Supporting Information

Light-Driven Visualization of Endogenous Cysteine, Homocysteine, and Glutathione Near-Infrared Fluorescent Probe

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Experimental section

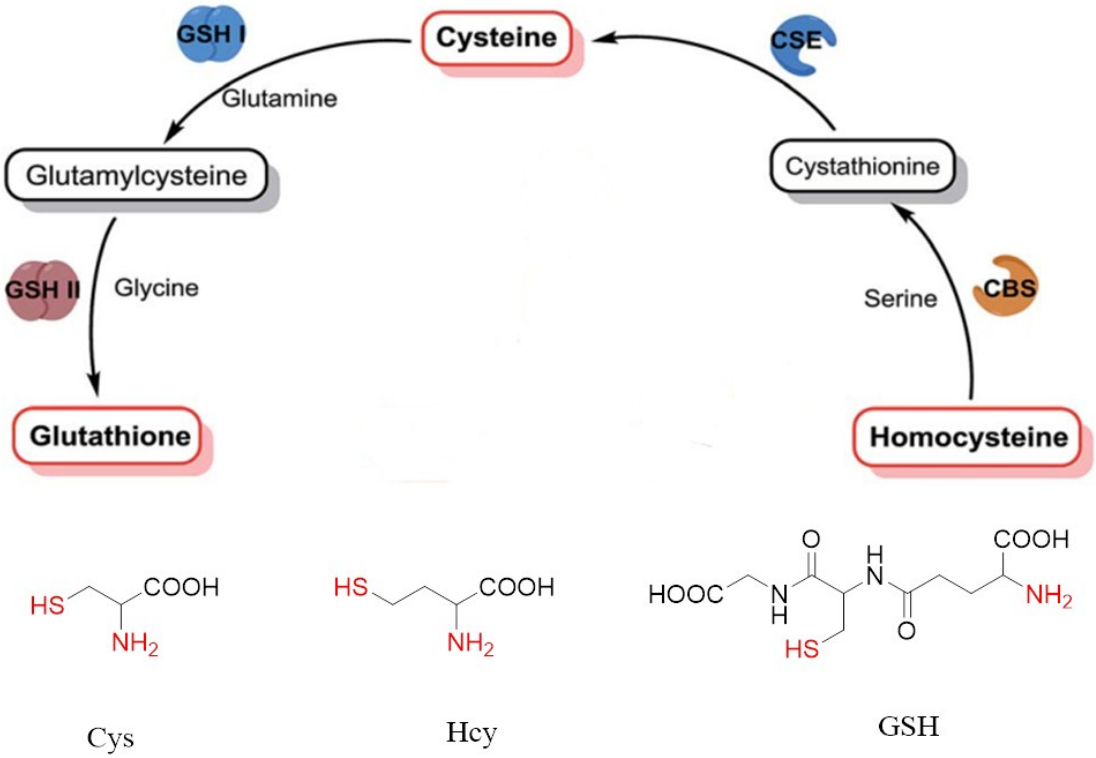
Experimental Details. Unless otherwise noted, all of the chemicals for syntheses were obtained from ordinary supplier. Mass spectra (ESI) were performed on a Bruker Daltonics Esquire6000 spectrometer with a LQC system. High resolution mass spectra (HRMS) were carried out on a Bruker microTOF-Q II mass spectrometer. NMR spectra were measured on a JEOL-ECS-400 M instrument using tetramethylsilane (TMS) as an internal standard. Fluorescence spectra were carried out on a Hitachi F-7000 spectrofluorimeter. UV-Vis spectra were determined on a Varian Cary UV-Cary 100 spectrophotometer. Blue channel: E_x = 405 nm, E_m=475-515 nm; green channel: E_x = 515 nm, E_m=540-600 nm; red channel: E_x = 515 nm, E_m=700-780 nm. B/G: merge of blue and green channels; B/R: merge of blue and red channels; G/R: merge of green and red channels; B/G/R: merge of blue, green, and red channels; B/B/G/R: merge of bright field, blue, green, and red channels.

Cytotoxicity Tests. The MTT assays were carried out to evaluate the cytotoxicity of probe **1**. HeLa cells were placed at 1×10^4 cells per well in 96-well plates and then incubated for one day in a humidified CO_2 incubator (5% CO_2). Subsequently, the cells were added with a series concentrations of probe **1** (0, 2.5 5, 10, 20, 30, 50 μM) and cultured for another day. Then 20 μL of MTT dye solution (5 mg/mL) was added in each well. After 4 h, 150 μL of dimethyl sulfoxide was added to dissolve the MTT formazan. Finally, an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad, Model 550) was used to measure the OD570 (absorbance value) of each well referenced at 450 nm.

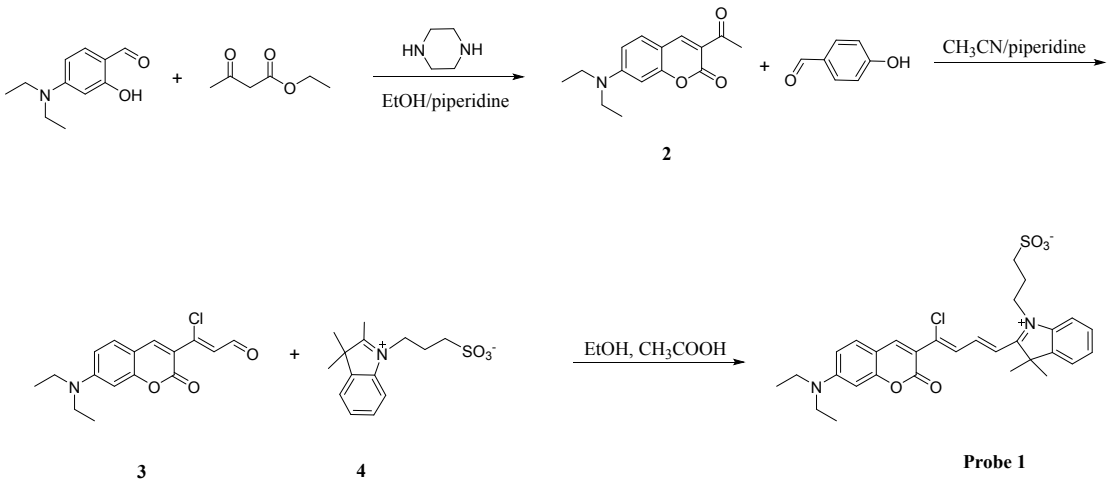
The detection limit calculation. The detection limit for thiophenols based on the IUPAC definition (signal-to-noise ratio S/N=3) is calculated by the linear function and the following equation:

$$\text{Detection limit} = \frac{3\sigma}{k} \times 10^{-5}\ \text{mol}\cdot\text{L}^{-1}$$

Where σ is the standard derivation of absorbance experiments of 13 blank solutions; k is the slope of the linear calibration curve in Figure S1-S3; the concentration of probe **1** is 1×10^{-5} mol·L^{-1}.



Scheme S1 The Metabolic Relationship of Biothiols and its Structure.



Scheme S2 Synthetic route of probe **1**.

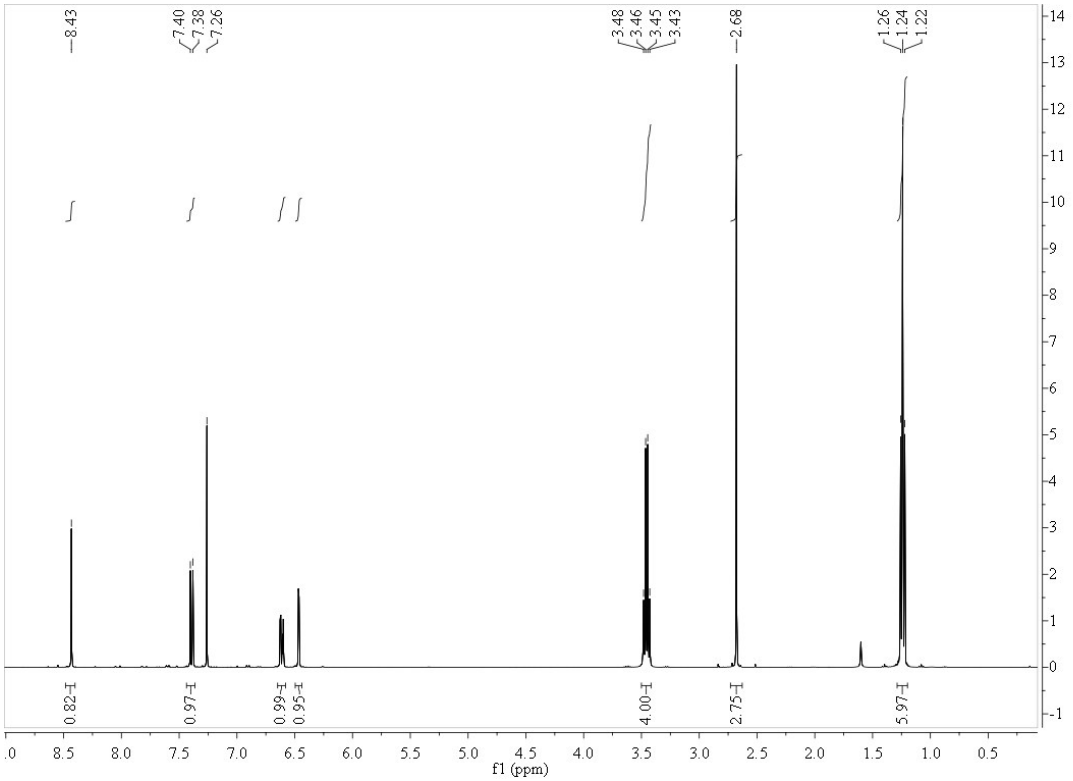


Fig. S1 ^{1}H NMR spectra of compound **2** in $CDCl_3$.

