Electronic Supplementary Information (ESI) for

Coumarin-based fluorescent probe with 4-phenylselenium as the active site for multi-channel discrimination of biothiols

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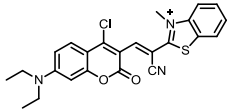
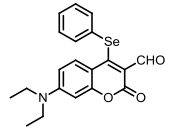
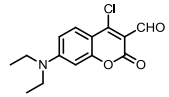
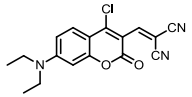
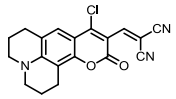
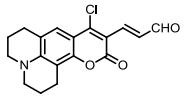
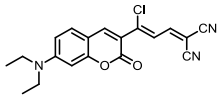
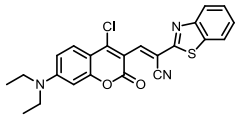
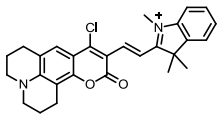
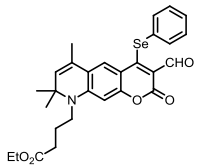
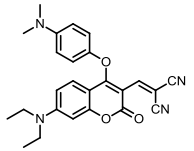
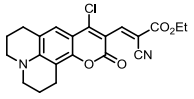
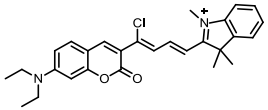
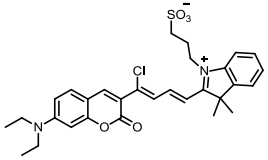
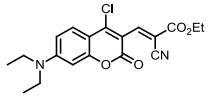
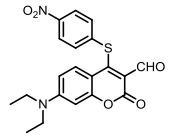
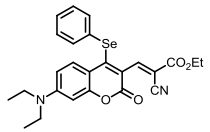
Table S1. Summary of coumarin-based fluorescent probes for the detection of biothiols.

Fluorescent probes	Test condition	Distinguish detection	Emission wavelength /nm	Response time /min	LOD /nM	References
	PBS buffer (pH 7.4, 10 mM, 1 mM CTAB)	Cys GSH	420 512	60 60	/ 50	*J. Am. Chem. Soc.*, 2014, **136**, 574-577
	PBS buffer (pH 7.4, 10 mM, 25% DMSO)	GSH	550	/	270	*Chem. Sci.*, 2015, **6**, 5435–5439
	PBS buffer (pH 7.4,)	Cys Hcy GSH	470 470 546	34 60 40	180 90 80	*Anal. Chim. Acta.*, 2015, **900**, 103-110
	PBS buffer (pH 7.4, 10 mM, 30% DMF)	H_2S GSH	564 517	6 6	42 87	*Chem. Commun.*, 2016, **52**, 4628-4631
	PBS buffer (pH 7.4, 10 mM, 30% CH_3CN)	Cys GSH	498 560	40	190 430	*Biosens. Bioelectron.*, 2017, **90**, 117
	PBS buffer (pH 7.4, 10 mM, 20% DMSO)	Cys Hcy	480 542	60 60	1990 620	*Chem. Asian. J.*, 2017, **12**, 2098-2103
	PBS buffer (pH 7.4, 10 mM, 50% CH_3CN)	H_2S GSH	490 505	30 7	75 280	*Analyst.*, 2018, **143**, 440-448
	PBS buffer (10 mM, pH 7.4, 60% DMSO)	Cys Hcy GSH	457 559 529	4 15 5	0.5 3.6 6.9	*Angew. Chem. Int. Ed.*, 2018, **57**, 4991-4994

	PBS buffer (pH 7.4, 10 mM, 1% DMSO)	Cys Hcy GSH	475 475 575	25 55 50	14 81 97	*New. J. Chem*., 2018, **42**, 12615-12620
	HEPES (pH 7.4, 10 mM, 10% DMSO)	Cys Hcy GSH	510 510 590	15 / 30	17.0 / 1270	*Anal. Chem*., 2018, **90**, 2648-2654
	PBS buffer (pH 7.4, 10 mM, 20% DMSO)	Biothiols H_2S H_2S_2	469 508 576	10 60 15	Cys: 210/GSH: 170 420 60	*Chem. Commun*., 2019, **55**, 8130-8133
	PBS buffer (10 mM, pH 7.4, 40% DMSO)	Cys Hcy GSH	503 467 568	15 15 15	0.2 0.7 1.0	*Angew. Chem. Int. Ed*., 2019, **58**, 4557-4561
	PBS buffer (pH 7.4, 10 mM, 20% CH_3OH)	Cys Hcy GSH	489/564/600 489/564/600 489/564/600	60 90 80	2965 6140 6847	*Anal. Chem*., 2019, **91**, 1472-1478
	PBS buffer (pH 7.4, 10 mM)	Cys Hcy GSH	498/573/612 498/573/612 498/573/612	60 90 70	2200 2080 1890	*J. Mater. Chem. B*., 2019, **7**, 7723-7728
	PBS buffer (10 mM, pH 7.4, 30% DMSO)	Cys Hcy GSH	462 449 547	15	30 60 200	*Talanta*., 2020, **219**, 121353
	PBS buffer (pH 7.4, 10 mM, 5% EtOH)	Cys Hcy GSH	495 495 565	25 25 20	106 82 57	*Anal. Chem*., 2021, **93**, 2244-2253

	PBS buffer (10 mM, pH 7.4, 40% DMSO)	Cys Hcy GSH	465 542 533	2 15 2	3.0 39.5 9.7	This work

“/” No available data.

2. Time-dependent UV/Vis absorption spectral response of probe toward biothiols

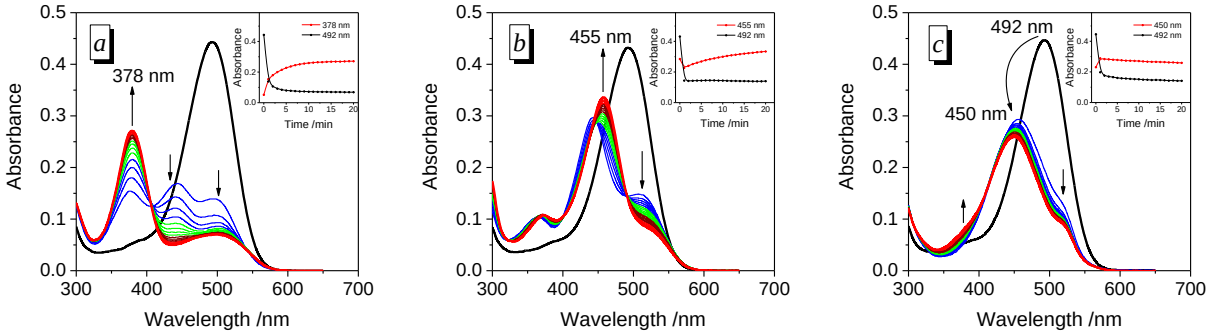


Fig. S1 Time-dependent UV/Vis absorption spectra of 10 μM CouSePh upon addition of (a) 10 μM Cys, (b) 10 μM Hcy or (c) 10 μM GSH in PBS buffer (10 mM, pH 7.4, 40% DMSO). Inset: time-dependent absorbance varies at 378/492 nm from (a) Cys, 455/492 nm from (b) Hcy or 450/492 nm from (c) GSH.

3. Time-dependent fluorescence spectral response of probe toward Hcy upon excitation at 455 nm

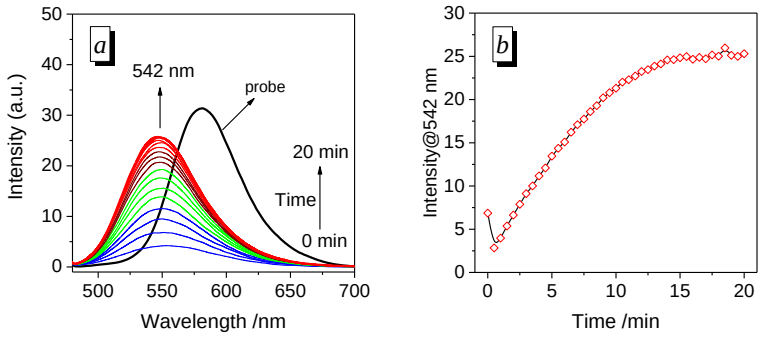


Fig. S2 Time-dependent fluorescence spectra of 10 μM probe CouSePh upon addition of 10 μM Hcy in PBS buffer (10 mM, pH 7.4, 40% DMSO), λ_{ex} =455 nm.

