

## Supporting Information

### Simultaneous Fluorescence Sensing of Cys and GSH from Different Emission Channels

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## Experimental Section

**1. General information and methods.** All reagents and solvents were purchased from commercial sources and were of the highest grade. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography (FC) was performed using silica gel 60 (200–300 mesh). Absorption spectra were taken on an Agilent 8453 spectrophotometer. Fluorescence spectra were taken on SHIMADZU RF-5301PC fluorescence spectrometer unless otherwise noted. The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded at 600 and 150 MHz, respectively. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer.

### 2. Procedures for biothiols sensing

The solutions of amino acids, reduced glutathione (GSH) and various anions were prepared in deionized water. A stock solution of **1** (1 mM) was prepared in  $\text{CH}_3\text{CN}$ . The stock solution of **1** was then diluted to the corresponding concentration (1  $\mu\text{M}$  or 2  $\mu\text{M}$  or 10  $\mu\text{M}$ ) with PB buffer (10 mM, pH 7.4, containing 1 mM CTAB). Spectra data were recorded in an indicated time after the addition of amino acids.

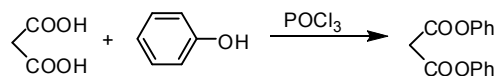
**3. Cell culture and fluorescence imaging:** The COS-7 cell line was provided by Institute of Biotechnology of Shanxi University. Cells were grown in CMEM medium supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37 °C in humidified environment of 5%  $\text{CO}_2$ . Cells were plated on 6-well plate at  $5 \times 10^6$  cells per well and allowed to adhere for 12 hours. The cells were washed with phosphate buffered saline (PBS) and then incubated with **1** (10  $\mu\text{M}$ ) and CTAB (1 mM) in CMEM medium for 30 min at 37 °C and washed 3 times with PBS. For the control experiment, the cells were pretreated with 0.5 mM Cys (or GSH) for 30 min at 37 °C in a humidified incubator. After washing with PBS, the cells were further incubated

with 10  $\mu\text{M}$  of **1** and 1 mM of CTAB in culture media for 30 min. Cell imaging was then carried out after washing cells with PBS. Fluorescence imaging was performed with a LEICA TCS-SP5 Laser Scanning Confocal Microscope with a 60  $\times$  oil-immersion objective lens. Emission was collected at 415-440 nm for blue channel (excited at 405 nm) and at 495-580 nm for red channel (excited at 458 nm).

**4. Cytotoxicity assays.** COS-7 Cells were grown in CMEM medium supplemented with 10 % FBS (Fetal Bovine Serum) and 1% antibiotics at 37  $^{\circ}\text{C}$  in humidified environment of 5%  $\text{CO}_2$ . Immediately before the experiment, the cells well placed in a 96-well plate, followed by addition of increasing concentrations of probe **1**. The final concentrations of the probe were kept from 0 to 10  $\mu\text{M}$ . The cells were then incubated at 37  $^{\circ}\text{C}$  in an atmosphere of 5%  $\text{CO}_2$  and 95% air for 24 h, followed by MTT assays (n= 5). Untreated assay with CMEM (n = 5) was also conducted under the same conditions.

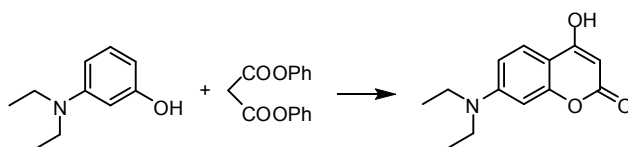
## 5. Synthesis

### 5.1 Synthesis of diphenyl malonate



To a mixture of malonic acid (11.0g, 106mmol) and phenol (19.95 g, 212 mmol) was slowly added POCl<sub>3</sub> (11.5 mL, 123 mmol) at 0 $^{\circ}\text{C}$ . The mixture was heated at 115  $^{\circ}\text{C}$  until strong release of HCl ceased (about 1.5 h). The upper layer was poured into 150 mL of water and three extractions with EtOAc followed by the usual work up gave diphenyl malonate (20.6 g, 98% yield) as a pale brown oil which was pure enough to be used without further purification in the next step.

### 5.2 Synthesis of 7-(diethylamino)-4-hydroxy-coumarin



To a solution of diphenyl malonate (12.8 g, 50 mmol) in toluene (50 mL) was added

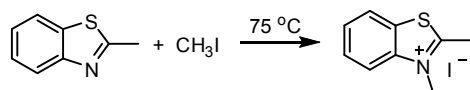
3-N,N-diethylaminophenol (8.25 g, 50 mmol). The reaction mixture was refluxed for 7 h. After completion of the reaction, the cake was filtered and washed with hexanes. The product was dried under vacuum to give a light yellow solid (8.7 g, 74% yield).

### 5.3 Synthesis of 4-chloro-7-diethylaminocoumarin-3-aldehyde



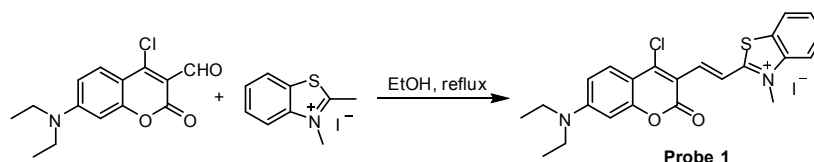
Under nitrogen, fresh distilled DMF (2.8 mL) was added dropwise to POCl<sub>3</sub> (2.8 mL) at 20-50°C and stirred for 30 minutes to yield a red solution. Then a portion of 7-(diethylamino)-4-hydroxy-coumarin (2.33 g, 10 mmol, dissolved in 13.2 mL DMF) was added dropwise to the above solution and yield a scarlet suspension. The mixture was stirred at 60 °C for 12 hours and then poured into 100 mL of ice water. NaOH solution (20 %) was added to adjust the pH of the mixture to obtain a large amount of precipitate. The crude product was filtered, washed with water, dried and recrystallized with absolute ethanol giving 4-chloro-7-diethylaminocoumarin-3-aldehyde (1.20 g, 88.2% yield). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 10.31 (s, 1H), 7.87 (d, *J* = 9.3 Hz, 1H), 6.71 (d, *J* = 7.2 Hz, 1H), 6.46 (s, 1H), 3.50 (q, *J* = 7.2 Hz, 4H), 1.27 (t, *J* = 7.2 Hz, 6H).

### 5.4 Synthesis of 2,3-dimethylbenzothiazolium iodid



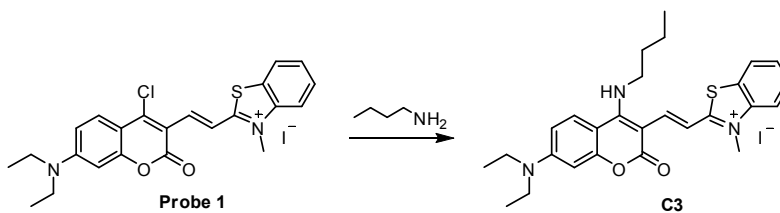
To a solution of 2-methylbenzothiazole (1.49 g, 10 mmol) in CH<sub>3</sub>CN (20 mL) was added methyl iodide (3.548 g, 25 mmol). The mixture was refluxed under an N<sub>2</sub> atmosphere for 24 h. After cooled to room temperature, the solid was filtered off and dried under vacuum to afford a white solid **2** (2.67 g, 91.8% yield), which was then used for next step reaction without further purification.

### 5.5 Synthesis of the probe **1**



4-Chloro-7-diethylaminocoumarin-3-aldehyde (280 mg, 1.0 mmol) and 2,3-dimethylbenzothiazolium iodide (291 mg, 1.0 mmol) were dissolved in 20 mL EtOH. The reaction mixture was refluxed with stirring for 12 h and then cooled to r.t., The precipitate was collected by filter and then was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 20:1 v/v) to give the probe as dark blue solid (0.47 g, 85% yield). <sup>1</sup>H NMR (600 Hz, DMSO-*d*<sub>6</sub>) δ 8.37 (d, *J* = 9.0 Hz, 1H), 8.29(d, *J* = 15 Hz, 1H), 8.26 (d, *J* = 9.0 Hz, 1H), 8.06 (d, *J* = 15 Hz, 1H), 7.84 (t, *J* = 8.3 Hz, 1H), 7.76 (m, 2H), 6.95 (dd, *J*<sub>1</sub> = 2.4 Hz, *J*<sub>2</sub> = 9.6 Hz, 1H), 6.69 (d, *J* = 2.2 Hz, 1H), 4.22 (s, 3H), 3.53(q, *J* = 7.0 Hz, 4H), 1.16(t, *J* = 7.0 Hz, 6H); <sup>13</sup>C NMR (150 MHz, DMSO-*d*<sub>6</sub>) δ 175.2, 161.3, 158.5, 157.0, 155.1, 145.5, 142.6, 132.8, 132.0, 131.7, 131.0, 127.5, 120.1, 117.7, 115.1, 113.0, 110.8, 99.7, 58.2, 48.2, 39.5, 15.8; ESI-MS: calcd for 425.1085, Found 425.1088.

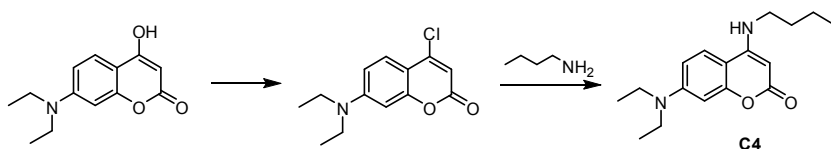
### 5.6 Synthesis of control compound C3



Compound **1** (55 mg, 0.1 mmol) and n-BuNH<sub>2</sub> (73 mg, 1 mmol) were dissolved in 15 mL anhydrous DMF in a 50 mL round bottom flask. The mixture was stirred at 60-70 °C under N<sub>2</sub> for 2 h. Then it was cooled and added to 100 mL of water with violent stirring. The precipitate was collected by filter and then purified by chromatography on silica gel with (CH<sub>2</sub>Cl<sub>2</sub>/MeOH=20:1) as the eluent to give **C3** as a dark red solid (25 mg, 42.4 % yield). <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 8.40 (s, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 8.08 (s, 1H), 8.07 (d, *J* = 14.3 Hz, 1H), 8.04 (d, *J* = 9.0 Hz, 1H), 7.90 (d, *J* = 14.3 Hz, 1H), 7.74 (t, *J* = 7.8 Hz, 1H), 7.63 (t, *J* = 7.8 Hz, 1H), 6.78 (dd, *J*<sub>1</sub> = 2.4 Hz,

$J_2 = 9.6$  Hz, 1H), 6.5 (d,  $J = 1.8$  Hz, 1H), 4.08 (s, 3H), 3.73 (q,  $J = 6.6$  Hz, 2H), 3.48 (q,  $J = 7.2$  Hz, 4H), 1.80 (m, 2H), 1.36 (m, 2H), 1.15 (t,  $J = 7.2$  Hz, 6H), 0.91 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (150 MHz, DMSO- $d_6$ )  $\delta$  175.5, 163.6, 162.3, 158.2, 155.5, 148.3, 145.3, 132.1, 130.4, 129.6, 126.9, 118.9, 112.5, 108.1, 105.6, 100.0, 96.7, 52.1, 47.5, 38.5, 35.1, 22.8, 16.9, 15.8; ESI-MS: calcd for 462.2209, Found 462.2211.

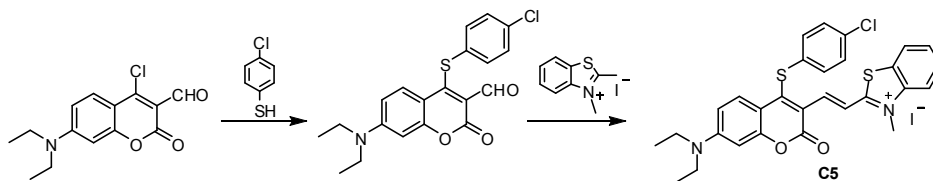
### 5.7 Synthesis of control compound **C4**



7-(Diethylamino)-4-hydroxy-coumarin (2.0 g, 8.6 mmol) was refluxed in 12 mL of POCl<sub>3</sub> for 4h. The mixture was poured onto crushed ice (100 g), and the solution was justified to pH = 7. The light green precipitate were collected and the washed with water. The resulting solid was purified by flash chromatography with CH<sub>2</sub>Cl<sub>2</sub> as the eluent, furnishing 7-(diethylamino)-4-chloro-coumarin as a light green solid (2.34 g, 86 % yield).

