Supporting Information

Organelle-targeting activity-based hemicyanine derivatives for enhanced and selective type-I photodynamic therapy under hypoxia

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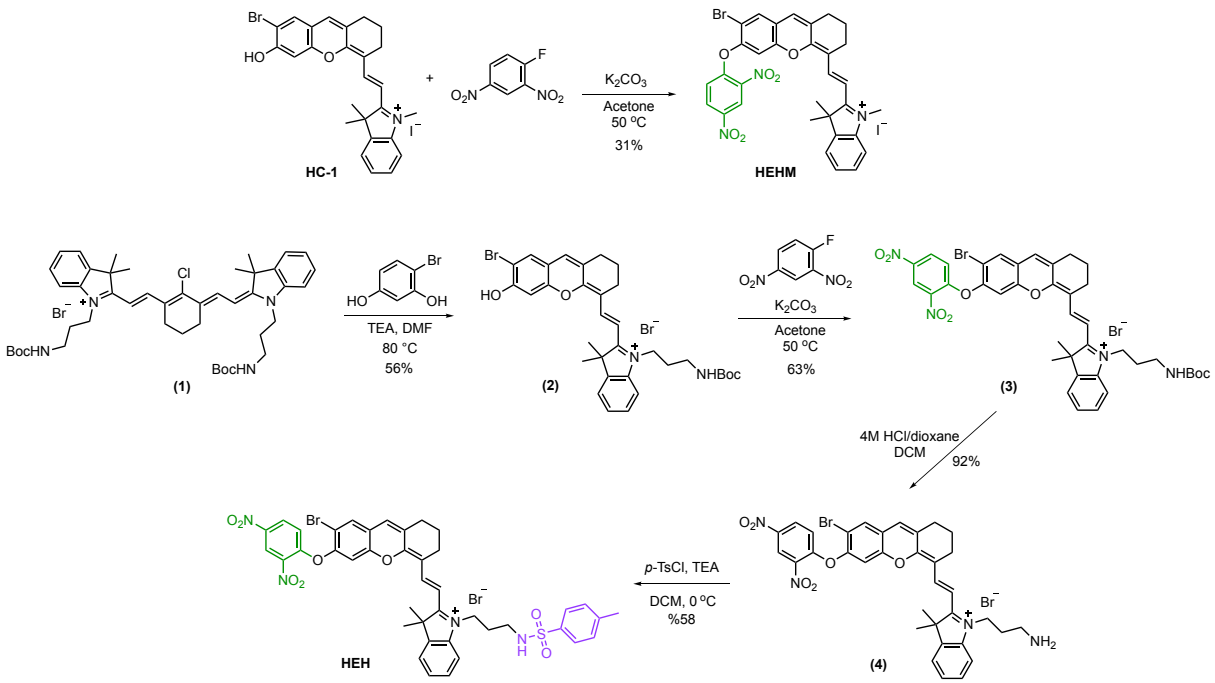
1. General

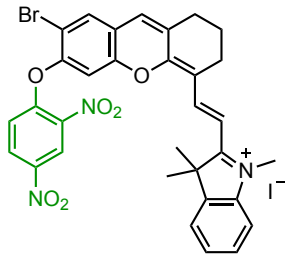
All reagents were commercially available and used without further purification unless otherwise noted. All dry solvents used in reactions were obtained by using standard procedures. Reactions performed at the inert atmosphere were done in Schlenk line and Argon was used as an inert gas. Thin layer chromatography (TLC Merck Silica Gel 60 F254) was performed by using commercially prepared 0.25 mm silica gel plates and visualization of the compounds were done by irradiation with UV. Column chromatography was performed by using thick-walled glass columns and silica Gel 60 (Merck 230-400 mesh). The relative proportions of solvents in chromatography solvent mixtures refer to the volume:volume ratio. ^{1}H NMR and ^{13}C NMR analysis were recorded by 500 MHz Bruker Ascend magnet equipped with Avance NEO console spectrometer using $CDCl_3$, and MeOD 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. Mass spectra were recorded on Waters Vion IMS QTOF high resolution mass spectrometer. In LC-QTOF-MS analyses, Waters UPLC (ACQUITY H-Class PLUS Core System) coupled with Waters Vion IMS QTOF mass detector was used. Absorption and fluorescence measurements were acquired on Biotek Synergy H1 MF microplate reader and Agilent Cary Eclipse spectrophotometers respectively. Fluorescence quantum yield measurements were performed on Edinburgh Instruments FS5 spectrofluorometer. qRT-PCR analyses were done by using Roche Lightcycler 480. Biotek Synergy H1 MF microplate reader was used in cell viability experiments.

2. Synthesis



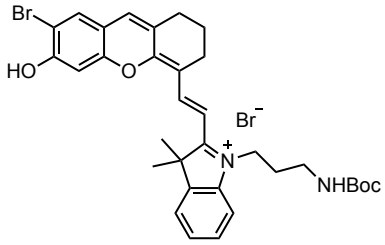
Scheme S1. Synthesis of **HEHM** and **HEH**.

Synthesis of HEHM:



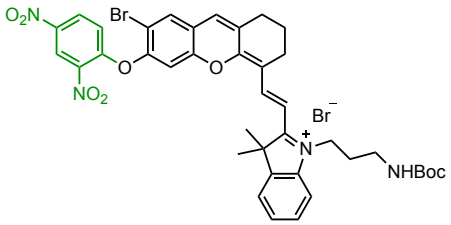
Potassium carbonate (K_2CO_3) (46.3 mg, 0.34 mmol) was added to a solution of **HC-1**[1] (100 mg, 0.17 mmol) in dry acetone (1.70 mL). After 10 minutes, 1-fluoro-2,4-dinitrobenzene (126 mg, 0.68 mmol) was added, and the reaction was stirred overnight at 50 °C. Then, the solvent was removed under reduced pressure, and the residue was purified by column chromatography using DCM:MeOH (100:0 to 100:10, v/v) to give **HEHM** with 31% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.91 (d, *J* = 2.7 Hz, 1H), 8.61 (d, *J* = 15.4 Hz, 1H), 8.42 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.63 (s, 1H), 7.53 – 7.49 (m, 1H), 7.47 – 7.44 (m, 3H), 7.24 (s, 1H), 7.09 (d, *J* = 9.2 Hz, 1H), 6.95 (s, 1H), 6.85 (d, *J* = 15.4 Hz, 1H), 4.22 (s, 3H), 2.88 (t, *J* = 6 Hz, 2H), 2.76 – 2.71 (m, 2H), 1.99 – 1.91 (m, 2H), 1.78 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.85, 152.83, 146.41, 142.06, 132.57, 131.85, 129.60, 128.69, 127.65, 122.60, 122.44, 118.39, 116.63, 113.70, 111.04, 108.89, 51.51, 35.85, 29.78, 27.90, 25.02, 20.15. HRMS: calcd. for $C_{32}H_{27}BrN_3O_6^+$ [M^+]: 628.1078; HRMS found: m/z 628.1082 [M^+].

Synthesis of Compound (2):



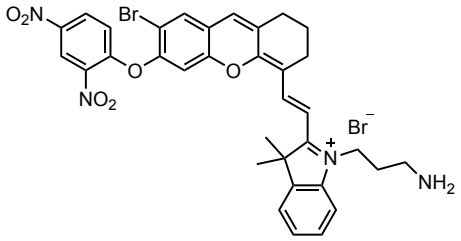
4-Bromo-1,2-dihydroxybenzene (0.18 g, 0.94 mmol), and triethylamine (TEA) (0.5 mL) were dissolved in 5 mL anhydrous DMF, and the solution was stirred at r.t. under nitrogen atmosphere for 10 min. Then, compound **(1)**[2] (0.36 g, 0.47 mmol) in 1 mL anhydrous DMF was added to the reaction dropwise and stirred at 80 ℃ for 5h. The crude material was diluted with DCM and washed with water. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was then purified via column chromatography initially using 1:9 MeOH/DCM (v/v) and then 1:2 MeOH/DCM (v/v) to obtain compound **(2)** (Yield: 56%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (d, *J* = 13.2 Hz, 1H), 7.66 (s, 1H), 7.32 – 7.27 (m, 3H), 7.24 (*br.* s, 1H), 7.06 (t, *J* = 7.4 Hz, 1H), 6.81 (d, *J* = 7.8 Hz, 1H), 6.65 (s, 1H), 5.62 (d, *J* = 13.2 Hz, 1H), 4.67 (*br.* s, 1H), 3.83 (*br.* s, 2H), 3.25 (*br.* s, *J* = 5.2 Hz, 2H), 2.68 (t, *J* = 5.8 Hz, 2H), 2.61 (t, *J* = 5.7 Hz, 2H), 1.98 – 1.94 (m, 2H), 1.92 – 1.89 (m, 2H), 1.67 (s, 6H), 1.44 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.34, 165.75, 160.43, 158.23, 156.26, 143.13, 139.75, 139.58, 133.34, 131.01, 128.34, 122.67, 122.28, 116.81, 116.24, 116.06, 108.13, 103.37, 94.64, 79.64, 47.71, 38.42, 28.75, 28.51, 28.04, 27.34, 24.51, 21.37. MS: calcd. for $C_{33}H_{38}BrN_2O_4^+$ [M^+]: 605.2009; HRMS found: m/z 605.2011.

Synthesis of Compound (3):



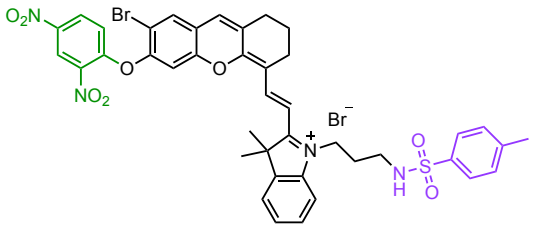
To a solution of compound **(2)** (0.06 g, 0.09 mmol) in 10 mL anhydrous acetone, K_2CO_3 (0.02 g, 0.2 mmol) was added and stirred at room temperature for 10 min. Then, 1-fluoro-2,4-dinitrobenzene (0.09 g, 0.5 mmol), was added to the reaction. The reaction was stirred overnight at 50 ℃ and monitored by TLC. The solid was collected and dried under vacuum and further purified via column chromatography using 5:95 MeOH/DCM (v/v) to obtain compound **(3)** (Yield: 63%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (d, *J* = 2.7 Hz, 1H), 8.57 (d, *J* = 15.2 Hz, 1H), 8.41 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.68 (s, 1H), 7.52 – 7.41 (m, 4H), 7.19 (s, 1H), 7.06 (d, *J* = 9.2 Hz, 1H), 7.03 (s, 1H), 6.81 (d, *J* = 15.3 Hz, 1H), 5.77 (*br.* s, 1H), 4.68 (t, *J* = 7.7 Hz, 2H), 3.45 – 3.39 (m, 2H), 2.88 (t, *J* = 5.9 Hz, 2H), 2.74 (t, *J* = 5.8 Hz, 2H), 2.20 – 2.15 (m, 2H), 1.96 – 1.91 (m, 2H), 1.76 (s, 6H), 1.40 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.99, 158.30, 156.58, 154.40, 152.73, 151.04, 146.55, 142.36, 142.23, 140.95, 138.94, 132.44, 132.03, 129.64, 129.56, 128.66, 128.14, 122.72, 122.38, 122.34, 118.26, 116.62, 113.83, 111.30, 110.88, 107.86, 79.31, 51.48, 45.21, 37.84, 29.67, 28.53, 28.45, 28.12, 25.06, 20.07.

Synthesis of Compound (4):



To a solution of compound **(3)** (0.01 g, 0.01 mmol) in 2 mL DCM, 4M HCl in dioxane (2 mL) was added and stirred at room temperature under argon atmosphere. The reaction monitored by TLC. Upon completion, the mixture was precipitated in diethyl ether (Et_2O). The resulting precipitate that formed was collected via filtration and dried under vacuum to obtain compound **(4)** (Yield: 92%). ^{1}H NMR (500 MHz, MeOD) δ 8.97 (d, *J* = 2.8 Hz, 1H), 8.81 (d, *J* = 15.1 Hz, 1H), 8.48 (dd, *J* = 9.3, 2.8 Hz, 1H), 7.95 (s, 1H), 7.68 (dd, *J* = 7.4, 3.9 Hz, 2H), 7.62 – 7.56 (m, 2H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.34 (s, 1H), 7.22 (d, *J* = 9.2 Hz, 1H), 6.69 (d, *J* = 15.1 Hz, 1H), 4.56 (t, *J* = 7.6 Hz, 2H), 3.20 – 3.17 (m, 2H), 2.84 (t, *J* = 5.6 Hz, 2H), 2.80 (t, *J* = 6.0 Hz, 2H), 2.28 – 2.22 (m, 2H), 2.0 – 1.95 (m, 2H), 1.80 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 181.19, 161.02, 155.33, 154.36, 153.29, 148.61, 148.59, 144.06, 143.98, 142.36, 133.48, 133.05, 131.09, 130.48, 130.34, 129.49, 124.08, 123.49, 123.20, 119.76, 117.07, 114.37, 112.35, 111.77, 107.28, 52.75, 43.81, 38.05, 30.43, 28.13, 26.95, 25.22, 21.41.

Synthesis of HEH:



To a solution of compound **7** in anhydrous DCM (10 mL), *p*-toluenesulfonyl chloride (0.02 g, 0.08 mmol) was added at 0°C and stirred under argon atmosphere. After 15 min, TEA (0.02 mL) was added to the reaction mixture and kept on stirring at room temperature overnight. Upon completion of the reaction, revealed by TLC, the crude product was purified via silica gel flash column chromatography using 2:98 MeOH/DCM (v/v) (Yield: 58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (d, *J* = 2.7 Hz, 1H), 8.59 (d, *J* = 15.3 Hz, 1H), 8.53 (t, *J* = 5.8 Hz, 1H), 8.38 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.83 (d, *J* = 8.2 Hz, 2H), 7.62 (s, 1H), 7.50 – 7.44 (m, 4H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.14 (s, 1H), 7.11 (d, *J* = 15.4 Hz, 1H), 6.97 (d, *J* = 9.2 Hz, 1H), 6.89 (s, 1H), 4.81 (t, *J* = 7.5 Hz, 2H), 3.08 (m, 2H), 3.02 (t, *J* = 5.8 Hz, 2H), 2.71 (t, *J* = 5.6 Hz, 2H), 2.37 (s, 3H), 2.22 (m, 2H), 1.97 (t, *J* = 5.9 Hz, 2H), 1.76 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 179.04, 158.23, 154.52, 152.81, 150.94, 146.88, 143.27, 142.39, 142.28, 141.01, 139.04, 136.65, 133.15, 131.91, 129.78, 129.72, 129.38, 128.60, 127.62, 127.40, 122.69, 122.53, 122.47, 117.94, 117.77, 113.72, 111.26, 110.87, 108.72, 51.43, 44.40, 39.76, 29.90, 29.82, 28.39, 28.22, 24.97, 21.65, 20.12. HRMS: calcd. for $C_{41}H_{38}BrN_4O_8S^+$ [M^+]: 825.1588; HRMS found: m/z 825.1596.

