Electronic Supplementary Information (ESI†)

Naphthalimide-based Fluorescent Probe for Selectively and Specifically Detecting Glutathione in Lysosome of Living Cells

Meijiao Cao, Haiyan Chen,* Dan Chen, Zhiqiang Xu, Shenghua Liu, Xiaoqiang Chen,* and Jun Yin*

1. Experiment section

1.1. Instruments and reagents

All reactions were taken place by using standard Schlenk techniques under an argon atmosphere, unless elaborated. All reagents and materials were purchased commercially and without further purification. Column chromatography was performed over silica gel (200-300 mesh). 400 MHz and their chemical shifts are relative to TMS. Electrospray (EI) mass spectra were carried on Firmigan Trace. UV-Vis spectra were obtained on U-3310 UV Spectrophotometer. Fluorescence spectra were taken on Perkin-Elmer, LS-55 fluorescence Spectrophotometer.

1.2. Characterizations

A stock solution of **1a** or **1b** (1 mM) was prepared in DMSO and a stock solution of amino acids (10 mM) was dissolved in distilled water. We selected a wide range of amino acids, such as Ala, Arg, Cys, Glu, Gly, GSH, Hcy, His, Lys, Met, Ser and Tyr. For selectivity, the work solution of **1a** or **1b** (10 μM) was prepared by mixing 1 mM of **1a** or **1b** and 0.1 mM of amino acids (10 mM) in 0.02 M HEPES buffer/DMSO (9/1, v/v). For titration, the work solution of **1b** (10 μM) was obtained by mixing 1 mM of **1b** in 0.02 M HEPES buffer/DMSO (9/1, v/v) with varying concentration of GSH from 0 to 200 μM. For all fluorescence spectra were collected from 390 to 720 nm excitation with 370 nm, slit width at 5/4 nm except for the fluorescence spectra of **1a**.

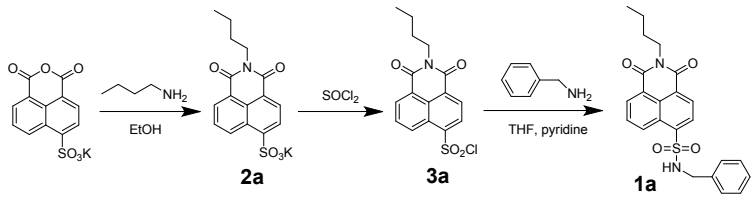
1.3. DFT calculation

All the DFT calculations were carried out at the B3LYP/6-31G level, using the Gaussian 09 program.

1.4. Cell culture and imaging

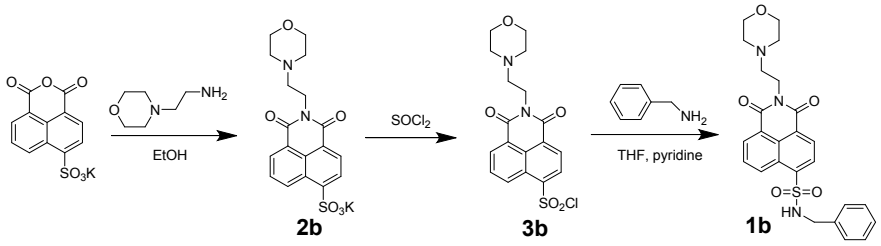
The human hepatoma cells cell lines HepG2 were obtained from American Type Culture Collection (ATCC, USA). The lysosome red probe LysoRed was obtained from KeyGEN BioTECH (Nanjing, China). All the fluorescence spectrums were collected by LSCM (FV 1000, Olympus, Japan). The cell lines were cultured in DMEM medium supplemented with 10% (v/v) calf serum, penicillin (100 U/mL) and streptomycin (100 mg/mL). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

