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Electronic Supplementary Information (ESI)

for

A colorimetric and near-infrared fluorescent probe for biothiols and its application in living cells

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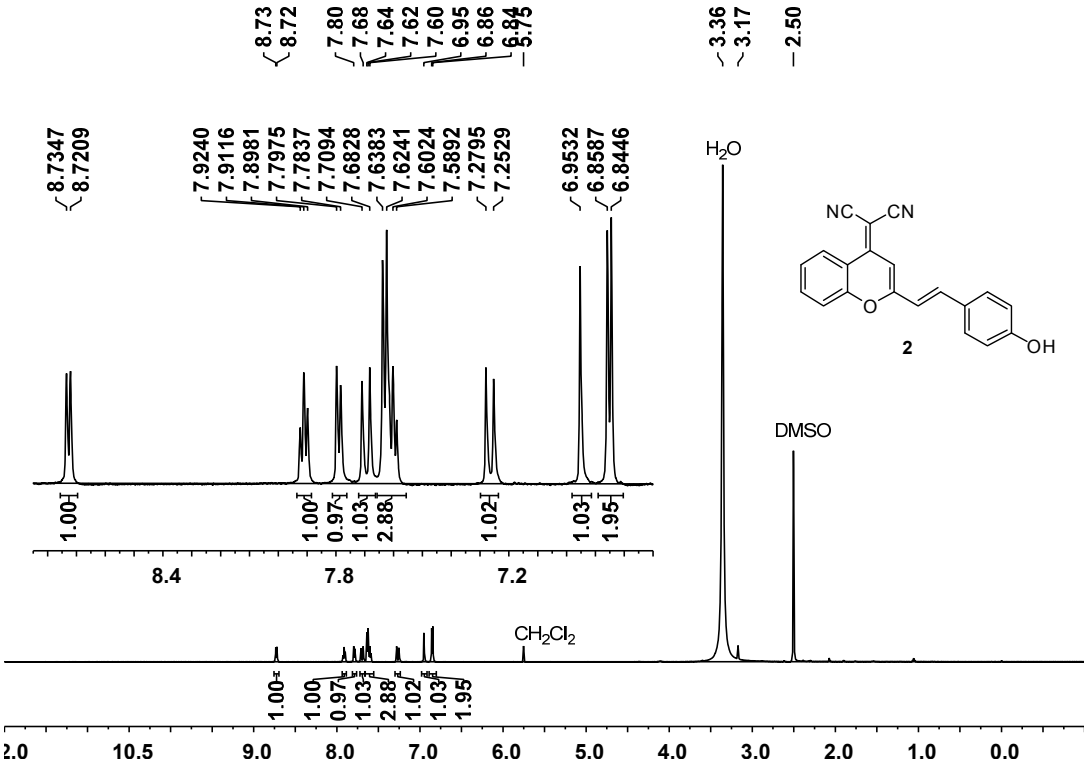
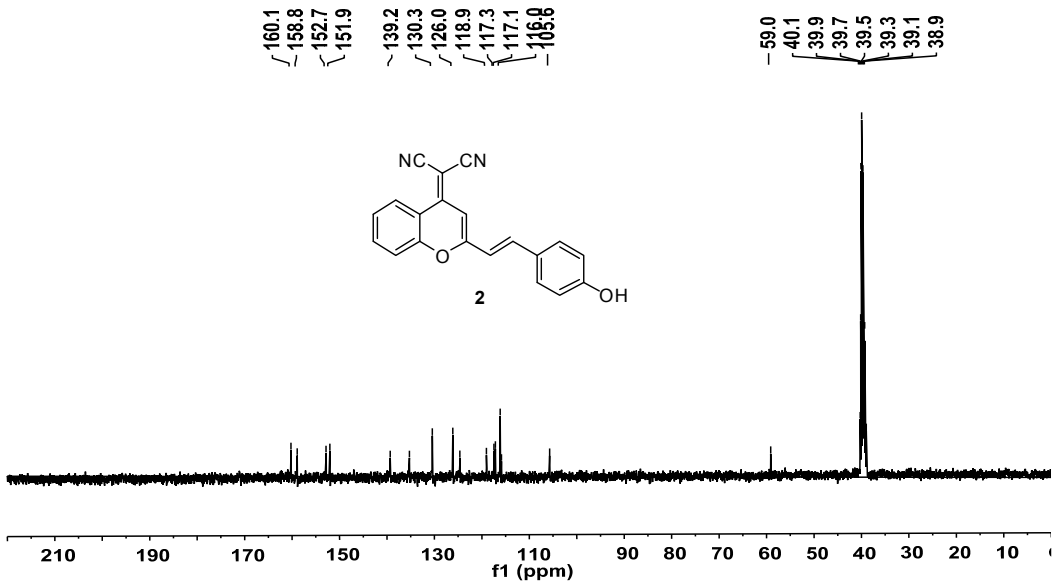
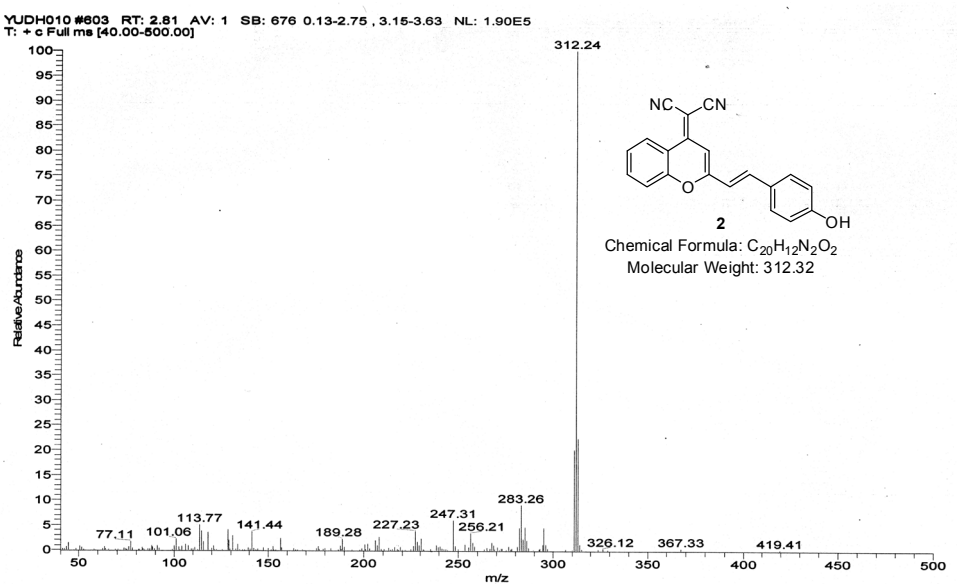
1. Structure characterizations of compound 2 and probe 1



