Electronic Supplementary Information (ESI)

for

A low dose, highly selective and sensitive colorimetric and fluorescent probe for biothiols and its application for bioimaging

Qiong Zhang, Dehuan Yu, Shuangshuang Ding and Guoqiang Feng*

Key Laboratory of Pesticide and Chemical Biology of Ministry of Education, College of Chemistry, Central China Normal University,152 Luoyu Road, Wuhan 430079, P. R. China. E-mail: gf256@mail.ccnu.edu.cn

Contents

1. Experimental general

All reagents were purchased from commercial suppliers and used without further purification. All aqueous solutions and buffers were prepared with using distilled water that had been passed through a Millipore-Q ultrapurification system. TLC analysis was performed using precoated plates. Melting points were determined using an X-4 apparatus and are not corrected. NMR spectra were measured on a Varian Mercury 400 instrument, operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrophotometer as KBr pellets and were reported in cm^{-1}. Electrospray mass spectra (ESI-MS) were acquired on Agilent 1100 Series LC/MS ion trap mass spectrometers and 6530 Accurate-Mass QTOF spectrometer coupled to an Agilent HPLC 1200 series. UV-vis spectra and fluorescent spectra were recorded on an Agilent Cary 100 UV-vis spectrophotometer and an Agilent Cary Eclipse fluorescence spectrophotometer, respectively. Both spectrophotometers are equipped with a temperature controller. Standard quartz cuvettes with a 10 mm lightpath were used for all optical measurements. Cell imaging was performed in an Olypus IX71 inverted fluorescence microscopy with a 20× objective lens.

Optical measurements: Stock solutions of probe **1** (1 mM) were prepared in DMSO (HPLC grade). Stock solutions (0.01-10 M) of the analytes were prepared in ultrapure water. For optical measurements, a solution of probe **1** (10 μM or as stated) was prepared in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v). Then 3.0 mL of the probe **1** solution was placed in a quartz cell until the temperature reached at 37 °C over a few minutes. The UV-vis or fluorescent spectra were then recorded upon addition of various analytes.

Determination of the fluorescence quantum yield： In our system, the fluorescence quantum yields of probe **1** (Φ = 0.09) and compound **2** (Φ = 0.56) were determined in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v), using rhodamine B (Φ_f = 0.89 in ethanol) as standard. The quantum yield was calculated using the following equation:

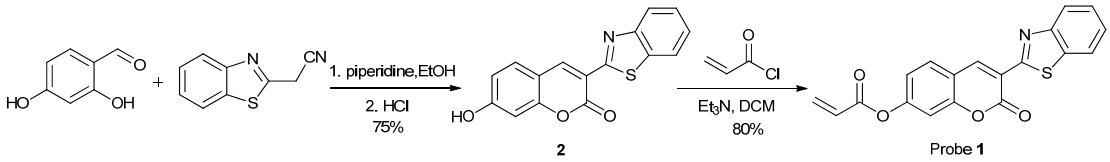
$$\Phi_x = \Phi_s(A_sF_x / A_xF_s) (n_x^2/n_s^2)$$

where, A_x and A_s are the absorbance of the sample and the reference, respectively, at

the same excitation wavelength, F_x and F_s 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.

Cell imaging experiments: HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum), 100mg/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2, water saturated incubator at 37 °C, and then were seeded in a 12-well culture plate for one night before cell imaging experiments. For living cells imaging experiment of probe **1**, cells were incubated with 1μM (or 10 μM) of probe **1** (with 0.2% DMSO, v/v) for 60 min at 37 °C and washed three times with prewarmed PBS, and then imaged immediately. For N-ethylmaleimide (NEM) treated experiments, HeLa cells were pretreated with 0.5 mM NEM for 60 min at 37 °C, washed three times with prewarmed PBS, and then incubated with 10 μM probe **1** for 60 min at 37 °C. Cell imaging was then carried out after washing cells with prewarmed PBS buffer.

2. Synthesis of compound 2 and probe 1

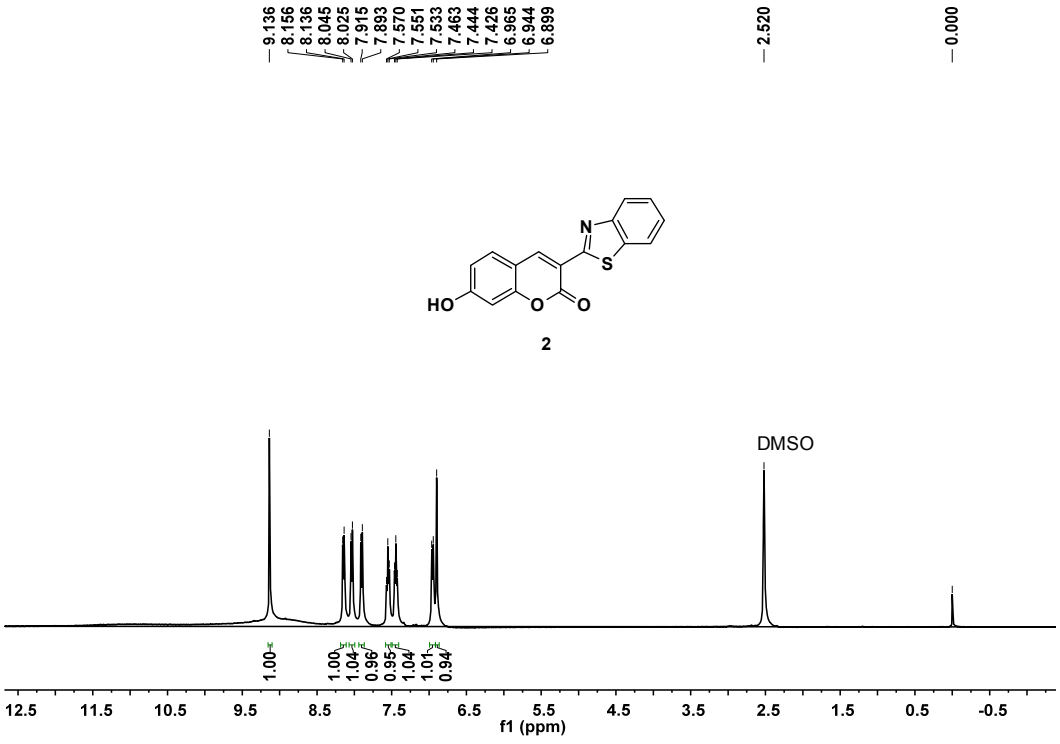


Scheme S1. Synthesis of probe **1**.