Synthesis of H:

Compound **(H)** was obtained by treating the solution of **(HEH)** (5 mM) in acetonitrile with Na_2S aqueous solution (5 equiv.). The reaction mixture was diluted with water and washed with with DCM (5 mL x 3). The combined organic phases dried with Na_2SO_4 and DCM was removed under vacuum. Compound **(H)** was separated *via* preparative thin layer chromatography using 10:90 MeOH/DCM (v/v) as blue colored solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.17 (d, *J* = 13.7 Hz, 1H), 7.54 (s, 1H), 7.33 – 7.29 (m, 4H), 7.18 – 7.13 (m, 4H), 6.98 (d, *J* = 7.7 Hz, 1H), 6.71 (s, 1H), 5.75 (d, *J* = 13.7 Hz, 1H), 4.01 (*br.* s, 2H), 3.53 (*br.* s, 2H), 2.59 (*br.* s, 2H), 2.51 (*br.* s, 2H), 2.19 – 2.11 (m, 2H), 2.08 (s, 3H), 1.82 (*br.* s, 2H), 1.63 (s, 6H). HRMS: calculated $C_{35}H_{36}BrN_2O_4S^+$ [M^+]: 659.1574; HRMS found: m/z 659.1579.

Synthesis of HC-1:

HC-1 was synthesized according to literature.[1]

HC-1: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, *J* = 13.4 Hz, 1H), 7.63 (s, 1H), 7.30 – 7.28 (m, 3H), 7.06 (t, *J* = 7.4 Hz, 1H), 6.85 (d, *J* = 7.8 Hz, 1H), 6.66 (s, 1H), 5.59 (d, *J* = 13.4 Hz, 1H), 3.35 (s, 3H), 2.68 (t, *J* = 5.9 Hz, 2H), 2.61 (t, *J* = 5.8 Hz, 2H), 1.95 – 1.84 (m, 2H), 1.66 (s, 6H).

3. Photophysical Characterization

HEH and **HEHM** were dissolved in dimethyl sulfoxide (DMSO) to obtain a 5 mM stock solution. Absorption spectra were recorded on Biotek Synergy H1 MF microplate reader. Fluorescence spectra of the probes were acquired on an Agilent Cary Eclipse fluorescence spectrophotometer. The excitation and emission slit widths were set to 5 nm. The experiments were performed in a DMSO solution containing 2% PBS. H_2S stock solutions were freshly prepared from sodium sulfide nonahydrate ($Na_2S{\cdot}9H_2O$) (Sigma-Aldrich) and varying concentrations of Na_2S were prepared by diluting Na_2S stock solution.

In the case of active **HC-1** and **H** cores, measurements were performed either in DMSO (2% PBS, pH 7.4) or in PBS (1% DMSO, pH 7.4). Absorption measurements were acquired on a Shimadzu UV-Vis-NIR spectrophotometer. Fluorescence spectra of the probes were acquired on an Agilent Cary Eclipse fluorescence spectrophotometer.

Fluorescence Quantum Yield:

For fluorescence quantum yield measurement, an integrating sphere (Edinburgh Instruments) was placed inside the sample compartment of the spectrometer. The internal cavity of the sphere was coated with a PTFE-like material to enable a reflectance of approximately >99% over the wavelength range between 400 and 1500 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 sample, a blank spectrum was measured by using the reference solvents DMSO (2% PBS, pH 7.4). Both PSs were treated with Na_2S before the measurement. For both 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. To cover a scattering range, the emission scans were started from 20 nm below the actual excitation wavelength (726 nm) and finished at 900 nm. Furthermore, the step size and the integration time of the measurements were set to 1 nm and 0.2 seconds, respectively. After 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

$$\boldsymbol{QY} = \frac{\boldsymbol{E_s - E_B}}{\boldsymbol{S_B - S_s}} \qquad \textit{(Eq. 1)}$$

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

4. LC-QTOF-MS Analyses

HEHM (10 μM) was treated with Na_2S (5 eq.) in DMSO (2% PBS, pH 7.4). After initiating the reaction, 120 μL of samples were taken from the reaction medium at different times (60, 120, 180, and 240 sec) and immediately quenched with $ZnCl_2$ (200 μM) (in MEOH). Prior to LC-MS analysis, all samples were diluted with equal amounts (500 μL) of MeOH. Finally, LC-MS experiments were conducted by monitoring the active core **HC-1** (m/z calcd. for $C_{26}H_{25}Br^{35}NO_2^+$: 462.1063) and **HEHM** (calcd. for $C_{32}H_{27}Br^{35}N_3O_6^+$: 628.1078).

5. Detection of ROS Generation in Solution

Singlet oxygen generation capability of **HEH**, **HEHM, HC-1** and **H** were evaluated using singlet oxygen sensor green (SOSG). The emission of SOSG (5 μM) was measured in the presence of Na_2S-treated **HEH** (10 μM) or **HEHM** (10 μM) in PBS (1% DMSO, pH 7.4). In the case of active cores **HC-1** (10 μM) and **H** (10 μM) in PBS (1% DMSO, pH 7.4) were used. The solutions were put under light irradiation for 2 minutes, and the fluorescence intensity of SOSG was recorded at 20-second intervals. ($\lambda_{ex/em}$ = 504/528 nm).

Singlet oxygen generation capability of **HEH**, **HEHM, HC-1** and **H** were also evaluated using 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA). The absorption change of ABDA at 380 nm was measured in the presence of Na_2S-treated **HEH** (10 μM) or **HEHM** (10 μM) in PBS (1% DMSO, pH 7.4). In the case of active cores **HC-1** (10 μM) and **H** (10 μM) in PBS (1% DMSO, pH 7.4) were used. The solutions were put under light irradiation for 2 minutes, and the absorption change at 390 nm was recorded at 20-second intervals.

Superoxide radical anion generation potential of **HEH**, **HEHM, HC-1** and **H** were investigated using dihydroethidium (DHE). The emission of DHE (30 μM) was recorded in the presence of H_2S treated **HEH** (10 μM) or **HEHM** (10 μM) in PBS solution containing calf thymus DNA (200 μg mL^{-1}). In the case of active cores **HC-1** (10 μM) and **H** (10 μM) were used. The solutions were put under light irradiation for 2 minutes, and the fluorescence intensity of DHE was recorded at 20-second intervals. ($\lambda_{ex/em}$ = 510/576 nm).

6. Cell Culture Studies

SH-SY5Y neuroblastoma cells and Vero kidney epithelial cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. The cells were incubated in a humidified Eppendorf Galaxy 170S incubator at 37 °C, 5% CO_2. The cells were subcultured every 3-4 days at a 70-80% confluency. Luzchem LED Illuminator – LEDi-KIT3 (640 nm, 66.7 mW/cm^2) was used as a light source in cell studies. All experiments were performed in triplicates (n=3).

Cell Viability under Normoxia:

Cell viability was evaluated using Cell Titer-Glo® (CTG) Luminescent Cell Viability Assay. The cells were seeded into 96-well black-sided culture plates ($1x10^4$ cells per well) and incubated at 37 °C, 5% CO_2 for 24 h. Then, cells were treated with various concentrations of **HEH** or **HEHM** (0-8 μM, 1% DMSO, v/v) for 3 hours. The cells were put under light irradiation (640 nm, 66.7 mW/cm^2) for 10 minutes or left in dark conditions. 24 hours after the initial treatment, the media was replaced with CTG solution and shaken for 2 minutes to induce cell lysis and then incubated for an additional 8 minutes at room temperature. Following the incubation period, the luminescence of each well was recorded on a Biotek Synergy H1 MF microplate reader. The percentage of viable cells was calculated relative to the untreated control groups.

Cell Viability Under Hypoxia:

SH-SY5Y cells were seeded on poly-L-lysine coated 96-well plates at a density of 10,000 cells/well. The cells were incubated overnight to allow them to adhere to the bottom of the wells. Then media of cells was changed into DMEM (without phenol red, L-glutamine and Glucose) and cells were incubated in a hypoxia incubator (1% O_2) for 3 hours. Then, cells were incubated with **HEH** and, **HEHM** at varying concentrations (0-8 μM, 1% DMSO, v/v) for 1 hour. At the end of the incubation, the cells were put under light irradiation (640 nm, 66.7 mW cm^{-2})

for 10 minutes or left in dark conditions. After light treatment, the media was replaced with DMEM (with L-glutamine and high Glucose) and incubation continued under normoxic conditions for 24 hours. 24 hours after the initial treatment, the media was replaced with CTG solution and shaken for 2 minutes to induce cell lysis and then incubated for an additional 8 minutes at room temperature. Following the incubation period, the luminescence of each well was recorded on a microplate reader. The percentage of viable cells was calculated relative to the untreated control groups.

***In Vitro* Imaging:**

35 mm glass bottom dishes were coated with poly-L-lysine and cells were seeded at $1x10^4$ density and incubated at 37 °C, 5% CO_2 for 24 h. The cells were treated with **HEH** or **HEHM** (1 μM) for 30 minutes and then washed with PBS. Then, cells were stained with tracker dyes according to the manufacturer's advised protocol. Incubation durations were 15, 30, 30, and 10 min for ER-Tracker Green (500 nM), Mitotracker Green (200 nM), Lysotracker Green (200 nM), and Hoechst 33342 (1 μg mL^{-1}), respectively. The cells were washed three times with PBS and then monitored using a Leica DMI8 SP8 Inverted Confocal Microscope. Similar protocol was followed for inhibition and induction experiments. The cells were divided into three groups and subjected to a pretreatment with an inhibitor, enhancer or inducer: $ZnCl_2$ (300 μM) for 10 min, NaHS (1 mM) for 1 h or L-Cys (200 μM) for 1 h. As control, the cells were either treated with **HEH** or **HEHM** (4 μM) or remained blank.

Intracellular ROS Generation Assay:

After the treatment with **HEH** or **HEHM** (4 μM), the cells were washed with PBS and incubated with DCF-DA (10 μM) for 30 minutes. Following the incubation, the cells were put under light irradiation (66.7 mW/cm^2) for 10 minutes or left in dark conditions. The cells were washed with PBS and monitored using Leica DMI8 SP8 Inverted Confocal Microscope.

SH-SY5Y cells were seeded into 35 mm glass bottom dishes. The cells were incubated with DHE (10 μM), HPF (10 μM) or SOSG (10 μM) for 30 min at 37 °C. Following the incubation period, the media was removed, and the cells were washed three times with PBS. DHE: $\lambda_{ex/em}$ = 514/545-620 nm; HPF: $\lambda_{ex/em}$ = 488/520-620; SOSG: $\lambda_{ex/em}$ = 488/520-620 nm.

Assessment of Mitochondrial Membrane Potential:

SH-SY5Y cells were seeded into 35 mm glass bottom dishes. The cells were incubated with JC-1 dye (10 μg mL^{-1}) for 15 minutes following the PDT period. The control experiments were performed in the dark and/or in the absence of **HEHM**. The cells were washed three times with PBS, and the images were captured under confocal microscopy. Green channel: $\lambda_{ex/em}$ = 488/505-550 nm; Red channel: $\lambda_{ex/em}$ = 561/575-630 nm.

Evaluation of Endoplasmic Reticulum Stress under Confocal Microscopy:

SH-SY5Y cells were seeded into 35 mm glass bottom dishes. After the PDT action, the cells were incubated with Tht (1.6 μM) for 10 minutes. The control experiments were conducted in the dark and/or in the absence of **HEH**. Following the incubation period, the media was replaced, and the images were captured under confocal microscopy. $\lambda_{ex/em}$ = 458/475-550 nm.

Protein extraction, Immunoblot analysis and antibodies:

Protein extraction was performed with RIPA buffer (50 mM TRIS-HCl pH 7.4, 150 mM NaCl, 1% NP40, 0.25% Na-deoxycholate) supplemented with a complete protease inhibitor cocktail

(Roche, 04-693-131-001) and 1mM phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich, P7626). For phosphorylated proteins, protein extraction was performed with RIPA buffer supplemented with complete protease inhibitor cocktail and phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich, P7626) and 100 nM okadaic acid, 1 µM cyclosporine A, 1 mM NaF, 50 mM β-glycerophosphate. Cell extracts (30-50µg) were separated in 15-10 % SDS-polyacrylamide gels and transferred to Nitrocellulose membranes (Amersham, GE10600002). Immunoblotting was performed as previously described.[3] Primary antibodies (ab) were used in this study: anti-EIF2AK3 (CST, #3192, 1:1000), anti-p-EIF2AK3 (Thr 980) (CST, #3179, 1:1000), anti-EIF2A ab (Santa Cruz, sc-133227, 1:1000), anti-p-EIF2A (Ser 51) ab (CST, #3597,1:1000), anti-Cleaved Caspase-3 (Asp175) ab (CST, 9661, dilution 1:1000), anti-PARP ab (CST, #9542, 1:1000) anti-β-actin/ACTB ab (Sigma-Aldrich, A5441, dilution 1:10.000), anti-CHOP ab (Santa Cruz, sc-7351, 1:1000), anti-HIF1α ab (CST, #14179, 1:1000). Protein bands were revealed with chemiluminescence under Chemidoc MP. Band intensities were quantified using ImageJ software.

RNA isolation and qRT-PCR analysis:

Total RNA was extracted using TRIzol reagent (Sigma-Aldrich, T9424) according to the manufacturer's instructions. cDNA was reverse transcribed from total RNA (DNase treated) as previously described.[4] SYBR Green Quantitative RT-PCR kit (Roche, 04-913-914-001) and Roche Light Cycler 480 were used for single-step qRT-PCR reactions. To activate the SYBR green, an initial cycle of 95°C, 10 min was performed followed by, PCR reactions: 40 cycles of 95°C for 15 sec and 60°C for 1 min. Then a thermal denaturation protocol was used to generate the dissociation curves for the verification of amplification specificity (a single cycle of 95°C for 60 sec, 55°C for 60 sec and 80 cycles of 55°C for 10 sec). Changes in mRNA levels were quantified using the 2-ΔΔCT method using GAPDH (Gyceraldehyde-3- phosphate dehydrogenase) mRNA as control. Reactions were performed in duplicates and the number of independent experiments (n) was marked. Primers were purchased from Macrogen.

