Electronic Supplementary Material (ESI) for Analyst.

A Novel NIR Fluorescent Probe for Visualizing Hydrogen Sulfide in Alzheimer's Disease

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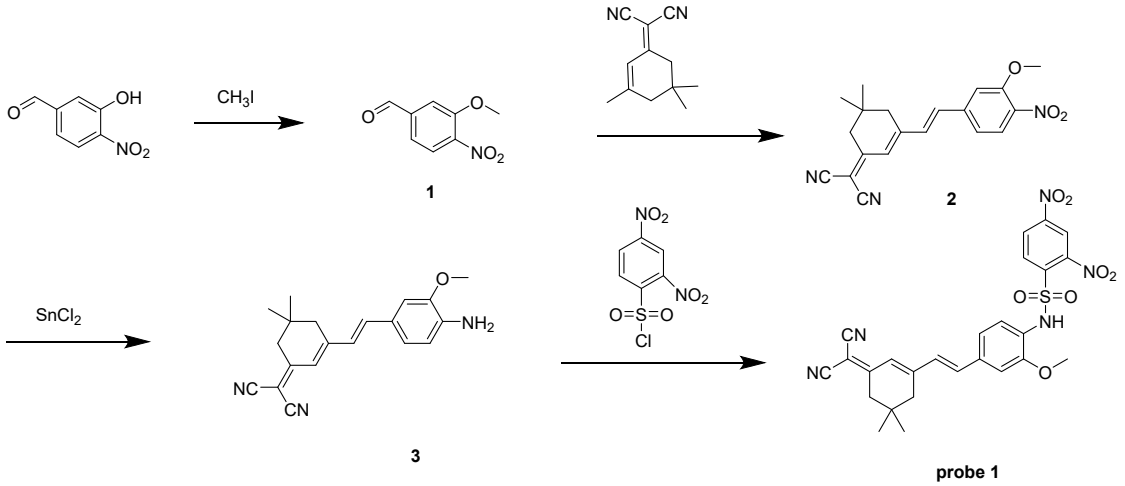
Scheme S1. synthesis route of probe 1.

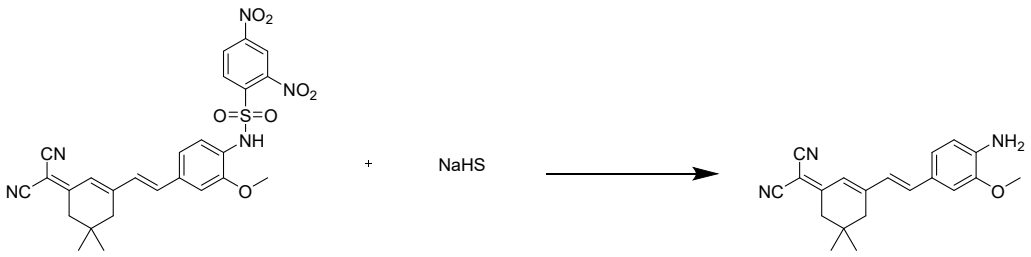


Synthesis of compound 1. 3-hydroxy-4-nitrobenzaldehyde (2.00 g, 11.97 mmol) and methyl iodide (6.79 g, 47.87 mmol) were added into the mixed solvent of anhydrous acetonitrile and N, N-dimethylformamide (DMF), then 1.65 g (11.97 mmol) potassium carbonate was added and the solution reacted at room temperature for 24 hours, then filtered and concentrated to afford the crude which was further washed with deionized water for three times. The white solid precipitated was filtered and dried under vacuum to obtain compound **1** (1.85 g, 85.35% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ10.06 (s, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.61 (d, *J* = 1.5 Hz, 1H), 7.56 (dd, *J* = 8.1, 1.5 Hz, 1H), 4.04 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ190.4, 153.0, 143.3, 139.7, 125.9, 122.6, 112.6, 56.8.

Synthesis of compound 2. Compound 1 (1.85 g, 10.21 mmol) and (2.09 g, 11.23 mmol) 2-(3,5,5-trimethylcyclohex-2-en-1-yl) malononitrile were added into acetonitrile, then added 2 drops of piperidine. The reaction mixture was stirred at room temperature for 12 h, and the cake was filtered to obtain a yellow solid. The crude product was purified by recrystallization from acetonitrile to obtain the yellow solid compound **2** (1.51 g, 42.32% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ7.88 (d, *J* = 8.3 Hz, 1H), 7.21 - 7.11 (m, 2H), 7.10 - 6.95 (m, 2H), 6.93 (s, 1H), 4.01 (s, 3H), 2.62 (s, 2H), 2.47 (s, 2H), 1.09 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ168.9, 153.5, 152.4, 141.8, 139.3, 134.1, 132.8, 126.6, 125.5, 119.3, 113.1, 112.4, 111.7, 80.5, 56.6, 42.9, 39.1, 32.1, 28.0.

Synthesis of compound 3. Under the protection of argon, 1.51 g (4.32 mmol) of compound **2** and 1.17 g (5.19 mmol) of stannous chloride were added to absolute ethanol, and the reaction was refluxed for 12 hours. The reaction solution was filtered to remove stannous chloride, and the filtrate was further purified by passed through the column chromatography. After purification, the solid was dried under vacuum to give the red compound 3 (721 mg, 52.23% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ7.02 - 6.96 (m, 2H), 6.95 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.80 (d, *J* = 15.9 Hz, 1H), 6.76 (s, 1H), 6.66 (d, *J* = 7.9 Hz, 1H), 4.18 (s, 2H), 3.91 (s, 3H), 2.55 (s, 2H), 2.43 (s, 2H), 1.05 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ169.4, 155.0, 147.2, 139.1, 138.3, 126.1, 125.1, 123.3, 121.9, 114.2, 114.1, 113.4, 108.0, 76.3, 55.6, 43.0, 39.2, 32.0, 28.1.

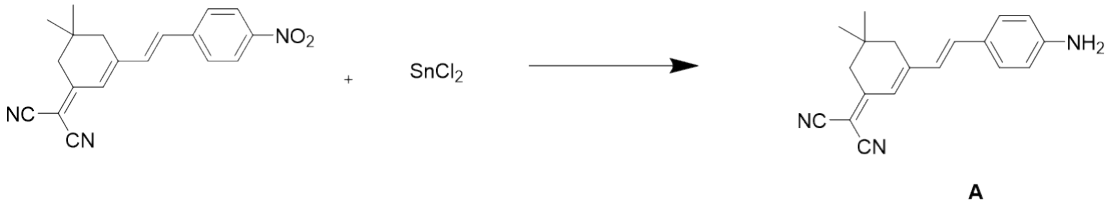
Scheme S2. The product from the reaction between probe 1 with NaHS (1-H_2S (3)).



probe 1 1-H_2S(3)

To a solution of probe **1** (40 mg, 72.8 μmol) in 10 mL acetonitrile was added 2 mL NaHS solution [8 mg (142.7 μmol) NaHS was dissolved in 2 mL PBS buffer (pH = 7.4)]. The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, the mixture was concentrated to afford the crude product, which was purified by flash column chromatography to afford the black solid (11.2 mg, 48.1% yield). ^{1}H NMR (500 MHz,) δ 7.02 – 6.96 (m, 2H), 6.95 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.80 (d, *J* = 15.9 Hz, 1H), 6.76 (s, 1H), 6.66 (d, *J* = 7.9 Hz, 1H), 4.18 (s, 2H), 3.91 (s, 3H), 2.55 (s, 2H), 2.43 (s, 2H), 1.05 (s, 6H).

Scheme S3. Preparation of compound A.



A

Under the protection of argon, 500 mg (1.57 mmol) of (E)-2-(5,5-dimethyl-3-(4-nitrostyryl) cyclohex-2-en-1-ylidene) malononitrile and 890 mg (4.70 mmol) of stannous chloride were added to absolute ethanol, and the reaction was refluxed for 12 hours. The reaction solution was filtered to remove stannous chloride, and the filtrate was passed through the column. After purification and purification, the solid was dried under vacuum to give the red product **A** (142 mg, 31.34% yield). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.3 (d, *J* = 8.2 Hz, 2H), 7.0 (d, *J* = 15.9 Hz, 1H), 6.8 (d, *J* = 16.0 Hz, 1H), 6.7 (s, 1H), 6.7 (d, *J* = 8.1 Hz, 2H), 4.2 - 3.9 (m, 2H), 2.6 (s, 2H), 2.4 (s, 2H), 1.1 (s, 6H). ^{13}C NMR (126 MHz, Chloroform-d) δ 169.4, 155.1, 148.5, 137.8, 129.5, 126.0, 125.2, 121.9, 115.1, 114.1, 113.3, 43.0, 39.2, 32.0, 28.1.

The preparation of Aβ42 aggregates.

To a solution of NaH_2PO_4 (39.0 mg) and $EDTANa_2$ (8.4 mg) in 25 mL ultrapure water was added 0.5-0.6 mg of A*β* peptide. The mixture was shaken at 37 °C for 48 hours (120 r/min,). Then, the resulting Aβ aggregates solution was stored in a 4°C refrigerator. The concentration of A*β* aggregates is approximately 220 μg/mL.