4. The concentration dependent UV/Vis absorption spectral response of probe toward biothiols

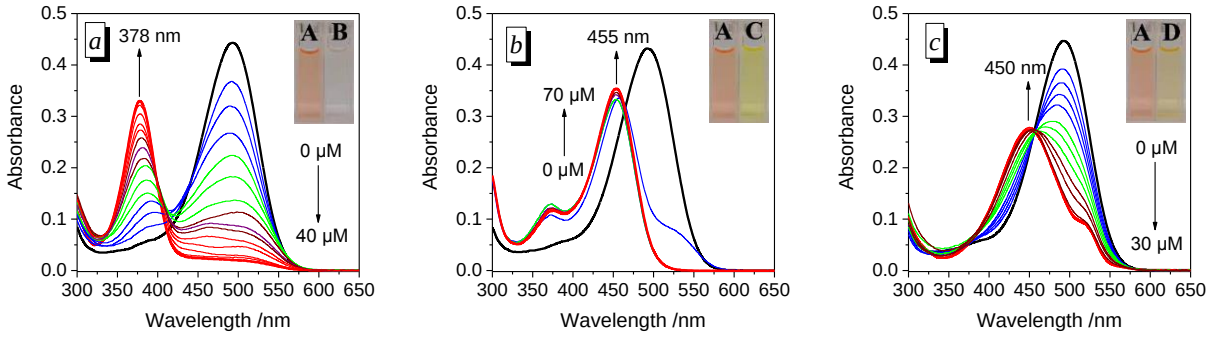


Fig. S3 UV/Vis absorption spectra of 10 μM probe CouSePh upon addition of increasing concentrations of (a) 0-40 μM Cys, (b) 0-70 μM Hcy or (c) 0-30 μM GSH in PBS buffer (10 mM, pH 7.4, 40% DMSO). Insets: photos of 10 μM probe solution before (A) and after (B/C/D) addition of corresponding biothiol under room light.

5. The spectral response of probe toward NAC

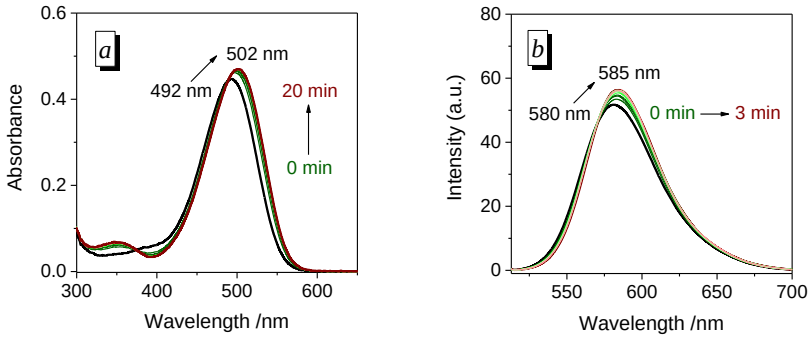
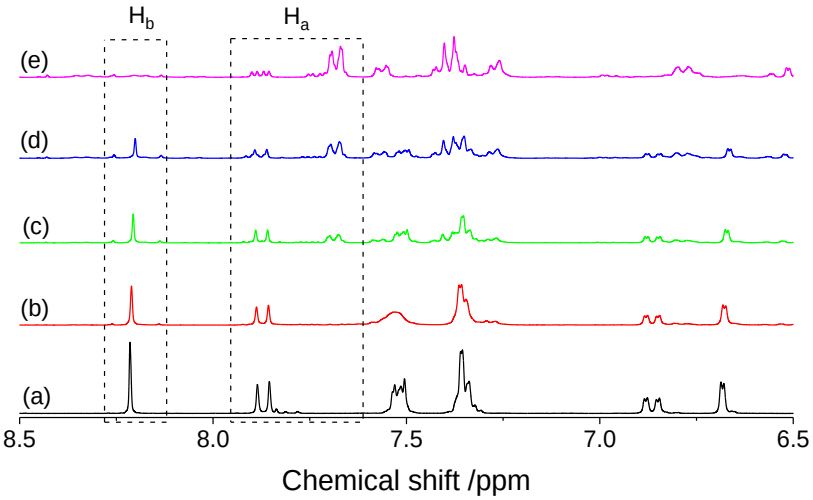


Fig. S4 Time-dependent (a) UV/Vis absorption and (b) fluorescence spectra of 10 μM probe CouSePh upon addition of 10 μM NAC in PBS buffer (10 mM, pH 7.4, 40% DMSO), λ_{ex} =492 nm.

6. The evidence of sensing products for probe with different biothiols



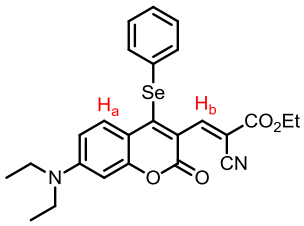


Fig. S5 The partial ^{1}H NMR spectra of probe CouSePh in DMSO-d_6 upon addition of increasing Cys.

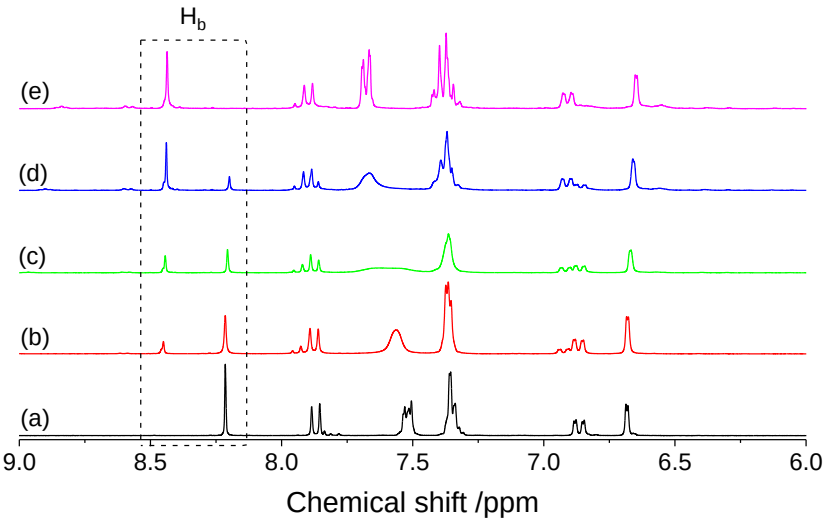


Fig. S6 The partial ^{1}H NMR spectra of probe CouSePh in DMSO-d_6 upon addition of increasing GSH.

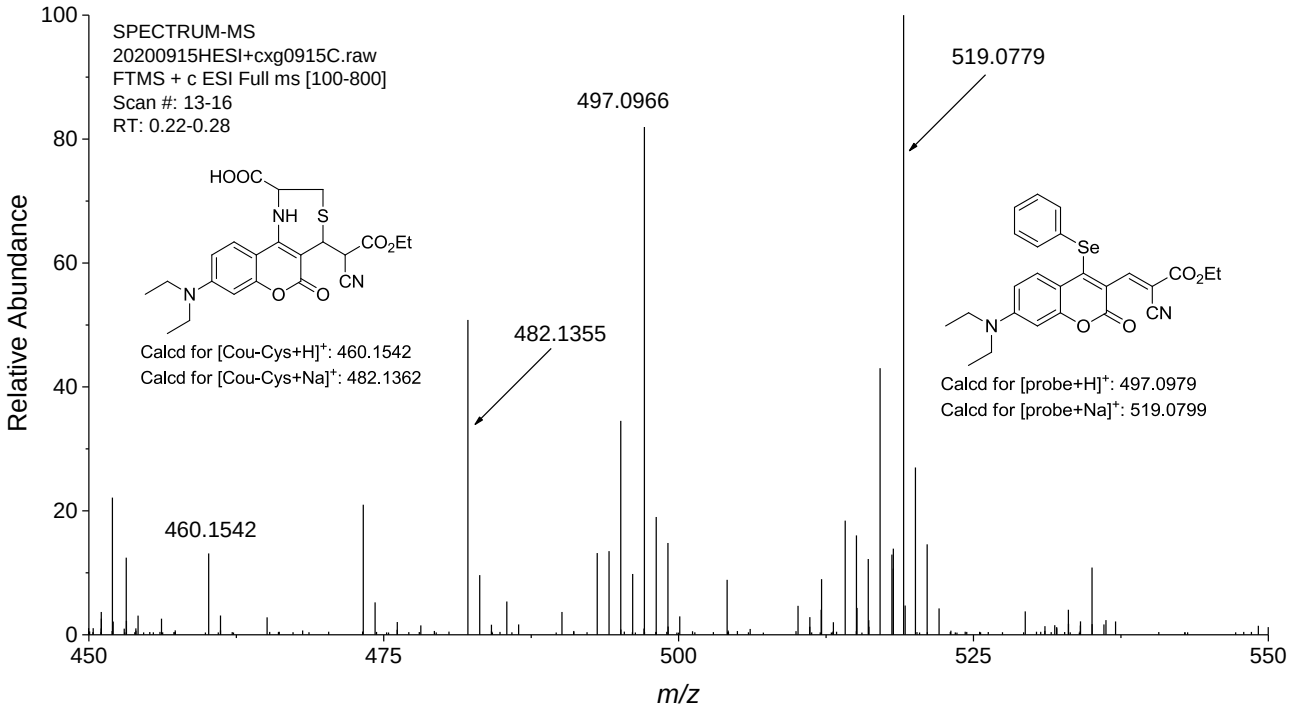


Fig. S7 The high-resolution mass spectrum (HRMS) for the mixture of probe CouSePh with Cys.