1.5. Synthesis of 1a and 1b



Synthesis of **1a**: To a solution of 4-sulfo-1,8-naphthalic anhydride potassium salt (2 g, 6 mmol) in ethanol (100 ml) under an argon atmosphere was added N-butylamine (3 ml, 12 mmol). After the reaction mixture was refluxed for 3 h, the residue was cooled to room temperature, filtered and washed with ethanol. The obtained cream residue **2a** in 65% yield was without further purified according to the previous literatures. To a solution of **2a** (250 mg, 0.85 mmol) under an argon atmosphere was added $SOCl_2$ (20 ml) and a catalytic amount of DMF. After the result solution was refluxed for 12 h, the solvent removal under reduced pressure. The residue **3a** was used directly to the next reaction. To a solution of benzylamine (111 mg, 0.85mmol) in anhydrous THF (20 ml) under argon atmosphere was added the pyridine (2 ml). After the reaction mixture was stirred for 5 min, a solution of **3a** (300 mg, 0.85 mmol) in THF (20 ml) was added dropwise at 0℃. After the result solution was refluxed for 16 h, the solvent removal under reduced pressure. The residue was purified by silica column hexane:dichloromethane=1:9 to obtain **1a** (237 mg, 0.56 mmol) as pale-yellow solid in 66% yield. ^{1}H NMR (400 MHZ, $CDCl_3$): δ (ppm) = 9.02 (d, *J*=8 Hz, 1H, Ar-H), 8.70 (d, *J*=8 Hz, 1H, Ar-H), 8.60 (d, *J*=8 Hz, 1H, Ar-H), 8.41 (d, *J*=8

Hz, 1H, Ar-H), 7.92 (t, *J*=8 Hz, 1H, Ar-H), 7.16-7.14 (m, 3H, Ph-H), 7.05-7.03 (m, 2H, Ph-H), 5.06 (t, *J*=4 Hz, 1H, NH), 4.21-4.17 (m, 4H, N-CH_2), 1.74-1.69 (m, 2H, CH_2), 1.48-1.43 (m, 2H, CH_2), 1.01 (t, *J*=8 Hz, 3H, CH_3). ^{13}C NMR (100 MHZ, $CDCl_3$): δ (ppm) = 163.43, 162.84, 140.46, 135.37, 131.65, 130.57, 129.38, 129.13, 128.74, 128.43, 127.85, 127.68, 126.70, 126.54, 123.11, 47.33, 40.46, 30.01, 20.26, 13.67. ESI-MS: m/z= 422.47 $[M]^+$; calculated exact mass= 422.13.



Synthesis of **1b**: the synthesis of **1b** was the similar to **1a** affording a pale-yellow solid in 60% yield. ^{1}H NMR (400 MHZ, $CDCl_3$): δ (ppm) = 9.03 (d, *J*=8 Hz, 1H, Ph-H), 8.70 (d, *J*=8 Hz, 1H, Ar-H), 8.60 (d, *J*=8 Hz, 1H, Ar-H), 8.42 (d, *J*=8 Hz, 1H, Ar-H), 7.93 (t, *J*=8 Hz, 1H, Ar-H), 7.16-7.15 (m, 3H, Ph-H), 7.06-7.05 (m, 2H, Ph-H), 5.18 (t, *J*=4 Hz, 1H, NH), 4.37 (t, *J*=8 Hz, 2H, N-CH_2), 4.20 (d, *J*=4 Hz, 2H, NH-CH_2), 3.67 (t, *J*=4 Hz, 4H, N-CH_2), 2.72 (t, *J*=8 Hz, 2H, N-CH_2), 2.60 (t, *J*=8 Hz, 2H, O-CH_2). ^{13}C NMR (100 MHZ, $CDCl_3$): δ (ppm) = 163.74, 163.17, 141.02, 135.74, 131.98, 131.02, 129.75, 129.39, 129.15, 129.03, 128.78, 128.21, 128.03, 127.08, 126.76, 123.37, 67.22, 56.23, 53.99, 47.64, 37.68. ESI-MS: m/z= 479.15 $[M]^+$; calculated exact mass= 479.15.

2. The absorption and fluorescence spectra of 1a

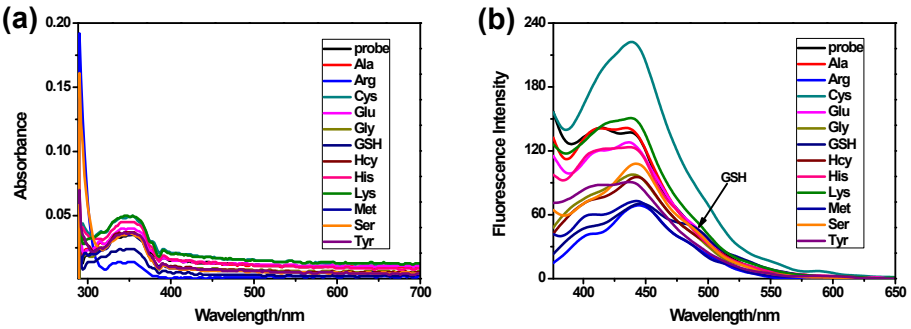


Fig. S1 The absorption (a) and fluorescence (b) spectra of **1a** (10 μM) with various amino acids (100 μM in HEPES buffer (0.02 M, pH= 7.4) containing 10% DMSO, λ_{ex} = 356 nm.

