 10 | | |
| | 3 | 0.996 | 0.978 | 0.988 | 0.976 | 3 | | 3 | 0.041 | 0.033 | 0.063 | 0.047 | 6 | | |
| | Mean | 0.990 | 0.974 | 0.984 | 0.969 | 1 | | Mean | 0.041 | 0.032 | 0.064 | 0.048 | 7 | Inconclusive | Non-photoreactive |
| FDSC-15 | 1 | 0.996 | 0.974 | 0.983 | 0.967 | 8 | FDSC-15 | 1 | 0.043 | 0.033 | 0.060 | 0.054 | 2 | | |
| | 2 | 1.004 | 0.975 | 0.993 | 0.971 | 6 | | 2 | 0.043 | 0.032 | 0.058 | 0.048 | 0 | | |
| | 3 | 1.005 | 0.982 | 0.994 | 0.977 | 6 | | 3 | 0.044 | 0.035 | 0.062 | 0.043 | 3 | | |
| | Mean | 1.002 | 0.977 | 0.990 | 0.972 | 7 | | Mean | 0.043 | 0.033 | 0.060 | 0.048 | 2 | Inconclusive | Non-photoreactive |
| FDSC-22 | 1 | 0.984 | 0.980 | 0.970 | 0.974 | 8 | FDSC-22 | 1 | 0.043 | 0.031 | 0.054 | 0.040 | -3 | | |
| | 2 | 0.989 | 0.982 | 0.976 | 0.974 | 7 | | 2 | 0.044 | 0.033 | 0.056 | 0.057 | -2 | | |
| | 3 | 0.993 | 0.991 | 0.980 | 0.986 | 7 | | 3 | 0.046 | 0.032 | 0.054 | 0.042 | -6 | | |
| | Mean | 0.989 | 0.984 | 0.975 | 0.978 | 7 | | Mean | 0.044 | 0.032 | 0.055 | 0.046 | -4 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 5 | Mean for 3 assays | | - | - | - | - | 2 | Inconclusive *3 | Non-photoreactive*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | PABA |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-04 | 1 | 0.964 | 0.972 | 0.953 | 0.963 | 1 | FDSC-04 | 1 | 0.031 | 0.032 | 0.042 | 0.050 | -6 | | |
| | 2 | 0.969 | 0.970 | 0.955 | 0.959 | 4 | | 2 | 0.032 | 0.032 | 0.040 | 0.049 | -9 | | |
| | 3 | 0.973 | 0.975 | 0.962 | 0.963 | 1 | | 3 | 0.033 | 0.033 | 0.039 | 0.049 | -11 | | |
| | Mean | 0.969 | 0.972 | 0.957 | 0.962 | 2 | | Mean | 0.032 | 0.032 | 0.040 | 0.049 | -9 | Negative | Non-photoreactive |
| FDSC-13 | 1 | 0.965 | 0.970 | 0.957 | 0.970 | 3 | FDSC-13 | 1 | 0.032 | 0.034 | 0.041 | 0.043 | 0 | | |
| | 2 | 0.966 | 0.970 | 0.960 | 0.962 | 1 | | 2 | 0.032 | 0.034 | 0.040 | 0.043 | -1 | | |
| | 3 | 0.960 | 0.972 | 0.952 | 0.965 | 3 | | 3 | 0.033 | 0.033 | 0.037 | 0.044 | -5 | | |
| | Mean | 0.964 | 0.971 | 0.956 | 0.966 | 2 | | Mean | 0.032 | 0.034 | 0.039 | 0.043 | -2 | Negative | Non-photoreactive |
| FDSC-20 | 1 | 0.966 | 0.974 | 0.918 | 0.949 | 29 | FDSC-20 | 1 | 0.030 | 0.032 | 0.043 | 0.046 | 1 | | |
| | 2 | 0.971 | 0.972 | 0.963 | 0.954 | -11 | | 2 | 0.030 | 0.032 | 0.042 | 0.045 | 0 | | |
| | 3 | 0.976 | 0.980 | 0.954 | 0.966 | 3 | | 3 | 0.032 | 0.034 | 0.039 | 0.045 | -5 | | |
| | Mean | 0.971 | 0.975 | 0.945 | 0.956 | 7 | | Mean | 0.031 | 0.033 | 0.041 | 0.045 | -1 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 4 | Mean for 3 assays | | - | - | - | - | -4 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | SDS |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-04 | 1 | 0.945 | 0.972 | 0.931 | 0.963 | 4 | FDSC-11 | 1 | 0.033 | 0.031 | 0.050 | 0.049 | -2 | | |
| | 2 | 0.960 | 0.970 | 0.941 | 0.959 | 9 | | 2 | 0.035 | 0.032 | 0.059 | 0.055 | 5 | | |
| | 3 | 0.950 | 0.975 | 0.937 | 0.963 | 3 | | 3 | 0.035 | 0.034 | 0.054 | 0.050 | 0 | | |
| | Mean | 0.952 | 0.972 | 0.936 | 0.962 | 5 | | Mean | 0.034 | 0.032 | 0.054 | 0.051 | 1 | Inconclusive | (Non-photoreactive) |
| FDSC-09 | 1 | 0.977 | 0.984 | 0.959 | 0.975 | 12 | FDSC-17 | 1 | 0.033 | 0.032 | 0.047 | 0.053 | -4 | | |
| | 2 | 0.989 | 0.984 | 0.981 | 0.980 | 2 | | 2 | 0.034 | 0.033 | 0.052 | 0.052 | 0 | | |
| | 3 | 0.985 | 0.994 | 0.969 | 0.987 | 10 | | 3 | 0.035 | 0.032 | 0.046 | 0.046 | -7 | | |
| | Mean | 0.984 | 0.987 | 0.970 | 0.981 | 8 | | Mean | 0.034 | 0.032 | 0.048 | 0.050 | -4 | Inconclusive | (Non-photoreactive) |
| FDSC-11 | 1 | 0.961 | 0.977 | 0.919 | 0.965 | 34 | FDSC-18 | 1 | 0.034 | 0.032 | 0.050 | 0.043 | 3 | | |
| | 2 | 0.974 | 0.972 | 0.968 | 0.967 | -2 | | 2 | 0.035 | 0.032 | 0.048 | 0.047 | 0 | | |
| | 3 | 0.971 | 0.979 | 0.958 | 0.971 | 5 | | 3 | 0.036 | 0.033 | 0.050 | 0.045 | 1 | | |
| | Mean | 0.969 | 0.976 | 0.948 | 0.968 | 12 | | Mean | 0.035 | 0.032 | 0.049 | 0.045 | 1 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | 8 | Mean for 3 assays | | - | - | - | - | -1 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 2 |
| Chemical Name | : | UV-571 |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| FDSC-10 | 1 | 0.997 | 0.982 | 0.994 | 0.976 | -6 | FDSC-10 | 1 | 0.034 | 0.032 | 0.054 | 0.047 | 1 | | |
| | 2 | 0.996 | 0.992 | 0.997 | 0.975 | -10 | | 2 | 0.034 | 0.032 | 0.055 | 0.053 | 2 | | |
| | 3 | 0.999 | 0.987 | 1.003 | 0.984 | -13 | | 3 | 0.034 | 0.034 | 0.057 | 0.055 | 4 | | |
| | Mean | 0.997 | 0.987 | 0.998 | 0.978 | -10 | | Mean | 0.034 | 0.033 | 0.055 | 0.052 | 2 | Inconclusive | (Non-photoreactive) |
| FDSC-16 | 1 | 0.988 | 0.986 | 0.989 | 0.996 | -1 | FDSC-16 | 1 | 0.034 | 0.031 | 0.052 | 0.041 | 7 | | |
| | 2 | 0.993 | 0.982 | 0.994 | 0.980 | -1 | | 2 | 0.033 | 0.032 | 0.053 | 0.044 | 9 | | |
| | 3 | 0.998 | 0.993 | 0.976 | 0.986 | 22 | | 3 | 0.034 | 0.033 | 0.053 | 0.043 | 8 | | |
| | Mean | 0.993 | 0.987 | 0.986 | 0.987 | 7 | | Mean | 0.034 | 0.032 | 0.053 | 0.043 | 8 | Inconclusive | (Non-photoreactive) |
| FDSC-26 | 1 | 0.956 | 0.959 | 0.954 | 0.954 | -4 | FDSC-26 | 1 | 0.033 | 0.037 | 0.045 | 0.042 | 1 | | |
| | 2 | 0.964 | 0.955 | 0.961 | 0.949 | -3 | | 2 | 0.033 | 0.033 | 0.045 | 0.050 | 1 | | |
| | 3 | 0.964 | 0.964 | 0.963 | 0.956 | -5 | | 3 | 0.034 | 0.033 | 0.044 | 0.043 | -1 | | |
| | Mean | 0.961 | 0.959 | 0.959 | 0.953 | -4 | | Mean | 0.033 | 0.034 | 0.045 | 0.045 | 0 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | -2 | Mean for 3 assays | | - | - | - | - | 3 | Inconclusive *3 | (Non-photoreactive)*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Acridine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-001 | 1 | 0.988 | 1.005 | 0.757 | 1.000 | 228 | MT-001 | 1 | 0.029 | 0.030 | 0.227 | 0.035 | 191 | | |
| | 2 | 1.005 | 1.009 | 0.778 | 1.005 | 225 | | 2 | 0.030 | 0.030 | 0.229 | 0.035 | 192 | | |
| | 3 | 0.994 | 1.010 | 0.775 | 1.012 | 217 | | 3 | 0.029 | 0.029 | 0.228 | 0.036 | 192 | | |
| | Mean | 0.996 | 1.008 | 0.770 | 1.006 | 223 | | Mean | 0.030 | 0.029 | 0.228 | 0.036 | 192 | Positive | Photoreactive |
| MT-009 | 1 | 0.980 | 0.994 | 0.756 | 0.989 | 221 | MT-009 | 1 | 0.029 | 0.029 | 0.203 | 0.034 | 169 | | |
| | 2 | 0.997 | 0.997 | 0.771 | 0.994 | 222 | | 2 | 0.030 | 0.029 | 0.205 | 0.035 | 171 | | |
| | 3 | 0.988 | 0.999 | 0.765 | 0.995 | 219 | | 3 | 0.029 | 0.028 | 0.209 | 0.034 | 176 | | |
| | Mean | 0.988 | 0.997 | 0.764 | 0.993 | 221 | | Mean | 0.029 | 0.029 | 0.206 | 0.034 | 172 | Positive | Photoreactive |
| MT-016 | 1 | 0.983 | 0.996 | 0.751 | 0.992 | 228 | MT-016 | 1 | 0.028 | 0.029 | 0.205 | 0.034 | 173 | | |
| | 2 | 0.982 | 0.988 | 0.757 | 0.985 | 221 | | 2 | 0.028 | 0.029 | 0.200 | 0.033 | 168 | | |
| | 3 | 0.986 | 0.989 | 0.764 | 0.984 | 219 | | 3 | 0.029 | 0.029 | 0.196 | 0.033 | 163 | | |
| | Mean | 0.984 | 0.991 | 0.757 | 0.987 | 223 | | Mean | 0.028 | 0.029 | 0.200 | 0.033 | 168 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 222 | Mean for 3 assays | | - | - | - | - | 177 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Acridine HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-001 | 1 | 0.995 | 1.005 | 0.774 | 1.000 | 219 | MT-001 | 1 | 0.031 | 0.030 | 0.212 | 0.035 | 175 | | |
| | 2 | 1.001 | 1.009 | 0.785 | 1.005 | 214 | | 2 | 0.030 | 0.030 | 0.212 | 0.035 | 175 | | |
| | 3 | 1.003 | 1.010 | 0.788 | 1.012 | 213 | | 3 | 0.029 | 0.029 | 0.215 | 0.036 | 179 | | |
| | Mean | 0.999 | 1.008 | 0.782 | 1.006 | 215 | | Mean | 0.030 | 0.029 | 0.213 | 0.036 | 176 | Positive | Photoreactive |
| MT-009 | 1 | 0.982 | 0.994 | 0.757 | 0.989 | 221 | MT-009 | 1 | 0.028 | 0.029 | 0.213 | 0.034 | 180 | | |
| | 2 | 0.987 | 0.997 | 0.770 | 0.994 | 213 | | 2 | 0.029 | 0.029 | 0.214 | 0.035 | 180 | | |
| | 3 | 0.996 | 0.999 | 0.780 | 0.995 | 212 | | 3 | 0.028 | 0.028 | 0.217 | 0.034 | 184 | | |
| | Mean | 0.988 | 0.997 | 0.769 | 0.993 | 215 | | Mean | 0.029 | 0.029 | 0.215 | 0.034 | 181 | Positive | Photoreactive |
| MT-016 | 1 | 0.981 | 0.996 | 0.755 | 0.992 | 222 | MT-016 | 1 | 0.029 | 0.029 | 0.199 | 0.034 | 166 | | |
| | 2 | 0.990 | 0.988 | 0.770 | 0.985 | 216 | | 2 | 0.029 | 0.029 | 0.196 | 0.033 | 163 | | |
| | 3 | 0.992 | 0.989 | 0.771 | 0.984 | 217 | | 3 | 0.028 | 0.029 | 0.196 | 0.033 | 164 | | |
| | Mean | 0.988 | 0.991 | 0.765 | 0.987 | 218 | | Mean | 0.029 | 0.029 | 0.197 | 0.033 | 164 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 216 | Mean for 3 assays | | - | - | - | - | 174 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Amiodarone HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-010 | 1 | 0.979 | 0.998 | 0.938 | 0.994 | 37 | MT-010 | 1 | 0.032 | 0.029 | 0.037 | 0.034 | 0 | | |
| | 2 | 1.007 | 1.002 | 0.965 | 0.997 | 38 | | 2 | 0.032 | 0.029 | 0.038 | 0.035 | 1 | | |
| | 3 | 1.017 | 1.001 | 0.981 | 0.996 | 32 | | 3 | 0.032 | 0.028 | 0.038 | 0.034 | 0 | | |
| | Mean | 1.001 | 1.000 | 0.961 | 0.996 | 36 | | Mean | 0.032 | 0.029 | 0.038 | 0.034 | 0 | Positive | (Photoreactive) |
| MT-016 | 1 | 0.990 | 0.996 | 0.936 | 0.992 | 50 | MT-016 | 1 | 0.032 | 0.029 | 0.037 | 0.034 | 1 | | |
| | 2 | 0.990 | 0.988 | 0.934 | 0.985 | 53 | | 2 | 0.031 | 0.029 | 0.037 | 0.033 | 1 | | |
| | 3 | 0.991 | 0.989 | 0.938 | 0.984 | 49 | | 3 | 0.031 | 0.029 | 0.039 | 0.033 | 3 | | |
| | Mean | 0.990 | 0.991 | 0.936 | 0.987 | 51 | | Mean | 0.031 | 0.029 | 0.038 | 0.033 | 2 | Positive | (Photoreactive) |
| MT-023 | 1 | 0.991 | 1.002 | 0.936 | 0.997 | 51 | MT-023 | 1 | 0.031 | 0.029 | 0.038 | 0.036 | 0 | | |
| | 2 | 0.997 | 0.998 | 0.943 | 0.993 | 50 | | 2 | 0.031 | 0.029 | 0.038 | 0.036 | 1 | | |
| | 3 | 0.996 | 0.998 | 0.949 | 0.995 | 43 | | 3 | 0.030 | 0.029 | 0.038 | 0.036 | 0 | | |
| | Mean | 0.995 | 0.999 | 0.943 | 0.995 | 48 | | Mean | 0.031 | 0.029 | 0.038 | 0.036 | 0 | Positive | (Photoreactive) |
| Mean for 3 assays | | - | - | - | - | 45 | Mean for 3 assays | | - | - | - | - | 1 | Positive [*3] | (Photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Chlorpromazine HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-005 | 1 | 0.971 | 1.000 | 0.986 | 0.997 | -20 | MT-005 | 1 | 0.029 | 0.029 | 0.119 | 0.034 | 85 | | |
| | 2 | 0.973 | 1.003 | 0.992 | 1.000 | -23 | | 2 | 0.029 | 0.029 | 0.121 | 0.034 | 87 | | |
| | 3 | 0.973 | 1.002 | 0.993 | 0.998 | -23 | | 3 | 0.029 | 0.029 | 0.122 | 0.034 | 88 | | |
| | Mean | 0.972 | 1.002 | 0.990 | 0.998 | -22 | | Mean | 0.029 | 0.029 | 0.121 | 0.034 | 87 | Positive | Photoreactive |
| MT-013 | 1 | 0.963 | 0.994 | 0.994 | 0.988 | -35 | MT-013 | 1 | 0.028 | 0.029 | 0.117 | 0.034 | 84 | | |
| | 2 | 0.966 | 0.995 | 0.992 | 0.991 | -30 | | 2 | 0.029 | 0.029 | 0.119 | 0.035 | 84 | | |
| | 3 | 0.970 | 0.995 | 0.998 | 0.990 | -31 | | 3 | 0.029 | 0.028 | 0.119 | 0.034 | 85 | | |
| | Mean | 0.966 | 0.994 | 0.994 | 0.990 | -32 | | Mean | 0.029 | 0.029 | 0.118 | 0.034 | 84 | Positive | Photoreactive |
| MT-020 | 1 | 0.973 | 1.003 | 0.992 | 1.003 | -21 | MT-020 | 1 | 0.029 | 0.030 | 0.113 | 0.035 | 78 | | |
| | 2 | 0.981 | 1.000 | 1.006 | 0.998 | -26 | | 2 | 0.029 | 0.030 | 0.113 | 0.035 | 78 | | |
| | 3 | 0.977 | 0.999 | 0.997 | 0.997 | -22 | | 3 | 0.029 | 0.031 | 0.113 | 0.036 | 79 | | |
| | Mean | 0.977 | 1.001 | 0.998 | 0.999 | -23 | | Mean | 0.029 | 0.030 | 0.113 | 0.036 | 78 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | -26 | Mean for 3 assays | | - | - | - | - | 83 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Doxycycline HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-006 | 1 | 0.978 | 1.002 | 0.805 | 0.996 | 167 | MT-006 | 1 | 0.036 | 0.029 | 0.280 | 0.034 | 240 | | |
| | 2 | 0.995 | 1.006 | 0.824 | 0.999 | 165 | | 2 | 0.037 | 0.029 | 0.292 | 0.034 | 250 | | |
| | 3 | 0.987 | 1.007 | 0.817 | 1.001 | 165 | | 3 | 0.036 | 0.028 | 0.291 | 0.033 | 250 | | |
| | Mean | 0.987 | 1.005 | 0.815 | 0.999 | 166 | | Mean | 0.036 | 0.029 | 0.288 | 0.034 | 247 | Positive | Photoreactive |
| MT-014 | 1 | 0.980 | 1.007 | 0.814 | 1.003 | 162 | MT-014 | 1 | 0.035 | 0.029 | 0.282 | 0.035 | 241 | | |
| | 2 | 0.999 | 1.012 | 0.837 | 1.008 | 159 | | 2 | 0.036 | 0.029 | 0.293 | 0.035 | 251 | | |
| | 3 | 0.992 | 1.010 | 0.828 | 1.009 | 160 | | 3 | 0.035 | 0.029 | 0.296 | 0.037 | 255 | | |
| | Mean | 0.991 | 1.010 | 0.826 | 1.006 | 160 | | Mean | 0.035 | 0.029 | 0.290 | 0.035 | 249 | Positive | Photoreactive |
| MT-021 | 1 | 0.984 | 1.008 | 0.816 | 1.001 | 163 | MT-021 | 1 | 0.035 | 0.029 | 0.297 | 0.035 | 257 | | |
| | 2 | 0.982 | 1.004 | 0.820 | 1.000 | 157 | | 2 | 0.035 | 0.030 | 0.307 | 0.035 | 266 | | |
| | 3 | 0.987 | 1.004 | 0.823 | 1.001 | 159 | | 3 | 0.034 | 0.029 | 0.300 | 0.035 | 260 | | |
| | Mean | 0.985 | 1.005 | 0.820 | 1.000 | 160 | | Mean | 0.035 | 0.029 | 0.302 | 0.035 | 261 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 162 | Mean for 3 assays | | - | - | - | - | 252 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Fenofibrate |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-002 | 1 | 1.011 | 1.011 | 0.831 | 1.005 | 175 | MT-002 | 1 | 0.038 | 0.029 | 0.033 | 0.033 | -9 | | |
