

Supporting Information

A highly selective and sensitive fluorescent thiol probe through dual-reactive and dual-quenching groups

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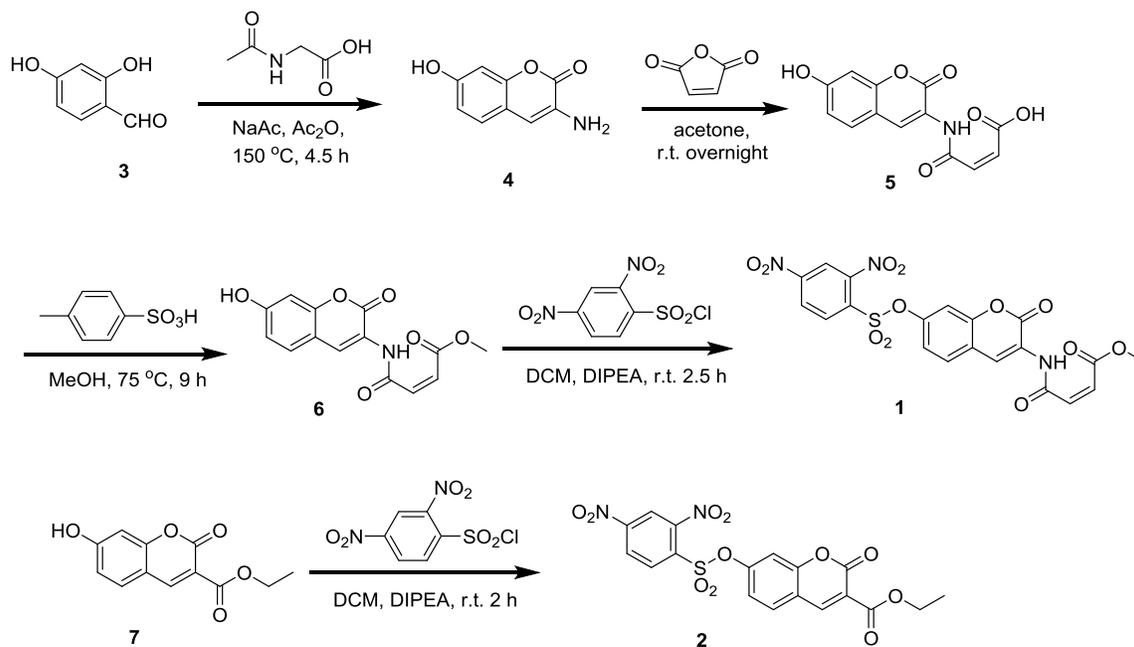
1. Material and Methods

1.1 Reagents and instruments

All chemicals and solvents were purchased from commercial suppliers and applied directly in the experiment without further purification. The progress of the reaction was monitored by TLC on pre-coated silica plates (Merck 60 F254, 250 μm thickness), and spots were visualized by KMnO_4 , UV light or iodine. Merck silica gel 60 (70-200 mesh) was used for column chromatography purification. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker model DPX-400 MHz NMR spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ($\text{Si}(\text{CH}_3)_4 = 0.00$ ppm) or residual solvent peaks ($\text{DMSO}-d_6 = 2.50$ ppm). ^1H NMR coupling constants (J) are reported in Hertz (Hz), and multiplicity is indicated as the following: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublet). High resolution mass spectra (HR-MS) were obtained on a Varian 7.0 T FTICR-MS. Fluorescence signal was

recorded with a FluoroMax-4 fluorescence photometer. Fluorescence images were acquired using a Leica TCS SPE Confocal Scanning Microscope.

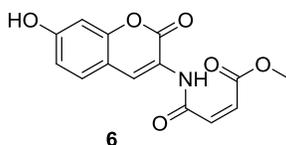
1.2 Synthesis of probe 1 and probe 2



Scheme S1. Synthetic route for probe **1** and **2**.

*Compounds 4-7 were synthesized according to the literatures with a little modification. ^[1]

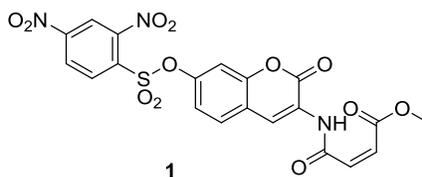
Synthesis of methyl (Z)-4-((7-hydroxy-2-oxo-2H-chromen-3-yl)amino)-4-oxobut-2-enoate (**6**)



Compound **5** (222 mg, 0.8 mmol) and 4-methylbenzenesulfonic acid (34 mg, 0.2 mmol) was added to a 50 mL round bottle flask containing 15 mL methanol. The mixture was refluxed at 75 °C for 9 hour. After cooling, the solvent was evaporated with rotary evaporator. The crude product was separated by silica gel column chromatography with

DCM / EtOAc = 1 / 5 to get compound **6** as yellow solid (181 mg, 78%). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.31 (s, 1H), 10.01 (s, 1H), 8.58 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 6.70 (d, *J* = 9.6 Hz, 2H), 6.41 (d, *J* = 12 Hz, 1H), 3.69 (s, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.98, 163.10, 159.80, 157.68, 151.64, 130.50, 129.82, 129.02, 126.42, 120.58, 113.62, 111.25, 101.93, 51.55; HR-MS: calcd. for C₁₄H₁₅NO₆ [M+H]⁺ 290.0665; found, 290.0670.

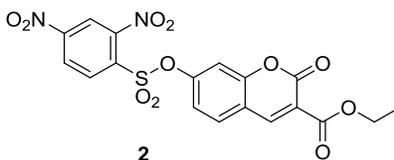
Synthesis of methyl (Z)-4-((7-(((2,4-dinitrophenyl)sulfonyl)oxy)-2-oxo-2H-chromen-3-yl)amino)-4-oxobut-2-enoate (1**)**



5 mL DCM containing compound **6** (150 mg, 0.52 mmol) was added to 10 mL DCM containing 2,4-dinitrobenzenesulfonyl chloride (207 mg, 0.78 mmol). Then, DIPEA (267 μL, 1.55 mmol) was added to the above DCM solution drop-by-drop at ice bath. The mixture solution was stirred at room temperature for 2.5 hours. After that, the reaction solution was washed with water and brine. The organic phase was dried by Na₂SO₄. Then, solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography with DCM / MeOH = 200 / 1 to get 221 mg yellow powder **1** with yield 82%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 10.27 (s, 1H), 9.05 (d, *J* = 2.4 Hz 1H), 8.68 (s, 1H), 8.60 (dd, *J* = 2.4, 8.8 Hz, 1H), 8.29 (d, *J* = 8.8 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.30 (d, *J* = 2.4 Hz, 1H), 7.14 (dd, *J* = 2.4, 8.4 Hz, 1H), 6.74 (d, *J* = 12 Hz, 1H), 6.45 (d, *J* = 12 Hz, 1H), 3.69 (s, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.82, 163.61, 156.68, 151.40, 149.98, 148.27, 148.16, 133.69, 131.03, 130.85,

129.60, 129.55, 127.46, 124.77, 123.18, 121.13, 119.54, 118.65, 109.98, 51.58; HR-MS: calcd. for C₂₀H₁₄N₃O₁₂S [M+H]⁺ 520.0298; found, 520.0297.

