

SUPPORTING INFORMATION

A platinum complex that binds non-covalently to DNA and induces cell death via a different mechanism than cisplatin

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S1. Spectroscopy characterisation

^1H and COSY NMR spectra were recorded on a BrukerAvance 400 MHz Ultrashield NMR spectrometer. The variable temperature ^1H NMR studies were conducted on a BrukerAvance 500 MHz Ultrashield NMR spectrometer. Electron spray ionisation mass spectra were recorded on a BrukerDaltonics Esquire 3000 spectrometer at Imperial College London. Elemental Analysis of the compounds prepared was performed by Mr. Alan Dickerson (University of Cambridge).

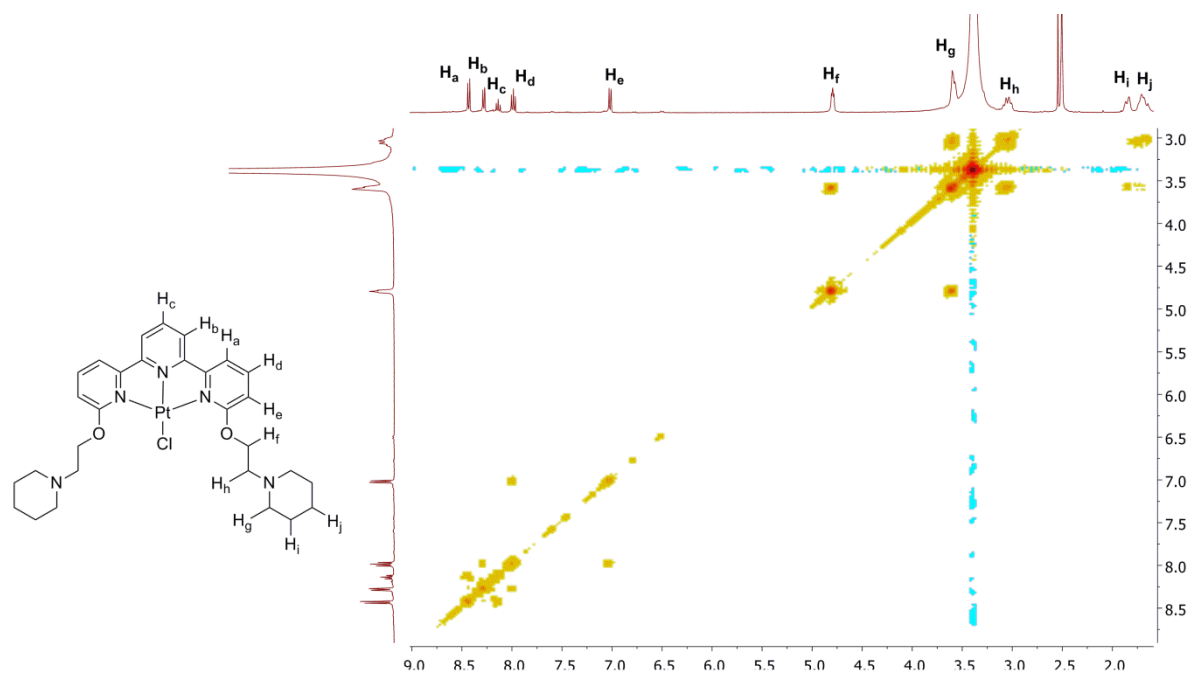


Figure S-1. ^1H COSY spectrum of **1** in DMSO-d_6 .

S2. Stability studies: VT ^1H NMR and UV-vis spectroscopy

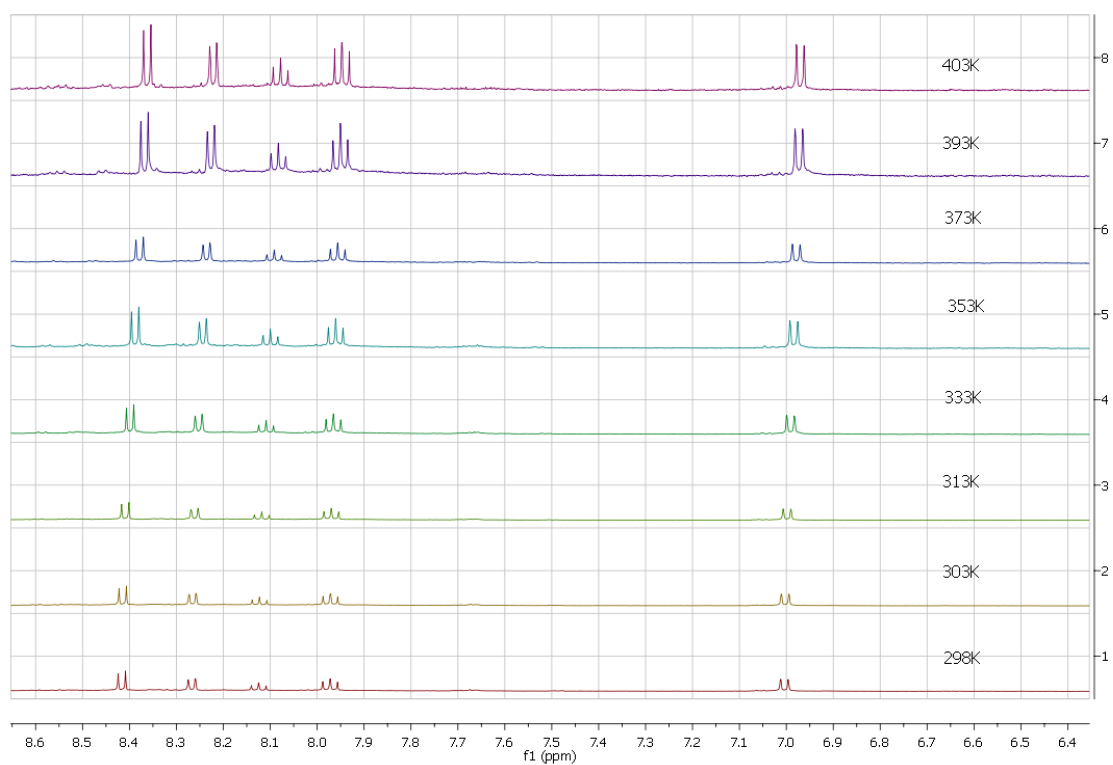


Figure S-2. VT ^1H NMR spectrum of **1** (between 298 and 403 K) in DMSO-d_6 .

