Electronic Supplementary Information (ESI) for Chemical Communications

Aryl-thioether substituted nitrobenzothiadiazole probe for selective detection of cysteine and homocysteine

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General Methods.

Unless otherwise noted, materials were obtained from Aldrich and were used without further purification. Melting points were measured using a Büchi 530 melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 300 MHz or Varian 500 MHz. Chemical shifts are given in ppm and coupling constants (J) in Hz. UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu)

Cell culture.

HeLa cells (human epithelial adenocarcinoma) were purchased from Korean Cell Line Bank (Seoul, Korea). Cells were grown in DMEM (Dulbecco Modified Eagle Medium) supplemented with 10 % heat-inactivated fetal bovine serum, 100 U/mL penicillin and 100 U/mL streptomycin. All cells were maintained in an incubator at 37°C and 5% CO_2/ air environment.

Confocal microscopy imaging.

Cells were seeded in a 35-mm glass bottomed dishes at a density of 3×10^5 cells per dish in DMEM media. After overnight culture, HeLa cells were incubated with 1 mM NEM (N-ethylmaleimide) for 30 min and washed with DPBS and incubated with 300 μM cysteine, homocysteine, GSH-MEE for 30 min. After washing with DPBS, cells were incubated with 3 μM probe **1** for 30 min and fluorescence images were acquired by confocal laser microscopy (Fluoview 1200, Olympus, Japan). Fluorescence images were acquired by excitation at 473 nm laser diode and detected at BA 490 - 590 nm. Except for overnight culture, cell experiments were tested in HBSS (Hanks' balanced salt solution) media.

Cytotoxicity experiments

Cells were seeded in a 96-well plate with culture media. After overnight culture, cells were incubated with probe **1** for 24 h at 37 ℃. After washing with DPBS, 0.5 mg/mL of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, Sigma) containing media was added to the cells for 4 h at 37 ℃ and the produced formazan was dissolved in 0.1 mL of dimethylsulfoxide (DMSO) and read at OD 650 nm with a Spectramax Microwell plate reader. All experiments were presented as the mean ± SD from three independent experiments.

Synthesis of probe 1. To a solution of 4-aminothiophenol (125 mg, 1.0 mm) and triethylamine (303 mg, 3.0 mm) in dry THF (20 mL), 4-chloro-7-nitrobenzo[c][1,2,5]thiadiazole (215 mg, 1.0 mm) was added. The mixture was refluxed for 4 h under nitrogen atmosphere. After cooing to room temperature, the solvent was removed, and washed with water and extracted with dichloromethane. The crude

product was purified by column chromatography (eluent: hexane/ dichloromethane 1：1), affording the desired product. Yield, 93%. mp 182˚C (dec.); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.66 (d, 1H), 6.83 (d, 2H), 7.22 (d, 2H), 8.24 (d, 1H), 4.10 (NH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.36, 148.34, 145.12, 142.55, 137.22, 130.83, 120. 82, 116.43, 112.31; FAB-MS m/z =305.9 $[M+H]^+$, calcd for $C_{12}H_8N_4O_2S_2$ =304.35.

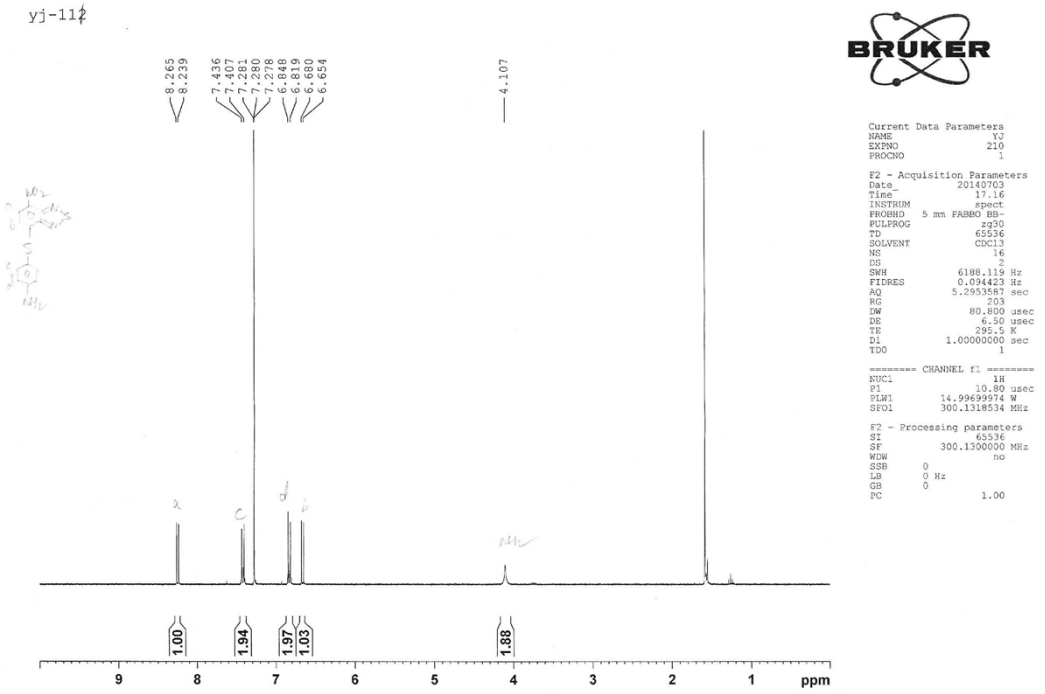


Figure S1. ^{1}H NMR (300 MHz) of compound probe **1** in $CDCl_3$.