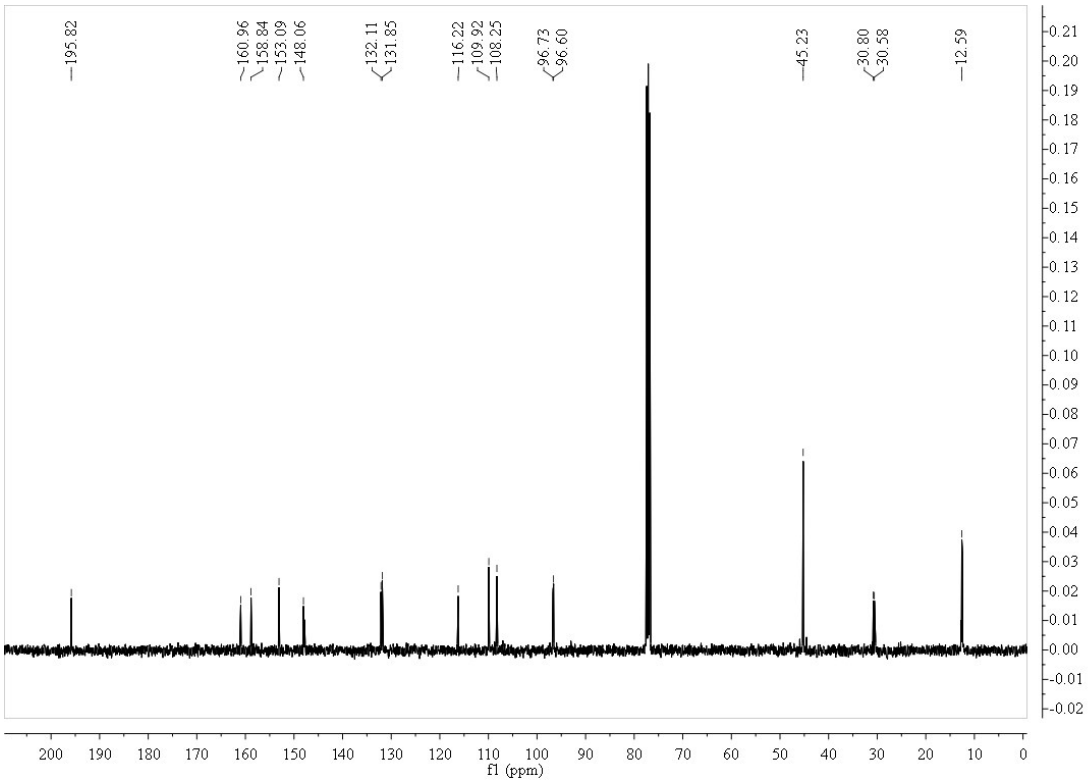


Fig. S2 ^{13}C NMR spectra of compound **2** in $CDCl_3$.

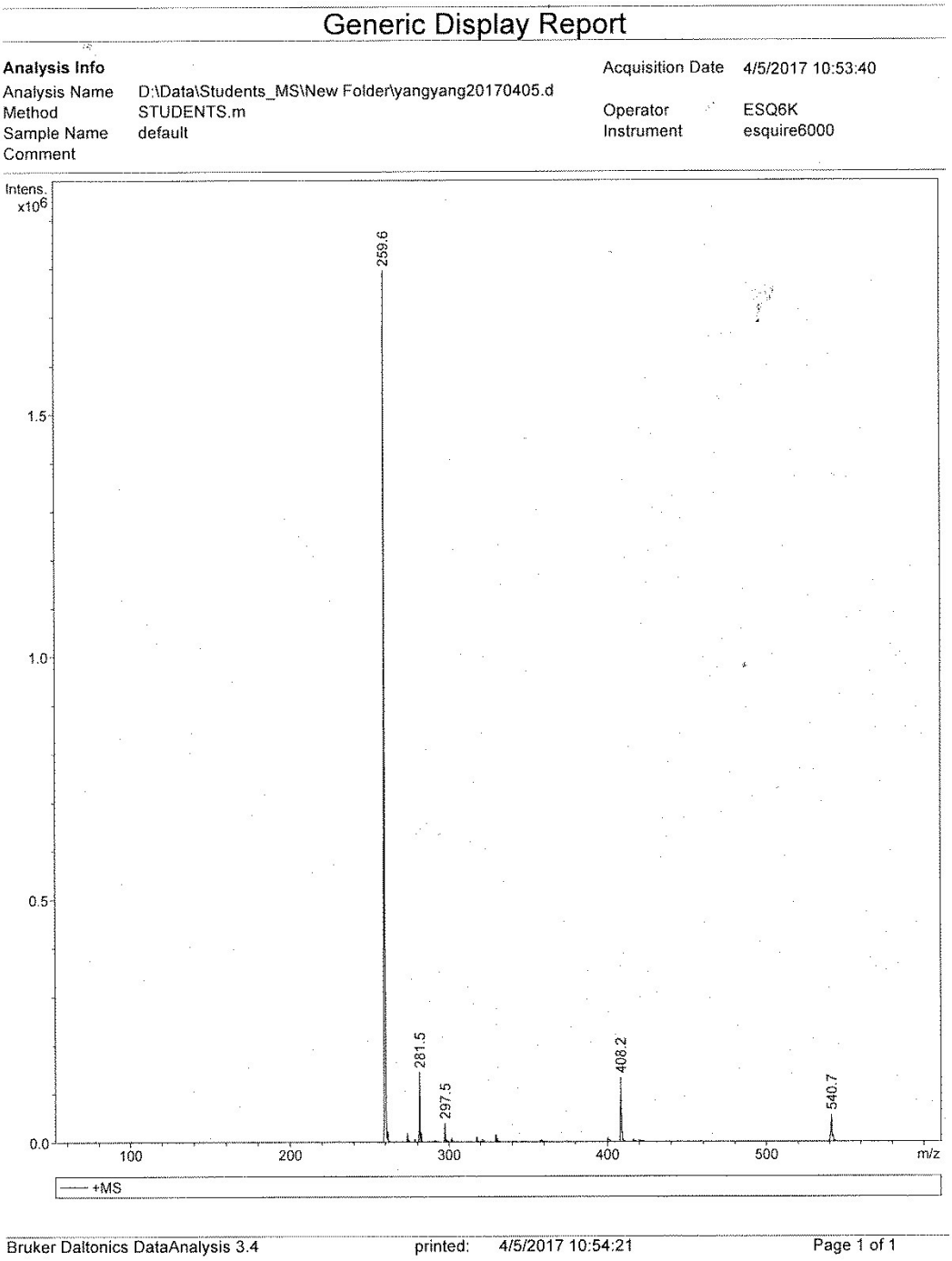


Fig. S3 ESI-MS spectra of compound **2**.

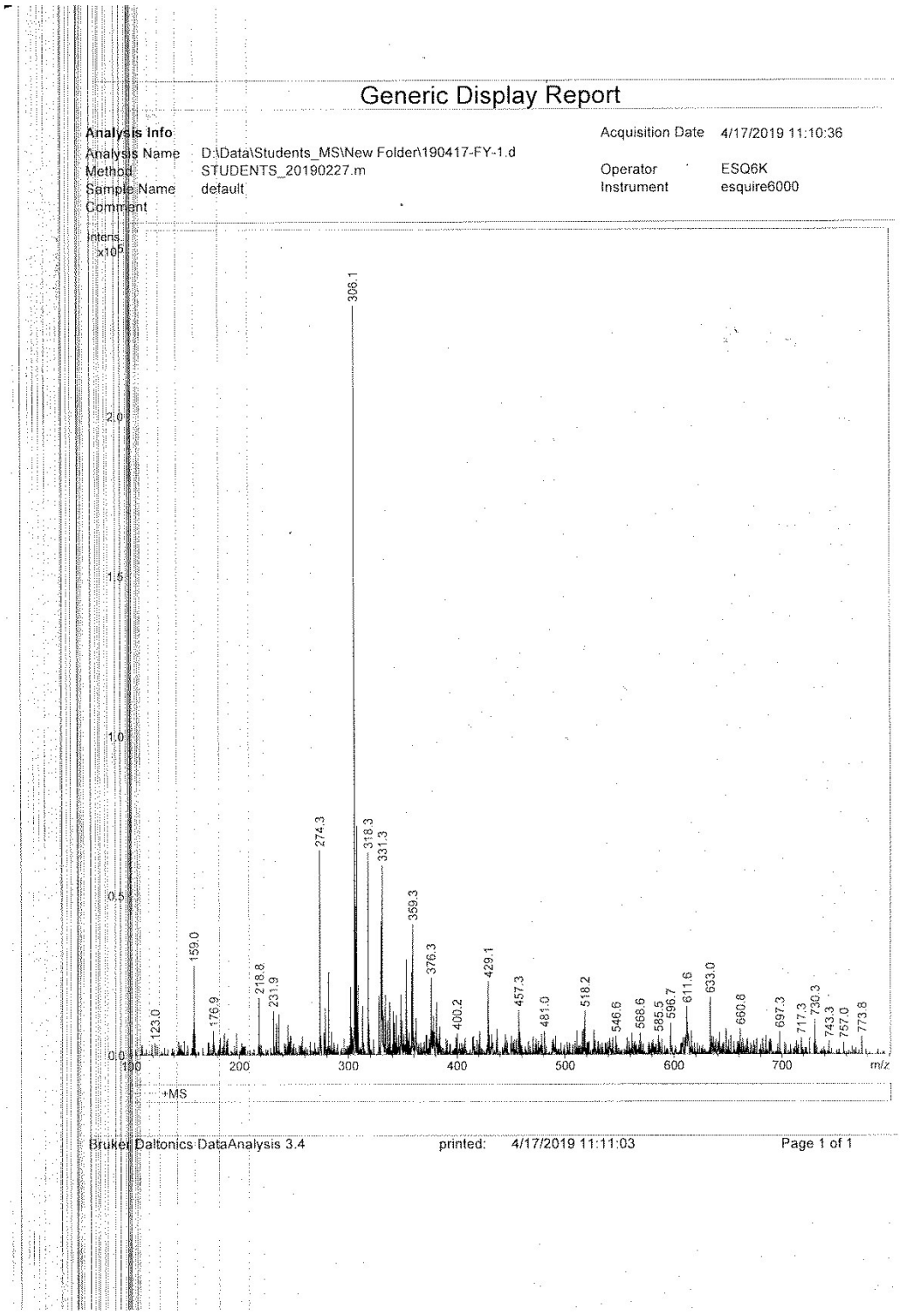


Fig. S4 ESI-MS spectra of compound **3**.

