Supporting Information

A diketopyrrolopyrrole–based fluorescence turn–on probe for the detection of Pb$^{2+}$ in aqueous solution and living cells

Xiaofeng Yang a,b,*, Yan Zhang a, Yexin Li a, Xiaolei Liu a, Jiaxin Mao a, Yuan Yuan a, Yu Cui a,*, Guoxin Sun a, Guangyou Zhang a

a School of Chemistry and Chemical Engineering, University of Jinan, No. 336, West Road of Nan Xinzhuang, Jinan 250022, Shandong, China
b Shandong Provincial Key Laboratory of Fluorine Chemistry and Chemical Materials, University of Jinan, No. 336, West Road of Nan Xinzhuang, Jinan 250022, Shandong, China

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Experimental Section

1. General Methods:

2-Hydrazinobenzothiazole was purchased from Aladdin Shanghai Reagent Company. Other reagents were used without further purification. Reactions were monitored by TLC. Flash chromatography separations were carried out using silica gel (200-300 mesh). $^1$H NMR and $^{13}$C NMR spectra were collected on a Bruker Avance II 400 MHz spectrometer. UV-vis spectra were recorded on a Shimadzu 3100 spectrometer. Fluorescence measurements were carried out using an Edinburgh Instruments Ltd–FLS920 fluorescence spectrophotometer.

2. Synthesis Section

Probe DPP‒HBT was prepared according to the method we reported previously by a simple reaction of 4,4’–(2,5–bis(2–(2–(2–methoxyethoxy)ethoxy)ethyl)–3,6–dioxo–2,3,5,6–tetrahydropyrrolo[3,4–c]pyrrole–1,4–diyl)dibenzaldehyde (DPP–AL) and 2–hydrazinobenzothiazole[1]. To 20 mL of compound DPP–AL (318 mg, 0.5 mmol) in ethanol, was added 5 mL of 2–hydrazinobenzothiazole (182 mg, 1.1 mmol) in ethanol dropwise. After the reaction solution was refluxed for 3 h, the rust red solid was precipitated, collected, and wash with ethanol to afford compound DPP–HBT (302 mg, 65%). $^1$H NMR (DMSO–$d_6$, 400 MHz) δ (ppm) 12.56 (br, 2H,), 8.22 (s, 2H), 8.04 (d, $J$ = 8.4 Hz, 4H), 7.87 (d, $J$ = 8.4 Hz, 4H), 7.79 (d, $J$ = 4.8 Hz, 2H), 7.46 (d, $J$ = 4.8 Hz, 2H), 7.32 (t, $J$ = 8.0 Hz, 2H), 7.14 (t, $J$ = 7.2 Hz, 2H), 3.92 (t, $J$ = 6.0 Hz, 4H), 3.56 (t, $J$ = 6.0 Hz, 4H), 3.41–3.36 (m, 16H), 3.18 (s, 6H). $^{13}$C NMR (DMSO–$d_6$, 100 MHz) δ (ppm) 167.73, 162.28, 148.28, 137.36, 133.52, 130.16, 130.03, 129.37, 128.69, 127.02, 126.56, 122.35, 122.07, 109.62, 71.74, 71.70, 70.30, 70.09, 68.43, 68.37, 58.50, 42.01; HRMS–ESI: $m/z$ calcld (%) for C$_{48}$H$_{51}$N$_8$O$_8$S$_2$: 931.3271 [M+H]$^+$; Found: 931.3242; Element analysis for C$_{48}$H$_{50}$N$_8$O$_8$S$_2$ (%): C 61.88, H 5.37, N 12.14, calculated C 61.92, H 5.41, N 12.03 (Fig. S9).
3. Cell Imaging

A549 Human Lung Adenocarcinoma cell lines were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO$_2$ at 37 °C. The cells were plated on 12 mm cover glasses in 6-well plate and allowed to grow for 24 h. Before the experiments, the cells were washed with PBS buffer, and then the cells were incubated DPP–HBT (10 μM) for 20 min at 37 °C then washed with PBS three times. After incubating with Pb$^{2+}$ (20 μM) for 30 min at 37 °C, cells were rinsed with PBS three times. Then, the fluorescence images were acquired through an Olympus IX71 fluorescence microscope.