II. Supplementary Spectra

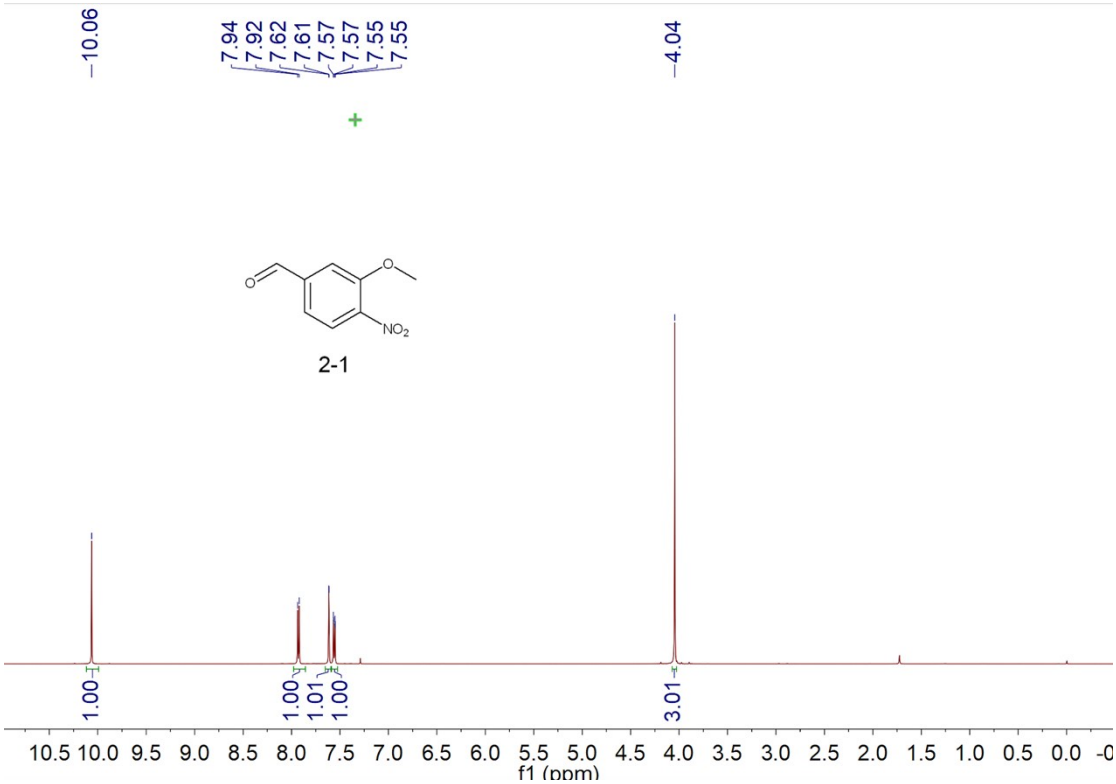


Fig. S1. ^{1}H NMR spectrum of Compound **1** in $CDCl_3$.

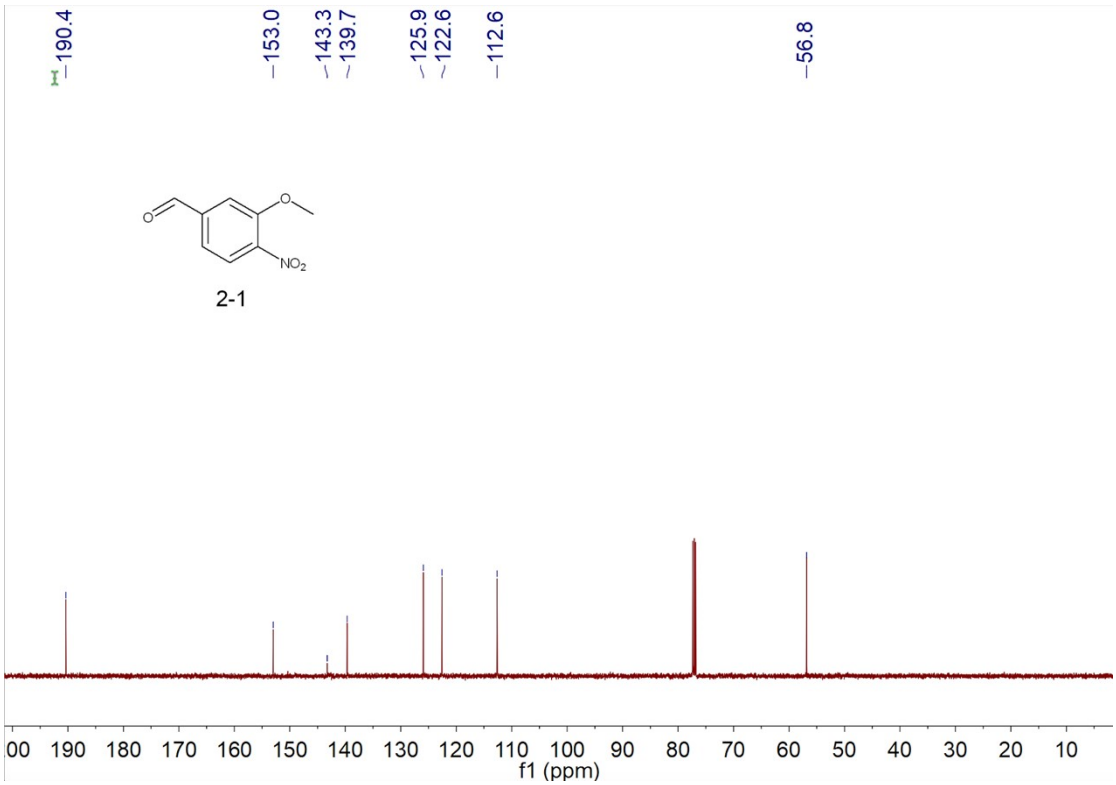


Fig. S2. ^{13}C NMR spectrum of Compound **1** in $CDCl_3$.

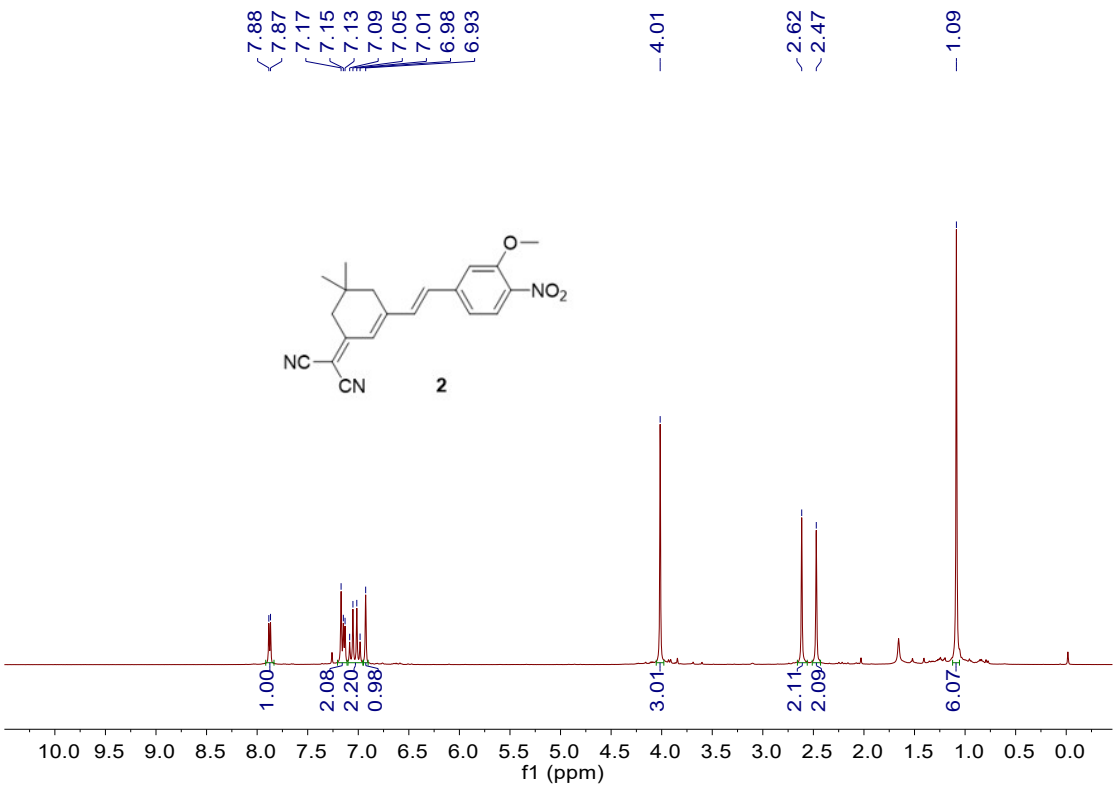


Fig. S3. ^{1}H NMR spectrum of Compound **2** in $CDCl_3$.