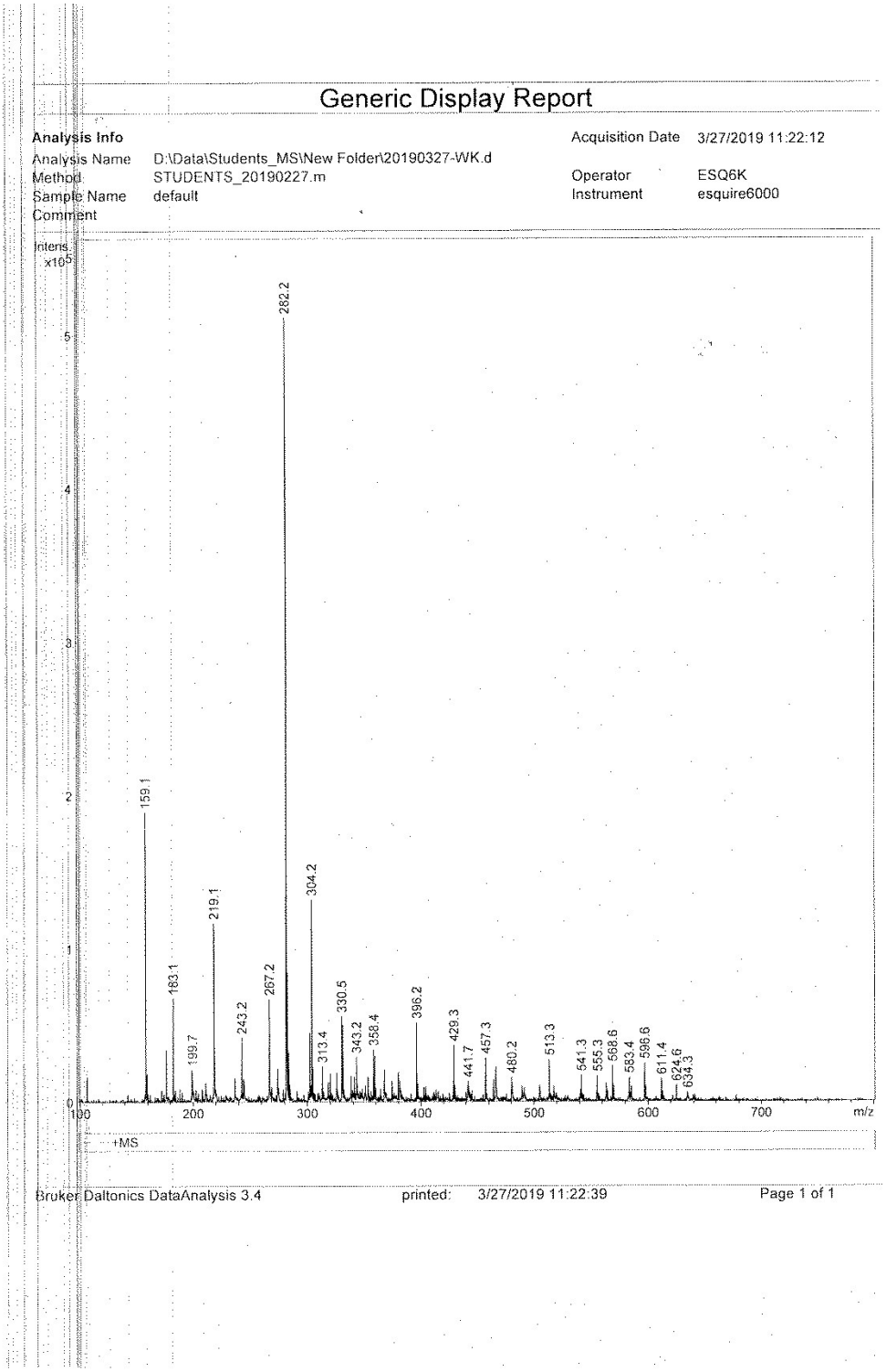


Fig. S5 ESI-MS spectra of compound **4**.

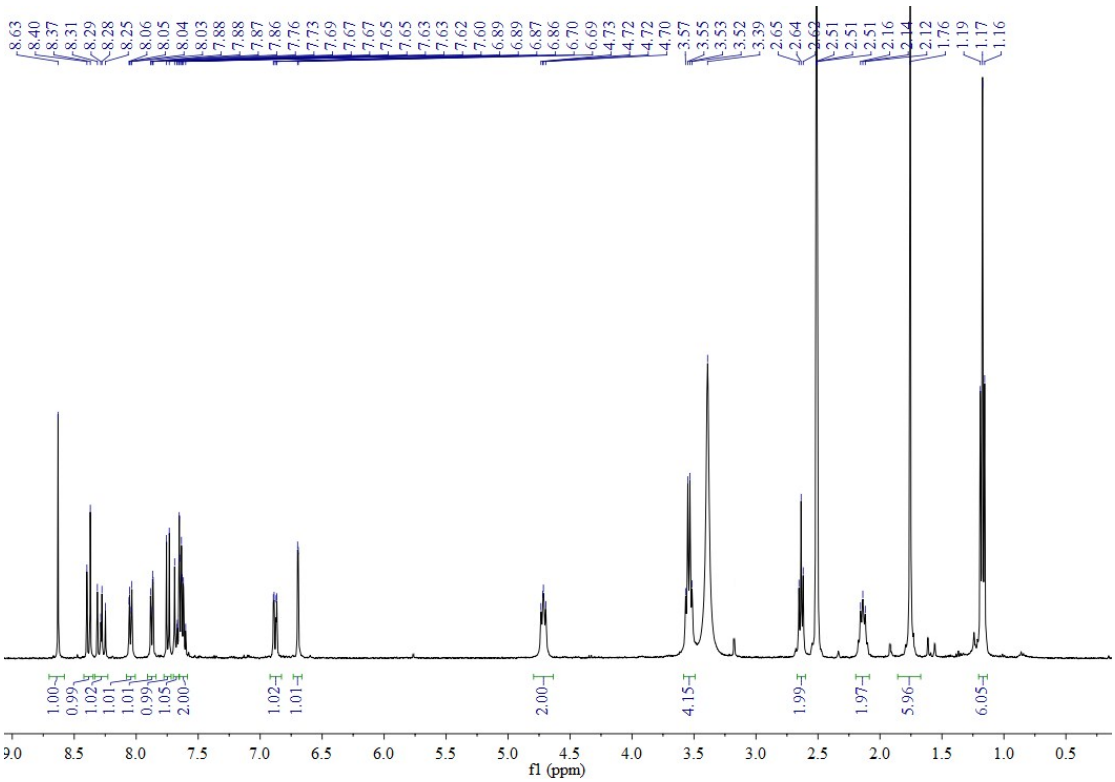


Fig. S6 ^{1}H NMR spectra of probe **1** in DMSO-d_6.

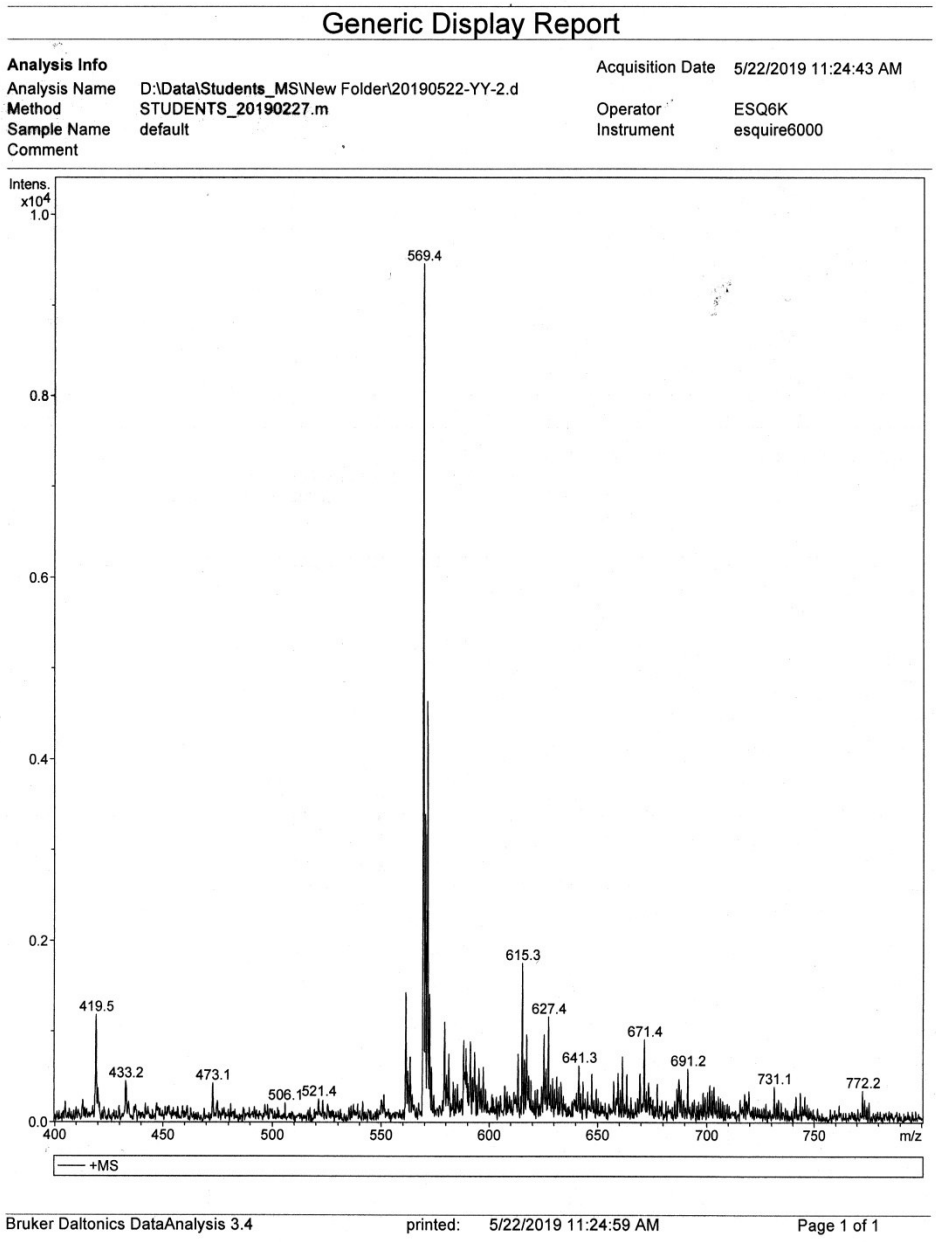


Fig. S7 ESI-MS spectra of probe **1**.

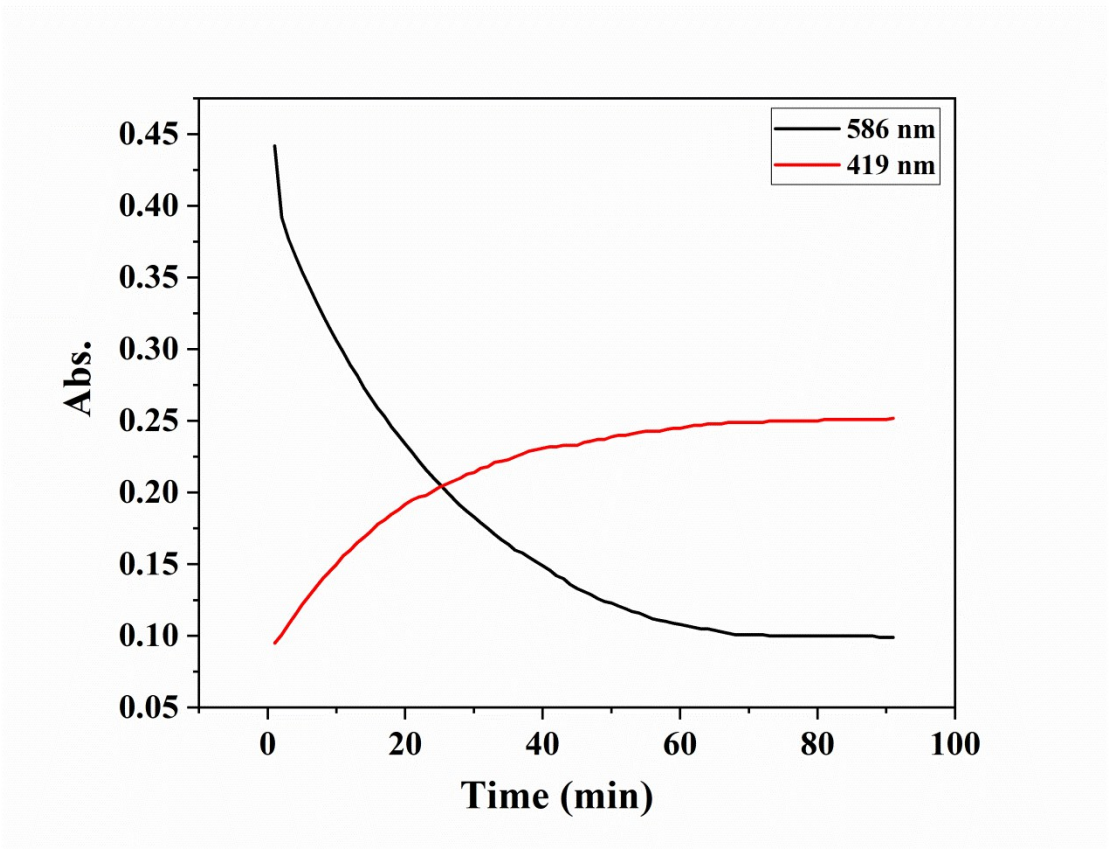


Fig. S8 The absorption intensity of probe **1** (10 μM) as a funciton of time at 586 nm and 419 nm in the presence of Cys (250 μM).

