A "dual-key-and-lock" ratiometric fluorescent probe with biocompatibility and selectivity for imaging vicinal dithiol proteins

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Electronic Supplementary Information

Materials

3,5-diaminobenzoic acid, acetonitrile and zinc powder were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China, https://www.aladdin-e.com/). Maleic anhydride, ethanol, methanol, $CHCl_3$, anhydride, THF, DIPEA and sodium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China, https://www.sinoreagent.com/). $TiCl_4$, HATU and BSA were purchased from Macklin Reagent Co., Ltd. (Shanghai, China, http://www.macklin.cn/). Fetal bovine serum (FBS) was supplied by Hyclone. DMEM, RPMI 1640 medium and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Dalian Meilun Biotechnology (Dalian, China, http://www.meilune.com/). HepG2 cells were obtained from National Collection of Authenticated Cell Cultures (Shanghai, China, https://www.cellbank.org.cn/). All of chemicals were used without further purification. Deionized (DI) water of 18 MΩ cm was used to prepare all aqueous solutions.

Apparatus and characterization

NMR experiments were performed on a Bruker Avance NEO spectrometer. Photoluminescence spectra were obtained using a Shimadzu RF-6000 fluorescence spectrophotometer. MTT assay was conducted by using a BioTek Synergy H1 ELISA plate reader at 570 nm. Confocal laser scanning microscope (CLSM) images were obtained on an Olympus FV-1200 confocal laser scanning microscope.

Cell culture and MTT assay

HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium

was supplemented with 10% fetal bovine serum and appropriate amounts of antibiotics (penicillin and streptomycin).

The viability of cells treated with TMR-TPE and PAO-EDT was determined by an MTT assay with 3-(4,5-dimethylthiazole-2-yl) 2,5-phenyltetrazolium bromide. HepG2 cells were cultured in a 96-well plate at 37°C under 5% CO_2 for 12 h. Then, cells were treated with different concentrations of TMR-TPE and PAO-EDT, respectively. After 24 h incubation, 20 μL of 5 mg mL^{-1} MTT solution was added into each well for 4 h. Then, the medium was aspirated out, and 150 μL of DMSO was added to dissolve the formazan. Absorbance was measured at 570 nm. Cell viability (%) was determined by the following equation: Viability = (mean Abs. of treated wells/mean Abs. of control wells) × 100%.

Intracellular imaging of VDPs

HepG2 cells were seeded in confocal dishes and incubated at 37 °C under 5% CO_2 for 12 h, and then incubated with TMR-TPE (25 μM) for 120 min. For negative control experiment, the cells were pretreated with MBA (30 μM) or PAO (30 μM) for 30 min and then incubated with TMR-TPE (25 μM) for 120 min. Fluorescent images of cells were obtained by using FV-1200 confocal laser scanning microscope. (Green channel: λ_{ex} = 405 nm, λ_{em} = 460-500 nm; red channel: λ_{ex} = 559 nm, λ_{em} = 575-630 nm.).

Evaluation of the effect of oxidative stress on intracellular VDPs

HepG2 cells were seeded in in confocal dishes and incubated at 37°C under 5% CO_2 for 12 h. Next, the cells were pretreated with H_2O_2 (100 μM) for 30 min and then

incubated with TMR-TPE (25 μM) for 120 min. Fluorescent images of cells were obtained by using FV-1200 confocal laser scanning microscope. (Green channel: λ_{ex} = 405 nm, λ_{em} = 460-500 nm; red channel: λ_{ex} = 559 nm, λ_{em} = 575-630 nm.).

Imaging of intracellular VDPs in DILI model

HepG2 cells were seeded in confocal dishes and incubated at 37°C under 5% CO_2 for 12 h. Next, the cells were pretreated with APAP (200 μM or 1 mM) for 12 h and then incubated with TMR-TPE (25 μM) for 120 min. For evaluating the effect of the therapeutic drugs, cells were pretreated with GSH (500 μM), NAC (500 μM), and Glu (500 μM) for 1 h before co-incubation with APAP (1 mM) for 12 h. Fluorescent images of cells were obtained by using FV-1200 confocal laser scanning microscope. (Green channel: λ_{ex} = 405 nm, λ_{em} = 460-500 nm; red channel: λ_{ex} = 559 nm, λ_{em} = 575-630 nm.).

Synthesis of compound 1

3,5-diaminobenzoic acid (1000 mg; 6.55 mmol) and maleic anhydride (1924 mg; 19.65 mmol) was dissolved in $CHCl_3$ (60 mL) and heated to reflux for 20 h. The resulting precipitate was filtered, and acetic anhydride (50 mL) and sodium acetate (175.6 mg; 2.14 mmol) were added. The mixture was heated for another 1.5 h at 100°C. Cold water (150 mL) was added to the resulting clear solution, and the mixture was vigorously stirred for 2 h. The precipitate was filtered and washed with water (200 mL) and ethanol (50 mL) to afford compound 1. ^{1}H NMR (DMSO-*d6*): 7.98 (d, 2H), 7.65 (t, 1H), 7.23 (s, 4H). ^{13}C NMR (DMSO-*d6*): 170.04, 166.39, 135.30, 132.83, 132.48, 129.02,

126.81.

Synthesis of compound 2

$TiCl_4$ (6.59 ml, 50 mmol) was added to an anhydrous THF (60 mL) solution of zinc powder (3.92 g, 60 mmol) and 4-aminohenzophenone (3.92 g, 60 mmol) under N_2 atmosphere at 0℃. Then the mixture was heated to reflux for 5 h. The solution was cooled to room temperature and quenched by addition of aqueous K_2CO_3. The mixture was washed by dichloromethane for three times. After solvent evaporation, the residue was purified on a silica-gel column using CH_2Cl_2: MeOH 75:1 as solvent to yield pure compound 2. ^{1}H NMR (DMSO-*d6*): 7.12 (t, 4H), 7.06 (t, 2H), 6.98 (d, 4H), 6.54 (d, 4H), 6.26 (d, 4H), 4.97 (s, 4H). ^{13}C NMR (DMSO-*d6*): 147.24, 145.36, 139.06, 132.12, 131.67, 131.38, 128.00, 126.20, 113.63.

Synthesis of compound 3

Compound 1 (156.1 mg, 0.5 mmol)), compound 2 (181.2 mg, 0.5 mmol), HATU (228.1 mg, 0.6mmol) and DIPEA (87 μL, 0.5mmol) was dissolved in $CHCl_2$ (5 mL) and stirred at room temperature for 30 min. The mixture was purified on a silica-gel column using CH_2Cl_2: ethyl acetate 10:1 as solvent to yield pure compound 3. ^{1}H NMR (DMSO-*d6*): 10.29 (s, 1H), 7.95 (d, 2H), 7.59 (t, 1H), 7.48 (d, 2H), 7.26 (s, 4H), 7.19-7.09 (m, 6H), 7.01 (d, 4H), 6.89 (d, 2H), 6.59 (d, 2H), 6.29 (d, 2H), 5.06 (s, 2H). ^{13}C NMR (DMSO-*d6*): 170.04, 164.03, 147.73, 144.71, 144.58, 141.47, 140.19, 137.91, 137.09, 136.68, 135.35, 132.64, 132.12, 131.63, 131.41, 131.29, 130.87, 128.26, 128.16, 126.74, 126.53, 125.66, 120.14, 113.59.