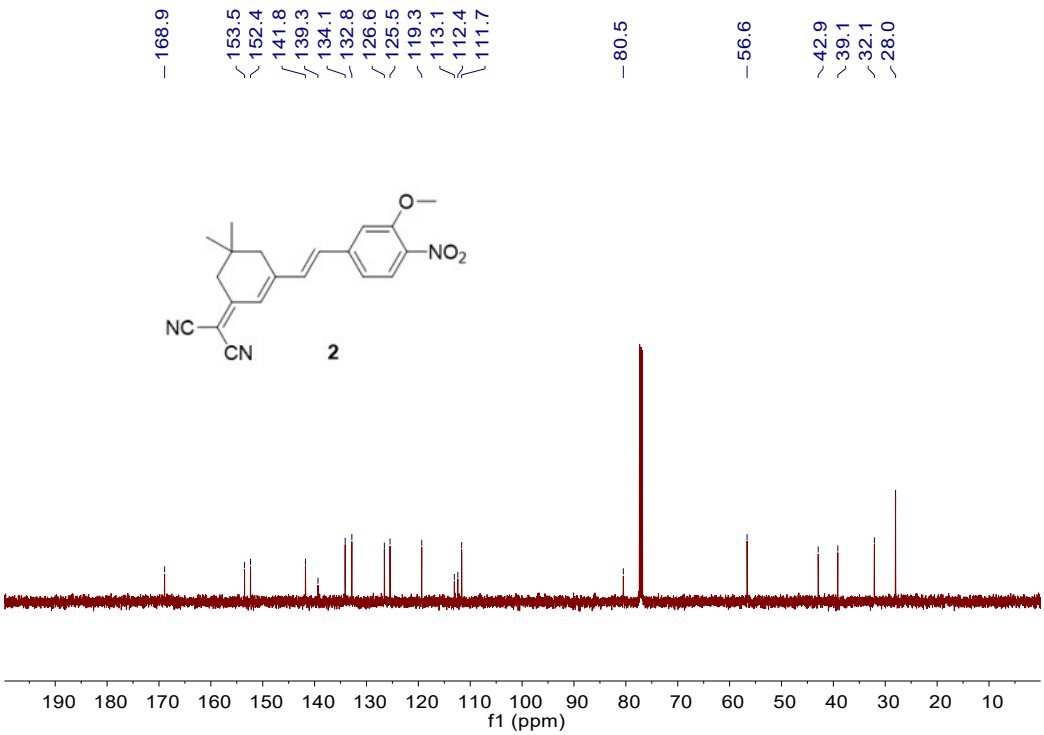


Fig. S4. ^{13}C NMR spectrum of Compound **2** in $CDCl_3$.

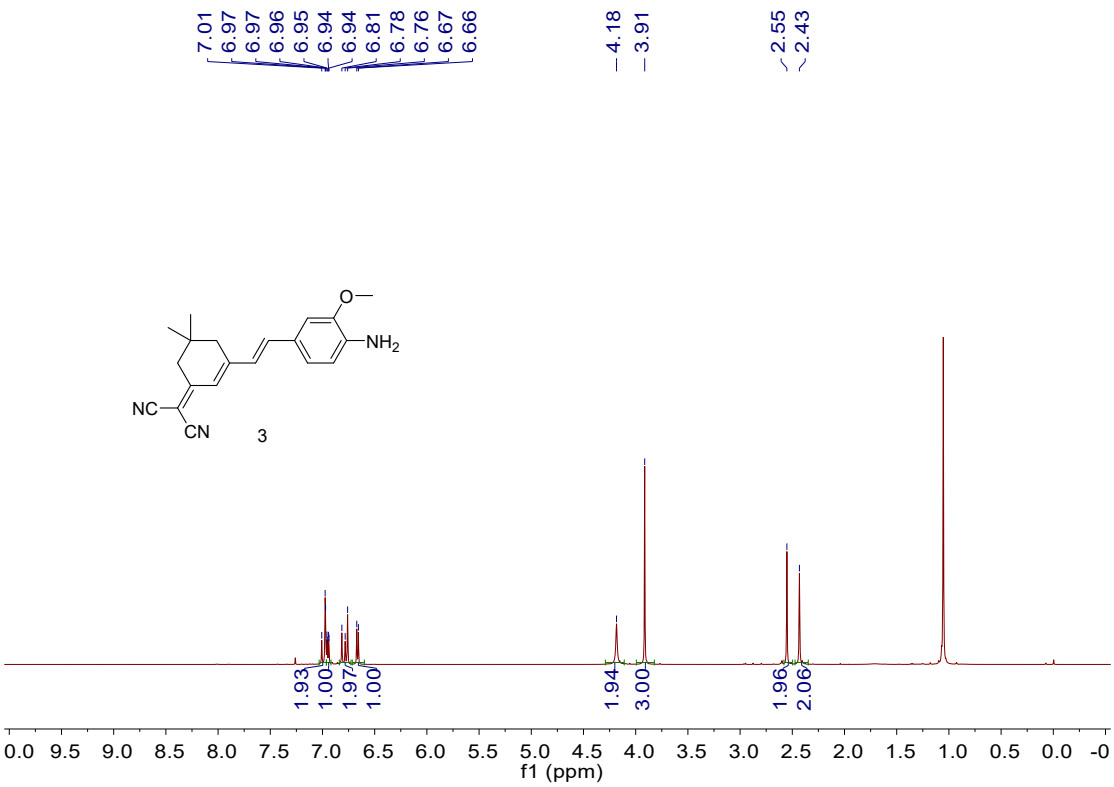


Fig. S5. ^{13}C NMR spectrum of Compound **3** in $CDCl_3$.

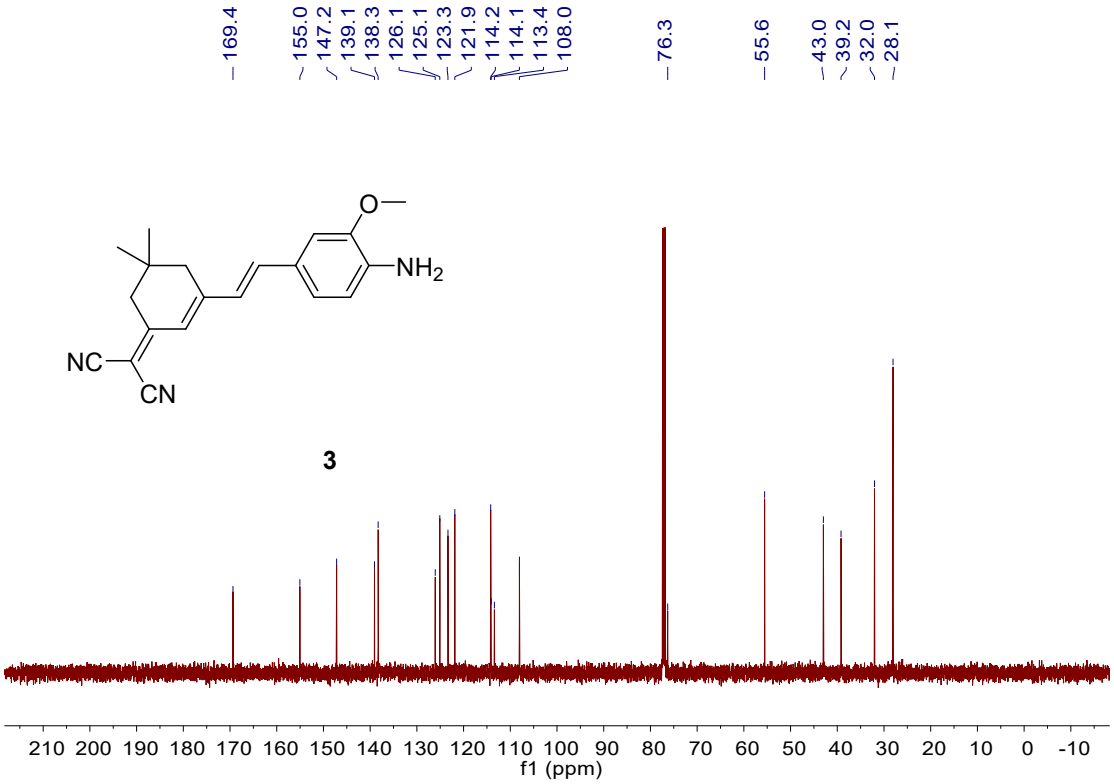


Fig. S6. ^{13}C NMR spectrum of Compound **3** in $CDCl_3$.

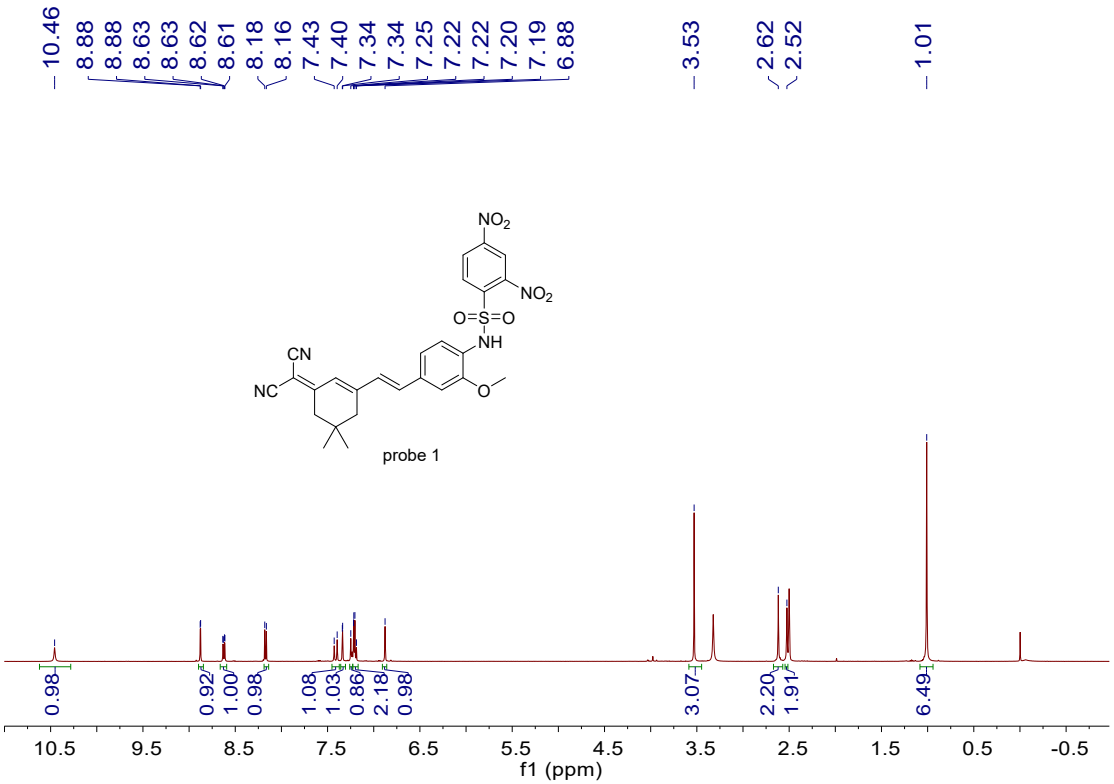


Fig. S7. ^{1}H NMR spectrum of probe 1 in DMSO-d_6.