| | 2 | 1.009 | 1.016 | 0.851 | 1.011 | 153 | | 2 | 0.038 | 0.030 | 0.033 | 0.034 | -9 | | |
| | 3 | 1.009 | 1.018 | 0.850 | 1.013 | 154 | | 3 | 0.037 | 0.028 | 0.034 | 0.033 | -8 | | |
| | Mean | 1.009 | 1.015 | 0.844 | 1.010 | 161 | | Mean | 0.038 | 0.029 | 0.034 | 0.034 | -9 | Positive | Photoreactive |
| MT-011 | 1 | 1.004 | 0.998 | 0.839 | 0.993 | 160 | MT-011 | 1 | 0.039 | 0.030 | 0.033 | 0.034 | -12 | | |
| | 2 | 1.007 | 1.005 | 0.839 | 0.999 | 162 | | 2 | 0.039 | 0.029 | 0.033 | 0.035 | -11 | | |
| | 3 | 1.004 | 1.003 | 0.838 | 1.000 | 161 | | 3 | 0.038 | 0.028 | 0.033 | 0.035 | -12 | | |
| | Mean | 1.005 | 1.002 | 0.839 | 0.997 | 161 | | Mean | 0.039 | 0.029 | 0.033 | 0.035 | -12 | Positive | Photoreactive |
| MT-019 | 1 | 1.013 | 1.009 | 0.846 | 1.001 | 159 | MT-019 | 1 | 0.039 | 0.030 | 0.033 | 0.035 | -12 | | |
| | 2 | 1.015 | 1.002 | 0.845 | 0.992 | 161 | | 2 | 0.040 | 0.030 | 0.033 | 0.035 | -12 | | |
| | 3 | 1.017 | 1.003 | 0.845 | 0.994 | 163 | | 3 | 0.039 | 0.030 | 0.033 | 0.035 | -11 | | |
| | Mean | 1.015 | 1.005 | 0.845 | 0.996 | 161 | | Mean | 0.039 | 0.030 | 0.033 | 0.035 | -12 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 161 | Mean for 3 assays | | - | - | - | - | -11 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Furosemide |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-004 | 1 | 0.993 | 0.999 | 0.838 | 0.992 | 150 | MT-004 | 1 | 0.032 | 0.029 | 0.087 | 0.034 | 50 | | |
| | 2 | 1.002 | 1.003 | 0.850 | 0.997 | 146 | | 2 | 0.033 | 0.029 | 0.088 | 0.034 | 50 | | |
| | 3 | 0.998 | 1.001 | 0.854 | 0.996 | 138 | | 3 | 0.031 | 0.029 | 0.092 | 0.034 | 56 | | |
| | Mean | 0.998 | 1.001 | 0.847 | 0.995 | 145 | | Mean | 0.032 | 0.029 | 0.089 | 0.034 | 52 | Positive | Photoreactive |
| MT-012 | 1 | 0.990 | 0.992 | 0.836 | 0.989 | 150 | MT-012 | 1 | 0.031 | 0.030 | 0.084 | 0.034 | 48 | | |
| | 2 | 0.997 | 0.999 | 0.843 | 0.994 | 150 | | 2 | 0.032 | 0.029 | 0.085 | 0.035 | 48 | | |
| | 3 | 0.992 | 0.999 | 0.850 | 0.996 | 139 | | 3 | 0.031 | 0.029 | 0.089 | 0.034 | 53 | | |
| | Mean | 0.993 | 0.997 | 0.843 | 0.993 | 146 | | Mean | 0.031 | 0.029 | 0.086 | 0.034 | 50 | Positive | Photoreactive |
| MT-018 | 1 | 0.998 | 0.997 | 0.841 | 0.993 | 154 | MT-018 | 1 | 0.031 | 0.029 | 0.089 | 0.033 | 53 | | |
| | 2 | 0.989 | 0.992 | 0.845 | 0.987 | 140 | | 2 | 0.031 | 0.028 | 0.090 | 0.033 | 53 | | |
| | 3 | 0.989 | 0.990 | 0.846 | 0.987 | 138 | | 3 | 0.031 | 0.028 | 0.091 | 0.033 | 55 | | |
| | Mean | 0.992 | 0.993 | 0.844 | 0.989 | 144 | | Mean | 0.031 | 0.028 | 0.090 | 0.033 | 54 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 145 | Mean for 3 assays | | - | - | - | - | 52 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Ketoprofen |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-001 | 1 | 1.010 | 1.005 | 0.779 | 1.000 | 229 | MT-001 | 1 | 0.030 | 0.030 | 0.115 | 0.035 | 78 | | |
| | 2 | 1.011 | 1.009 | 0.788 | 1.005 | 221 | | 2 | 0.031 | 0.030 | 0.117 | 0.035 | 79 | | |
| | 3 | 1.014 | 1.010 | 0.788 | 1.012 | 223 | | 3 | 0.030 | 0.029 | 0.119 | 0.036 | 82 | | |
| | Mean | 1.012 | 1.008 | 0.785 | 1.006 | 224 | | Mean | 0.030 | 0.029 | 0.117 | 0.036 | 80 | Positive | Photoreactive |
| MT-009 | 1 | 0.996 | 0.994 | 0.770 | 0.989 | 222 | MT-009 | 1 | 0.029 | 0.029 | 0.120 | 0.034 | 86 | | |
| | 2 | 1.002 | 0.997 | 0.776 | 0.994 | 222 | | 2 | 0.030 | 0.029 | 0.123 | 0.035 | 88 | | |
| | 3 | 1.000 | 0.999 | 0.782 | 0.995 | 215 | | 3 | 0.029 | 0.028 | 0.124 | 0.034 | 89 | | |
| | Mean | 1.000 | 0.997 | 0.776 | 0.993 | 220 | | Mean | 0.030 | 0.029 | 0.122 | 0.034 | 88 | Positive | Photoreactive |
| MT-016 | 1 | 0.992 | 0.996 | 0.781 | 0.992 | 207 | MT-016 | 1 | 0.030 | 0.029 | 0.120 | 0.034 | 86 | | |
| | 2 | 0.994 | 0.988 | 0.784 | 0.985 | 207 | | 2 | 0.030 | 0.029 | 0.121 | 0.033 | 88 | | |
| | 3 | 0.996 | 0.989 | 0.788 | 0.984 | 204 | | 3 | 0.029 | 0.029 | 0.121 | 0.033 | 87 | | |
| | Mean | 0.994 | 0.991 | 0.784 | 0.987 | 206 | | Mean | 0.030 | 0.029 | 0.121 | 0.033 | 87 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 217 | Mean for 3 assays | | - | - | - | - | 85 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | 6-methylcoumarine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-006 | 1 | 0.999 | 1.002 | 0.885 | 0.996 | 108 | MT-006 | 1 | 0.029 | 0.029 | 0.096 | 0.034 | 62 | | |
| | 2 | 1.007 | 1.006 | 0.896 | 0.999 | 105 | | 2 | 0.029 | 0.029 | 0.095 | 0.034 | 61 | | |
| | 3 | 1.008 | 1.007 | 0.898 | 1.001 | 104 | | 3 | 0.029 | 0.028 | 0.098 | 0.033 | 64 | | |
| | Mean | 1.005 | 1.005 | 0.893 | 0.999 | 106 | | Mean | 0.029 | 0.029 | 0.097 | 0.034 | 62 | Positive | Photoreactive |
| MT-014 | 1 | 1.004 | 1.007 | 0.905 | 1.003 | 95 | MT-014 | 1 | 0.029 | 0.029 | 0.104 | 0.035 | 69 | | |
| | 2 | 1.014 | 1.012 | 0.914 | 1.008 | 96 | | 2 | 0.030 | 0.029 | 0.105 | 0.035 | 69 | | |
| | 3 | 1.009 | 1.010 | 0.910 | 1.009 | 96 | | 3 | 0.029 | 0.029 | 0.107 | 0.037 | 72 | | |
| | Mean | 1.009 | 1.010 | 0.909 | 1.006 | 96 | | Mean | 0.029 | 0.029 | 0.105 | 0.035 | 70 | Positive | Photoreactive |
| MT-021 | 1 | 1.005 | 1.008 | 0.905 | 1.001 | 96 | MT-021 | 1 | 0.030 | 0.029 | 0.101 | 0.035 | 65 | | |
| | 2 | 1.005 | 1.004 | 0.902 | 1.000 | 98 | | 2 | 0.030 | 0.030 | 0.102 | 0.035 | 66 | | |
| | 3 | 1.006 | 1.004 | 0.899 | 1.001 | 102 | | 3 | 0.030 | 0.029 | 0.105 | 0.035 | 70 | | |
| | Mean | 1.005 | 1.005 | 0.902 | 1.000 | 99 | | Mean | 0.030 | 0.029 | 0.103 | 0.035 | 67 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 100 | Mean for 3 assays | | - | - | - | - | 66 | Positive *3 | Photoreactive*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | 8-MOP |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-001 | 1 | 0.994 | 1.005 | 0.925 | 1.000 | 67 | MT-001 | 1 | 0.031 | 0.030 | 0.061 | 0.035 | 23 | | |
| | 2 | 1.002 | 1.009 | 0.933 | 1.005 | 68 | | 2 | 0.030 | 0.030 | 0.059 | 0.035 | 22 | | |
| | 3 | 1.001 | 1.010 | 0.938 | 1.012 | 61 | | 3 | 0.030 | 0.029 | 0.060 | 0.036 | 23 | | |
| | Mean | 0.999 | 1.008 | 0.932 | 1.006 | 65 | | Mean | 0.030 | 0.029 | 0.060 | 0.036 | 23 | Positive | Photoreactive |
| MT-009 | 1 | 0.990 | 0.994 | 0.909 | 0.989 | 77 | MT-009 | 1 | 0.030 | 0.029 | 0.064 | 0.034 | 29 | | |
| | 2 | 0.994 | 0.997 | 0.910 | 0.994 | 80 | | 2 | 0.030 | 0.029 | 0.064 | 0.035 | 29 | | |
| | 3 | 0.993 | 0.999 | 0.915 | 0.995 | 74 | | 3 | 0.030 | 0.028 | 0.065 | 0.034 | 31 | | |
| | Mean | 0.992 | 0.997 | 0.911 | 0.993 | 77 | | Mean | 0.030 | 0.029 | 0.065 | 0.034 | 30 | Positive | Photoreactive |
| MT-016 | 1 | 0.986 | 0.996 | 0.911 | 0.992 | 70 | MT-016 | 1 | 0.030 | 0.029 | 0.068 | 0.034 | 34 | | |
| | 2 | 0.989 | 0.988 | 0.912 | 0.985 | 73 | | 2 | 0.029 | 0.029 | 0.064 | 0.033 | 31 | | |
| | 3 | 0.984 | 0.989 | 0.912 | 0.984 | 68 | | 3 | 0.030 | 0.029 | 0.062 | 0.033 | 29 | | |
| | Mean | 0.986 | 0.991 | 0.912 | 0.987 | 70 | | Mean | 0.030 | 0.029 | 0.065 | 0.033 | 31 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 71 | Mean for 3 assays | | - | - | - | - | 28 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Nalidixic acid |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-002 | 1 | 1.009 | 1.011 | 0.866 | 1.005 | 138 | MT-002 | 1 | 0.030 | 0.029 | 0.351 | 0.033 | 316 | | |
| | 2 | 1.020 | 1.016 | 0.881 | 1.011 | 134 | | 2 | 0.030 | 0.030 | 0.317 | 0.034 | 282 | | |
| | 3 | 1.014 | 1.018 | 0.880 | 1.013 | 130 | | 3 | 0.029 | 0.028 | 0.318 | 0.033 | 284 | | |
| | Mean | 1.014 | 1.015 | 0.875 | 1.010 | 134 | | Mean | 0.029 | 0.029 | 0.329 | 0.034 | 294 | Positive | Photoreactive |
| MT-011 | 1 | 0.999 | 0.998 | 0.859 | 0.993 | 135 | MT-011 | 1 | 0.029 | 0.030 | 0.386 | 0.034 | 351 | | |
| | 2 | 1.011 | 1.005 | 0.876 | 0.999 | 130 | | 2 | 0.030 | 0.029 | 0.397 | 0.035 | 362 | | |
| | 3 | 1.005 | 1.003 | 0.874 | 1.000 | 126 | | 3 | 0.029 | 0.028 | 0.388 | 0.035 | 354 | | |
| | Mean | 1.005 | 1.002 | 0.870 | 0.997 | 130 | | Mean | 0.029 | 0.029 | 0.390 | 0.035 | 356 | Positive | Photoreactive |
| MT-019 | 1 | 1.004 | 1.009 | 0.877 | 1.001 | 118 | MT-019 | 1 | 0.029 | 0.030 | 0.339 | 0.035 | 305 | | |
| | 2 | 1.003 | 1.002 | 0.875 | 0.992 | 119 | | 2 | 0.029 | 0.030 | 0.362 | 0.035 | 328 | | |
| | 3 | 1.007 | 1.003 | 0.878 | 0.994 | 120 | | 3 | 0.029 | 0.030 | 0.344 | 0.035 | 310 | | |
| | Mean | 1.005 | 1.005 | 0.877 | 0.996 | 119 | | Mean | 0.029 | 0.030 | 0.348 | 0.035 | 314 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 128 | Mean for 3 assays | | - | - | - | - | 321 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Nalidixic acid (Na salt) |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-002 | 1 | 1.007 | 1.011 | 0.873 | 1.005 | 129 | MT-002 | 1 | 0.029 | 0.029 | 0.331 | 0.033 | 297 | | |
| | 2 | 1.015 | 1.016 | 0.886 | 1.011 | 124 | | 2 | 0.030 | 0.030 | 0.342 | 0.034 | 308 | | |
| | 3 | 1.012 | 1.018 | 0.886 | 1.013 | 121 | | 3 | 0.029 | 0.028 | 0.327 | 0.033 | 293 | | |
| | Mean | 1.011 | 1.015 | 0.882 | 1.010 | 125 | | Mean | 0.029 | 0.029 | 0.334 | 0.034 | 299 | Positive | Photoreactive |
| MT-011 | 1 | 0.993 | 0.998 | 0.862 | 0.993 | 126 | MT-011 | 1 | 0.030 | 0.030 | 0.367 | 0.034 | 331 | | |
| | 2 | 1.006 | 1.005 | 0.874 | 0.999 | 127 | | 2 | 0.030 | 0.029 | 0.389 | 0.035 | 353 | | |
| | 3 | 1.002 | 1.003 | 0.877 | 1.000 | 120 | | 3 | 0.029 | 0.028 | 0.376 | 0.035 | 340 | | |
| | Mean | 1.000 | 1.002 | 0.871 | 0.997 | 124 | | Mean | 0.030 | 0.029 | 0.377 | 0.035 | 341 | Positive | Photoreactive |
| MT-019 | 1 | 1.008 | 1.009 | 0.879 | 1.001 | 120 | MT-019 | 1 | 0.031 | 0.030 | 0.327 | 0.035 | 291 | | |
| | 2 | 1.007 | 1.002 | 0.881 | 0.992 | 116 | | 2 | 0.030 | 0.030 | 0.397 | 0.035 | 362 | | |
| | 3 | 1.005 | 1.003 | 0.876 | 0.994 | 120 | | 3 | 0.030 | 0.030 | 0.342 | 0.035 | 308 | | |
| | Mean | 1.007 | 1.005 | 0.879 | 0.996 | 119 | | Mean | 0.030 | 0.030 | 0.355 | 0.035 | 320 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 123 | Mean for 3 assays | | - | - | - | - | 320 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Norfloxacin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-005 | 1 | 0.995 | 1.000 | 0.826 | 0.997 | 164 | MT-005 | 1 | 0.031 | 0.029 | 0.184 | 0.034 | 148 | | |
| | 2 | 0.999 | 1.003 | 0.832 | 1.000 | 163 | | 2 | 0.030 | 0.029 | 0.181 | 0.034 | 147 | | |
| | 3 | 0.997 | 1.002 | 0.828 | 0.998 | 164 | | 3 | 0.030 | 0.029 | 0.186 | 0.034 | 152 | | |
| | Mean | 0.997 | 1.002 | 0.829 | 0.998 | 164 | | Mean | 0.030 | 0.029 | 0.184 | 0.034 | 149 | Positive | Photoreactive |
| MT-013 | 1 | 0.989 | 0.994 | 0.811 | 0.988 | 174 | MT-013 | 1 | 0.030 | 0.029 | 0.182 | 0.034 | 147 | | |
| | 2 | 0.995 | 0.995 | 0.820 | 0.991 | 171 | | 2 | 0.030 | 0.029 | 0.182 | 0.035 | 148 | | |
| | 3 | 0.992 | 0.995 | 0.819 | 0.990 | 169 | | 3 | 0.029 | 0.028 | 0.187 | 0.034 | 153 | | |
| | Mean | 0.992 | 0.994 | 0.816 | 0.990 | 171 | | Mean | 0.030 | 0.029 | 0.184 | 0.034 | 149 | Positive | Photoreactive |
| MT-020 | 1 | 1.008 | 1.003 | 0.832 | 1.003 | 174 | MT-020 | 1 | 0.030 | 0.030 | 0.175 | 0.035 | 139 | | |
| | 2 | 1.009 | 1.000 | 0.841 | 0.998 | 167 | | 2 | 0.030 | 0.030 | 0.177 | 0.035 | 142 | | |
| | 3 | 1.003 | 0.999 | 0.834 | 0.997 | 166 | | 3 | 0.030 | 0.031 | 0.173 | 0.036 | 137 | | |
| | Mean | 1.007 | 1.001 | 0.836 | 0.999 | 169 | | Mean | 0.030 | 0.030 | 0.175 | 0.036 | 139 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 168 | Mean for 3 assays | | - | - | - | - | 146 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Ofloxacin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-006 | 1 | 0.999 | 1.002 | 0.863 | 0.996 | 130 | MT-006 | 1 | 0.029 | 0.029 | 0.450 | 0.034 | 416 | | |
| | 2 | 1.003 | 1.006 | 0.870 | 0.999 | 127 | | 2 | 0.030 | 0.029 | 0.462 | 0.034 | 427 | | |
| | 3 | 1.007 | 1.007 | 0.880 | 1.001 | 121 | | 3 | 0.029 | 0.028 | 0.450 | 0.033 | 416 | | |
| | Mean | 1.003 | 1.005 | 0.871 | 0.999 | 126 | | Mean | 0.030 | 0.029 | 0.454 | 0.034 | 420 | Positive | Photoreactive |
| MT-014 | 1 | 1.002 | 1.007 | 0.870 | 1.003 | 128 | MT-014 | 1 | 0.030 | 0.029 | 0.468 | 0.035 | 432 | | |
| | 2 | 1.003 | 1.012 | 0.875 | 1.008 | 124 | | 2 | 0.031 | 0.029 | 0.489 | 0.035 | 452 | | |
| | 3 | 1.007 | 1.010 | 0.881 | 1.009 | 122 | | 3 | 0.030 | 0.029 | 0.469 | 0.037 | 433 | | |
| | Mean | 1.004 | 1.010 | 0.875 | 1.006 | 125 | | Mean | 0.030 | 0.029 | 0.475 | 0.035 | 439 | Positive | Photoreactive |
| MT-021 | 1 | 0.993 | 1.008 | 0.870 | 1.001 | 118 | MT-021 | 1 | 0.030 | 0.029 | 0.480 | 0.035 | 445 | | |
| | 2 | 1.002 | 1.004 | 0.881 | 1.000 | 116 | | 2 | 0.030 | 0.030 | 0.486 | 0.035 | 450 | | |
| | 3 | 1.007 | 1.004 | 0.886 | 1.001 | 116 | | 3 | 0.029 | 0.029 | 0.479 | 0.035 | 444 | | |
| | Mean | 1.001 | 1.005 | 0.879 | 1.000 | 117 | | Mean | 0.030 | 0.029 | 0.482 | 0.035 | 446 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 123 | Mean for 3 assays | | - | - | - | - | 435 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Piroxicam |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-002 | 1 | 1.008 | 1.011 | 0.825 | 1.005 | 178 | MT-003 | 1 | 0.033 | 0.029 | 0.043 | 0.036 | 4 | | |