Table S1. Primers were used in this study

Gene	Forward	Reverse
ERN1	CACAGTGACGCTTCCTGAAAC	GCCATCATTAGGATCTGGGAGA
ATF6	AGCAGCACCCAAGACTCAAAC	GCATAAGCGTTGGTACTGTCTGA
ATF4	ATGACCGAAATGAGCTTCCTG	GCTGGAGAACCCCATGAGGT
DDIT3	GGAAACAGAGTGGTCATTCCC	CTGCTTGAGCCGTTCATTCTC
IL1β	AACCTCTTCGAGGCACAAGG	AGCCATCATTTCACTGGCGA
HMGB1	GCGAAGAAACTGGGAGAGATGTG	GCATCAGGCTTTCCTTTAGCTCG
GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC

7. Supplementary Figures:

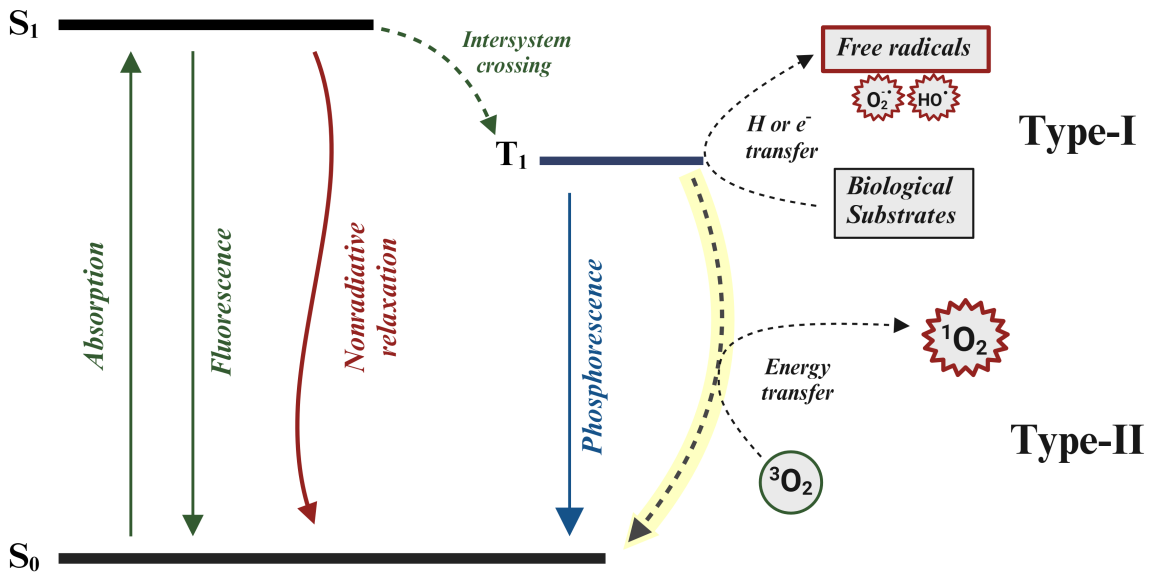


Figure S1. Modified Jablonski diagram showing type-I and type-II PDT mechanisms.

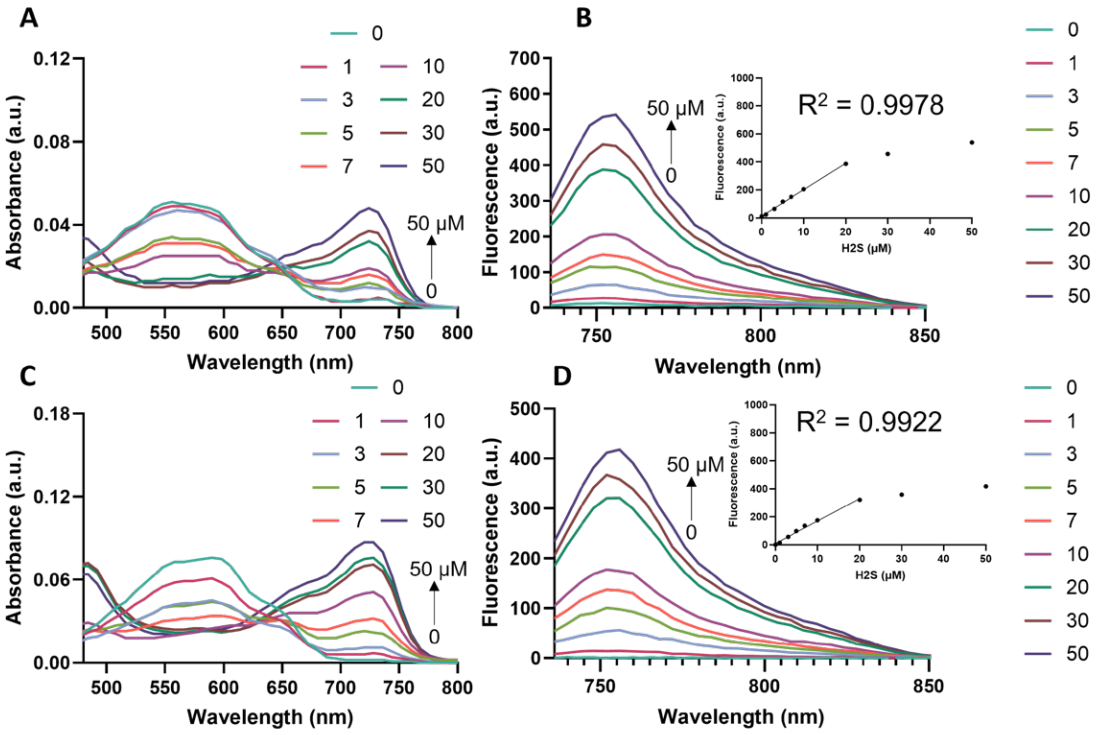


Figure S2. Absorbance spectra of (A) **HEHM** (10 μM) and (C) **HEH** (10 μM) in the presence of varying concentrations of Na_2S (0-50 μM). Fluorescence spectra of (B) **HEHM** (10 μM) and (D) **HEH** (10 μM) in the presence of varying concentrations of Na_2S (0-50 μM). λ_{ex} = 726 nm.

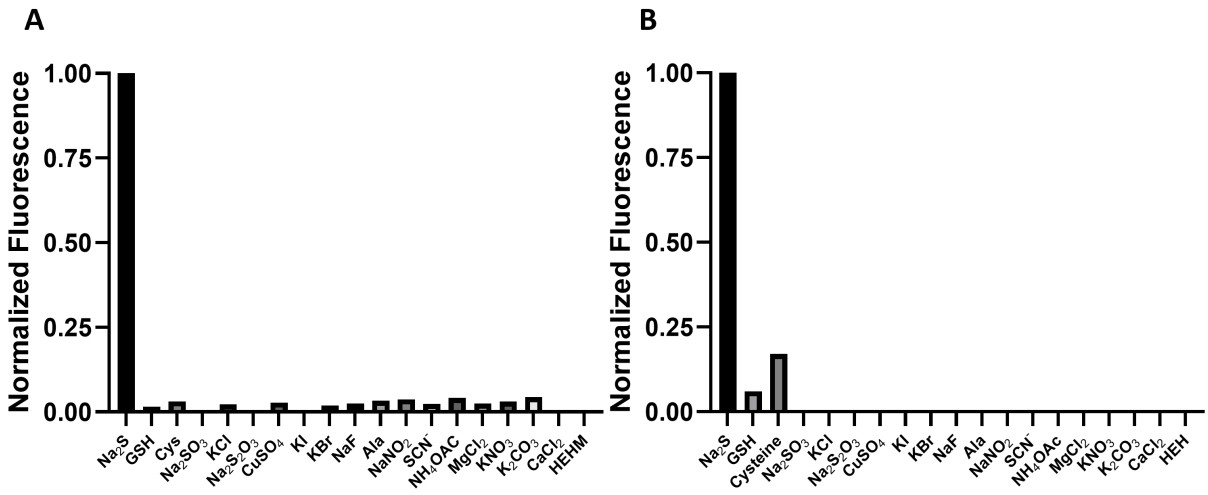


Figure S3. Normalized fluorescence of (A) **HEHM** (10 μM) or (B) **HEH** (10 μM) in the presence of different analytes in PBS (pH 7.4).

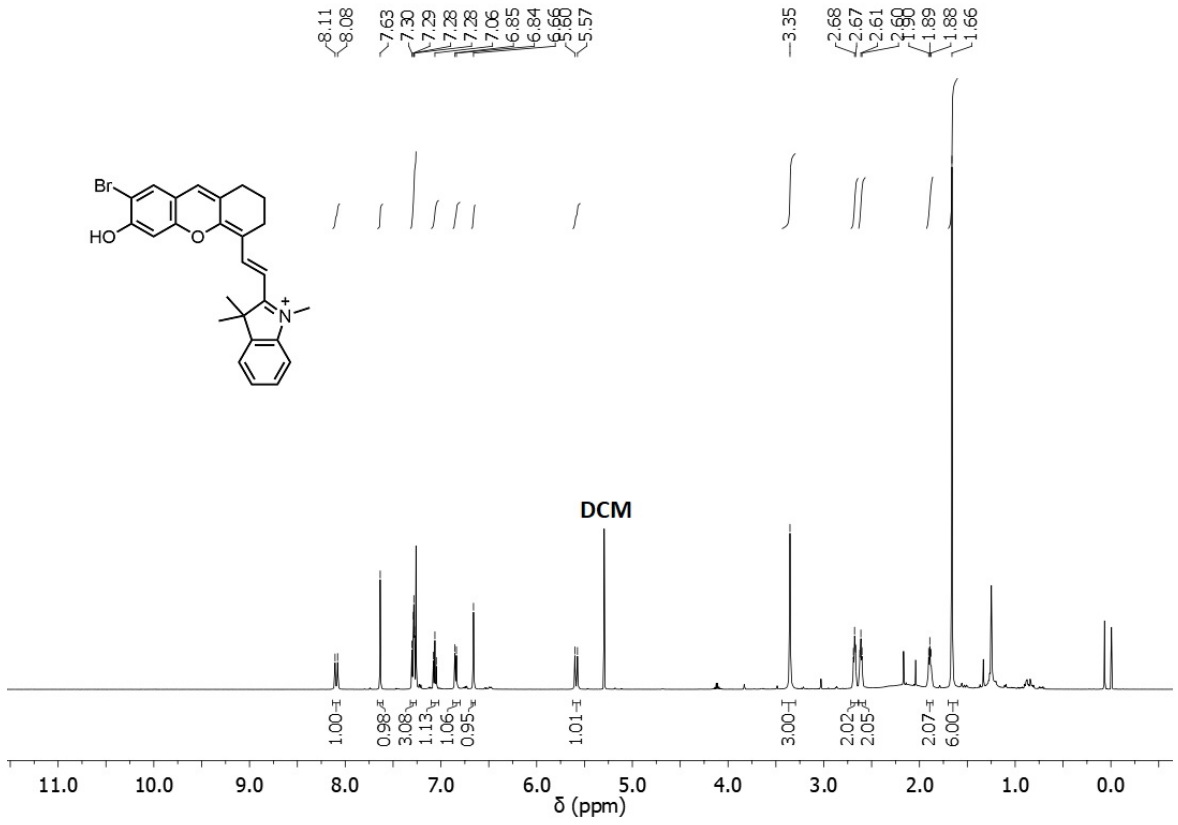


Figure S4. ^{1}H NMR spectrum of photoactive product **HC-1**.

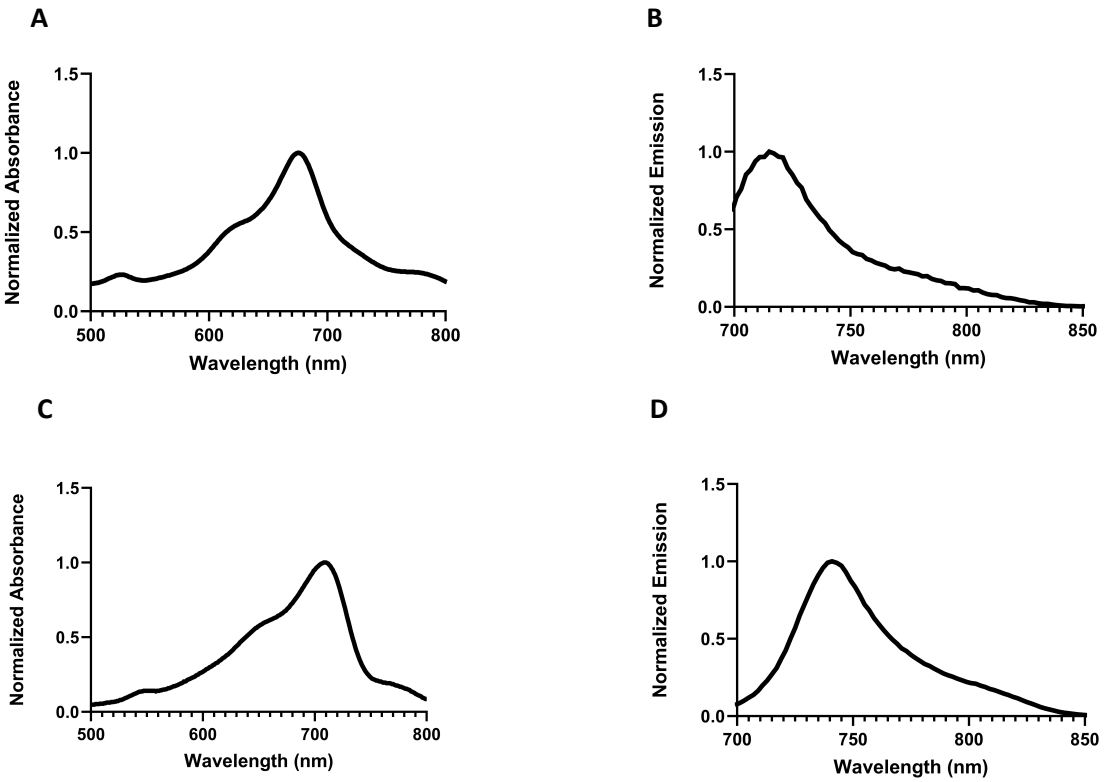
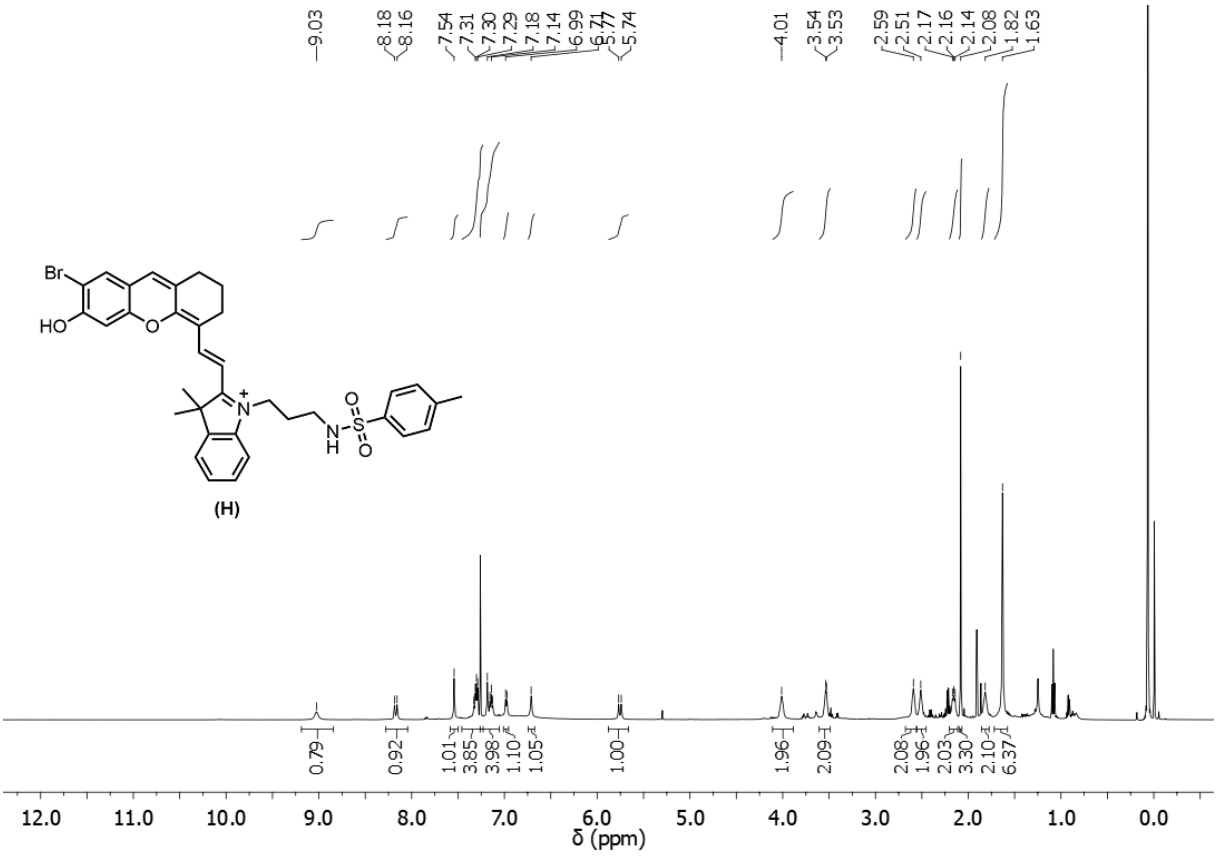


