Electronic Supplementary Information (ESI)

for

Aldehyde group assisted thiolysis of dinitrophenyl ether: a new promising approach for efficient hydrogen sulfide probes

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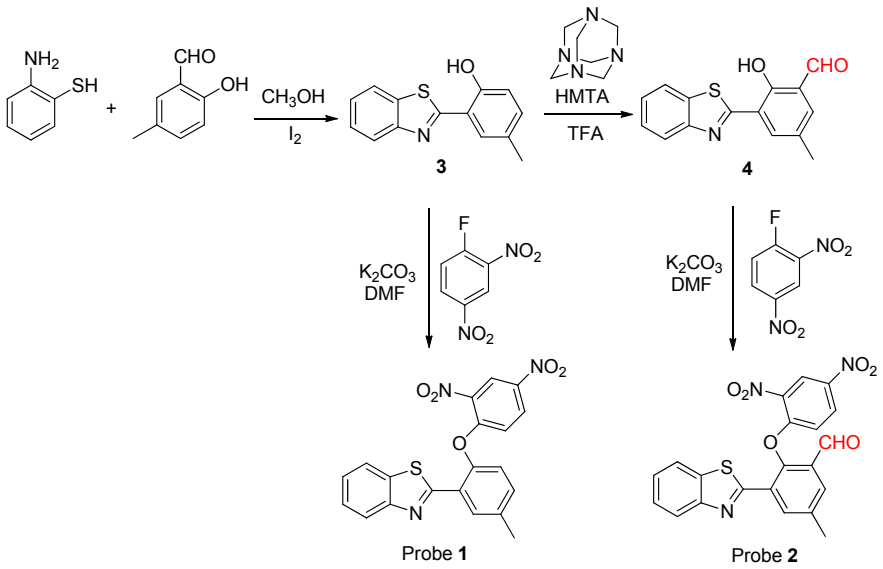
1. Experimental

1.1 General

Starting materials were purchased from commercial suppliers and used without further purification. All solvents were purified prior to use. The stock solutions of probe **1** and **2** were prepared in HPLC grade CH_3CN. All other solutions and buffers were prepared with distilled water that had been passed through a Millipore-Q water ultrapurification system. TLC analysis was performed using precoated silica plates. IR spectra were recorded on a FT-IR spectrophotometer as KBr pellets and were reported in cm^{-1}. NMR spectra were measured on Varian Mercury 400 and 600 instruments and coupling constants (*J* values) are reported in hertz. The low-resolution MS spectra were performed on an electron ionization mass spectrometer. HR-MS data were obtained with an LC/Q-TOF MS spectrometer. UV-vis and fluorescence spectra were recorded on an Agilent Cary 100 UV-vis spectrophotometer and Agilent Cary Eclipse fluorescence spectrophotometer (with temperature controller), respectively. Standard quartz cuvettes with a 10 mm lightpath were used for all UV-vis spectra and fluorescent spectra measurements.

