Supplementary Information:

A monoadduct generating Ru(II) complex induces ribosome biogenesis stress and is a molecular mimic of phenanthriplatin

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Experimental Methods

Figures:

Experimental methods

Materials and instrumentation

Materials for synthesis were purchased from commercial sources and were used without further purification. The water used for chemical and biological studies was obtained from a Milli-Q® water purification system. All NMR spectra were recorded on either a JOEL ECZr spectrometer operating at 500 MHz, or Bruker Avance NEO spectrometer operating at 400 MHz. Proton chemical shifts are reported in ppm (δ) relative to the residual solvent peak of CD_3CN (δ 1.93 ppm). Electrospray ionization (ESI) mass spectra were obtained on a Thermo-Scientific Q-Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization source. Ultraviolet/visible light absorption spectra were obtained from either an Agilent G1315A diode array detector coupled to an HPLC, a BMG Labtech FLUOstar Omega micro-plate reader, or an Agilent Cary 60 UV/Vis spectrophotometer. Agarose gels were run on a Bio-Rad horizontal gel electrophoresis system, imaged on a ChemiDoc MP System (Bio-Rad), and analyzed using Image Lab Software Version 6.1. The 6-well plates used for the clonogenic assay were imaged using ChemiDoc MP system and quantified using Image Lab. GraphPad Prism 9 and Microsoft excel were used to analyze and plot data. The 1 kb DNA Ladder, 6x Loading Dye, EcoR1, Nt.BspQ1 and all associated buffers were purchased from New England BioLabs Inc. The DNA Gyrase kit was purchased from TopoGen and ATP concentration was determined with CellTiter-Glo 3D from Promega. Schrödinger Maestro 2018-4 was utilized for all docking experiments. A Seahorse Flux Analyzer was used for bioenergetic measurements that report on mitochondrial function.

Synthesis and Characterization of Phenanthriplatin, 1, and 2

Phenanthriplatin:

Phenanthriplatin was synthesized as previously described and verified by Electrospray Ionization Mass Spectrometry (ESI-MS). ESI-MS calcd for $C_{13}H_{15}ClN_3Pt$ 443.8; found 444.0.[1]

Compound 1: [Ru(tpy)(dip)Cl]Cl

$[Ru(tpy)Cl_3]$ (204 mg, 0.46 mmol) and 4,7-diphenyl-1,10-phenanthroline (151 mg, 0.45 mmol) were added to degassed solution of ethanol:water (2:1) in a pressure tube, and refluxed at 90 °C, producing a red solution. After 4 h, the solution was cooled to room temperature and transferred into ~50 mL of water. Subsequently, 1 to 2 mL of saturated aqueous (aq) KPF_6 was added to the solution to produce a reddish precipitate which was isolated by filtration. The precipitate was washed with ~10 mL of water three times, and then ~10 mL of diethyl ether. The product was purified by flash chromatography, using a MeCN:water gradient with 0.1% saturated aqueous KNO_3. The red product eluted around 8% water in MeCN, and fractions containing the product were collected and dried *in vacuo*. The solid was redissolved in water with minimal acetonitrile, converted to the PF_6 salt by addition of a saturated solution of KPF_6 (aq), and extracted in DCM. The DCM was removed *in vacuo* to obtain the pure product. Yield: 166 mg, 0.24 mmols (52%). ^{1}H NMR (CD_3CN): δ 10.53 (d, J = 5.2 Hz, 1H), 8.59 (d, J = 8.0 Hz, 2H), 8.45 (d, J = 8.0 Hz, 2H), 8.30 – 8.27 (m, 2H), 8.17 (t, J = 8.0 Hz, 1H), 8.06 (d, J = 9.2 Hz, 1H), 7.91 – 7.85 (m, 4H), 7.78 – 7.68 (m, 6H), 7.56 – 7.54 (m, 3H), 7.45 – 7.43 (m, 2H), 7.27 – 7.21 (m, 3H). ^{13}C NMR (CD_3CN): δ 158.70, 158.08, 152.56, 152.45, 152.21, 149.78, 147.93, 147.64, 146.60, 136.99, 136.54, 135.75, 133.73, 129.98, 129.63, 129.46, 129.32, 129.24, 128.94, 128.60, 128.17, 127.09, 126.06, 125.89, 125.37, 124.90, 123.51, 122.50. ESI MS calcd for $C_{39}H_{27}ClN_5Ru$ [M]+ 702.20; Found 702.2. Purity by HPLC = 97%. λ_{max} nm (λ_{max}, ε x 10^3) (275 nm, 56.0), (315 nm, 31.5), (440 nm, 10.8), (505 nm, 13.0).

Compound 2: [Ru(ph-tpy)(dip)Cl]Cl

$[Ru(ph\text{-}tpy)Cl_3]$ (200 mg, 0.38 mmol) and 4,7-diphenyl-1,10-phenanthroline (133 mg, 0.40 mmol) were added to ethylene glycol in a pressure tube. Ethylene glycol was used in place of ethanol:water (2:1) due to improved reagent solubility at higher temperatures. The mixture was refluxed at 135 °C for 1 hour. The resulting red solution was cooled to room temperature and transferred to a flask with ~50 mL of water. Approximately 1 to 2 mL of a saturated solution of KPF_6 (aq) was added to the solution to produce a reddish precipitate which was separated from the solvent by filtration. The precipitate was washed with ~10 mL of water three times then ~10 mL of diethyl ether. The product was purified by flash chromatography, using a MeCN:water gradient with 0.1% KNO_3. The product eluted around 6% water in MeCN, and the pure fractions were collected and dried in vacuo. The compound was redissolved in water with minimal acetonitrile and converted to the PF_6 salt by addition of saturated solution of KPF_6 (aq), then

extracted in DCM. The DCM-dissolved product was dried in vacuo to obtain the dried pure product. The product was dried in vacuo to obtain the dried pure product. Yield: 201 mg, 0.26 mmols (68%).^{1}H NMR (CD_3CN): δ 10.57 (d, J = 5.2, 1H), 8.83 (s, 2H), 8.57 (d, J = 8.0, 2H), 8.29 – 8.24 (m, 2H), 8.15 (d, J = 7.4, 2H), 8.03 (d, J = 9.6, 1H), 7.88 – 7.83 (m, 4H), 7.79 – 7.59 (m, 9H), 7.53 – 7.51 (m, 3H), 7.43 – 7.40 (m, 2H), 7.24 – 7.18 (m, 3H). ^{13}C NMR (CD_3CN): δ 158.81, 158.14, 152.52, 152.10, 149.83, 147.95, 147.65, 146.64, 146.10, 137.10, 136.94, 136.55, 135.75, 129.99, 129.96, 129.64, 129.46, 129.32, 129.24, 128.92, 128.60, 128.19, 127.66, 127.10, 126.10, 125,90, 125.38, 124.89, 123.71, 120.52. ESI MS calcd for $C_{45}H_{31}ClN_5Ru$ [M]+ 778.13; Found 778.13. Purity by HPLC = 96%. λ_{max} nm (λ_{max}, ε x 10^3) (280 nm, 54.8), (315 nm, 23.5), (440 nm, 7.9), (515 nm, 11.8).

Counterion Exchange

Compounds **1** and **2** were converted to Cl^- salts by dissolving 15–30 mg of each complex as the PF_6 salt in 1–2 mL of methanol. The dissolved compound was loaded onto an Amberlite IRA-410 chloride ion exchange column, eluted with methanol, and then the methanol was removed in vacuo.

HPLC Analysis for Purity

Phenanthriplatin and compounds **1** and **2** were analyzed using an Agilent 1100 series HPLC system equipped with a G1322A degasser, G1311A quaternary pump, and G1315A UV Diode Array Detector. The HPLC system was controlled with ChemStation software version B.01.03 (Agilent Technologies). Final data was exported and processed in Excel and Graphpad Prism 9. The chromatographic conditions were optimized on a Phenomenex Luna 5 µm C18(2) 100 Å column fitted with a Phenomenex C18 guard column. Injections (20 µL) of 100 µM solutions for each compound were used. The mobile phases for HPLC consisted of (A) 0.1% formic acid in diH_2O and (B) 0.1% formic acid in HPLC grade acetonitrile. Both mobile phases were degassed under helium. The following mobile phase gradient was used: 98−95% A from 0 to 5 min; 95−70% A from 5 to 15 min; 70−40% A from 15 to 20 min; 40−5% A from 20 to 30 min; 5−98% A from 30 to 35 min; re-equilibration at 98% A from 35 to 40 min with a flow rate of 1.0 mL/min. The detection wavelength was 280 nm.

Bacteria Maintenance

pET-45b plasmid DNA was transformed into *E. coli* BL21(DE3) to confer resistance to ampicillin, and plated onto agar plates with 100 µg/mL ampicillin. The plates were incubated for 16 hours at 37 °C before colonies were selected and cultured in Luria Broth (LB) at 37 °C with 180 rpm shaking. Following incubation, cells were prepared for bacterial assays.

Cell Line Maintenance

All parental cell lines were obtained from the ATCC. The HL-60 cells were maintained in IMDM media, and supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL). The A549, DU145 and MIA PaCa-2 cell lines were maintained in DMEM media and supplemented with 10% FBS containing penicillin (100 U/mL) and streptomycin (100 µg/mL). The HEL299 cells were maintained in Opti-MEM™ supplemented with 5% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL). All cell lines were cultured at 37 °C with 5% CO2.

Bacteria Growth Inhibition

E. coli BL21(DE3) cells transformed with pET-45b plasmid were plated in M63 minimal medium at 4×10^5 cells/well in 96-well flat bottom Greiner tissue culture treated plates. Cells were dosed with compounds from 0 – 100 µM and incubated for 16 h at 37 °C. Following incubation, resazurin (final concentration 73 µM) was added to each well, and cell viability was then quantified by measuring fluorescence emission at 595 nm (λ_{ex} : 535 nm) on a SpectraFluor Plus plate reader (Tecan). The minimum inhibitory concentration (MIC) was determined from the lowest compound concentration that resulted in no resorufin turnover.

Cell Cytotoxicity

The HL-60 cells were cultured in IMDM media supplemented with 2% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL) and plated in 96-well plates at 30,000 cells/well. The A549, DU145, and MIA PaCa-2 cells were seeded at 2,000 cells/ well in DMEM media supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL) and allowed to adhere to the well overnight. The HEL299 cells were seeded in in opti-MEM™ supplemented with 5% FBS, penicillin (100 U/mL), and streptomycin

(100 µg/mL) at 2,000 cells/well and allowed to adhere to the well overnight. Media was aspirated and replaced with Opti-MEM™ supplemented with 2% FBS the following day for each cell line. Compounds were serially diluted in Opti-MEM™ supplemented with 2% FBS penicillin (100 U/mL), and streptomycin (100 µg/mL), and then added to the cells. For **3**, an extracellular solution was used in place of opti-MEM™ to prevent cellular damage from light irradiation. The extracellular solution was made with 10 mM HEPES, 10 mM glucose, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 3.3 mM KH_2PO_4, 0.83 mM K_2HPO_4, and 145 mM NaCl in deionized water.

Cells were incubated with each compound for 72 h followed by the addition of resazurin (73 µM final concentration). Cells were further incubated for 3 h to allow for reduction of resazurin to resorufin by viable cells, and data collected as described above. Measurements were taken in triplicate for all cytotoxicity data.

Zebrafish Embryo Toxicity

Animal studies were approved under the University of Kentucky's Institutional Animal Care and Use Committee, protocol 2019-3399. Healthy 2-day post fertilization (dpf) Casper strain zebrafish embryos were pipetted into 96-well plates, at 1 larvae per well in 150 µL 1X E3 media (5 mM NaCl, 0.17 mM KCl, 0.33 mM $MgSO_4$ in diH_2O). Compounds were prepared at 2X of the desired concentration in E3 media and 150 µL was added to each well. Plates were incubated in the dark for 168 h, with drug refreshed during media change at 72 h. Animals were imaged using a EVOS FL Imaging System (Thermo Fisher Scientific). Each compound was tested in triplicate at three concentrations.

Resistance/Sensitivity Profiles in Different Cancer Cell Lines

Resistance and sensitivity profiles for each compound were generated in order to correlate specific cell line sensitivity to the compound's potential MoA. The National Cancer Institute's Developmental Therapeutics Program COMPARE analysis was used as the model for this analysis.[2]

National Cancer Institute 60-Cell Line Panel Analysis

Approximately 20 mg of compounds **1** and **2** were sent to the National Cancer Institute (NCI) for 60-cell line analysis. Cell growth was measured after 48 h treatment with 10 µM of each compound. The data was plotted as mean growth percent subtracted by individual cell growth percent. Following single-dose experiments, both compounds were selected for five-dose response screening. Cell cytotoxicity was measured after 48 h treatment with each compound at the concentrations indicated in Fig. S11 and S12.

Clonogenic Assay

E. coli were treated with phenanthriplatin, cisplatin, rifampicin, tetracycline, **1**, and **2** at the minimum inhibitory concentration (MIC) or 4x the MIC determined by the bacterial growth inhibition assay for 16 h at 37 °C. Following treatment, 2,500 cells were pulled from the culture, washed three times with cold PBS, and spread on agar plates. After a 16 h incubation at 37 °C, the colonies on the plate were imaged and quantified.

Cellular Uptake

E. coli were cultured as described above. Then, bacteria were centrifuged and Luria broth was aspirated. Afterwards, $4x10^7$ cells were suspended in M63 minimal media and dosed with 20 µM of each compound. Cells treated with **3** were irradiated with 7 J/cm^2 405 nm light or were protected from light. Cells were collected 24 h after compound addition by centrifugation at 5,000 rpm for 10 min. The culture medium was separated for analysis, and cells were washed twice with cold PBS and pelleted. Both the cell content and 100 µL of medium were pre-pared for analysis by heating at 100 °C for 1 h with 150 µL of HNO_3 and 50 µL of H_2O_2, followed by a second digestion step with 50 µL of HCl added to the acidified solutions. Following digestion, the samples were diluted to 5 mL with diH_2O for analysis.

Trace-elements were quantified by inductively coupled plasma mass spectroscopy (ICP-MS). The ICP-MS was equipped with an octopole reaction system (Agilent 7900, Santa Clara, CA, USA). The octopole was operated in standard mode (no gas) for platinum (Pt) and ruthenium (Ru). Indium (In) was added as an internal standard for a final concentration of 10 ng In/ L. Calibration standards were matrix-matched and prepared using a certified reference standard (Agilent Technologies). The calibration curve was validated by analyzing a standard from the same source but with a different lot number after every 10

samples and after every calibration. The calibration curve was considered valid if the observed concentration for the independent standard was within 10% of the expected concentration. Spike recovery was determined on randomly selected samples during each analytical run. The detection limits for both Pt and Ru ranged from 0.007–0.010 ng/L, and spike recovery values averaged 104.0 % for Ru and 102.5 % for platinum.

