# A novel 3, 6-diamino-1, 8-naphthalimide derivative as a highly selective fluorescent "turn-on" probe for thiols

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Supporting Information

## Experimental

***Generals***. $^{1}H$ and $^{13}C$ NMR spectra were recorded in $d_6$-DMSO, $CD_3OD$, $CDCl_3$ or $d_6$-DMSO-$D_2O$ (5/2, v/v) on a Varian Mercury 400 spectrometer. LR-ESI-MS and HR-ESI-MS spectra were measured on Waters UPLC/Quattro Premier XE and Agilent 6460 Triple Quadrupole mass spectrometers, respectively. Silica gel 60 Å (reagent pure, Qingdao Haiyang Chemical Co. Ltd) was used for column chromatography. Analytical thin-layer chromatography was performed on silica gel plates 60 GF254 (chemical pure, Qingdao Haiyang Chemical Co. Ltd). Detection on TLC was made by use of a UV lamp (254 or 365 nm). Fluorescence spectra were measured on a Shimadzu RF-5301PC spectrofluorimeter. UV-Vis spectra were measured on a TU-1901 spectrophotometer. Fluorescence images of HepG2 cells were undertaken with Olympus FV1000 confocal laser scanning microscopy.

*N*-butyl-3, 6-dinitro-1, 8-naphthalimide **3** was prepared according to the reported literature. [1] All reagents and solvents were purchased from commercial sources and were of analytical grade. Solvents were dried according to standard procedures.

**Synthesis of compounds 1 and 2**

*Compound* ***2***. To a solution of compound **3** (155 mg, 0.45 mmol) in ethanol (10 mL) was added 20 mg of 10% Pd/C in ethanol (3 mL). The resulting mixture was stirred under the atmosphere of hydrogen at 40 ° C for 3 h, and then concentrated under reduced pressure. The obtained residue was purified by chromatography on a silica gel column, eluted with $CHCl_3/CH_3OH$ (400/1, v/v) to afford compound **2** (108 mg, 85%) as a yellowish solid having $^1$H NMR (400 MHz, $d_6$-DMSO) $\delta$ 7.58 (s, 2H), 6.93 (s, 2H), 5.68 (s, 4H), 3.98 (t, $J$ = 7.2 Hz, 2H), 1.57 (m, 2H), 1.35 (m, 2H), 0.92 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $d_6$-DMSO) $\delta$ 164.3, 148.0, 135.9, 122.7, 117.4, 110.0, 30.1, 20.2, 14.1 and ESI-MS *m/z*: 284.2 ($[M+H]^+$).

*Compound* **1**. A solution of compound **2** (28 mg, 0.10 mmol) and 2, 6-dimethylpyridine (54 mg, 0.50 mmol) in anhydrous $CH_2Cl_2$ (20 mL) was stirred at 0 ºC for 15 min and then added to a solution of 2, 4-dinitrobenzenesulfonyl chloride (133 mg, 0.50 mmol) in anhydrous $CH_2Cl_2$ (10 mL). The resulting mixture was stirred at room temperature for 45 h and concentrated under reduced pressure. The obtained residue was purified by chromatography on a silica gel column, eluted with $CHCl_3/CH_3OH$ (200/1, v/v) to afford compound **1** (14 mg, 28%) as a yellow solid having $^1$H NMR (400 MHz, $d_6$-DMSO) $\delta$ 8.89 (d, $J$ = 2.0 Hz, 1H), 8.58 (m, 1H), 8.30 (d, $J$ = 3.2 Hz, 1H), 7.87 (d, $J$ = 2.4 Hz, 1H), 7.86 (d, $J$ = 2.4 Hz, 1H), 7.69 (d, $J$ = 2.0 Hz, 1H), 7.14 (d, $J$ = 2.0 Hz, 1H), 3.97 (t, $J$ = 7.2 Hz, 2H), 1.56 (m, 2H), 1.32 (m, 2H), 0.91 (t, $J$ = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, $d_6$-DMSO) $\delta$ 163.7, 163.5, 150.6, 149.0, 148.3, 136.4, 135.4, 134.6, 132.1, 127.7, 123.6, 123.0, 121.6, 120.8, 120.6, 119.4, 118.5, 111.2, 30.0, 20.2, 14.1; ESI-MS *m/z*: 514.2 ($[M+H]^+$) and HR-ESI-MS for $C_{22}H_{19}N_5O_8S$ ($[M+H]^+$) calcd: 514.1033, found: 514.1029.

**Determination of quantum yields**

The quantum yield of compound **2** was measured by using the protocol described in literature.[2] Thus, the absorption and emission spectra of compound **2** were measured in ethanol. It showed absorption and emission maxima at 415 nm ($\varepsilon$ = 8300 $M^{-1}\cdot cm^{-1}$) and 560 nm, respectively. Coumarin **4** was chosen as a standard because its absorption and emission

spectra largely overlap those of compound **2** in ethanol. Fluorescence quantum yield $\Phi_X$ of compound **2** was calculated according to eq (1).

$$\Phi_X = \Phi_S \times [Abs_S / Abs_X] \times [A_{FX} / A_{FS}] \times [N_X / N_S]^2 \quad (1)$$

Where, $\Phi_S$ (= 0.78) is the reported quantum yield of coumarin **4** in ethanol; $Abs_S$ and $Abs_X$ are the absorbance at the excitation wavelengths of coumarin **4** and compound **2**, respectively; $A_{FS}$ and $A_{FX}$ are the areas under the emission spectra of coumarin **4** and compound **2**, respectively; and $N_S$ and $N_X$ are the refractive indexes of the solvents used for coumarin **4** and compound **2**, respectively. Here ethanol was used in both cases, thus $N_S = N_X = 1.3611$. It has been reported that *N*-butyl-4-amino-1, 8-naphthalimide has the absorption and emission maxima at 430 nm ($\varepsilon = 11600\ M^{-1}\cdot cm^{-1}$) and 541 nm ($\Phi = 0.13$), respectively. [3]

**Table 1** Results for coumarin **4** and compound **2**

| Sample | Abs | $A_F$ | $\Phi$ |
|---|---|---|---|
| Coumarin **4** | 0.0464 | 31189 | 0.78 |
| Compound **2** | 0.0160 | 819 | 0.0594 |

**Procedures of thiols sensing**

A stock solution of compound **1** (1 mM) was prepared in DMSO and then diluted to the corresponding concentration (25 μM) with 1 : 1 DMSO-PBS buffer (0.01 M, pH 7.4). Fluorescence spectra were recorded in an indicated time after the addition of amino acids and different kinds of common species ($\lambda_{ex}$ = 415 nm, ex/em = 5 nm/5 nm).

**Effect of pH value**

The effect of pH on the fluorescence intensities of compound **1** (25 μM) was determined in the absence and presence of Cys (250 μM), respectively. The reaction of compound **1** and Cys was carried out at room temperature for 120 min, in 1 : 1 DMSO-PBS buffer (0.01 M) of varying pH from 3.0 to 9.0. The corresponding fluorescence spectrum at each pH was recorded ($\lambda_{ex}$ = 415 nm.). The fluorescence intensities at 560 nm were plotted against pH, which is shown in Fig. S9.

