Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Self-reporting Intracellular Delivery Agent for Singlet Oxygen

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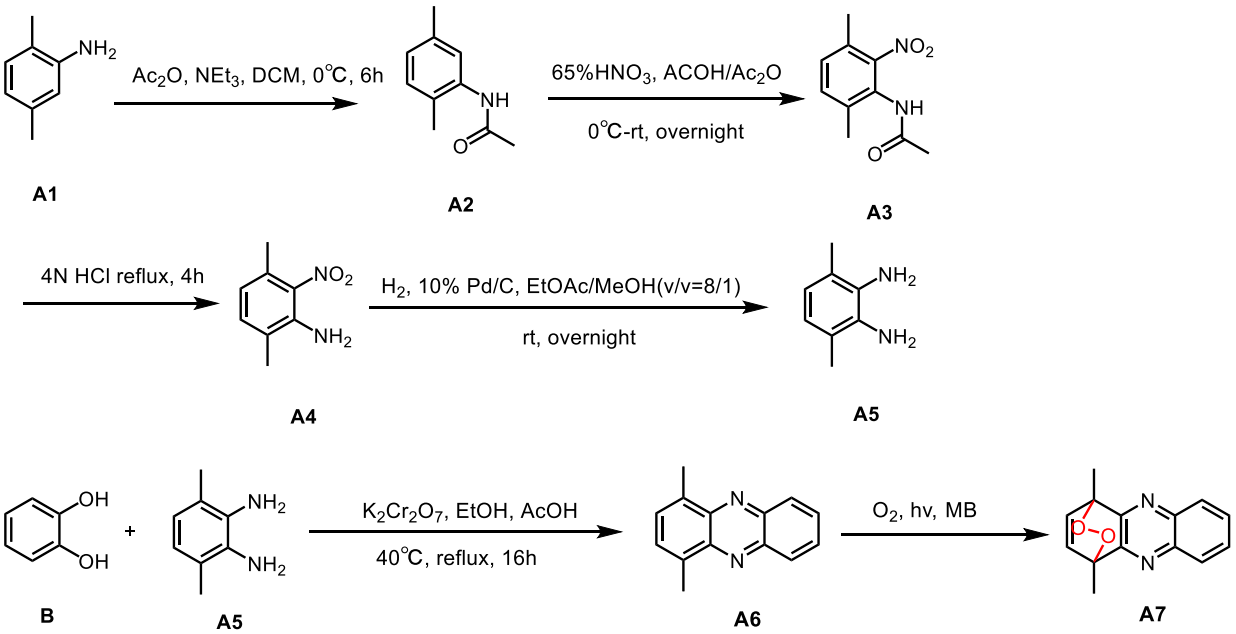
1.Synthesis of 1,4-dimethylphenazine endoperoxide (A7)



Scheme S1. The synthesis of **1,4-dimethylphenazine endoperoxide（A7）**.

Synthesis of **A2:** To a solution of **A1** (1.2 g, 10 mmol) mixed solvent of acetic anhydride (1 mL) and trethylamine (1.6 mL) at 0 °C in an atmosphere of aryon. This mixture was stirred at room temperature for 2 hours. The mixture was poured into ice and stirred rigrously for another 30min. The organic layer was then washed sequentially with HCl (2 mol/L), $NaHCO_3$ and brine, dried by Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (Hexane : EtOAc = 1:1, v/v) to afford **A2** with 15% yield.

^{1}H NMR (400 MHz, CD_3OD) δ 7.11 – 7.07 (m, 2H), 6.94 (d, J = 7.6 Hz, 1H), 2.27 (s, 3H), 2.17 (s, 3H), 2.11 (s, 3H).

Synthesis of **A3:** To a solution of **A2** (500mg, 3.06 mmol) mixed solvent of acetic acid (1.5 mL) and acetic anhydride (1.5 mL) at 0 °C, 65 % nitric acid (275 μL) was added dropwise. This mixture was stirred overnight at room temperature and then poured onto crushed ice, extracted with ethyl acetate. The combined extracts were washed with aqueous $NaHCO_3$ and brine, dried, concentrated, and purified by column chromatography (Hexane : EtOAc = 3:1, v/v) to afford **A3** with 63% yield.

^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (d, J = 7.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 2.27 (s, 3H), 2.23 (s, 3H), 2.09 (s, 3H).

Synthesis of **A4*:*** A mixture of **A3** (100 mg, 0.56 mmol) in 4 N hydrochloride (3.7 mL) was heated to reflux for 4 h. After cooled to room temperature, the mixture was neutralized with aqueous $NaHCO_3$ and extracted with ethyl acetate. The extracts were washed with brine, dried, and concentrated to give **A4** with 97% yield.

1H NMR (400 MHz, CD_3OD) δ 7.02 (d, J = 7.5 Hz, 1H), 6.44 (d, J = 7.5 Hz, 1H), 2.28 (s, 3H), 2.13 (s, 3H).

Synthesis of **A5***:* To a solution of **A4** (90 mg, 0.54 mmol) in mixed solvent of ethyl acetate (0.7 mL) and methanol (88 μL), 8 mg of 10 % Pd/C was added. This mixture was vacuumed and backfilled with hydrogen and stirred overnight at room temperature before filtration. The filtrate was concentrated to **A5** with 99% yield.

^{1}H NMR (400 MHz, CD_3OD) δ 6.40 – 6.38 (s, 2H) 2.08 (s, 6H).

Synthesis of **A6***:* The *o*-benzoquinone was freshly prepared by stirring a solution of catechol (60 mg, 0.43 mmol) in $CHCl_3$ (8.62 mL) into a solution of $K_2Cr_2O_7$ (253 mg, 0.86 mmol) in 1 M H_2SO_4 (4.3 mL). After stirring for 10 min at room temperature, the organic fraction was collected, the solvent was removed under reduced pressure, and the benzoquinone was reacted with **A5** (30 mg, 0.22 mmol) along with AcOH (130 μL), using DCM (2.5 mL) as solvent. The reaction mixture was heated to 40 °C and stirred overnight at this temperature.The solution was then extracted with saturated aqueous $NaHCO_3$ and DCM. The organic fractions were collected and dried over sodium sulfate, the solvent was removed under reduced pressure, and the product was purified by silica gel chromatography (Hexane : CH_2Cl_2 = 3:1, v/v) to afford **A6** with 15% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, J = 10.1 Hz, 2H), 7.73 (d, J = 10.1 Hz, 2H), 7.46 (s, 2H), 2.81 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.2, 141.1, 134.3, 128.8, 128.7, 128.5, 16.6.

Synthesis of **A7: A6** (30 mg, 0.14 mmol) was dissolved in $CDCl_3$ (1 mL). The reaction mixture was cooled to 0 °C in the ice bath. Methylene blue (10 mg, 0.03 mmol) was added into the solution and mixture was stirred for 8 h under oxygen atmosphere. During the reaction, 18 W, 630 nm red light was used. After removal of the solvent by rotary evaporator. Activated carbon was used to remove the methylene blue. The product was white solid from with 90% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 9.7 Hz, 2H), 7.67 (d, J = 9.7 Hz, 2H), 6.76 (s, 2H), 1.94 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.1, 139.2, 138.5, 128.7, 128.2, 80.1, 13.7.

2.Optical Measurements

UV absorption and fluorescence emission of **A6** and **A7** at different concentrations in solution.

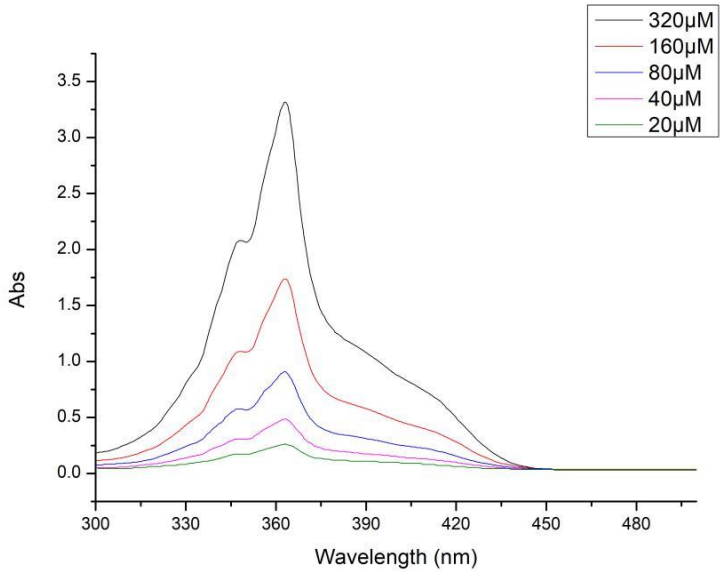


Figure S1. Electronic absorption spectra of **A6** at different concentrations in solution (DMF/MeCN = 1:2, v/v)