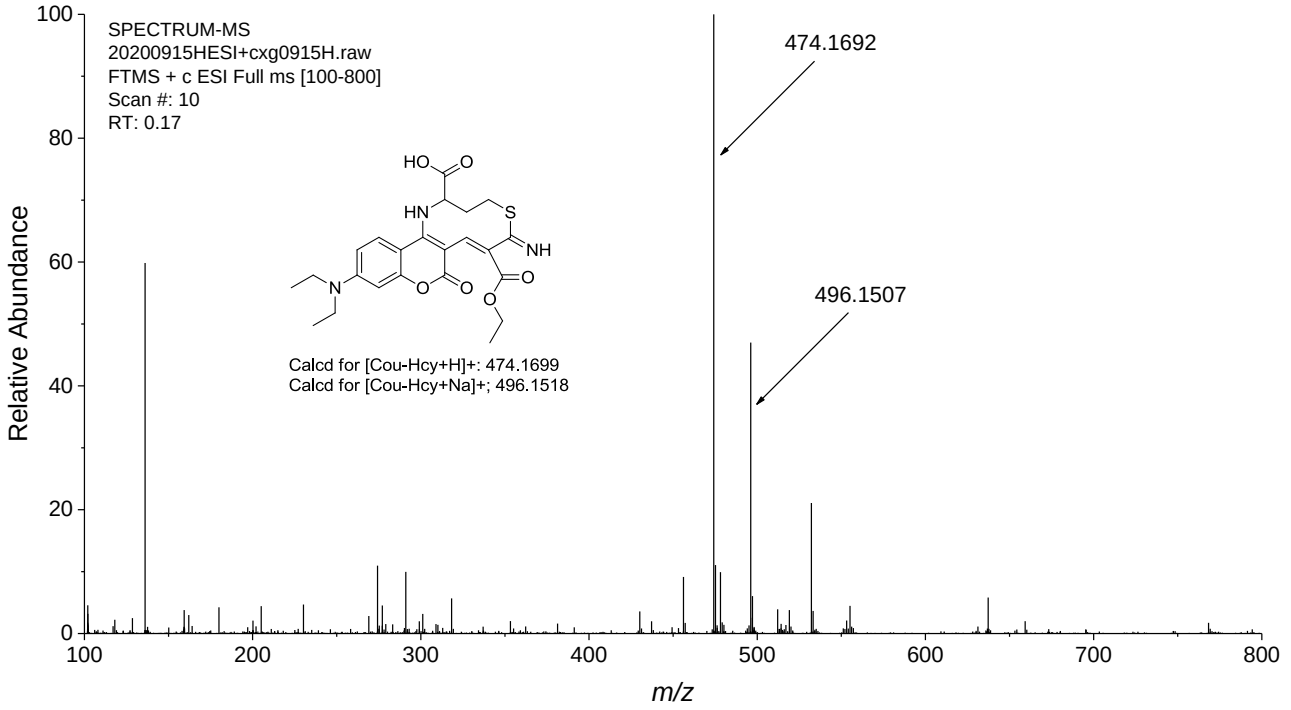


Fig. S8 The high-resolution mass spectrum (HRMS) for the mixture of probe CouSePh with Hcy.

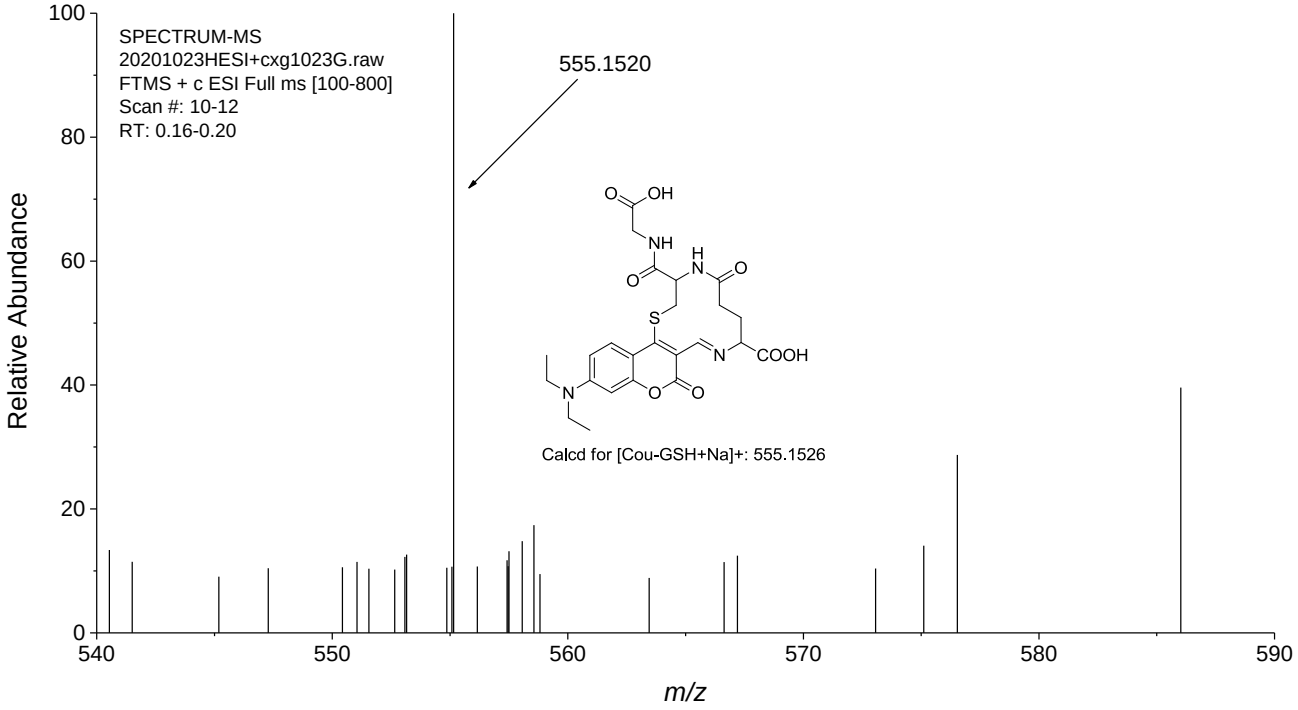


Fig. S9 The high-resolution mass spectrum (HRMS) for the mixture of probe CouSePh with GSH.

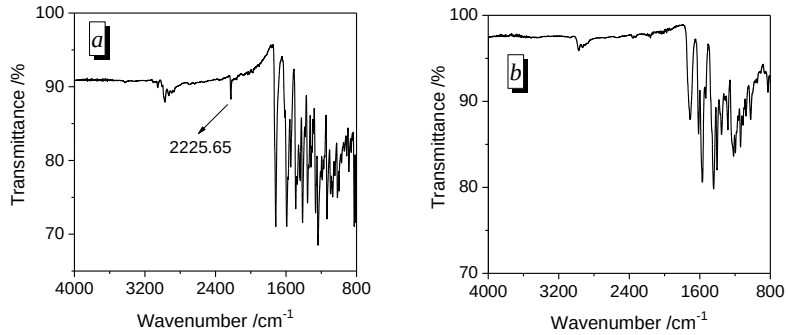


Fig. S10 The infrared spectra of (a) probe CouSePh and (b) sensing product Cou-Hcy.

