Highly Promising Discrimination of Various Catecholamine by Ratiometric Fluorescence under Intermolecular Self-Association of Two Sensing Elements

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Supplementary data
(16 pages)

1. Synthesis of crown ether/coumarin based sensor

1.1 Preparation of 7-diethylamino-2-oxo-2H-chromen-3-carboxylic acid (I)

\[
\begin{align*}
\text{4-diethylaminosalicylaldehyde (0.386 g, 2 mmol), diethylmalonate (0.61 mL, 4 mmol), and piperidine (0.2 mL) were dissolved in absolute ethanol (6 mL). The reaction mixture was stirred and refluxed for 6 hours under nitrogen atmosphere. Then 10\% NaOH (6 mL) solution was added to the reaction and refluxed for 15 min. The solution was cooled to room temperature and acidified to pH 2 using concentrated hydrochloric acid at 0\degree C affording a precipitate deposit which was filtered out, washed with water, then recrystallized with ethanol to give an orange crystal (I, 74\%).} \\
&\text{\^{1}H-NMR (400 MHz, CDCl}_{3}\text{): }\delta \text{ (in ppm) } = 12.37 \text{ (s, 1H, COO}H)\text{,} 8.67 \text{ (s, 1H, Ar}H)\text{,} 7.46 \text{ (d, } J = 9.2 \text{ Hz, 1H, Ar}H)\text{,} 6.71 \text{ (dd, } J = 4.27 \text{ Hz, 1H, Ar}H)\text{,} 6.53 \text{ (d, } J = 2.0 \text{ Hz, 1H, Ar}H)\text{,} 3.49 \text{ (q, } J = 7.07 \text{ Hz, 4H, CH}_{2}\text{CH}_{3})\text{,} 1.26 \text{ (t, } J = 7.2 \text{ Hz, 6H, CH}_{2}\text{CH}_{3}). \text{^13C-NMR (400 MHz, CDCl}_{3}\text{): }\delta \text{ (in ppm) } = 165.52, 164.42, 158.07, 153.78, 150.27, 131.94, 110.91, 108.59, 105.70, 96.89, 45.34, 12.39.}
\end{align*}
\]

Scheme S1 Synthesis of compound I
MALDI-TOF mass: Anal. Calcd for [C_{14}H_{15}NO_{4}]^+ m/z = 261.10  
Found m/z = 261.32

1.2 Preparation of 7-diethylamino-2-oxo-2H-chromen-3-carboxylic chloride (2)

![Scheme S2 Synthesis of compound 2](image)

Into a two-neck round bottom flask equipped with a magnetic bar, 1 (0.187 g, 0.7 mmol) was added to dry thionyl chloride (3 mL) and the suspension was stirred at room temperature for 3 hours under nitrogen atmosphere. The precipitate was washed with dichloromethane under vacuum to give a yellow solid 2 in a quantitative yield.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ (in ppm) = 8.68 (s, 1H, ArH), 7.49 (d, $J$ = 4.4 Hz, 1H, ArH), 6.76 (m, 1H, ArH), 6.59 (s, 1H, CCH), 3.50 (q, $J$ = 6.9 Hz, 4H, CH$_2$CH$_3$), 1.28 (t, $J$ = 3.4 Hz, 6H, CH$_3$CH$_3$)

2. Characterization of the compounds
**Figure S1** The $^1$H-NMR spectrum of sensor NB in DMSO-$d_6$ at 400 MHz

**Figure S2** The $^{13}$C-NMR spectrum of sensor NB in DMSO-$d_6$ at 400 MHz
**Figure S3** MALDI-TOF mass spectrum of sensor NB shown at 317.379 m/z

**Figure S4** The $^1$H-NMR spectrum of 1 in CDCl$_3$ at 400 MHz
Figure S5 The $^{13}$C-NMR spectrum of 1 in CDCl$_3$ at 400 MHz

Figure S6 MALDI-TOF mass spectrum of 1 shown at 261.322 m/z
Figure S7 The $^1$H-NMR spectrum of 2 in CDCl$_3$ at 400 MHz

Figure S8 The $^1$H-NMR spectrum of sensor CC in CDCl$_3$ at 400 MHz
**Figure S9** The $^{13}\text{C}$-NMR spectrum of sensor CC in CDCl$_3$ at 400 MHz

**Figure S10** The ESI-High Resolution Mass spectrum of sensor CC at 560.25 m/z
Figure S11 IR spectrum of sensor CC

3. Job’s plots studies

![Graph of Job's plots studies](image)
Figure S12 Job’s plots for 1:1 complex of sensor NB with (A) DA (B) NE at $1 \times 10^{-5}$ M in DMSO:phosphate buffer (0.01 M, pH 7.4, 1:9, v/v)