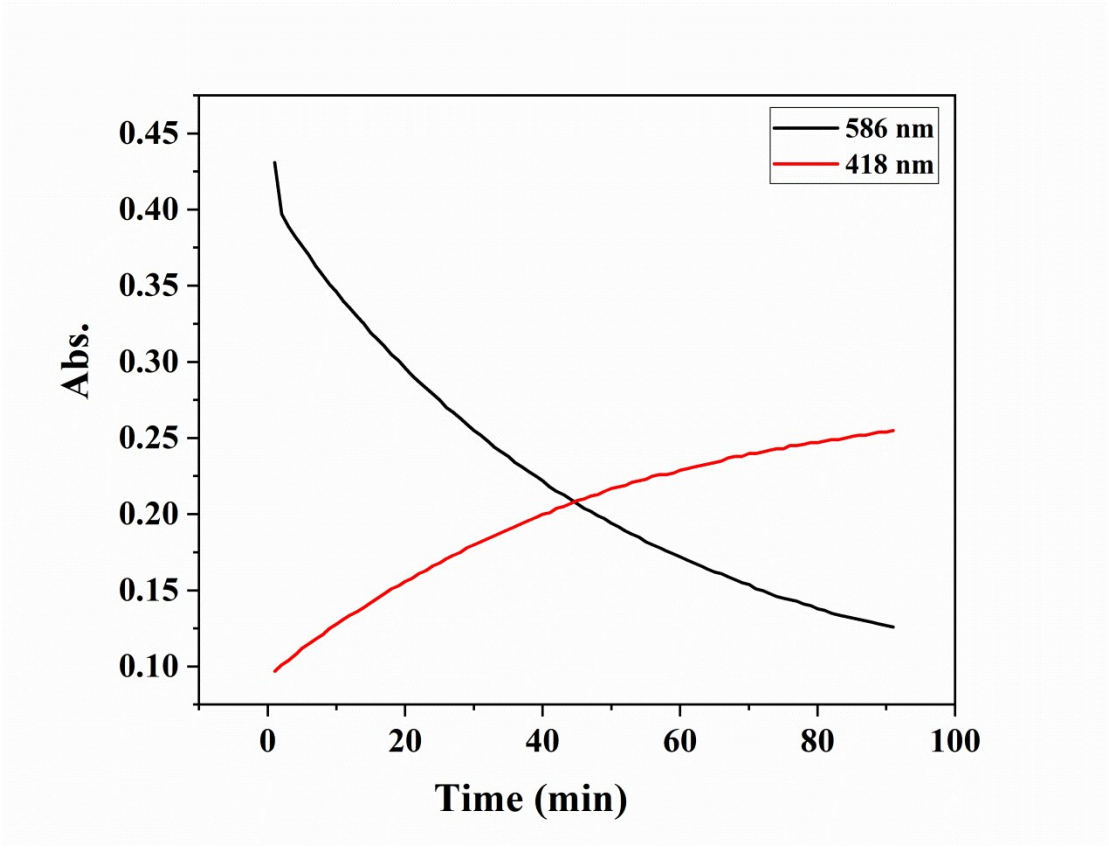


Fig. S9 The absorption intensity of probe **1** (10 μM) as a funciton of time at 586 nm and 419 nm in the presence of Hcy (250 μM).

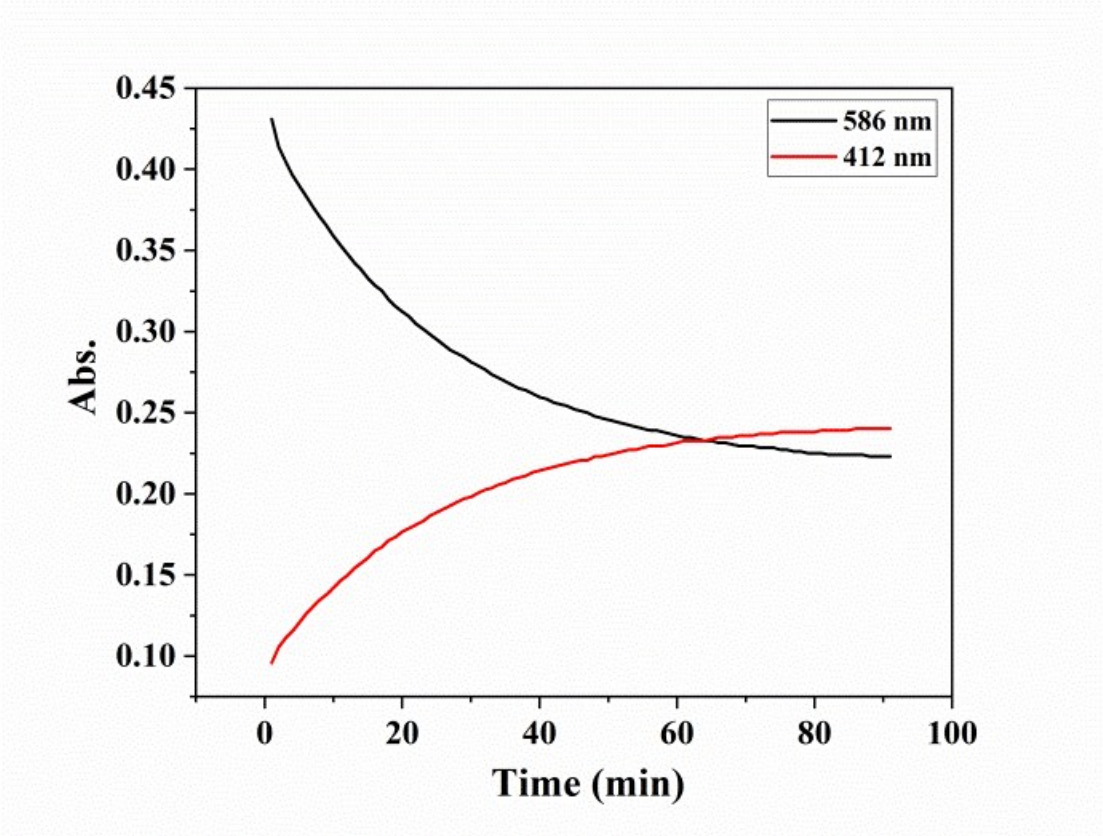


Fig. S10 The absorption intensity of probe **1** (10 μM) as a funciton of time at 586 nm and 419 nm in the presence of GSH (250 μM).

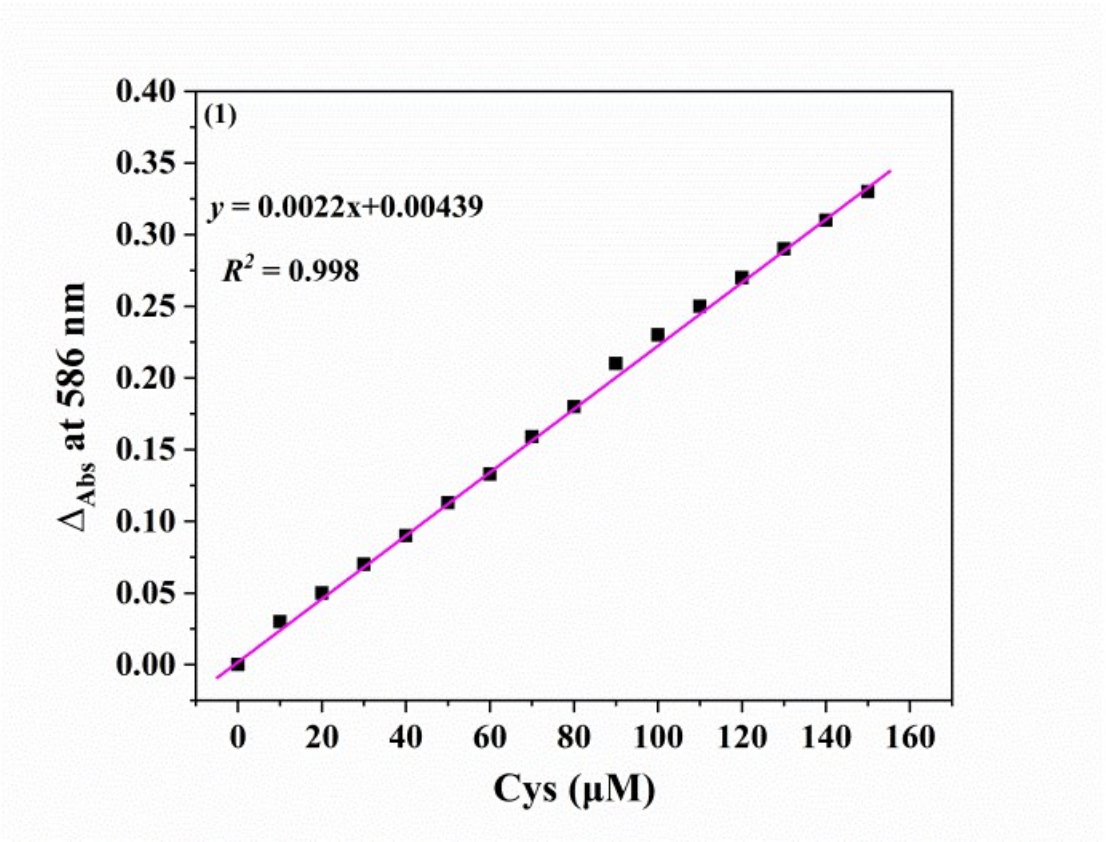


Fig. S11 The absorption response of **1** at 586 nm and as a function of Cys concentration.

