Red fluorescent probes based on a BODIPY analogue for selective and sensitive

detection of selenols in solutions and in living systems

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### Table S1. Comparison table of key properties of fluorescent probes for Sec in literatures and in this work

<table>
<thead>
<tr>
<th>References</th>
<th>Structures of probes</th>
<th>Wavelength maxima (\lambda_{\text{abs}}/\lambda_{\text{em}})</th>
<th>LOD</th>
<th>Selectivity (I_{\text{Sec}}/I_{\text{DTT}}) for incubation time</th>
<th>Photostability</th>
<th>Animal imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACIE 2006, 45, 1810.</td>
<td><img src="image" alt="Structure" /></td>
<td>450/520 nm</td>
<td>-</td>
<td>&gt;200 for 10 min at pH 5.8</td>
<td>no test</td>
<td>No</td>
</tr>
<tr>
<td>CC 2015, 51, 3102.</td>
<td><img src="image" alt="Structure" /></td>
<td>460/580 nm</td>
<td>7.0 nM</td>
<td>2.5-fold for F/F&lt;sub&gt;0&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; 30 min</td>
<td>Yes 500 s</td>
<td>Mice</td>
</tr>
<tr>
<td>JACS 2015, 137, 757.</td>
<td><img src="image" alt="Structure" /></td>
<td>370/502 nm</td>
<td>62 nM</td>
<td>20.2 for 5 min</td>
<td>no test</td>
<td>No</td>
</tr>
<tr>
<td>CEJ 2015, 21, 11696.</td>
<td><img src="image" alt="Structure" /></td>
<td>650/712 nm</td>
<td>-</td>
<td>65-fold for F/F&lt;sub&gt;0&lt;/sub&gt;</td>
<td>no test</td>
<td>Mice</td>
</tr>
<tr>
<td>AC 2016, 88, 6084.</td>
<td><img src="image" alt="Structure" /></td>
<td>380/535 nm</td>
<td>18 nM</td>
<td>120-fold for F/F&lt;sub&gt;0&lt;/sub&gt;</td>
<td>no test</td>
<td>Zebrafish</td>
</tr>
<tr>
<td>This work</td>
<td><img src="image" alt="Structure" /></td>
<td>650/663, 655 nm</td>
<td>16 nM 9 nM</td>
<td>20.9 (Sel-p2) 170-, 40-fold for F/F&lt;sub&gt;0&lt;/sub&gt; 15 min</td>
<td>Yes 60 min</td>
<td>Mice</td>
</tr>
</tbody>
</table>

<sup>a</sup>F/F<sub>0</sub> expresses the fluorescence increment before and after addition of Sec.
The comparison table (Table S1) displays five reported fluorescent probes for Sec: one sulfonate probe, three sulfonamide probes and one benzoselenadiazole probe. It is well known that both sulfonates and sulfonamides are photolabile. So, the photostability of the four probes hasn’t been tested. Among five reported fluorescent probes only one case is a near-IR fluorescent probe (HD-Sec). The selectivity (I_{Sec}/I_{DTT}) of fluorescent probes depends the incubation time, i.e. the longer incubation time, the lower selectivity. The value of F/F₀ expresses the fluorescence increment before and after addition of Sec, and the longer incubation time, the larger value of F/F₀ before finishing the sensing reaction.

II. Photophysical properties of probes and compounds 4 and 5

![UV/vis absorption spectra](a) and fluorescence spectra (dash) of Sel-p1, Sel-p2 (black) and compounds 4 and 5 (red), and excitation spectra (dot) of compounds 4 and 5 (blue), in PBS (pH 7.4) buffered water-DMSO (v/v, 1:1).

Figure S1. UV/vis absorption spectra (solid) and fluorescence spectra (dash) of Sel-p1, Sel-p2 (black) and compounds 4 and 5 (red), and excitation spectra (dot) of compounds 4 and 5 (blue), in PBS (pH 7.4) buffered water-DMSO (v/v, 1:1).
III. Time-dependent UV/vis absorption spectra of the reaction of Sel-p1/Sel-p2 with Sec

Figure S2. Time-dependent UV/vis absorption spectra of $5 \mu$M Sel-p1 (a) and Sel-p2 (b) by the treatment of equal molar (Sec)$_2$ with DTT (25 $\mu$M for Sel-p1 and 12.5 $\mu$M for Sel-p2) in the PBS (pH 7.4)–DMSO solution mixture (v/v, 1:1).

Figure S3. UV/vis absorption spectra of $5 \mu$M Sel-p1 (a) and Sel-p2 (b) in the presence of different concentrations of equal molar (Sec)$_2$ with DTT (0-25 $\mu$M for Sel-p1 and 0-12.5 $\mu$M for Sel-p2) recorded after 15 min.
IV. HRMS confirmation of the sensing product of Sel-p2 with Sec

Figure S4. Mass spectrum of the reaction mixture of Sel-p2 incubated with equal molar (Sec)$_2$ and DTT in PBS (pH 7.4) buffered water–DMSO (v/v, 1:1)
V. Measurements of pKa values of compounds 4 and 5

Figure S5. UV/Vis absorption spectra of various solutions of compounds 5 (a) and 4 (e) in the pH range; Plots of absorbance at 480 nm (b) and 655 nm (c) for 5, and 465 nm for 4 (f) vs pH values; Fluorescence spectra of various solutions of compounds 5 (d) and 4 (g) in the pH range.
VI. pH effects on the sensing reaction of Sel-p1/Sel-p2 with Sec

![Graph a](imagea.png)

![Graph b](imageb.png)

**Figure S6.** Effects of pH on fluorescence intensity of the probe (5 μM) and the reaction solutions of the probe (5 μM) with equal molar (Sec)$_2$ with DTT (25 μM for Sel-p1 and 12.5 μM for Sel-p2) in PBS (pH 7.4) buffered water–DMSO (v/v, 1:1).
VII. Photostability and thermostability of Sel-p1 and Sel-p2

Figure S7. The time courses of UV/vis and fluorescence spectra of probes (5 μM) in the presence of equal molar (Sec)₂ with DTT (25 μM) in aqueous solution (DMSO/PBS, v/v 1:1, pH 7.4) recorded for 60 min.

Figure S8. Fluorescence changes of 5 μM probes and the probe (5 μM) in the presence of equal molar (Sec)₂ with DTT (25 μM for Sel-p1 and 12.5 μM for Sel-p2) in aqueous solution (DMSO/PBS, v/v 1:1, pH 7.4) respectively under continuous 600 nm irradiation by a xenon lamp (150 W).
VIII. The selectivity of the fluorescent probe for Sec, and the pKa of its related fluorophore

Table S2. The selectivity of the probe (\( \text{Fl-H} \)), and the pKa of its related fluorophore (Fl-H)

<table>
<thead>
<tr>
<th>Fl-H</th>
<th>pKa</th>
<th>Sec(^a) (F/F(_0))</th>
<th>DTT(^a) (F/F(_0))</th>
<th>Selectivity(^a) (Sec/DTT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.8[1], 7.91[2]</td>
<td>16.5</td>
<td>1.5</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>7.26[3]</td>
<td>20.1</td>
<td>2.0</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>7.02[4]</td>
<td>72.1</td>
<td>1.5</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>5.8[5]</td>
<td>25.0</td>
<td>1.2</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>6.8(^b)</td>
<td>42.9(^b)</td>
<td>2.2(^b)</td>
<td>19.5(^b)</td>
</tr>
</tbody>
</table>

\(^a\) From B. Zhang, C. Ge, J. Yao, Y. Liu, H. Xie and J. Fang, *J. Am. Chem. Soc.*, 2015, 137, 757-769. \(^b\) From this work.

References
IX. Cytotoxicity of Sel-p2 and fluorescence imaging of mice

Figure S9. MTT assay of MCF-7 cells in the presence of different concentrations of Sel-p2.

Figure S10. Representative fluorescent images of selenocysteine in mice (a) only the probe Sel-p2 (20 μM), (b) equal molar (Sec)₂ with DTT (20 μM in 100 μL saline) and followed Sel-p2 after 1 h, (c) 4 h, (d) fluorescence intensity from the abdominal area of groups a-c.
X. Copies of NMR of related compounds.