3. Photograph of 1b under UV light and absorption spectra of 1b

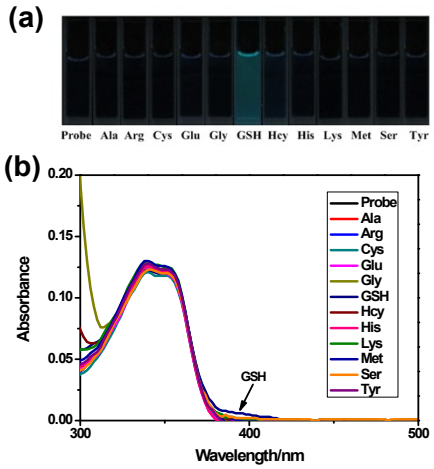


Fig. S2 (a) Photograph of **1b** with various amino acids under UV light. (b) The absorption spectra of **1b** (10 μM) with various amino acids (100 μM) in HEPES buffer (0.02 M, pH= 7.4) containing 10% DMSO. λ_{ex} = 370 nm.

4. The competitive fluorescence assay of 1b and the absorption titration spectrum of 1b

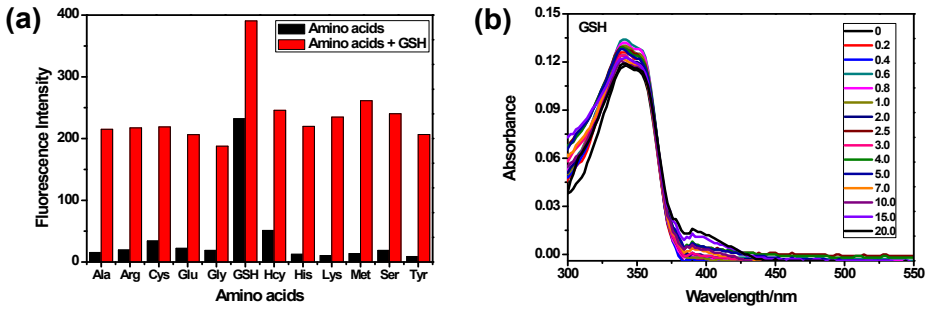


Fig. S3 (a) The fluorescence change of **1b** with GSH in the presence of various amino acids. Black bars are **1b** with other amino acids; red bars are **1b** with GSH in the present of other amino acids. **(b)** The absorption spectra of **1b** (10 μM) in the presence of 0 - 20 equiv of GSH in HEPES buffer (0.02 M, pH= 7.4) containing 10% DMSO.

5. TD-DFT calculation for 1a, 1b and 1b+GSH

Table S1 Major electronic excitations for **1a**, **1b** and **1b+GSH**

Compound	Excited state	λ/nm [eV]	Osc. str (f)	Major contributions
1a	$S_0 \rightarrow S_4$	339.42 [3.65]	0.2659	HOMO-2→LUMO (68%)
	$S_0 \rightarrow S_5$	328.09 [3.78]	0.0134	HOMO-4→LUMO (39%)
1b	$S_0 \rightarrow S_5$	354.27 [3.50]	0.0314	HOMO-1→LUMO (49%)
	$S_0 \rightarrow S_8$	340.13 [3.65]	0.2319	HOMO-3→LUMO (57%)
1b+GSH	$S_0 \rightarrow S_2$	362.15 [3.42]	0.2304	HOMO-1→LUMO (64%)
	$S_0 \rightarrow S_6$	327.23 [3.79]	0.0973	HOMO-3→LUMO (60%)