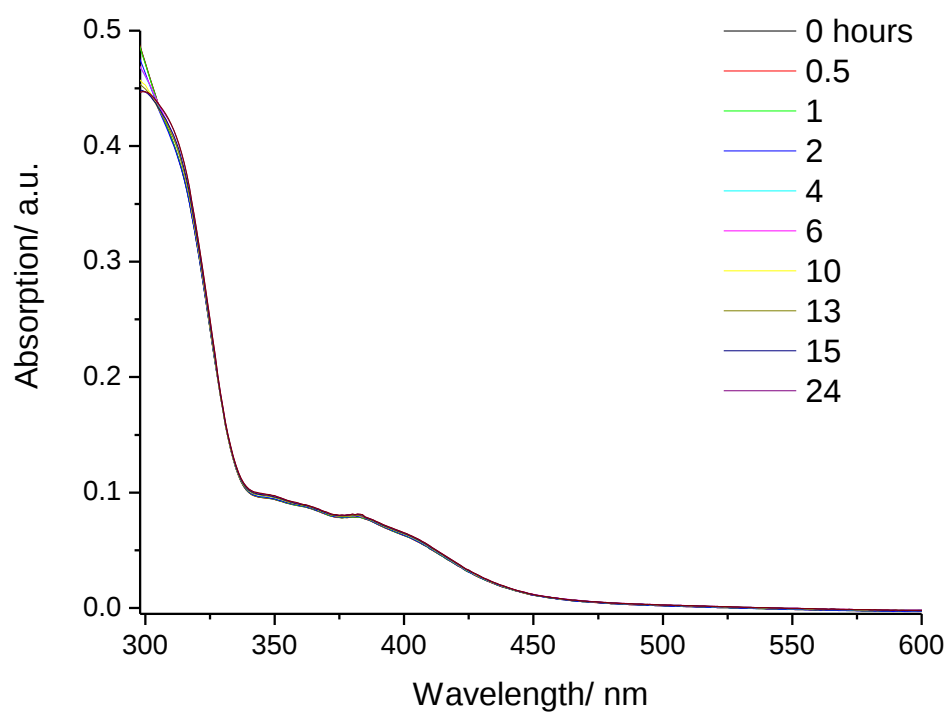


Figure S-3. UV-Vis spectrum of **1** in Tris-HCl 10 mM/ KCl 100 mM over a 24 hour period.

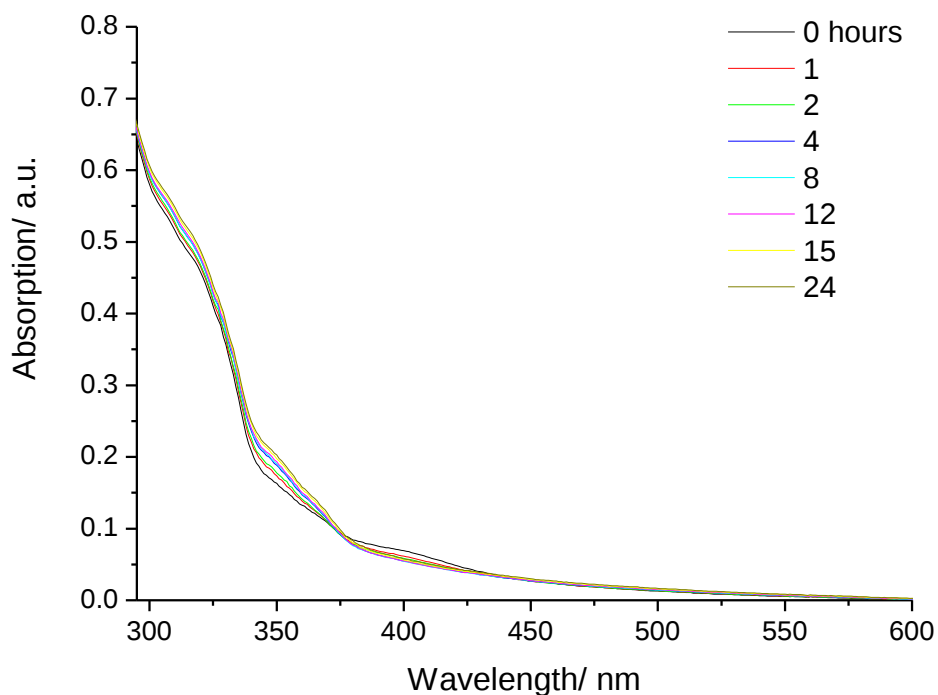


Figure S-4. UV-Vis spectrum of **1** in Tris-HCl 10 mM/ KCl 100 mM in the presence of U2OS cell lysate (3.4 mg of protein) over a 24 hour period.