| | 2 | 1.011 | 1.016 | 0.840 | 1.011 | 166 | | 2 | 0.034 | 0.029 | 0.044 | 0.034 | 4 | | |
| | 3 | 1.011 | 1.018 | 0.846 | 1.013 | 160 | | 3 | 0.033 | 0.028 | 0.043 | 0.034 | 4 | | |
| | Mean | 1.010 | 1.015 | 0.837 | 1.010 | 168 | | Mean | 0.033 | 0.029 | 0.044 | 0.035 | 4 | Positive | Photoreactive |
| MT-011 | 1 | 1.012 | 0.998 | 0.840 | 0.993 | 167 | MT-011 | 1 | 0.032 | 0.030 | 0.041 | 0.034 | 3 | | |
| | 2 | 1.021 | 1.005 | 0.858 | 0.999 | 158 | | 2 | 0.034 | 0.029 | 0.042 | 0.035 | 3 | | |
| | 3 | 1.026 | 1.003 | 0.866 | 1.000 | 155 | | 3 | 0.033 | 0.028 | 0.041 | 0.035 | 3 | | |
| | Mean | 1.020 | 1.002 | 0.855 | 0.997 | 160 | | Mean | 0.033 | 0.029 | 0.042 | 0.035 | 3 | Positive | Photoreactive |
| MT-019 | 1 | 1.001 | 1.009 | 0.835 | 1.001 | 157 | MT-019 | 1 | 0.033 | 0.030 | 0.045 | 0.035 | 7 | | |
| | 2 | 1.002 | 1.002 | 0.841 | 0.992 | 152 | | 2 | 0.033 | 0.030 | 0.045 | 0.035 | 7 | | |
| | 3 | 1.003 | 1.003 | 0.843 | 0.994 | 151 | | 3 | 0.033 | 0.030 | 0.046 | 0.035 | 8 | | |
| | Mean | 1.002 | 1.005 | 0.840 | 0.996 | 153 | | Mean | 0.033 | 0.030 | 0.045 | 0.035 | 7 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 160 | Mean for 3 assays | | - | - | - | - | 5 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Promethazine HCl |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-004 | 1 | 0.981 | 0.999 | 0.933 | 0.992 | 43 | MT-004 | 1 | 0.030 | 0.029 | 0.070 | 0.034 | 34 | | |
| | 2 | 0.985 | 1.003 | 0.935 | 0.997 | 43 | | 2 | 0.030 | 0.029 | 0.069 | 0.034 | 34 | | |
| | 3 | 0.985 | 1.001 | 0.935 | 0.996 | 43 | | 3 | 0.029 | 0.029 | 0.071 | 0.034 | 37 | | |
| | Mean | 0.984 | 1.001 | 0.935 | 0.995 | 43 | | Mean | 0.030 | 0.029 | 0.070 | 0.034 | 35 | Positive | Photoreactive |
| MT-012 | 1 | 0.974 | 0.992 | 0.922 | 0.989 | 48 | MT-012 | 1 | 0.030 | 0.030 | 0.067 | 0.034 | 32 | | |
| | 2 | 0.978 | 0.999 | 0.925 | 0.994 | 49 | | 2 | 0.030 | 0.029 | 0.067 | 0.035 | 32 | | |
| | 3 | 0.978 | 0.999 | 0.932 | 0.996 | 42 | | 3 | 0.029 | 0.029 | 0.068 | 0.034 | 34 | | |
| | Mean | 0.977 | 0.997 | 0.926 | 0.993 | 46 | | Mean | 0.030 | 0.029 | 0.067 | 0.034 | 33 | Positive | Photoreactive |
| MT-018 | 1 | 0.976 | 0.997 | 0.938 | 0.993 | 34 | MT-018 | 1 | 0.029 | 0.029 | 0.068 | 0.033 | 34 | | |
| | 2 | 0.979 | 0.992 | 0.936 | 0.987 | 39 | | 2 | 0.029 | 0.028 | 0.069 | 0.033 | 35 | | |
| | 3 | 0.973 | 0.990 | 0.926 | 0.987 | 43 | | 3 | 0.029 | 0.028 | 0.072 | 0.033 | 38 | | |
| | Mean | 0.976 | 0.993 | 0.933 | 0.989 | 39 | | Mean | 0.029 | 0.028 | 0.070 | 0.033 | 36 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 43 | Mean for 3 assays | | - | - | - | - | 35 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Rosiglitazone |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-006 | 1 | 0.962 | 1.002 | 0.901 | 0.996 | 54 | MT-006 | 1 | 0.032 | 0.029 | 0.051 | 0.034 | 14 | | |
| | 2 | 0.968 | 1.006 | 0.904 | 0.999 | 57 | | 2 | 0.032 | 0.029 | 0.053 | 0.034 | 16 | | |
| | 3 | 0.966 | 1.007 | 0.900 | 1.001 | 61 | | 3 | 0.032 | 0.028 | 0.053 | 0.033 | 16 | | |
| | Mean | 0.965 | 1.005 | 0.902 | 0.999 | 57 | | Mean | 0.032 | 0.029 | 0.052 | 0.034 | 15 | Positive | |
| MT-014 | 1 | 0.966 | 1.007 | 0.907 | 1.003 | 55 | MT-014 | 1 | 0.032 | 0.029 | 0.055 | 0.035 | 17 | | |
| | 2 | 0.969 | 1.012 | 0.909 | 1.008 | 56 | | 2 | 0.032 | 0.029 | 0.055 | 0.035 | 17 | | |
| | 3 | 0.968 | 1.010 | 0.912 | 1.009 | 52 | | 3 | 0.031 | 0.029 | 0.053 | 0.037 | 16 | | |
| | Mean | 0.968 | 1.010 | 0.910 | 1.006 | 54 | | Mean | 0.032 | 0.029 | 0.054 | 0.035 | 17 | Positive | |
| MT-021 | 1 | 0.965 | 1.008 | 0.909 | 1.001 | 51 | MT-021 | 1 | 0.031 | 0.029 | 0.053 | 0.035 | 16 | | |
| | 2 | 0.973 | 1.004 | 0.912 | 1.000 | 56 | | 2 | 0.030 | 0.030 | 0.051 | 0.035 | 15 | | |
| | 3 | 0.966 | 1.004 | 0.906 | 1.001 | 56 | | 3 | 0.031 | 0.029 | 0.052 | 0.035 | 15 | | |
| | Mean | 0.968 | 1.005 | 0.909 | 1.000 | 54 | | Mean | 0.031 | 0.029 | 0.052 | 0.035 | 15 | Positive | |
| Mean for 3 assays | | - | - | - | - | 55 | Mean for 3 assays | | - | - | - | - | 16 | Positive [*3] | |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Tetracycline |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-004 | 1 | 0.999 | 0.999 | 0.858 | 0.992 | 135 | MT-004 | 1 | 0.034 | 0.029 | 0.185 | 0.034 | 146 | | |
| | 2 | 1.000 | 1.003 | 0.860 | 0.997 | 134 | | 2 | 0.034 | 0.029 | 0.187 | 0.034 | 148 | | |
| | 3 | 0.995 | 1.001 | 0.857 | 0.996 | 133 | | 3 | 0.034 | 0.029 | 0.182 | 0.034 | 143 | | |
| | Mean | 0.998 | 1.001 | 0.858 | 0.995 | 134 | | Mean | 0.034 | 0.029 | 0.185 | 0.034 | 146 | Positive | Photoreactive |
| MT-012 | 1 | 0.989 | 0.992 | 0.857 | 0.989 | 129 | MT-012 | 1 | 0.036 | 0.030 | 0.144 | 0.034 | 103 | | |
| | 2 | 0.998 | 0.999 | 0.864 | 0.994 | 131 | | 2 | 0.033 | 0.029 | 0.141 | 0.035 | 103 | | |
| | 3 | 0.991 | 0.999 | 0.860 | 0.996 | 127 | | 3 | 0.033 | 0.029 | 0.134 | 0.034 | 97 | | |
| | Mean | 0.993 | 0.997 | 0.860 | 0.993 | 129 | | Mean | 0.034 | 0.029 | 0.140 | 0.034 | 101 | Positive | Photoreactive |
| MT-018 | 1 | 0.980 | 0.997 | 0.850 | 0.993 | 127 | MT-018 | 1 | 0.034 | 0.029 | 0.164 | 0.033 | 125 | | |
| | 2 | 0.989 | 0.992 | 0.853 | 0.987 | 131 | | 2 | 0.034 | 0.028 | 0.166 | 0.033 | 127 | | |
| | 3 | 0.986 | 0.990 | 0.854 | 0.987 | 128 | | 3 | 0.034 | 0.028 | 0.157 | 0.033 | 118 | | |
| | Mean | 0.985 | 0.993 | 0.852 | 0.989 | 129 | | Mean | 0.034 | 0.028 | 0.162 | 0.033 | 123 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 131 | Mean for 3 assays | | - | - | - | - | 123 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Anthracene |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-008 | 1 | 1.011 | 1.010 | 0.991 | 0.995 | 4 | MT-008 | 1 | 0.031 | 0.031 | 0.041 | 0.035 | 4 | | |
| | 2 | 1.007 | 1.012 | 0.986 | 0.997 | 6 | | 2 | 0.032 | 0.031 | 0.041 | 0.036 | 4 | | |
| | 3 | 1.012 | 1.011 | 0.992 | 0.997 | 5 | | 3 | 0.031 | 0.030 | 0.040 | 0.035 | 4 | | |
| | Mean | 1.010 | 1.011 | 0.990 | 0.996 | 5 | | Mean | 0.032 | 0.031 | 0.041 | 0.036 | 4 | Inconclusive | (Non-photoreactive) |
| MT-015 | 1 | 1.006 | 1.002 | 0.995 | 0.997 | 4 | MT-015 | 1 | 0.028 | 0.030 | 0.037 | 0.035 | 5 | | |
| | 2 | 1.013 | 1.007 | 1.002 | 1.004 | 4 | | 2 | 0.029 | 0.030 | 0.037 | 0.035 | 4 | | |
| | 3 | 1.014 | 1.006 | 1.004 | 0.995 | 2 | | 3 | 0.028 | 0.029 | 0.036 | 0.034 | 4 | | |
| | Mean | 1.011 | 1.005 | 1.000 | 0.998 | 3 | | Mean | 0.029 | 0.030 | 0.037 | 0.034 | 4 | Inconclusive | (Non-photoreactive) |
| MT-022 | 1 | 0.996 | 1.001 | 0.988 | 0.994 | 2 | MT-022 | 1 | 0.029 | 0.030 | 0.037 | 0.034 | 4 | | |
| | 2 | 1.000 | 1.000 | 0.988 | 0.992 | 6 | | 2 | 0.028 | 0.030 | 0.037 | 0.035 | 4 | | |
| | 3 | 1.001 | 0.996 | 0.990 | 0.993 | 5 | | 3 | 0.028 | 0.029 | 0.037 | 0.034 | 3 | | |
| | Mean | 0.999 | 0.999 | 0.988 | 0.993 | 4 | | Mean | 0.028 | 0.029 | 0.037 | 0.034 | 4 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | 4 | Mean for 3 assays | | - | - | - | - | 4 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Avobenzone |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-005 | 1 | 1.011 | 1.000 | 0.950 | 0.997 | 56 | MT-005 | 1 | 0.066 | 0.029 | 0.084 | 0.034 | 13 | | |
| | 2 | 1.024 | 1.003 | 0.960 | 1.000 | 61 | | 2 | 0.066 | 0.029 | 0.084 | 0.034 | 13 | | |
| | 3 | 1.016 | 1.002 | 0.955 | 0.998 | 58 | | 3 | 0.065 | 0.029 | 0.083 | 0.034 | 13 | | |
| | Mean | 1.017 | 1.002 | 0.955 | 0.998 | 58 | | Mean | 0.066 | 0.029 | 0.084 | 0.034 | 13 | Positive | Photoreactive |
| MT-013 | 1 | 1.014 | 0.994 | 0.955 | 0.988 | 55 | MT-013 | 1 | 0.071 | 0.029 | 0.085 | 0.034 | 9 | | |
| | 2 | 1.026 | 0.995 | 0.960 | 0.991 | 61 | | 2 | 0.072 | 0.029 | 0.086 | 0.035 | 9 | | |
| | 3 | 1.020 | 0.995 | 0.951 | 0.990 | 65 | | 3 | 0.071 | 0.028 | 0.086 | 0.034 | 10 | | |
| | Mean | 1.020 | 0.994 | 0.956 | 0.990 | 60 | | Mean | 0.071 | 0.029 | 0.086 | 0.034 | 9 | Positive | Photoreactive |
| MT-020 | 1 | 1.031 | 1.003 | 0.966 | 1.003 | 63 | MT-020 | 1 | 0.058 | 0.030 | 0.083 | 0.035 | 18 | | |
| | 2 | 1.032 | 1.000 | 0.964 | 0.998 | 66 | | 2 | 0.059 | 0.030 | 0.081 | 0.035 | 17 | | |
| | 3 | 1.038 | 0.999 | 0.969 | 0.997 | 67 | | 3 | 0.058 | 0.031 | 0.083 | 0.036 | 19 | | |
| | Mean | 1.034 | 1.001 | 0.966 | 0.999 | 65 | | Mean | 0.058 | 0.030 | 0.082 | 0.036 | 18 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 61 | Mean for 3 assays | | - | - | - | - | 13 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Bithionol |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-006 | 1 | 0.955 | 1.002 | 0.869 | 0.996 | 80 | MT-006 | 1 | 0.083 | 0.029 | 0.122 | 0.034 | 34 | | |
| | 2 | 0.960 | 1.006 | 0.875 | 0.999 | 79 | | 2 | 0.083 | 0.029 | 0.122 | 0.034 | 34 | | |
| | 3 | 0.963 | 1.007 | 0.874 | 1.001 | 84 | | 3 | 0.082 | 0.028 | 0.122 | 0.033 | 35 | | |
| | Mean | 0.959 | 1.005 | 0.872 | 0.999 | 81 | | Mean | 0.082 | 0.029 | 0.122 | 0.034 | 34 | Positive | Photoreactive |
| MT-014 | 1 | 0.928 | 1.007 | 0.857 | 1.003 | 67 | MT-014 | 1 | 0.081 | 0.029 | 0.117 | 0.035 | 30 | | |
| | 2 | 0.942 | 1.012 | 0.870 | 1.008 | 68 | | 2 | 0.081 | 0.029 | 0.116 | 0.035 | 29 | | |
| | 3 | 0.936 | 1.010 | 0.862 | 1.009 | 70 | | 3 | 0.080 | 0.029 | 0.116 | 0.037 | 30 | | |
| | Mean | 0.935 | 1.010 | 0.863 | 1.006 | 68 | | Mean | 0.080 | 0.029 | 0.116 | 0.035 | 30 | Positive | Photoreactive |
| MT-021 | 1 | 0.935 | 1.008 | 0.857 | 1.001 | 72 | MT-021 | 1 | 0.080 | 0.029 | 0.119 | 0.035 | 33 | | |
| | 2 | 0.946 | 1.004 | 0.870 | 1.000 | 71 | | 2 | 0.080 | 0.030 | 0.119 | 0.035 | 33 | | |
| | 3 | 0.945 | 1.004 | 0.868 | 1.001 | 72 | | 3 | 0.080 | 0.029 | 0.120 | 0.035 | 34 | | |
| | Mean | 0.942 | 1.005 | 0.865 | 1.000 | 72 | | Mean | 0.080 | 0.029 | 0.120 | 0.035 | 33 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 74 | Mean for 3 assays | | - | - | - | - | 32 | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Hexachlorophene |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-005 | 1 | 0.911 | 1.000 | 0.682 | 0.997 | 225 | MT-005 | 1 | 0.056 | 0.029 | 0.072 | 0.034 | 10 | | |
| | 2 | 0.927 | 1.003 | 0.696 | 1.000 | 227 | | 2 | 0.056 | 0.029 | 0.072 | 0.034 | 10 | | |
| | 3 | 0.938 | 1.002 | 0.704 | 0.998 | 230 | | 3 | 0.055 | 0.029 | 0.071 | 0.034 | 11 | | |
| | Mean | 0.925 | 1.002 | 0.694 | 0.998 | 227 | | Mean | 0.056 | 0.029 | 0.072 | 0.034 | 10 | Positive | Photoreactive |
| MT-013 | 1 | 0.875 | 0.994 | 0.650 | 0.988 | 221 | MT-013 | 1 | 0.054 | 0.029 | 0.067 | 0.034 | 8 | | |
| | 2 | 0.901 | 0.995 | 0.668 | 0.991 | 229 | | 2 | 0.055 | 0.029 | 0.067 | 0.035 | 8 | | |
| | 3 | 0.909 | 0.995 | 0.677 | 0.990 | 228 | | 3 | 0.054 | 0.028 | 0.067 | 0.034 | 8 | | |
| | Mean | 0.895 | 0.994 | 0.665 | 0.990 | 226 | | Mean | 0.054 | 0.029 | 0.067 | 0.034 | 8 | Positive | Photoreactive |
| MT-020 | 1 | 0.911 | 1.003 | 0.694 | 1.003 | 215 | MT-020 | 1 | 0.056 | 0.030 | 0.068 | 0.035 | 6 | | |
| | 2 | 0.926 | 1.000 | 0.705 | 0.998 | 219 | | 2 | 0.055 | 0.030 | 0.068 | 0.035 | 6 | | |
| | 3 | 0.932 | 0.999 | 0.713 | 0.997 | 217 | | 3 | 0.055 | 0.031 | 0.068 | 0.036 | 7 | | |
| | Mean | 0.923 | 1.001 | 0.704 | 0.999 | 217 | | Mean | 0.055 | 0.030 | 0.068 | 0.036 | 6 | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 223 | Mean for 3 assays | | - | - | - | - | 8 | Positive *3 | Photoreactive*3 |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Rose bengal |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-005 | 1 | 1.201 | 1.000 | 0.581 | 0.997 | 617 | MT-005 | 1 | 2.728 | 0.029 | 2.679 | 0.034 | No data[*4] | | |
| | 2 | 1.207 | 1.003 | 0.599 | 1.000 | 604 | | 2 | 2.759 | 0.029 | 2.689 | 0.034 | No data[*4] | | |
| | 3 | 1.208 | 1.002 | 0.602 | 0.998 | 602 | | 3 | 2.729 | 0.029 | 2.687 | 0.034 | No data[*4] | | |
| | Mean | 1.205 | 1.002 | 0.594 | 0.998 | 608 | | Mean | 2.738 | 0.029 | 2.685 | 0.034 | No data[*4] | Positive | Photoreactive |
| MT-013 | 1 | 1.154 | 0.994 | 0.563 | 0.988 | 587 | MT-013 | 1 | 2.720 | 0.029 | 2.672 | 0.034 | No data[*4] | | |
| | 2 | 1.169 | 0.995 | 0.571 | 0.991 | 595 | | 2 | 2.757 | 0.029 | 2.686 | 0.035 | No data[*4] | | |
| | 3 | 1.164 | 0.995 | 0.574 | 0.990 | 586 | | 3 | 2.736 | 0.028 | 2.682 | 0.034 | No data[*4] | | |
| | Mean | 1.162 | 0.994 | 0.569 | 0.990 | 589 | | Mean | 2.738 | 0.029 | 2.680 | 0.034 | No data[*4] | Positive | Photoreactive |
| MT-020 | 1 | 1.193 | 1.003 | 0.580 | 1.003 | 611 | MT-020 | 1 | 2.750 | 0.030 | 2.706 | 0.035 | No data[*4] | | |
| | 2 | 1.205 | 1.000 | 0.597 | 0.998 | 606 | | 2 | 2.735 | 0.030 | 2.703 | 0.035 | No data[*4] | | |
| | 3 | 1.199 | 0.999 | 0.593 | 0.997 | 603 | | 3 | 2.742 | 0.031 | 2.691 | 0.036 | No data[*4] | | |
| | Mean | 1.199 | 1.001 | 0.590 | 0.999 | 607 | | Mean | 2.742 | 0.030 | 2.700 | 0.036 | No data[*4] | Positive | Photoreactive |
| Mean for 3 assays | | - | - | - | - | 601 | Mean for 3 assays | | - | - | - | - | No data[*4] | Positive [*3] | Photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

