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ORIGINAL ARTICLE

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Platinum-acridinylthiourea conjugates show cell line-specific cytotoxic enhancement in H460 lung carcinoma cells compared to cisplatin

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Abstract Recently, we reported a new class of DNA-targeted hybrid platinum-acridine agents. The parent intercalator, ACRAMTU, a 9-aminoacridine derivative, intercalates into the minor groove of DNA, causing the corresponding prototypical conjugate, PT-ACRAMTU (type I/$n=2$), to form DNA adducts dissimilar to traditional platinum drugs. Both these agents show cytotoxic activity in leukemic and ovarian cancer cells. Following the use of clonogenic survival assays, we report on the cytotoxic effects of ACRAMTU, PT-ACRAMTU, and three PT-ACRAMTU derivatives, on additional cell lines including colon (RKO), lung (H460), and cisplatin-sensitive (A2780) and cisplatin-resistant (A2780/CP) ovarian cells. While a dose-dependent effect was observed with both ACRAMTU and PT-ACRAMTU, an enhanced cytotoxic effect was seen with PT-ACRAMTU in all cell lines. PT-ACRAMTU appeared to have a similar IC_{50} value to cisplatin except in H460 lung cancer cells in which PT-ACRAMTU had a twofold lower IC_{50} value. PT-ACRAMTU appeared to act in a time-dependent manner. In H460 cells the IC_{50} value of PT-ACRAMTU was 235-fold higher following a 1-h incubation than following a 24-h incubation (0.27 μ*M*), while following an 8-h incubation the IC_{50} value was 0.41 μ*M*. Three derivatives of PT-ACRAMTU were also tested. A tetraalkylated derivative, type II/$n=2$, generated the highest IC_{50} values in all cell lines, while the trialkylated derivative, type III/$n=2$, generated IC_{50} values similar to its isomer, PT-ACRAMTU. PT-ACRAMTU with an added CH_2 group in the thiourea linker (type I/$n=3$) showed IC_{50} values similar to the type I/$n=2$ prototype in H460 lung cells. An apoptotic response to PT-ACRAMTU appeared to be generated in H460 cells as evidenced by DNA laddering. These results suggest that type I/$n=2$ and type I/$n=3$ may be promising agents for the treatment of lung cancer and should be pursued in animal models.

Keywords Platinum · Acridine · Cytotoxicity · Apoptosis · DNA laddering

Abbreviations ACRAMTU: 1-[2-(acridin-9-ylamino-ethyl]-1,3-dimethylthiourea · PT-ACRAMTU: $[PtCl(en)(ACRAMTU)](NO_3)_2$ ·

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Introduction

Traditional platinum based therapies including cisplatin have been used to treat a variety of cancers with some efficacy; however, several problems have been associated with these therapies including intrinsic and acquired cellular resistance, as well as toxicity [7, 12, 17, 21, 24, 26]. Although over 3000 platinum compounds have been synthesized, only cisplatin, carboplatin, and oxaliplatin are currently being used clinically in the USA [11]. These drugs predominantly form intrastrand crosslinks at adjacent guanine/guanine or guanine/adenine residues and are believed to inhibit replication and transcription [14, 18, 22]. Although some success has been found with

these agents clinically, their usefulness is limited due to some of the issues mentioned above.

We have developed a new class of platinum compounds composed of a monofunctional platinum metalating unit tethered through a thiourea linkage to an acridine intercalator (ACRAMTU) [4, 20]. Our prototypical drug, PT-ACRAMTU (type I/$n=2$, where n refers to the number of methylene groups within the linker chain that connects the thiourea nitrogen with the 9-aminoacridine unit), binds predominantly to guanine residues (80%) at the N7 position [5] in the major groove of DNA in a similar manner to other platinum drugs [18]. However, it also exhibits unprecedented binding to adenine residues in the minor groove [3]. ACRAMTU and PT-ACRAMTU can generate a cytotoxic response in cisplatin-sensitive and cisplatin-resistant ovarian cells, as well as leukemic cells [20]. The presence of the platinum moiety provides an enhanced cytotoxic response and is able to partially relieve cisplatin resistance in C13* ovarian cancer cells [20].

