Supporting Information

A colorimetric and ratiometric fluorescent probe for rapid, sensitive and visual detection of metabisulfite in food and living cells

Chong Duan, a, b Jun-Feng Zhang, a Yubo Hu, a Ruiting Hao, a, * Lintao Zeng b, * and Rui Xu a, *

a Key Laboratory of Renewable Energy Advanced Materials and Manufacturing Technology Ministry of Education, Yunnan Normal University, Kunming 650092, PR China. E-mail: ruitinghao@semi.ac.cn (R. Hao), ecowatch_xr@163.com (R. Xu)
b School of Chemistry and Chemical Engineering, Tianjin University of Technology, Tianjin 300384, PR China. E-mail: zlt1981@126.com (L. Zeng)

Table of contents

1. Comparison of ratiometric fluorescent probes for SO₂ derivatives....................... Table S1

2. Fluorescence spectra changes of CZBI.............................................................. Fig. S1

3. Selectivity of CZBI.......................................................................................... Fig. S2-S3

4. Cytotoxicity of CZBI ........................................................................................ Fig. S4

5. Cell imaging of CZBI ......................................................................................... Fig. S5

6. Subcellular localization of CZBI .......................................................................... Fig. S6

7. ¹H NMR, ¹³C NMR and HR-MS characterization of CZBI..................................... Fig. S7-S9

8. HR-MS characterization of CZBI-HSO₃............................................................ Fig. S10
Table S1. Comparison of ratiometric fluorescent probes for SO\(_2\) derivatives.

<table>
<thead>
<tr>
<th>Probes</th>
<th>(\lambda_{\text{ex}}) /nm</th>
<th>(\lambda_{\text{em}}) /nm</th>
<th>Solvent</th>
<th>Detection limit/nM</th>
<th>Sensitivity</th>
<th>Response time/s</th>
<th>Detection object</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMB</td>
<td>360</td>
<td>430</td>
<td>PBS buffer (5% DMSO)</td>
<td>43.0</td>
<td>good</td>
<td>&lt; 100</td>
<td>HSO(_3)(^-)</td>
<td>Living cells</td>
</tr>
<tr>
<td>T</td>
<td>488</td>
<td>690</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHB-ID</td>
<td>404</td>
<td>467</td>
<td>PBS buffer (30% DMSO)</td>
<td>58.6</td>
<td>good</td>
<td>&lt; 90</td>
<td>HSO(_3)(^-)</td>
<td>Living cells</td>
</tr>
<tr>
<td>probe 1</td>
<td>420</td>
<td>485</td>
<td>PBS buffer (0.25% EtOH)</td>
<td>34.0</td>
<td>good</td>
<td>&lt; 300</td>
<td>SO(_3)(^2-)</td>
<td>Living cells</td>
</tr>
<tr>
<td>probe 2</td>
<td>466</td>
<td>523</td>
<td>HEPES buffer</td>
<td>0.27</td>
<td>good</td>
<td>&lt; 90</td>
<td>SO(_3)(^2-)</td>
<td>Living cells</td>
</tr>
<tr>
<td>CP</td>
<td>380</td>
<td>450</td>
<td>PBS buffer (5% DMSO)</td>
<td>390</td>
<td>good</td>
<td>&lt; 5</td>
<td>HSO(_3)(^-)</td>
<td>Living Cells, brain tissues, and zebrafishes</td>
</tr>
<tr>
<td>probe 1</td>
<td>500</td>
<td>560</td>
<td>PBS buffer (10% DMF)</td>
<td>87</td>
<td>good</td>
<td>&lt; 30</td>
<td>HSO(_3)(^-)</td>
<td>Living cells and food samples</td>
</tr>
<tr>
<td>CZBI</td>
<td>322</td>
<td>462</td>
<td>PBS buffer (30% EtOH)</td>
<td>6.8</td>
<td>good</td>
<td>&lt; 30</td>
<td>S(_2)O(_5)(^2-)</td>
<td>Living cells and food samples</td>
</tr>
</tbody>
</table>

Fig. S1 Fluorescence spectra changes of CZBI in EtOH/PBS solution ($v/v = 3/7$, pH 7.4, 10 mM) upon addition of increasing amount of $S_2O_5^{2-}$ (0–30 μM). Each spectrum was recorded after CZBI was incubated with $S_2O_5^{2-}$ for 1 min. (a) $\lambda_{em} = 462$ nm, $\lambda_{ex} = 322$ nm, slits: 2.5/2.5 nm; (b) $\lambda_{em} = 588$ nm, $\lambda_{ex} = 510$ nm, slits: 2.5/10 nm.

Fig. S2 (a) Absorbance ratios ($A_{510}/A_{322}$) and (b) Fluorescence intensity ratios ($F_{462}/F_{588}$) of CZBI (10 μM) in the presence of various analytes (100.0 μM) in EtOH/PBS solution ($v/v = 3/7$, pH 7.4, 10 mM) at 25 °C. Each spectrum was recorded after CZBI was incubated with $S_2O_5^{2-}$ for 1 min.
Fig. S3 Fluorescence response of CZBI (10 μM) towards $S_2O_5^{2-}$ in the presence of various analytes (100.0 μM) in EtOH/PBS solution (v/v = 3/7, pH 7.4, 10 mM) at 25 °C. Each spectrum was recorded after the analytes were incubated with CZBI (10 μM) for 1 min.

Fig. S4 Cytotoxicity of CZBI. HeLa cells were incubated with CZBI (0–25 μM) for 24 h. Results are mean ± SD, n = 5.
**Fig. S5** Confocal fluorescence images of CZBI in HeLa cells incubated with different concentrations of S$_2$O$_5^{2-}$. HeLa cells were incubated with CZBI (10 μM) at 37 °C for 30 min, and then further treated with different amounts of S$_2$O$_5^{2-}$ for 10 min. Fluorescence images of HeLa cells from blue channel ($\lambda_{ex} = 403$ nm, $\lambda_{em} = 425–475$ nm) and red channel ($\lambda_{ex} = 543$ nm, $\lambda_{em} = 552–617$ nm). The ratiometric images ($F_{blue}/F_{red}$) were obtained by mediating the blue channel image with the related red channel image. Scale bar: 20 μm.

**Fig. S6** Confocal fluorescence images of HeLa cells stained with (a) 10 μM CZBI (red channel: $\lambda_{ex} = 543$ nm, $\lambda_{em} = 560–610$ nm) and (b) 200 nM MitoTracker Green FM (green channel: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500–530$ nm) at 37 °C for 30 min (c) Merged image of (a) and (b). (d) Bright field image. (e) Correlation plot of MitoTracker Green FM and CZBI intensities. (f) Intensity profile of regions of interest (ROI) across HeLa cells. Scale bar: 10 μm.
Fig. S7 $^1$H NMR spectrum of compound CZBI in DMSO-$d_6$ (400 MHz).

Fig. S8 $^{13}$C NMR spectrum of CZBI in DMSO-$d_6$ (100 MHz).
Fig. S9 HR-MS spectrum of CZBI.

Fig. S10 HR-MS spectrum of CZBI-HSO$_3$. 