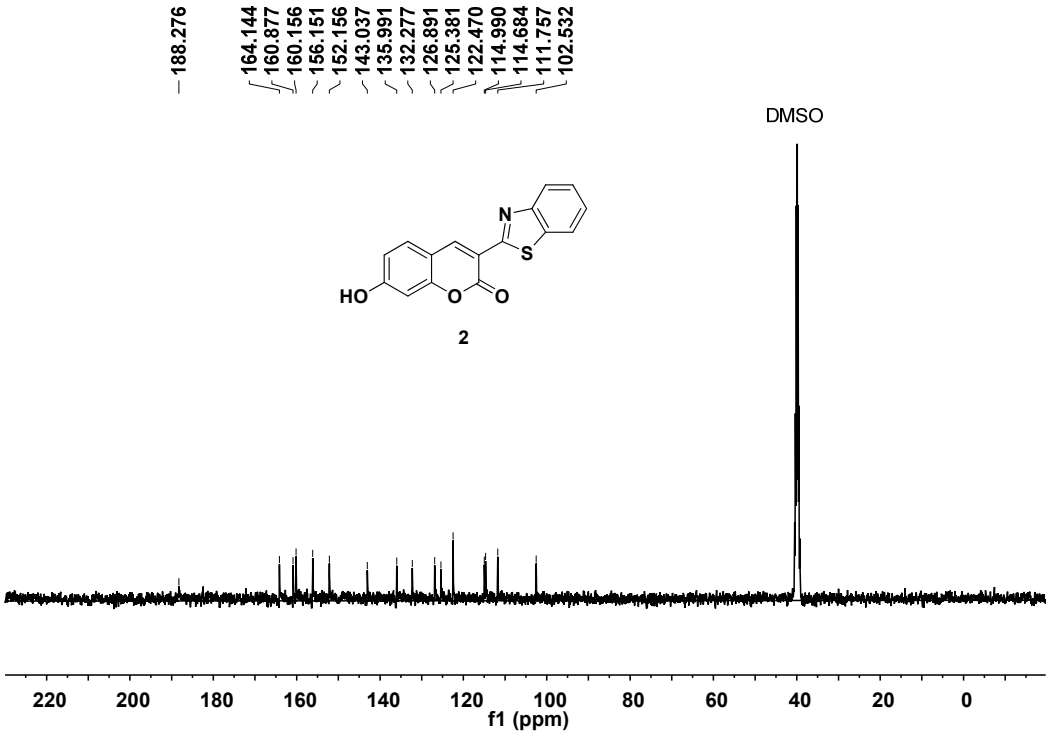
Synthesis of 3-benzothiazolyl-7-hydroxycoumarin (2). Compound **2** was synthesized in 75 % yield according to a previously reported method (Ref. W. Lin, L. Long and W. Tan, *Chem. Commun*., 2010, **46**, 1503–1505). M.p. 304-306 ˚C. TLC (silica plate): R_f 0.3 (petroleum ether : ethyl acetate 2:1, v/v); ^{1}H NMR (400 MHz, d_6-DMSO, Me_4Si): δ (ppm) 9.14 (d, J = 1.7 Hz, 1H), 8.15 (d, J = 7.9 Hz, 1H), 8.04 (d, J = 8.2 Hz, 1H), 7.92 – 7.89 (m, 1H), 7.55 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.1 Hz, 1H), 6.95 (d, J = 8.6 Hz, 1H), 6.90 (s, 1H). ^{13}C NMR (100 MHz, d_6-DMSO, Me_4Si): δ (ppm) 188.3, 164.2, 160.9, 160.2, 156.2, 152.2, 143.0, 136.0, 132.3, 126.9, 125.4, 122.5, 115.0, 114.7, 111.8, 102.5. IR (KBr) ν_{max} (cm^{-1}): 2965, 2737, 1721 (s), 1707, 1615, 1599, 1585, 1558, 1525, 1452 (s), 1344, 1298, 1257 (s), 858, 755, 508. EI-MS: m/z found 295.15 (M^+, 100%), 267.14 (M^+ - C_2H_4, 35%).

Synthesis of probe 1. To a solution of compound **2** (147 mg, 0.5 mmol) in dry dichloromethane (5 mL) was added acryloyl chloride (90 μL) and Et_3N (150 μL). The resulting mixture was stirred at room temperature until the reaction was complete (monitored by TLC in a silica plate, the Rf of the starting material 2 is 0.3 using petroleum ether : ethyl acetate 1:1 (v/v) as mobile phase. Water (10 mL) was used to wash the resulting solution three times, and the dichloromethane phase was dried over Na_2SO_4. After filtered and removal of the organic solvent, a yellow solid product was formed, which can be further purified by recrystallization from ethanol to afford the pure product (140 mg, 80%). M.p. 227-229 °C; TLC (silica plate): R_f 0.7 (petroleum ether : ethyl acetate 2:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) : δ (ppm) 9.06 (s, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 7.9 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.43 (t, J = 7.5 Hz, 1H), 7.30 (s, 1H), 7.21 (dd, J = 8.4, 1.6 Hz, 1H), 6.68 (d, J = 17.3 Hz, 1H), 6.36 (dd, J = 17.2, 10.4 Hz, 1H), 6.12 (d, J = 10.4 Hz, 1H). ^{13}C NMR (100 MHz, d_6-DMSO) δ 182.1, 164.2, 160.3, 159.8, 154.6, 154.5, 152.5, 142.2, 136.5, 135.1, 131.9, 127.8, 127.3, 126.1, 123.1, 120.0, 119.5, 117.5, 110.7, 55.49. IR (KBr) ν_{max} (cm^{-1}): 3440 (br s), 2978, 2942, 2739, 2677, 2604, 2494, 1732 (s), 1610, 1477, 1398, 1156, 1037, 767; EI-MS: m/z found 349.17 (M^+, 40%), 295.19 (M^+ - C_2H_3CO, 100%). HR-MS Calc. for $C_{19}H_{12}NO_4S^+$ ($M + H^+$) 350.04815, found 350.04805.

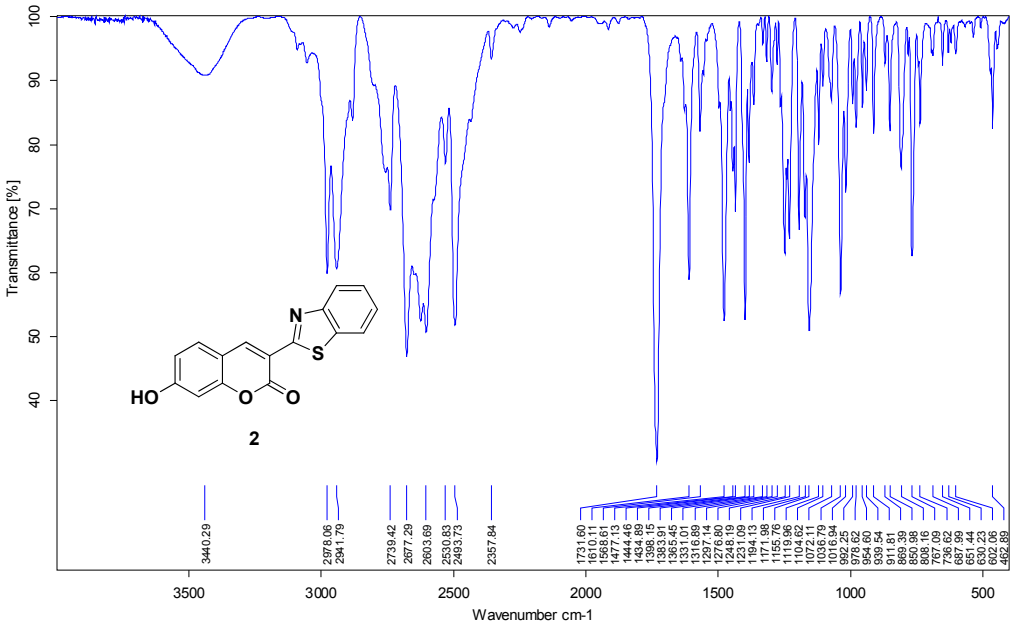
Structure characterizations for compound 2 and probe 1



