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

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1. Experiment section
2. The absorption and fluorescence spectra of 1a with various amino acids
3. Photograph of 1b under UV light and absorption spectra of 1b
4. The competitive fluorescence assay of 1b and the absorption titration spectrum of 1b
5. TD-DFT calculation for 1a, 1b and 1b+GSH
6. ESI Mass spectrometry of 1b in the presence of GSH, Cys and Hcy
7. ^1H NMR, ^13C NMR, ESI-MS spectrum
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 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% CO2.

1.5. Synthesis of 1a and 1b

![Synthesis of 1a and 1b](image_url)

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 SOCl2 (20 ml) and a catalytic amount of DMF. After the reflux of 3 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 3 h, a solution of SOCl2 (20 ml) was added and this mixture 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. 1H NMR (400 MHz, CDCl3): 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), 8.41 (d, J=8 Hz, 1H, Ar-H), 8.41 (d, J=8 Hz, 1H, Ar-H), 8.41 (d, J=8 Hz, 1H, Ar-H), 8.41 (d, J=8 Hz, 1H, Ar-H), 8.41 (d, J=8 Hz, 1H, Ar-H), 8.41 (d, J=8 Hz, 1H, Ar-H).
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-CH₂), 1.74-1.69 (m, 2H, CH₂), 1.48-1.43 (m, 2H, CH₂), 1.01 (t, J=8 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃): 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. ¹H NMR (400 MHz, CDCl₃): 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-CH₂), 4.20 (d, J=4 Hz, 2H, NH-CH₂), 3.67 (t, J=4 Hz, 4H, N-CH₂), 2.72 (t, J=8 Hz, 2H, N-CH₂), 2.60 (t, J=8 Hz, 2H, O-CH₂). ¹³C NMR (100 MHz, CDCl₃): 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, 47.64, 37.68. ESI-MS: m/z= 479.15 [M]+; calculated exact mass= 479.15.

2. The absorption and fluorescence spectra of 1a

![Absorption Spectra](image)

**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, λₑₓ = 356 nm.

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

![Photograph of 1b](image)
4. The competitive fluorescence assay of 1b and the absorption titration spectrum of 1b

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

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

![NMR spectrum with chemical shifts and peaks]

[Chemical structure images]

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Page 56