Electronic Supplementary Information

Near-infrared Fluorescence Probes Detect Reactive Oxygen Species for Keloid Diagnosis

Penghui Cheng, a Jianjian Zhang, b Jiaguo Huang, a Qingqing Miao, a Chenjie Xu a and Kanyi Pu * a

a School of Chemical and Biomedical Engineering, Nanyang Technological University, 637457 Singapore. Email: kypu@ntu.edu.sg

b Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry and Materials Science, Northwest University, Xi’an, Shaanxi 710127, People’s Republic of China

Table of Contents:

1. UV/Vis absorption and fluorescence spectra in titration experiments
2. Time-dependent fluorescence spectra
3. HPLC experiments
4. Cytotoxicity
5. Stability test
6. NMR and MS Data
7. HPLC condition
1. UV/Vis absorption and fluorescence spectra in titration experiments

CyTF

(a) UV-Vis absorption spectra and (b) fluorescence of CyTF (20 µM) upon titrating different concentrations of ONOO⁻. (c) UV-Vis absorption and (d) fluorescence of CyBA (20 µM) upon titrating different concentrations of ONOO⁻. (e) UV-Vis absorption and (f) fluorescence of CyBA (20 µM) upon titrating different concentrations of H₂O₂. Experiments were performed at 25 °C in PBS (1 ×, pH = 7.4) containing 20% DMSO. Excitation: 640 nm.

Fig. S1
2. Time-dependent fluorescence spectra

![Time-dependent fluorescence spectra](image)

**Fig. S2** Time-dependent fluorescence changes of CyTF (20 µM) and CyBA (20 µM) upon addition of 25 µM ONOO⁻.

3. HPLC experiments

![HPLC experiments](image)

**Fig. S3** High performance liquid chromatography (HPLC) traces of the incubation mixture of CyBA in the absence (upper panel) or presence (middle panel) of H₂O₂ (500 µM), and HPLC traces of CyOH in water (lower panel). Wavelength: 600 nm.
4. **Cytotoxicity**

**Fig. S4** MTS assay for the relative viability of Normal Dermal Fibroblasts (NDFs) and Keloid-derived fibroblasts (KFs) treated with various concentrations of CyTF and CyBA. Error bars represent the standard deviation of 5 trials.
5. Stability test

Fig. S5 HPLC traces of 10 µM CyTF and CyBA in DMEM after incubation for 0, 0.5 and 1 h.

6. NMR and MS Data

Compound 2:
Compound 3:

Compound 4:
Compound 5:

CyTF:
CyBA:
7. HPLC condition for ROS cleavage experiment and stability test

<table>
<thead>
<tr>
<th>Time (minute)</th>
<th>Flow (ml/min.)</th>
<th>H₂O %</th>
<th>CH₃CN %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>35</td>
<td>1.0</td>
<td>30</td>
<td>90</td>
</tr>
<tr>
<td>37</td>
<td>1.0</td>
<td>30</td>
<td>90</td>
</tr>
<tr>
<td>38</td>
<td>1.0</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>1.0</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>