Supporting Information

Dual-quenching NBD-based fluorescent probes for separate detection of H_2S and Cys/Hcy in living cells

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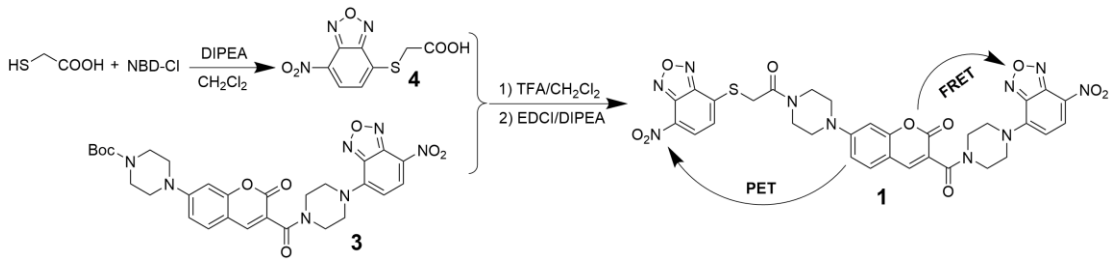


Fig. S1 Synthetic route of probe **1**.

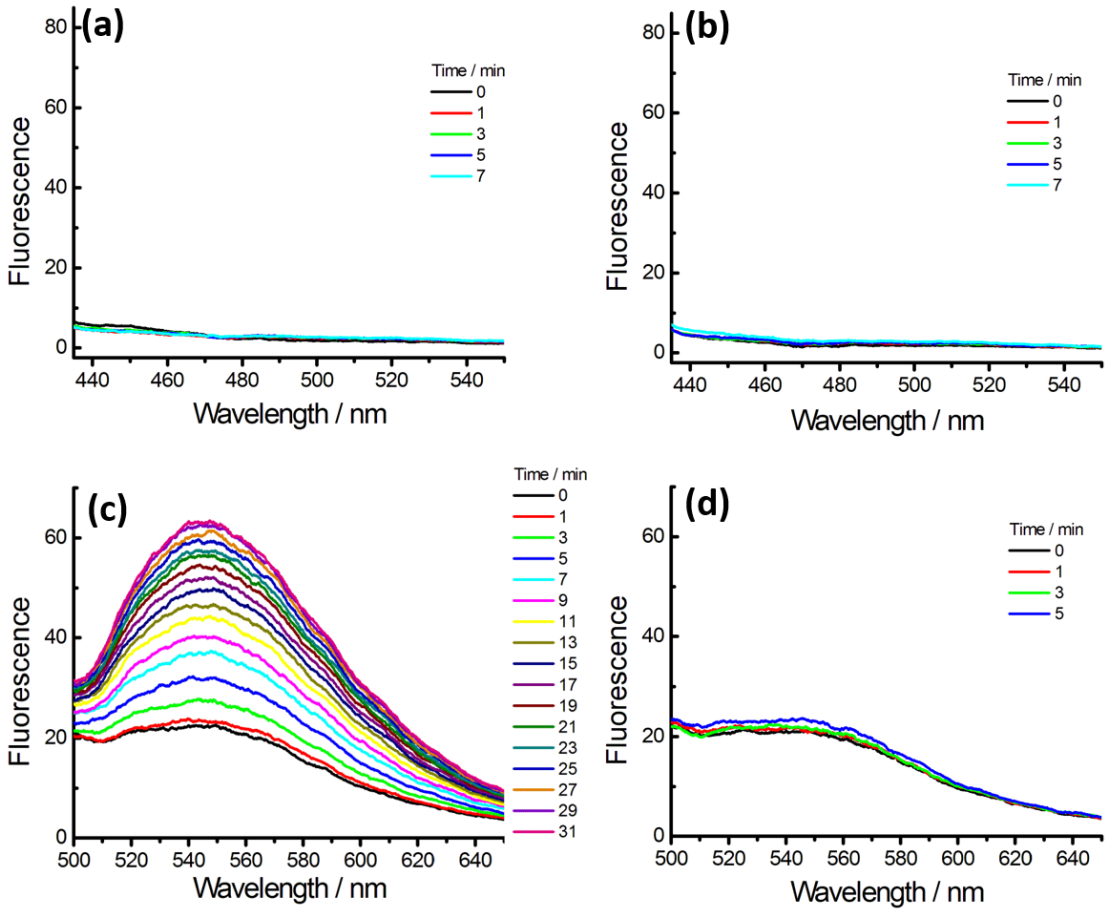


Fig. S2 Time-dependent fluorescence spectra of probe **1** (1 μM) toward Hcy (100 μM, a) and GSH (100 μM, b) upon the excitation of 405 nm. Time-dependent fluorescence spectra of probe **1** (1 μM) toward Hcy (100 μM, c) and GSH (100 μM, d) upon the excitation of 470 nm.

compound	Absorption/nm	ε/$M^{-1}cm^{-1}$	Quantum Yield
2	350, 480	9.76×10^3, 11.11×10^3	0.0015[a], 0.0058[b]
6	390	13.76×10^3	0.21[a]
8	480	23.92×10^3	0.023[b]

[a]Excitation at 390 nm. [b]Excitation at 485 nm.

Table S1 Optical properties of compounds **2**, **6** and **8** in PBS buffer.

