

## Brief Articles

### Design, Synthesis, and Biological Activity of a Novel Non-Cisplatin-type Platinum–Acridine Pharmacophore

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Platinum–acridine conjugates were prepared from [PtCl<sub>2</sub>(ethane-1,2-diamine)] and the novel acridinylthioureas MeHNC(S)NMeAc (6) and MeHNC(S)NMe(CH<sub>2</sub>CH<sub>2</sub>)NHAc (15) by replacing one chloro leaving group in the cisplatin analogue with thiourea sulfur. In HL-60 leukemia cells, IC<sub>50</sub> values for 7 (Pt-tethered 6) and 16 (Pt-tethered 15) were 75 and 0.13 μM, respectively. In the ovarian cell lines 2008 and C13\*, 16 was active at micromolar concentrations and showed only partial cross-resistance with clinical cisplatin. Possible structure–activity relationships are discussed.

#### Introduction

Platinum coordination compounds, such as *cis*-diamminedichloroplatinum(II) (cisplatin) and *cis*-diammine-1,1-cyclobutanedicarboxylatoplatinum(II) (carboplatin), are in clinical use for the treatment of malignancies of the urogenital tract and other cancers.<sup>1</sup> The overall clinical success of cisplatin, as evidenced by the number of long-term survivors among individuals afflicted with advanced testicular cancer,<sup>2</sup> is somewhat diminished by intrinsic and acquired tumor resistance.<sup>3</sup> The development of thousands of direct cisplatin derivatives—most recently accomplished by high-throughput synthesis and screening methods<sup>4</sup>—reflects the common notion, that the formation of bifunctional covalent adducts on DNA is a prerequisite for cytotoxicity in platinum-based agents.<sup>5</sup> The 1,2 intrastrand cross-link between adjacent purine bases formed by cisplatin is considered the principal cytotoxic lesion of the drug.<sup>5</sup> Structural analogues of cisplatin, however, damage DNA in a way similar to the parent drug and therefore cause similar biological effects.<sup>6</sup> In fact, to be considered for clinical evaluation, a new platinum drug would need to demonstrate unique biological properties that eliminate multifactorial drug resistance and lead to a spectrum of activity different from that of the clinical agents. Therefore, future platinum drug candidates are unlikely to be discovered by following the classical structure–activity relationships for cisplatin analogues.

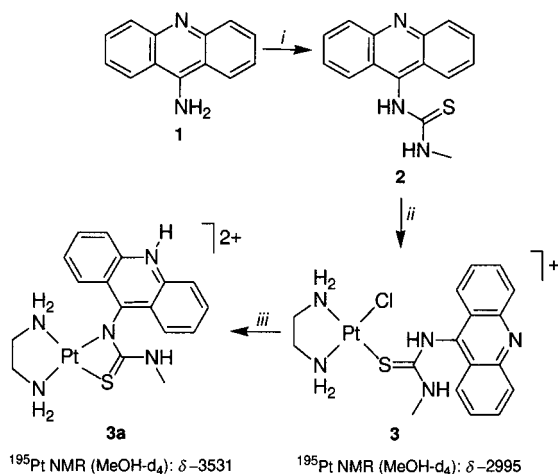
Duplex DNA is the biological target of the acridinium group with intercalation being the predominant mode of association.<sup>7</sup> Intercalator-based drug conjugates were

