Supplementary data

A highly selective turn-off fluorescent probe for Cu(II) based on dansyl derivative and its application in living cell imaging

Jiaguo Huanga, Min Liua, Xiaoqian Mab, Qiong Dongb, Bin Yeb, Wei Wanga, Wenbin Zenga,*

aSchool of Pharmaceutical Sciences, Central South University.
bThe Third Xiangya Hospital, Central South University

Correspondence information: Wenbin Zeng, School of Pharmaceutical Sciences, Central South University, 172 Tongzipo road, Changsha, 410013, P. R. China. Fax: 0086-731-82650459; Tel: 0086-731-82650459; E-mail: wbzeng@hotmail.com.

Contents

1. Optimization of condition for probe 1 measurement……………………………………..S1
2. The UV response of 1 to various metal ions……………………………………………S2
3. The titration experiments of probe 1……………………………………………………S3
4. The fluorescent intensity change of 1 to various anions……………………………………S4
5. Binding Constant………………………………………………………………………………S5
6. Job plot of the complexation between the probe 1 and Cu2+……………………………S6
7. The effect of pH…………………………………………………………………………………S7-S8
8. Fluorescence quantum yields………………………………………………………………..Table S1
9. Reference
10. 1H NMR, 13C NMR and ESI or HRMS spectra
1. Optimization of condition for probe 1 measurement

Fig. S1. Influence of the HEPES buffer solution concentration on the fluorescence intensity of 20 μM probe 1. (pH =7.0, λ_ex/λ_em = 348 nm/515 nm).

2. The UV response of 1 to various metal ions

Fig. S2. UV-vis absorption spectra of probe 1 (16 μM) towards various metal ions (800μM) in CH3CN/HEPES buffer (8/2, v/v, pH =7.0).
3. The titration experiments of probe 1

(a) [Graph showing fluorescence intensity vs. wavelength (nm) with various concentrations of probe 1.]

(b) [Graph showing fluorescence intensity vs. wavelength (nm) with various concentrations of probe 1.]

(c) [Graph showing absorbance (A) vs. wavelength (nm) with various concentrations of probe 1.]
Fig. S3. (a) Fluorescence spectra of 8 μM probe 1 with Cu$^{2+}$ between 0 and 2 equiv (CH$_3$CN/HEPES buffer = 8/2, v/v, pH=7.0, $\lambda_{ex}/\lambda_{em}$ = 348 nm /515 nm); (b) Fluorescence spectra of 20 μM probe 1 with Cu$^{2+}$ between 0 and 10 equiv (only HEPES buffer, pH=7.0, $\lambda_{ex}$ = 348 nm); (c) UV-vis absorption spectra of probe 1 (16 μM) with Cu$^{2+}$ between 0 and 1.1 equiv ((CH$_3$CN/HEPES buffer = 8/2, v/v, pH=7.0); (d) spectra of (c) from 260 nm to 450 nm.

4. The fluorescent intensity change of 1 to various anions

Fig. S4. The fluorescence variation of probe 1 (16 μM) to various anions at 800 μM concentration in CH$_3$CN/HEPES buffer (8/2, v/v, pH=7.0, $\lambda_{ex}/\lambda_{em}$ = 348 nm /515 nm).
5. Binding Constant

The binding constant was calculated from the emission intensity - titration curves. According to the equation:

\[
\frac{F-F_0}{F_0} = f \left[ 1 + \frac{1}{K_S [Cu^{2+}]} \right],
\]

where \( F_0 \) is the emission intensity of probe 1 at 515 nm, \( F \) is the emission intensity of 1 at 515 nm upon the addition of different concentration of Cu (II), \( f \) is the fraction of the initial fluorescence which is accessible to the sensor, \([Cu^{2+}]\) is the concentration of Cu\(^{2+}\).

