Support Information

1. Materials and instruments

Unless otherwise stated, all chemicals were purchased from commercial suppliers and used without further purification. Deionized water was used throughout the experiments. Fluorescence spectra were measured using an F-7000 fluorescence spectrophotometer (Hitachi, Japan). UV-vis absorption spectra were recorded with a UV-2450 ultraviolet spectrophotometer (Shimadzu, Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE-600 MHz and 150 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). A Thermo Scientific Q Exactive LC–MS/MS system (Thermo Fisher Scientific, US) was used for HRMS spectra. The fluorescent images of probe were acquired with a ZEISS LSM 880 confocal laser scanning microscope (ZEISS, Germany).

2. HeLa cell culture and imaging

HeLa cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 10% Fetal Bovine Serum and 1% antibiotics at 37 °C in humidified environment of 5% CO_2. Cells were plated on 6-well plate and allowed to adhere for 24 h. Before the experiments, cells were washed 3 times with PBS (pH 7.4). The fluorescence excited at 458 nm for the green channel (475-535 nm) and at 488 nm for the yellow channel (540-600 nm).

3. Zebrafish culture and imaging

The zebrafish were kept at 28 °C and cultured in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 5-10% methylene blue, pH 7.5). The fluorescence excited at 458 nm for the green channel (475-535 nm) and at 488 nm for the yellow channel (540-600 nm).

4. Experimental section

Synthesis of *compound 1*. 4-(N-N-diethylamino) salicylaldehyde (1.93 g, 10 mmol), ethyl acetoacetate (1.52 mL, 12 mmol) and piperidine (99 μL, 1 mmol) were dissolved in 20 mL ethanol. The mixture was further stirred at room temperature for 12 h. After that, the precipitate was filtered and dried to obtain a yellow solid (2.28 g, yield 88%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.49 (s, 1H), 7.66 (d, J = 9.0 Hz, 1H), 6.79 (dd, J = 9.0, 2.3 Hz, 1H), 6.57 (d, J = 2.0 Hz, 1H), 3.49 (q, J = 7.0 Hz, 4H), 1.42-0.88 (m, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 194.70, 160.37, 158.75, 153.42, 148.15, 132.93, 115.41, 110.60, 107.94, 96.25, 44.88, 30.66, 12.81 (Fig. S4).

Synthesis of *compound 2*. *Compound 1* (2 mmol, 0.519 g) and p-hydroxybenzaldehyde (2.4 mmol, 0.293 g) were dissolved in 10 mL ethanol. 150 μL piperidine was added and the mixture was refluxed for 36 h. After cooling to room temperature, the mixture was filtered under reduced pressure and dried to obtain an orange powder (0.181 g, yield 25%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.09 (s, 1H), 8.58 (s, 1H), 7.80 (d, J = 15.7 Hz, 1H), 7.69 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 15.7 Hz, 1H), 7.58 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 6.81 (dd, J = 9.0, 1.8 Hz, 1H), 6.61 (s, 1H), 3.50 (d, J = 7.0 Hz, 4H), 1.15 (t, J = 7.0 Hz, 6H). ^{13}C NMR (150 MHz,

DMSO-d_6) δ 185.80, 160.43, 160.24, 158.60, 153.32, 148.65, 143.07, 132.72, 130.98, 126.40, 122.00, 116.42, 116.28, 110.61, 108.35, 96.34, 44.90, 12.84 (Fig. S5).