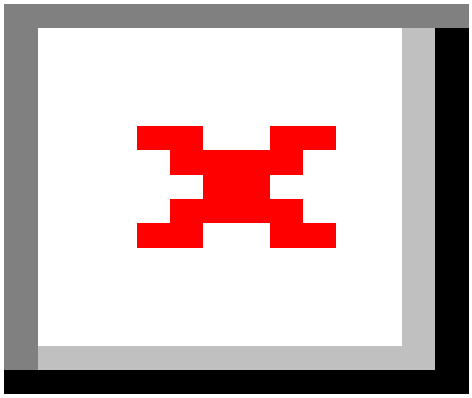


Figure S2. ^{13}C NMR (75 MHz) of compound probe **1** in $CDCl_3$.

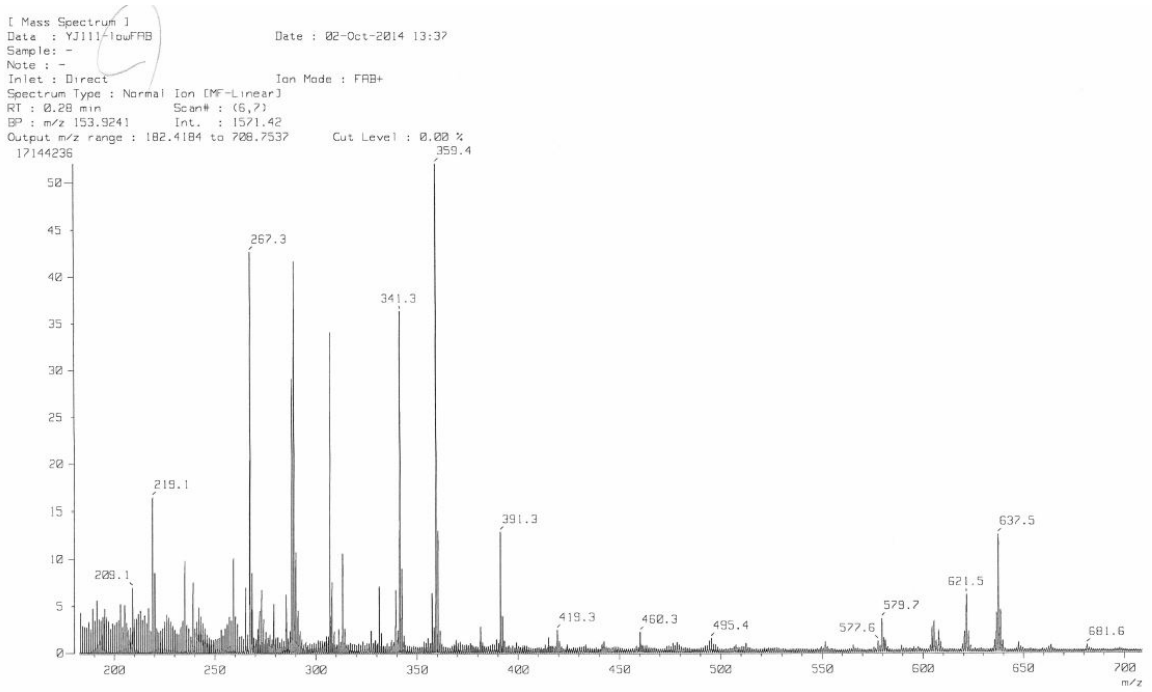
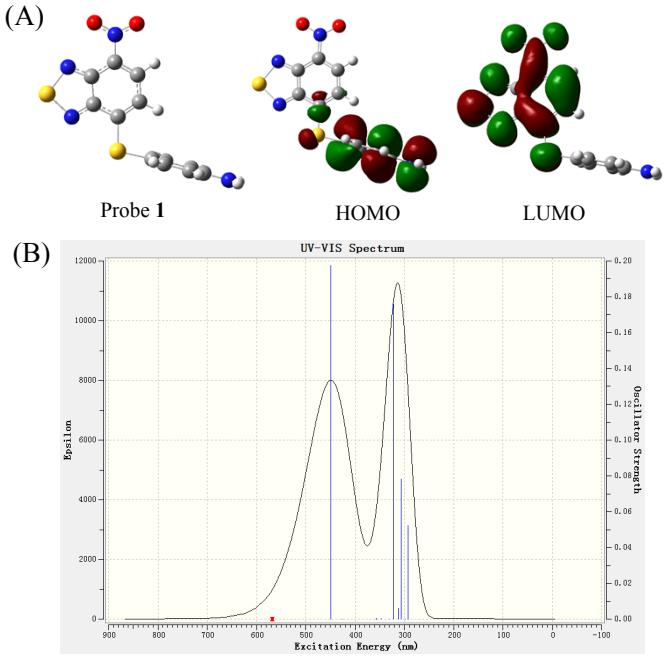


Figure S3. The FAB mass spectrum of probe **1**.