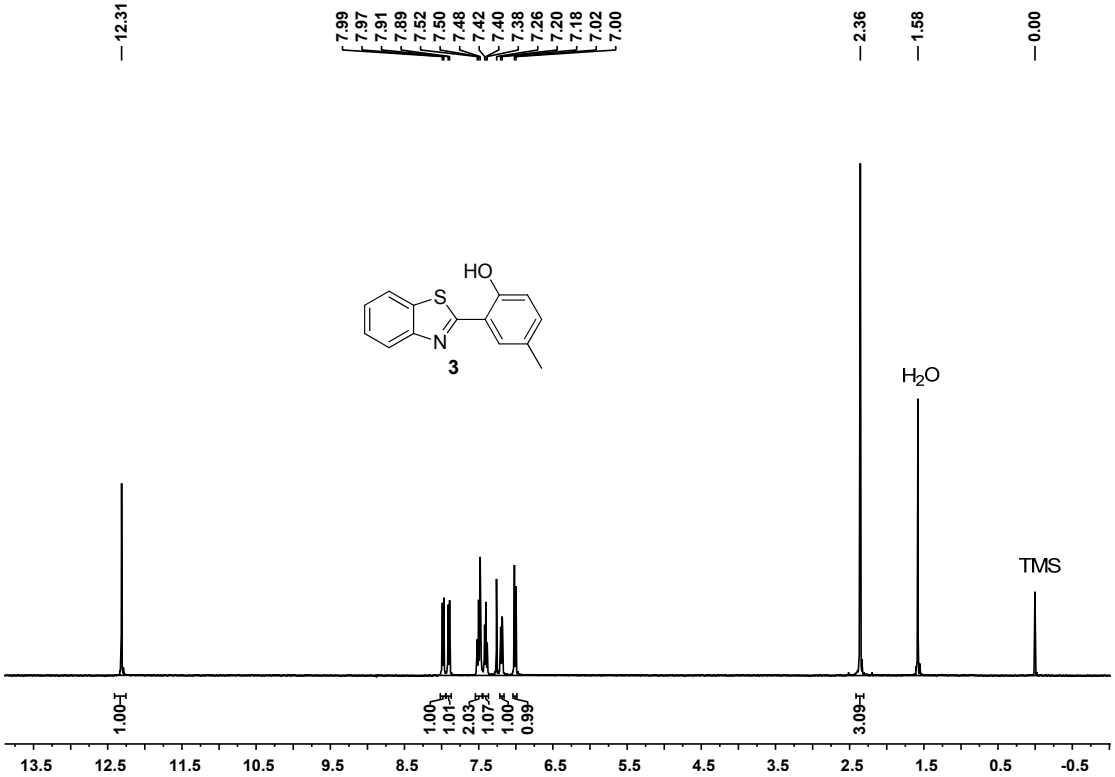
1.2 Synthesis

1.2.1 Synthesis of probe 1 and probe 2

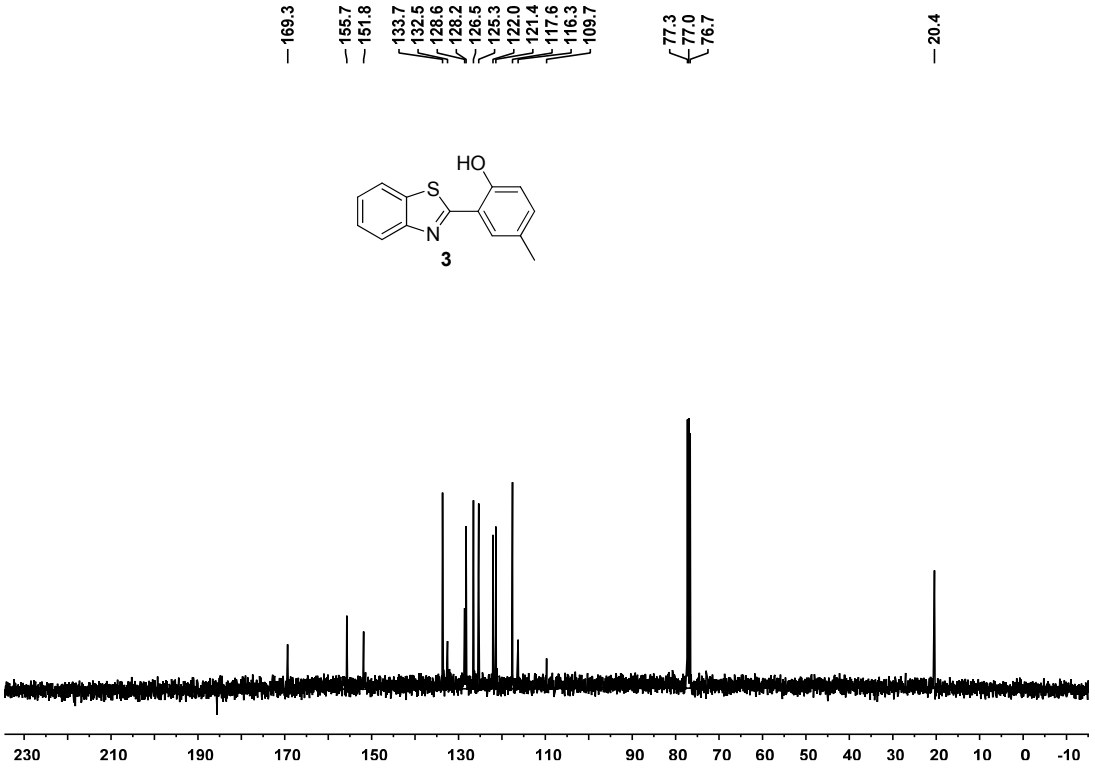


Scheme **S1**. Synthesis of probe **1** and probe **2**

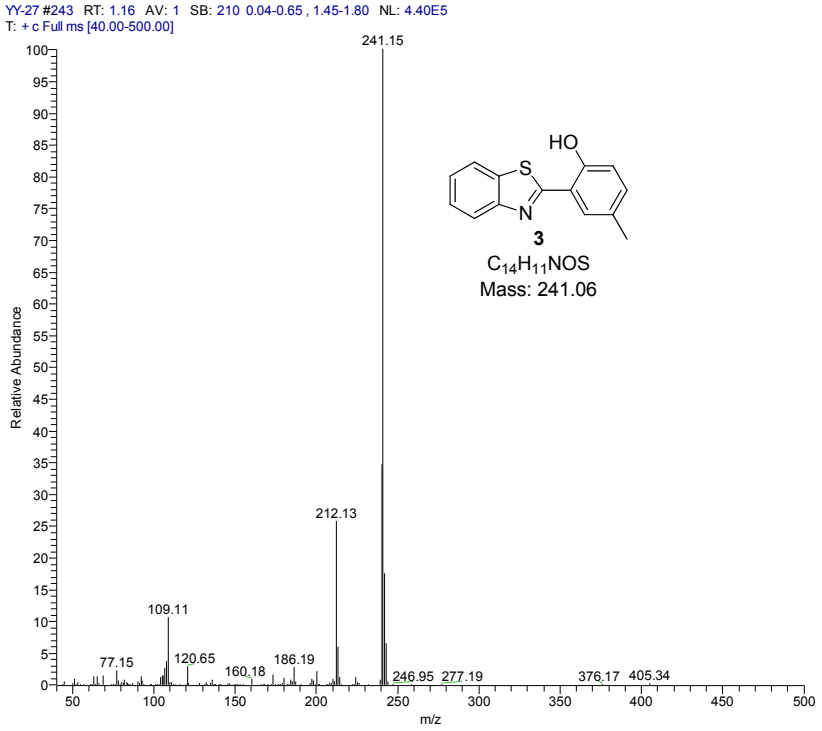
Synthesis of compound 3: To a solution of 2-aminobenzenethiol (109 mg, 1.00 mmol) and 2-hydroxy-5-methylbenzaldehyde (136 mg, 1 mmol) in anhydrous CH_3OH (10 mL) was added I_2 (126 mg, 0.5 mmol). The mixture was stirred at room temperature. After about 5 min, yellow-green precipitate generated gradually. The reaction mixture was further stirred for another 2 h. The solid was collected on a filter and washed with cold CH_3OH. Further dried in a vacuum afforded **3** as a pale-yellow solid (120 mg, yield 50.1%). Mp 127-128 ºC. TLC (silica plate): R_f = 0.78 (hexane:ethyl acetate = 5:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.31 (s, 1H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.54–7.45 (m, 2H), 7.40 (t, *J* = 7.2 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 155.7, 151.9, 133.7, 132.5, 128.6, 128.3, 126.5, 125.3, 122.0, 121.4, 117.6, 116.3, 20.4. IR (KBr) ν_{max} (cm^{-1}): 3057, 2914, 1780, 1653 (s), 1501 (s), 1457(s), 1222,1271, 1182, 1067, 995, 789, 755, 723; EI-MS: m/z found 241.15 (M^+, 100%). This compound has been reported by K. Anthony, *et al*., in *J. Chem. Soc., Perkin Trans. II*, 1984, 2111-2118.



^{1}H NMR spectrum of **3** in $CDCl_3$.

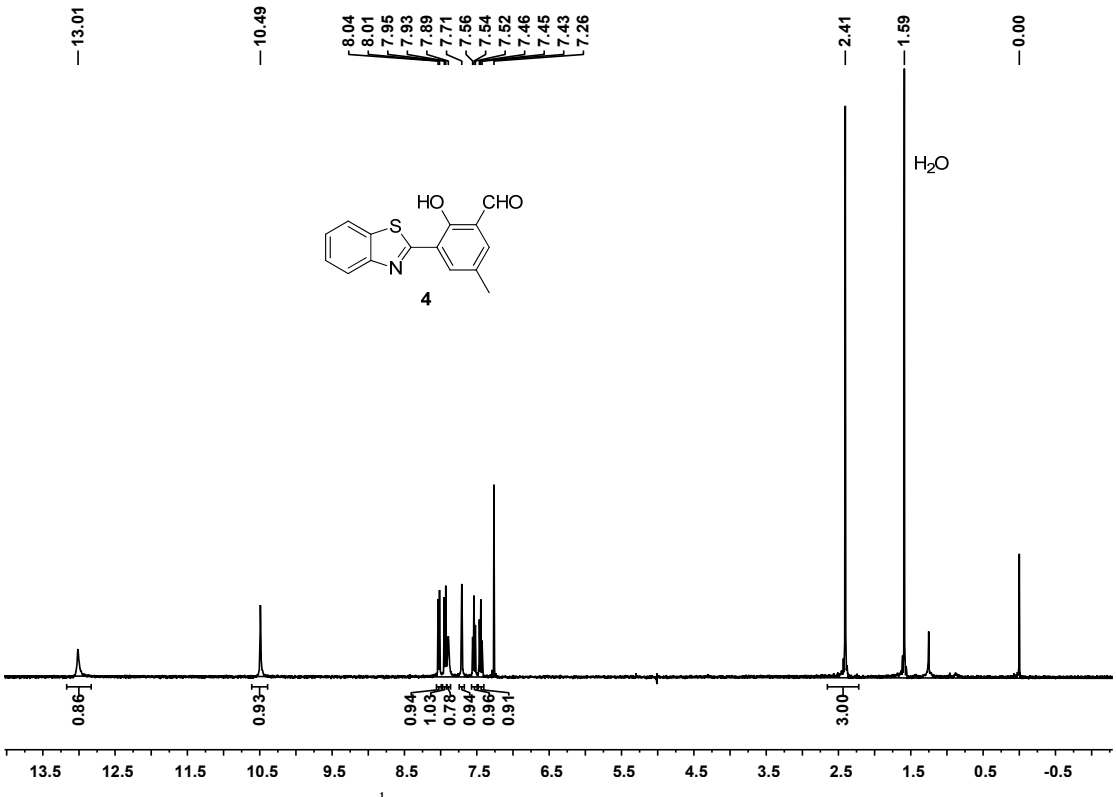


^{13}C NMR spectrum of **3** in $CDCl_3$.

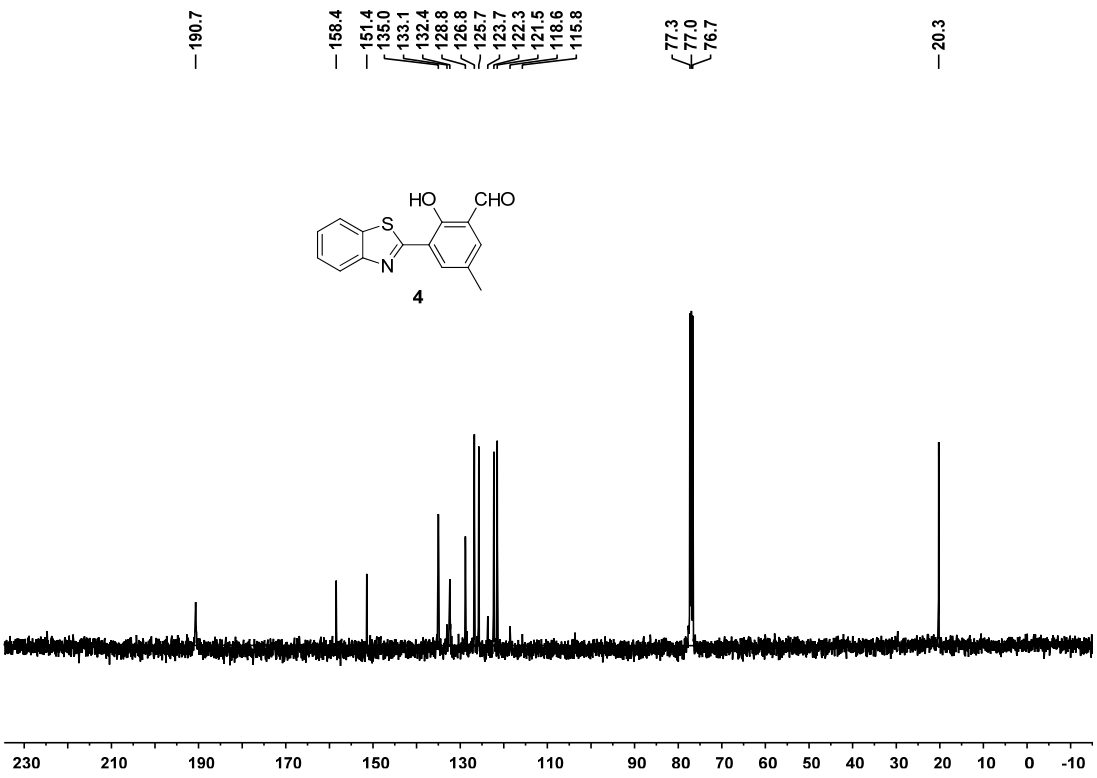


MS (EI) spectrum of **3**.