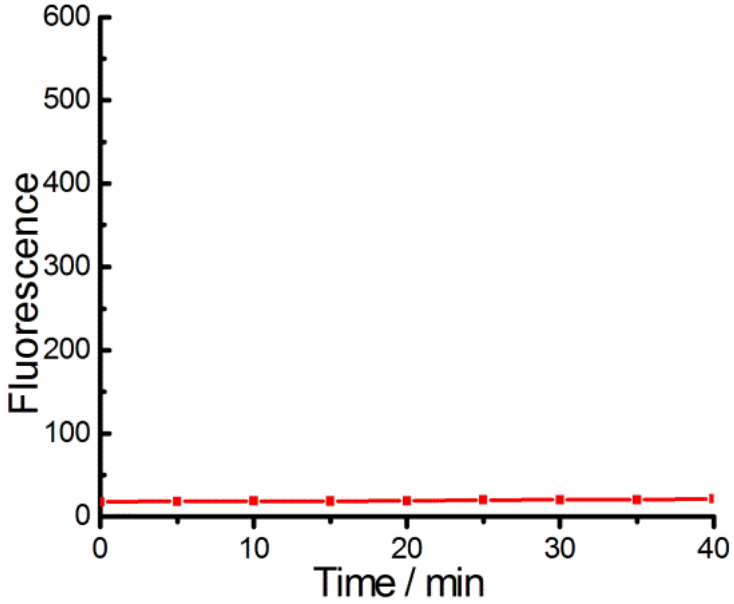


Fig. S3 Emissions of probe **2** (1 μM) at 455 nm within 40 min in PBS buffer.

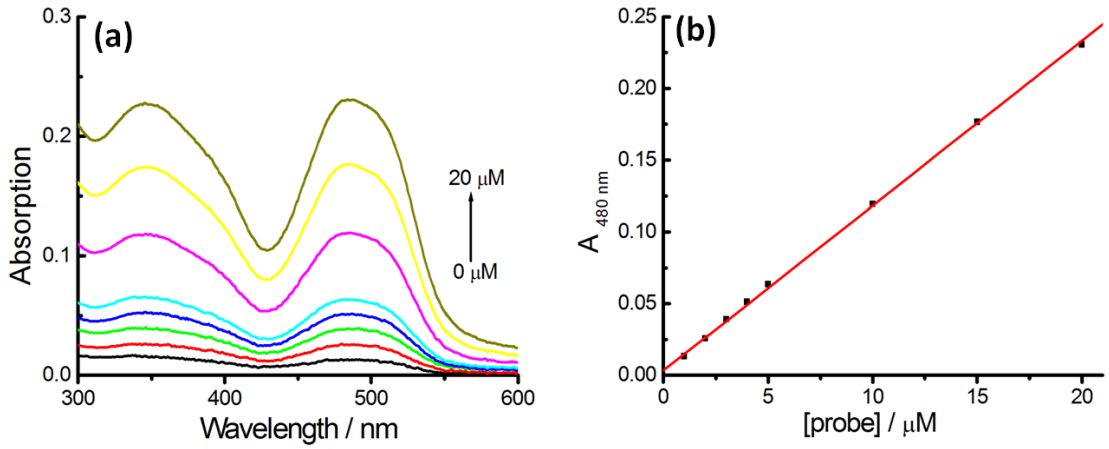


Fig. S4 (a) Absorption profiles of probe **2** at different concentrations in PBS buffer. (b) The linear relationship of absorbance of probe **2** at 480 nm versus probe concentration.

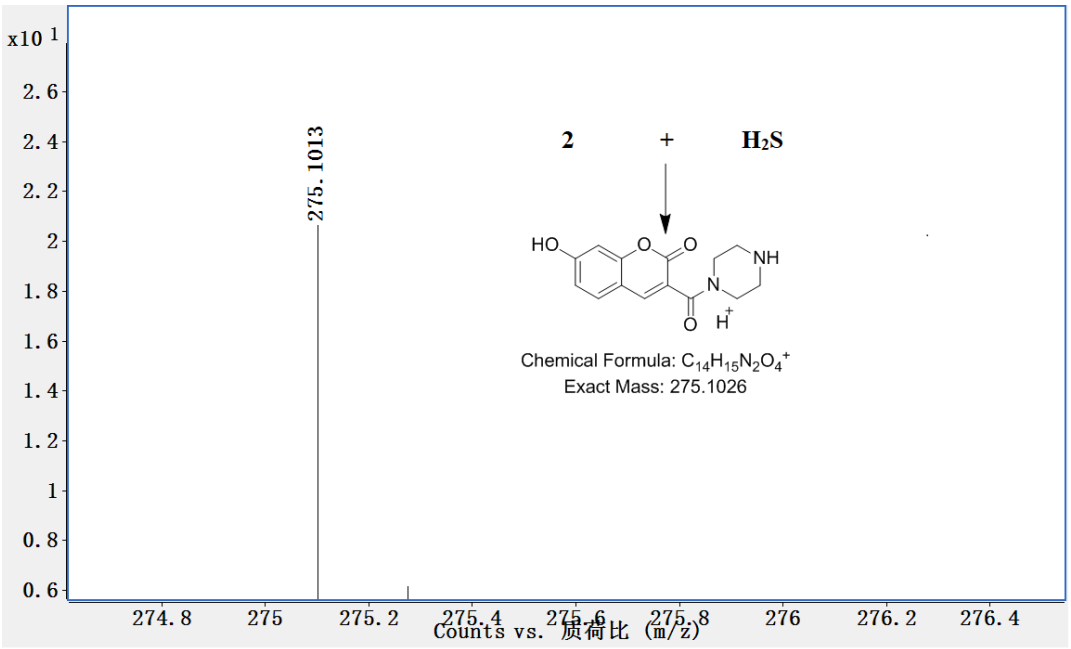


Fig. S5 HRMS spectra of compound **6** from the reaction of **2** with H_2S. **2** (200 μM) was incubated with H_2S (2.5 mM) in PBS buffer (pH=7.4, 50 mM, 30% CH_3CN) at room temperature for 1 h in microcentrifuge tube.