DNA Metalation

E. coli were cultured in M63 minimal medium as described above and dosed with 20 μM compound. Cells were collected after 24 h by centrifugation at 8,000 rpm for 5 min. Genomic DNA was isolated using a GE Healthcare genomic DNA kit. The DNA in each sample was quantified, and the DNA samples were digested for ICP analysis as described above. The data was normalized to the DNA content in each sample.

Bacterial Cytological Profiling

E. coli culture, treatment, and filamentous growth. BL21(DE3) cells were cultured in 1x Luria Broth (LB) at 37°C with 250 rpm shaking and plated in M63 minimal media at $4x10^5$ cells per well in a 96-well flat bottom transparent microplate (Greiner Bio-One). Compounds were serially diluted 1:3 in M63 minimal media and added to the cells such that the final concentration ranged from 0–100 μM. The dosed cells were incubated at 37°C with 250 rpm of shaking for 24 hours and protected from light prior to imaging.

E. coli imaging and quantification. After the 24-hour incubation, cell density (OD_{600}) was measured using a FlexStation 3 Multi-Mode Microplate Reader. Approximately 350,000 cells treated at the compound MIC were sampled and suspended in 100 μL of 1x PBS. The cell suspension was seeded on a 35 x 1.5 mm glass bottom dish coated with poly-d-lysine (PDL). Bacterial cells were adhered to the coated surface by centrifugation at 1,000 rpm for 10 minutes. Following this, cells were washed with PBS, and the fluorescent dye FM4-64 was added for a final concentration of 5 μg/mL. Imaging was carried out on a Nikon A1R confocal microscope equipped with Galvano scanner and GaAsP detectors. Images for treated samples were captured using a 60X (1.40 NA) infinity corrected oil immersion objective. Untreated samples were collected with a 100X (1.49 NA) infinity corrected oil immersion objective. The 561 nm channel was used to visualize FM4-64 fluorescence. Each image was captured at 1024 x1024 pixel resolution. FM4-64 was visualized on the 561 nm excitation channel with 60–63% laser power, 68–73% gain, and 0% offset. For the *E. coli* phenotypic distribution analysis, the filament length was quantitated using Nikon Elements Analysis software, and histograms were plotted with Gaussian distribution regression in GraphPad Prism 9.0.0. Histograms and filamentous population percentages represent filaments greater than or equal to 6 μm in length. Although assumptions based on doubling time could be made about cells greater than the 2 μm average are undergoing mitosis, there is no way to determine cell status with this analysis, so a 6 μm cutoff was used to identify filamentous bacteria. This ensured a higher confidence that the stress phenotype was resultant of compound treatment. This threshold accounts for doubling of 95% of the healthy cell population.

Nucleophosmin Redistribution

Cell culture and treatment for nucleophosmin redistribution. A549 human lung carcinoma cells were plated at 40,000 cells per 35 mm glass bottom dish. The cells were grown overnight at 37°C with 5% CO_2 in DMEM containing 10% FBS, 100 U penicillin, and 100 μg/mL streptomycin. Cells were treated with 0.5 μM phenanthriplatin, 0.5 μM compound **2**, 5 μM cisplatin, 5 μM compound **1**, and 5 nM actinomycin D. Compounds were prepared from DMSO stocks such that final concentrations of DMSO did not exceed 0.1% (v/v) in media. Concentrations were selected based on 72-hour IC_{50} values. Cells were incubated with compound for 24 hours at 37°C with 5% CO_2.

Immunofluorescence. Following compound treatment and incubation, cells were washed with PBS. Cells were fixed with 4% paraformaldehyde in PBS for 20 minutes at room temperature then permeabilized with 0.5% Triton-X in PBS for 20 minutes at room temperature. Cells were blocked in two 10 minute steps with 1% BSA in PBST. Cells were incubated with primary antibody (NPM1 Monoclonal Antibody, FC-61991, Thermo Fisher, 1:200 dilution in PBST with 1% BSA) for 2 hours and secondary antibody (Goat Anti-Mouse IgG H&L Alexa Fluor 488, ab150113, Abcam, 1:1000 dilution in PBST with 1% BSA) for 1 hour. Cells were counterstained with 5 μM DRAQ5 in PBST for 30 minutes. Triplicate wash steps were performed with PBST in between each incubation.

Imaging and quantification. Images were captured with a Nikon A1R confocal microscope using either a 60X (1.40 NA) or 100X (1.49 NA) infinity corrected oil immersion objective. NPM1 was visualized on the 488 nm excitation channel with 3% laser power, 55% gain, and 0% offset. DRAQ5 was visualized on the 640 nm excitation channel with 8% laser power, 130% gain, and -10% offset. For delocalized NPM1 phenotype analysis, cells lacking distinct fluorescent foci and/or the presence of rounded nucleoli with NPM1 at the periphery were quantified as a percentage of the total population and data was plotted in GraphPad Prism 9.0.0. Over 100 cells were analyzed for each compound treatment. Representative pixel intensity plots were generated using ImageJ.

DNA Gel Electrophoresis

Linear and open coil DNA controls were prepared by digesting pUC19 plasmid DNA with EcoR1 and Nt.BspQ1 following the manufacturer's protocol. Compounds **1** and **2** were serially diluted 1:2, giving final concentrations of 7.8, 15.6, 31.3, 62.5, 125, 250, and 500 μM. These solutions were then mixed with 40 μg/mL pUC19 in 10 mM phosphate buffer pH 7.4 and 133 μg bovine serum albumin (BSA). The BSA was not added to the matrix for the samples in Fig. S18A and B. Phenanthriplatin was serially diluted 1:4, giving final concentrations of 0.031, 0.12, 0.49, 1.95, 7.8, 31.3, 125, and 500 μM, and then subsequently mixed with 40 μg/mL pUC19 in 10 mM phosphate buffer pH 7.4. The samples were incubated for 16 hours at 37 °C before they were resolved on a 1% agarose gel prepared in 1X Tris-Acetate-EDTA (TAE) buffer with 0.3 μg of pUC19 per lane. Electrophoresis was performed at 100 V for approximately 90 min. Afterwards, the gels were stained with 0.5 μg/mL ethidium bromide (EtBr) in TAE buffer for 40 min, destained with TAE buffer for 30 min, then imaged on a ChemiDoc MP System (Bio-Rad).

Inhibition of DNA Gyrase Activity

A DNA supercoiling assay was performed using an *E. coli* and relaxed DNA kit from TopoGEN (TG2000G-1KIT) in the presence of phenanthriplatin, **1**, and **2**, and ciprofloxacin (positive control). The general protocol was adapted from TopoGen. Circular plasmid relaxed DNA pHOT-1 was used as the substrate. For *E.coli* gyrase assays, a mixture containing ~500 ng of relaxed pHOT-1 and 1 U of E. coli DNA gyrase was prepared by adding 4 μL of assay buffer (The 5X assay buffer was provided in a DNA gyrase kit and diluted to 1X for the reaction.1X buffer: 35 mM Tris-Cl, pH 7.5, 24 mM KCl, 4mM $MgCl_2$, 2 mM dithiothreitol, 1.8 mM spermidine, 1 mM ATP, 6.5% glycerol, 0.1 mg BSA/mL) and the required amount of each compound. The final volume was adjusted to 20 μL by adding autoclaved deionized water. The reaction was carried out at 37 ºC for 60 min and then quenched by addition of 5 μL stopping buffer (The 5X stopping buffer was added to solution to achieve final concentration 1X. 5X buffer: 5% sarkosyl, 0.125% bromophenol blue, 25% glycerol). Proteinase K was added for a final concentration of 50 μg/mL to digest the gyrase to prevent affecting DNA mobility. Then, 20 μL of Phenol: Chloroform: isoamyl alcohol (25:24:1) was added, the sample was vortexed, and spun down. This resulted in two layers of solution. The aqueous phase was removed and analyzed by electrophoresis using a 1% agarose gel prepared in Tris-Borate-EDTA (TBE) buffer. The gel was run for 2 h at 70 V. After electrophoresis, the gels were stained with 0.5 μg/mL EtBr in TBE buffer for 45 min followed by a 30 min destaining with TBE. To improve topoisomer separation, TBE was used in place of TAE. Gel images were obtained on a ChemiDoc imaging system (Bio-Rad).

Hydrophobicity Measurements

The hydrophobicity of **1** and **2** were quantified by measuring the logarithm of their partition coefficient (log P values) using the shake flask method. Each compound was analyzed at 40 μM concentrations. The solutions were inverted 100 times in a mixture of octanol and water, then allowed to equilibrate for 24 hours. For samples with BSA, 133 μg of protein was added to each compound in water prior to inversion. Following equilibration, each phase was placed into a 96-well plate and the absorbance was measured at the peak of the metal to ligand charge transfer (MLCT) transition. Log P values were determined by the logarithm of the quotient of the octanol MLCT absorbance over the water MLCT absorbance.

Aqueous Stability

The aqueous stability of **1** and **2** was studied at 37 °C by UV/Vis spectroscopy. The compounds were analyzed at 40 μM concentrations in solutions both in the presence and absence of 133 μg BSA in a clear 96-well plate. Measurements were taken over the course of 72 hours. Breathe-Easy® membranes and plate covers, which were removed prior to each measurement, were placed over the 96-well plates to slow solvent evaporation. All measurements were performed in triplicate.

Docking Analysis

Topoisomerase docking was performed based on a previous report where mitoxantrone was docked into the active site of topoisomerase IIα.[3]We docked mitoxantrone as a control with our system, which exhibited agreement with the results previously reported.[3]

Dickerson dodecamer coordinates were imported from the RCSB Protein Data Bank (PDBID: 4C64). The protein preparation wizard was used to process the imported structure in the following ways: Bond orders were assigned using the CCD database. Original hydrogen atoms were removed and re-added to account for potentially missing hydrogen atoms in the published coordinates. Zero-order bonds to metals were created. Waters beyond 5 Å from het groups were removed. Hydrogen bonds were assigned and optimized, and waters with less than 3 hydrogen bonds to non-waters were removed. Heavy atoms were converged to an RMSD of 0.30 Å using the OPLS3e force field. Phenanthriplatin and **2** were prepared as follows: Structures were built using the "build" function in Maestro, adding individual atoms and fragments as appropriate to account for all atoms, with the correct connectivity, in each structure. Ligands were manually positioned in a reasonable geometric arrangement using published structures of similar complexes in the Cambridge Crystallographic Data Center (CCDC) as a reference (e.g., CCDC: ACIDIA). Bonds to the metal center were manually decreased to zero-order bonds, and formal charges were assigned. The "minimize selected atoms" function was used to ensure no large deviations were present in the geometry of the structure, and the resulting complex was used in the docking analyses.

Before investigating potential interactions of **2** with the target DNA sequence, phenanthriplatin was used as a control, as this complex has known interactions with the target DNA sequence.[4] Due to the smaller size of this coordination complex, and the aforementioned published computational evaluations of phenanthriplatin, a simpler docking experiment with fewer degrees of freedom was performed as follows: The receptor grid was generated with a center X = 4, Y= 7, Z= 13. The gridbox range for X, Y, and Z was set at 17, and the gridbox ligand range was set at 10. The phenanthriplatin complex was docked using this created receptor grid with the following settings: van der Waals radii were set to a scaling factor of 0.80 with a partial charge cutoff of 0.15. Standard precision was used, and the ligand sampling was flexible. A total of 5,000 poses were kept for the initial phase of docking, and the scoring window for keeping initial poses was 100. The best 400 poses were kept for energy minimization, with the dielectric constant set to 2.0 and the maximum number of minimization steps equal to 100. The OPLS3e force field was used. The complex had 10 poses written, and post-docking minimization was performed on all 10 poses.

Phenanthriplatin was docked into the Dickerson dodecamer (4C64), showing a pose in which the exchangeable chloride ligand was positioned near N7 of dG. This calculation was performed as a control evaluation of the system prior to further, more complex, calculations with compound **2**. Encouraged by these results, a more intensive induced-fit calculation was executed for the interaction of **2** and the DNA target sequence. The "box center" was defined by "picking" the centroid of a workspace ligand. Since there are no ligands bound to the structure used (4C64), a placeholder atom was positioned in the center of the double helix molecule using the build function and selected to define the box location. This atom, when selected, is automatically excluded from the induced-fit calculation, and does not impact the structure of the target sequence. The box size was defined to dock ligands with length ≤ 36 Å to allow for a broad sampling space on the target sequence. The "standard" protocol was used with the OPLS3e force field, generating up to 20 poses. Side chains were trimmed automatically (based on B-factor) with receptor van der Waals scaling set to 0.70, ligand van der Waals set to 0.50, and the maximum number of poses set to 20. Residues within 5 Å of ligand poses were refined, and side chains were optimized. For glide redocking, structures within 30.0 kcal/mol of the best structure, and within the top 20 structures overall were redocked with standard precision.

Ligand Exchange Kinetics

For ligand exchange analysis, 100 μM of each compound was monitored in diH_2O. To evaluate the effect of specific nucleophiles, either 5 mM deoxyguanosine (dG) or 5 mM glutathione (GSH) was added to the compound. For kinetic measurements by HPLC, 20 μL of sample was injected at each timepoint. Samples were incubated at 23 °C. For kinetic measurements by UV/VIS, 200 μL of 50 μM 2 were analyzed in diH_2O in the presence or absence of 5 mM GSH/dG. The UV/Vis samples were incubated at 37 °C for the time indicated.

In Vitro Complex of Enzyme (ICE) Assay

Approximately $2x10^6$ A549 human lung carcinoma cells were seeded into 60 mm petri dishes in DMEM supplemented with 10% FBS, 100 U penicillin, and 100 µg/mL streptomycin, and incubated for 16 h at 37 °C with 5% CO_2. Following incubation, the DMEM was aspirated and Opti-MEM with 2% FBS, 100 U penicillin, and 100 µg/mL streptomycin was added to the petri dish. Then, etoposide (100 µM), phenanthriplatin (50 µM), or **2** (50 µM and 100 µM) was added to the cells and incubated for 90 min at 37 °C with 5% CO_2. The ccDNA was extracted from the cells using an ICE Assay Kit (TopoGEN, TG1020-2A) with manufacturer instructions with a minor modification. Instead of lysing the cells on the dish, they were scraped from the plate in PBS and transferred to a sterile 1.7 mL tube and lysed there. The DNA-TopIIα complex was blotted on a Bio-RAD dot blot apparatus at 250 ng, 500 ng, 1 µg, and 5 µg of DNA. The ccDNA was detected by immunoblotting using a topoisomerase IIα polyclonal rabbit antibody (TG1020-2a).