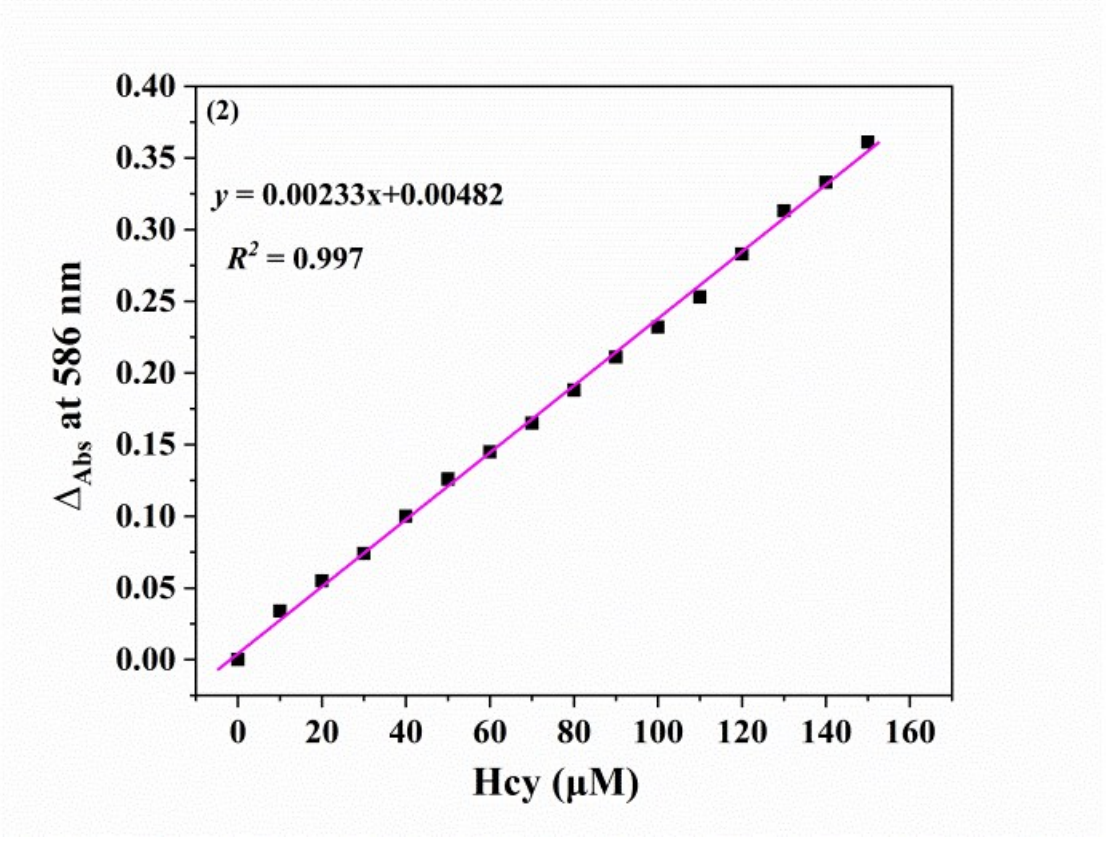


Fig. S12 The absorbtion response of **1** at 586 nm and as a function of Hcy concentration.

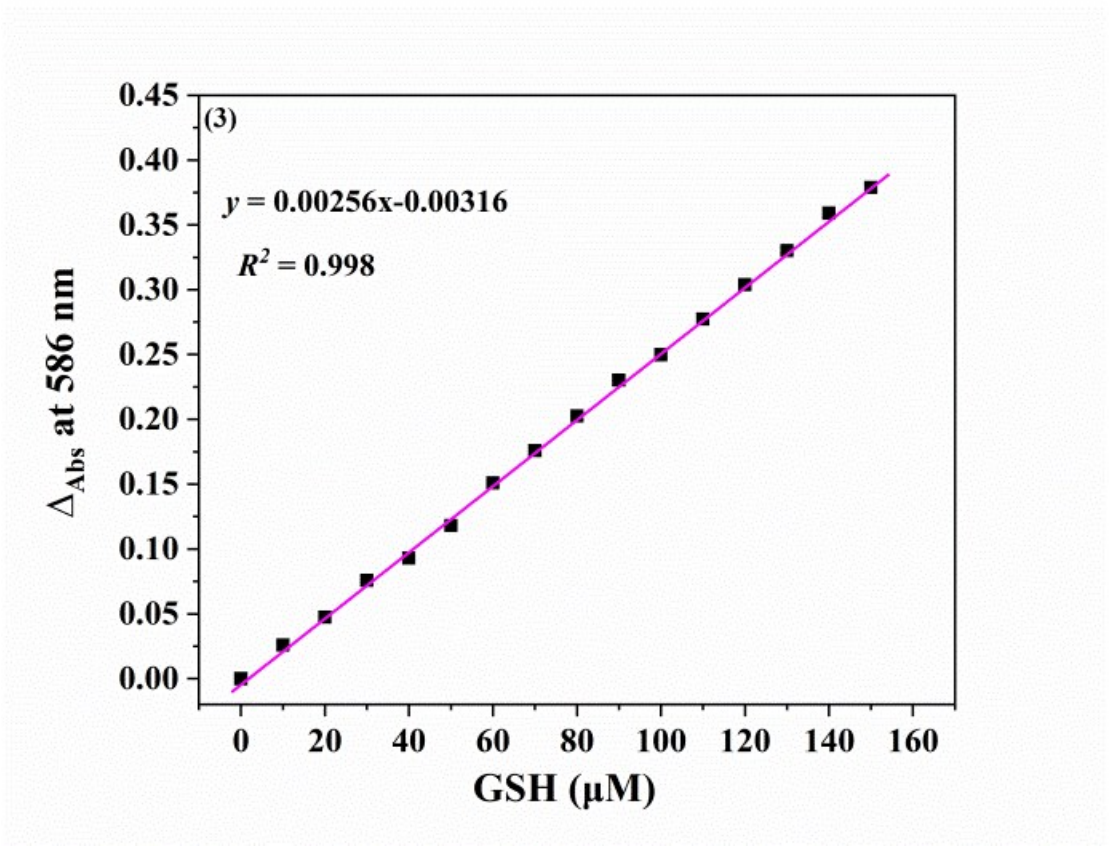


Fig. S13 The absorption response of **1** at 586 nm and as a function of GSH concentration.

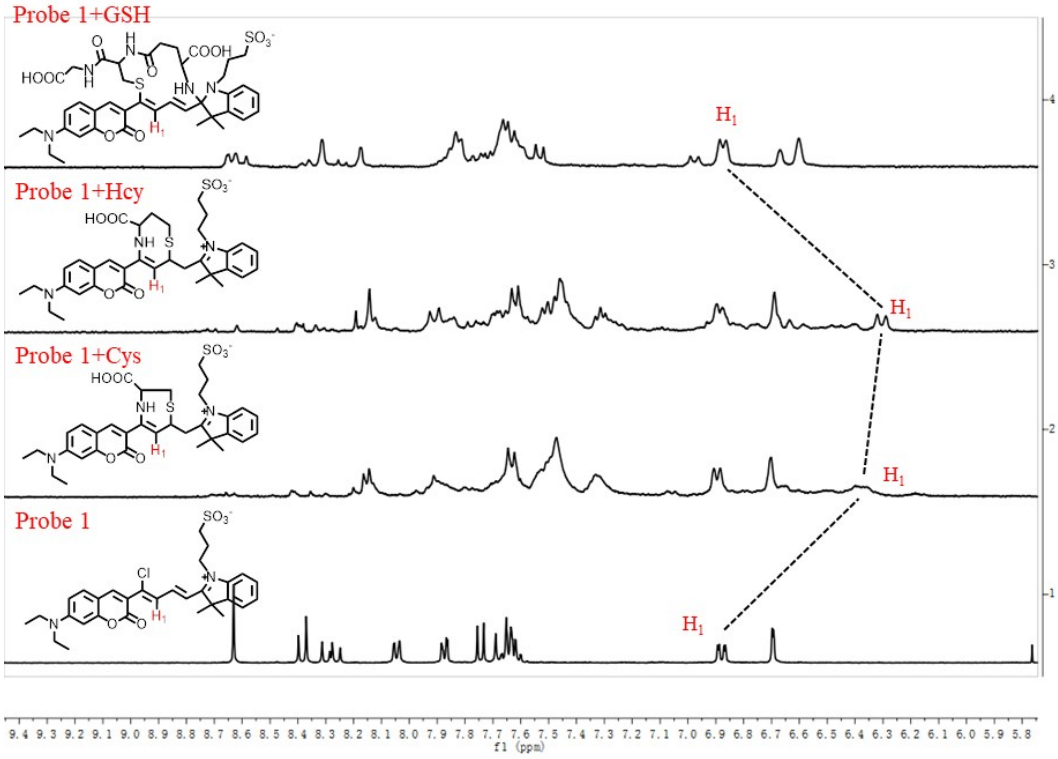
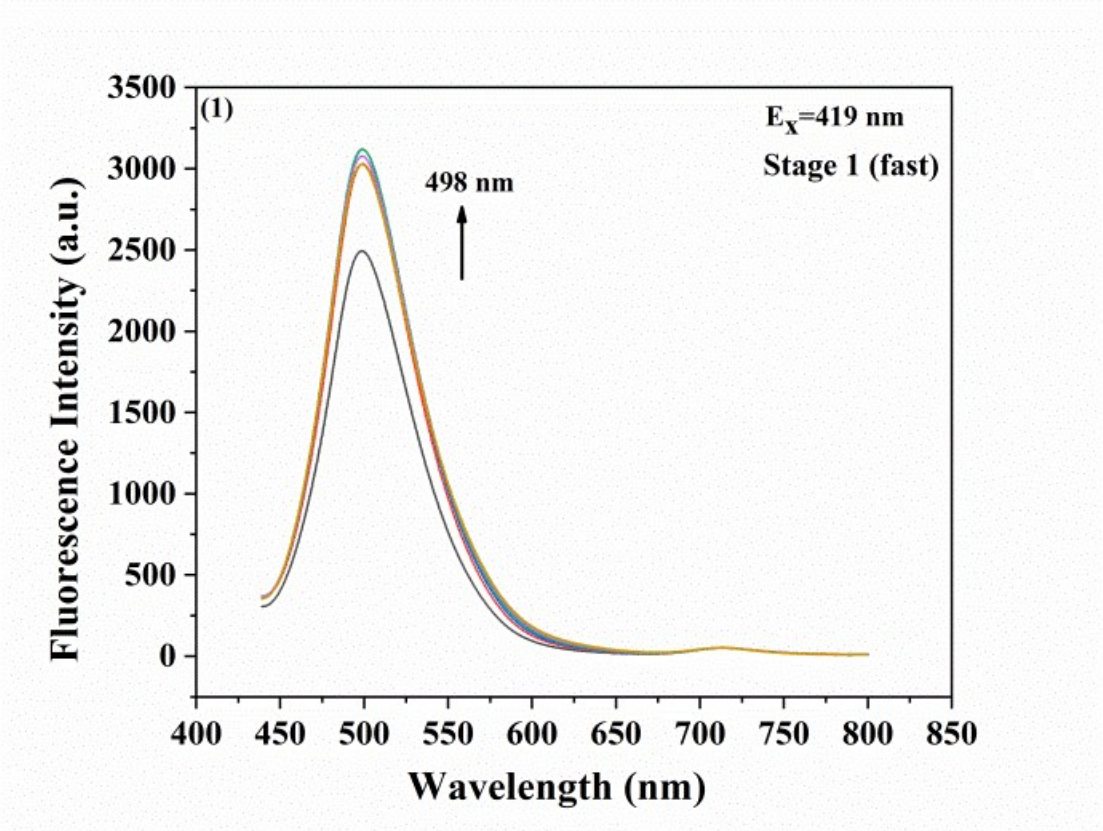
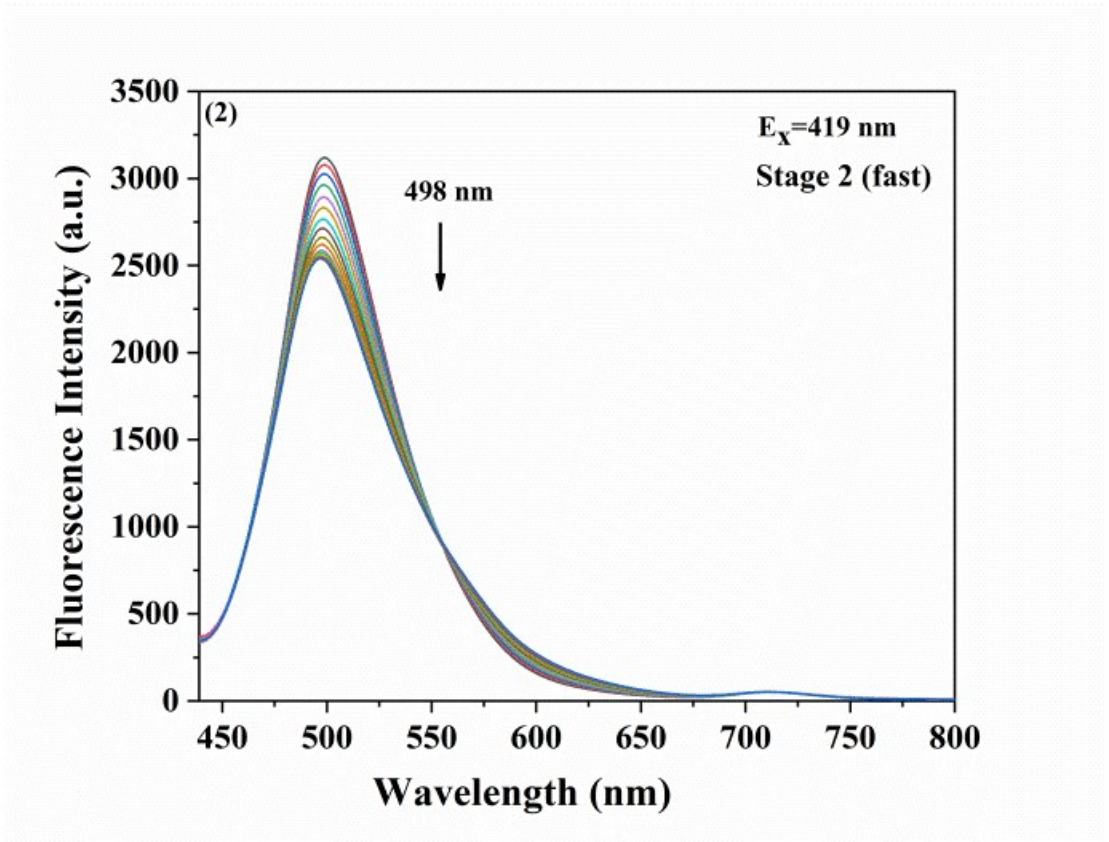


Fig. S14 ^{1}H NMR titration spectra of **1** in the presence of Cys, Hcy, and GSH.





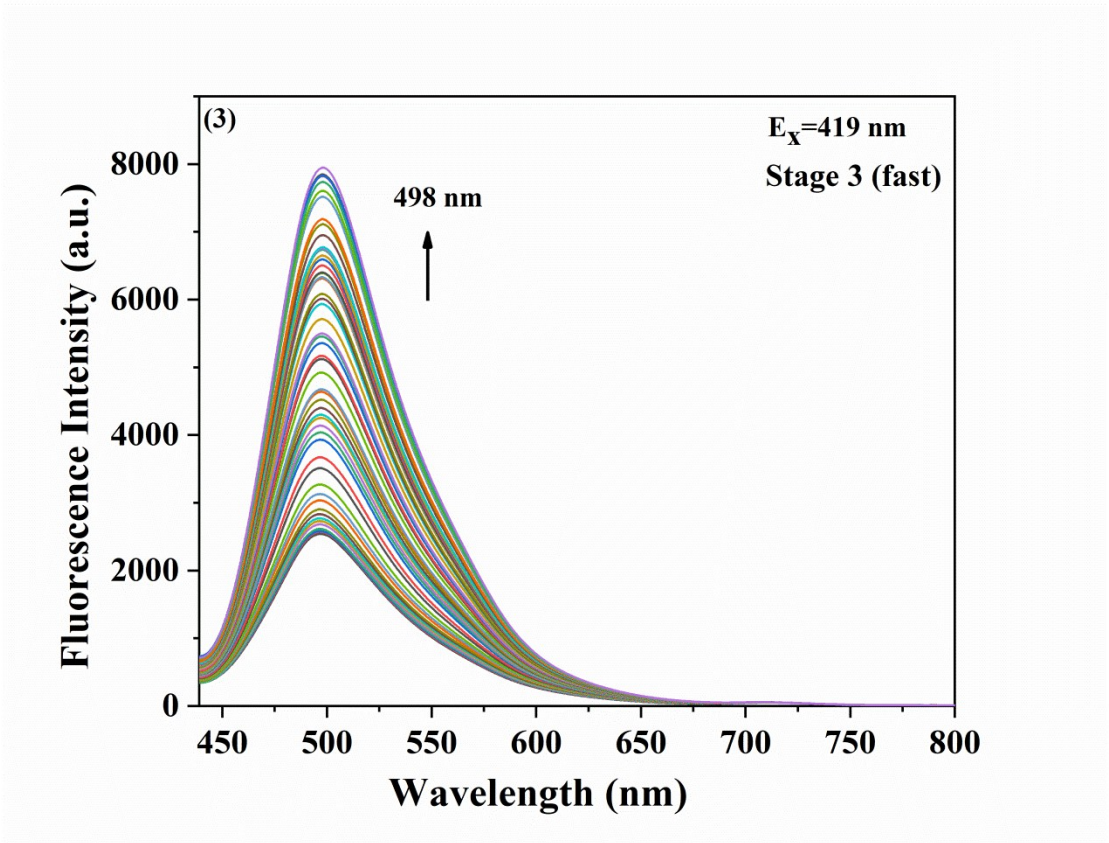
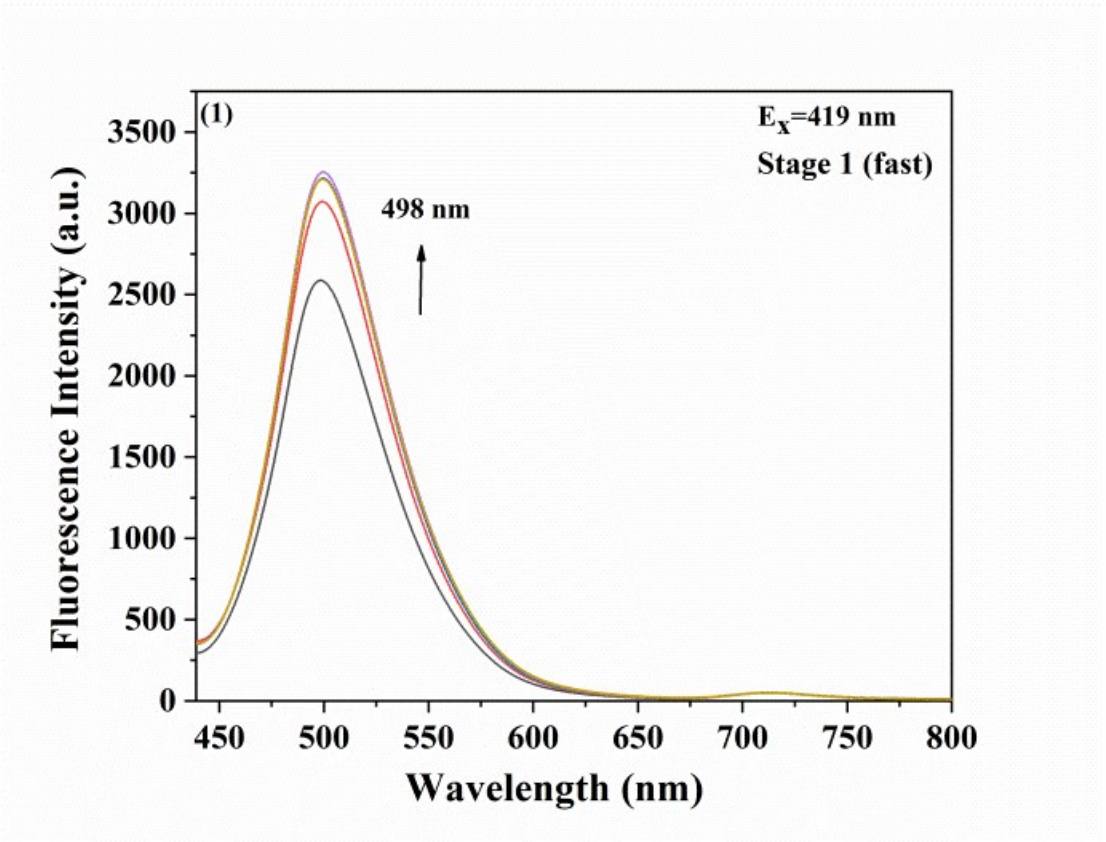