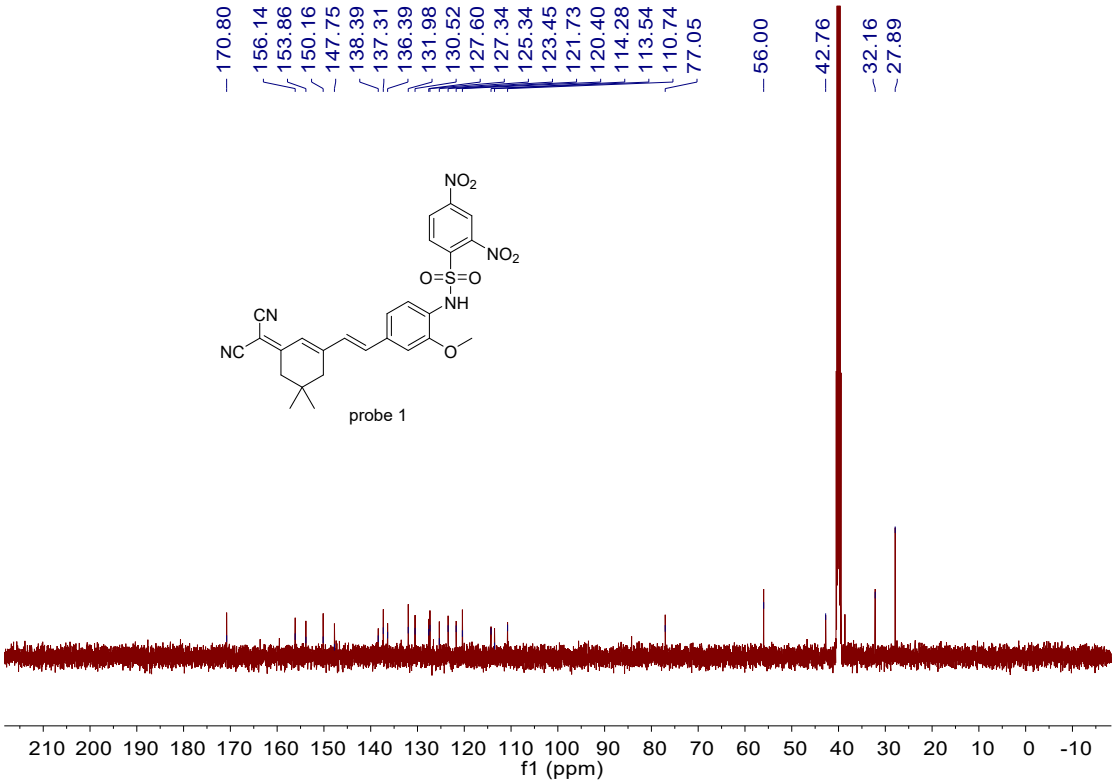


Fig. S8. ^{13}C NMR spectrum of probe **1** in DMSO-d_6.

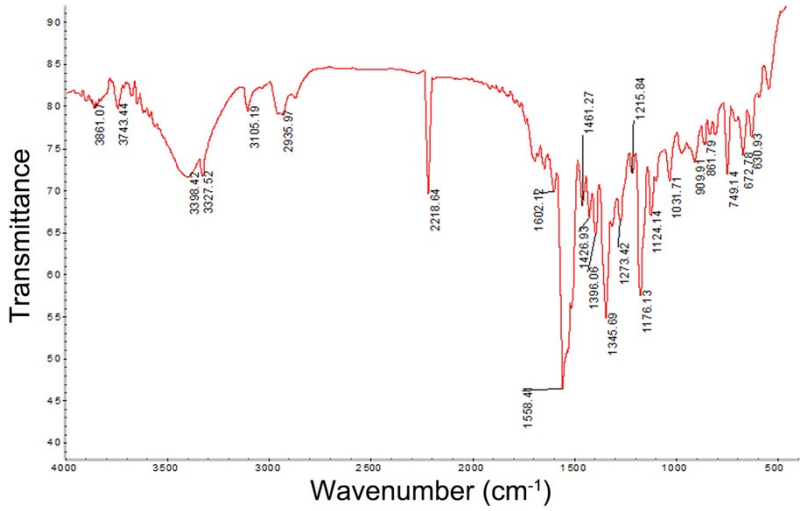


Fig. S9. FT-IR spectrum of the probe **1**.

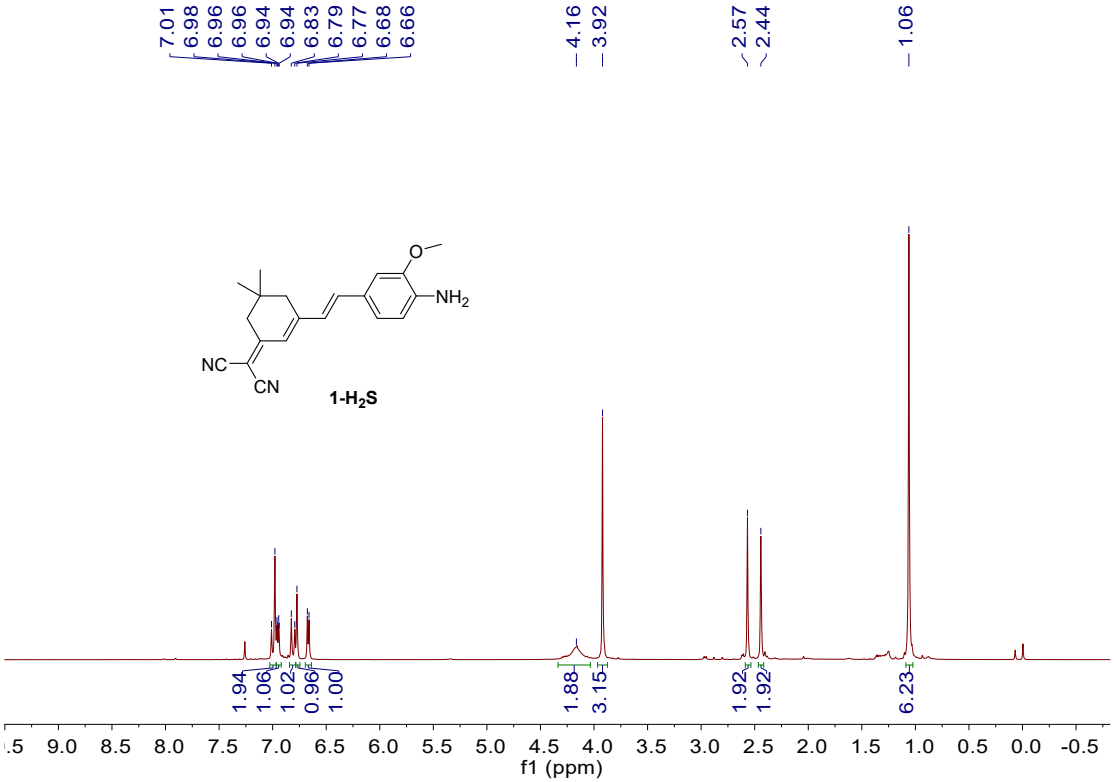


Fig. S10. ^{1}H NMR spectrum of the isolated product **1-H_2S** (**3**) from the reaction between probe **1** with NaHS in $CDCl_3$.

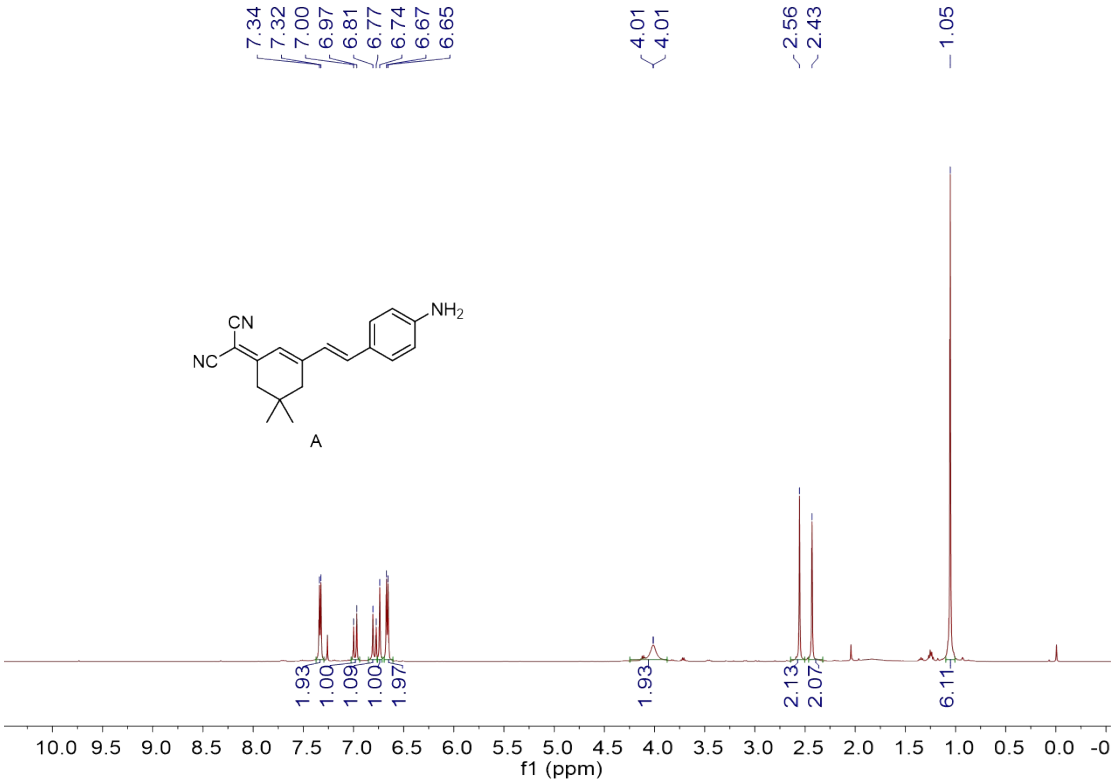


Fig. S11. ^{1}H NMR spectrum of Compound **A** in $CDCl_3$.

