Supporting Information for "Probing Platinum–Adenine-N3 Adduct Formation with DNA Minor-Groove Binding Agents"

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Experimental Procedures

Materials. The DNA sequences were synthesized and HPLC-purified by IDT (Coralville, IA). Buffers were prepared from biochemical grade chemicals in ultrapure laboratory grade water obtained from a Milli-Q A10 synthesis water purification system. The complementary sequences 5´-GGCCTTTAATATGTGCATGGTTAGTTAAACATGGTTATGAATCAAC (**46top**) and 5´-GTTGATTCATAACCATGTTTAACTAACCATGCACATATTAAAGGCC (**46bot**) were dissolved in 20 mM Tris buffer (pH 7.2) and stored at −20 °C. DNA concentrations were determined spectrophotometrically using the extinction coefficients provided by the manufacturer. Cisplatin was synthesized according to the procedure developed by Dhara (*1*). PT-ACRAMTU, [Pt(en)(ACRAMTU-*S*)Cl]$(NO_3)_2$ (en = ethane-1,2-diamine, ACRAMTU = 1-[2-(acridine-9-ylamino)ethyl]-1,3-dimethylthiourea, acridinium cation), was synthesized according to the published procedure (*2*) and dissolved in Milli-Q water and stored in the dark at −20°C. Platinum concentrations were determined by UV-visible spectroscopy (*3*). Me-lex$^{py/py}$ (methyl-3-(1-methyl-5-(1-methyl-5-(propylcarbamoyl)-1*H*-pyrrol-3-ylcarbamoyl)-1*H*-pyrrol-3-ylamino)-3-oxopropane-1-sulfonate) was provided by Prof. Barry Gold (Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh). Me-lex$^{py/py}$ was stored on desiccant at −80 °C and reconstituted in Milli-Q water containing 10% DMF as a 1 mM stock solution, which was appropriately diluted with Tris buffer prior to incubation with DNA samples.

Chemical Footprinting Assay. The sequence **46top** was labeled with [γ-^{32}P]-ATP (Perkin Elmer, Waltham, MA) at the 5´ end using T4 polynucleotide kinase (New England Biolabs, Ipswich, MA). The labeled DNA was mixed with unlabeled **46top** and **46bot** to produce a final strand concentration of 5 μM. The solution was heated to 90 °C and slowly cooled to room temperature to allow the DNA strands to hybridize. Footprinting reactions were assembled as follows: Maxam–Gilbert G and G+A lanes were generated using a standard protocol (*4*). Incubations of unmodified DNA with Me-lex$^{py/py}$ were performed at two different concentrations of alkylating agent (20 and 50 μM). The mixtures were incubated at 37 °C for 16 h. In another set of reactions, the DNA was incubated in the dark at 37 °C for 24 h with 10, 20, 40, and 60 μM PT-ACRAMTU to generate DNA modified with varying levels of platinum (r_b, drug-to-nucleotide ratio). The platinated DNA samples were then reacted with 50 μM Me-lex$^{py/py}$ at 37 °C for 16 h. From each reaction, a volume of 10 μL was removed and diluted with 190 μL of 20 mM Tris buffer, and 4 μL of sonicated calf thymus DNA (1 mg/mL) were added. The samples were heated at 90 °C for 15 min to induce depurination of methylated nucleobase and immediately quenched on ice. To precipitate the DNA, 50 μL of sodium acetate (0.3 M) and 900 μL of ethanol were added. After the mixtures were stored at −20 °C for 15 min, the samples were centrifuged and the supernatant decanted. Platinum-modified samples were incubated with 20 μL of sodium cyanide (1 M) in the dark at 37 °C for 16 h. The subsequent induction of DNA strand breaks was achieved using standard manipulations described in the Maxam–Gilbert assay (*4*). Finally, all samples were heat-denatured and separated on 16% polyacrylamide gel containing 8 M urea. The gels were dried and scanned on a BioRad FX-Pro plus phosphorimager (Hercules, CA). Band intensities were integrated using the Quantity One 1-D Analysis Software (version 4.1.1, Bio-Rad Laboratories, Hercules, CA).

Cell Proliferation Assay. The cell proliferation assay was performed according to a published procedure (*5, 6*) using the Celltiter 96 Aqueous Non-Radioactive Cell Proliferation Assay kit (Promega, Madison, WI). NCI-H460 were incubated with netropsin for 30 min prior to treatment with platinum complex. Cells were incubated with platinum for 72 h. Drug–response curves were generated by fitting the data sets to the appropriate sigmoidal function, y = min+[(max-min)/[1+10^(log IC_{50}-x)*hillslope*], in SigmaPlot 9.0 (Systat Software Inc., San Jose, CA).