S3. UV-VIS spectra & reciprocal plots of $D/\Delta\epsilon_{ap}$ versus D

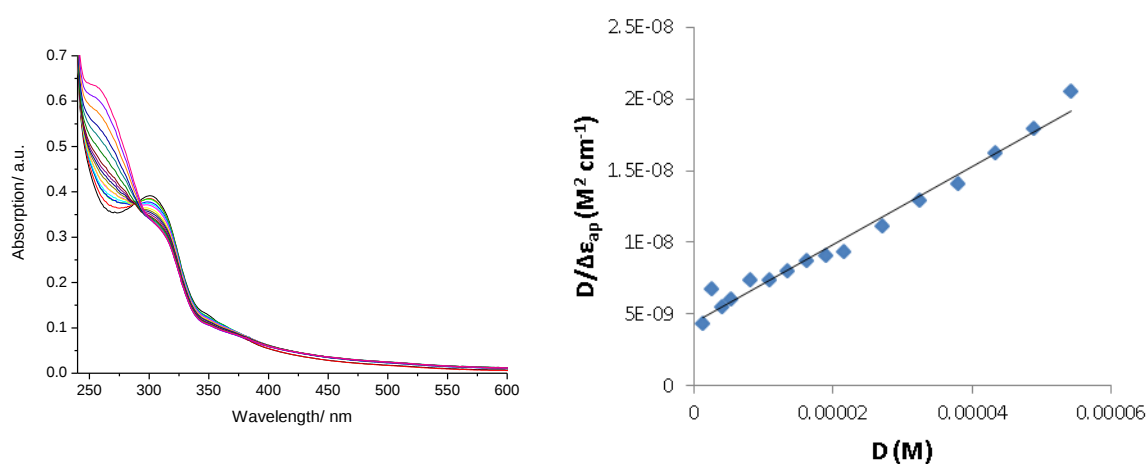


Figure S-5. UV-Vis titration and reciprocal plot of $D/\Delta\epsilon_{ap}$ versus D for **1** (20 μ M) upon addition of ct-DNA.

F

S4. Fluorescent intercalator displacement (FID) assay

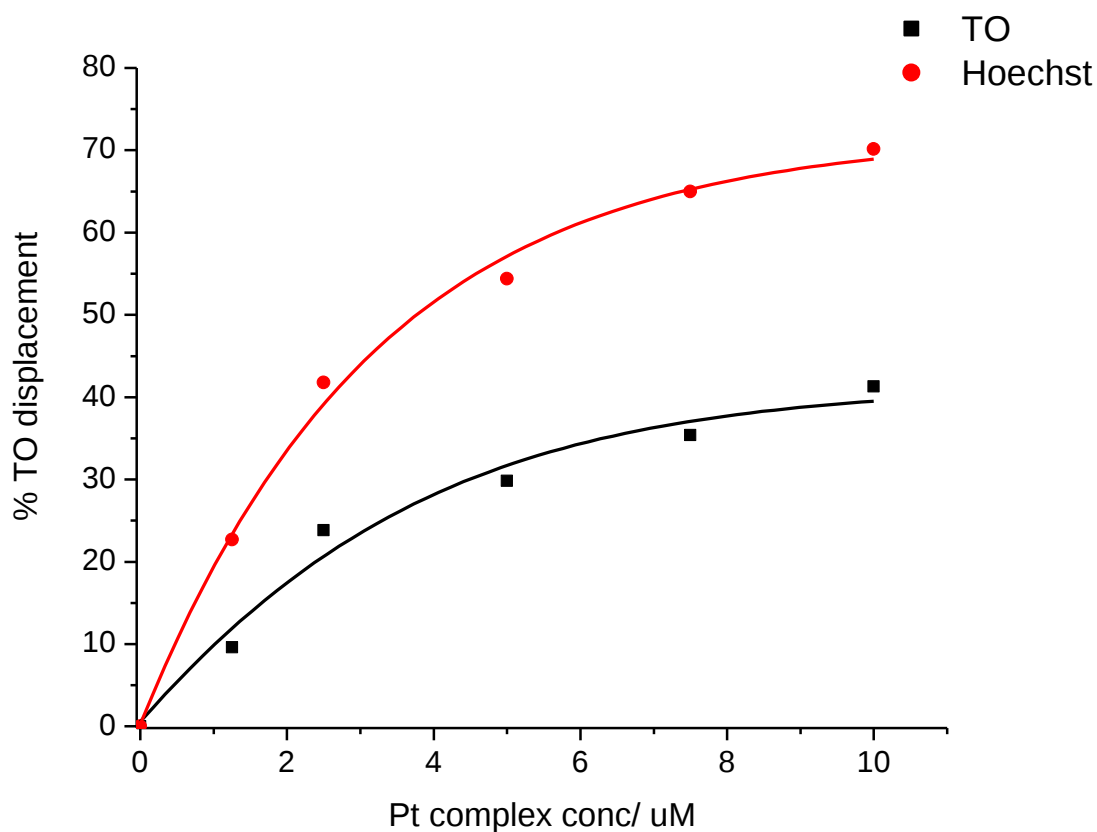


Figure S-6. Graphical representation of TO/ Hoechst displacement from duplex 26-mer DNA upon increasing concentration of **1** from 1.25 to 10 μM.

Table S-1. DC₅₀ values obtained for the platinum(II) complex, **1** against TO and Hoechst dyes given in μM. The values are an average of three independent measurements. All error values are within 5%.