Figure S5. Normalized absorption and fluorescence spectra of **HC-1** (10 μM) (A, B) in PBS (1% DMSO, pH 7.4) and (C, D) in DMSO (2% PBS, pH 7.4).



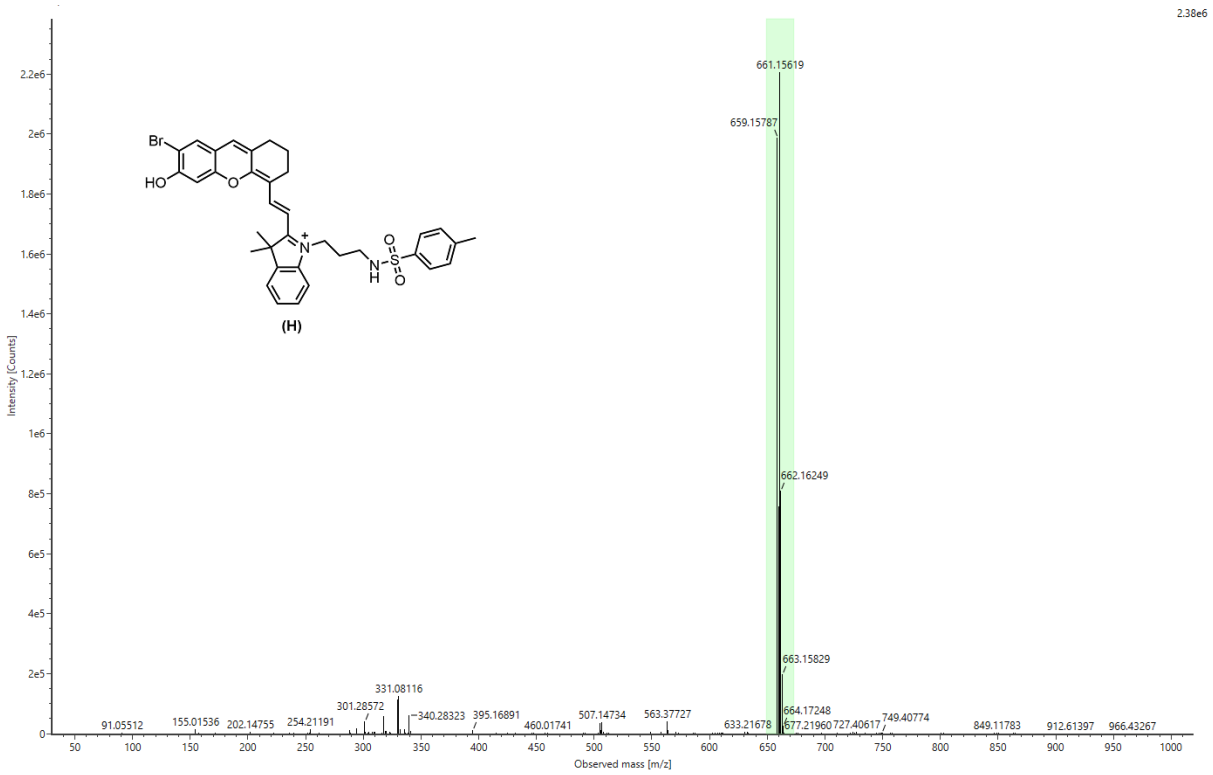


Figure S6. ^{1}H NMR and HRMS spectra of photoactive product **H**.

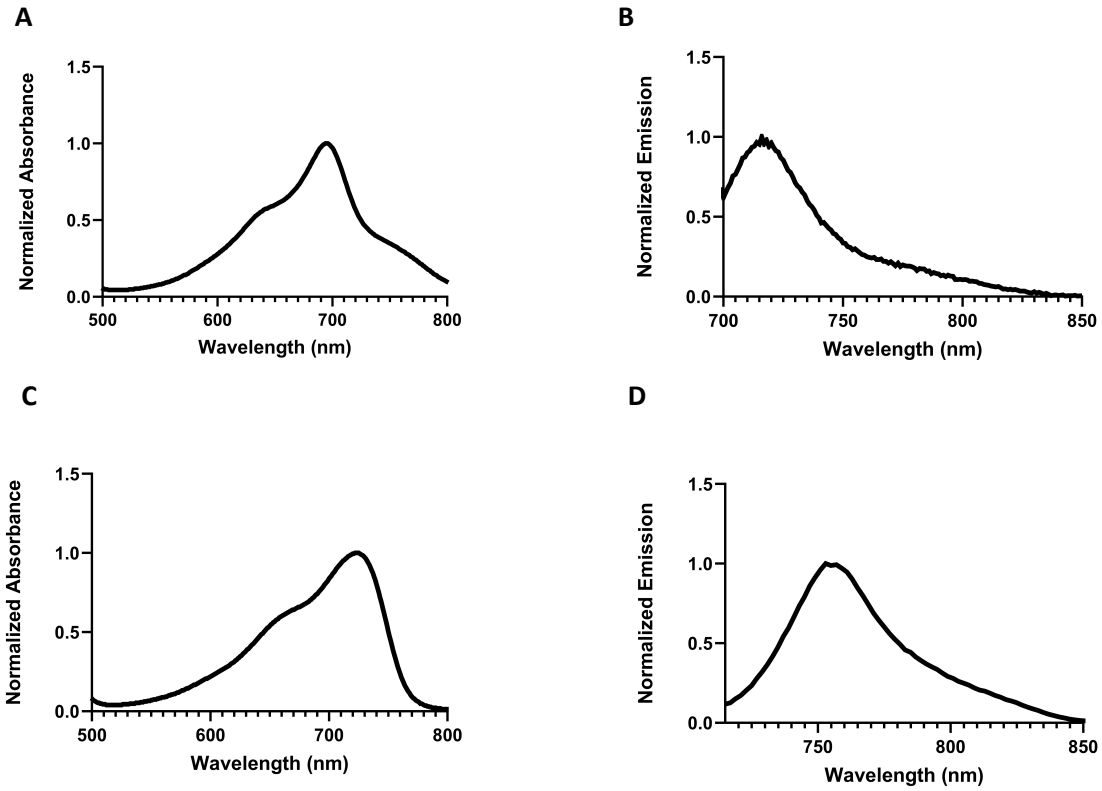


Figure S7. Normalized absorption and fluorescence spectra of **H** (10 μM) (A, B) in PBS (1% DMSO, pH 7.4) and (C, D) in DMSO (2% PBS, pH 7.4).

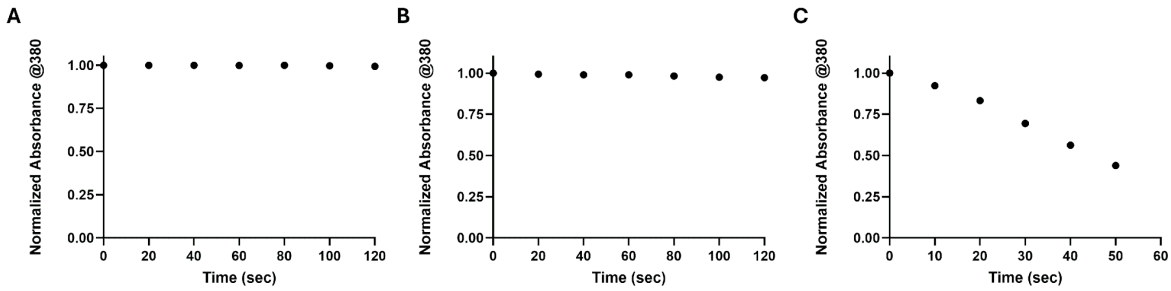


Figure S8. Normalized absorption signal of the ABDA solution containing Na_2S-treated (A) **HEHM** (10 μM) or (B) **HEH** (10 μM) and (C) methylene blue (10 μM) at 380 nm in PBS (1% DMSO, pH 7.4). The solutions were exposed to LED (640 nm, 66.7 mW cm^{-2}) irradiation.

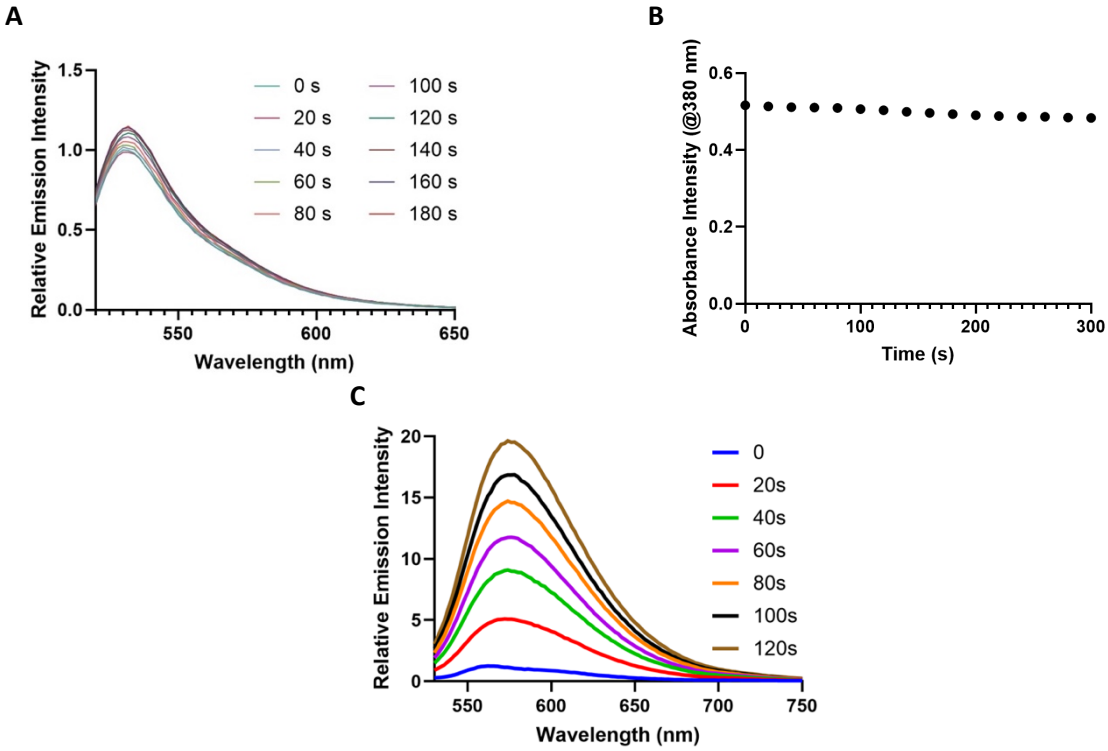


Figure S9. ROS generation capacity of **HC-1**. (A) Normalized fluorescence spectra of SOSG, (B) absorption signal of ABDA at 380 nm and (C) fluorescence spectra of DHE upon irradiation of **HC-1** (10 mM) in PBS (1% DMSO, pH 7.4). The solutions were exposed to light irradiation (640 nm, 66.7 mW cm^{-2}). DHE assay contains ctDNA (200 μg mL^{-1}). DHE: λ_{ex} = 510 nm; SOSG: λ_{ex} = 504 nm.