Synthesis of ethyl 7-(((2,4-dinitrophenyl)sulfonyl)oxy)-2-oxo-2H-chromene-3-carboxylate (**2**)



2,4-dinitrobenzenesulfonyl chloride (147 mg, 0.55 mmol) was added to 6 mL DCM. Then, 5 mL DCM containing compound **7** (123 mg, 0.52 mmol) and DIPEA (285 μ L, 1.60 mmol) was added to the above solution. The mixture solution was stirred at room temperature for 2 hours. After that, the reaction solution was washed with water and brine. The organic phase was dried by Na₂SO₄. Then, solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography with DCM / MeOH = 200 / 1 to get 208 mg light yellow powder **2** with yield 86%. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 9.07 (s, 1H), 8.75 (s, 1H), 8.62 (d, *J* = 8.0 Hz, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 8.4 Hz, 1H), 7.35 (s, 1H), 7.23 (d, *J* = 8.0, 1H), 4.29 (q, *J* = 7.2, 2H), 1.31 (t, *J* = 7.2, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 162.10, 155.15, 155.10, 151.57, 151.45, 148.11, 147.54, 133.65, 132.16, 130.76, 127.53, 121.18, 118.60, 118.15, 117.60, 110.14, 61.27, 13.94; HR-MS: calcd. for C₁₈H₁₃N₂O₁₁S [M+H]⁺ 465.0240; found, 465.0235.

1.3 Procedure for fluorescence measurement

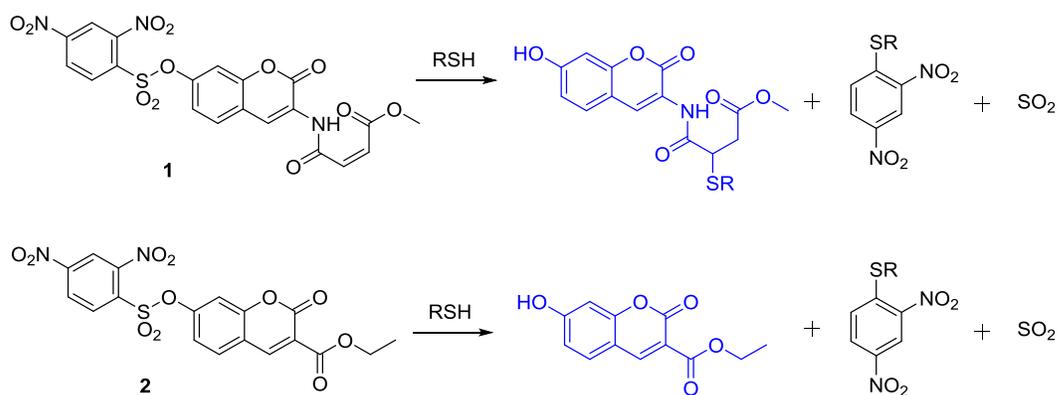
Probe **1** or **2** were dissolved in DMSO for preparing 10 mM stock solution. Then, the probes were diluted in PBS (10 mM, pH 7.4) buffer to afford the final concentration of 1 μ M. The amino acids were prepared as stock solutions of 100 mM in Millipore water.

Appropriate amount of cysteine and other amino acids were added to separate portions of the solution and mixed thoroughly. The reaction mixture was shaken uniformly before emission spectra measurement. The fluorescence spectra were conducted by using a FluoroMax-4 fluorescence photometer with a 10 mm quartz cuvette.

1.4 Fluorescence microscope experiment

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and appropriate amount of antibiotic (penicillin and streptomycin). Approximately 10^5 cells were seeded in a confocal dish (35 mm) with the medium at 37 °C. To prepare the experiment setup, the cells were allowed to adhere to the dish for 24 hours. They were then incubated with probe **1** (1 μ M) for different time intervals at 37 °C. Fluorescence images were taken by a Leica TCS SPE Confocal Scanning Microscope. Emission was collected at blue channel with 405 nm excitation.

2. Supplementary Figures



Scheme S2. Reaction mechanism of probes **1** and **2** toward thiol RSH.

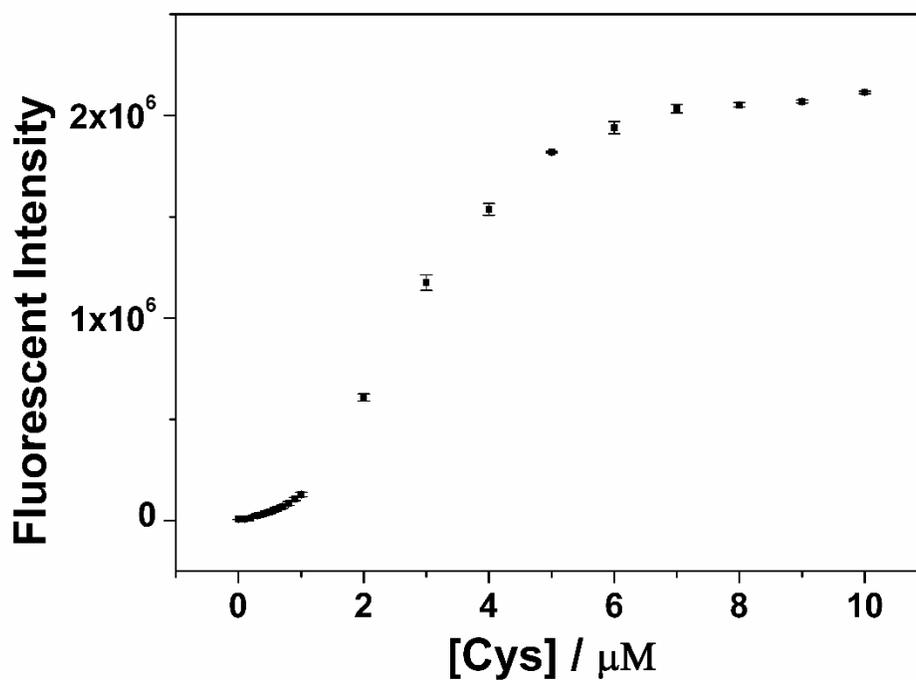


Figure S1. Fluorescence intensity changes at 470 nm during the reaction of probe **1** with different concentration of cysteine (0-10 μM) in PBS buffer.