Compound	Excited state	λ/nm [eV]	Osc. str (f)	Major contributions
Probe 1	S_2	448.86 [3.86]	0.1977	HOMO-2→LUMO (70%)
	S_8	321.06 [2.76]	0.1759	HOMO-8→LUMO (68%)

Figure S4. Frontier molecular orbital profiles and optimized structures (bottom) based on TD-DFT (B3LYP/6-31G*) calculations of probe **1**.

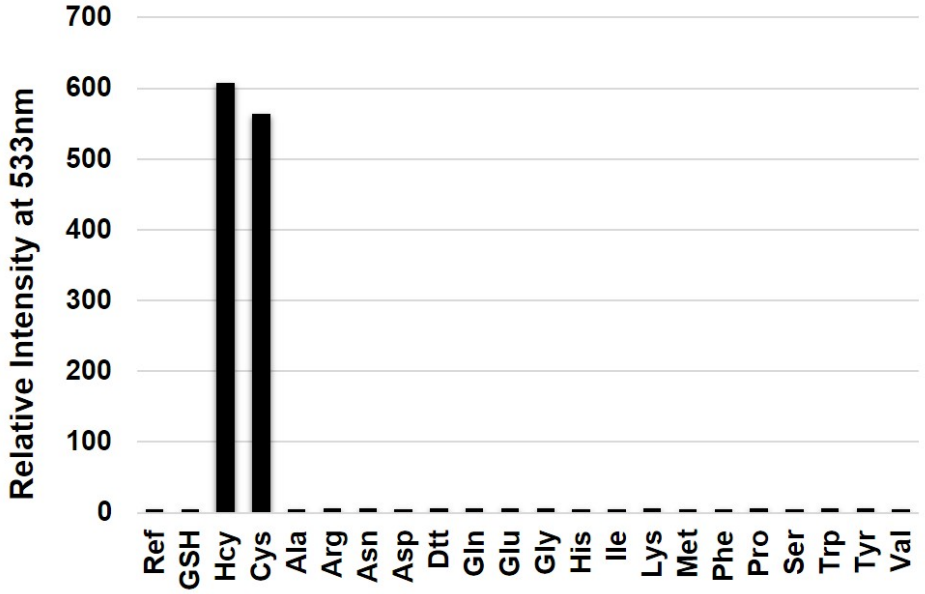


Figure S5. Selective Response of probe **1** to Cys & HCy. Relative fluorescence intensities of probe **1** (10 μM) upon addition of 10 equiv. of various amino acids or GSH in HEPES (0.01M, pH 7.4) containing 1% DMSO at 533nm. (Excitation wavelength: 475nm)

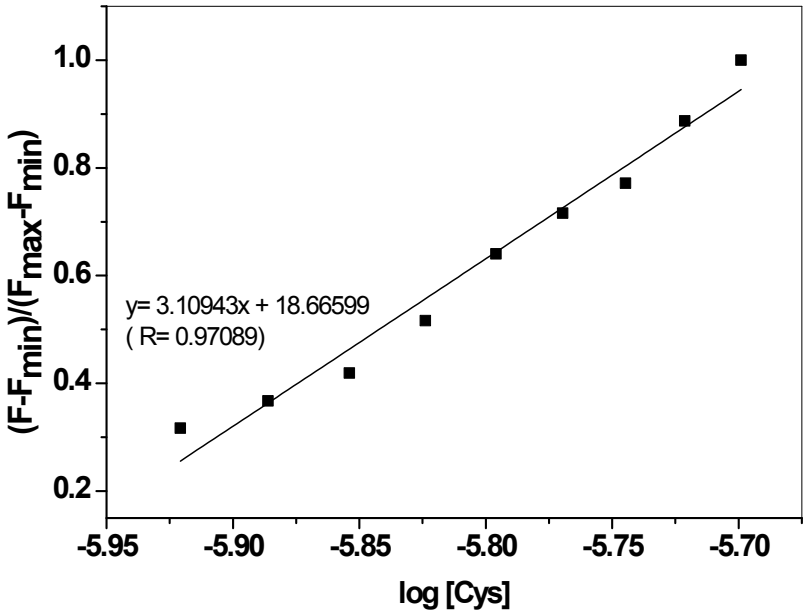


Figure S6. Normalized fluorescence responses of probe **1** (1 μM) to changing Cys concentrations in DMSO-HEPES (0.01 M, pH 7.4) (1:99, v/v). (Detection limit = 9.93056×10^{-7} M)