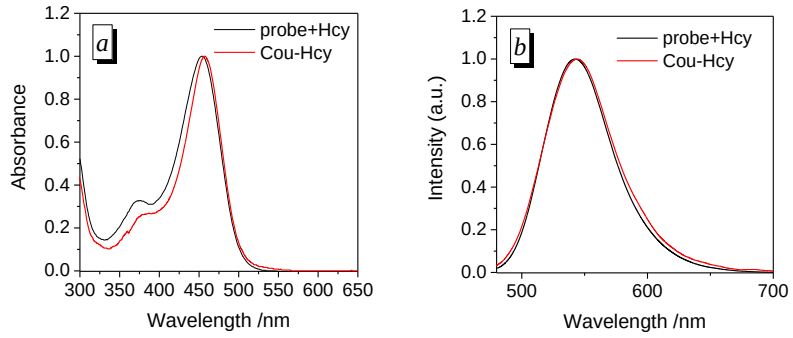


Fig. S11 Normalized (a) UV/Vis absorption and (b) fluorescence spectra of 10 μM probe CouSePh upon addition of Hcy and 10 μM sensing product Cou-Hcy in PBS buffer (10 mM, pH 7.4, 40% DMSO), λ_{ex} =455 nm.

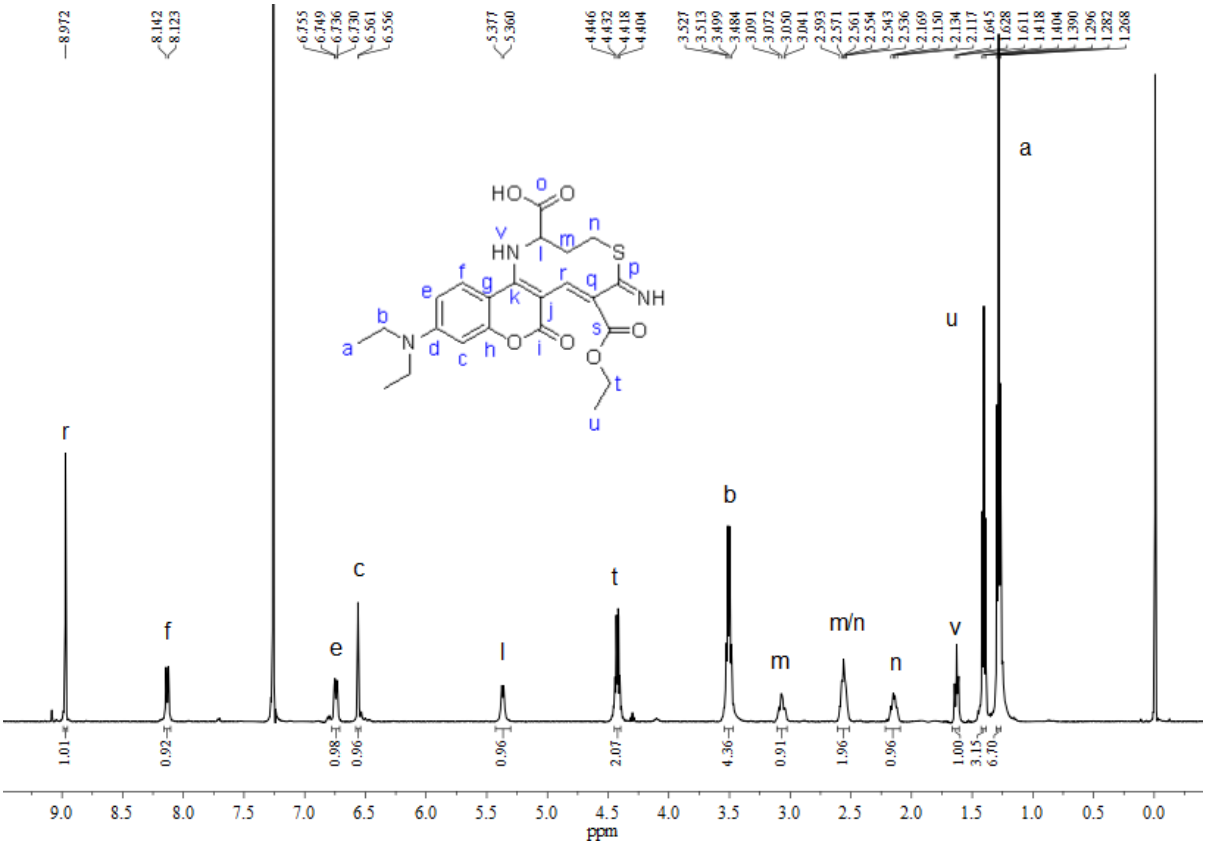


Fig. S12 The ^{1}H NMR spectrum of sensing product Cou-Hcy ($CDCl_3$, 500 MHz).

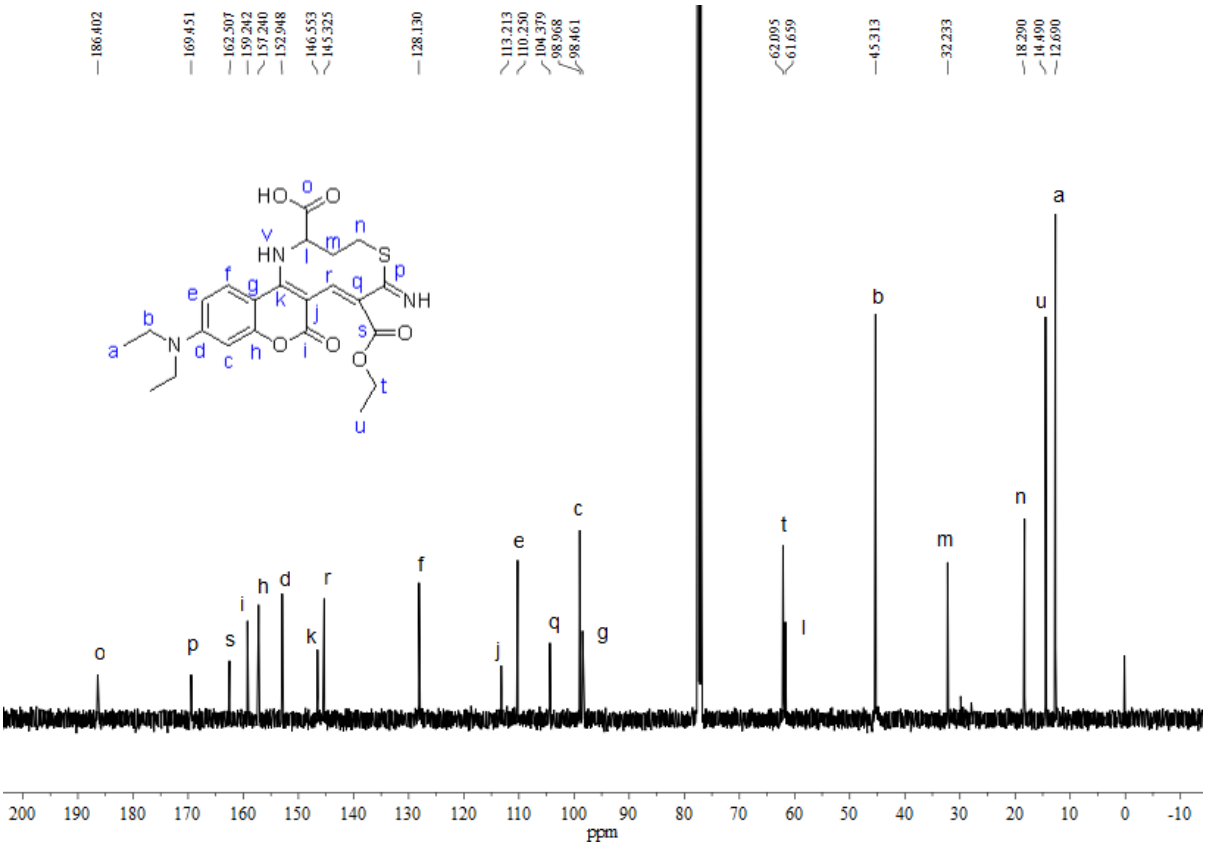


Fig. S13 The ^{13}C NMR spectrum of sensing product Cou-Hcy ($CDCl_3$, 125 MHz).