**Kinetic study**

The kinetic profiles of the reaction were examined under *pseudo-first-order* conditions with a large excess of Cys, Hcy, or GSH over compound **1** in 1 : 1 DMSO-PBS (0.01 M, pH 7.4) at room temperature. The *pseudo-first-order* rate constant $k$ was calculated according to eqn (2):

$$\ln[F_{max}/(F_{max} - F_t)] = kt \qquad (2)$$

Wherein $F_t$ and $F_{max}$ are the fluorescence intensity at 560 nm at time $t$ and when the reaction is complete, respectively. $k$ is the *pseudo-first-order* rate constant. [4]

**Detection limit**

The detection limit was calculated based on the fluorescence titration. The slit was adjusted to 10 nm. To determine the S/N ratio, the emission intensity of compound **1** (25 μM) in the absence of Cys was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the Cys concentration was obtained in the range of 0~100 μM (R = 0.9998, Fig. S11). The detection limit was then calculated with the equation: detection limit = $3\sigma_{bi}/m$, wherein $\sigma_{bi}$ is the standard deviation of blank measurements and $m$ is the slope of the straight line between the fluorescence intensity and the concentration of Cys. [5] The detection limit was calculated to be $2.0\times10^{-7}$ M at S/N = 3 (signal-to-noise ratio of 3 : 1) for Cys. Similarly, the detection limit was calculated to be $4.3\times10^{-7}$ M for GSH (Fig. S12) and $1.2\times10^{-6}$ M for Hcy (Fig. S13) at S/N = 3, respectively.

**Fluorescence Imaging**

HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% double resistant (50 U/mL penicillin, 50 μg/mL streptomycin) at 37 ºC under a humidified atmosphere containing 5% $CO_2$. Exponentially growing cells were placed in 15 mm culture dish ($1\times10^6$ cells/mL) and incubated at 37 °C for 12 h for attachment. HepG2 cells were treated with 5 μM of compound **1** in culture media for 2 h at 30 ºC and washed 3 times with PBS buffer (0.01 M, pH 7.4). For the control experiment, the cells were

treated with *N*-ethylmaleimide (NEM, 20 μM) in culture media for 30 min at 30 ºC. After washed with PBS buffer to remove the remaining NEM, the cells were further incubated with 5 μM of compound **1** in culture media for 2 h at 30 ºC. [6]

**References**


1. M. S. Alexiou and J. H. P. Tyman, *J. Chem. Res. (S)* 2001, **2**, 59.
2. W. Xuan, R. Pan, Y. Cao, K. Liu, W. Wang, *Chem. Commun.* 2012, **48**, 10669.
3. S. Fery-Forgues, D. Lavabre, *J. Chem. Educ.* 1999, **76**, 1260.
4. L. Yuan, W. Y. Lin and Y. T.Yang, *Chem. Commun.* 2011, **47**, 6275.
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6. Y. H. Chen, J. Z. Zhao, H. M. Guo and L. J. Xie, *J. Org. Chem*. 2012, **77**, 2192.

