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Bis(acridinylthiourea)platinum(II) Complexes: Synthesis, DNA Affinity, and Biological Activity in Glioblastoma Cells

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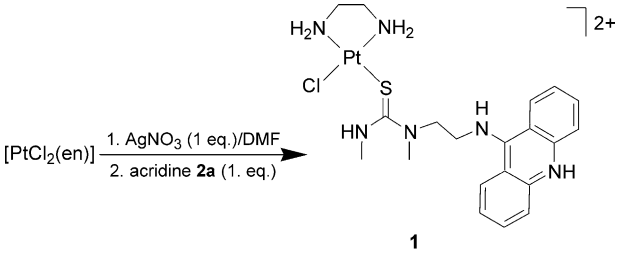
Abstract—The preparation of two novel bis(acridine)platinum(II) complexes is reported. The 4+ charged conjugates associate strongly with double-stranded native DNA ($K_i > 10^6$), possibly through bisintercalation. A cell viability assay was used to demonstrate that both compounds are capable of mediating cytotoxicity at micromolar concentrations in SNB19 brain tumor cells.

Recently, we have reported a new class of DNA-targeted hybrid platinum–acridine agents that show cytotoxic activity at nano-to-micromolar concentrations in solid tumor and leukemia cancer cell lines.[1] The prototype, conjugate **1**, was synthesized by replacing *one* chloro leaving group in [$PtCl_2$(en)] (en = ethane-1,2-diamine), a cisplatin analogue, with the novel 9-aminoacridine derivative, 1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea (**2a**).[1] Unlike intercalator-tethered *cis*-diaminedichloro complexes reported previously,[2–6] **1** does not induce bifunctional covalent adducts (cross-links) in DNA but acts through a mechanism that involves monofunctional platination and intercalation of the planar chromophore into the DNA base stack. This type of adduct, which causes local unwinding of double-stranded DNA by 21°, is considered a potential cytotoxic lesion of the drug.[7] The sequence and groove specificity of platinum binding are currently under investigation.

In unpurified preparations of conjugate **1**, obtained from reactions of equimolar amounts of platinum precursor and acridine, a minor impurity was observed. We were now able to identify the side product as the corresponding bis(acridinylthiourea)platinum(II) complex, **3a**. Monoactivation of [$PtCl_2$(en)], achieved by abstraction of one chloro ligand with silver ion, obviously does not completely prevent the unwanted substitution of the second chloride by **2a** (Scheme 1). The formation of **3a** in the above reaction mixtures is most likely due to the high nucleophilicity of thiourea sulfur.[8] Sulfur donors exhibit a high affinity to divalent platinum and are known to replace chloro ligands without prior solvolysis of the Pt-Cl bond.[9]

To investigate the DNA interactions and possible biological effects of bis(acridinylthiourea)platinum(II) complexes we synthesized **3a** and the new derivative, **3b**, containing an acridinylthiourea with an extended propylene flexible linker chain (**2b**). The tethering of two acridine moieties to platinum was achieved after removal of chloride in the precursor with two equivalents of silver nitrate, affording both complexes as their nitrate salts (Scheme 2). The new acridine derivative, 1-[3-(acridin-9-ylamino)propyl]-1,3-dimethylthiourea (**2b**, HNO_3 salt), was generated using the synthetic scheme developed for the prototype, **2a**.[1] Briefly, the synthesis involved selective protection of the primary and



1
+ **3a** (minor impurity)

Scheme 1.