developed to enhance the DNA affinity of well-established therapeutics, such as nitrogen mustards<sup>8</sup> and platinum drugs.<sup>9</sup> In the case of targeted platinum, the classical *cis*-[PtX<sub>2</sub>A<sub>2</sub>] (X = leaving group, A<sub>2</sub> = diamine nonleaving group) unit has been linked to various intercalating groups via flexible alkyl chains of varying length.<sup>9</sup> Temple et al.<sup>10</sup> recently demonstrated that the *cis*-diamminedichloroplatinum(II) moiety in 9-aminoacridine–platinum complexes has a tendency to dictate the sequence specificity of covalent attachment of platinum on DNA. This results in cisplatin-like binding of the conjugates to adjacent purines, unless prohibited by steric strain in the polymethylene linkage. Our working hypothesis for the discovery of structurally novel Pt-containing pharmacophores demands that, to produce non-cisplatin behavior, the metal needs to be completely prevented from forming cross-links within extended runs of consecutive purine bases on DNA. Toward this objective, we have developed platinum complexes derived from [PtCl<sub>2</sub>(en)] (en = ethane-1,2-diamine), a cisplatin analogue, by replacing one of the chloro leaving groups with novel acridinylthioureas. The use of bidentate en instead of simple NH<sub>3</sub> prevents trans-labilization and undesired displacement of the nonleaving group by sulfur and nitrogen donors.<sup>11</sup> Thiourea sulfur was used to covalently link acridine to platinum. The thermodynamic stability of the Pt–S bond renders thiourea a typical nonleaving group that should not be displaced by DNA nucleophiles.<sup>11</sup> As a consequence, platinum is turned into a monofunctional DNA metalating group. Monofunctional platinum chloroam(m)ine complexes, such as [PtCl(NH<sub>3</sub>)<sub>3</sub>]<sup>+</sup>, however, are known to be therapeutically inactive,<sup>6</sup> and the cytotoxic potential of sulfur-modified platinum would greatly rely on alternative lesions, such as a dual-binding mode involving covalent and intercalative association.<sup>12</sup>

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Scheme 1<sup>a</sup>

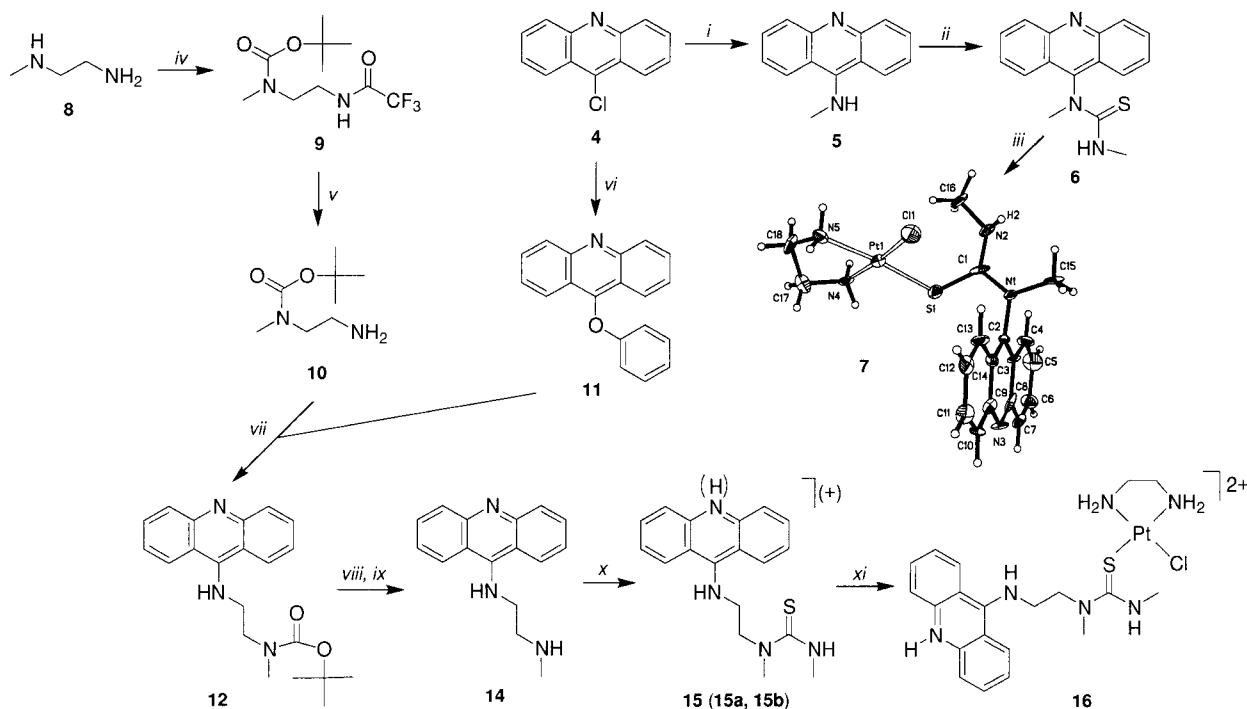
<sup>a</sup> Counter ions not shown. Reagents and conditions: (i) MeNCS/EtOH/Δ. (ii) [PtCl<sub>2</sub>(en)], AgNO<sub>3</sub>/DMF, dark, rt. (iii) aqueous solution.

