Supporting Information

Resorufin Enters the Photodynamic Therapy Arena: A Monoamine Oxidase Activatable Agent for Selective Cytotoxicity

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1. Materials and instruments

All reagents were commercially available and used without further purification unless otherwise noted. All dry solvents used in reactions were directly obtained from the Mbraun MBSPS5 solvent drying system. The inert atmosphere was obtained by Argon. The ^{1}H and ^{13}C-NMR spectra were recorded on a Varian (500 MHz) or Bruker Avance III Ultrashield (400 MHz) spectrometers using $CDCl_3$ or d_6-DMSO as the solvents. The chemical shifts are reported in parts per million (ppm) downfield from an internal TMS (trimethylsilane) reference. Coupling constants (J) are reported in hertz (Hz), and the spin multiplicities were specified by the following symbols: s (singlet), d (doublet), t (triplet), and m (multiplet). NMR spectra were processed with MestReNova program. Column chromatography was performed by using thick walled glass columns and silica Gel 60 (Merck 230-400 mesh). Thin layer chromatography (TLC Merck Silica Gel 60 F254) was performed by using commercially prepared 0.25 mm silica gel plates and visualization was provided by UV lamp. The relative proportions of solvents in chromatography solvent mixtures refer to the volume: volume ratio. Electronic absorption spectra in solution were acquired using a Shimadzu Uv-3600 Uv-Vis-NIR spectrophotometer. Fluorescence spectra were determined on Agilent Cary Eclipse fluorescence spectrophotometer. Fluorescence quantum yields of the samples were investigated by using a fluorescence spectrometer (FLS 1000, Edinburgh Instruments) with an integrating sphere accessory. Mass spectra were recorded on Waters Synapt G1 High Definition mass spectrometer. HPLC analyses were carried with Agilent 1260 Infinity II system consisted of a quad pump (1260 Quad Pump VL), an autosampler, a diode array detector (1260 DAD WR) and a ChemStation software. The purity of biologically evaluated compound was ≥95% as determined by HPLC.

2. Activation mechanism

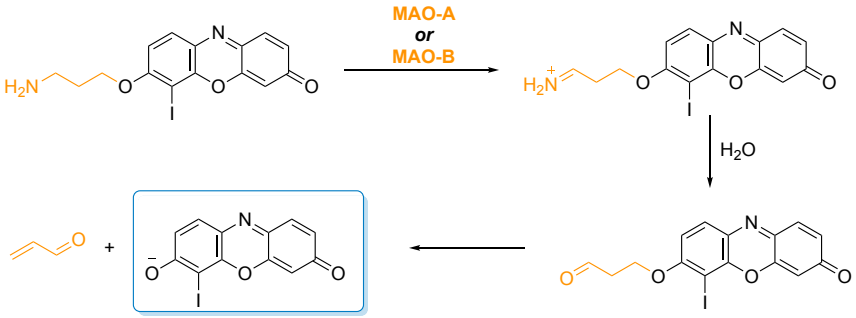
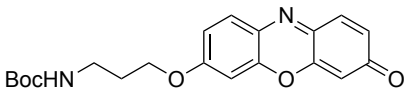


Figure S1. Activation mechanism of R1 in the presence of MAO isoforms.

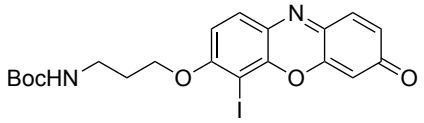
3. Synthesis

Compound 1:



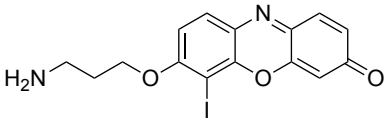
Resorufin (300 mg, 1.41 mmol, 0.1 M) and Cs_2CO_3 (915 mg, 2.82 mmol, 0.2 M) were dissolved in 14 mL dry DMF and stirred at room temperature. N-aminotertbutyloxycarbonyl-3-propylbromide (671 mg, 2.82 mmol, 0.2 M) was added slowly and reaction was stirred overnight at 50 °C. All content was transferred to the extraction funnel and diluted with 200 mL ethyl acetate. Then it was washed with brine. Organic layer was separated, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (Hex:EtOAc, 1:2, R_f = 0.52). The product was obtained as orange crystal (368 mg, 71 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, *J* = 8.9 Hz, 1H), 7.42 (d, *J* = 9.8 Hz, 1H), 6.93 (dd, *J* = 8.9, 2.6 Hz, 1H), 6.86 – 6.78 (m, 2H), 6.32 (d, *J* = 2.0 Hz, 1H), 4.78 (s, 1H), 4.13 (t, *J* = 6.1 Hz, 2H), 3.36 (q, *J* = 6.2 Hz, 2H), 2.05 (p, *J* = 6.2 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.3, 162.8, 156.0, 149.8, 145.64, 145.58, 134.7, 134.2, 131.6, 128.4, 113.9, 106.7, 100.5, 79.5, 66.7, 37.6, 29.5, 28.4. HRMS m/z calc. for $C_{20}H_{22}N_2O_5$: 370.1529; found: 370.1535 [M].

Compound 2:



(1) (200 mg, 0.54 mmol, 0.013 M) was dissolved in 40 mL ethanol and stirred for 15 minutes. Iodic acid solution in 2 mL water (47.5 mg, 0.27 mmol, 0.006 M) and iodine (354 mg, 1.39 mmol, 0.033 M) were added to the reaction mixture. The mixture was heated and refluxed for 3 hours. By monitoring TLC, reaction was cooled down to room temperature and EtOH was removed under reduced pressure. Remaining solid was diluted with 130 mL EtOAc and washed with sodium thiosulfate. Organic layer was separated, dried under Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (Hex:EtOAc 1:1, R_f = 0.50). The product was obtained as red crystal (134 mg, 53 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.7 Hz, 1H), 7.42 (d, *J* = 9.7 Hz, 1H), 7.03 – 6.96 (m, 3H), 4.77 (s, 1H), 4.18 (t, *J* = 6.1 Hz, 2H), 3.37 (q, *J* = 6.1 Hz, 2H), 2.13 – 2.01 (m, 2H), 1.46 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 180.6, 163.4, 156.0, 151.0, 145.9, 144.1, 134.6, 132.0, 131.4, 128.5, 115.0, 100.7, 85.1, 79.5, 66.9, 37.6, 29.5, 28.4. HRMS m/z calc. for $C_{20}H_{22}N_2O_5I$: 496.0495 $[M+H]^+$; found: 497.0574.

Compound R1:



(2) (80 mg, 0.16 mmol, 0.04 M) was dissolved in 4 mL DCM. 1.23 mL TFA was added (16 mmol, 4.0 M) to the reaction mixture dropwise and it was stirred at room temperature for 5 hours. Then, DCM was removed in vacuo, and product was purified in column chromatography on silica gel. (0-18 % MeOH/EtOAc, R_f = 0.1). The product was obtained as light red crystal (57 mg, 90 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (d, J = 8.9 Hz, 1H), 7.82 (bs, 2H), 7.57 (d, J = 9.7 Hz, 1H), 7.18 (d, J = 2.6 Hz, 1H), 7.12 (dd, J = 8.9, 2.6 Hz, 1H), 6.99 (d, J = 9.7 Hz, 1H), 4.29 (t, J = 6.2 Hz, 2H), 3.01 (t, J = 7.3 Hz, 2H), 2.07 (p, J = 5.0 Hz, 2H) ^{13}C NMR (126 MHz, DMSO-d6) δ 180.9, 163.3, 151.6, 146.1, 144.6, 135.5, 132.0, 131.7, 128.7, 115.5, 101.1, 85.4, 66.6, 36.7, 27.1. HRMS m/z calc. for $C_{15}H_{14}N_2O_3I$: 395.9971 $[M+H]^+$; found: 397.0050.