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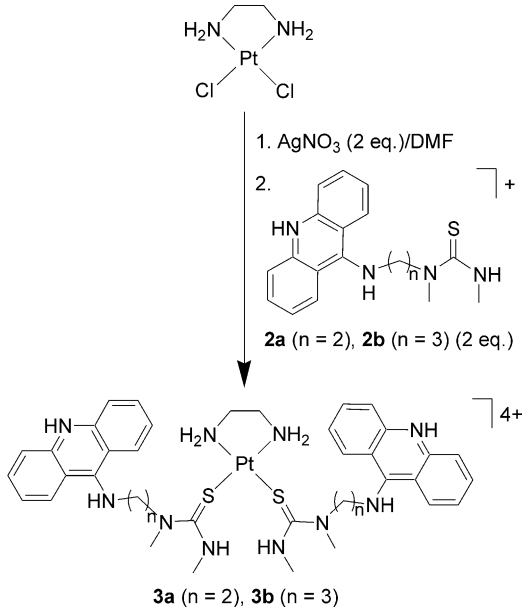
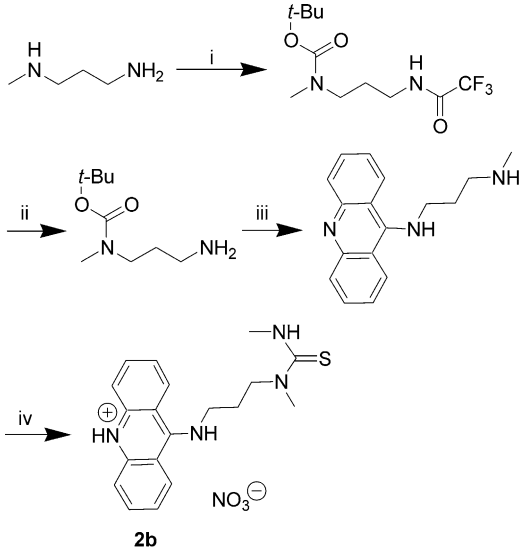
secondary amino groups in N^1-methylpropane-1,3-diamine and attachment of the deprotected primary amino nitrogen to C(9) in the acridine precursor, 9-phenoxyacridine. After removal of the *tert*-butyl carbamate (Boc) group, the secondary amino group was reacted with methylisothiocyanate followed by treatment of the reaction mixture with HNO_3 to give the desired hydronitrate salt of the acridinylthiourea, **2b** (Scheme 3).

^{1}H NMR spectroscopy, combustion analyses, and, where appropriate, ^{195}Pt NMR spectroscopy were used to characterize the new compounds, **2b**, **3a**, and **3b**.[10] ^{195}Pt chemical shifts of −3362 and −3345 ppm were found for **3a** and **3b**, respectively, characteristic of a $[N_2S_2]$ environment of platinum(II).[11] The combined NMR data confirm the formation of symmetrical bisintercalator complexes with the two intercalators linked to the metal center via thiourea sulfur.

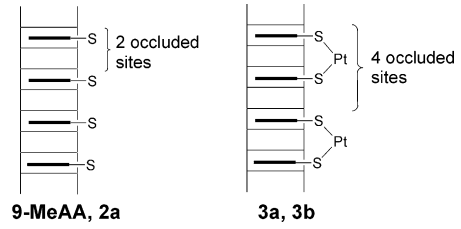


Scheme 2.



Scheme 3. Reagents and conditions: (i) 1. CF_3COOEt, 2. $(BOC)_2O$, THF/0 °C; (ii) OH^-/MeOH; (iii) 1. 9-PhOAcr/THF; 2. HCl/AcOH, 3. 2 M NH_3; (iv) 1. MeNCS, 2. HNO_3.

Under physiological conditions, compounds **3a** and **3b** exist as 4+ cations with protonated acridine moieties ($pK_a \approx 9.8$[1]) and a 2+ charge localized on the divalent platinum center. Strong DNA binding is predicted for these compounds despite their inability to undergo covalent DNA interactions due to the lack of substitution-labile chloride. Both agents have the potential to associate with double-stranded DNA via bisintercalation. Additional electrostatic forces and hydrogen bonding involving thiourea- and en-NH groups may also contribute to the DNA binding. Spectrophotometric equilibrium binding titrations[12] showed that **3a** and **3b** bind to calf thymus DNA with high affinity. Upon addition of nucleic acid to buffered solutions of **3a** and **3b**, a red shift and decrease in absorbance of acridine-based bands in the 350–450 nm region was observed. From Scatchard-type plots[13] we determined the association constants (K_i) and the size of the binding sites (n, number of nucleotides; $n/2$, number of base pairs) for both complexes. The binding isotherms in the intercalation region (at low drug-to-DNA base pair ratios, r) were fitted to the neighbor exclusion model of McGhee and von Hippel.[14] The data are summarized in Table 1. The bisacridines prove to be stronger binders than the simple monointercalators 9-(methylamino) acridine (9-MeAA) and **2a**.[7] Complex **3a** showed an approx. 5-fold higher affinity than **3b**, possibly indicating more favorable intercalative DNA interactions of the $n=2$ derivative. Two interesting trends emerged for the set of compounds listed in Table 1 that deserve further discussion. First, an increase in n is noted for **3a** and **3b** compared to the simple acridines, 9-MeAA and **2a**.[7] While the values observed for the latter species are consistent with each drug molecule occupying 4 nucleotides (2 base pairs), indicative of classical intercalation,[13] the increased (approximately doubled) binding site size for **3a** and **3b** suggests a bisintercalative binding mode:



