Electronic Supplementary Information (ESI)

A reaction-based ratiometric fluorescent sensor for detection of Hg(II) ions both in cells and bacteria†

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Contents

1. Experimental Section ........................................................................................................2
   1.1. General .........................................................................................................................2
   1.2. Synthetic procedure ....................................................................................................2
      1.2.1. 7-(diethylamino)-4-hydroxycoumarin (1) .........................................................2
      1.2.2. 4-bromo-7-(diethylamino)-3-formylcoumarin (2) ................................................2
      1.2.3. 7-(diethylamino)-3-formyl-4-(2-nitrophenyl)coumarin (3) .................................3
      1.2.4. 7-(diethylamino)-3-(1,3-dithiolan-2-yl)-3-formyl-4-(2-nitrophenyl) coumarin (4) .................................................................................................................3
      1.2.5. 4-(2-aminophenyl)-7-(diethylamino)-3-(1,3-dithiolan-2-yl)-3-formylcoumarin (ATC-Hg) ....................................................................................................................4
   1.3. General procedure for the spectra measurement .......................................................4
   1.4. Evidence for the signaling process ..............................................................................4
   1.5. Cell culture and imaging ............................................................................................5
   1.6. E. coli cultivation and imaging ..................................................................................5
2. Photophysical data of ATC-Hg .......................................................................................5
3. NMR Data .........................................................................................................................9
4. ESI(+)-MS Data .............................................................................................................14
1. Experimental Section

1.1. General

Unless otherwise stated, all reagents and solvents were obtained from commercial suppliers, and were used without further purification. Column chromatography was performed on silica gel (Qingdao haiyang) 200–300 mesh. All solvents used in spectra test systems were chromatographically pure. MeHgCl was purchased from Aladdin Industrial Corporation.

$^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AMX-400 with chemical shifts expressed in parts per million (in CDCl$_3$ or DMSO-d$_6$, Me$_4$Si as internal standard). The High-resolution mass spectra were obtained on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer. TLC analyses were performed on silica gel GF 254 using UV light as visualizing agent. The pH values were determined by a Leici pH3c (digital display) pH meter. UV-vis absorption spectra were recorded on a Hitachi PharmaSpec UV-1900 UV-visible spectrophotometer. Fluorescence spectra were determined using a HITAI F-7000 Spectro fluorophotometer. The confocal laser scanning microscopy (CLSM) and the bacterial imaging experiments were performed on a ZEISS LSM 780 confocal laser scanning microscope.

1.2. Synthetic procedure

\[
\begin{align*}
\text{COOH} & \quad \text{POCl}_3 \quad \text{COOH} \\
\text{CHO} & \quad \text{Tolune} \quad \text{CHO} \\
\text{CHO} & \quad \text{Zn, NH}_4Cl \quad \text{CHO} \\
\text{HO} & \quad \text{H}_2\text{O} \quad \text{HO} \\
\text{ATC-Hg} & \quad \text{Cl}_2\text{H}_2\text{O} \quad \text{Cl}_2\text{H}_2\text{O} \\
\end{align*}
\]

Scheme S1. Synthetic scheme of probe ATC-Hg.

1.2.1. 7-(diethylamino)-4-hydroxycoumarin (I)

I was synthesized following literature $^{[1]}$. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 11.92 (s, 1H), 7.50 (d, J = 8.8 Hz, 1H), 6.62 (d, J = 9.2 Hz, 1H), 6.41 (s, 1H), 5.21 (s, 1H), 3.38 (q, J = 7.2 Hz, 4H), 1.07 (t, J = 7.2 Hz, 6H).

1.2.2. 4-bromo-7-(diethylamino)-3-formylcoumarin (2)

Under nitrogen, fresh distilled DMF (5 mL) was added dropwise to POBr₃ (8.6 g, 30 mmol) at 20-50°C and stirred for 30 minutes. Then a portion of compound 1 (2.33 g, 10 mmol, dissolved in 13 mL DMF) was added dropwise to the above solution. The mixture was stirred at 60 °C for 12 hours and then poured into 100 mL ice water. NaOH solution (20 %) was added to adjust the pH of the mixture to obtain a large amount of precipitate. The crude product was filtered, washed with water, dried under vacuum giving compound 2 (2.49 g, 92% yield).

1H NMR (400 MHz, Chloroform-d) δ 10.22 (s, 1H), 7.86 (d, J = 9.6 Hz, 1H), 6.67 (dd, J = 9.2, 2.5 Hz, 1H), 6.40 (d, J = 2.5 Hz, 1H), 3.47 (q, J = 7.2 Hz, 4H), 1.24 (t, J = 7.2 Hz, 6H).