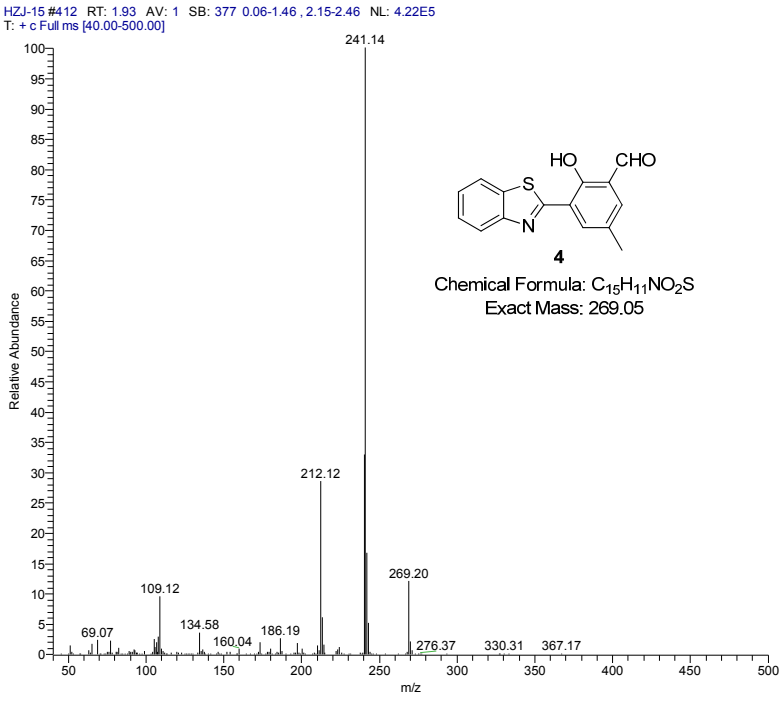
Synthesis of compound 4: Compound **3** (241 mg, 1 mmol) was dissolved in 10 mL of trifluoroacetic acid and then hexamethylenetetramine (168 mg, 1.2 mmol) was added. The mixture was refluxed over night until all the starting material was consumed. The reaction mixture was then cooled to room temperature and poured into 6 M HCl (30 mL) and extracted with CH_2Cl_2. The combined organic extracts were washed with saturated brine. Next purification was done by column chromatography (ethyl acetate: petroleum ether = 1:5) to afford the pure product as a yellow solid (237 mg, yield 87%). Mp 176-178 ºC. TLC (silica plate): R_f = 0.6 (hexane:ethyl acetate 5:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.01 (s, 1H, CHO), 10.49 (s, 1H, OH), 8.02 (d, *J* = 8.1 Hz, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.89 (s, 1H), 7.71 (s, 1H), 7.54 (t, *J* = 7.7 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 2.41 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.7, 158.4, 151.4, 135.0, 133.1, 132.4, 128.8, 126.8, 125.7, 123.7, 122.3, 121.5, 118.6, 115.8, 20.3. IR (KBr) ν_{max} (cm^{-1}): 2918, 2860, 1686, 1609, 1559 (s), 1492 (s), 1471, 1439, 1432, 1343, 779, 756, 629. EI-MS: m/z found 269.20 (M^+, 12%), 241.14 (M^+ - CHO, 100%). HR-MS Calc. for $C_{15}H_{12}NO_2^+$ (M + H^+) 270.0583, found 270.0589.

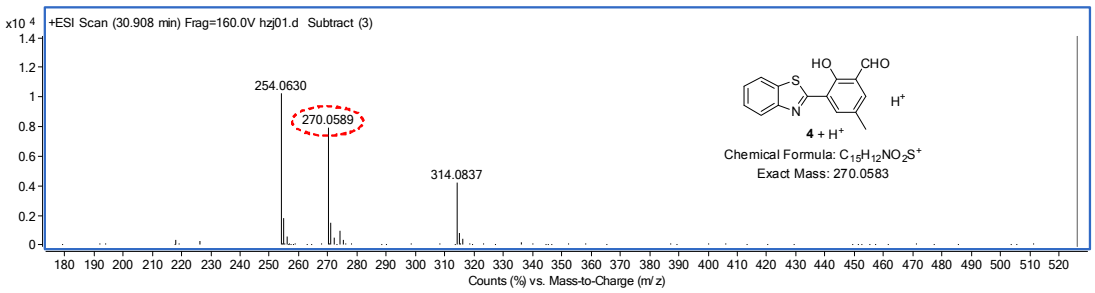


^{1}H NMR spectrum of **4** in $CDCl_3$.



^{13}C NMR spectrum of **4** in $CDCl_3$.





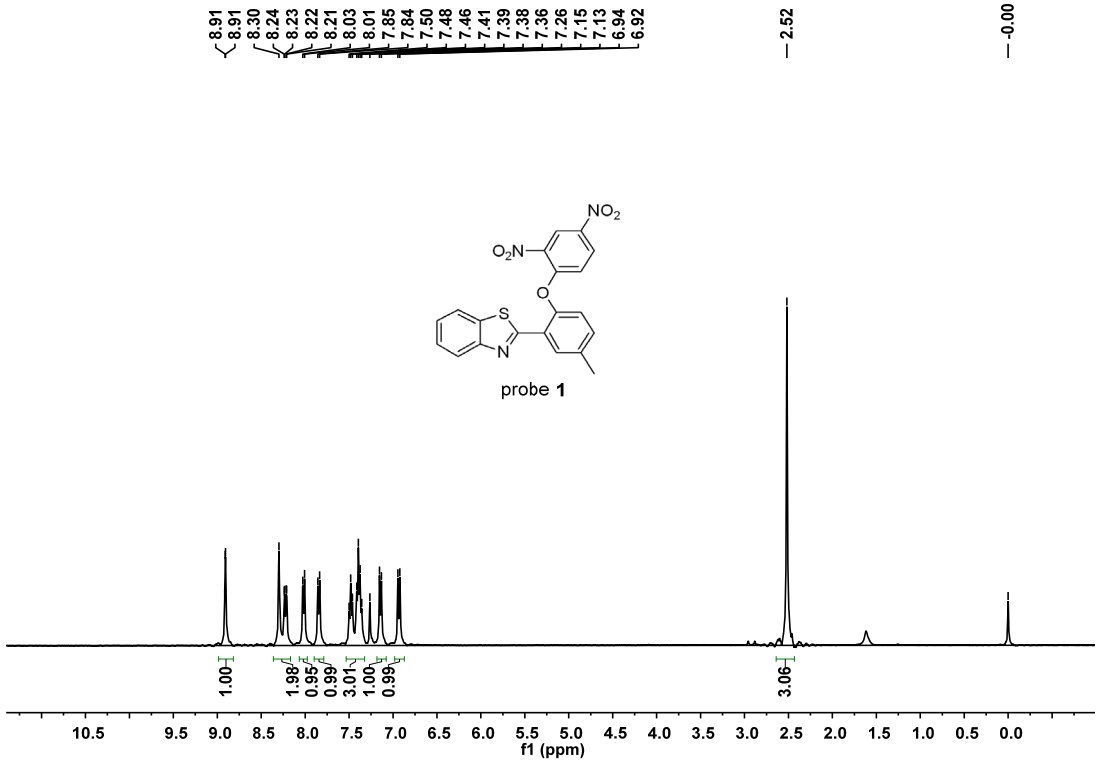
Synthesis of probe 1:

A mixture of compound **3** (241 mg, 1 mmol), 1-fluoro-2, 4-dinitrobenzene (205 mg, 1.1 mmol), and K_2CO_3 (274 mg, 2 mmol) in DMF (5 mL) was stirred at room temperature. After the reaction completes (about 1 h, monitored by TLC), the reaction mixture was poured into ice water. The precipitate was collected by filtration and washed with cold water to afford an offwhite solid (378 mg, 93.5%). Mp: 179-180 °C; TLC (silica plate): R_f = 0.45 (hexane:ethyl acetate = 5:1, v/v); ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.91 (d, *J* = 1.8 Hz, 1H), 8.36 – 8.17 (m, 2H), 8.02 (d, *J* = 8.1 Hz, 1H), 7.84 (d, *J* = 7.9 Hz, 1H), 7.53 – 7.32 (m, 3H), 7.14 (d, *J* = 8.2 Hz, 1H), 6.93 (d, *J* = 9.2 Hz, 1H), 2.52 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 155.8, 152.5, 148.4, 141.6, 139.4, 137.5, 135.4, 133.1, 131.2, 128.8, 126.4, 126.0, 125.5, 123.3, 122.1, 121.9, 121.4, 117.5, 20.9. IR (KBr) ν_{max} (cm^{-1}): 3110, 3045, 2919, 1611, 1545, 1524, 1501, 1478, 1316, 1244, 983, 806, 765,735; EI-MS: m/z found 407.23 (M^+, 41%), 361.18 (M^+ - NO_2, 100%); HR-MS Calc. for $C_{20}H_{14}N_3O_5S^+$ (M + H^+) 408.06487, found 408.06488.

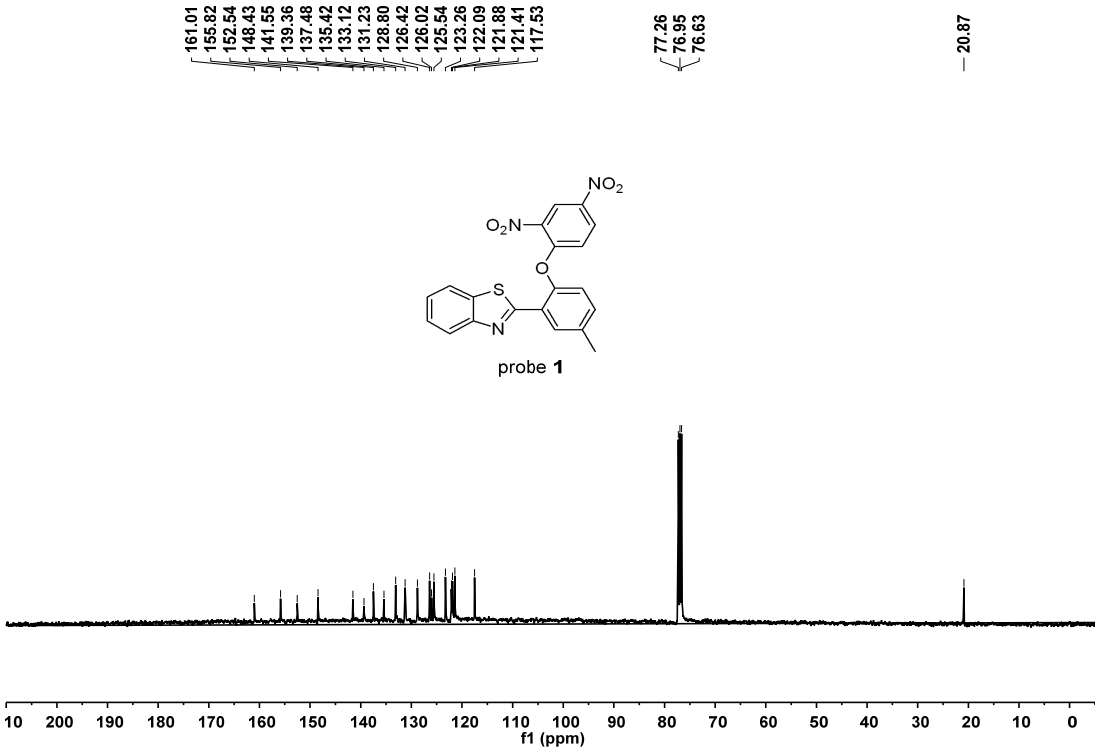
Synthesis of probe 2:

A mixture of compound **4** (269 mg, 1 mmol), 1-fluoro-2, 4-dinitrobenzene (205 mg, 1.1 mmol), and K_2CO_3 (274 mg, 2mmol) in DMF (8 mL) was stirred at room temperature. After the reaction completes (about 1 h, monitored by TLC), the reaction mixture was poured into ice water. The precipitate was collected by filtration and washed with water. The crude product was further purified by recrystallisation from dichloromethane to afford a colorless solid (369 mg, 85%). Mp: 254-256 °C; TLC (silica plate): R_f = 0.24 (hexane:ethyl acetate = 5:1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si): δ (ppm) 10.16 (s, 1H, CHO), 8.96 (d, *J* = 2.7 Hz, 1H, ArH), 8.55 (s, 1H, ArH), 8.18 (dd, *J* = 9.3 and 2.7 Hz, 1H, ArH), 8.02 (d, *J* = 8.1 Hz, 1H, ArH), 7.94 (s, 1H, ArH), 7.51 (t, *J* = 7.8 Hz, 1H, ArH), 7.41 (t, *J* = 7.5 Hz, 1H, ArH), 6.75 (d, *J* = 9.3 Hz, 1H, ArH), 2.61 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 192.4, 159.8, 157.8, 155.1, 151.9, 147.4, 142.2, 141.6, 138.2, 138.2, 136.4, 134.9, 129.7, 128.60, 127.0, 123.1, 122.2, 116.7, 113.1.50, 112.9, 20.2; IR (KBr) ν_{max} (cm^{-1}): 3115, 2740, 1701, 1601, 1595 (s), 1536 (s), 1481, 1449, 1430, 1343, 803, 761, 703; MS (EI) m/z found 435.20 (M^+, 2%), 418.20 (M^+ - OH, 15%), 407.19 (M^+ - CHO, 100%); HR-MS (ESI) calculated for $C_{21}H_{14}N_3O_6S^+$ (M + H^+) 436.05978, found 436.05951.

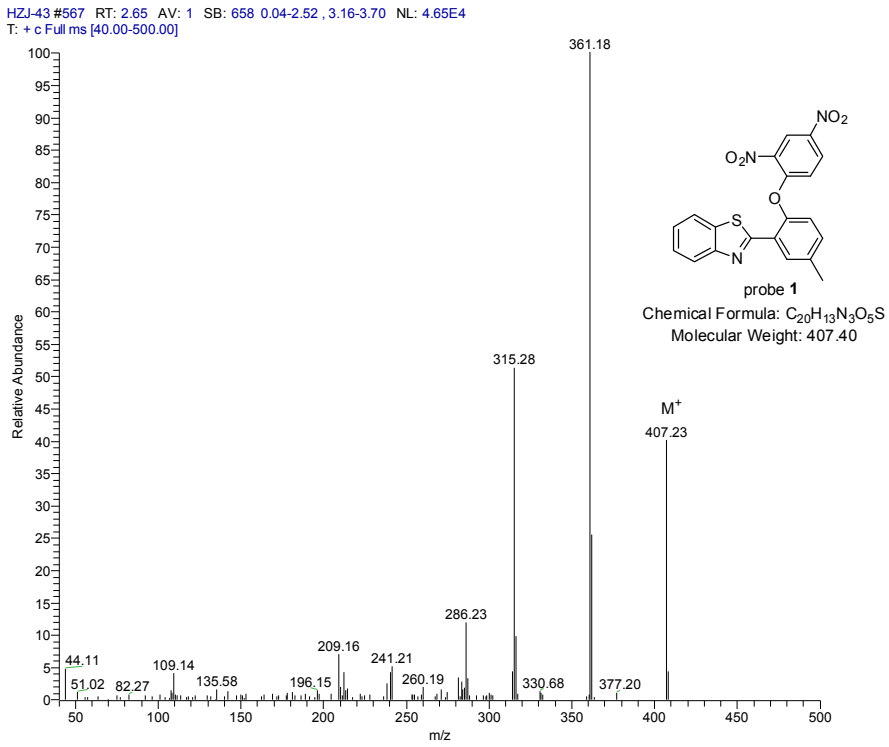
1.2.2 Structure characterizations of probe 1 and probe 2



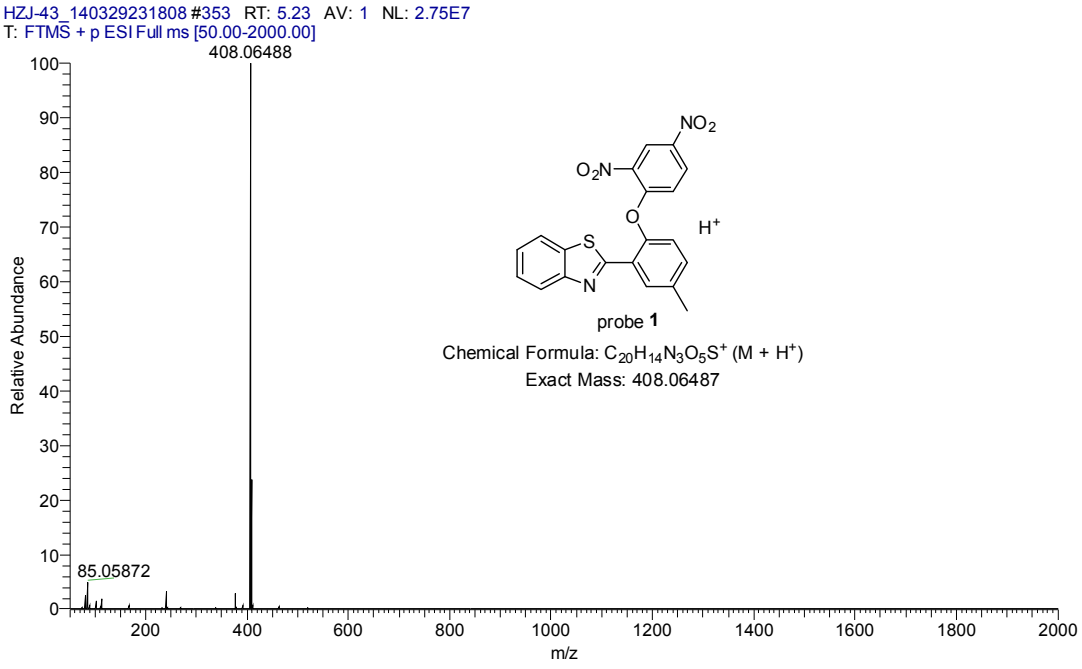
^{1}H-NMR spectrum of probe **1** in $CDCl_3$



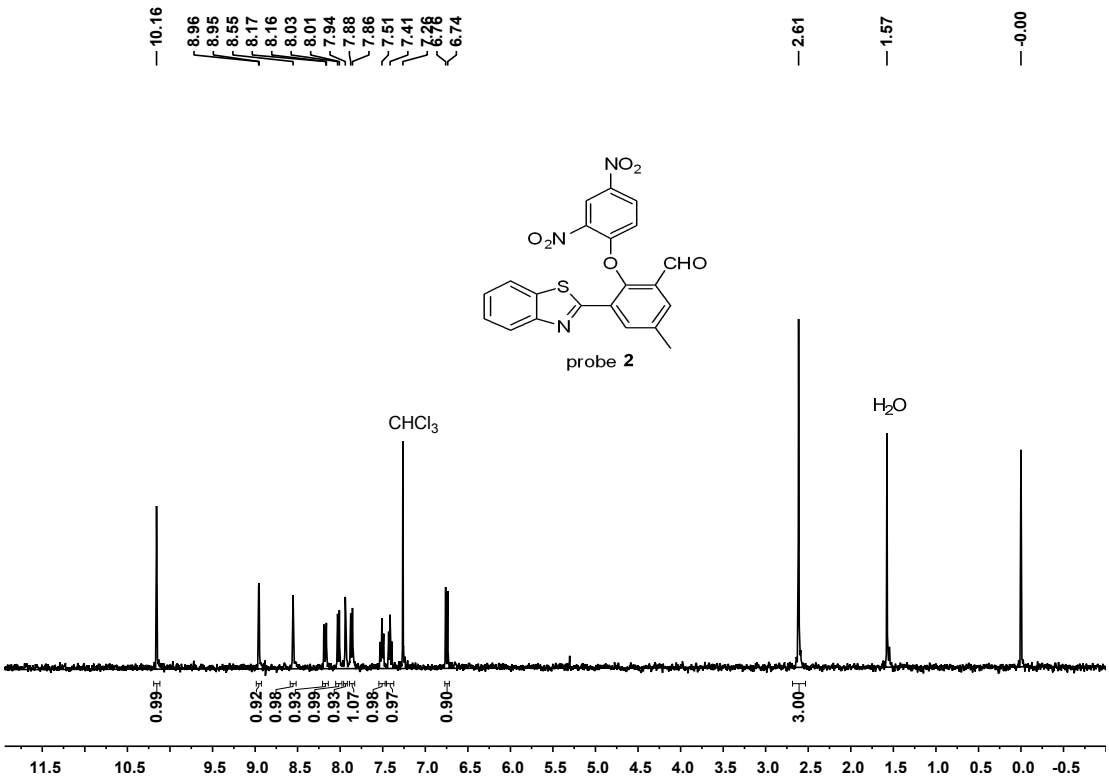
^{13}C-NMR spectrum of probe **1** in $CDCl_3$