Synthesis of compound 4

6-carboxyl tetramethylrhodamine (430.4 mg, 1.0 mmol) was dissolved in CH_3CN (50 mL), then triethylamine (208 μL, 1.5 mmol), HATU (456.3 mg, 1.2 mmol), and TTDDA-BOC (320.4 mg, 1.0 mmol) were added. The mixture was stirred at room temperature for 2 h. After solvent evaporation, the residue was purified on a silica-gel column using CH_2Cl_2 : MeOH = 5 : 1 as solvent to yield pure compound 4. ^{1}H NMR (DMSO-*d6*): 13.36 (s, 1H), 8.72 (t, 1H), 8.22 (t, 1H), 7.82 (s, 1H), 6.97-6.73 (m, 6H), 3.49-3.30 (m, 16H), 3.19 (s, 12H), 2.94 (d, 2H), 1.74 (t, 2H), 1.57 (t, 2H), 1.36 (s, 9H). ^{13}C NMR (DMSO-*d6*): 168.73, 164.94, 152.63, 152.43, 141.08, 129.63, 128.97, 125.14, 122.69, 109.55, 106.13, 98.44, 77.84, 70.19, 69.97, 68.65, 68.53, 46.03, 37.68, 37.38, 30.16, 29.59, 28.72, 9.30.

Synthesis of compound 5

2.2 mL TFA was added to an anhydrous CH_2Cl_2 (10 mL) solution of compound 4 (147.0 mg, 0.2 mmol). Then the mixture was stirred at room temperature for 5 h. After solvent evaporation, triethylamine (139 μL, 1.0 mmol), succinic anhydride (100.0 mg, 1.0 mmol) and MeCN (20 mL) were added. The mixture was stirred at room temperature for 12 h. After solvent evaporation, the residue was purified on a silica-gel column using CH_2Cl_2 : MeOH = 20 : 3 (0.1% TFA) as solvent to yield pure compound 5.

Synthesis of TPE-TMR

Compound 5 (73.5 mg, 0.10 mmol) was dissolved in CH_3CN (10 mL), then triethylamine (21 μL, 0.15 mmol), HATU (45.6 mg, 0.12 mmol) and compound 3 (65.7

mg, 0.10 mmol) were added. The mixture was stirred at room temperature for 2 h. After solvent evaporation, the residue was purified on a silica-gel column using CH_2Cl_2 : MeOH = 10 : 1 as solvent to yield TPE-TMR. ^{1}H NMR (DMSO-*d6*): 10.66 (s, 1H), 10.34-10.29 (m, 1H), 9.90-9.85 (m, 1H), 7.95 (d, 3H), 7.60 (d, 1H), 7.56-7.42 (m, 4H), 7.25 (s, 4H), 7.22-7.06 (m, 10H), 7.05-6.94 (m, 10H), 6.80 (d, 2H), 3.52-3.37 (m, 6H), 3.33 (s, 12H), 3.29-3.20 (m, 2H), 2.97-2.77 (m, 16H).

Synthesis of DAN-NH_2

Dansulfoyl chloride (487.4 mg, 1.8 mmol) was dissolved in 20 mL CH_2Cl_2, and ethylenediamine (6 mL, 90 mmol) was dissolved in 50 mL CH_2Cl_2. The above solutions were mixed in 0℃ and stirred at room temperature for 1 h. Then, 150 mL of 1 M HCl was added for extraction, the aqueous phase was collected and neutralized by 5 M NaOH, and 100 mL of dichloromethane was added for extraction to collect the organic phase to afford DAN-NH_2. ^{1}H NMR ($CDCl_3$): 8.56 (d, 1H), 8.33 (d, 1H), 8.28 (d, 1H), 7.57 (ddd, 2H), 7.21 (d, 1H), 2.94-2.91 (m, 8H), 2.72 (t, 2H), 1.26 (t, 1H).

Synthesis of reference probe

Compound 1 (218.6 mg, 0.7 mmol) was dispersed in 20 mL acetonitrile, then HATU (380.2 mg, 1.0 mmol) and DIPEA (174 μL) were added. After dissolving, DAN-NH_2 (205.4 mg, 0.7 mmol) was added. The mixture was stirred at room temperature for 1 h. After solvent evaporation, the residue was purified on a silica-gel column using EA : PE = 5 : 1 as solvent to yield pure reference probe. ^{1}H NMR (DMSO-*d6*): 8.57 (t, 1H), 8.45 (d, 1H), 8.28 (d, 1H), 8.12-8.07 (m, 2H), 7.81 (d, 2H), 7.63-7.53 (m, 3H), 7.24-

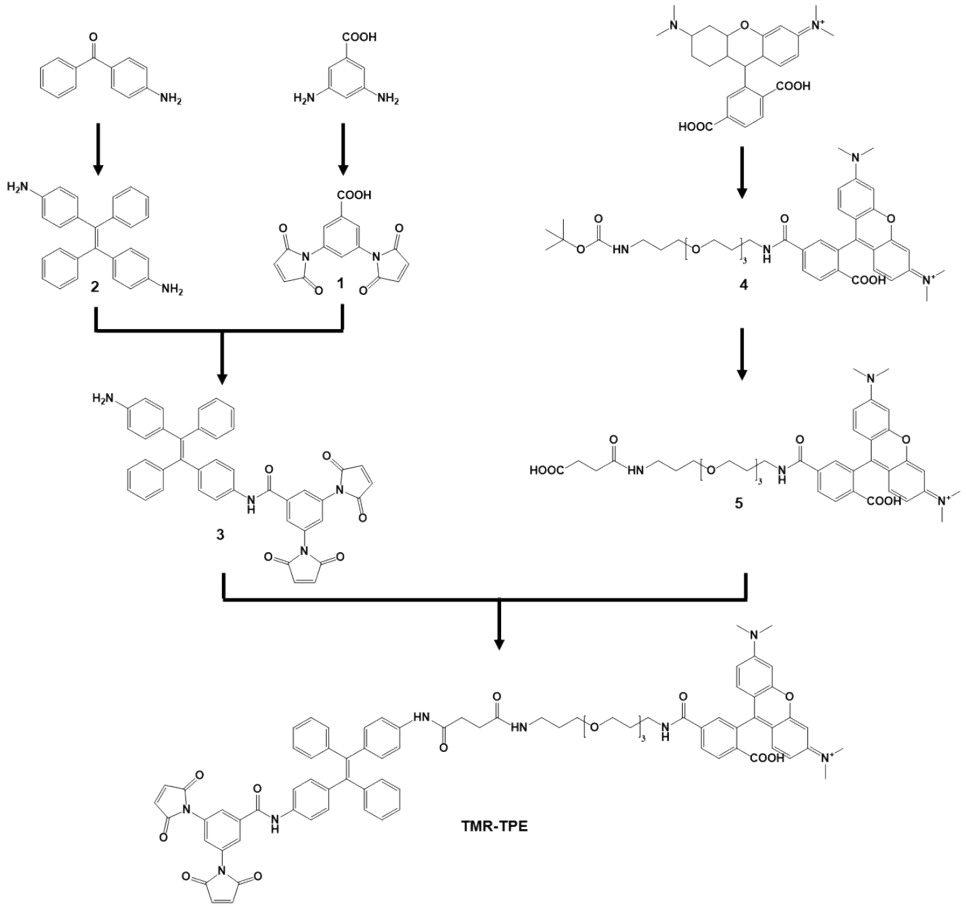
7.22 (m, 5H), 3.29 (q, 2H), 2.96 (q, 2H), 2.82 (s, 6H).

