Supporting Information

for

*Monofunctional Platinum(II) Anticancer Complexes Based on Multidentate Phenanthridine-Containing Ligand Frameworks*

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**EXPERIMENTAL DETAILS**

Air-sensitive manipulations were carried either in a N₂-filled glove box or using standard Schlenk techniques under Ar. 4-amino-(2-tert-butyl)phenanthridine\(^1\) and 4-amino-(2-trifluoromethyl)phenanthridine\(^2\) were synthesized according to literature procedures. All other reagents were purchased from commercial suppliers as noted and used without further purification. Organic solvents were dried and distilled using appropriate drying agents, and distilled water was degassed prior to use. 1- and 2D NMR spectra were recorded on Bruker Avance 300 MHz or Bruker Avance – III 500 MHz spectrometers. \(^1\)H and \(^{13}\)C\(^{1}\)H\(^{1}\) NMR spectra were referenced to residual solvent peaks. Infrared spectra of the proligands were collected using a Bruker Invenio R FTIR spectrometer. High resolution mass spectra of Pt(II) complexes were recorded using a Bruker microOTOF-QIII. Elemental analysis was performed at the University of Manitoba using a Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer.

**Synthesis of tBu-phenanthridine-nacac proligand (L1):** A Teflon-stoppered flask was charged with 4-amino-(2-tert-butyl)phenanthridine (1.01 g, 3.03 mmol), acetylacetone (1.30 mL, 12.7 mmol), p-tosic acid (0.050 g, 0.27 mmol), toluene (10 mL) and 4 Å molecular sieves. The reaction was heated to reflux for 48 h, then cooled to room temperature and filtered over Celite. The solvent was then evaporated to leave an oily dark brown residue, which was redissolved in a minimal amount of CH₂Cl₂ and passed through a short plug of silica. The solvent was evaporated to isolate a beige solid. Yield = 0.858 g (64%). \(^1\)H NMR (CDCl\(_3\), 300 MHz, 22 °C): \(\delta\) 13.44 (s, 1H; NH), 9.31 (s, 1H; C\(_6\)-H), 8.63 (d, \(J_{HH} = 8.3\) Hz, 1H; C\(_{10}\)-H), 8.32 (d, \(J_{HH} = 1.9\) Hz, 1H; C\(_1\)-H), 8.06 (d, \(J_{HH} = 7.3\) Hz, 1H; C\(_7\)-H), 7.86 (ddd, \(J_{HH} = 1.3\) Hz, 7.7
Hz, 8.5 Hz, 1H; C₈-H), 7.71 (dd overlapped, J_HH = 0.9 Hz, 7.5 Hz, 8.5 Hz, 1H; C₉-H), 7.60 (d, J_HH = 1.9 Hz, 1H; C₃-H), 5.35 (s, 1H; C₁₅-H), 2.23 (s, 3H; C₁₃-H), 2.18 (s, 3H; C₁₇-H), 1.49 ppm (s, 9H; C₁₂-H). ¹³C{¹H} NMR (CDCl₃, 75 MHz, 22 °C): δ 196.33 (C₁₆), 158.65 (C₁₄), 152.42 (C₆), 149.74 (C₂), 136.91 (C₄), 136.12 (C₄a), 132.74 (C₄b), 130.97 (C₈), 129.06 (C₇), 127.65 (C₉), 126.83 (C₆a), 124.39 (C₆b), 122.16 (C₁₀), 119.68 (C₃), 113.88 (C₁), 99.74 (C₁₅), 35.46 (C₁₁), 31.57 (C₁₂), 29.54 (C₁₇), 21.09 ppm (C₁₃). IR (ATR): ν max 3100-2800 (C-H stretch, b, w), 1617 (C=O stretch, n, m), 1570 cm⁻¹ (N-H bend, n, s).

