A novel near-infrared xanthene fluorescent probe for detection of thiophenol in vitro and in vivo

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![Graph showing linearity between fluorescence intensity at 740 nm versus PhSH concentration in the range of 2−18 μM.](image)

**Fig. S1** Linearity between the fluorescence intensity at 740 nm versus PhSH concentration in the range of 2−18 μM. \( \lambda_{ex} = 670 \text{ nm}, \lambda_{em} = 740 \text{ nm}. \)

![Graph showing pH-dependent fluorescence response of NOF (10 μM) towards PhSH (30 μM).](image)

**Fig. S2** The pH-dependent fluorescence response of NOF (10 μM) towards PhSH (30 μM). \( \lambda_{ex} = 670 \text{ nm}, \lambda_{em} = 740 \text{ nm}. \)
Fig. S3 Changes of fluorescence intensity of NOF irradiated at 670 nm for 60 min, $\lambda_{em} = 740$ nm.
**Table S1.** Comparison of representative fluorescent probes for thiophenol (PhSH)

<table>
<thead>
<tr>
<th>References</th>
<th>λ&lt;sub&gt;ex&lt;/sub&gt;/nm</th>
<th>λ&lt;sub&gt;em max&lt;/sub&gt;/nm</th>
<th>Detection limit/μM</th>
<th>Imaging applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lin. W. et al. Chem. Commun., 2010, 46, 1503.</td>
<td>405</td>
<td>494</td>
<td>0.0018</td>
<td>HeLa cells</td>
</tr>
<tr>
<td>Zhang, W. et al. J. Mater. Chem. C, 2015, 3, 8248.</td>
<td>380</td>
<td>517</td>
<td>0.0103</td>
<td>A549 cells</td>
</tr>
<tr>
<td>Xiong, L. et al. ACS Sens., 2017, 2, 599.</td>
<td>538</td>
<td>645</td>
<td>0.0099</td>
<td>Not given</td>
</tr>
<tr>
<td>Guo S. et al. Talanta, 2018, 185, 359.</td>
<td>595</td>
<td>653</td>
<td>0.015</td>
<td>HeLa Cells</td>
</tr>
<tr>
<td>Zhou S. et al. Sens. Actuators B Chem., 2018, 276, 361.</td>
<td>488</td>
<td>590</td>
<td>0.036</td>
<td>HeLa cells</td>
</tr>
<tr>
<td>Li Y. et al. Talanta, 2019, 199, 355.</td>
<td>540</td>
<td>658</td>
<td>0.220</td>
<td>HeLa cells</td>
</tr>
<tr>
<td>Liu, Q. et al. Sens. Actuators B Chem., 2019, 283, 820.</td>
<td>543</td>
<td>624</td>
<td>0.0081</td>
<td>HepG2 cells</td>
</tr>
<tr>
<td>Xu T. et al. ACS Sustain. Chem. Eng., 2020, 8, 6413.</td>
<td>470</td>
<td>585</td>
<td>0.0028</td>
<td>Not given</td>
</tr>
<tr>
<td>Yang L. et al. Dyes Pigments, 2020, 175, 108154.</td>
<td>574</td>
<td>620</td>
<td>0.550</td>
<td>Not given</td>
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<tr>
<td>This work</td>
<td>670</td>
<td>740</td>
<td>0.120</td>
<td>MCF-7 cells mice</td>
</tr>
</tbody>
</table>

**Note:** This work indicates the detection limit for thiophenol in mice.
**Fig. S4** Fluorescence intensity of NOF (10 μM) in the presence of various analytes. Black bars represent the addition of a single analyte (50 μM). Red bars represent the subsequent addition of thiophenol (30 μM) to the mixture. $\lambda_{ex} = 670$ nm, $\lambda_{em} = 740$ nm.

**Fig. S5** Fluorescence intensity of NOF (10 μM) in the presence of various metal ions. Black bars represent the addition of a single metal ion (50 μM). Red bars represent the subsequent addition of thiophenol (30 μM) to the mixture. $\lambda_{ex} = 670$ nm, $\lambda_{em} = 740$ nm.

**Fig. S6** (a) Absorption and (b) fluorescence spectra of NOF and the product of NOF reacted with PhSH. $\lambda_{ex} = 670$ nm, $\lambda_{em \ max} = 740$ nm.
Fig. S7 Representations of the frontier molecular orbitals (MOs) for the $S_0$ geometry of NOF and NOF-OH as determined at the DFT//B3LYP/6-31G* level.

Table S2 Frontier molecular orbital energies (in eV) calculated at the DFT//B3LYP/6-31G* level.

<table>
<thead>
<tr>
<th>Sample</th>
<th>HOMO-2</th>
<th>HOMO-1</th>
<th>HOMO</th>
<th>LUMO</th>
<th>LUMO+1</th>
<th>LUMO+2</th>
<th>GAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOF</td>
<td>-9.57</td>
<td>-8.81</td>
<td>-8.18</td>
<td>-5.60</td>
<td>-4.58</td>
<td>-4.24</td>
<td>2.58</td>
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<tr>
<td>NOF-OH</td>
<td>-9.56</td>
<td>-8.73</td>
<td>-8.06</td>
<td>-5.54</td>
<td>-3.88</td>
<td>-3.82</td>
<td>2.52</td>
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</tbody>
</table>

Fig. S8 Viable cells after treatment with indicated concentrations of probe NOF after 24 hours, the cell viability was observed via MTT assay.
Fig. S9 $^1$H NMR (400 MHz, CDCl$_3$) spectrum of compound 1.

Fig. S10 $^1$H NMR (400 MHz, CD$_3$OD) spectrum of NOF.
Fig. S11 HR-MS of NOF.

Fig. S12 $^1$H NMR (400 MHz, CD$_3$OD) spectrum of NOF-OH.
**Fig. S13** HR-MS of NOF-OH.