Because platinum-based treatment strategies are used to treat several different types of cancer, we further explored the cytotoxic effects of ACRAMTU and PT-ACRAMTU in other solid tumor cell lines including lung, colon, and additional ovarian cell lines to determine if there is a cancer-specific response. We also assessed the importance of length of drug incubation on these cells. Finally, derivatives of the prototypical drug were tested for their in vitro cytotoxicity to assess the effects of structural modifications of the acridine moiety on the biological activity of the conjugates.

Materials and methods

Drugs

The synthesis of ACRAMTU, PT-ACRAMTU (type I/$n=2$), type II/$n=2$, and type III/$n=2$ has been reported elsewhere [1, 20]. Cisplatin was purchased from Sigma Chemicals. All drugs were serially diluted in 0.9% saline immediately prior to the incubations. Stock solutions of each drug were aliquoted and stored at −20°C.

Synthesis of the type I/$n=3$ conjugate

The 9-aminoacridine precursor, 1-[3-(acridin-9-ylamino)propyl]-1,3-dimethylthiourea·HNO_3, was synthesized and characterized as described previously [2]. The corresponding conjugate was generated as follows. A mixture of 163 mg (0.500 mmol) of [$PtCl_2$(en)], where en represents ethane-1,2-diamine, and 82.4 mg (0.485 mmol) of $AgNO_3$ in 10 ml dry dimethylformamide (DMF) was stirred in the dark for 14 h. Precipitated AgCl was filtered off, and to the filtrate was added dropwise within 2 h 191 mg (0.475 mmol) of the acridinylthiourea in 5 ml DMF. After stirring for another hour at room temperature the solvent was removed in vacuum at a temperature < 35°C. The oily residue was redissolved in 25 ml dry MeOH, and the solution was treated with activated carbon for 15 min and passed through a Celite pad. The filtrate was concentrated to a volume of 5–8 ml and subjected to chromatographic purification on a 100×2.5 cm Sephadex LH-20 column using dry MeOH as eluent to remove small amounts of *bis*(acridinylthiourea) complex [2]. The second fraction was collected, solvent removed in vacuum to a final volume of 10 ml, and 50 ml diethyl ether added to precipitate the type I/$n=3$ conjugate in its dinitrate salt form. The yellow microcrystalline product was dried in vacuum at 65°C for 6 h. Yield 150 mg (42%). ^{1}H NMR (D_2O, 300 MHz): δ 2.22 (q, 2H), 2.47 (s w/^{195}Pt satellites, 4H), 2.94 (s, 3H), 2.95 (s, 3H), 4.04 (t, 2H), 4.13 (t, 2H), 7.51 (t, 2H), 7.64 (d, 2H), 7.90 (t, 2H), 8.24 (d, 2H). Satisfactory C, H, N, Cl, and S elemental analyses.

Cell cultures

The following cell lines were used: NCI-H460, RKO, A2780, and A2780/CP. The NCI-H460 and RKO cell lines were obtained from the American Type Culture Collection (ATCC). The NCI-H460 lung cancer cells were grown in a modified RPMI medium containing 10 m*M* HEPES, 4.5 g/l glucose, and 1 m *M* sodium pyruvate, while RK0 colon cancer cells were cultured in McCoy's 5A medium. A2780 cisplatin-sensitive and A2780/CP cisplatin-resistant ovarian cell lines were obtained from Dr. Stephen G. Chaney, Department of Biochemistry and Biophysics at the University of North Carolina at Chapel Hill, and were cultured in RPMI medium. All culture media were supplemented with 10% fetal bovine serum (FBS) and 20% antibiotic/antimycotic solution (penicillin, streptomycin, and amphotericin B).