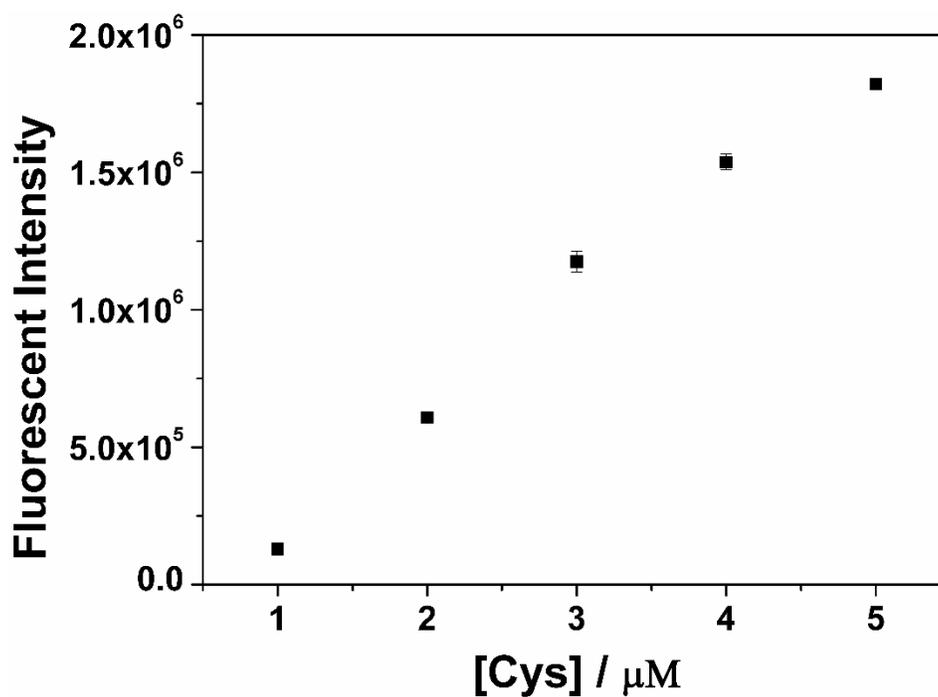


Figure S2. Fluorescence intensity changes at 470 nm during the reaction of probe **1** with different concentration of cysteine (1-5 μM).

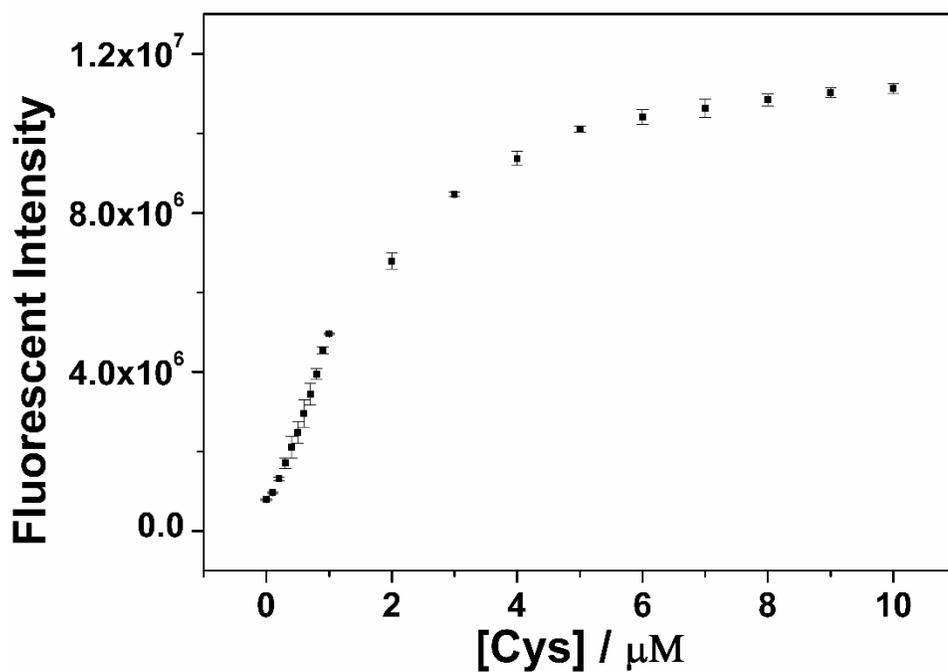


Figure S3. Fluorescence intensity changes at 440 nm during the reaction of probe 2 with different concentration of cysteine (0-10 μM).

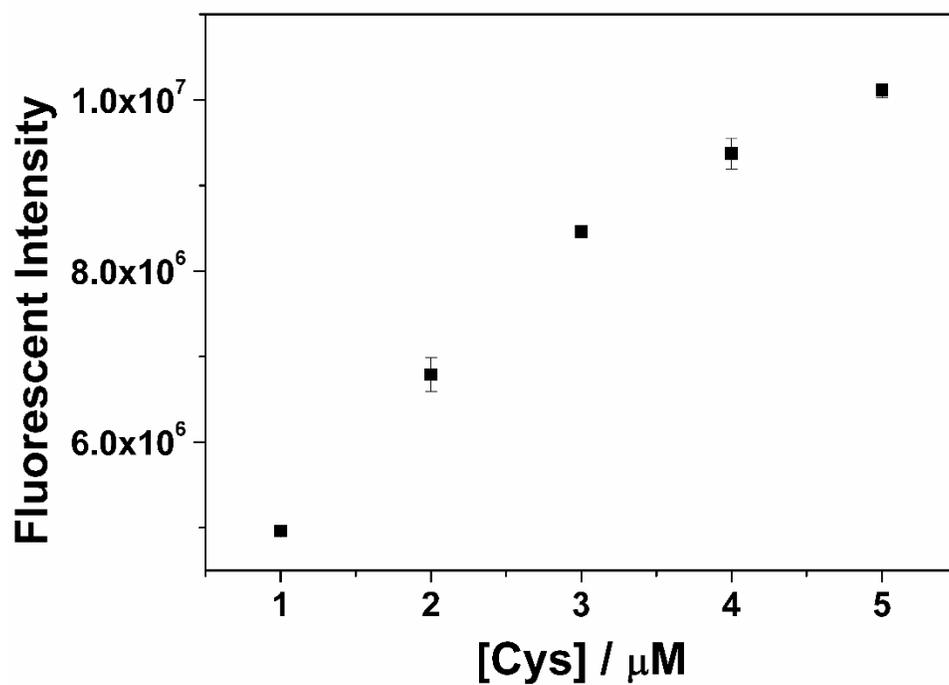


Figure S4. Fluorescence intensity changes at 440 nm during the reaction of probe 2 with different concentration of cysteine (1-5 μM).