7-(Diethylamino)-4-chloro-coumarin (0.126 g, 0.5 mmol) and n-BuNH<sub>2</sub> (0.365 g, 5 mmol) were dissolved in 10 mL anhydrous DMF in a 50 mL round bottom flask. The mixture was stirred at 60-70°C under N<sub>2</sub> for 2 h. The solvent was removed by rotary evaporation and the residue was purified by chromatography on silica gel with CH<sub>2</sub>Cl<sub>2</sub>/MeOH=50:1 as the eluent to give **C4** as a light yellow oil (90 mg, 62.5% yield).  $^1\text{H}$  NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  7.44 (d,  $J = 9.0$ Hz, 1H), 6.53 (dd,  $J_1 = 2.4$  Hz,  $J_2 = 9.0$  Hz, 1H), 6.43 (d,  $J = 2.4$  Hz, 1H), 5.68 (s, 1H), 5.08 (s, 1H), 3.50 (q,  $J = 7.2$  Hz, 4H), 3.24 (d,  $J = 6.6$  Hz, 2H), 1.67 (m, 2H), 1.41 (m, 2H), 1.17 (t,  $J = 7.2$  Hz, 6H), 0.94 (t,  $J = 7.8$  Hz, 3H);  $^{13}\text{C}$  NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  167.2, 158.7, 156.8, 153.3, 124.2, 110.9, 107.3, 101.3, 82.9, 47.6, 45.5, 33.6, 23.1, 16.6, 15.3; ESI-MS: calcd for 289.1910, Found 289.1912.

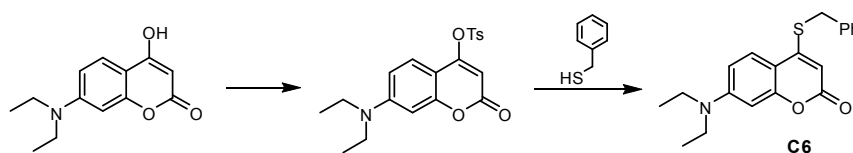
### 5.8 Synthesis of control compound **C5**



4-Chloro-7-diethylaminocoumarin-3-aldehyde (280 mg, 1.0 mmol) and  $\text{Cs}_2\text{CO}_3$  (0.6 g, 2.0 mmol) were measured into a flame-dried round bottom flask. To the starting material was added B (144 mg, 1 mmol). The solution was stirred at r.t. for 19 h, and then a brick-red precipitate was gained by addition of cold water (50 mL). The precipitate was collected by filter and then purified by chromatography on silica gel with  $(\text{CH}_2\text{Cl}_2/\text{MeOH}, 30:1 \text{ v/v})$  as the eluent to give 4-(4-chlorophenylthiol)-7-diethylaminocoumarin-3-aldehyde as an orange solid (0.14 g, 36.2% yield).

4-(4-Chlorophenylthiol)-7-diethylaminocoumarin-3-aldehyde (96 mg, 0.25 mmol) and 2,3-dimethylbenzothiazolium iodid (73 mg, 0.25 mmol) were dissolved in 5 mL EtOH. The reaction mixture was refluxed with stirring for 12 h and then cooled to r.t.. The precipitate was collected by filter and then was purified by column chromatography on silica gel ( $\text{CH}_2\text{Cl}_2/\text{MeOH}, 10:1 \text{ v/v}$ ) to give **C5** as dark blue solid (15 mg, 91% yield).  $^1\text{H}$  NMR(600 MHz,  $\text{DMSO-}d_6$ )  $\delta$  8.41 (d,  $J = 15.0$  Hz, 1H), 8.34 (d,  $J = 8.4$  Hz, 1H), 8.25 (d,  $J = 15.0$  Hz, 1H), 8.24 (d,  $J = 8.4$  Hz, 1H), 7.86-7.83 (q, 2H), 7.75(t,  $J = 7.7$  Hz, 1H), 7.48 (d,  $J = 8.4$  Hz, 2H), 7.43 (d,  $J = 8.4$  Hz, 2H), 6.88 (dd,  $J_1 = 1.8$  Hz,  $J_2 = 9.0$  Hz, 1H), 6.72 (d,  $J = 1.8$  Hz, 1H), 4.18 (s, 3H), 3.54 (q,  $J = 7.0$  Hz, 4H), 1.16 (t,  $J = 7.0$  Hz, 6H);  $^{13}\text{C}$  NMR (150 MHz,  $\text{DMSO-}d_6$ )  $\delta$  161.6, 158.2, 156.7, 156.3, 145.6, 145.3, 136.8, 135.7, 133.8, 133.6, 133.3, 132.8, 131.6, 130.8, 127.5, 120.0, 118.1, 117.5, 114.8, 113.8, 100.0, 48.1, 39.4, 15.9; ESI-MS: calcd for 552.1621, Found 552.1629.

### 5.9 Synthesis of control compound **C6**

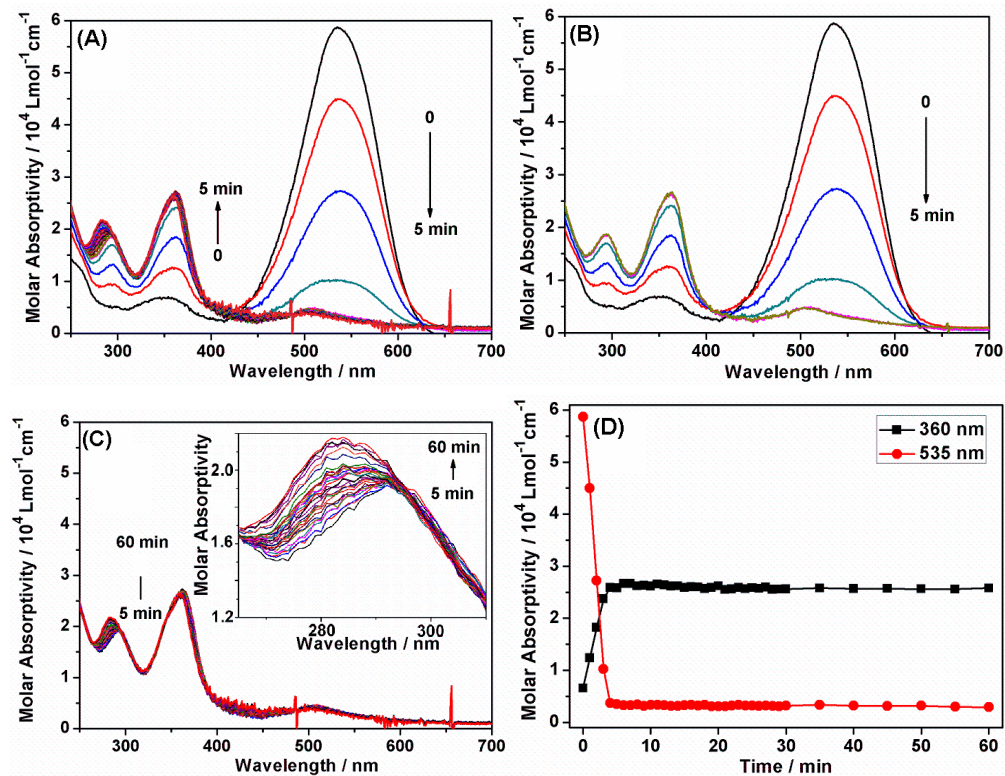


To a solution of 7-(Diethylamino)-4-hydroxy-coumarin (233 mg, 1.0 mmol) in

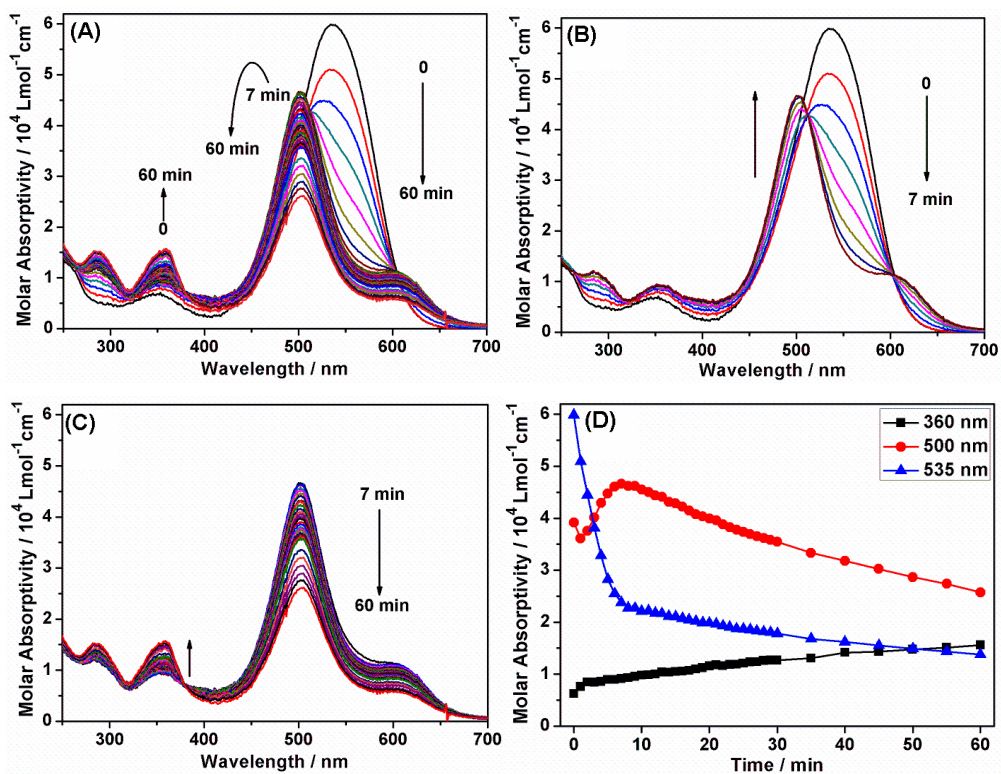
CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added Et<sub>3</sub>N (2 eq) at r.t.. TsCl (1.2 eq) was added dropwise to the solution at 0 °C under N<sub>2</sub>. After 10 min, the ice-bath was removed and the reaction was stirred further for 5 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was separated, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated to give 7-(diethylamino)-coumarin-4-yl 4-methylbenzenesulfonate, which was then used for next step reaction without further purification.

Benzyl mercaptan (0.148 g, 1.2 mmol) was added to a solution of 7-(diethylamino)-coumarin-4-yl 4-methylbenzenesulfonate (0.387 g, 1 mmol) and Et<sub>3</sub>N (0.242 g, 2.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) under N<sub>2</sub>. The reaction mixture was stirred at r.t., monitored by TLC, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (10 mL), and filtered through a short silica gel bed. The filtrate was concentrated to a residue that was purified by CH<sub>2</sub>Cl<sub>2</sub> to afford **C6** a light yellow solid (0.1 g, 29.5% yield). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 7.50 (d, *J* = 9.0 Hz, 1H), 7.43 (d, *J* = 7.2 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 1H), 6.57 (d, *J* = 8.4 Hz, 1H), 6.48 (s, 1H), 5.92 (s, 1H), 4.22 (s, 2H), 3.40 (q, *J* = 7.0 Hz, 4H), 1.19 (t, *J* = 7.0 Hz, 6H); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 163.2, 159.0, 157.6, 153.4, 137.3, 131.8, 131.3, 127.7, 111.8, 110.5, 104.7, 100.9, 48.0, 38.5, 15.2; ESI-MS: calcd for 340.1365, Found 340.1368.