Clonogenic survival assay

Cells were plated at different cell densities (100, 250, 500, 1000, 1500, 2000, 4000, and 8000 cells) in 60-mm dishes and allowed to attach overnight. Drug was then added at various concentrations (0.1–80 μ*M*) for 1, 8, 24, or 48 h. At the appropriate time, the drug was removed and dishes were washed twice with 1× phosphate-buffered saline (PBS). Fresh medium (3 ml) was then added to each dish. The plates were incubated for between 8 and 14 days at 37°C in an atmosphere containing 5% CO_2. They were then fixed using a 3:1 methanol/acetic acid solution and stained using a 0.4% crystal violet solution. All colonies of 50 cells or more were then counted and the concentration that inhibited cell growth by 50% (IC_{50}) was determined using CalcuSyn (BIOSOFT, Ferguson, Mo.). The results are presented in all graphs as the means ± SEM from at least three trials.

DNA laddering assay

H460 lung cancer cells were plated and grown to 75% confluency and then treated with various concentrations (0.05–2 μ*M*) of PT-ACRAMTU for 24 h. A no-treatment control, a 50 μ*M* etoposide (Sigma) control and a 10 Gy radiation control were also plated. Cells were irradiated using a ^{137}Cs irradiator (JL Sheppard and Associates) at a dose rate of 451 rad/min. At 24 h, the medium was collected and attached cells were harvested. Following centrifugation at 1000 rpm in a Beckman Coulter Allegra 6R centrifuge, the supernatant was aspirated and the pellet resuspended in 1 ml PBS. DNA was then extracted from the cells using a Promega Wizard genomic DNA purification kit. DNA was quantitated using a Beckman Coulter DU640 spectrophotometer. DNA (50 μg) was then resolved on a 1.5% agarose gel run at 60 V for approximately 2–3 h. The gel was stained using 0.5 μg/ml of ethidium bromide in dH_2O.

Results

In order to determine if ACRAMTU and PT-ACRAMTU (Fig. 1) generated a cytotoxic response in additional solid tumor cell lines, clonogenic survival assays were performed in cisplatin-sensitive and cisplatin-resistant ovarian cancer cells, A2780 and A2780/CP, respectively, H460 lung cancer cells, and RKO colon cancer cells. Cisplatin (Fig. 1) was used as a control. Figure 2 shows clonogenic survival data of A2780 ovarian cells and H460 lung cancer cells treated with each of the three drugs. A dose-dependent response was observed in both cell lines, as well as the A2780CP and RKO cells (data not shown). ACRAMTU was not as effective as either cisplatin or PT-ACRAMTU at reducing cell survival. PT-ACRAMTU appeared to be as cytotoxic as cisplatin in A2780 cells, and more cytotoxic than cisplatin in H460 cells. IC_{50} data are presented for all four cell lines in Table 1. While ACRAMTU had approximately twofold higher IC_{50} values than cisplatin or PT-ACRAMTU in RKO cells, a tenfold higher IC_{50} was observed in ovarian cells, and IC_{50} values between 13- and 30-fold higher, respectively, were observed in cisplatin- or PT-ACRAMTU-treated lung cells. Both PT-ACRAMTU and cisplatin had IC_{50} values in the low micromolar range (0.17–0.70 μ*M*) in ovarian and lung cells, but higher IC_{50} values in colon cells (>2 μ*M*). While there appeared to be little difference in the IC_{50} values of PT-ACRAMTU and cisplatin in ovarian and colon cells, PT-ACRAMTU had a twofold lower IC_{50} in H460 cells than cisplatin.