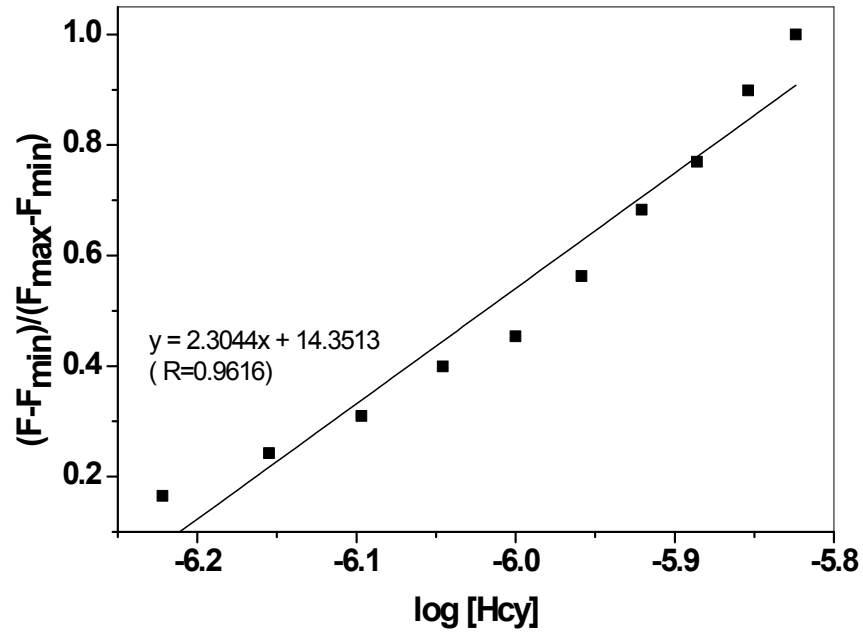


Figure S7. Normalized fluorescence responses of probe **1** (1 μM) to changing Hcy concentrations in DMSO-HEPES (0.01M, pH 7.4) (1:99, v/v). (Detection limit = 5.91741×10^{-7}M)

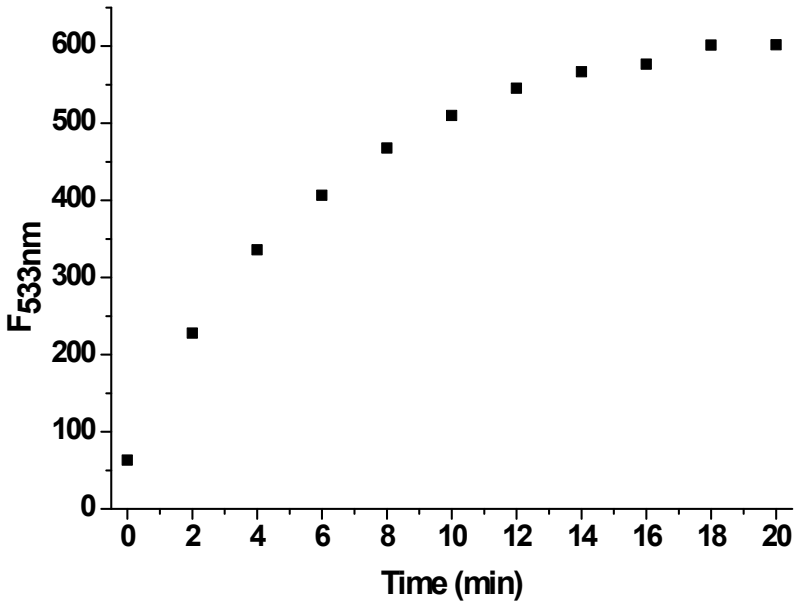


Figure S8. Time-dependent change of probe **1** (10 μM) with the addition of 10 equiv. of Cys in DMSO-HEPES (0.01 M, pH 7.4) (1:99, v/v). (Excitation wavelength: 475 nm) (Slit: 3×5 nm)

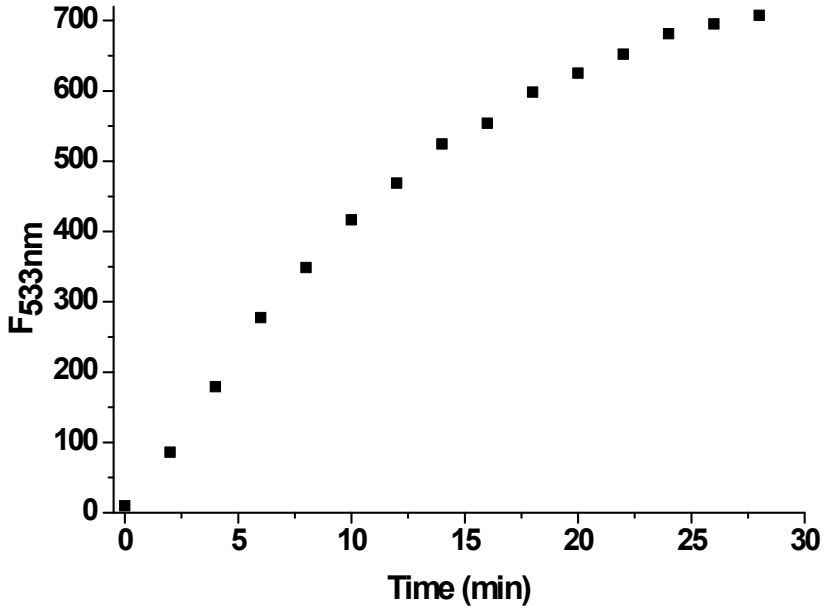


Figure S9. Time-dependent change of probe **1** (10 μM) with the addition of 10 equiv. of Hcy in DMSO-HEPES (0.01M, pH 7.4) (1:99, v/v). (Excitation wavelength: 475 nm) (Slit: 3×5 nm)