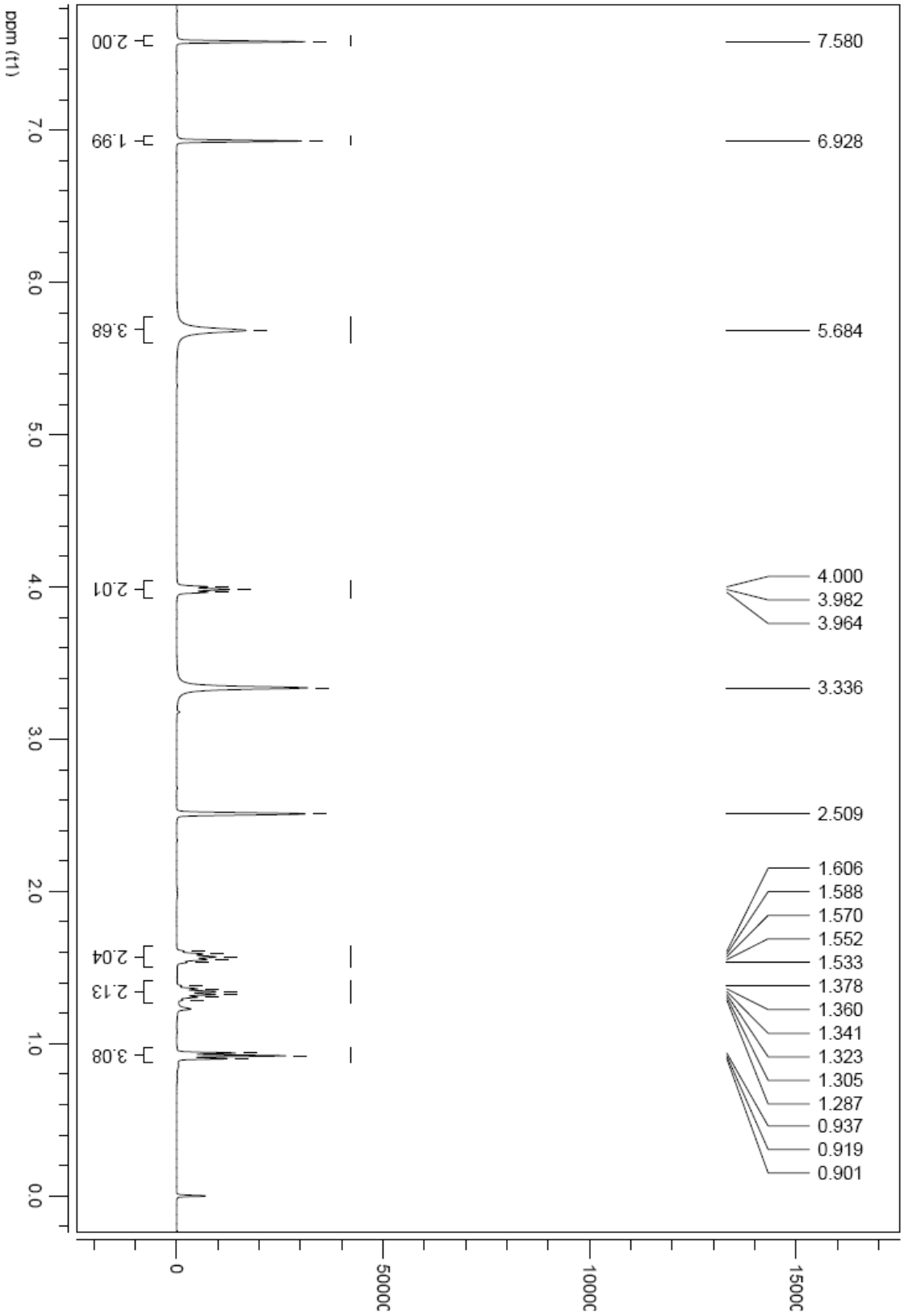


**Fig. S1** $^{1}$H NMR (400 MHz) of compound **2** in $d_6$-DMSO.

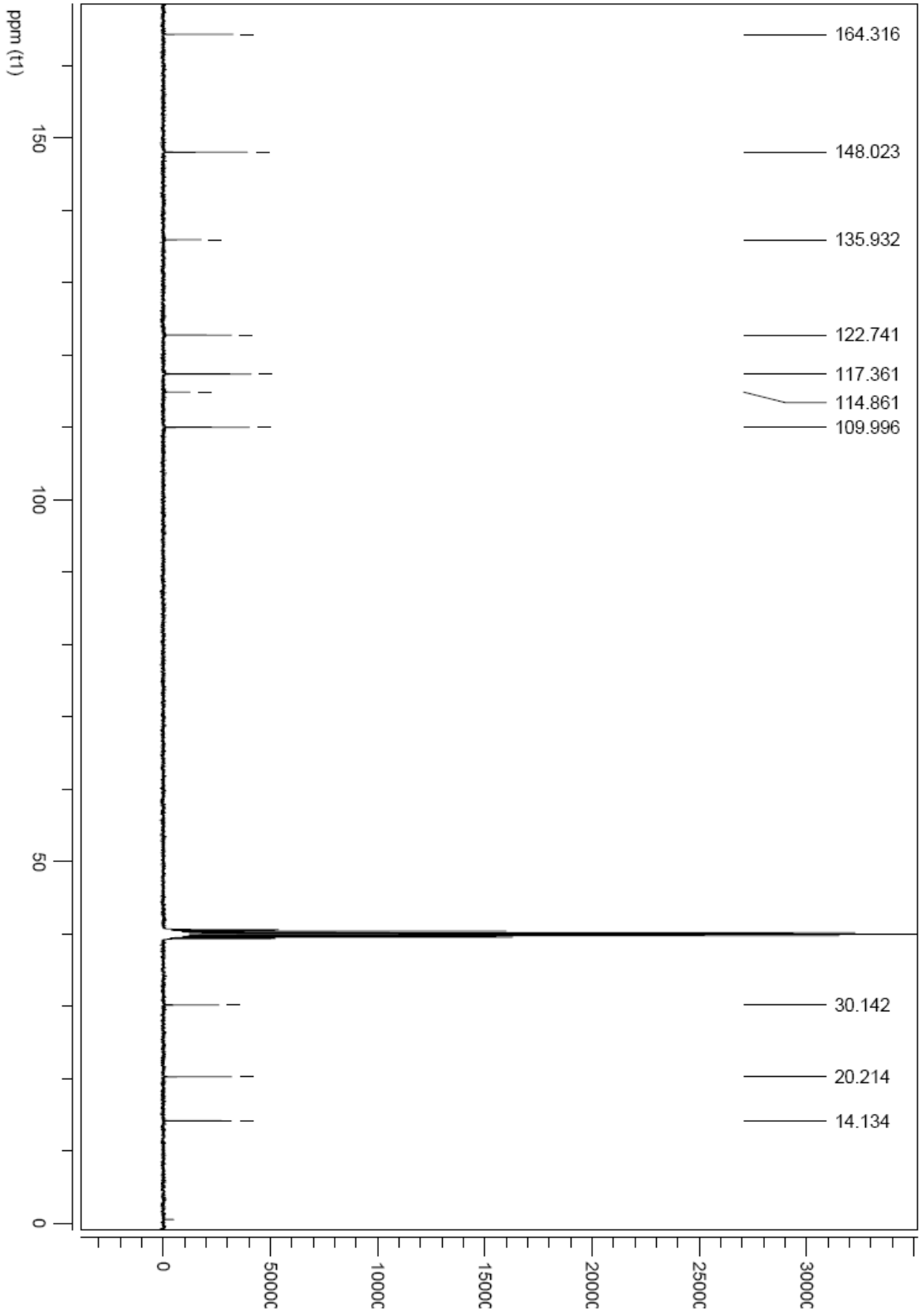


**Fig. S2** $^{13}$C NMR (100 MHz) of compound **2** in $d_6$-DMSO.

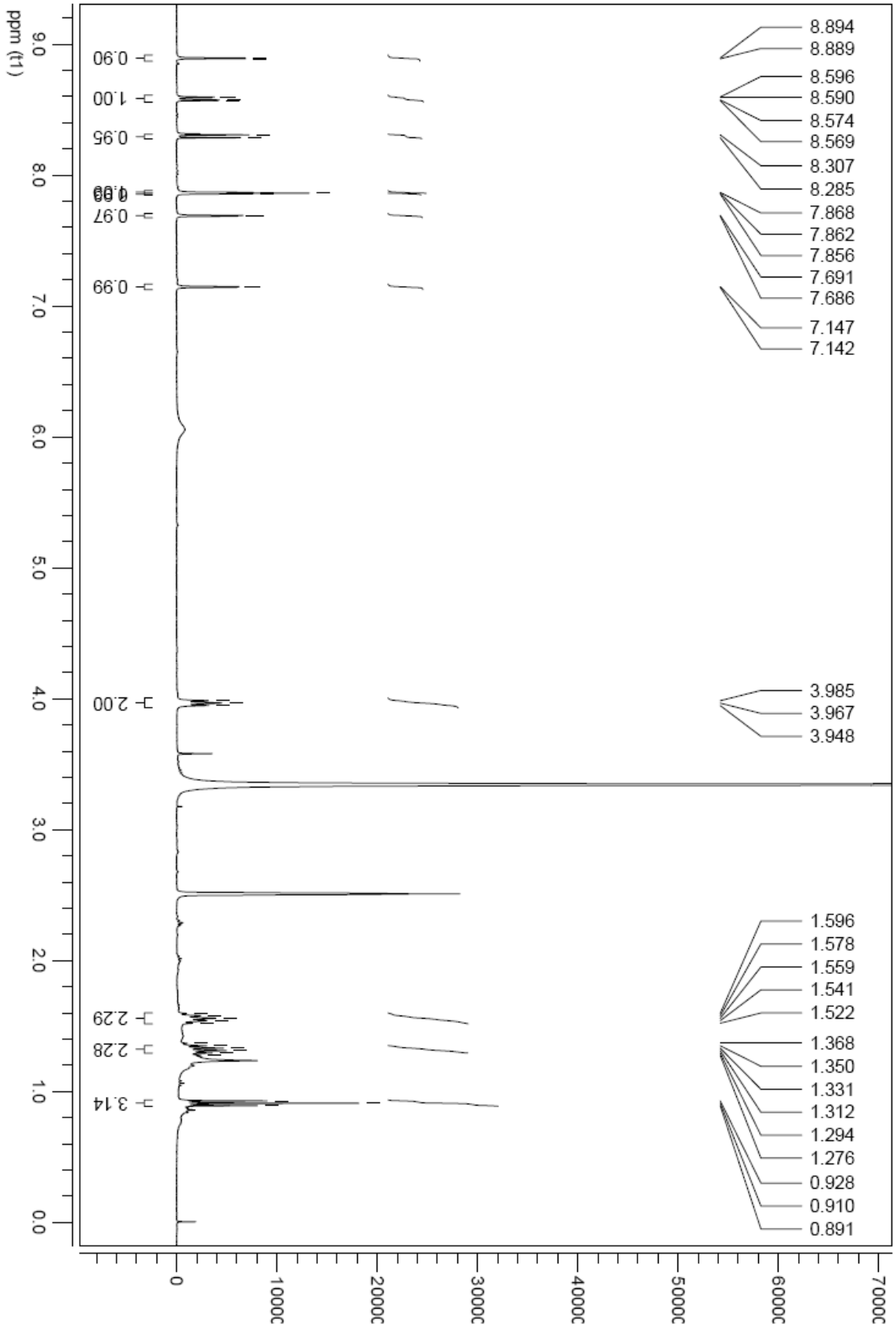


**Fig. S3** $^{1}$H NMR (400 MHz) of compound **1** in $d_6$-DMSO.

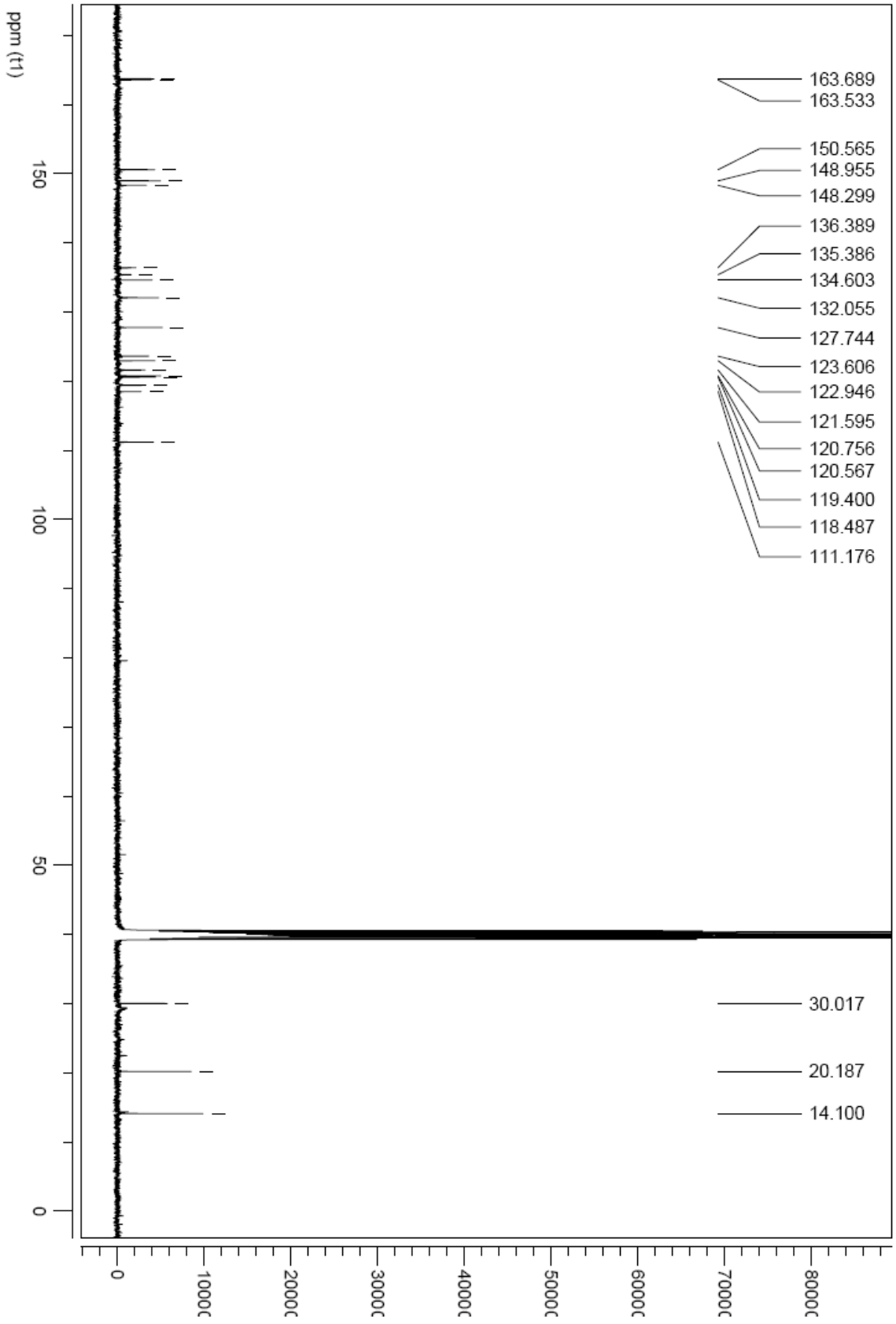


**Fig. S4** $^{13}$C NMR (100 MHz) of compound **1** in $d_6$-DMSO.

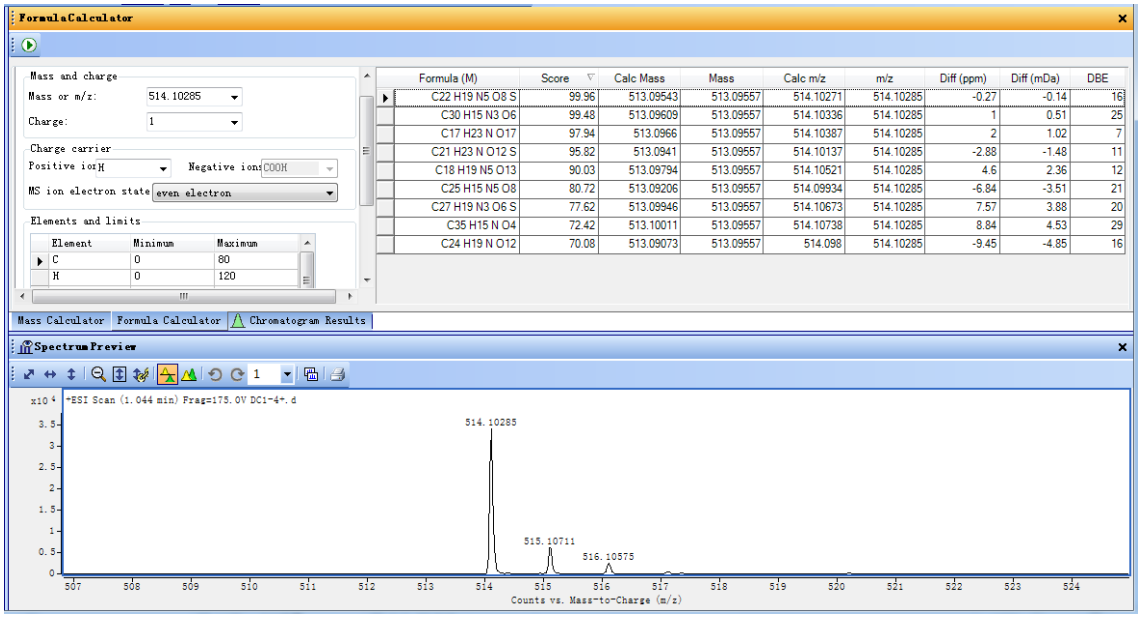


**Fig. S5** HR-ESI-MS of compound **1**.

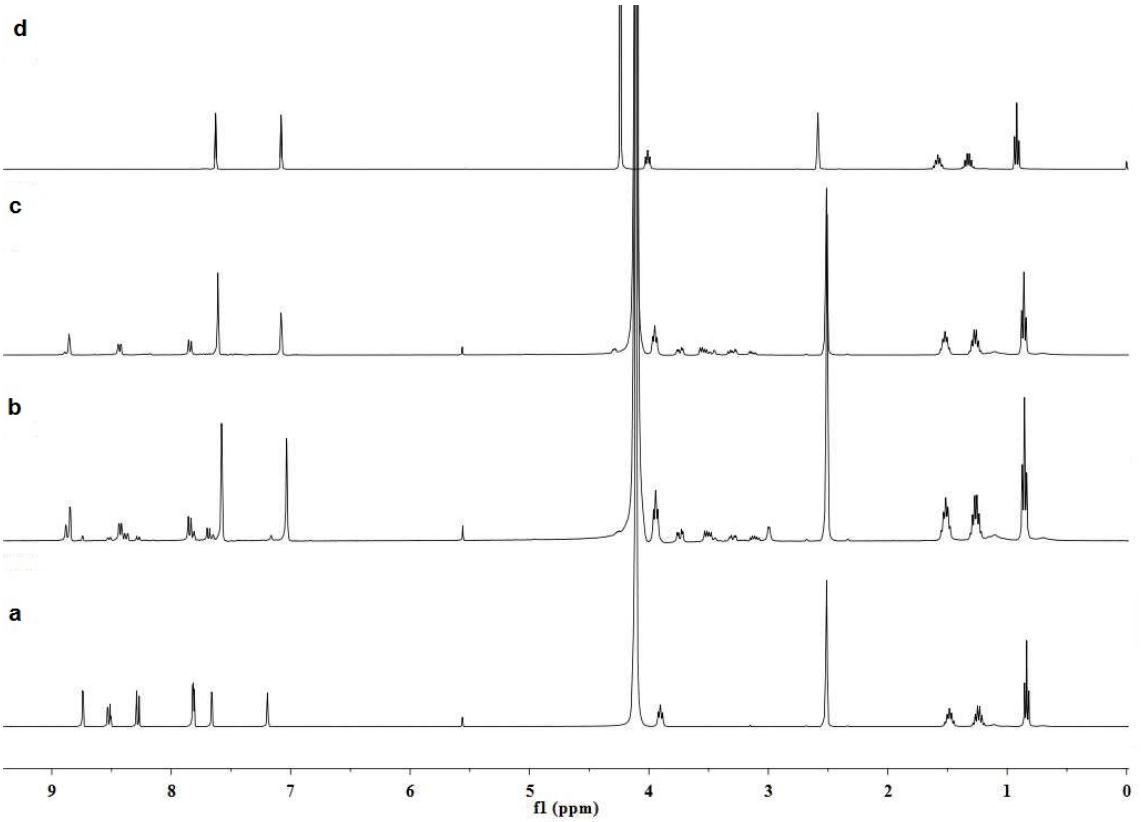


**Fig. S6** $^{1}$H NMR spectra of compound **1** in the absence (a), presence of Cys for 18 h (b) and for 72 h (c), and of compound **2** (d) in $d_6$-DMSO/$D_2O$ (5/2, v/v).

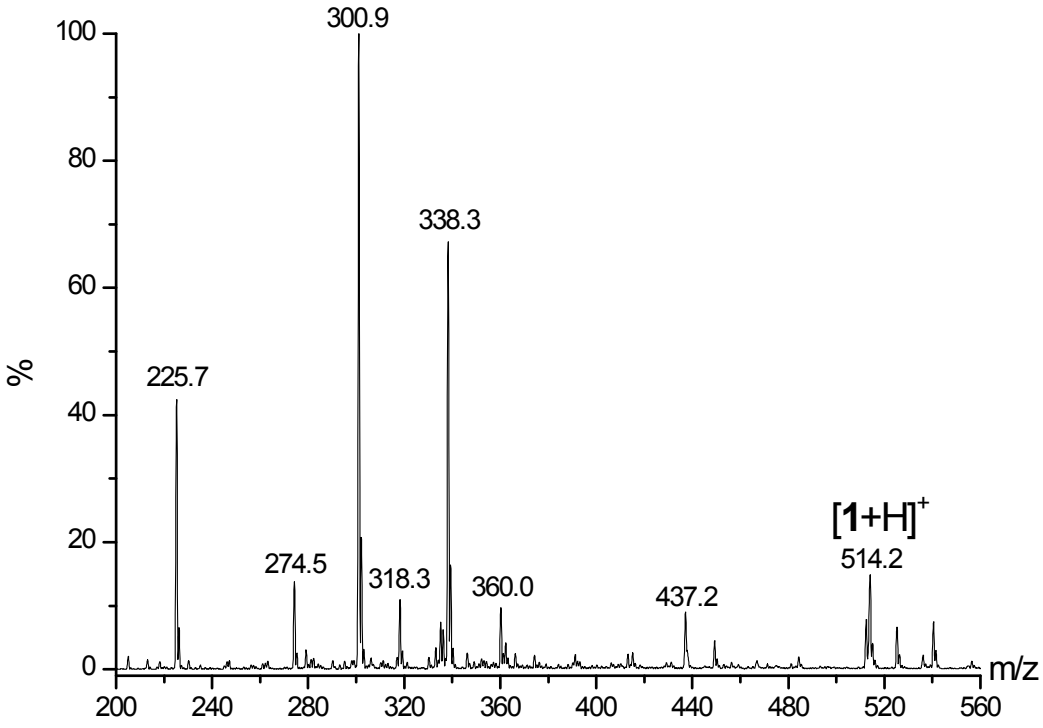


**Fig. S7** LR-ESI-MS of compound **1**.

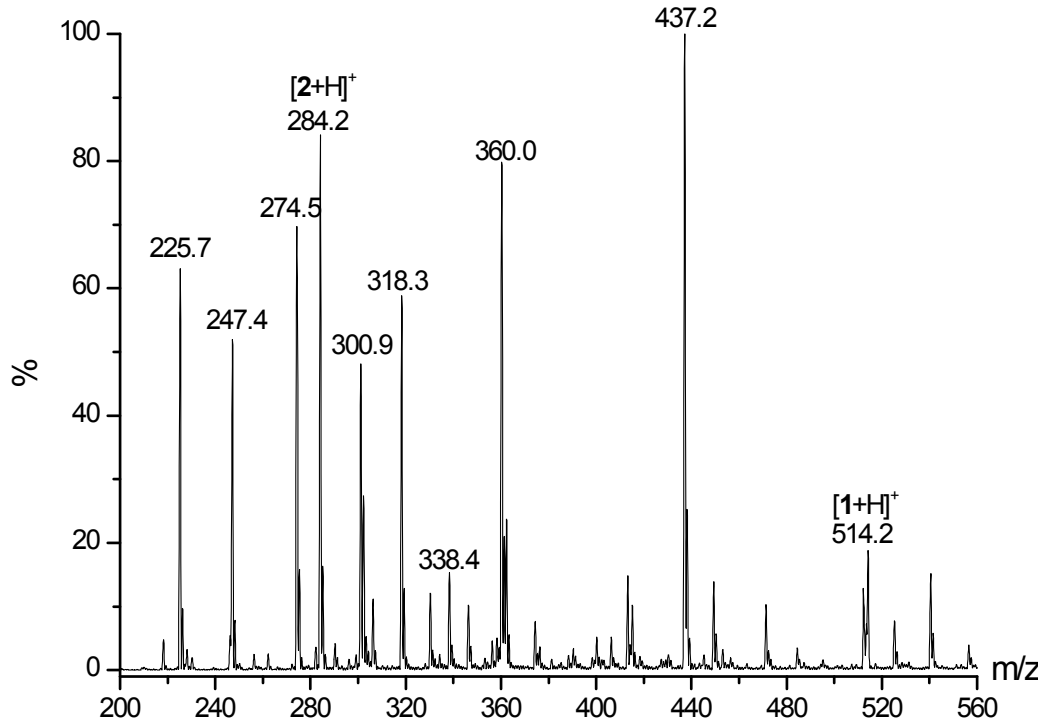


**Fig. S8** LR-ESI-MS of compound **1** titrated with Cys.

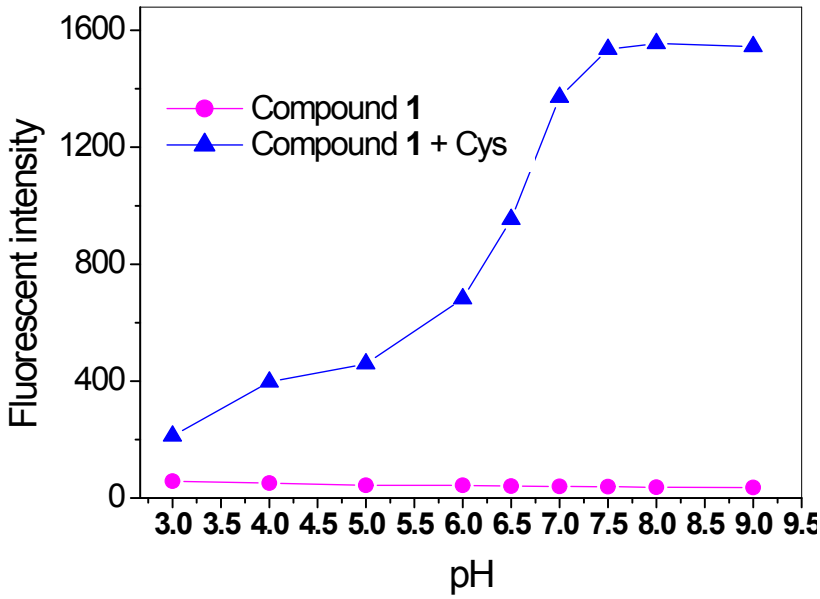


**Fig. S9** Effect of pH on the fluorescence intensities of compound **1** (25 μM) in the absence (●) and presence (▲) of Cys (250μM). The reaction of compound **1** and Cys was carried out at room temperature for 120 min, in 1 : 1 DMSO-PBS buffer (0.01 M) of varying pH from 3 to 9. $\lambda_{ex}/\lambda_{em}$ = 415/560 nm.

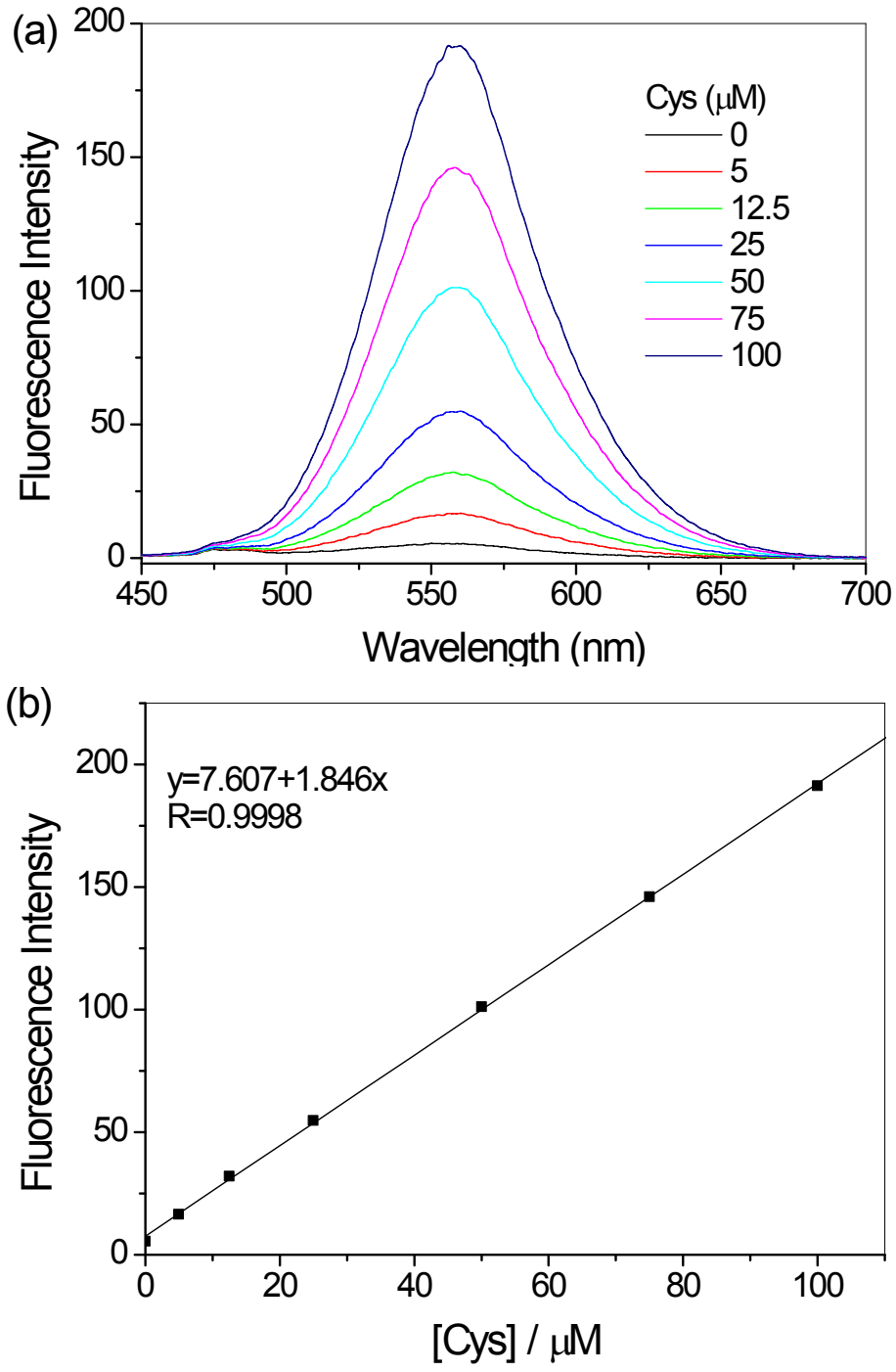


**Fig. S10** (a) Fluorescence response of compound **1** (25 μM) to Cys of varying concentrations in 1 : 1 DMSO-PBS buffer (0.01 M, pH 7.4) ($\lambda_{ex}/\lambda_{em}$ = 415/560 nm, ex/em 5/5 nm). (b) Fluorescence intensity at 560 nm of compound **1** (25 μM) upon the addition of Cys (0~100 μM).

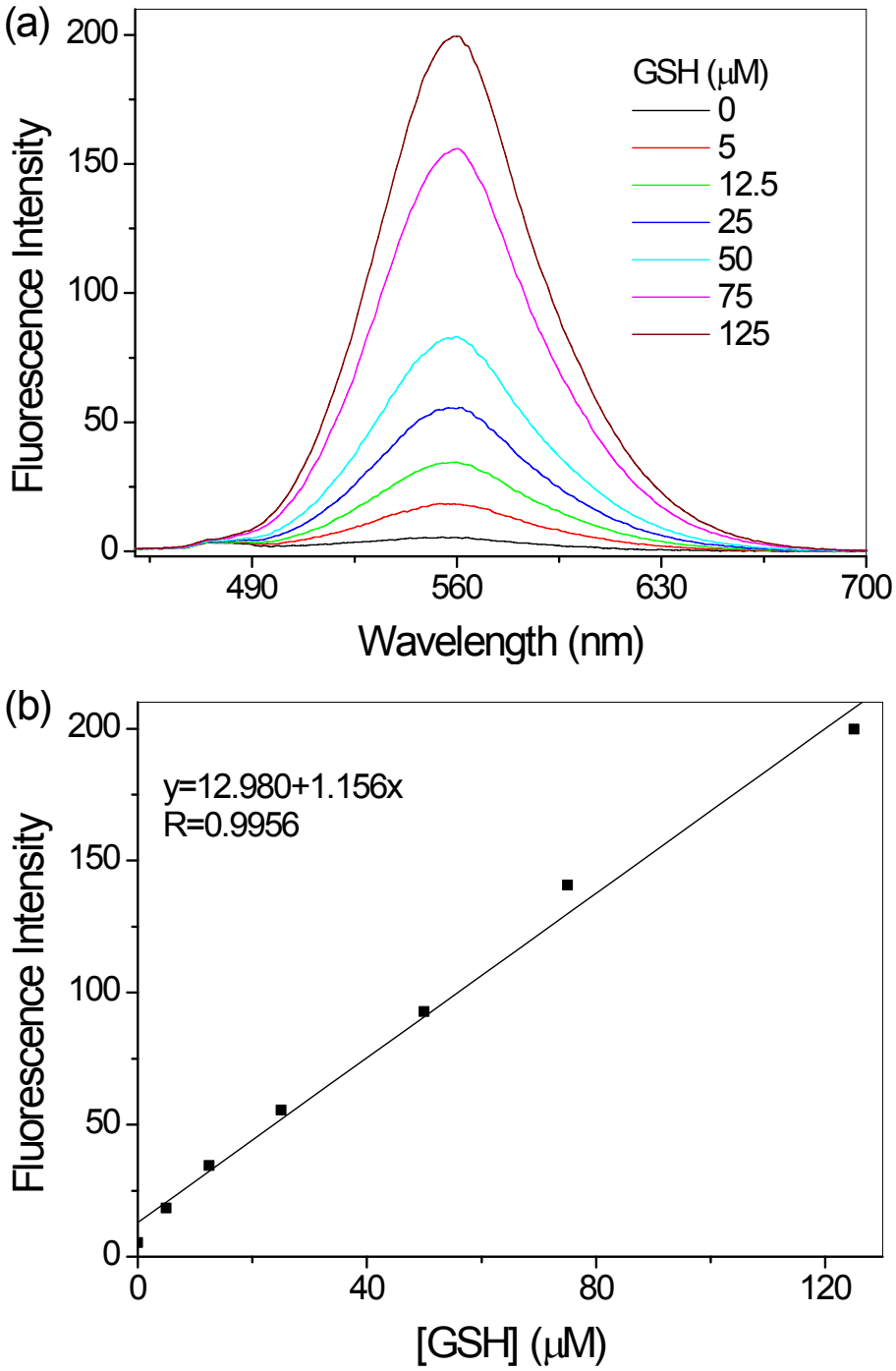


**Fig. S11** (a) Fluorescence response of compound **1** (25 μM) to GSH of varying concentrations in 1 : 1 DMSO-PBS buffer (0.01 M, pH 7.4) ($\lambda_{ex}/\lambda_{em}$ = 415/560 nm, ex/em 5/5 nm). (b) Fluorescence intensity at 560 nm of compound **1** (25 μM) upon the addition of GSH (0~150 μM).

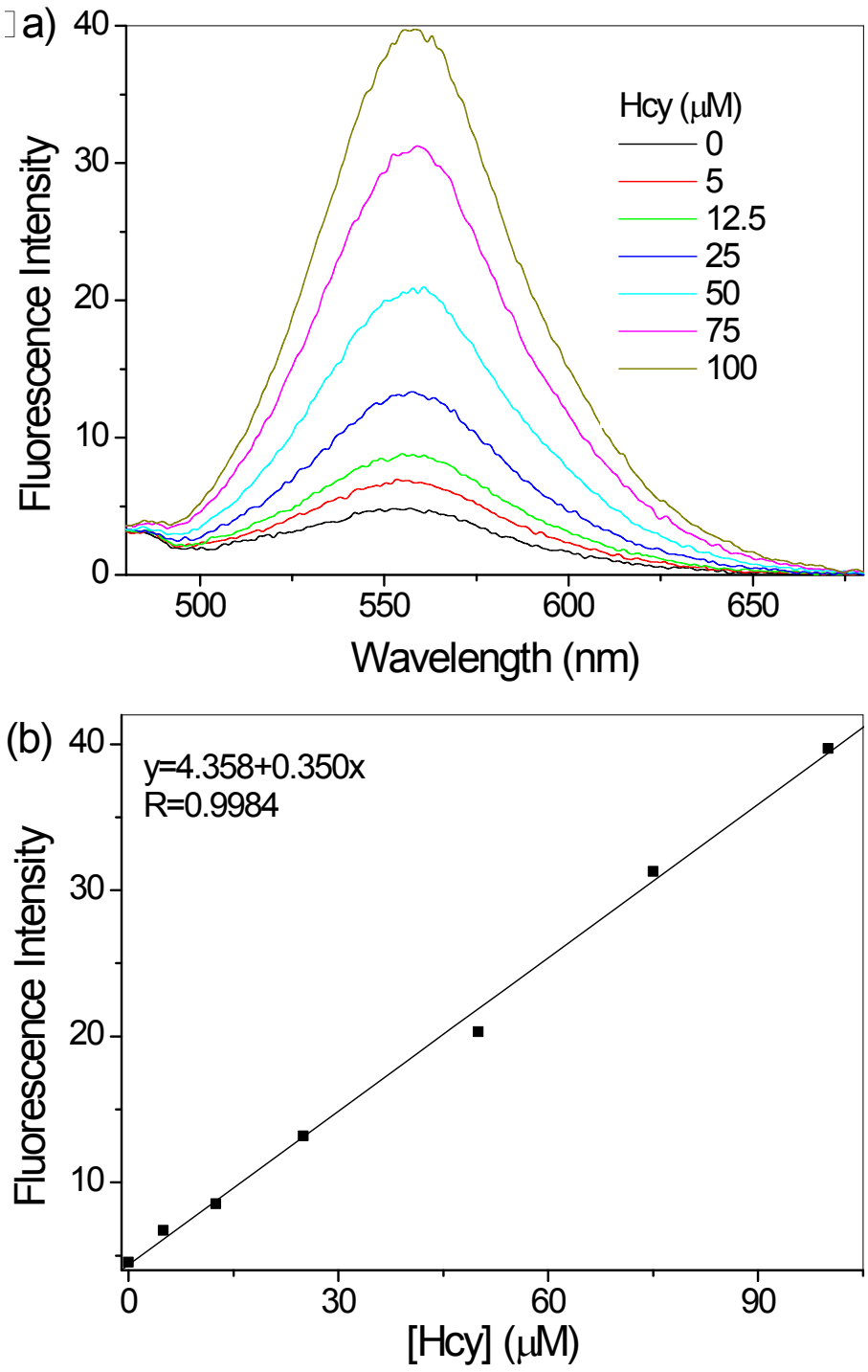


**Fig. S12** (a) Fluorescence response of compound **1** (25 μM) to Hcy of varying concentrations in 1 : 1 DMSO-PBS buffer (0.01 M, pH 7.4) ($\lambda_{ex}/\lambda_{em}$ = 415/560 nm, ex/em 5/5 nm). (b) Fluorescence intensity at 560 nm of compound **1** (25 μM) upon the addition of Hcy (0~150 μM).

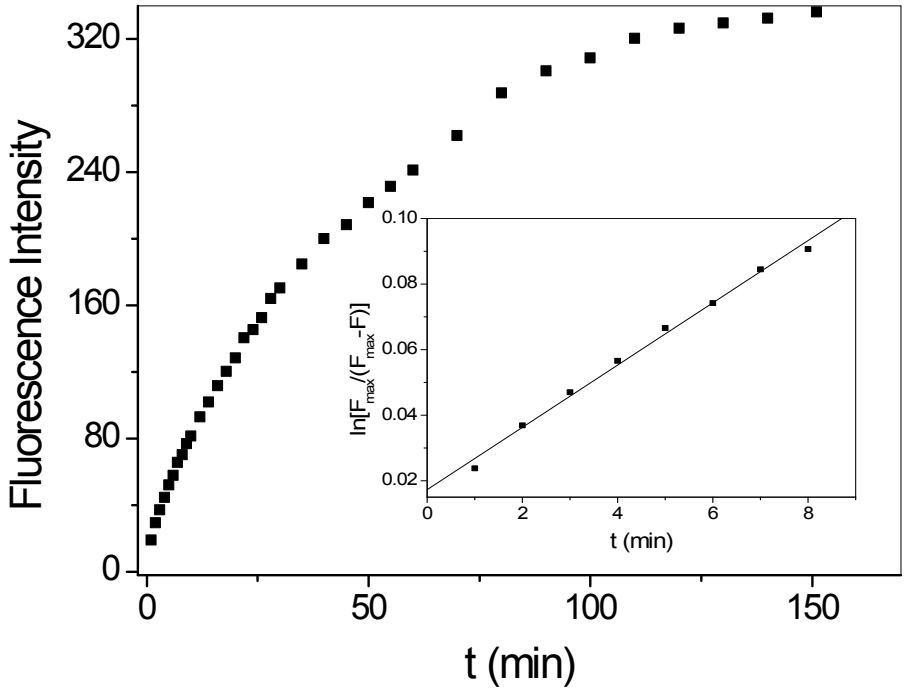


**Fig. S13** Time-dependent fluorescence response of compound **1** (25 μM) to GSH (1 mM) at room temperature in 1 : 1 DMSO-PBS buffer (0.01 M, pH 7.4).

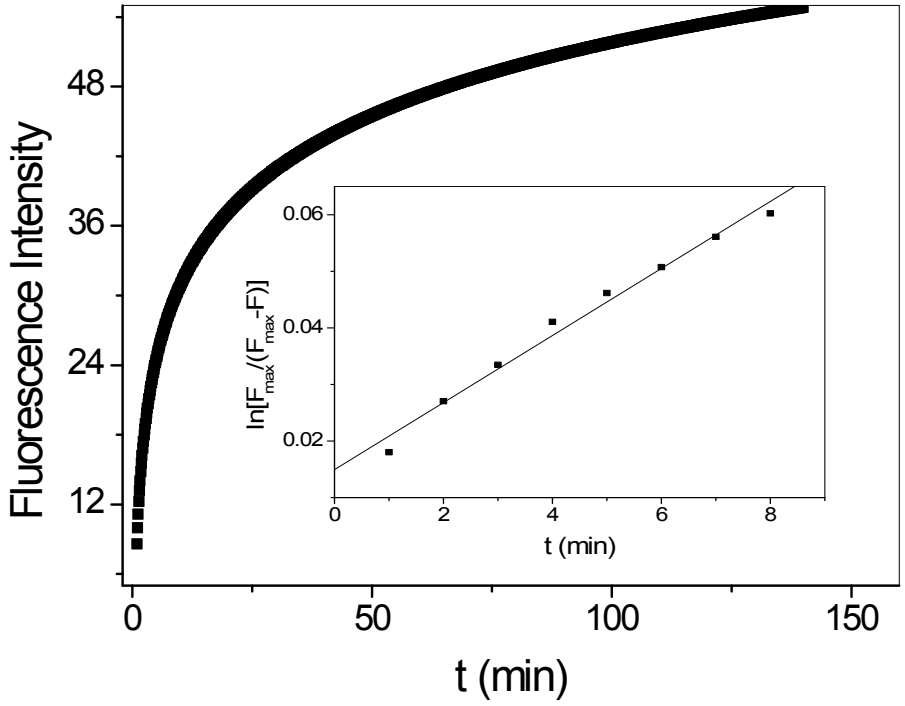


**Fig. S14** Time-dependent fluorescence response of compound **1** (25 μM) to Hcy (1 mM) at room temperature in 1 : 1 DMSO-PBS buffer (0.01 M, pH 7.4).