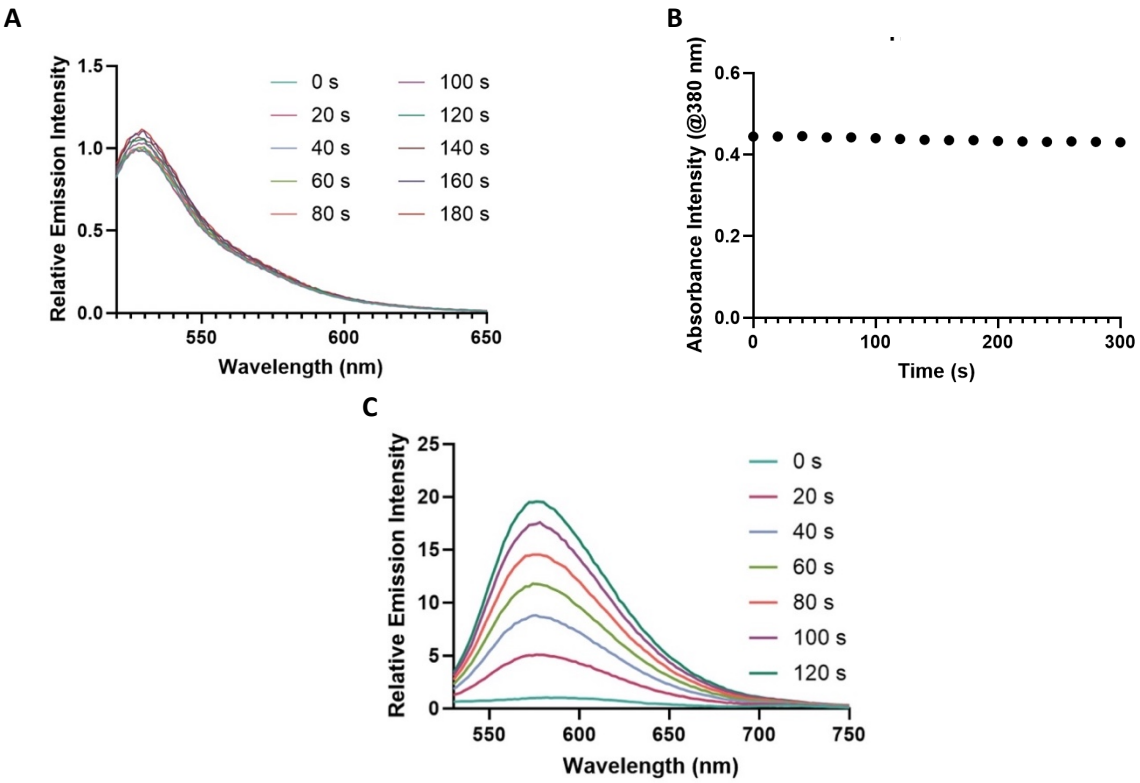
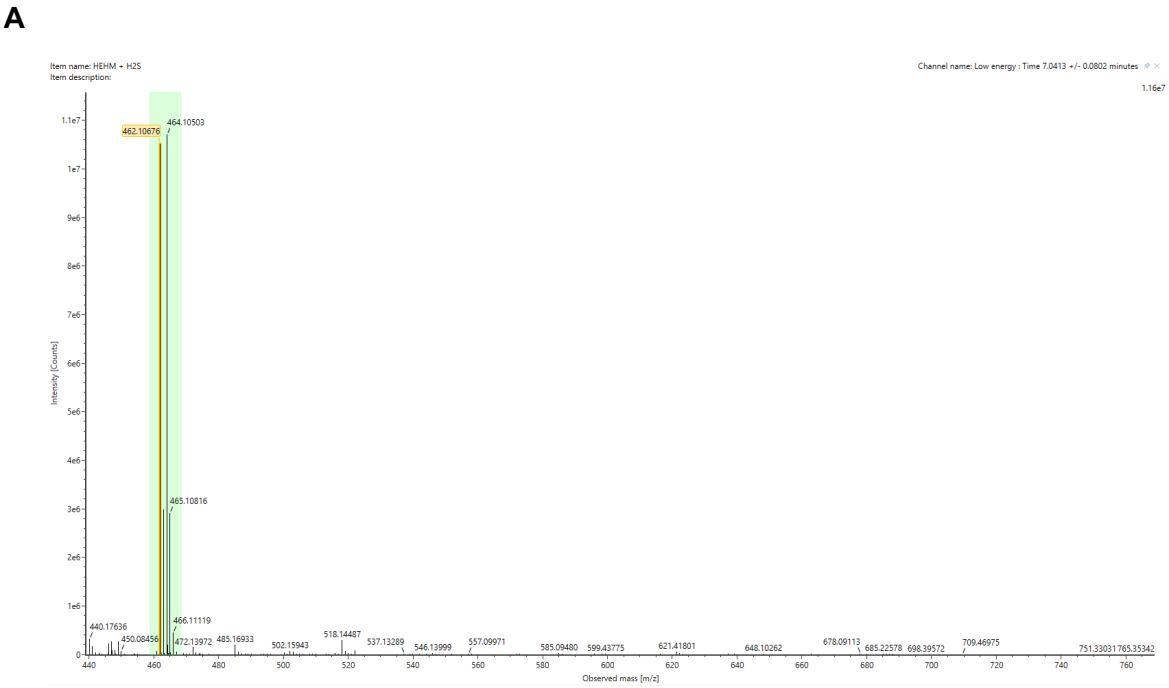


Figure S10. ROS generation capacity of **H**. (A) Normalized fluorescence spectra of SOSG, (B) absorption signal of ABDA at 380 nm and (C) fluorescence spectra of DHE upon irradiation of **H** (10 mM) in PBS (1% DMSO, pH 7.4). The solutions were exposed to light irradiation (640 nm, 66.7 mW cm^{-2}). DHE assay contains ctDNA (200 μg mL^{-1}). DHE: λ_{ex} = 510 nm; SOSG: λ_{ex} = 504 nm.



B

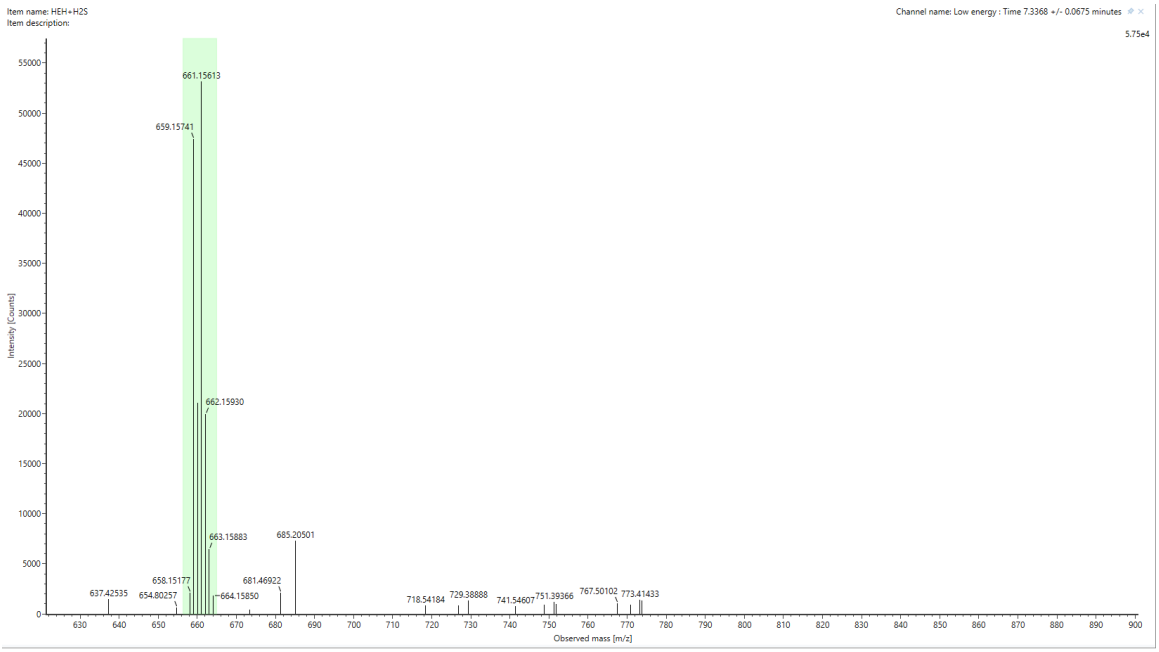


Figure S11. HR-MS spectrum of (A) **HEHM** + Na_2S (m/z calcd. for $C_{26}H_{25}Br^{35}NO_2^+$: 462.1063; HRMS found: m/z 462.1068 [M^+]) and (B) **HEH** + Na_2S (m/z calcd. for $C_{35}H_{36}Br^{35}N_2O_4S^+$: 659.1574; HRMS found: m/z 659.1574 [M^+]).

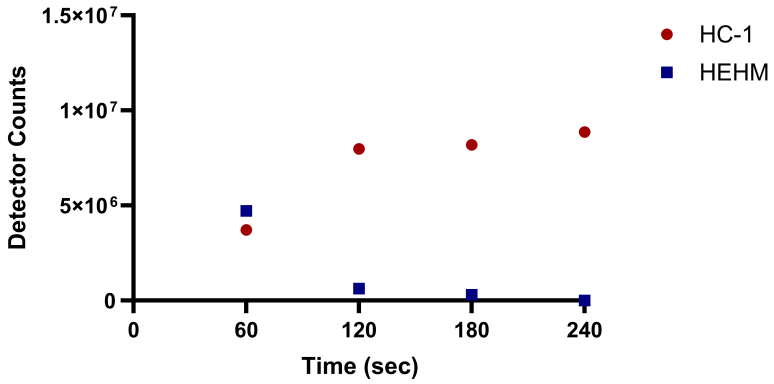


Figure S12. LC-QTOF-MS detector counts vs. time graph for **HEHM** + Na_2S. Formation of **HC-1** and consumption of **HEHM** are monitored during the reaction.

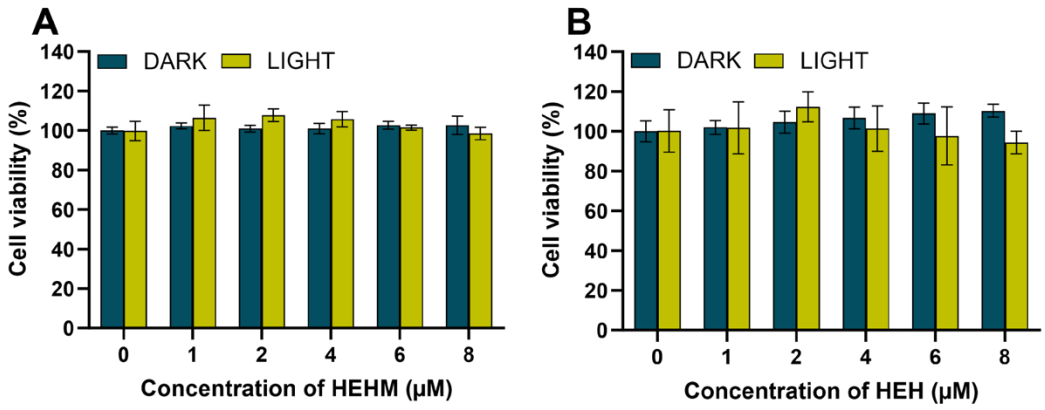


Figure S13. Cell viability of VERO cells incubated with varying concentrations (0-8 μM) of (A) **HEHM** or (B) **HEH** for 3 h at 37 °C, followed by light irradiation (640 nm, 66.7 mW cm^{-2}) for 10 min and dark incubation for additional 21 h. The dark group incubated for 24h in the absence of light.

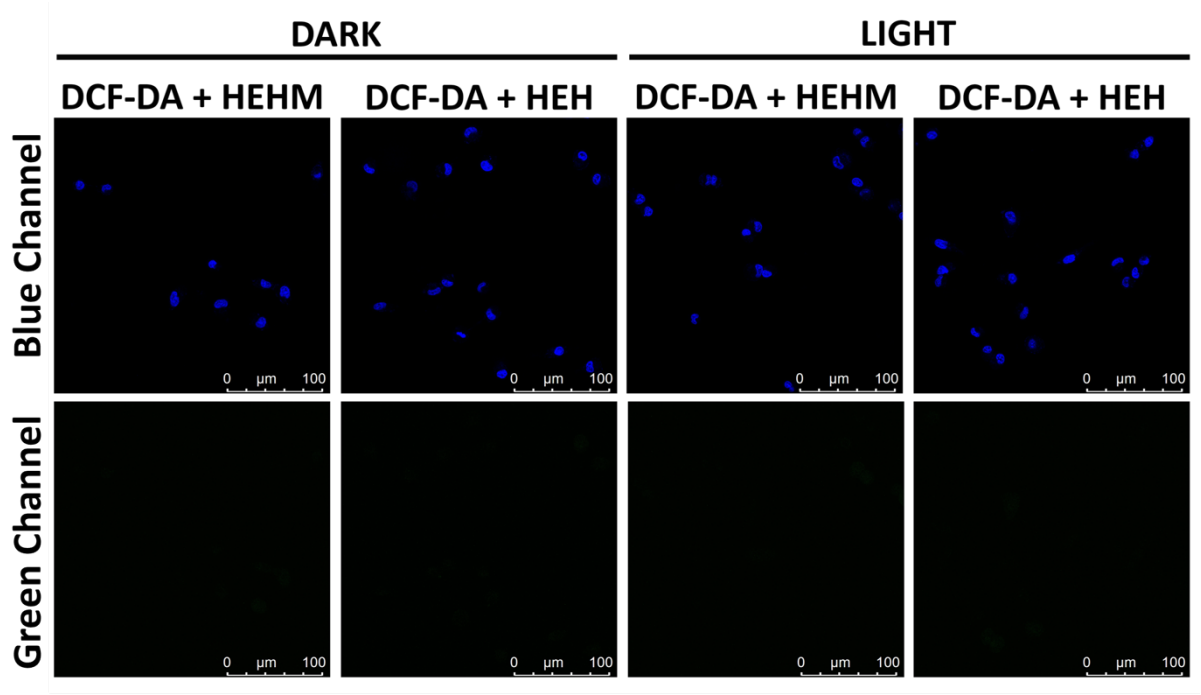


Figure S14. Confocal images of VERO cells incubated with **HEHM** (4 μM) or **HEH** (4 μM) for 30 min and stained with DCF-DA (10 μM) for 45 min. The cells were incubated in the dark or put under light exposure (640 nm, 66.7 mW cm^{-2}) for 10 min. Blue Channel: Hoechst 33342; Green channel: DCF. Scale bar 100 μm.

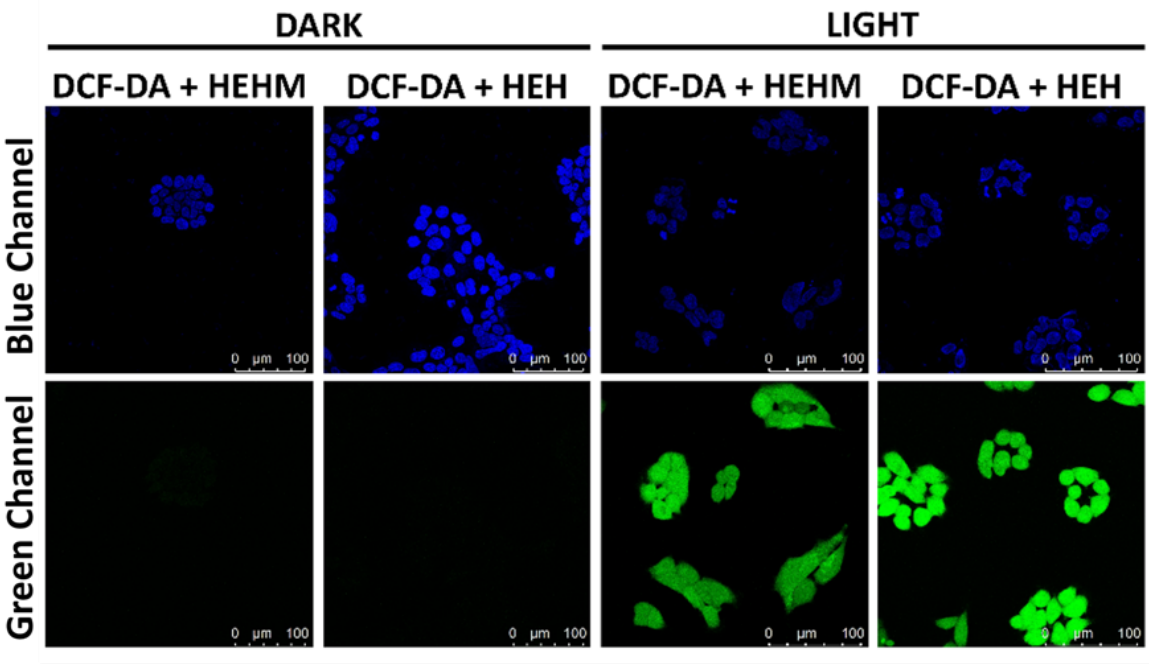


Figure S15. Confocal images of SH-SY5Y cells incubated with **HEHM** (4 µM) or **HEH** (4 µM) for 30 min and stained with DCF-DA (10 µM) for 45 min. The cells were incubated in the dark or put under light exposure (640 nm, 66.7 mW cm^{-2}) for 10 min. Blue Channel: Hoechst 33342; Green channel: DCF. Scale bar 100 µm.