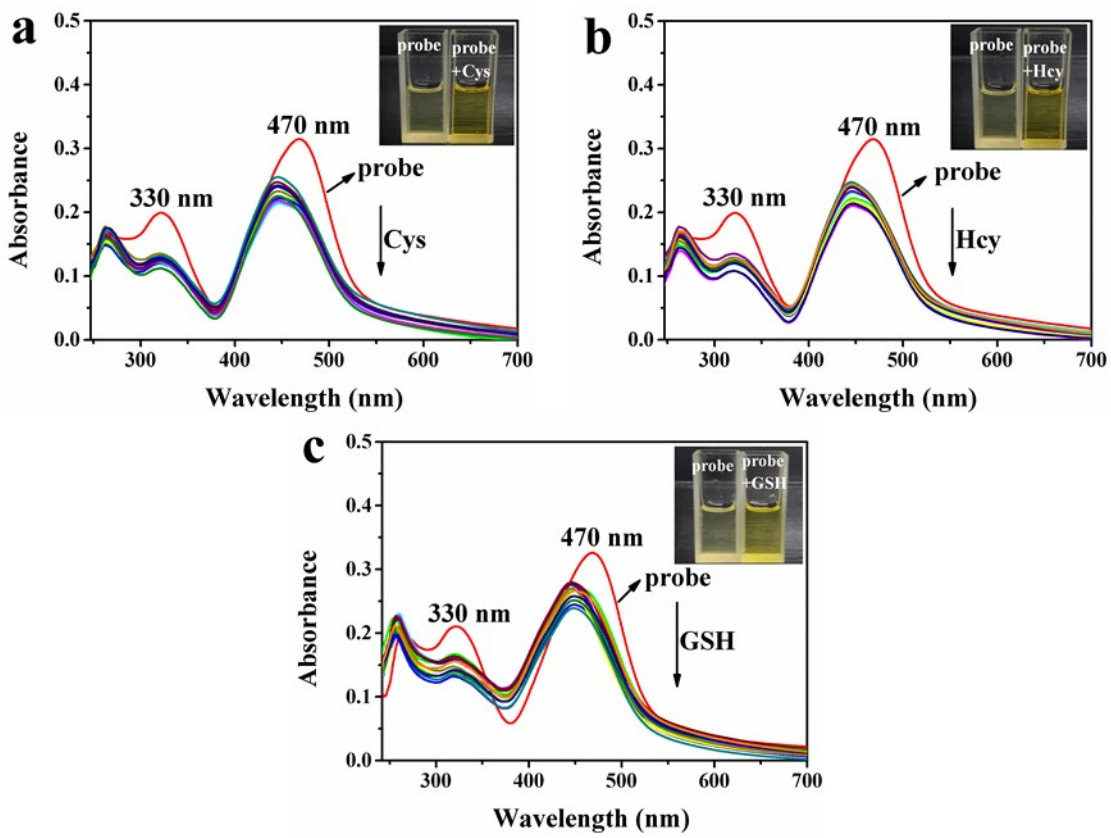


Fig. S1. The UV-vis absorption spectra of ***CB*** (20 μM) after addition of thiols (0-100 μM) in DMSO/PBS (v/v, 6/4, pH 7.40); Insert: color change of the solution in day light.

Fig. S2. Time response of ***CB*** to thiols.