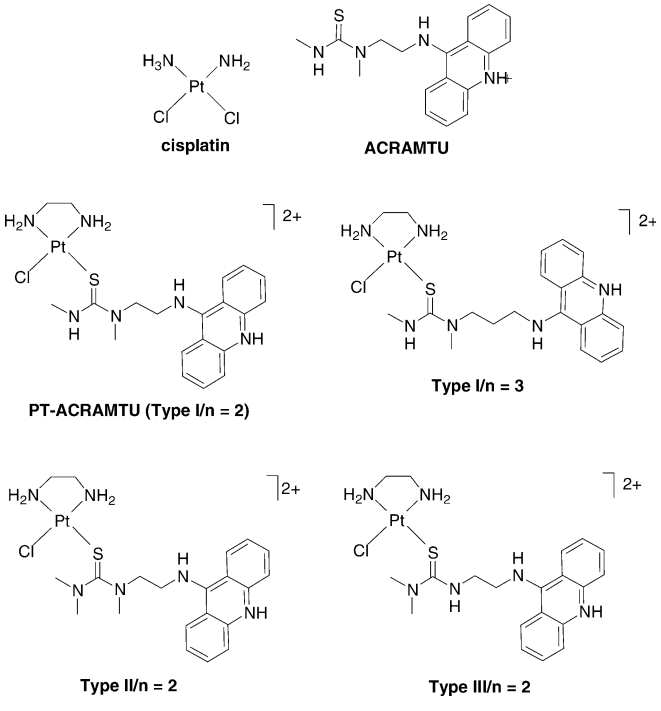

Fig. 1 Structures of the traditional drug cisplatin, the novel parent drug, ACRAMTU, and its platinum derivatives, PT-ACRAMTU (type I/$n=2$), type I/$n=3$, type II/$n=2$, and type III/$n=2$

The proposed mechanism of these conjugates involves rapid intercalative preassociation with nuclear DNA followed by a slower binding step in which the platinum center irreversibly attaches to nucleobase nitrogen at the site of intercalation [5]. To determine if the length of PT-ACRAMTU exposure affects cell survival, clonogenic survival assays were conducted in H460 lung carcinoma cells. Previous experiments used a 24-h incubation time, as did initial chemical and biochemical studies [4, 5, 20]. Cells were treated for various incubation times (1, 8, and 24 h) with PT-ACRAMTU at various concentrations. Both a time- and dose-dependent response was observed following PT-ACRAMTU treatment with the lowest IC_{50} observed following the 24-h incubation (Fig. 3). A 1-h incubation with PT-ACRAMTU was relatively ineffective with an IC_{50} (63.4 μ*M*) 235 times higher than that following the 24-h incubation (0.27 μ*M*). The IC_{50} following the 8-h incubation was 154 times lower than following the 1-h incubation. The IC_{50} value (0.41 μ*M*) was more similar to that observed following the 24-h incubation (0.27 μ*M*). While the response to cisplatin showed a similar time course to the response to PT-ACRAMTU in that cisplatin was more cytotoxic following the 24-h incubation (IC_{50} 0.63 μ*M*) than following the 1-h incubation (IC_{50} 11.2 μ*M*), the cytotoxic response to ACRAMTU was significantly delayed compared to the response to the corresponding conjugate, with IC_{50} values ranging from >220 μ*M* at 1 h to 8.4 μ*M* at 24 h (data not shown).

Acridinylthiourea acts like a typical nonleaving group, which is not replaced by nucleobase nitrogen in reactions of the conjugates with double-stranded DNA. Since the conjugates act through a dual covalent-intercalative mechanism, the nature of the thiourea linkage can be predicted to play a critical role in the DNA