4. Photophysical Characterization

Fluorescence quantum yield calculation:

Fluorescence quantum yields of the samples were investigated by using a fluorescence spectrometer (FLS 1000, Edinburgh Instruments) with an integrating sphere accessory. A continuous-wave xenon lamp was used as the excitation source and the emitted fluorescence was detected with a standard photomultiplier (PMT-900) covering a wavelength range of 200-800 nm. During the measurements, the PMT was cooled down to -20 ^{0}C by using a built-in housing to reduce the undesired dark current noise.
For quantum yield measurement, an integrating sphere (Edinburgh Instruments) was placed inside the sample compartment of the spectrometer. Internal cavity of the sphere was coated with a PTFE-like material to enable a reflectance of approximately >99% (>95%) over the wavelength range between 400 and 1500 nm (250 and 2500 nm). The sphere had two ports which were 90^o apart. The excitation beam was sent to the sample through the excitation port and the fluorescence was collected from the emission port. The excitation port of the sphere consisted of a lens to effectively focus the beam on the sample. The emission port was open aperture.
Prior to the experiments performed with the samples, the blank spectra were measured by using the reference solvents (PBS (pH 7.4, 1% DMSO)). For both of the measurements (blank and sample), two identical quartz cuvettes with equal volumes were used. First, the reference sample was placed inside the sphere and the emission/excitation slits were adjusted at the excitation wavelength so that the response of the PMT remained linear during the measurements. In order to cover a scattering range, the emission scans were started from 20 nm below the actual excitation wavelengths and finished at 750 nm. Furthermore, the step size and the integration time of the measurements were set to 1 nm and 0.2 seconds, respectively. After the all the emission measurements of the samples and references were complete, the quantum yields of the samples were determined by using the Fluoracle® software. The built-in analysis tool calculates the quantum yield (QY) as

$$QY = \frac{E_s - E_B}{S_B - S_s}$$

where E_s (E_B) and S_s (S_B) are the selected areas for the emitted and scattered signals of the sample (blank).

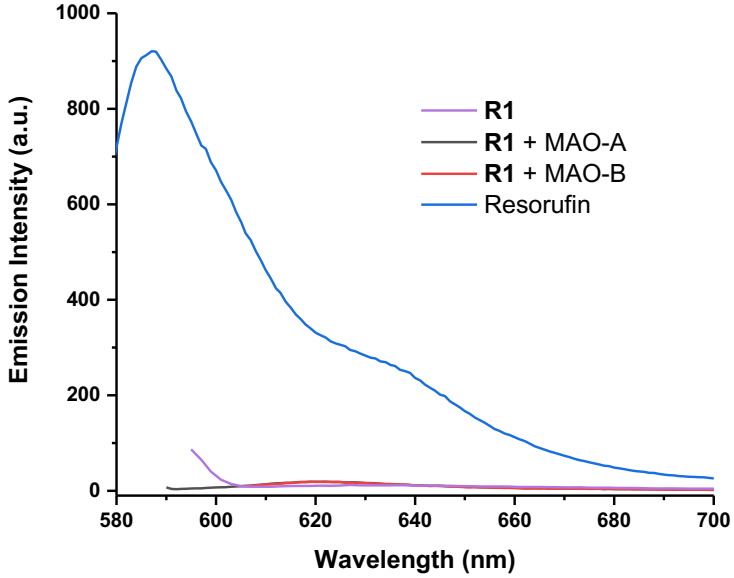


Figure S2. Fluorescence spectra of **Resorufin** (10 µM) and **R1** (10 µM) before and after addition of MAO-A and MAO-B (20 µg/mL) in PBS buffer (pH 7.4, 1% DMSO). Resorufin, R1+MAOA/B were irradiated from their absorption maxima. R1 was irradiated at 590 nm.

Table S1. Photophysical properties and $^1O_2$ quantum yields of the resorufin derivatives.

	λ_{abs} [nm][(a)]	ε [$M^{-1}cm^{-1}$][(a)]	λ_{ems} [nm][(a)]	φ_F [%][(a,b)]	φ_Δ [%][(a,c)]
R1	500, 408	18000, 13500	620	n.d. [(d)]	n.d. [(d)]
R1 + MAO-A	588	25000	620	0.23	37
R1 + MAO-B	595	16000	620	0.25	28

[a] measured in PBS buffer (pH 7.4, 1% DMSO) [b] calculated via spectrophotometer with an integrated sphere detector [c] methylene blue was used as a reference in PBS buffer (Φ_Δ = 0.52) [d] not determined.

Photostability:

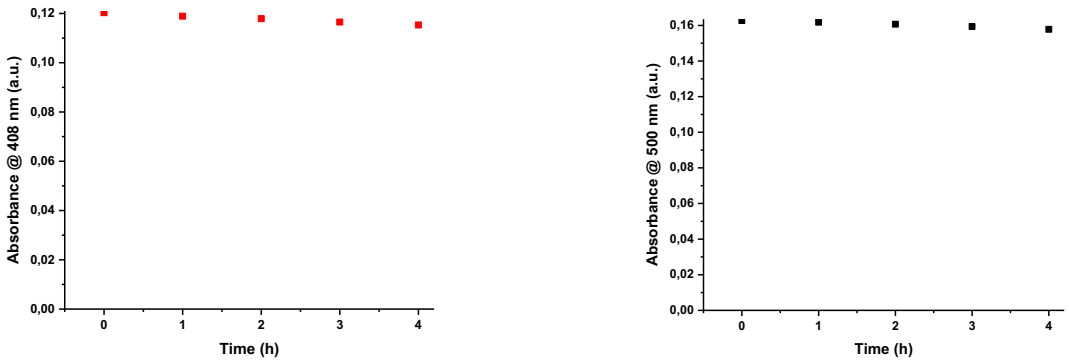


Figure S3. Absorbance measurements of **R1** (10 µM) in PBS (pH 7.4, 1% DMSO) representing the stability at 408 and 500 nm maximum bands upon irradiation with a 595 nm LED for 4 hours.

Reaction between R1 and H_2O_2:

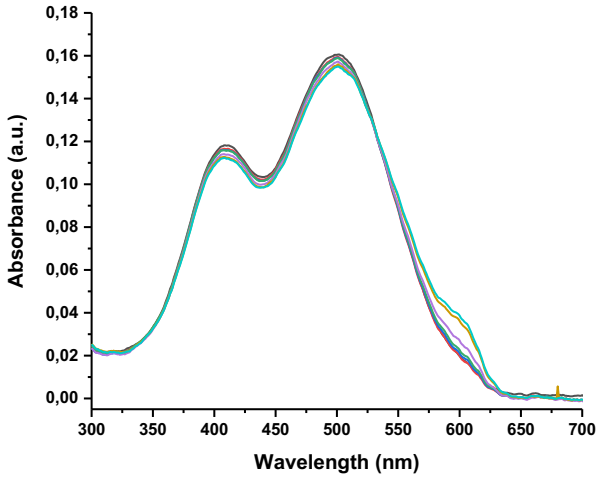


Figure S4. Treatment of **R1** (10 µM) with 100 µM H_2O_2 in PBS (pH 7.4, 1% DMSO). Absorption measurements were taken in each 30 minutes for 4 hours.

5. Singlet Oxygen Detection Experiments

Chemical detection of singlet oxygen:

Singlet oxygen generation yield of **R1** was evaluated by using a water-soluble trap molecule 2,2'-(anthracene-9,10-diyl)bis(methylene)dimalonic acid (ADMDA) in aqueous solutions (PBS pH 7.4, 1% DMSO). Methylene blue ($\Phi\Delta$ = 0.52 in PBS buffer) was employed as a reference compound for singlet oxygen quantum yield calculations. PDT agent (**R1**) (10 µM) and ADMDA (O.D. = 0.6-1.5) were mixed in oxygen bubbled PBS (pH 7.4, 1% DMSO). Initially, several measurements were taken in dark and then the solution was exposed to the LED light (595 nm, 20 mW/cm^2) repeatedly from a distance of 10 cm. After each irradiation, absorbance of the ADMDA was recorded. For each photosensitizer, slope of absorbance maxima of ADMDA at 380 nm versus time graph were drawn. Finally, singlet oxygen quantum yields were calculated according to the equation given below:

$$\Phi_\Delta(PS) = \Phi_\Delta(ref) \times \frac{m(PS)}{m(ref)} \times \frac{F(ref)}{F(PS)} \times \frac{PF(ref)}{PF(PS)}$$

where PS and ref represent **R1** and methylene blue, respectively. m is the slope of absorbance maxima of ADMDA at 380 nm versus time graph, F is the correction factor, which is given by $F = 1 - 10^{-OD}$ (OD at the irradiation wavelength, which is 595 nm), and PF is absorbed photonic flux in µEinstein $dm^{-3}s^{-1}$. PF was ignored in the calculations as both **R1** and MB were irradiated with the same light source (595 nm LED).