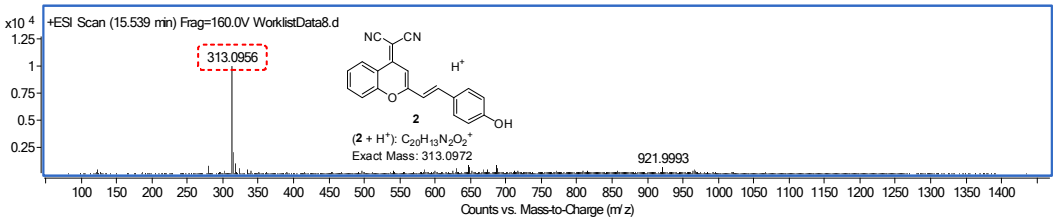
^{1}H-NMR spectrum of compound **2** in d_6-DMSO



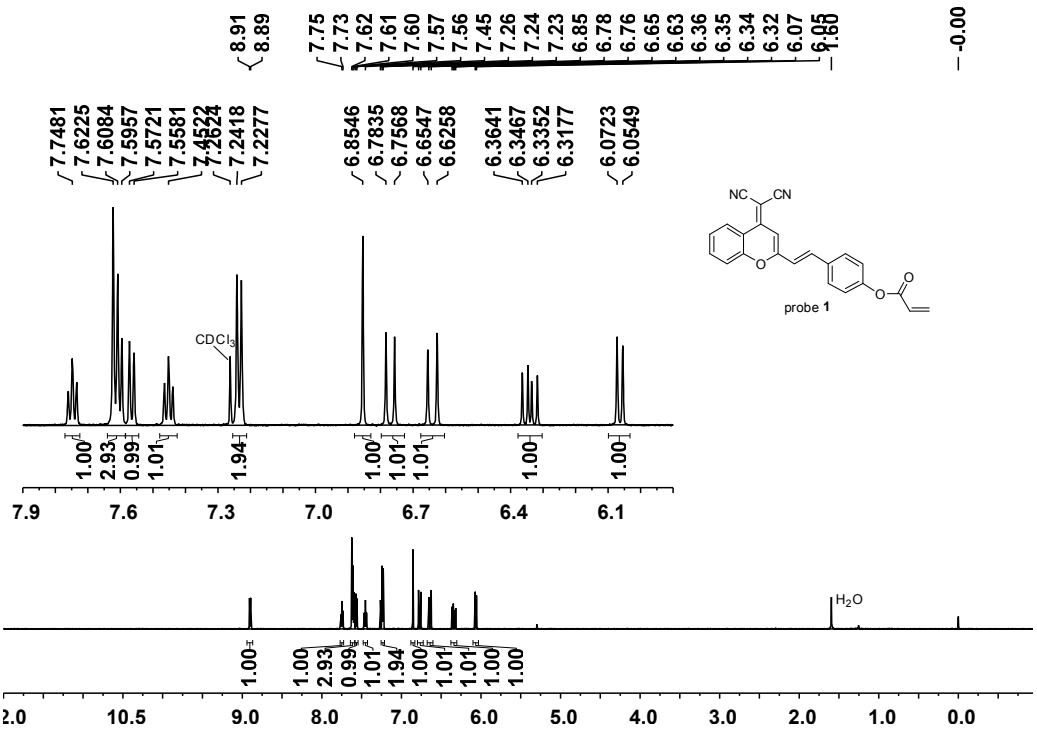
^{13}C-NMR spectrum of compound **2** in d_6-DMSO