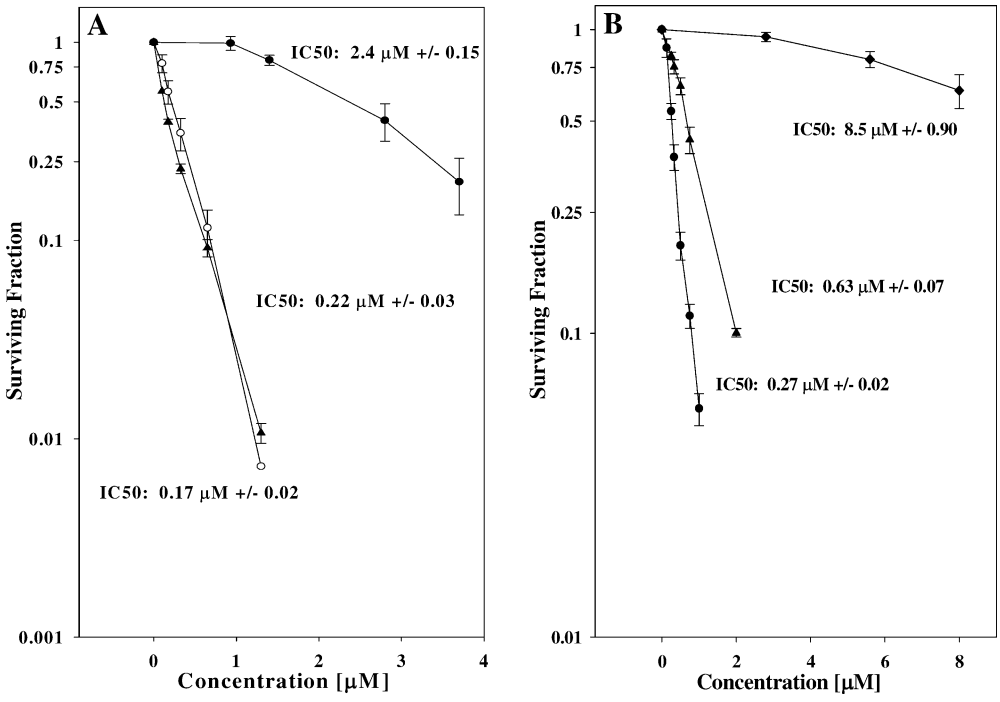

Fig. 2 Clonogenic survival assays of ACRAMTU, PT-ACRAMTU, and cisplatin in (**a**) A2780 cisplatin-sensitive ovarian cancer cells, and (**b**) H460 lung cancer cells. Cells were plated and treated with ACRAMTU (*solid circles*), PT-ACRAMTU (*solid triangles*), and cisplatin (*open circles*) for 24 h at various concentrations. IC_{50} values were determined using CalcuSyn. Each IC_{50} is the average ± SEM of three individual experiments

perturbations caused by adducts of these agents and ultimately their cytotoxicity. To determine to what extent suitably modified derivatives of the prototypical compound affect drug efficacy, additional clonogenic survival assays were conducted. IC_{50} data are presented in Table 1. Structural changes were made both to the bulkiness and conformational flexibility of the thiourea linker region to generate the type II/$n=2$, type III/$n=2$ [1], and type I/$n=3$ derivatives (Fig. 1). Like our type I prototypical PT-ACRAMTU, the type III/$n=2$ derivative contains a trialkylated thiourea group, while the type II/$n=2$ derivative contains a tetraalkylated thiourea (Fig. 1). While type I/$n=2$ and type III/$n=2$ can be considered isomers, PT-ACRAMTU, and type II and type III drugs all have an ethylene chain ($n=2$) that links the thiourea to the acridine unit. An additional CH_2 group was added in the aliphatic chain to make the third derivative, type I/$n=3$ (Fig. 1). Similar IC_{50} values were obtained with the prototype type I/$n=2$ and its isomer type III/$n=2$ in all four cell lines. The type II/$n=2$ derivative showed higher IC_{50} values in all four cell lines. Type I ($n=2$, 3) and type III ($n=2$) conjugates showed a marked advantage over cisplatin (about 2.5-fold) and ACRAMTU (about 30-fold) only in H460 lung cancer cells of the cell lines tested.

Finally, in order to gain further insight into the mechanism of action of PT-ACRAMTU, DNA ladder experiments were conducted to assess if an apoptotic response was generated following drug treatment. H460 cells were treated with either PT-ACRAMTU (0.05–2 μ*M*) or, as controls, 10 Gy ionizing radiation or 50 μ*M* etoposide. Figure 4 shows that with increasing concentrations of PT-ACRAMTU, fragmented DNA appeared in a characteristic ladder pattern indicative of an apoptotic response. Comparable DNA fragmentation was also seen with 10 Gy ionizing radiation and 50 μ*M* etoposide.