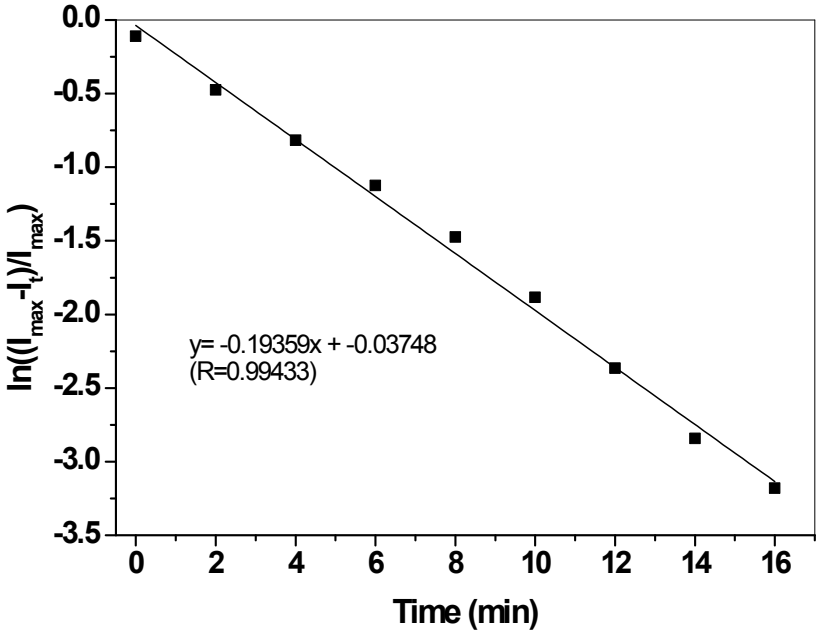


Figure S10. Pseudo first-order kinetic plot of probe **1** (10 μM) with the addition of 10 equiv. of Cys in DMSO-HEPES (0.01 M, pH 7.4) (1:99, v/v). (slope = - 0.19459, so K'= 0.19359 min^{-1})

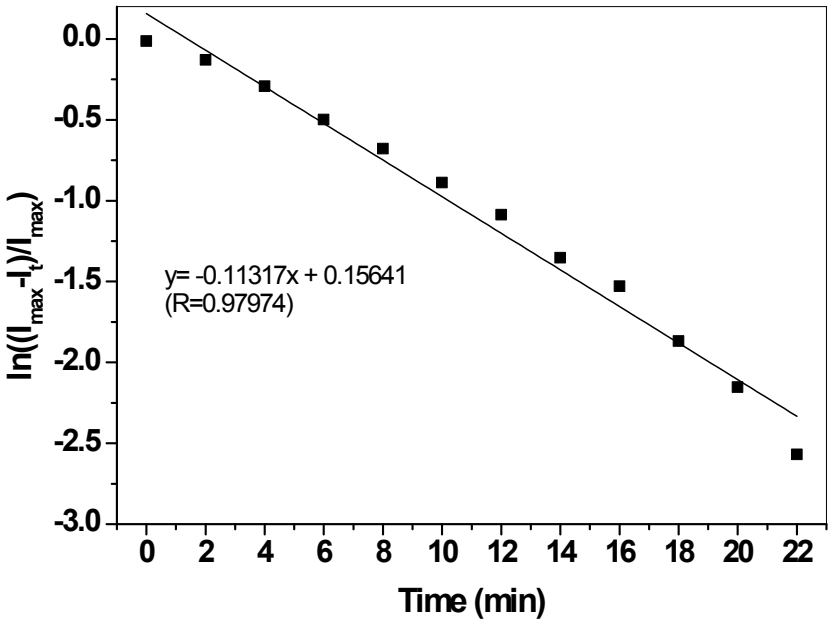


Figure S11. Pseudo first-order kinetic plot of probe **1** (10 μM) with the addition of 10 equiv. of Hcy in DMSO-HEPES (0.01 M, pH 7.4) (1:99, v/v). (slope = - 0.11317, so K'= 0.11317 min^{-1})

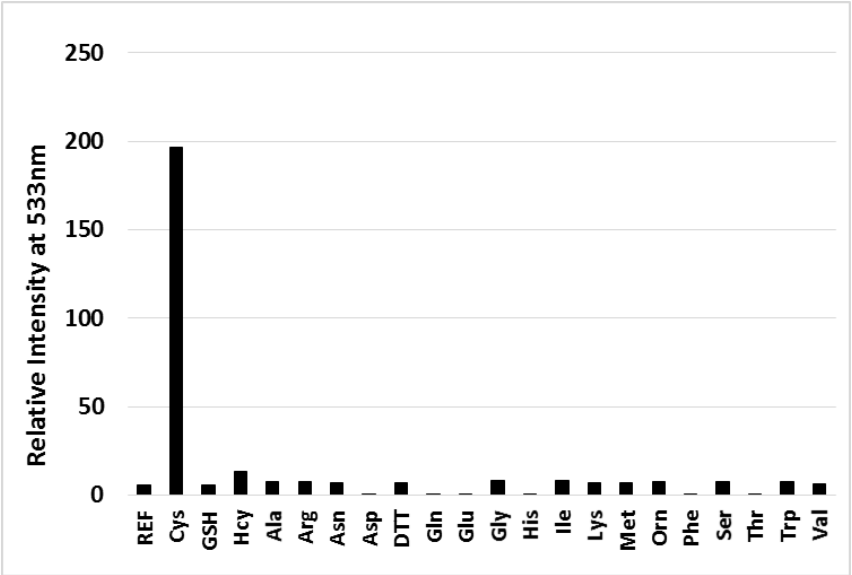


Figure S12. Selective Response of probe **1** to Cys. Relative fluorescence intensities of probe **1** (10 μM) upon addition of 10 equiv. of various amino acids or Hcy or GSH in citric acid-Na_2HPO_4 (0.01M, pH 6.0) containing 1% DMSO at 533nm. (Excitation wavelength: 475nm)