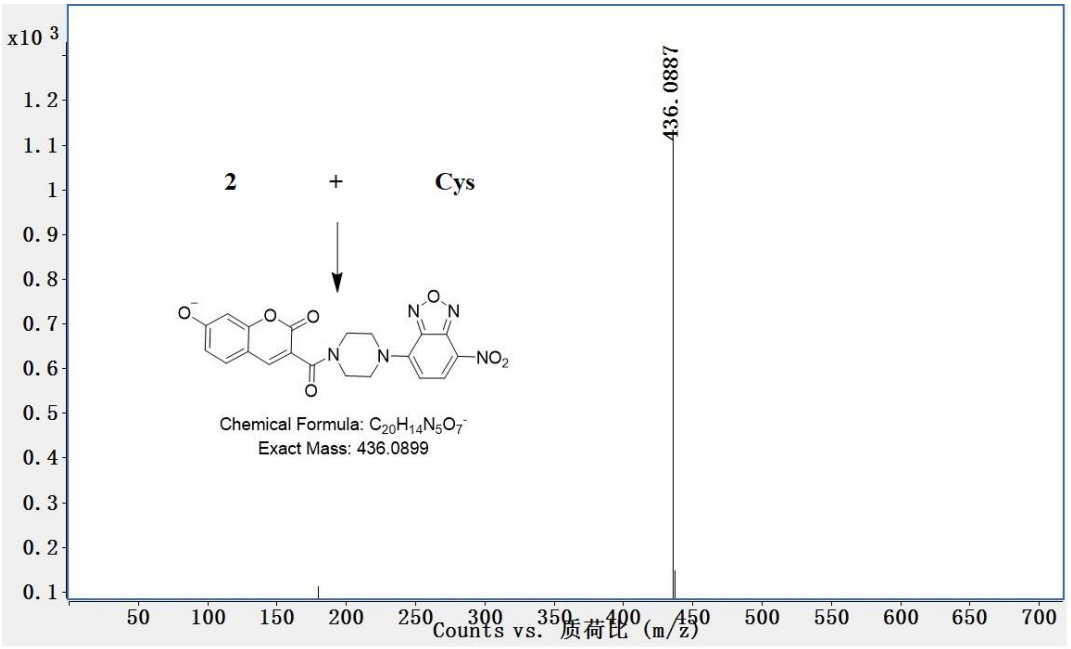


Fig. S6 HRMS spectra of compound **7** from **2** and Cys. **2** (200 μM) was incubated with Cys (2.5 mM) in PBS buffer (pH=7.4, 50 mM, 30% CH_3CN) at room temperature for 1 h in microcentrifuge tube.

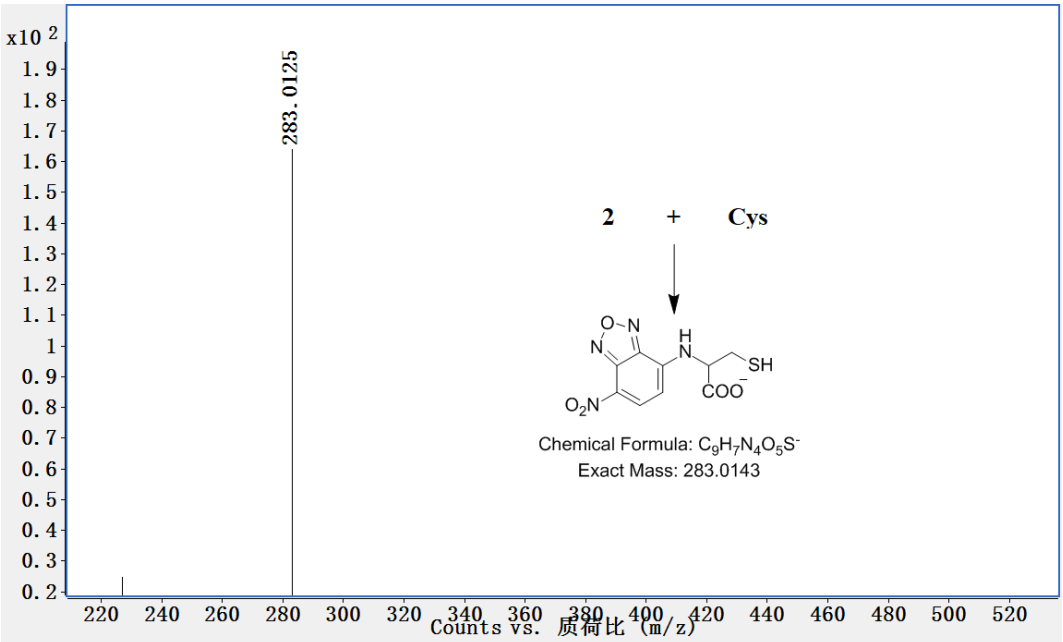


Fig. S7 HRMS spectra of compound **8** from **2** with Cys. Under N_2 protection, to a solution of **2** (10 mg, 0.017 mmol) in 2 mL CH_3CN, 2 mL degassed PBS and Cys (10 mg) were added one by one. This mixture was stirred for 4 h at room temperature and then characterized.

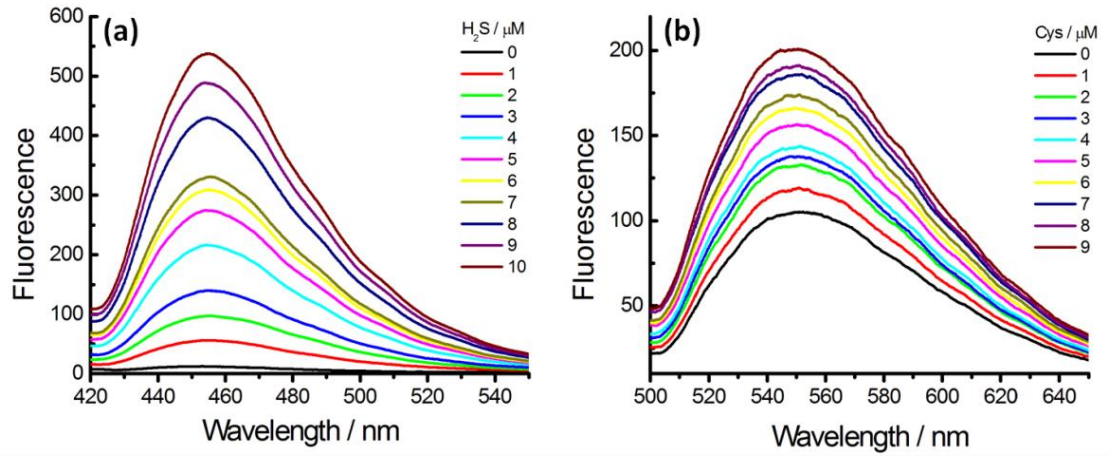


Fig. S8 (a) Fluorescence spectra of probe **2** (1 μM) toward different concentrations of H_2S (0-10 μM) upon excitation of 405 nm. (b) Fluorescence spectra of probe **2** (1 μM) toward different concentrations of Cys (0-9 μM) upon excitation of 470 nm.