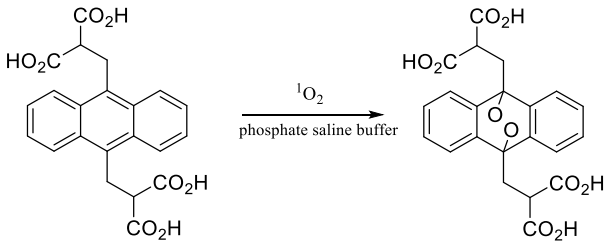


Figure S5. Cycloaddition reaction between $^1O_2$ and 2,2'-(anthracene-9,10-diyl)bis(methylene)dimalonic acid (ADMDA).

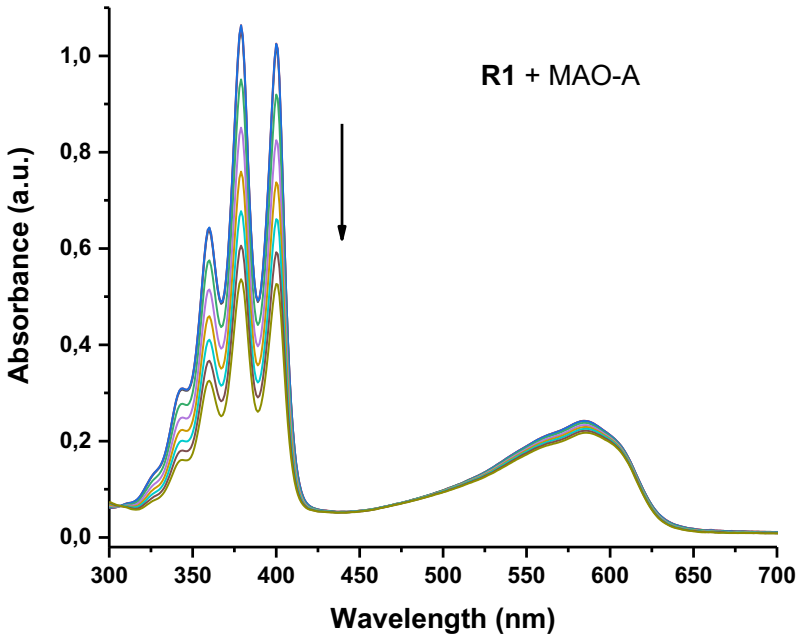


Figure S6. Decrease in the absorbance of ADMDA in PBS (pH 7.4, 1% DMSO) upon irradiation of **R1** (10 μM) with a 595 nm LED in the presence of MAO-A (20 µg/mL).

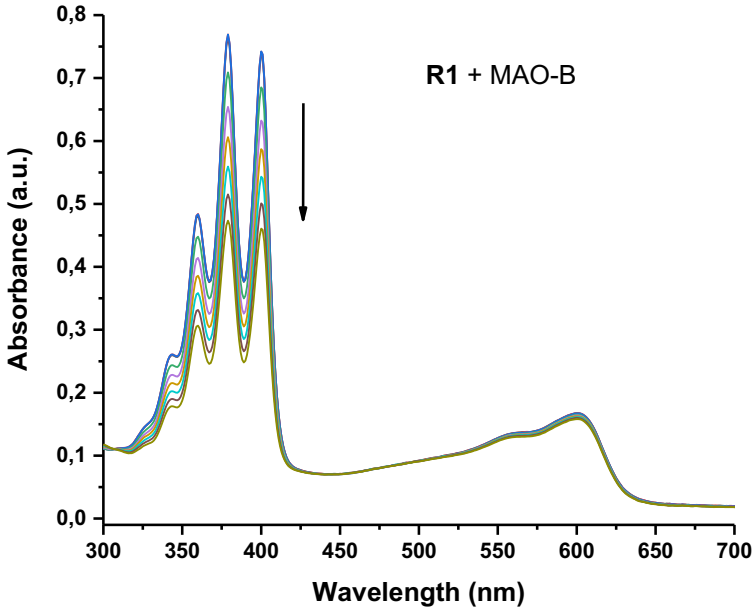


Figure S7. Decrease in the absorbance of ADMDA in PBS (pH 7.4, 1% DMSO) upon irradiation of **R1** (10 μM) with a 595 nm LED in the presence of MAO-B (20 µg/mL).

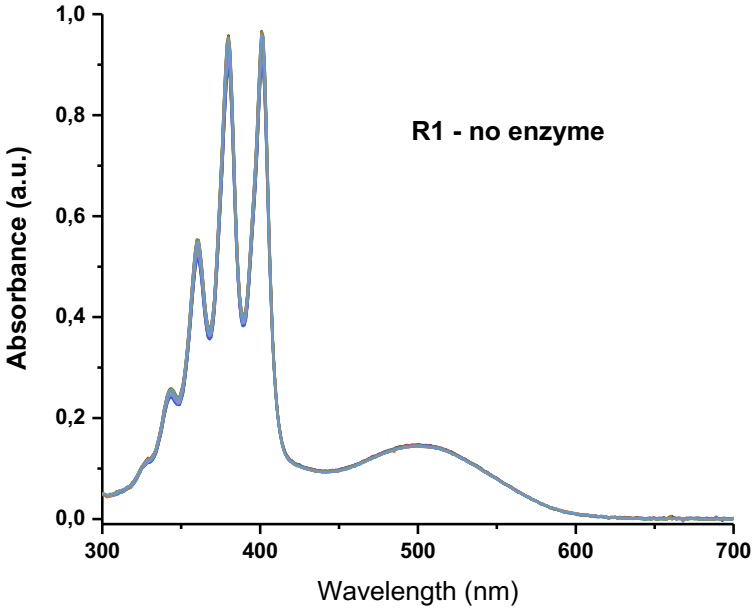


Figure S8. Decrease in the absorbance of ADMDA in PBS (pH 7.4, 1% DMSO) upon irradiation of **R1** (10 μM) with a 595 nm LED.

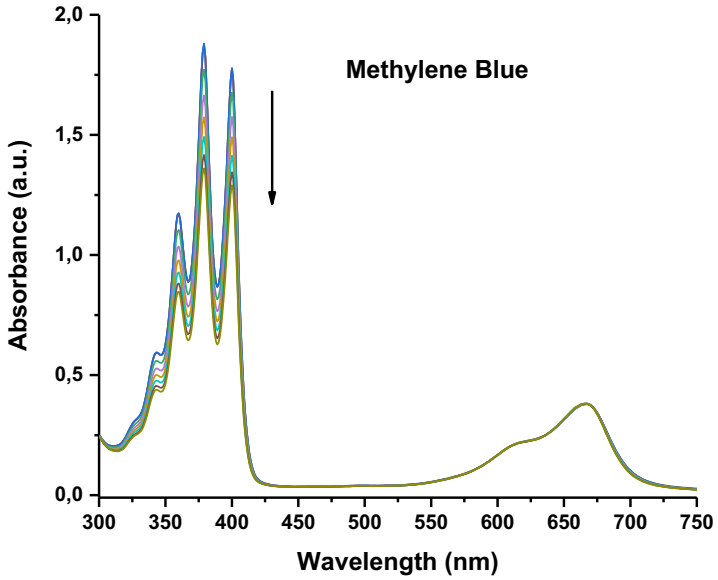


Figure S9. Decrease in the absorbance of ADMDA in PBS (pH 7.4, 1% DMSO) upon irradiation of **methylene blue** (4 μM) with a 595 nm LED.

Singlet oxygen phosphorescence measurements:

In the experiments, the 600-nm output of a tunable optical parametric amplifier (Spectra-Physics, TOPAS Prime) which was pumped with a 1-kHz, 100-fs, 800-nm Ti^{3+}:sapphire regenerative amplifier (Spectra-Physics, Spitfire Ace) was used to optically excite the samples. The near-infrared emission characteristics of the samples were further investigated by using a fluorescence spectrometer (Edinburgh Instruments, FLS 1000) with a liquid-nitrogen-cooled near-infrared photomultiplier tube (NIR-PMT, kept at -80 ˚C). During the measurements, the 600-nm pulse energy incident on the samples was around 38 µJ and the emission bandwidth of the monochromator was set to 30 nm to further increase the signal to noise ratio.