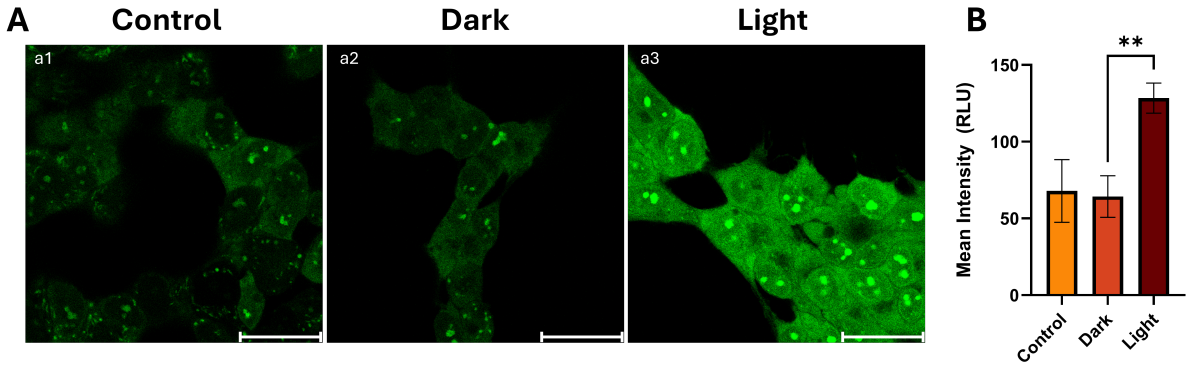


Figure S16. Intracellular monitoring of ER stress with Thioflavin T. (A) SH-SY5Y cells treated with Tht (1.6 µM) for 10 min in the absence (a1) or presence (a2-a3) of **HEH** after dark incubation (a1-a2) or PDT (a3) period. (B) Mean intensities harvested from SH-SY5Y cells in A. $\lambda_{ex/em}$ = 458/475-550 nm. Scale bar: 25 µm. Statistical analysis was performed using two-tailed t-test, **: $p < 0.01$.

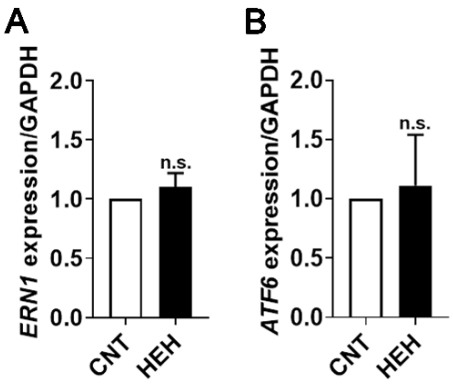


Figure S17. qRT-PCR analyses of *ERN1* and *ATF6* in SH-SY5Y cells upon PDT. qRT-PCR analyses of (A) *ERN1* and (B) *ATF6* level in SH-SY5Y cells upon PDT. (mean ± SD of independent experiments, n = 3, statistical analysis was performed using Student t-test, n.s.: p>0.05).

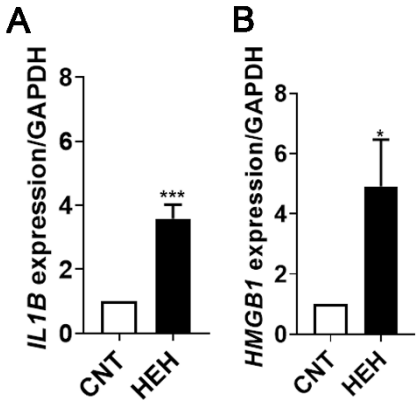


Figure S18. qRT-PCR analyses of *IL1β* and *HMGB1* in SH-SY5Y cells upon PDT. qRT-PCR analyses of (A) *IL1β* and (B) *HMGB1* level in SH-SY5Y cells upon PDT. (mean ± SD of independent experiments, n = 3, statistical analysis was performed using Student t-test, *: p < 0.05, **: p < 0.01; ***: p < 0.001; ****:p<0.0001).

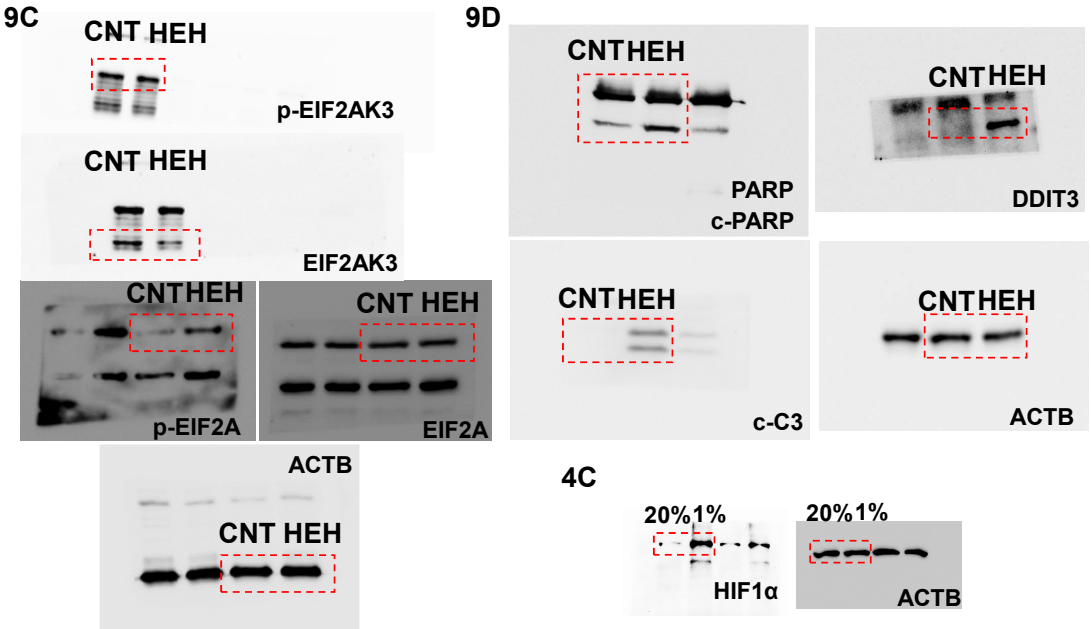


Figure S19. Complete uncropped Immunoblot images for the experiments given in Figures 4C, 9C and 9D.

8. NMR and HR-MS Spectra

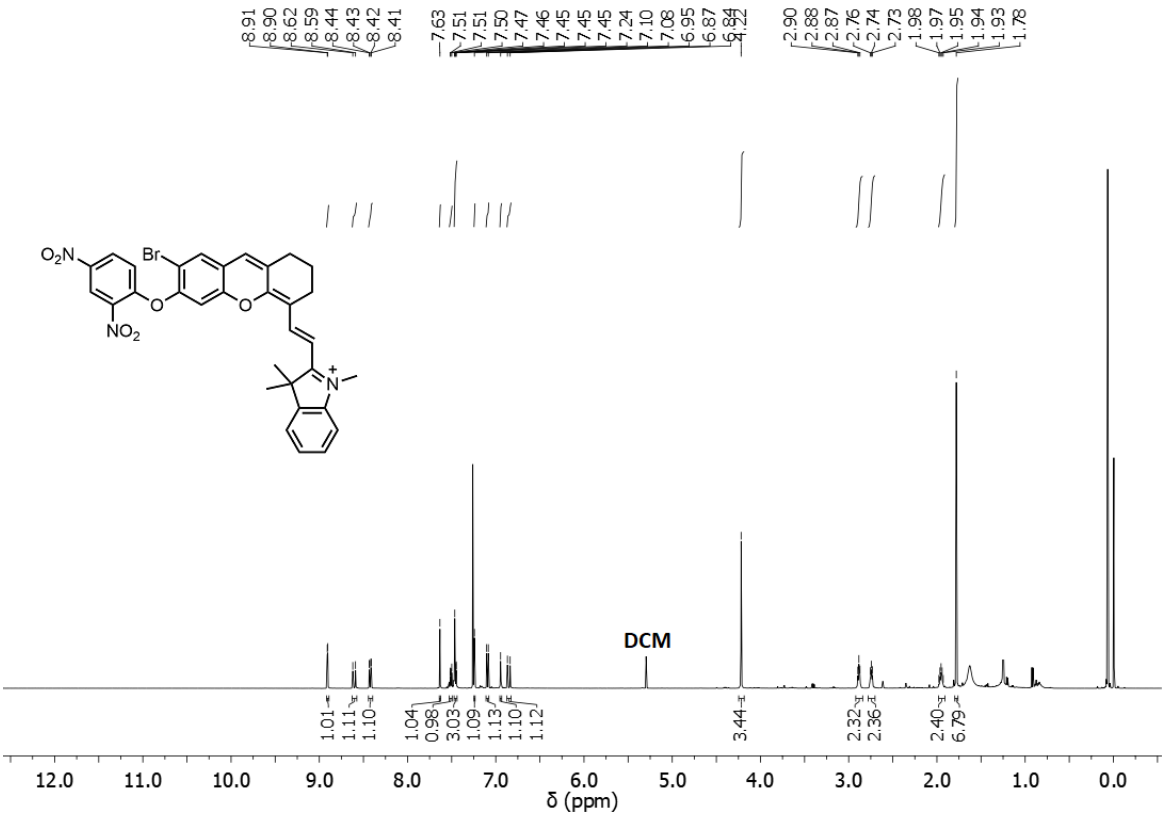


Figure S20. ^{1}H NMR spectrum of compound **HEHM** in $CDCl_3$.

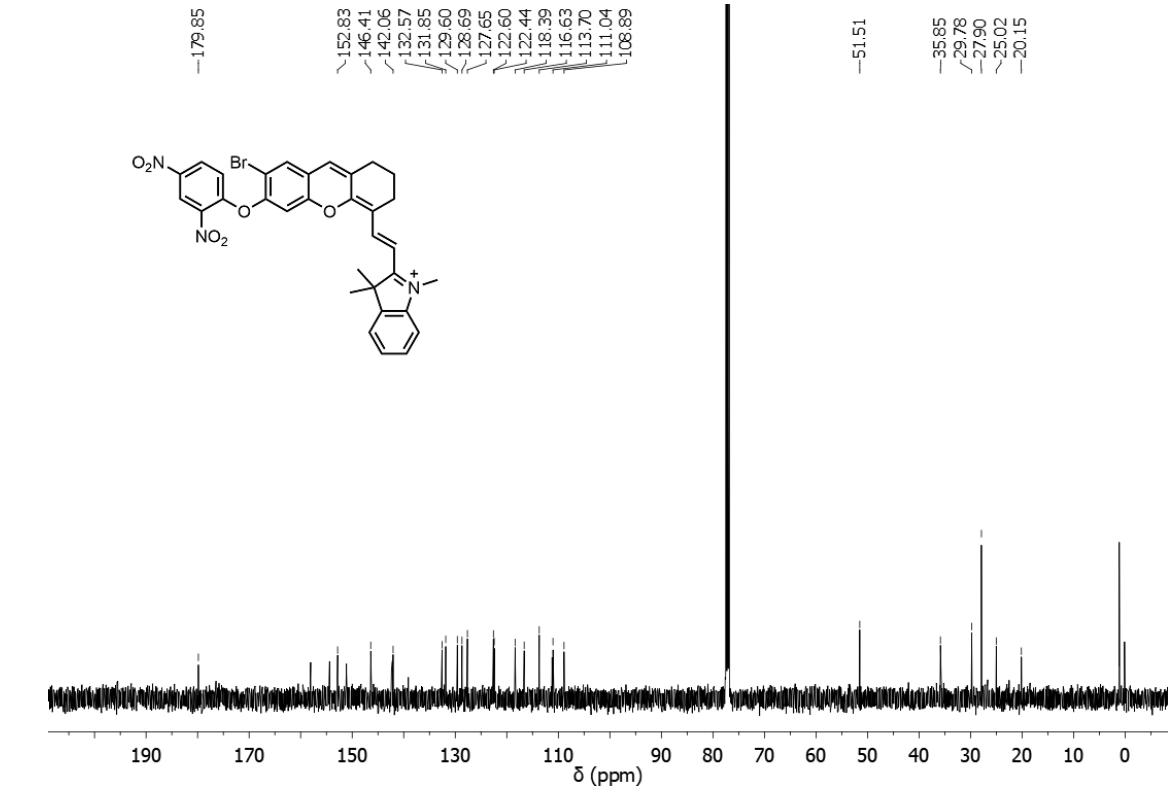


Figure S21. ^{13}C NMR spectrum of compound **HEHM** in $CDCl_3$.