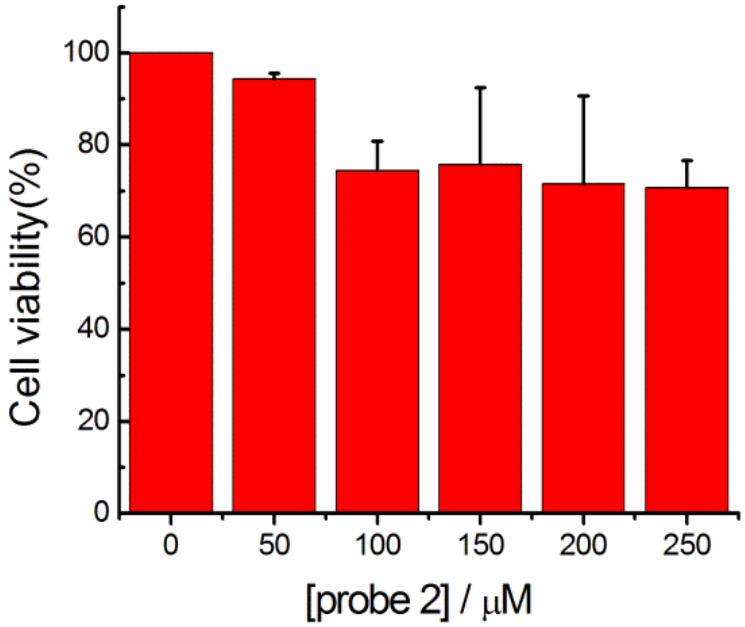


Fig. S9 Cytotoxicity assessment of probe **2**.

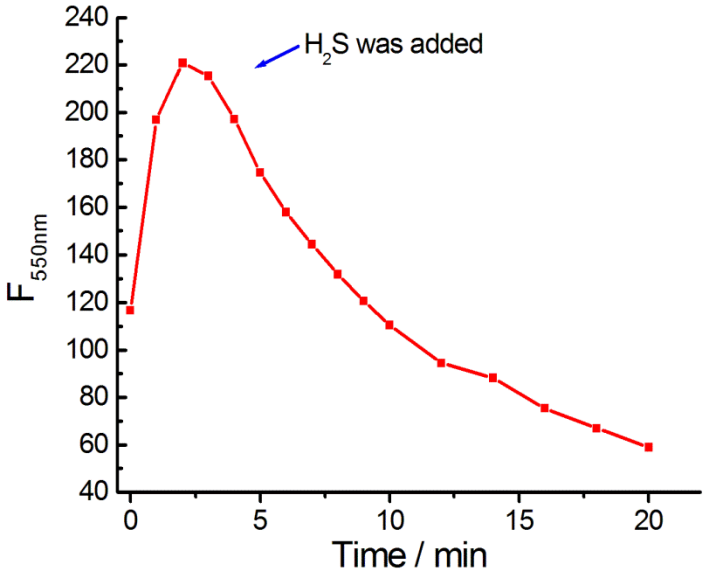


Fig. S10 Emission of probe **2** in the presence of Cys and H_2S. **2** (1 μM) was incubated with H_2S (100 μM) for 4 min, then Cys (100 μM) was added. Excitation: 470 nm.

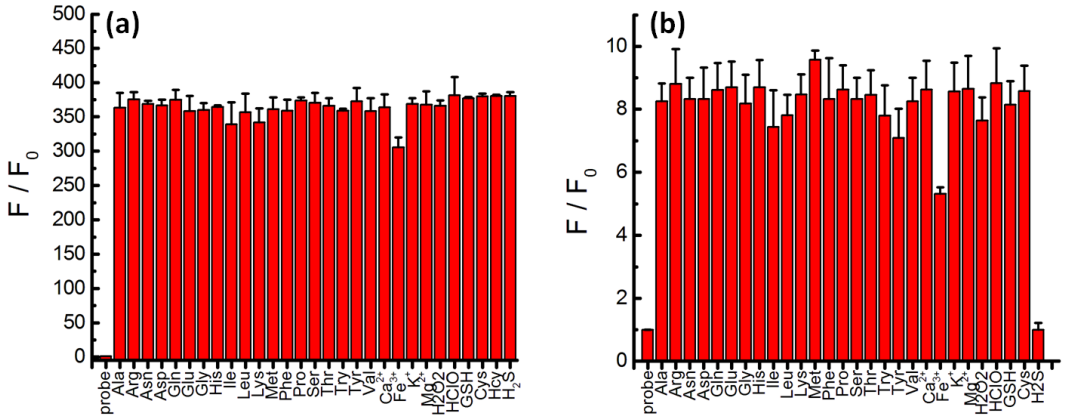


Fig. S11 Relative fluorescence of **2** (1 μM) at (a) 455 nm toward different types of amino acids and biothiols in the presence of H_2S; (b) 550 nm toward different types of amino acids and biothiols in the presence of Cys in PBS buffer. Excitation, (a) 405 nm; (b) 470 nm. All reactions were incubated for 30 min. Biothiols were all 100 μM, and all other species were 1 mM.