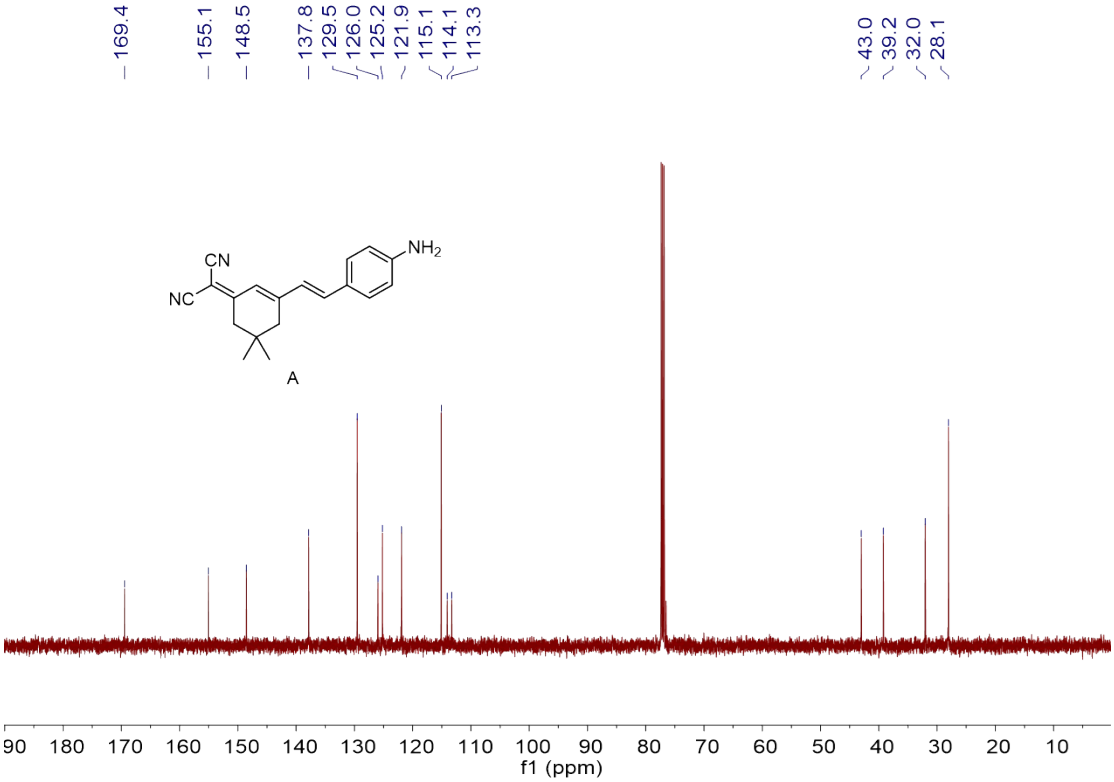


Fig. S12. ^{13}C NMR spectrum of Compound **A** in $CDCl_3$.

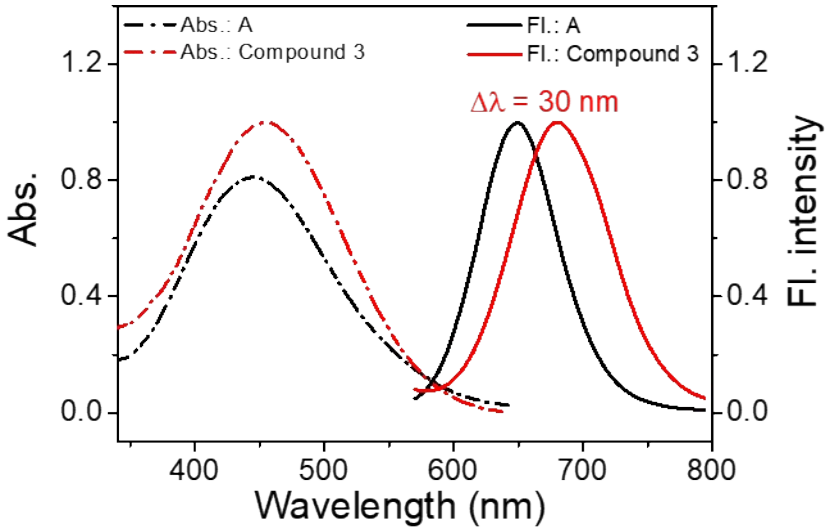


Fig. S13. Normalized absorption (dashed lines) and fluorescence (solid lines) spectra of compound **A** and compound **3** in DMSO-PBS (pH 7.4, 10 mM, v/v/, 1/9) at 37 °C.

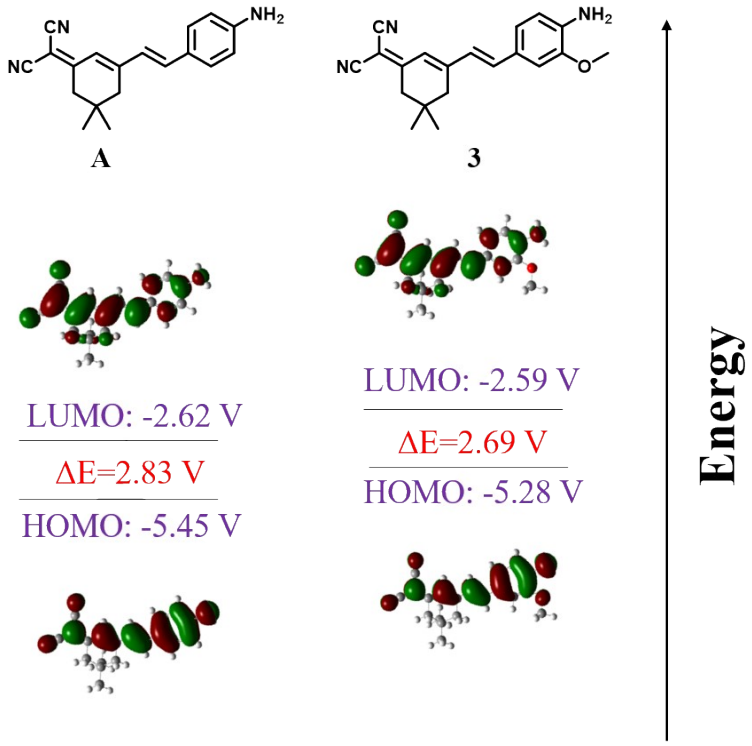


Fig. S14. HOMO and LUMO orbitals of compound **A** and compound **3**. Calculations were performed with density functional theory (DFT) with the B3LYP/6-311G method using the Gaussian 09 package.

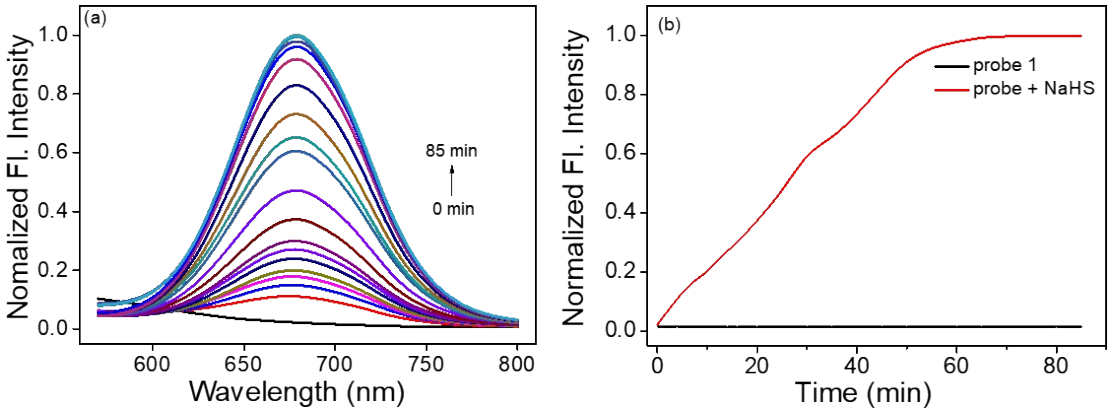


Fig. S15. (a) Time-dependent normalized fluorescent spectra responses of the probe **1** (10 μM). (b) the corresponding normalized intensity changes at 680 nm of probe **1** (10 μM) in the absence and presence of 10 equiv. of NaHS in DMSO-PBS (pH 7.4, 10 mM, v/v/, 1/9) at 37 ℃. λ_{ex} = 457 nm, λ_{em} = 680 nm, slit (nm): 5.0/10.0.