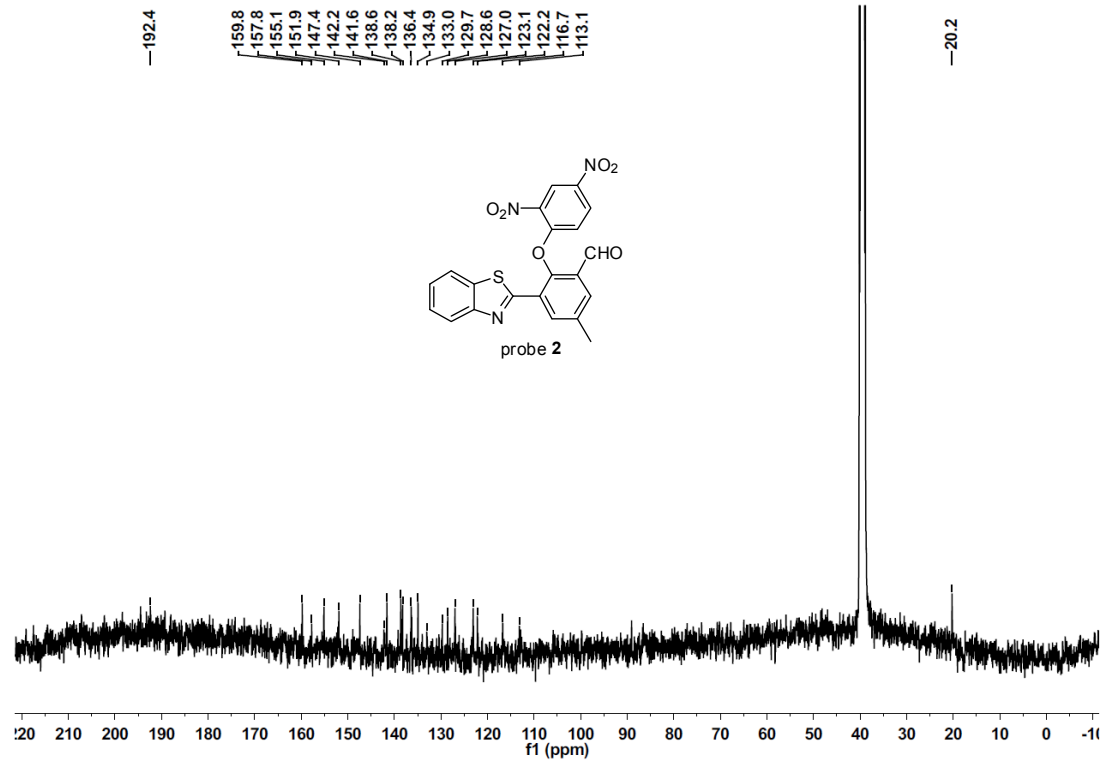
MS (EI) spectrum of probe **1**



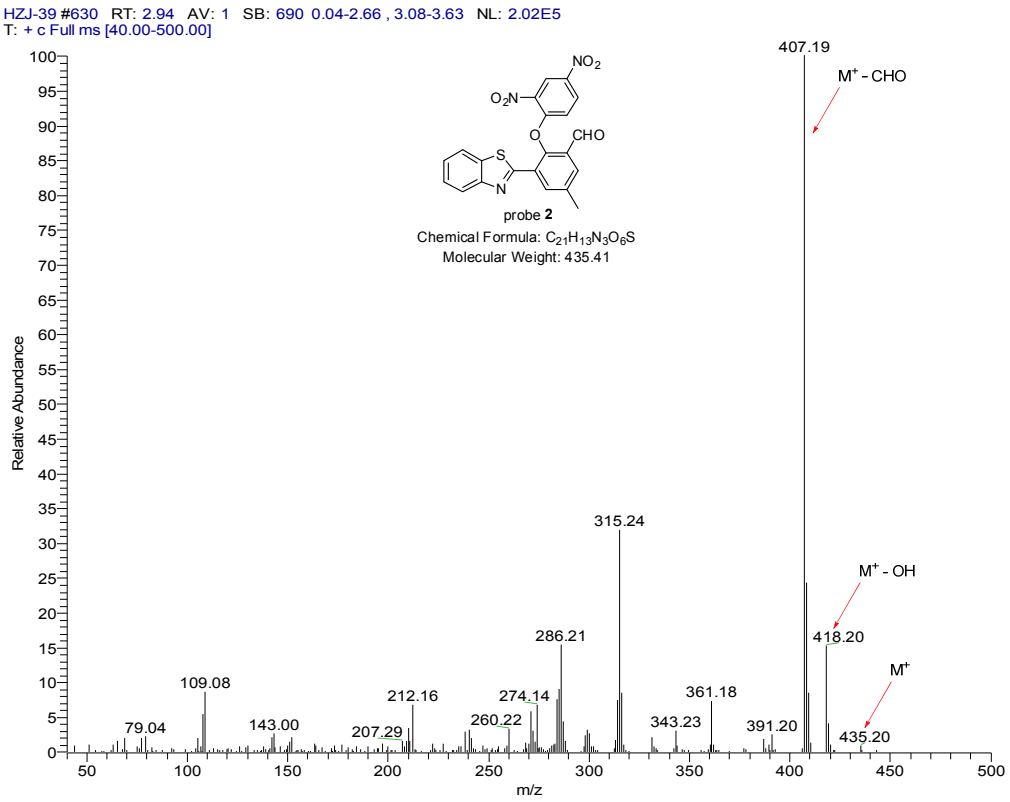
HR-MS spectrum of probe **1**



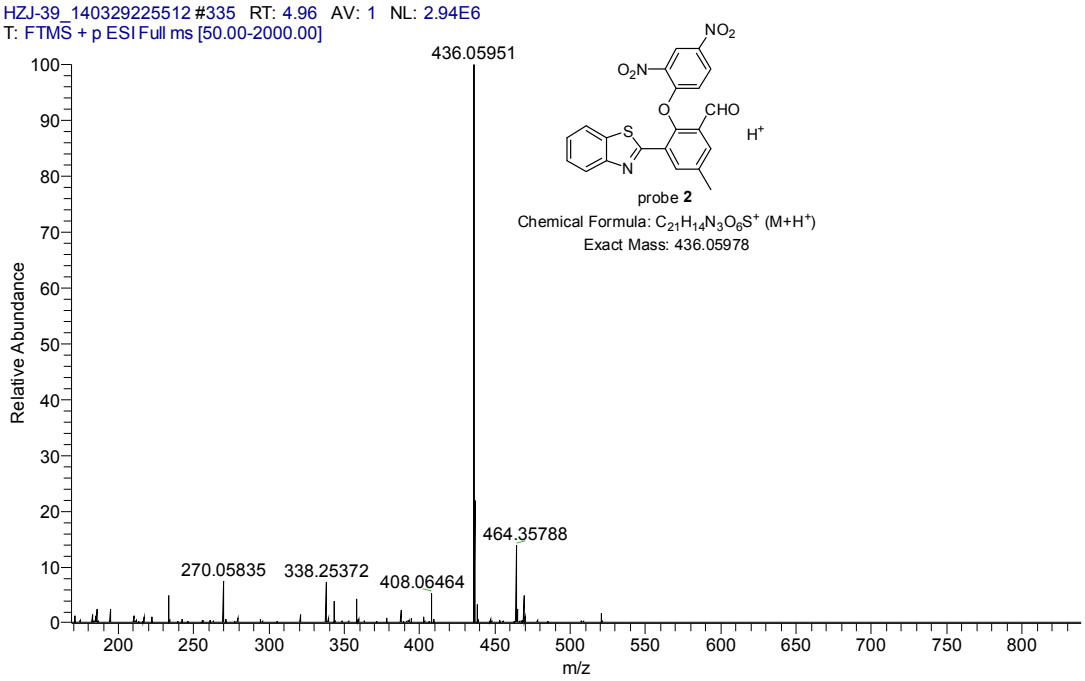
^{1}H-NMR spectrum of probe **2** in $CDCl_3$



^{13}C NMR spectrum of probe **2** in DMSO-d_6. (Note: This spectrum is not perfect due to the low solubility of probe **2**, however, the carbon signals can be assigned and agree well with probe **2**. MS spectra further proved its structure, see below.)



