Supporting Information

A novel quinoline-based two-photon fluorescent probe for detecting Cd$^{2+}$ in vitro and in vivo

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1. Cell cytotoxicity
2. Spectroscopic properties of APQ and APQ-Cd$^{2+}$ complex
3. MALDI-TOF MS spectra of APQ、APQ-Cd$^{2+}$ complex and APQ-Zn$^{2+}$ complex
4. $^1$H NMR spectra of APQ and APQ-Cd$^{2+}$ complex
5. NMR spectra
1. Cell cytotoxicity

To ascertain the cytotoxic effect, the MTT (5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide) assay was performed as previously reported. HeLa cells were passed and plated to ~ 70% confluence in 96-well plates 24h before treatment. Prior to APQ treatment, the DMEM was removed and replaced with fresh DMEM, and aliquots of APQ stock solutions (5 mM DMSO) were added to obtain final concentrations of 10, 30, and 50 μM. The treated cells were incubated for 24 h at 37 °C and under 5% CO2. Subsequently, the cells were treated with 5 mg/mL MTT (40 μL /well) and incubated for an additional 4 h (37oC, 5% CO2). Then the cells were dissolved in DMSO (150 μL/well), and the absorbance at 570 nm was recorded. The cell viability (%) was calculated according to the following Equation: Cell viability% = OD570(sample)/OD570(control)×100, where OD570 (sample) represents the optical density of the wells treated with various concentration of 6-MPQ and OD570(control) represents that of the wells treated with DMEM+10% FCS. percent cell survival values are relative to untreated control cells.

Table S1. Cytotoxicity Data of APQ (HeLa, 24 h)

<table>
<thead>
<tr>
<th>Probe concentration</th>
<th>0μM</th>
<th>10μM</th>
<th>30μM</th>
<th>50μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell survival %</td>
<td>97.37±6.7%</td>
<td>93.25±6.9%</td>
<td>52.75±7.10%</td>
<td>43.68±7.0%</td>
</tr>
</tbody>
</table>
2. Spectroscopic Properties of APQ

Fluorescence quantum yields were determined using the quinine sulfate (in 0.1 N H₂SO₄, Φ = 0.55) as the standard (excited by 320 nm).

\[
Φ_U = \frac{Φ_S(F_U A_S)}{F_SA_U}
\]

The quantum yields are calculated by the following equation:

Au and As are the UV absorption of analyte and the standard. Fu and Fs are integrated fluorescence emission of analyte and the standard.

The dissociation constant \(K_d\) is calculated by following equation:

\[
\frac{F}{F_0} = 1 + \left( \frac{F_\text{max}}{2F_0} - \frac{1}{2} \right) \left[ 1 + \frac{C_M}{C_L} \left( K_S - \frac{1}{4} \frac{C_M}{C_L} \right) ^2 \right] ^{-1}
\]

where \(F\) and \(F_0\) are the fluorescence intensity of APQ in the presence and absence of Cd²⁺, \(C_M\) and \(C_L\) are the concentrations of Cd²⁺ and APQ; \(K_S\) is the stability constant. \(K_d\) is the reciprocal of \(K_S\).

Table S2 Spectroscopic Properties of APQ and APQ-Cd²⁺

<table>
<thead>
<tr>
<th>absorption maxima (\lambda) (nm), (ε\times10^4) (M⁻¹cm⁻¹)</th>
<th>emission maxima (\lambda) (nm)</th>
<th>fluorescence quantum yields (Φ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APQ</td>
<td>APQ⁺Cd²⁺</td>
<td>APQ</td>
</tr>
<tr>
<td>27044</td>
<td>23980</td>
<td>480</td>
</tr>
</tbody>
</table>

Fig S1. Fluorescence emission spectra of APQ (25 μM) with the excitation at 320 nm upon the titration of Cd²⁺ (0-1.5 equiv) in the methanol-water solutions (1:9, v/v, 50 mM HEPES buffer, pH = 7.4).
3. MALDI-TOF MS spectra of APQ, APQ-Cd\(^{2+}\) complex and APQ-Zn\(^{2+}\) complex

Fig S2. MALDI TOF MS of APQ (DCTB as matrix)

Fig S3. MALDI-TOF MS spectra of APQ-Cd\(^{2+}\) complex
Fig S4. MALDI-TOF MS spectra of APQ-Zn\(^{2+}\) complex

4. \(^1\)H NMR spectra of APQ and APQ-Cd\(^{2+}\) complex

Fig S5. The \(^1\)H-NMR spectra of the APQ (A) and APQ+1.2eq Cd\(^{2+}\) (B) in DMSO-d6.
5. NMR spectra
$^1$HNMR