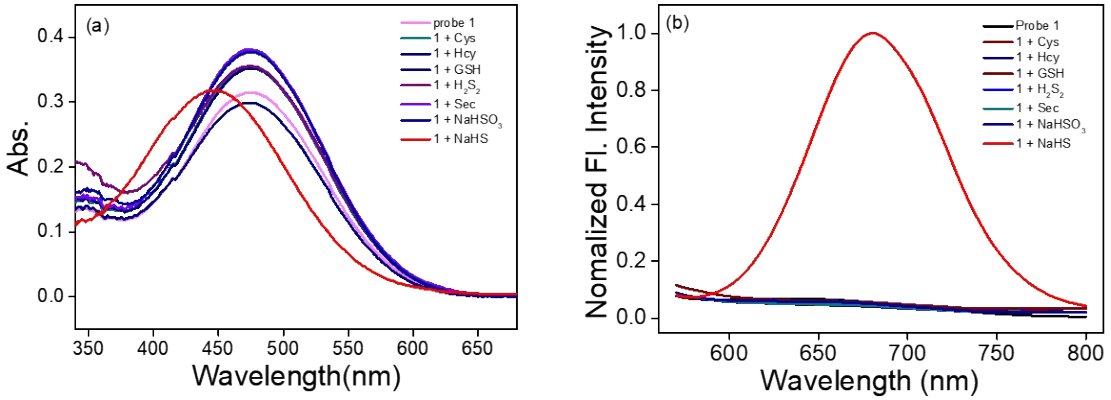


Fig. S16. (a) Absorption spectra of probe **1** (10 μM) upon addition of 10 equiv. of Cys, Hcy, GSH, H_2S_2, Sec, $NaHSO_3$ and NaHS (100 μM) in DMSO-PBS (10 mM, pH = 7.4, v/v, 1/9) at 37°C for 60 min. (b) Normalized fluorescence spectra of probe **1** (10 μM) upon addition of 10 equiv. of Cys, Hcy, GSH, NaHS, H_2S_2, Sec and $NaHSO_3$ (100 μM) in DMSO-PBS (10 mM, pH = 7.4, v/v, 1/9). at 37 °C for 30 min. λ_{ex} = 457 nm, λ_{em} = 680 nm. slit (nm): 5.0/10.0.

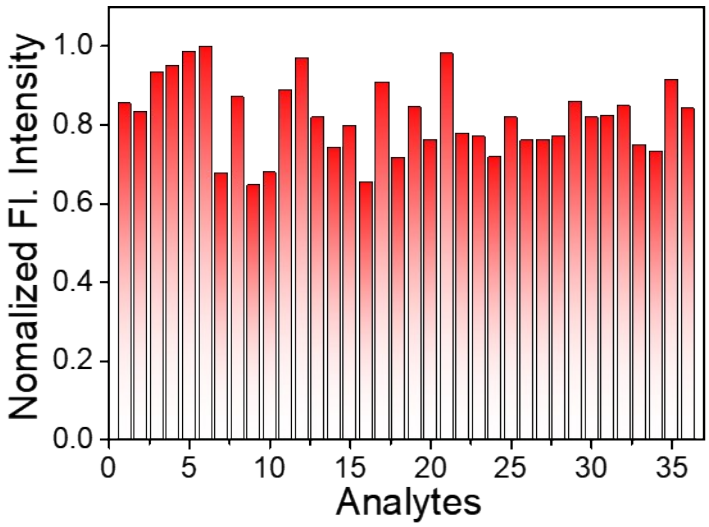


Fig. S17. Normalized fluorescent spectra responses of the probe **1** (10 μM) in the presence of Cys (200 μM), GSH (200 μM), and other analytes (100 μM) with NaHS (100 μM) mutual common at 680 nm. Analytes including: 1. NaHS, 2. H_2O_2, 3. $ONOO^-$,4. NO, 5. $O_2^{\cdot-}$, 6.$^1O_2$, 7. tBuOO, 8. SO_2, 9. NAC, 10. Hcy, 11. GSH, 12. Cys, 13. H_2S_2, 14. Sec, 15. Na^+, 16. Ag^+, 17. K^+, 18. Co^{2+}, 19. Zn^{2+}, 20. Fe^{2+}, 21. Ca^{2+}, 22. Ba^{2+}, 23. Val, 24. His, 25. Pro, 26. Gly, 27. Lys, 28. Glu, 29. Ser, 30. Thr, 31. Asp, 32. Arg, 33. Leu, 34.Trp, 35. HPO_4^{2-}, 36. HCO_3^-. λ_{ex} = 457 nm, λ_{em} = 680 nm. slit (nm): 5.0/10.0.

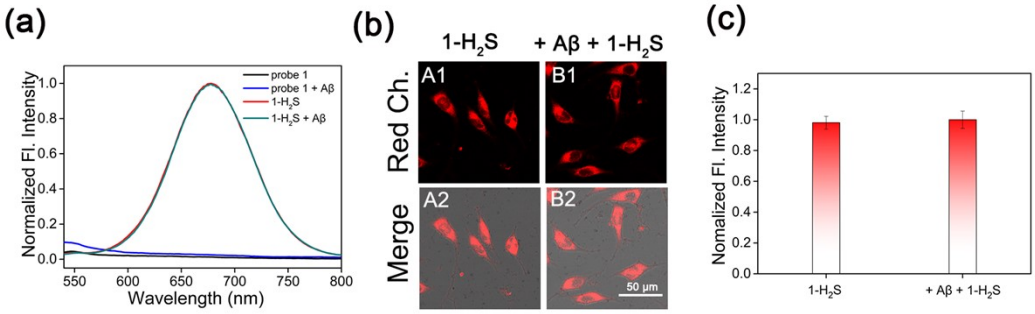


Figure S18. (a) The fluorescence spectra of the isolated product **1-H_2S** (10 μM) upon addition of Aβ_{42} aggregates (20 μg/mL) in DMSO-PBS (pH 7.4, 10 mM, v/v, 1/9) at 25 °C for 60 min. λ_{ex} = 457 nm, λ_{em} = 680 nm, slit (nm): 5.0/10.0. (b) Confocal fluorescence images of **1-H_2S** in SH-SY5Y cells. (A1-A2) The cells were treated with **1-H_2S** (5 μM) for 60 min, (B1-B2) cells were pretreated with Aβ_{42} aggregates (10 μM) for 30 min, and then incubated with **1-H_2S** (5 μM) for 60 min. (c) The corresponding fluorescence intensities of red channel. (λ_{ex} = 458 nm, λ_{em} = 600-750 nm for the red channel). Scale bar: 50 μm.

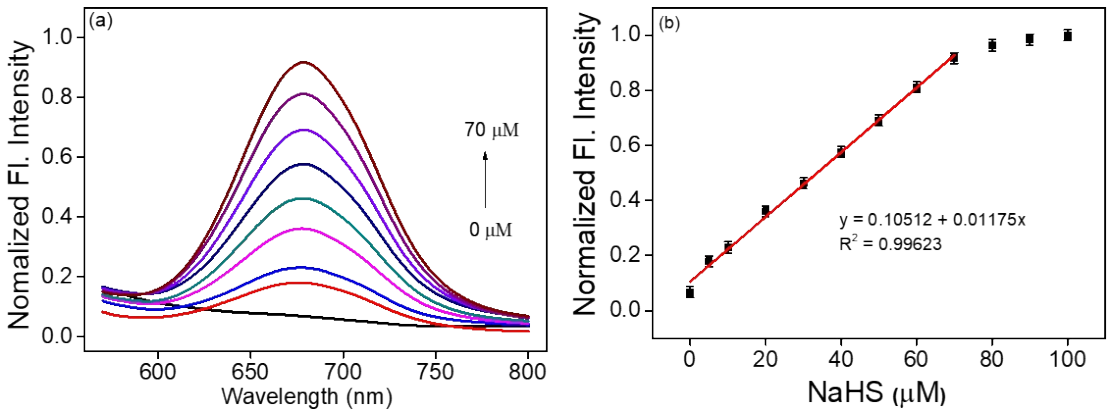


Fig. S19. (a) Fluorescence intensity spectra of probe **1** (10 μM) in the presence of 0 μM to 70 μM of NaHS in DMSO/PBS (10 mM, pH = 7.4, v/v, 1/9) at 37 ℃ for 60 min. λ_{ex} = 457 nm, λ_{em} = 680 nm, slit (nm): 5.0/10.0. (b) The linear changes of the fluorescence intensity of probe at 680 nm and as a function of NaHS concentration.

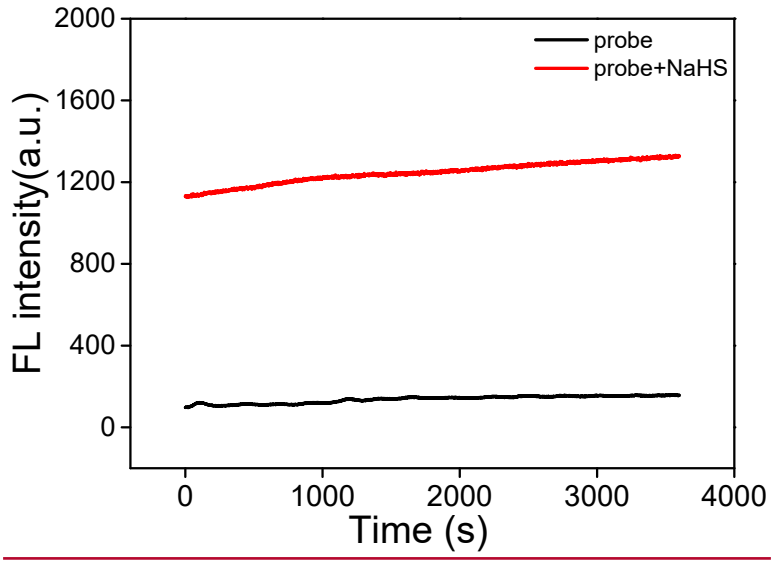


Fig. S20. Time-dependent normalized fluorescent spectra of the probe (10 μM) in the absence and presence of 10 equiv. of NaHS in DMSO-PBS (pH 7.4, 10 mM, v/v/, 1/9) at 37 ℃. λ_{ex} = 457 nm, λ_{em} = 680 nm, slit (nm): 5.0/10.0.