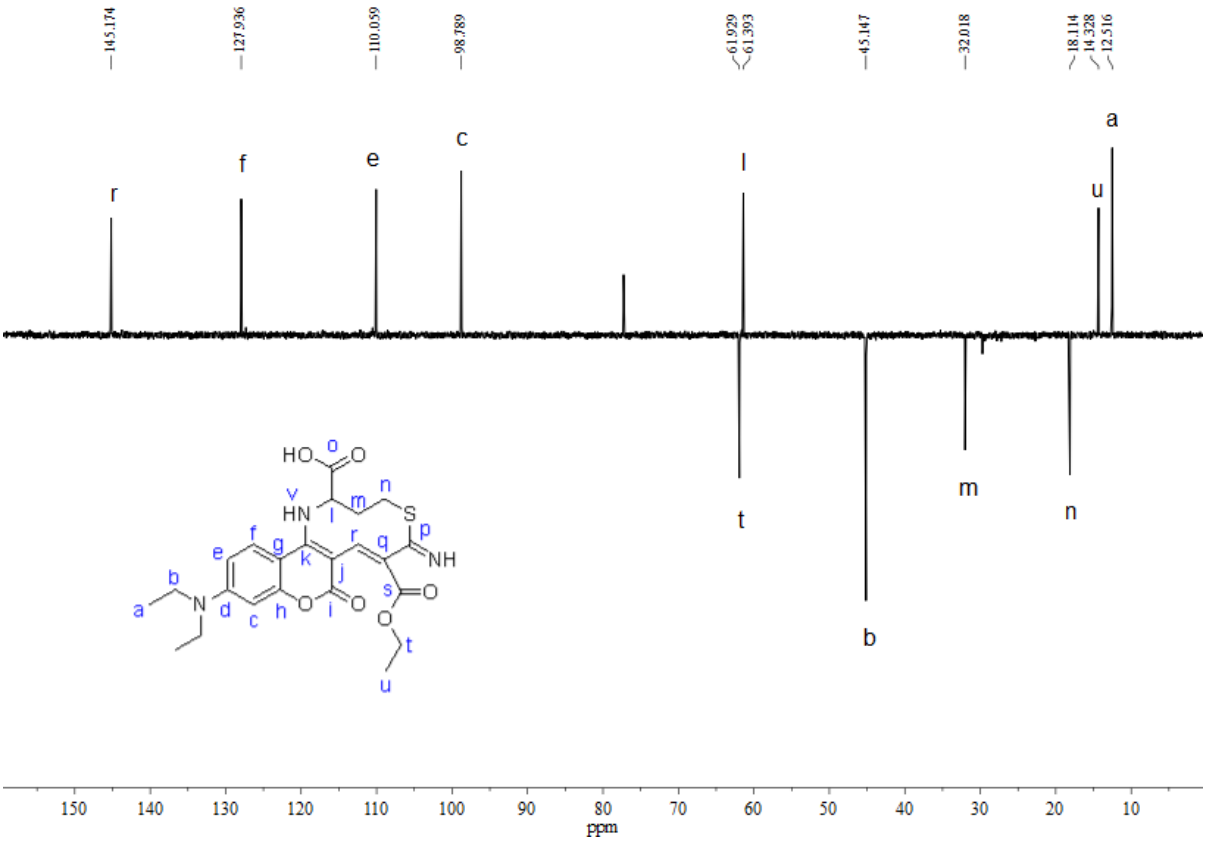


Fig. S14 The ^{13}C-DEPT 135 NMR spectrum of sensing product Cou-Hcy.

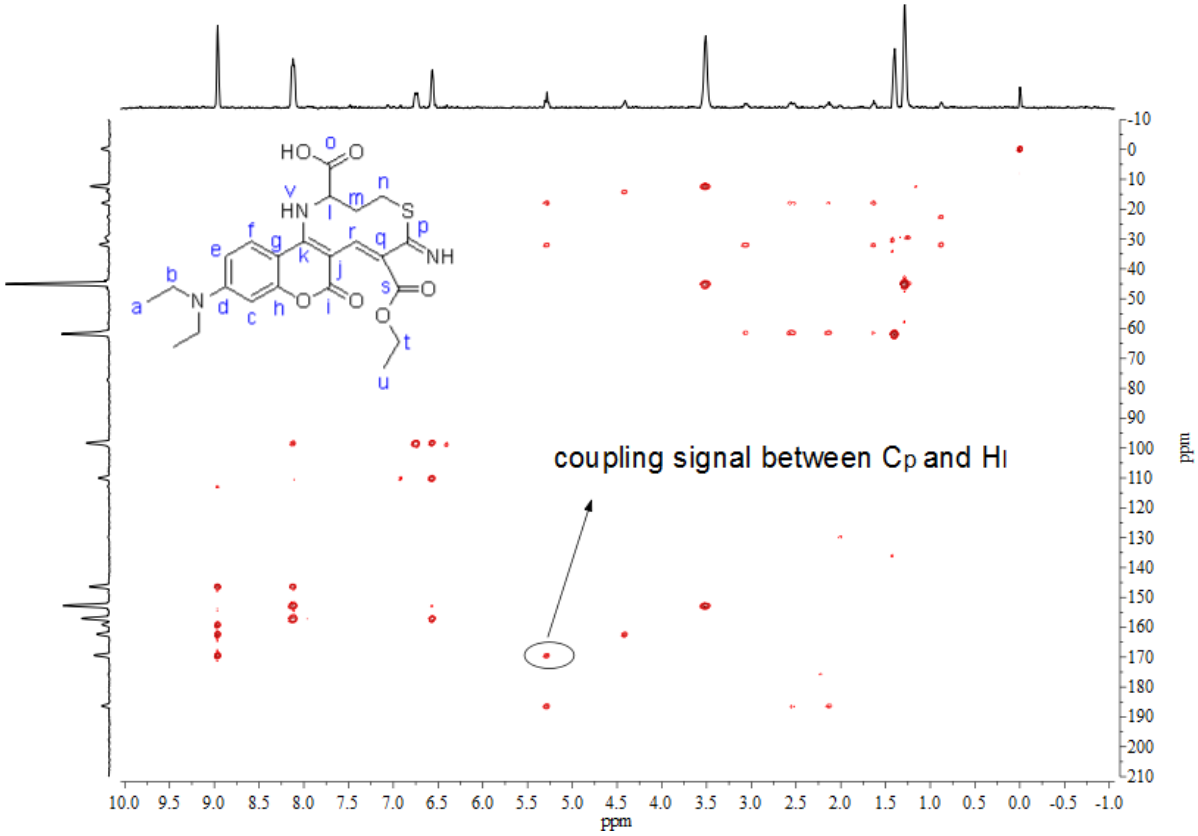


Fig. S15 The ^{1}H-^{13}C HMBC NMR spectrum of the sensing product Cou-Hcy.

7. Time-dependent spectral response of probe toward H_2S

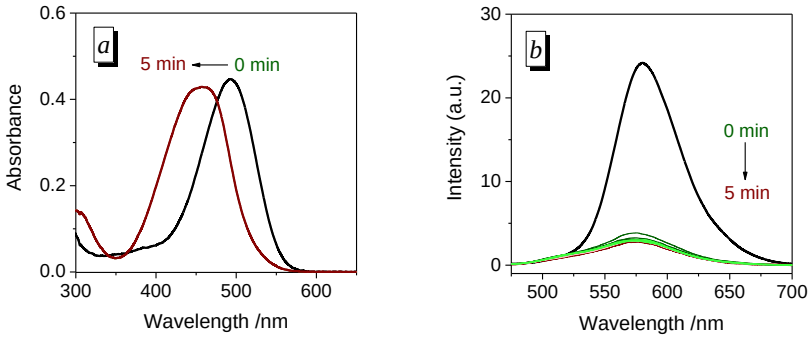


Fig. S16 Time-dependent (a) UV/Vis absorption and (b) fluorescence spectra of 10 μM probe CouSePh upon addition of 10 μM Na_2S in PBS buffer (10 mM, pH 7.4, 40% DMSO), λ_{ex} =450 nm.