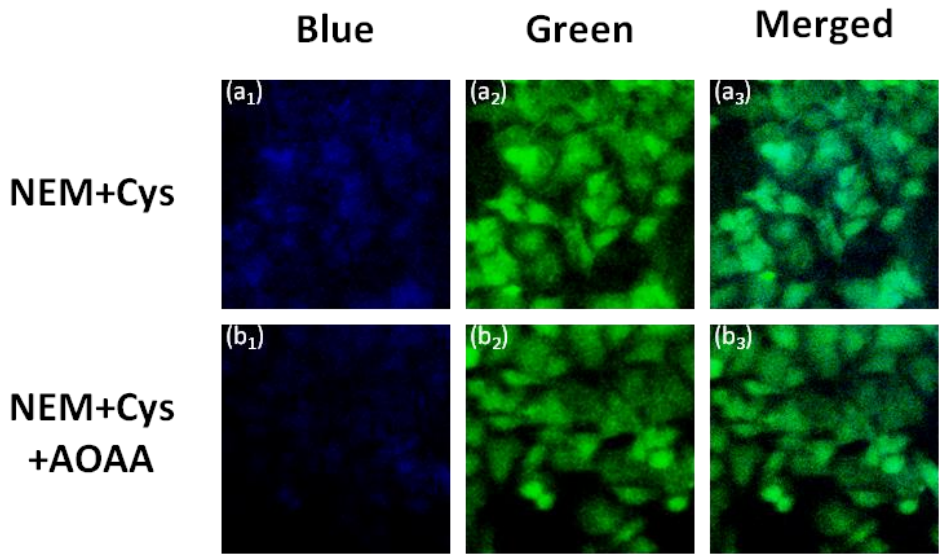
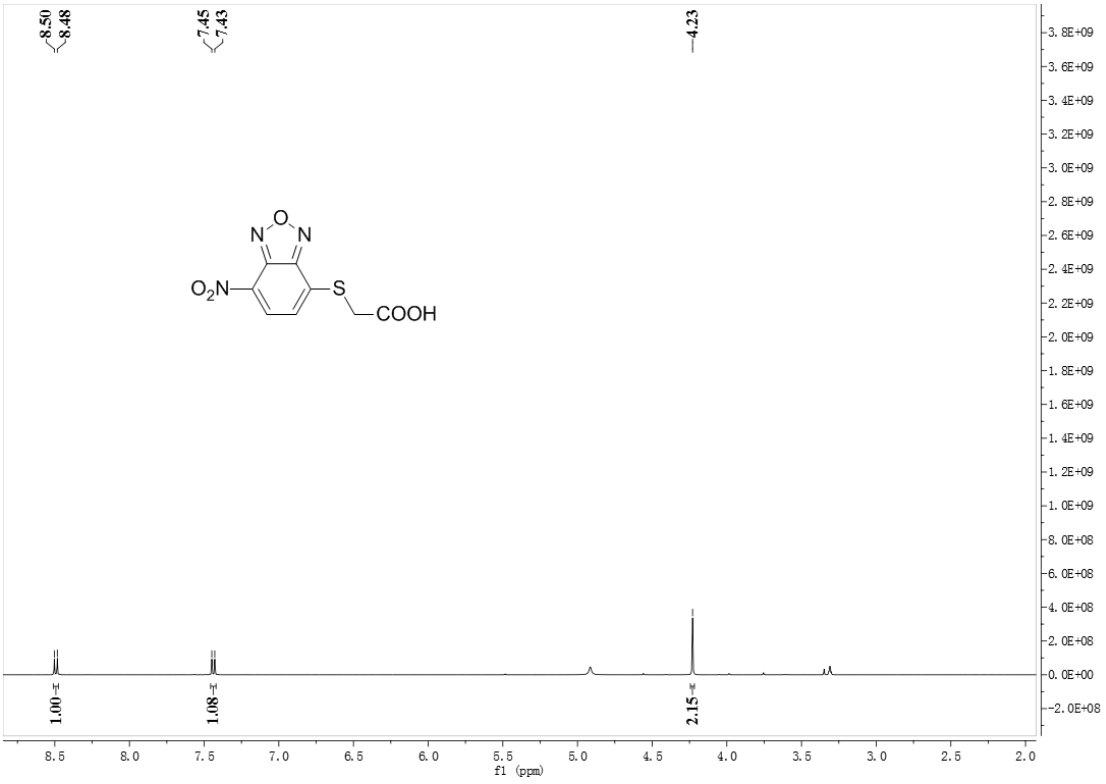
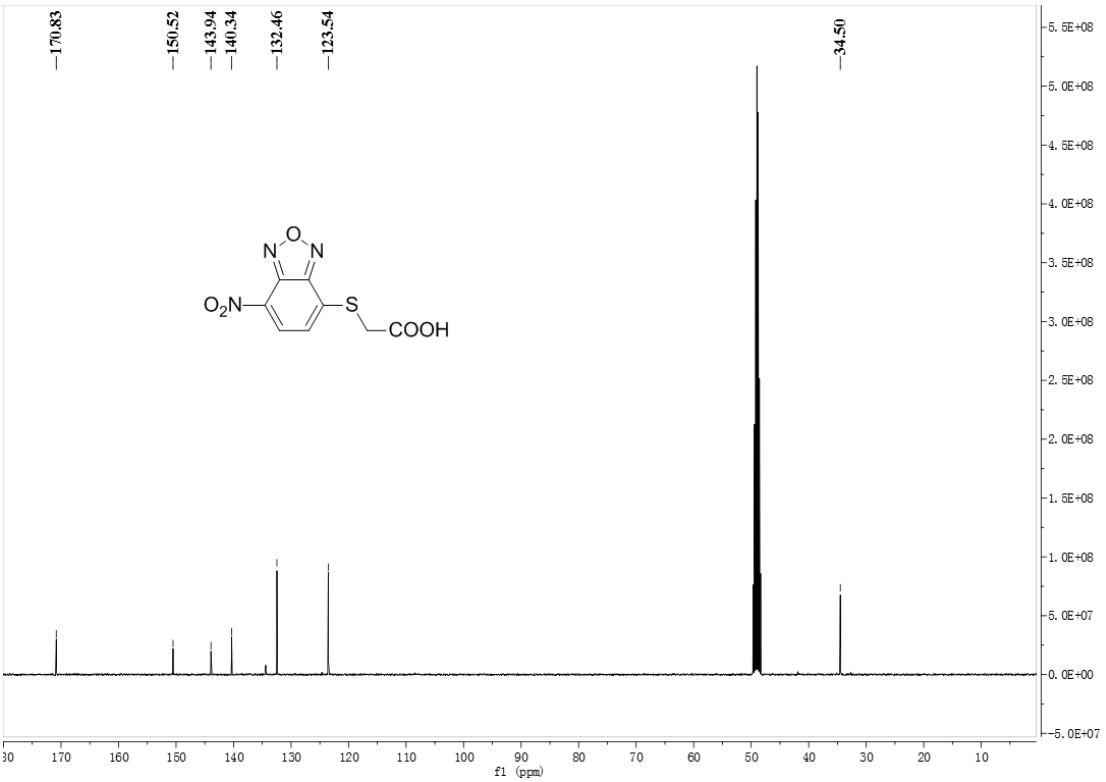


