Supporting information for

Fluorescence enhancement, cellular imaging and biological investigation of chiral pyrrolidinols modified naphthalimide derivatives

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Scheme 1. 1) N,N-dimethylethylene diamine, EtOH; 2) 2-methoxyethanol, DIPEA, amino alcohols (L-prolinol, D-prolinol, R-3-hydroxypyrrolidine and S-3-hydroxypyrrolidine).

1. Experimental part

1.1 Measurements

1H NMR and 13C NMR spectra were recorded on a Bruker 600 spectrometer. HRMS analysis was performed on an Apex Ultra 7.0T FT-MS (Bruker Dalonik Company). UV–Vis spectra were
recorded in a quartz cell (light path 10 mm or 5 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a S-1700 temperature controller. Fluorescence spectra were performed on F-7000 (Hitachi Instruments). The fluorescence images were obtained using Olympus confocal laser scanning microscopy (Olympus Fluoview FV1000).

1.2 MTT assay

The compounds NI1-4 were dissolved in phosphate buffered saline (PBS) and diluted to the required concentration with culture medium. The cytotoxicity was evaluated by MTT assay. Briefly, cells were plated in 96-well microassay culture plates (10^4 cells per well) and grown overnight at 37 °C in a 5% CO₂ incubator. The compounds NI1-4 were then added to the wells to achieve final concentrations ranging from 10⁻⁷ to 10⁻⁴ M. Wells containing culture medium without cells were used as control blanks; wells containing culture medium and cisplatin or amonofide was used as positive control. The plates were incubated at 37 °C in a 5% CO₂ incubator for 48 h. Upon completion of the incubation, stock MTT (Sigma) dye solution (20 μL, 5 mg/μL) was added to each well. After 4 h incubation, 2-propanol (100 μL) was added to solubilize the MTT formazan. The optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC₅₀ value was determined from the plot of % viability against dose of complexes added.

1.3 Confocal microscopy study

The A549 cells were seeded 1 day before experiments in a 6-well plate at 4.0 × 10⁵ cells/well. Then cells were incubated with NI1 (5.0 μM), NI2 (4.0 μM), NI3 (2.0 μM) and NI4 (1.0 μM) at 37 °C for 12 h. After incubation, the unbound molecules were washed third with PBS buffer.

1.4 Synthesis procedures and analytical data

General synthesis of NI1-4: In a 50-mL flask, 500 mg (1.44 mmol) of compound M-1 was dissolved in 10 mL of 2-methoxyethanol. Then, 2.88 mmol of amino alcohols (L-prolinol, D-prolinol, R-3-hydroxypyrrolidine and S-3-hydroxypyrrolidine) and 3 mL of DIPEA were added, and the mixture was heated under an N₂ atmosphere at 120°C for 24 h or 48 h. After the mixture was cooled to room temperature, the solvent was removed in vacuo. The solid obtained was purified by column chromatography to afford pure NI1-4 with the yields of 62.6%, 58.8%, 50.8% and 39.1%.

NI1: m. p. 92.4-94.2 °C ¹H NMR (CDCl₃, 600 MHz): δ 1.82 (m, 1H), 2.09 (m, 1H), 2.16 (m, 1H),
2.31 (m, 1H), 2.72 (m, 6H), 3.11 (t, 2H, \( J = 6.0 \) Hz), 3.62 (t, 1H, \( J = 8.4 \) Hz), 3.75 (dd, 1H, \( J = 3.0 \) Hz, 11.4 Hz), 3.82 (dd, 1H, \( J = 4.8 \) Hz, 11.4 Hz), 4.13 (m, 1H), 4.31 (m, 1H), 4.38 (m, 2H), 7.06 (d, 1H, \( J = 8.4 \) Hz, Ar-H), 7.52 (t, 1H, \( J = 7.8 \) Hz, Ar-H), 8.28 (d, 1H, \( J = 8.4 \) Hz, Ar-H), 8.43 (d, 1H, \( J = 7.2 \) Hz, Ar-H), 8.48 (d, 1H, \( J = 8.4 \) Hz, Ar-H); \(^{13}\)C NMR (CDCl\(_3\), 150 MHz): \( \delta \) 25.64, 28.47, 36.33, 44.64, 56.19, 57.45, 61.26, 62.23, 110.81, 111.80, 122.08, 123.73, 124.34, 130.74, 130.78, 131.28, 132.13, 133.00, 153.47, 153.75, 164.51; HRMS (ESI): calcd. for C\(_{21}\)H\(_{26}\)N\(_3\)O\(_3\): 368.1974, found 368.1969.