MS (EI) spectrum of probe **2**



HR-MS spectrum of probe **2**

1.3 Determination of the fluorescence quantum yield

In our system, the fluorescence quantum yields of **3**, **4**, probe **1** and probe **2** were determined to be Φ = 0.04, 0.178, 0.002 and 0.006 in PBS buffer (20 mM, pH 7.4) containing 20% CH_3CN (v:v) at 25˚C, using quinine sulfate (Φ_f= 0.58 in 1N H_2SO_4) as standard. The quantum yield was calculated using the following equation:

$$\Phi_x = \Phi_s(A_s F_x / A_x F_s)\,(n_x^2/n_s^2)$$

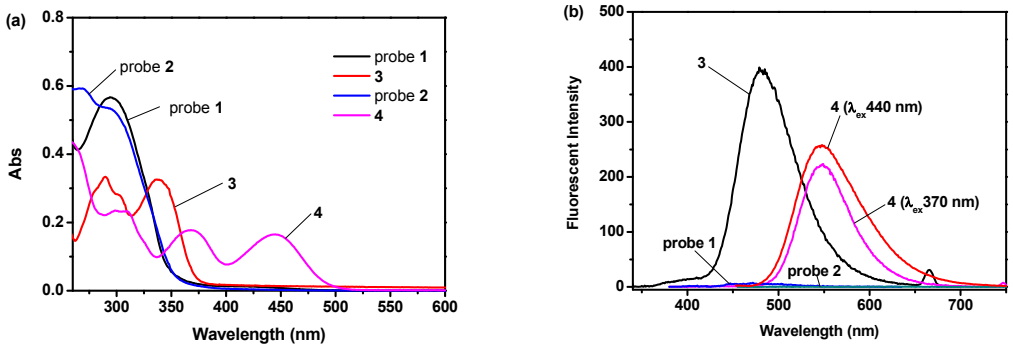
where, A_x and A_s are the absorbance of the sample and the reference, respectively, at the same excitation wavelength, F_x and Fs are the corresponding relative integrated fluorescence intensities, and n is the refractive index of the solvent. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05.

1.4 Cell culture and imaging

HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum), 100 mg/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, water saturated incubator at 37 °C. Before cell imaging experiments, HeLa cells were seeded in 12-well culture plate for one night. For living cells imaging experiment of probe **1**, as a control experiment, cells were incubated with 20 μM probe **1** (4% content of acetonitrile is containing due to stock solutions of probe **1** was prepared in acetonitrile) for 20 min at 37 °C and washed three times with prewarmed PBS buffer, and then imaged immediately (Fig. 4, A and B). HS^- imaging in celles was then investigated. In this case, HeLa cells were pretreated with 20 μM probe **1** for 20 min at 37 °C, washed three times with prewarmed PBS buffer, and then incubated with 200 μM NaHS for 30 min at 37 °C. Cell imaging was then carried out after washing cells with prewarmed PBS buffer (Fig. 4, C and D).

2 Additional Data

2.1 UV-vis and fluorescence spectra of probe 1, probe 2, 3 and 4.



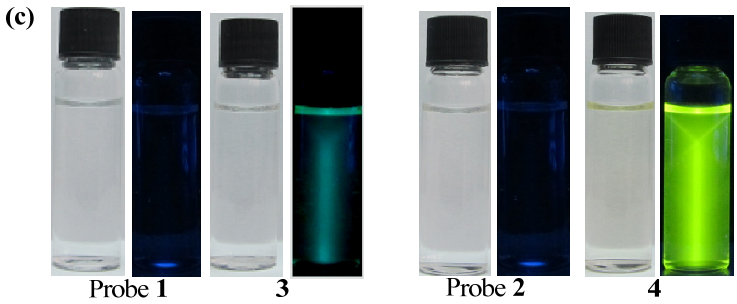


Fig. S1 (a) UV-vis spectra of probe **1**, probe **2**, **3** and **4**; (b) Fluorescence spectra of probe **1**, probe **2**, **3** and **4**; (c) Color and fluorescence color for probe **1**, **3**, probe **2** and **4**. All spectra were measured in PBS buffer solution (20 mM, pH 7.4, 25% CH_3CN, v/v) at 25˚C and the concentrations of probe **1**, probe **2**, **3** and **4** are used 20 μM, respectively. Fluorescence measure conditions for **3** and probe **1**: λ_{ex} = 330 nm, d_{em}= d_{ex}= 5 nm, and for **4** and probe **2**: λ_{ex} = 440 nm, slit width: d_{ex}= d_{em}= 5 nm, respectively. The fluorescence spectrum of **4** when exited at 370 nm is also shown (Note: in this case, a smaller slit width was set at d_{ex}= 2.5 nm, d_{em}= 5 nm).

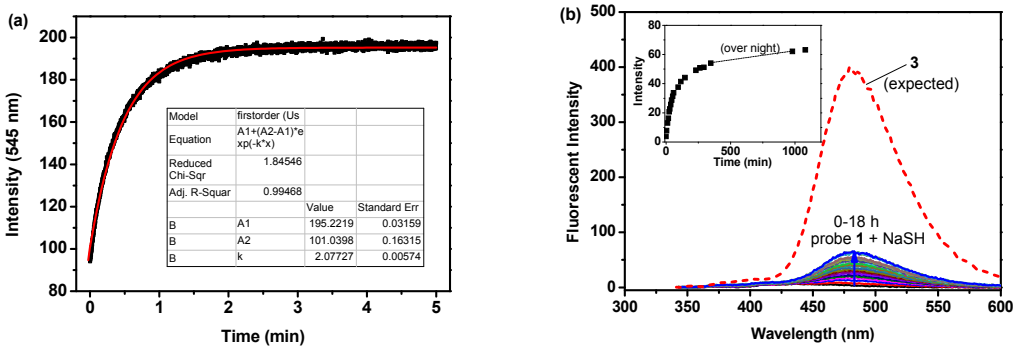


Fig. S2 (a) Kinetics of fluorescent intensity changes of probe **2** (20 μM) at 545 nm in the presence of HS^- (200 μM) in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v) at 25˚C. λ_{ex} = 440 nm, slit width: d_{ex} = 5 nm, d_{em} = 5 nm. The data curve is fitted (red line) by a first order reaction scheme (see equation inserted, where A1 and A2 are the final and initial fluorescent intensity, respectively. The observed pseudo-first-order rate constant k_{obs} was determined to be about 2.077 ± 0.006 min^{-1}). (b) Fluorescent spectra changes of probe **1** (20 μM) in the presence of HS^- (200 μM) in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v) at 25˚C. λ_{ex} = 330 nm, slit width: d_{ex} = d_{em} = 5 nm. This experiment indicates that the reaction between probe **1** and HS^- is much slower than that of probe **2**.

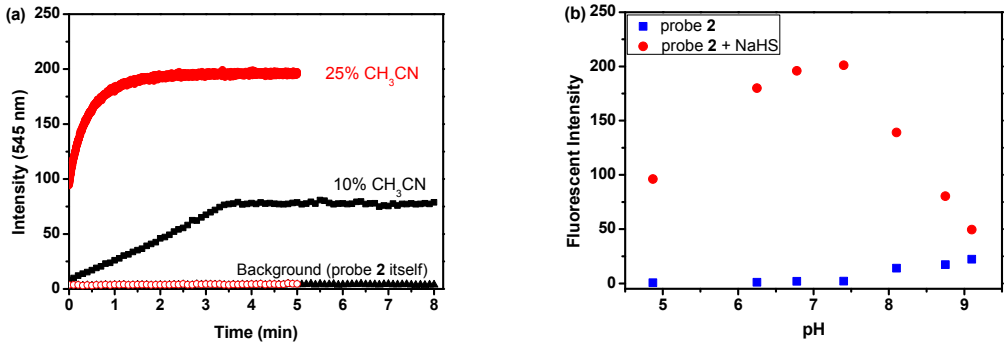


Fig. S3 (a) A comparison of kinetics of fluorescence intensity changes of probe **2** (20 μM) upon addition of 10 equiv NaHS in PBS buffer (20 mM) containing 25% CH_3CN (v:v) and 10% CH_3CN (v:v) at 25˚C. Although this result indicates that reducing the amount of CH_3CN is also working for probe **2**, this will reduce the fluorescence intensity and the reaction rate as well, so 25% of CH_3CN was used in the following experiments. (b) The effect of pH on the fluorescence intensity changes of probe **2** (20 μM) upon addition of 10 equiv NaHS in PBS buffer (20 mM) containing 25% CH_3CN (v:v) at 25˚C. All the data are monitored at λ_{em} = 545 nm with λ_{ex} = 440 nm and acquired 5 min after the mixture standing at 25˚C. This result indicates that probe **2** can work over a wide range pH, and the optimal fluorescence signal responses for HS^- were observed around pH 7.0.