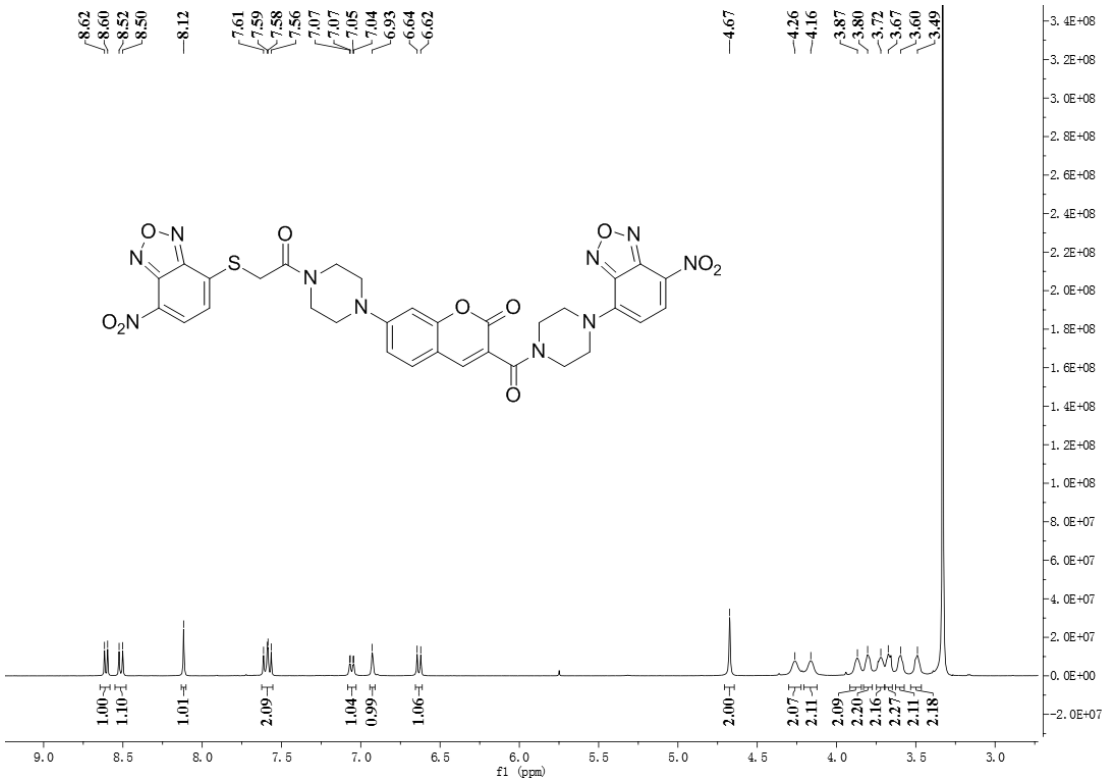
Fig. S12 Fluorescence images of probe **2** for detection of Cys-induced H_2S in living cells. HeLa cells were incubated with (a) NEM, Cys, and probe **2** one by one; (b) NEM, AOAA (400 μM), Cys, and probe **2** one by one. Probe **2** was 5 μM; NEM was 1 mM; Cys was 500 μM. The incubation time was 30 min, respectively. Emissions were collected at the green channel (500-550 nm) with 488 nm excitation and the blue channel (440-490 nm) with 405 nm excitation, respectively.



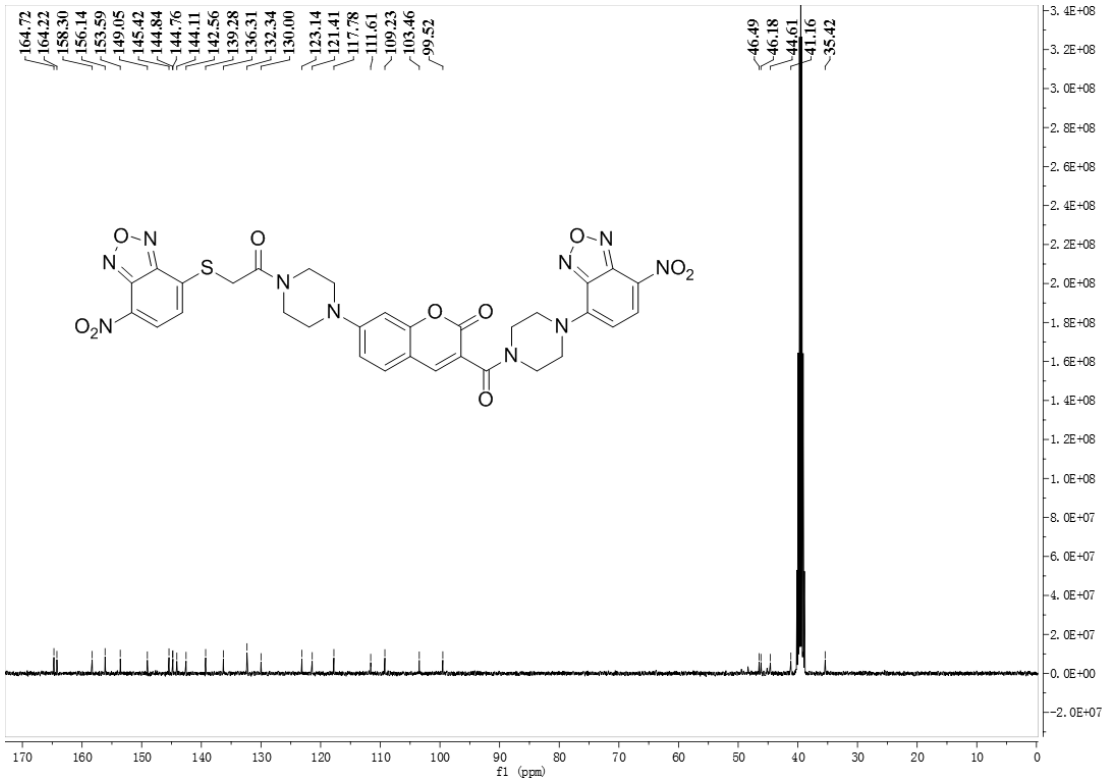
^{1}H NMR spectrum of compound **4**.