HeLa Parental and XPA Knockout Cytotoxicity

The HeLa parental cells were purchased from the ATCC. The HeLa XPA knockouts were graciously donated by the Wood Lab at MD Anderson. To validate homozygous knockout, HeLa XPA knockout cells were lysed and immunoblotted for XPA with a monoclonal mouse XPA antibody purchased from Abcam (ab65963). After the HeLa XPA knockout was validated, the parental and knockout cells were placed in DMEM media supplemented with 10% FBS, 100 U penicillin, and 100 µg/mL streptomycin, plated in 96-well plates at 5,000 cells/well, and allowed to adhere to the wells overnight. Media was aspirated and replaced with Opti-mem the following day. Compounds were serially diluted in Opti-MEM supplemented with 2% FBS, 100 U penicillin, and 100 µg/mL streptomycin, and then added to the cells. Cells were incubated with each compound for 72 h followed by the addition of resazurin. Cells were further incubated for 2 h to allow for reduction of resazurin to resorufin by viable cells, and data collected as described above. Measurements were collected in triplicate.

Immunoblotting

Wild-type and XPA knockout HeLa cells were cultured as described above. Once the plates were confluent, 3×10^6 cells were collected and washed with ice-cold PBS followed by the addition of ice-cold lysis buffer (20 mM Tris pH 7.5, 1% Triton X100, 5 mM EDTA, 5 mM sodium pyrophosphate, 5 mM sodium fluoride, 150 mM NaCl, 2 mM sodium vanadate, 1 mM PMSF, and 1× Roche complete protease inhibitor cocktail). The cells were lysed on ice for 15 min, followed by centrifugation for 10 min at 20,800 g at 4 °C. The supernatant was transferred to a 1.7 mL tube, an aliquot removed for protein concentration determination by BCA, with SDS sample buffer added to the remaining lysate and boiled at 95 °C for 5 min then stored at −20 °C.

The HeLa WT and XPA knockout cell lysates were resolved on a 4–12% Bis-Tris gel at increasing concentrations (5 µg, 10 µg, and 15 µg), followed by transfer to a nitrocellulose membrane. The membrane was blocked for 1 h in PBST (PBS with 0.1% Tween 20) with 5% nonfat milk, followed by the addition of the XPA primary antibody (acbam, ab180618) at a 1:1000 dilution and incubated overnight at 4 °C. The membranes were washed for 5 min with PBST and repeated for a total of four washes, then incubated with secondary antibody (Jackson ImmunoResearch) for 1 h at room temperature, washed 4× with PBST, followed by the addition of Luminal (Clarity, Bio-Rad) and imaged on a Chemi-Doc system (Bio-Rad).

Tubulin Polymerization

Cell culture and treatment for α tubulin polymerization/depolymerization. A549 human lung carcinoma cells were plated at 40,000 cells per 35 mm glass bottom dish. The cells were grown overnight at 37°C with 5% CO_2 in DMEM containing 10% FBS, 100 U penicillin, and 100 µg/mL streptomycin. Cells were treated with 1 µM paclitaxel, 0.2 µM vinblastine sulfate, and 1 µM compound **2**. Cells were incubated with compound for 16 h at 37°C with 5% CO_2.

Immunofluorescence. Following compound treatment and incubation, cells were washed with PBS. Cells were fixed with 4% paraformaldehyde in PBS for 20 minutes at room temperature then permeabilized with 0.5% Triton-X in PBS for 20 minutes at room temperature. Cells were blocked in two 10 minutes steps with 1% BSA in PBST. Cells were incubated with primary antibody (α-Tubulin Monoclonal Antibody, ab7750, Abcam 1:200 dilution in PBST with 1% BSA) for 2 hours and secondary antibody (Goat Anti-Mouse IgG H&L Alexa Fluor 488, ab150113, Abcam, 1:1000 dilution in PBST with 1% BSA) for 1 hour.

Cells were counterstained with 5 μM DRAQ5 in PBST for 30 minutes. Triplicate wash steps were performed with PBST in between each incubation.

Imaging and quantification. Images were captured with a Nikon A1R confocal microscope using a 60X (1.40 NA) infinity corrected oil immersion objective. α-tubulin was visualized on the 488 nm excitation channel. DRAQ5 was visualized on the 640 nm excitation channel.

Cellular Bioenergetics

All Seahorse XF96 experiments were performed with A549 cells. The A549 cells were seeded in a Seahorse XF 96-well plate (Agilent) at $2.5x10^4$ cells in 100 μL of DMEM supplemented with 10% FBS and 100 μg/mL pen-strep per well. The plates were set at room temperature for 1 hour to allow cells to settle and then placed in a 37 °C incubator with 5% CO_2 overnight. The next day, DMEM was aspirated from the cells and replaced with 100 μL **2** in extracellular buffer. Each condition was prepared to have a final concentration of 1% DMSO. The cells were incubated with **2** for one hour. Following incubation, the extracellular buffer was aspirated and Opti-MEM supplemented with 2% FBS, 100 U penicillin, and 100 μg/mL streptomycin was added to the cells. After fifteen minutes, the media was aspirated and replaced with Seahorse XF DMEM Medium (pH 7.4) supplemented with 25 mM XF Glucose, 2 mM XF Glutamine, and 1 mM XF Pyruvate and cells were incubated at 37 °C for 1 hour in a CO_2-free incubator before running the assay. The assay was performed using a pneumatic injection for oligomycin, carbonyl cyanide-p-trifluoro-methoxy-phenylhydrazone (FCCP), rotenone, and Antimycin A . Recordings were initiated and oligomycin (1.0 μM) was injected at 18 minutes. This was followed by injection of FCCP (1.2 μM) at 36 minutes and rotenone/antimycin A (1.0 μM) at 54 minutes. Quadruplicate replicates were measured. Seahorse experiments were done at the University of Kentucky Markey Cancer Center Redox Metabolism Facility.

Nucleophosmin Redistribution

Cell culture and treatment for nucleophosmin redistribution. A549 human lung carcinoma cells were plated at 40,000 cells per 35 mm glass bottom dish. The cells were grown overnight at 37ºC with 5% CO_2 in DMEM containing 10% FBS, 100 U penicillin, and 100 μg/mL streptomycin. Cells were treated with 0.5 μM phenanthriplatin, 0.5 μM compound **2**, 5 μM cisplatin, 5 μM compound **1**, and 5 nM actinomycin D. Compounds were prepared from DMSO stocks such that final concentrations of DMSO did not exceed 0.1% (v/v) in media. Concentrations were selected based on 72-hour IC_{50} values. Cells were incubated with compound for 24 hours at 37ºC with 5% CO_2.

Immunofluorescence. Following compound treatment and incubation, cells were washed with PBS. Cells were fixed with 4% paraformaldehyde in PBS for 20 minutes at room temperature then permeabilized with 0.5% Triton-X in PBS for 20 minutes at room temperature. Cells were blocked in two 10 minutes steps with 1% BSA in PBST. Cells were incubated with primary antibody (NPM1 Monoclonal Antibody, FC-61991, Thermo Fisher, 1:200 dilution in PBST with 1% BSA) for 2 hours and secondary antibody (Goat Anti-Mouse IgG H&L Alexa Fluor 488, ab150113, Abcam, 1:1000 dilution in PBST with 1% BSA) for 1 hour. Cells were counterstained with 5 μM DRAQ5 in PBST for 30 minutes. Triplicate wash steps were performed with PBST in between each incubation.

Imaging and quantification. Images were captured with a Nikon A1R confocal microscope using either a 60X (1.40 NA) or 100X (1.49 NA) infinity corrected oil immersion objective. NPM1 was visualized on the 488 nm excitation channel with 3% laser power, 55% gain, and 0% offset. DRAQ5 was visualized on the 640 nm excitation channel with 8% laser power, 130% gain, and -10% offset. For delocalized NPM1 phenotype analysis, cells lacking distinct fluorescent foci and/or the presence of rounded nucleoli with NPM1 at the periphery were quantified as a percentage of the total population and data was plotted in GraphPad Prism 9.0.0. Over 100 cells were analyzed for each compound treatment. Representative pixel intensity plots were generated using ImageJ.

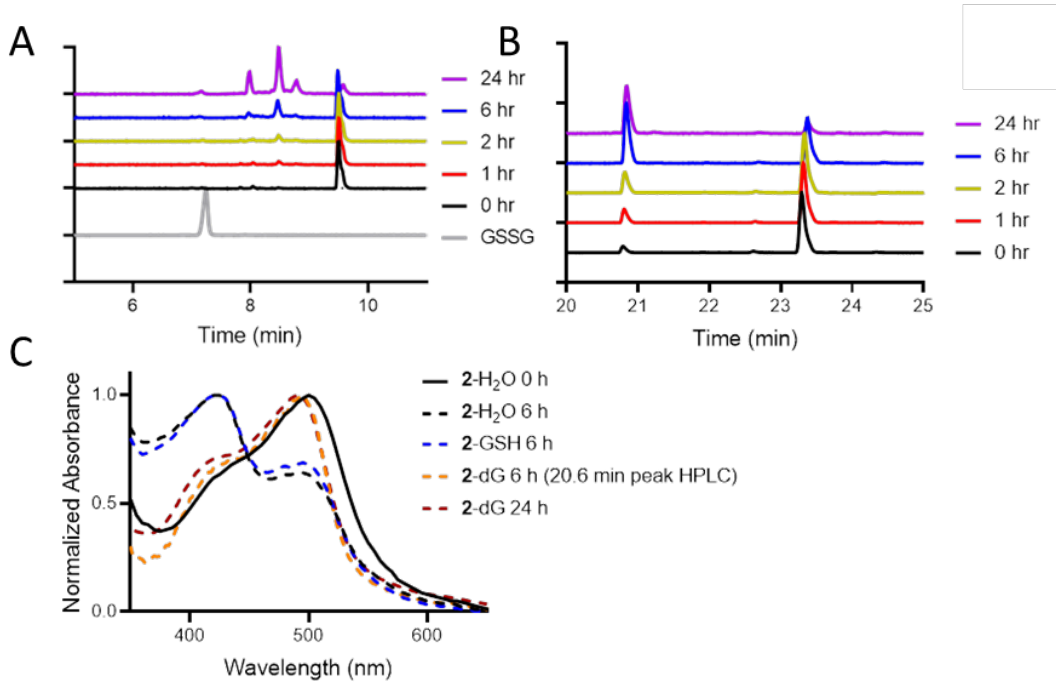

Figure S1. HPLC and UV/Vis Glutathione reaction kinetics**.** HPLC kinetics of A) 100 μM phenanthriplatin with 5 mM GSH and B) 100 μM **2** with 5 mM glutathione (GSH). C) UV/Vis kinetics of **2** in diH_2O, 5 mM dG, and 5 mM GSH after 6 h. In A, oxidized glutathione (GSSG) was used as a glutathione control.

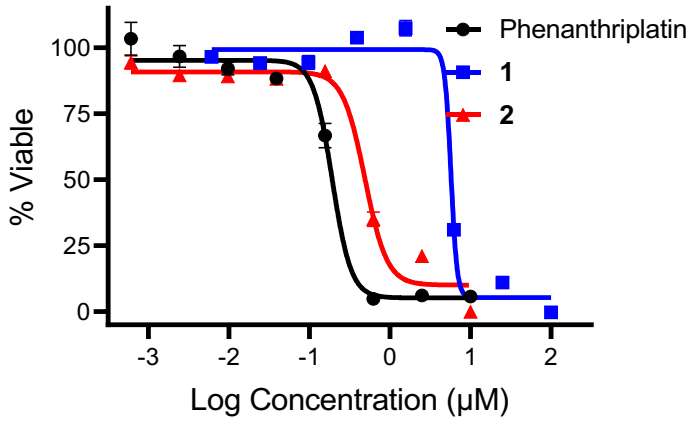

Figure S2. Dose response in A549 cells with phenanthriplatin, **1**, and **2**. Viability was determined 72 h after compound treatment. The measurements were collected in triplicate.

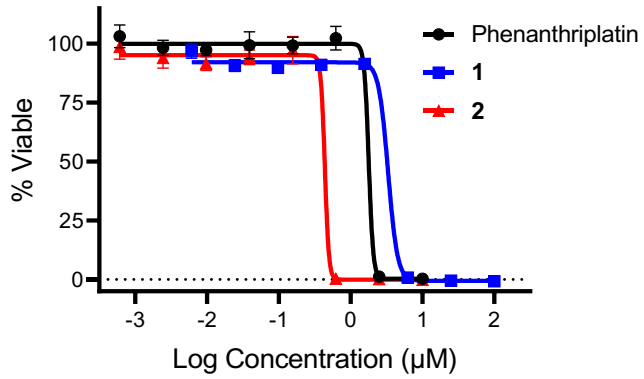

Figure S3. Dose response in HL-60 cells with phenanthriplatin, **1**, and **2**. Viability was determined 72 h after compound treatment. The measurements were collected in triplicate.

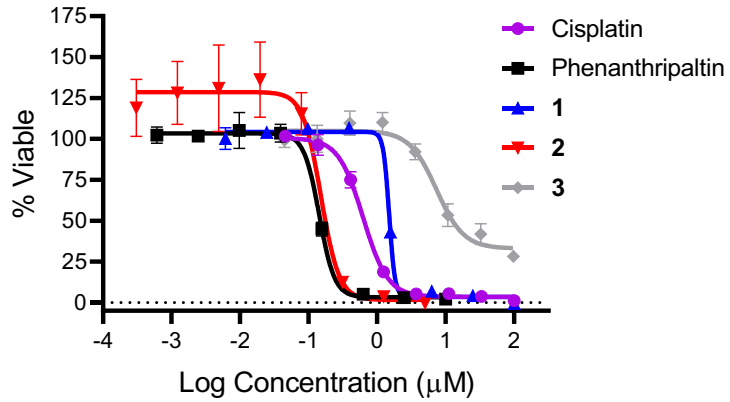

Figure S4. Dose response in DU145 cells with cisplatin, phenanthriplatin, **1**, **2**, and **3**. Viability was determined 72 h after compound treatment. Compound **3** was treated with 457 nm light (29 J/cm^2). The measurements were collected in triplicate.