Discussion

In this study, the cytotoxic effects of five compounds were determined in cell lines from solid tumors that are traditionally treated with platinum-based chemother-

Table 1 IC_{50} values (μ*M*) in solid tumor cell lines determined in clonogenic survival experiments. Cells were treated for 24 h. The values presented are means ± SEM from an average of at least three experiments. Numbers in parentheses are resistance factors: IC_{50}(resistant)/IC_{50}(sensitive)

Compound	H460 (lung)	A2780 (ovarian)	A2780/CP (ovarian)	RKO (colon)
ACRAMTU	8.5 ± 0.90	2.4 ± 0.15	6.75 ± 1.05 (2.8)	5.3 ± 0.56
Cisplatin	0.63 ± 0.07	0.22 ± 0.03	0.70 ± 0.12 (3.18)	2.8 ± 0.32
PT-ACRAMTU type I/$n=2$	0.27 ± 0.02	0.17 ± 0.02	0.65 ± 0.08 (3.82)	2.2 ± 0.80
PT-ACRAMTU type I/$n=3$	0.23 ± 0.01	0.17 ± 0.03	0.66 ± 0.06 (3.88)	9.7 ± 0.24
Type II/$n=2$	3.3 ± 0.22	0.73 ± 0.19	1.1 ± 0.17 (1.5)	9.9 ± 0.60
Type III/$n=2$	0.31 ± 0.04	0.24 ± 0.017	0.52 ± 0.016 (2.16)	3.1 ± 0.32

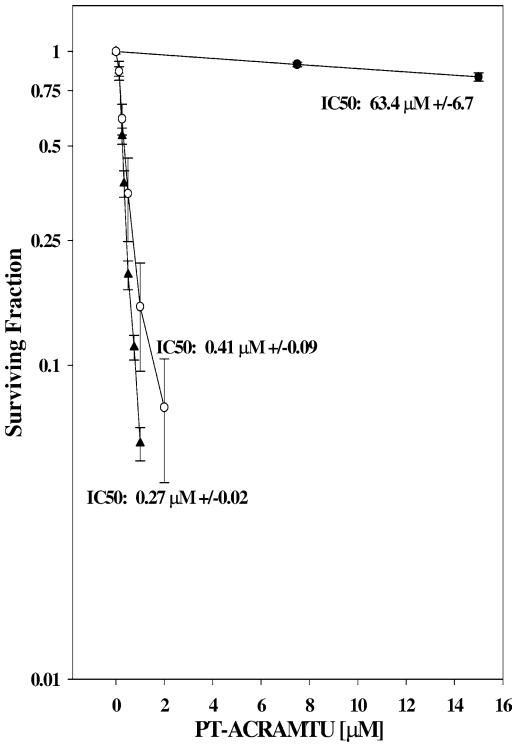

Fig. 3 Clonogenic survival assay. H460 lung cancer cells were plated at various densities and exposed to PT-ACRAMTU for 1, 8 or 24 h. No treatment controls were also plated. Drug was then removed and following incubation for10–14 days, and cells were fixed and stained. IC_{50} values were determined using CalcuSyn. The values presented are means ± SEM from an average of at least three experiments

apy. There was a time- and dose-dependent effect in cells treated with Pt-ACRAMTU. A 24-h incubation with PT-ACRAMTU generated the best cytotoxic response