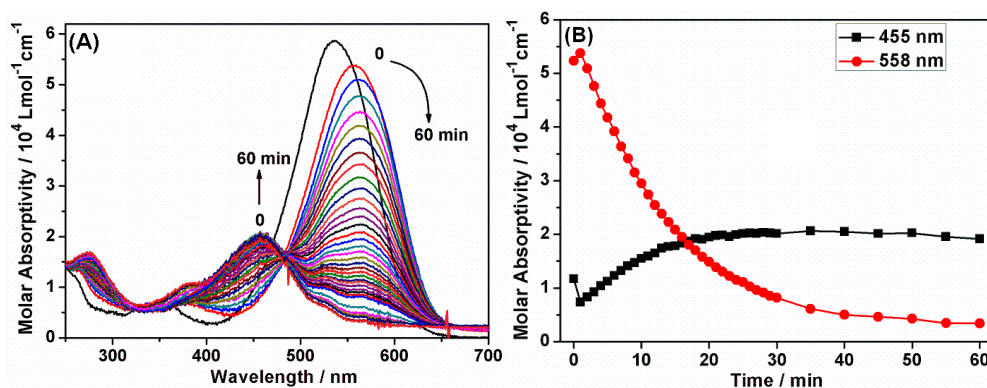
## 6. Supplementary Spectra



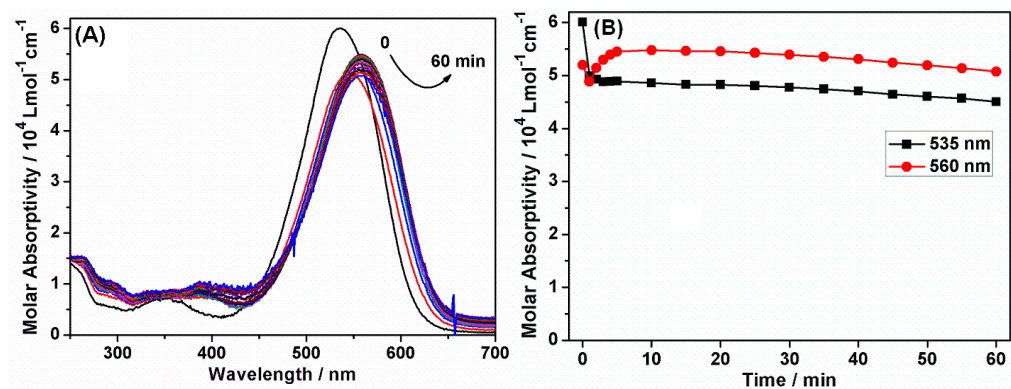
**Figure S1.** Time-dependent absorption spectra of **1** (2 μM) at different time scales upon addition of Cys (20 μM) in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at 25 °C. (A) 0–60 min; (B) 0–5 min; (C) 5–60 min; (D) the corresponding time-dependent absorption intensity changes.



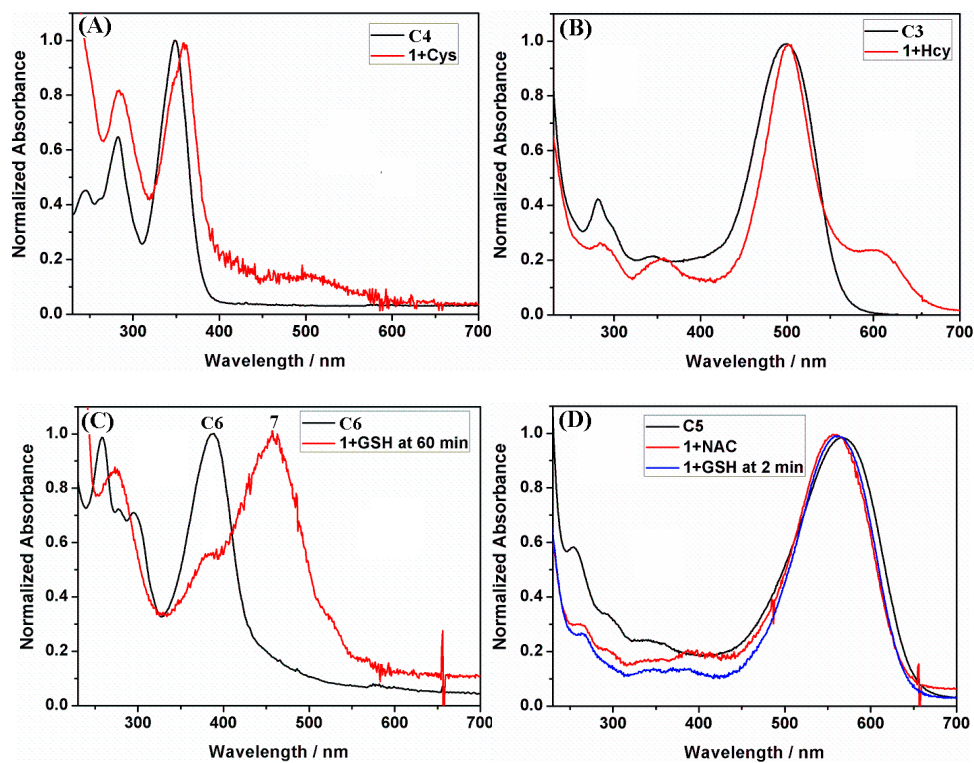
**Figure S2.** Time-dependent absorption spectra of probe 1 (2 μM) at different time scales upon addition of Hcy (20 μM) in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at 25 °C. (A) 0–60 min; (B) 0–5 min; (C) 5–60 min; (D) the corresponding time-dependent absorption intensity changes.



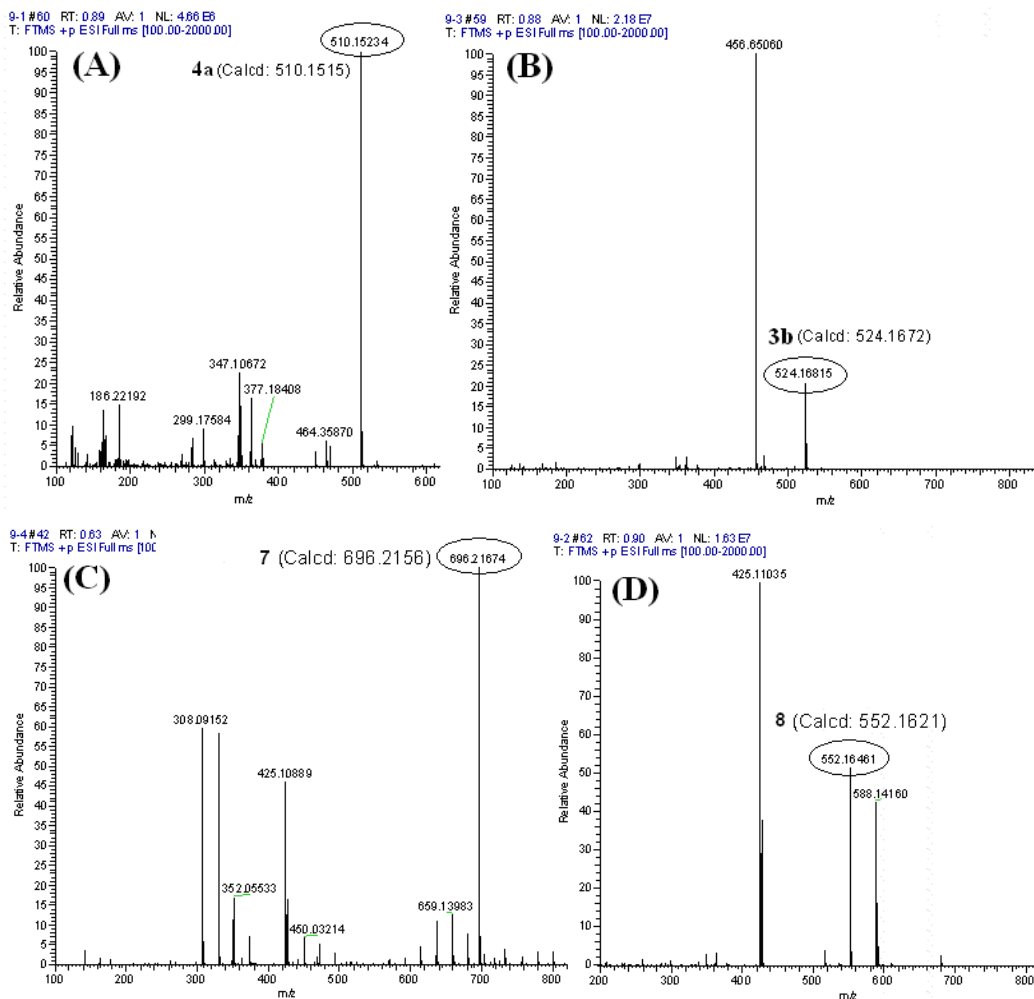
**Figure S3.** Time-dependent absorption spectra of probe 1 (2 μM) in the presence of 10 equiv of GSH (A) and the corresponding time-dependent absorption intensity changes (B) in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at 25 °C.



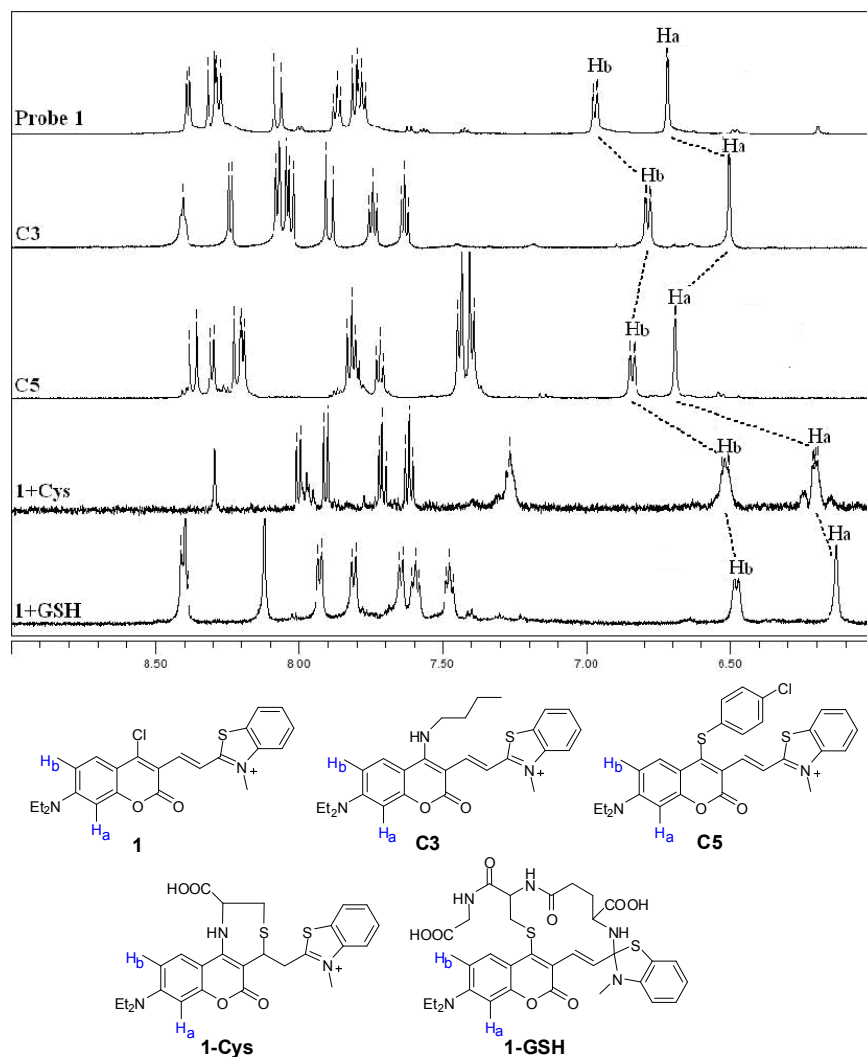
**Figure S4.** Time-dependent absorption spectra of probe **1** (2  $\mu\text{M}$ ) in the presence of 10 equiv of NAC (A) and the corresponding time-dependent absorption intensity changes (B) in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at 25  $^{\circ}\text{C}$ .