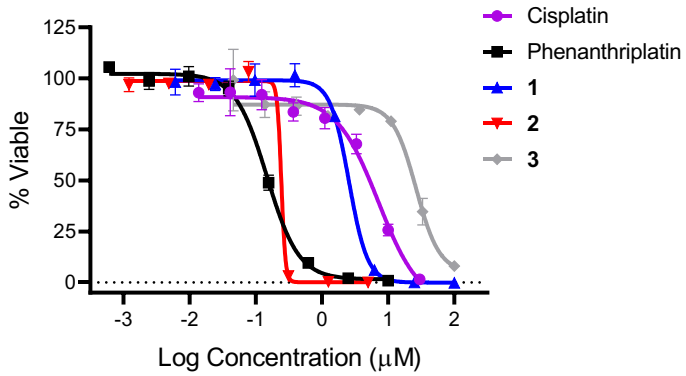

Figure S5. Dose response in MIA PaCa2 cells with cisplatin, phenanthriplatin, **1**, **2**, and **3**. Compound **3** was treated with 457 nm light (29 J/cm^2). Viability was determined 72 h after compound treatment. The measurements were collected in triplicate.

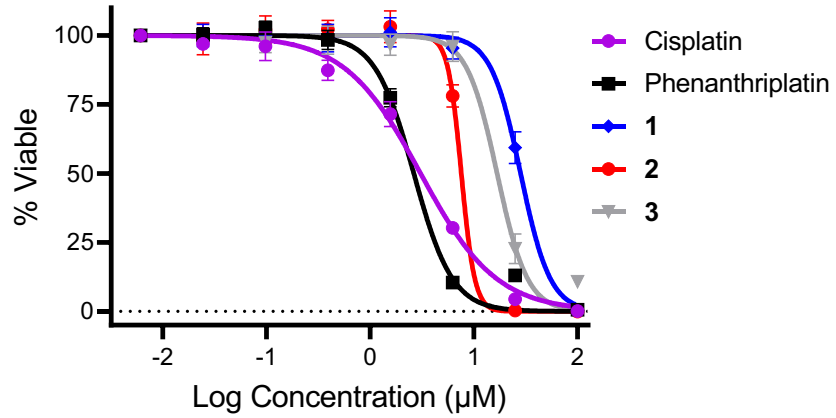

Figure S6. Dose response in HEL299 cells with cisplatin, phenanthriplatin, **1**, **2**, and **3**. Viability was determined 72 h after compound treatment. Compound **3** was treated with 457 nm light (29 J/cm^2). The measurements were collected in triplicate.

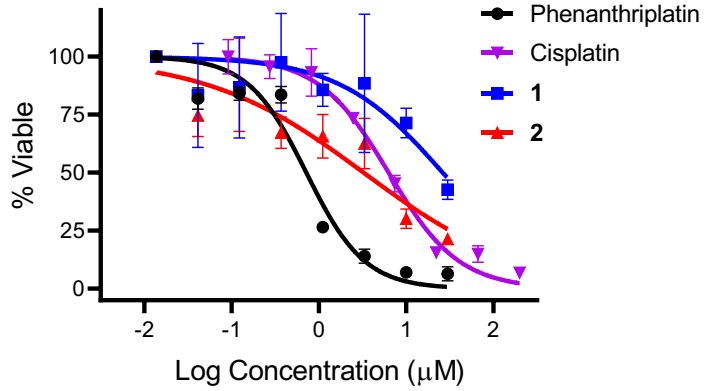

Figure S7. Dose response in DU145 spheroids with cisplatin, phenanthriplatin, **1**, **2**, and **3**. Viability was determined 72 h after compound treatment. The measurements were collected in triplicate.

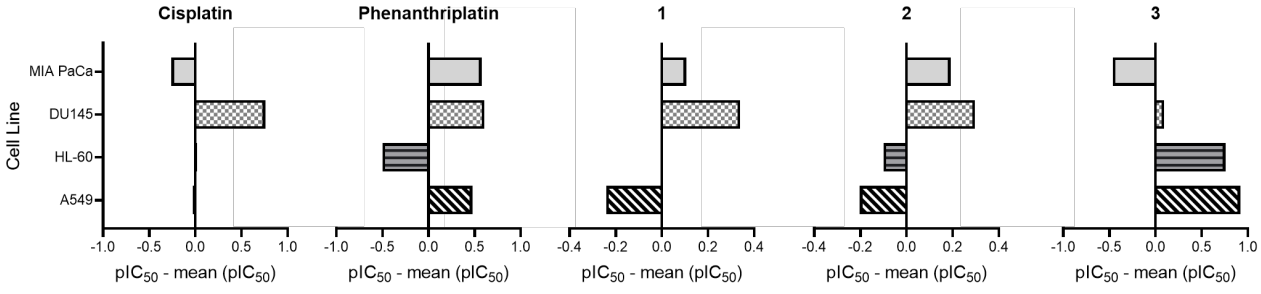

Figure S8. Sensitivity-resistance cytotoxicity difference plot display the sensitivity or resistance to various compounds for each cell line. Sensitivity is indicated by a positive value, whereas resistance is indicated by a negative value. The vertical line indicates the average pIC_{50}.

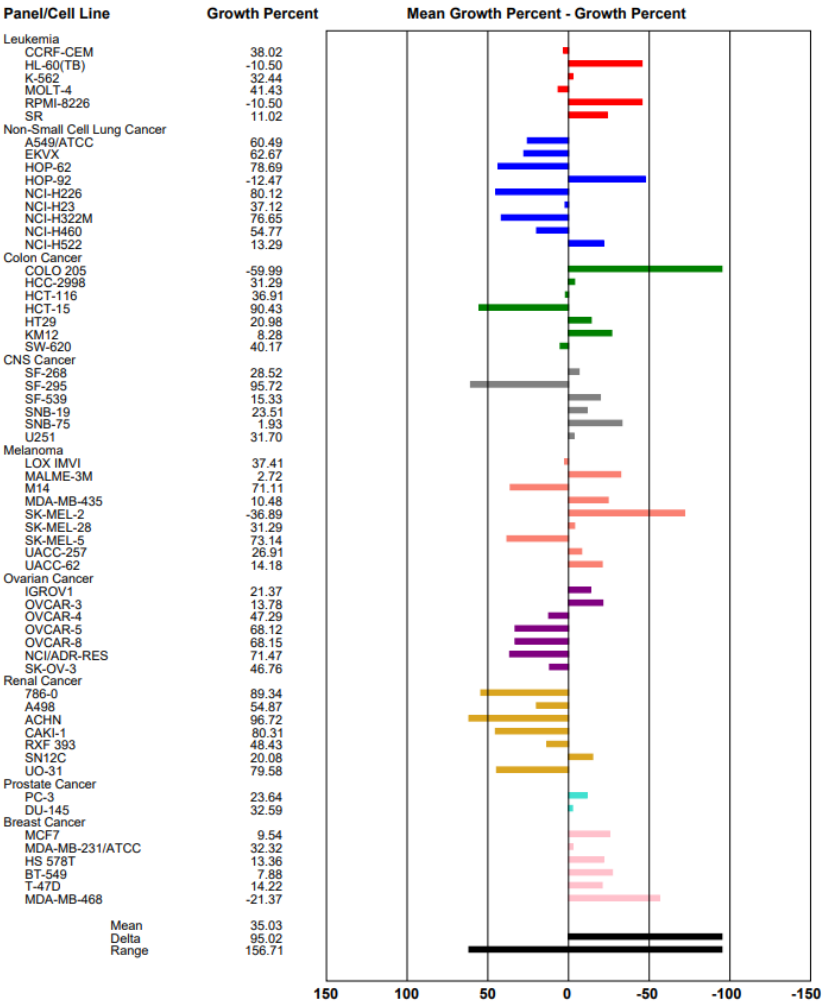

Figure S9. Single dose sensitivity profile for compound **1**.

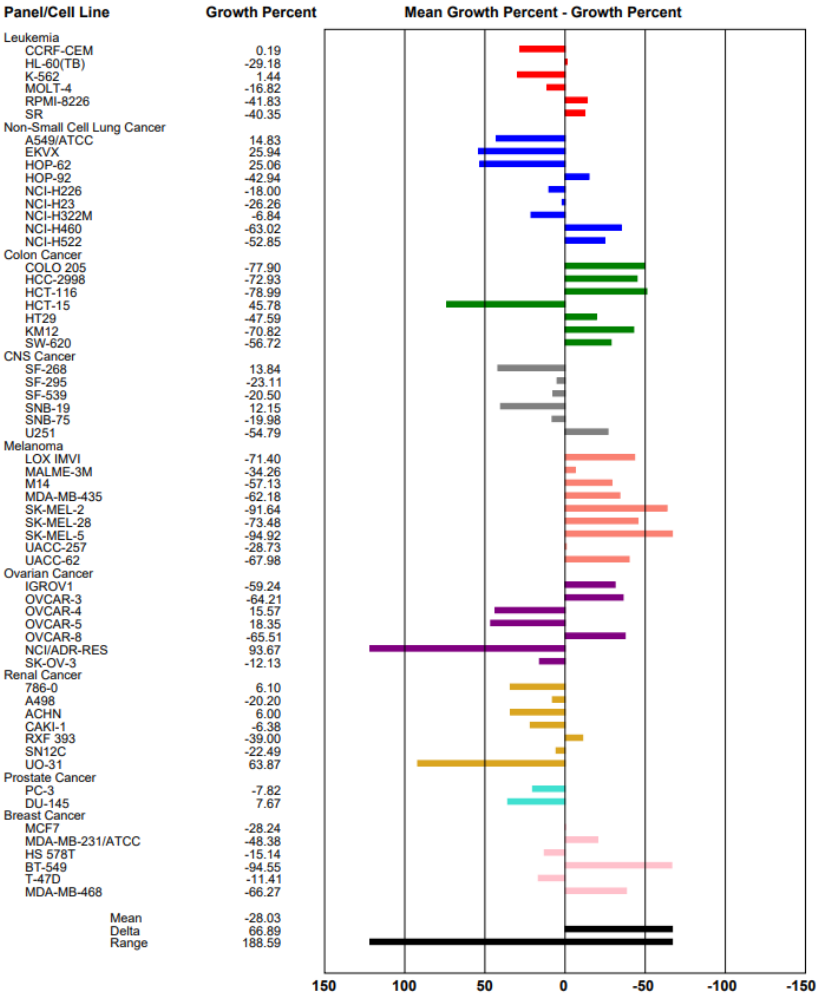

Figure S10. Single dose sensitivity profile for compound **2.**

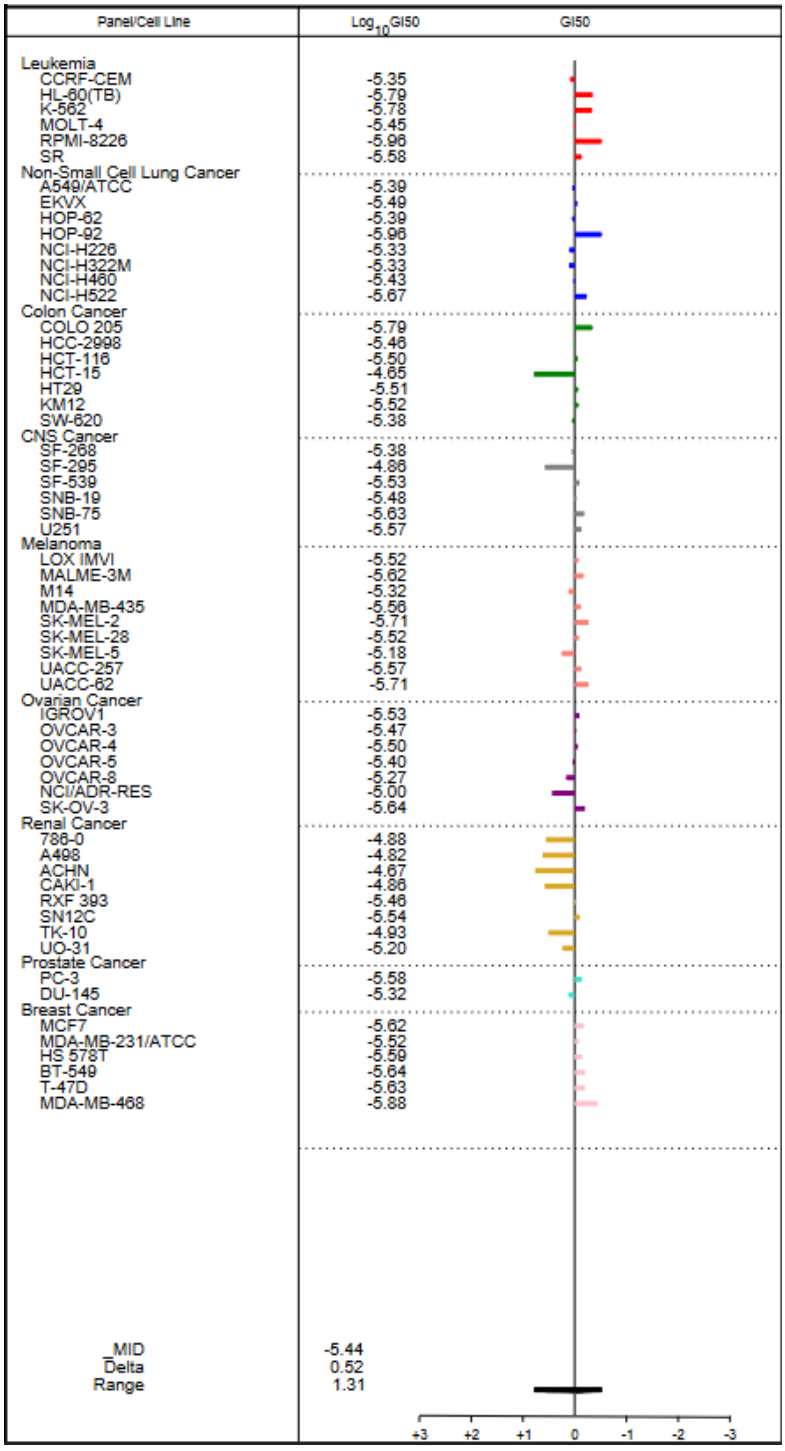

Figure S11. Five dose response sensitivity profile for compound **1**.