**Synthesis of CF₃-phenanthridine-nacac proligand (L₂):** An identical procedure to the synthesis of L₁ was followed using 4-amino-(2-trifluoromethyl)phenanthridine (1.09 g, 3.15 mmol), acetylacetone (1.30 mL, 12.7 mmol), p-tosisic acid (0.040 g, 0.23 mmol), toluene (10 mL), and 4 Å molecular sieves. L₂ was isolated as an off-white solid. Yield = 0.911 g (64%). ¹H NMR (CDCl₃, 300 MHz, 22 °C): δ 13.72 (s, 1H; NH), 9.44 (s, 1H; C₆-H), 8.49 (s, 1H; C₁-H), 8.12 (d, J_HH = 7.9 H, 1H; C₇-H), 7.93 (dd, J_HH = 1.2 Hz, 7.7 Hz, 8.7 Hz, 1H; C₈-H), 7.79 (dd overlapped, J_HH = 0.8 Hz, 7.5 Hz, 8.7 Hz, 1H; C₉-H), 7.68 (d, J_HH = 1.4 Hz, 1H; C₃-H), 5.41 (s, 1H; C₁₄-H), 2.36 (s, 3H; C₁₂-H), 2.21 ppm (s, 3H; C₁₆-H). ¹³C{¹H} NMR (CDCl₃, 75 MHz, 22 °C): δ 197.22 (C₁₅), 156.59 (C₁₃), 154.58 (C₆), 138.73 (C₄), 137.90 (C₂), 132.34 (C₆a), 131.90 (C₈), 129.32 (C₇), 128.73 (C₉), 126.91 (C₆b), 124.71 (C₄a), 122.47 (C₄b), 122.34 (C₁₀), 114.08 (q overlapped, C₁), 113.94 (q overlapped, C₃), 101.79 (C₁₄), 29.84 (C₁₆), 21.59 ppm (C₁₂), C₁₁ - not observed. ¹⁹F NMR (CDCl₃, 470 MHz, 22 °C): δ -62.25 ppm. IR (ATR): ν max 3100-2800 (C-H stretch, b, w), 1634 (C=O stretch, n, s), 1579 cm⁻¹ (N-H bend, n, s).
Synthesis of [Pt(tBu-phenanthridine-nacac)Cl] (1): A solution of L1 (0.501 g, 1.51 mmol) in THF (25 mL) was added to a suspension of PtCl₂ (0.407 g, 1.53 mmol), Ag₂O (0.179 g, 0.774 mmol) and 4 Å molecular sieves in THF (25 mL). The mixture was protected from light and heated to reflux at 60 °C for 16 h. The mixture was then cooled and filtered over Celite. The solvent was then evaporated under vacuum to leave an orange solid, which was redissolved in CHCl₃ and recrystallized via diffusion of diethylether vapours into the filtered solution. Yield = 0.739 g (87%). ¹H NMR (CDCl₃, 500 MHz, 22°C): δ 10.03 (s, 1H, 3JₚH = 39 Hz; C₆-H), 8.57 (d, 3JₚH = 8.3 Hz, 1H; C₁₀-H), 8.10 (d, 3JₚH = 1.2 Hz, 1H; C₁-H), 8.07 (d, 3JₚH = 7.9 Hz, 1H; C₇-H), 7.99 (ddd, 3JₚH = 7.5 H, 9 Hz, 1H; C₅-H), 7.78 (d, 3JₚH = 1.3 Hz, 1H; C₃-H), 7.74 (ddd, 3JₚH = 7.5 Hz, 8.5 Hz, 1H; C₉-H), 5.32 (s, 1H; C₁₄-H), 2.46 (s, 3H; C₁₂-H), 2.07 (s, 3H; C₁₆-H), 1.50 ppm (s, 9H; C₁₇-H). ¹³C{¹H} NMR (CDCl₃, 125 MHz, 22 °C): 179.80 (C₁₅), 157.85 (C₁₃), 155.01 (C₆), 151.96 (C₂), 148.78 (C₄), 139.22 (C₄₆), 133.54 (C₅), 132.36 (C₆₆), 130.18 (C₇), 128.63 (C₉), 126.38 (C₆₉), 125.19 (C₆₇), 122.48 (C₁₀), 119.57 (C₃), 114.02 (C₁), 106.66 (C₁₄), 35.72 (C₁₁), 31.61 (C₁₇), 25.98 (C₁₆), 25.66 ppm (C₁₂). HRMS (APCI-TOF) m/z: [M + H]⁺ Caled for C₂₂H₂₃ClN₂OPt 561.1198; Found 561.1085.