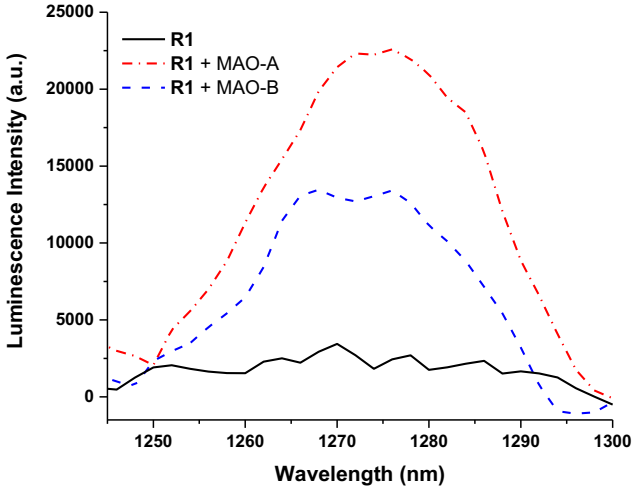


Figure S10. Singlet oxygen phosphorescence signals of **R1** (10 μM) before and after addition of MAO-A and MAO-B (20 μg/mL) in PBS buffer (pH 7.4, 1% DMSO). **R1** was treated with MAO-A and MAO-B separately for 3 hours at 37 °C before running both experiments.

6. Cell Culture and Imaging

Table S2. IC50 values and phototoxicity indexes (PI) of **R1**. ND: not determined. OD: optical density

R1 IC50 VALUES						
CELL TYPES	**SH-SY5Y**		**NIH/3T3**		**MCF-7**	
IRRADIATION TIME	**DARK**	**LIGHT**	**DARK**	**LIGHT**	**DARK**	**LIGHT**
4 HOURS	-	0.42 μM	-	6.50 μM	7.56 μM	4.00 μM
30 MINS	-	1.90 μM	-	-	ND	ND
PHOTOTOXICITY INDEXES						
CELL TYPES	**SH-SY5Y**		**NIH/3T3**		**MCF-7**	
IRRADIATION TIME	**R1**		**R1**		**R1**	
4 HOURS	0.042		0.041		0.228	
30 MINS	0.032		0.004		ND	

Phototoxicity index (PI) of LED= (OD_{Dark} - OD_{Light}) / OD_{Dark}

Cell culture:

NIH/3T3 cells were cultured in DMEM, High Glucose medium (Gibco, USA), and SH-SY5Y and MCF-7 cells were cultured in DMEM/F-12 medium (Lonza, Switzerland), both of which were supplemented with 10% fetal bovine serum (Gibco, USA) and 1% Pen/Step (Gibco, USA). All cells were grown in a humidified incubator at 37°C with 5% CO_2 level.

Cell viability assay:

Cell viability was detected by MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay. Cells ($4x10^5$ cells/well for NIH/3T3 and MCF-7 cells, $3x10^5$ cells/well for SH-SY5Y cells) were seeded in 96-

well flat bottom cell culture plates in 100 µL medium (Corning, MA, USA) as triplicates for each condition 12 hours prior to the experiment. The experiment group was subjected to 8 different concentrations of the drug (**R1**) provided in the figures for 3 hours, then exposed to the LED light source (595 nm, 20 mW/cm^2) either for 4 hours (288 J/cm^2) or 30 minutes (36 J/cm^2) from a distance of 10 cm, which was followed by incubation in the dark for 20 hours. Hence, the cells were subjected to the drug for 24 hours in total. During light irradiation temperature rise on the LED setup was avoided with the help of cooling blocks attached to LED source. The drug stock was first serially diluted in DMSO. Then equal volumes from these stocks were added to the media to keep final DMSO concentration constant at each well (0.1 %). After that an equal volume (100 µL) of drug solutions were added to each well. The control group was also subjected to the same 8 serial dilutions of the drug for 24 hours without irradiation by a LED light source. To calculate % survival with respect to untreated cells both in the illuminated plates and plates kept in the dark, the cells were kept in medium with 0.1 % DMSO at separate wells. At the end of 24 hours incubation period, 3 mg/µL MTT (Sigma-Aldrich, USA) was added to each well and incubated for 4 hours. The medium was then replaced with DMSO (dimethyl sulfoxide - Merck Millipore, USA) to dissolve the formazan crystals and the absorbance was measured at 570 nm in a microplate reader (BioTek's Synergy H1, Winooski, VT, USA). All incubations including the exposure to LED light source were performed in a humidified incubator at 37°C with 5% CO_2 level. All experiments were repeated three times (N=3).

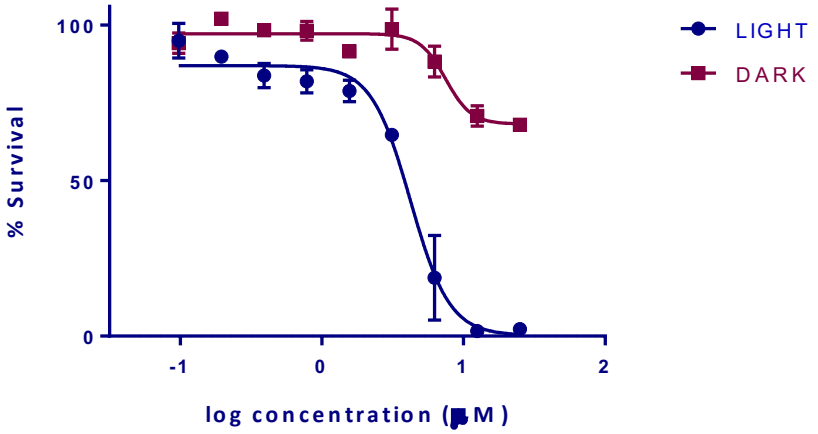


Figure S11. *In vitro* cell viability of MCF-7 cells. The cells were incubated with varying concentrations of **R1** and either kept in dark or irradiated for 4 hours with a 595 nm LED light source (20 mW/cm^2).

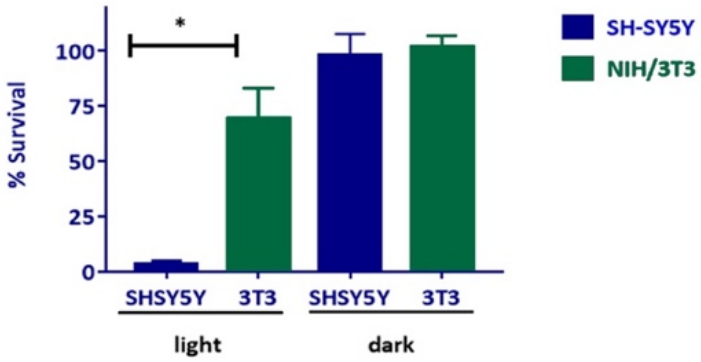


Figure S12. Cell viability difference between cancerous SH-SY5Y and healthy NIH/3T3 cells when they were incubated with 2.5 µM **R1** (*: $p < 0.05$).