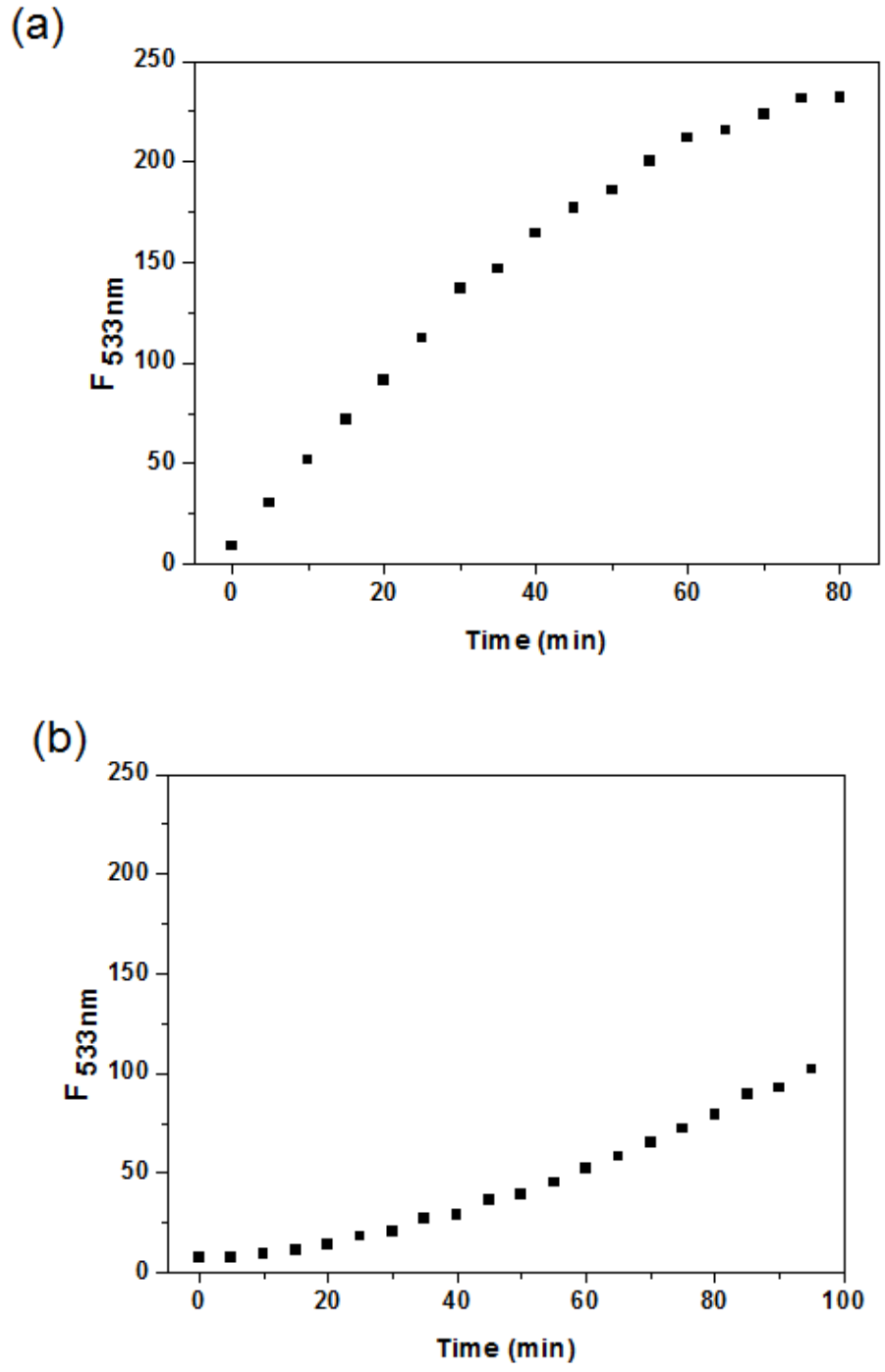


Figure S13. (a) Time-dependent change of probe **1** (10 μM) with the addition of 10 equiv. of Cys in in citiric acid-Na_2HPO_4 (0.01M, pH 6.0) containing 1% DMSO. (Excitation wavelength: 475 nm) (Slit: 3×5 nm) (b) Time-dependent change of probe **1** (10 μM) with the addition of 10 equiv. of Hcy in citiric acid-Na_2HPO_4 (0.01M, pH 6.0) containing 1% DMSO. (Excitation wavelength: 475 nm) (Slit: 3×5 nm)