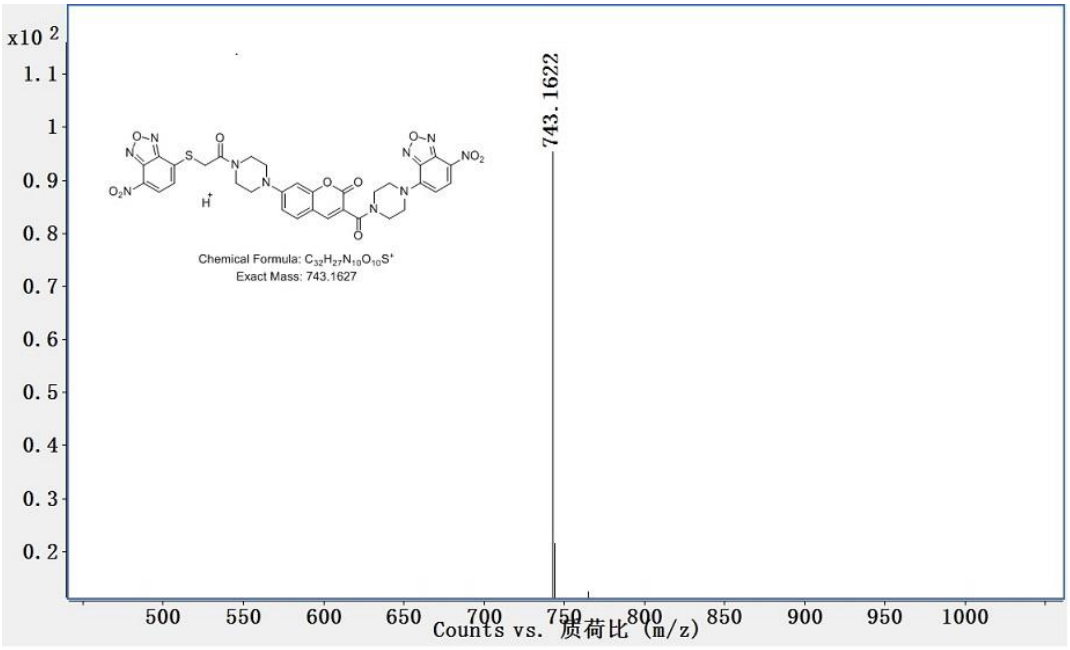
^{13}C NMR spectrum of compound **4**.



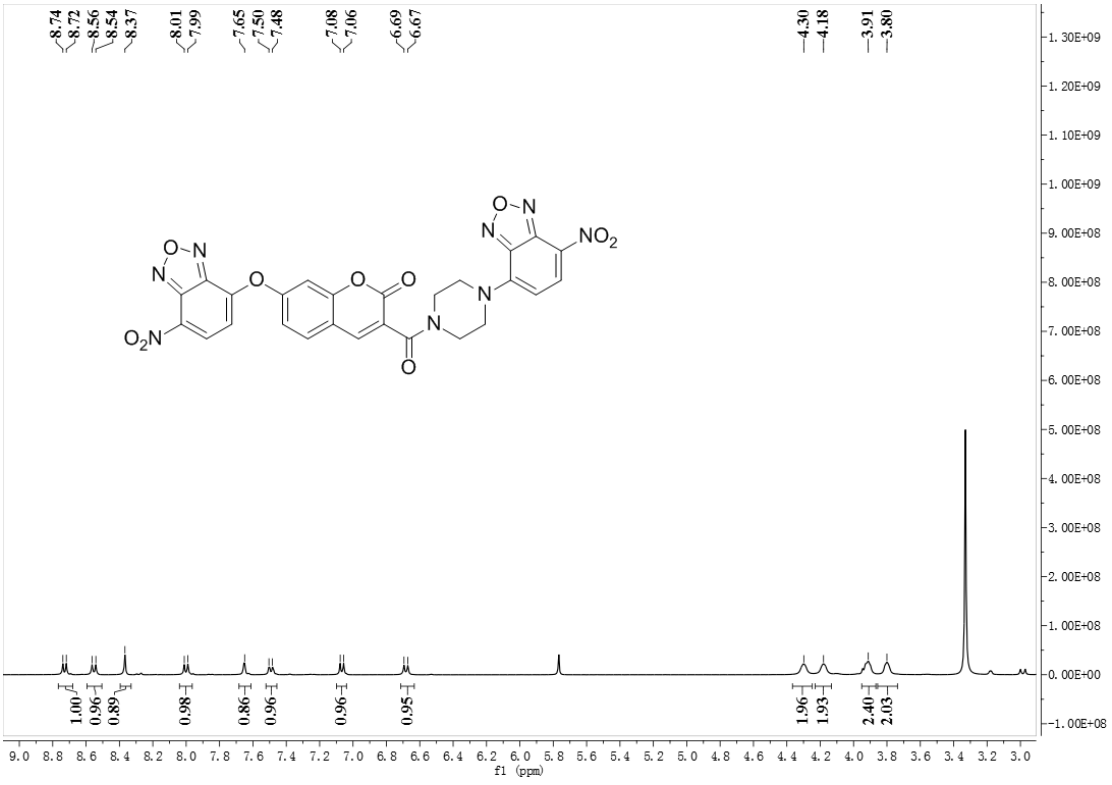
^{1}H NMR spectrum of probe **1**.