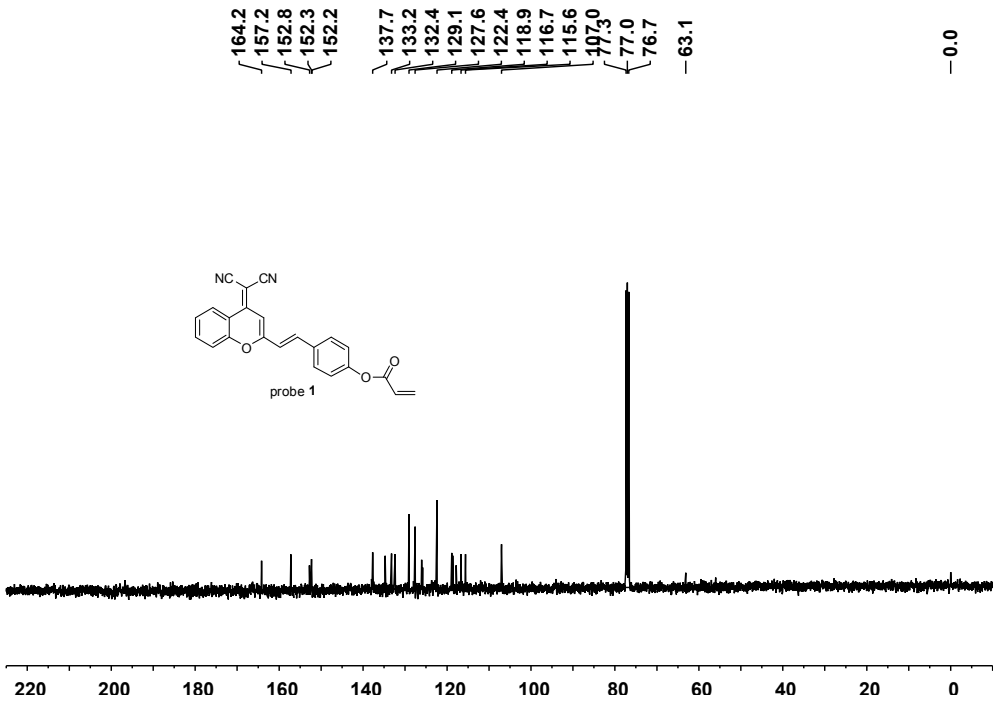
MS (EI) spectrum of compound **2**



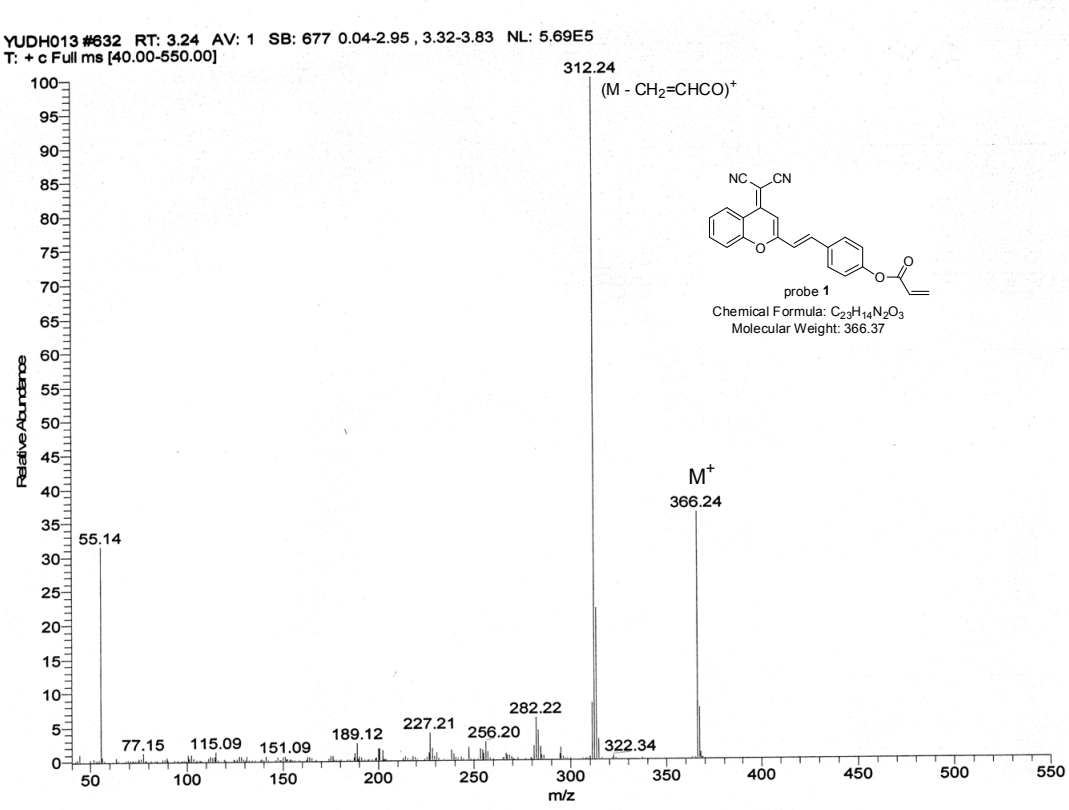
HR-MS spectrum of compound **2**



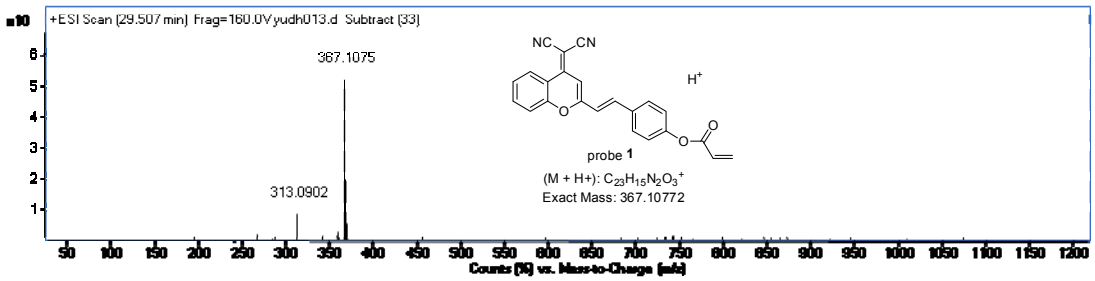
^{1}H-NMR spectrum of probe **1** in $CDCl_3$