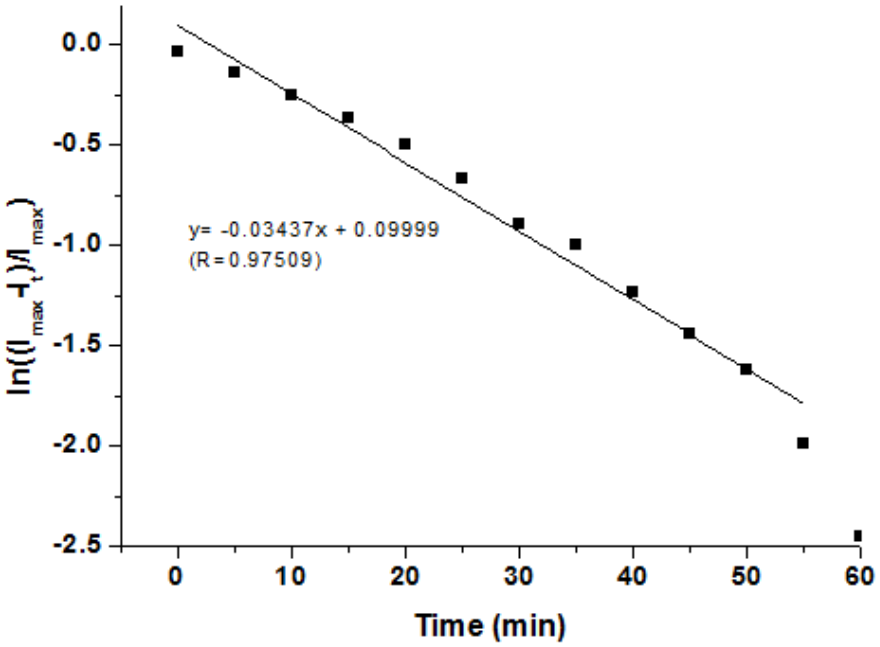


Figure S14. Pseudo first-order kinetic plot of probe **1** (10 μM) with the addition of 10 equiv. of Cys in citiric acid-Na_2HPO_4 (0.01M, pH 6.0) containing 1% DMSO. (slope = - 0.03437, so K'= - 0.03437 min^{-1})

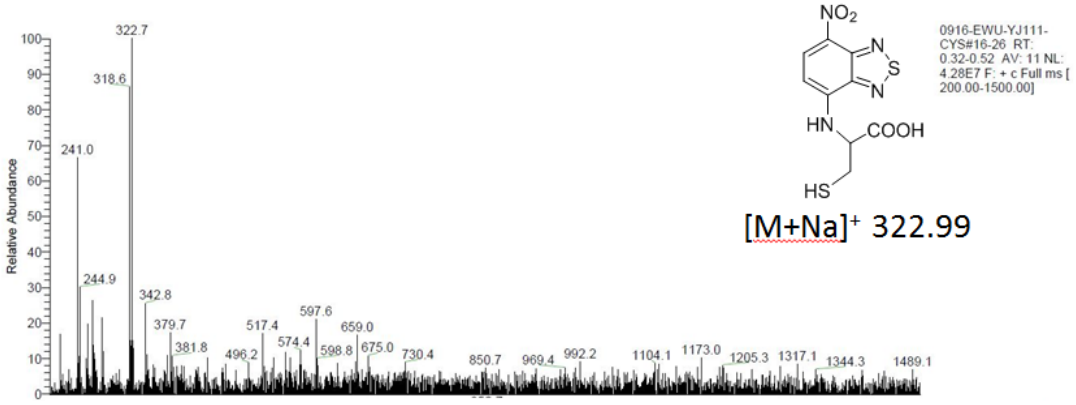


Figure S15. The ESI mass spectrum of probe **1**+Cys.

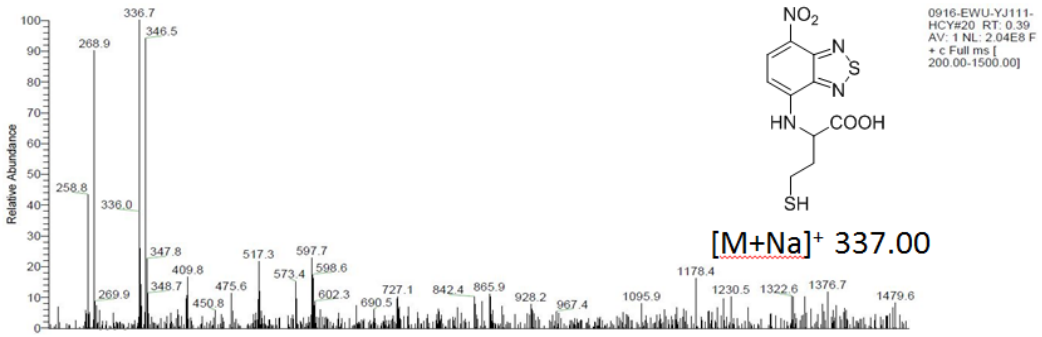


Figure S16. The ESI mass spectrum of probe **1**+Hcy.