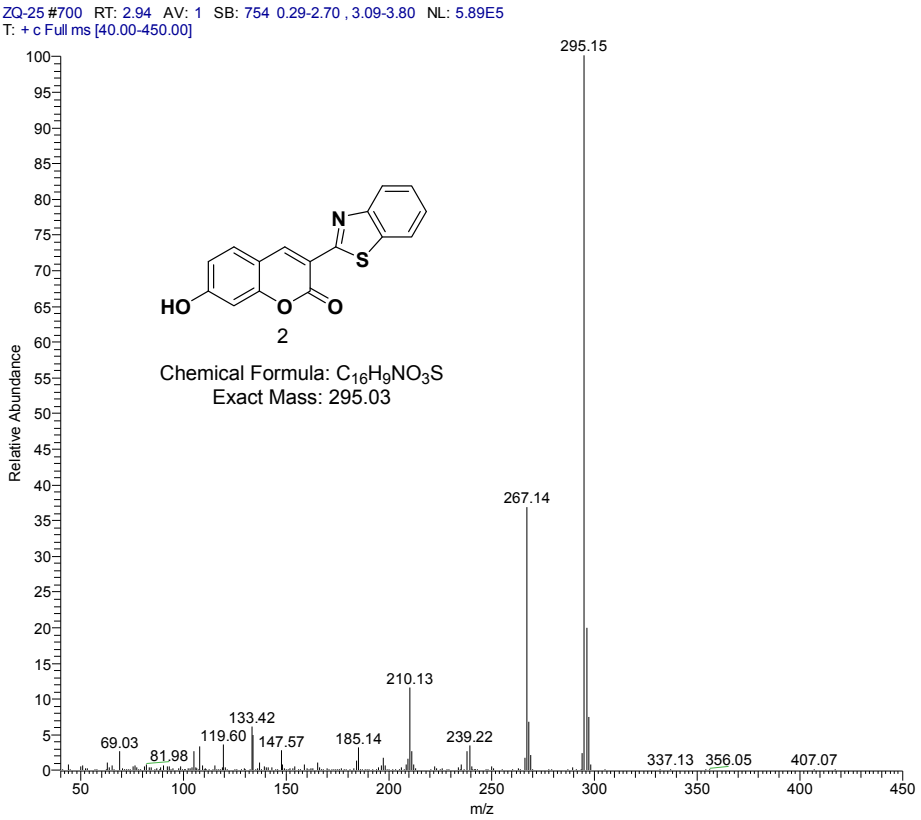
^{1}H-NMR spectrum of compound **2** in d_6-DMSO



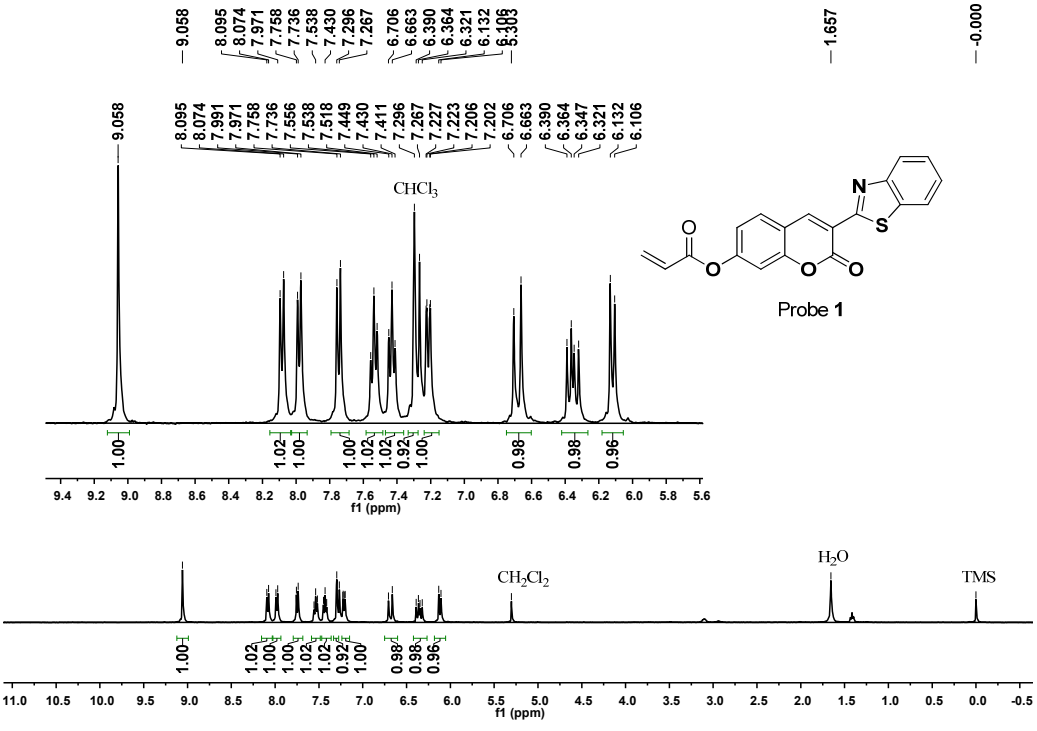
^{13}C-NMR spectrum of compound **2** in d_6-DMSO