^{13}C-NMR spectrum of probe **1** in d_6-DMSO



MS (EI) spectrum of probe **1**



HR-MS spectrum of probe **1**

2. Additional spectra

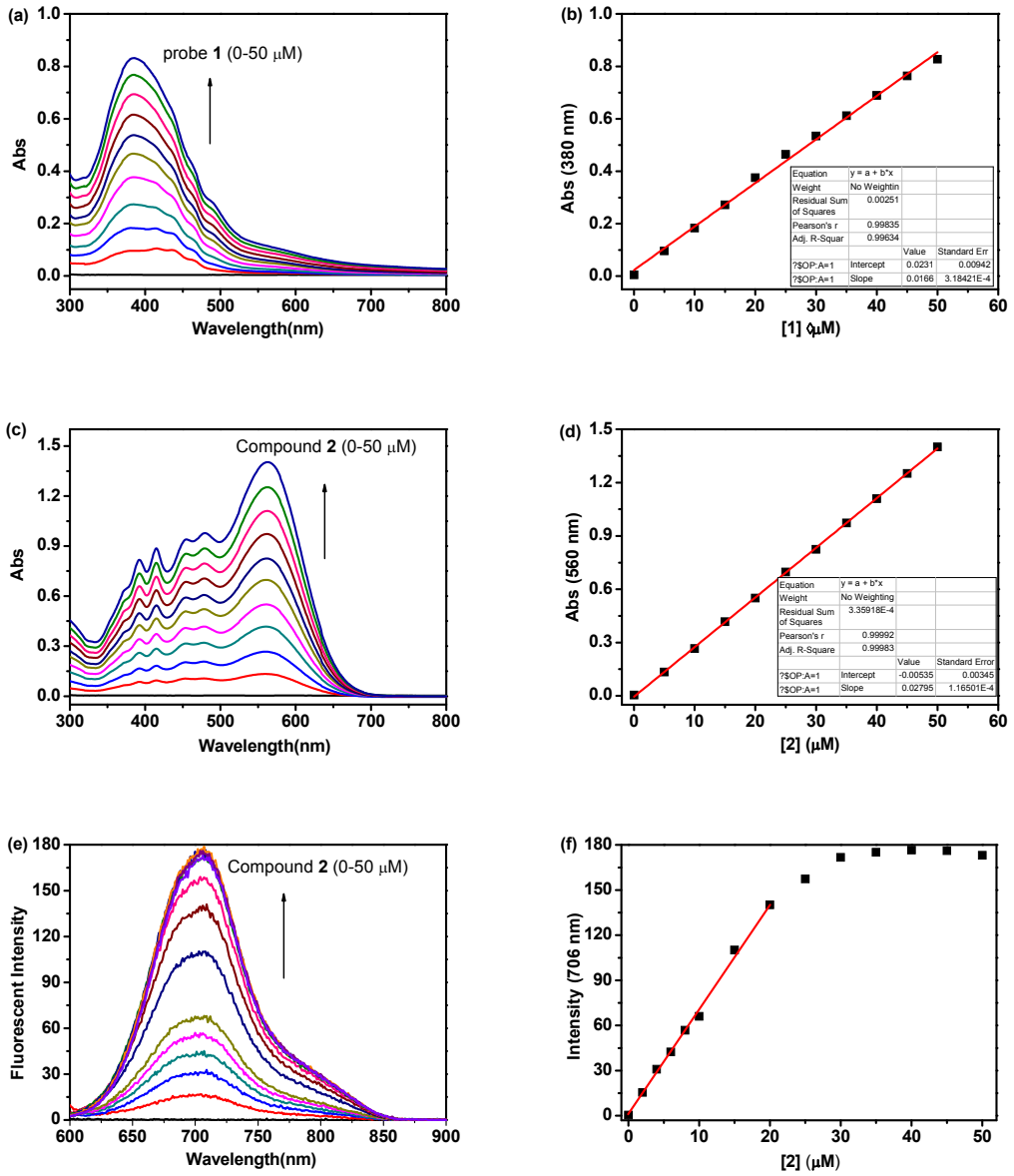


Fig. S1 (a) Absorption spectra of probe **1** at different concentrations. (b) Absorbance intensity changes at 380 nm of probe **1** as a function of its concentration from 0-50 μM. (c) Absorption

spectra of compound **2** at different concentrations. (d) Absorbance intensity changes at 560 nm of compound **2** as a function of its concentration from 0-50 μM. (e) Fluorescence spectra of compound **2** at different concentrations. (f) Fluorescence intensity changes of compound **2** at 706 nm as a function of its concentration from 0-50 μM. All spectra were collected in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37 °C. For fluorescence measurement, λ_{ex} = 560 nm with slit width: (10, 10).