*4 : Over the OD criteria

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 3
Chemical Name : Aspirin

Solubility
Singlet oxygen 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Superoxide anion 200 μM : Solution 20 μM : Not determined 2 μM : Not determined
Test concentration
Singlet oxygen 200 μM
Superoxide anion 200 μM

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-004 | 1 | 1.001 | 0.999 | 0.996 | 0.992 | -1 | MT-004 | 1 | 0.029 | 0.029 | 0.033 | 0.034 | -1 | | |
| | 2 | 1.003 | 1.003 | 0.998 | 0.997 | -2 | | 2 | 0.030 | 0.029 | 0.034 | 0.034 | -1 | | |
| | 3 | 1.005 | 1.001 | 1.002 | 0.996 | -2 | | 3 | 0.029 | 0.029 | 0.033 | 0.034 | -1 | | |
| | Mean | 1.003 | 1.001 | 0.999 | 0.995 | -2 | | Mean | 0.029 | 0.029 | 0.033 | 0.034 | -1 | Negative | Non-photoreactive |
| MT-012 | 1 | 0.994 | 0.992 | 0.988 | 0.989 | 2 | MT-012 | 1 | 0.029 | 0.030 | 0.033 | 0.034 | -1 | | |
| | 2 | 0.998 | 0.999 | 0.989 | 0.994 | 4 | | 2 | 0.030 | 0.029 | 0.035 | 0.035 | 0 | | |
| | 3 | 1.002 | 0.999 | 0.996 | 0.996 | 2 | | 3 | 0.029 | 0.029 | 0.033 | 0.034 | -1 | | |
| | Mean | 0.998 | 0.997 | 0.991 | 0.993 | 3 | | Mean | 0.029 | 0.029 | 0.034 | 0.034 | -1 | Negative | Non-photoreactive |
| MT-018 | 1 | 0.992 | 0.997 | 0.987 | 0.993 | 1 | MT-018 | 1 | 0.029 | 0.029 | 0.034 | 0.033 | 0 | | |
| | 2 | 0.996 | 0.992 | 0.989 | 0.987 | 3 | | 2 | 0.029 | 0.028 | 0.034 | 0.033 | 0 | | |
| | 3 | 0.995 | 0.990 | 0.993 | 0.987 | -2 | | 3 | 0.029 | 0.028 | 0.034 | 0.033 | 0 | | |
| | Mean | 0.994 | 0.993 | 0.990 | 0.989 | 1 | | Mean | 0.029 | 0.028 | 0.034 | 0.033 | 0 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 1 | Mean for 3 assays | | - | - | - | - | -1 | Negative *3 | Non-photoreactive*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Benzocaine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-006 | 1 | 1.005 | 1.002 | 0.998 | 0.996 | 1 | MT-006 | 1 | 0.028 | 0.029 | 0.033 | 0.034 | 0 | | |
| | 2 | 1.005 | 1.006 | 0.999 | 0.999 | 0 | | 2 | 0.029 | 0.029 | 0.034 | 0.034 | 0 | | |
| | 3 | 1.014 | 1.007 | 1.008 | 1.001 | 0 | | 3 | 0.028 | 0.028 | 0.034 | 0.033 | 1 | | |
| | Mean | 1.008 | 1.005 | 1.002 | 0.999 | 0 | | Mean | 0.028 | 0.029 | 0.034 | 0.034 | 0 | Negative | Non-photoreactive |
| MT-014 | 1 | 1.008 | 1.007 | 0.998 | 1.003 | 6 | MT-014 | 1 | 0.029 | 0.029 | 0.036 | 0.035 | 1 | | |
| | 2 | 1.010 | 1.012 | 1.002 | 1.008 | 4 | | 2 | 0.030 | 0.029 | 0.036 | 0.035 | 1 | | |
| | 3 | 1.010 | 1.010 | 1.003 | 1.009 | 3 | | 3 | 0.029 | 0.029 | 0.035 | 0.037 | 0 | | |
| | Mean | 1.009 | 1.010 | 1.001 | 1.006 | 4 | | Mean | 0.029 | 0.029 | 0.036 | 0.035 | 1 | Negative | Non-photoreactive |
| MT-021 | 1 | 1.003 | 1.008 | 0.995 | 1.001 | 3 | MT-021 | 1 | 0.029 | 0.029 | 0.035 | 0.035 | 0 | | |
| | 2 | 1.004 | 1.004 | 0.997 | 1.000 | 3 | | 2 | 0.029 | 0.030 | 0.035 | 0.035 | 0 | | |
| | 3 | 1.009 | 1.004 | 1.000 | 1.001 | 3 | | 3 | 0.029 | 0.029 | 0.035 | 0.035 | 0 | | |
| | Mean | 1.005 | 1.005 | 0.997 | 1.000 | 3 | | Mean | 0.029 | 0.029 | 0.035 | 0.035 | 0 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 2 | Mean for 3 assays | | - | - | - | - | 0 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Erythromycin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-007 | 1 | 0.958 | 0.996 | 0.955 | 0.992 | 0 | MT-007 | 1 | 0.030 | 0.029 | 0.038 | 0.036 | 2 | | |
| | 2 | 0.965 | 1.002 | 0.961 | 0.998 | 1 | | 2 | 0.031 | 0.029 | 0.038 | 0.035 | 1 | | |
| | 3 | 0.970 | 1.000 | 0.968 | 0.997 | 0 | | 3 | 0.030 | 0.028 | 0.038 | 0.034 | 1 | | |
| | Mean | 0.964 | 0.999 | 0.961 | 0.996 | 0 | | Mean | 0.030 | 0.029 | 0.038 | 0.035 | 1 | Negative | Non-photoreactive |
| MT-015 | 1 | 0.974 | 1.002 | 0.968 | 0.997 | -2 | MT-015 | 1 | 0.030 | 0.030 | 0.038 | 0.035 | 4 | | |
| | 2 | 0.980 | 1.007 | 0.978 | 1.004 | -5 | | 2 | 0.031 | 0.030 | 0.038 | 0.035 | 3 | | |
| | 3 | 0.987 | 1.006 | 0.987 | 0.995 | -6 | | 3 | 0.030 | 0.029 | 0.037 | 0.034 | 4 | | |
| | Mean | 0.980 | 1.005 | 0.978 | 0.998 | -4 | | Mean | 0.030 | 0.030 | 0.038 | 0.034 | 4 | Negative | Non-photoreactive |
| MT-022 | 1 | 0.955 | 1.001 | 0.953 | 0.994 | -4 | MT-022 | 1 | 0.030 | 0.030 | 0.037 | 0.034 | 2 | | |
| | 2 | 0.971 | 1.000 | 0.968 | 0.992 | -3 | | 2 | 0.030 | 0.030 | 0.037 | 0.035 | 2 | | |
| | 3 | 0.968 | 0.996 | 0.966 | 0.993 | -3 | | 3 | 0.030 | 0.029 | 0.037 | 0.034 | 2 | | |
| | Mean | 0.965 | 0.999 | 0.962 | 0.993 | -3 | | Mean | 0.030 | 0.029 | 0.037 | 0.034 | 2 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -2 | Mean for 3 assays | | - | - | - | - | 2 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 3