NI2: m.p. 89.2-90.6 °C. \(^1\)H NMR (CDCl\(_3\), 600 MHz): \( \delta \) 1.84 (m, 1H), 2.10 (m, 1H), 2.16 (m, 1H), 2.32 (m, 1H), 2.77 (m, 6H), 3.17 (t, 2H, \( J = 6.0 \) Hz, -CH\(_2\)), 3.64 (t, 1H, \( J = 8.4 \) Hz), 3.77 (dd, 1H, \( J = 3.0 \) Hz, 11.4 Hz), 3.82 (dd, 1H, \( J = 4.8 \) Hz, 11.4 Hz), 4.13 (m, 1H), 4.32 (m, 1H), 4.43 (m, 2H), 7.08 (d, 1H, \( J = 8.4 \) Hz, Ar-H), 7.54 (t, 1H, \( J = 7.8 \) Hz), 8.33 (d, 1H, \( J = 8.4 \) Hz, Ar-H), 8.47 (d, 1H, \( J = 7.2 \) Hz, Ar-H), 8.49 (d, 1H, \( J = 8.4 \) Hz, Ar-H); \(^{13}\)C NMR (CDCl\(_3\), 150 MHz): \( \delta \) 25.63, 28.54, 35.62, 44.18, 55.97, 57.42, 61.32, 62.39, 111.01, 123.86, 124.48, 130.91, 131.45, 132.32, 133.18, 153.65, 163.86, 164.71; HRMS (ESI): calcd. for C\(_{21}\)H\(_{26}\)N\(_3\)O\(_3\): 368.1974, found 368.1963.

NI3: m.p. 102.3-104.6 °C. \(^1\)H NMR (CD\(_2\)OD, 600 MHz): \( \delta \) 2.17 (m, 1H), 2.22 (m, 1H), 2.57 (s, 6H), 2.93 (m, 2H), 3.67 (d, 1H, \( J = 10.8 \) Hz), 3.77 (t, 1H, \( J = 7.8 \) Hz), 4.10 (m, 2H), 4.34 (t, 2H, \( J = 7.2 \) Hz, -CH\(_2\)), 4.62 (m, 1H), 6.88 (d, 1H, \( J = 8.4 \) Hz, Ar-H), 7.59 (t, 1H, \( J = 7.8 \) Hz, Ar-H), 8.30 (d, 1H, \( J = 9.0 \) Hz, Ar-H), 8.48 (d, 1H, \( J = 7.2 \) Hz, Ar-H), 8.72 (d, 1H, \( J = 9.0 \) Hz, Ar-H); \(^{13}\)C NMR (CD\(_2\)OD, 150 MHz): \( \delta \) 37.23, 40.24, 47.71, 54.29, 60.45, 64.61, 73.70, 112.39, 113.03, 125.58, 126.22, 126.81, 134.86, 135.15, 136.71, 137.33, 157.23, 168.18, 169.02; HRMS (ESI): calcd. for C\(_{20}\)H\(_{24}\)N\(_3\)O\(_3\): 354.1818, found 354.1813.