Complex	TO ds ²⁶ DC ₅₀	Hoechst ds ²⁶ DC ₅₀
1	> 10	3.69

S5. Circular dichroism spectra

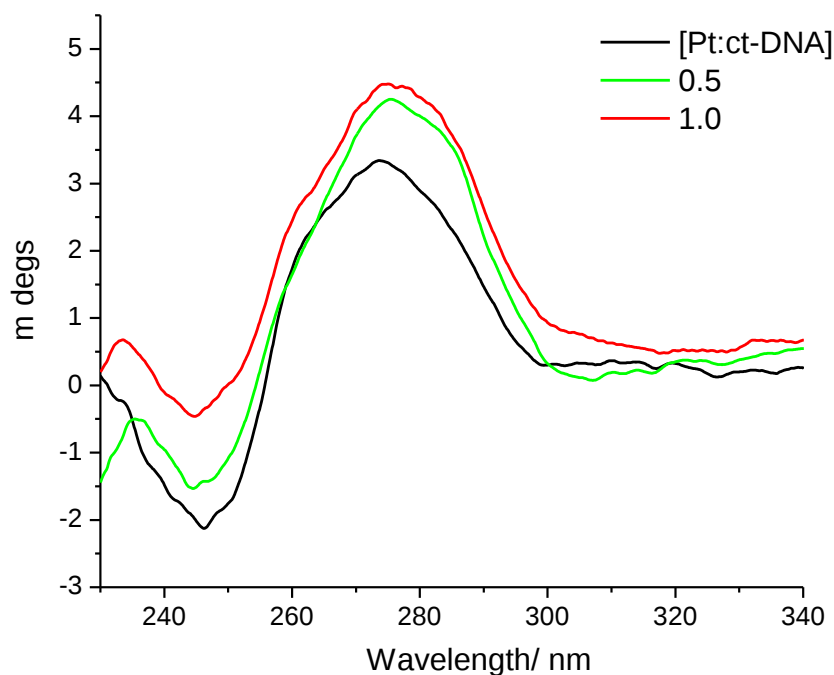


Figure S-7. CD spectrum of ct-DNA (200 μ M) treated with platinum complex (**1**: base pair = 0.5, 1)

S6. Selective ct-DNA precipitation

The amount of platinum(II) complexes, **1** and **2** covalently bound to ct-DNA was determined using a previously reported protocol [*Chem. Commun.*, **2012**, 48, 67, *Inorg. Chem.*, **2003**, 42, 4394 and *J. Am. Chem. Soc.* **1985**, 107, 708]. A solution of ct-DNA (0.5 mM) was incubated with **1** and **2** (at a bp:metal complex ratio of 5:1) at 37°C in 10 mM phosphate buffer (pH 7). At certain time points, a 250 μ L aliquot was removed and the DNA was precipitated by adding 10 μ L of NaCl (5 M) and 1.0 mL of EtOH (stored at 4 °C). The solution was centrifuged to remove the DNA, and the concentration of unbound metal complex was determined spectroscopically, using controls that did not contain ct-DNA.

The synthesis of **2** has previously been reported by us. [*Inorg Chem*, 48, **19**, 2009] The structure is provided below.