Chemical Name : Penicillin G

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-006 | 1 | 1.004 | 1.002 | 0.998 | 0.996 | 0 | MT-006 | 1 | 0.031 | 0.029 | 0.041 | 0.034 | 5 | | |
| | 2 | 1.006 | 1.006 | 0.999 | 0.999 | 0 | | 2 | 0.031 | 0.029 | 0.040 | 0.034 | 4 | | |
| | 3 | 1.013 | 1.007 | 1.006 | 1.001 | 1 | | 3 | 0.031 | 0.028 | 0.040 | 0.033 | 4 | | |
| | Mean | 1.007 | 1.005 | 1.001 | 0.999 | 0 | | Mean | 0.031 | 0.029 | 0.040 | 0.034 | 4 | Negative | Non-photoreactive |
| MT-021 | 1 | 1.004 | 1.008 | 0.993 | 1.001 | 5 | MT-021 | 1 | 0.031 | 0.029 | 0.049 | 0.035 | 11 | | |
| | 2 | 1.007 | 1.004 | 0.997 | 1.000 | 4 | | 2 | 0.032 | 0.030 | 0.049 | 0.035 | 12 | | |
| | 3 | 1.009 | 1.004 | 0.999 | 1.001 | 6 | | 3 | 0.031 | 0.029 | 0.047 | 0.035 | 10 | | |
| | Mean | 1.007 | 1.005 | 0.996 | 1.000 | 5 | | Mean | 0.031 | 0.029 | 0.048 | 0.035 | 11 | Negative | Non-photoreactive |
| MT-023 | 1 | 1.001 | 1.002 | 0.992 | 0.997 | 5 | MT-023 | 1 | 0.031 | 0.029 | 0.055 | 0.036 | 17 | | |
| | 2 | 1.003 | 0.998 | 0.996 | 0.993 | 4 | | 2 | 0.031 | 0.029 | 0.055 | 0.036 | 17 | | |
| | 3 | 1.003 | 0.998 | 0.996 | 0.995 | 3 | | 3 | 0.032 | 0.029 | 0.054 | 0.036 | 15 | | |
| | Mean | 1.003 | 0.999 | 0.994 | 0.995 | 4 | | Mean | 0.031 | 0.029 | 0.055 | 0.036 | 16 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 3 | Mean for 3 assays | | - | - | - | - | 10 | Negative [*3] | Non- photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Phenytoin |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-004 | 1 | 0.994 | 0.999 | 0.985 | 0.992 | 3 | MT-004 | 1 | 0.031 | 0.029 | 0.070 | 0.034 | 34 | | |
| | 2 | 1.003 | 1.003 | 0.996 | 0.997 | 1 | | 2 | 0.032 | 0.029 | 0.071 | 0.034 | 34 | | |
| | 3 | 0.998 | 1.001 | 0.989 | 0.996 | 3 | | 3 | 0.031 | 0.029 | 0.073 | 0.034 | 37 | | |
| | Mean | 0.998 | 1.001 | 0.990 | 0.995 | 2 | | Mean | 0.031 | 0.029 | 0.071 | 0.034 | 35 | Positive | Weakly photoreactive |
| MT-012 | 1 | 0.987 | 0.992 | 0.980 | 0.989 | 3 | MT-012 | 1 | 0.031 | 0.030 | 0.052 | 0.034 | 16 | | |
| | 2 | 1.001 | 0.999 | 0.990 | 0.994 | 7 | | 2 | 0.032 | 0.029 | 0.053 | 0.035 | 17 | | |
| | 3 | 0.994 | 0.999 | 0.983 | 0.996 | 7 | | 3 | 0.031 | 0.029 | 0.053 | 0.034 | 17 | | |
| | Mean | 0.994 | 0.997 | 0.984 | 0.993 | 6 | | Mean | 0.031 | 0.029 | 0.053 | 0.034 | 17 | Negative | Non-photoreactive |
| MT-018 | 1 | 0.987 | 0.997 | 0.979 | 0.993 | 4 | MT-018 | 1 | 0.030 | 0.029 | 0.059 | 0.033 | 24 | | |
| | 2 | 0.979 | 0.992 | 0.972 | 0.987 | 3 | | 2 | 0.031 | 0.028 | 0.061 | 0.033 | 25 | | |
| | 3 | 0.984 | 0.990 | 0.976 | 0.987 | 4 | | 3 | 0.030 | 0.028 | 0.065 | 0.033 | 29 | | |
| | Mean | 0.983 | 0.993 | 0.975 | 0.989 | 4 | | Mean | 0.030 | 0.028 | 0.062 | 0.033 | 26 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 4 | Mean for 3 assays | | - | - | - | - | 26 | Positive [*3] | Weakly photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Bumetrizole |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-002 | 1 | 1.021 | 1.011 | 1.023 | 1.005 | -7 | MT-002 | 1 | 0.067 | 0.029 | 0.070 | 0.033 | -2 | | |
| | 2 | 1.026 | 1.016 | 1.027 | 1.011 | -6 | | 2 | 0.069 | 0.030 | 0.072 | 0.034 | -2 | | |
| | 3 | 1.023 | 1.018 | 1.024 | 1.013 | -6 | | 3 | 0.070 | 0.028 | 0.073 | 0.033 | -2 | | |
| | Mean | 1.023 | 1.015 | 1.025 | 1.010 | -6 | | Mean | 0.068 | 0.029 | 0.072 | 0.034 | -2 | Inconclusive | Non-photoreactive |
| MT-011 | 1 | 1.017 | 0.998 | 1.028 | 0.993 | -16 | MT-011 | 1 | 0.066 | 0.030 | 0.075 | 0.034 | 2 | | |
| | 2 | 1.022 | 1.005 | 1.033 | 0.999 | -16 | | 2 | 0.066 | 0.029 | 0.074 | 0.035 | 2 | | |
| | 3 | 1.024 | 1.003 | 1.035 | 1.000 | -16 | | 3 | 0.066 | 0.028 | 0.074 | 0.035 | 3 | | |
| | Mean | 1.021 | 1.002 | 1.032 | 0.997 | -16 | | Mean | 0.066 | 0.029 | 0.074 | 0.035 | 2 | Inconclusive | Non-photoreactive |
| MT-019 | 1 | 1.028 | 1.009 | 1.037 | 1.001 | -18 | MT-019 | 1 | 0.055 | 0.030 | 0.069 | 0.035 | 9 | | |
| | 2 | 1.031 | 1.002 | 1.042 | 0.992 | -19 | | 2 | 0.056 | 0.030 | 0.070 | 0.035 | 9 | | |
| | 3 | 1.026 | 1.003 | 1.039 | 0.994 | -21 | | 3 | 0.056 | 0.030 | 0.070 | 0.035 | 9 | | |
| | Mean | 1.028 | 1.005 | 1.039 | 0.996 | -19 | | Mean | 0.056 | 0.030 | 0.070 | 0.035 | 9 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -14 | Mean for 3 assays | | - | - | - | - | 3 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Camphor sulfonic acid |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-001 | 1 | 1.012 | 1.005 | 1.006 | 1.000 | 4 | MT-001 | 1 | 0.031 | 0.030 | 0.034 | 0.035 | -4 | | |
| | 2 | 1.013 | 1.009 | 1.009 | 1.005 | 2 | | 2 | 0.031 | 0.030 | 0.034 | 0.035 | -3 | | |
| | 3 | 1.010 | 1.010 | 1.004 | 1.012 | 4 | | 3 | 0.031 | 0.029 | 0.034 | 0.036 | -4 | | |
| | Mean | 1.012 | 1.008 | 1.007 | 1.006 | 3 | | Mean | 0.031 | 0.029 | 0.034 | 0.036 | -4 | Negative | Non-photoreactive |
| MT-009 | 1 | 1.002 | 0.994 | 0.997 | 0.989 | 1 | MT-009 | 1 | 0.030 | 0.029 | 0.034 | 0.034 | -1 | | |
| | 2 | 1.003 | 0.997 | 0.998 | 0.994 | 2 | | 2 | 0.030 | 0.029 | 0.034 | 0.035 | -1 | | |
| | 3 | 1.001 | 0.999 | 0.996 | 0.995 | 1 | | 3 | 0.030 | 0.028 | 0.034 | 0.034 | -1 | | |
| | Mean | 1.002 | 0.997 | 0.997 | 0.993 | 1 | | Mean | 0.030 | 0.029 | 0.034 | 0.034 | -1 | Negative | Non-photoreactive |
| MT-016 | 1 | 0.993 | 0.996 | 0.985 | 0.992 | 3 | MT-016 | 1 | 0.030 | 0.029 | 0.034 | 0.034 | 0 | | |
| | 2 | 0.997 | 0.988 | 0.988 | 0.985 | 4 | | 2 | 0.031 | 0.029 | 0.035 | 0.033 | 0 | | |
| | 3 | 0.999 | 0.989 | 0.991 | 0.984 | 4 | | 3 | 0.029 | 0.029 | 0.033 | 0.033 | -1 | | |
| | Mean | 0.996 | 0.991 | 0.988 | 0.987 | 4 | | Mean | 0.030 | 0.029 | 0.034 | 0.033 | 0 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 3 | Mean for 3 assays | | - | - | - | - | -2 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Chlorhexidine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-004 | 1 | 0.932 | 0.999 | 0.900 | 0.992 | 25 | MT-004 | 1 | 0.033 | 0.029 | 0.052 | 0.034 | 14 | | |
| | 2 | 0.932 | 1.003 | 0.901 | 0.997 | 25 | | 2 | 0.034 | 0.029 | 0.051 | 0.034 | 12 | | |
| | 3 | 0.928 | 1.001 | 0.902 | 0.996 | 20 | | 3 | 0.033 | 0.029 | 0.050 | 0.034 | 12 | | |
| | Mean | 0.931 | 1.001 | 0.901 | 0.995 | 23 | | Mean | 0.033 | 0.029 | 0.051 | 0.034 | 13 | Negative | Non-photoreactive |
| MT-012 | 1 | 0.917 | 0.992 | 0.893 | 0.989 | 20 | MT-012 | 1 | 0.033 | 0.030 | 0.046 | 0.034 | 8 | | |
| | 2 | 0.922 | 0.999 | 0.894 | 0.994 | 24 | | 2 | 0.034 | 0.029 | 0.045 | 0.035 | 7 | | |
| | 3 | 0.925 | 0.999 | 0.898 | 0.996 | 22 | | 3 | 0.033 | 0.029 | 0.044 | 0.034 | 6 | | |
| | Mean | 0.921 | 0.997 | 0.895 | 0.993 | 22 | | Mean | 0.033 | 0.029 | 0.045 | 0.034 | 7 | Negative | Non-photoreactive |
| MT-018 | 1 | 0.911 | 0.997 | 0.890 | 0.993 | 17 | MT-018 | 1 | 0.032 | 0.029 | 0.049 | 0.033 | 12 | | |
| | 2 | 0.919 | 0.992 | 0.888 | 0.987 | 27 | | 2 | 0.033 | 0.028 | 0.048 | 0.033 | 10 | | |
| | 3 | 0.918 | 0.990 | 0.891 | 0.987 | 23 | | 3 | 0.033 | 0.028 | 0.047 | 0.033 | 9 | | |
| | Mean | 0.916 | 0.993 | 0.889 | 0.989 | 22 | | Mean | 0.033 | 0.028 | 0.048 | 0.033 | 10 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 22 | Mean for 3 assays | | - | - | - | - | 10 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Cinnamic acid |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-007 | 1 | 0.999 | 0.996 | 0.995 | 0.992 | 1 | MT-007 | 1 | 0.029 | 0.029 | 0.044 | 0.036 | 9 | | |
| | 2 | 1.012 | 1.002 | 1.010 | 0.998 | -1 | | 2 | 0.029 | 0.029 | 0.044 | 0.035 | 8 | | |
| | 3 | 1.007 | 1.000 | 1.002 | 0.997 | 1 | | 3 | 0.028 | 0.028 | 0.043 | 0.034 | 9 | | |
| | Mean | 1.006 | 0.999 | 1.002 | 0.996 | 0 | | Mean | 0.029 | 0.029 | 0.044 | 0.035 | 9 | Negative | Non-photoreactive |
| MT-015 | 1 | 1.008 | 1.002 | 1.002 | 0.997 | 0 | MT-015 | 1 | 0.029 | 0.030 | 0.043 | 0.035 | 11 | | |
| | 2 | 1.024 | 1.007 | 1.017 | 1.004 | 0 | | 2 | 0.029 | 0.030 | 0.043 | 0.035 | 10 | | |
| | 3 | 1.014 | 1.006 | 1.009 | 0.995 | -2 | | 3 | 0.029 | 0.029 | 0.043 | 0.034 | 10 | | |
| | Mean | 1.016 | 1.005 | 1.009 | 0.998 | -1 | | Mean | 0.029 | 0.030 | 0.043 | 0.034 | 10 | Negative | Non-photoreactive |
| MT-022 | 1 | 1.000 | 1.001 | 0.992 | 0.994 | 2 | MT-022 | 1 | 0.029 | 0.030 | 0.041 | 0.034 | 7 | | |
| | 2 | 0.997 | 1.000 | 0.990 | 0.992 | 2 | | 2 | 0.030 | 0.030 | 0.041 | 0.035 | 6 | | |
| | 3 | 1.002 | 0.996 | 0.994 | 0.993 | 3 | | 3 | 0.029 | 0.029 | 0.042 | 0.034 | 7 | | |
| | Mean | 1.000 | 0.999 | 0.992 | 0.993 | 2 | | Mean | 0.029 | 0.029 | 0.041 | 0.034 | 7 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 0 | Mean for 3 assays | | - | - | - | - | 9 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Drometrizole |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-010 | 1 | 0.998 | 0.998 | 0.988 | 0.994 | 6 | MT-010 | 1 | 0.030 | 0.029 | 0.041 | 0.034 | 5 | | |
| | 2 | 1.012 | 1.002 | 0.999 | 0.997 | 10 | | 2 | 0.031 | 0.029 | 0.041 | 0.035 | 6 | | |
| | 3 | 1.015 | 1.001 | 1.002 | 0.996 | 9 | | 3 | 0.030 | 0.028 | 0.041 | 0.034 | 6 | | |
| | Mean | 1.009 | 1.000 | 0.996 | 0.996 | 8 | | Mean | 0.030 | 0.029 | 0.041 | 0.034 | 6 | Inconclusive | (Non-photoreactive) |
| MT-016 | 1 | 0.996 | 0.996 | 0.989 | 0.992 | 3 | MT-016 | 1 | 0.030 | 0.029 | 0.041 | 0.034 | 8 | | |
| | 2 | 1.000 | 0.988 | 0.994 | 0.985 | 3 | | 2 | 0.029 | 0.029 | 0.041 | 0.033 | 8 | | |
| | 3 | 1.000 | 0.989 | 0.995 | 0.984 | 1 | | 3 | 0.029 | 0.029 | 0.039 | 0.033 | 6 | | |
| | Mean | 0.999 | 0.991 | 0.992 | 0.987 | 2 | | Mean | 0.029 | 0.029 | 0.041 | 0.033 | 7 | Inconclusive | (Non-photoreactive) |
| MT-023 | 1 | 1.004 | 1.002 | 0.995 | 0.997 | 5 | MT-023 | 1 | 0.030 | 0.029 | 0.043 | 0.036 | 6 | | |
| | 2 | 1.003 | 0.998 | 0.996 | 0.993 | 4 | | 2 | 0.029 | 0.029 | 0.043 | 0.036 | 7 | | |
| | 3 | 1.004 | 0.998 | 0.997 | 0.995 | 3 | | 3 | 0.029 | 0.029 | 0.043 | 0.036 | 7 | | |
| | Mean | 1.004 | 0.999 | 0.996 | 0.995 | 4 | | Mean | 0.029 | 0.029 | 0.043 | 0.036 | 7 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | 5 | Mean for 3 assays | | - | - | - | - | 7 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | L-Histidine |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-005 | 1 | 0.995 | 1.000 | 0.987 | 0.997 | 4 | MT-005 | 1 | 0.030 | 0.029 | 0.086 | 0.034 | 51 | | |
| | 2 | 0.997 | 1.003 | 0.990 | 1.000 | 4 | | 2 | 0.030 | 0.029 | 0.085 | 0.034 | 50 | | |
| | 3 | 0.995 | 1.002 | 0.987 | 0.998 | 4 | | 3 | 0.029 | 0.029 | 0.086 | 0.034 | 52 | | |
| | Mean | 0.996 | 1.002 | 0.988 | 0.998 | 4 | | Mean | 0.029 | 0.029 | 0.085 | 0.034 | 51 | Positive | Weakly photoreactive |
| MT-013 | 1 | 1.011 | 0.994 | 1.006 | 0.988 | 1 | MT-013 | 1 | 0.030 | 0.029 | 0.083 | 0.034 | 48 | | |
| | 2 | 1.011 | 0.995 | 1.001 | 0.991 | 7 | | 2 | 0.030 | 0.029 | 0.082 | 0.035 | 47 | | |
| | 3 | 1.012 | 0.995 | 1.006 | 0.990 | 2 | | 3 | 0.029 | 0.028 | 0.082 | 0.034 | 48 | | |
| | Mean | 1.011 | 0.994 | 1.004 | 0.990 | 3 | | Mean | 0.030 | 0.029 | 0.082 | 0.034 | 48 | Positive | Weakly photoreactive |
| MT-020 | 1 | 1.015 | 1.003 | 1.010 | 1.003 | 3 | MT-020 | 1 | 0.029 | 0.030 | 0.083 | 0.035 | 48 | | |
| | 2 | 1.018 | 1.000 | 1.012 | 0.998 | 4 | | 2 | 0.029 | 0.030 | 0.083 | 0.035 | 48 | | |
| | 3 | 1.021 | 0.999 | 1.014 | 0.997 | 5 | | 3 | 0.029 | 0.031 | 0.083 | 0.036 | 49 | | |
| | Mean | 1.018 | 1.001 | 1.012 | 0.999 | 4 | | Mean | 0.029 | 0.030 | 0.083 | 0.036 | 48 | Positive | Weakly photoreactive |
| Mean for 3 assays | | - | - | - | - | 4 | Mean for 3 assays | | - | - | - | - | 49 | Positive *3 | Weakly photoreactive*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Methylbenzylidene camphor |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-002 | 1 | 1.011 | 1.011 | 1.010 | 1.005 | -4 | MT-002 | 1 | 0.029 | 0.029 | 0.032 | 0.033 | -2 | | |
| | 2 | 1.008 | 1.016 | 1.008 | 1.011 | -4 | | 2 | 0.029 | 0.030 | 0.032 | 0.034 | -2 | | |
| | 3 | 1.005 | 1.018 | 1.004 | 1.013 | -4 | | 3 | 0.028 | 0.028 | 0.031 | 0.033 | -2 | | |
| | Mean | 1.008 | 1.015 | 1.007 | 1.010 | -4 | | Mean | 0.029 | 0.029 | 0.032 | 0.034 | -2 | Inconclusive | Non-photoreactive |
| MT-011 | 1 | 0.998 | 0.998 | 0.996 | 0.993 | -3 | MT-011 | 1 | 0.029 | 0.030 | 0.032 | 0.034 | -2 | | |
| | 2 | 1.002 | 1.005 | 1.001 | 0.999 | -4 | | 2 | 0.029 | 0.029 | 0.033 | 0.035 | -3 | | |
| | 3 | 0.999 | 1.003 | 0.997 | 1.000 | -3 | | 3 | 0.028 | 0.028 | 0.032 | 0.035 | -3 | | |
| | Mean | 1.000 | 1.002 | 0.998 | 0.997 | -3 | | Mean | 0.029 | 0.029 | 0.032 | 0.035 | -3 | Inconclusive | Non-photoreactive |
| MT-019 | 1 | 0.999 | 1.009 | 0.997 | 1.001 | -7 | MT-019 | 1 | 0.028 | 0.030 | 0.032 | 0.035 | -1 | | |
| | 2 | 1.003 | 1.002 | 1.001 | 0.992 | -8 | | 2 | 0.028 | 0.030 | 0.032 | 0.035 | -1 | | |
| | 3 | 1.001 | 1.003 | 1.000 | 0.994 | -8 | | 3 | 0.028 | 0.030 | 0.033 | 0.035 | 0 | | |
| | Mean | 1.001 | 1.005 | 0.999 | 0.996 | -8 | | Mean | 0.028 | 0.030 | 0.033 | 0.035 | -1 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -5 | Mean for 3 assays | | - | - | - | - | -2 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Octrizole |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-002 | 1 | 1.033 | 1.011 | 1.040 | 1.005 | -12 | MT-002 | 1 | 0.063 | 0.029 | 0.071 | 0.033 | 3 | | |
| | 2 | 1.028 | 1.016 | 1.036 | 1.011 | -13 | | 2 | 0.066 | 0.030 | 0.075 | 0.034 | 4 | | |
| | 3 | 1.033 | 1.018 | 1.042 | 1.013 | -14 | | 3 | 0.066 | 0.028 | 0.077 | 0.033 | 5 | | |
| | Mean | 1.031 | 1.015 | 1.040 | 1.010 | -13 | | Mean | 0.065 | 0.029 | 0.074 | 0.034 | 4 | Inconclusive | Non-photoreactive |
| MT-011 | 1 | 1.011 | 0.998 | 1.017 | 0.993 | -11 | MT-011 | 1 | 0.060 | 0.030 | 0.072 | 0.034 | 6 | | |
| | 2 | 1.011 | 1.005 | 1.016 | 0.999 | -11 | | 2 | 0.062 | 0.029 | 0.073 | 0.035 | 5 | | |
| | 3 | 1.013 | 1.003 | 1.020 | 1.000 | -12 | | 3 | 0.061 | 0.028 | 0.072 | 0.035 | 6 | | |
| | Mean | 1.012 | 1.002 | 1.018 | 0.997 | -11 | | Mean | 0.061 | 0.029 | 0.072 | 0.035 | 6 | Inconclusive | Non-photoreactive |
| MT-019 | 1 | 1.023 | 1.009 | 1.029 | 1.001 | -14 | MT-019 | 1 | 0.063 | 0.030 | 0.079 | 0.035 | 11 | | |
| | 2 | 1.032 | 1.002 | 1.037 | 0.992 | -15 | | 2 | 0.063 | 0.030 | 0.079 | 0.035 | 11 | | |
| | 3 | 1.031 | 1.003 | 1.044 | 0.994 | -22 | | 3 | 0.063 | 0.030 | 0.079 | 0.035 | 11 | | |
| | Mean | 1.029 | 1.005 | 1.037 | 0.996 | -17 | | Mean | 0.063 | 0.030 | 0.079 | 0.035 | 11 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -14 | Mean for 3 assays | | - | - | - | - | 7 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Octyl methacrylate |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-013 | 1 | 0.989 | 0.994 | 0.974 | 0.988 | 11 | MT-013 | 1 | 0.030 | 0.029 | 0.034 | 0.034 | 0 | | |
| | 2 | 0.996 | 0.995 | 0.981 | 0.991 | 10 | | 2 | 0.030 | 0.029 | 0.035 | 0.035 | 0 | | |
| | 3 | 0.992 | 0.995 | 0.980 | 0.990 | 8 | | 3 | 0.030 | 0.028 | 0.035 | 0.034 | -1 | | |
| | Mean | 0.992 | 0.994 | 0.979 | 0.990 | 10 | | Mean | 0.030 | 0.029 | 0.035 | 0.034 | 0 | Inconclusive | Non-photoreactive |
| MT-020 | 1 | 1.008 | 1.003 | 1.002 | 1.003 | 4 | MT-020 | 1 | 0.030 | 0.030 | 0.035 | 0.035 | -1 | | |
| | 2 | 1.005 | 1.000 | 1.001 | 0.998 | 2 | | 2 | 0.029 | 0.030 | 0.036 | 0.035 | 0 | | |
| | 3 | 1.006 | 0.999 | 0.998 | 0.997 | 7 | | 3 | 0.029 | 0.031 | 0.035 | 0.036 | 0 | | |
| | Mean | 1.006 | 1.001 | 1.000 | 0.999 | 4 | | Mean | 0.029 | 0.030 | 0.035 | 0.036 | 0 | Inconclusive | Non-photoreactive |
| MT-023 | 1 | 1.001 | 1.002 | 0.991 | 0.997 | 7 | MT-023 | 1 | 0.029 | 0.029 | 0.035 | 0.036 | -1 | | |
| | 2 | 1.001 | 0.998 | 0.991 | 0.993 | 6 | | 2 | 0.028 | 0.029 | 0.035 | 0.036 | 0 | | |
| | 3 | 1.007 | 0.998 | 0.998 | 0.995 | 4 | | 3 | 0.028 | 0.029 | 0.035 | 0.036 | -1 | | |
| | Mean | 1.003 | 0.999 | 0.993 | 0.995 | 6 | | Mean | 0.029 | 0.029 | 0.035 | 0.036 | -1 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 7 | Mean for 3 assays | | - | - | - | - | 0 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

Laboratory : 3
Chemical Name : Octyl methoxycinnamate

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*1 | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results*2 | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-004 | 1 | 1.009 | 0.999 | 1.002 | 0.992 | 2 | MT-004 | 1 | 0.045 | 0.029 | 0.046 | 0.034 | -5 | | |
| | 2 | 1.012 | 1.003 | 1.003 | 0.997 | 3 | | 2 | 0.046 | 0.029 | 0.046 | 0.034 | -5 | | |
| | 3 | 1.012 | 1.001 | 1.003 | 0.996 | 3 | | 3 | 0.045 | 0.029 | 0.046 | 0.034 | -5 | | |
| | Mean | 1.011 | 1.001 | 1.003 | 0.995 | 3 | | Mean | 0.046 | 0.029 | 0.046 | 0.034 | -5 | Inconclusive | Non-photoreactive |
| MT-012 | 1 | 1.010 | 0.992 | 1.005 | 0.989 | 1 | MT-012 | 1 | 0.046 | 0.030 | 0.046 | 0.034 | -5 | | |
| | 2 | 1.017 | 0.999 | 1.010 | 0.994 | 3 | | 2 | 0.046 | 0.029 | 0.047 | 0.035 | -4 | | |
| | 3 | 1.016 | 0.999 | 1.011 | 0.996 | 1 | | 3 | 0.046 | 0.029 | 0.046 | 0.034 | -5 | | |
| | Mean | 1.014 | 0.997 | 1.009 | 0.993 | 2 | | Mean | 0.046 | 0.029 | 0.046 | 0.034 | -5 | Inconclusive | Non-photoreactive |
| MT-018 | 1 | 1.011 | 0.997 | 1.003 | 0.993 | 4 | MT-018 | 1 | 0.046 | 0.029 | 0.047 | 0.033 | -4 | | |
| | 2 | 1.009 | 0.992 | 1.001 | 0.987 | 4 | | 2 | 0.046 | 0.028 | 0.046 | 0.033 | -5 | | |
| | 3 | 1.011 | 0.990 | 1.004 | 0.987 | 3 | | 3 | 0.046 | 0.028 | 0.047 | 0.033 | -4 | | |
| | Mean | 1.010 | 0.993 | 1.003 | 0.989 | 4 | | Mean | 0.046 | 0.028 | 0.047 | 0.033 | -4 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 3 | Mean for 3 assays | | - | - | - | - | -5 | Inconclusive *3 | Non-photoreactive*3 |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | Octyl salicylate |

Solubility

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Solution | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 20 μM |
| Superoxide anion | 20 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-007 | 1 | 1.004 | 0.996 | 1.004 | 0.992 | -3 | MT-007 | 1 | 0.036 | 0.029 | 0.042 | 0.036 | 0 | | |
| | 2 | 1.014 | 1.002 | 1.012 | 0.998 | -1 | | 2 | 0.037 | 0.029 | 0.042 | 0.035 | 0 | | |
| | 3 | 1.010 | 1.000 | 1.011 | 0.997 | -4 | | 3 | 0.036 | 0.028 | 0.042 | 0.034 | 0 | | |
| | Mean | 1.009 | 0.999 | 1.009 | 0.996 | -3 | | Mean | 0.036 | 0.029 | 0.042 | 0.035 | 0 | Inconclusive | Non-photoreactive |
| MT-015 | 1 | 1.009 | 1.002 | 1.005 | 0.997 | -3 | MT-015 | 1 | 0.036 | 0.030 | 0.041 | 0.035 | 1 | | |
| | 2 | 1.020 | 1.007 | 1.016 | 1.004 | -3 | | 2 | 0.037 | 0.030 | 0.041 | 0.035 | 0 | | |
| | 3 | 1.017 | 1.006 | 1.015 | 0.995 | -4 | | 3 | 0.036 | 0.029 | 0.041 | 0.034 | 0 | | |
| | Mean | 1.015 | 1.005 | 1.012 | 0.998 | -3 | | Mean | 0.037 | 0.030 | 0.041 | 0.034 | 0 | Inconclusive | Non-photoreactive |
| MT-022 | 1 | 1.014 | 1.001 | 1.011 | 0.994 | -3 | MT-022 | 1 | 0.036 | 0.030 | 0.041 | 0.034 | 0 | | |
| | 2 | 1.012 | 1.000 | 1.008 | 0.992 | -3 | | 2 | 0.037 | 0.030 | 0.040 | 0.035 | -1 | | |
| | 3 | 1.013 | 0.996 | 1.010 | 0.993 | -4 | | 3 | 0.036 | 0.029 | 0.041 | 0.034 | 0 | | |
| | Mean | 1.013 | 0.999 | 1.010 | 0.993 | -3 | | Mean | 0.036 | 0.029 | 0.041 | 0.034 | 0 | Inconclusive | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -3 | Mean for 3 assays | | - | - | - | - | 0 | Inconclusive [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | PABA |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-007 | 1 | 0.997 | 0.996 | 0.994 | 0.992 | 1 | MT-007 | 1 | 0.030 | 0.029 | 0.034 | 0.036 | -2 | | |
| | 2 | 1.001 | 1.002 | 1.014 | 0.998 | -16 | | 2 | 0.029 | 0.029 | 0.034 | 0.035 | -1 | | |
| | 3 | 1.000 | 1.000 | 0.996 | 0.997 | 1 | | 3 | 0.029 | 0.028 | 0.033 | 0.034 | -1 | | |
| | Mean | 1.000 | 0.999 | 1.001 | 0.996 | -5 | | Mean | 0.029 | 0.029 | 0.034 | 0.035 | -1 | Negative | Non-photoreactive |
| MT-015 | 1 | 1.007 | 1.002 | 1.002 | 0.997 | -2 | MT-015 | 1 | 0.030 | 0.030 | 0.034 | 0.035 | 0 | | |
| | 2 | 1.013 | 1.007 | 1.009 | 1.004 | -3 | | 2 | 0.030 | 0.030 | 0.034 | 0.035 | 0 | | |
| | 3 | 1.012 | 1.006 | 1.008 | 0.995 | -3 | | 3 | 0.029 | 0.029 | 0.033 | 0.034 | 0 | | |
| | Mean | 1.011 | 1.005 | 1.006 | 0.998 | -3 | | Mean | 0.030 | 0.030 | 0.033 | 0.034 | 0 | Negative | Non-photoreactive |
| MT-022 | 1 | 1.003 | 1.001 | 0.993 | 0.994 | 4 | MT-022 | 1 | 0.029 | 0.030 | 0.033 | 0.034 | -1 | | |
| | 2 | 1.005 | 1.000 | 0.996 | 0.992 | 3 | | 2 | 0.030 | 0.030 | 0.033 | 0.035 | -1 | | |
| | 3 | 1.000 | 0.996 | 0.991 | 0.993 | 3 | | 3 | 0.030 | 0.029 | 0.033 | 0.034 | -1 | | |
| | Mean | 1.003 | 0.999 | 0.993 | 0.993 | 3 | | Mean | 0.030 | 0.029 | 0.033 | 0.034 | -1 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | -2 | Mean for 3 assays | | - | - | - | - | -1 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | SDS |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |
| Superoxide anion | 200 μM : | Solution | 20 μM : | Not determined | 2 μM : | Not determined |

Test concentration

| | |
|---|---|
| Singlet oxygen | 200 μM |
| Superoxide anion | 200 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-007 | 1 | 0.982 | 0.996 | 0.975 | 0.992 | 4 | MT-007 | 1 | 0.132 | 0.029 | 0.145 | 0.036 | 7 | | |
| | 2 | 0.986 | 1.002 | 0.977 | 0.998 | 5 | | 2 | 0.132 | 0.029 | 0.143 | 0.035 | 5 | | |
| | 3 | 0.983 | 1.000 | 0.973 | 0.997 | 7 | | 3 | 0.132 | 0.028 | 0.141 | 0.034 | 3 | | |
| | Mean | 0.984 | 0.999 | 0.975 | 0.996 | 5 | | Mean | 0.132 | 0.029 | 0.143 | 0.035 | 5 | Negative | Non-photoreactive |
| MT-015 | 1 | 0.993 | 1.002 | 0.981 | 0.997 | 6 | MT-015 | 1 | 0.115 | 0.030 | 0.125 | 0.035 | 6 | | |
| | 2 | 0.996 | 1.007 | 0.983 | 1.004 | 6 | | 2 | 0.115 | 0.030 | 0.125 | 0.035 | 6 | | |
| | 3 | 0.991 | 1.006 | 0.980 | 0.995 | 4 | | 3 | 0.115 | 0.029 | 0.124 | 0.034 | 5 | | |
| | Mean | 0.994 | 1.005 | 0.981 | 0.998 | 5 | | Mean | 0.115 | 0.030 | 0.125 | 0.034 | 6 | Negative | Non-photoreactive |
| MT-022 | 1 | 0.978 | 1.001 | 0.966 | 0.994 | 6 | MT-022 | 1 | 0.097 | 0.030 | 0.117 | 0.034 | 15 | | |
| | 2 | 0.983 | 1.000 | 0.968 | 0.992 | 9 | | 2 | 0.098 | 0.030 | 0.116 | 0.035 | 14 | | |
| | 3 | 0.985 | 0.996 | 0.970 | 0.993 | 9 | | 3 | 0.097 | 0.029 | 0.114 | 0.034 | 12 | | |
| | Mean | 0.982 | 0.999 | 0.968 | 0.993 | 8 | | Mean | 0.097 | 0.029 | 0.116 | 0.034 | 14 | Negative | Non-photoreactive |
| Mean for 3 assays | | - | - | - | - | 6 | Mean for 3 assays | | - | - | - | - | 8 | Negative [*3] | Non-photoreactive[*3] |

*1 : decrease of A440 $x10^3$ = (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 $x10^3$ = (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)

Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 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

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

## Appendix 7 Individual data of Phase 2 study

| | | |
|---|---|---|
| Laboratory | : | 3 |
| Chemical Name | : | UV-571 |

Solubility

| | | | | | | |
|---|---|---|---|---|---|---|
| Singlet oxygen | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |
| Superoxide anion | 200 μM : | Precipitation | 20 μM : | Precipitation | 2 μM : | Solution |

Test concentration

| | |
|---|---|
| Singlet oxygen | 2 μM |
| Superoxide anion | 2 μM |

| Singlet oxygen | | | | | | | Superoxide anion | | | | | | | Judgement | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A440(-) | | A440(+) | | | | | A560(-) | | A560(+) | | | | |
| Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*1] | Experimental No. | Run# | Test Chemical | Blank | Test Chemical | Blank | Results[*2] | Judged by Original Criteria | Judged by Criteria for Proposed Protocol |
| MT-008 | 1 | 1.009 | 1.010 | 0.999 | 0.995 | -6 | MT-008 | 1 | 0.032 | 0.031 | 0.038 | 0.035 | 1 | | |
| | 2 | 1.015 | 1.012 | 1.004 | 0.997 | -4 | | 2 | 0.032 | 0.031 | 0.038 | 0.036 | 1 | | |
| | 3 | 1.010 | 1.011 | 0.999 | 0.997 | -4 | | 3 | 0.032 | 0.030 | 0.038 | 0.035 | 1 | | |
| | Mean | 1.011 | 1.011 | 1.001 | 0.996 | -5 | | Mean | 0.032 | 0.031 | 0.038 | 0.036 | 1 | Inconclusive | (Non-photoreactive) |
| MT-015 | 1 | 1.013 | 1.002 | 1.008 | 0.997 | -2 | MT-015 | 1 | 0.031 | 0.030 | 0.037 | 0.035 | 2 | | |
| | 2 | 1.016 | 1.007 | 1.013 | 1.004 | -4 | | 2 | 0.031 | 0.030 | 0.036 | 0.035 | 2 | | |
| | 3 | 1.012 | 1.006 | 1.010 | 0.995 | -5 | | 3 | 0.030 | 0.029 | 0.035 | 0.034 | 1 | | |
| | Mean | 1.014 | 1.005 | 1.011 | 0.998 | -4 | | Mean | 0.031 | 0.030 | 0.036 | 0.034 | 2 | Inconclusive | (Non-photoreactive) |
| MT-022 | 1 | 1.002 | 1.001 | 1.001 | 0.994 | -5 | MT-022 | 1 | 0.032 | 0.030 | 0.038 | 0.034 | 1 | | |
| | 2 | 1.004 | 1.000 | 1.003 | 0.992 | -4 | | 2 | 0.030 | 0.030 | 0.036 | 0.035 | 1 | | |
| | 3 | 1.005 | 0.996 | 1.002 | 0.993 | -3 | | 3 | 0.030 | 0.029 | 0.036 | 0.034 | 1 | | |
| | Mean | 1.004 | 0.999 | 1.002 | 0.993 | -4 | | Mean | 0.031 | 0.029 | 0.037 | 0.034 | 1 | Inconclusive | (Non-photoreactive) |
| Mean for 3 assays | | - | - | - | - | -4 | Mean for 3 assays | | - | - | - | - | 1 | Inconclusive [*3] | (Non-photoreactive)[*3] |

*1 : decrease of A440 x$10^3$ = (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 x$10^3$ = (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)

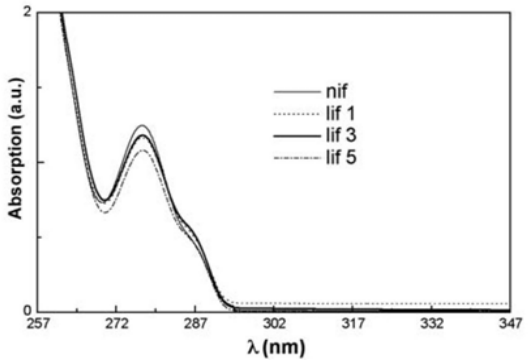
Judged by Original Criteria:
Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 μM
Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 μM
Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for 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: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Weakly photoreactive : Singlet oxygen results <25 and Superoxide results ≥20, <70 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
Non-photoreactive : Singlet oxygen results <25 and Superoxide results <20 at 200 μM: 20 μM without photodegradation is used for judgment only when precipitation or coloration is observed at 200 μM
(Photoreactive), (Weakly photoreactive), (Non-photoreactive): Ros assay was conducted at 2 μM due to precipitation at 20μM and 200μM. Although, the results met the photoreactive criteria (Singlet oxygen results ≥25 and Superoxide results ≥70, Singlet oxygen results <25 and Superoxide results ≥70, Singlet oxygen results ≥25 and Superoxide results <70), weakly photoreactive criteria (Singlet oxygen results <25 and Superoxide results ≥20, <70), or Non-photoreactive criteria (Singlet oxygen results <25 and Superoxide results <20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

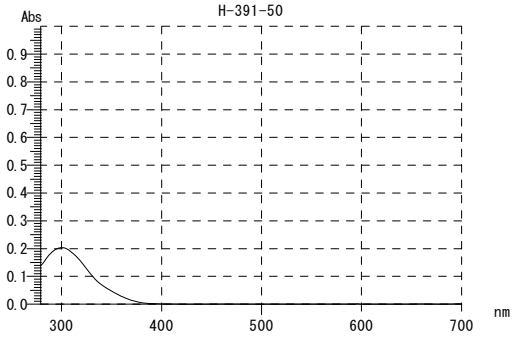
*3 : Final judgment based on the mean value of three assays

# Appendix 8 UV spectrum of test chemicals

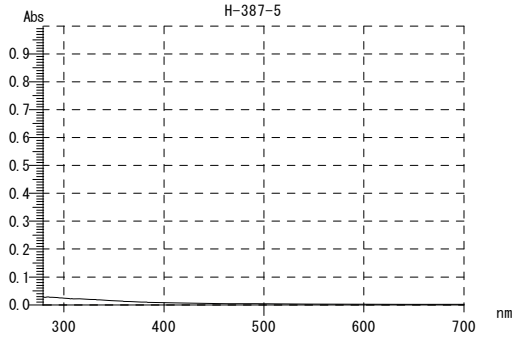
| Chemical Name | : | 5-FU[1)] |
|---|---|---|
| Solvent | : | Saline(adjusted to pH8.4) |
| Test concentration | : | 100 μM |



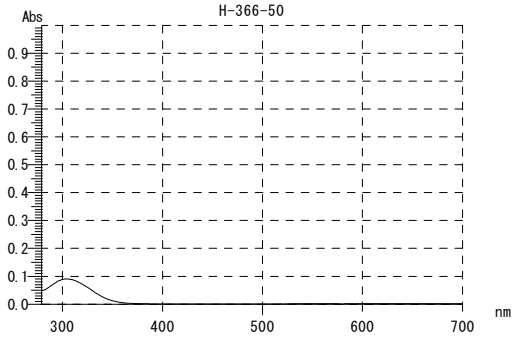

| Chemical Name | : | 8-MOP |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



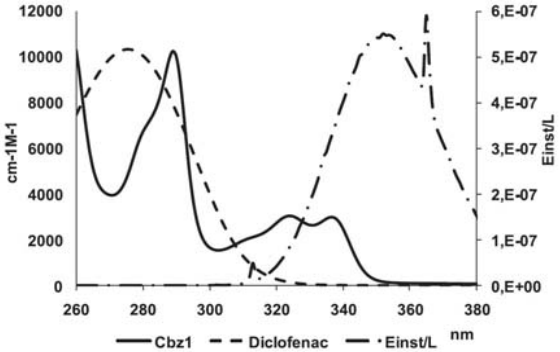

| Chemical Name | : | Amiodarone HCl |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 5 μM |



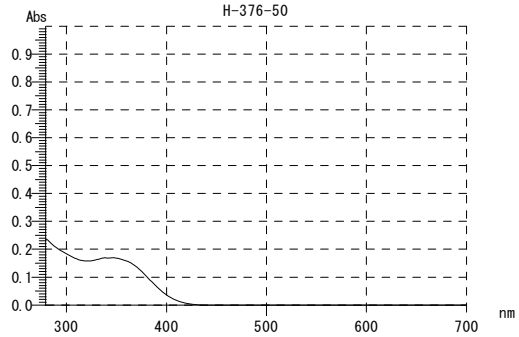

| Chemical Name | : | Chlorpromazine HCl |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |




| Chemical Name | : | Diclofenac[2)] |
|---|---|---|
| Solvent | : | Phosphate buffer(pH8.5) |
| Sample Concentration | : | <5 μM |




| Chemical Name | : | Doxycycline HCl |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |

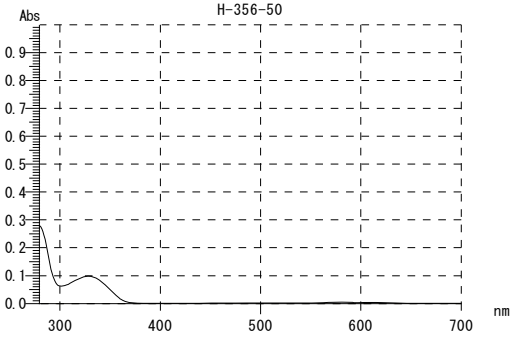



Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%.
UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was emloyed.

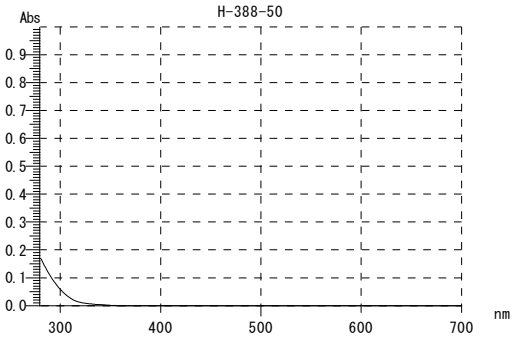
1)UV spectrum of 5-FU(normal line (nif)) was extracted from the article of M. L. Pascu, M. Brezeanu, L. Voicu, A. Staicu, B. Carstocea, R. A. Pascu (2005) *in vivo* , 19, 215-220
2)UV spectrum of Diclocenac (dashed line) was extracted from the article of J. Eriksson, J. Svanfelt, L. Kronberg (2010) *Photochemistry and Photobiology* , 86, 528-532

## Appendix 8 UV spectrum of test chemicals

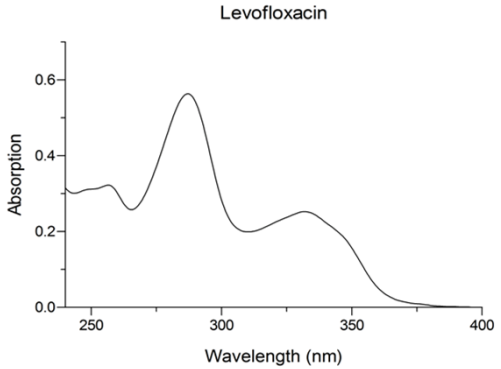
| Chemical Name | : | Furosemide |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



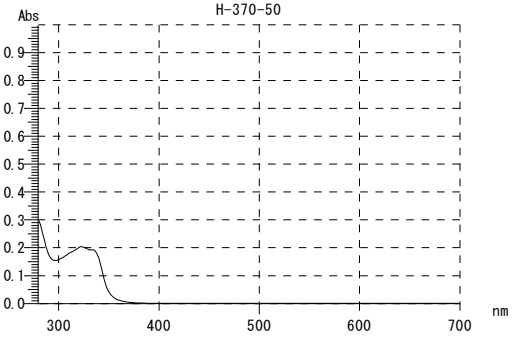

| Chemical Name | : | Ketoprofen |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



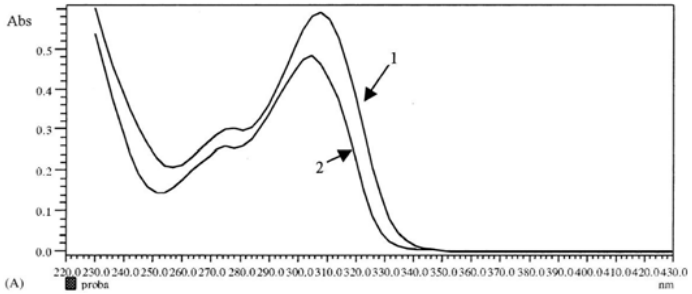

| Chemical Name | : | Levofloxacin[1)] |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4) |
| Test concentration | : | 20 μM |



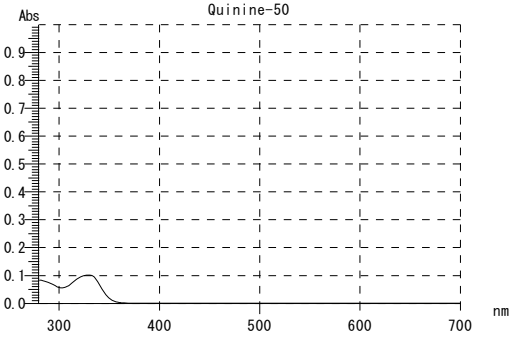

| Chemical Name | : | Norfloxacin |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |




| Chemical Name | : | Omeprazole[2)] |
|---|---|---|
| Solvent | : | Methanol /NH3 4.0% v/v (pH* 9.0) |
| Sample Concentration | : | 8.64 μg/mL(23.5 μM) |




| Chemical Name | : | Quinine HCl |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |

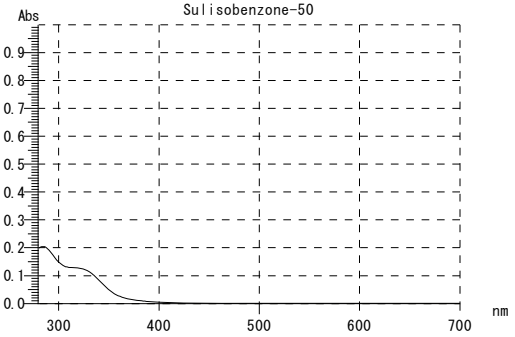



Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%.
UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was emloyed.