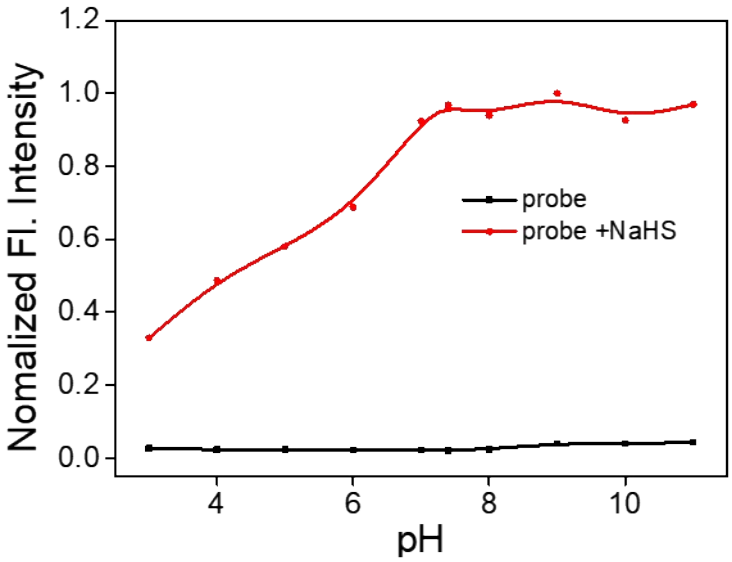


Fig. S21. Profile of pH dependence of the fluorescence intensity of free probe **1** (10 μM) and probe **1** (10 μM) with 10 equiv. of NaHS (0.1 mM) in DMSO-PBS (pH 7.4, 10 mM, v/v, 1/9) at 37 °C for 60 min. (a) λ_{ex} = 457 nm, (b) λ_{ex} = 680 nm, slit (nm):5.0/10.0.

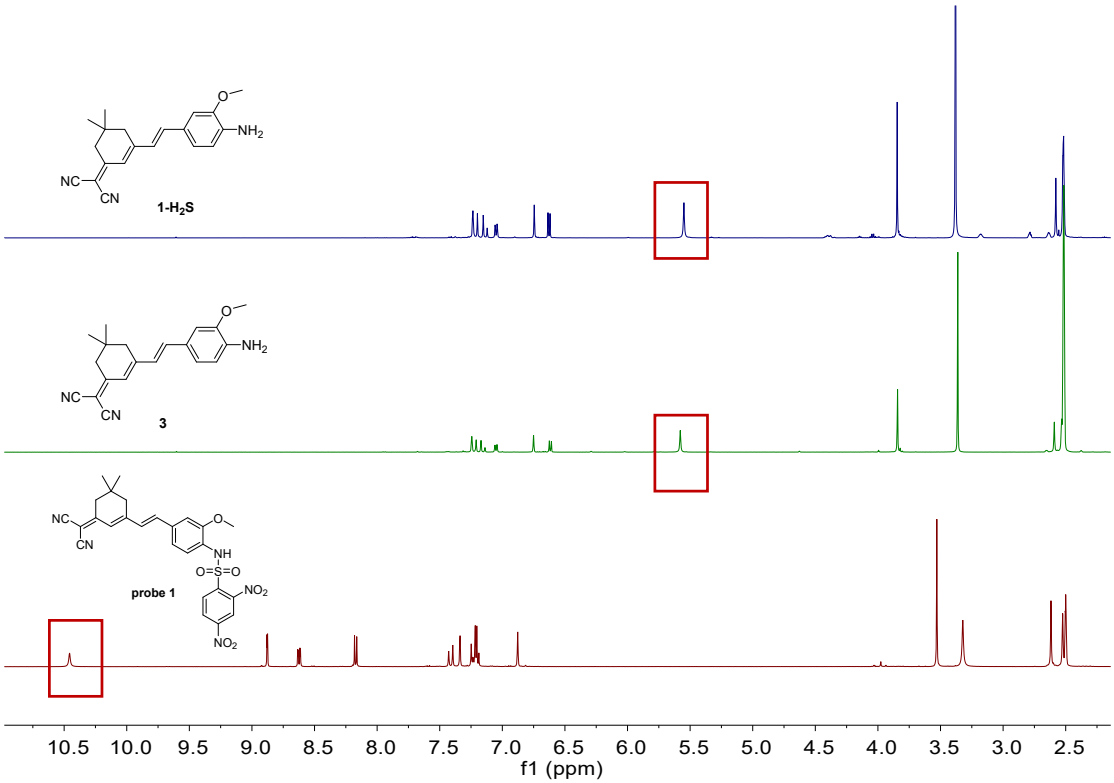


Fig. S22. ^{1}H NMR (500 MHz) spectra of probe **1**, compound **3** and the isolated product of probe **1** + NaHS in DMSO-d_6.

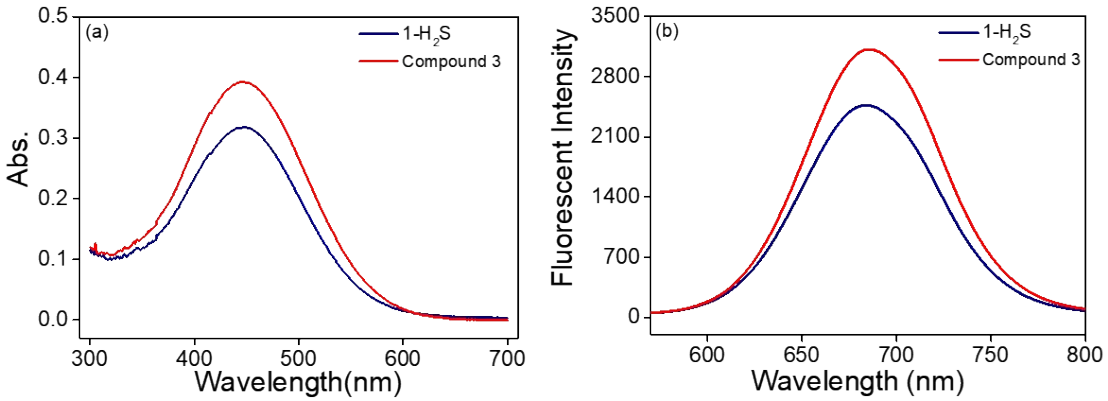


Fig. S23. (a) Absorption spectra of Compound **3** and **1-H_2S** (10 μM) in DMSO-PBS (10 mM, pH = 7.4, v/v, 1/9). (b) Fluorescence spectra of compound **3** and **1-H_2S** (10 μM) in DMSO-PBS (10 mM, pH = 7.4, v/v, 1/9). λ_{ex} = 457 nm, λ_{em} = 680 nm. slit (nm): 5.0/10.0.

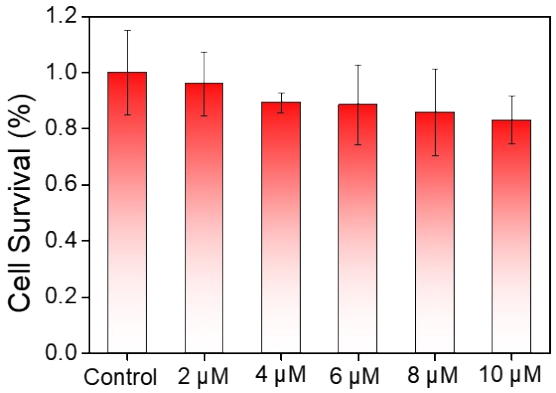


Fig. S24. MTT assay for the survival rate of living Hela cells treated with various concentrations of probe **1** for 24 h.

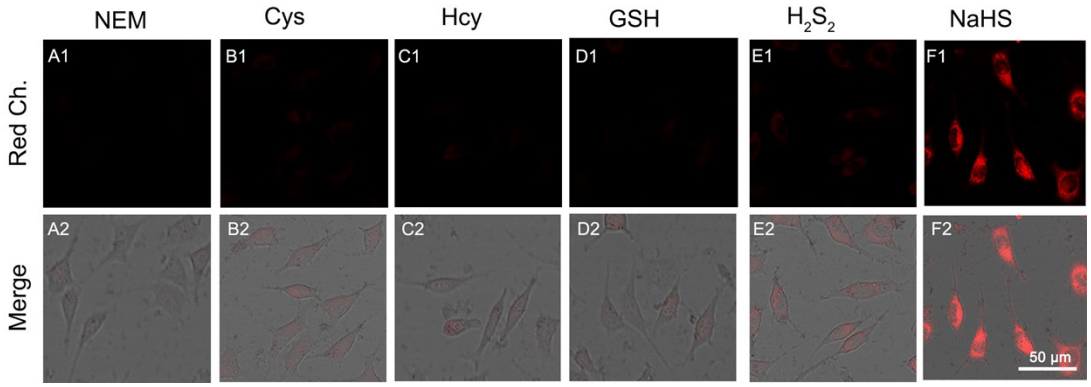


Fig. S25. Confocal fluorescence imaging performance toward exogenous reactive sulfur species in living SH-SY5Y cells. In the initial status group (A1), cells were pretreated with NEM (200 μM) for 30 min, subsequently incubated with probe **1** (5 μM) for 30 min. (B1-F1) Cells were pretreated with NEM (200 μM) for 30 min, subsequently incubated with Cys (200 μM) /Hcy (200 μM) /GSH/ (200 μM)/ H_2S_2 (100 μM)/NaHS (100 μM) for 30 min, and finally incubated with probe **1** (5 μM) for 30 min. A2-F2 are the corresponding bright-field images of A1-F1. (λ_{ex} = 458 nm, λ_{em} = 600-750 nm for the red channel). Scale bar: 50 μm.