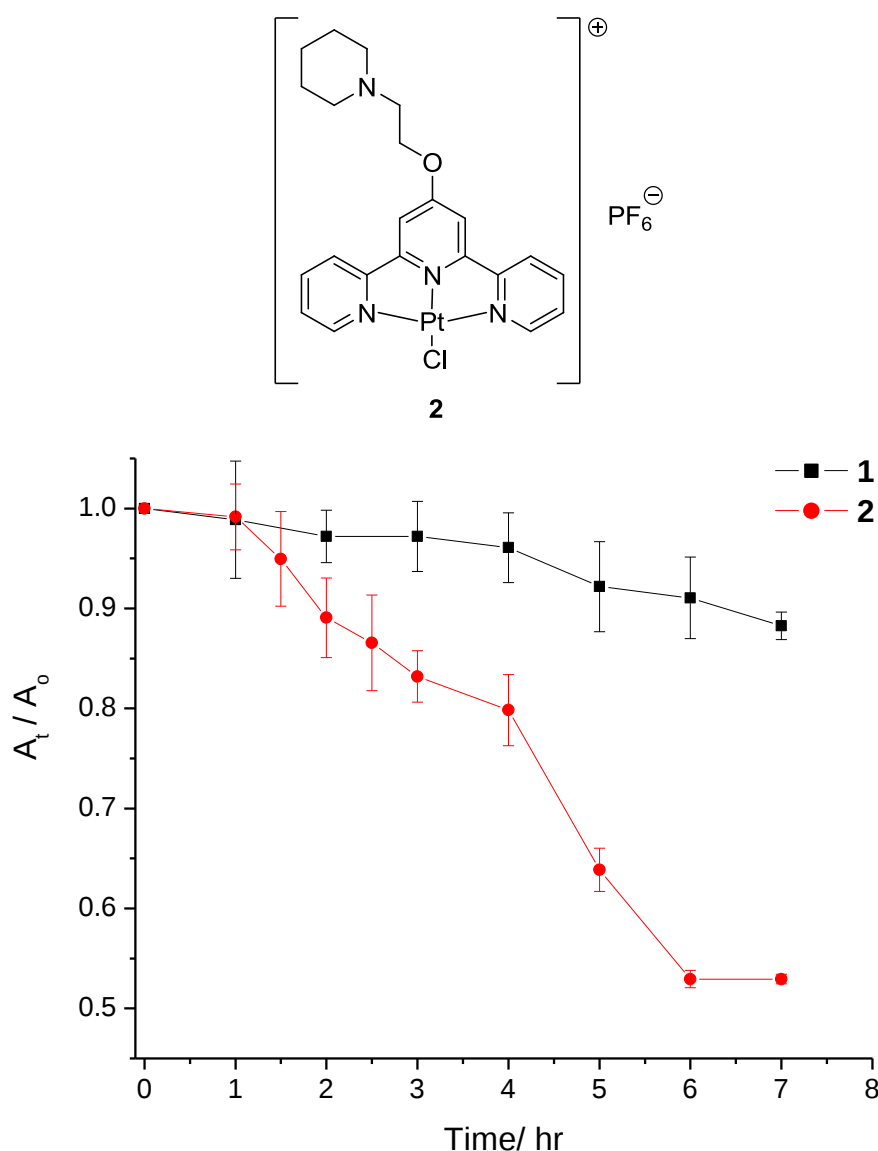


Figure S-8. Graphical representation of the change in platinum complex (**1** and **2**) present in the supernatant at various time points after expose to ct-DNA.

S7. Agarose gel electrophoresis

Plasmid DNA (pUC19) was purchased from Sigma Aldrich. A DNA stock solution of 419 µg/mL in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA storage buffer was prepared. The nicking ability of **1** was determined by monitoring the conversion of supercoiled plasmid DNA (form I) to nicked circular DNA (form II) using agarose gel electrophoresis. The migration pattern was monitored to give insight into covalent binding. To probe the effect of complex concentration on platination and nicking, solutions containing 256 ng (30 µM) of DNA and 0, 10, 20, 50, 100 and 200 µM of **1** with a total reaction volume of 15 µL were incubated at 37°C for 24 h. After this time period, loading buffer (5 µL, containing 0.25% bromophenol blue, 0.25% xylene cyanol and 60% glycerol) was added and reaction mixtures were immediately loaded onto 1% agarose gels containing 1.0 µg mL^{−1} of ethidium bromide. The DNA fragments were separated by applying 40 V for 1 h in Tris-acetate EDTA (TAE) buffer. The DNA bands were analysed under UV light using the Fujifilm Image Reader LAS-3000.

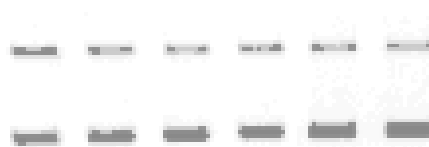


Figure S-9. Agarose gel electrophoresis of pUC19 DNA treated with **1** and after 24 h incubation at 37°C. (a) Lane 1: DNA only; lanes 2-6: DNA + 10, 20, 50, 100 and 200 μM of **1**.

S8. ¹H NMR studies with guanosine

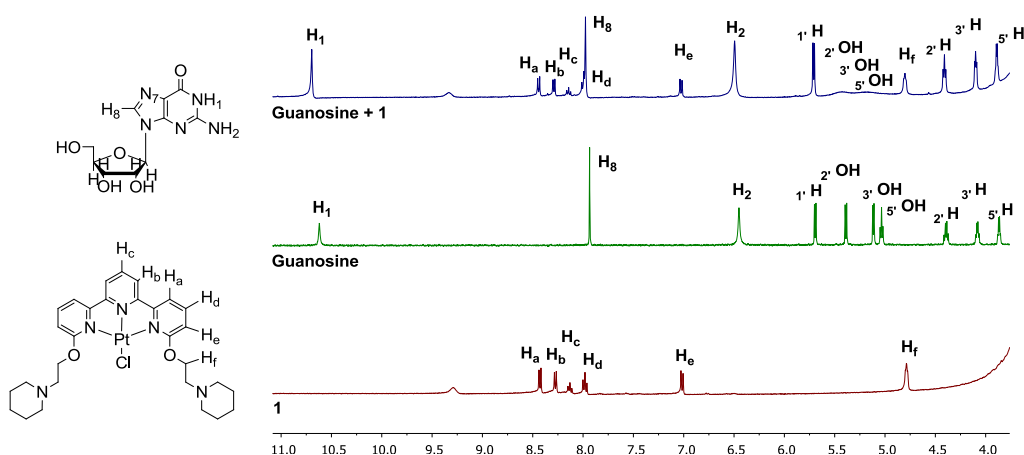


Figure S-10. ¹H NMR spectrum of (bottom to top): **1** alone, guanosine alone and **1** in the presence of guanosine (2 eq.). The broadening of the hydroxyl protons (2', 3', 5' OH) can be attributed to hydrogen bonding interactions with the piperidine side arms.

S9. Computational docking studies

DFT: The molecular structure of **1** was optimized by DFT calculations using the Gaussian 03 program at the BP86 level of theory. [*Dalton Trans.*, **2012**, 41, 10724] The LANL2DZ basis set was used for the platinum atom; other atoms were described by a 6-31G** basis set.

The optimized structure of **1** shows a Pt-Cl distance of 2.37 Å with the Pt metal perfectly placed in the centre of the ligand (Pt-N_{cis} distance 2.10 Å; Pt-N_{trans} 1.97 Å). These values are in good agreement with the values reported in the literature for terpy-Pt-Cl complexes. [Gaussian03, Gaussian, Inc., Pittsburgh PA, 2003] The pyridine rings are non-planar with respect to each other, which is probably due to the electrostatic repulsion of the negatively charged Cl atom with the oxygen atoms (Cl-O distance 2.95 Å).