On the other hand, the notably reduced red shift and hypochromicities found for **2a** and the platinum-bridged species **3a** and **3b** compared to simple 9-MeAA possibly indicate that groove interactions of the thiourea and platinum–thiourea linker groups allow only partial intercalation of the planar chromophores into the DNA base stack. Minor groove interactions have recently been established for the thiourea residue in **2a** (distamycin A competition displacement assays,[7] 2-D NMR spectroscopy[15]). In summary, it appears that alternative (non-specific) DNA–drug interactions compensate for the reduced tendency of the acridine moiety

Table 1. DNA binding data for acridines and bisacridines in calf thymus DNA deduced from UV–visible equilibrium titrations[a]

Compd	K_i (M^{-1})	n	$\Delta\lambda$ (nm)	% H	Literature
9-MeAA	$(1.1\pm0.2)\times10^5$	4.4	6	46	Ref 7
2a	$(1.5\pm0.2)\times10^6$	3.8	5	36	Ref 7
3a	$(1.0\pm0.1)\times10^7$	7.3	3	32	This work
3b	$(2.2\pm0.3)\times10^6$	7.4	3	32	This work

[a]Data were fitted to $r/C_f = K_i(1-nr)[(1-nr)/(1-(n-1)r]^{n-1}$ with n, number of nucleotides occupied by a single drug molecule; r, occupied binding sites/total number of sites; C_f, concentration of free drug; K_i, intrinsic binding constant, giving standard deviations (values are means of two experiments). Spectral changes were followed at $\lambda_{max} = 413$ nm; $\Delta\lambda$ = bathochromic shift; H = hypochromicity.

in **2a**, **3a**, and **3b** to insert into the DNA base stack. Cooperative binding of the acridine chromophores in **3a** and **3b**, as previously reported for simple polymethylene-bridged bisacridines,[16] was not observed.

Reversible DNA intercalators and bisintercalators are of potential interest for their use as anticancer therapies.[17] The cytotoxic effect of most intercalators belonging to the acridine family is closely related to the ability of these compounds to inhibit (poison) DNA topoisomerases.[17] Previous studies have shown that **2a** and the corresponding conjugate **1** produce topo-mediated DNA damage.[18] To assess the biological activity of the complexes **3a** and **3b** in cancer cells, we studied the effect of both compounds on the viability of SNB19 glioblastoma cells using the trypan blue exclusion assay.[19] In a parallel set of experiments, rat astrocytes[20] were exposed to drug to assess the effect of the new agents on normal brain cells. Cisplatin, the clinical agent, was included in this study for comparison of effectiveness. Both cell lines were incubated at a drug concentration of 2 μM. At this concentration, **3a** and **3b** decreased the cell viability by 67 and 60%, respectively, while leaving the astrocytes unaffected. Cisplatin was markedly less active in SNB19, producing only 20% non-viable cells. The data are summarized in Figure 1. The cytotoxic effect observed in this assay has now been confirmed in clonogenic survival assays performed in our laboratory. In these experiments, which will be reported in detail elsewhere, compounds **3a** and **3b** showed IC_{50} values in the low micromolar concentration range.

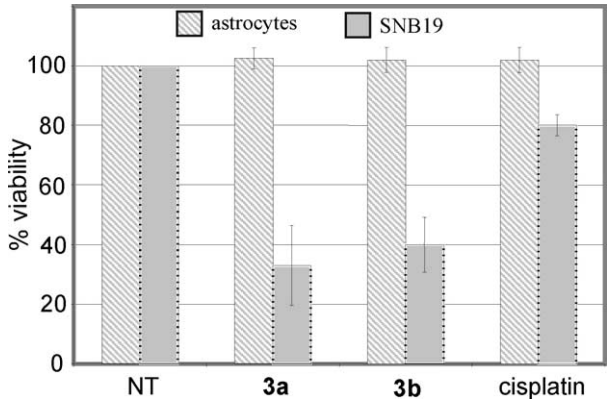


Figure 1. Assessment of cell viability using the trypan blue exclusion assay. Columns represent the average of two experiments. NT denotes untreated cells. The incubation concentration was 2 μM in all cases.

