Supporting information

Design of a ratiometric fluorescent probe for benzenethiols based on thiol-sulfoxide reaction

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General Methods and Instruments

All chemicals were purchased from commercial suppliers and used without further purification unless otherwise specified. 2-Chloro-5-benzoyl-pyrrole\(^1\), 2,4-dimethyl-3-ethylpyrrole\(^2\), 3-chloro-5,7-dimethyl-6-ethyl-8-phenyl-BODIPY\(^3\) were prepared according to literature procedures.

\(^1\)H NMR and \(^{13}\)C NMR spectra were recorded on spectrometers operating at 400 MHz and 100 MHz, respectively. Deuterated chloroform was used as the solvent, TMS as internal standard. Mass spectra were measured on a HP 1100 LC-MS spectrometer. UV-vis spectra were measured using a Shimadzu UV-2550 spectrophotometer. Fluorescence spectroscopic measurements were conducted on a Varian Cary Eclipse fluorescence spectrophotometer. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

For absorption or fluorescence measurements, compounds were dissolved in EtOH to obtain stock solutions (2-5 mM). These stock solutions were diluted with aqueous solutions to the desired concentration.


Experiential Section

Scheme S1. Synthesis of BSFO and BSFI.

Synthesis of 3-(4-methylphenyl)sulfenyl-5,7-dimethyl-6-ethyl-8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BSFI). A reaction mixture of 1 (0.358 g, 1 mmol), p-thiocresol (0.149 g, 1.2 mmol) and Et$_3$N (3 mL) in 20 mL CH$_3$CN was stirred for 24h at room temperature. Excess CH$_3$CN was removed under vacuum, and the residue was dissolved in ethyl acetate, washed H$_2$O, dried over Na$_2$SO$_4$. The crude product was purified by flash chromatography (silica gel) to afford BSFI (0.289 g, 65%). $^1$H NMR (400MHz, CDCl$_3$) $\delta$ 7.51 (d, 2H), 7.46-7.43 (m, 3H), 7.32-7.27 (m, 2H), 7.20 (d, 2H), 6.21 (d, $J = 4$ Hz, 1H), 5.77 (d, $J = 4$ Hz, 1H), 2.62 (S, 3H), 2.38-2.32 (m, 5H), 1.41 (S, 3H), 1.02 (t, $J = 8$ Hz, 3H); $^{13}$C NMR (100MHz, CDCl$_3$) $\delta$ 158.8, 152.2, 139.9, 139.3, 139.0, 134.3, 130.2, 129.0, 128.3, 127.7, 127.0, 116.9, 21.3, 17.1, 14.4, 13.0, 12.1; HRMS (ESI) calcd for C$_{26}$H$_{24}$N$_2$BF$_2$S [M - H]: 445.1721. Found: 445.1760.
Synthesis of 3-(4-methylphenyl)lsulfinyl-5,7-dimethyl-6-ethyl-8-phenyl-4,4-difluoro-4-bora- 3a,4a-diaza-s-indacene (BSFO). TO a solution of BSFI (0.223 g, 0.5 mmol) in 20 mL dichloromethane was added 0.99 equivalents of m-CPBA (0.085 g, 0.49 mmol) at 0 ºC. The reaction was then stirred for 30 minutes. Excess potassium carbonate was added to neutralize any acids, and washed with water. The organic layers were dried over anhydrous Na₂SO₄, and evaporated in vacuo. The crude product was further purified by flash chromatography (silica gel) to afford BSFO (0.169 g, 73%). ¹H NMR (400MHz, CDCl₃) δ 7.79 (d, 2H), 7.52-7.47 (m, 4H), 7.35-7.28 (m, 3H), 6.73 (d, J = 4 Hz, 1H), 6.26 (d, J = 4 Hz, 1H), 2.69 (s, 3H), 2.38 (m, 5H), 1.47 (S, 3H), 1.04 (t, J = 7.6 Hz, 3H); ¹³C NMR (100MHz, CDCl₃) δ 166.6, 152.0, 143.5, 141.9, 141.6, 141.3, 137.8, 137.8, 135.9, 134.88, 133.5, 129.8, 129.6, 128.8, 128.6, 128.6, 128.5, 125.2, 124.4, 114.2, 21.4, 17.1, 14.0, 13.7, 12.4; HRMS (ESI) calcd for C₂₆H₂₄N₂BF₂SO [M - H]⁻: 461.1670. Found: 461.1445.
Figure S1. (a) Normalized absorption spectra and (b) Normalized fluorescent emission spectra of **BSFO** and **BSFI** in a phosphate buffer (pH 6.1, 25 mM sodium phosphate buffer, 50% EtOH as co-solvent) at room temperature.
Figure S2. HRMS spectrum of BSFO + benzenethiols.
Figure S3. Partial $^1$H NMR spectra of (a) BSFO, (b) the isolated product of BSFO + benzenethiols, (c) BSFI.
Figure S4. Visible color changes (up) and fluorescence color changes (bottom) of BSFO after the addition of 100 equiv of various related species (from left to right represents BSFO only, glycine, PhNH₂, PhOH, D-xylose, CH₃(CH₂)₁₁SH, PhCH₂SH, cystein, and p-thiocresol respectively).
Figure S5. Calibration plot of the emission response to benzenethiols concentrations. The Standard Deviation was obtained by fluorescence responses to be $\sigma = 2.26 \times 10^{-3}$, therefore, the detection limit was calculated by the formula $(3\sigma/k)$ and gave a result as $7.41 \times 10^{-7}$ M.
Figure S6. Time course kinetic measurement of the emission response of BSFO to benzenethiols. Data (I\textsubscript{568}/I\textsubscript{536}) were collected under pseudo-first-order conditions (10 μM BSFO, 1 mM thiophenol) with excitation at λ\textsubscript{ex} = 484 nm.
Figure S7. HPLC analysis of BSFI.
Figure S8. HPLC analysis of BSFO.
Table S1. Measurement of benzenethiol concentrations in water samples from Huangpu River.

The amount of benzenethiols in the Huangpu river sample was estimated by using the internal standard method with standard benzenethiols solution (5 mM stock) in ethanol.

The crude water samples from Huangpu River were adjusted to pH 6.1 phosphate buffer, and different concentrations of benzenethiol (0.3, 1, 5, 10, 30, 100 μM) were obtained by diluting the stock solution into a solvent mixture of ethanol and PB buffer (3 mL, V/V = 1:1, pH 6.1). The resulting samples were further treated with probe BSFO (10 μM). The resulting solution was shaken well. After 30 min, the emission was recorded. The ratiometric emission response (I\text{568}/I\text{520}) of BSFO was plotted against different concentrations of benzenethiols, a calibration curve revealing a good linear relationship was obtained, with R-value = 0.99. Then the unknown concentration of benzenethiols in the Huangpu river sample was determined by the calibration curve.

<table>
<thead>
<tr>
<th>sample</th>
<th>Concentration of benzenethiols added (mol/L)</th>
<th>Concentration of benzenethiols determined by BSFO (mol/L)</th>
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<tbody>
<tr>
<td>1</td>
<td>2.40×10^{-4}</td>
<td>2.82×10^{-4}</td>
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<tr>
<td>2</td>
<td>6.84×10^{-5}</td>
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<tr>
<td>3</td>
<td>3.42×10^{-6}</td>
<td>3.10×10^{-6}</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>No detection</td>
</tr>
</tbody>
</table>