Fig. S15 The time-dependent emission spectra of **1** (10 μM) with 419 nm excitation upon addition of 250 μM in phosphate-buffered saline (pH 7.4, 10 mM).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E_X=419 nm
Stage 1 (fast)
498 nm
Fluorescence Intensity (a.u.)
Wavelength (nm)
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1000
1500
2000
2500
3000
3500
450
500
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600
650
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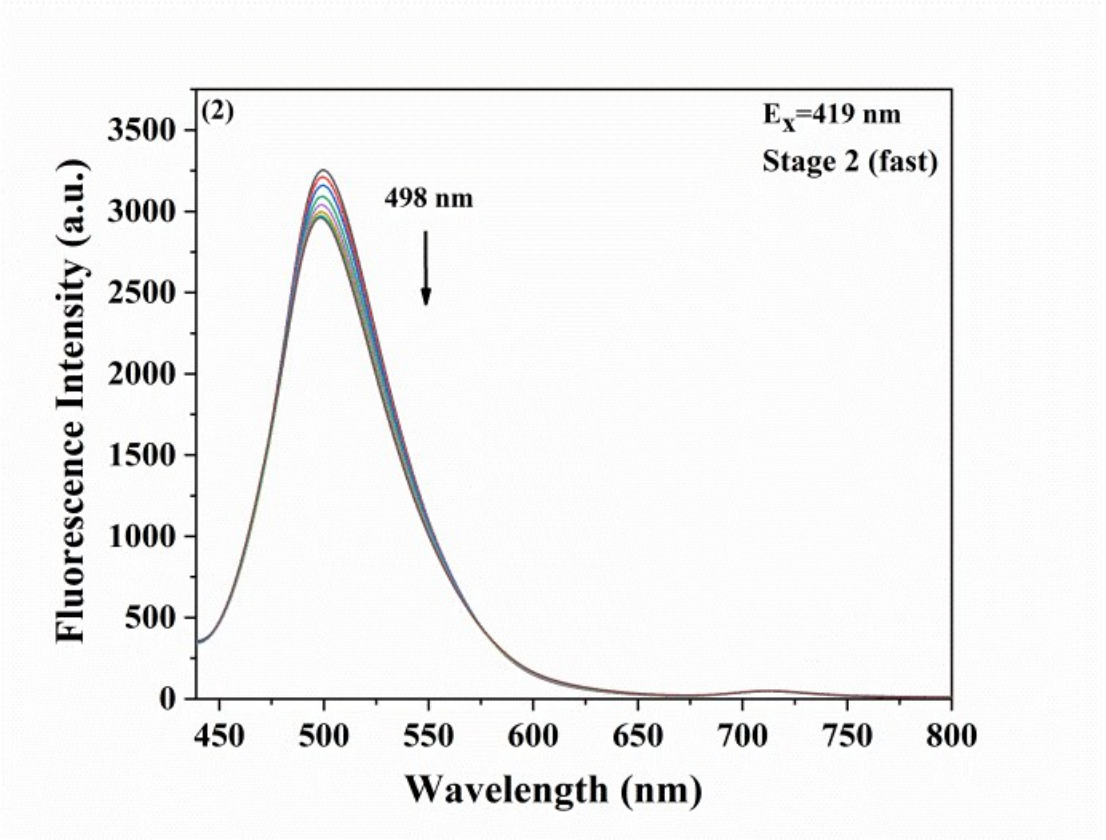
(2)
E_X=419 nm
Stage 2 (fast)
498 nm
Fluorescence Intensity (a.u.)
Wavelength (nm)
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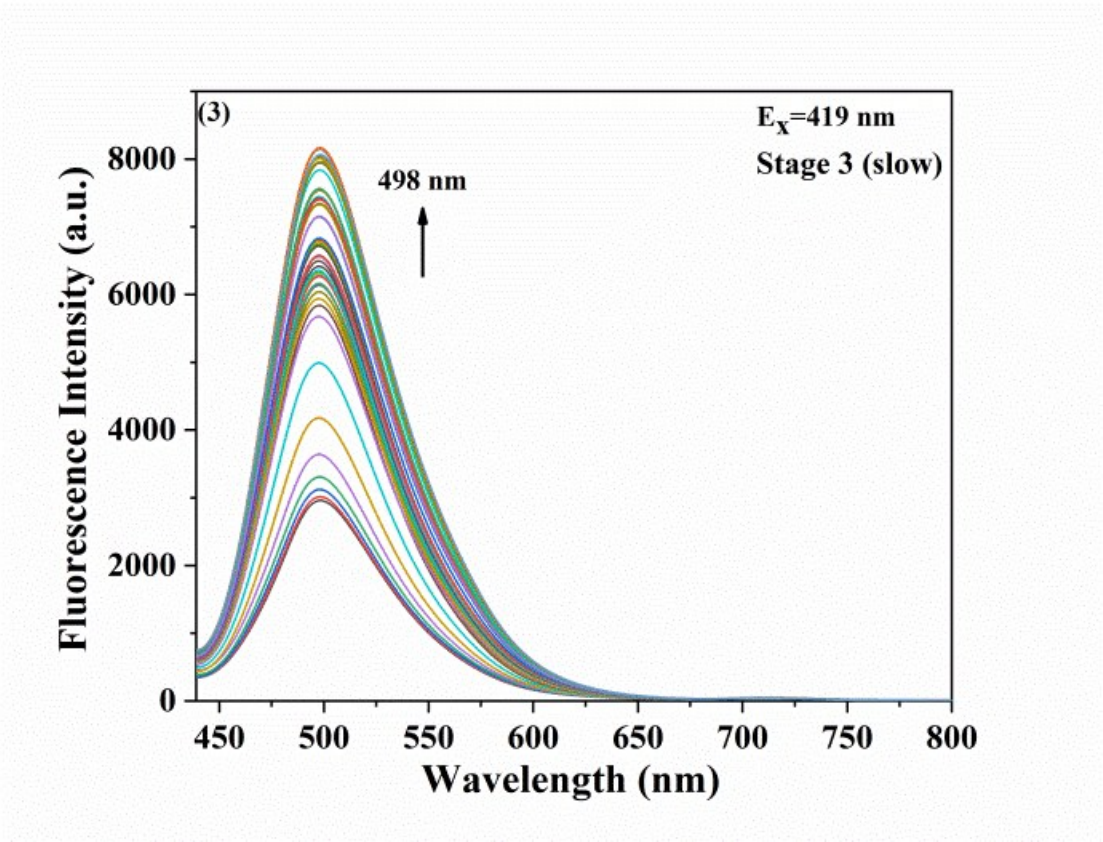
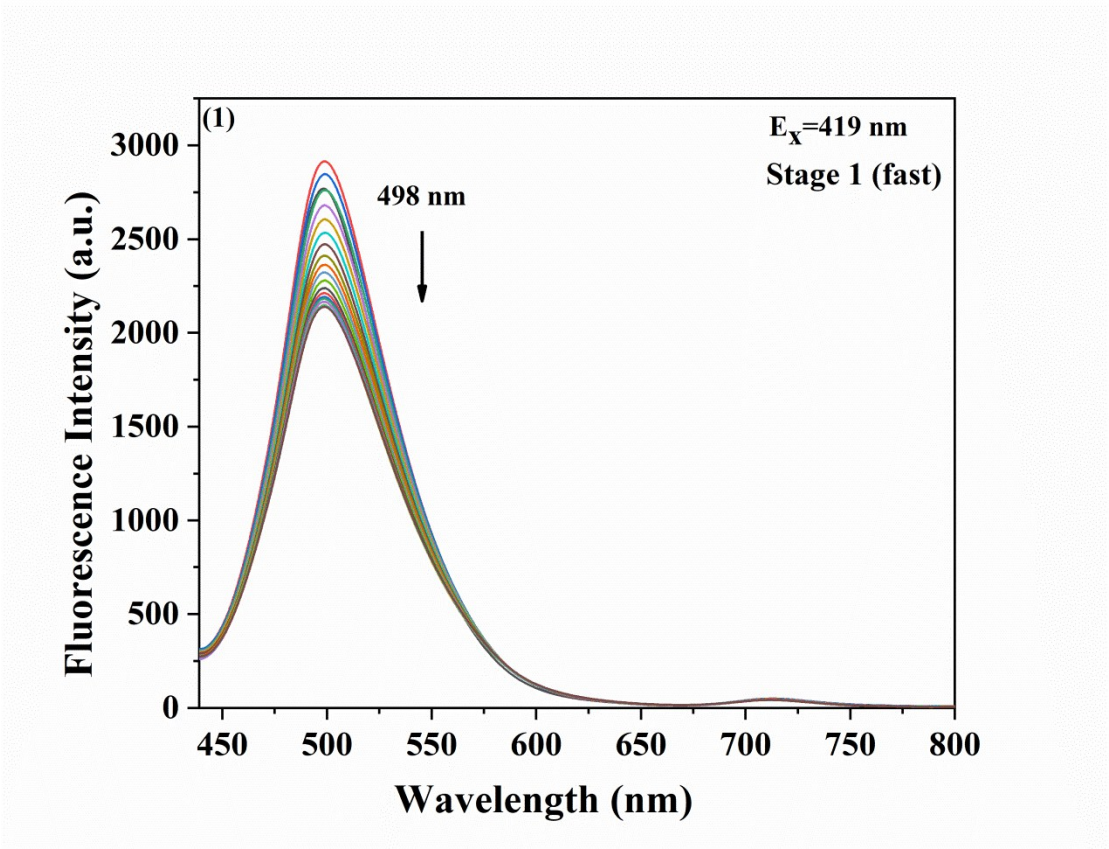


Fig. S16 The time-dependent emission spectra of **1** (10 μM) with 419 nm excitation upon addition of 250 μM Hcy in phosphate-buffered saline (pH 7.4, 10 mM).



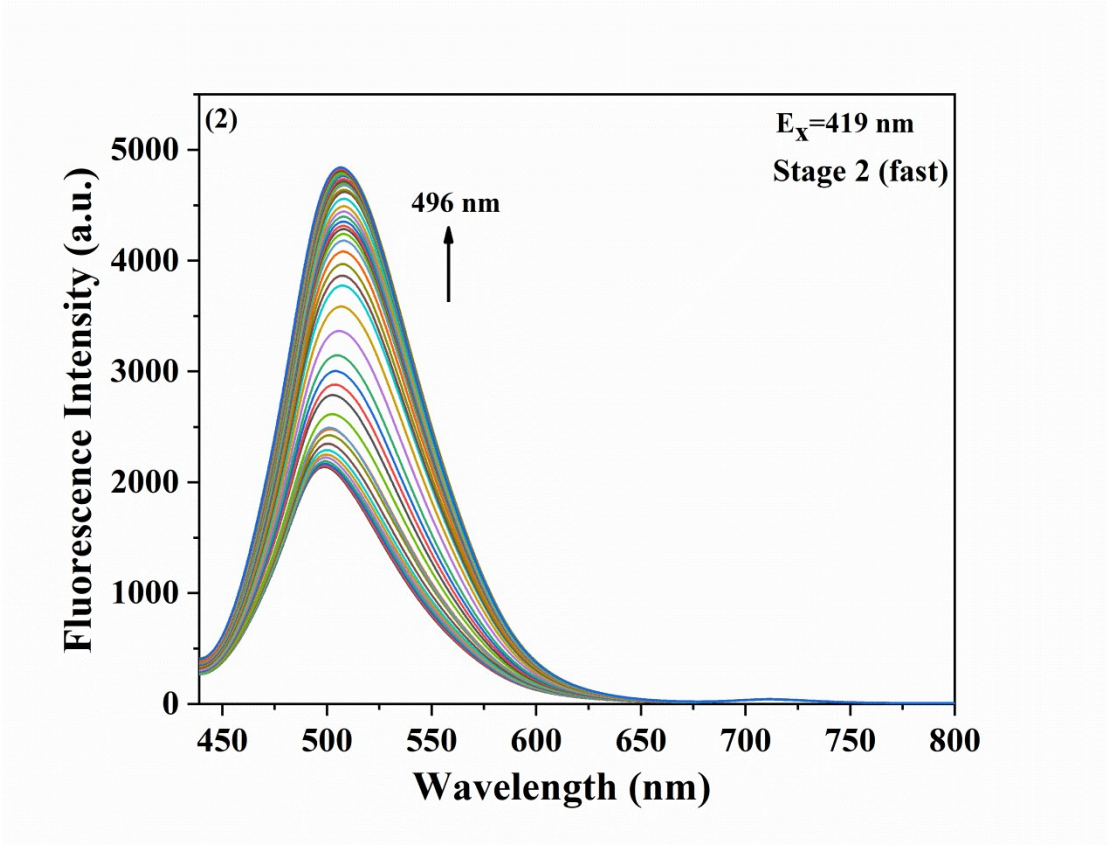


Fig. S17 The time-dependent emission spectra of **1** (10 μM) with 419 nm excitation upon addition of 250 μM GSH in phosphate-buffered saline (pH 7.4, 10 mM).