Synthesis of PAO

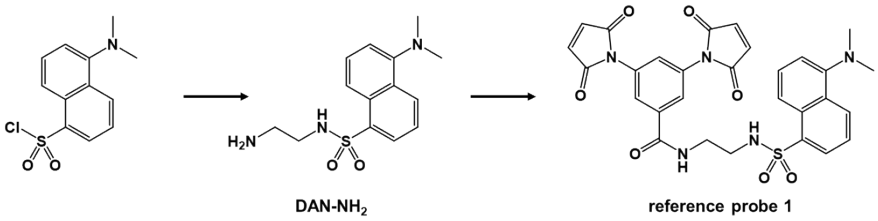
P-arsanilic acid (10.85 g, 50 mmol) was dissolved in anhydrous methanol (60 mL), and the solution was heated to reflux. Thereafter, phenylhydrazine (10.3 mL, 100 mmol) was added dropwisely in 10 min. The mixture was stirred for 1 h. After solvent evaporation, 85 mL water (85℃) and 60 mL NaOH solution (0.1 M) were added, followed by washing with ether (150 mL). After removing the ether phase, 40 mL of NH_4Cl solution (5 M) was added and the mixture was kept at 0℃ for 1 h to produce white precipitate of PAO. The precipitate was filtered through a Buchner funnel, followed by washing with 50 mL of ice water to yield pure PAO.

Synthesis of PAO-EDT

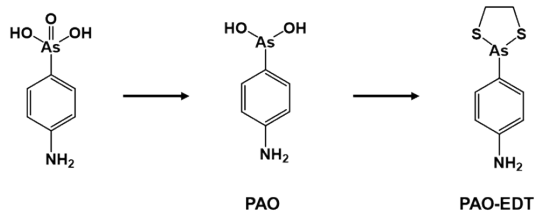
PAO (1.29 g, 6.42 mmol) was dissolved in 10 mL ethanol, and EDT (0.65 mL, 8.46 mmol) was added dropwisely under reflux. The reaction mixture was then stirred and refluxed for 30 min. The excess supernatant was removed after the reaction mixture was chilled in ethanol/ice. The PAO-EDT precipitate was recrystallized with 10 mL of ethanol, which was then filtered followed by washing with 10 mL of ice water to yield pure PAO-EDT. ^{1}H NMR (DMSO-*d6*): 7.27 (d, 2H), 6.56 (d, 2H), 5.41 (s, 2H), 3.35-3.30 (m, 2H), 3.26-3.21 (m, 2H).



Scheme S1. Synthetic routes of TMR-TPE.



Scheme S2. Synthetic routes of reference probe 1.



Scheme S3. Synthetic routes of PAO-EDT.

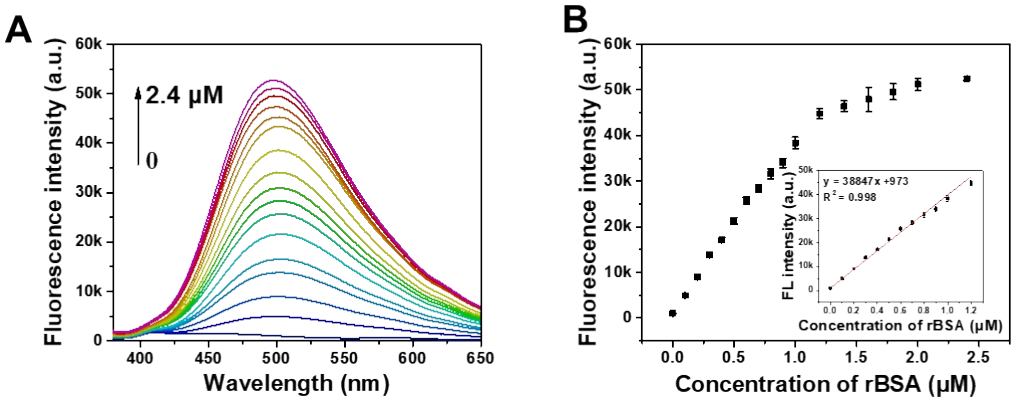


Fig. S1. (A) Variation of fluorescence spectra of Compound 3 (10 μM) at λ_{ex} = 360 nm with increasing the concentration of rBSA (0-2.4 μM). (B) Fluorescence intensity at 502 nm in response to rBSA at various concentrations and linear relationship between fluorescence intensity at 502 nm and rBSA concentration (inset).

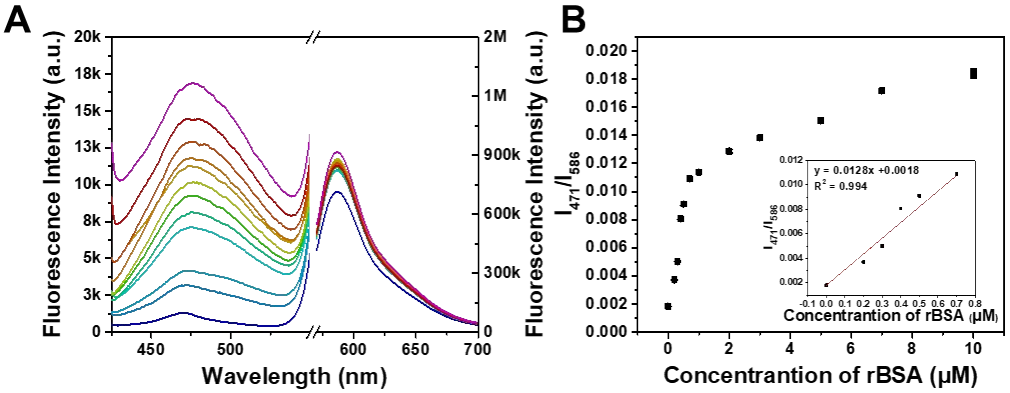


Fig. S2. (A) Variation of fluorescence spectra of TMR-TPE (10 μM) at λ_{ex} = 405 nm and 559 nm with increasing the concentration of rBSA (0-10 μM). (B) Fluorescence intensity ratio (I_{471}/I_{586}) of TMR-TPE in response to rBSA at various concentrations and linear relationship between I_{471}/I_{586} and rBSA concentration (inset).

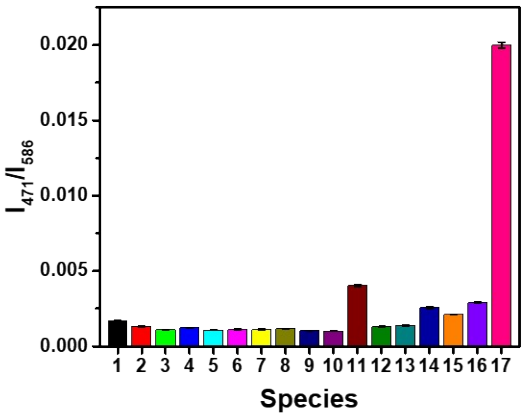


Fig. S3. Fluorescence intensity ratio (I_{471}/I_{586}) of the TMR-TPE at λ_{ex} = 405 and 559 nm upon the addition of various species (1. blank, 2. arginine, 3. threonine 4. histidine, 5. methionine, 6. cysteine, 7. homocysteine, 8. glutathione, 9. TCEP, 10. DTT, 11. lipase, 12. lactate dehydrogenase, 13. lysozyme, 14. ovalbumin, 15. BSA, 16. HSA, 17. rBSA. The concentration of small molecules is 1 mM, and proteins are 5 μM.)