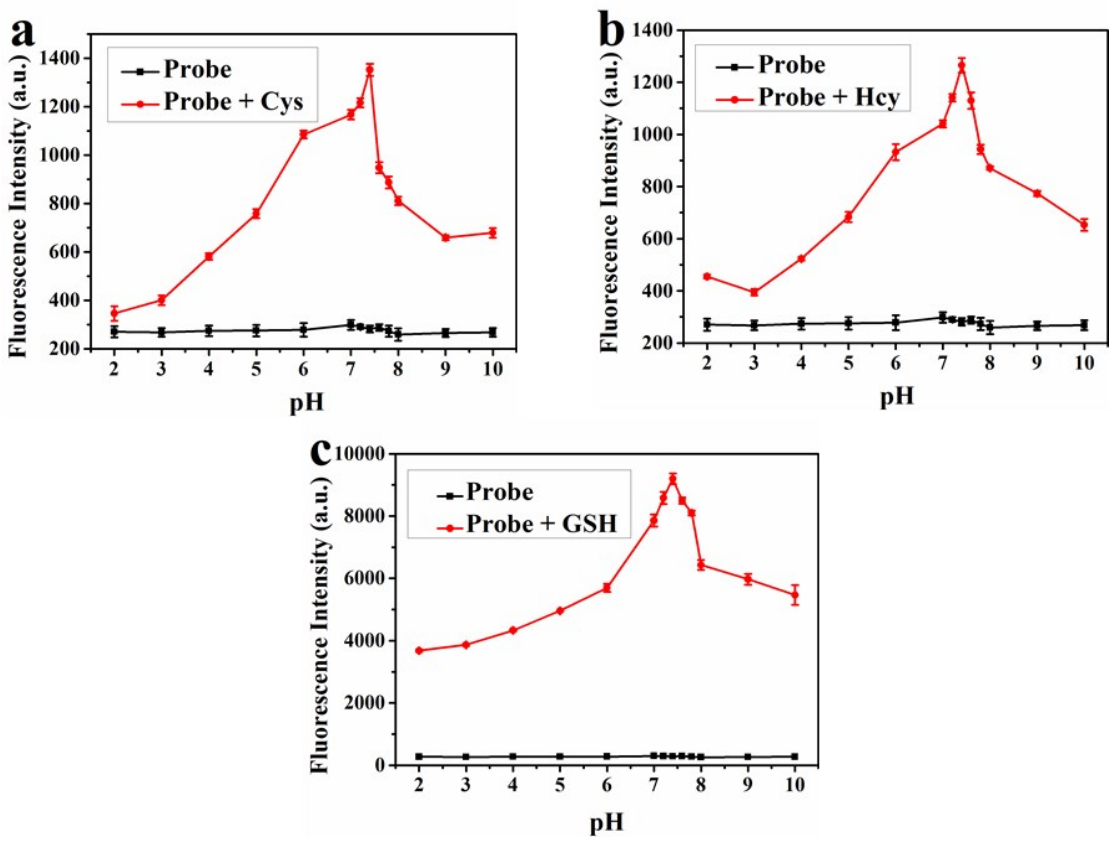
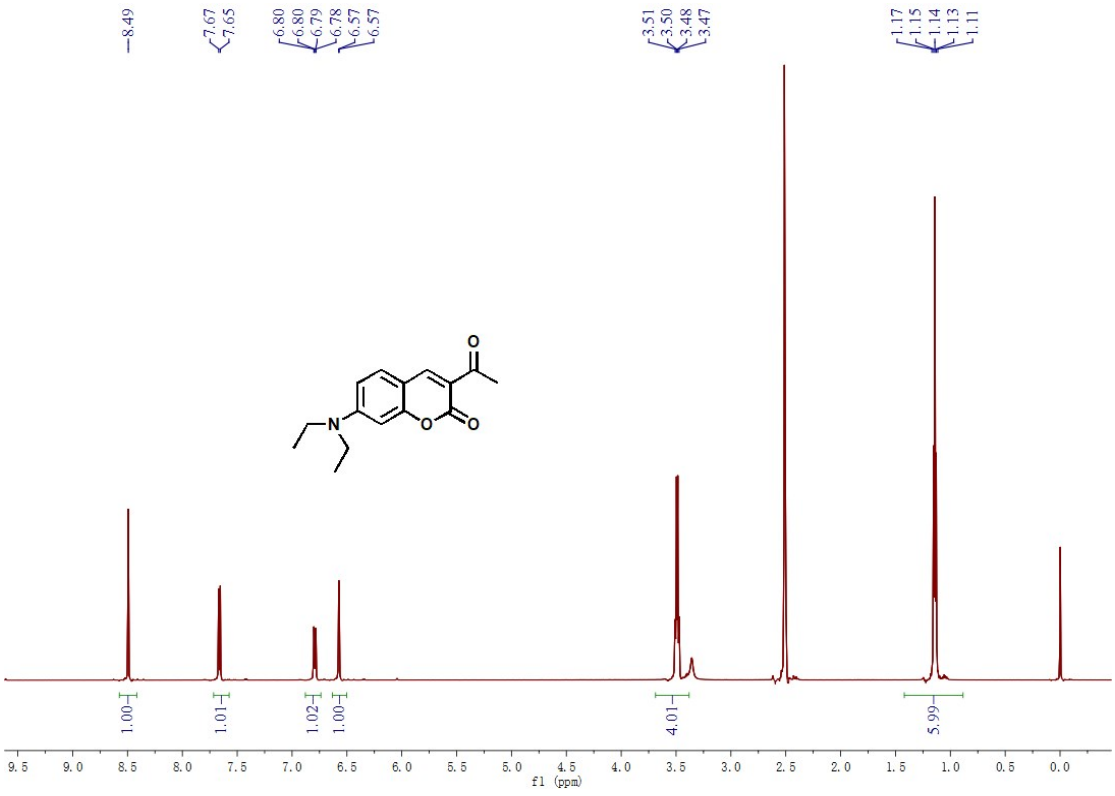
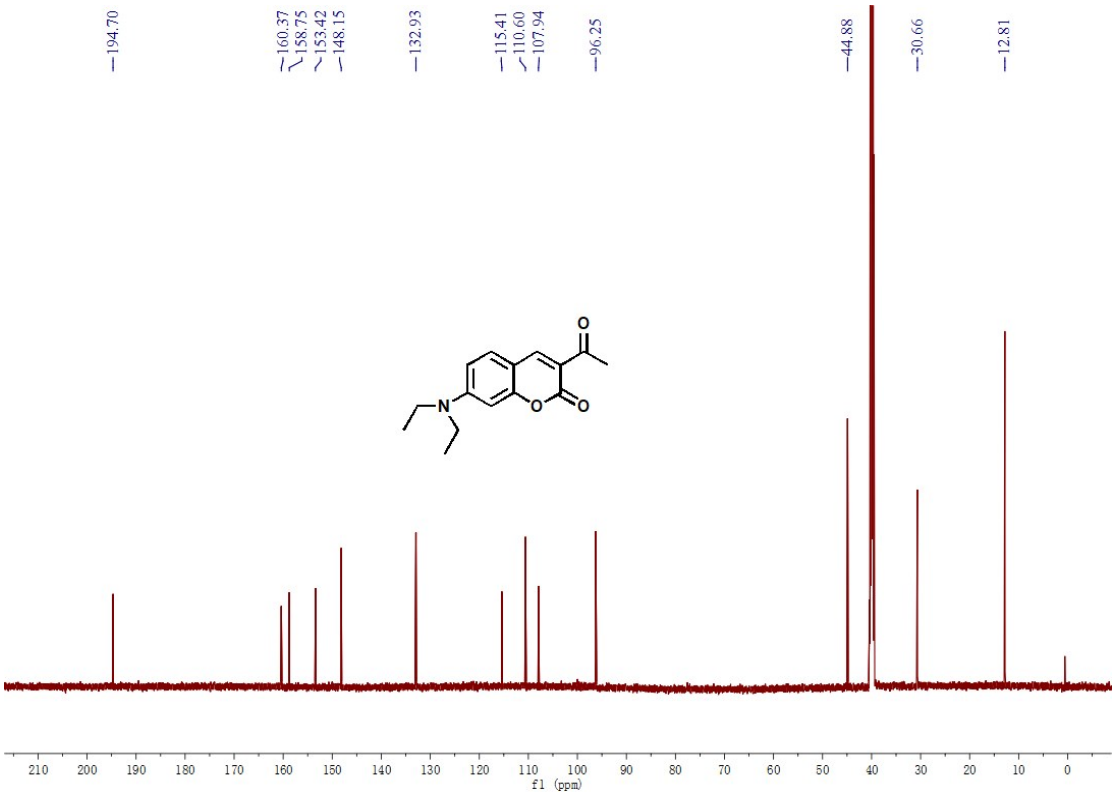


Fig. S3. PH-dependence fluorescence spectra of ***CB*** in the presence and absence of thiols.

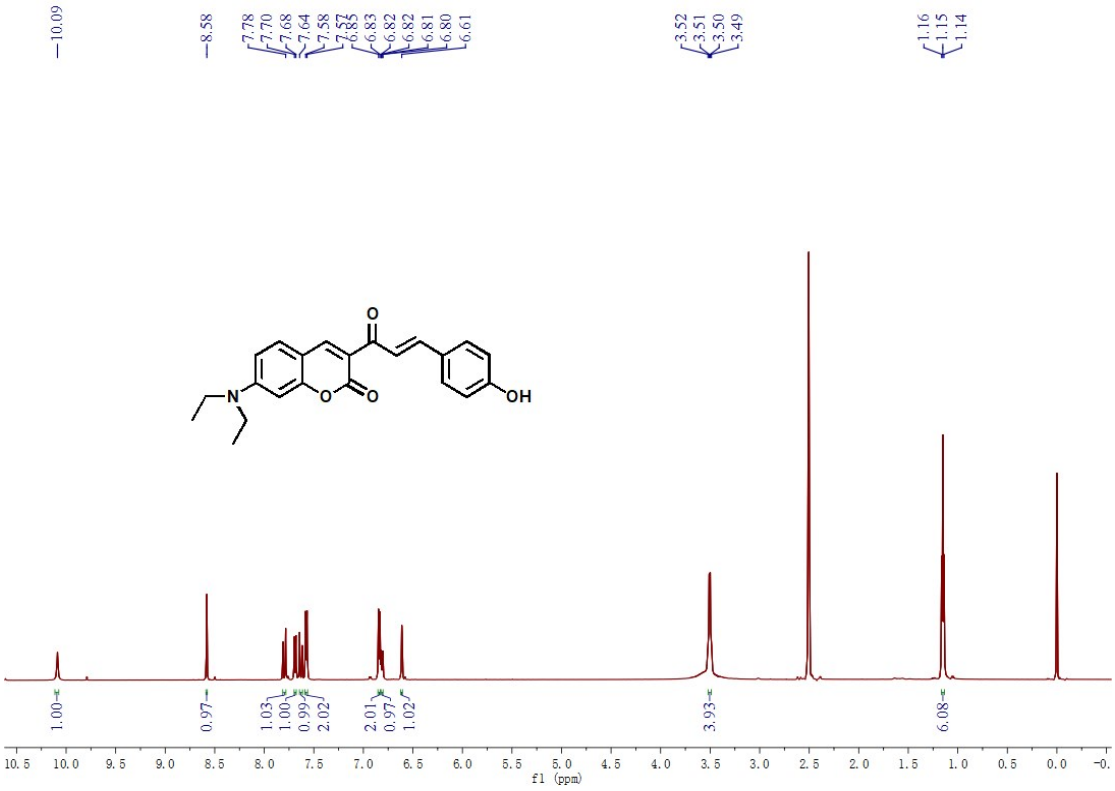


^{1}H NMR (600 MHz) of ***compound 1*** in DMSO-$d_{6.}$

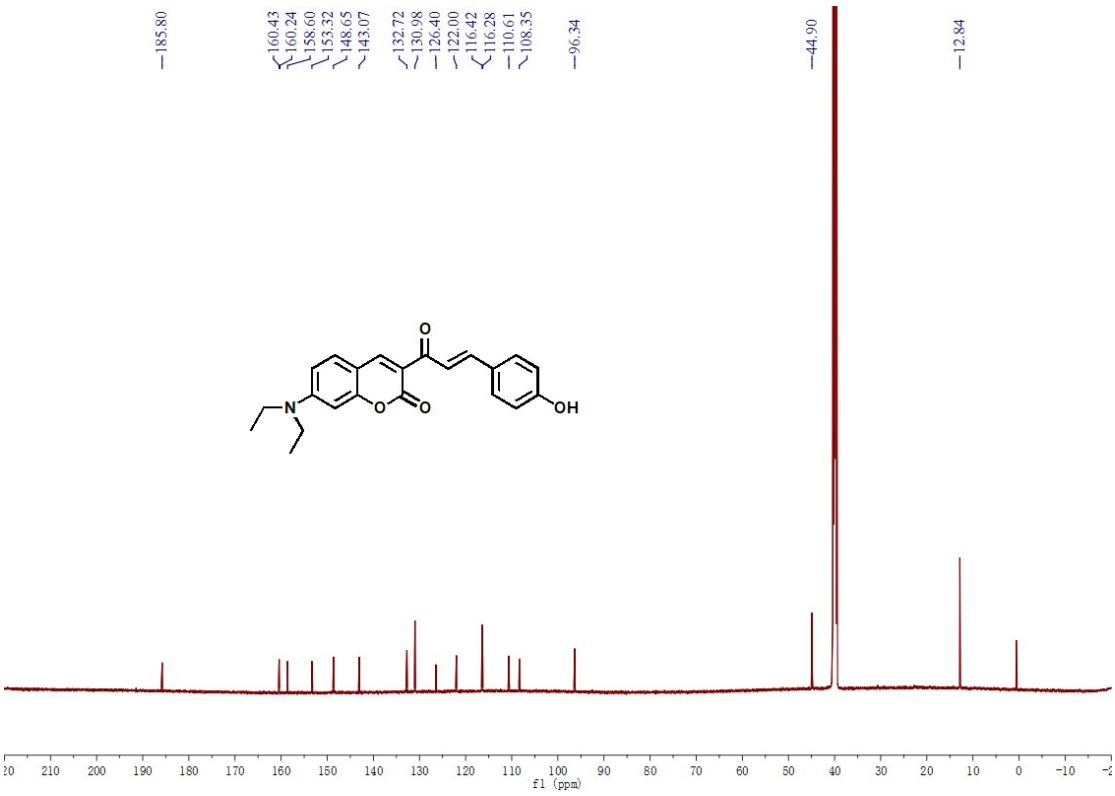


^{13}C NMR (150 MHz) of ***compound 1*** in DMSO-$d_{6.}$

Fig. S4. NMR characterization of ***compound 1***.

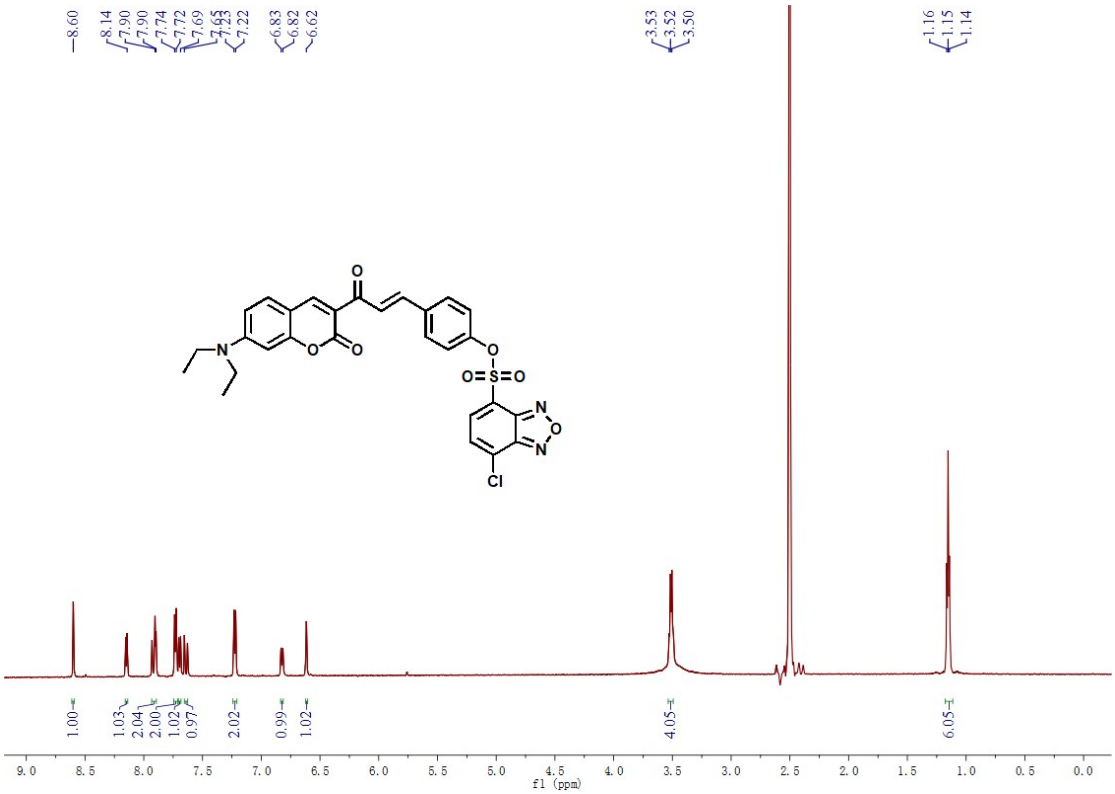


^{1}H NMR (600 MHz) of ***compound 2*** in DMSO-$d_{6.}$

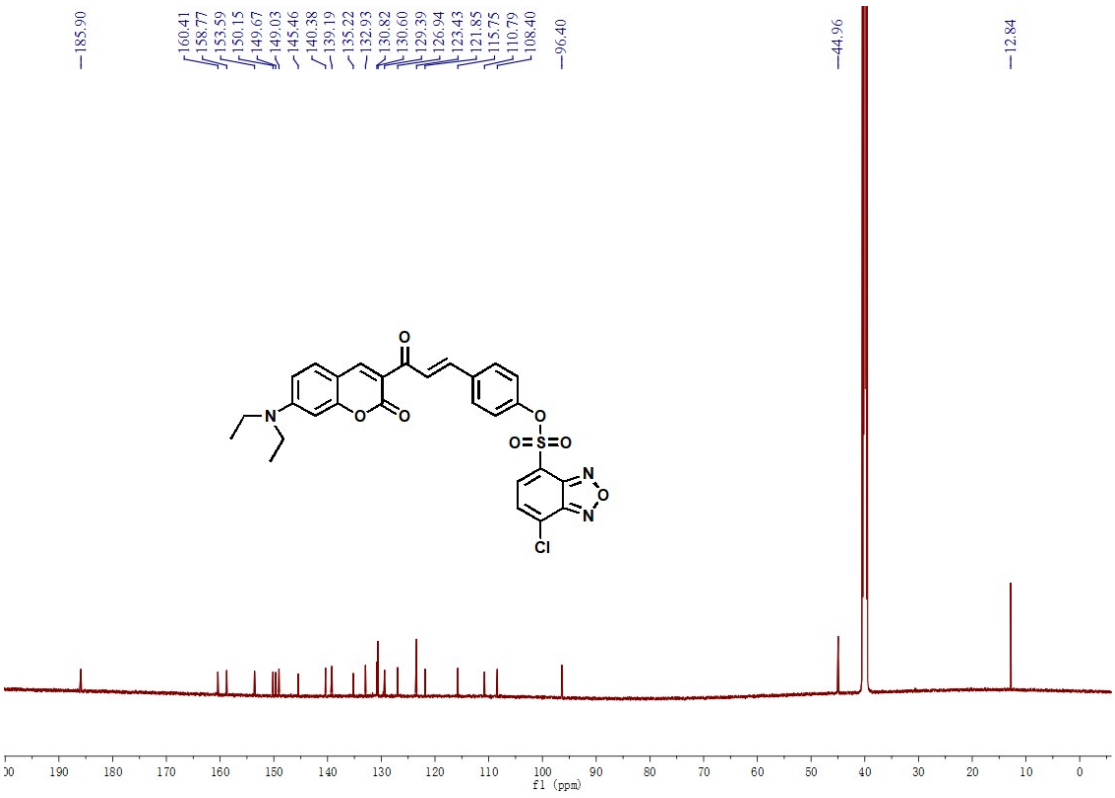


^{13}C NMR (150 MHz) of ***compound 2*** in DMSO-$d_{6.}$

Fig. S5. NMR characterization of ***compound 2***.

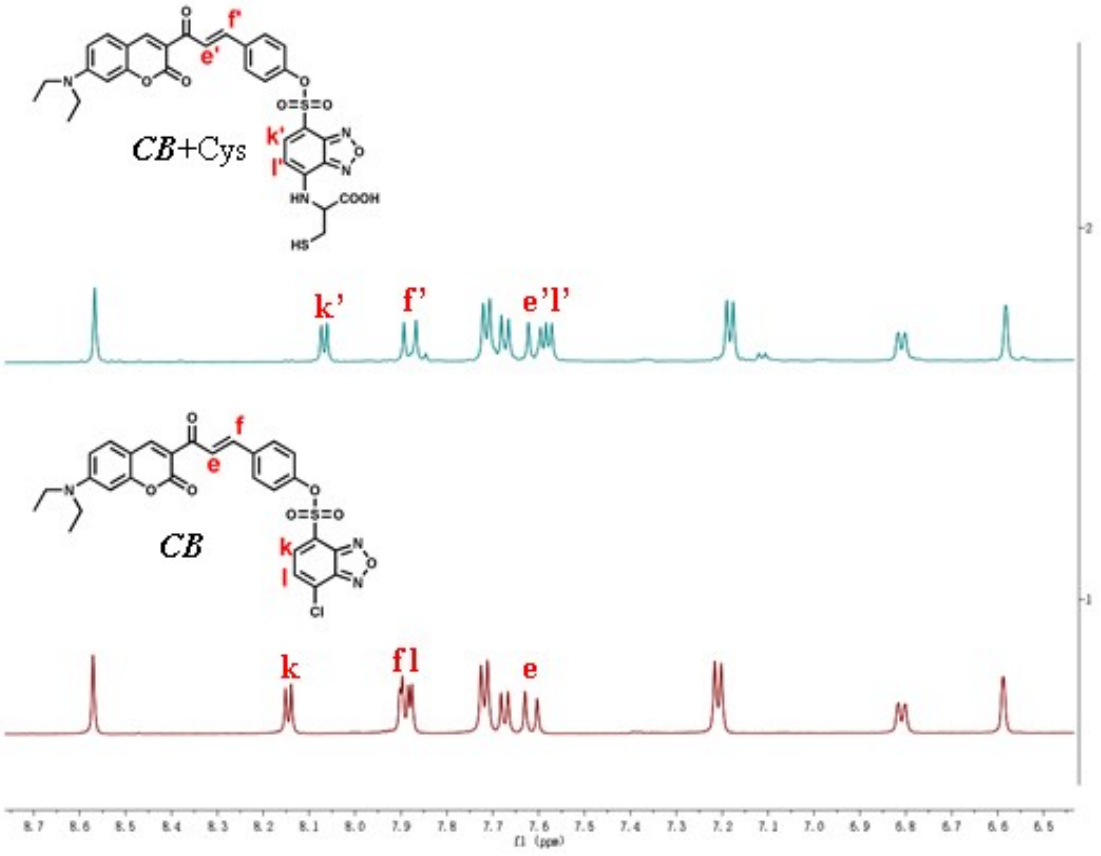


^{1}H NMR (600 MHz) of ***CB*** in DMSO-d_6.

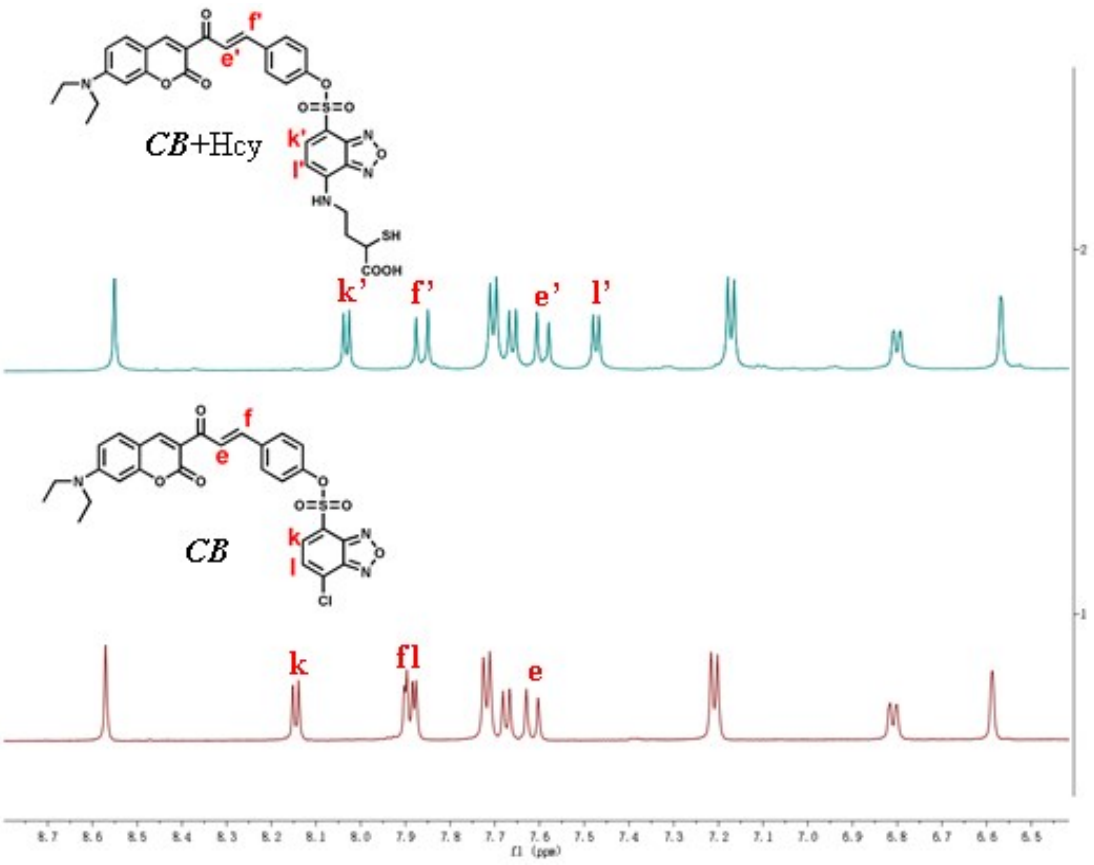


^{13}C NMR (150 MHz) of ***CB*** in DMSO-d_6.