In conclusion, we have synthesized a novel type of platinum-based bisacridine agent that strongly associates with double-stranded DNA, possibly through bisintercalation. The preliminary biological data suggest that **3a** and **3b** may have potential utility as DNA-targeted agents in cancer chemotherapy. Platinum drugs have been among the primary chemotherapeutic agents used against malignant gliomas but have shown poor response rates.[21] The platinum-acridines **1**, **3a**, and **3b** are likely to act through a mechanism different from that of current platinum-based cross-linkers and may be more effective treatments against this aggressive type of brain cancer. Although high-molecular-weight polycationic compounds such as **3a** and **3b** are unlikely to pass the blood–brain barrier, novel strategies of local drug delivery (interstitial chemotherapy) hold considerable promise of improving the clinical potential of such agents in neuro-oncology.[22] Multimodality therapy, even though not curative, is still the best strategy for the long-term management of this disease.[21] Another future goal will therefore be to explore the use of the above conjugates as radiation sensitizers in combination chemotherapy.

Acknowledgements

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10. Synthesis and characterization of compounds. **1-[3-(Acridin-9-ylamino)propyl]-1,3-dimethylthiourea·HNO$_3$** (**2b**). This derivative was synthesized from the precursor *N*-acridin-9-yl-*N*′-methylpropane-1,3-diamine analogous to the procedure

reported for **2a** (see ref 1). The purity of intermediates was assessed by ^{1}H NMR spectroscopy. **2b**: Yield (final step, Scheme 3, iv): 66%. ^{1}H NMR (300 MHz, MeOH-d_4) δ 2.27 (2H, q), 2.95 (3H, s), 3.07 (3H, s), 4.11 (2H, t), 4.19 (2H, t), 7.54 (2H, t), 7.76 (2H, d), 7.94 (2H, t), 8.50 (2H, d). Elemental analysis for $C_{19}H_{23}N_5O_3S{\cdot}0.5H_2O$ (410.49), Found: C, 55.90; H, 5.84; N, 16.96; S, 7.75. Calcd: C, 55.59; H, 5.89; N, 17.06; S, 7.81. **Complexes 3a and 3b (tetranitrate salts)**. A mixture of 326 mg (1.00 mmol) of [$PtCl_2$(en)] and 338 mg (2.00 mmol) of $AgNO_3$ in 10 mL DMF was stirred for 20 h in the dark. AgCl was filtered off, and 2.00 mmol of the appropriate acridinylthiourea was added to the filtrate. The mixture was allowed to stir for 6 h in the dark. DMF solvent was distilled off in vacuum at room temperature, and the oily residue was redissolved in approximately 250–300 mL of hot dry MeOH. The solution was treated with activated carbon (100 mg) for 10 min and filtered through Celite while hot. After the solutions were concentrated to a final volume of 50 mL and stored at 4 °C for 12 h, a brownish-yellow crystal mass was obtained. The crude products were recrystallized twice from hot methanol to afford bright-yellow needles, which were collected and dried at 60 °C in vacuum. **3a**: Yield: 550 mg (45%). ^{1}H NMR (300 MHz, D_2O) δ 2.66 (4H, t), 3.04 (6H, s), 3.31 (6H, s), 3.80 (4H, t), 4.17 (4H, t), 7.24 (4H, d), 7.40 (4H, t), 7.72 (4H, t), 7.90 (4H, d). ^{195}Pt NMR (107.5 MHz, D_2O): δ −3362 (vs. $Na_2[PtCl_6]$ standard). Elemental analysis for $C_{38}H_{50}N_{14}O_{12}PtS_2$ (1154.10), Found: C, 38.80; H, 4.48; N, 16.50; S, 5.40. Calcd: C, 39.55; H, 4.37; N, 16.99; S, 5.56. **3b**: Yield: 520 mg (47%). ^{1}H NMR (300 MHz, D_2O) δ 2.01 (4H, q), 3.05 (6H, s), 3.27 (6H, s), 3.61 (4H, t), 3.84 (4H, t), 7.20 (4H, d), 7.27 (4H, t), 7.66 (4H, t), 7.77 (4H, d). ^{195}Pt NMR (107.5 MHz, D_2O) δ −3345 (vs $Na_2[PtCl_6]$ standard). Elemental analysis for $C_{40}H_{54}N_{14}O_{12}PtS_2$ (1182.15), Found: C, 40.39; H, 4.64; N, 16.26; S, 5.05. Calcd: C, 40.64; H, 4.60; N, 16.59; S, 5.43.

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