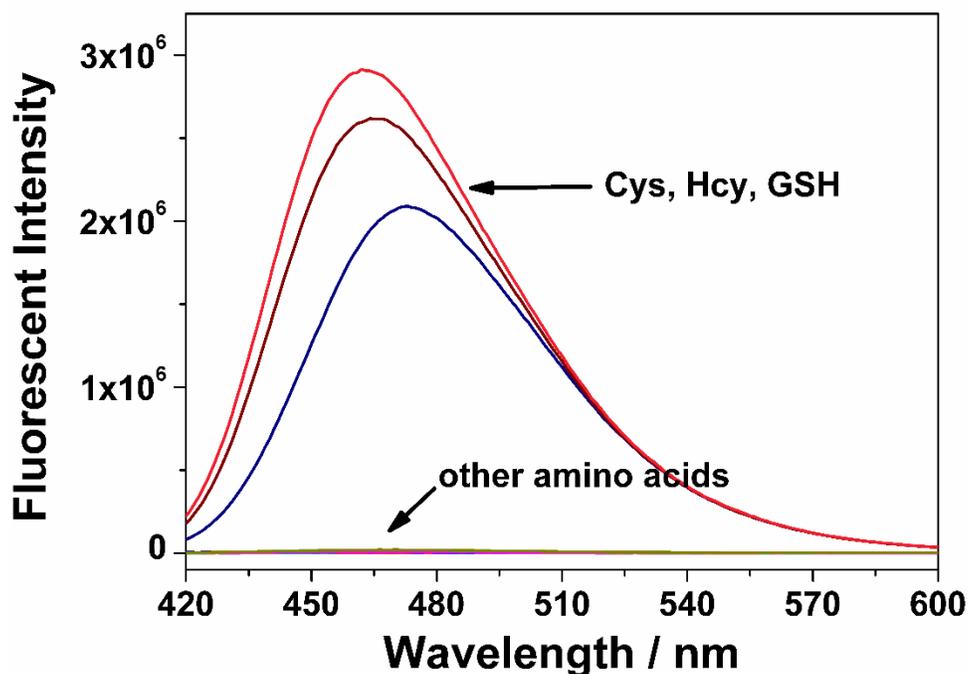


Figure S5. Selectivity experiments of 1 μM probe **1** assayed with 19 natural amino acids (1 mM), Cys (10 μM), GSH (10 μM) and Hcy (10 μM) in PBS buffer (10 mM, pH=7.4).

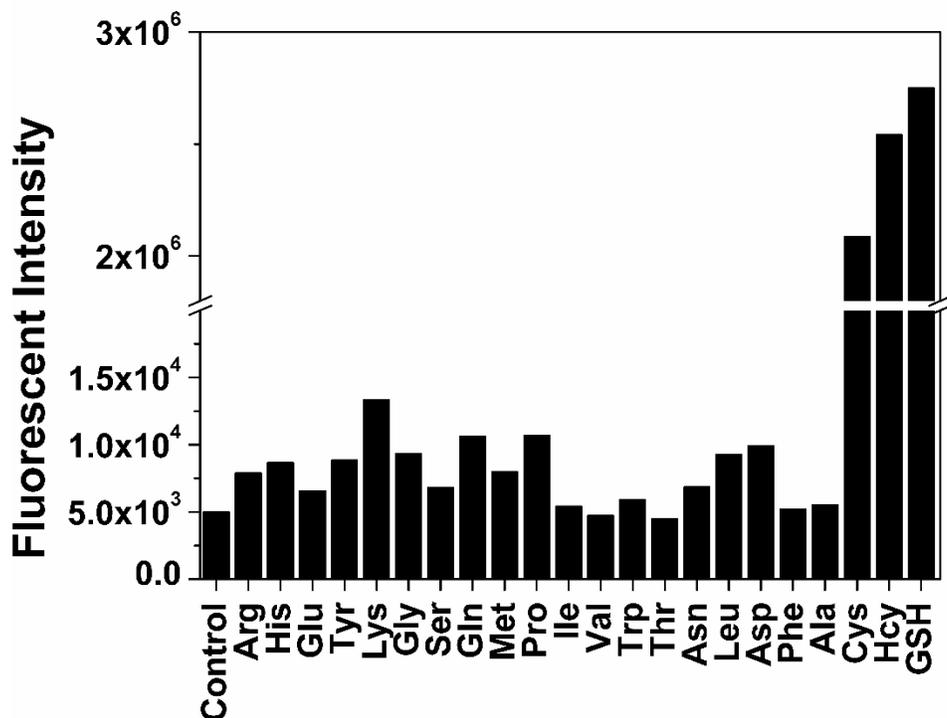


Figure S6. Fluorescence intensity at 470 nm of 1 μM probe **1** assayed with 19 natural amino acids (1 mM), Cys (10 μM), GSH (10 μM) and Hcy (10 μM) in PBS buffer (10 mM, pH=7.4).

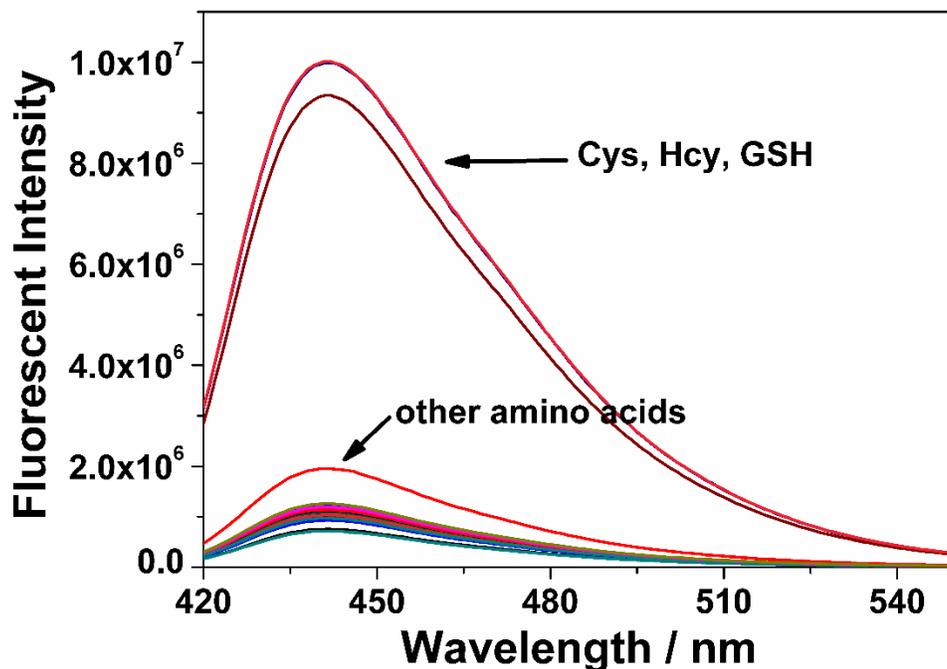


Figure S7. Selectivity experiments of 1 μM probe 2 assayed with 19 natural amino acids (1 mM), Cys (10 μM), GSH (10 μM) and Hcy (10 μM) in PBS buffer (10 mM, pH =7.4).