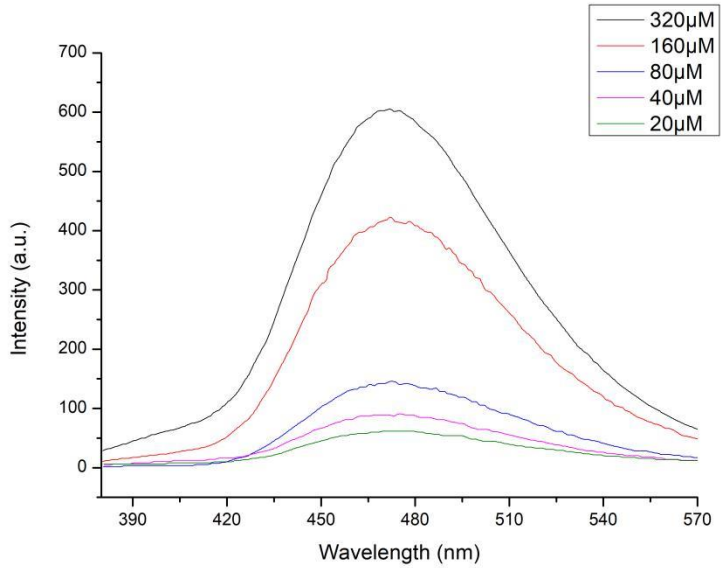


Figure S2. Fluorescence spectra of **A6** at different concentrations in solution (DMF/MeCN = 1:2, v/v) (λ_{ex}=383 nm)

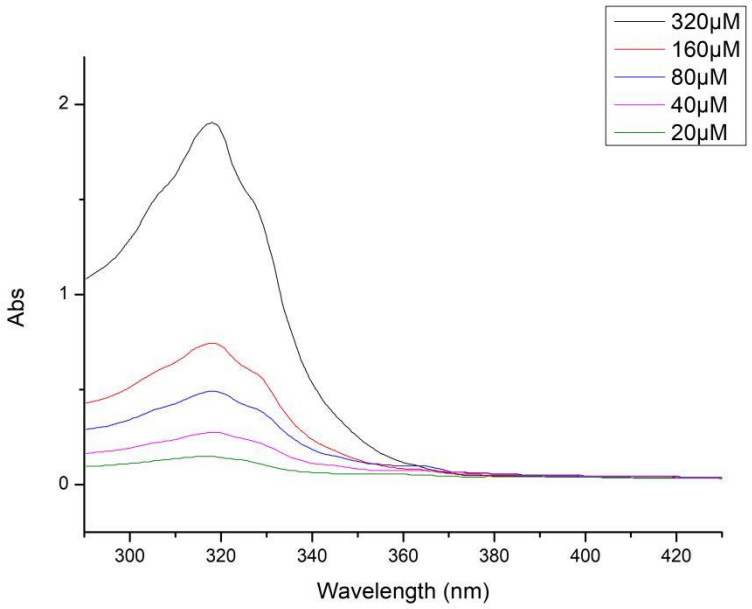


Figure S3. Electronic absorption spectra of **A7** at different concentrations in solution (DMF/MeCN= 1:2, v/v).

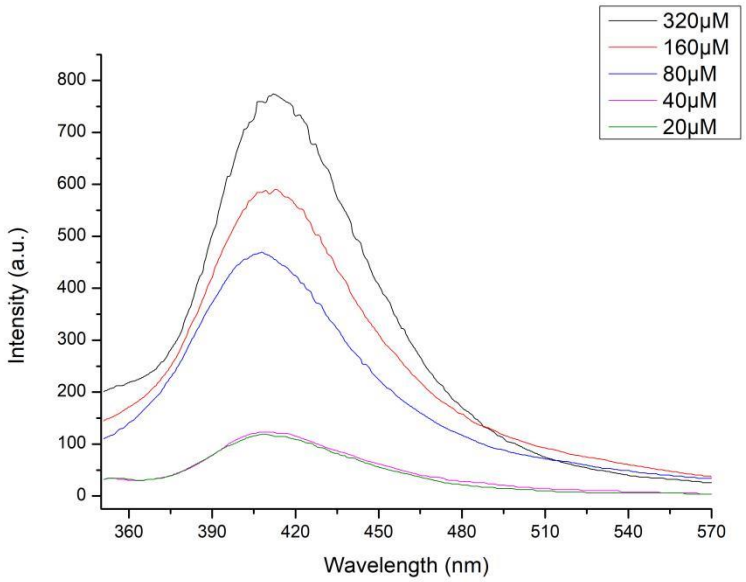


Figure S4. Fluorescence spectra of **A7** at different concentrations in solution (DMF/MeCN = 1:2, v/v) (λ_{ex} 313 nm)

Dissolve **A7** in the above solution (DMF/MeCN = 1:2, v/v) and place the reaction bottle in a 37 ^{o}C water bath with dark condition. Monitor UV and fluorescence changes during the decay process of **A7** at 37 ^{o}C within 96 hours. The changes in UV and fluorescence spectra confirm that under this condition, **A7** can spontaneously transform into **A6**.

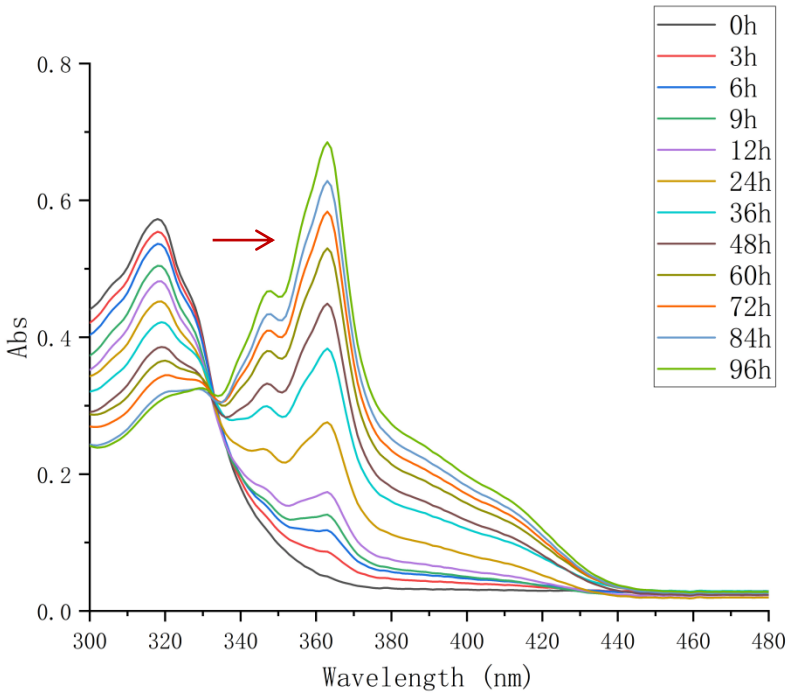


Figure S5. UV spectra variation of **A7** (200 μM) during decay at 37 °C.

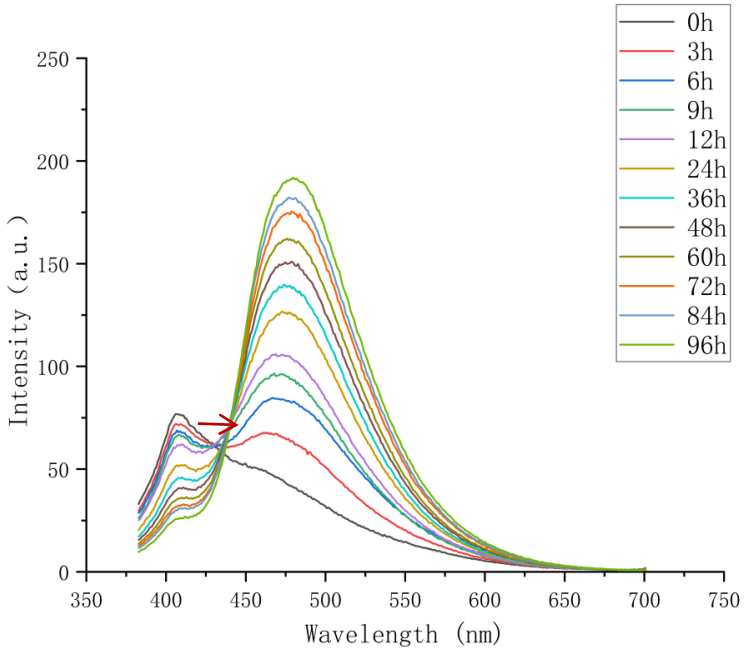


Figure S6. Fluorescence spectra variation of **A7** (200 μM) during decay at 37 °C.

3.Detection of Singlet Oxygen Release

In singlet oxygen generation experiments, 1,3-Diphenylisobenzofuran (DPBF) was used as chemical singlet oxygen trap molecule in DMSO. This procedure includes approximately 6 mM **A7**, each mixed with trap compound (approximately 40 μM) in DMF. Measurements were taken at 20 minutes intervals at 37 ºC in dark conditions. Absorbance decrease of trap molecules at 417 nm was monitored, revealing singlet oxygen generation.