![Fig. S5. Fitting of Fluorescence titration curve of 1 in CH\(_3\)CN/HEPES (8: 2, v/v, pH =7.0). The binding constant is \( K_S = 5.08 \times 10^4 \) M\(^{-1}\).](image)

6. Job plot of the complexation between the probe 1 and Cu\(^{2+}\)

![Fig. S6. Job plot of the complexation between the probe 1 and Cu\(^{2+}\) (CH\(_3\)CN/HEPES buffer = 8/2, v/v, pH=7.0, \(\lambda_{ex}/\lambda_{em}= 348 \text{ nm }/515 \text{ nm}\). The total molar concentration of 1 and Cu\(^{2+}\) is 10\(\mu\)M;](image)
7. The effect of pH

![Graphs showing fluorescence intensity vs. wavelength at different pH values.](image)

Fig. S7. (a) Influence of pH on fluorescence spectra of free 1 (16 μM) in CH$_3$CN/H$_2$O solution (8/2, v/v); (b) Influence of pH from 1.0 to 3.0; (c) Influence of pH 4.0 to 13.0.
Fig. S8. (a) Influence of pH on fluorescence spectra of I/Cu\textsuperscript{2+} adduct (16 μM I and 80 μM Cu\textsuperscript{2+}) in CH\textsubscript{3}CN/H\textsubscript{2}O solution (8/2, v/v); (b) Influence of pH from 1.0 to 2.0; (c) Influence of pH from 3.0 to 11.0; (d) Influence of pH from 12.0 to 13.0.

8. Fluorescence quantum yield

Table S1. Photophysical Data\textsuperscript{a}

<table>
<thead>
<tr>
<th>Sample</th>
<th>(F_{515})</th>
<th>(\Phi_f) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>178.03</td>
<td>8.2</td>
</tr>
<tr>
<td>I + Cu\textsuperscript{2+}</td>
<td>9.23</td>
<td>0.42</td>
</tr>
</tbody>
</table>

\textsuperscript{a}F\textsubscript{515}: Fluorescence intensity at 515nm. \(\Phi_f\): Fluorescence quantum yield.

9. Reference

10. $^1$H NMR, $^{13}$C NMR, ESI-MS or HRMS spectra

5-(1, 3-dioxoisindolin-2-yl)naphthalene-1-sulfonic acid (3)

$^1$H NMR (400 MHz, CDCl$_3$)
5-(1, 3-dioxoisindolin-2-yl)naphthalene-1-sulfonic acid (3)

$^{13}$C NMR (400 MHz, CDCl$_3$)
Elemental Composition Report

Single Mass Analysis
Tolerance = 100.0 PPM  /  DBE: min = -1.6, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron loss
12 formula(e) evaluated with 1 results within limits (all results up to 1000 for each mass)
Elements Used:
C: 24-24  H: 20-20  N: 0-3  S: 0-2  Na: 0-1

454.1562
XFX2.2 (0.064)

Minimum: 5.0  100.0  50.0
Maximum: -2.8

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>1-FIT</th>
<th>1-FIT (Norm)</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>477.1447</td>
<td>477.1460</td>
<td>-1.3</td>
<td>-2.7</td>
<td>12.5</td>
<td>11.0</td>
<td>0.0</td>
<td>C24H26N2O5SNa</td>
</tr>
</tbody>
</table>
Tert-butyl4-(5-(1,3-dioxoisooindolin-2-yl)naphthalene-1-sulfonamido)piperidine-1-carboxylate (5)

$^1$H NMR (400 MHz, CDCl$_3$)
Tert-butyl4-(5-(1,3-dioxoisoindolin-2-yl)naphthalene-1-sulfonamido) piperidine-1-carboxylate (5)

$^{13}$C NMR (400 MHz, CDCl$_3$)
### Elemental Composition Report

**Single Mass Analysis**

Tolerance = 10.0 PPM / DBE: min = -1.8, max = 50.0

Element prediction: Off

Number of isotope peaks used for I-FIT = 3

Monoisotopic Mass, Even Electron Ions (SZ formulation) evaluated with 1 results within limits (all results up to 1000 for each mass)