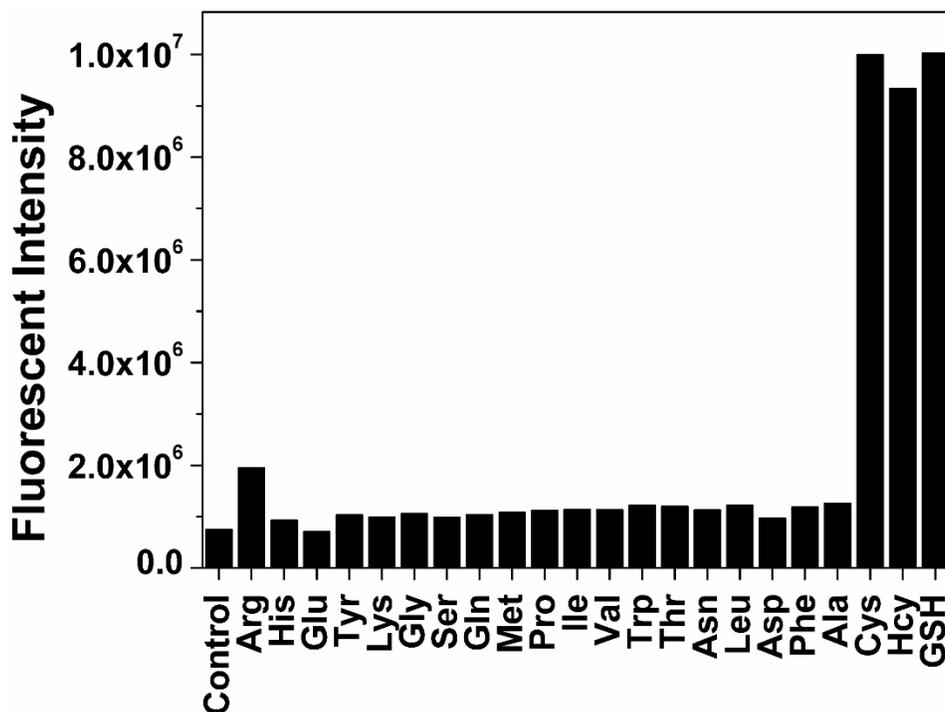


Figure S8. Fluorescence intensity at 440 nm of 1 μM probe 2 assayed with 19 natural amino acids (1 mM), Cys (10 μM), GSH (10 μM) and Hcy (10 μM) in PBS buffer (10 mM, pH =7.4).

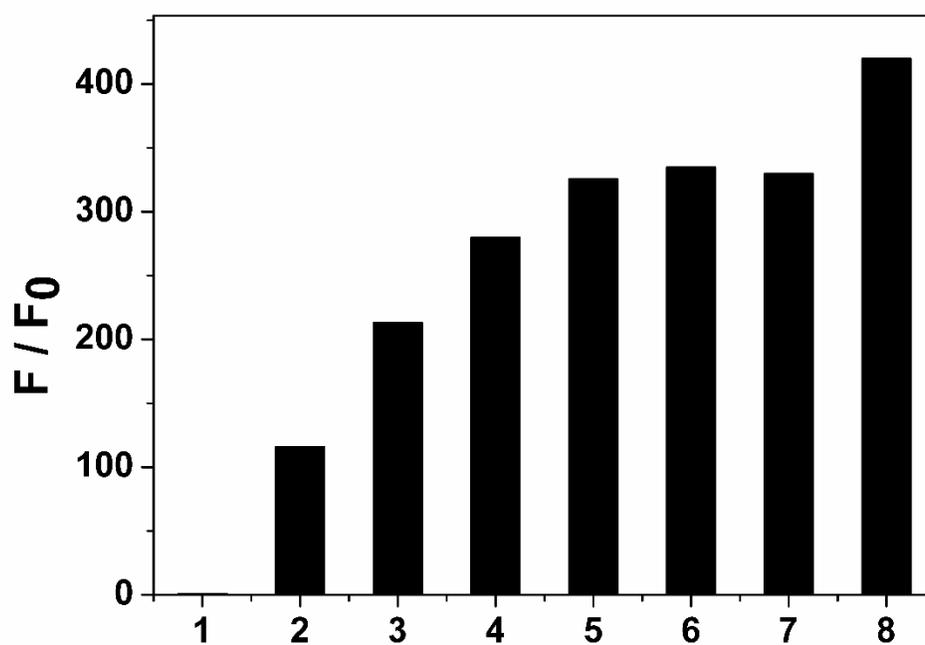


Figure S9. Relative fluorescence intensity of 1 μ M probe **1** assayed with different concentrations of H₂S and Cys (Em :470 nm). 1, control; 2, 10 μ M H₂S; 3, 50 μ M H₂S; 4, 100 μ M H₂S; 5, 200 μ M H₂S; 6, 500 μ M H₂S; 7, 1mM H₂S; 8, 10 μ M Cys.

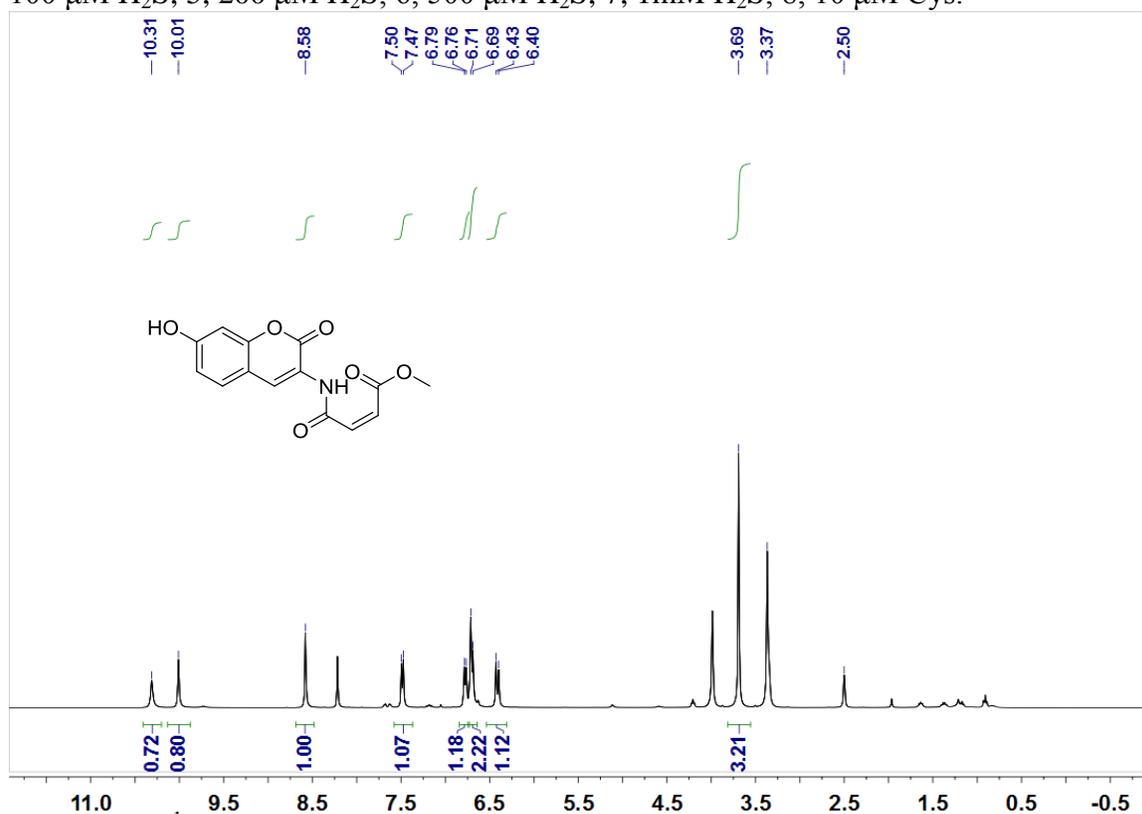


Figure S10. ¹H NMR spectrum of **6**.