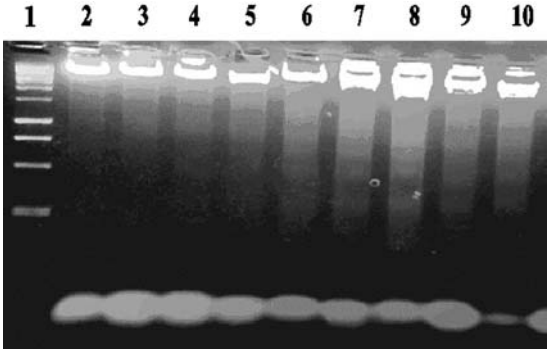

Fig. 4 DNA laddering experiment. H460 lung cancer cells were plated and grown to 75% confluency and then treated with various concentrations (0.05–2 µ*M*) of Pt-ACRAMTU for 24 h. A no-treatment control, a 50 µ*M* etoposide control and a 10 Gy radiation control were also plated. At 24 h, all cells were harvested and the DNA extracted using a Promega Wizard genomic DNA purification kit. DNA (50 µg) was resolved on a 1.5% agarose gel run at 60 V for approximately 2–3 h and stained with ethidium bromide. *Lane 1* ladder; *lane 2* no treatment; *lanes 3–8* 0.05, 0.1, 0.25, 0.5, 1 and 2 µ*M* Pt-ACRAMTU, respectively; *lane 9* 10 Gy radiation control; *lane 10* 50 µ*M* etoposide control

compared to 1-h and 8-h incubations. Similar results have been observed with SNB19 brain tumor cells [16]. Due to its positive charge, ACRAMTU, both as a free ligand and as nonleaving group in PT-ACRAMTU, intercalates quickly into the DNA base stack. While simple intercalation produces a significant cytotoxic effect, as evidenced by the micromolar IC_{50} values found for ACRAMTU, the additional covalent binding of the platinum in PT-ACRAMTU to DNA appears to generate a significantly enhanced cytotoxic response, i.e., lower IC_{50} values. This suggests that the cellular damage caused by PT-ACRAMTU is indeed produced at the DNA level in a two-step process involving reversible intercalation (rapid) and irreversible platination (slow) of the biopolymer. The data also correlate well with biochemical data generated in cell-free systems. *Eco*RI cleavage studies by Budiman et al., for instance, have shown that PT-ACRAMTU protects plasmid DNA from cleavage at the enzyme's restriction sites in a time-dependent manner [9]: e.g., protection from *Eco*RI cleavage does not occur efficiently until PT-ACRAMTU has been incubated for at least 6–12 h. Enhanced cellular uptake of PT-ACRAMTU compared to ACRAMTU may be one of the contributors to the distinctly different time-courses of cytotoxicity of the two drugs. Intracellular drug accumulation, however, may be less critical for these agents due to their high affinity to nuclear DNA and reduced residence times in the cytosol. It should be noted that both drugs are positively charged at physiological pH (2+ for PT-ACRAMTU, 1+ for ACRAMTU). Considering the extra positive charge on the conjugate and its ability to irreversibly bind DNA, and on the basis of the above-mentioned biochemical work in cell-free systems [9], it appears that the differential target interactions of the drugs control the time-course of the cytotoxic response.

PT-ACRAMTU appears to be a more cytotoxic drug than ACRAMTU. This confirms earlier results obtained in leukemic cells [20] and glioblastoma cells [16]. In ovarian cells, however, there appears to be a cell line difference. The 2008/C13* pair were the only two cell lines in which ACRAMTU appeared to have a lower IC_{50} value than PT-ACRAMTU [20]. In contrast, our data suggest that PT-ACRAMTU is a more cytotoxic drug than ACRAMTU in A2780 and A2780/CP cell lines. The IC_{50} values observed are more similar to IC_{50} values that have been obtained for other solid tumor cell lines [1, 16]. However, in the 2008 and C13* cells, both ACRAMTU and PT-ACRAMTU partially alleviated cisplatin resistance by a factor of 7- and 4.8-fold, respectively; this was not observed in the A2780 pair with either drug. Previous studies have shown that there is increased repair of cisplatin adducts in the A2780CP cells and that fewer lesions are present [15, 23]. This suggests that in ovarian cells, the sensitization to our hybrid compound may be cell line specific and dependent on the genetic background of the cells.