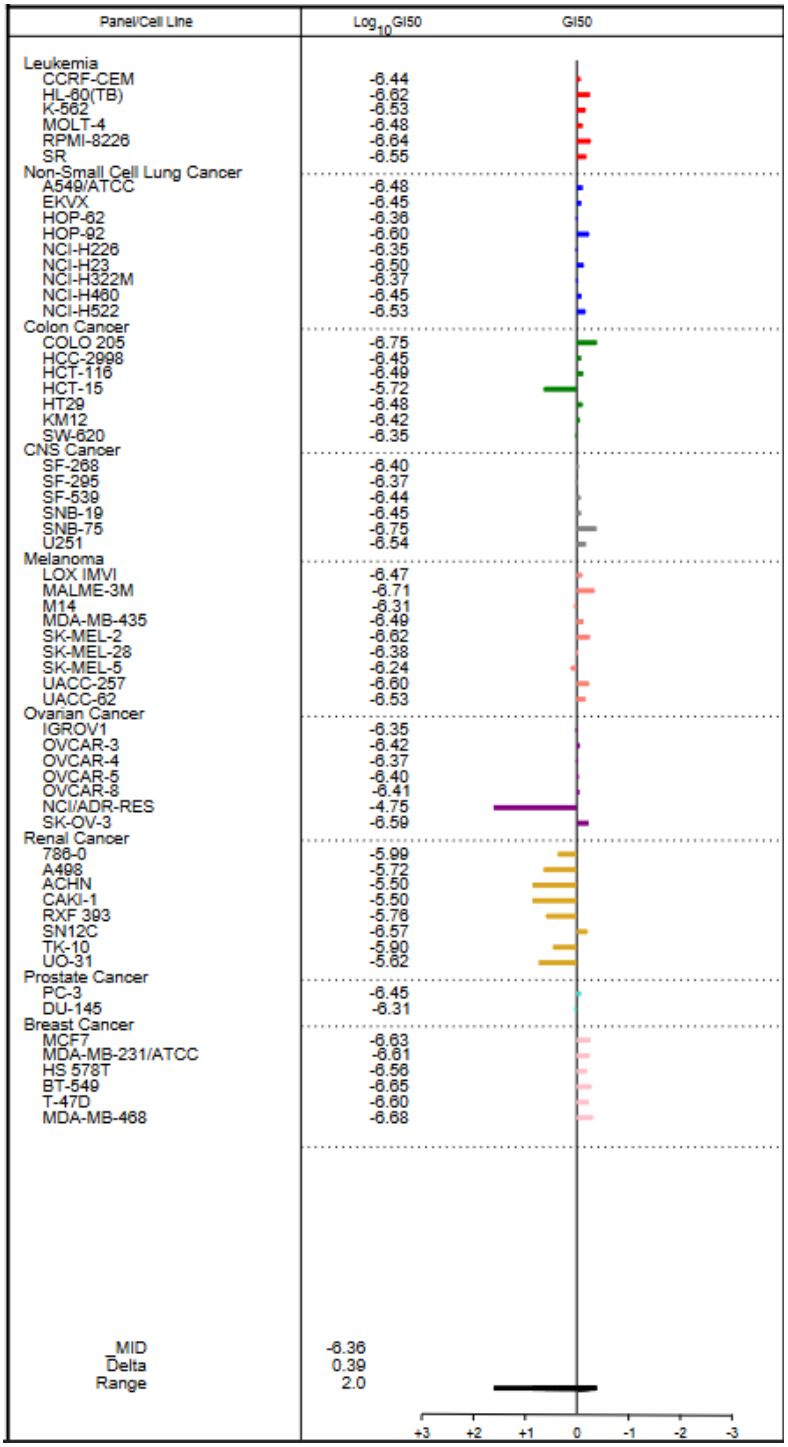

Figure S12. Five dose response sensitivity profile for compound **2.**

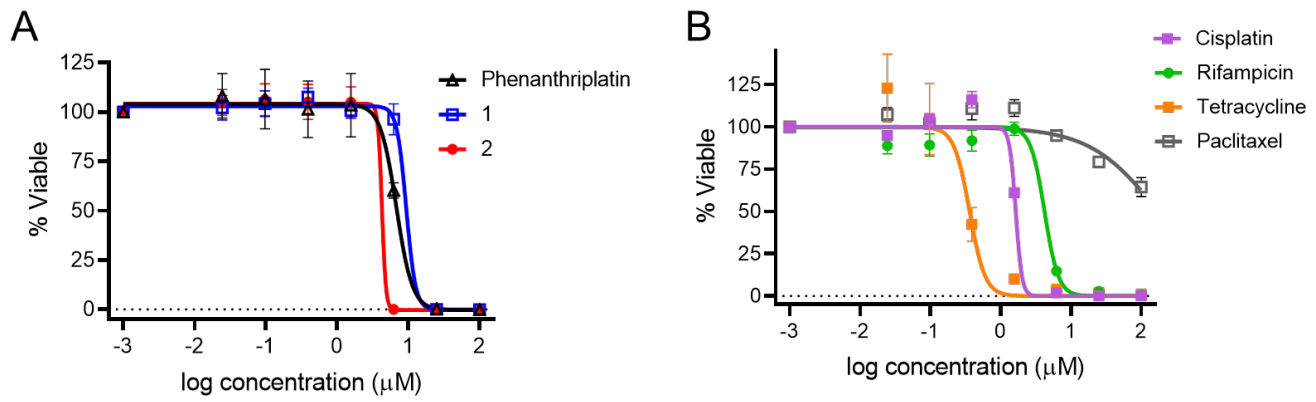

Figure S13. Bacterial growth inhibition. Bacterial dose response for A) phenanthriplatin, **1**, and **2**, B) cisplatin, rifampicin, paclitaxel, and tetracycline. Viability was measured 16 h after compound treatment. The measurements were collected in triplicate.

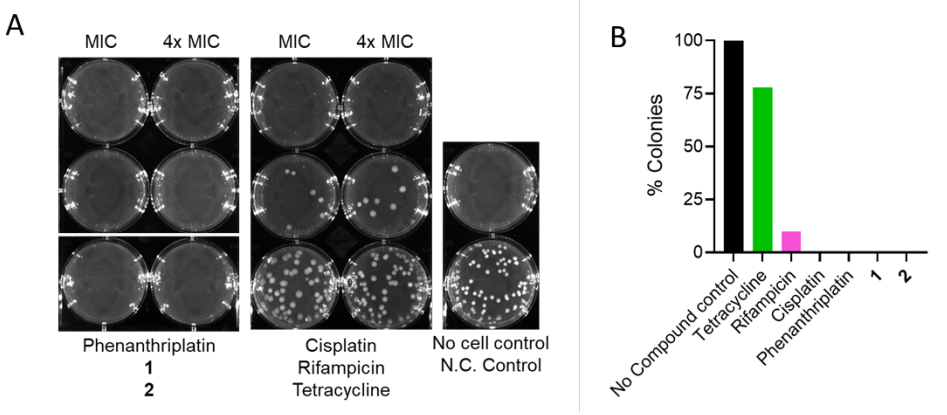

Figure S14. Clonogenic assay of phenanthriplatin, cisplatin, rifampicin, tetracycline, **1**, and **2**. A) For colony formation, ~2,500 cells were plated following 16 hr incubation with each compound at the MIC (left) or 4x the MIC (right). B) The colony number was plotted as a percentage of colonies in the sample vs. the colonies in the no compound control following compound treatment at the MIC.

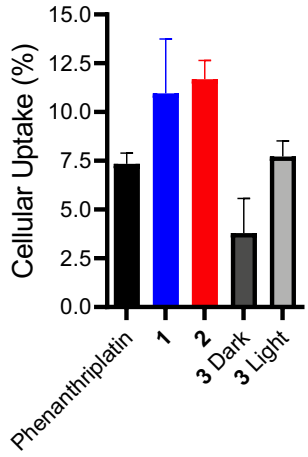

Figure S15. Cellular uptake of phenanthriplatin, **1**, **2,** and **3** in *E.coli*. Phenanthriplatin, **1**, and **2** were run in triplicate; dark and light samples of compound **3** were run in duplicate.

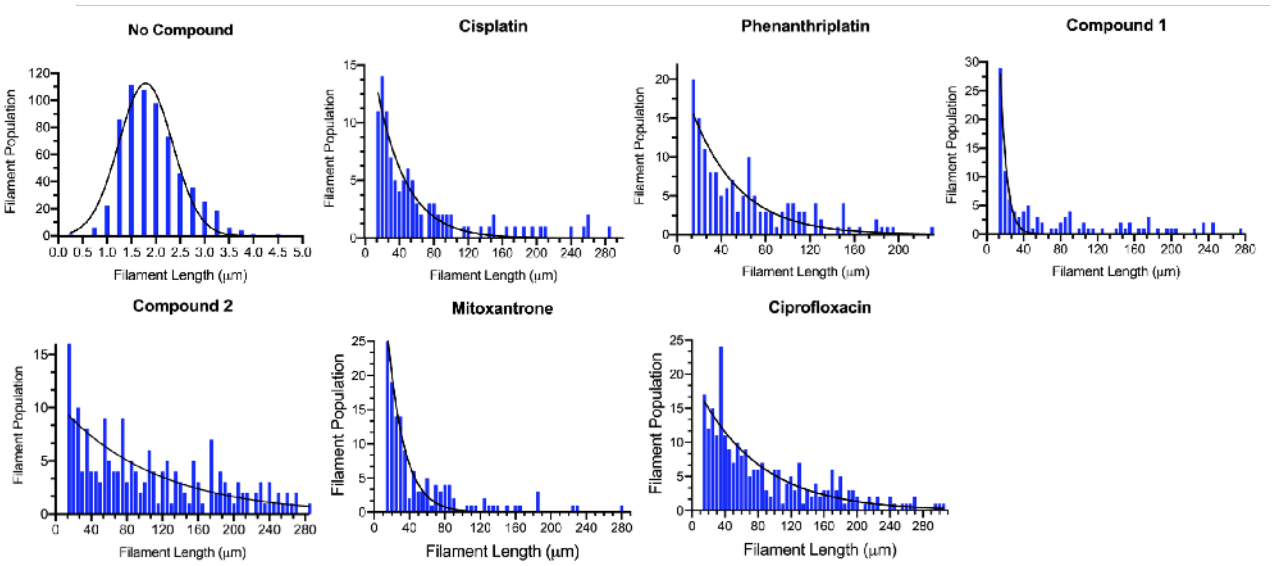

Figure S16. *E. coli* filament size distribution. The size distribution for 100 *E. coli* cells was quantified to measure stress response following a 16 h treatment with each compound indicated. The bacteria were treated at the MIC of each compound (See Fig. 2F).

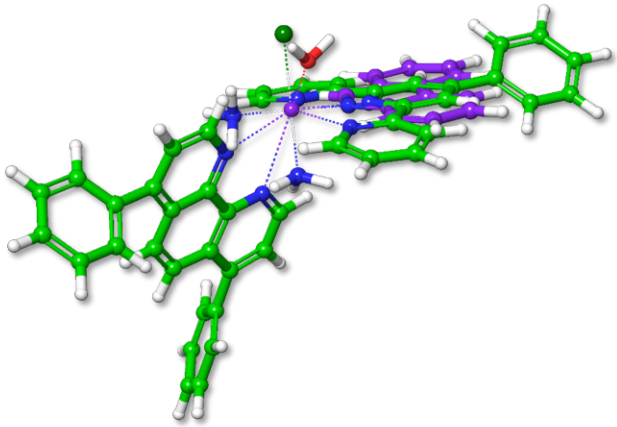

Figure S17. Docking overlay of **2** and phenanthriplatin. Compound **2** (green, modeled as the aqua species) and phenanthriplatin (purple) were overlayed, with the labile ligand for each positioned at the top. In this orientation, the extended tpy ligand projects over the phenanthridine ligand.

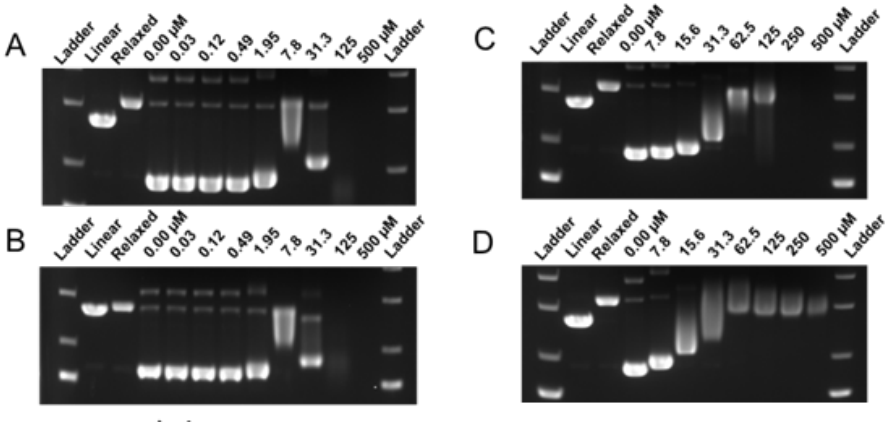

Figure S18. DNA damage gels. A) Agarose gels for DNA incubated with phenanthriplatin, B) phenanthriplatin with BSA, C) **1** with BSA, and D) **2** with BSA. All samples were incubated for 16 h at 37 °C before gel electrophoresis. The measurements were collected in duplicate for phenanthriplatin and **2**.

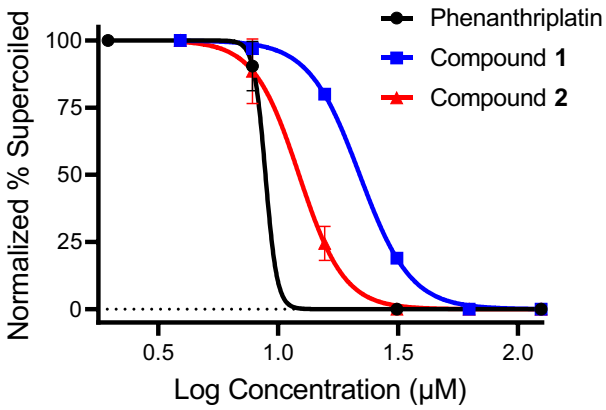

Figure S19. DNA damage gel quantification. Supercoiled DNA was quantified by densitometry and normalized to determine the percent of supercoiled DNA remaining following treatment with each compound at the concentrations indicated. The data was collected in duplicate except in the case for **1**.