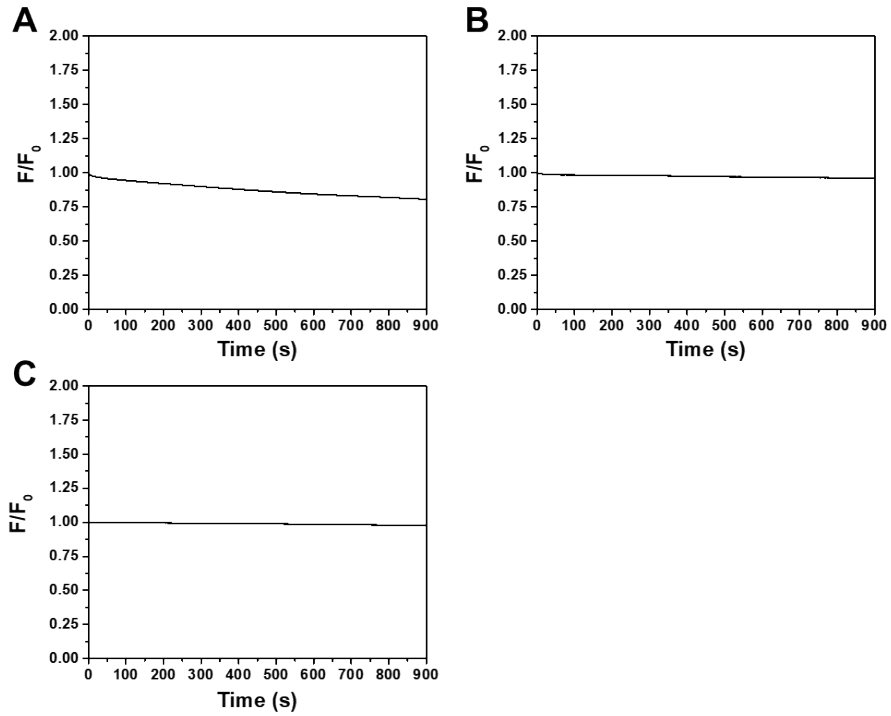


Fig. S4. Fluorescence intensity of TMR-TPE under continuous irradiation with a xenon lamp for 15 min at (A) $\lambda_{ex/em}$=360/471 nm, (B) $\lambda_{ex/em}$=405/471 nm and (C) $\lambda_{ex/em}$=559/586 nm.

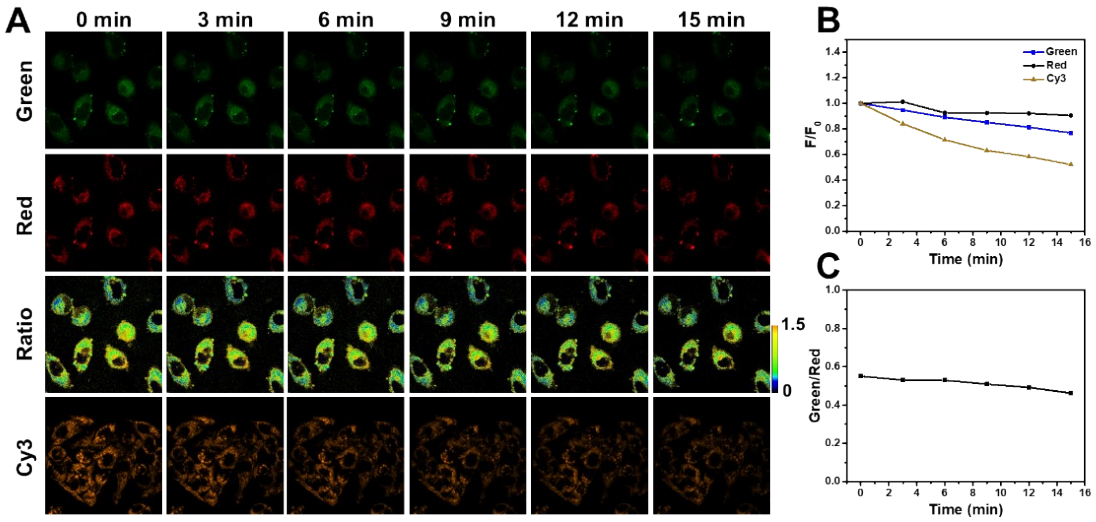


Fig. S5. (A) CLMS images of HepG2 cells stained by TMR-TPE or Cy3 under continuous irradiation with 405 and 559 nm laser of confocal microscopy for 15 min. Green channel: λ_{ex} = 405 nm, λ_{em} = 460-500 nm; red channel: λ_{ex} = 559 nm, λ_{em} = 575-630 nm; Cy3: λ_{ex} = 559 nm, λ_{em} = 575-630 nm. (B) Optical density at different time. (C) F_{green}/F_{red} ratios at different time.

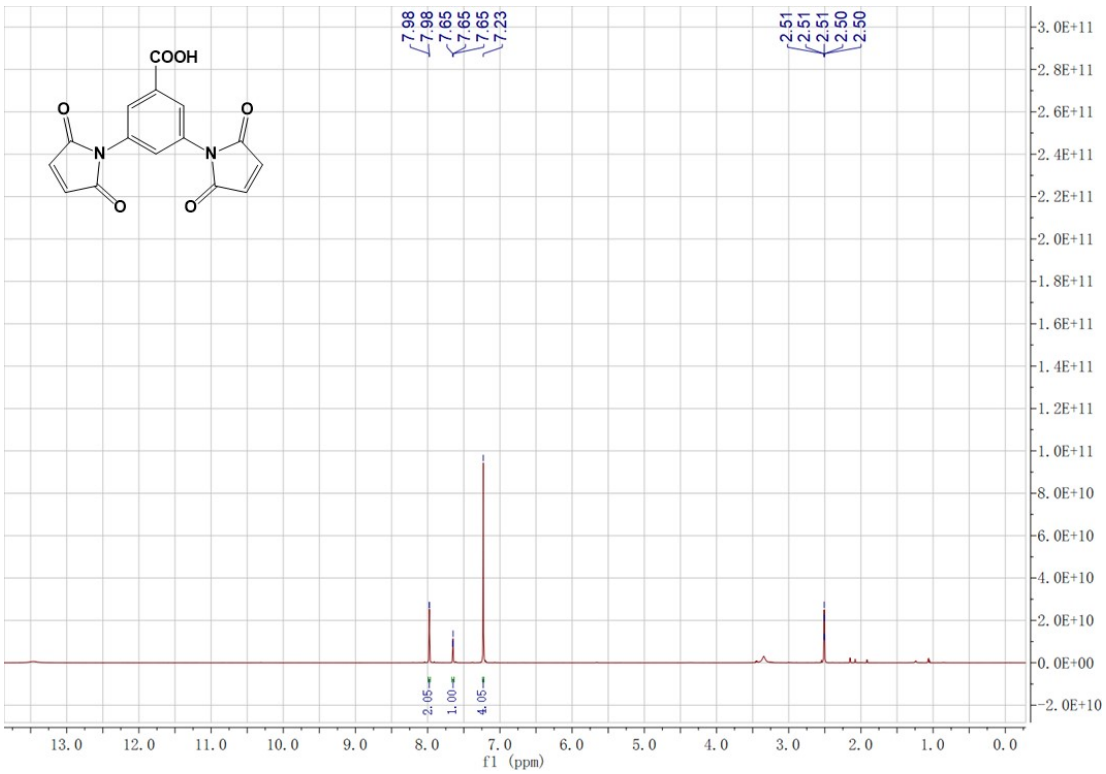


Fig. S6. ^{1}H NMR Spectrum of compound 1 in DMSO-*d6*.

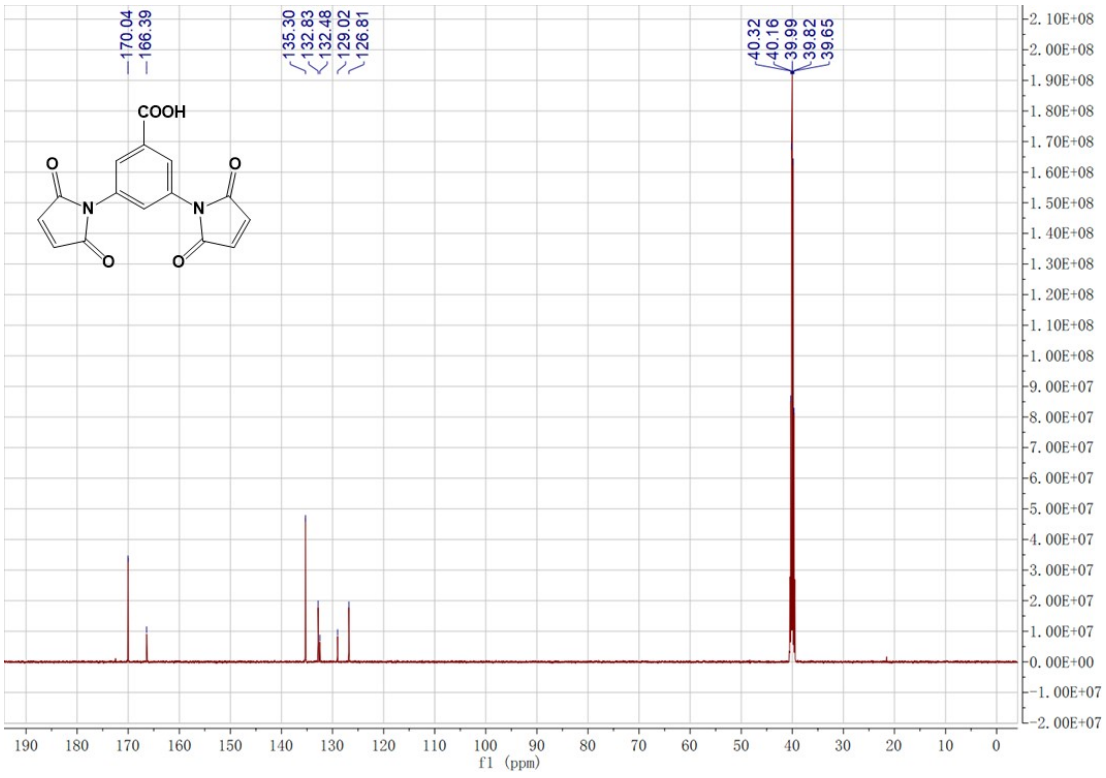


Fig. S7. ^{13}C NMR Spectrum of compound 1 in DMSO-*d6*.

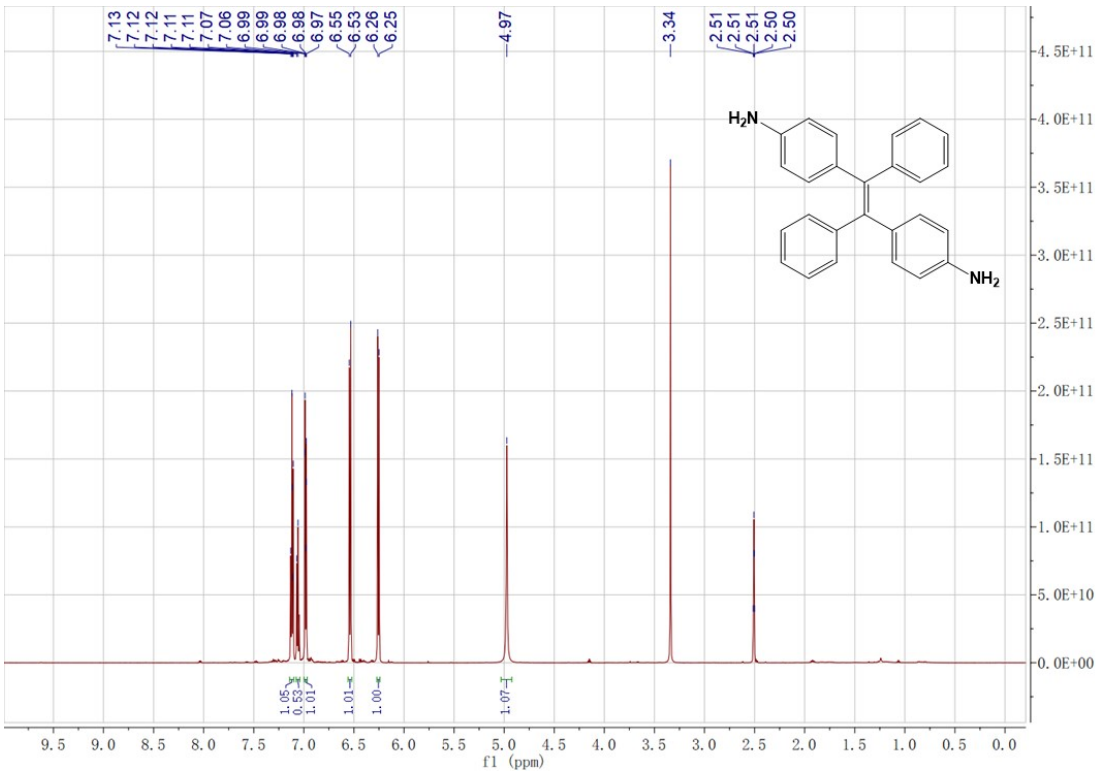


Fig. S8. ^{1}H NMR Spectrum of compound 2 in DMSO-*d6*.

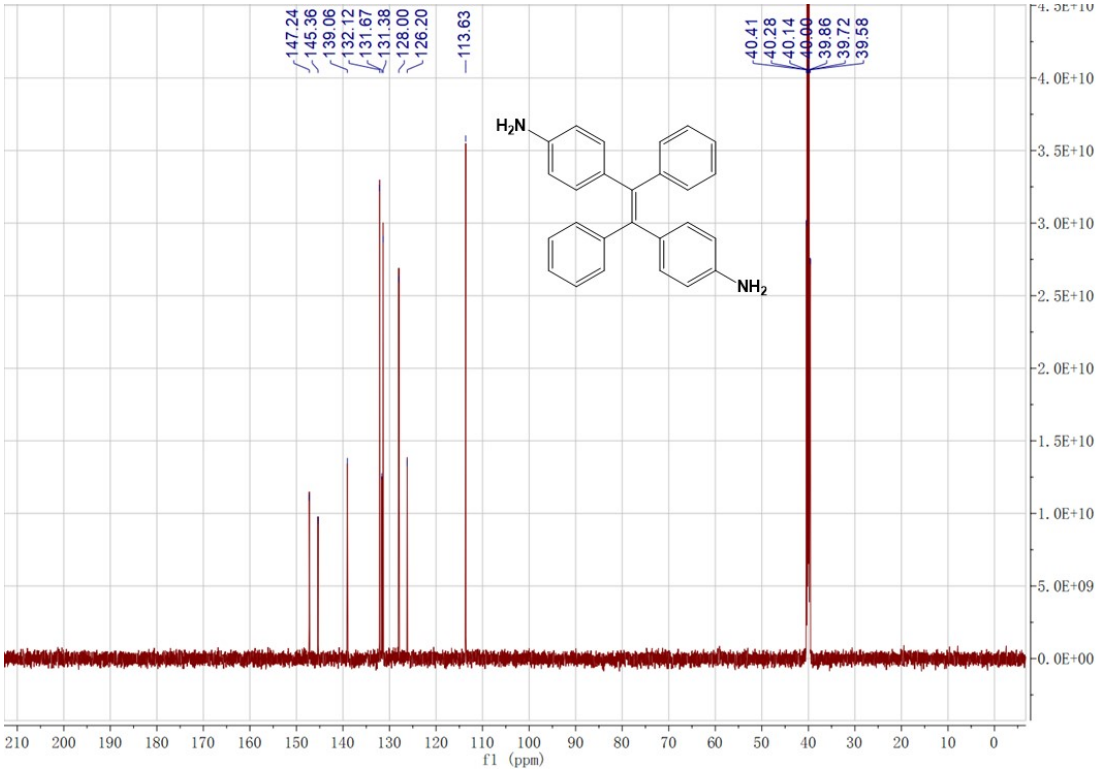


Fig. S9. ^{13}C NMR Spectrum of compound 2 in DMSO-*d6*.