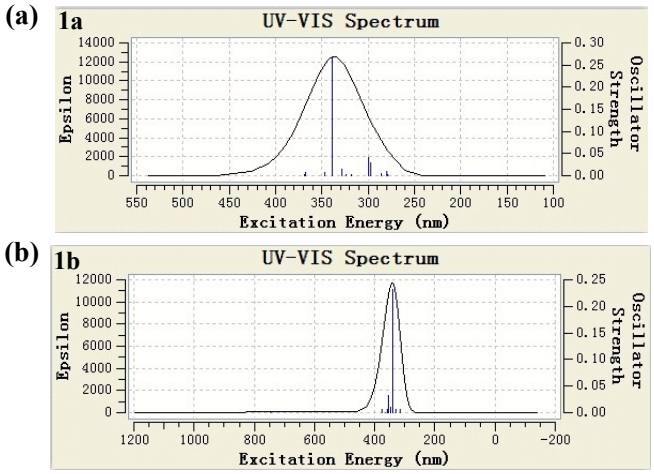


Fig. S4 UV-Vis Spectrum of **1a** and **1b** using TD-DFT calculation.

6. ESI Mass spectrometry of 1b in the presence of GSH, Cys and Hcy

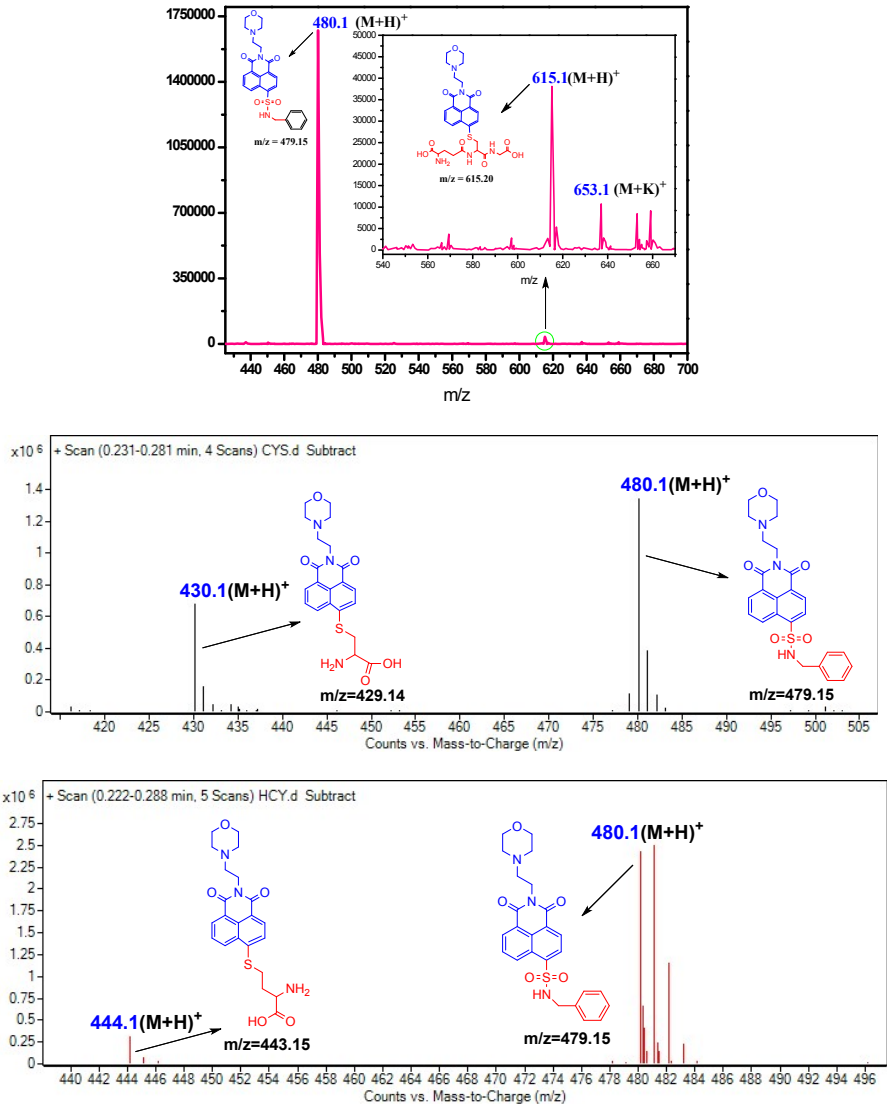