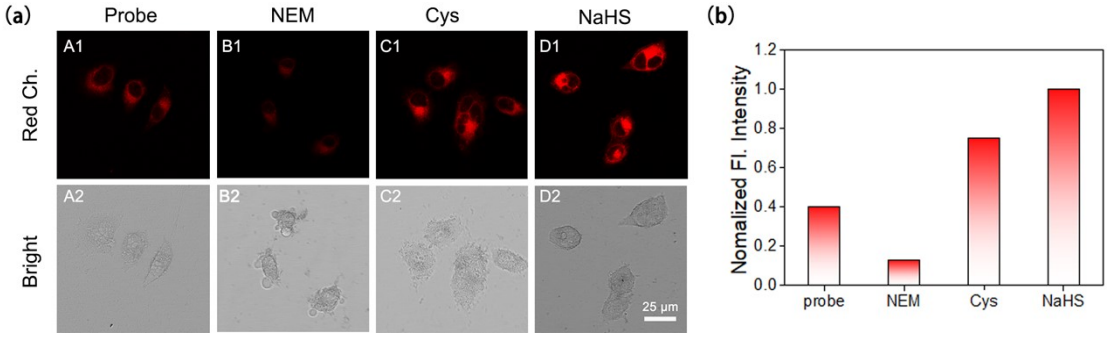


Fig. S26. (a) Confocal fluorescence imaging of endogenous and exogenous H_2S in HeLa cells. (A1-A2) Cells were incubated with probe **1** (5 μM) for 30 min. (B1-B2) The cells were pretreated with NEM (200 μM) for 30 min, and then incubated with probe **1** (5 μM, 30 min). (C1-C2) The cells were pretreated with Cys (200 μM) for 30 min, and then incubated with probe **1** (5 μM, 30 min). (D1-D2) The cells were pretreated with NEM (0.2 mM) for 30 min, subsequently incubated with NaHS (100 μM, 30 min), and finally incubated with probe **1** (5 μM) for 30 min. (b) Relative pixel intensity in panel (a). (λ_{ex} = 458 nm, λ_{em} = 600 - 750 nm for the red channel). Scale bar: 25 μm.

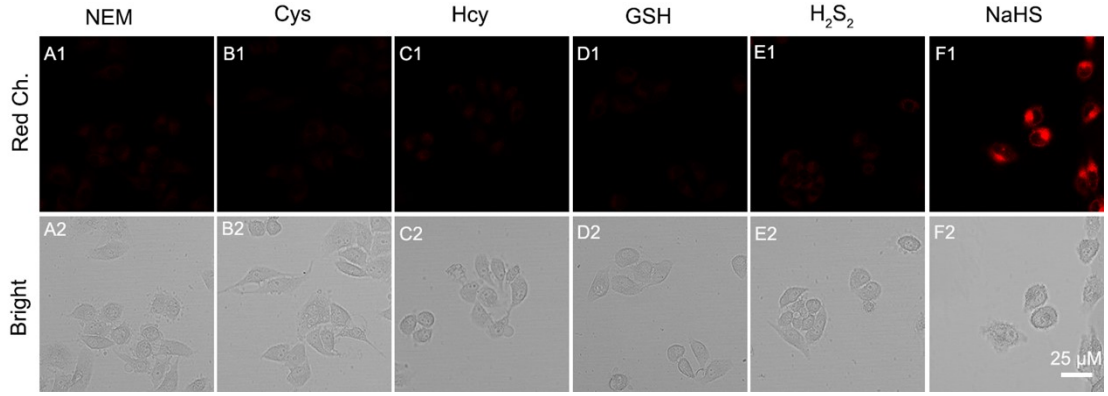


Fig. S27. Confocal fluorescence imaging performance toward exogenous ractive sulfur species in living HeLa cells. In the initial status group (A1), cells were pretreated with NEM (200 μM) for 30 min, subsequently incubated with probe **1** (5 μM) for 30 min. (B1-F1) Cells were pretreated with NEM (200 μM) for 30 min, subsequently incubated with Cys (200 μM) /Hcy (200 μM) /GSH/ (200 μM)/ H_2S_2 (100 μM)/NaHS (100 μM) for 30 min, and finally incubated with probe **1** (5 μM) for 30 min. A2-F2 are the corresponding bright-field images of A1-F1. (λ_{ex} = 458 nm, λ_{em} = 600-750 nm for the red channel).

Scale bar: 25 μm.

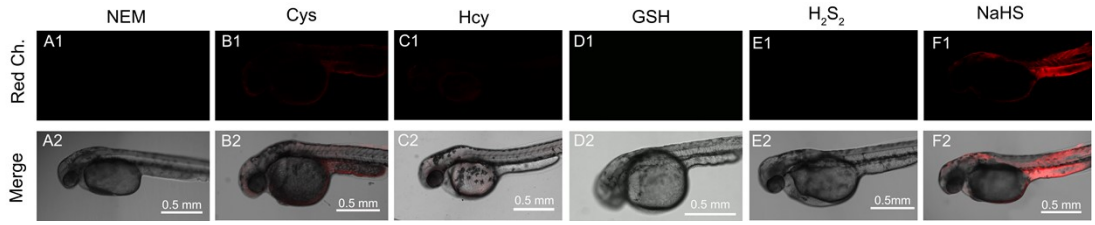


Fig. S28. Confocal fluorescence imaging performance toward exogenous ractive sulfur species in zebrafish. In the initial status group (A1), zebrafish were pretreated with NEM (200 μM) for 30 min, subsequently incubated with probe **1** (10 μM) for 60 min. (B1-F1) zebrafish were pretreated with NEM (200 μM) for 30 min, subsequently incubated with Cys (100 μM) /Hcy (100 μM) /GSH/ (100 μM)/ H_2S_2 (100 μM)/NaHS (100 μM) for 30 min, and finally incubated with probe **1** (10 μM) for 30 min. A2-F2 are the corresponding merge images of A1-F1. (λ_{ex} = 458 nm, λ_{em} = 600-750 nm for the red channel). Scale bar: 0.5 mm

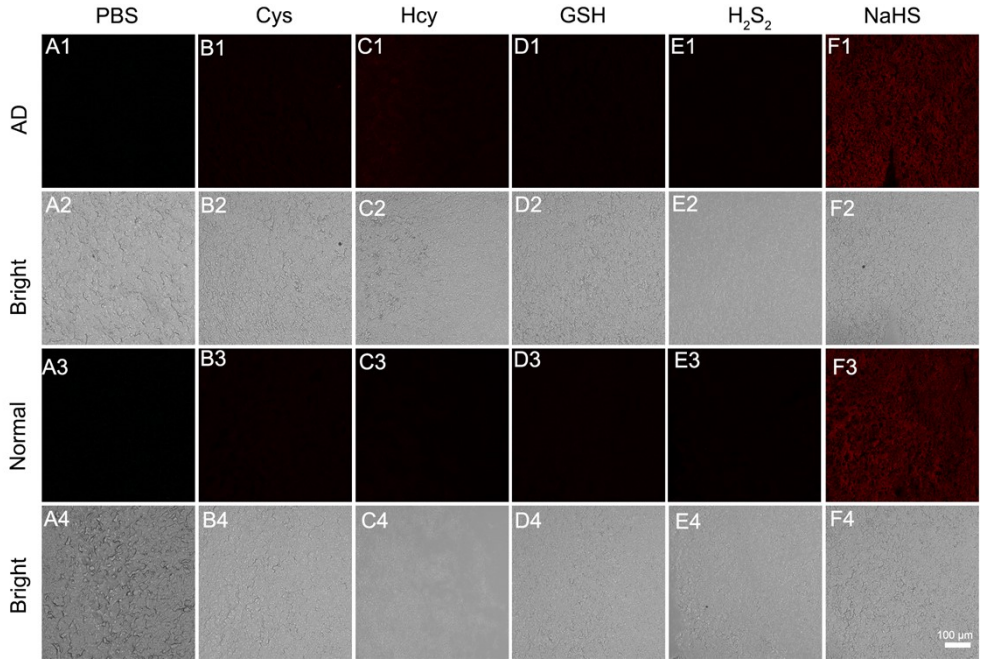


Fig. S29. Confocal fluorescence images of probe **1** against reactive sulfur species in hippocampus tissues of mice. (A)The fresh normal and AD mice brain hippocampus tissues pretreated with PBS (30 min). (B-F) Hippocampus tissues were pretreated with NEM (200 μM) for 30 min, subsequently incubated with Cys(200 μM)/ Hcy(200 μM)/ GSH(200 μM)/ H_2S_2 (200 μM)/ NaHS (200 μM) for 30 min, and finally incubated with probe **1** (10 μM) for 30 min. (A1-F1, A2-F2) Hippocampus tissues of Alzheimer's disease mice. (A3-F3, A4-F4) Hippocampus tissues of normal mice. (λ_{ex} = 458 nm, λ_{em} = 600-750 nm for the red channel). Scale bar: 100 μm.