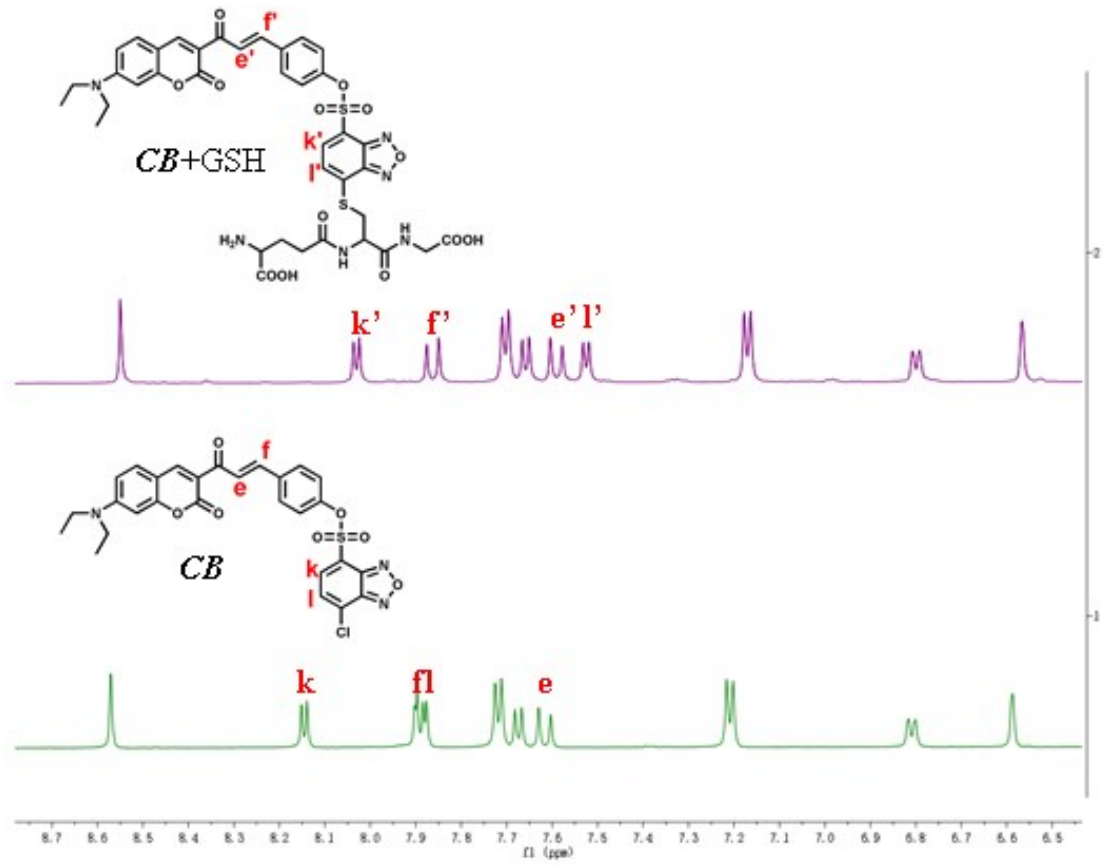
Fig. S6. NMR characterization of ***CB***.



^{1}H NMR titration of ***CB*** + Cys in DMSO-d_6/D_2O (v/v, 20/1).



^{1}H NMR titration of ***CB*** + Hcy in DMSO-d_6/D_2O (v/v, 20/1).



^{1}H NMR titration of ***CB*** + GSH in DMSO-d_6/D_2O (v/v, 20/1).

Fig. S7. NMR titration characterization of ***CB*** + thiols.

HRMS analysis of ***CB.***

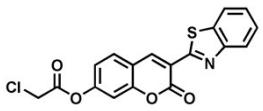
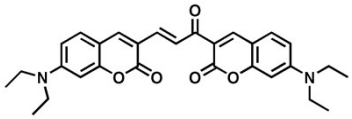
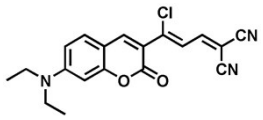
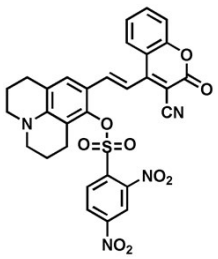
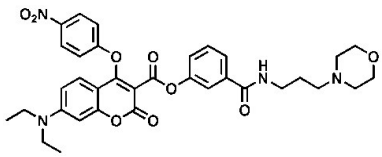
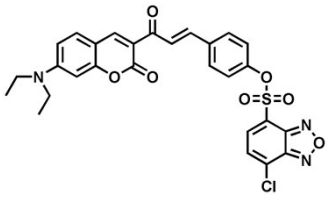
HRMS analysis of ***CB***+Cys.

HRMS analysis of ***CB***+Hcy.

HRMS analysis of ***CB***+GSH.

Fig. S8. HRMS analysis of ***CB*** and ***CB***+thiols.

Table S1. Comparison of some previous coumarin-based work for detection of thiols.

Probes	Distinguishing targets for detection	Detection limits	Biological system	Ref.
	Cys	0.06 μM	HeLa cells	[1]
	Hcy	3.5 μM	HeLa cells	[2]
	GSH	0.30 μM	A549 cells	[3]
	Cys/Hcy/GSH	7.9 nM (Cys), 10.2 nM (Hcy), 4.2 nM (GSH)	A549 cells	[4]
	Cys/Hcy and GSH	0.86 μM (Cys), 1.52 μM (Hcy), 1.75 μM (GSH)	HeLa cells	[5]
	Cys/Hcy and GSH	0.22 μM (Cys), 0.40 μM (Hcy), 0.3 nM (GSH)	HeLa cells Zebrafish	This work

References

[1] J. Yang, Y. Yu, B. Wang and Y. Jiang, *Journal of Photochemistry and Photobiology A: Chemistry*, 2017, **338**,178-182.

[2] K. Wang, S. Xu, Y. Lei, W. Zheng, Q. Zhang, S. Chen, H. Hu and Z. Hu, *Talanta*, 2019, **196**, 243-248.

[3] X. Li, F. Huo, Y. Yue, Y. Zhang and C. Yin, *Sensors and Actuators B: Chemical*, 2017, **253**, 42-49.

[4] J. Wang, F. Zhang, L. Yang, B. Wang and X. Song, *Journal of Luminescence*, 2021, **234**, 117994.

[5] H. Zhang, L. Xu, W. Li, W. Chen, Q. Xiao, J. Huang, C. Huang, J. Sheng and X. Song, *RSC Adv*., 2019, **9**, 7955-7960.