4. Absorption spectra of DPP–HBT with Pb$^{2+}$

![Absorption spectra of DPP–HBT](image)

**Figure S1.** Absorption spectra of DPP–HBT (10 μM) and Pb$^{2+}$ (0 - 2 equiv.) in CH$_3$CN/0.01 M PBS buffer (v/v, 1:1, pH 7.4).
5. Job’s Plot

![Job's plot for DPP‒HBT and Pb²⁺ in CH₃CN/0.01 M PBS buffer (v/v, 1:1, pH 7.4), λₑₓ: 475 nm.](image)

Figure S2. Job’s plot for **DPP‒HBT** and Pb²⁺ in CH₃CN/0.01 M PBS buffer (v/v, 1:1, pH 7.4), λₑₓ: 475 nm.
6. Binding constants

Figure S3. Benesi–Hildebrand plot of probe DPP–HBT (10 μM) using 1:2 stoichiometry for association between probe and Pb$^{2+}$, $\lambda_{ex}$: 475 nm.

The binding constants were calculated from the spectral titration data by Benesi–Hildebrand equation:

$$\frac{1}{I} - \frac{1}{I_0} = \frac{1}{K(I_{max} - I_0)[Pb^{2+}]^2} + \frac{1}{I_{max} - I_0}$$

Where, $I_0$ was the fluorescence intensity of probe DPP–HBT, $I$ the fluorescence intensity obtained with Pb$^{2+}$, $I_{max}$ the fluorescence intensity obtained with excess amount of Pb$^{2+}$, $K$ the association constant, [Pb$^{2+}$] the concentration of Pb$^{2+}$ added. $K$ was calculated by dividing intercept with slope from the B–H plot by considering 1:2 binding mode between probe DPP–HBT and Pb$^{2+}$. Linear fitting of the titration profiles resulted in a good linearity (correlation coefficient was over 0.996) (Fig. S3), which strongly supported the 1:2 binding stoichiometry of probe DPP–HBT, and the binding constant was calculated to be $1.59 \times 10^8$ M$^{-2}$ for probe DPP–HBT.
7. Determination of detection limit of Pb$^{2+}$

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of DPP–HBT (10 μM) without Pb$^{2+}$ was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and Pb$^{2+}$ concentration could be obtained in the 0-0.7 μM (correlation coefficient was 0.9927). The detection limit is then calculated with the equation: detection limit = 3σ/k, where σ is the standard deviation of blank measurements; k is the slope of linear calibration curve. The detection limit was measured to be 0.23 nM at S/N = 3.

Figure S4. (a) Fluorescence changes of DPP–HBT (10 μM) upon addition of Pb$^{2+}$. (b) Fluorescence spectra of DPP–HBT (10 μM) in the presence of Pb$^{2+}$ (0.23 nM) in CH$_3$CN/0.01 M PBS buffer (v/v, 1:1, pH 7.4) (λ$_{ex}$: 475 nm).
8. Time-dependent fluorescence changes of DPP‒HBT with Pb$^{2+}$

![Time-dependent fluorescence changes of DPP‒HBT](image)

**Figure S5.** Time-dependent fluorescence spectral changes of DPP‒HBT (10 μM) upon addition of 2 equiv. of Pb$^{2+}$ in CH$_3$CN/0.01 M PBS buffer (v/v, 1:1, pH 7.4), $\lambda_{ex}$: 475 nm. Inset: time dependent fluorescence intensity changes (at 585 nm) of probe DPP‒HBT with Pb$^{2+}$.