2.2 Data for investigation of the sensing mechanism

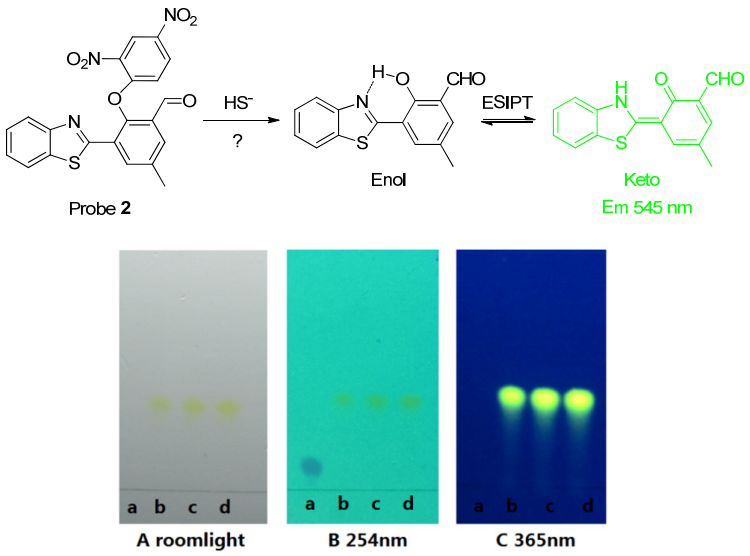


Fig. S4 The pictures of the thin layer chromatography TLC plates under different light used to compare probe **2**, the reference sample of **4** and the isolated product from the reaction of probe **2** with NaHS in 1:1 H_2O-CH_3CN (v/v). (A) under room light, (B) under light of 254 nm, (C) under light of 365 nm. Spots on the TLC plate are: a. probe **2**; b. the reaction product of probe **2** and NaHS; c. mixture of b and **4**; d. the reference sample of **4**. The eluent for TLC: hexane:ethyl acetate = 5:1 (v/v). This indicates that the reaction of probe **2** with NaHS produced **4**. The green fluorescence at 545 nm indicates an ESIPT process of the product (see S. Goswami, A. Manna, S. Paul, A. K. Das, K. Aich and P. K. Nandi, *Chem. Commun*., 2013, **49**, 2912-2914.)

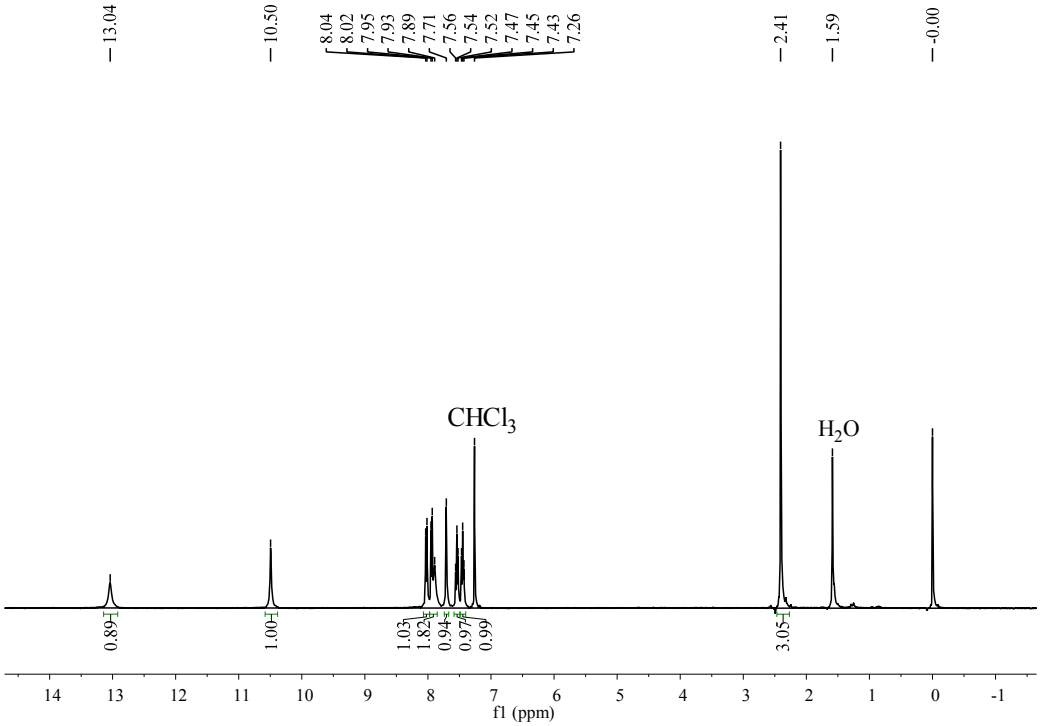


Fig. S5 ^{1}H NMR spectrum of the isolated product from the reaction of probe **2** with NaHS. It is identical to the ^{1}H NMR spectrum of the reference sample of **4** (see above). This further proved that the reaction of probe **2** with NaHS produced compound **4**.

2.3 Others

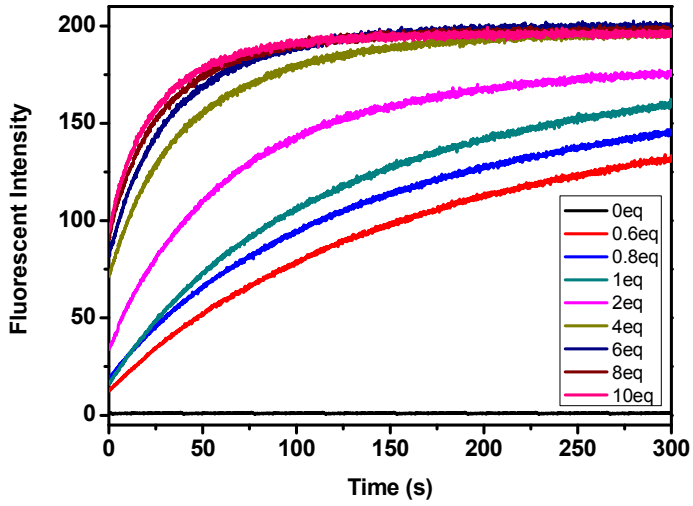


Fig. S6 Kinetics of fluorescent intensity changes of probe **2** (20 μM) at 545 nm in the presence of HS^- at different concentrations (12, 20, 40, 60, 80, 100, 120, 160, 200 μM, respectively). The experiments were performed in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v) at 25˚C with λ_{ex} = 440 nm, slit width: $d_{ex} = d_{em}$ = 5 nm.

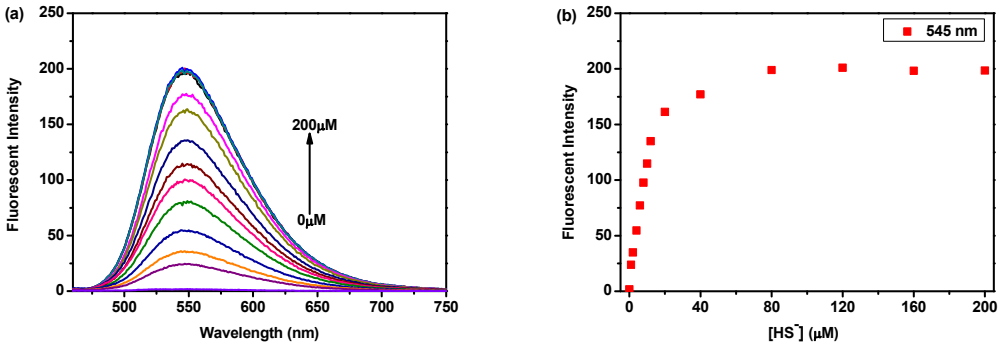


Fig. S7 (a) Fluorescence spectra changes of probe **2** (20 μM) upon addition of different concentrations of HS^- in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v). The final concentrations of HS^- are 0, 2, 4, 6, 8, 10, 12, 14, 16, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 μM, respectively. (b) Fluorescent intensity changes of probe **2** at 545 nm against concentration of HS^-. Each spectrum was collected at 5 min after HS^- addition. λ_{ex}= 440 nm, slit width: $d_{ex} = d_{em} = 5$ nm.

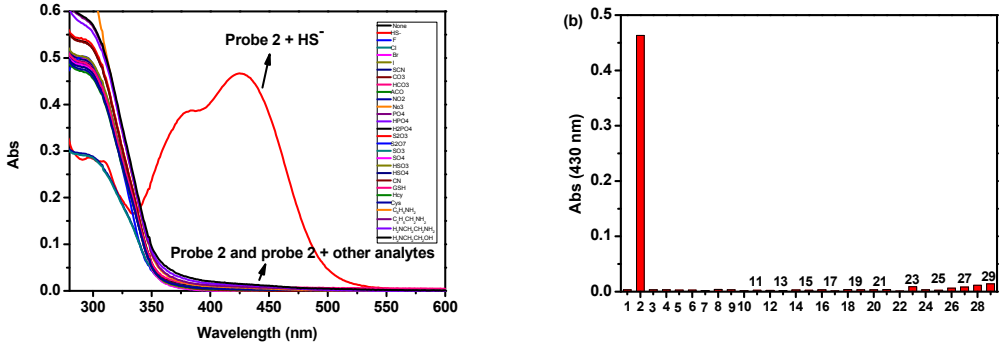


Fig. S8 (a) UV-vis spectra changes of probe **2** (20 μM) in the presence of various anions (10 equiv.). (b) Absorbance changes of probe **2** at 430 nm for various analytes (10 equiv.). The number (1-29) represent probe **2** with analytes: 1. None, 2. HS^-, 3. F^-, 4. Cl^-, 5. Br^-, 6. I^-, 7. SCN^-, 8. CO_3^{2-}, 9. AcO^-, 10. NO_3^-, 11. NO_2^-, 12. PO_4^{3-}, 13. HPO_4^{2-}, 14. $H_2PO_4^-$, 15. $S_2O_3^{2-}$, 16. $S_2O_7^{2-}$, 17. SO_4^{2-}, 18. SO_3^{2-}, 19. HSO_3^-, 20. HSO_4^-, 21. CN^-, 22. GSH, 23. Hcy, 24. Cys, 25, NAC, 26. $C_6H_5NH_2$, 27. $C_6H_5CH_2NH_2$, 28. $H_2NCH_2CH_2NH_2$, 29. $HOCH_2CH_2NH_2$, respectively.