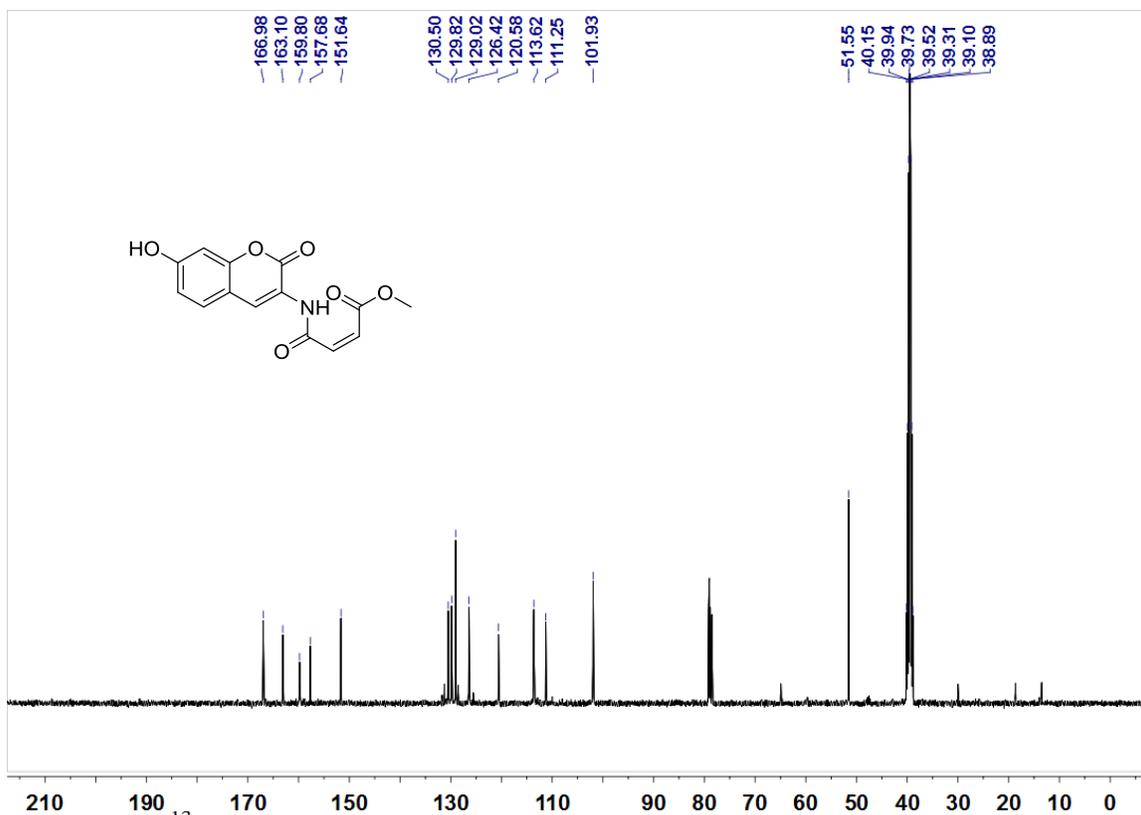


Figure S11. ^{13}C NMR spectrum of 6.

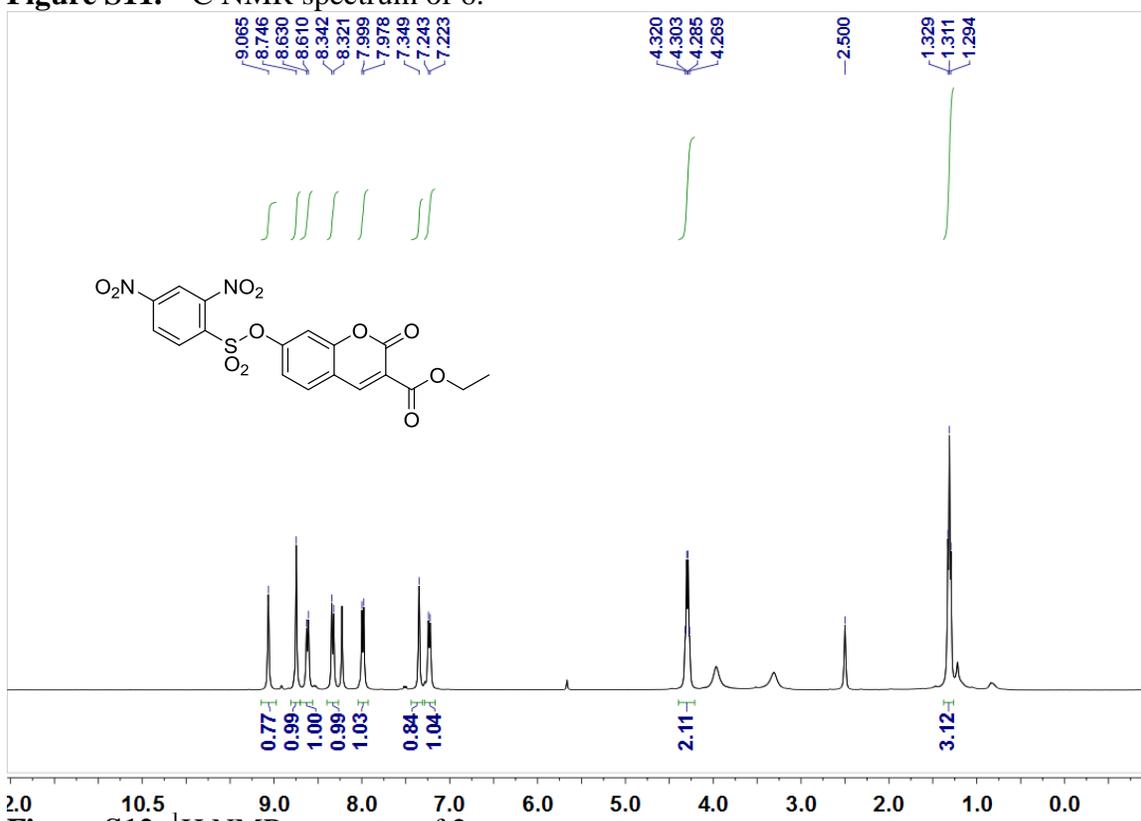


Figure S12. ^1H NMR spectrum of 2.