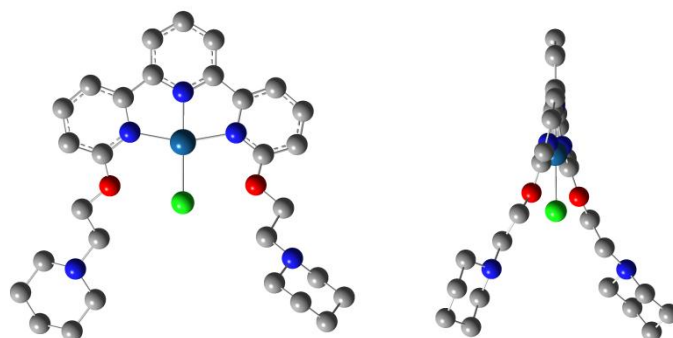


Figure S-11. DFT optimized structure of **1**.

Density Functional Theory: Density functional theory calculations were performed on the Gaussian 03 program. The geometry of **1** was optimized without geometry constraints, using the functional bp86. The basis set 6-31g(d,p) was used for C, H, O, N and Cl atoms and lanl2dz for Pt atom. Frequency calculations were run to confirm this structure to be energy minima.

Docking: Docking studies were carried out using Autodock (Version 4.2) docking software. The structure of B-DNA dodecamer (CGCGAATTCGCG)₂ (PDB:1BNA) was used as a model to study the interaction between the metal complex and DNA. The structure of the metal complex was obtained by DFT optimization. Autodock Tools was used to generate the pdbqt files required for both the DNA and the complex. All the polar hydrogens were added and non-polar hydrogens emerged in these files. The DNA structure was kept rigid during the docking while the metal complex was allowed to have rotatable bonds. DNA was enclosed in the grid box defined by Auto Grid (dimensions 40" × 40" × 60" Å) used for dispersion/repulsion, hydrogen bonding, electrostatics, and desolvation, respectively. Auto Grid performed a pre-calculated atomic affinity grid maps for each atom type in the ligand plus an electrostatics map and a separate desolvation map present in the substrate molecule. Then, during the AutoDock calculation, the energetics of a particular ligand configuration is evaluated using the values from the grids. Ligand docking was carried out with the AutoDock 4.2 Lamarckian Genetic Algorithm (LGA) with an initial population of 150 randomly placed individuals, a maximum number of 25×10^6 energy evaluations and a probability of performing local search on individual of 0.15. One hundred independent docking runs were performed and the results clustered with a RMSD of 2.0 Å. Other parameters were set as default. After clustering all the docking solutions, the most populated cluster was the most favourable by energy of binding.

S10. Cellular Studies: General

The U2OS human osteosarcoma, HEK 293T embryonic kidney, GM05757 normal human fibroblast, SH-SY5Y neuroblastoma, NIH 3T3 mouse embryo fibroblast cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM- low glucose) or RPMI-1640 which was supplemented with 10% fetal bovine serum or newborn calf serum and 1% penicillin/streptomycin. All cells were grown at 310 K in a humidified atmosphere containing 5% CO₂.

S11. Flow cytometry

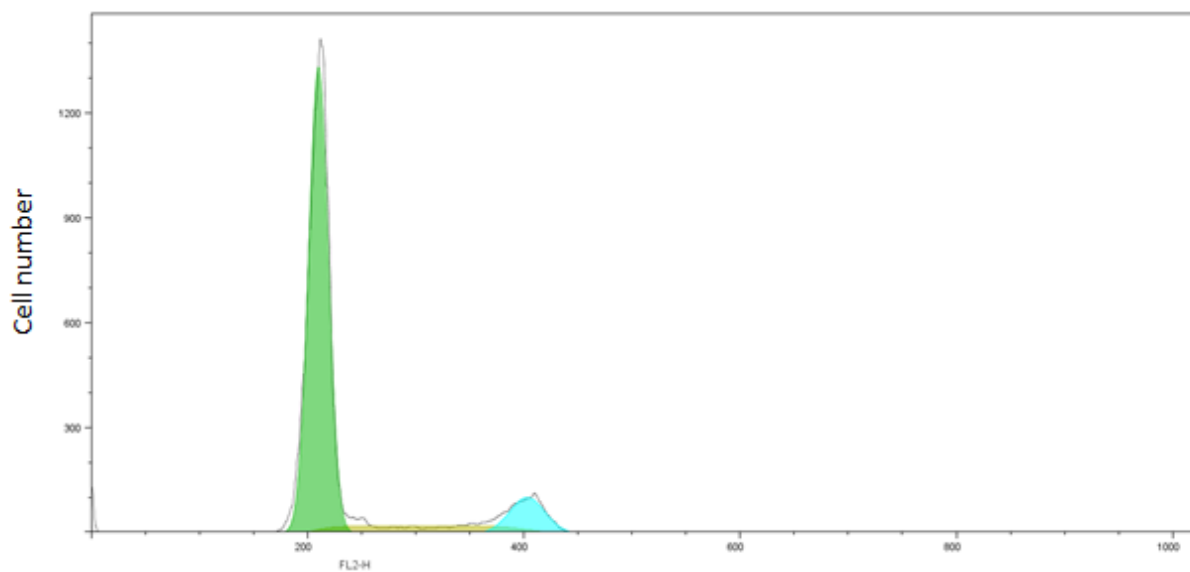


Figure S-12. Histogram representing the different phases of the cell cycle for NIH 3T3 cells under normal (proliferating) conditions. Sub G1: 2.47%, G1: 69.64%, S: 10.31% and G2/M: 12.45%.