8. Time-dependent spectral response of probe toward the mixture of biothiols

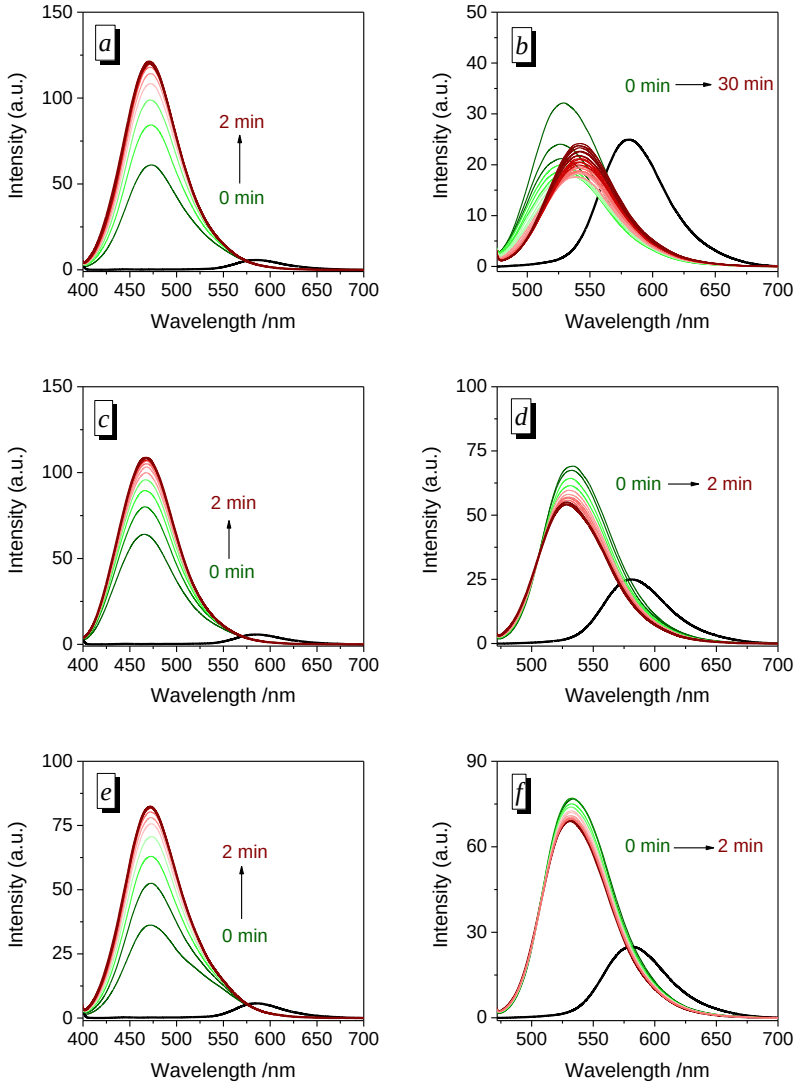


Fig. S17 Time-dependent fluorescence spectra of 10 μM probe CouSePh upon addition of the mixture of Cys/Hcy/GSH: (a/b) 10 μM/10 μM/10 μM, (c/d) 10 μM/10 μM/100 μM and (e/f) 5 μM/5 μM/100 μM, excitated at (a/c/e) 378 nm and (b/d/f) 450 nm.

9. pH effect studies and MTT assay

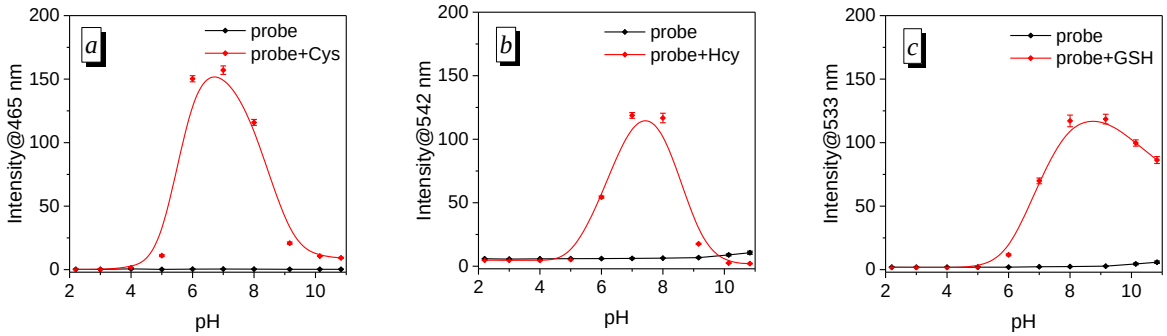


Fig. S18 The effect of pH on the fluorescence intensity of 10 μM probe CouSePh in the absence or presence of (a) Cys (λ_{ex} =378 nm), (b) Hcy (λ_{ex} =455 nm) or (c) GSH (λ_{ex} =450 nm) in PBS buffer (10 mM, 40% DMSO).

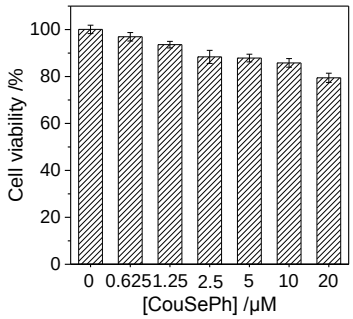
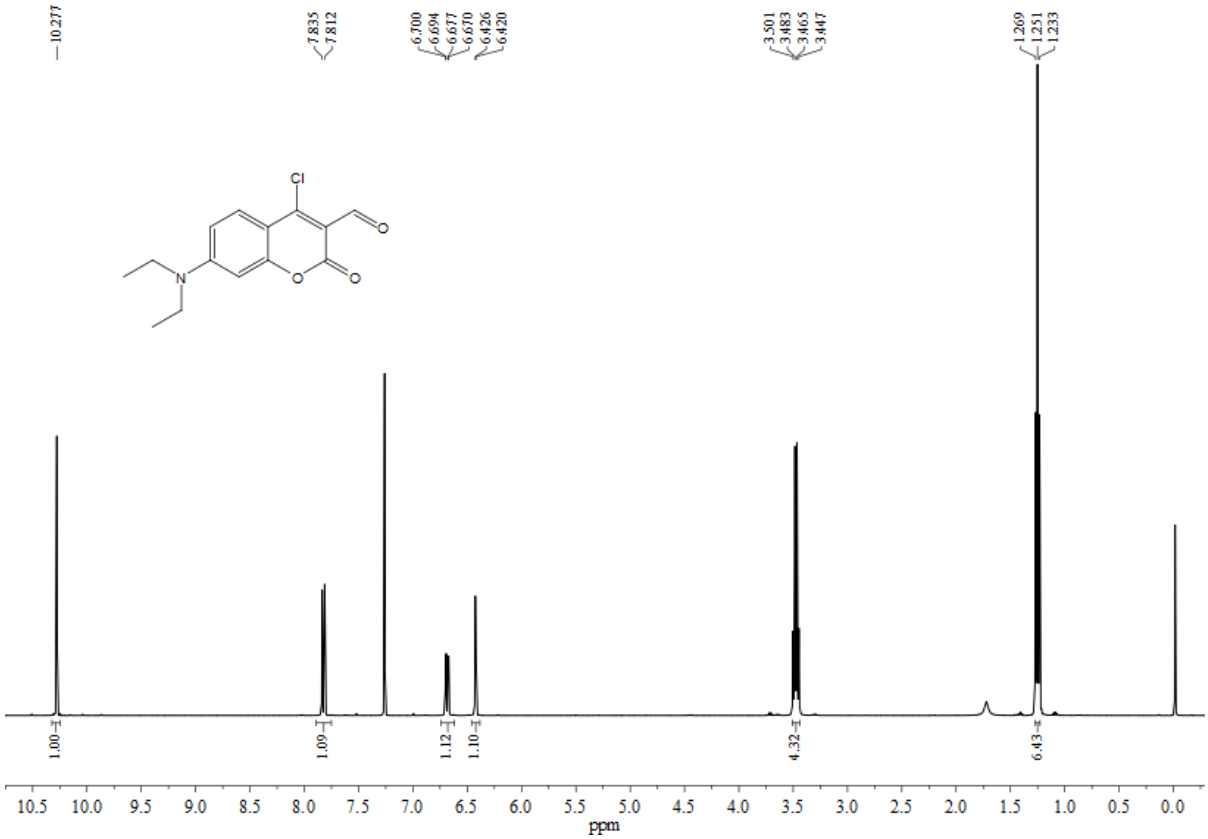