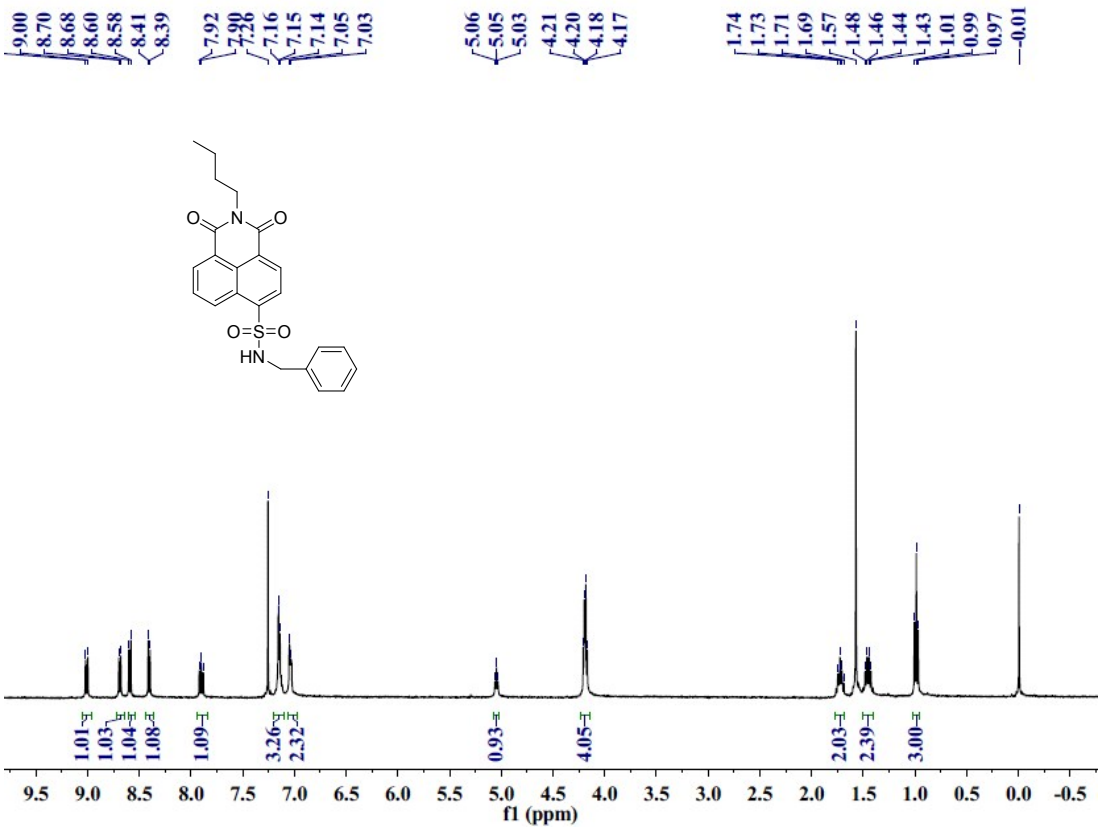
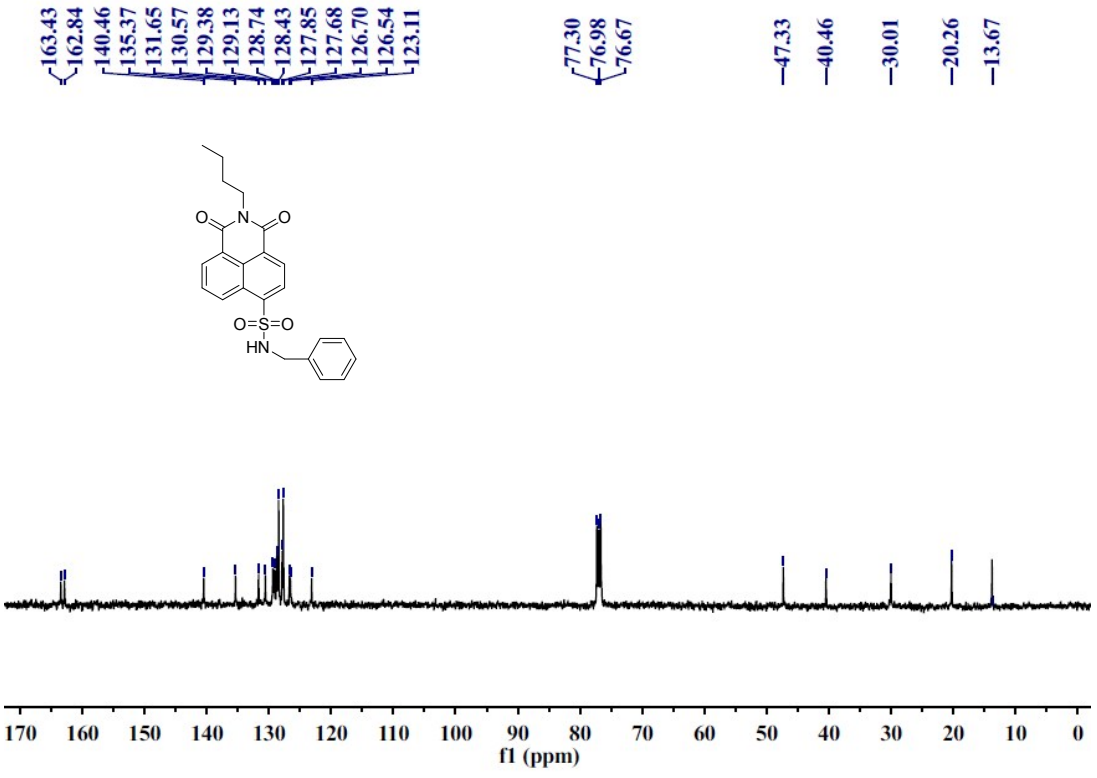
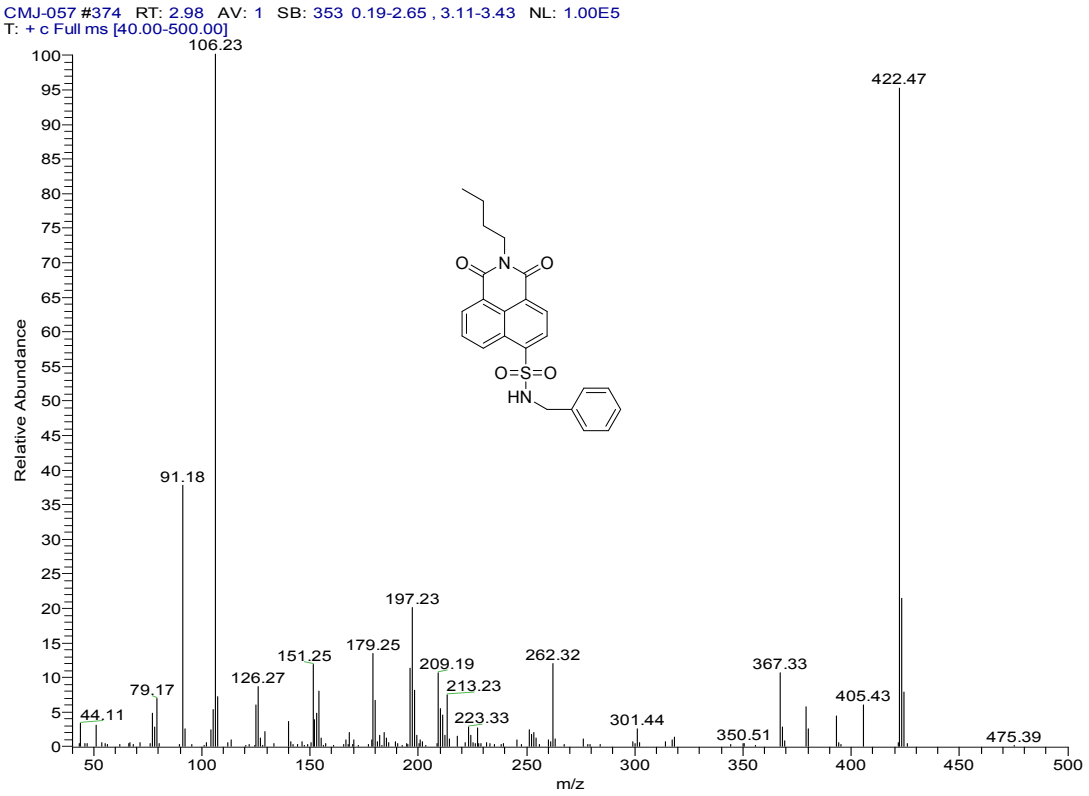