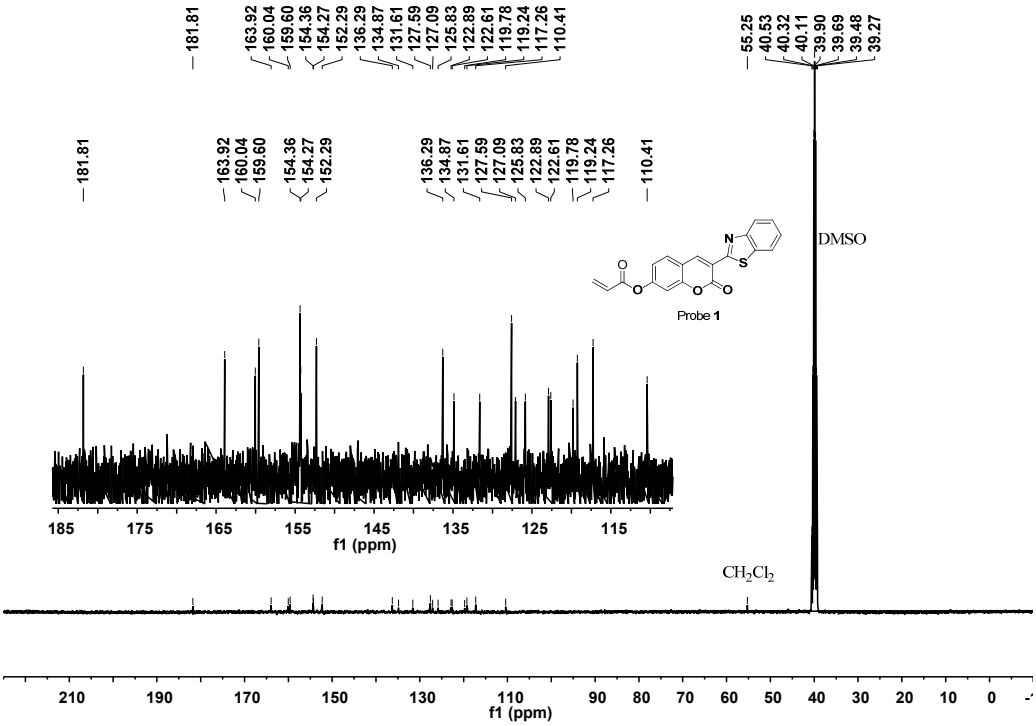
IR spectrum of compound **2**



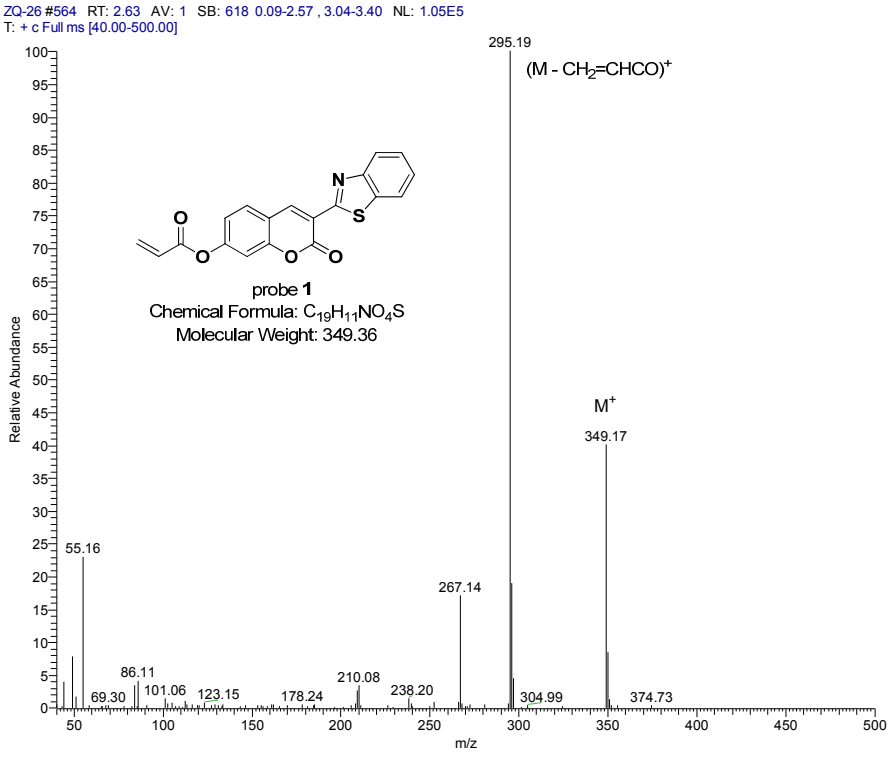
Mass spectrum of compound **2**



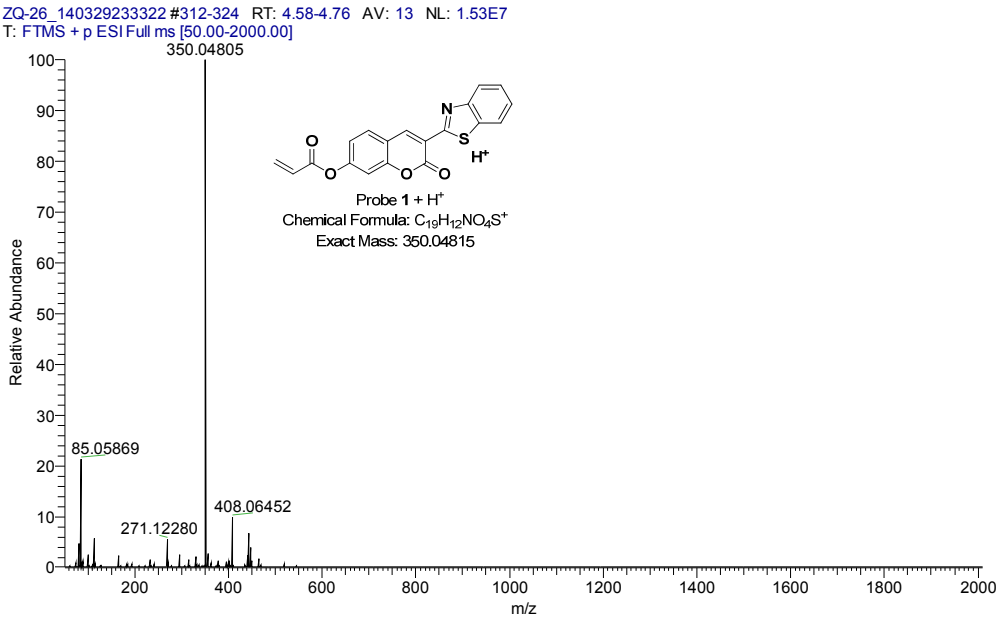
^{1}H-NMR spectrum of probe **1** in $CDCl_3$



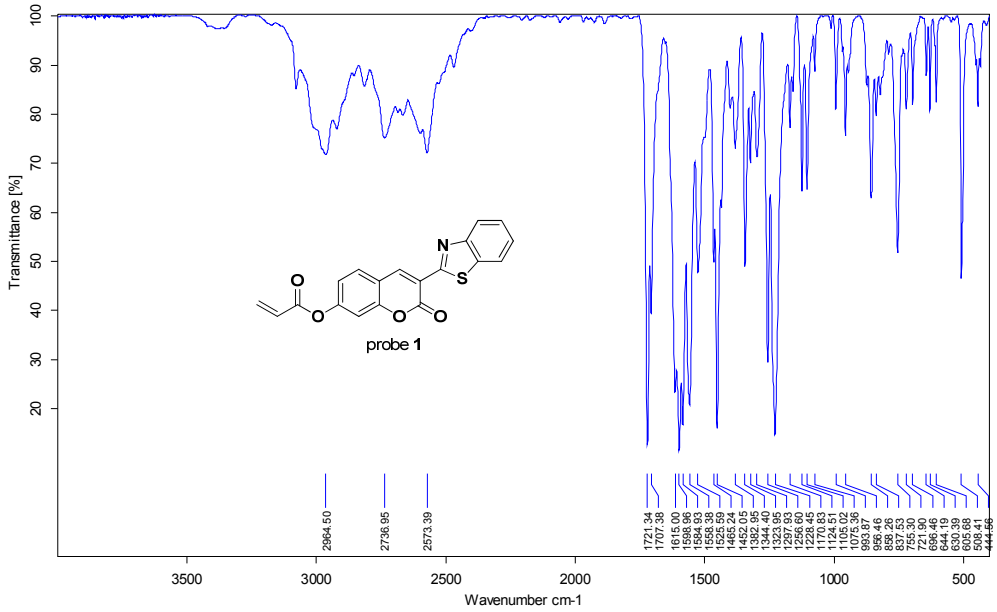
^{13}C-NMR spectrum of probe **1** in d_6-DMSO