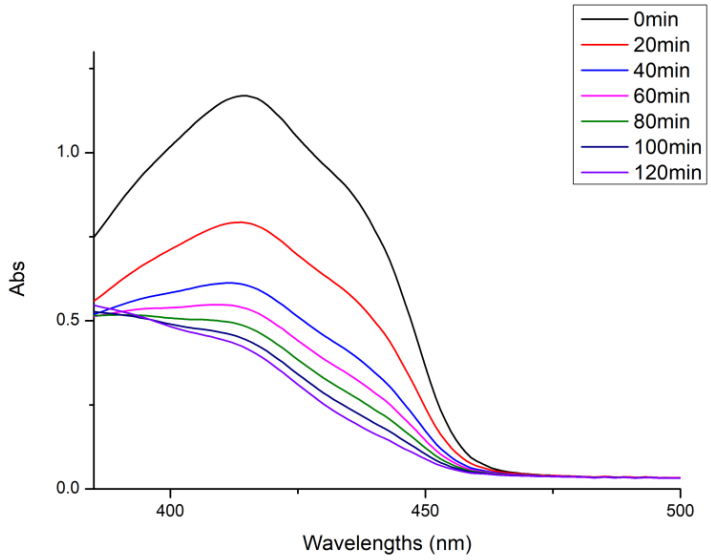


Figure S7. Decreasing absorbance peak for the singlet oxygen trap DPBF at 417 nm with time in DMSO in response to the addition of **A7.**

4.Temporal Evolution of A7 ^{1}H NMR at 37 °C

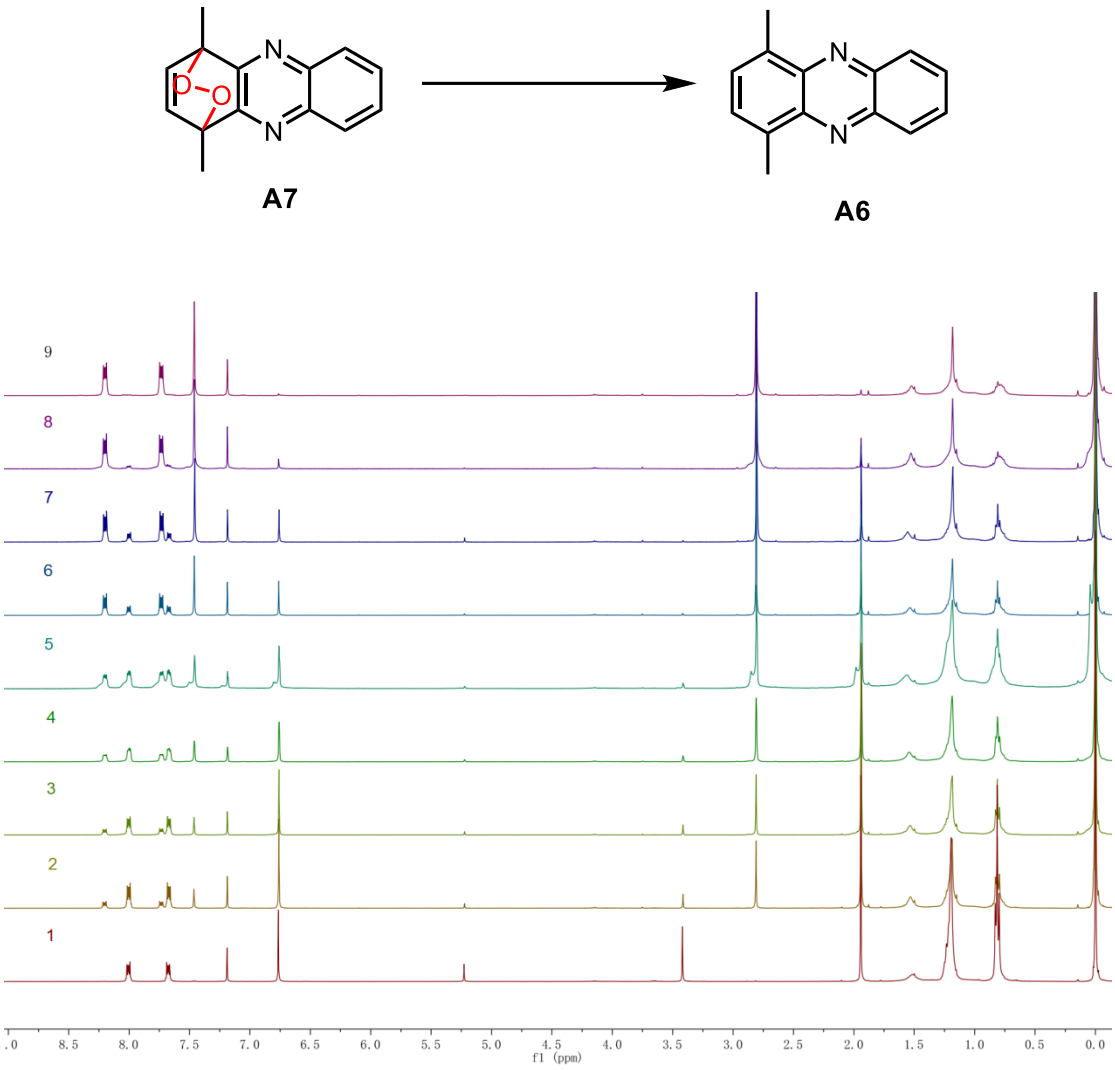


Figure S8. Temporal evolution of **A7** ^{1}H NMR at 37 °C in $CDCl_3$. (from bottom to top, 1: 0 hours, 2: 19 hours, 3: 24 hours, 4: 42 hours, 5: 67 hours, 6: 144 hours, 7: 187 hours, 8: 361 hours, 9: **A6**).

The half-life of **A7** was calculated in accordance to the first-order reaction and the ^{1}H NMR integral ratios and peak integrals between 6.8 to 7.5 ppm was used for this calculation. The formula are given below:

$\ln[A] = -kt + \ln[A]0$, $t1/2 = 0.693/k$

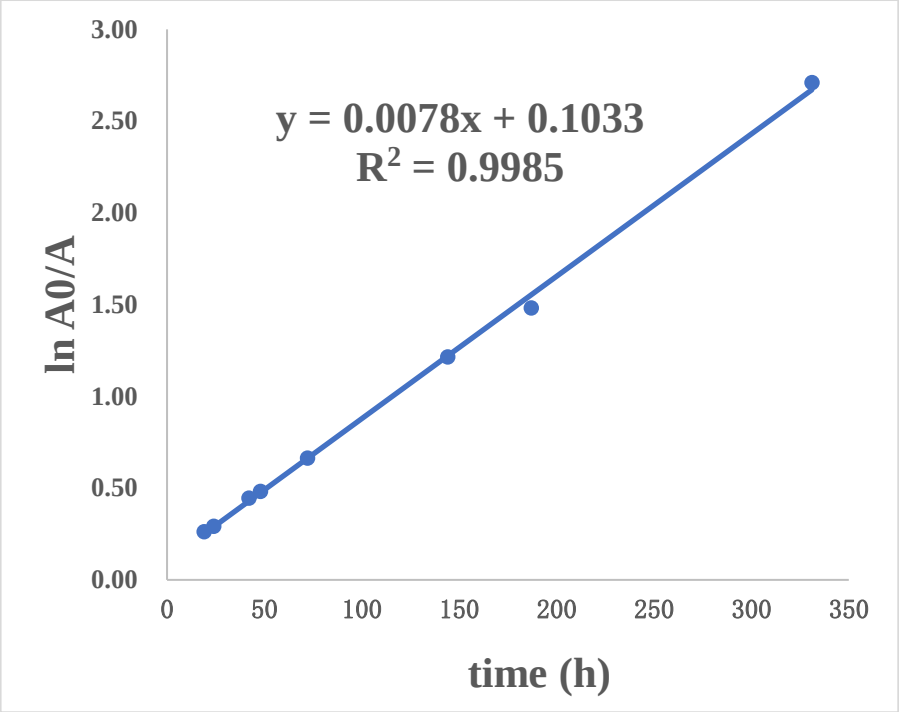


Figure S9. Half-life calculation of **A7**: 88.8 hours (at 37 ^{o}C in $CDCl_3$)

5.MTT Assay

4T1/ SKOV3/ MCF-7/ HepG2/ Hela/ A549 cells were plated at 96-well plates (8×10^3 cells per well in 100 µL medium containing 10% FBS and 1% penicillin), then incubated at 37 ^{o}C in a humidified atmosphere composed of 2% O_2 and 5% CO_2 for 24 h. The cells were treated with **A6** and **A7** at different concentrations (5, 10, 20, 30, 40, 60, 80, and 100 µM) for 24 h. Then, MTT solution (20 µL of 0.5 mg/ml in PBS) was added to each well and the cells were incubated at 37 ^{o}C for 4 h. Subsequently, the medium was removed and 150 µL DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm with a microplate reader.

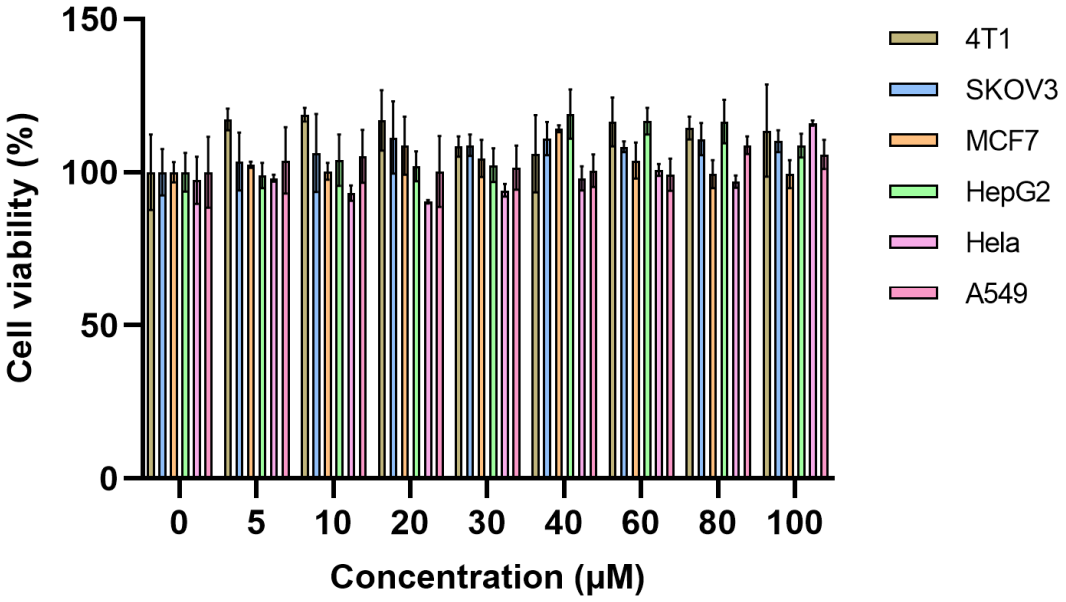


Figure S10. Cell viability of 4T1/ SKOV3/ MCF-7/ HepG2/ Hela/ A549 cells after incubation with various concentrations of **A6** for 24 hours. Data were assessed as mean ± SD (n = 6).