Synthesis of [Pt(CF₃-phenanthridinenacac)Cl] (2): An identical procedure to the synthesis of 1 was followed using L₂ (0.501 g, 1.46 mmol) in THF (25 mL) and a suspension of PtCl₂ (0.398 g, 1.50 mmol), Ag₂O (0.174 g, 0.752 mmol) and 4 Å molecular sieves in THF (25 mL). Dark red crystals were obtained by diffusion of diethylether vapours into a CHCl₃ solution of 1. Yield = 0.722 g (86%). ¹H NMR (CDCl₃, 500 MHz,
**X-Ray Crystallography Experimental**

X-ray crystal structure data was collected from multi-faceted crystals of suitable size and quality selected from a representative sample of crystals of the same habit using an optical microscope. Crystals were mounted on MiTiGen loops with data collection carried out in a cold stream of nitrogen (150 K; Bruker D8 QUEST ECO). Diffractometer manipulations were carried out using Bruker APEX3 software. Structure solution and refinement was carried out using XS, XT and XL software, embedded within the Bruker SHELXTL suite. For each structure, the absence of additional symmetry was confirmed using ADDSYM incorporated in the PLATON program. CCDC Nos. 1959703 (L1) and 1959704 (1) contain the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.
Crystal structure data for L1 (CCDC Deposit #1959703): X-ray quality crystals were grown following diffusion of diethylether vapor into CHCl₃ at room temperature. Crystal structure parameters: C₂₂H₂₄N₂O₁ 332.43 g/mol, triclinic, space group P2₁/c; a = 13.0840(8) Å, b = 15.8238(9) Å, c = 8.6839(5) Å, α = γ = 90°, β = 98.385(3)°, V = 1778.68(18) Å³; Z = 4, ρcalcd = 1.241 g cm⁻³; crystal dimensions 0.300 x 0.100 x 0.090 mm; diffractometer Bruker D8 QUEST ECO CMOS; Mo Kα radiation, 150(2) K, 2θmax = 2.698 to 27.568°; 23438 reflections, 4082 independent (Rint = 0.0479), direct methods; absorption coeff (μ = 0.076 mm⁻¹), absorption correction semi-empirical from equivalents (SADABS); refinement (against F₀²) with SHELXTL V6.1, 231 parameters, 0 restraints, R₁ = 0.0594 (I > 2σ) and wR₂ = 0.1499 (all data), Goof = 1.101, residual electron density 0.266/−0.245 e Å⁻³.

Crystal structure data for 1 (CCDC Deposit #1959704): X-ray quality crystals were grown following diffusion of diethylether vapor into CHCl₃ at room temperature. Crystal structure parameters: C₂₃H₂₄Cl₄N₂O₁Pt₁ 681.33 g/mol, monoclinic, space group P2₁/n; a = 6.9467(3) Å, b = 18.7502(6) Å, c = 18.4435(6) Å, α = γ = 90°, β = 100.0770(10)°, V = 2365.24(15) Å³; Z = 4, ρcalcd = 1.913 g cm⁻³; crystal dimensions 0.150 x 0.059 x 0.047 mm; diffractometer Bruker D8 QUEST ECO CMOS; Mo Kα radiation, 150(2) K, 2θmax = 2.243 to 27.535°; 44379 reflections, 5419 independent (Rint = 0.0646), direct methods; absorption coeff (μ = 6.403 mm⁻¹), absorption correction semi-empirical from equivalents (SADABS); refinement (against F₀²) with SHELXTL V6.1, 285 parameters, 0 restraints, R₁ = 0.0326 (I > 2σ) and wR₂ = 0.0677 (all data), Goof = 1.075, residual electron density 1.442/−1.154 e Å⁻³.
**Theoretical Calculations**

DFT calculations were performed using ORCA version 4.1.2. Tight convergence criteria (TightSCF and TightOpt) were used and the SCF and energy integration grids were set to 4 and 5, respectively (Grid4 Finalgrid5). The ligands were optimized in the gas phase using the PBE0 functional and the def2-TZVP basis set on all atoms using the crystal structure coordinates of L1 as starting input. The resolution of identity (split-RI-J) with chain-of-spheres approximation (RIJCOSX) was employed to speed up the calculation (Intaccx:4.01,4.01,4.34; Gridx: 1,1,2). Frequency analyses were subsequently performed to confirm all structures were at a minimum on the ground state potential energy surface, and to interpret experimental IR spectra.

**Cell Cultures**

A2780 and A2780cis cell lines were cultured in RPMI 1640 with L-glutamine supplemented with 10% FBS and 1% PS. SKOV-3, MDA-MB-231, A549, and HEK293 cell line were cultured in DMEM 1g/L glucose, with L-glutamine & sodium pyruvate supplemented with 10% FBS and 1% PS. MET5A cell line was cultured in M199 supplemented with 10% FBS, 1% PS, insulin, 0.1% Trace Elements B, 4 mM Hydrocortisone, and 4 ng/mL EGF. All cell lines were incubated at 37°C under an atmosphere containing 5% CO₂. Cells were passaged by trypsinization and split in a 1:5 ratio.

**Cytotoxicity Assays**

MTT assays were completed to determine the cytotoxicity of compounds. 100 µL of cell solution containing 2 x 10⁴ cells/mL was seeded in a 96-well plate. The plate was incubated for at 37 °C under an atmosphere containing 5% CO₂ for 24 hr to allow for adherence of cells. 50 µL of cell
medium with varying concentrations of compounds was added, and the cells were incubated for 
72 hr. After 72 hr, 30 µL of 5.0 mg/mL MTT in PBS was added to the cell medium. After 2-4 h, 
the medium was aspirated, and 200 µL of DMSO was added to each well. DMSO content in 
media was kept below 0.5 % at all times. The plate was shaken for 10 minutes at room 
temperature. The absorbance of purple formazan was recorded at 562 nm with a BioTek ELx800 
plate reader. IC_{50} values were determined using Origin software. All experiments were 
performed in triplicate.

**Uptake**

SKOV-3 cells were plated in a 6-well and at 37 °C under an atmosphere containing 5% CO₂ for 
24 hr. Cells were then treated with 2 µM Pt compound for 24 hr. After incubation with Pt, cells 
were collected by trypsinization and washed with 1 mL PBS. Cells were counted, recollected, 
and resuspended in 200 µL 65% nitric acid. Samples were stored shaking at room temperature 
overnight. After the cell pellet was dissolved, the solutions were diluted with water and analyzed 
by GFAAS. All experiments were performed in triplicate.

**Apoptosis**

SKOV-3 cells were seeded in a 6-well plate at a concentration of 4 x 10⁵ cells/well. Cells were 
then incubated at 37°C under an atmosphere containing 5% CO₂ for 24 hr. Cells were treated 
with compound for 72 hr (15 µM cisplatin, 1 µM phenanthriplatin, 1 µM 2). Cells were 
harvested, the supernatant was then discarded, and cells were resuspended in 1 mL PBS. Cells 
were resuspended in 1X binding buffer using the FITC Annexin V Apoptosis Detection Kit 1 
(BD Biosciences, Franklin Lakes, NJ, USA) reaching a concentration of 10⁶ cells/ mL. 100 µL of
this cell solution was transferred to a 5 mL culture tube. Then, 5 µL of Annexin V-FITC and 10 µL propidium iodide (PI) solutions were added to cells. Cells were incubated for 15 min at RT in the dark and then brought to a final volume of 400 µL with 1X binding buffer. Cells were then analyzed with FITC and PerCP-Cy5-5 channels on FACSARia™II (BD Biosciences, Franklin Lakes, NJ, USA) and data was processed using FlowJo software.
FIGURES

Figure S1. $^1$H NMR (300 MHz, 22°C) spectrum of L1 in CDCl₃.

Figure S2. $^{13}$C{$^1$H} NMR (75 MHz, 22°C) spectrum of L1 in CDCl₃.
Figure S3. $^1$H-$_1^1$H COSY spectrum of L1 in CDCl$_3$.

Figure S4. HSQC spectrum L1 in CDCl$_3$. 
Figure S5. HMBC spectrum of L1 in CDCl₃.

Figure S6. ATR-IR spectrum of L1.

$V_{\text{N-H, bend}}$
1570.5 cm⁻¹ (exp)
1642.4 cm⁻¹ (Calc. gas phase)

$V_{\text{C=O, stretch}}$
1615.4 cm⁻¹ (exp)
1709.2 cm⁻¹ (Calc. gas phase)
**Figure S7.** $^1$H NMR (300 MHz, 22°C) spectrum of L2 in CDCl$_3$.

**Figure S8.** $^{13}$C$\{^1$H$\}$ NMR (75 MHz, 22°C) spectrum of L2 in CDCl$_3$. 
Figure S9. $^1$H-$^1$H COSY spectrum of L2 in CDCl3.

Figure S10. HSQC spectrum of L2 in CDCl3.
**Figure S11.** HMBC spectrum of L2 in CDCl₃.

**Figure S12.** ATR-IR spectrum of L2.

- $v_{C=O, \text{stretch}}$: 1615.4 cm⁻¹ (exp) 1709.2 cm⁻¹ (Calc. gas phase)
- $v_{N-H, \text{bend}}$: 1576.6 cm⁻¹, 1586.8 cm⁻¹ (exp) 1646.0 cm⁻¹, 1648.4 cm⁻¹ (Calc. gas phase)
Figure S13. $^1$H NMR (500 MHz, 22°C) spectrum of 1 in CDCl$_3$.

Figure S14. $^{13}$C{$^1$H} NMR (125 MHz, 22°C) spectrum of 1 in CDCl$_3$. 
Figure S15. $^1$H-$^1$H COSY spectrum of 1 in CDCl$_3$.

Figure S16. HSQC spectrum of 1 in CDCl$_3$. 
Figure S17. HMBC spectrum of 1 in CDCl₃.

Figure S18. HRMS spectrum of 1.
Figure S19. $^1$H NMR (500 MHz, 22°C) spectrum of 2 in CDCl$_3$.

Figure S20. $^{13}$C\{$^1$H\} NMR (125 MHz, 22°C) spectrum of 2 in CDCl$_3$. 
Figure S21. $^1$H–$^1$H COSY spectrum of 2 in CDCl$_3$.

Figure S22. HSQC spectrum of 2 in CDCl$_3$. 
**Figure S23.** HMBC spectrum of 2 in CDCl$_3$.

**Figure S24.** 1D selective gradient TOCSY (120 ms, freq: 2.07 ppm, 128 transients, 4 Hz line broadening) spectrum of 1 in CDCl$_3$. 
**Figure S25.** 1D selective gradient TOCSY (120 ms, freq: 2.46 ppm, 128 transients, 4 Hz line broadening) spectrum of 2 in CDCl₃.

**Figure S26.** HRMS spectrum of 2.
Table S1. MTT cytotoxicity assay data A2780 and A2780cis cell lines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>A2780 IC$_{50}$ (µM)</th>
<th>A2780cis IC$_{50}$ (µM)</th>
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<tbody>
<tr>
<td>cisplatin</td>
<td>1.41 ± 0.54</td>
<td>32.94 ± 8.82</td>
</tr>
<tr>
<td>1</td>
<td>2.81 ± 0.168</td>
<td>4.57 ± 1.41</td>
</tr>
<tr>
<td>2</td>
<td>0.48 ± 0.17</td>
<td>0.55 ± 0.22</td>
</tr>
<tr>
<td>L2</td>
<td>7.22 ± 2.07</td>
<td>20.1 ± 7.17</td>
</tr>
<tr>
<td>4-amino-(2-tert-butyl)phenanthridine</td>
<td>8.38 ± 2.83</td>
<td>20.5 ± 6.07</td>
</tr>
<tr>
<td>4-amino-(2-trifluoromethyl)phenanthridine</td>
<td>19.3 ± 12.1</td>
<td>&gt;100 µM</td>
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Table S2. Cellular uptake of cisplatin, phenthriplatin, and 2 in SKOV3 ovarian cancer cells ([Pt] = 2 µM, 24 h at 37 °C, 5% CO$_2$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>log([Pt]) Conc (pmol/million cells)</th>
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<tbody>
<tr>
<td>cisplatin</td>
<td>2.12 ± 0.129</td>
</tr>
<tr>
<td>phenanthriplatin</td>
<td>2.88 ± 0.023</td>
</tr>
<tr>
<td>2</td>
<td>4.09 ± 0.138</td>
</tr>
</tbody>
</table>

References