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



HR-MS spectrum of probe **1**



IR spectrum of probe **1**

3. Additional studies

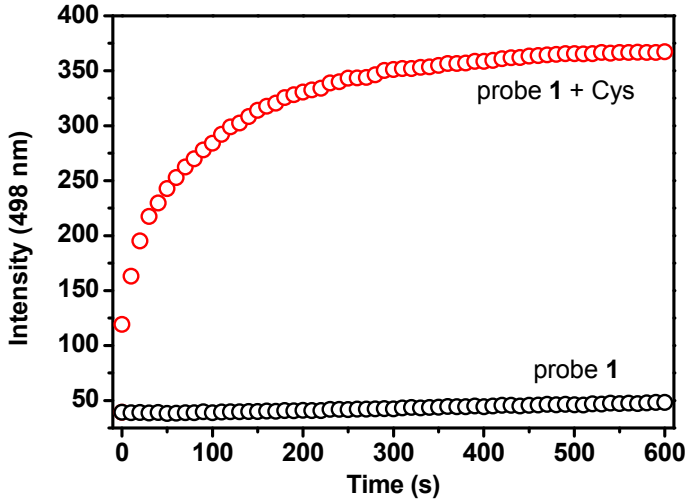
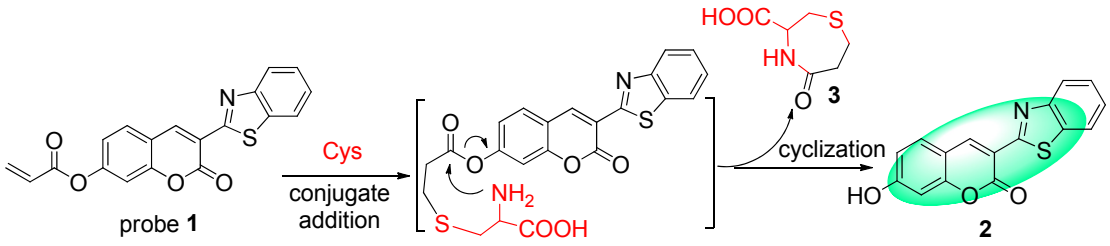


Fig. S1 Fluorescent kinetics of probe **1** (10 μM) in the absence and presence of 50 μM of Cys in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v) at 37 °C. The spectra were monitored at 498 nm and collected at10 s intervals, respectively, with λ_{ex} = 458 nm; d_{ex} = d_{em} = 2.5 nm.

Sensing mechanism of probe 1 for Cys



Scheme S2. A proposed mechanism for sensing of Cys by probe **1**.

To a 150 mL flask, probe **1** (0.14 g, 0.4 mmol) and Cys (1.25 eq) were combined in 100 mL of MeOH : H_2O (90 : 10, v/v) solution, and the mixture stirred at room temperature for 1 h. Then, Et_3N (30 μL) was added and the solution stirred ca. 2 h. The solvents was removed under reduced pressure and the crude product was subjected to column chromatography (eluted with CH_2Cl_2: MeOH, 5 : 1, v/v) to afford (58 mg, 49.2%) of product **A** and 18 mg of product **B** as an off-white solid. Product **A** was checked by TLC, ^{1}H NMR and Mass spectra, which was proved to be **2** by comparison with those of reference sample of compound **2**. Product **B** was proved to

be **3** by its ^{1}H NMR spectrum with comparison to that of previously reported spectrum of **3**. See below.

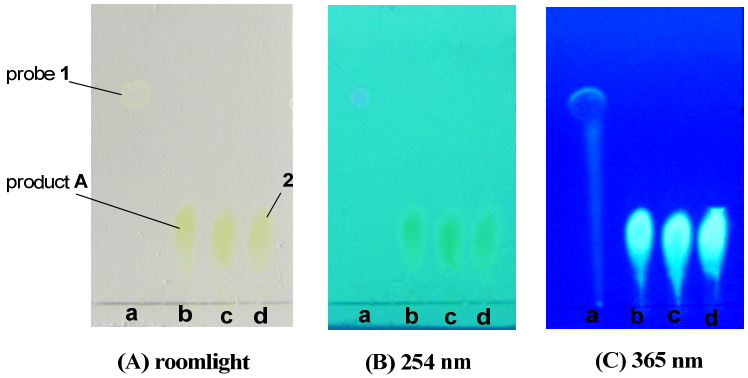


Fig. S2 TLC analysis of the isolated product **A** from the reaction of probe **1** and Cys. plate under different light used to compare probe **1**, the reference sample of compound **2** and the isolated reaction product of probe **1** and Cys. (A) under room light, (B) under light of 254 nm, (C) under light of 365 nm. Spots on the TLC plate are: a. probe **1**, b. the reaction product **A**, c. mixture of product **A** and the reference sample of compound **2**, d. the reference sample of compound **2**. The eluent for TLC: petroleum ether : ethyl acetate = 2 : 1 (v/v).