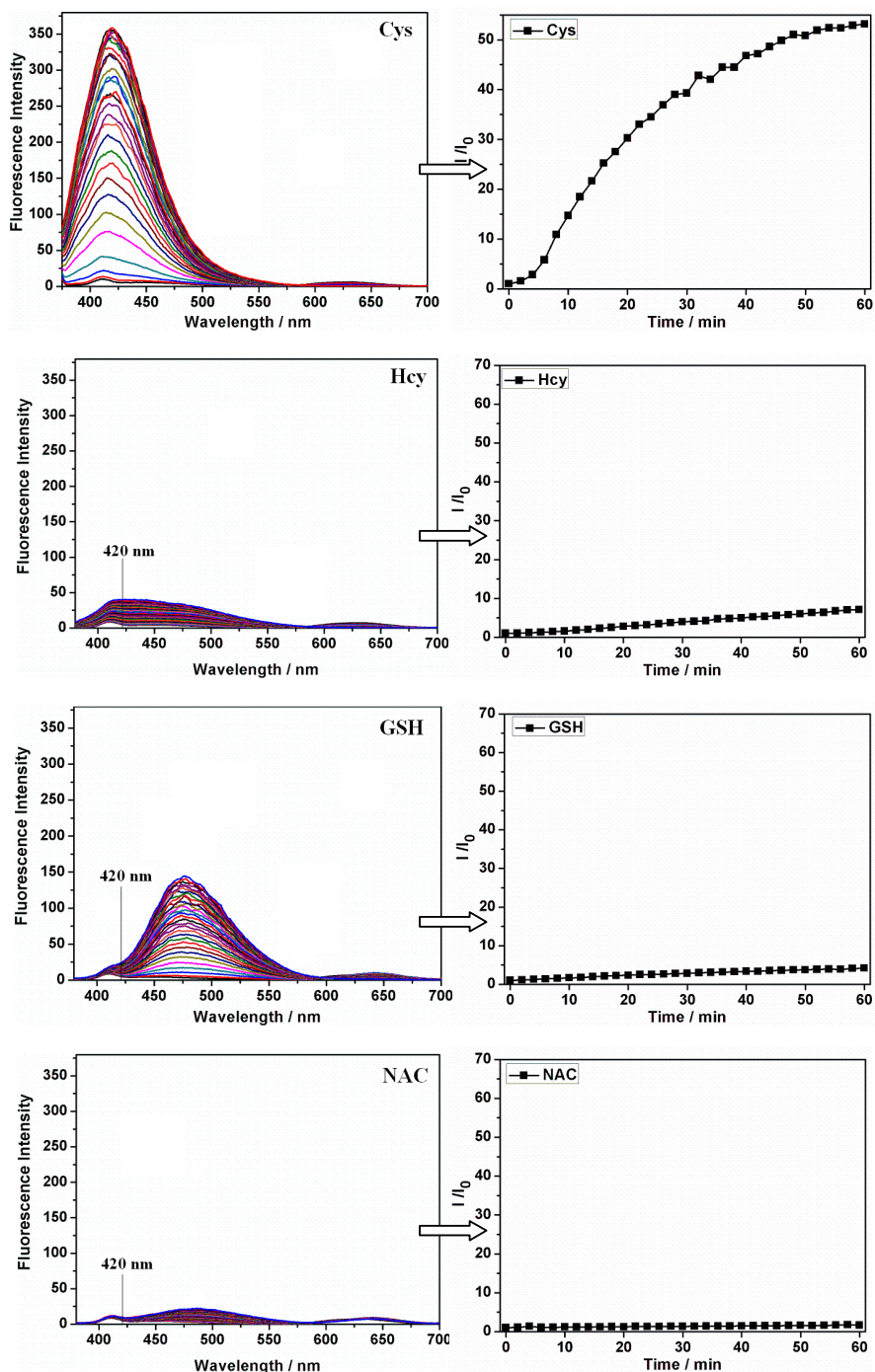
**Figure S5.** Normalized absorption spectra of control compounds vs **1**-thiol adducts. (A) **C4** and **1+Cys**; (B) **C3** and **1+Hcy**; (C) **C6** and **1+GSH** (at 60 min); (D) **C5**, **1+NAC**, and **1+GSH** (at 2 min).



**Figure S6.** HRMS charts of probe **1** upon addition of excess Cys (A), Hcy (B), GSH (C), and NAC (D) in PB buffer (10 mM, pH 7.4, containing 1 mM CTAB).

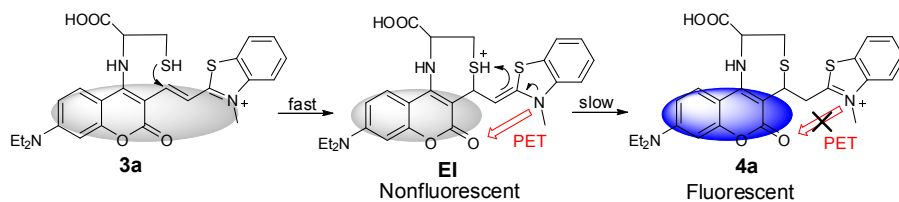


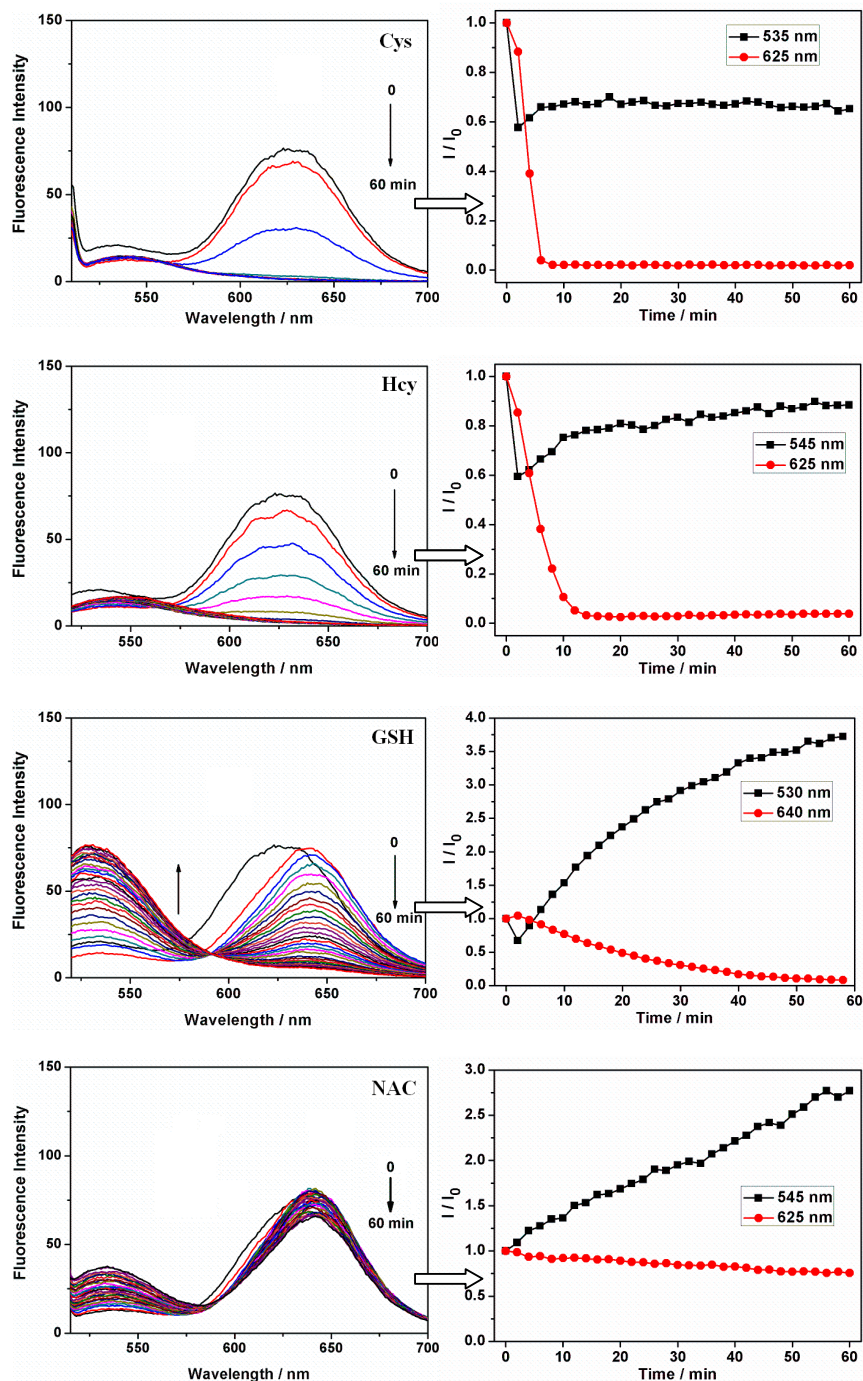
**Figure S7.** Partial  $^1\text{H}$  NMR spectra comparison of **1**, **C3**, **C5**, **1+Cys**, and **1+GSH**. The  $^1\text{H}$  NMR spectra of **1**, **C3**, and **C5** were obtained in  $\text{DMSO-}d_6\text{-D}_2\text{O}$  (8:2, v/v). The  $^1\text{H}$  NMR spectra of **1+Cys** and **1+GSH** were obtained in  $\text{DMSO-}d_6\text{-D}_2\text{O}$  (8:2, v/v, containing excess of CTAB). In order to ensure reaction completion, excess of CTAB and Cys (or GSH) were used. In the case, only the aromatic proton signals are clear. It was found that the chemical shifts of coumarin protons  $\text{H}_a$  and  $\text{H}_b$  in **1-Cys** and **1-GSH** displayed the obvious upfield shifts compared with those of **1**, **C3**, and **C5**. The observed upfield shifts in **1-Cys** or **1-GSH** indicated that the intramolecular cyclization reaction (Scheme 2 in our manuscript) breaks the conjugation between 7-diethylamino-coumarin moiety and electron-withdrawing benzothiazolium cation, resulting in the increased electron density around  $\text{H}_a$  and  $\text{H}_b$  in **1-Cys** or **1-GSH**, and thus the upfield chemical shifts. These results are in good agreement with our proposed reaction mechanism.



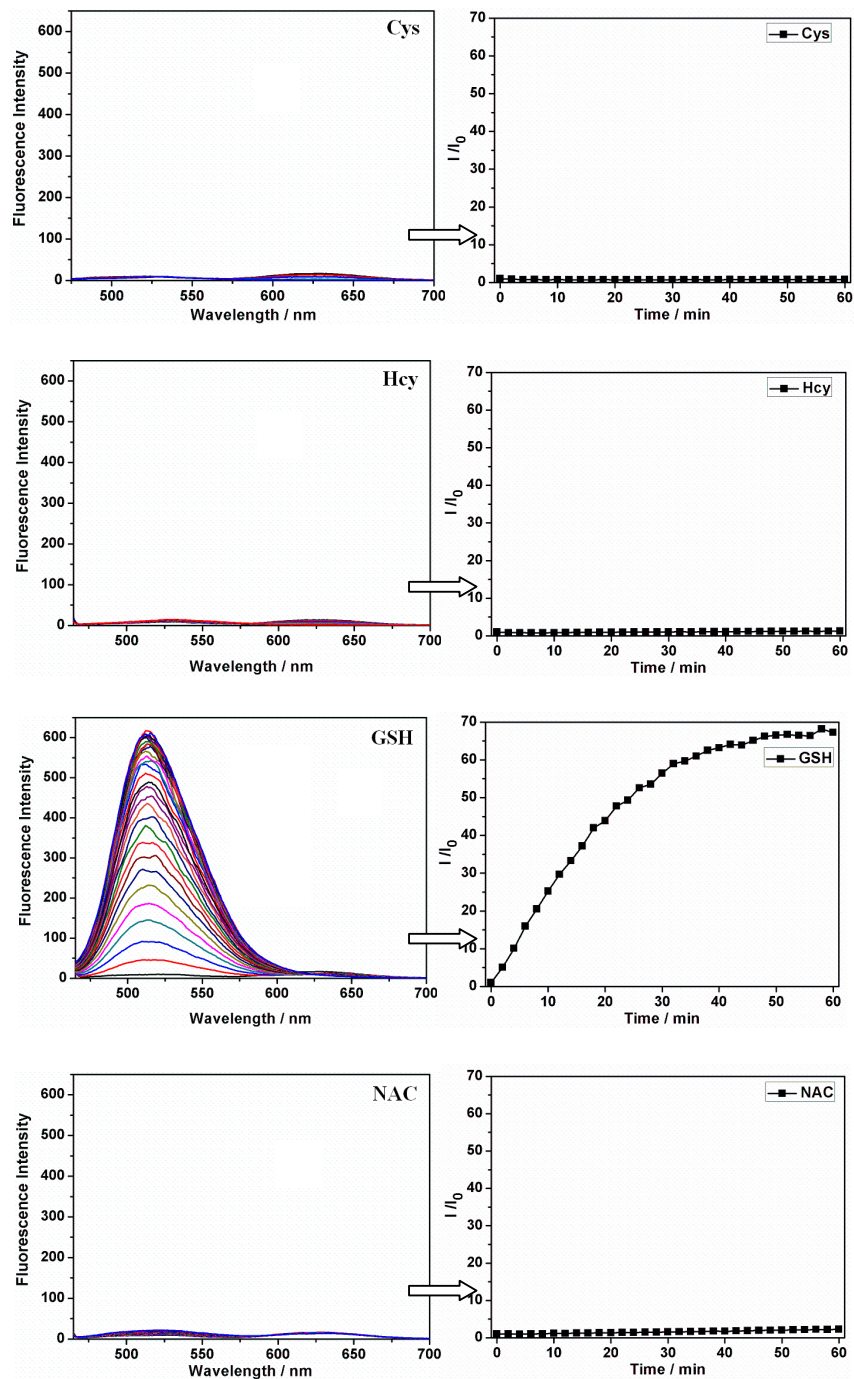
**Figure S8.** Time-dependent fluorescence spectra of probe **1** ( $1 \mu\text{M}$ ) in the presence of 10 equiv of Cys, Hcy, GSH, and NAC and the corresponding time-dependent fluorescence intensity changes in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at  $25 \text{ }^\circ\text{C}$ .  $\lambda_{\text{ex}} = 360 \text{ nm}$ . It is worth mentioning that the time-dependent absorption spectra of **1** in the presence of Cys indicate that the reaction appears to be completed within 5 min (Fig. S1); however, the corresponding time-dependent fluorescence spectra revealed that the obvious fluorescence enhancement occur after 5 min and