Reactivity of PT-ACRAMTU with Netropsin. Since the reduced potency (higher IC_{50} values) could also have been the result of PT-ACRAMTU being consumed in a direct reaction with netropsin, experiments to rule out this possibility were performed:

Netropsin was incubated with PT-ACRAMTU in physiological phosphate-buffered saline (PBS) for 72 h at 37 °C (conditions used in the cell proliferation assay) in D_2O, and the reaction was monitored by ^1H NMR spectroscopy. The spectra of netropsin and PT-ACRAMTU were virtually unchanged and no new sets of peaks were observed, indicating that no reaction had occurred. To further rule out platinum–netropsin adduct formation, the mixtures were analyzed by in-line liquid chromatography and electrospray mass spectrometry in negative and positive ion modes. As in the NMR experiment, there were no signs of platinum–netropsin adduct formation, and the only new species that had formed was assigned to the hydrolysis product of PT-ACRAMTU. Based on these data, adduct formation between platinum and netropsin can be firmly excluded.

Summary of Cell Proliferation Assay.

Table S1. Summary of Cytotoxicity Data.

	IC_{50}	SE
PT-ACR[a] + 0 μM Net[c]	0.068 μM	±0.003
PT-ACR + 6.25 μM Net	0.139 μM	±0.009
PT-ACR + 12.5 μM Net	0.138 μM	±0.006
PT-ACR + 25 μM Net	0.163 μM	±0.013
Cisplatin[b] + 0 μM Net	1.506 μM	±0.16
Cisplatin + 6.25 μM Net	1.552 μM	±0.08
Cisplatin + 12.5 μM Net	1.311 μM	±0.08
Cisplatin + 25 μM Net	1.245 μM	±0.09

[a] Average of 3 experiments performed in triplicate ± SE.

[b] Average of 2 experiments performed in triplicate ± SE.

[c] The cell viability (% of control) in incubations with netropsin (net) in the absence of platinum drug was 100% for [net] = 6.25–25 μM, 92.5% for [net] = 50 μM, and 57.4% for [net] = 75 μM.

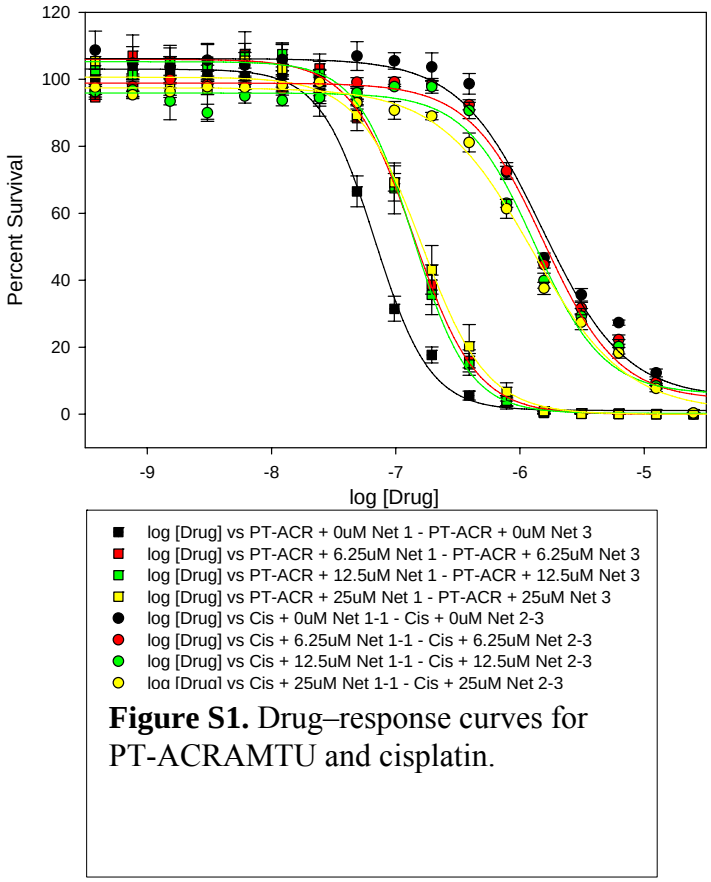

Figure S1. Drug–response curves for PT-ACRAMTU and cisplatin.

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