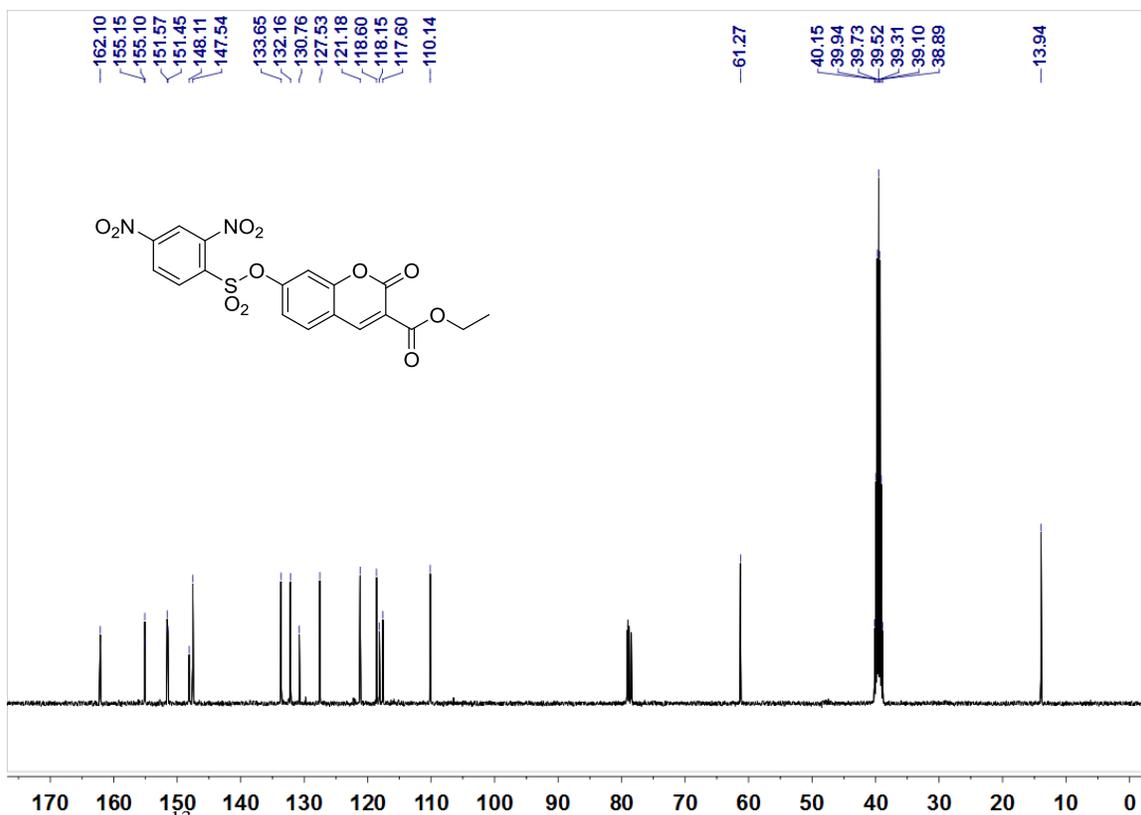


Figure S13. ^{13}C NMR spectrum of 2.

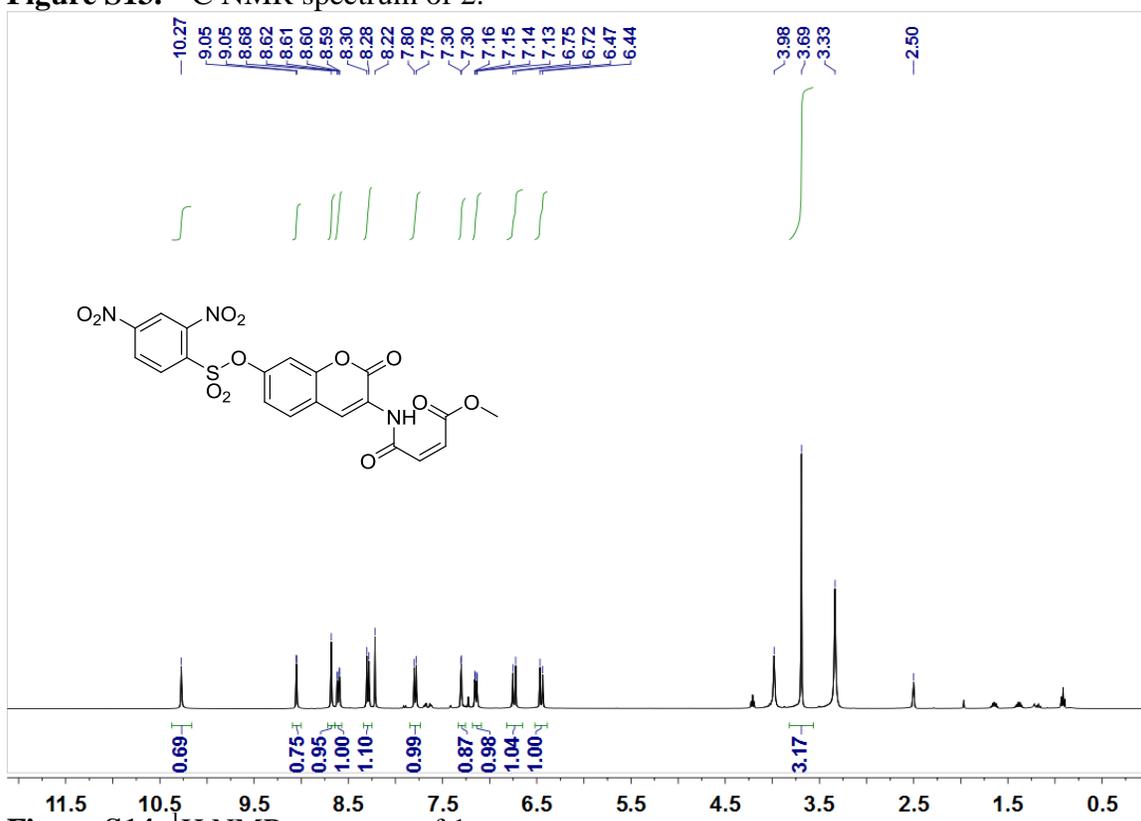


Figure S14. ^1H NMR spectrum of 1.

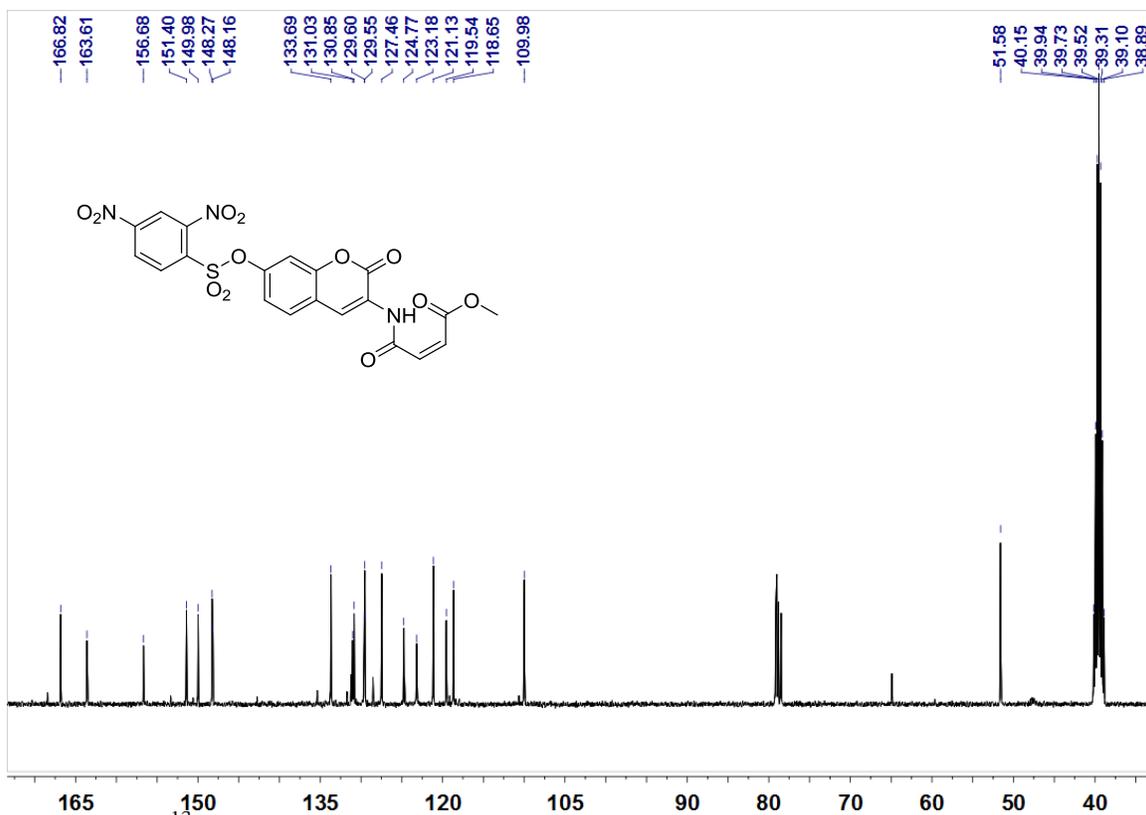


Figure S15. ¹³C NMR spectrum of 1.