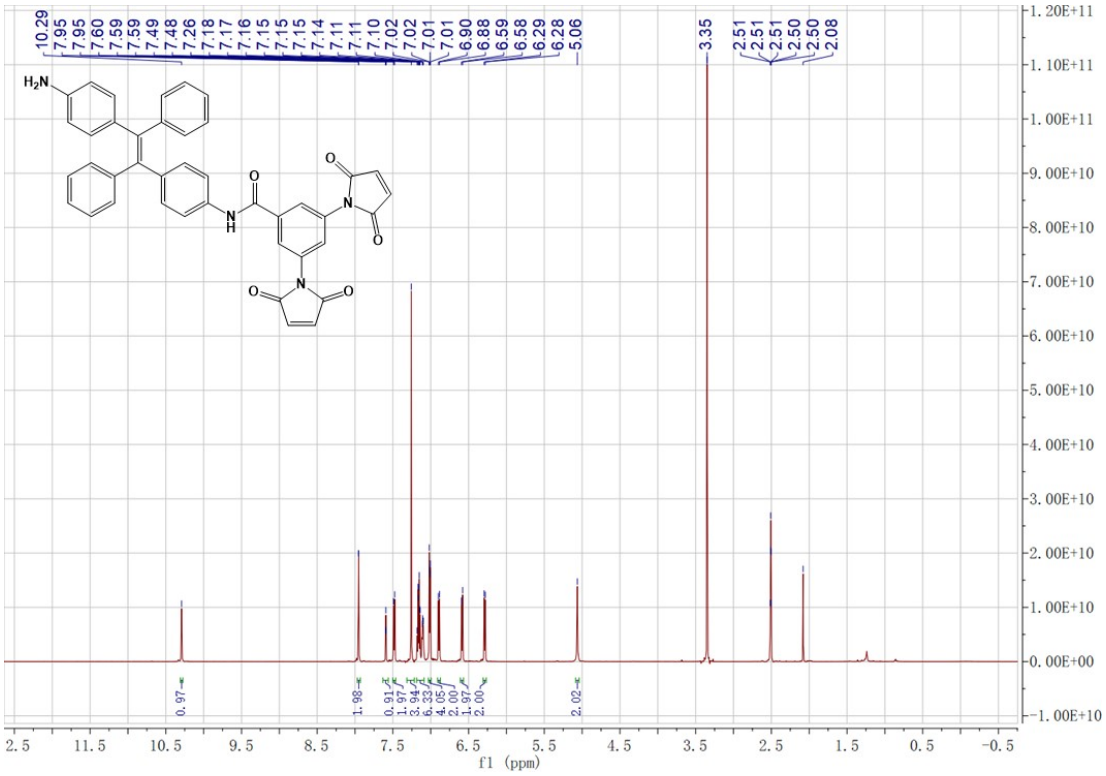


Fig. S10. ^{1}H NMR Spectrum of compound 3 in DMSO-*d6*.

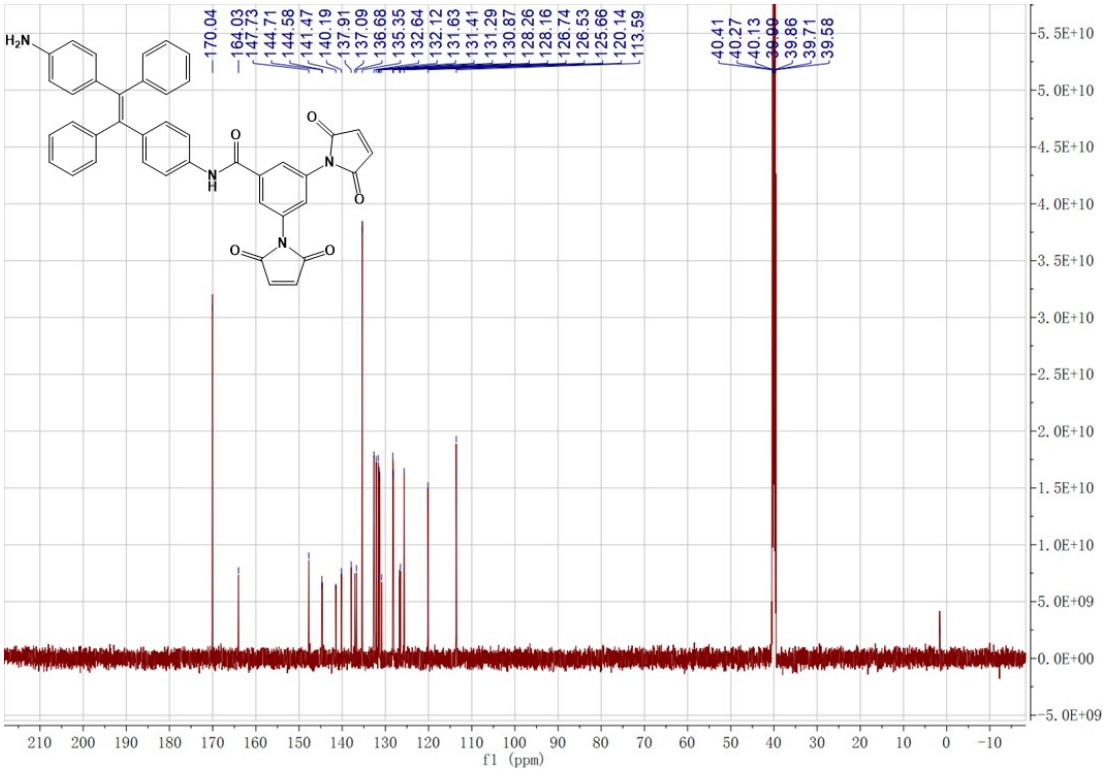


Fig. S11. ^{13}C NMR Spectrum of compound 3 in DMSO-*d6*.

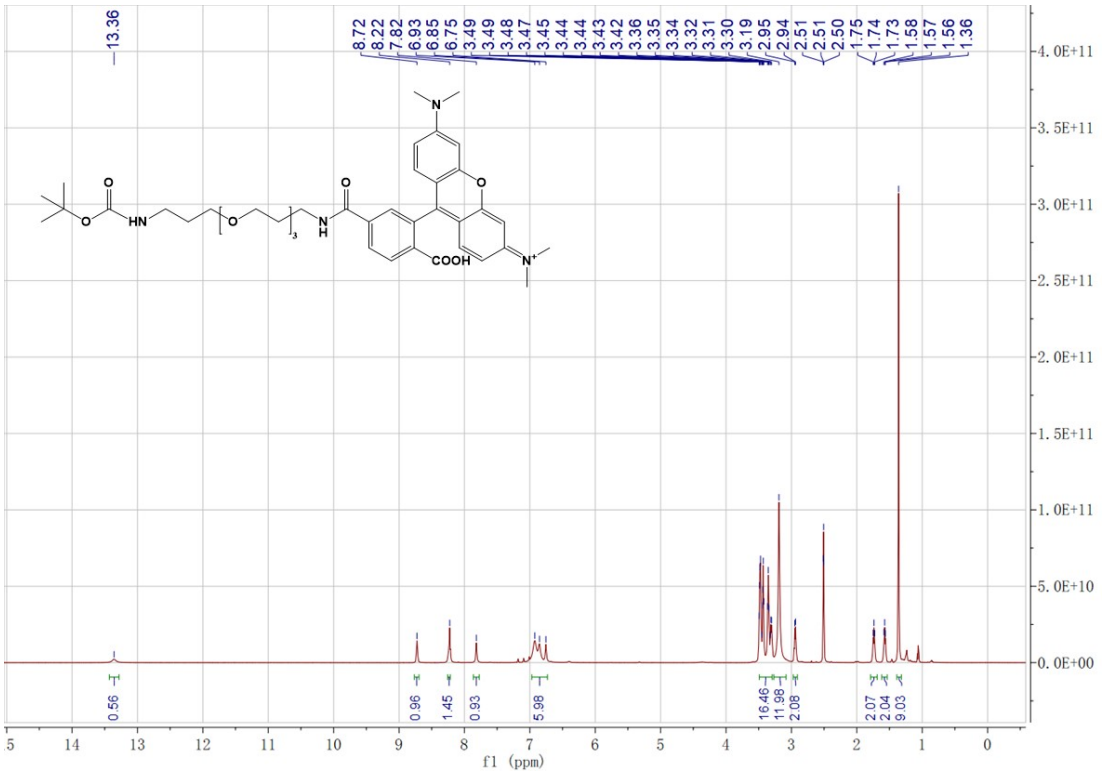


Fig. S12. ^{1}H NMR Spectrum of compound 4 in DMSO-*d6*.