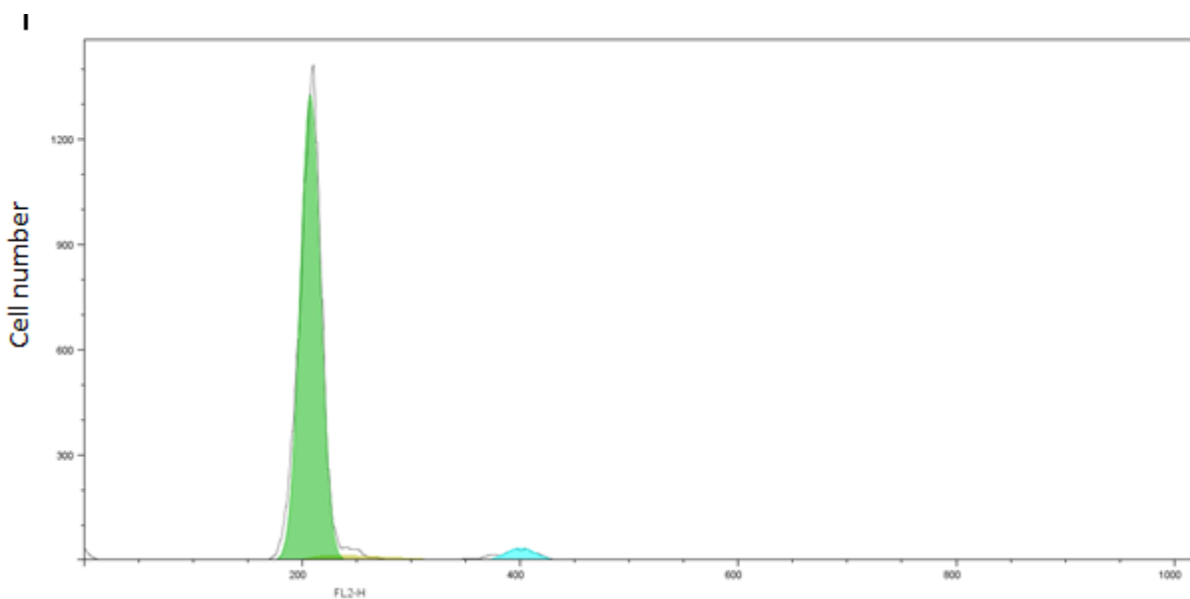


Figure S-13. Histogram representing the different phases of the cell cycle for NIH 3T3 cells under starved (non-proliferating) conditions. Sub G1: 3.58%, G1: 84.85%, S: 4.18% and G2/M: 3.68%.

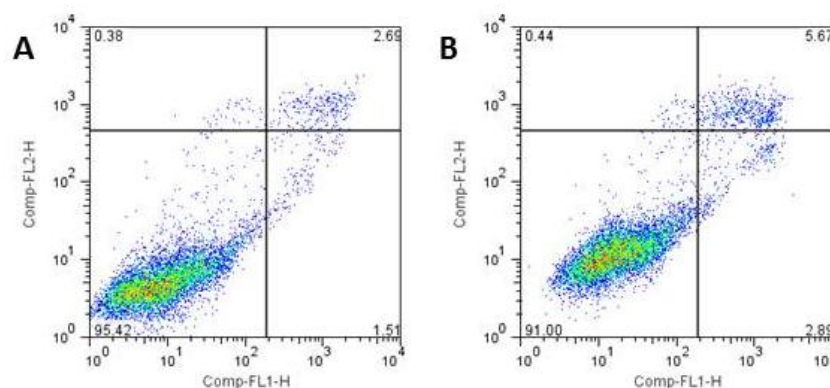


Figure S-14. FITC Annexin V/PI binding assay plots of (A) untreated cells, (B) cells treated with **1**.

S12. Immunoblot Analysis

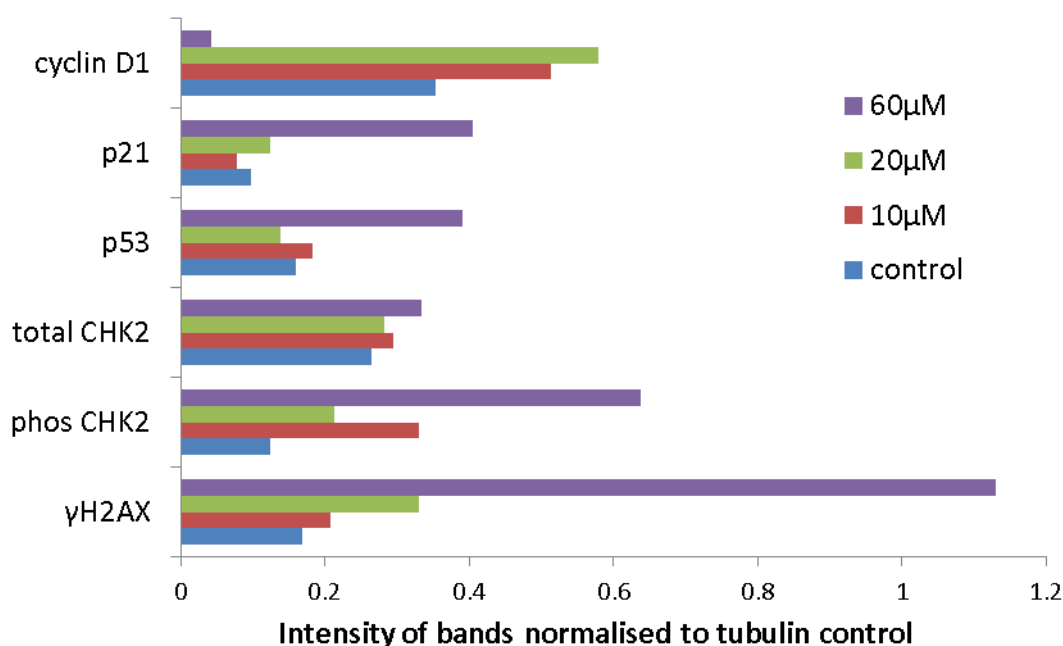


Figure S-15. The relative ratios of the band intensities normalised against the loading control (α -tubulin). This was carried out using the JImage software. Note that although in the experiment shown there appears to be a small increase in cyclin D1 at 10 and 20μM **1**, when data were averaged over three independent repeats no statistical differences were observed. Thus, although we see an increase in cells in the G1 phase of the cell cycle after treatment with **1**, this appears not to be a result of changes in either cyclin D1 or p21 levels. This data is consistent with other studies of G1 arrest prior to necrosis (Sané et al, Cancer Res. 1999 59:3565-9; Greay et al, Cancer Chemother Pharmacol. 2010 65:877-88).