## Results and Discussion

**Chemistry.** The synthetic chemistry used in this study is summarized in Schemes 1 and 2. Two types of acridine-thioureas were prepared. The derivatives 1-acridin-9-yl-3-methylthiourea (**2**) and 1-acridin-9-yl-1,3-dimethylthiourea (**6**) were obtained by reaction of the 9-amino nitrogen in the appropriate 9-aminoacridines with isothiocyanate. 1-[2-(Acridin-9-ylamino)ethyl]-1,3-dimethylthiourea (**15**) was prepared by attaching the primary amino nitrogen in *N*-methylethylenediamine (**8**) to the planar chromophore via nucleophilic aromatic substitution. In **15**, the thiourea group and the 9-amino nitrogen on acridine are separated by an ethylene

linker. The synthesis involved selective protection<sup>13</sup> of the amino groups in **8** and subsequent transformation of the dangling, deprotected secondary amine in intermediate **14** into thiourea. The rationale behind the use of **8** instead of en was that the resulting trialkylated thiourea derivative would show greater chemical robustness than an analogous dialkylated species. In the presence of transition metal ions, mono- and disubstituted thioureas have been shown to undergo deprotonation<sup>14</sup> and desulfurization<sup>15</sup> of the SCN<sub>2</sub> framework. To produce the platinum-acridines, **2**, **6**, and **15a** (the HNO<sub>3</sub> salt of **15**) were reacted with the monoactivated form of [PtCl<sub>2</sub>(en)], [PtCl(DMF-O)(en)]<sup>+</sup>. Bidentate en instead of simple NH<sub>3</sub> was introduced to avoid trans-labilization of the nonleaving group by sulfur. The reaction of **6** and **15a** with platinum gave the desired conjugates **7** and **16** in 62% and 58% yield, respectively. S,N chelate formation precluded the isolation of an analogous complex of **2** (Scheme 1). The decomposition of target compound **3** to **3a** in reaction mixtures of **6** and platinum was detected by <sup>195</sup>Pt NMR spectroscopy.

<sup>1</sup>H NMR spectroscopy, elemental analyses, and <sup>195</sup>Pt NMR spectroscopy, where applicable, were used to identify the structures of the target compounds (see Experimental Section). In addition, the single-crystal X-ray structure of conjugate **7** was determined (Scheme 2). The structure confirms the formation of a 1:1 conjugate with **6** being linked to square-planar platinum through thiourea sulfur. <sup>195</sup>Pt NMR spectra taken over a period of 2 days indicate that the [PtN<sub>2</sub>SCl] coordination (for both **7** and **16**) persists in solution.<sup>11</sup> In the solid state, the sterically demanding acridin-9-yl group on N1 is oriented cis to sulfur and perpendicular to the planar SCN<sub>2</sub> thiourea framework (angle between planes is

Scheme 2<sup>a</sup>

<sup>a</sup> Counter ions and crystal solvent not shown for clarity. Reagents and conditions: (i) MeNH<sub>2</sub>/MeOH/2 M NH<sub>3</sub>. (ii) MeNCS/EtOH/Δ. (iii) [PtCl<sub>2</sub>(en)], AgNO<sub>3</sub>/DMF/dark, rt. (iv) 1, CF<sub>3</sub>COOEt/THF/<10 °C; 2, (Boc)<sub>2</sub>O/THF/<10 °C. (v) dilute NaOH/48 h, 35 °C. (vi) NaOC<sub>6</sub>H<sub>5</sub>, Δ. (vii) THF/Δ. (viii) HCl, CH<sub>3</sub>COOH/rt. (ix) 2 M NH<sub>3</sub>. (x) **15**, MeNCS/EtOH/Δ; **15a,b**, from **15** and 1 M HNO<sub>3</sub> and 1 M HCl, respectively/MeOH. (xi) **15a**, [PtCl<sub>2</sub>(en)], AgNO<sub>3</sub>/DMF/dark, rt.

**Table 1.** Cytotoxicity Data for **7**, **15b**, and **16**

compd	IC <sub>50</sub> , μM		
	HL-60	2008	C13* <sup>a</sup>
<b>7</b>	75	>100	>100
<b>15b</b>	11	2.2	3.7 (1.7) <sup>b</sup>
<b>16</b>	0.13	3.8	9.6 (2.5)

<sup>a</sup> C13\* is the cisplatin-resistant variant of the 2008 cell line.

<sup>b</sup> Values in parentheses are resistance factors, IC<sub>50,resistant</sub>/IC<sub>50,sensitive</sub>.

88.0°). The bulkiness of the planar residue has a pronounced effect on the molecular dynamics of **7**, which is slow on the NMR time scale, as evidenced by the multiplicity and temperature dependence of the signals in the <sup>1</sup>H and <sup>195</sup>Pt NMR spectra (see Supporting Information). This effect is not observed for conjugate **16**.

A critical difference between the acridinylthioureas **6** and **15** emerged with respect to their acid–base chemistry. The basicity of endocyclic acridine nitrogen is an important parameter that determines the protonation state of the chromophore at physiological pH. Cationic planar heterocycles usually exhibit high DNA affinity.<sup>16</sup> We determined a pK<sub>a</sub> of 9.8 (± 0.1) for **15**. In contrast, the apparent pK<sub>a</sub> of **6** in 25% MeOH was found to be 3.6 (± 0.2), which extrapolates to a pK<sub>a</sub> around 4.0 in water.<sup>17</sup> The lowered basicity of **6** as compared to **15** is ascribed to the less efficient conjugation between the “anilino” and endocyclic nitrogens as a result of the orthogonal orientation of the thiourea and acridine π-systems (see Scheme 2). Under physiological conditions, **6** will be deprotonated, while **15** will exist as the cationic acridinium form. This crucial difference should be reflected in the DNA binding and biological activity of the acridines and the corresponding platinum conjugates.

**Cytotoxicity.** **7**, **15b**, and **16** were studied for potential antitumor activity against HL-60 leukemia cells and human ovarian 2008 (wild type) and C13\* (cisplatin-resistant) cell lines. **2** and **6** could not be solubilized with *N*-dimethylformamide and dimethyl sulfoxide and were not included in this study. The results are summarized in Table 1. Conjugate **7** was inefficient at inhibiting cell growth in the cell lines tested. In contrast, **15b** (the acridinium chloride salt of **15**) and its platinum conjugate, **16**, proved to be cytotoxic at micromolar concentrations. While **15b** appears to be only moderately active in HL-60 cells, tethering of the [PtCl(en)]<sup>+</sup> fragment to thiourea sulfur (giving **16**) causes a dramatic increase in cytotoxicity (approximately 85-fold) in this cell line. **15b** was 5-fold more potent in the 2008 ovarian cell line than in HL-60 cells. The opposite effect was observed for **16**, which was slightly less cytotoxic in the 2008 cell line than the unmodified acridine, **15b**. In C13\*, a cell line possessing acquired cisplatin resistance, **15b** and **16**, were markedly active and showed only low levels of cross resistance with the clinical agent.

From the structures of **7** and **16**, it can be inferred that the DNA binding of these agents must be distinctly different from that of cisplatin, potentially involving platination of nucleophilic sites and interactions of the planar acridines with the base stack. Interestingly, **16**, which contains a high-pK<sub>a</sub>, flexibly linked acridine, is ca. 580 times more potent in HL-60 cells than **7**, which

carries a rigidly linked, low-pK<sub>a</sub> acridine. Although the mechanistic basis of this observation is still to be explored, we suggest that the reason for the inactivity of **7** may lie at the DNA level. The inability of acridine in **7** to efficiently interact with the duplex, for electrostatic and/or steric reasons discussed above, may lead to only minor structural alterations in DNA that ultimately render the monofunctional adducts repairable and noncytotoxic. Thus, the ethylene linker in **16** may be a structural prerequisite for a sterically feasible and electronically favored dual-binding mode. The magnitude of changes in DNA conformation resulting from such “quasibifunctional” adducts may ultimately trigger downstream effects that lead to cell death.