reach equilibrium after 60 min (this Figure, first row). Thus, some details may be omitted in the time-dependent absorption spectra studies of **1** upon addition of Cys. Given the slight absorption changes at around 280 nm from 5 min to 60 min (Fig. 1A, inset), we proposed an enamine intermediate (**EI**, see below), resulted from the intramolecular cyclization of **3a**. In fact, **EI** has similar chemical structure to **4a**, but is expected to be non-fluorescent due to the photo-induced electron transfer (PET). The slow proton transfer of **EI** leads to **4a** that is fluorescent due to removing the PET process.

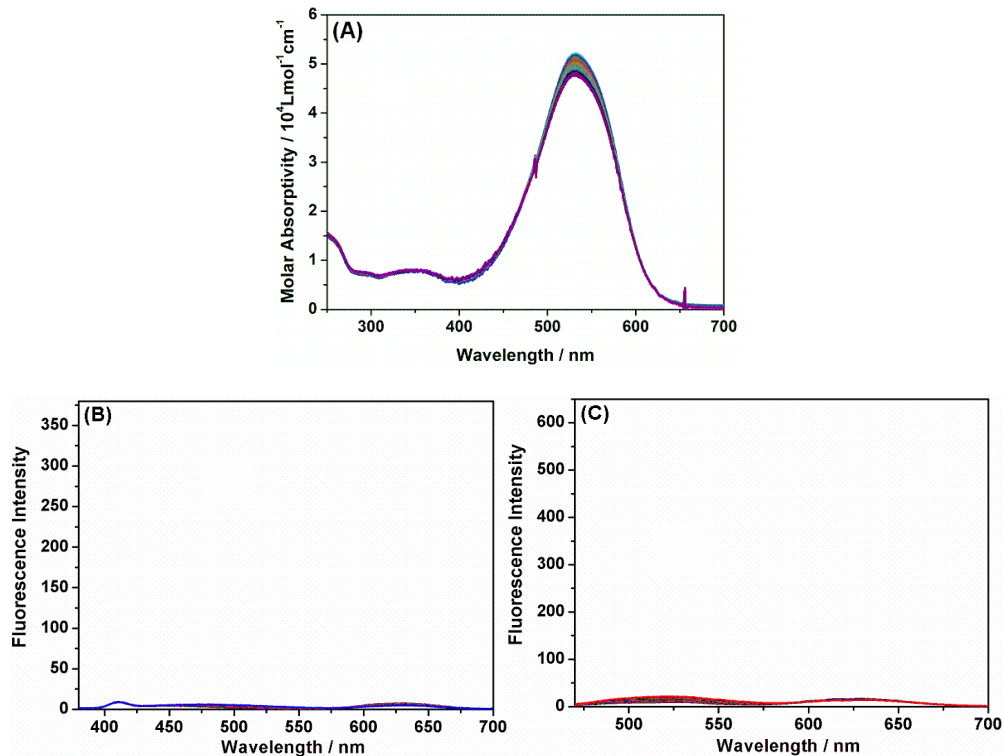




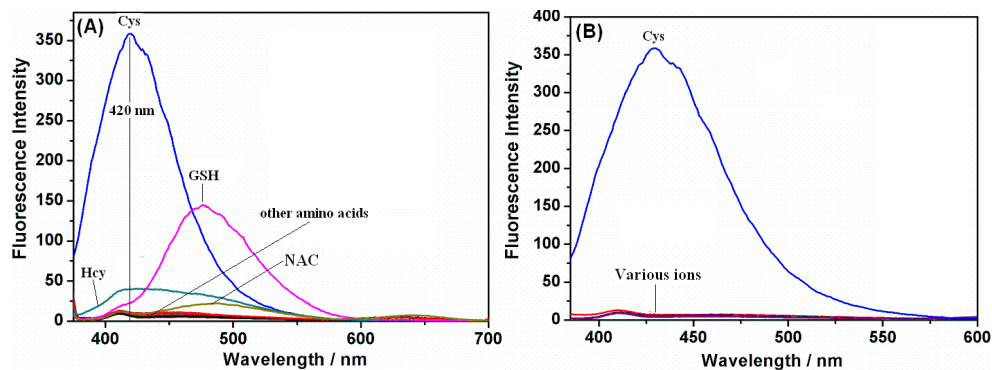
**Figure S9.** Time-dependent fluorescence spectra of probe **1** ( $1 \mu\text{M}$ ) in the presence of 10 equiv of Cys, Hcy, GSH, and NAC and the corresponding time-dependent fluorescence intensity changes in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at  $25^\circ\text{C}$ .  $\lambda_{\text{ex}} = 500 \text{ nm}$ .



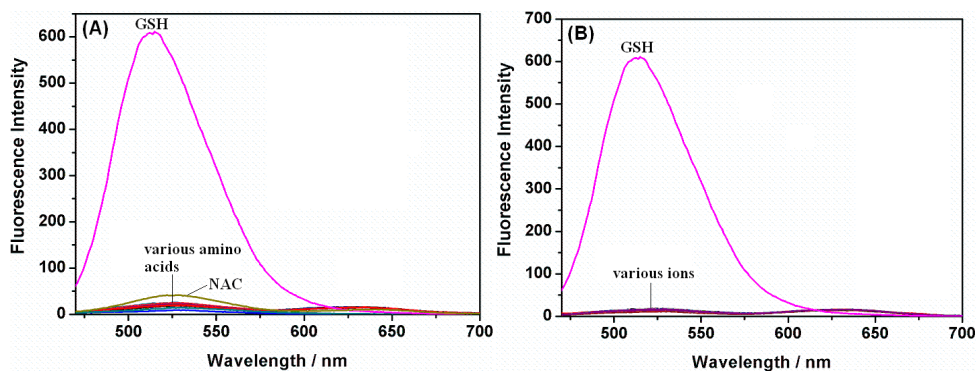
**Figure S10.** Time-dependent fluorescence spectra of probe **1** (1  $\mu$ M) in the presence of 10 equiv of Cys, Hcy, GSH, and NAC and the corresponding time-dependent fluorescence intensity changes in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at 25  $^{\circ}$ C.  $\lambda_{\text{ex}} = 450$  nm.



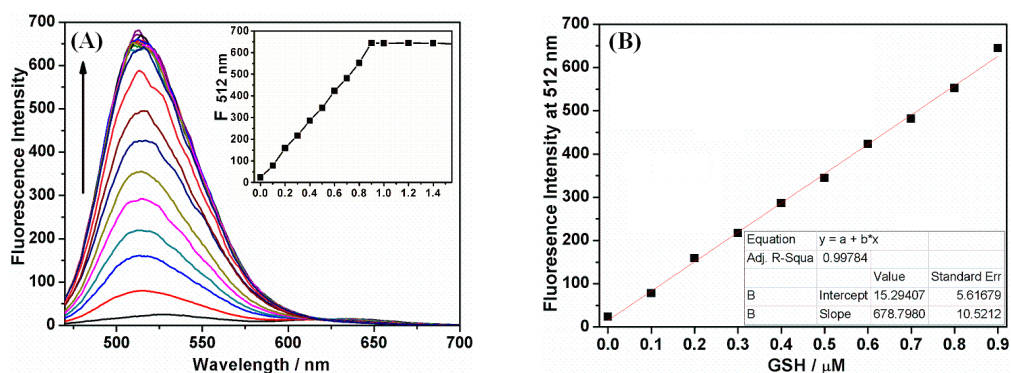
**Figure S11.** (A) Time-dependent absorption spectra of **1** (2  $\mu\text{M}$ ). (B) Time-dependent fluorescence spectra of **1** (1  $\mu\text{M}$ ) excited at 360 nm. (C) Time-dependent fluorescence spectra of **1** (1  $\mu\text{M}$ ) excited at 450 nm. Time scale: 0–60 min.



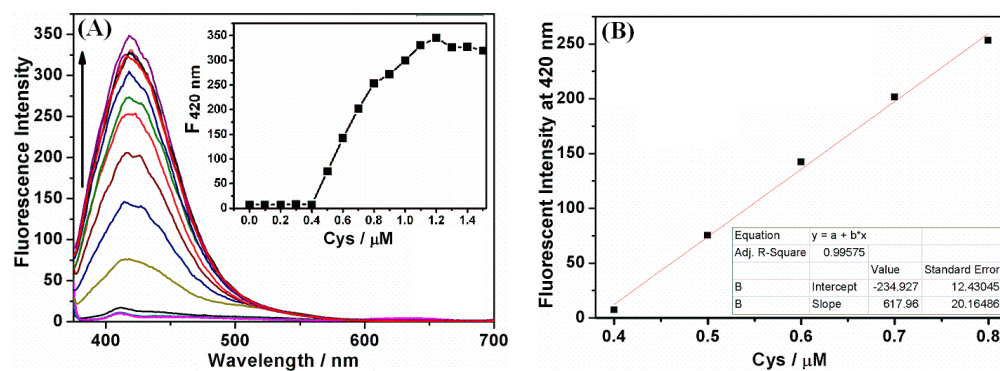
**Figure S12.** Fluorescence spectra of **1** (1  $\mu\text{M}$ ) upon addition of various amino acids, GSH, and NAC (10 equiv) (A) as well as representative ions (B) (200 equiv) in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at 25  $^{\circ}\text{C}$ . Each spectrum was recorded 60 min after addition of the corresponding species. Amino acid: Cys, Hcy, GSH, Ala, Arg, Asp, Gln, Glu, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Try, Tyr, Val. Ions:  $\text{AcO}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PPi}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ .  $\lambda_{\text{ex}} = 360 \text{ nm}$ .



**Figure S13.** Fluorescence spectra of **1** (1 μM) upon addition of various amino acids, GSH, and NAC (10 equiv) (A) as well as representative ions (B) (200 equiv) in PB buffer (pH 7.4, 10 mM, containing 1 mM CTAB) at 25 °C. Each spectrum was recorded 60 min after addition of the corresponding species. Amino acid: Cys, Hcy, GSH, Ala, Arg, Asp, Gln, Glu, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Try, Tyr, Val. Ions:  $\text{AcO}^-$ ,  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{PPi}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ .  $\lambda_{\text{ex}} = 450 \text{ nm}$ .