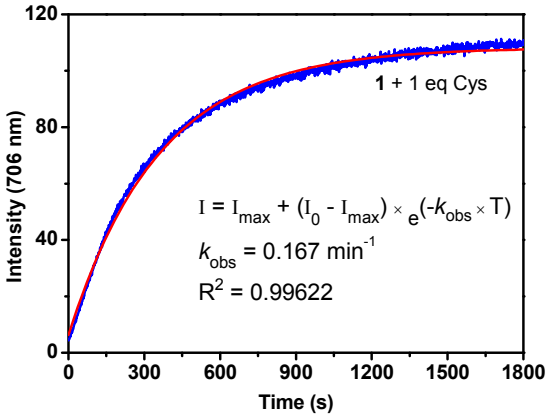


Fig. S2 Time-dependent of fluorescence kinetics spectra of probe **1** (20 μM) upon addition of Cys (20 μM) in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37˚C. The reaction is monitored at 706 nm. λ_{ex} = 560 nm, slit width: (10, 10).

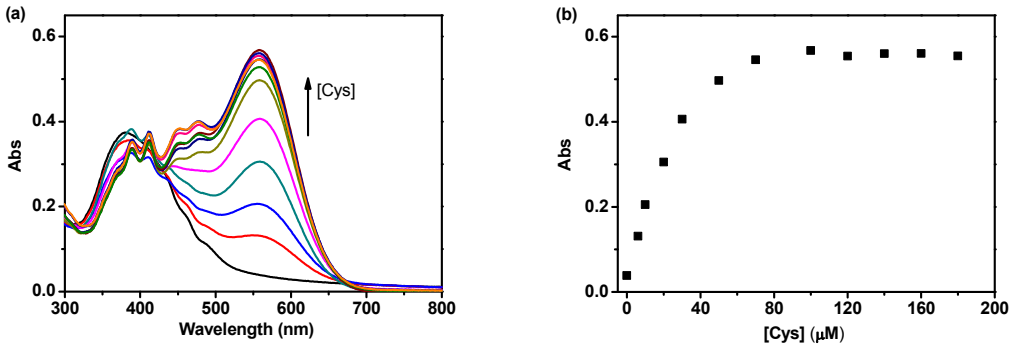


Fig. S3 (a) Absorption spectra changes of probe **1** (20 μM) upon addition of different concentrations of Cys in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37 °C. Final concentration of Cys: 0, 6, 10, 30, 40, 50, 60, 70, 100, 120, 140, 160 and 180 μM, respectively. (b) Absorbance changes at 560 nm of probe **1** (20 μM) against concentration of Cys. Each spectrum was obtained 30 min after Cys addition.

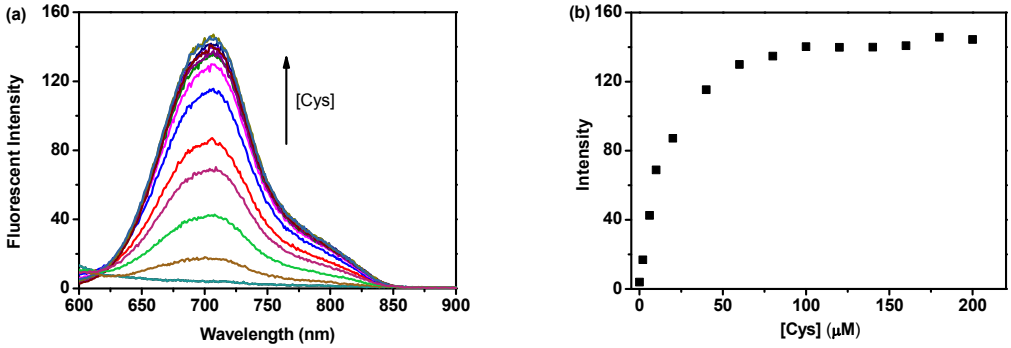


Fig. S4 (a) Fluorescence spectra changes of probe **1** (20 μM) upon addition of different concentrations of Cys in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37 °C. Final concentration of Cys: 0, 2, 6, 10, 20, 40, 60, 80, 100, 120, 140, 160 and 180 μM, respectively. (b) Fluorescence intensity changes at 706 nm of probe **1** (20 μM) against concentration of Cys. Each spectrum was obtained 30 min after Cys addition. λ_{ex} = 560 nm, slit width: (10, 10).

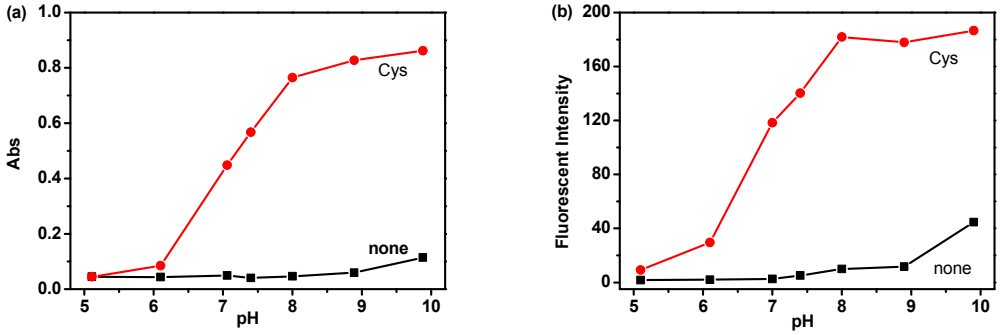
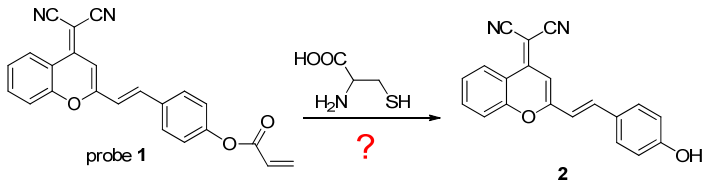


Fig. S5 (a) Absorbance responses of probe **1** (20 μM) at 560 nm in the presence and absence of Cys (5 eq) under different pHs. (b) Fluorescence intensity responses of probe **1** (20 μM) at 706 nm in the presence and absence of Cys (5 eq) under different pHs. All experiment was performed in PBS buffer (10 mM) with 50% DMSO at 37 °C and each data was obtained 30 min after addition of Cys. For fluorescence measurements, λ_{ex} = 560 nm, slit width: (10, 10).

Data for investigation of the sensing mechanism



This experiment was performed according to a reported procedure (see Ref: X. Yang, Y. Guo and R. Strongin, *Angew. Chem., Int. Ed.*, 2011, **50**, 10690–10693). To a 100 mL flask, probe **1** (50 mg, 0.14 mmol) and Cys (21 mg, 1.25 eq) were combined in 30 mL of MeOH: H_2O (90: 10, v/v) solution, and the mixture stirred at room temperature for 1 h. Then, Et_3N (30 μL) was added and the solution stirred overnight. The solvents was removed under reduced pressure and the crude

product was subjected to column chromatography (eluted with CH_2Cl_2: MeOH, 5:1, v/v) to afford a red solid (23 mg), which was proved to be compound **2** (see below).

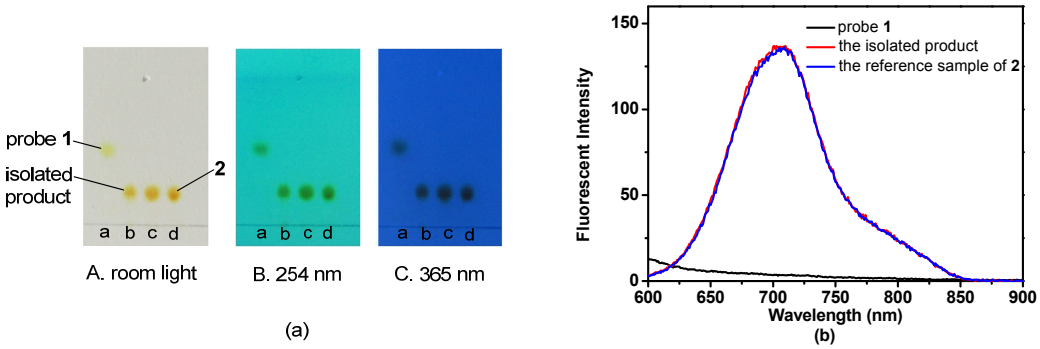


Fig. S6 (a) The thin layer chromatography (TLC) plate under different light to compare probe **1**, the reference sample of **2** and the isolated product from the reaction mixture of probe **1** with Cys. Spots on the TLC plate are: a. probe **1**; b. the isolated reaction product of probe **1** and Cys; c. mixture of b and **2**; d. the reference sample of **2**. The eluent for TLC: hexane:ethyl acetate = 3:1 (v/v). (b) Fluorescent spectra of probe **1**, the isolated product and the reference sample of **2** in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37 °C. λ_{ex} = 560 nm, slit width: (10,10).

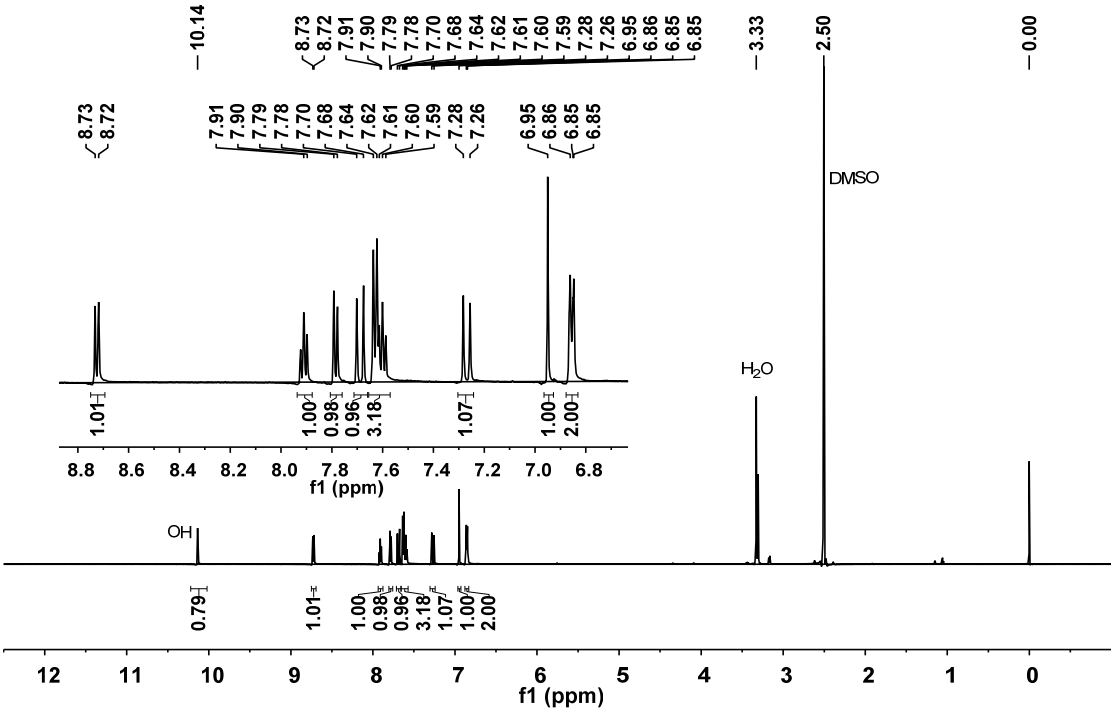


Fig. S7 ^{1}H NMR spectrum of the isolated product from the reaction of probe **1** and Cys, which is identical to that of the reference sample of **2** (see above).