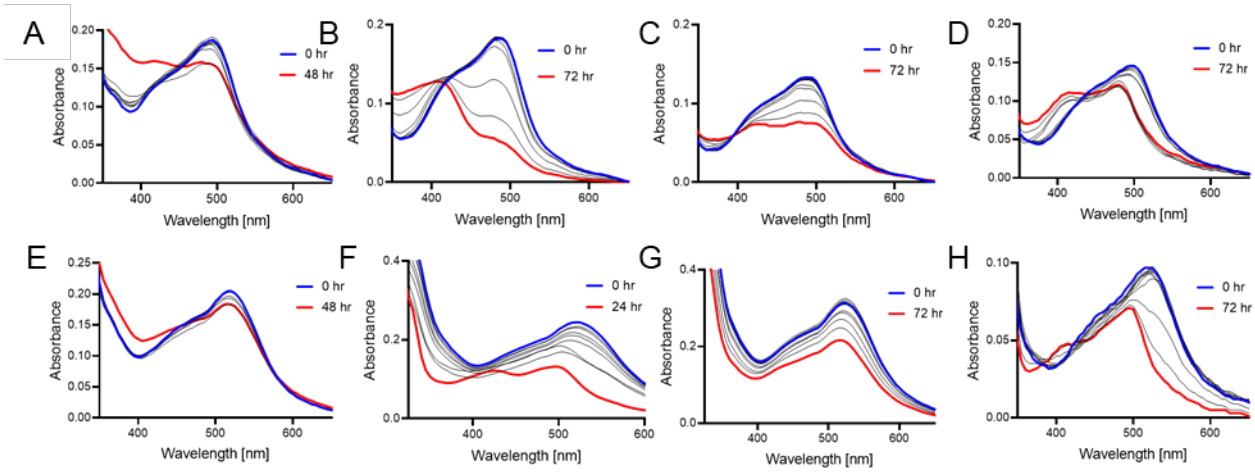

Figure S20. Aqueous stability assessment of **1** and **2**. Aqueous stability of **1** in A) Opti-MEM, B) 10 mM phosphate buffer, C) 10 mM phosphate buffer with 133 µg BSA, and D) 10 mM phosphate buffer, 133 µg of BSA, and 133 µg CT-DNA. Aqueous stability of **2** in E) Opti-MEM, F) 10 mM phosphate buffer), G) 10 mM phosphate buffer with 133 µg BSA, and H) 10 mM phosphate buffer, 133 µg of BSA, and 133 µg CT-DNA at 200 µL final volume. Different endpoints for A and E were due to media evaporation, resulting in unreliable data. The different endpoint for F was due to **2** precipitating at the 48 h and 72 h timepoints. The measurements were collected in triplicate.

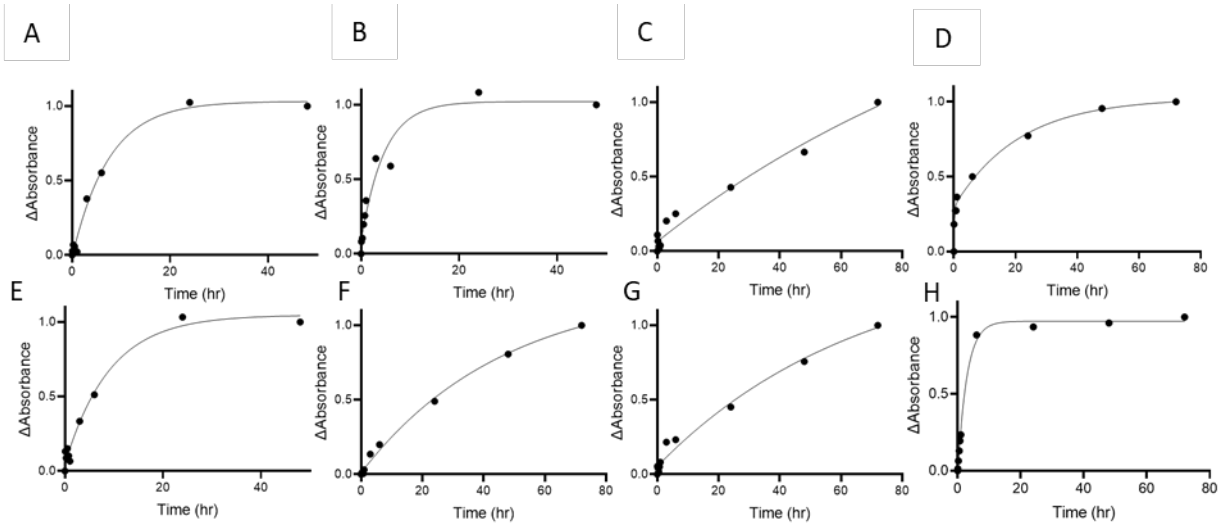

Figure S21. Normalized change in absorbance from the aqueous stability studies. The data reflects the stability of the compounds under differing conditions. The change in absorption at 505 nm of **1** in A) Opti-MEM, B) 10 mM phosphate buffer, C) 10 mM phosphate buffer with 133 µg BSA, and D) 10 mM phosphate buffer, 133 µg of BSA, and 133 µg CT-DNA. Normalized change in absorbance at 520 nm of **2** in E) Opti-MEM, F) 10 mM phosphate buffer, G) 10 mM phosphate buffer with 133 µg BSA, and H) 10 mM phosphate buffer, 133 µg of BSA, and 133 µg CT-DNA. The reason for the different endpoints for A, E, and F are described above. The measurements were collected in triplicate.

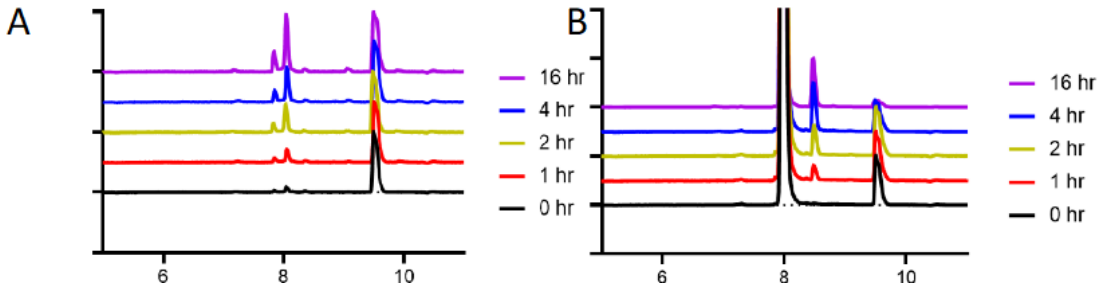

Figure S22. HPLC binding kinetics for phenanthriplatin. HPLC kinetics of 100 μM phenanthriplatin in A) diH_2O only; B) with 5 mM deoxyguanosine (dG). The peak at 7.8 minutes corresponds to deoxyguansine. Phenanthriplatin eluted at 9.5 mins. The detection wavelength was 280 nm.

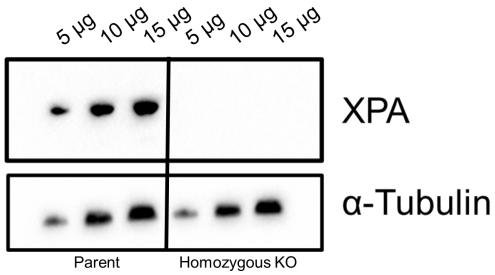

Figure S23. Western blot of XPA in HeLa parental and homozygous knockout. Increasing amounts of cell lysates (5–15 μg) were analyzed for presence of XPA to verify homozygous knockout for the XPA KO HeLa cell line. The loading control is α-tubulin.

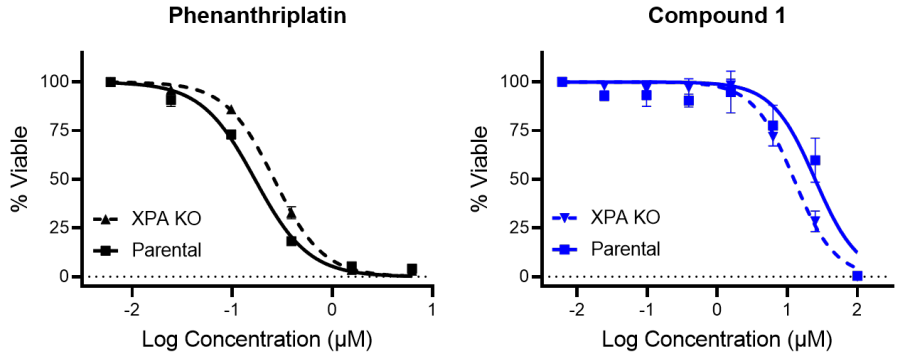

Figure S24. Cell cytotoxicity in HeLa parental and XPA knockout. Cytotoxicity was determined following a 72 h treatment with phenanthriplatin and compound **1**. The measurements were collected in triplicate.

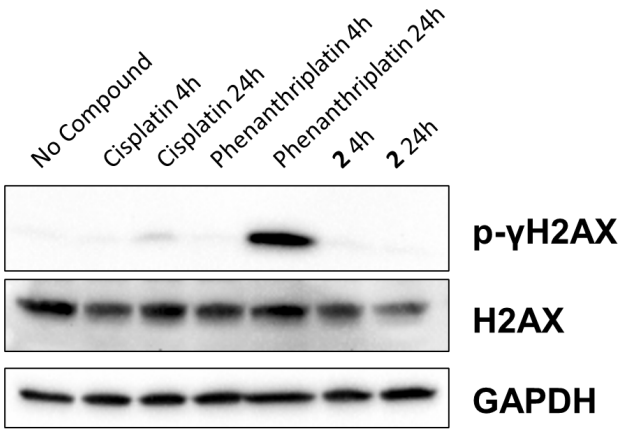

Figure S25. Immunoblotting of p-γH2AX, H2AX, and GAPDH following treatment with cisplatin, phenanthriplatin, and **2**. Approximately 10^6 A549 cells treated were with 5 μM cisplatin, 0.5 μM phenanthriplatin, or 0.5 μM **2** for the hours indicated. At 24 h, phosphorylation of H2AX was present for phenanthriplatin-treated cells, and to a much lower extent, cisplatin-treated cells.

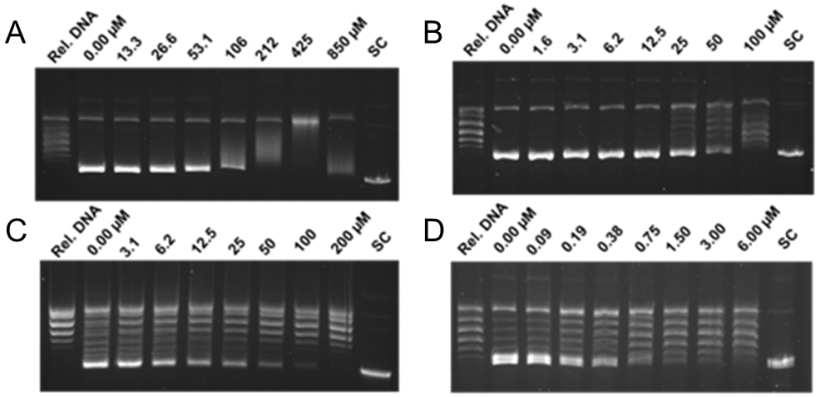

Figure S26. DNA gyrase inhibition gels. DNA Gyrase activity inhibition with A) phenanthriplatin, B) **1**, C) **2**, and D) ciprofloxacin. The reaction was performed for 1 h at 37 °C before quenching and gel electrophoresis. Note the different concentrations used in the dose responses. The measurements were collected in duplicate.

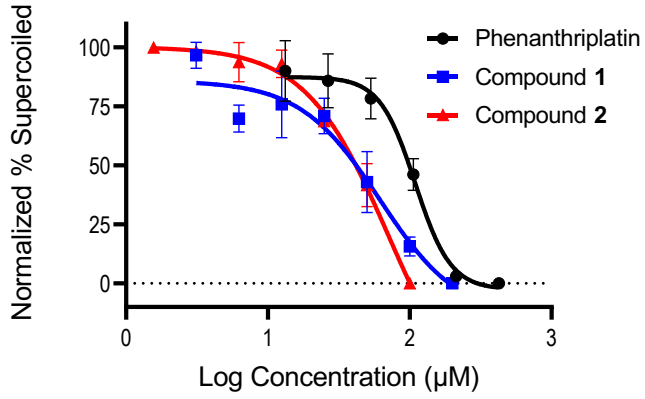

Figure S27. DNA gyrase activity inhibition quantification of **1**, **2**, and phenanthriplatin. Supercoiled DNA was quantified by densitometry and normalized to determine the percent of supercoiled DNA remaining following treatment with each compound at the concentrations indicated. Measurements were performed in duplicate.

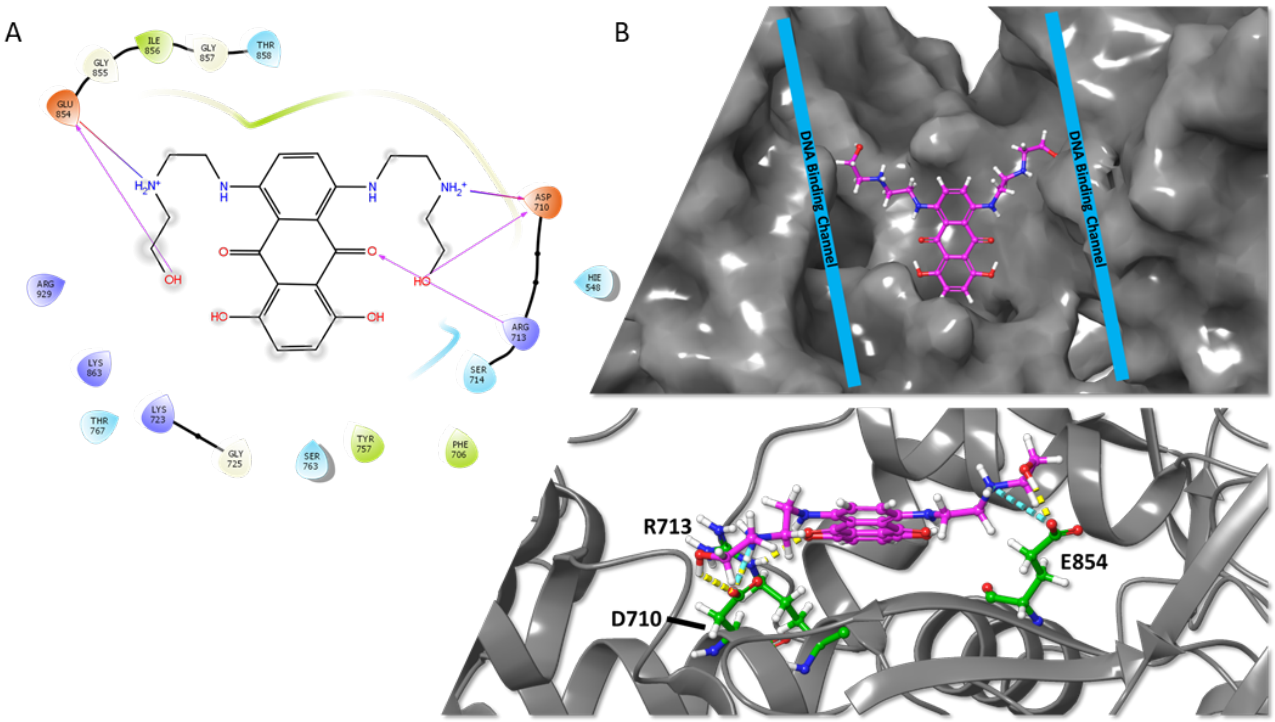

Figure S28. Rigid body docking of mitoxantrone in Topoisomerase IIα. A) Substrate-enzyme contact diagram. B) Mitoxantrone in the DNA binding channel and 3D amino acid contacts. Topoisomerase IIα from the crystal structure 4FM9 was used as previously reported.