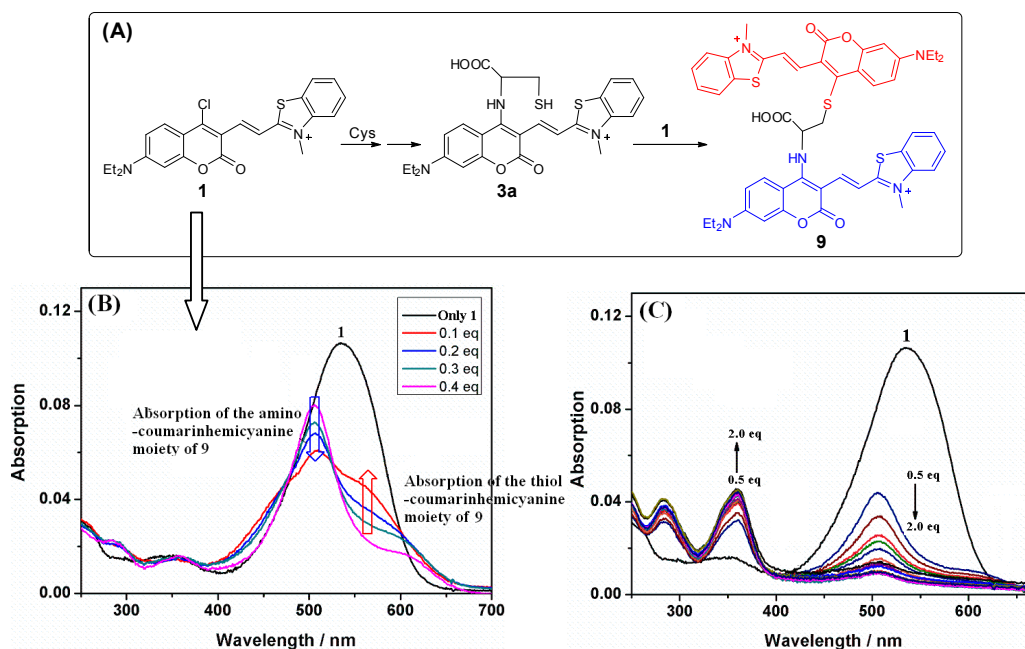


**Figure S14.** (A) Fluorescence spectra changes of **1** (1 μM) upon addition 0 to 1.5 equiv of GSH in PB buffer (10 mM, pH 7.4, containing 1 mM CTAB). Each spectrum was recorded after 60 min. (B) Fluorescence response of **1** at 512 nm to GSH concentration. Slits: 10/10 nm.  $\lambda_{\text{ex}} = 450 \text{ nm}$ .

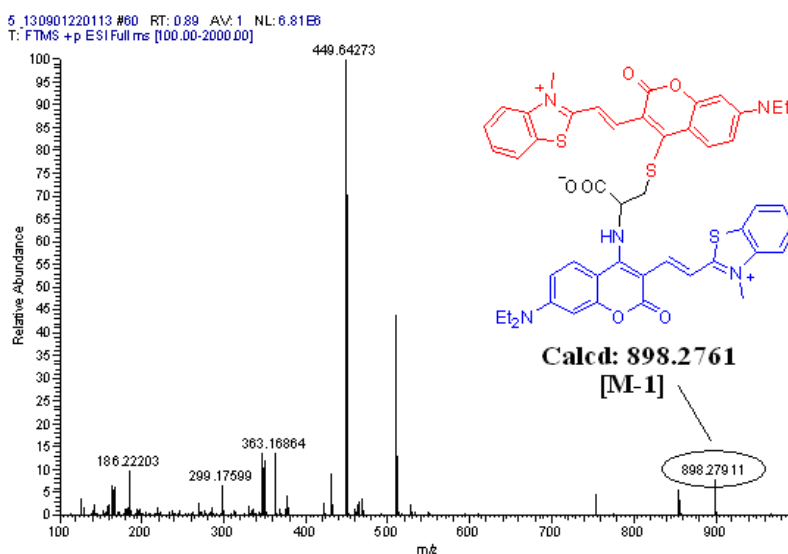


**Figure S15.** (A) Fluorescence spectra changes of **1** (1  $\mu\text{M}$ ) upon addition 0 to 1.5 equiv of GSH in PB buffer (10 mM, pH 7.4, containing 1 mM CTAB). Each spectrum was recorded after 60 min. (B) Fluorescence response of **1** at 420 nm to Cys concentration. Slits: 10/10 nm.  $\lambda_{\text{ex}} = 360$  nm.

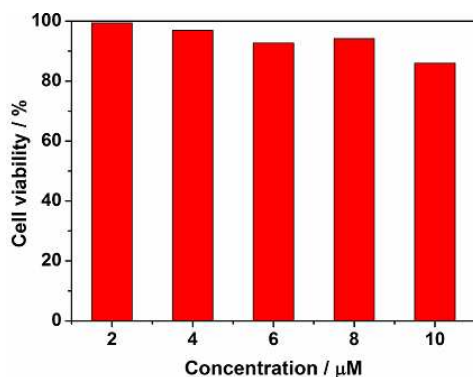
Note that the initial fluorescence silence is probably due to an intermolecular thiol-halogen nucleophilic substitution between intermediate **3a** and unreacted **1**, which results in a nonfluorescent 2:1 **1**-Cys adduct **9** (see Figures S16, S17). That is to say, when less than 0.4 equiv of Cys is added to the solution of **1**, less than 0.4 equiv of intermediate **3a** could be produced. Theoretically, the produced **3a** would either react with the remaining **1** (> 0.6 equiv) to produce adduct **9** or undergo an intramolecular cyclization to produce **4a**. In fact, the thiol-halogen  $\text{S}_{\text{N}}\text{Ar}$  nucleophilic substitution between **3a** and **1** to produce **9** is faster than the intramolecular cyclization of **3a** to produce **4a**. Thus, when less than 0.4 equiv of Cys is added to the solution of **1**, only the nonfluorescent **9** is produced. However, the more Cys is used, the less **1** is left, and thus the more **3a** exists, which will undergo an intramolecular cyclization to produce fluorescent **4a**.



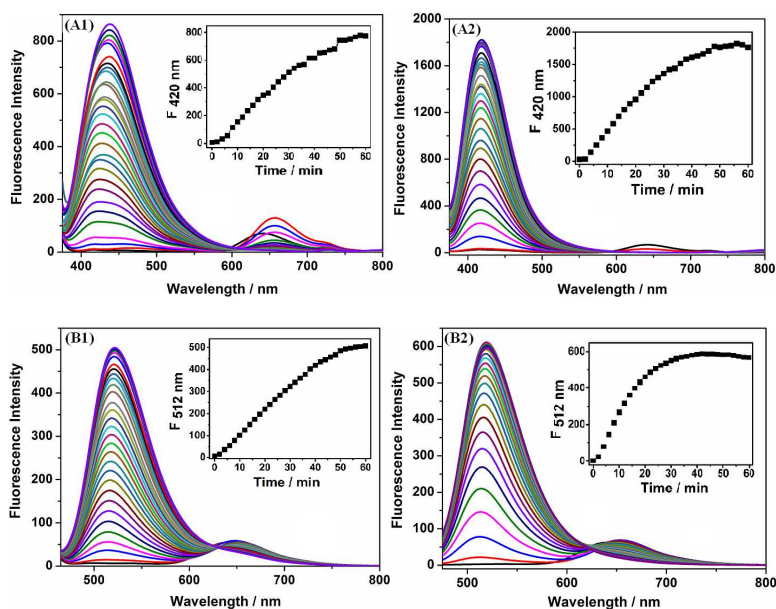
**Figure S16.** (A) Proposed reaction mechanism of **1** with 0 to 0.4 equiv of Cys. (B) UV-vis spectra changes of **1** (2  $\mu$ M) upon addition 0 to 0.4 equiv of Cys. (C) UV-vis spectra changes of **1** (2  $\mu$ M) upon addition 0.5 to 2.0 equiv of Cys.



**Figure S17.** HRMS charts of probe **1** upon addition of 0.4 equiv Cys in PB buffer (10 mM, pH 7.4, containing 1 mM CTAB).



**Figure S18.** Percentage of viable COS-7 cells after treatment with indicated concentrations of probe **1** after 24 hours.



**Figure S19.** Time-dependent fluorescence response of **1** (10 μM) in the presence of Cys/Hcy/GSH (100 μM/100 μM/1 mM) (A1) or pure Cys (100 μM) (A2) excited at 360 nm in PB buffer (10 mM, pH 7.4, 1 mM CTAB) at 25 °C. Time-dependent fluorescence response of **1** (10 μM) in the presence of Cys/Hcy/GSH (1 μM/1 μM/10 μM) (B1) or pure GSH (10 μM) (B2) excited at 450 nm in PB buffer (10 mM, pH 7.4, 1 mM CTAB) at 25 °C. Fluorescence spectra were taken on HITACHI F-7000 fluorescence spectrometer. Slits: 10/10 nm.

Note that in the mixture experiment, both the fluorescent intensity of Cys and GSH decreased (A1 and B1) when compared with those of pure Cys and GSH (A2 and B2) due to the competitive reaction kinetics.

7.  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and HRMS chart of probe 1 and control compounds C3–C6.

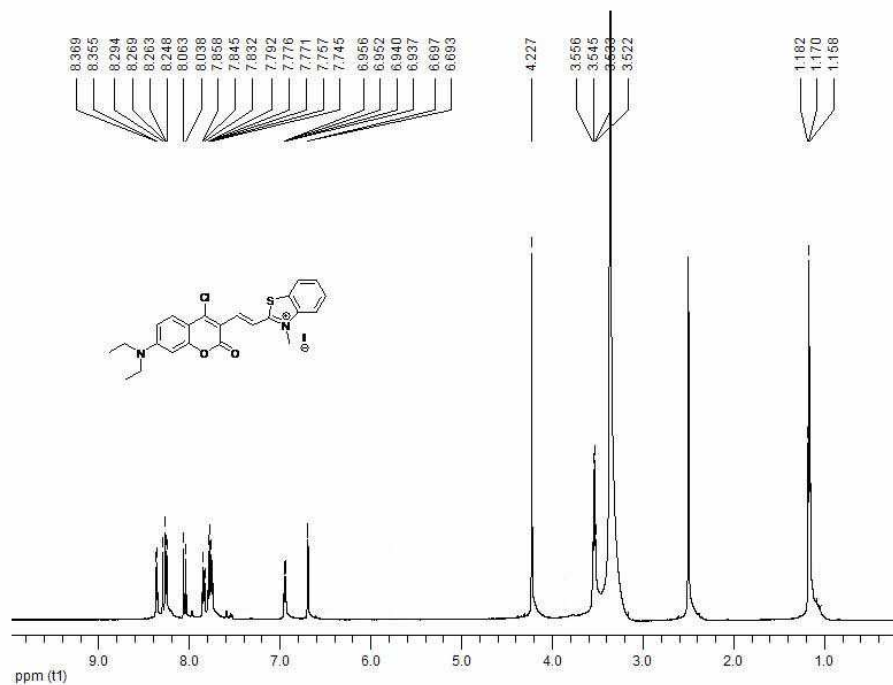


Figure S20A.  $^1\text{H}$  NMR chart of probe 1 (DMSO- $d_6$ , 600 MHz).