While PT-ACRAMTU has shown promise in several cancer cell lines [20], two strategies were employed to

modify PT-ACRAMTU to modulate the target binding of platinum and possibly increase cytotoxicity: (1) optimizing the steric effects of alkylated thiourea in the coordination sphere of the metal, and (2) addition of a CH_2 group to the aliphatic chain that links the platinum and acridine chromophore. The bulkiness of the leaving and nonleaving groups is known to control side reactions of the square-planar metal [25]. Addition of methyl groups to the thiourea linker may slow unwanted reactions of platinum with detoxifying sulfur nucleophiles, such as glutathione [10]. The type I/$n=2$ and type III/$n=2$ drugs, both trialkylated compounds, showed similar IC_{50} values in all four cell lines tested. Although the type II/$n=2$ was not as cytotoxic as the trialkylated derivatives, it was able to reduce cisplatin resistance in the A2780/CP cells twofold. While the type I/$n=2$ compound did not have an effect on cisplatin resistance, the type III/$n=2$ compound reduced cisplatin resistance 1.5-fold. In model reactions using 2′-deoxyguanosine (dGuo), mimicking the covalent DNA binding step, the type II/$n=2$ derivative showed a longer half-life than either the type I/$n=2$ or type III/$n=2$ drugs [1]. This may be due to the increased steric hindrance produced by the tetraalkylated thiourea in type II/$n=2$ conjugate. The second modification, variation of the aliphatic chain in the thiourea linker, may potentially alter the sequence-specificity and kinetics of DNA damage and, ultimately, the cytotoxicity of the adducts. The added CH_2 group in type I/$n=3$ introduces more flexibility into the platinum–acridine linker, which might facilitate binding of the metal in a preintercalated complex to nucleobase nitrogen and/or result in adducts structurally dissimilar to and more cytotoxic than those formed by PT-ACRAMTU.

Previous studies with PT-DMACRTU (DMACRTU = 1-acridin-9-yl-1,3-dimethylthiourea), a compound similar to PT-ACRAMTU but containing a rigidly tethered acridine lacking the ethylene bridge, causes only minor untwisting of plasmid DNA (indicating inefficient intercalation) and has been proved to be biologically inactive [4]. Addition of the CH_2 group to the linker giving type I/$n=3$ did not lead to a more effective drug. Studies by Gean et al. [13] have shown similar results with a cationic platinum–triamine complex linked to an anthraquinone intercalator: in vitro assays to assess cytotoxic activity in P388 Adriamycin-sensitive and Adriamycin-resistant leukemic cells indicated that increasing the length of the linker chain between the platinum and intercalator does not result in increased cytotoxic activity. We hypothesized that instead of partial intercalation of the acridine like as observed for the type II/$n=2$ [6], full integration of the acridine in the DNA base stack may cause a larger degree of duplex unwinding, which may translate into increased cytotoxicity. Previous unwinding studies had shown that a direct relationship exists for this type of compound between unwinding angle and cytotoxicity [4]. Future biophysical studies will therefore revisit the relationship between DNA unwinding and biological activity of type I/$n=3$ and longer-chain length analogues. The prototype also shows low micromolar IC_{50} values in the lung cancer cell lines A549 and SK-LU-1 (unpublished results). All three lung cell lines have an activated *ras* mutation, in contrast to the ovarian cells, A2780 and A2780/CP. Previous groups have shown that activated *ras* sensitizes cells to drugs that inhibit topoisomerase II enzymes through an enhanced apoptotic mechanism [19]. We were able to show through DNA laddering experiments that PT-ACRAMTU treatment generates an apoptotic response. Since previous in vitro studies in our laboratory have indicated that PT-ACRAMTU may act as a topoisomerase poison, this suggests a potential mechanism of action for our compounds [8], which in the light of the data available, might be most efficient for the type I and type III compounds.

In conclusion, we showed that a novel intercalator-linked platinum agent, PT-ACRAMTU, shows dose-dependent cytotoxicity in ovarian, colon, and lung cancer cells, and that this compound is more active than the simple intercalator, ACRAMTU. In addition, we demonstrated a time-dependent response to PT-ACRAMTU in H460 lung cancer cells. We studied the effects of an additional CH_2 group in the linker of PT-ACRAMTU and variations of the bulkiness of the sulfur-linked nonleaving group. While type I and type III analogs of PT-ACRAMTU may be promising compounds to study further in lung cancer cells, more dramatic modifications of the prototypical drug are needed to produce similar effects in other cancer cell lines.

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