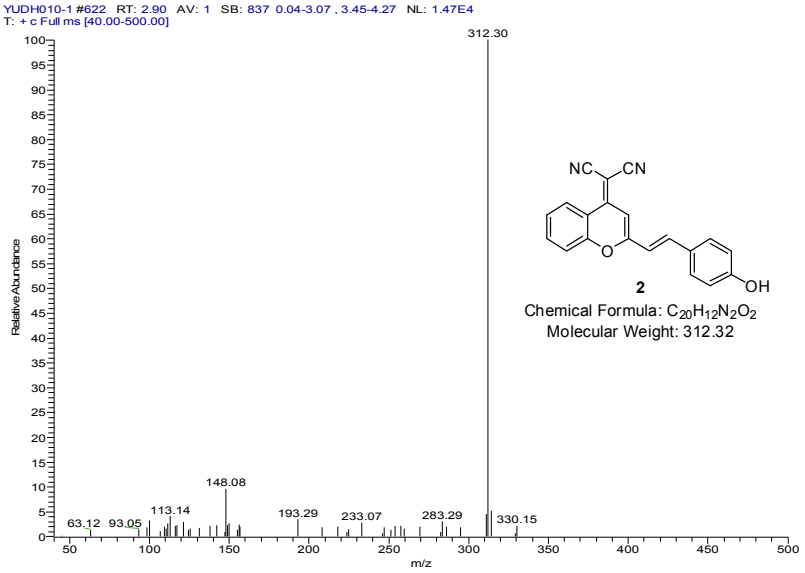


Fig. S8 MS spectrum of the isolated product from the reaction of probe **1** and Cys, which showed the expected mass of **2**.

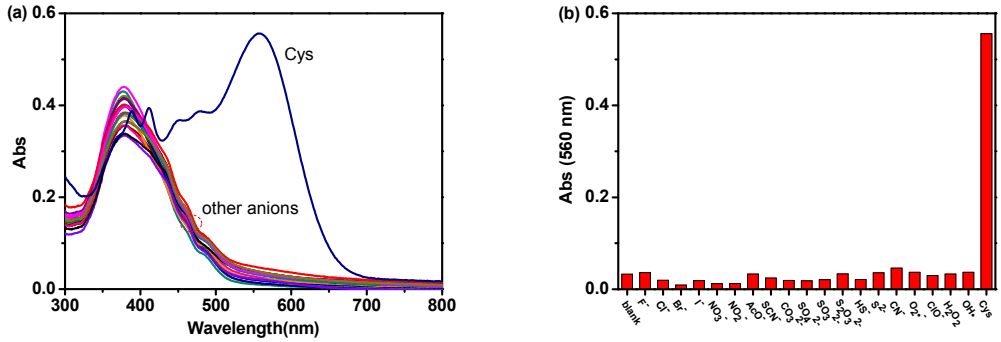


Fig. S9 (a) Absorption spectral responses of probe **1** (20 μM) upon addition of 100 μM various anions and Cys. Anions are F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, AcO^-, SCN^-, CO_3^{2-}, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, HS^-, S^{2-}, CN^-, $O_2\bullet^-$, ClO^-, H_2O_2 and OH•. (b) A comparison of absorbance intensity changes of probe **1** (20 μM) at 560 nm upon addition of 100 μM various anions and Cys. All experiment was performed in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37 °C and each spectrum was obtained 30 min after addition of various anions.

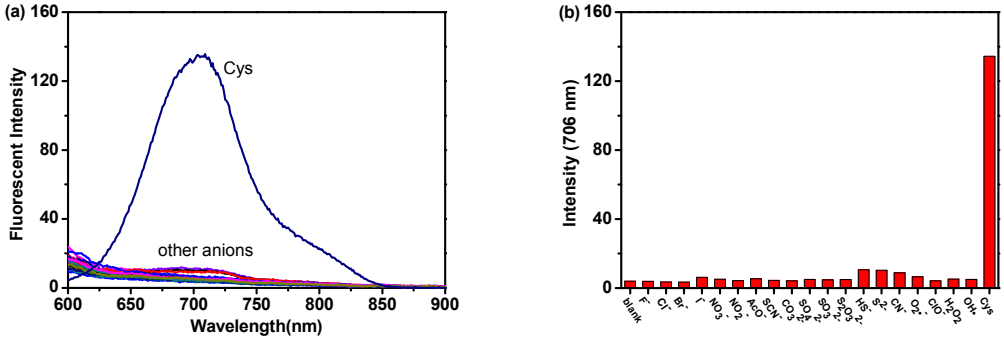


Fig. S10 (a) Fluorescent spectral responses of probe **1** (20 μM) upon addition of 100 μM various anions and Cys. Anions are F^-, Cl^-, Br^-, I^-, NO_3^-, NO_2^-, AcO^-, SCN^-, CO_3^{2-}, SO_4^{2-}, SO_3^{2-},

$S_2O_3^{2-}$, HS^-, S^{2-}, CN^-, $O_2^{\bullet-}$, ClO^-, H_2O_2 and OH•. (b) A comparison of fluorescence intensity changes of probe **1** (20 μM) at 706 nm upon addition of 100 μM various anions and Cys. All experiment was performed in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37 °C and each spectrum was obtained 30 min after addition of various anions. λ_{ex} = 560 nm , slit width: (10, 10).