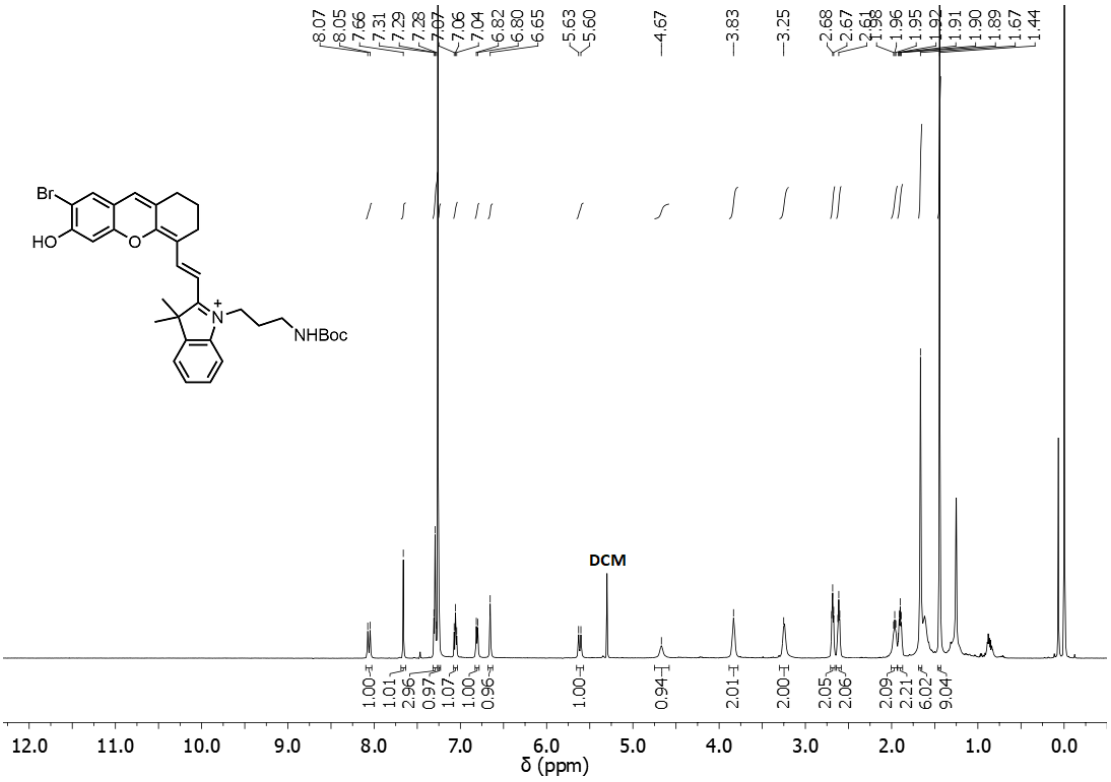


Figure S22. ^{1}H NMR spectrum of compound **(2)** in $CDCl_3$.

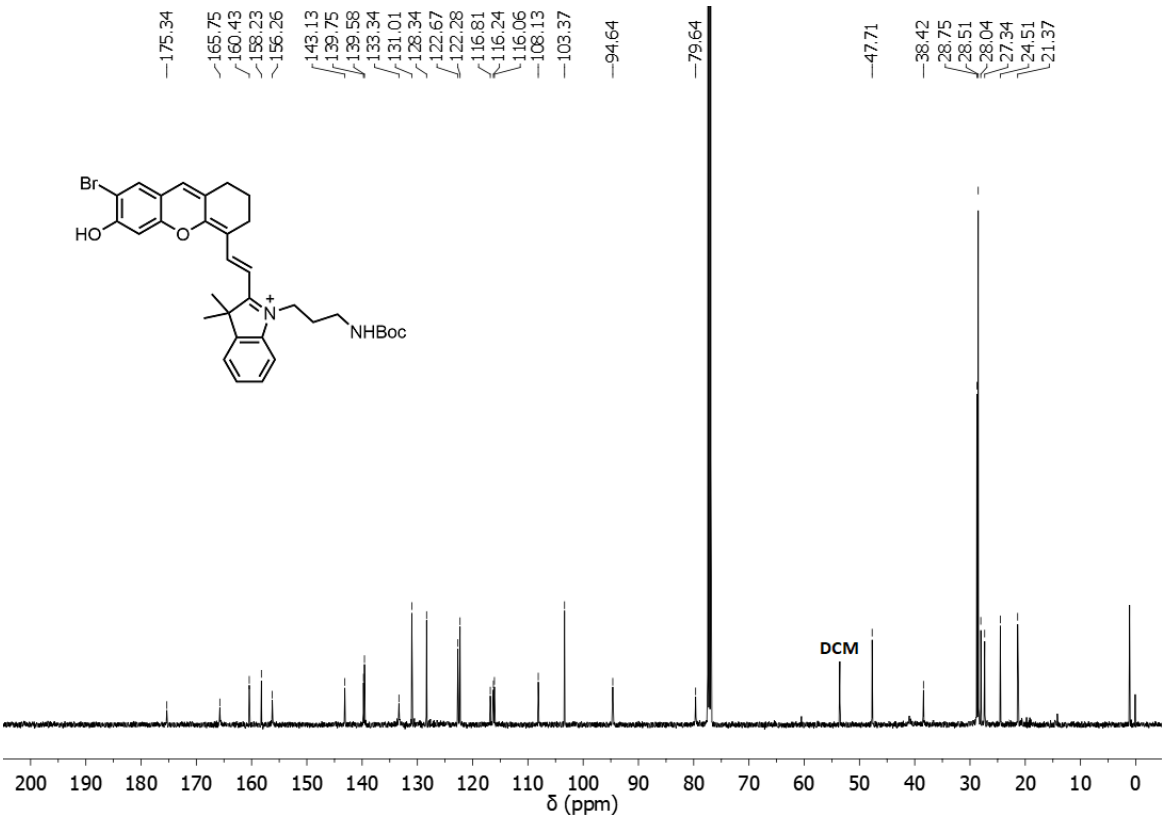


Figure S23. ^{13}C NMR spectrum of compound **(2)** in $CDCl_3$.

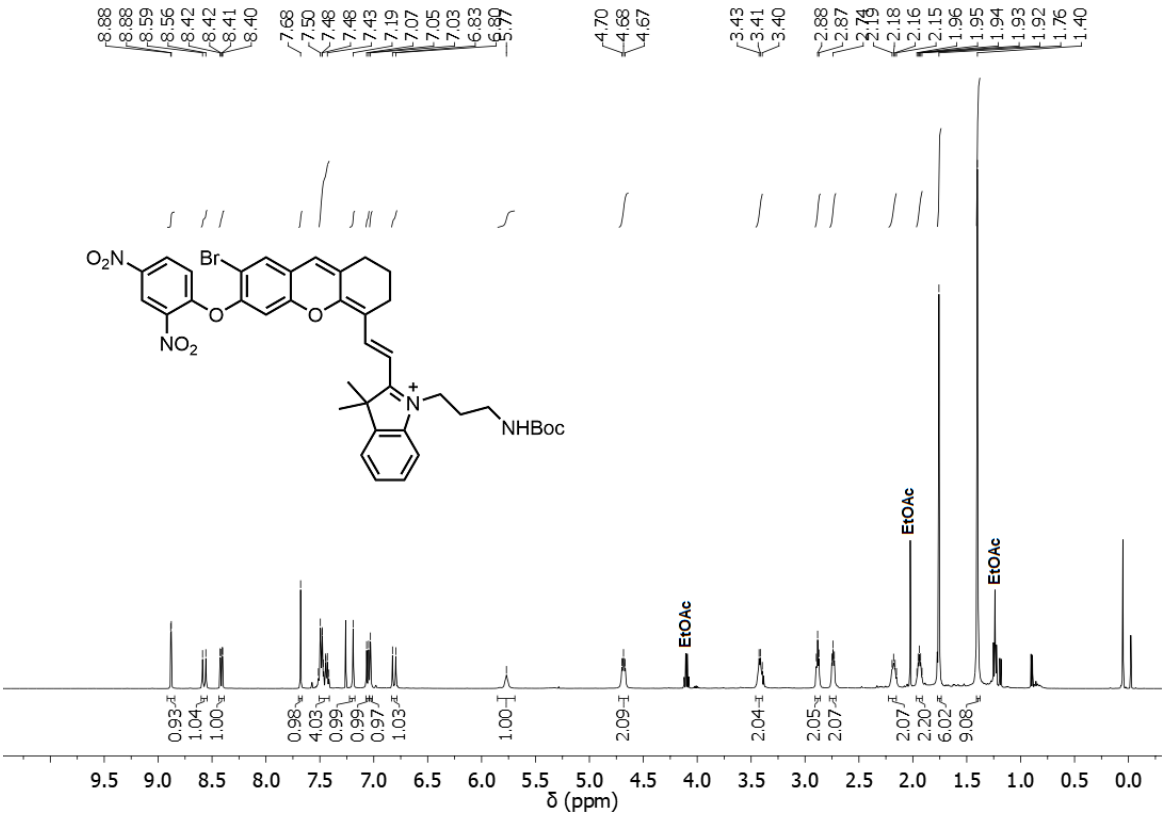


Figure S24. ^{1}H NMR spectrum of compound **(3)** in $CDCl_3$.

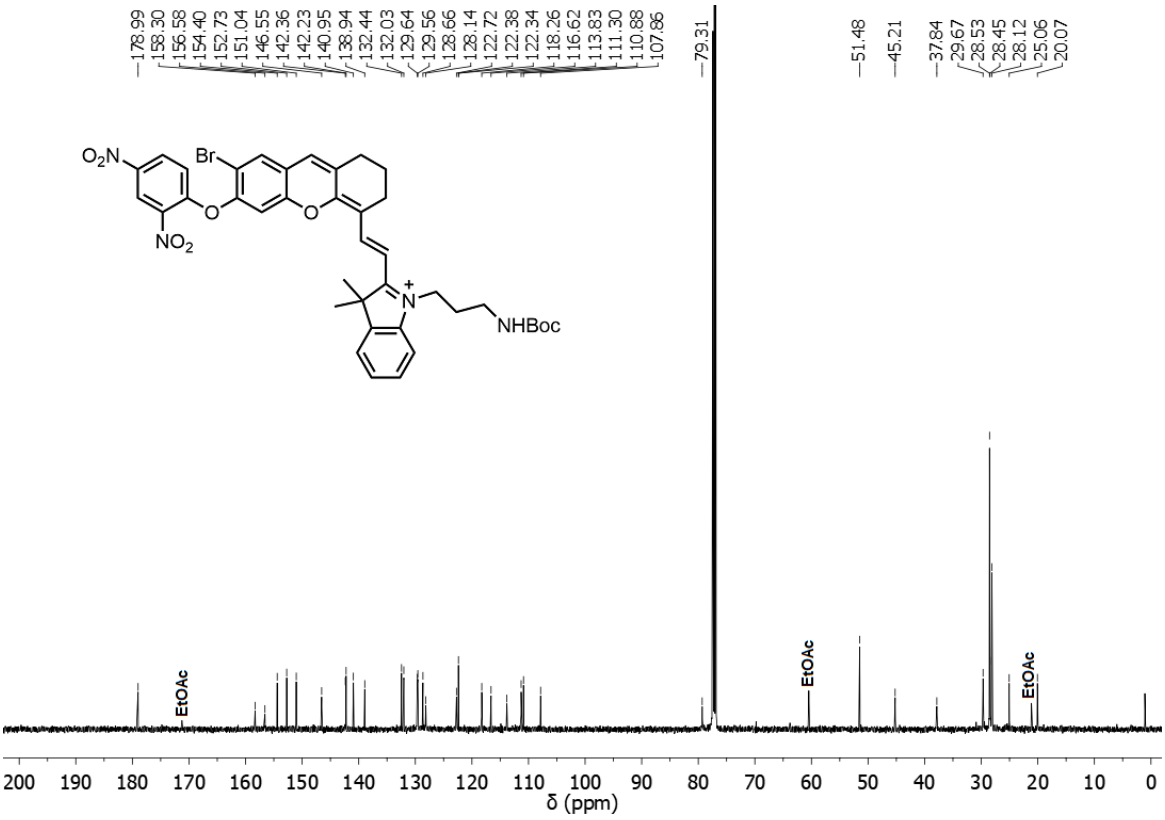


Figure S25. ^{13}C NMR spectrum of compound **(3)** in $CDCl_3$.

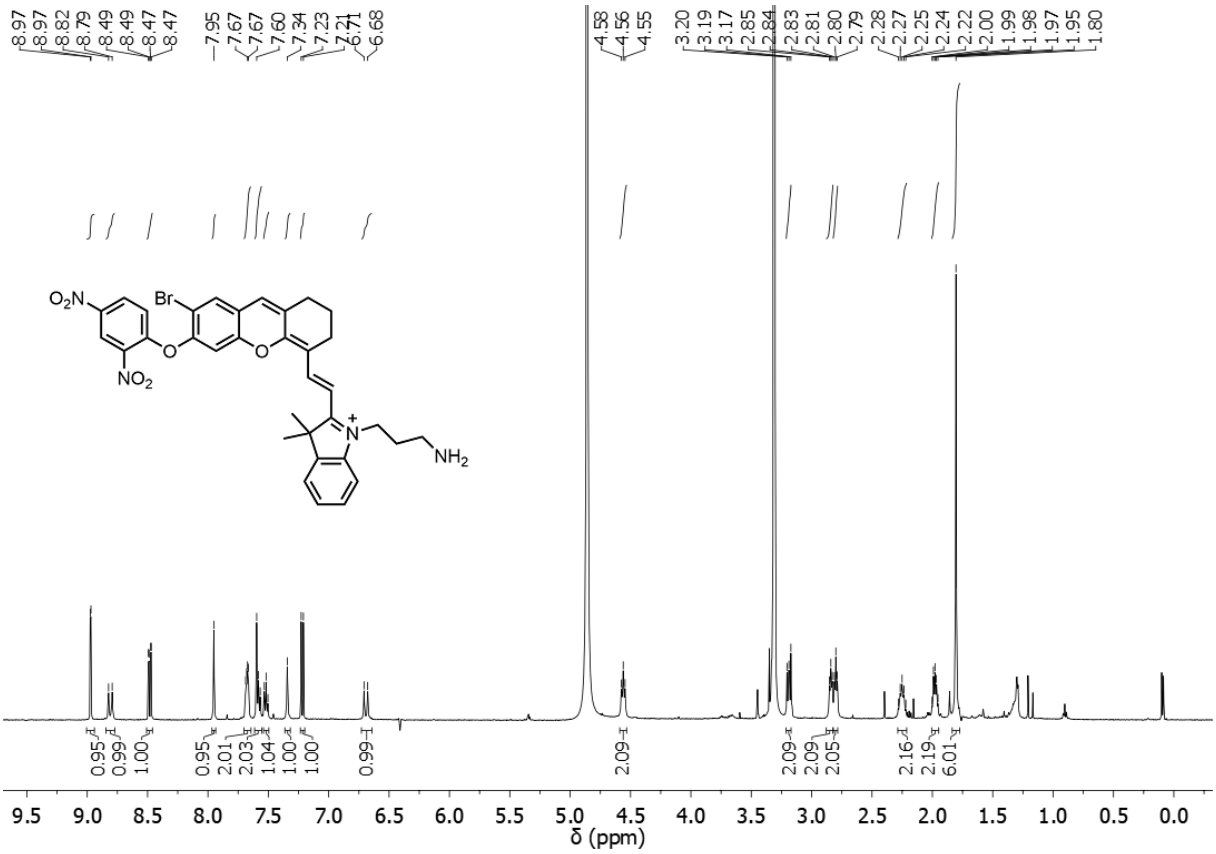


Figure S26. ^{1}H NMR spectrum of compound **(4)** in MeOD.

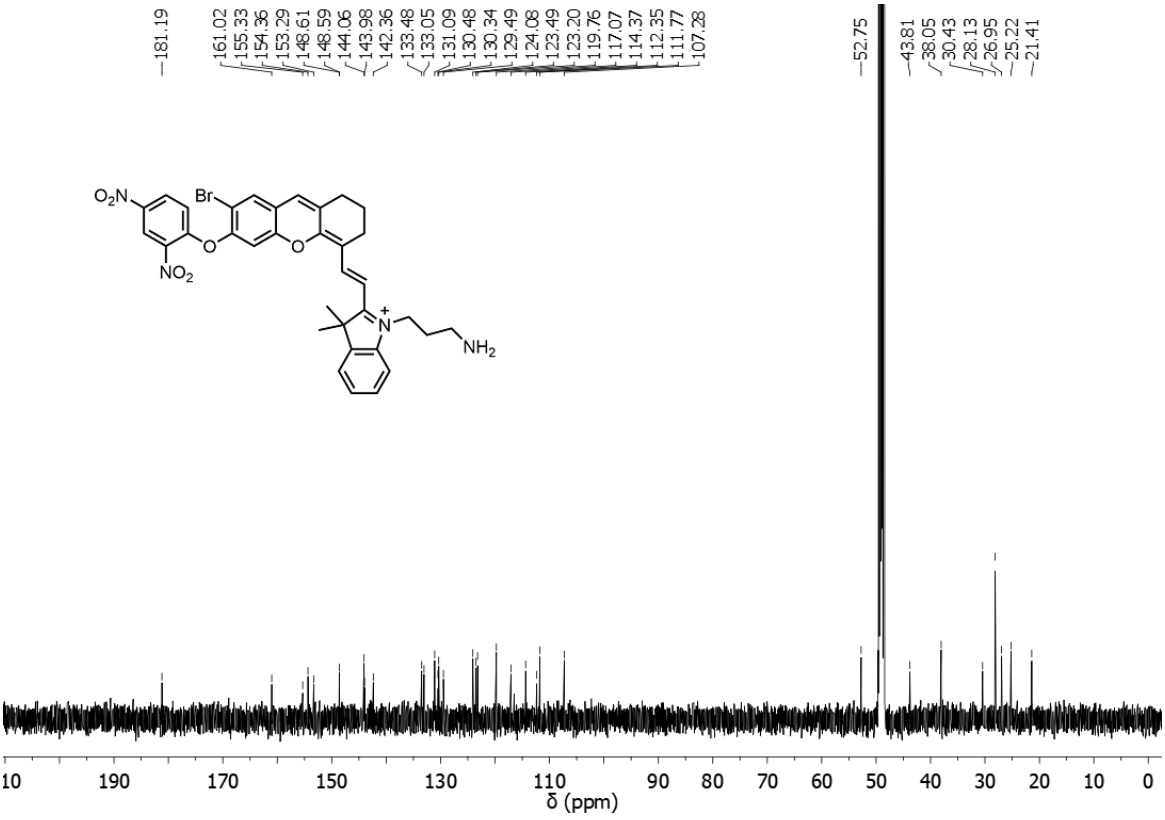


Figure S27. ^{13}C NMR spectrum of compound **(4)** in MeOD.

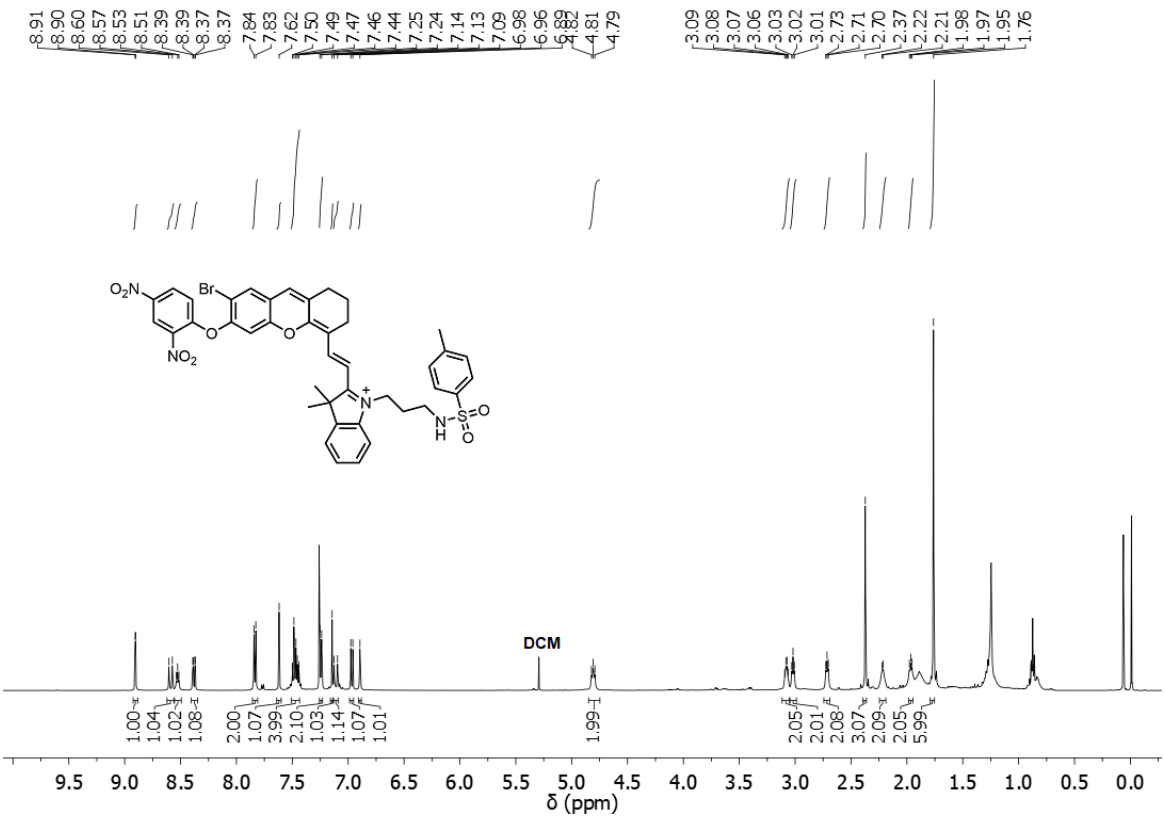


Figure S28. ^{1}H NMR spectrum of compound **HEH** in $CDCl_3$.

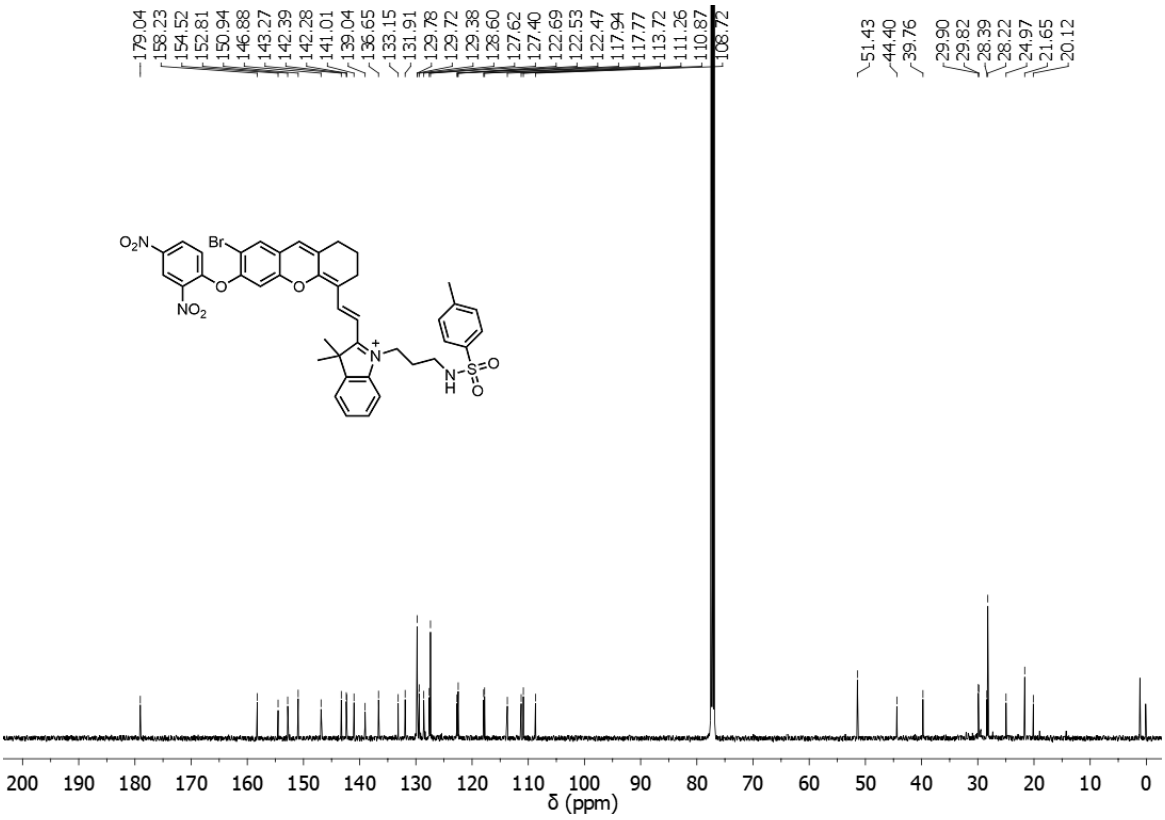


Figure S29. ^{13}C NMR spectrum of compound **HEH** in $CDCl_3$.

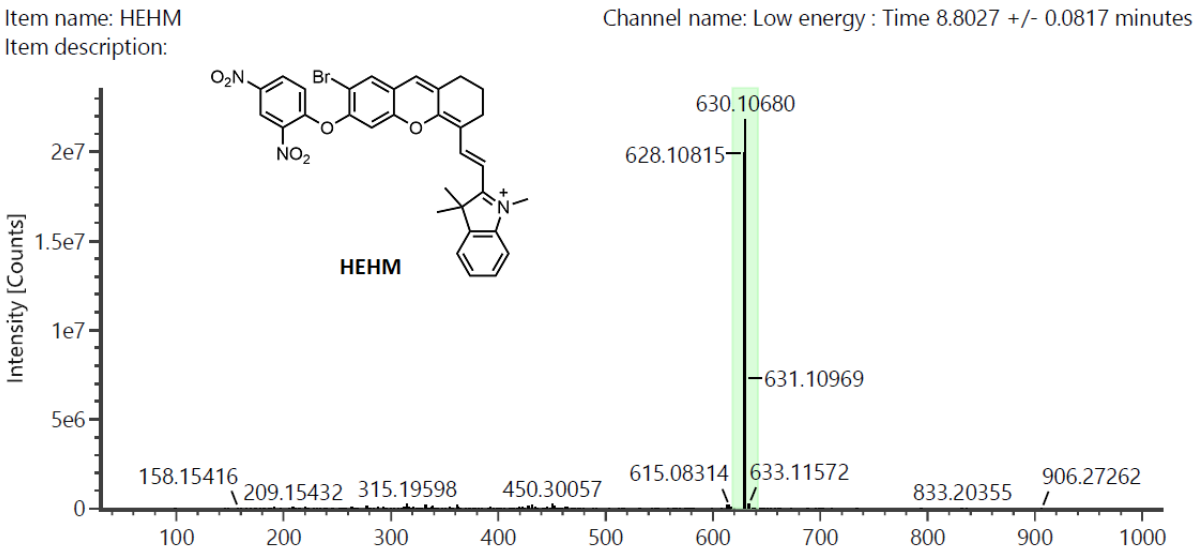


Figure S30. HRMS spectrum of compound **HEHM**.

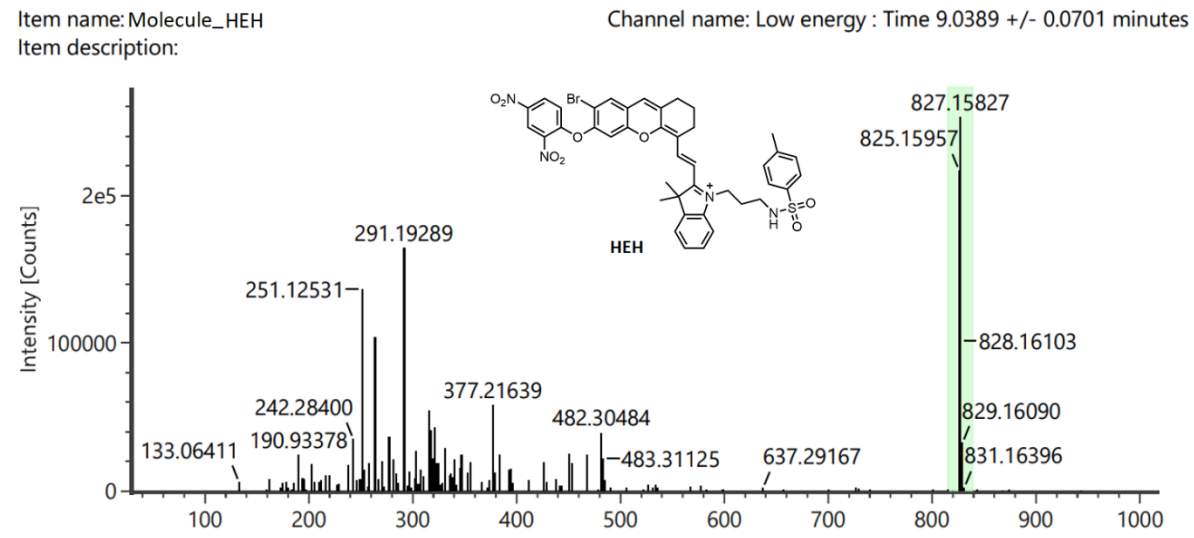


Figure S31. HRMS spectrum of compound **HEH**.

References:

1. H. Gunduz, K. Bilici, S. Cetin, A. Muti, A. Sennaroglu, H. Y. Acar, S. Kolemen, *Journal of J. Photochem. Photobiol. B, Biol.* **2021,** *217,* 112171.
2. Z. Luo, Z. Huang, K. Li, Y. Sun, J. Lin, D. Ye, H.-Y. Chen, *Anal. Chem.* **2018**, *90,* 2875-2883.
3. Y. Akkoc, L. Lyubenova, H. Grausgruber, D. Janovská, A. Yazici, I. Cakmak, D. Gozuacik Minor cereals exhibit superior antioxidant effects on human epithelial cells compared to common wheat cultivars Journal of Cereal Science, 85 (2019), pp. 143-152.
4. Akkoc, Y., Dalci, K., Karakas, H. E., Erbil-Bilir, S., Yalav, O., Sakman, G., … Gozuacik, D. (2022). Tumor-derived CTF1 (cardiotrophin 1) is a critical mediator of stroma-assisted and autophagy-dependent breast cancer cell migration, invasion and metastasis. Autophagy, 19(1), 306–323.