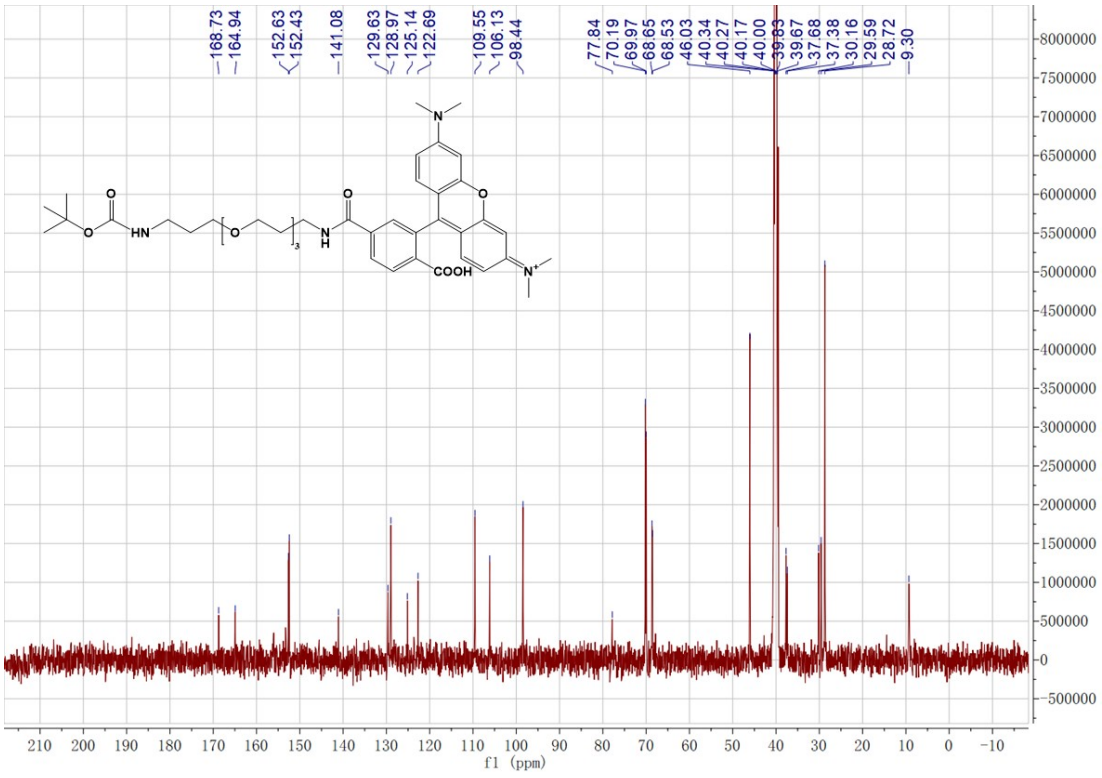


Fig. S13. ^{13}C NMR Spectrum of compound 4 in DMSO-*d6*.

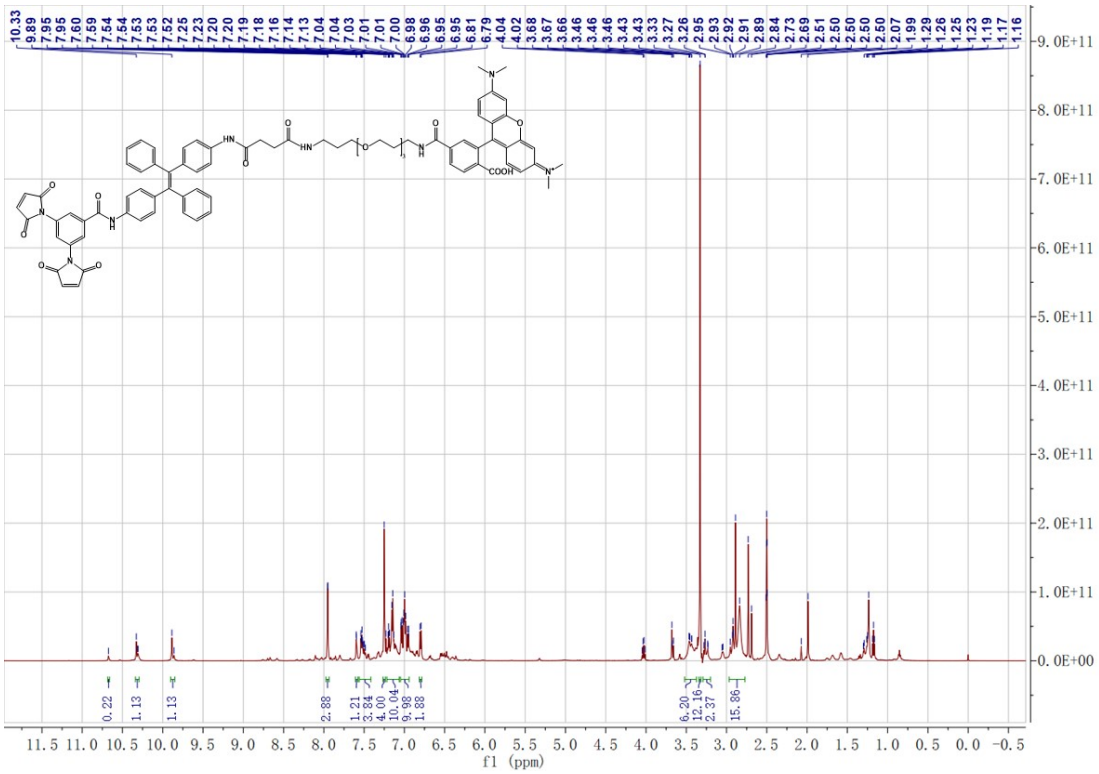


Fig. S14. ^{1}H NMR Spectrum of TMR-TPE in DMSO-*d6*.