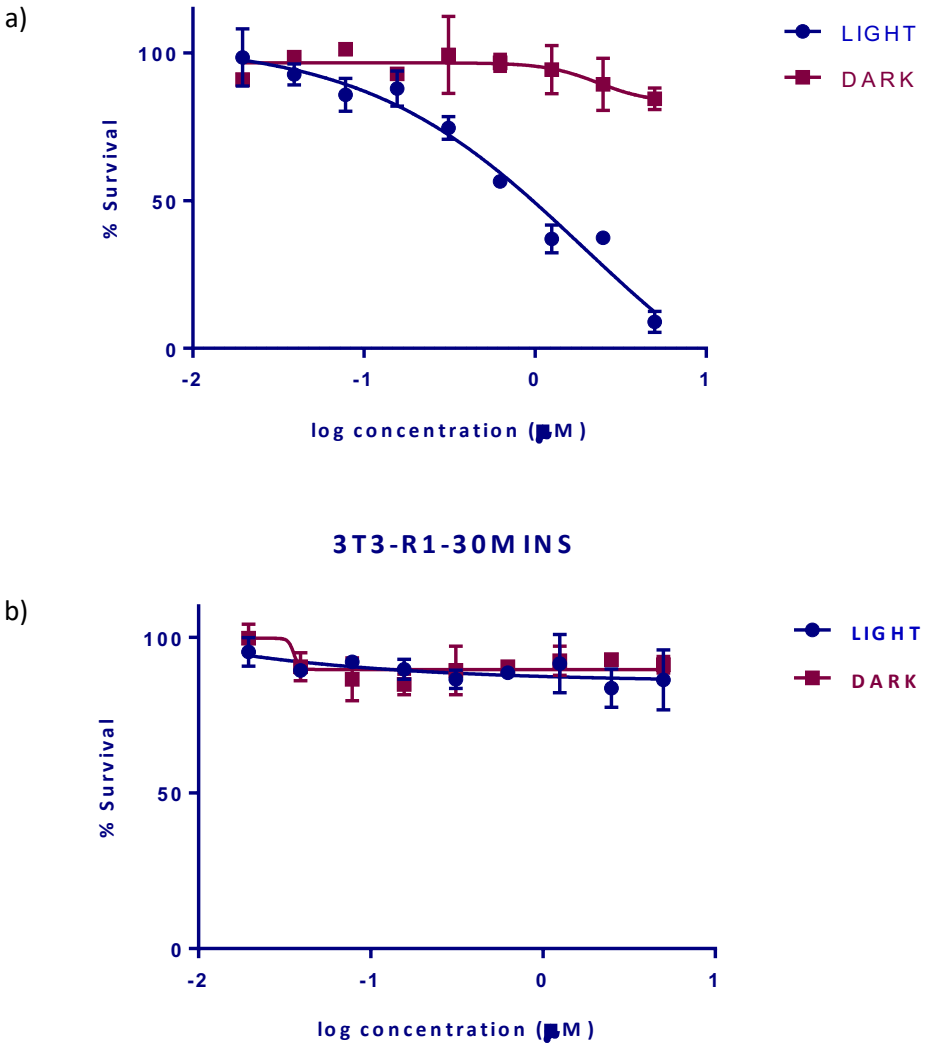
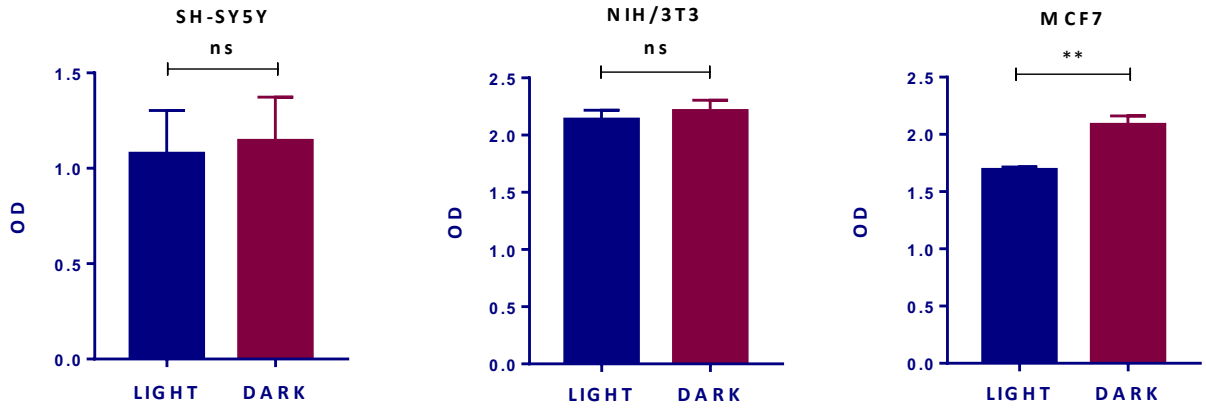


Figure S13. *In vitro* cell viability of (a) SH-SY5Y and (b) NIH-3T3 cells. The cells were incubated with varying concentrations of **R1** and either kept in dark or irradiated for 30 minutes with a 595 nm LED light source (20 mW/cm^2).



SHSY5Y-p value 0.4878, unpaired t-test, two-tailed

3T3-p value 0.1004, unpaired t-test, two-tailed

MCF7-p value 0.0012, $p < 0.05$, unpaired t-test, two-tailed

Figure S14. Cell viabilities of SH-SY5Y, NIH-3T3 and MCF-7 under 4 hours LED light (20 mW/cm^2) irradiation.

ROS imaging:

SH-SY5Y cells were seeded in 27-mm glass bottom dishes ($1x10^6$ cells/dish) 24 hours before the experiment. Treatment with 2.5 μM **R1** was performed in a control group (no light) and two different experimental groups for 3 hours at 37°C in the dark. All groups were rinsed once with DPBS followed by incubation in the dark with 10 μM ROS sensor (2',7'–Dichlorofluorescein diacetate – Sigma-Aldrich, USA) prepared in DPBS for 45 mins at 37°C. After washing DPBS, only medium was added to one experimental group and the control group, and 10 mM NaN_3 containing medium was added to the other experimental group. Both experimental groups were exposed to LED light source for 30 mins and the control group was kept in the dark. Dishes were rinsed with DPBS and 2 mL DPBS was added to each dish just before imaging with Leica DMI8 SP8 CS/DLS microscope (Leica Microsystems, Germany) at 20x magnification at 37°C.
To be able to validate the results, singlet oxygen imaging was repeated on SH-SY5Y cells in the presence of clorgyline (N-Methyl-N-propargyl-3-(2,4-dichlorophenoxy)propylamine hydrochloride, MAO-A inhibitor – Sigma-Aldrich, USA) or on NIH/3T3 cells in the absence of clorgyline which intrinsically have low level of MAO-A enzyme. Both cell types were seeded in 27-mm glass bottom dishes (1x106 cells/dish). After 24 hours, 0.1 μM clorgyline was applied to only SH-SY5Y cells for 30 mins at 37°C.Then both SH-SY5Y and NIH/3T3 cells were treated with 2.5 μM **R1**. Cells were rinsed once with DPBS and then incubated with 10 μM ROS sensor for 45 mins at 37°C in the dark, which was followed by the exposure to LED light source for 30 mins at 37°C. Cells were visualized by Leica DMI8 SP8 CS/DLS microscope at 20x magnification at 37°C.

Annexin V-FTIC and PI staining:

SH-SY5Y cells were seeded into 27-mm glass bottom dishes (ThermoFisher, USA) as $1x10^6$ cells/dish 24 hours before the experiment. The control and experiment groups were treated with 2.5 μM **R1** for 24 hours. During this period, the experiment group was exposed to LED light source for 4 hours, and the control group was kept in the dark. At the end of 24 hours, cells were washed once with cold Cell Staining Buffer (Biolegend, UK). Then, 1.5 mL Annexin V Binding Buffer with 75 μL Annexin V conjugated to FITC (Biolegend, UK) was added to each well and incubated for 15 mins at 37°C in the dark. After that, cells were washed once with DPBS, then incubated with 150 μL PI (Biolegend, UK) prepared in 1.5 ml Annexin V Binding Buffer for 10 mins at 37°C in the dark. Dishes were rinsed once with DPBS and 2 ml DPBS was added to each dish just before imaging with Leica DMI8 SP8 CS/DLS microscope (Leica Microsystems, Germany) at 20x magnification at 37°C.

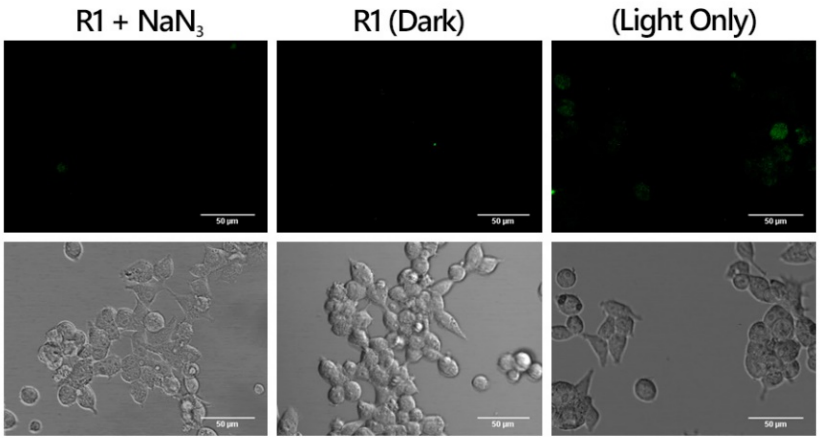


Figure S15. Monitoring ROS production by using senor $DCFH_2$-DA (10 μM) in SH-SY5Y cells. (Left): Cells were treated with NaN_3 (10 mM) before **R1** 2.5 (μM) incubation (3 hours) and light exposure. (Middle): Cells were incubated with **R1** 2.5 (μM) for 3 hours but kept under dark. (Right): Cells were irradiated with light in the absence of **R1**. Scale: 50 μM.