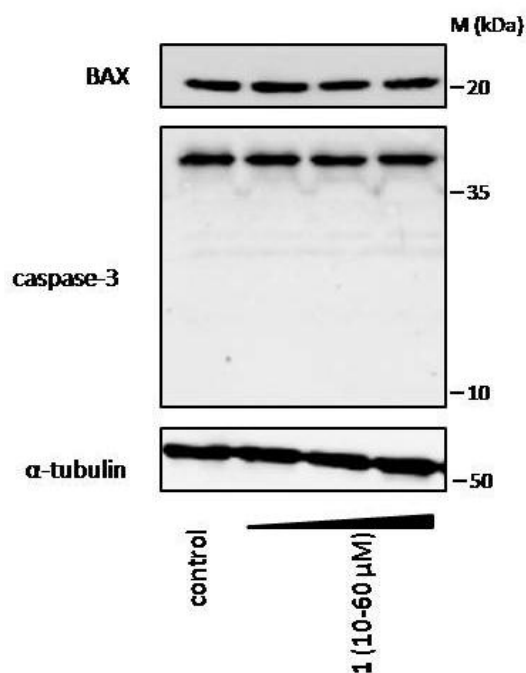


Figure S-16. Analysis of protein expression in U2OS cells following treatment with **1** (10-60 μ M) after 24 h incubation. Whole cell lysates were resolved by SDS-PAGE and analysed by immunoblotting against BAX, caspase-3 and α -tubulin (loading control). Results are representative of three independent experiments. MW: Protein Molecular Weight Marker.

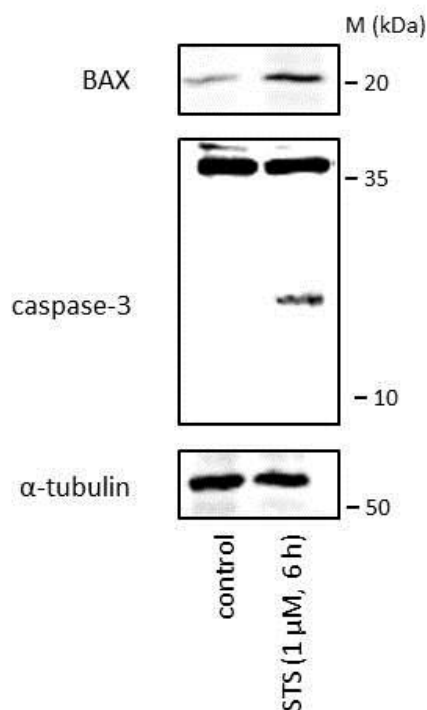


Figure S-17. Analysis of protein expression in U2OS cells following treatment with staurosporine, STS (1 μ M) after 6 h incubation. Whole cell lysates were resolved by SDS-PAGE and analysed by immunoblotting against BAX, caspase-3 and α -tubulin (loading control). MW: Protein Molecular Weight Marker.

S13. Extraction of HMGB1 released into culture media

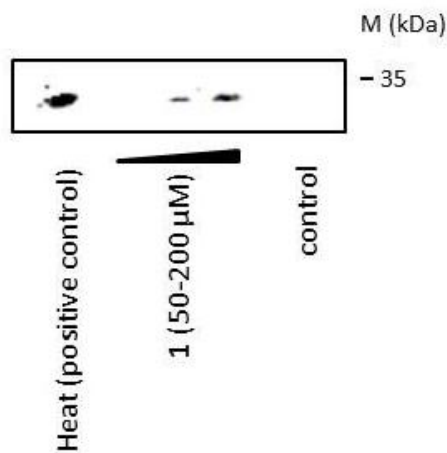


Figure S-18. Analysis of HMGB1 protein expression in culture media following treatment of U2OS cells with **1** (50-200 μM) after 48 h incubation. Precipitated proteins were resolved by SDS-PAGE and analysed by immunoblotting against HMGB1. MW: Protein Molecular Weight Marker.

S14. Cellular uptake

Table S-2. Values for cellular uptake reported as ng of platinum per mg of protein. The reported values are an average of four repeats. The corresponding errors are provided in each case.

Cell type	Whole cell (ng/mg)	Cytoplasm (ng/mg)	Nucleus (ng/mg)
U2OS	4.01 ± 0.86	2.43 ± 0.69	2.26 ± 0.22
SH-SY5Y	4.64 ± 0.45	1.68 ± 0.52	2.54 ± 0.23
NIH 3T3	2.71 ± 0.23	1.59 ± 0.56	1.20 ± 0.62
HEK 293T	2.34 ± 0.48	1.68 ± 0.12	0.69 ± 0.31
GMO5757	1.15 ± 0.36	0.82 ± 0.25	0.47 ± 0.11
NIH 3T3 - quiescent	0.25 ± 0.12	0.19 ± 0.05	0.11 ± 0.10