Elements Used:
- C: 28.28
- H: 29.329
- N: 0.6
- O: 6.6
- S: 0.1

536.1777

**FX-001 3 (0.083) Cm (2:34)**

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>m/z</th>
<th>PPM</th>
<th>DBE</th>
<th>i-PIT</th>
<th>i-PIT (Norm)</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>536.1846</td>
<td>536.1855</td>
<td>-0.9</td>
<td>-1.7</td>
<td>15.5</td>
<td>16.8</td>
<td>0.0</td>
<td>C28 H30 N3 O6 S</td>
</tr>
</tbody>
</table>

**Minimum:**
-1.8

**Maximum:**
5.0  10.0  50.0
5-(1,3-dioxoisindolin-2-yl)-N-(piperidin-4-yl)naphthalene-1-sulfonamide (6)

$^1$H NMR (400 MHz, CDCl$_3$)
5-(1,3-dioxoisindolin-2-yl)-N-(piperidin-4-yl)naphthalene-1-sulfonamide (6)

ESI-MS

Mass Spectrum Deconvolution Report

Analysis Info
Analysis Name D:\Data\20120329-Jack-09.d
Method .\20111121-TEA.m
Sample Name 20120329-Jack-09
Comment 20120329-Jack-09

Acquisition Parameter
Ion Source Type ESI
Ion Polarity Positive
Positive Acquiring ion Polarity off
Scan Range 105 m/z
Scan Speed 1000 m/z
Detect Cell 113.5 Volt
Detected Cell 45.6 Volt
Accumulation Time 1162 ms
Averaging 2 Spectra
Auto MS/MS on

# 1 2 3 4 5 6 7 8 9 10
1 116.3 117.0 118.3 119.3 117.0 118.3 119.3 120.0 121.2 124.0
2 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0
3 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0
4 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0
5 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0
6 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0
7 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0
8 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0
9 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0
10 130.3 131.0 132.6 134.2 138.4 141.1 141.1 142.2 144.0 147.0

4-(5-(1,3-dioxoisindolin-2-yl)naphthalene-1-sulfonamido)-N-methylpiperidine-
4-(5-(1,3-dioxiisoindolin-2-yl)naphthalene-1-sulfonamido)-N-methylpiperidine-
1-carboxamide (7)

4-(5-aminonaphthalene-1-sulfonamido)-N-methylpiperidine-1-carboxamide (8)
$^1$H NMR (400 MHz, CD$_3$OD)

4-(5-aminonaphthalene-1-sulfonamido)-N-methylpiperidine-1-carboxamide (8)
$^{13}$C NMR (400 MHz, CD$_3$OD)

4-(5-aminonaphthalene-1-sulfonamido)-N-methylpiperidine-1-carboxamide (8)
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.8, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Exact Mass, Elements
54 formulae evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 17-17  H: 22-23  N: 0-6  O: 0-8  S: 0-1

363.1491
FX-004 2 (0.082) Cm (2:19)

Minimum:
Maximum:

Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  i-FIT (Norm)  Formula
363.1491  363.1491  0.0  0.0  8.5  30.5  0.0  C17 H23 N4 O3 S

1: TOF MS ES+ 1.50e+004
1-carboxamide (1)

$^1$H NMR (400 MHz, CD$_3$OD)

N-methyl-4-(5-(pyridin-2-ylmethylamino)naphthalene-1-sulfonamido)piperidine-
1-carboxamide (1)

$^{13}$C NMR (400 MHz, CD$_3$OD)

N-methyl-4-(5-(pyridin-2-ylmethylamino)naphthalene-1-sulfonamido)piperidine-
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.8, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
53 formula(s) evaluated with 1 results within limits (all results up to 1000) for each mass
Elements Used:
C: 18-24   H: 24-30   N: 0-6   O: 0-6   S: 0-1
453.1938
FX-908 9 (0.220)

Minimum:  5.0  10.0  -1.8
Maximum:  50.0

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>i-FIT (Norm)</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>454.1913</td>
<td>454.1913</td>
<td>0.0</td>
<td>0.0</td>
<td>12.5</td>
<td>13.4</td>
<td>0.0</td>
<td>C23 H28 N5 O3 S</td>
</tr>
</tbody>
</table>

1: TOF MS ES+ 1.72e+001