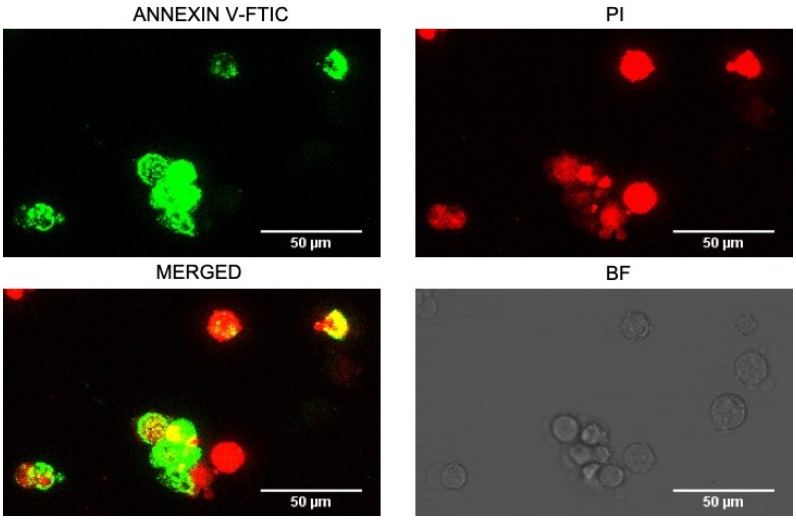


Figure S16. Confocal microscope images of Annexin V-FTIC and PI stained SH-SY5Y cells. Cells were incubated with **R1** (2.5 µM) and either kept under dark or irradiated with a 595 nm LED (20 mW/cm^2) for 4 hours. Scale: 50 µM.

7. LogP Calculation

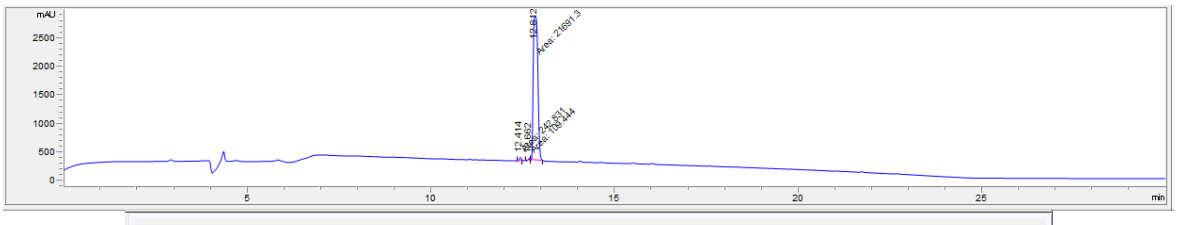
Calculation was done according to a literature example.[1] An equal volume (3 mL) of *n*-octanol and distilled water (DI) were added to a centrifuge tube, then **R1** was dissolved in that mixture to get a final concentration of 100 µM. The sample was incubated at room temperature with a brief vortex in every 5 min for 1 hour. In the next step, the mixture was centrifugated at 2000 rpm for 10 minutes. Octanol and water layers were separately transferred to quartz cells and characterized in UV-VIS spectroscopy to get maximum absorbance values. Known concentrations of **R1** in octanol and water were also measured to get molar absorptivity values in both solvents. Concentrations of **R1** in both solvents were then calculated by dividing absorbance values from centrifuge tubes to respective molar absorptivity values. The experiment was repeated three times (N=3).

$\log P\ (R1) = \log ([R1]_{oct} / [R1]_{water}) = 0.412 \pm 0.020$

$\text{LogP (resorufin)}^1 = 0.427 \pm 0.036$

8. HPLC

The purity of biologically tested **R1** was checked by HPLC. 20 µL of **R1** (dissolved at ~ 1 mg/mL in water) was injected into Agilent Poroshell 120 EC-C18 column (4 µl x 4.6 x 150 mm). Column temperature was set to 40°C. Gradient elution was carried out with a mobile phase of (A) water : %0.1 TFA and (B) acetonitrile : 0.08% TFA. The gradient program was ramped from %1 to %90 B in 20 min at a flow rate of 0.5 mL/min. Detector was set to 210 nm.



#	Time	Type	Area	Height	Width	Area%	Symmetry
1	12.414	MM	242.8	60	0.0674	1.102	1.431
2	12.662	MM	109.4	34.7	0.0526	0.496	1.248
3	12.812	MM	21691.3	2495	0.1449	98.402	0.594

Figure S17. HPLC analysis of **R1**.

9. NMR Spectra

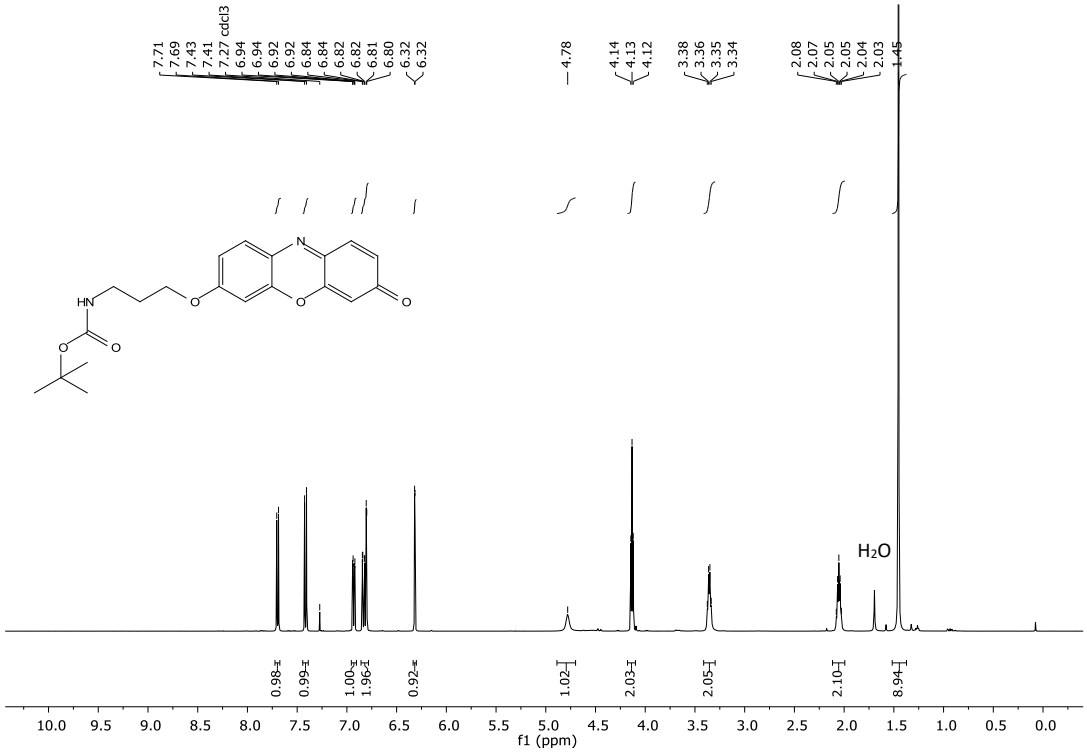


Figure S18. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

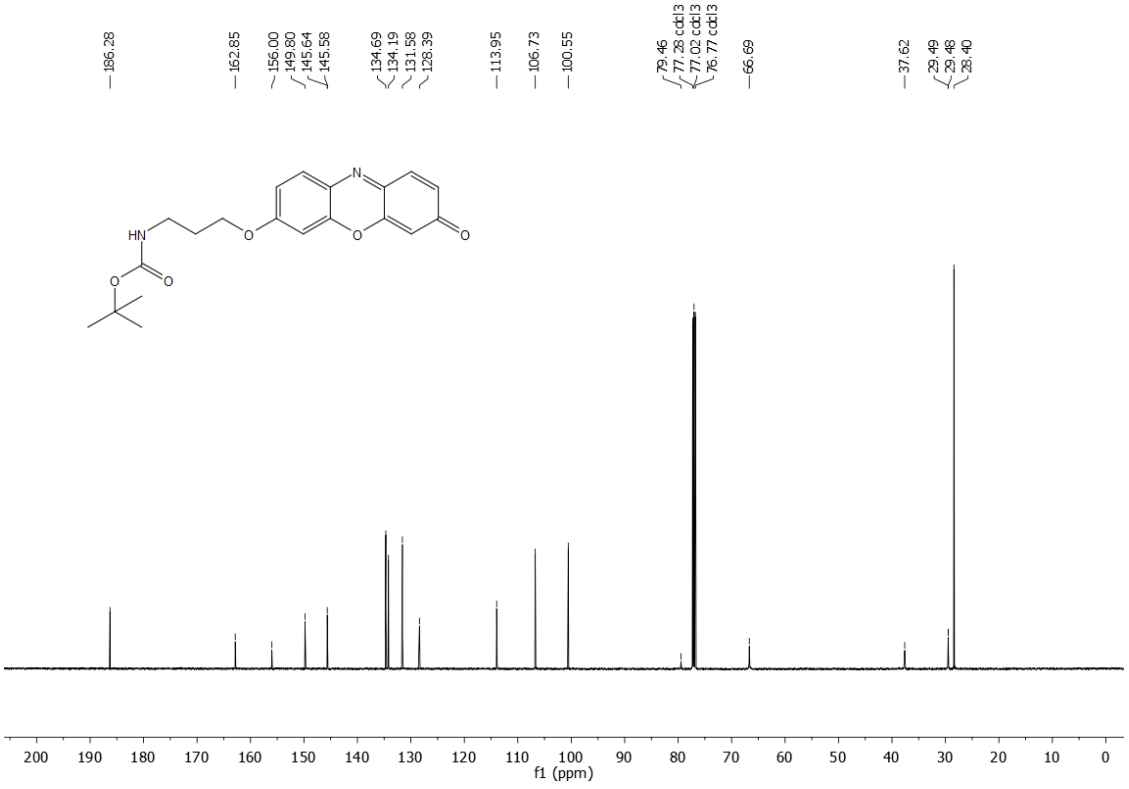


Figure S19. ^{13}C NMR spectrum of compound **1** in $CDCl_3$.

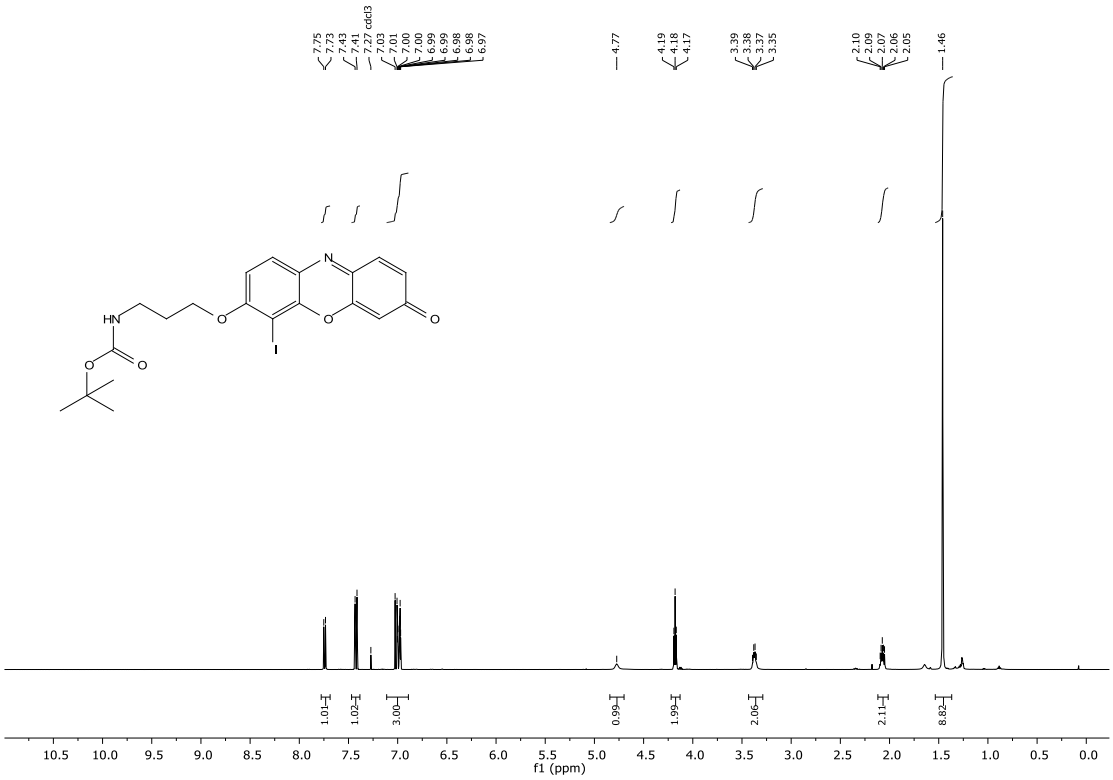


Figure S20. ^{1}H NMR spectrum of compound **2** in $CDCl_3$.

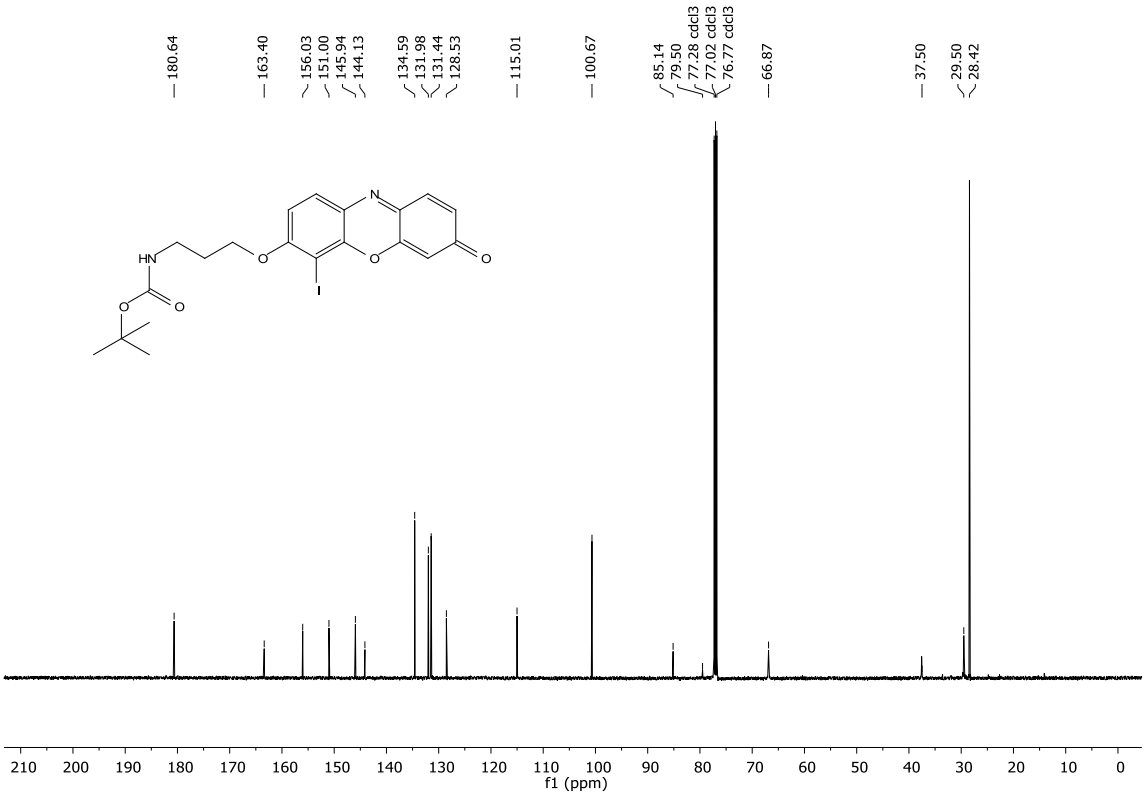


Figure 21. ^{13}C NMR spectrum of compound **2** in $CDCl_3$.