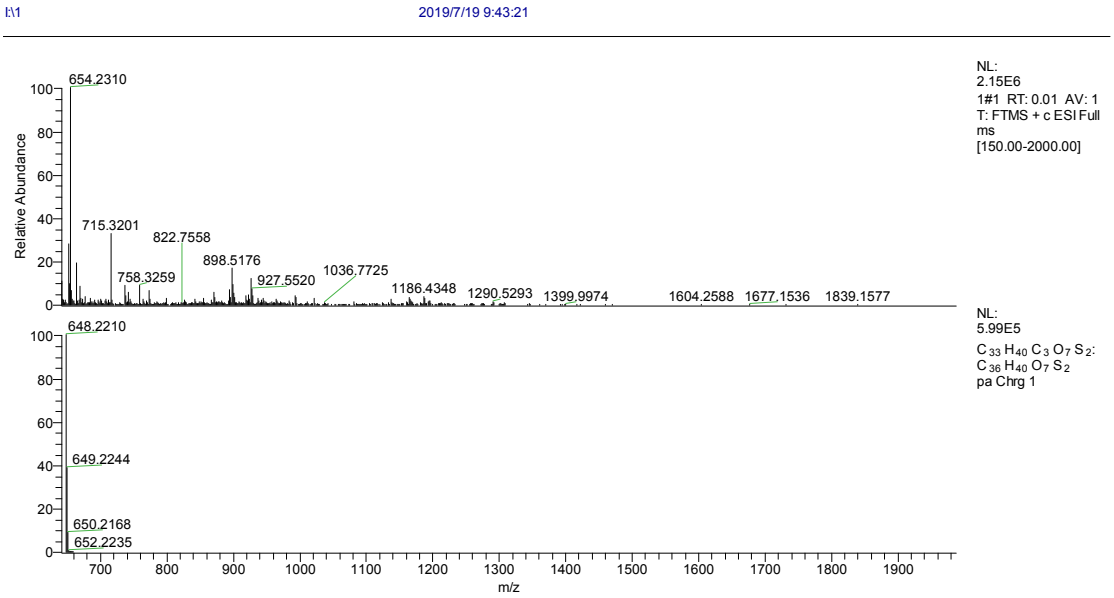


Fig. S18 The HRMS spectra of **1** with Cys.

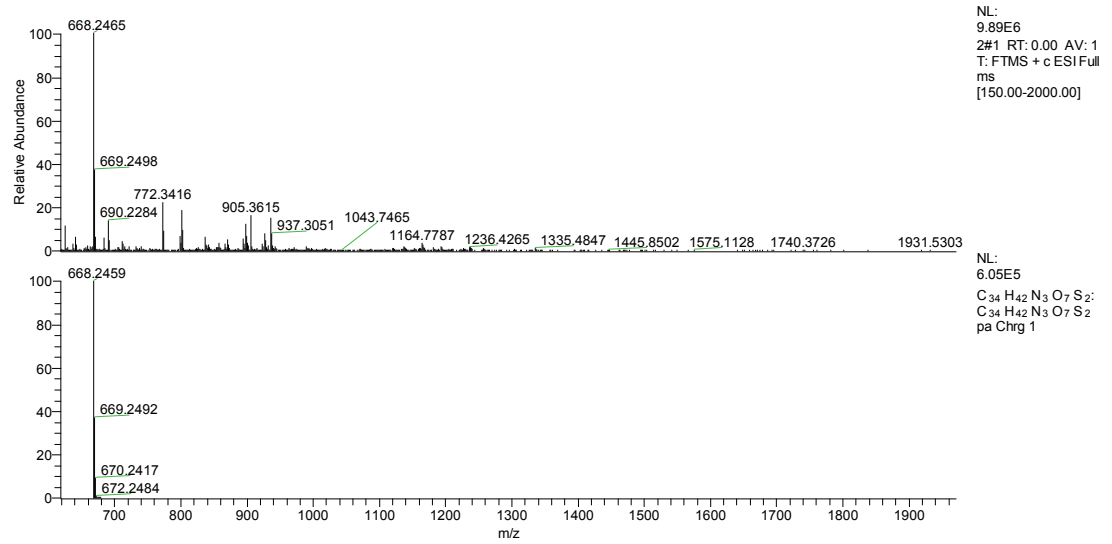


Fig. S19 The HRMS spectra of **1** with Hcy.

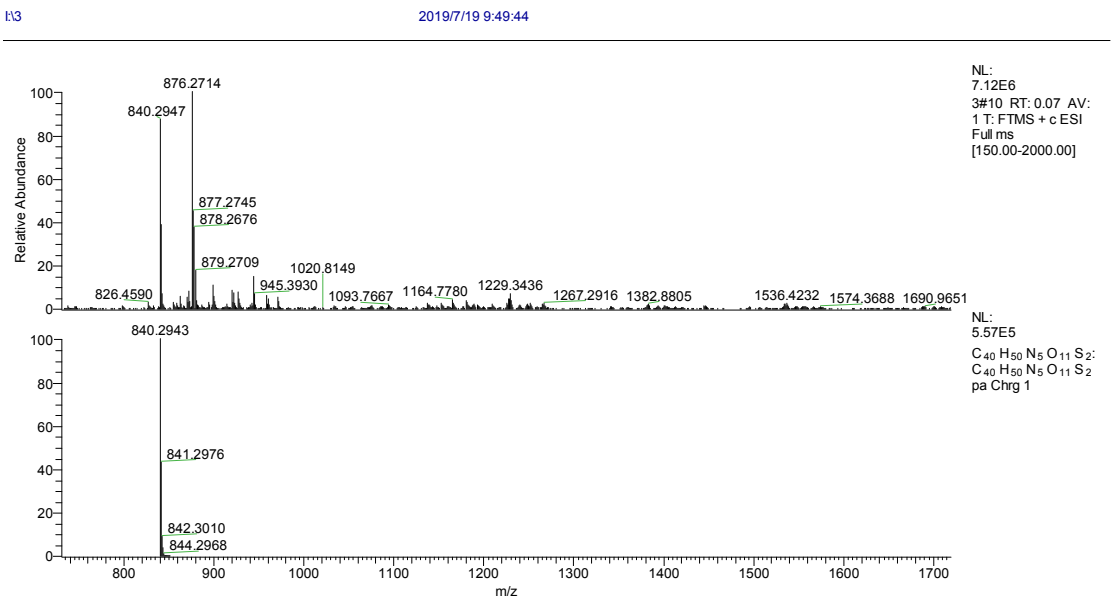


Fig. S20 The HRMS spectra of **1** with GSH.

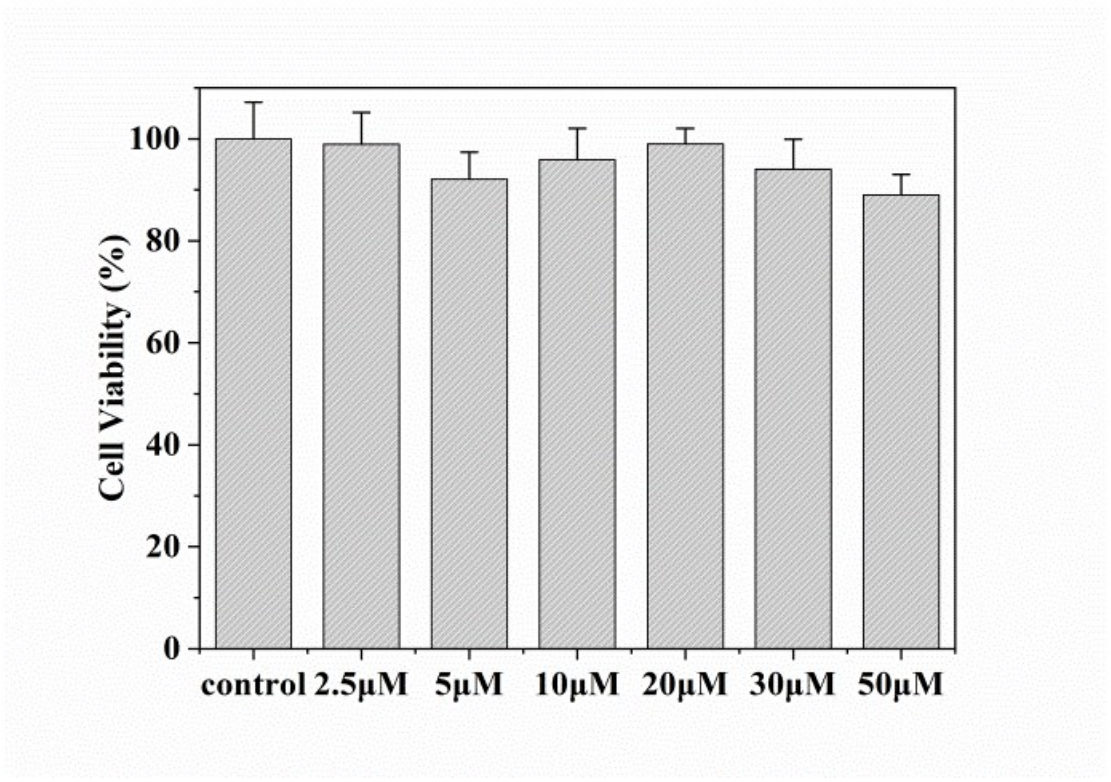


Fig. S21 Cell viability values (%) estimated by MTT assay with HeLa cells, which were cultured in the presence of 0-50 μM probe **1** for 24 h.