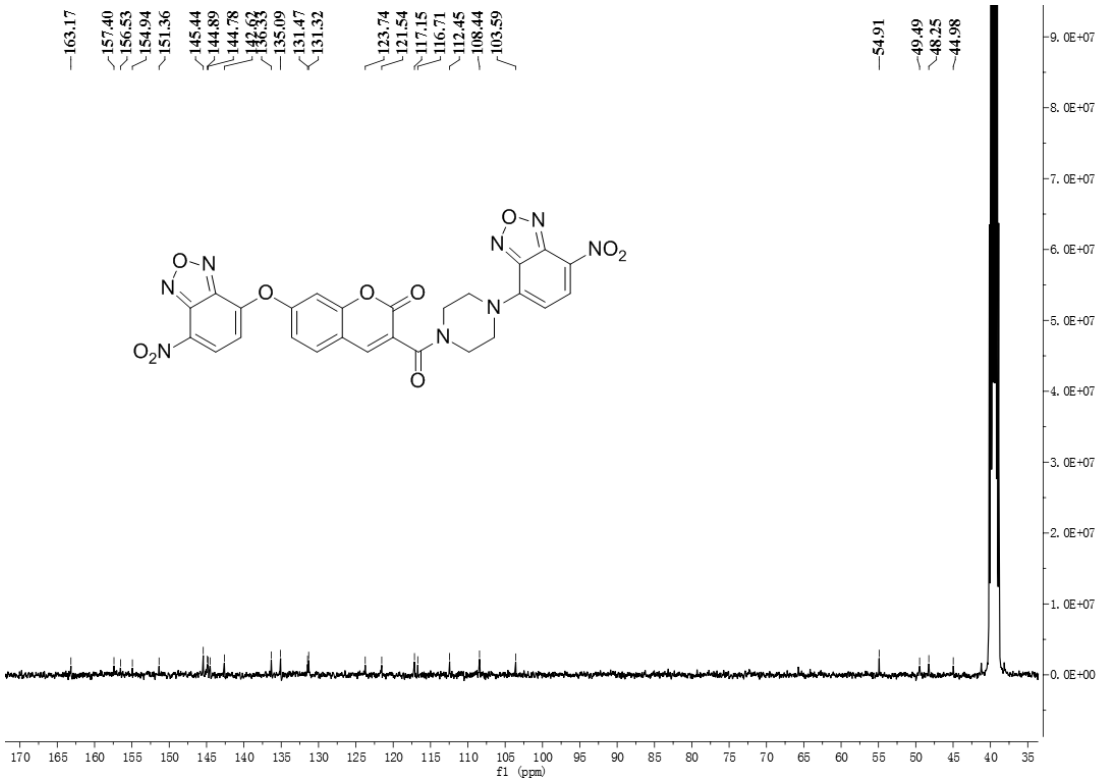
^{13}C NMR spectrum of probe **1**.



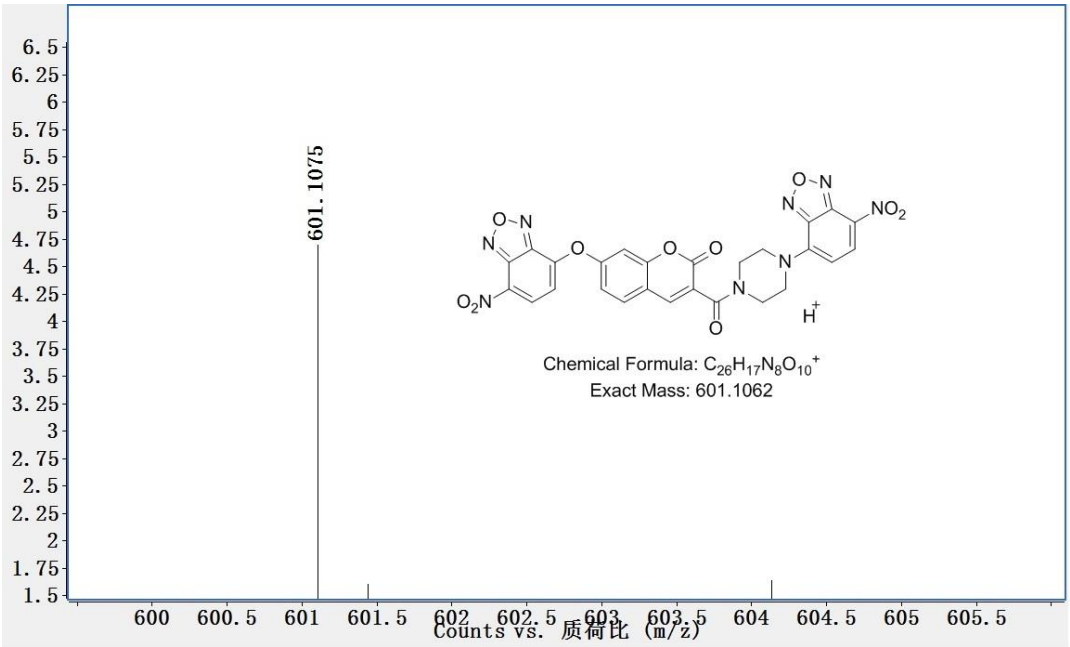
HRMS spectrum of probe **1**.



^{1}H NMR spectrum of probe **2**.



^{13}C NMR spectrum of probe **2**.



HRMS spectrum of probe **2**.