13C NMR (101 MHz, Chloroform-d) δ 188.2, 159.2, 155.8, 153.5, 147.3, 132.0, 112.7, 110.7, 109.2, 96.3, 45.3, 12.4. HRMS (ESI): m/z: Calcd for C₁₄H₁₅BrNO₃: 324.0235, 326.0215 [M+H]+; Found: 324.0248, 326.0235.

1.2.3. 7-(diethylamino)-3-formyl-4-(2-nitrophenyl)coumarin (3)

The mixture of compound 2 (1.718 g), compound 5 (1.5 g, 1.5 eq.), K₂CO₃ (2.536 g, 3.0 eq.), Pd(PPh₃)₄ (76 mg, 5 mol%), THF (20 mL) and H₂O (2 mL) was degassed by bubbling N₂ before heated to 68°C for 24 hours. After organic solvent was evaporated at reduced pressure and the residue was extracted with CH₂Cl₂. The organic lay was dried with Na₂SO₄, filtered and evaporated under reduced pressure to afford a crude product, which was purified via a flash chromatography with silica gel to give compound 3 (68 mg, 3.5% yield). 1H NMR (400 MHz, Chloroform-d) δ 10.10 (s, 1H), 8.33 (dd, J = 8.0, 1.0 Hz, 1H), 7.73 (td, J = 7.5, 1.3 Hz, 1H), 7.65 (td, J = 7.9, 1.5 Hz, 1H), 7.18 (dd, J = 7.6, 1.6 Hz, 1H), 6.66 (d, J = 9.2 Hz, 1H), 6.52 (d, J = 2.5 Hz, 1H), 6.44 (dd, J = 9.2, 2.8 Hz, 1H), 3.42 (q, J = 7.2 Hz, 4H), 1.21 (t, J = 7.2 Hz, 6H). 13C NMR (101 MHz, Chloroform-d) δ 188.6, 162.1, 157.8, 156.6, 153.0, 147.2, 133.9, 131.2, 129.9, 129.6, 129.5, 124.8, 110.6, 110.1, 108.4, 97.3, 45.2, 12.4. HRMS (ESI): m/z: Calcd for C₂₀H₁₉N₂O₅: 367.1294 [M+H]+; Found: 367.1289.

1.2.4. 7-(diethylamino)-3-(1,3-dithiolan-2-yl)-3-formyl-4-(2-nitrophenyl)coumarin (4)

Under nitrogen, compound 3 (60 mg) was dissolved in anhydrous CH₂Cl₂ (5 mL) with ethylenedithiol (49 mg, 3.2 eq.) with constant stirring before BF₃·Et₂O (31 mg, 1.3 eq.) was added at room temperature. The mixture was allowed to stir for 4 hours before a solution of NaHCO₃ (2 mL) was added. The resulting mixture was extracted with CH₂Cl₂. The organic layer was dried with Na₂SO₄, filtered and evaporated under reduced pressure to afford a crude product, which was purified via a flash chromatography with silica gel to give compound 4 (70 mg, 97% yield). 1H NMR (400 MHz, Chloroform-d) δ 8.25 (d, J = 8.0 Hz, 1H), 7.77 (t, J = 8.0 Hz, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.33 (d, J = 7.6 Hz, 1H), 6.48 (s, 1H), 6.40 – 6.30 (m, 2H), 5.34 (d, J = 2.0 Hz, 1H), 3.64-3.51 (m, 2H), 3.33 (q, J = 7.2 Hz, 4H), 3.14 (m, 2H), 1.13 (t, J = 7.2 Hz, 6H). 13C NMR (101 MHz, Chloroform-d) δ 159.2, 155.4, 150.5, 149.3, 147.7, 134.0, 131.4, 130.2, 130.1, 127.1, 125.0, 116.9, 108.8, 108.1, 97.2, 49.7, 44.8, 41.2.
1.2.4. HRMS (ESI): m/z: Calcd for C_{22}H_{22}N_{2}O_{4}S_{2} Na: 465.0919 [M+Na]^+; Found: 465.0928.

1.2.5. 4-(2-aminophenyl)-7-(diethylamino)-3-(1,3-dithiolan-2-yl)-3-formylcoumarin (ATC-Hg)