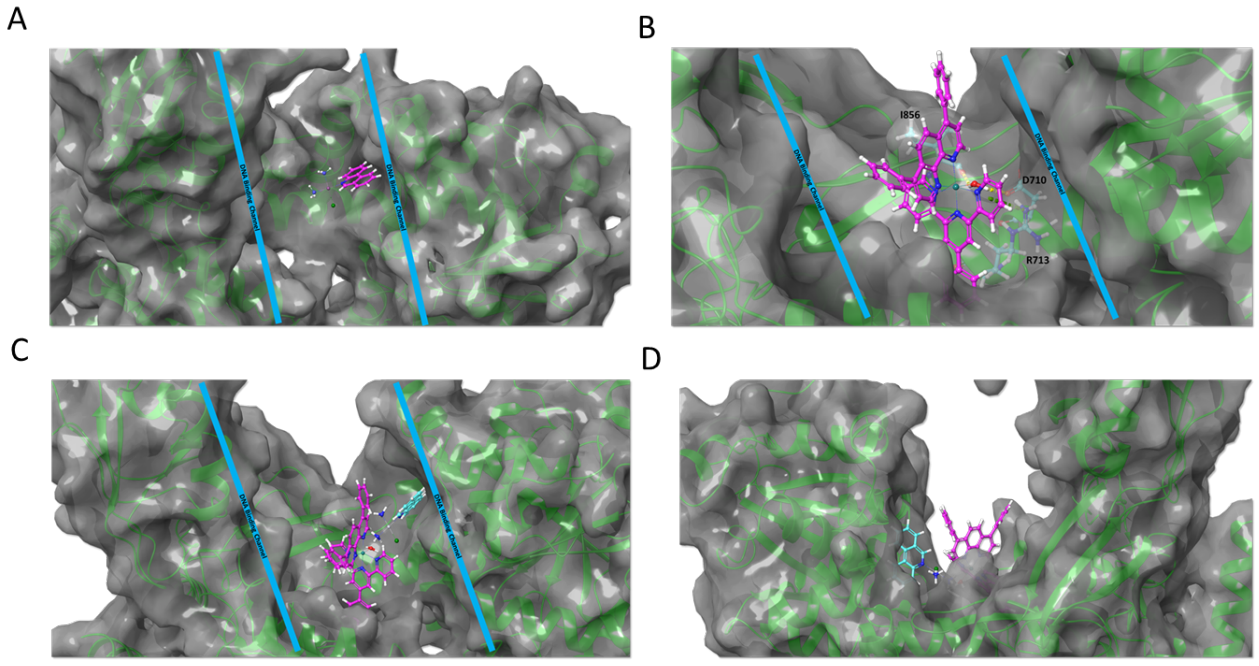

Figure S29. Rigid body docking of phenanthriplatin and **2** in Topoisomerase IIα**.** Rigid body docking of A) phenanthriplatin, B) compound **2**, C) both phenanthriplatin and **2** in the DNA binding pocket of Topoisomerase IIα**,** and D) side view of both phenanthriplatin and **2** in Topoisomerase IIα (crystal structure from PDB 4FM9).

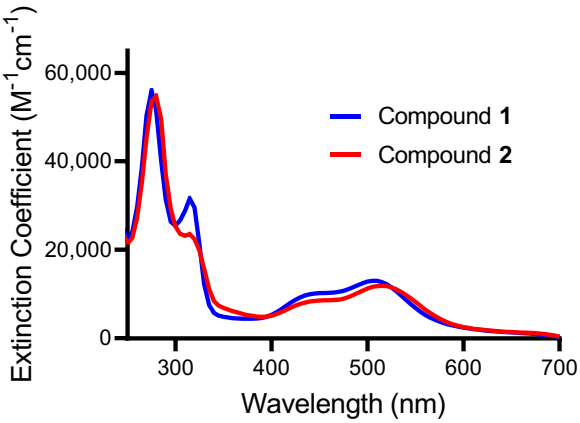

Figure S30. UV/Vis spectra of compounds **1** (blue) and **2** (red) in acetonitrile. The data is representative of triplicate measurements. The compounds were not emissive.

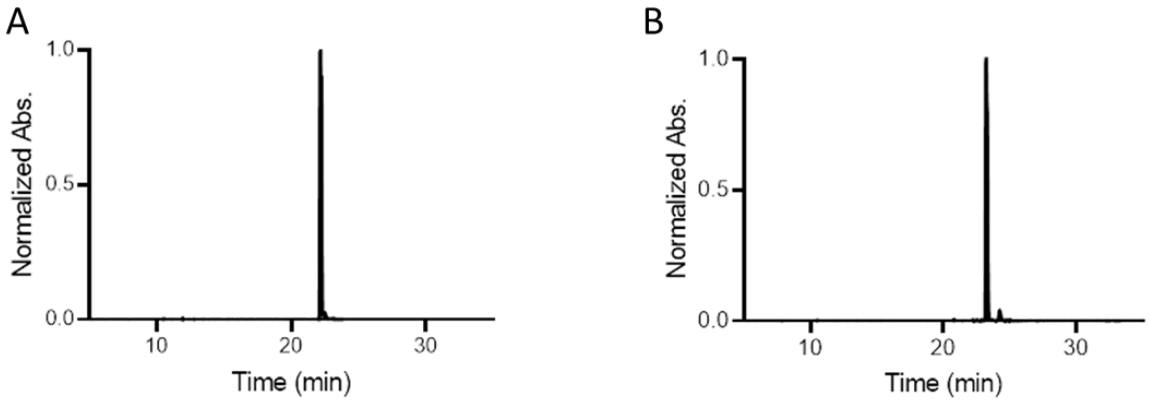

Figure S31. Analysis of purity of compounds by HPLC. A) Compound **1** (97 %) and B) compound **2** (96 %). The detection wavelength was 280 nm.

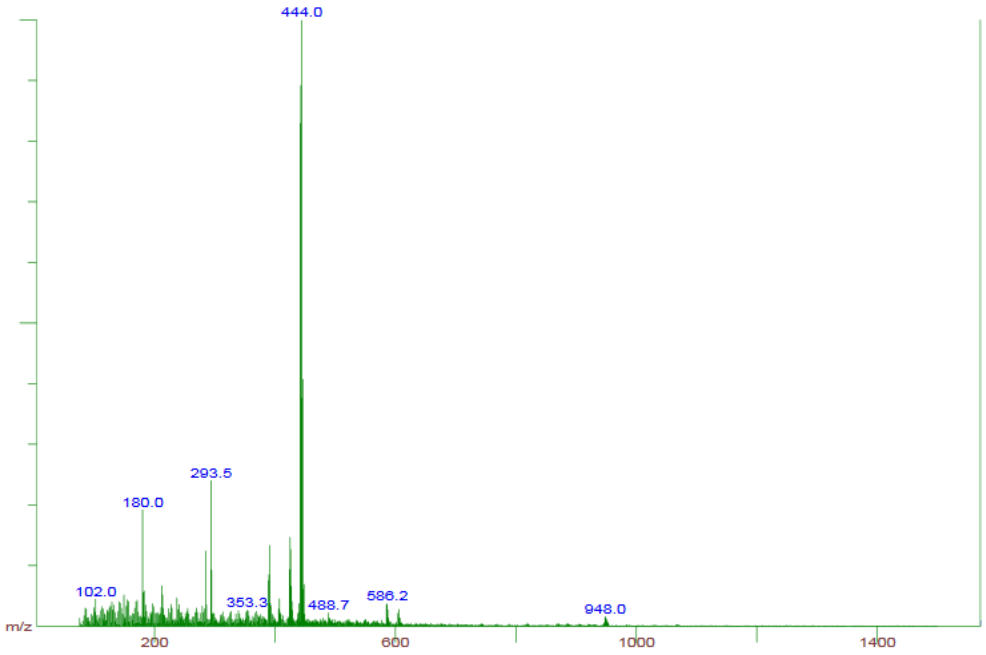

Figure S32. ESI-MS of phenanthriplatin.

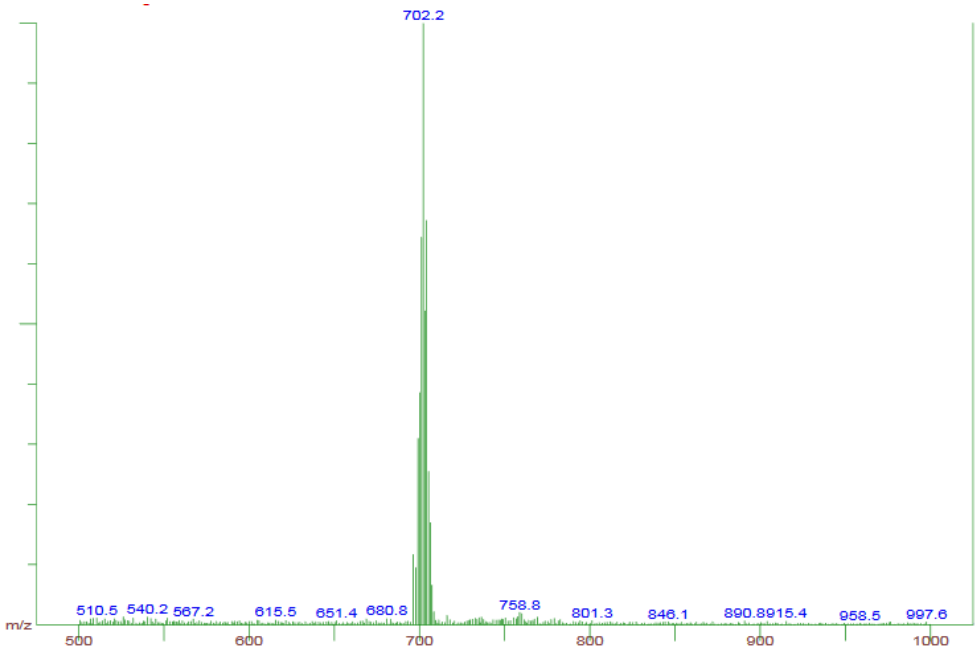

Figure S33. ESI-MS of compound **1**.