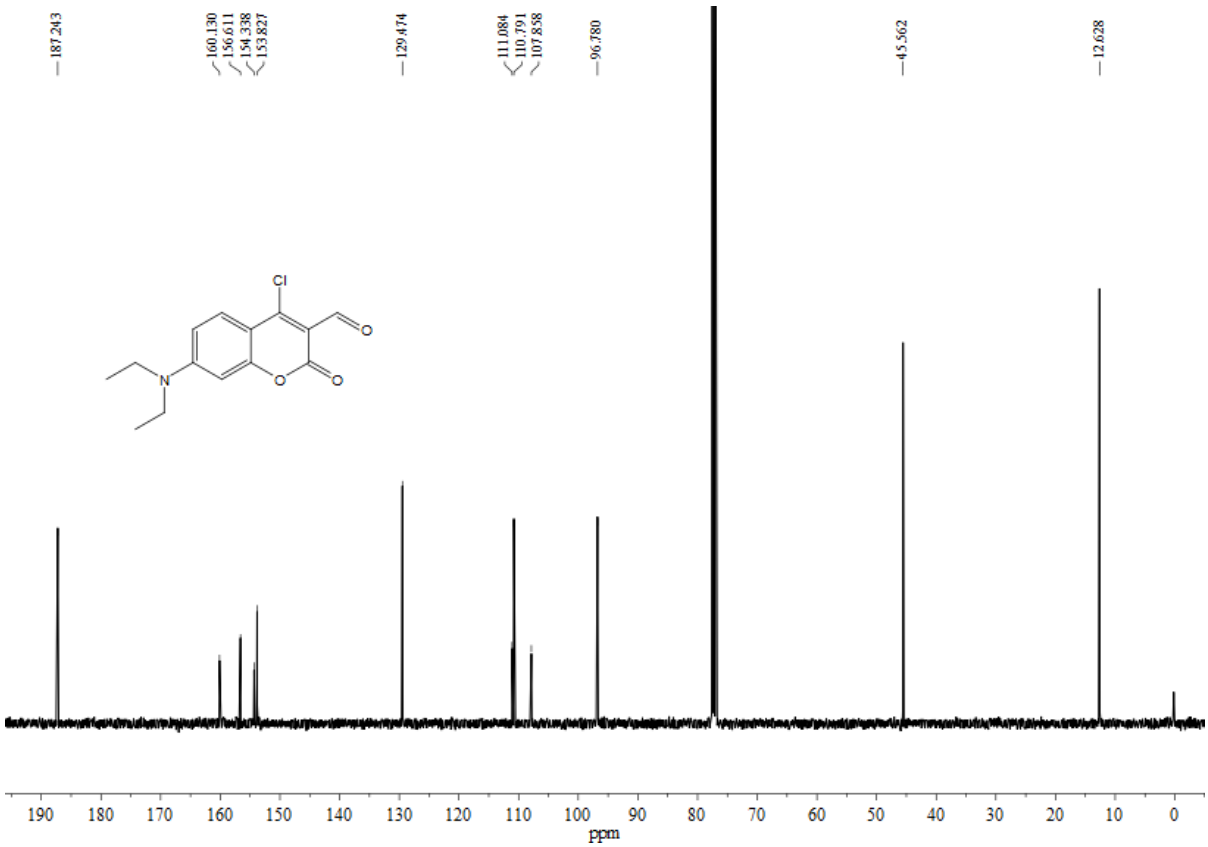
Fig. S19 MTT assay of HepG2 cells incubated with CouSePh medium (0-20 μM) for 24 h.

10. Copies of NMR spectra for related compounds

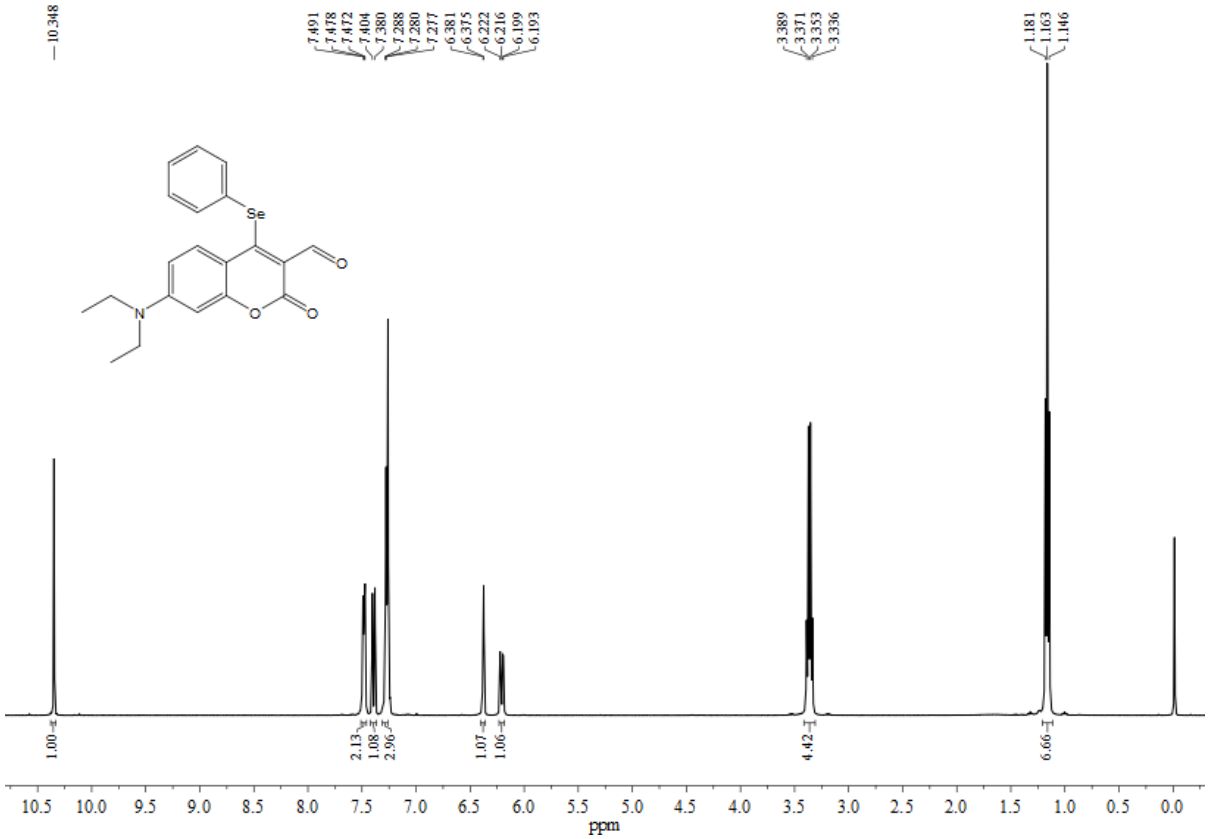
^{1}H NMR spectrum of compound **1** ($CDCl_3$, 400 MHz).



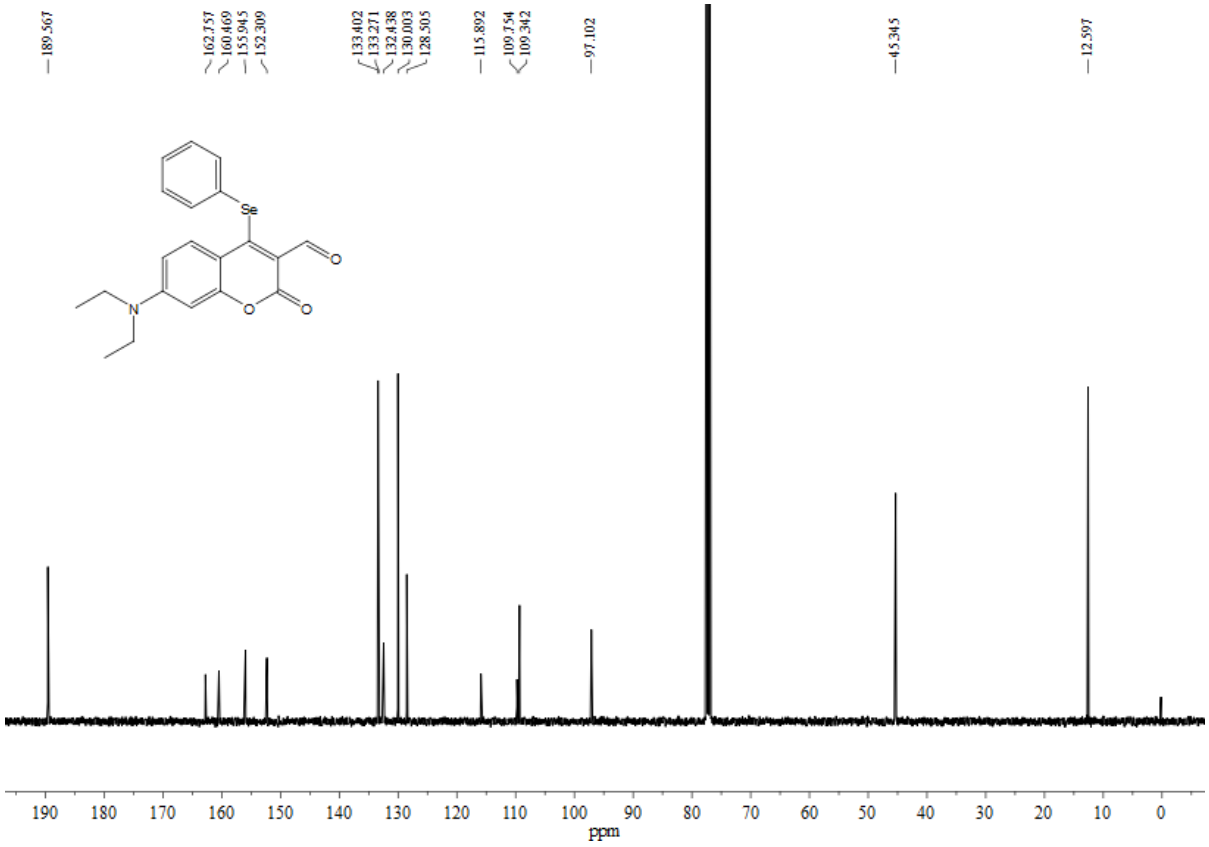
^{13}C NMR spectrum of compound **1** ($CDCl_3$, 100 MHz)