Compound 4 (70 mg) was dissolved in MeOH/CH_{2}Cl_{2} (3+3 mL). Zn dust (153 mg, 14.8 eq.) and saturated NH_{4}Cl solution (0.8 mL) was sequentially added with vigorous stirring. The mixture was allowed to react for 4 hours. Most of the organic solvents was evaporated off under reduced pressure before H_{2}O was added. The resulting mixture was extracted with CH_{2}Cl_{2}. The organic lay was dried with Na_{2}SO_{4}, filtered and evaporated under reduced pressure to afford a crude product, which was purified via a flash chromatography with silica gel to give ATC-Hg (35 mg, 54% yield). \( ^1\)H NMR (400 MHz, Chloroform-d) \( \delta \) 7.43 (q, J = 8.2 Hz, 2H), 7.09 (t, J = 7.1 Hz, 1H), 7.01 (d, J = 7.6 Hz, 1H), 6.68 (d, J = 8.8 Hz, 1H), 6.53 (s, 1H), 6.50 (d, J = 2.5 Hz, 2H), 6.40 (dd, J = 9.1, 2.5 Hz, 1H), 5.82 (s, 1H), 5.43 (s, 1H), 3.71 (m, 2H), 3.37 (q, J = 7.1 Hz, 4H), 3.18 (m, 2H), 1.17 (t, J = 6.8 Hz, 6H). \( ^{13}\)C NMR (101 MHz, Chloroform-d) \( \delta \) 159.4, 155.9, 150.7, 148.6, 146.5, 130.0, 128.8, 128.4, 121.9, 112.0, 118.9, 115.2, 108.9, 107.9, 97.1, 49.1, 44.8, 41.4, 41.3, 12.5. HRMS (ESI): m/z: Calcd for C_{22}H_{24}N_{2}O_{2}S_{2} Na: 435.1177 [M+Na]^+; Found: 435.1180.

1.3. General procedure for the spectra measurement

The stock solution of the probe ATC-Hg (5 mM) and MeHgCl (50 mM) was prepared in DMSO. The solutions of various testing species (50 mM) were prepared from AgNO_{3}, BaCl_{2}•2H_{2}O, CdCl_{2}•2.5H_{2}O, Cr(NO_{3})_{3}•6H_{2}O, CuCl_{2}•2H_{2}O, FeCl_{2}•4H_{2}O, FeCl_{3}, MnCl_{2}, NiCl_{2}•6H_{2}O, Pb(NO_{3})_{2}, CuCl_{2} and Hg(NO_{3})_{2}•H_{2}O (5 mM) in deionized water. The test solution of the probe ATC-Hg (5 \( \mu \)M) in 2 mL PBS buffer (10 mM, pH 7.4) was prepared by placing 2 \( \mu \)L of the probe ATC-Hg stock solution in 2 mL aqueous buffer. The resulting solution was shaken well and incubated with appropriate testing species for 4 min at room temperature before recording the spectra. Unless otherwise noted, for all measurements, the excitation wavelength was 405 nm, the excitation slit widths were 5 nm, and emission slit widths were 5 nm.

1.4. Evidence for the signaling process

To obtain evidence for the transformation of ATC-Hg to compound c-ql in the presence of Hg^{2+} ions, the \( ^1\)H NMR spectrum of the signaling mixture of ATC-Hg and Hg^{2+} was recorded. NMR sample was prepared by mixing Hg(NO_{3})_{2}•H_{2}O (6 mg, 2.4eq.) and ATC-Hg (3 mg, 1eq.) in DMSO-d_{6} (0.6 mL), and spectrum was obtained without any further treatment.

3-(diethylamino)-6H-chromeno[3,4-c]quinolin-6-one (c-ql) \( ^1\)H NMR (400 MHz, DMSO-d_{6}) \( \delta \) 8.85 (d, J = 8.5 Hz, 1H), 8.77 (s, 1H), 8.69 (d, J = 8.5 Hz, 1H), 8.28 (d, J = 9.3 Hz, 1H), 8.01 (t, J = 7.6 Hz, 1H), 7.89 (t, J = 7.6 Hz, 1H), 6.79 (dd, J = 9.3, 2.3 Hz, 1H), 6.64 (d, J = 2.4 Hz, 1H), 3.45 (q, J = 7.0 Hz, 4H), 1.13 (t, J = 6.9 Hz, 6H). \( ^{13}\)C
NMR (101 MHz, DMSO-d$_6$) $\delta$ 159.0, 154.7, 150.5, 143.2, 133.3, 132.9, 132.8, 130.4, 129.2, 128.7, 124.8, 120.3, 112.3, 110.2, 104.4, 98.4, 44.5, 12.8. HRMS (ESI): m/z: Calcd for C$_{20}$H$_{18}$N$_2$O$_2$ Na: 319.1447 [M+Na]$^+$; Found: 319.1446.

1.5. Cell culture and imaging

Hela cells were cultured in Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% Antibiotic–antimycotic at 37°C in a 5% CO$_2$/95% air incubator. For fluorescence imaging, cells (4 $\times$ 10$^3$/well) were passed on a 6-well plate and incubated for 24 h. Before the staining experiment, cells was immobilized using 4% paraformaldehyde solution, and was washed 3 times 15 minutes later to get rid of the paraformaldehyde. The immobilized cells was incubated with various concentrations of Hg$^{2+}$ (5, 10, 25μM) and 5 μM ATC-Hg, or only 5 μM ATC-Hg for 5 min at 37°C, then washed twice with PBS. The confocal fluorescent images were captured with an excitation light at 405 nm.