9. The fluorescence intensity changes of DPP‒HBT in the presence of Pb$^{2+}$ ions and EDTA

![Fluorescence intensity changes](image)

**Figure S6.** (a) Fluorescence intensity changes of DPP‒HBT (10 μM) in CH$_3$CN/0.01 M PBS buffer (v/v, 1:1, pH 7.4) at excitation at 475 nm after addition of 2 equiv. Pb$^{2+}$, 2 equiv. Pb$^{2+}$ + 4 equiv. EDTA and 2 equiv. Pb$^{2+}$ + 4 equiv. EDTA + 4 equiv. Pb$^{2+}$, respectively. (b) Stepwise complexation/decomplexation cycles carried out in CH$_3$CN with DPP‒HBT and Pb$^{2+}$ ion.
10. Effect of pH

**Figure S7.** Effect of pH on the fluorescence intensity of DPP‒HBT (10 μM) in CH$_3$CN/0.01 M PBS buffer (v/v, 1:1) in the absence (black dot) and presence of Pb$^{2+}$ (red dot). ($\lambda_{ex}$: 475 nm).
11. Cytotoxicity assay of DPP–HBT

*In vitro* cytotoxicity was measured by using the colorimetric methyl thiazolyl tetrazolium (MTT) assay against human lung adenocarcinoma (A549) cells. A549 Human Lung Adenocarcinoma cell lines were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO$_2$ at 37 °C. Cells were seeded onto 96-well tissue culture plates in presence of 500 μL DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO$_2$ at 37 °C for 24 h and then incubated for 24 h in presence of DPP–HBT at different concentrations (10-100 μM). Then, 20 μL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT (5 mg/mL) was added to each well and incubated for 4 hours. Next, violet formazan crystals were dissolved in 100 μL of DMSO. The absorbance of solution was measured at 570 nm using microplate reader. The cell viability was determined by assuming 100% cell viability for cells without DPP–HBT.

![Figure S8](image)

**Figure S8.** Cell viability of DPP–HBT at different concentrations against A549 cells after 24 h incubation.
12. $^1$H and $^{13}$C NMR of DPP–HBT

Figure S9. $^1$H NMR and $^{13}$C NMR spectrum of DPP–HBT (DMSO-$d_6$, 400 MHz).
13. Comparison of the present probe **DPP–HBT** and already available Pb\(^{2+}\) probes

The detection limit of compound **DPP–HBT** for Pb\(^{2+}\) was \(2.3 \times 10^{-10}\) M (Table S1, entry 19), much lower than that of existing organic small molecule probes (Table S1, entries 10–18). These results demonstrated that compound **DPP–HBT** could be used as a high sensitivity probe for Pb\(^{2+}\) ions detection.

Table S1. Comparison of the present probe with existing Au\(^{3+}\) probes

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<th>the type of probe</th>
<th>LOD</th>
<th>reference</th>
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<tr>
<td>1</td>
<td>not mentioned</td>
<td>nano–material</td>
<td>(1.0 \times 10^{-10}) M</td>
<td><em>ACS Appl. Mater. Interfaces</em>, 2014, 6, 2568–2575.</td>
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<td>3</td>
<td>not mentioned</td>
<td>nano–material</td>
<td>50 mg L(^{-1})</td>
<td><em>Analyst</em>, 2012, 137, 760–764.</td>
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<td>6</td>
<td>human serum</td>
<td>DNA</td>
<td>22.8 pM</td>
<td><em>Biosensors and Bioelectronics</em>, 2013, 43, 231–236.</td>
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<td>7</td>
<td>not mentioned</td>
<td>peptide</td>
<td>0.6 nM</td>
<td><em>Biosensors and Bioelectronics</em>, 2015, 68, 225–231.</td>
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<td>9</td>
<td>not mentioned</td>
<td>quantum dots</td>
<td>0.006 nmol L(^{-1})</td>
<td><em>Journal of Hazardous Materials</em>, 2013, 250–257, 45–52.</td>
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<td>13</td>
<td>not mentioned</td>
<td>organic small molecule</td>
<td>(1.32 \times 10^{-8}) M (2.7 (\mu)g L(^{-1}))</td>
<td><em>J. Org. Chem.</em>, 2009, 74, 4787–4796.</td>
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<td>15</td>
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<td>organic small molecule</td>
<td>(6.3 \times 10^{-5}) M</td>
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<td>16</td>
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<td>18</td>
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<td>2.7 (\mu)g L(^{-1})</td>
<td><em>Org. Lett.</em>, 2008, 10, 41–44.</td>
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<td>19</td>
<td>A549 cells</td>
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<td><strong>Present work</strong></td>
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