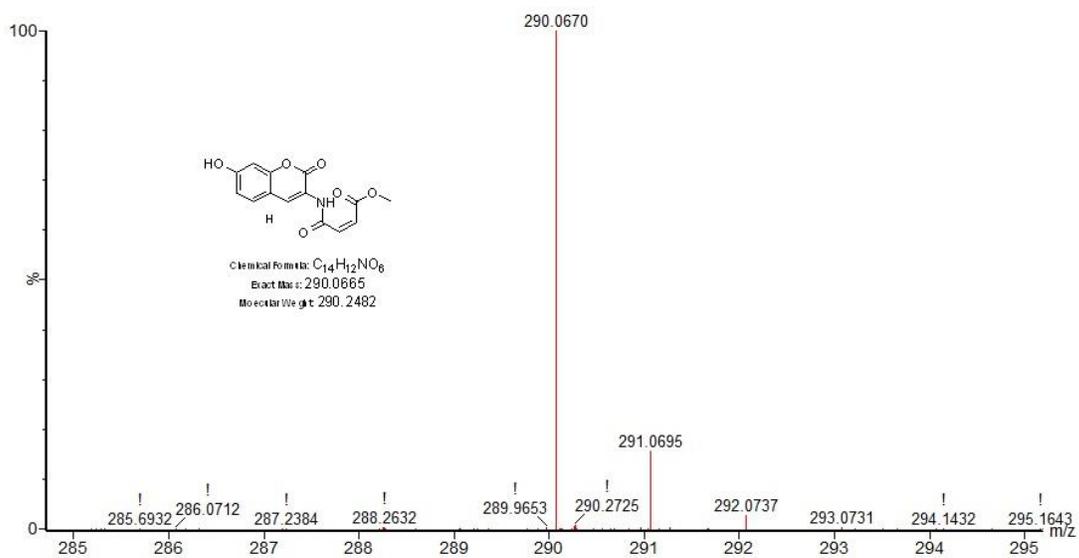


Figure S16. HR-MS spectrum of 6.

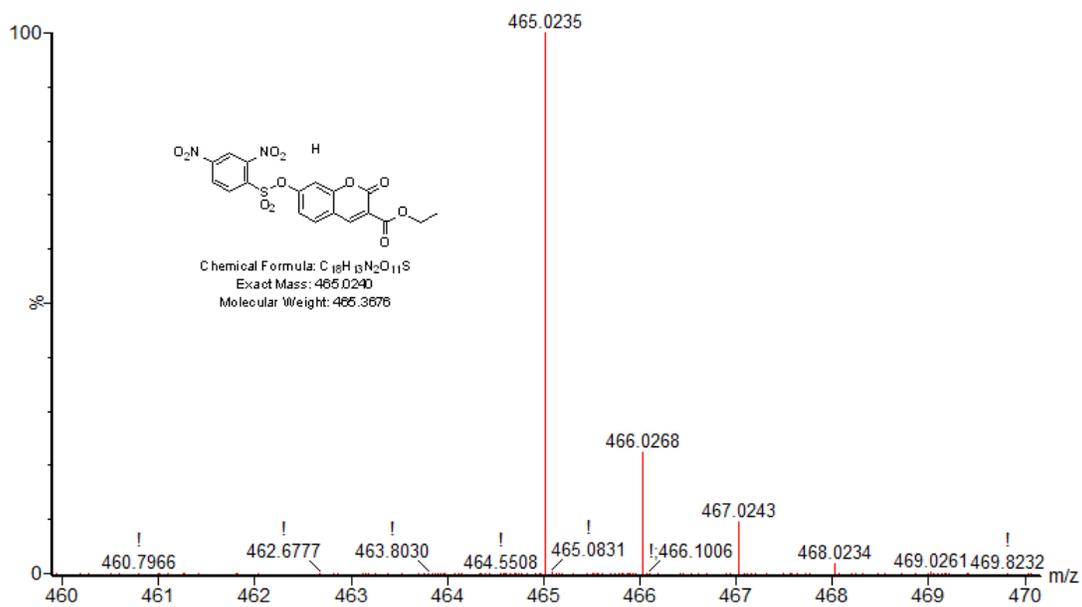


Figure S17. HR-MS spectrum of 2.

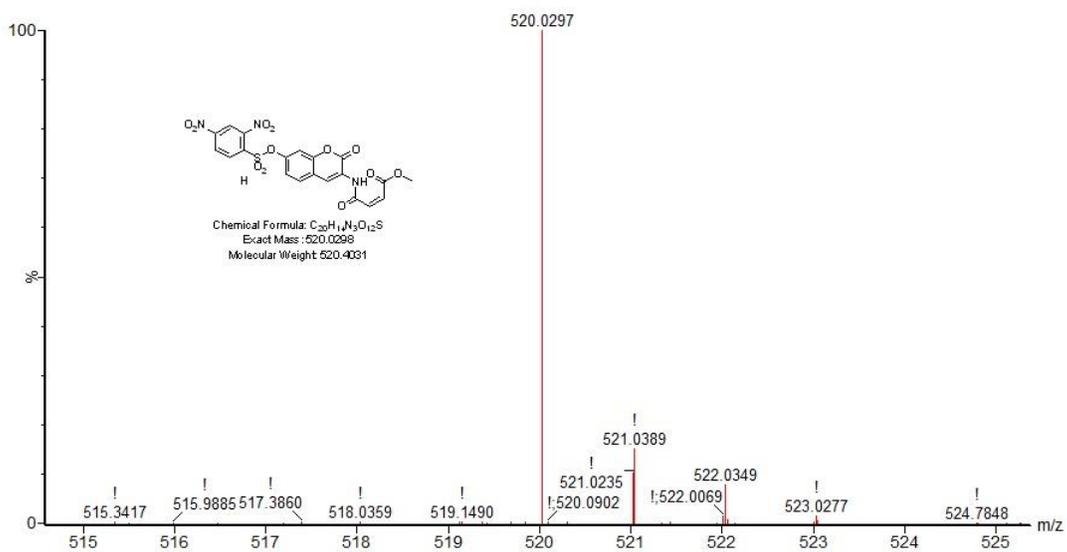


Figure S18. HR-MS spectrum of 1.

3. References

[1] Yi, L.; Li, H.; Sun, L.; Liu, L.; Zhang, C.; Xi, Z. *Angew. Chem., Int. Ed.*, **2009**, *48*, 4034.