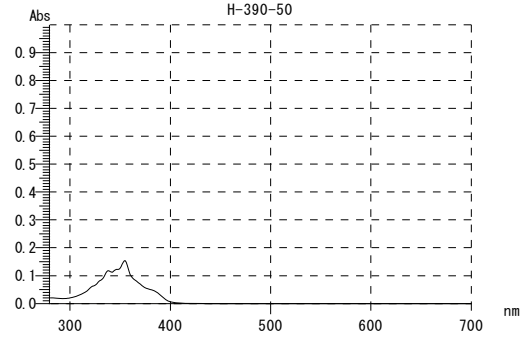
1)Test chemical was dissolved in 20 mM NaPB(pH7.4). UV/vis absorption spectra was recorded with a HITACHI U-2010 spectrophotometer (HITACHI, Tokyo, Japan).
2)Omeprazole (curve 1) was extracted from the article of K. K. Rajic, D. Novovic, V. Mrinkovic, D. Agbaba (2003) *Journal of Pharmaceutical and Biomedical Analysis* , 32, 1019-1027

# Appendix 8 UV spectrum of test chemicals

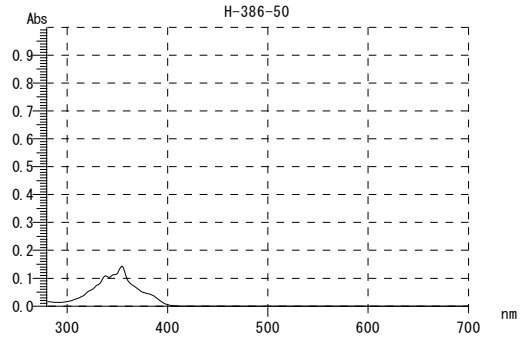
| Chemical Name | : | Sulisobenzone |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



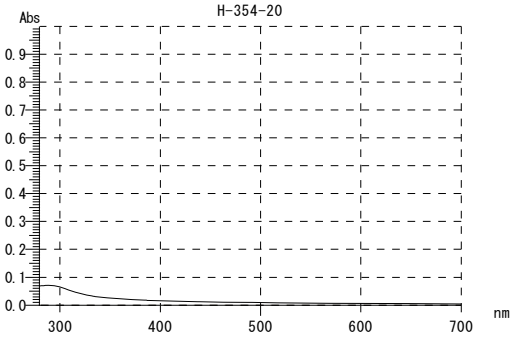

| Chemical Name | : | Acridine |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



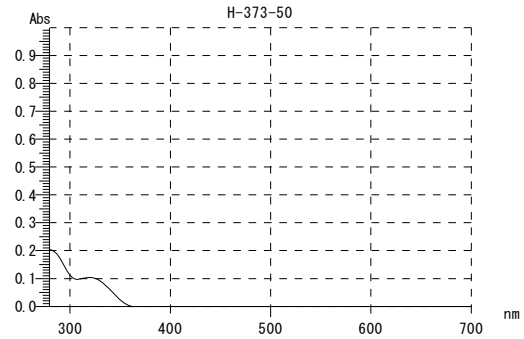

| Chemical Name | : | Acridine HCl |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



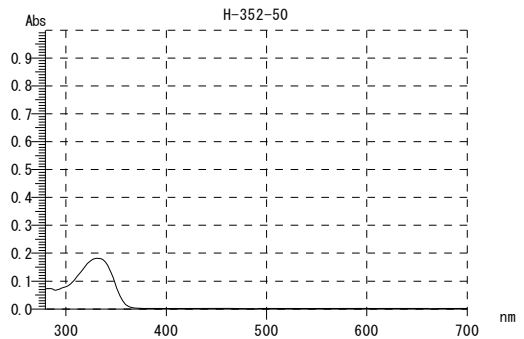

| Chemical Name | : | Fenofibrate |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 20 μM |




| Chemical Name | : | 6-methylcoumarine |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |



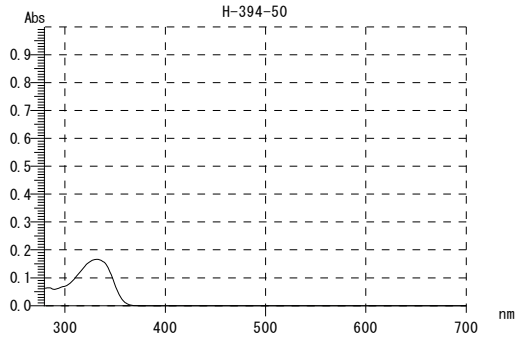

| Chemical Name | : | Nalidixic acid |
|---|---|---|
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |



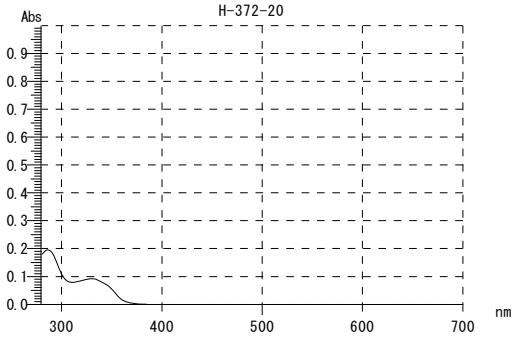

Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%.
UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was emloyed.

# Appendix 8 UV spectrum of test chemicals

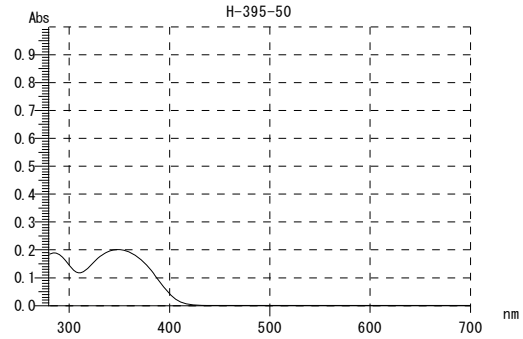
| | | |
|---|---|---|
| Chemical Name | : | Nalidixic acid (Na salt) |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



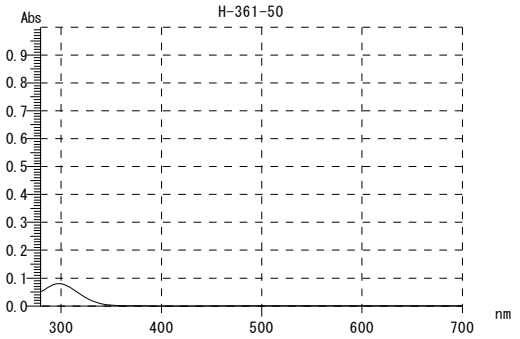

| | | |
|---|---|---|
| Chemical Name | : | Ofloxacin |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 20 μM |



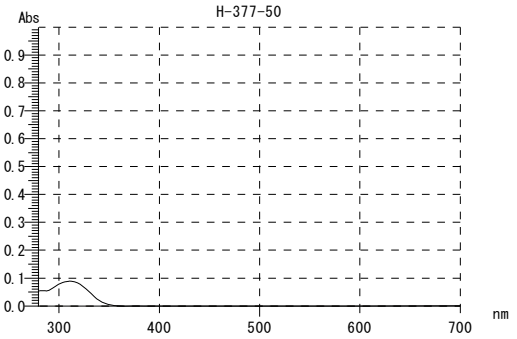

| | | |
|---|---|---|
| Chemical Name | : | Piroxicam |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



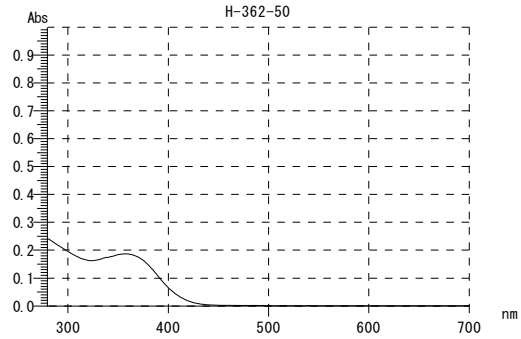

| | | |
|---|---|---|
| Chemical Name | : | Promethazine HCl |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |




| | | |
|---|---|---|
| Chemical Name | : | Rosiglitazone |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |



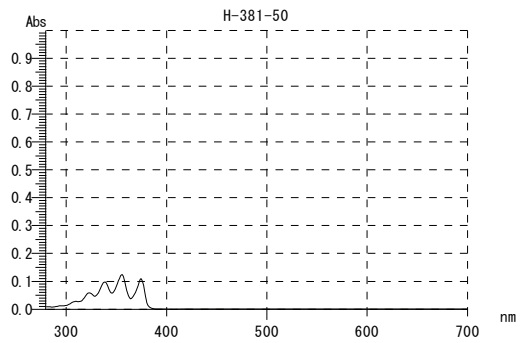

| | | |
|---|---|---|
| Chemical Name | : | Tetracycline |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |



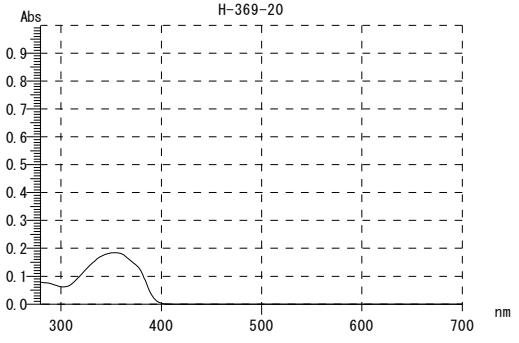

Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%.
UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was emloyed.

# Appendix 8 UV spectrum of test chemicals

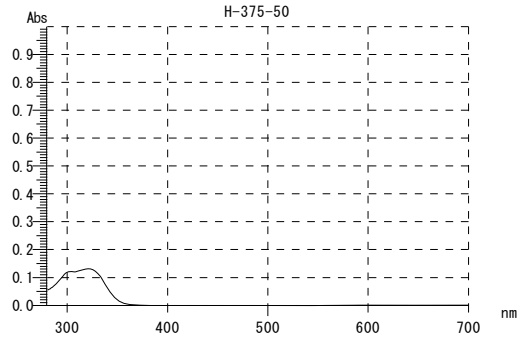
| | | |
|---|---|---|
| Chemical Name | : | Anthracene |
| Solvent | : | Methanol, 0.5% DMSO |
| Test concentration | : | 50 μM |



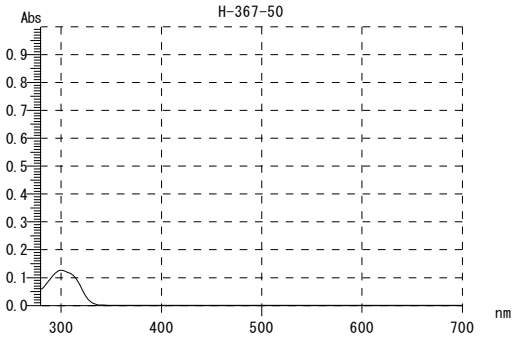

| | | |
|---|---|---|
| Chemical Name | : | Avobenzone |
| Solvent | : | Methanol, 0.5% DMSO |
| Test concentration | : | 20 μM |



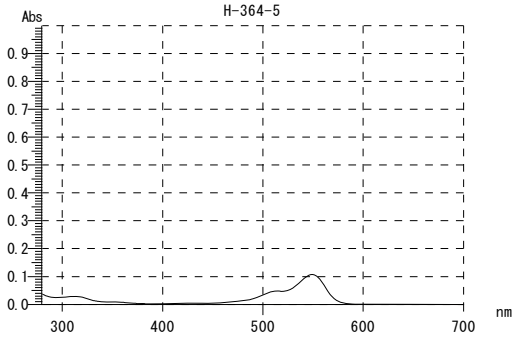

| | | |
|---|---|---|
| Chemical Name | : | Bithionol |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



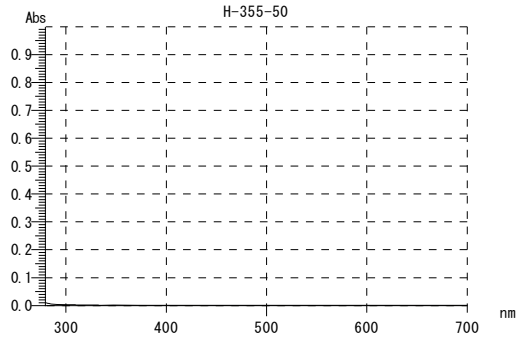

| | | |
|---|---|---|
| Chemical Name | : | Hexachlorophene |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |




| | | |
|---|---|---|
| Chemical Name | : | Rose bengal |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 5 μM |



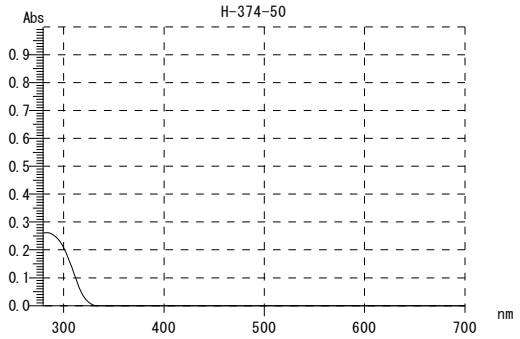

| | | |
|---|---|---|
| Chemical Name | : | Aspirin |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |



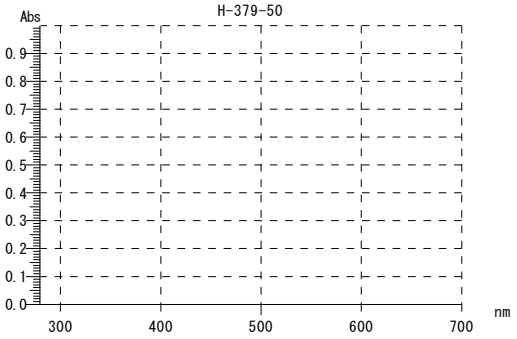

Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%.
UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was emloyed.

# Appendix 8 UV spectrum of test chemicals

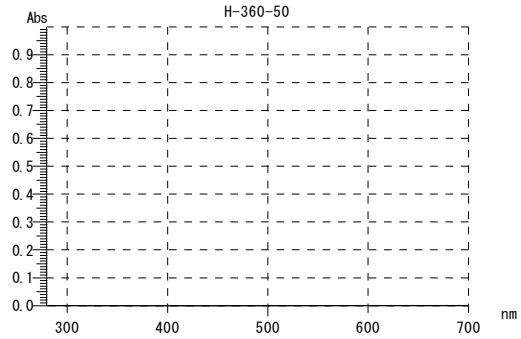
| | | |
|---|---|---|
| Chemical Name | : | Benzocaine |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



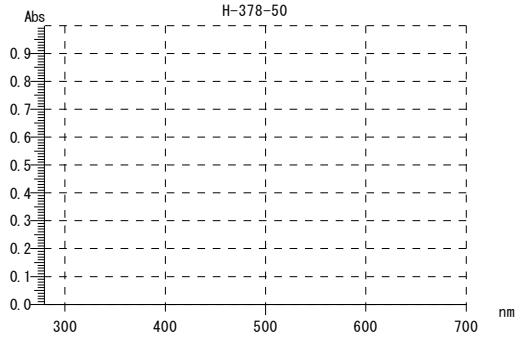

| | | |
|---|---|---|
| Chemical Name | : | Erythromycin |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



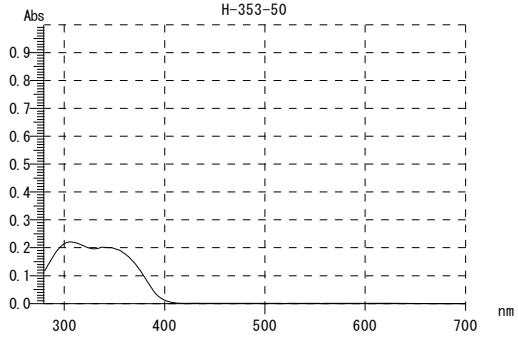

| | | |
|---|---|---|
| Chemical Name | : | Phenytoin |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



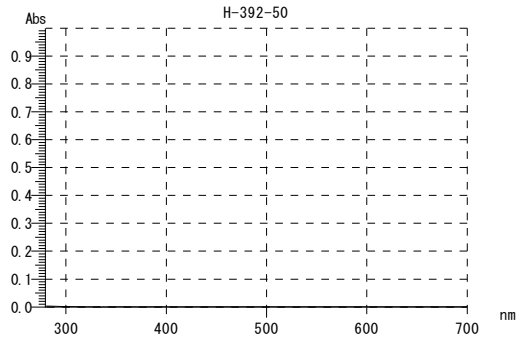

| | | |
|---|---|---|
| Chemical Name | : | Penicillin G |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |




| | | |
|---|---|---|
| Chemical Name | : | Bumetrizole |
| Solvent | : | Methanol, 0.5% DMSO |
| Sample Concentration | : | 50 μM |



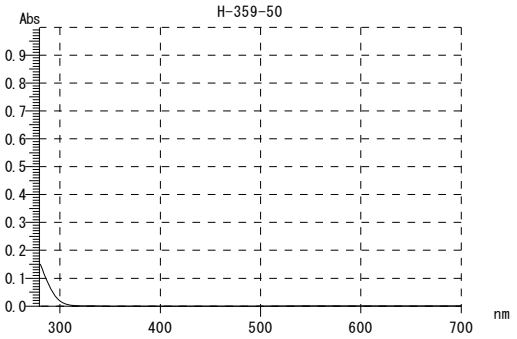

| | | |
|---|---|---|
| Chemical Name | : | Camphor sulfonic acid |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |



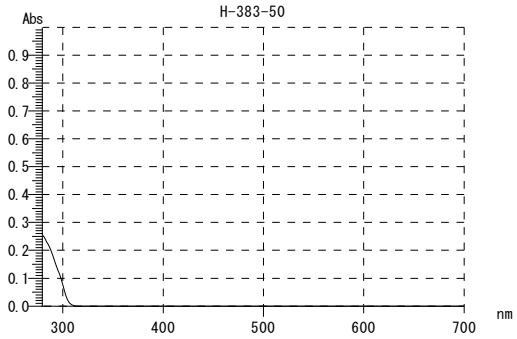

Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%.
UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was emloyed.