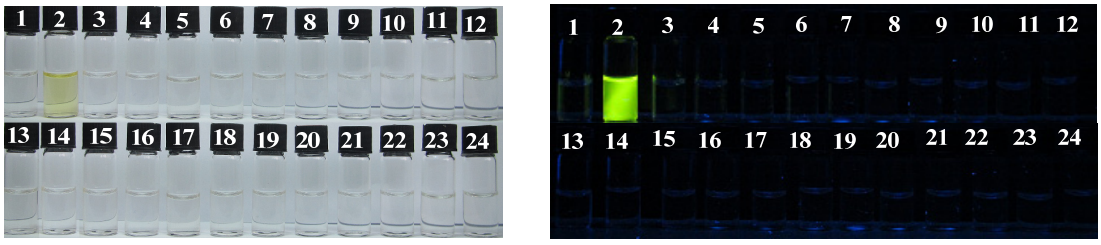


Fig. S9 (a) Color changes and (b) fluorescence color changes under a 365 nm light of probe **2** (20 μM) with 200 μM of different analytes (From 1 to 24: 1. None, 2. HS^-, 3. F^-, 4. Cl^-, 5. Br^-, 6. I^-, 7. SCN^-, 8. CO_3^{2-}, 9. AcO^-, 10. NO_3^-, 11. NO_2^-, 12. PO_4^{3-}, 13. HPO_4^{2-}, 14. $S_2O_3^{2-}$, 15. $S_2O_7^{2-}$, 16. SO_4^{2-}, 17. SO_3^{2-}, 18. HSO_3^-, 19. HSO_4^-, 20. CN^-, 21. GSH, 22. Hcy, 23. Cys, 24, NAC) in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v).

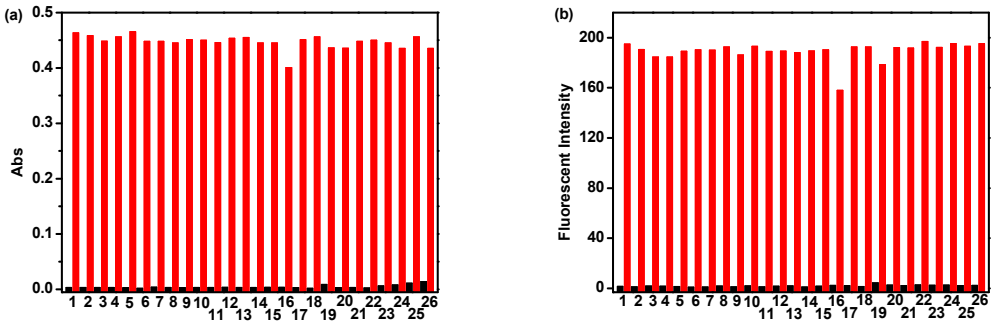


Fig. S10 (a) Absorbance of probe **2** (20 μM) at 430 nm toward various analytes. (b) Fluorescence intensity of probe **2** (20 μM) at 550 nm toward various analytes. Black bars represent the addition of a single analyte (200 μM, from **1-26**: 1. F^-, 2. Cl^-, 3. Br^-, 4. I^-, 5. SCN^-, 6. CO_3^{2-}, 7. AcO^-, 8. NO_3^-, 9. NO_2^-, 10. PO_4^{3-}, 11. HPO_4^{2-}, 12. $S_2O_3^{2-}$, 13. $S_2O_7^{2-}$, 14. SO_4^{2-}, 15. SO_3^{2-}, 16. HSO_3^-, 17. HSO_4^-, 18. CN^-, 19. GSH, 20. Hcy, 21. Cys, 22, NAC. 23. $C_6H_5NH_2$, 24. $C_6H_5CH_2NH_2$, 25. $H_2NCH_2CH_2NH_2$, 26. $HOCH_2CH_2NH_2$) Red bars represent the subsequent addition of HS^- (200 μM) to the mixture. All experiment was performed in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v) at 25˚C and each spectrum was obtained 5 min after addition of various analytes. λ_{ex}= 440 nm, slit width: d_{ex} = d_{em} = 5 nm.

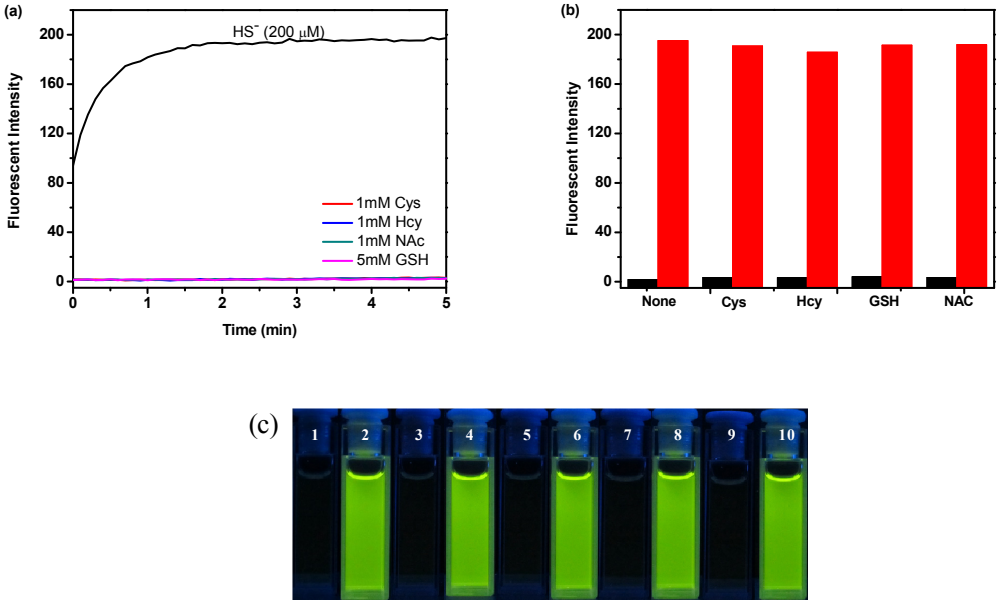


Fig. S11 (a) Fluorescence intensity changes of probe **2** (20 μM) at 545 nm upon addition of NaHS (200 μM), Cys (1 mM), Hcy (1 mM), NAc (1 mM) and GSH (5 mM) in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v), respectively. λ_{ex}= 440 nm, slit width: d_{ex} = d_{em} = 5 nm.
(b) Fluorescence responses of probe **2** (20 μM) to NaHS (200 μM) at 545 nm in the absence and presence of biothiols: Cys (1 mM), Hcy (1 mM), GSH (5 mM) and NAC (1 mM) in PBS buffer (20 mM, pH 7.4) containing 25% CH_3CN (v:v). Black bar: probe **2** or probe **2** + a single biothiol. Red bars represent the subsequent addition of NaHS (200 μM) to the mixture. Each data was collected after 5 min of mixing probe **2** and the analyte with λ_{ex} = 440 nm, slits: 5/5 nm.

(c) The corresponding fluorescent images of the above resulting solutions: 1. Probe **2** only; 2. Probe **2** + NaHS; 3. Probe **2** + Cys; (4) Subsequent addition of NaHS to probe **2** + Cys; 5. Probe **2** +Hcy; 6. Subsequent addition of NaHS to probe **2** + Hcy; 7. Probe **2** +GSH; 8. Subsequent addition of NaHS to probe **2** + GSH; 9. Probe **2** + NAC; 10. Subsequent addition of NaHS to probe **2** + NAC.

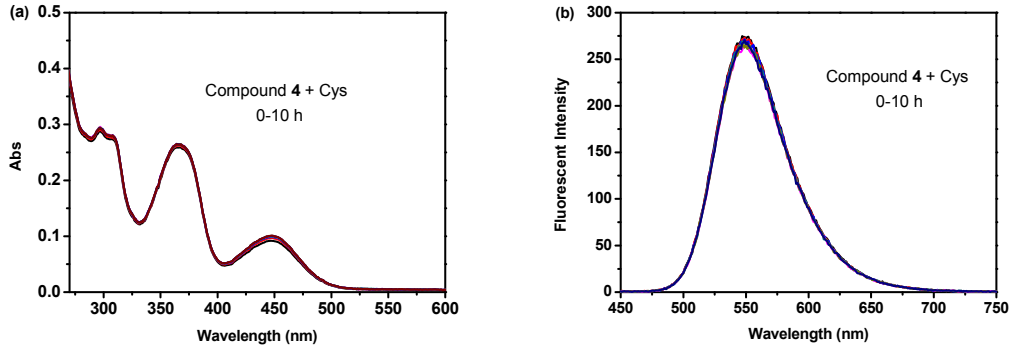


Fig. S12 (a) UV-vis spectra and (b) fluorescent spectra responses of compound **4** (20 μM) upon addition of 10 equiv of Cys monitored in a 10 hours time-scale in PBS buffer (20 mM, pH 7.4) with 50% CH_3CN (v/v) at 25˚C.