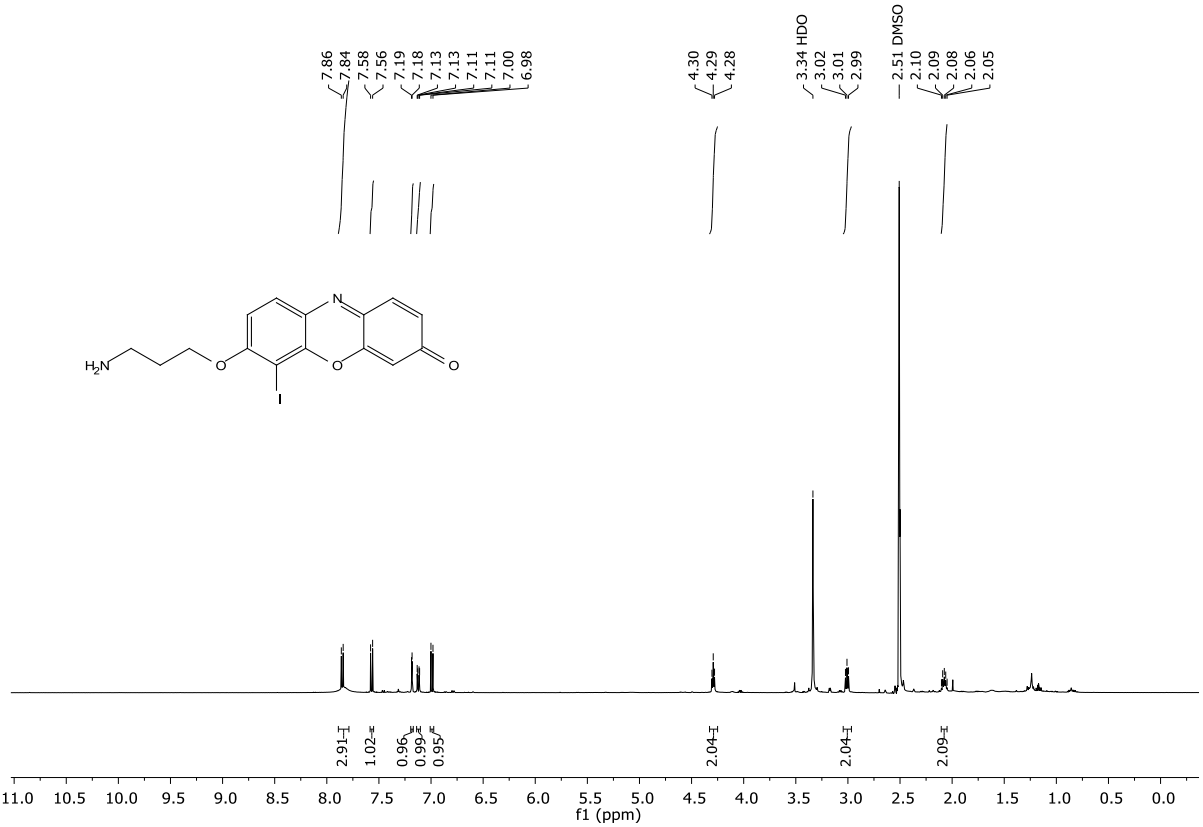


Figure S22. ^{1}H NMR spectrum of compound **R1** in DMSO-*d6*.

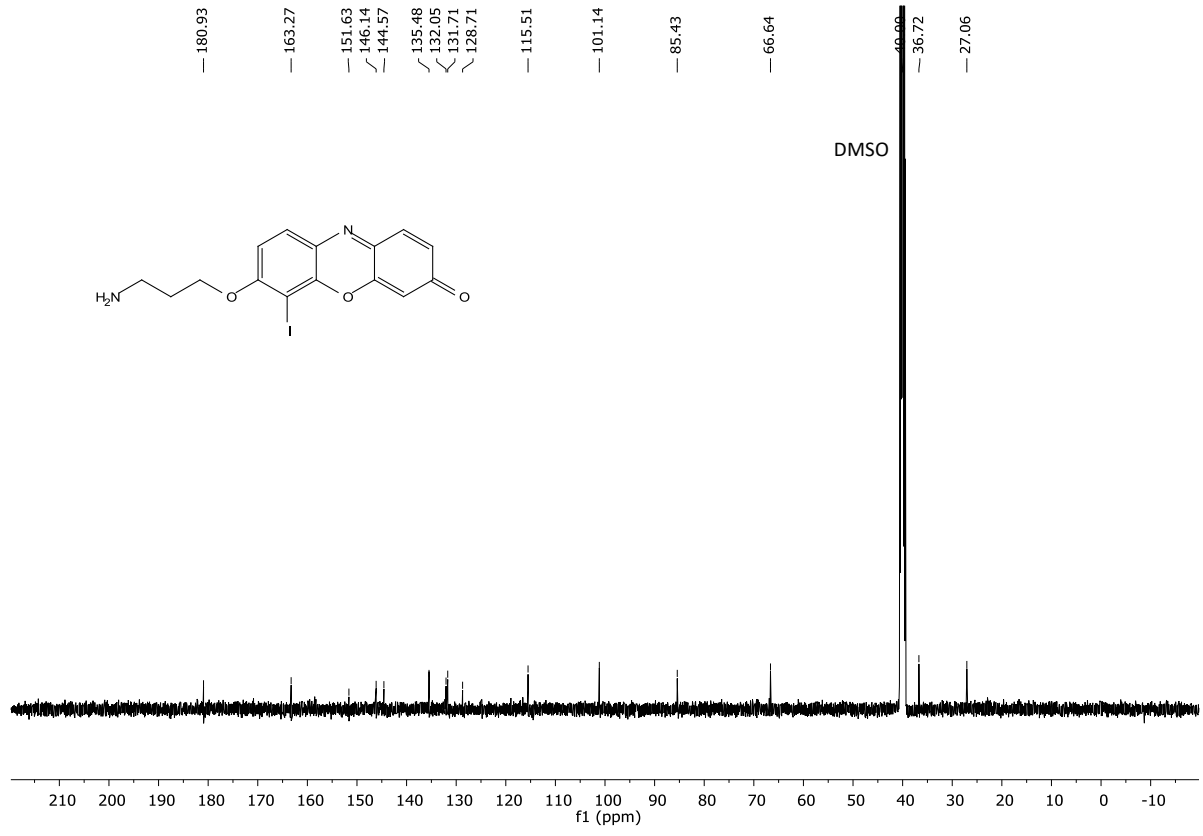


Figure S23. ^{13}C NMR spectrum of compound **R1** in DMSO-*d6*.

10. HRMS Spectra

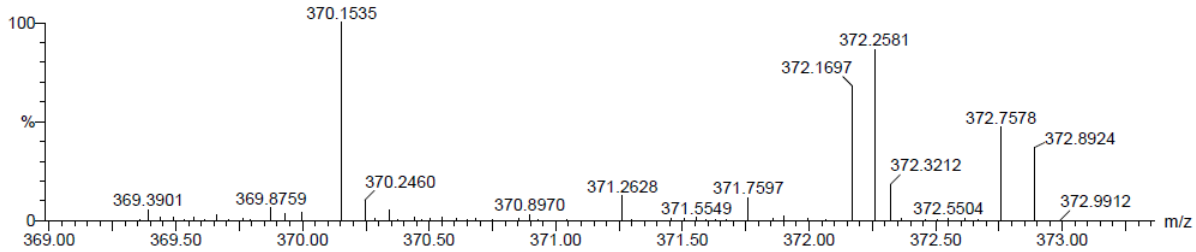


Figure S24. HRMS spectrum of compound **1**.

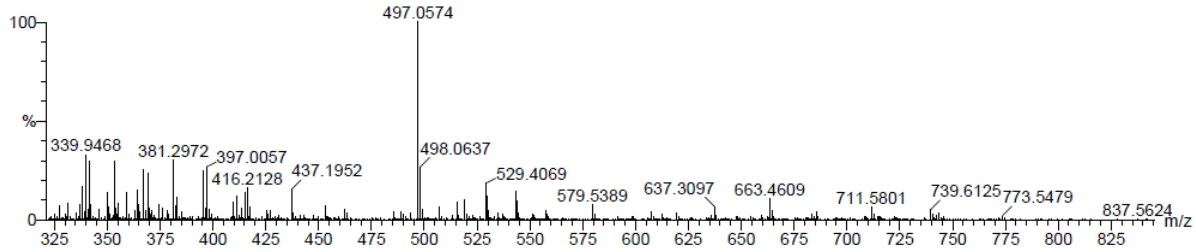


Figure S25. HRMS spectrum of compound **2**.

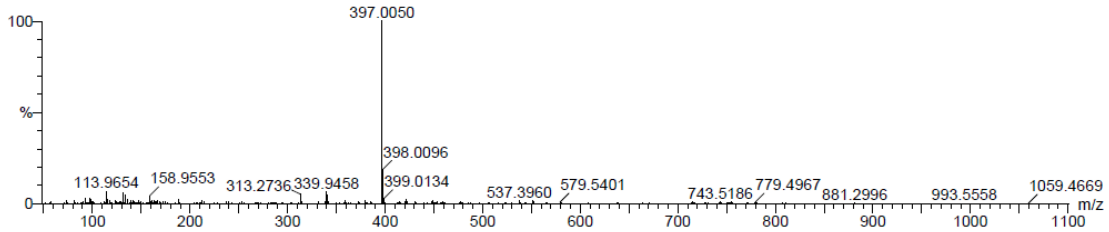


Figure S26. HRMS spectrum of compound **R1**.

References:

1. Han, B. H.; Zhou, M.; Vellimana, A. K.; Milner, E.; Kim, D. H.; Greenberg, J. K.; Chu, W.; Mach, R. H.; Zipfel, G. J. *Molecular Neurodegeneration*, **2011**, *6*, 86.