# Appendix 8 UV spectrum of test chemicals

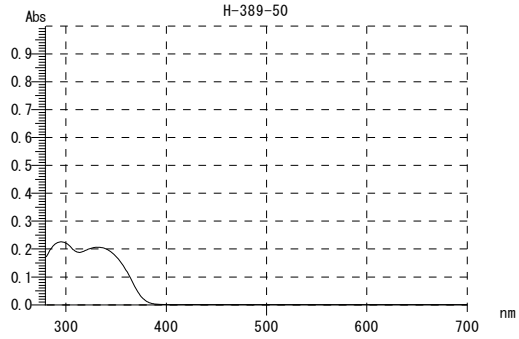
| | | |
|---|---|---|
| Chemical Name | : | Chlorhexidine |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



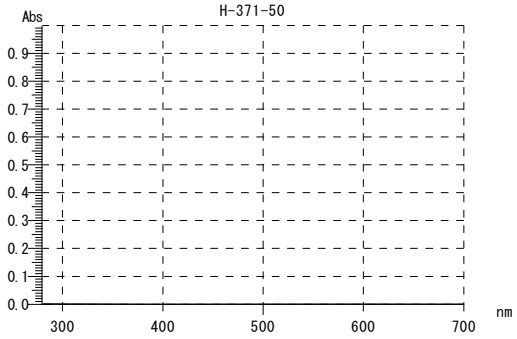

| | | |
|---|---|---|
| Chemical Name | : | Cinnamic acid |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 50 μM |



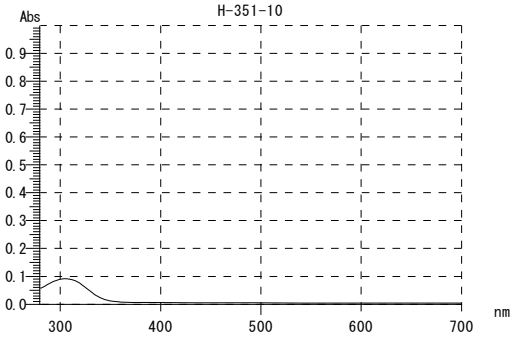

| | | |
|---|---|---|
| Chemical Name | : | Drometrizole |
| Solvent | : | Methanol, 0.5% DMSO |
| Test concentration | : | 50 μM |



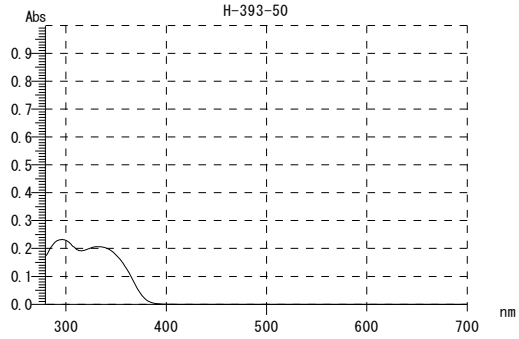

| | | |
|---|---|---|
| Chemical Name | : | L-histidine |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |




| | | |
|---|---|---|
| Chemical Name | : | Methylbenzylidenecamphor |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 10 μM |



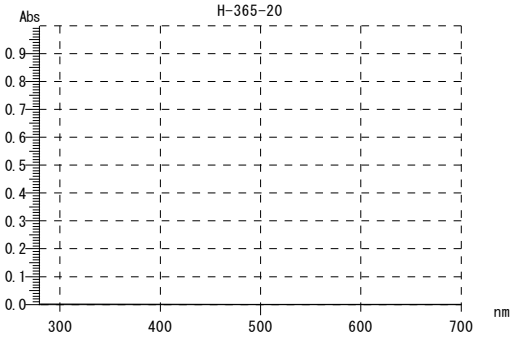

| | | |
|---|---|---|
| Chemical Name | : | Octrizole |
| Solvent | : | Methanol, 0.5% DMSO |
| Sample Concentration | : | 50 μM |



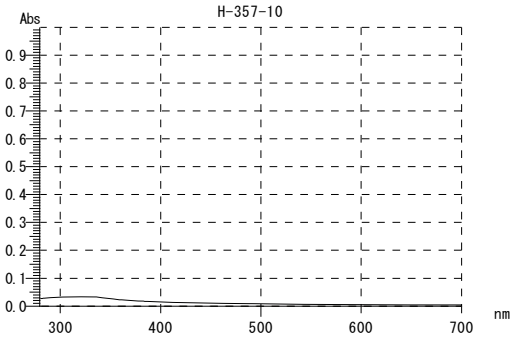

Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%.
UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was emloyed.

# Appendix 8 UV spectrum of test chemicals

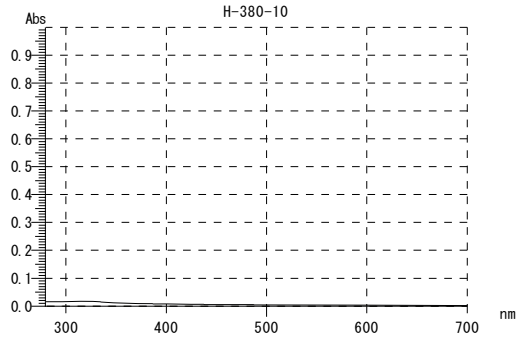
| | | |
|---|---|---|
| Chemical Name | : | Octyl methacrylate |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 20 μM |



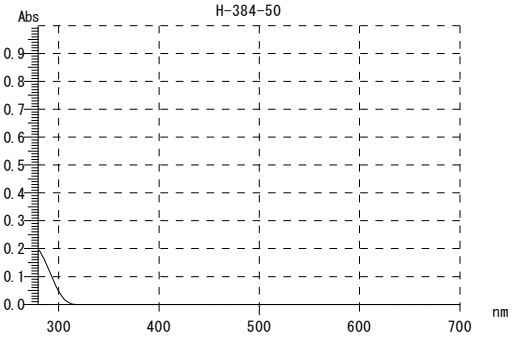

| | | |
|---|---|---|
| Chemical Name | : | Octylmethoxycinnamate |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 10 μM |



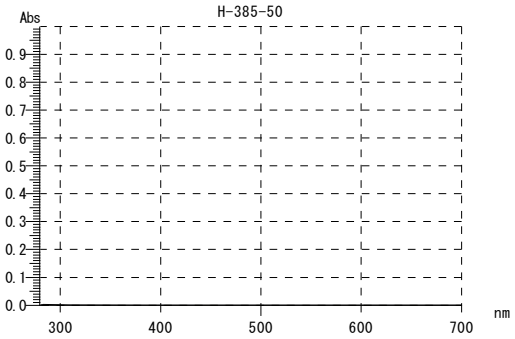

| | | |
|---|---|---|
| Chemical Name | : | Octyl salicylate |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Test concentration | : | 10 μM |



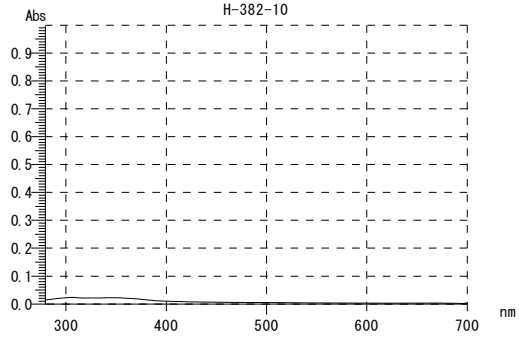

| | | |
|---|---|---|
| Chemical Name | : | PABA |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |




| | | |
|---|---|---|
| Chemical Name | : | SDS |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 50 μM |




| | | |
|---|---|---|
| Chemical Name | : | UV-571 |
| Solvent | : | 20 mM NaPB(pH7.4), 0.5% DMSO |
| Sample Concentration | : | 10 μM |




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 emloyed.

# Appendix 9 Preparation information and appearance of the stock solutions and the reaction mixtures

| Chemicals | | Lab 1 | | | | | | | | Lab 2 | | | | | | | | Lab 3 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Stock solution | | | | Reaction mixture | | | | Stock solution | | | | Reaction mixture | | | | Stock solution | | | | Reaction mixture | | | |
| N0. | Name | 1st Solvent | Concentration | | | Concentration [a] | | | | 1st Solvent | Concentration | | | Concentration [a] | | | | 1st Solvent | Concentration | | | Concentration [a] | | | |
| | | | 10 mM | 1 mM | 0.1 mM | | 200 μM | 20 μM | 2 μM | | 10 mM | 1 mM | 0.1 mM | | 200 μM | 20 μM | 2 μM | | 10 mM | 1 mM | 0.1 mM | | 200 μM | 20 μM | 2 μM |
| II - 1 | Acridine | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 2 | Acridine HCl | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 3 | Amiodarone HCl | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. |
| | | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sus. | Sol. |
| II - 4 | Chlorpromazine HCl | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 5 | Doxycycline HCl | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 6 | Fenofibrate | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sol. | - |
| II - 7 | Furosemide | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 8 | Ketoprofen | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 9 | 6-methylcoumarine | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 10 | 8-MOP | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 11 | Nalidixic acid | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 12 | Nalidixic acid (Na salt) | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 13 | Norfloxacin | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 14 | Ofloxacin | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 15 | Piroxicam | DMSO | Sol. | Sol. | - | SO | Sol. | - | - | DMSO | Sol. | Sol. | - | SO | Sol. | - | - | DMSO | Sol. | Sol. | - | SO | Sol. | - | - |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - |
| II - 16 | Promethazine HCl | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 17 | Rosiglitazone | DMSO | Sol. | Sol. | - | SO | Sol. | - | - | DMSO | Sol. | Sol. | - | SO | Sol. | - | - | DMSO | Sol. | Sol. | - | SO | Sol. | - | - |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - |
| II - 18 | Tetracycline | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 19 | Anthracene | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. |
| | | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sus. | Sol. |
| II - 20 | Avobenzone | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - |
| | | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sol. | - |
| II - 21 | Bithionol | DMSO | Sol. | Sol. | - | SO | Sol. | - | - | DMSO | Sol. | Sol. | Sol. | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sol. | - | - |
| II - 22 | Hexachlorophene | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | Sol. | Sol. | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sol. | - | - |
| II - 23 | Rose bengal | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | 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 1 and 0.1 mM were used to prepare 20 and 2 μM..

# Appendix 9 Preparation information and appearance of the stock solutions and the reaction mixtures (continued)

| Chemicals | | Lab 1 | | | | | | | | Lab 2 | | | | | | | | Lab 3 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Stock solution | | | | Reaction mixture | | | | Stock solution | | | | Reaction mixture | | | | Stock solution | | | | Reaction mixture | | | |
| N0. | Name | 1st Solvent | Concentration | | | | Concentration [a)] | | | 1st Solvent | Concentration | | | | Concentration [a)] | | | 1st Solvent | Concentration | | | | Concentration [a)] | | |
| | | | 10 mM | 1 mM | 0.1 mM | | 200 μM | 20 μM | 2 μM | | 10 mM | 1 mM | 0.1 mM | | 200 μM | 20 μM | 2 μM | | 10 mM | 1 mM | 0.1 mM | | 200 μM | 20 μM | 2 μM |
| II - 24 | Aspirin | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 25 | Benzocaine | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 26 | Erythromycin | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II – 27 | Penicillin G | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 28 | Phenytoin | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 29 | Bumetrizole | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - |
| | | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sol. | - |
| II - 30 | Camphor sulfonic acid | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 31 | Chlorhexidine | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 32 | Cinnamic acid | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 33 | Drometrizole | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - |
| II - 34 | L-Histidine | DMSO | Sus. | - | - | SO | - | - | - | DMSO | Sus. | - | - | SO | - | - | - | DMSO | Sus. | - | - | SO | - | - | - |
| | | | | | | SA | - | - | - | | | | | SA | - | - | - | | | | | SA | - | - | - |
| | | NaPB | Sol. | - | - | SO | Sol. | - | - | NaPB | Sol. | - | - | SO | Sol. | - | - | NaPB | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 35 | Methylbenzylidene camphor | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - |
| II - 36 | Octrizole | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - |
| | | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sol. | - |
| II - 37 | Octyl methacrylate | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - |
| II - 38 | Octyl methoxycinnamate | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sol. | - |
| II - 39 | Octyl salicylate | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | - | SO | Sus. | Sol. | - |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sol. | - |
| II - 40 | PABA | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - | | | | | SA | Sol. | - | - |
| II - 41 | SDS | DMSO | Sol. | Sol. | - | SO | Sol. | - | - | DMSO | Sol. | Sol. | Sol. | SO | Sol. | - | - | DMSO | Sol. | - | - | SO | Sol. | - | - |
| | | | | | | SA | Sus. | Sol. | - | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sol. | - | - |
| II - 42 | UV-571 | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sol. | - | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. | DMSO | Sol. | Sol. | Sol. | SO | Sus. | Sus. | Sol. |
| | | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | Sus. | Sus. | Sol. | | | | | SA | 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 1 and 0.1 mM were used to prepare 20 and 2 μM.

# INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING PHOTOTOXIC POTENTIAL OF CHEMICALS (VERSION 1.0)

**Issued by: the Validation Management Team (VMT)**
**Date: 22 April 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;

Singlet oxygen + Imidazole → [Peroxide intermediate] → Oxidized imidazole

[Peroxide intermediate] + RNO → RNO + Products

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.

Superoxide + NBT → $O_2$ + $NBT^+$

# 3. Materials

## 3.1. Control compounds

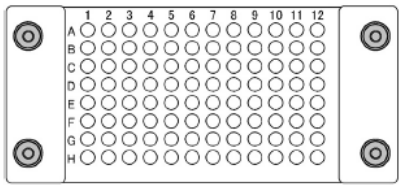
| Name | CAS No. | Manufucture | Molecular weight |
|---|---|---|---|
| Quinine monohydrochrolide dihydrate (Positive control) | 6119-47-7 | Sigma (Catalog No. 145920) | 396.91 |
| Sulisobenzone （Negative control） | 4065-45-6 | Tokyo Chemical Industry (Catalog No. H0466) | 308.31 |

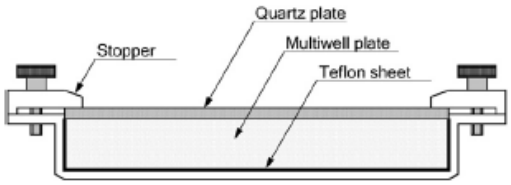
## 3.2. Solvent

Dimethylsulfoxide (DMSO): analytical grade

## 3.3. Instruments

| Instrument | Model |
|---|---|
| Solar simulator | Suntest CPS+, quipped UV (<290 nm) cut filter (Atlas Material Technology), or its equivalent<br>Suntest CPS, quipped UV (<290 nm) cut filter (Atlas Material Technology), or its equivalent |
| UVA detector | Topcon or Dr. Hönle |
| 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 $NaH_2PO_4 \cdot 2H_2O$ and 5.8 g of $Na_2HPO_4 \cdot 12H_2O$ 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 $2 \times 10^{-4}$ 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 $2 \times 10^{-2}$ M

Dilute the $2 \times 10^{-2}$ 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 $4 \times 10^{-4}$ 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℃). 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】 | |
|---|---|
| 20 mM NaPB | 480 μL |
| Imidazole | 250 μL |
| RNO | 250 μL |
| Compound | 20 μL |

↓

Mix (Vortex and Sonication for 5 – 10 min)

↓

Check solubility[1)]

↓

Add 200 μL of mixture to each well (n=3)

↓

Pre-read Abs at 440 nm after shaking for 5 sec

↓

Light exposure (250 W/$m^2$ for 1 hr at 25℃)[2)]

↓

Read Abs at 440 nm after shaking for 5 sec

↓

Check solubility[1)]

| 【Superoxide anion】 | |
|---|---|
| 20 mM NaPB | 855 μL |
| NBT | 125 μL |
| Compound | 20 μL |

↓

Mix (Vortex and sonication for 5-10 min)

↓

Check solubility[1)]

↓

Add 200 μL of mixture to each well (n=3)

↓

Pre-read Abs at 560 nm after shaking for 5 sec

↓

Light exposure (250 W/$m^2$ for 1 hr at 25℃)[2)]

↓

Read Abs 560 nm after shaking for 5 sec

↓

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. The solar simulator will be use at least 30 min after turning on the power supply at the indicator setting value of 250 W/m$^2$ (for CPS+). After the final experiment of the day, UVA intensity will be measured using a UVA detector. Temperature and light exposure will be written the set values by light exposure equipment (for CPS+). 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

**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 before after exposure)

### 6.2. Superoxide anion

**increase of A560 $\times 10^3$ = (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 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 to 500
Superoxide anion: 200 to 400

Negative control value at 200 μM (mean of 3 wells)
Singlet oxygen: -20 to 24
Superoxide anion: -20 to 19

## 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 (ATLAS VERSION 3.02)

**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;

Singlet oxygen + Imidazole → [Peroxide intermediate] → Oxidized imidazole

[Peroxide intermediate] + RNO → RNO + Products

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.

Superoxide + NBT → $O_2$ + $NBT^+$

# 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 monohydrochrolide 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.

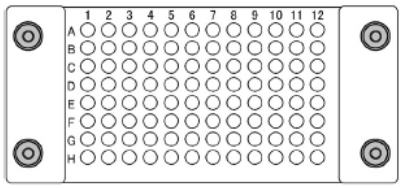
### 3.4. Reagents

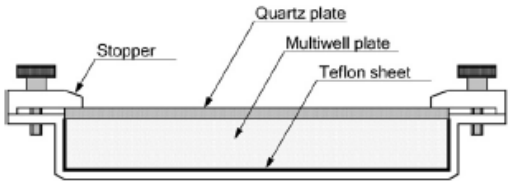
The following reagents will be used and stored according to the instructions of manufacturers.

- $NaH_2PO_4 \cdot 2H_2O$: e.g. Wako, catalog No. 192-02815
- $Na_2HPO_4 \cdot 12H_2O$: 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: Suntest CPS+ or CPS, equipped with a xenon arc lamp, UV (<290 nm) cut filter, and temperature control unit (Atlas Material Technology), 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 $NaH_2PO_4 \cdot 2H_2O$ and 5.8 g of $Na_2HPO_4 \cdot 12H_2O$, 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 $2 \times 10^{-4}$ 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 $2 \times 10^{-2}$ M.

Dilute the $2 \times 10^{-2}$ 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 $4 \times 10^{-4}$ 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 5.2.) will be set at 200 μM. When precipitation is observed at 20 μM in the reaction mixture

under a microscope, 1 mM of the test chemical solution should be prepared using DMSO. Furthermore, 0.1 mM of the test chemical solution should be prepared using DMSO when precipitation is observed at 2 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 5.2.) will be used at the maximum concentration without precipitation (2, 20, or 200 μM).

### 4.3. Positive and negative control chemicals

Stock solutions of qunine and sulisobenzone will be prepared at 10 mM each in DMSO (the final concentration of 200 μM) 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 will be set at an appropriate value (e.g. the indicator setting value of 250 W/m$^2$ for CPS+). The solar simulator and the temperature control unit (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, 1.8-2.2 mW/cm$^2$ of UVA intensity for the calibrated UVA detector will be set.

When the solar simulator has a temperature control unit, the temperature will be adjusted at 25°C. As for the solar simulator without a temperature control unit, a range of 20-29°C after light exposure is acceptable.

### 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 20 μM. Further experiments should be performed at 2 μM when precipitation is observed at 20 μM. When precipitation is observed at 2 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】 | |
|---|---|
| 20 mM NaPB | 480 μL |
| Imidazole | 250 μL |
| RNO | 250 μL |
| Chemical | 20 μL |

↓

Mix (Vortex and Sonication for 5 – 10 min)

↓

Add 200 μL of mixture to each well (n=3) [1)]

↓

Check solubility [2)]

↓

Pre-read Abs at 440 nm after shaking for 5 sec

↓

Light exposure for 1 hr [3)]

↓

Read Abs at 440 nm after shaking for 1 min

| 【Superoxide anion】 | |
|---|---|
| 20 mM NaPB | 855 μL |
| NBT | 125 μL |
| Chemical | 20 μL |

↓

Mix (Vortex and sonication for 5-10 min)

↓

Add 200 μL of mixture to each well (n=3) [1)]

↓

Check solubility [2)]

↓

Pre-read Abs at 560 nm after shaking for 5 sec

↓

Light exposure for 1 hr [3)]

↓

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

2) The reaction mixture will be checked solubility (solution or suspension) using a microscope (×100) before light exposure.

3) 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

**decrease of A440 $\times 10^3$ = (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 before after exposure)

### 6.2. Superoxide anion

**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 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 3.01**

Date: 29 August 2011

Items: 8. Criteria for Judgment (Page 8)

Positive (mean of 3 wells)

| | Version 3.0 | Version 3.01 |
|---|---|---|
| Singlet oxygen: | 150 or more | ---> 25 or more |
| Superoxide anion: | 200 or more | ---> 20 or more |

Reason: Mistake of description

**1. Version 3.02**

Date: 21 May 2012

Items: 8. Criteria for judgment (Page 8-9)

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.

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 μM 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.