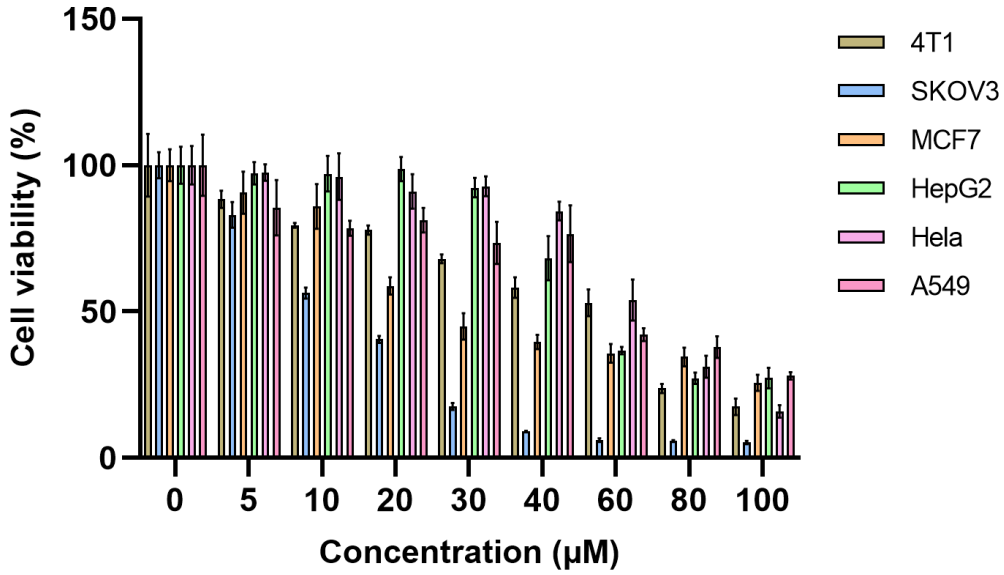


Figure S11. Cell viability of 4T1/ SKOV3/ MCF-7/ HepG2/ Hela/ A549 cells after incubation with various concentrations of **A7** for 24 hours. Data were assessed as mean ± SD (n = 6).

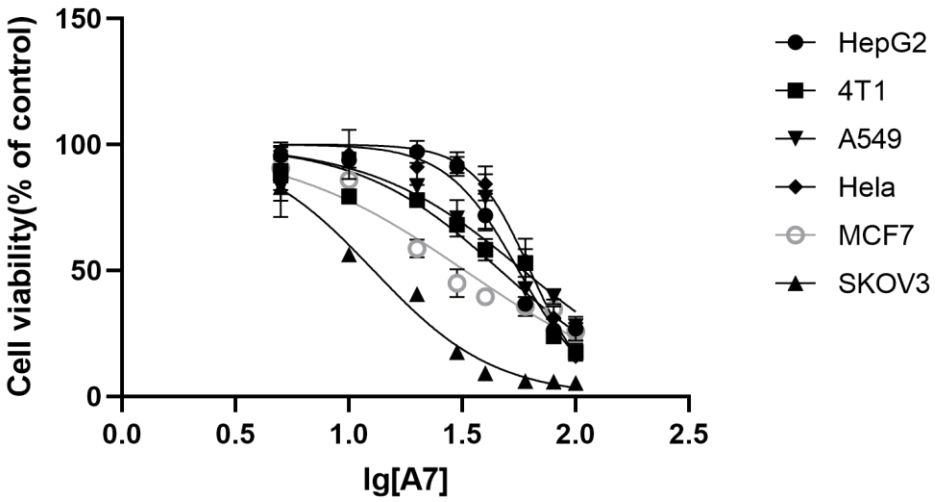


Figure S12. IC_{50} of 4T1/ SKOV3/ MCF-7/ HepG2/ Hela/ A549 cells after incubation with **A7**. (4T1: 46.6 μM; SKOV3: 12.9 μM; MCF-7: 32.4 μM; HepG2: 56.1 μM; Hela: 63.0 μM; A549: 58.7 μM).

7.Cell Migration by Scratch Test

HepG2 cells were plated at 24-well plates (5×10^4 cells per well in 1 mL DMEM medium containing 10% FBS and 1% penicillin), then incubated at 37 ^oC in a humidified atmosphere composed of 2% O_2 and 5% CO_2 for 24 h. On the back of 24-well plates, one straight line was equidistantly drawn using a ruler across each hole. Except the control group (no treatment), each well of cells were treated with **A6** and **A7** at 30 μM. The scratch condition at 0 h was recorded under a microscope for the convenience of subsequent analysis. At 24 h and 48 h after the scratch, the same location was selected to capture photos and record. The wound healing rate was calculated using the following equation:

Wound healing rate = (Initial scratch area - Scratch area at **t** time)/Initial scratch area

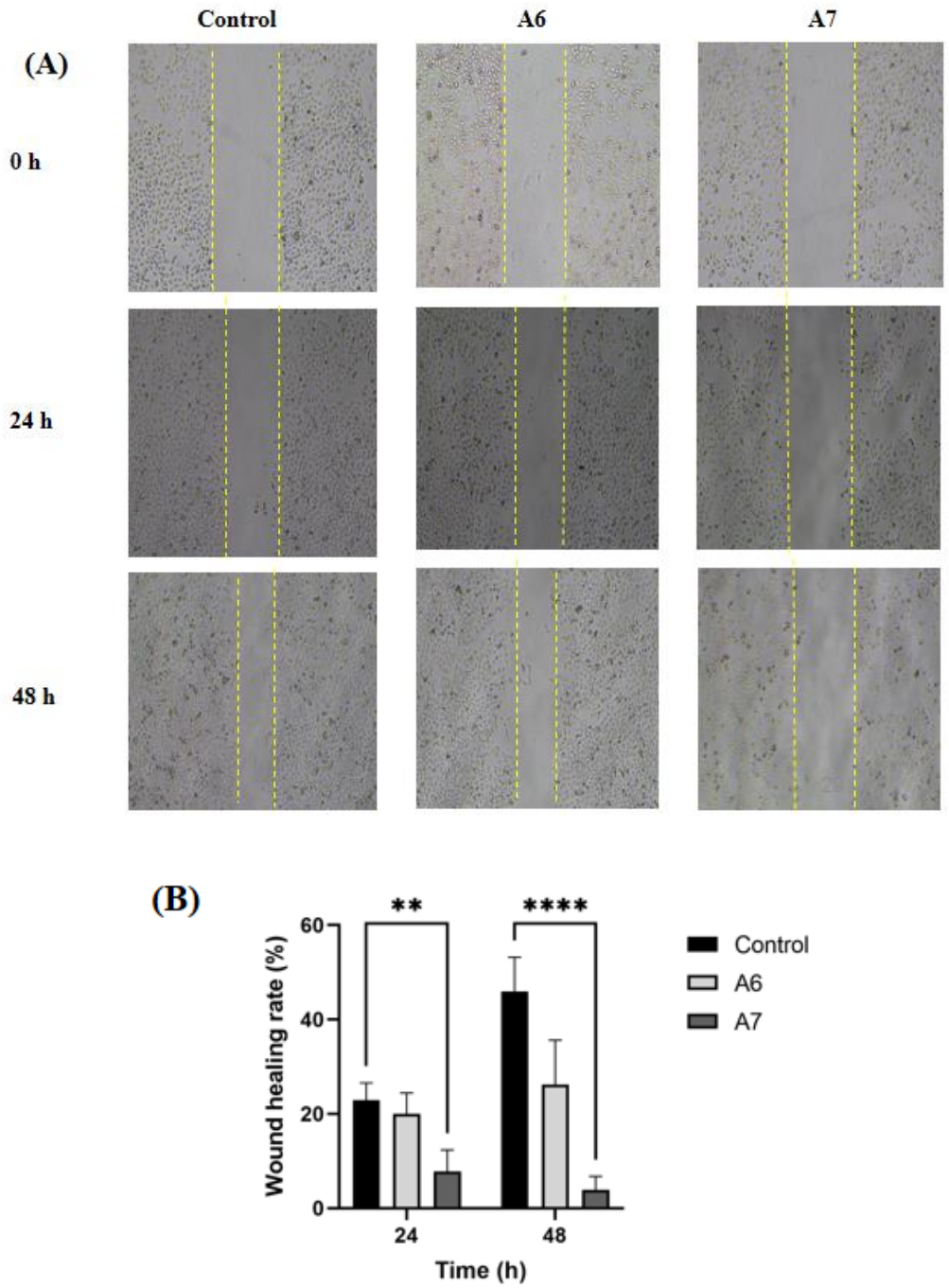


Figure S14. HepG2 cell migration determined by cell scratch test. **(A)** Representative images of HepG2 cell migration at 0, 24 h and 48 h in each group; **(B)** Quantitative statistics of cell healing rate at 24 h and 48 h in each group. (**p < 0.01; ****p < 0.0001)

8. Cell Migration by Transwell Assay

HepG2 cells were treated with **A6** and **A7** at 30 μM (no treatment in the control group) for 24 h. Cells were seeded into the upper chamber at 1×10^6 cells/mL while the lower chamber contained 600 μL of medium with 10% FBS. After 24 h the cells in the upper chamber were wiped off using cotton swabs, fixed in methanol for 30 min, and stained with 0.1% crystal violet for 10 min. After washing in PBS, the cells were observed under a microscope and immediately photographed in randomly-selected visual fields.