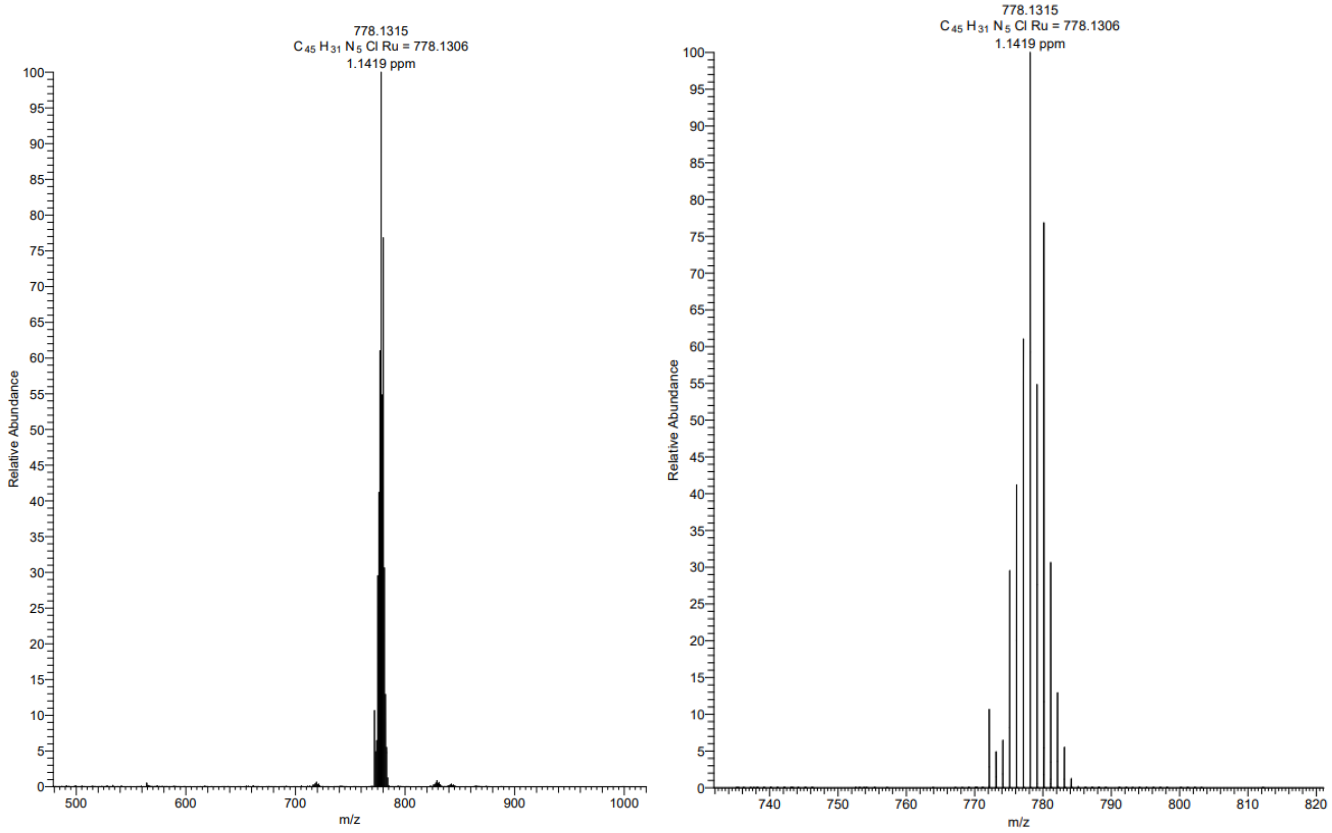

Figure S34. ESI-MS of compound **2** (aromatic region shown in inset)**.**

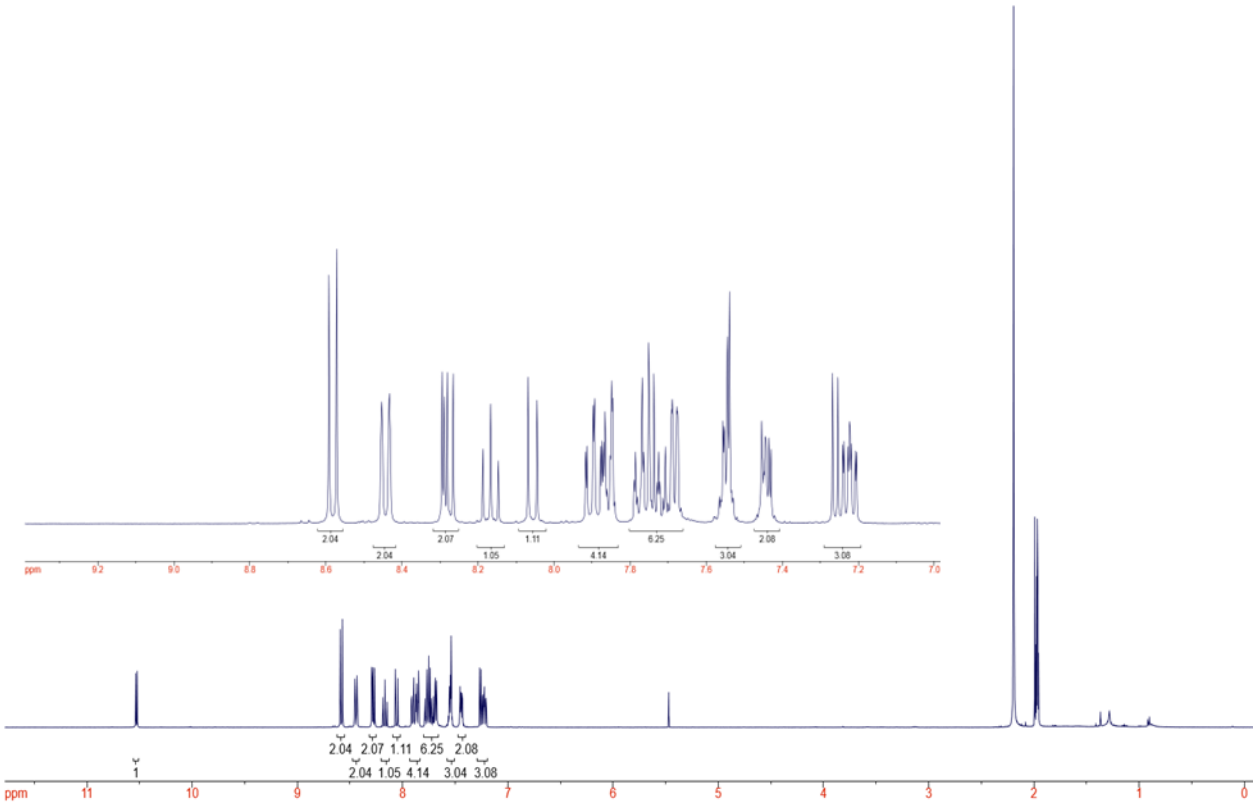

Figure S35. ^{1}H NMR of **1** in CD_3CN (aromatic region in inset).

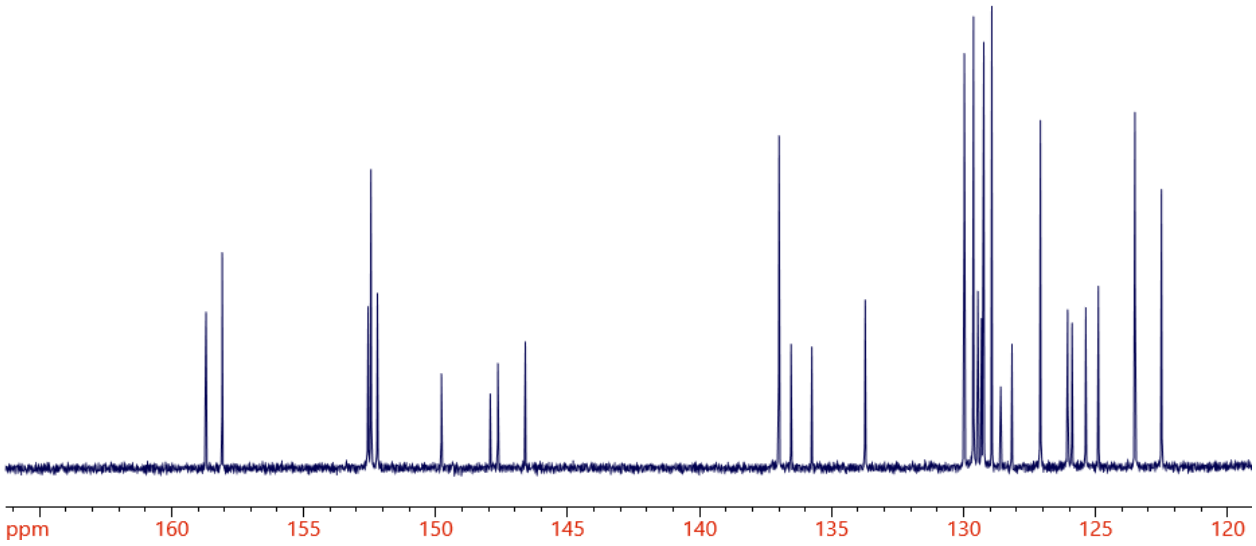

Figure S36. ^{13}C NMR of **1** in CD_3CN.

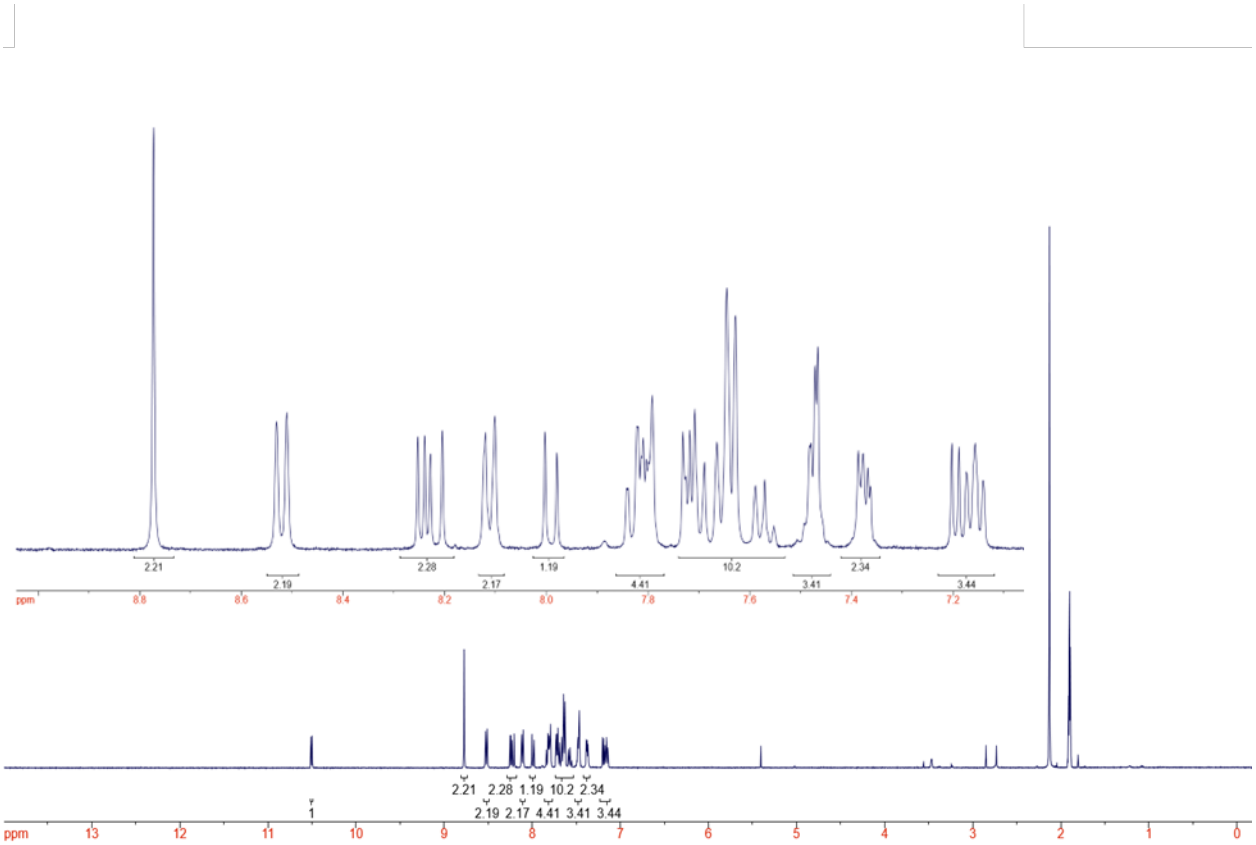

Figure S37. ^{1}H NMR of **2** in CD_3CN.

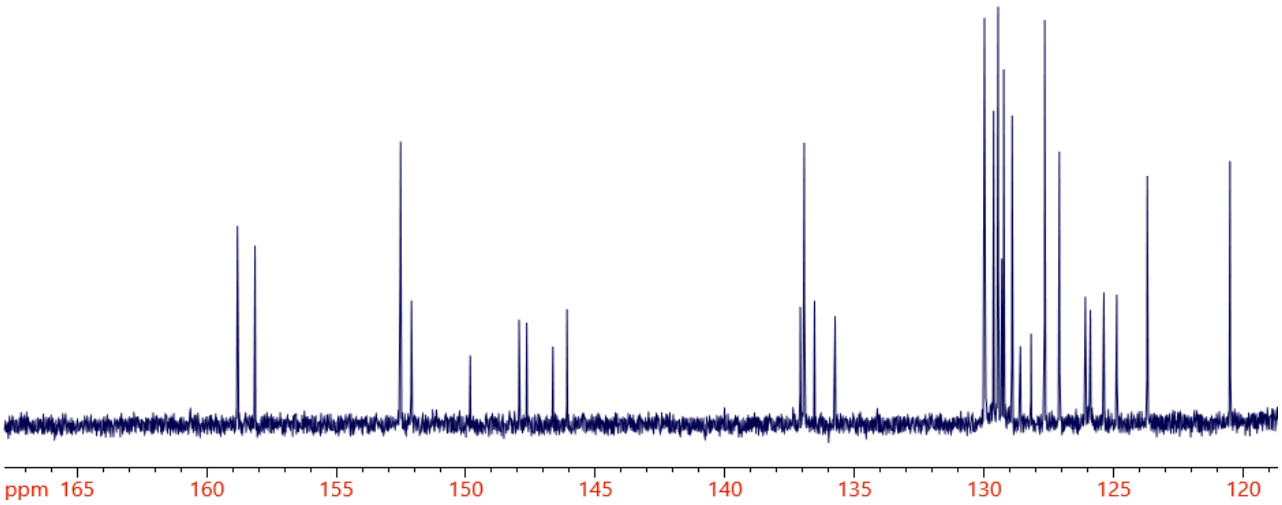

Figure S38. ^{13}C NMR of **2** in CD_3CN.

$$Average\ pIC_{50} = \frac{\Sigma\ cell\ line\ p(IC_{50})}{n_{cell\ lines}}$$

Equation 1. The average pIC_{50} calculation.

$$Specific\ cell\ line\ pIC_{50} - average\ pIC_{50}$$

Equation 2. Formula for generating sensitivity-resistance plot.

$$Cellular\ Uptake\ (\%) = \frac{metal\ in\ cells\ (ppb)}{metal\ in\ cells\ (ppb)\ + (dil.\ factor\ X\ metal\ in\ media\ (ppb))}\ X\ 100$$

Equation 3. Percent cellular uptake.

Table S1. COMPARE analysis results

Compound	**Correlation**
Compound **2**	*Reference*
Chromomycin A3	0.85
Paclitaxel	0.71
Actinomycin D	0.69
Vinblastine	0.64
Doxorubicin	0.55
Etoposide	0.39
Carboplatin	0.12
Cisplatin	-0.16
Compound **1**	N.D.
Oxaliplatin	N.D.

The correlation was obtained from a COMPARE Analysis of the five-dose data.

Table S2. Cellular uptake

Compound	Cellular Uptake %(st. dev)	Metal Atoms per Cell (st. dev)
Phenanthriplatin [a]	7.34 (0.55)	5.28×10^8 (4.6×10^7)
Compound **1** [a]	11.68 (0.96)	7.48×10^8 (9.9×10^7)
Compound **2** [a]	10.96 (2.78)	1.01×10^9 (5.5×10^8)
Compound **3** (dark) [b]	3.79 (1.78)	8.17×10^8 (1.9×10^8)
Compound **3** (light) [b]	7.72 (0.80)	1.73×10^9 (4.4×10^8)

[a] The average across triplicate measurements. [b] The average across duplicate measurements.

Table S3. DNA metalation [a]

Compound	nt/mc (± St. Dev.)
Cisplatin	540 (164)
Phenanthriplatin	345 (200)
Compound **1**	1,083 (598)
Compound **2**	3,137 (1,151)

[a] The average across triplicate measurements.

Table S4. DNA damage and topoisomerase inhibition

Compound	DNA Damage EC_{50}	DNA Gyrase EC_{50}	ICE (fold increase in intensity)
Phenanthriplatin	8.8 (0.5)	87.0 (8.4)	1.8
Compound **1**	21.9	35.1 (12.0)	n.d.
Compound **2**	12.2 (1.8)	37.8 (2.4)	0.9

DNA damage and DNA Gyrase EC_{50} values were determined by percent of supercoiled DNA present after each treatment concentration. The ccDNA was determined by the amount of DNA required to be loaded for immunodetection. Duplicate values were measured for DNA damage and DNA gyrase EC_{50} determination except in the case for compound **1**. For the ICE data, the values are reported as fold increase in intensity vs. the no treatment control. Values reported under 1.0 represent a decrease in intensity compared to the no treatment control. Not determined (n.d.) indicates that the sample was not assessed for ccDNA formation.

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