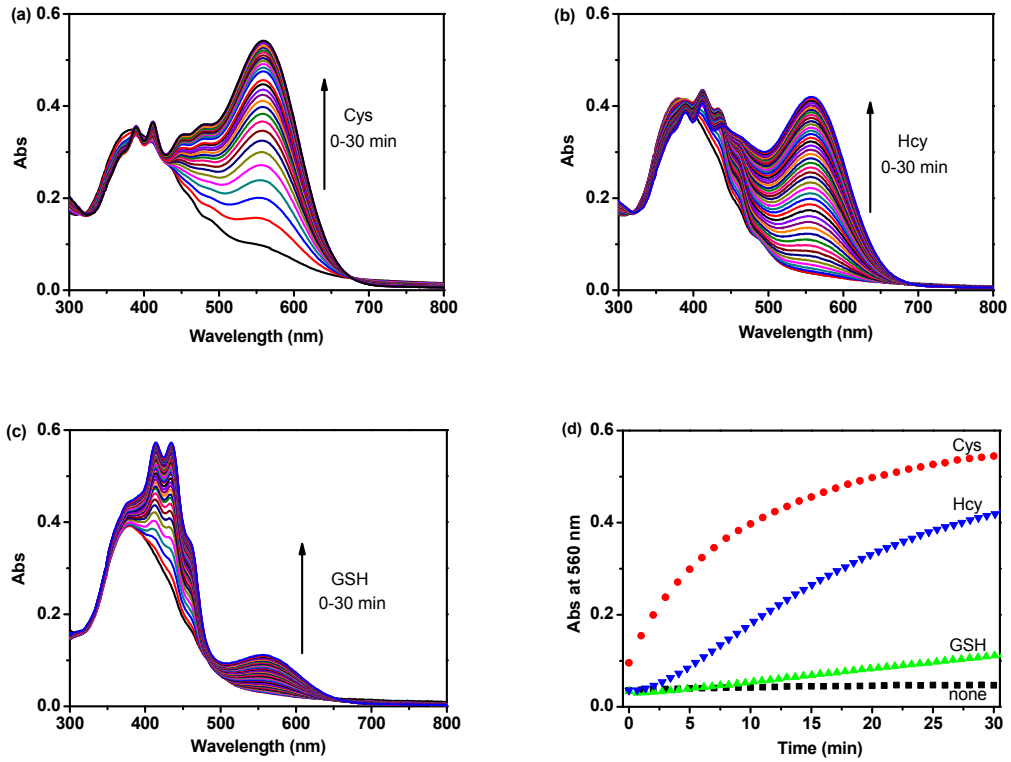


Fig. S11 (a)-(c): Scanning kinetics of the UV-vis spectra changes of probe **1** (20 μM) against time in the presence of 5 equiv of Cys (a), Hcy (b) and GSH (c), respectively. All spectra were measured in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37 °C. (d) The corresponding absorbance changes of probe **1** at 560 nm against time for Cys, Hcy and GSH.

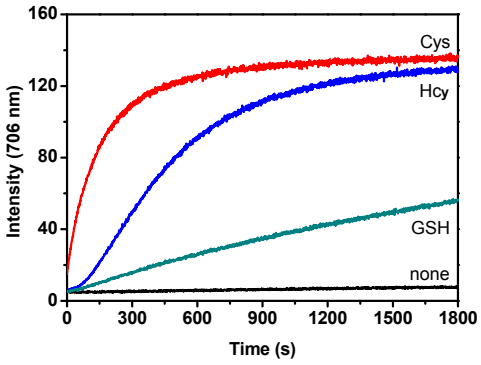


Fig. S12 Fluorescence kinetics of probe **1** (20 μM) at 706 nm in the absence and presence of biothiols (Cys, Hcy and GSH, 100 μM each). All spectra were measured in PBS buffer (10 mM, pH 7.4) with 50% DMSO at 37 °C. λ_{ex}= 560 nm, slit width (10,10).

Generation of reactive oxygen species (ROS)

ClO^-

The NaOCl solution was added to the probe **1** testing solution at 25 °C.

H_2O_2

H_2O_2 was added and the mixtures were stirred for 1 hour at 25 °C.

OH•

Hydroxyl radicals were generated by the addition of Fe^{2+} and H_2O_2 at room temperature in PBS buffer (pH 7.4) and the mixture was then stirred for 30 min. (Ref. Y. Zhou, J.-Y. Li, K.-H. Chu, K. Liu, C. Yao, J.-Y. Li, *Chem Commun*. 2012, **48**, 4677–4679.)

$O_2•^-$

O2•$^-$ was generated by an improved pyrogallol autoxidation method reported recently (Ref. X. Li, *J. Agric. Food Chem.*, 2012, **60**, 6418−6424).