4. Non-linear regression plots
Figure S13. Non-linear regression plots of sensor NB (1 x 10^{-5} M) toward 0-80 equiv. of (A) DA (B) NE and (C) EPI in DMSO:phosphate buffer (0.01 M, pH 7.4, 1:9, v/v) for calculation of binding constant ($K_b$)
Figure S14. UV-vis spectra titration with EPI
5. Calibration curves of detection limit for DA, NE and EPI

**Figure S15.** Linear plot of fluorescence intensity between sensor with a) DA b) NE c) EPI complexes and concentration of catecholamine guests
6. Complexation studies of sensor CC with various guests by fluorescence spectrophotometry technique

**Figure S16** Fluorescence spectra of sensor CC (5 x 10^{-5} M) in DMSO:phosphate buffer (0.1 M, pH 7.4, 1:9, v/v) in the present of various guest 100 equiv. (λ_{ex} = 340 nm)

**Figure 17.** UV-vis spectra of NB and CC 1x10^{-4} M
7. Principle component analysis (PCA) method for analysis of complexation

**Figure S18.** PCA score plot of (A) sensor NB (B) sensor CC and (C) the mixture sensors NB and CC upon addition of various guests (100 equiv.) in 1:9, v/v DMSO:phosphate buffer (pH 7.4). PCA score plot shows clustering for all 9 samples.
8. Complexation study of sensor NB with EPI in human urine sample

**Figure S19.** Calibration curve of sensor NB with the spiked EPI in the synthetic urine

**Figure S20.** PCA score plots of mixed NB and CC upon addition of various guests (100 equiv.) in urine samples and including fluorescence data of EPI for determining of PCA analysis
**Table S1** Composition of synthetic urine [1]

<table>
<thead>
<tr>
<th>Species</th>
<th>Concentration (g l⁻¹)</th>
<th>Concentration (mmol l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl₂·H₂O</td>
<td>0.65</td>
<td>Ca: 4.3</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>0.651</td>
<td>Mg:3.2</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.6</td>
<td>SO₄: 16</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>2.3</td>
<td>Citrate: 2.3</td>
</tr>
<tr>
<td>Na₃C₆H₇O₇·2H₂O</td>
<td>0.65</td>
<td>Oxalate: 0.149</td>
</tr>
<tr>
<td>Na₂C₂O₄</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>2.8</td>
<td>PO₄: 20.5</td>
</tr>
<tr>
<td>KCl</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>1</td>
<td>NH₄: 19</td>
</tr>
<tr>
<td>CO(NH₂)₂</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>C₄H₇N₅O</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

Total Na = 118 mEq
Total K = 42 mEq
pH = 5.8

**Table S2** Comparison of the sensitivity of sensory system for dopamine

<table>
<thead>
<tr>
<th>Sensing materials</th>
<th>Detection</th>
<th>Linear range</th>
<th>LOD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP-Si-ITO</td>
<td>Electrochemistry</td>
<td>2x10⁻⁶–8x10⁻⁴ M.</td>
<td>2x10⁻⁶ M.</td>
<td>2</td>
</tr>
<tr>
<td>Au at SiO₂-MIPs-GCE</td>
<td>Electrochemistry</td>
<td>4.8x10⁻⁸–5x10⁻⁵ M.</td>
<td>2x10⁻⁸ M</td>
<td>3</td>
</tr>
<tr>
<td>C₆₀-CNT/IL/GC electrode</td>
<td>Electrochemistry</td>
<td>0.06–25 µM</td>
<td>15 nM</td>
<td>4</td>
</tr>
<tr>
<td>Citrate-caped AuNPs</td>
<td>Colorimetric</td>
<td>2.5-20 µM</td>
<td>2500 nM</td>
<td>5</td>
</tr>
<tr>
<td>MBA- and DSP-modified AuNPsb</td>
<td>Colorimetric</td>
<td>5-180 nM</td>
<td>0.5 nM</td>
<td>6</td>
</tr>
<tr>
<td>Fe₃O₄ NPs</td>
<td>Fluorescence</td>
<td>0.01-0.4 µM</td>
<td>3 µM</td>
<td>7</td>
</tr>
<tr>
<td>Self-assembled PBA and CCc</td>
<td>Fluorescence</td>
<td>16.7-47.4 µM</td>
<td>1.47 µM</td>
<td>8</td>
</tr>
<tr>
<td>Phosphate-modified TiO₂ NPs</td>
<td>Fluorescence</td>
<td>0.5-100 µM</td>
<td>30 nM</td>
<td>9</td>
</tr>
<tr>
<td>Naphthalimide-boronic acid</td>
<td>Fluorescence</td>
<td>11.9-95.2 µM</td>
<td>7.71 µM</td>
<td>This work</td>
</tr>
</tbody>
</table>

*aSi-ITO: silanized indium tin oxide electrode.

*bMBA, 4-mercaptophenylboronic acid; DSP, dithiobis (succinimidyl propionate)

cPBA, Pyrene boronic acid; CC, crown-coumarin