Fig.S5 ESI mass spectrometry of **1b** in the presence of GSH, Cys and Hcy.

7. ^{1}H NMR, ^{13}C NMR, EI-MS spectrum



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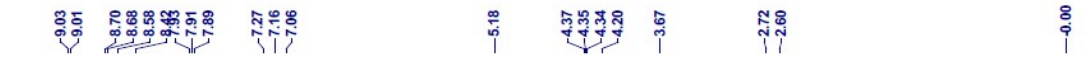
91.18
197.23
179.25
151.25
209.19
262.32
367.33
126.27
213.23
405.43
79.17
223.33
301.44
44.11
350.51
475.39
O
N
O
O=S=O
HN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9.01
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8.68
8.58
7.91
7.89
7.27
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7.06
5.18
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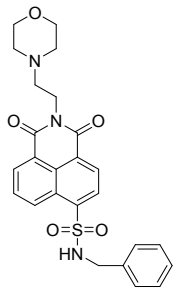
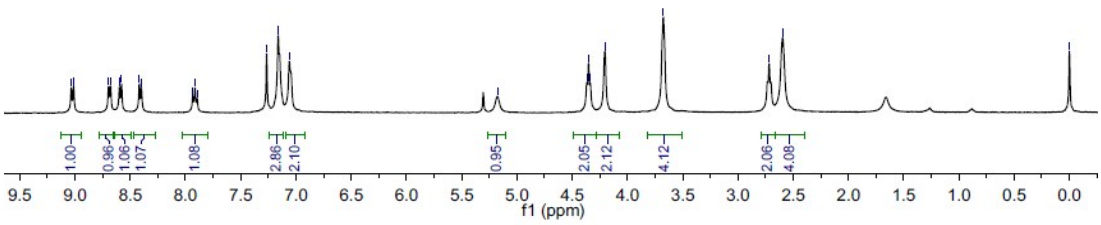
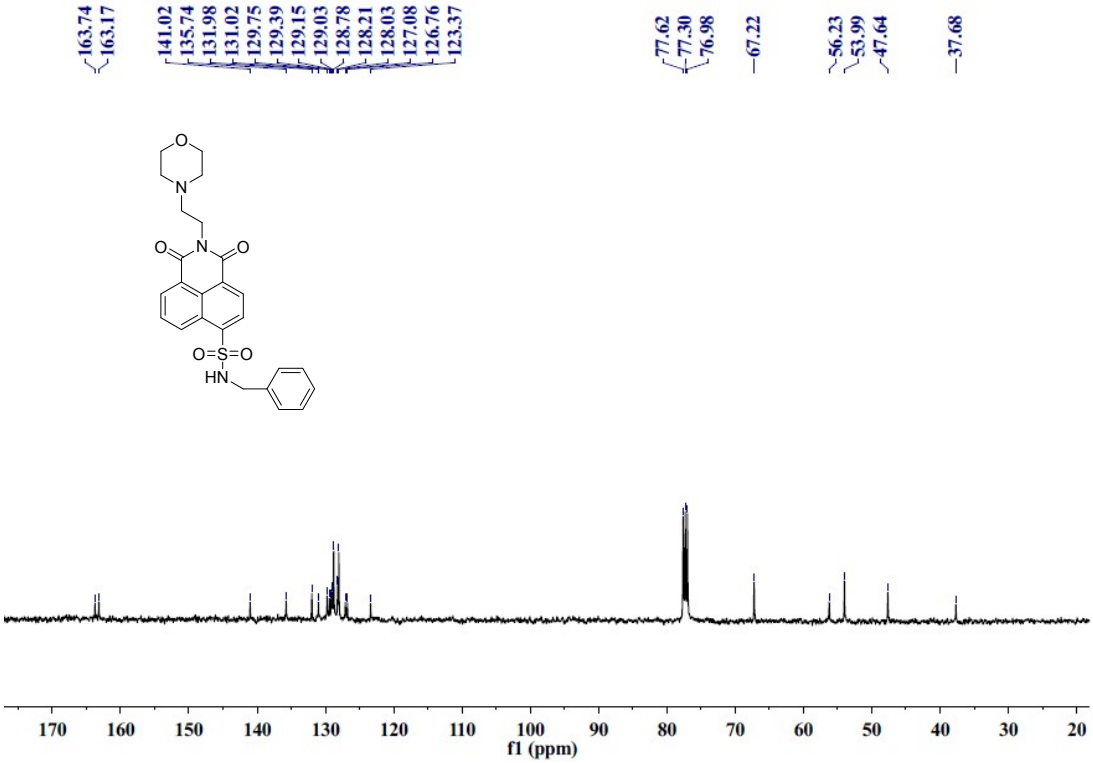
4.34
4.20
3.67
2.72
2.60
0.00


O
N
O
N
O
O=S=O
HN


1.00
0.96
1.06
1.07
1.08
2.86
2.10
0.95
2.05
2.12
4.12
2.06
4.08
f1 (ppm)


163.74
163.17
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135.74
131.98
131.02
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129.39
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129.03
128.78
128.21
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127.08
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123.37
77.62
77.30
76.98
67.22
56.23
53.99
47.64
37.68
O
N
O
N
O
O=S=O
HN
170
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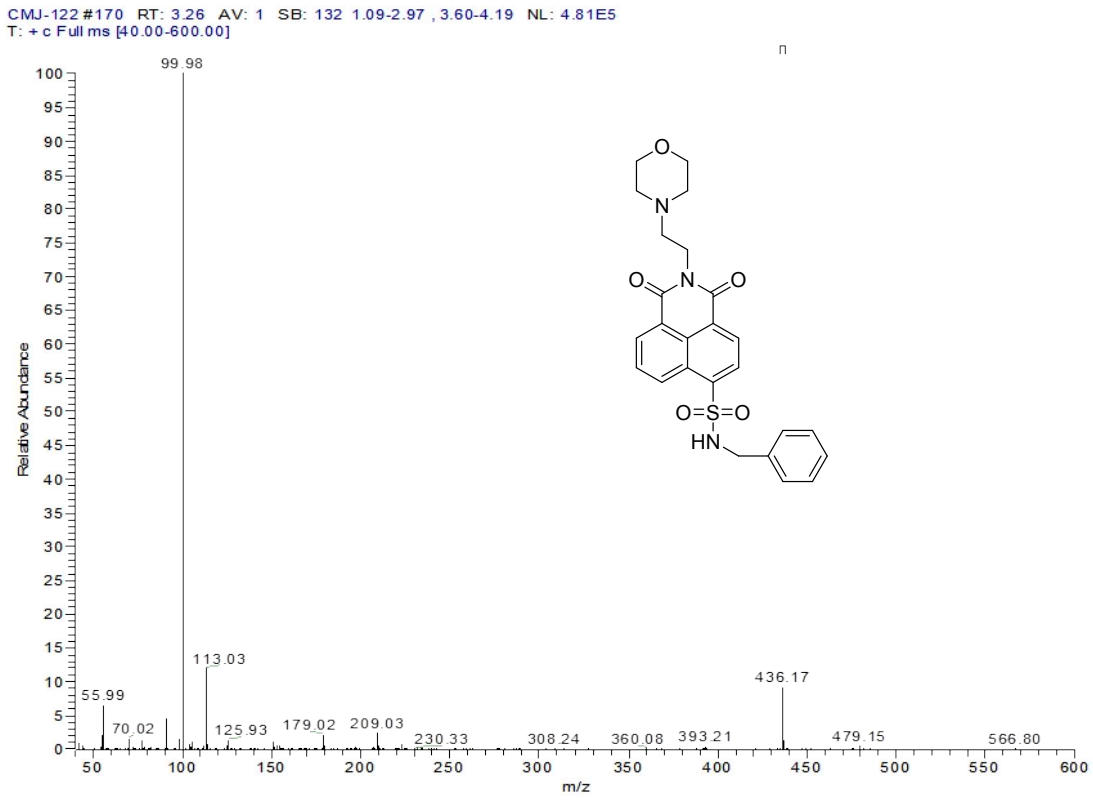
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)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55.99
70.02
125.93
179.02
209.03
230.33
308.24
360.08
393.21
436.17
479.15
566.80
O
N
O
N
O
O=S=O
HN