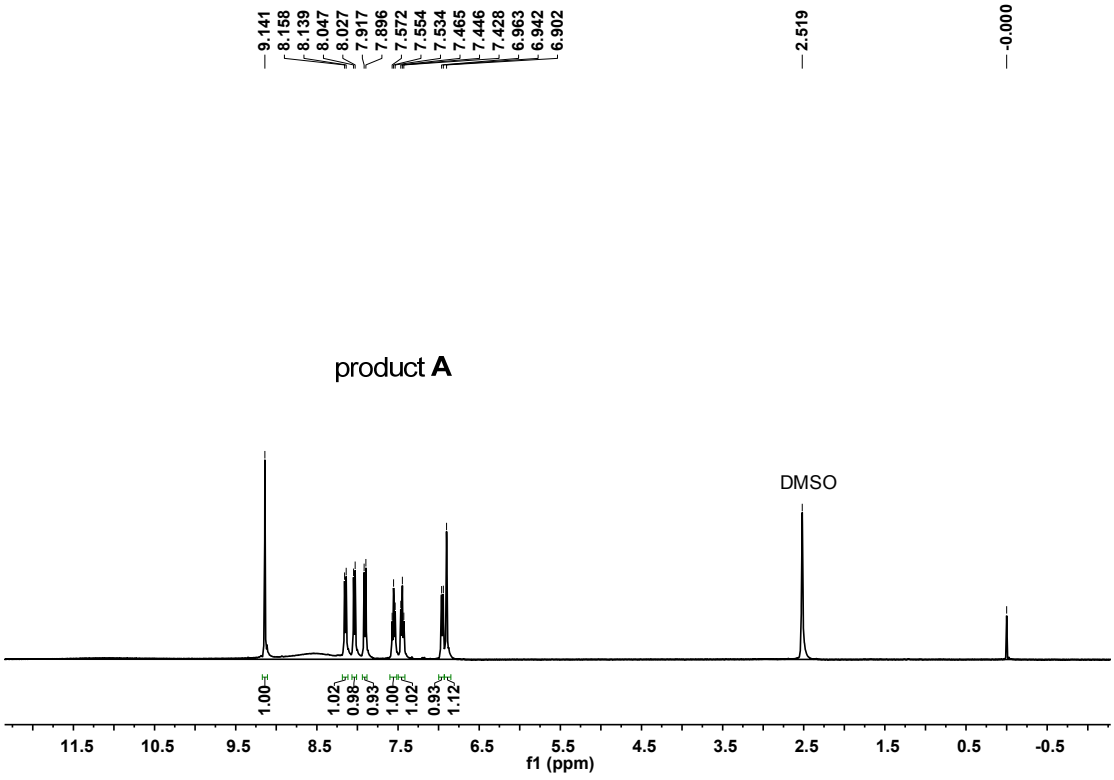


Fig. S3 ^{1}H NMR spectrum of product **A** in DMSO-d_6, which is identical to that of the reference sample of compound **2** (see above).

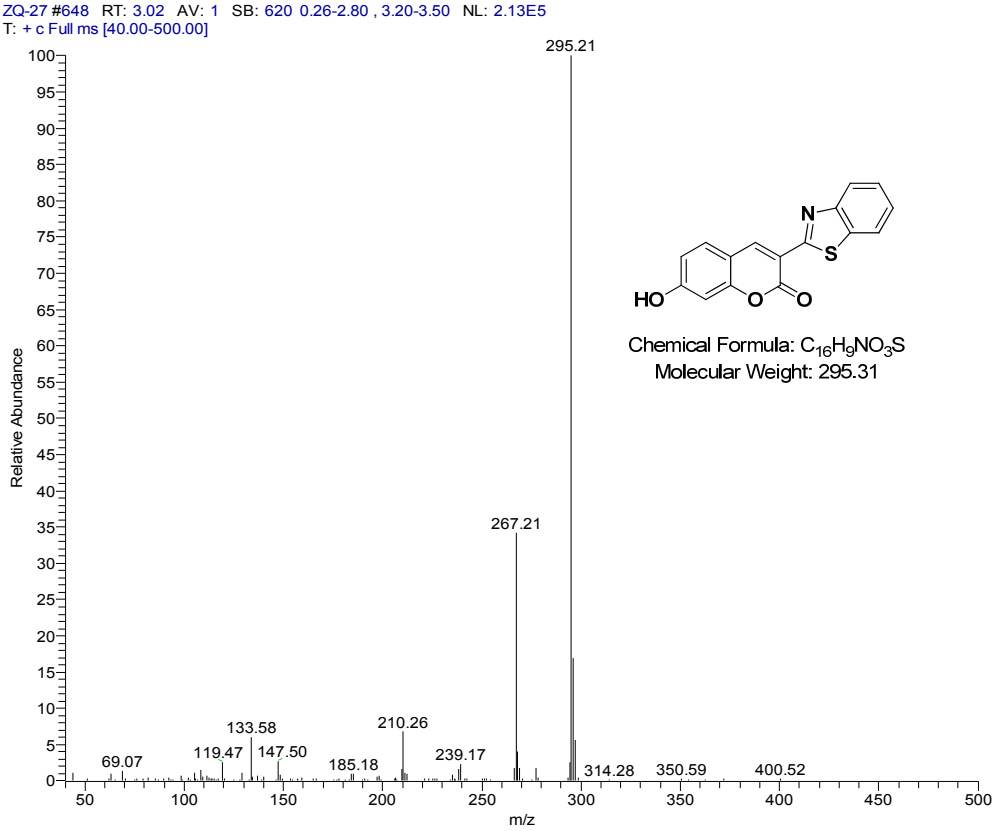


Fig. S4 Mass spectrum of product **A**, which shows the right mass of compound **2**

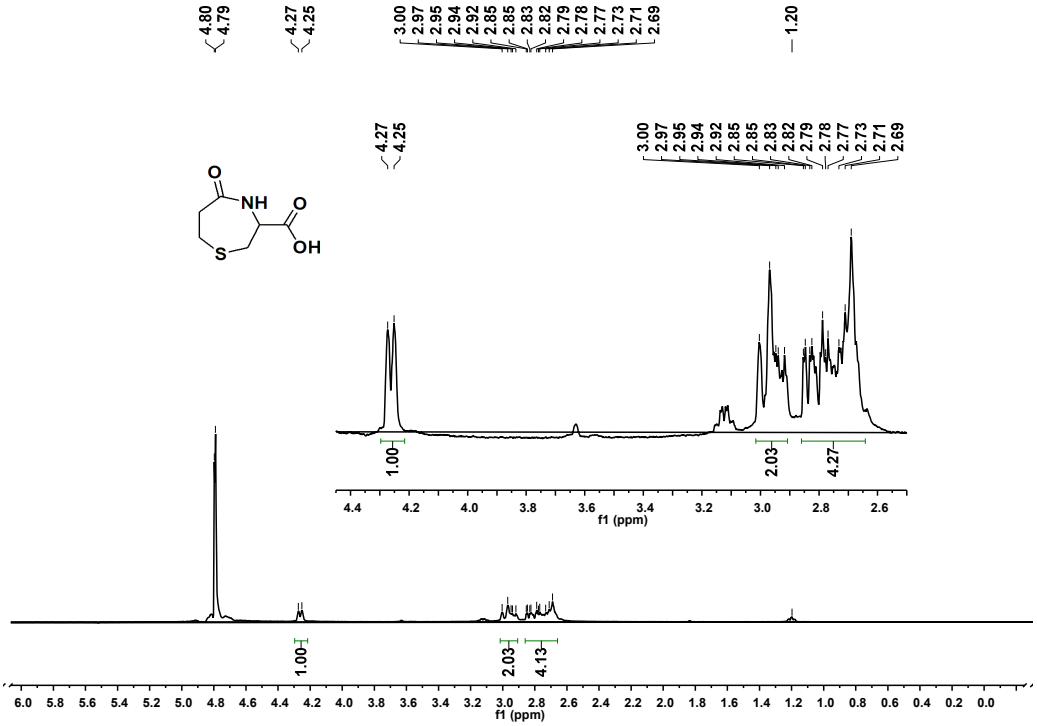


Fig. S5 ^{1}H-NMR spectrum of compound **B** in D_2O, which is identical to the previously reported spectrum of **3**, see Ref: X. Yang, Y. Guo and R. Strongin, *Angew. Chem., Int. Ed*., 2011, **50**, 10690–10693, Figure S28 in the ESI of this paper.

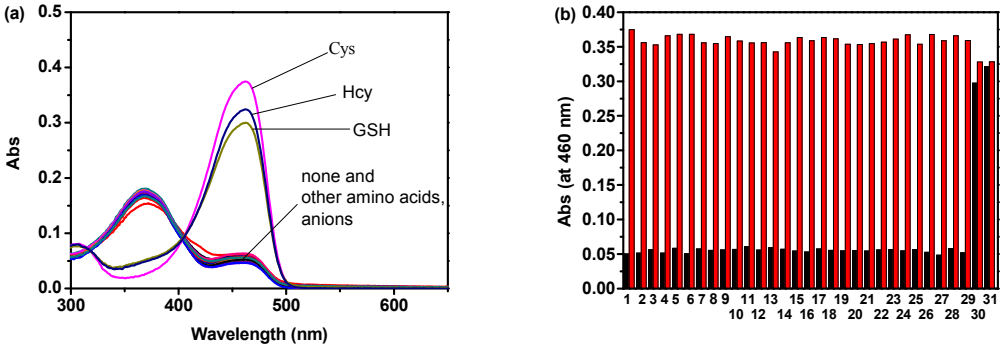


Fig. S6 (a) UV-vis spectra changes of probe **1** (10 μM) upon addition of various analytes (100 μM, Cys, Hcy, and GSH were used 50 μM). (b) Absorption changes of probe **1** (10 μM) at 460 nm for Cys (50 μM) in the presence of various analytes (100 μM). Black bars represent the addition of a single analyte. Red bars represent the subsequent addition of Cys to the mixture. Analytes 1-31: 1. none, 2. F^-, 3. Cl^-, 4. Br^-, 5. I^- 6. NO_3^-, 7. NO_2^-, 8. AcO^-, 9. SCN^-, 10. CO_3^{2-}, 11. SO_4^{2-}, 12. CN^-, 13. SO_3^{2-}, 14. $S_2O_3^{2-}$, 15. Ala, 16. Glu, 17. Thr , 18. Trp, 19. Phe, 20. Gln, 21. Gly, 22. Lys, 23. Arg, 24. Ile, 25. Asp, 26 .Leu, 27. Ser, 28. Met, 29. His, 30. GSH, 31. Hcy. Each spectrum was collected after 15 min of mixing each analyte with probe **1** in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v) at 37 °C.

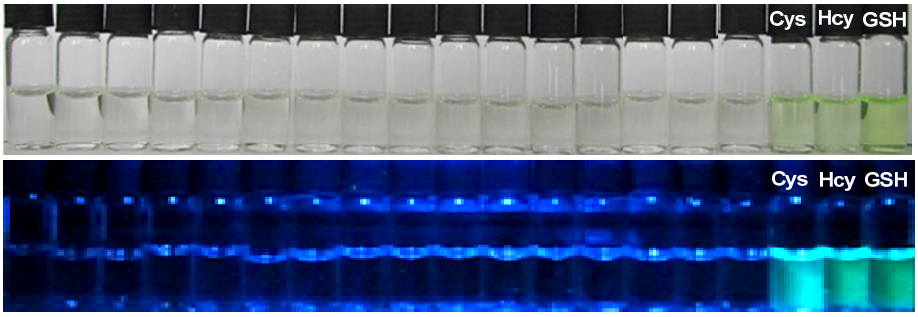


Fig. S7 Color changes and fluorescence changes (under a 365 nm UV lamp) of probe **1** (10 μM) upon addition of various analytes (from left to right: none, Ala, Glu, Arg, Asp, Gln, Gly, His, Leu, Lys, Met, Phe, Trp, Ser, Ile, Thr, Cys, Hcy, and GSH. Except Hcy, GSH and Cys were used 50 μM, others were use 100 μM.).

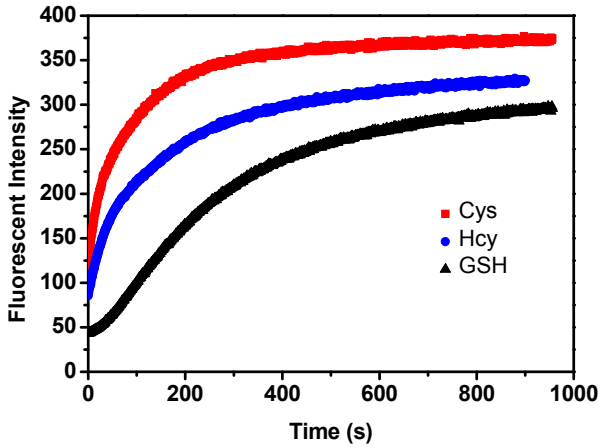


Fig. S8 Fluorescent kinetics of probe **1** (10 μM) with 50 μM of Cys, Hcy and GSH in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v) at 37 °C. The reactions are monitored at 498 nm with λ_{ex} = 458 nm, slit: d_{ex} = d_{em} = 2.5 nm.

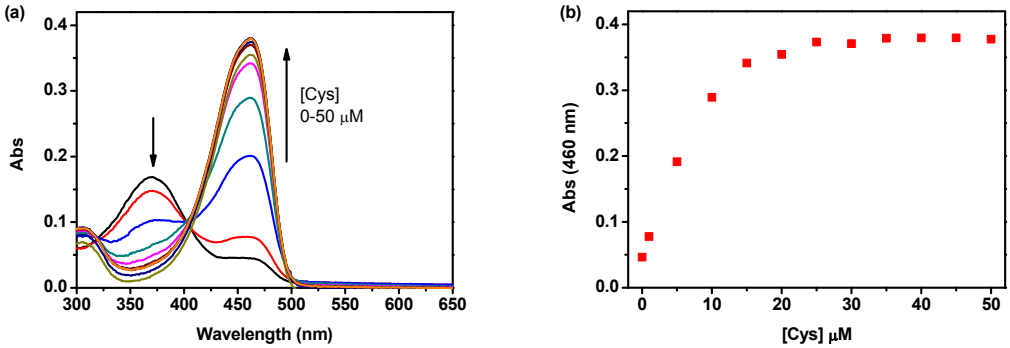


Fig. S9 (a) UV/Vis absorption spectra of probe **1** (10 μM) upon addition of different concentrations of Cys in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v) at 37 °C. Final concentration of Cys: 0, 1, 5, 10, 15, 20, 25, 30, 35 40, 45 and 50 μM, respectively. (b) The changes of the absorbance intensity at 460 nm of probe **1** (10 μM) against concentration of Cys. Each spectrum was obtained 15 min after Cys addition.

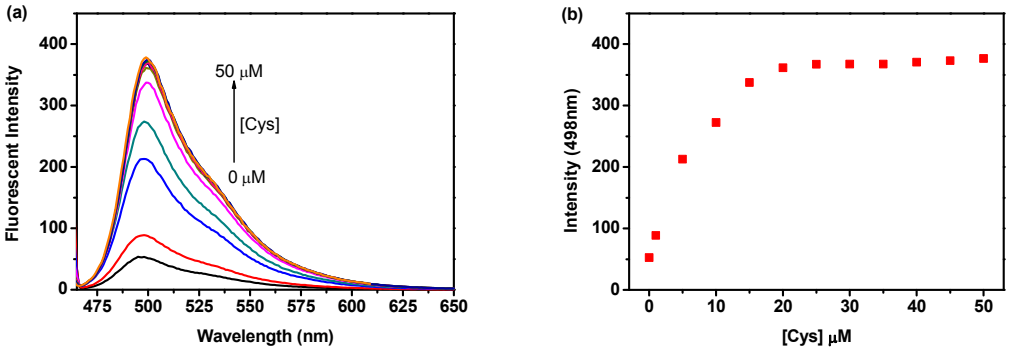


Fig. S10 (a) Fluorescent spectra changes of probe **1** (10 μM) upon addition of different concentrations of Cys in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v) at 37 °C. Final concentration of Cys: 0, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μM, respectively. (b) Fluorescent intensity changes of probe **1** (10 μM) at 498 nm against concentration of Cys. Each spectrum was obtained 15 min after Cys addition.

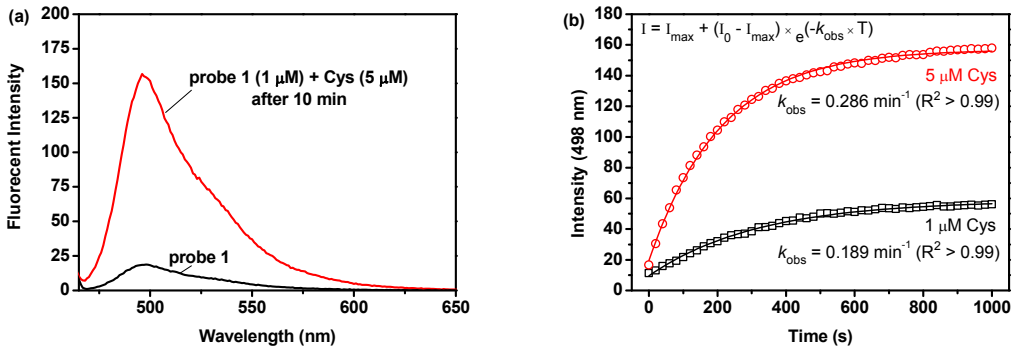


Fig. S11 (a) Fluorescent spectra changes of probe **1** (1 μM) upon addition of 5 μM Cys after 10 min. (b) Fluorescence kinetics of probe **1** (1 μM) monitored at 498 nm upon addition of Cys (1 μM and 5 μM) in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v) at 37 °C. The kinetics data are fitted (solid line) by a first-order reaction scheme as shown in the figure, and the observed pseudo-first-order rate constant k_{obs} was also shown. λ_{ex} = 458 nm, d_{ex} =2.5 nm, d_{em} = 5 nm.

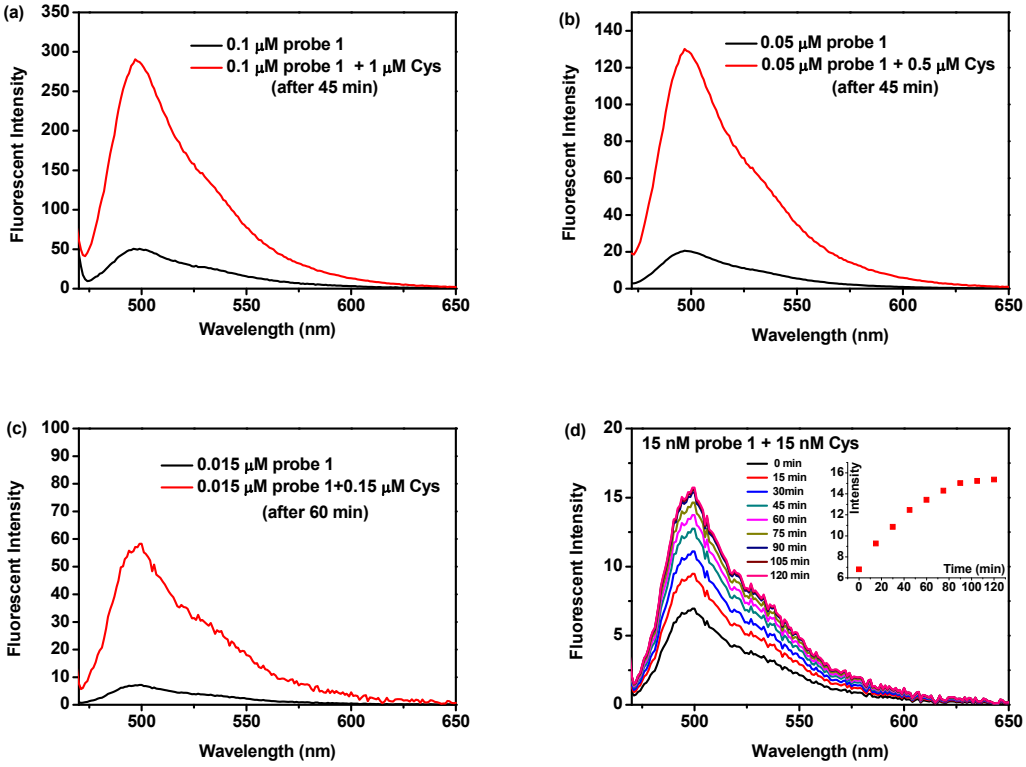


Fig. S12 Detection of Cys using very low concentrations of probe **1** in DMSO-PBS buffer (20 mM, pH 7.4, 1:1, v/v) at 37 °C. λ_{ex} = 458 nm, slit for (a) and (b): d_{ex} =5 nm, d_{em} = 10 nm, for (c) and (d): d_{ex} = d_{em} = 10 nm, respectively. Insert in (d): fluorescent intensity changes at 498 nm as a function of time.

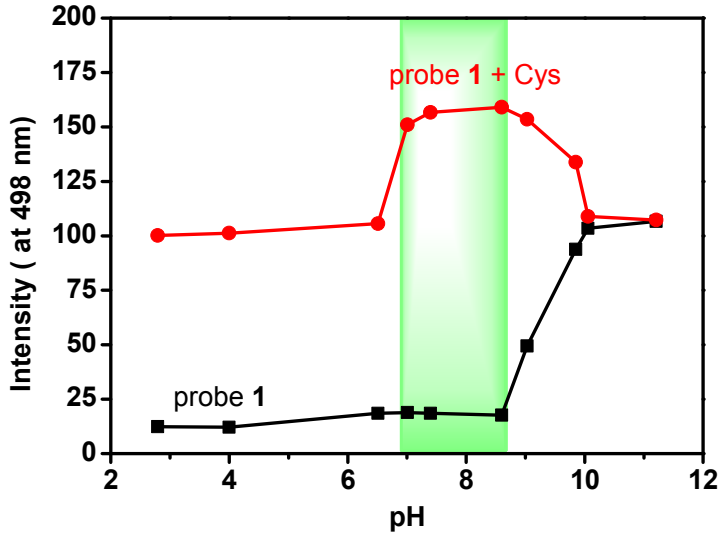


Fig. S13 The effect of pH on the fluorescence intensity changes of probe **1** (1 μM) at 498 nm in absence and presence of Cys (5 μM) in DMSO-PBS buffer (20 mM, 1:1, v/v) at 37 °C. All the data was obtained 15 min after mixing. λ_{ex} = 458 nm, d_{ex} =2.5 nm, d_{em} = 5 nm. Green color area indicates the best working pH range for probe **1**.