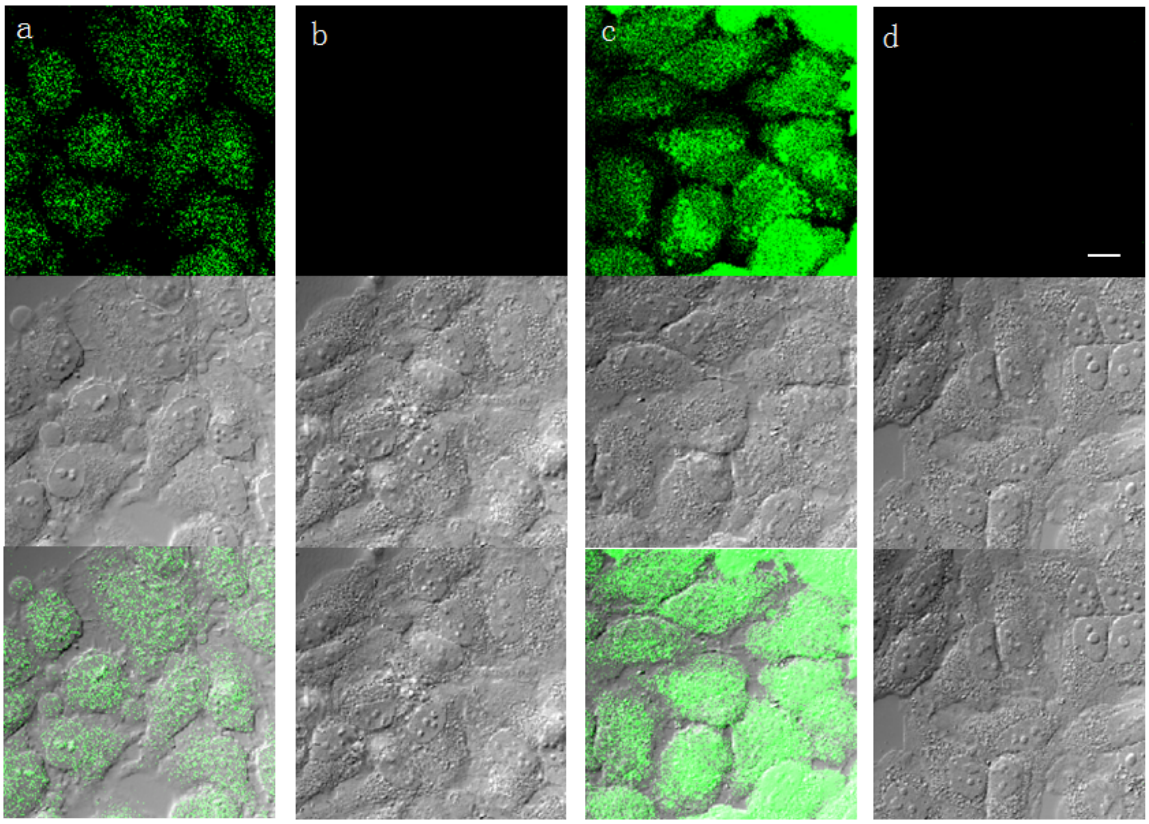


Figure S17. Fluorescence property of probe **1**. HeLa cells were incubated with 1 mM NEM for 30 min and incubated with 300 μM cysteine or homocysteine for 30min in HBSS. And cells were incubated with 3 μM probe **1** for 10 min in 10 mM citric acid/ Na_2HPO_4 pH 6.0 buffer. Only probe **1** (a), NEM (b), NEM + cysteine (c) and NEM + homocysteine (d). ex: 473 nm, em: 490-590 nm, upper: fluorescence image, middle: DIC, botton: merge. Scale bar: 10 μm.

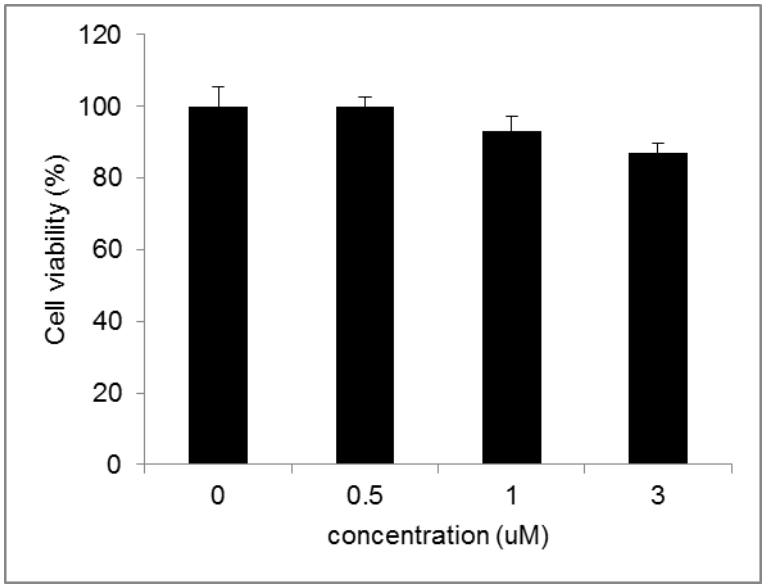


Figure S18. Cytotoxic effect of probe **1**. HeLa cells were incubated with each concentration of probe **1** for 24 h. Cell viability was measured by MTT assay. Results were expressed as mean ± standard deviation of three independent experiments.