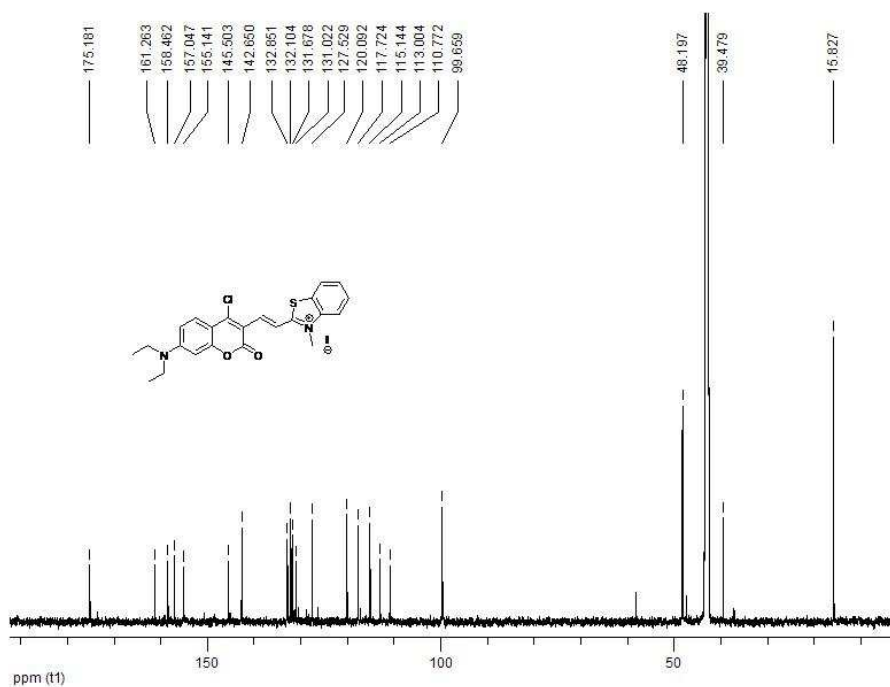
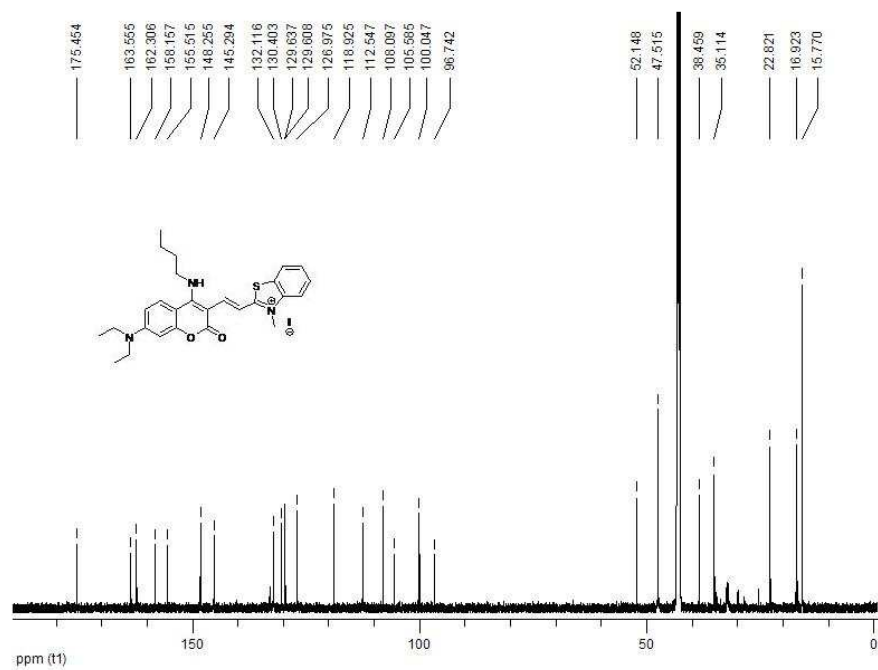
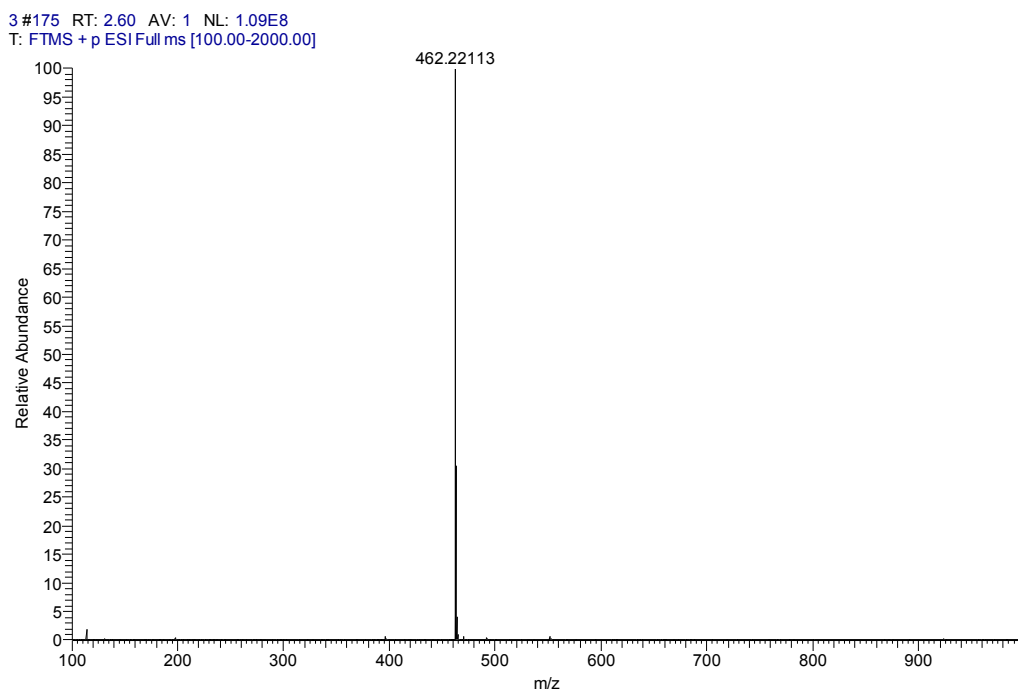


Figure S20B.  $^{13}\text{C}$  NMR chart of probe 1 (DMSO- $d_6$ , 150 MHz).

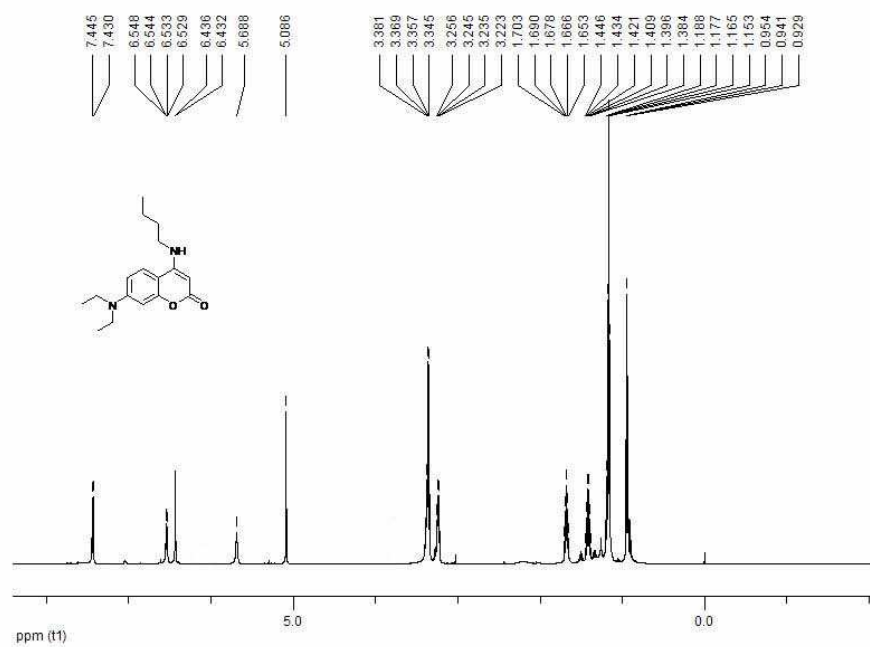




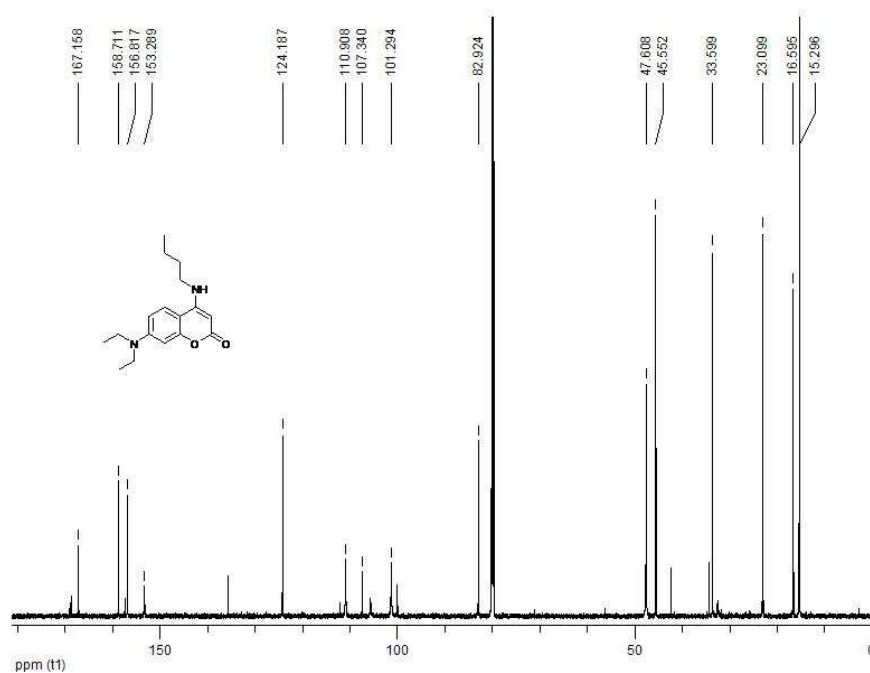
**Figure S21B.** <sup>13</sup>C NMR chart of control compound C3 (DMSO-*d*<sub>6</sub>, 600 MHz).



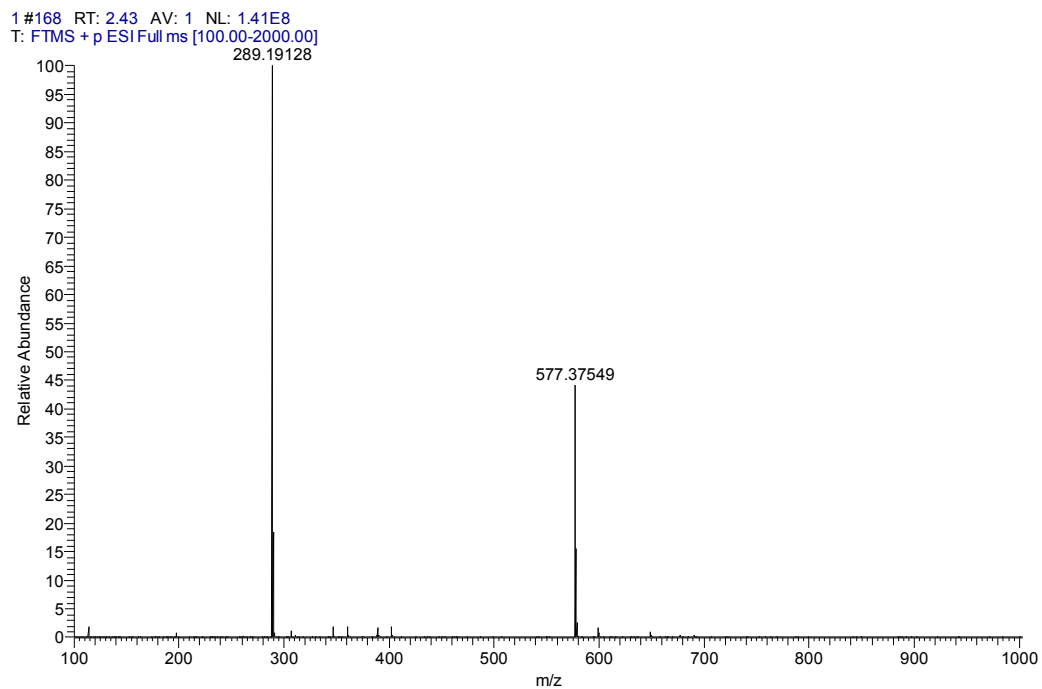
**Figure S21C.** HRMS chart of control compound C3.