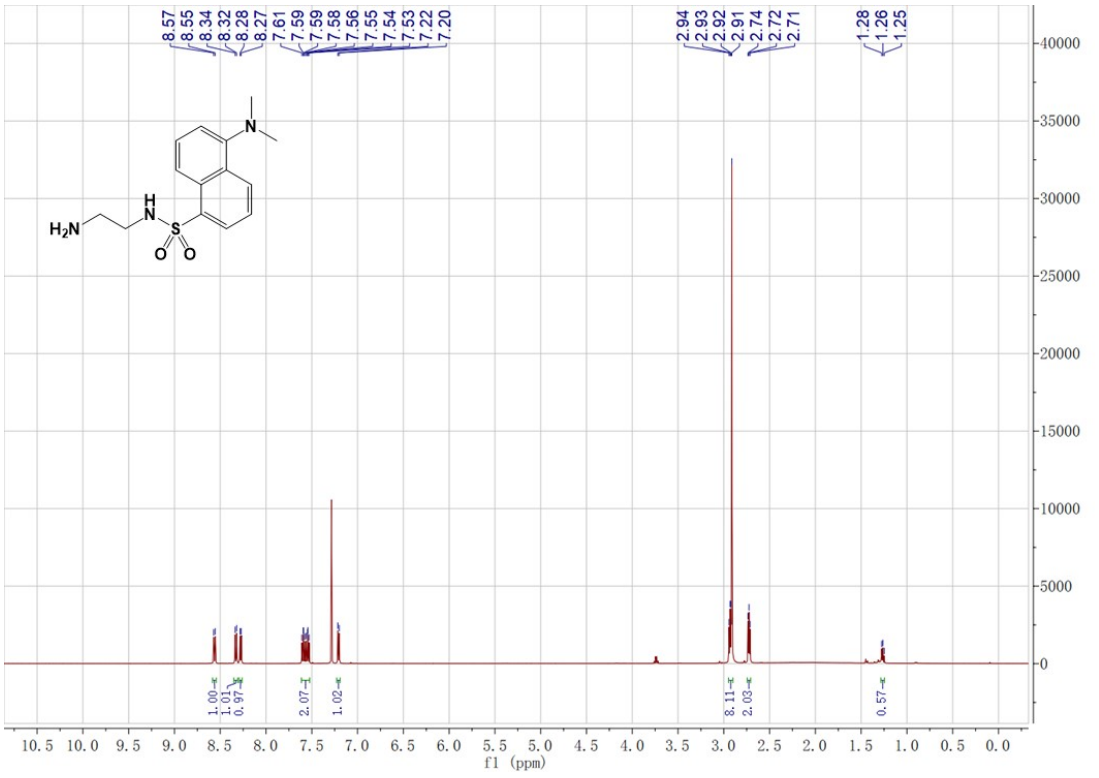


Fig. S15. ^{1}H NMR Spectrum of DAN-NH_2 in $CDCl_3$.

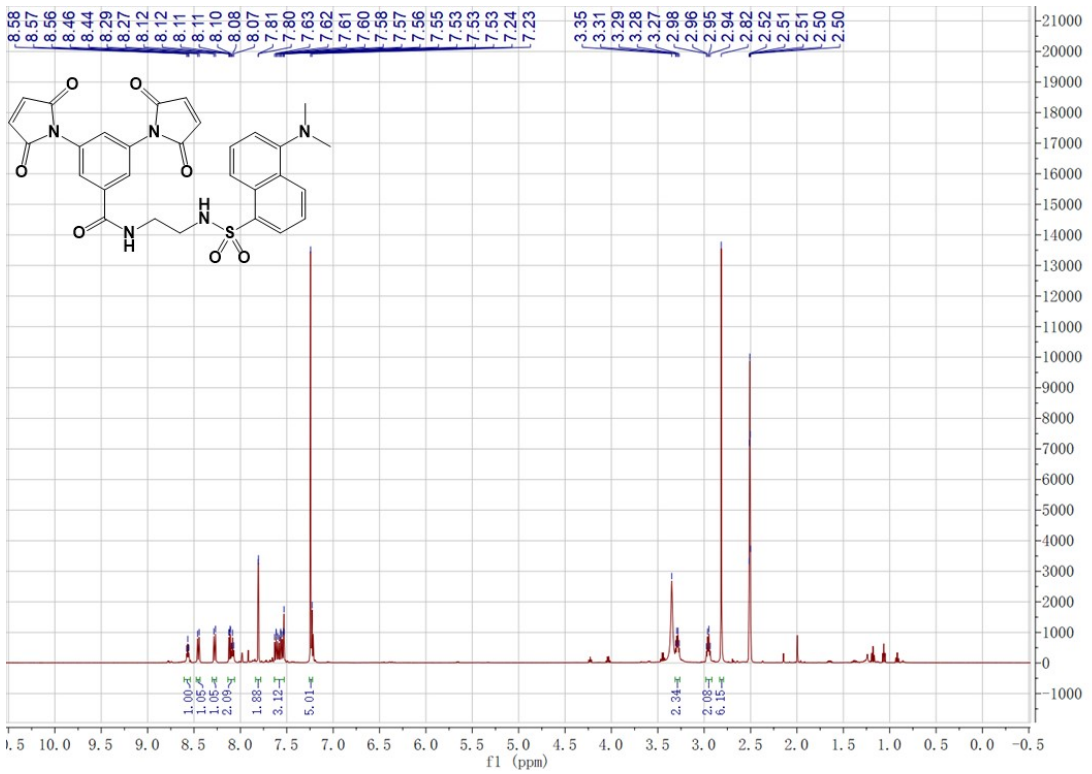


Fig. S16. ^{1}H NMR Spectrum of reference probe 1 in $CDCl_3$.

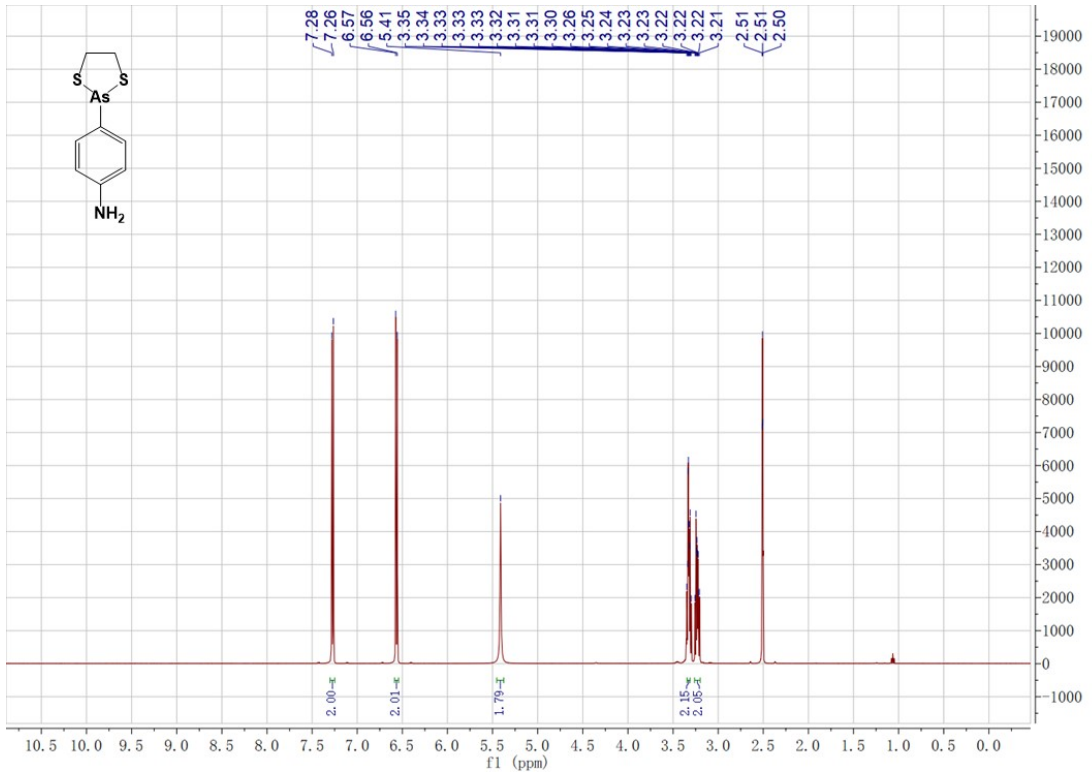


Fig. S17. ^{1}H NMR Spectrum of PAO-EDT in DMSO-*d6*.