Electronic Supplementary Information (ESI)

for

Highly sensitive and selective detection of biothiols by a new low dose colorimetric and fluorescent probe

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Contents

1. The optical spectra of probe 1 and compound 2………………………..page 2
2. Data for investigation of the sensing mechanism……………………….page 2
3. A colorimetric assay of probe 1 for biothiols……………………………page 3
4. Interference experiments of probe 1 for detection of biothiols………..page 4
5. Kinetics of probe 1 with Cys, Hcy and GSH…………………………..page 4
6. Additional data…………………………………………………………page 5-7
7. Structure characterizations of probe 1………………………………….page 8-9
1. The optical spectra of probe 1 and compound 2

![UV-Vis spectra of probe 1 and compound 2](image1)

*Fig. S1* (a) The UV-Vis spectra of probe 1 (5 μM) and compound 2 (5 μM) in PBS buffer (10 mM, pH 7.4, with 20% DMSO, v:v) at 37°C. (b) The fluorescence spectra of probe 1 (1 μM) and compound 2 (1 μM) in PBS buffer (10 mM, pH 7.4, with 20% DMSO, v:v) at 37°C. λ<sub>ex</sub> = 453 nm, slit width: d<sub>ex</sub> 2.5 nm/d<sub>em</sub> 5 nm. Color and emission color under a 365 nm light of 1 and 2 are inserted, respectively. The fluorescence quantum yield (Φ) for 1 and 2 under this experimental condition was determined to be 0.07 and 0.65, respectively, using rhodamine B as standard.

2. Data for investigation of the sensing mechanism

![UV-Vis absorption spectra](image2)

*Fig. S2* (a) UV-vis absorption spectra changes of probe 1 (5 μM) against time (0-20 min) upon addition of 5 equiv of Cys. (b) Fluorescence spectra changes of probe 1 (1 μM) against time (0-15 min) upon addition of 5 equiv of Cys. Data were collected in PBS buffer (10 mM, pH 7.4, 20% DMSO, v:v) at 37°C. For fluorescence measurement, λ<sub>ex</sub> = 453 nm, slit: d<sub>ex</sub> 2.5 nm/d<sub>em</sub> 5 nm. These data showed the conversion process of probe 1 to compound 2 in the presence of Cys.
Fig. S3 MS spectrum of the fluorescent product from the reaction of probe 1 with Cys, which confirmed that compound 2 was released.

3. A colorimetric assay of probe 1 for biothiols

![Image]

Fig. S4 A colorimetric assay of probe 1 (5 μM) for biothiols and other amino acids. (a) Color changes and (b) fluorescence changes (under a 365 nm UV lamp) of probe 1 upon addition of various analytes (from left to right: 1, Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, Cys, Hcy, and GSH) in PBS buffer (10 mM, pH 7.4, 20% DMSO, v/v). The concentration of biothiols was used 50 μM, the others were used 100 μM, respectively.
4. Interference experiments of probe 1 for detection of biothiols


5. Kinetics of probe 1 with Cys, Hcy and GSH

Fig. S6 Fluorescent kinetics of probe 1 (1 μM) in the absence and presence of 5 μM Cys, Hcy and GSH in DMSO-PBS buffer (10 mM, pH 7.4, 20% DMSO) at 37°C. The reactions are monitored at 493 nm with \(\lambda_{ex} = 453\) nm, slit: 2.5 nm/5 nm.
6. Additional data

Fig. S7 (a) Fluorescence spectra changes of probe 1 (1 μM) in DMSO-PBS buffer (10 mM, pH 7.4, 20% DMSO) at 37°C upon addition of different concentrations of Cys (0–10 μM). Each spectrum was obtained 15 min after Cys addition. Insert: Fluorescence intensity changes at 493 nm as a function of [Cys]. λex = 453 nm, slit: 2.5 nm/5 nm. (b): Linear relationship of fluorescence intensity at 493 nm as a function of Cys concentration (0-4.5 μM).
Fig. S8 (a) Fluorescence spectra changes of probe 1 (1 μM) in DMSO-PBS buffer (10 mM, pH 7.4, 20% DMSO) at 37°C upon addition of different concentrations of Hcy (0–10 μM). Each spectrum was obtained 15 min after Hcy addition. Insert: Fluorescence intensity changes at 493 nm as a function of [Hcy]. $\lambda_{ex} = 453$ nm, slit: 2.5 nm/5 nm. (b): Linear relationship of fluorescence intensity at 493 nm as a function of Hcy concentration (0–4 μM).
**Fig. S9** The fluorescence kinetics of probe 1 (1 μM) upon addition 5 equiv of GSH in PBS buffer (10 mM, pH 7.4, with 10-30% DMSO, v:v) at 37°C. λ_ex = 453 nm, slit width: δ_ex 2.5 nm/δ_em 5 nm.

**Fig. S10** Percentage of viable HeLa cells after treatment with indicated concentrations of probe 1 after 12 hours. The cell viability was observed via MTT assay.
7. Structure characterizations of probe 1

$^{1}$H-NMR spectrum of probe 1 in $d_6$-DMSO

$^{13}$C-NMR spectrum of probe 1 in $d_6$-DMSO
ZQ-38 #481
RT: 3.58
AV: 1
SB: 538 0.04-3.35 , 3.62-4.26
NL: 2.13E4
T: + c Full ms [40.00-800.00]

MS (EI) spectrum of probe 1

HR-MS spectrum of probe 1