NI4: m.p. 110.4-112.8 °C. \(^1\)H NMR (CDCl\(_3\), 600 MHz): \( \delta \) 2.19 (m, 2H), 2.39 (s, 6H), 2.69 (m, 2H), 3.61 (m, 2H), 3.73 (m, 1H), 3.99 (m, 1H), 4.06 (m, 1H), 4.33 (t, 2H, \( J = 7.2 \) Hz), 4.68 (s, 1H), 6.81 (d, 1H, \( J = 8.4 \) Hz, Ar-H), 7.55 (t, 1H, \( J = 7.2 \) Hz, Ar-H), 8.41 (d, 1H, \( J = 9.0 \) Hz, Ar-H), 8.54 (d, 1H, \( J = 8.4 \) Hz, Ar-H), 8.57 (d, 1H, \( J = 7.2 \) Hz, Ar-H); \(^{13}\)C NMR (CDCl\(_3\), 150 MHz): \( \delta \) 34.05, 37.59, 45.45, 50.56, 56.77, 60.95, 108.81, 123.28, 131.17, 131.89, 133.45, 152.72, 164.12, 164.92; HRMS (ESI): calcd. for C\(_{20}\)H\(_{24}\)N\(_3\)O\(_3\): 354.1818, found 354.1809.
Fig. S1 $^1$H NMR of compound N11 (600 MHz, CDCl$_3$).

Fig. S2 $^{13}$C NMR of compound N11 (150 MHz, CDCl$_3$).
**Fig. S3** HRMS (ESI) of compound NI1.

**Fig. S4** $^1$H NMR of compound NI2 (600 MHz, CDCl$_3$).
Fig. S5 $^{13}$C NMR of compound N12 (150 MHz, CDCl$_3$).

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**Fig. S6** HRMS (ESI) of compound N12.
Fig. S7 $^1$H NMR of compound N13 (600 MHz, CD$_3$OD).

Fig. S8 $^{13}$C NMR of compound N13 (150 MHz, CD$_3$OD).
Fig. S9 HRMS (ESI) of compound NI3.

Fig. S10 ¹H NMR of compound NI4 (600 MHz, CDCl₃).
Fig. S11. $^{13}$C NMR of compound NI4 (150 MHz, CDCl$_3$).

![Mass Spectrum SmartFormula Report](image)

Fig. S12 HRMS (ESI) of compound NI4.
**Table S1.** Cytotoxicity data for compounds NI1-4 (IC_{50}, μM)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Hela</th>
<th>MCF-7</th>
<th>SGC-7901</th>
<th>A549</th>
</tr>
</thead>
<tbody>
<tr>
<td>NI1</td>
<td>15.209±0.053</td>
<td>42.704±0.119</td>
<td>28.389±0.0499</td>
<td>5.4211±0.056</td>
</tr>
<tr>
<td>NI2</td>
<td>21.343±0.021</td>
<td>99.662±0.098</td>
<td>51.597±0.117</td>
<td>3.654±0.023</td>
</tr>
<tr>
<td>NI3</td>
<td>3.845±0.183</td>
<td>3.531±0.039</td>
<td>3.404±0.274</td>
<td>1.961±0.042</td>
</tr>
<tr>
<td>NI4</td>
<td>3.249±0.312</td>
<td>3.686±0.099</td>
<td>2.546±0.307</td>
<td>0.874±0.023</td>
</tr>
<tr>
<td>Amonofide</td>
<td>4.365±0.135</td>
<td>8.022±0.038</td>
<td>5.327±0.200</td>
<td>1.595±0.072</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>13.413±0.062</td>
<td>7.73±0.094</td>
<td>15.057±0.102</td>
<td>4.776±0.048</td>
</tr>
</tbody>
</table>

**Table S2.** Average T_m and ΔT_m for Ct-DNA in the absence and presence of NI1-4.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>T_m (°C)</th>
<th>ΔT_m (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ct-DNA</td>
<td>69.8</td>
<td>0</td>
</tr>
<tr>
<td>NI1</td>
<td>71.7</td>
<td>1.9</td>
</tr>
<tr>
<td>NI2</td>
<td>71.6</td>
<td>1.8</td>
</tr>
<tr>
<td>NI3</td>
<td>74.1</td>
<td>4.3</td>
</tr>
<tr>
<td>NI4</td>
<td>73.4</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Fig. S14. The $T_m$ curves of compounds NI1-4 ($5.0 \times 10^{-6}$ M) binding with Ct-DNA ($5.0 \times 10^{-5}$ M) (69.8, 71.8, 71.7, 73.4, 74.0) in phosphate buffer (1 mM, pH 7.4, 5 mM NaCl).