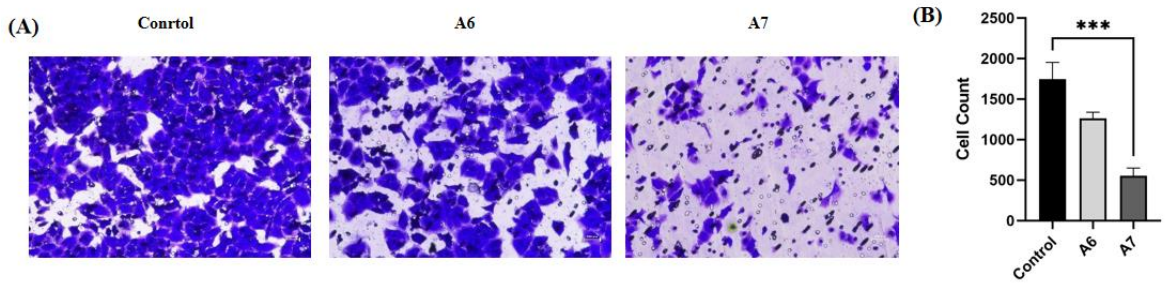


Figure S15. HepG2 cell migration determined by transwell assay. **(A)** Representative images of HepG2 cell migration in each group; **(B)** Analysis of cell migration in each group. (***$p < 0.001$)

9.Cell Imaging Experiment

HepG2 cells were plated at dishes (1×10^5 cells per well in 1 mL DMEM medium containing 10% FBS and 1% penicillin), then incubated at 37 ^oC in a humidified atmosphere composed of 2% O_2 and 5% CO_2. After 24 hours, the cells were incubated with **A7** (20 μM) for 0, 4, 8, 12, 24 h. Then cells were washed three times with DMEM medium and fluorescence was observed through the High Content Imaging.

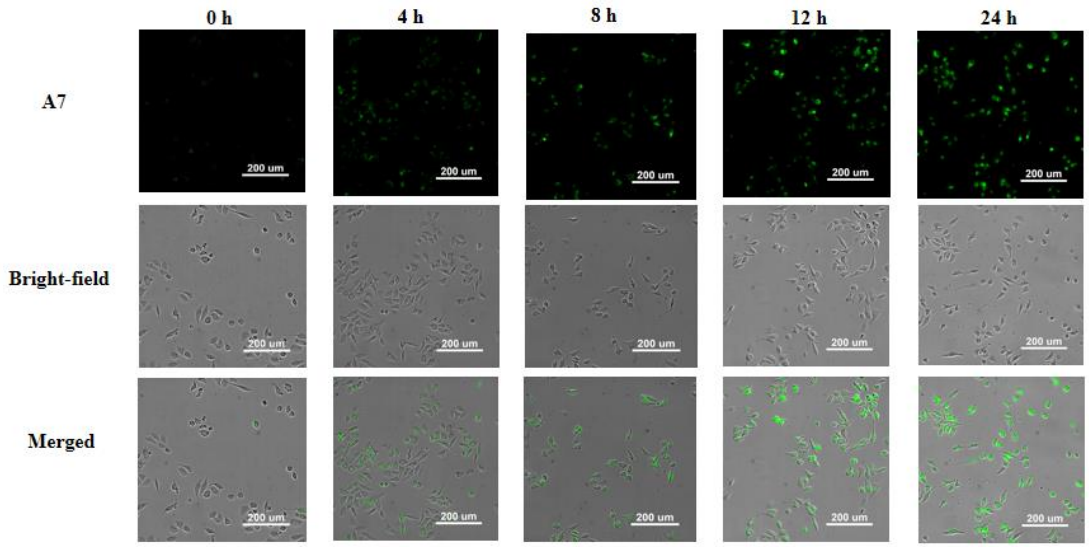


Figure S16. Confocal images showing the HepG2 cells treated with **A7** (20 μM) for 0, 4, 8, 12, 24 h. First row: **A7**, second row: bright field images, third column: merged images.

10.Intracellular Singlet Oxygen Generation

HepG2 cells were plated at dishes (1×10^5 cells per well in 1 mL DMEM medium containing 10% FBS and 1% penicillin), then incubated at 37 ^{o}C in a humidified atmosphere composed of 2% O_2 and 5% CO_2. After 24 hours, the cells were incubated with **A7** (50 μM) for 4 h (no treatment in the control group). Following this, the medium was replaced and washed with PBS. The cells were incubated for 20 min at 37°C after being treated with DCFH-DA (10 μM) in DMEM. The solution was removed after the incubation, and the cells were washed with PBS. The imaging was observed with the High Content Imaging.

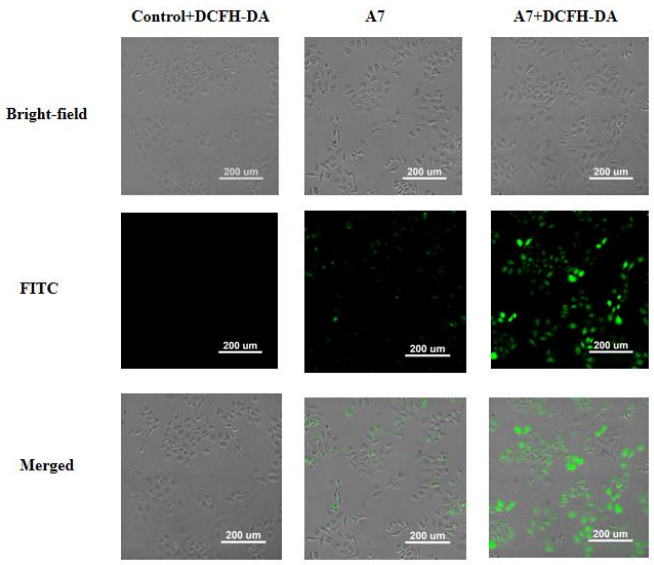


Figure S17. Confocal images of the singlet oxygen release with reactive oxygen species (ROS) probe DCFH-DA. HepG2 cells incubated with **A7** at 50 μM, merged images of DCFH-DA.

11.Apoptosis Experiment

HepG2 cells were plated at dishes (1×10^5 cells per well in 1 mL DMEM medium containing 10% FBS and 1% penicillin), then incubated at 37 ºC in a humidified atmosphere composed of 2% O_2 and 5% CO_2. After 24 hours, the cells were incubated with **A6** (160 μM) and **A7** (160 μM) for 4 h (no treament in the control group). After that, the cells were washed with PBS and stained according to the kit instructions, and the apoptosis of the cells was assessed using the High Content Imaging.

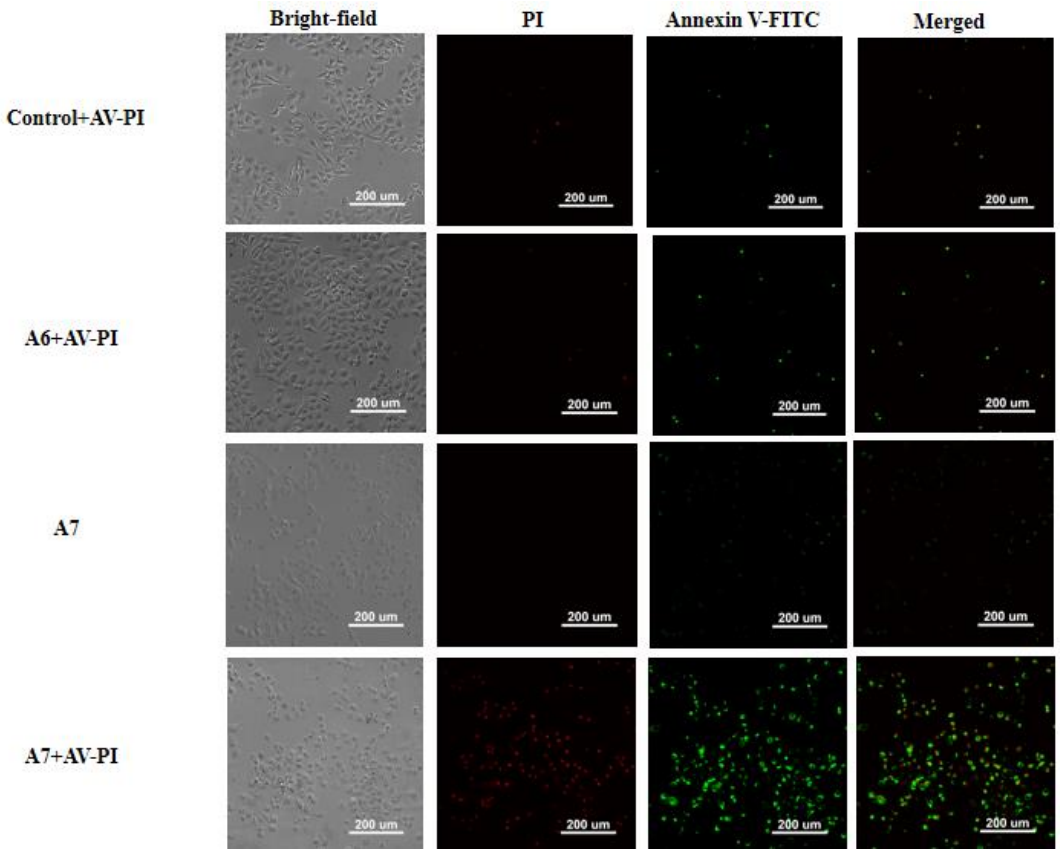


Figure S18. Confocal images of HepG2 cells with AnnexinV-FITC and propidiumiodide (PI). HepG2 cells incubated with **A6** and **A7** at 160 μM.

12.Live Dead Cell Staining Experiment

HepG2 cells were plated at dishes (1×10^5 cells per well in 1 mL DMEM medium containing 10% FBS and 1% penicillin), then incubated at 37 ºC in a humidified atmosphere composed of 2% O_2 and 5% CO_2. After 24 hours, the cells were incubated with **A6** (40 μM) and **A7** (40 μM, 160 μM) for 4 h (no treatment in the control group). After that, the cells were washed with PBS and stained according to the kit instructions, and the live dead condition of the cells was assessed using the High Content Imaging.

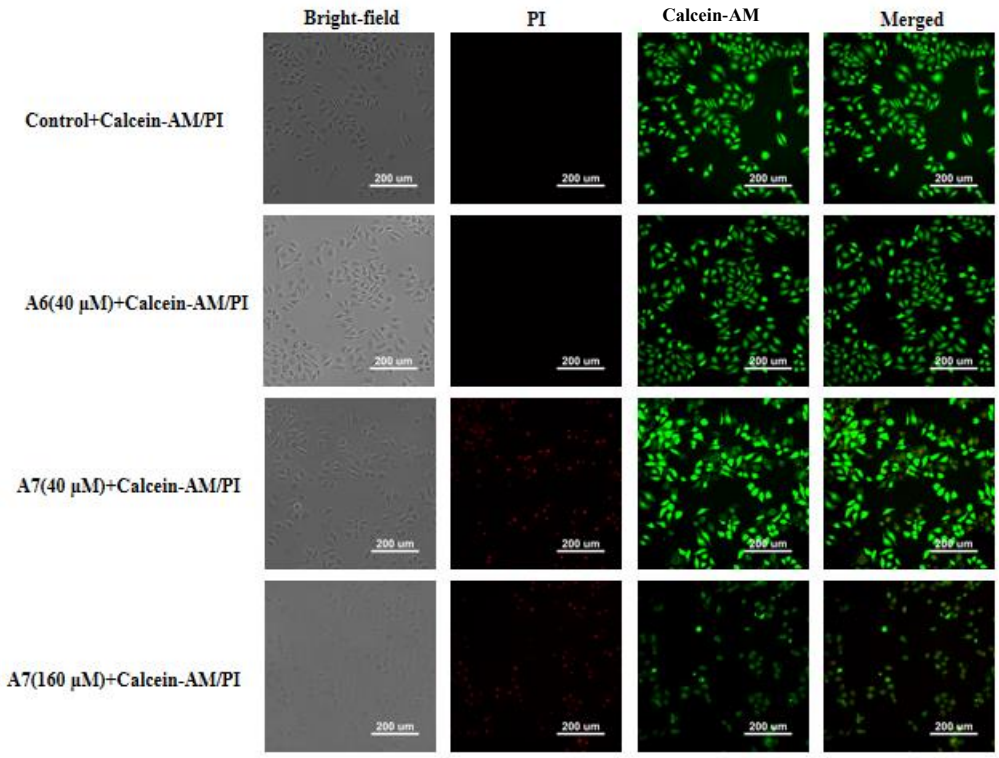


Figure S19. Confocal images of HepG2 cells with Calcein-AM and PI. HepG2 cells incubated with **A6** at 40 μM and **A7** at 40μM, 160 μM.

13.High Content Imaging of Multicellular Tumor Spheroids Intake Endoperoxide

5×10^3 per dish HepG2 cells were seeded with DMEM medium containing 10% FBS and 1% penicillin on Nunclon Sphera 96U Bottom Plate. The multicellular tumor spheroids were incubated at 37 ^oC in a humidified atmosphere composed of 2% O_2 and 5% CO_2. The culture medium was replaced every two days. The formation of the multicellular tumor spheroids was monitored by Nikon Model Eclipse Ts2-FL.

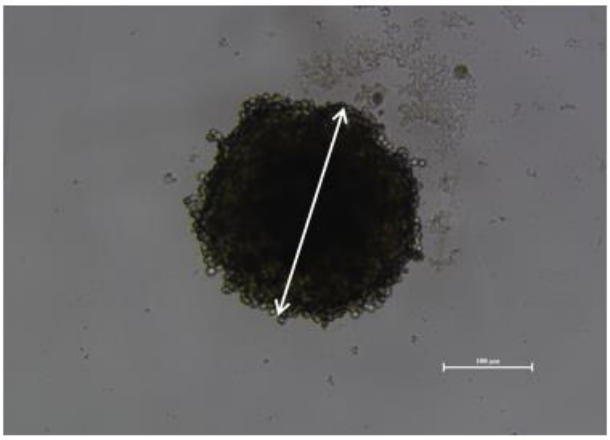


Figure S20. The formation of the multicellular tumor spheroids on the fifth day.

On the fifth day, the medium was replaced by new medium incubation with 30, 60, 100 µM **A7** at 37 ºC for 6 h. The Nunclon Sphera 96U Bottom Plate was taken in the High Content Imaging System.

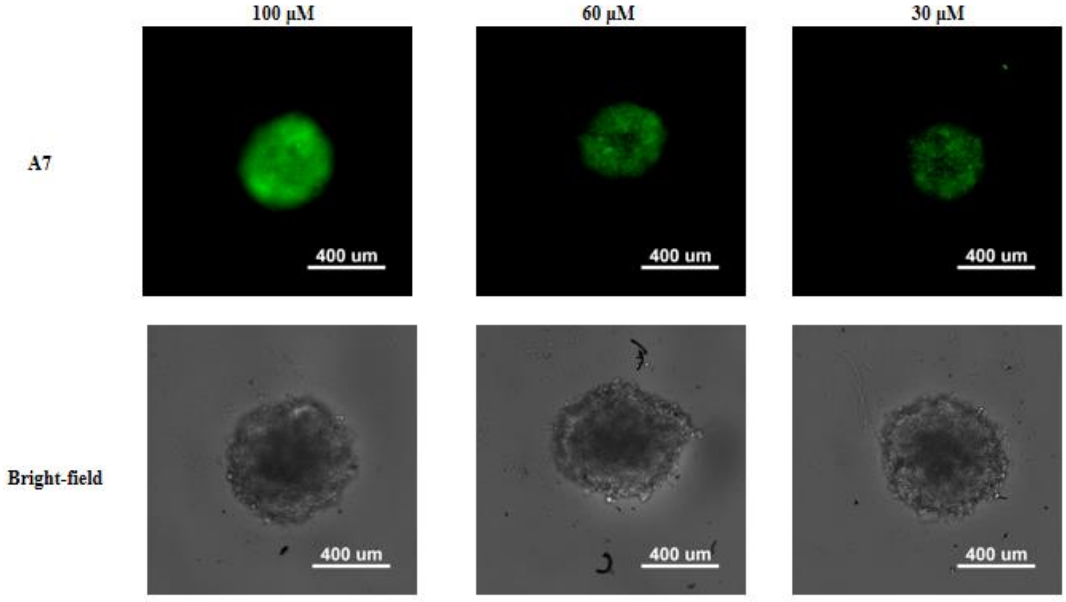


Figure S21. HCIS images of the multicellular tumor spheroids incubated with **A7** at 30, 60, 100 µM.

14.High Content imaging of multicellular tumor spheroids apoptosis

Annexin-FITC/PI Apoptosis Detection Kit was used for detection of **A7** induced multicellular tumor spheroids apoptosis. Firstly, the multicellular tumor spheroids were incubated onto the Nunclon Sphera 96U Bottom Plate for 5 days, then they were treated with following treatment: incubated with **A7** (100 μM) at 37 ^{o}C for 10 hours. After washing twice of PBS, the multicellular tumor spheroids were stained with Annexin-FITC/PI Apoptosis Detection Kit according to the manufacture instructions. The fluorescence was observed through the High Content Imaging.

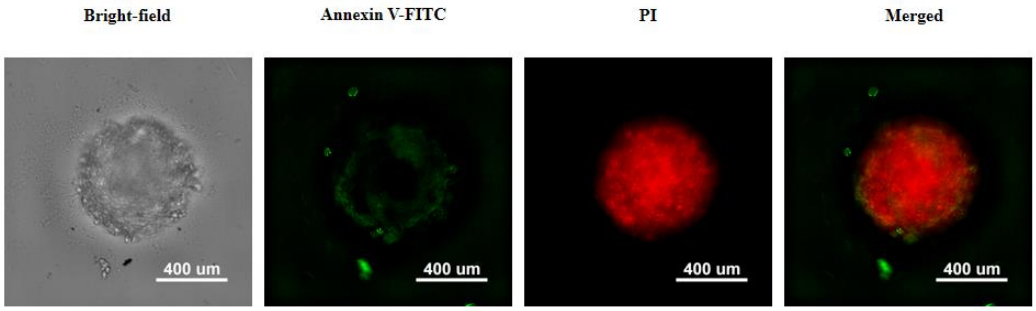


Figure S22. CLSM images of the multicellular tumor spheroids treated with fluorescein conjugate of Annexin V-FITC/PI. The multicellular tumor spheroids were incubated with **A7** (100 μM) at 37 ^{o}C for 10 hours.

13.NMR Spectra

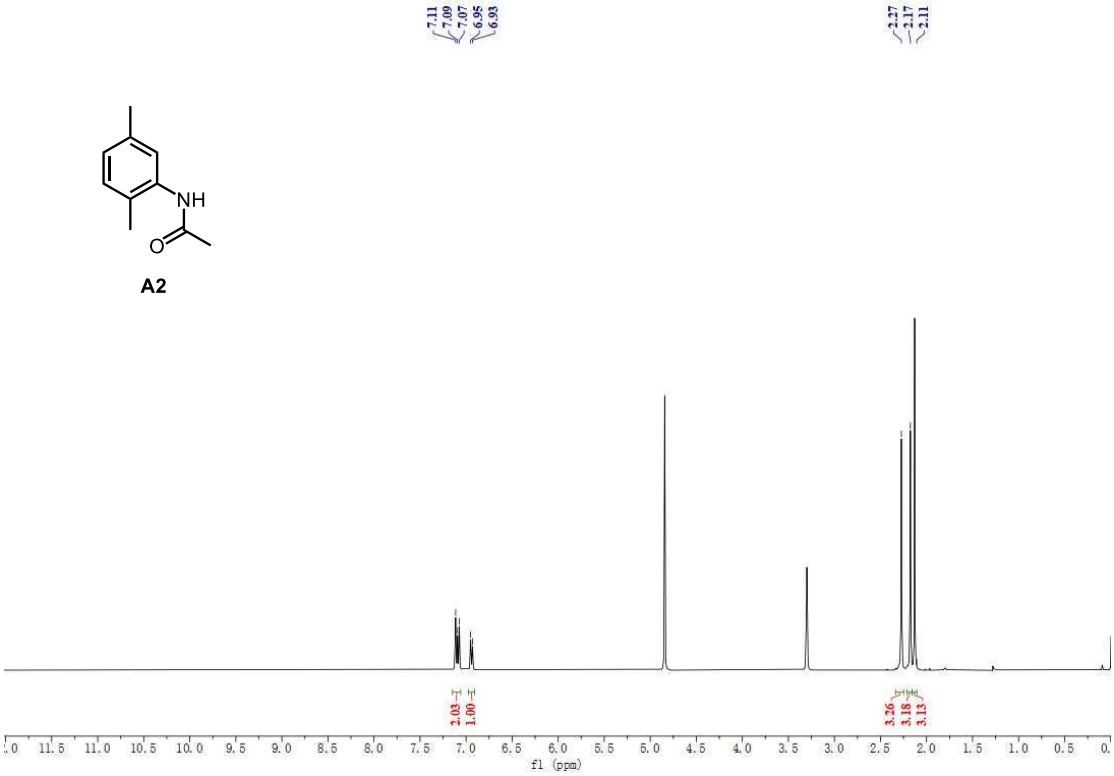


Figure S23. ^{1}H NMR Spectrum of compound **A2**

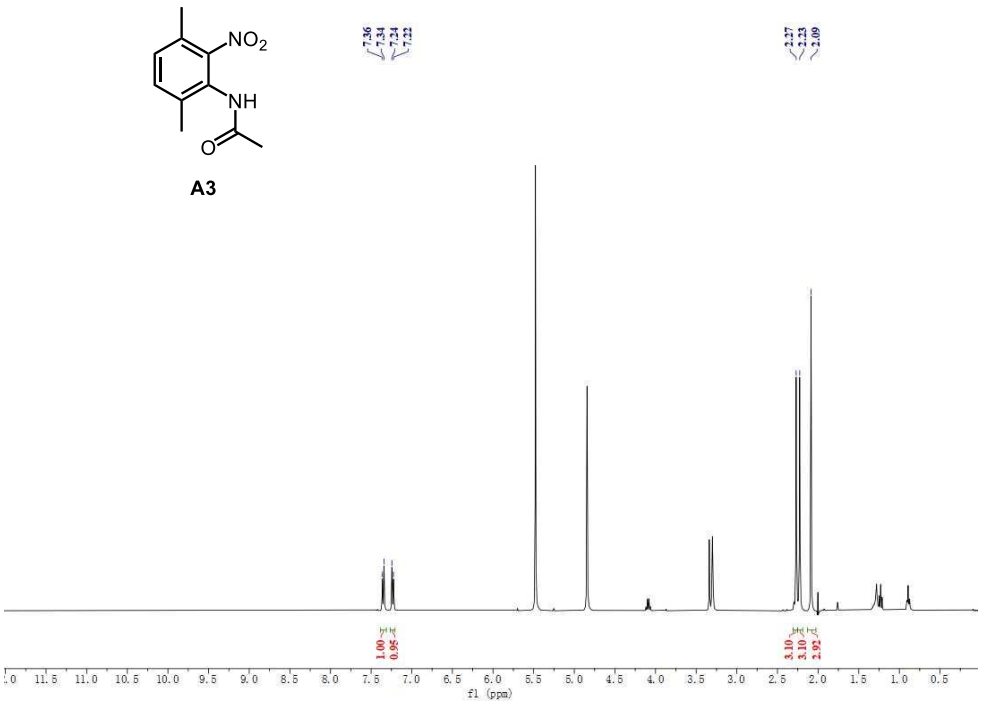


Figure S24. ^{1}H NMR Spectrum of compound **A3**

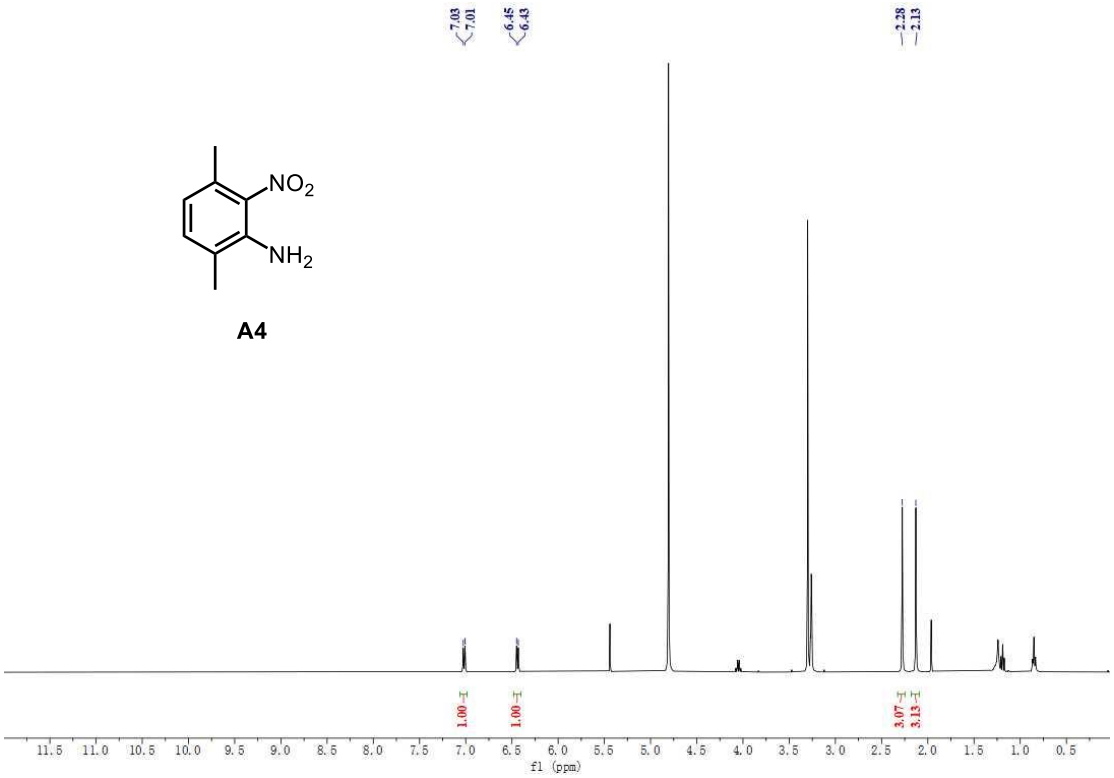


Figure S25. ^{1}H NMR Spectrum of compound **A4**

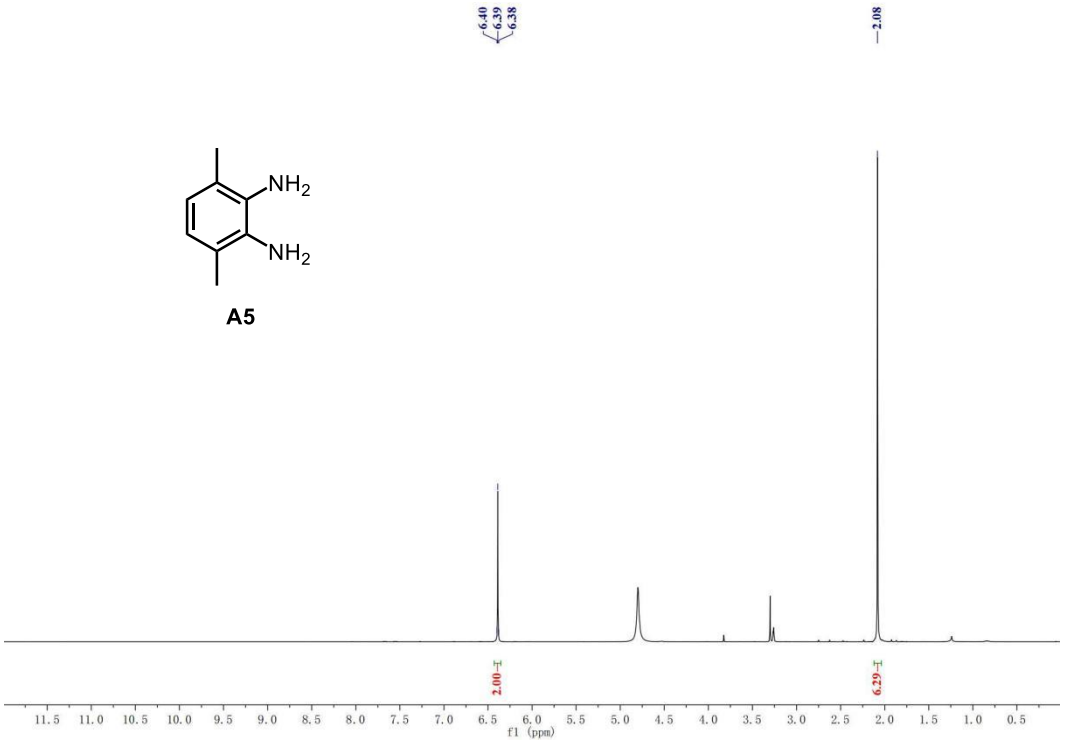


Figure S26. ^{1}H NMR Spectrum of compound **A5**

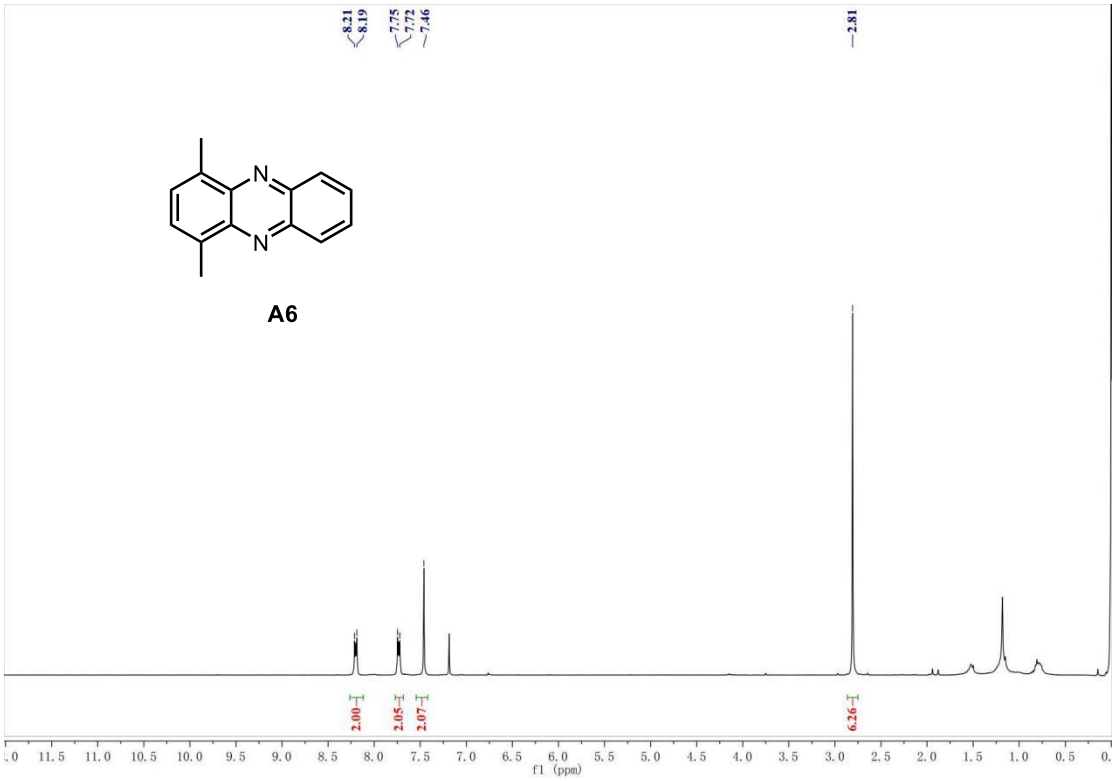


Figure S27. ^{1}H NMR Spectrum of compound **A6**

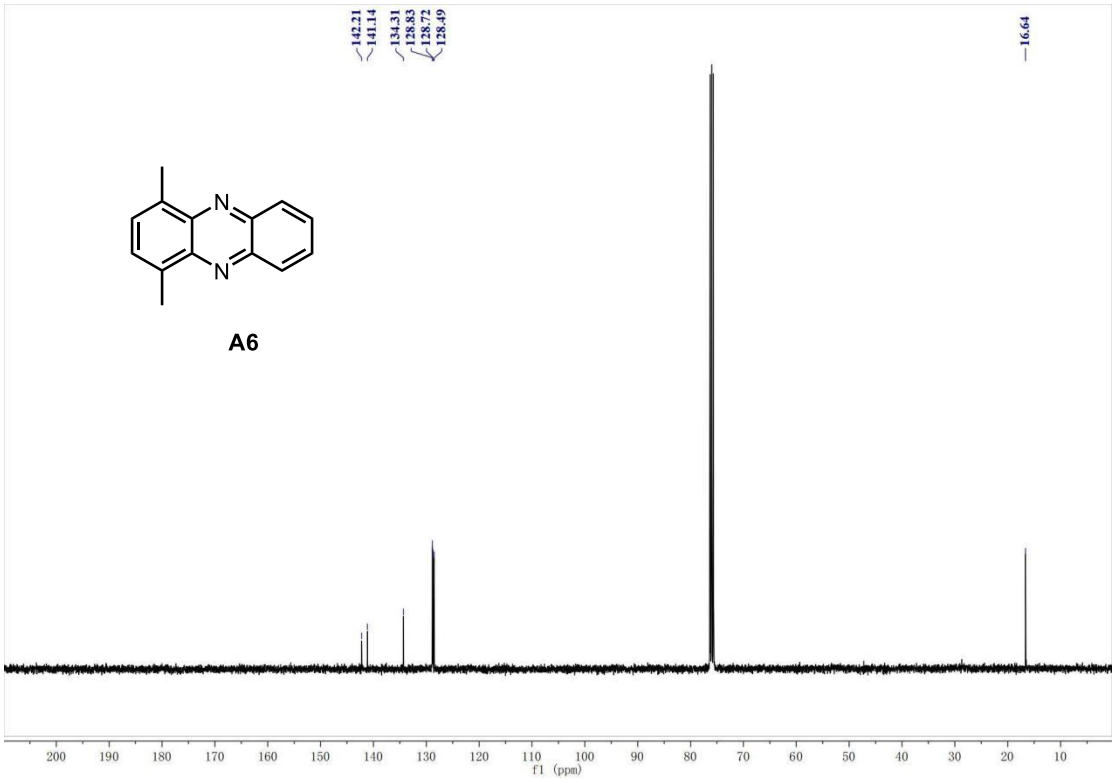


Figure S28. ^{13}C NMR Spectrum of compound **A6**

Figure S29. ^{1}H NMR Spectrum of compound **A7**

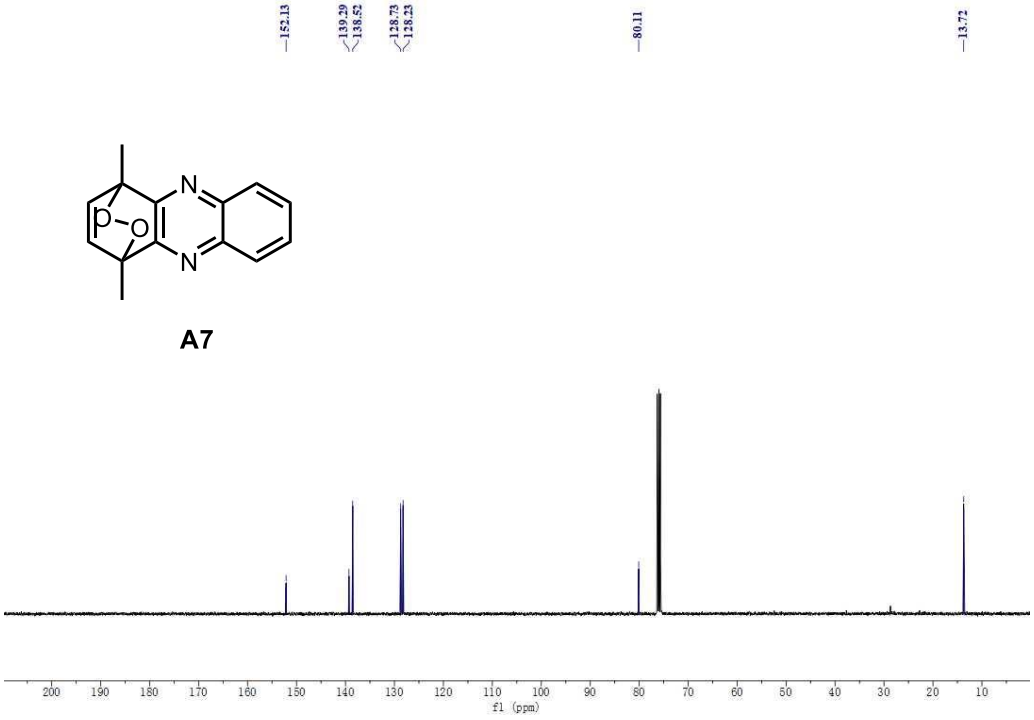


Figure S30. ^{13}C NMR Spectrum of compound **A7**