1.6. E. coli cultivation and imaging

Gram-negative bacteria E. coli as model bacteria was used in this study. Bacterial samples were transferred from the frozen state into a 15-mL centrifugal tube containing 10 mL sterile LB medium (Lysogeny Broth for E. coli) and incubated at 37 °C overnight in the shaker (200 rpm). After growth, the bacterial culture was centrifuged, and the supernatant was discarded. The E. coli in the bottom was then washed twice with PBS (pH 7.4, 10 mM) using the same procedure. The E. coli was divided into two groups, the control group and the experimental group. The experimental group was treated with various concentrations of Hg$^{2+}$ (5, 10, 25, 50, 100μM) for 30 min in the shaker at 37 °C and was then washed 3 times with PBS; after that, it was treated with 5 μM ATC-Hg for 5 min at 37 °C and was then washed 3 times with PBS. The control group was just treated with 5 μM ATC-Hg for 5 min at 37 °C and was then washed 3 times with PBS. The final solution of E. coli contained 60% PBS and 40% glycerol (v:v). The confocal fluorescent images were captured with an excitation light at 405 nm.
2. Photophysical data of ATC-Hg

Figure S1. (a). The UV-vis spectra titration of 5 μM ATC-Hg by Hg$^{2+}$. Spectra collected 4 min after Hg$^{2+}$ addition. (b). Dose-dependent the absorption ratios ($A_{480}/A_{410}$) enhancement with respect to Hg$^{2+}$ equivalence. (c). The UV-vis spectra titration of 5 μM ATC-Hg by MeHg$^+$. Spectra collected 30 min after MeHg$^+$ addition. (d). Dose-dependent the absorption ratios ($A_{480}/A_{410}$) enhancement with respect to MeHg$^+$ equivalence. Solvent: PBS buffer (10 mM, pH 7.4).

Figure S2. Fluorescence detection kinetics of ATC-Hg against Hg$^{2+}$(a-b) or MeHg$^+$ (c-d).

Figure S3. (a). Fluorescence titration of ATC-Hg by a low dose of Hg$^{2+}$. (b). Fluorescence titration of ATC-Hg by MeHg$^+$. $\lambda_{ex}$: 405 nm. Solvent: PBS buffer (10 mM, pH 7.4).
Figure S4. (a). Fluorescence spectra of 5 μM probe ATC-Hg upon addition of various metal ions (10eq. Ba^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, 0.1eq. Cu^{2+} in PBS buffer (10 mM, pH 7.4) and Ag^{+} in H_{2}O) without or (b) with 1eq. Hg^{2+} after 4 min. λ_ex: 405 nm. (c). The emission ratios (I_{572}/I_{492}) signal changes of (a) and (b).

Figure S5. The ^1H NMR spectrum of ATC-Hg + Hg^{2+}.
Figure S6. ESI(+)–Mass spectrum of ATC-Hg + Hg$^{2+}$.

<table>
<thead>
<tr>
<th>Sample a</th>
<th>Hg$^{2+}$ spiked / μM</th>
<th>Found / μM b</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water (FuNan River)</td>
<td>0</td>
<td>Not detected</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.096 ± 0.019</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.536 ± 0.025</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.010 ± 0.043</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.997 ± 0.156</td>
<td>100</td>
</tr>
</tbody>
</table>

Table S1. Application of ATC-Hg to the determination of Hg$^{2+}$ in local river water samples. (a The crude water samples from the FuNan River in ChengDu city were passed through a microfiltration membrane before use. b Relative standard deviations were calculated on the basis of three measurements.)
Figure S7. (a) Average intensity ratios from ratio images in Figure 4. Error bars represent standard deviation. (n = 8 fields of cells); (b) Fluorescent confocal microscopy images of normal *E. coli* stained with 5 μM ATC-Hg for 5 min in the presence of various concentrations of Hg^{2+} (0, 5, 10, 25, 50, 100μM) for 30 min in the shaker at 37°C. Green channel images: 470-520 nm; Red channel images: 560-590 nm; Ratio images of red/green. ex: 405 nm. (c) Average intensity ratios from ratio images in (b). Error bars represent standard deviation. (n = 8 fields of cells).
3. NMR Data

ATC-Hg:
Compound 2:
Compound 3:
Compound 4:
4. ESI(+) - MS Data

ATC-Hg:

Compound 2:

Compound 3:

Compound 4:
c-ql: