Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

The effect of geometric isomerism on the anticancer activity of the monofunctional platinum complex $[Pt(NH_3)_2(phenanthridine)Cl]NO_3$

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Contents

General considerations

All reagents were purchased from Strem, Aldrich, or Alfa and used without further purification. All reactions were carried out under normal atmospheric conditions. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 300/500 NMR spectrometer or a Bruker AVANCE-400 NMR spectrometer with a Spectro Spin superconducting magnet in the Massachusetts Institute of Technology Department of Chemistry Instrumentation Facility (MIT DCIF). Chemical shifts in ^{1}H and ^{13}C NMR spectra were internally referenced to solvent signals. Electrospray ionization mass spectrometry (ESI-MS) was performed on an Agilent Technologies 1100 series liquid chromatography/MS instrument. Fluorescence spectra were obtained on a Quanta Master 4 L-format scanning spectrofluorimeter (Photon Technology International) at room temperature. Graphite furnace atomic absorption spectroscopic (GFAAS) measurements were taken on a Perkin Elmer AAnalyst 600 spectrometer. Fluorescence images of A2780 cells were acquired using a Zeiss Axiovert 200M inverted epifluorescence microscope equipped with an EM-CCD digitalcamera (Hamamatsu) and a MS200 XY Piezo Z stage (Applied Scientific Instruments). The light source was an X-Cite 120 metal-halide lamp (EXFO). The microscope was operated with Volocity software (PerkinElmer). Images were processed and intensities were quantified with ImageJ software (NIH). Distilled water was purified by passage through a Millipore Milli-Q Biocel water purification system (18.2 MV) equipped with a 0.22 μm filter. Elemental microanalyses were performed by Intertek Pharmaceutical Services, Whitehouse, NJ.

Materials and Methods

Cisplatin was purchased from Strem Chemicals and oxaliplatin was from Council of Europe, EDQM. Phenanthriplatin was prepared following a published procedure.[1]

Synthesis of trans-DDP[2]

To a 250-mL round bottom flask, $K_2[PtCl_4]$ (1.2 g, 2.9 mmol) was added. A 20 mL portion of H_2O was added to dissolve the Pt(II) precursor. The solution was heated to 95 ^oC slowly. At 95 ^oC, 7 mL of $NH_3{\cdot}H_2O$ solution (25~30 wt%) was added. The reaction was stirred at 95 ^oC for 1.5 h and turned clear. The reaction was cooled to room temperature and the solvent was removed under vacuum. To this reaction, 50 mL of HCl (6 M in H_2O) was added. The reaction was stirred at 95 ^oC for 24 h. During this process, the *trans*-DDP precipitated as a yellow solid. The product was isolated by centrifuge. The yellow solid was washed with 10 mL H_2O, 10 mL EtOH twice, and 10 mL Et_2O twice. The yellow solid was dried under vacuum. (Yield: 800 mg, 92%)

Synthesis of trans-$[Pt(NH_3)_2(Phenanthridine)Cl]NO_3$

To a solution of trans-DDP (175 mg, 0.58 mmol) in 10 mL of DMF was added $AgNO_3$ (99 mg, 0.58 mmol) and the reaction was stirred in the dark at 55 °C. After 16 h, the AgCl precipitate was filtered. To the supernatant, phenanthriplatin (94 mg, 0.9 equiv) was added, and the reaction was stirred for 16 h at 55 °C. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in 30 mL of MeOH. Insoluble material was removed by filtration. The filtrate was stirred vigorously, and diethyl ether (100 ml) was then added to precipitate the desired compound as a solid. The compound was filtered and washed twice with 50 mL of diethyl ether. The compound was purified by redissolving in methanol and precipitating by adding it dropwise to vigorously stirred diethyl ether. The final compound was isolated by vacuum filtration and dried in vacuo. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.02 (s, 1H, Ar*H*), 9.72 (d, 1H, Ar*H*), 8.96 (dd, 2H, Ar*H*), 8.42 (d, 1H, Ar*H*), 8.16 (t, 1H, Ar*H*), 8.04 (t, 1H, Ar*H*), 7.95 (m, 2H, Ar*H*), 4.19 (t, 6H, NH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ 161.1, 142.8, 134.1, 132.0, 130.1, 129.7, 129.1, 128.9, 128.8, 126.6, 125.6, 123.3, 122.5. ESI-MS m/z calculated ($[M]^+$): 443.06, found: 443.2. Elemental Analysis calculated for $C_{13}H_{15}ClN_4O_3Pt$: C, 30.87; H, 2.99; N, 11.08; found: C, 30.77; H, 2.66; N, 10.81.

Cell line and cell line culture

Human ovarian carcinoma A2780, the cisplatin resistant A2780/CP70, and human breast carcinoma MCF-7 cells were incubated at 37 °C in 5% CO_2 and grown in RPMI medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Human lung carcinoma cell line A549 and human colorectal carcinoma HCT116 were incubated at 37 °C in 5% CO_2 and grown in DMEM

medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were passaged every 3 to 4 days and restarted from the frozen stock upon reaching passage number 25.

MTT assays

Cytotoxicity profiles of platinum compounds against human cancer cell lines were evaluated using MTT assays. Solutions of the cytotoxic compounds were freshly prepared in PBS then diluted using culture media to the desired concentrations. Cells were seeded on a 96-well culture plate (2000 cells per well) in 200 μL RPMI or DMEM media, and incubated for 24 h. The cells were treated with the platinum compounds, separately at varying concentrations, and incubated for 72 h at 37 °C in 5% CO_2. Then, the medium was removed and replaced by 200 μL fresh medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/mL) and incubated for 3 h. The medium was removed, 200 μL of DMSO was added to the wells, and the absorbance of the purple formazan was recorded at 570 nm using a BioTek Synergy HT multi-detection microplate plate reader. Each compound was tested in triplicate for each cell line.

Whole cell uptake studies

Cells (half a million) were seeded on 6-well culture plates and incubated for 24 h at 37 ^{0}C. The medium was then removed and 2.0 mL of fresh uptake medium containing the test compound (20 μM) were added. Cells were subsequently incubated for 5 h at 37 °C. The medium was then removed and cells were harvested and washed with PBS (2 mL X 3). Cells were then digested using 70% HNO_3 for 1 h at room temperature and the platinum content was analyzed by graphite furnace atomic absorption spectroscopy (GFAAS) to obtain the whole cell uptake. A control well was cultured for cell counting.

Cellular distribution study

Cells (2 million) were seeded on 60 mm x 10 mm petri dishes and incubated for 24 h at 37 ^{0}C. The medium was then removed and 3.0 mL of fresh uptake medium containing the tested compound (20 μM) were added. Cells were subsequently incubated for 5 h at 37 °C. The medium was then removed and cells were harvested and washed with PBS (3 mL X 3). Cells were then fractionated to isolate the membrane, cytoplasm and nuclei separately. Each portion was then digested using 70% HNO_3 for 1 h at room temperature and the platinum content was analyzed by GFAAS. A control well was cultured for cell counting.

LIVE/DEAD cell viability assay

A2780 cells were cultured on 35 mm sterile glass bottom culture dishes (MATTEK

Corporation) for 24 h at 37 °C and grown in RPMI supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were then treated with the tested compound (10μM) for 48 h at 37 °C. Before the assay, cells were carefully washed with 1 mL PBS and 1 mL dye-free RPMI to remove serum esterase activity generally present in serum-supplemented growth media. A 5 μL aliquot of calcein AM (4 mM in anhydrous DMSO) and 10 μL ethidium homodimer-1 (2 mM in DMSO/water, 1:4 vol/vol) were added to 10 mL of dye-free RPMI to produce a LIVE/DEAD working solution. A 2-mL aliquot of LIVE/DEAD working solution was carefully added to the petri dishes, which were then incubated at room temperature for 30 min. Subsequently, the medium of the samples was replaced with 1 mL dye-free RPMI before examination by fluorescence microscopy.

DNA unwinding

Unwinding of plasmid DNA (pUC19) by the platinum compounds was examined through agarose gel electrophoresis. The supercoiled pUC 19 DNA (15 μg/ml, 10 μl) in Tris-HCl (100 mM, pH 7.4) was incubated with platinum compounds at 30 μg/ml at 37 ℃ for 6 h. The DNA after incubation was loaded on a 0.7% agarose gel. Electrophoresis was carried out at 110 V for 80 min in TAE buffer and run in duplicate. The agarose gel was incubated in 1.0 μg/ml ethidium bromide for 30 min. DNA bands were visualized in UV light and then photographed.

DNA Platination Experiments

Two-million cells were seeded on 100 mm × 20 mm petri dishes and incubated for 24 h at 37 °C. These cells were then treated with the platinum compounds (10 μM) for 5 h at 37 °C. Afterward, fresh medium was added, followed by an additional 16 h of incubation at 37 °C. The medium was then removed and the cells were washed with PBS (2 mL x 2 mL), harvested by trypsinization (1 mL), and washed twice with 2 mL PBS. Solutions containing cells were centrifuged at 1500 rpm for 5 min at 4 °C. The cell pellet was suspended in DNAzol (1 mL, genomic DNA isolation reagent, MRC). The DNA was precipitated with pure ethanol (0.5 mL), washed with 75% ethanol (0.75 mL × 3), and re-dissolved in 0.4 mL of 8 mM NaOH. The DNA concentration was determined by UV−Vis spectroscopy and the platinum content was quantified by GFAAS.

Immunostaining of γH2AX

HCT116 cells were cultured on 35 mm sterile glass bottom culture dishes (MATTEK Corporation) for 24 h at 37 °C and grown in Medium supplemented with 10% FBS and 1% penicillin/streptomycin. The cells were then treated with 20 μM complex **1** for 24 h at 37 °C. Afterward, the cells were washed with PBS (2 mL) twice. A 1 mL 4% formaldehyde PBS solution was added to fix the cells upon 10 min incubation at R.T.

The fixed cells were washed with PBS (2 mL) twice and then incubated with blocking buffer (5% goat serum and 0.3% Triton X-100 in PBS) for 30 min for blocking. The cells were then incubated with 1 mL γH2AX primary antibody (1:1000) in the blocking buffer. Then the cells were washed with PBS (2mL) twice. A 1-mL portion of a secondary antibody (1:1000) in the blocking buffer was added and the cells were incubated at R.T. for 1h under dark. After washing with PBS (2 mL) to remove nonspecific binding, the cells were further incubated with Hoechst 33342 (5 μM in 1 mL PBS) for 10 min at R.T. Wash the cells with PBS (2 mL) twice. Afterward, the cells were replaced with 1 mL fresh PBS for examination by fluorescence microscopy.

Organoid selection and testing

Apc$^{\Delta/\Delta}$*;Kras*$^{G12D/+}$*;Trp53*$^{\Delta/\Delta}$ (AKP) small intestinal organoids were generated and selected for as described previously,[3] briefly, by culturing intestinal organoids from *Apc*$^{fl/fl}$*;Kras*$^{LSL\text{-}G12D/+}$*;Trp53*$^{fl/fl}$ mice and infecting in-vitro with Ad5CMV:Cre and selecting for with the aid of Wnt-deficient media and nutlin-3. The organoids were passaged by scratching the Matrigel from the well's surface and transferring into a 1.5 mL-eppendorf tube with 700 μL of phosphate-buffered saline (PBS) and centrifuging at 300 *g* for 5 min then removing the supernatant. 300 μL of TrypLE Express was mixed with the pellet and incubated for 1 minute at 37˚C. 700 uL of cold ADMEM was then added and the mixture was centrifuged at 300*g* for 5 min and the supernatant was removed. Matrigel was added to the pellet to allow for a density of 1,000-2,000 cells/μL and 10 μL of the mixture were plated at the center of wells in a 96-well clear-bottom plate. 200 uL of ADMEM and 1× B-27 supplement were added to each well mixed with varying concentrations of oxaliplatin, cisplatin, phenanthriplatin, or complex **1**. The organoids were incubated for 72 h at 37˚C and 5% CO_2, after which time pictures were taken. Cell-viability was assessed using CellTiterGlo (CTG) by adding 20 μL of CTG solution to each well and incubating for 25 min in the dark and measuring luminescence in each well using a plate reader.

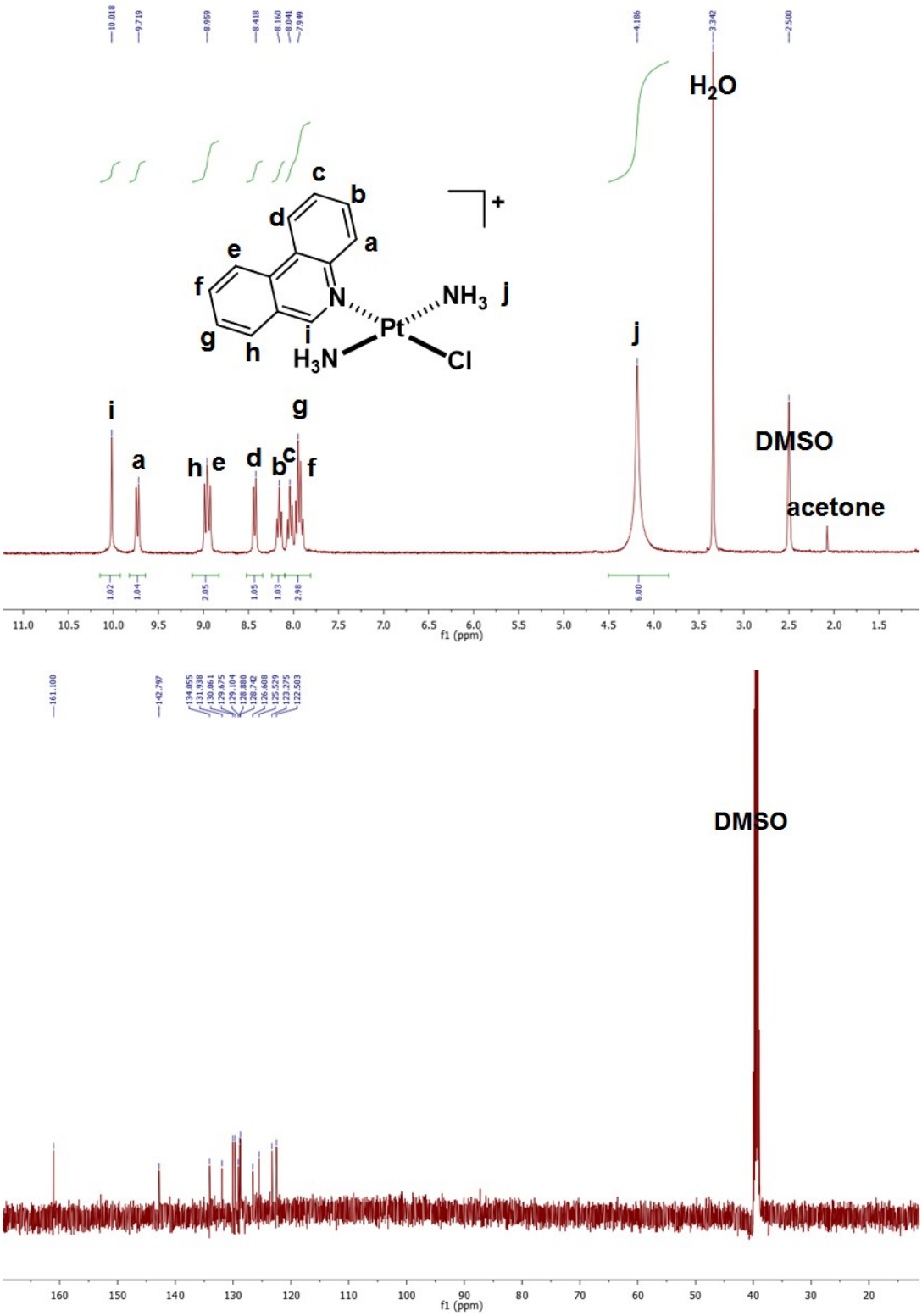


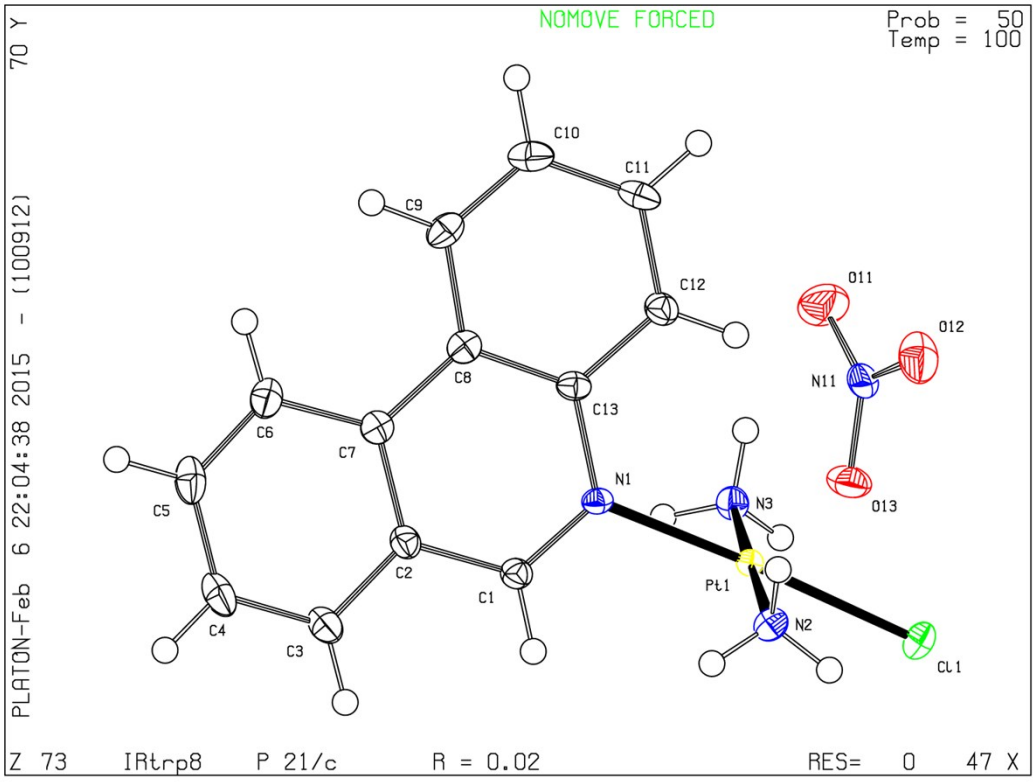
Figure S1. ^{1}H and ^{13}C NMR spectra of *trans*-[$Pt(NH_3)_2Cl$(phenanthridine)]NO_3

X-ray structure determination of [Pt(phen)(NH_3)$_2$Cl](NO_3), 1.

Table S1. Crystal data and structure refinement for [Pt(phen)(NH_3)$_2$Cl](NO_3).

Empirical formula	C13 H15 Cl N3 Pt . N1 O3
Formula weight	505.83
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 2_1/c
Unit cell dimensions	a = 7.4875(11) Å
	b = 15.363(2) Å β= 93.153(2)°.
	c = 13.1787(19) Å
Volume	1513.7(4) Å3
Z	4
Density (calculated)	2.220 Mg/m^3
Absorption coefficient	9.464 mm^{-1}
F(000)	960
Crystal size	0.15 x 0.08 x 0.05 mm^3
Theta range for data collection	2.038 to 29.621°.
Index ranges	-10<=h<=10, -21<=k<=21, -18<=l<=18
Reflections collected	31931
Independent reflections	4263 [R(int) = 0.0398]
Completeness to theta = 25.242°	100.0%
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4263 / 6 / 223
Goodness-of-fit on F^2	1.046
Final R indices [I>2sigma(I)]	R1 = 0.0189, wR2 = 0.0398
R indices (all data)	R1 = 0.0235, wR2 = 0.0415
Largest diff. peak and hole	1.864 and -0.800 e.Å^{-3}

Projection view of **1** with 50% probability ellipsoids:



Selected bond distances (Å): Pt(1)-N(1), 2.027(2); Pt(1)-N(2), 2.042(2); Pt(1)-N(3), 2.046(2); Pt(1)-Cl(1), 2.2980(8).

References

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