A fluorescent probe for rapid detection of low concentration mercury ions and its application in biological cells

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1. NMR and MS spectra

Fig. S1. $^1$H NMR (CDCl$_3$, 600 MHz) spectra of compound RBSH.

Fig. S2. $^{13}$C NMR (CDCl$_3$, 600 MHz) spectra of compound RBSH.
Fig. S3. ESI-MS spectrum of compound RBSH.

Fig. S4. $^1$H NMR (CDCl$_3$, 600 MHz) spectra of compound RBSH+Hg$^{2+}$. 
2. Experiment methods and graphs

Calculation of association constant

The association constant ($K_a$) of RBSH-Hg$^{2+}$ complex was determined by Benesi-Hildebrand Formula (1):

$$\Delta F = F - F_0 = \Delta F = [Hg^{2+}](F_{max} - F_0)/(\frac{1}{K_a} + [Hg^{2+}])$$

Where $F$ is the fluorescence intensity at 592 nm upon addition of different concentration of Hg$^{2+}$, $F_0$ is the fluorescence intensity at 592 nm in the absence of Hg$^{2+}$ and $F_{max}$ is the saturated intensity at 582 nm in the presence of Hg$^{2+}$. The association constant ($K_a$) was evaluated graphically by plotting $1/(F - F_0)$ against $1/([Hg^{2+}])$. Linear fit to the data according to the formula (1), through the slope and intercept, the binding constant of RBSH was calculated as $1.658\times10^5$ M$^{-1}$.

![Graph](image)

Fig. S6. Binding constant.
Determination of detection limit

According fluorescence titration experiments, we can also calculate the detection limit of RB
SH for Hg^{2+}. The fluorescence intensity of the blank samples was measured for 10 times, calculate the standard deviation of the fluorescence intensity at 592 nm. Then, make a curve based on the fluorescence intensity of RB
SH at 592 nm and the concentration of Hg^{2+} to obtain the slope. The detection limit was calculated according to the following formula:

\[
\text{Detection limit} = \frac{3 \times \text{SD}}{S} = \frac{1}{\sqrt{N-1}} \sum_{i=1}^{N} (X_i - \bar{X})^2
\]

Where SD is the standard deviation of the blank solution detected for 10 times; S is the slope of the calibration curve. Finally, the detection limit of RB
SH is calculated to be 5.9 nM.

MTT method

MTT method was used to perform cytotoxicity test to detect cell survival and growth. We used Hela cells as the experimental cells. Hela cells were placed in a 96-well plate and placed in atmosphere at 37 °C and 5% CO_{2} for 12 hours. Take the supernatant and add probe culture solution of different concentrations, then take the supernatant and add 5mg/mL MTT stock solution and leave it for 4h. Finally, take the supernatant again, add 150μL DMSO to each well plate. The absorbance of each well was measured at 550 nm using a microplate reader (Bio-Rad, Model 550). The experiment was repeated three times to get the average value. We selected Hela cells as the cells used for imaging experiments. Hela cells were placed in high glucose medium supplemented with 10% FBS (fetal bovine serum), 100 units/mL penicillin and 100 units/mL streptomycin temperature streptomycin in high glucose medium. The temperature was 37°C, and the culture environment was in an air atmosphere of 5% CO_{2}. Hela cells incubated in the incubator were seeded in 24-well plates at 1.5×10^5 cells per well. Six hours after the probe RB
SH was added, the cells were washed three times with PBS and live-cell imaging was performed. In addition, pretreatment with 10 μM Hg^{2+} for 1h, and then adding 10 μM probe RB
SH for 5h as a control group. Fluorescence images of cells were recorded under an inverted fluorescent microscope.

Fig. S7. Minimum detection limit
Fig. S8. Cytotoxicity assay of probe RB
for Hela cells by the MTT test. Hela cells were respectively cultured in the presence of different concentrations of RBSH (1.25, 2.5, 5, 10, and 20µM) at 37°C for 24h. For the control group, HELE cells were incubated under the same conditions but without the probe RBSH.

Mercury ion probe comparison

<table>
<thead>
<tr>
<th>Probe structures</th>
<th>Reagents</th>
<th>Detection Limit / nM</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Probe 2]</td>
<td>0.01 M acetic acid/ sodium acetate buffer</td>
<td>0.77*10⁻³</td>
<td>Y. Yu, L. R. Lin, K. B. Yang, X. Zhong, R. B. Huang and L. S. Zheng, Talanta, 2006, 69, 103-106.</td>
</tr>
<tr>
<td>Compounds</td>
<td>Solvents</td>
<td>LOD</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------</td>
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<tr>
<td><img src="image3.png" alt="Compound 3" /></td>
<td>EtOH/H$_2$O (1:3, v/v)</td>
<td>5.9</td>
<td>This work</td>
</tr>
</tbody>
</table>

Table S1 LOD comparison