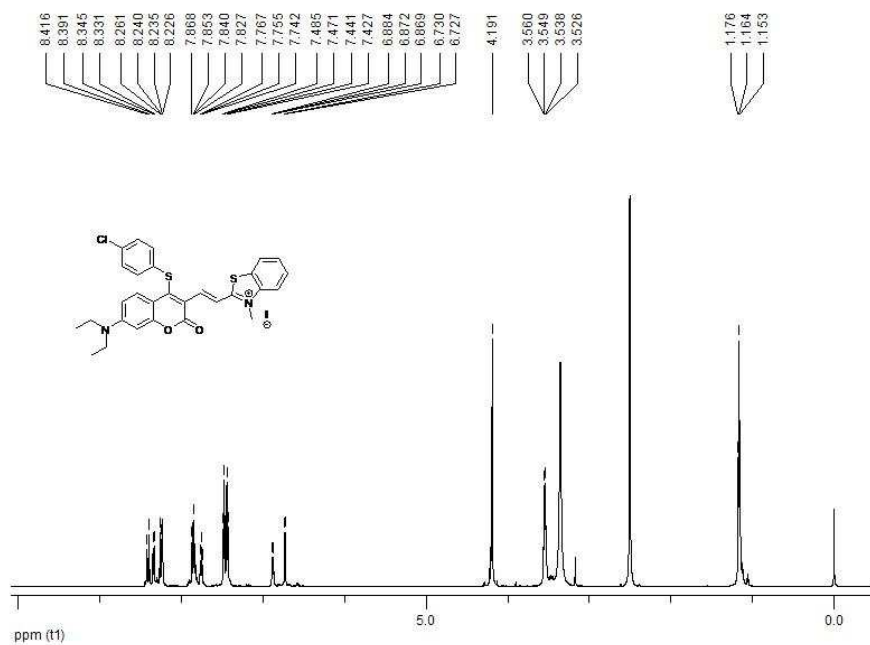
**Figure S22A.** <sup>1</sup>H NMR chart of control compound **C4** (CDCl<sub>3</sub>, 600 MHz).



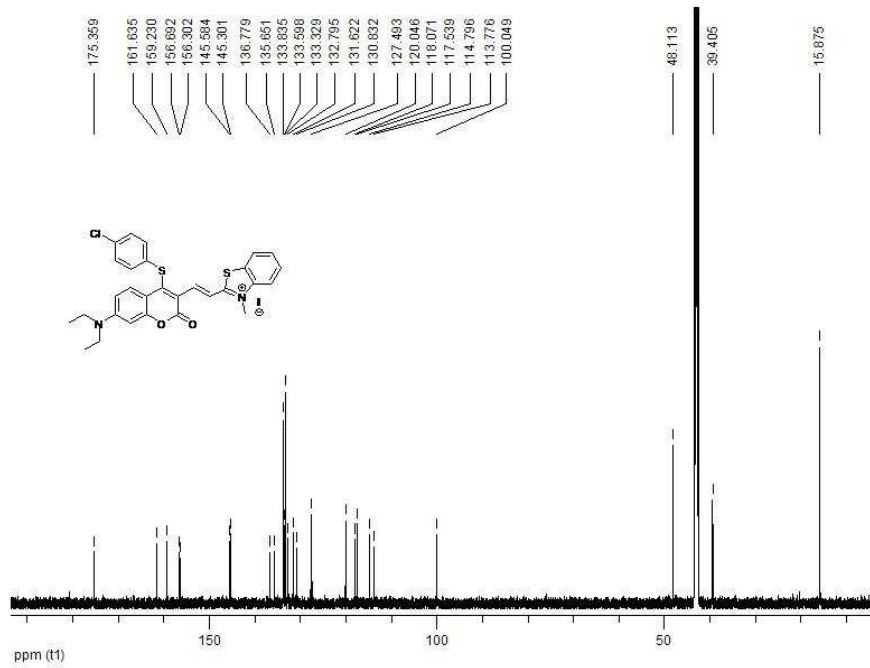
**Figure S22B.** <sup>13</sup>C NMR chart of control compound **C4** (CDCl<sub>3</sub>, 150 MHz).



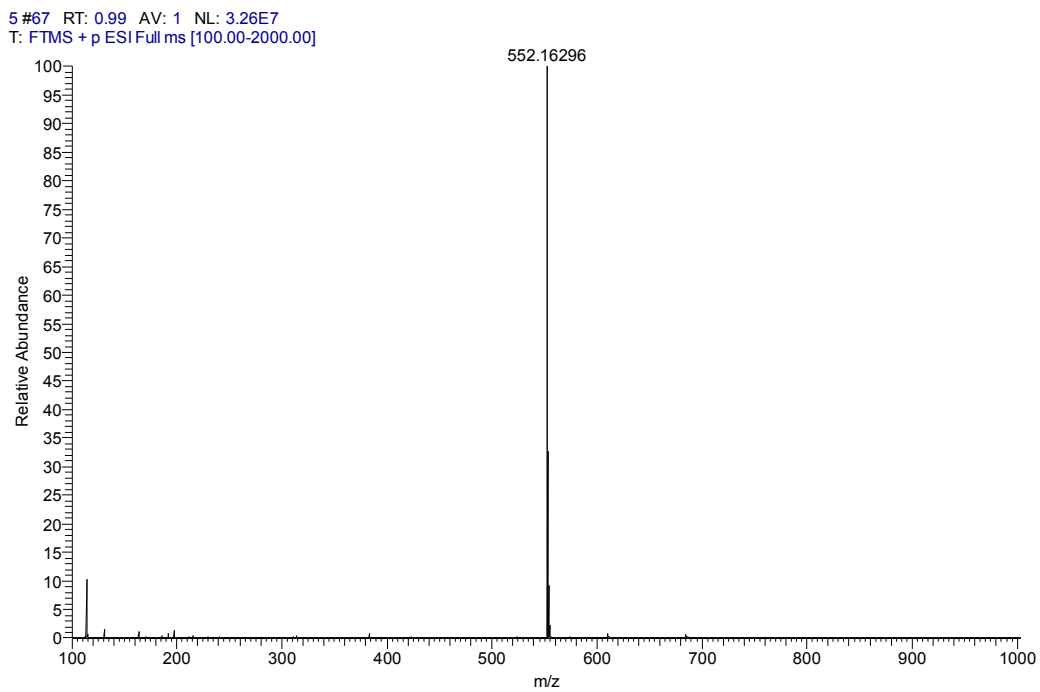
**Figure S22C.** HRMS chart of control compound **C4**.



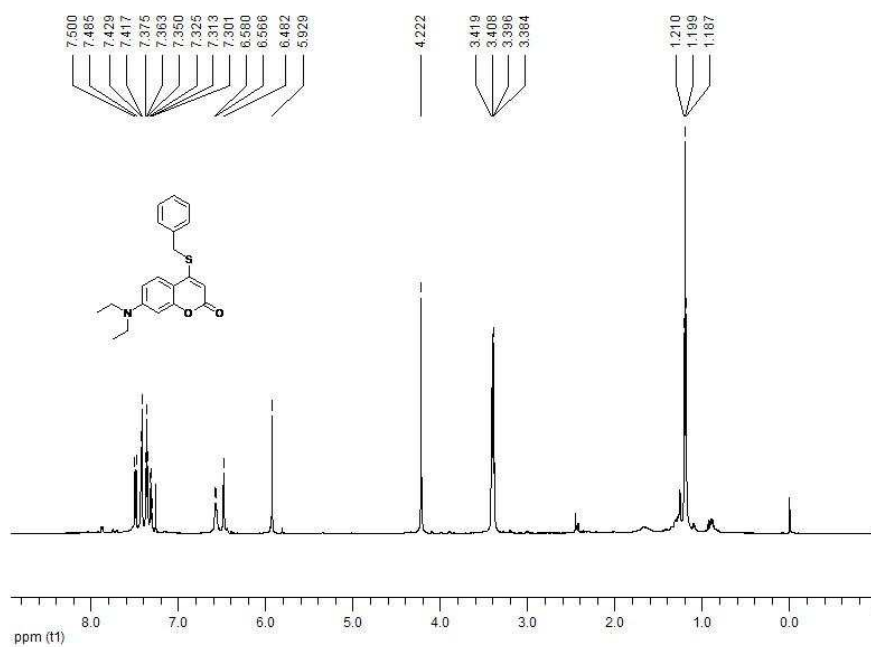
**Figure S23A.**  $^1\text{H}$  NMR chart of control compound **C5** ( $\text{DMSO-}d_6$ , 600 MHz).



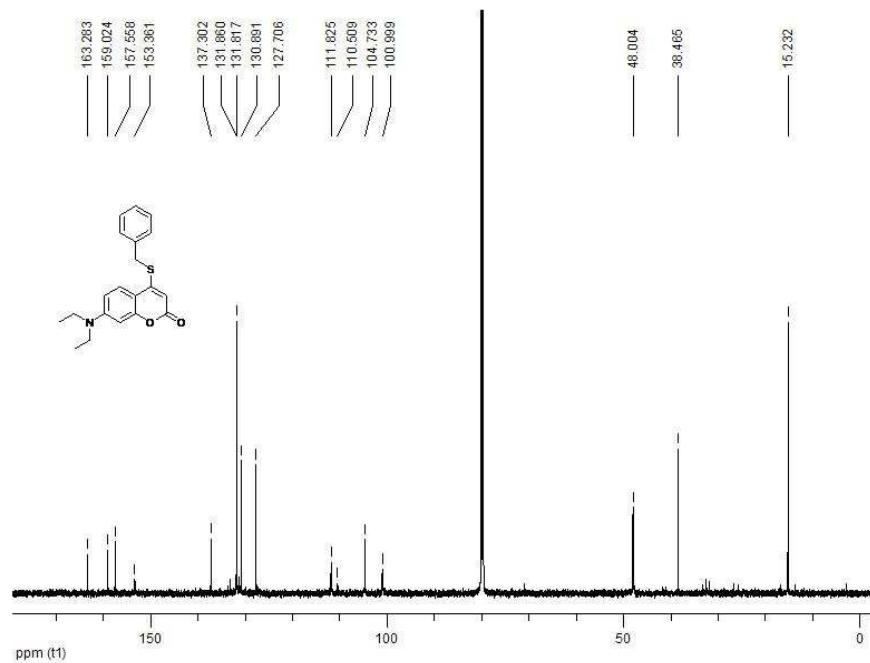
**Figure S23B.** <sup>13</sup>C NMR chart of control compound C5 (DMSO-*d*<sub>6</sub>, 600 MHz).



**Figure S23C.** HRMS chart of control compound C5.

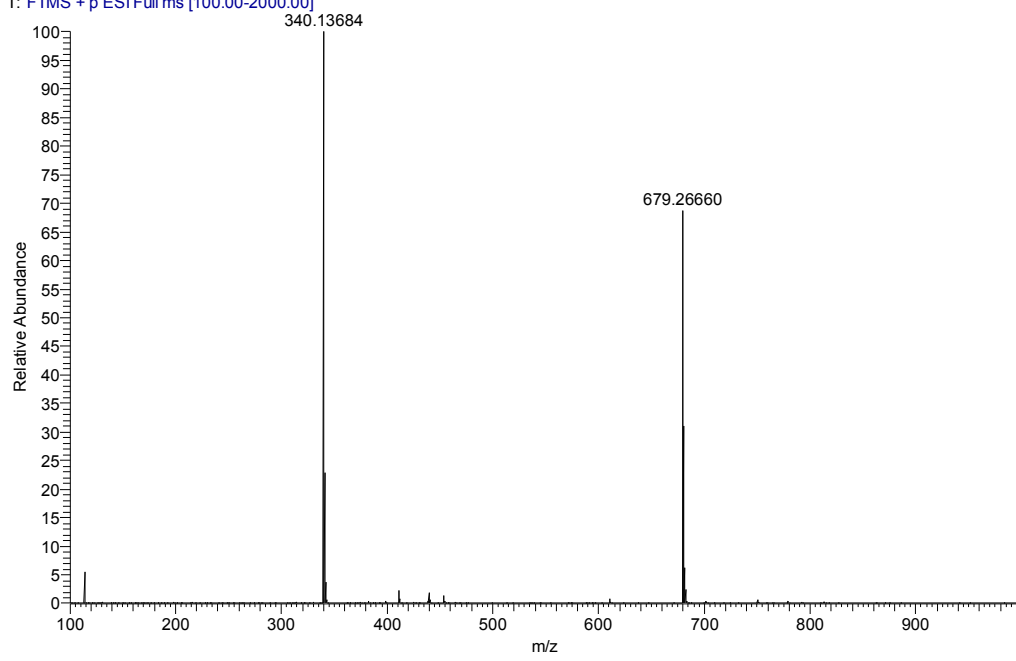


**Figure S24A.** <sup>1</sup>H NMR chart of control compound C6 (CDCl<sub>3</sub>, 600 MHz).



**Figure S24B.** <sup>13</sup>C NMR chart of control compound C6 (CDCl<sub>3</sub>, 600 MHz).

2#256 RT: 3.83 AV: 1 NL: 8.15E7  
T: FTMS + p ESI Full ms [100.00-2000.00]



**Figure S24C.** HRMS chart of control compound **C6**.