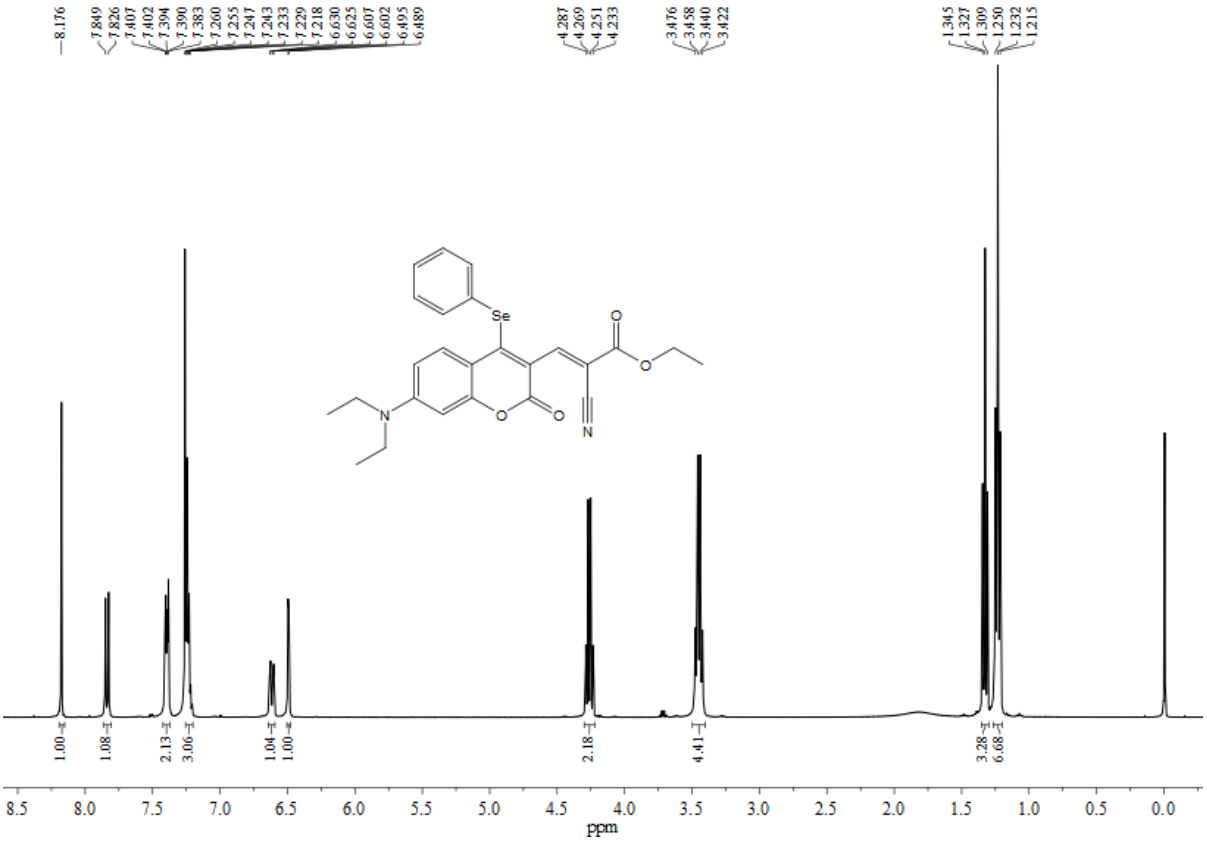
^{1}H NMR spectrum of compound **2** ($CDCl_3$, 400 MHz)



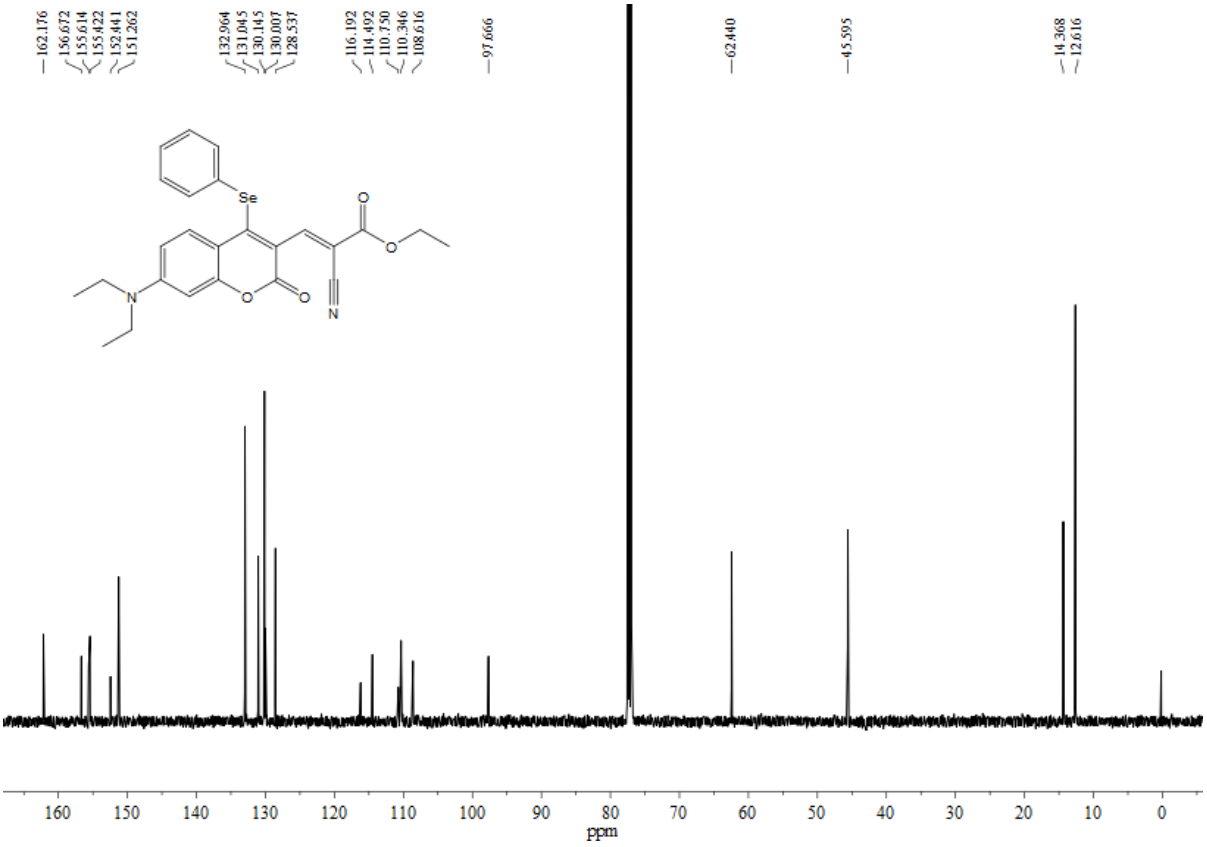
^{13}C NMR spectrum of compound **2** ($CDCl_3$, 100 MHz)



^{1}H NMR spectrum of probe CouSePh ($CDCl_3$, 400 MHz)



^{13}C NMR spectrum of probe CouSePh ($CDCl_3$, 100 MHz)



High resolution mass spectrum (HRMS) of probe CouSePh