Tumor resistance to cisplatin is multifactorial in nature and is usually mediated by elevated levels of glutathione, enhanced DNA repair, and impaired cellular accumulation of the drug.<sup>3</sup> The cisplatin-resistant cell line chosen, C13\*, exhibits a ca. 12-fold resistance as compared to the parent cell line, 2008.<sup>18</sup> Unmodified acridine, **15b**, and platinum-acridine, **16**, partially circumvent acquired resistance to cisplatin. Decreased drug accumulation and reduced ability to form intra-strand cross-links on DNA are the major determinants of cisplatin resistance in C13\*.<sup>19</sup> The level of cross-resistance established for **15b** and **16** (1.7- and 2.5-fold, respectively) appears to be in agreement with the observed ca. 2-fold impairment of drug uptake in the C13\* subline.<sup>20</sup> In the case of impaired uptake being the only contributor to the residual cross-resistance, our data would indicate that **16** acts through an alternative mechanism (possibly at the DNA level) not susceptible to cisplatin-specific detoxification and repair.

In summary, we have developed a prototypical platinum–acridine conjugate, **16**, that proved to be cytotoxic at micromolar concentrations. We have demonstrated that through simple modification of chemical structure, i.e., introduction of an ethylene spacer, an inactive derivative can be turned into a deadly cell poison. Although the cytotoxicity of **16** in 2008/C13\* ovarian cell lines does not indicate any advantage over that observed for unmodified acridine, **15b**, the reduced level of cross-resistance indicates that **16** partially circumvents acquired resistance to cisplatin *in vitro*. This finding and the high potency of **16** in HL-60 leukemia cells suggest potential clinical utility of this type of conjugate and warrant further DNA binding and structure–activity relationship studies within an extended series of structural derivatives and a broader range of cell lines.

## Experimental Section

**Chemistry. (a) Materials.** 9-Chloroacridine (**4**) and 9-phenoxyacridine (**11**) were synthesized according to known procedures.<sup>21</sup> All other reagents were obtained from common vendors and used as supplied. [PtCl<sub>2</sub>(en)] was prepared following the method described by Dhara<sup>22</sup> for cisplatin by simply replacing aqueous ammonia with ethane-1,2-diamine (en).

**(b) General Procedures.** <sup>1</sup>H NMR data were acquired on a Bruker Avance 300 spectrometer. Chemical shifts (δ, ppm) were referenced to residual solvent peaks. <sup>195</sup>Pt NMR spectra of **7** and **16** were recorded on a Bruker Avance 500 spectrometer at 107.5 MHz. Aqueous K<sub>2</sub>[PtCl<sub>4</sub>] was used as external standard, and <sup>195</sup>Pt chemical shifts are reported vs [PtCl<sub>6</sub>]<sup>2-</sup>. The decomposition of **3** in D<sub>2</sub>O was followed by <sup>195</sup>Pt NMR spectroscopy using a similar setup and a sweep width of

500 000 Hz. The  $pK_a$  values of **6** and **15** were determined spectrophotometrically. Melting points were determined on a Mel-Temp II apparatus and are uncorrected. Elemental analyses were performed by Quantitative Technologies, Inc., Madison, NJ.

**(c) Synthesis of Drug Prototypes.** 1-[2-(Acridin-9-ylamino)ethyl]-1,3-dimethylthiourea (**15**), 1-[2-(Acridin-9-ylamino)ethyl]-1,3-dimethylthiourea Hydronitrate (**15a**), and 1-[2-(Acridin-9-ylamino)ethyl]-1,3-dimethylthiourea Hydrochloride (**15b**). A solution of methylisothiocyanate (2.60 g, 35.6 mmol) in 50 mL of absolute ethanol was added dropwise within 15 min to a solution of 7.04 g (28.0 mmol) of **14** in 350 mL of absolute ethanol. The mixture was heated at reflux for 6 h and passed, while hot, through a Celite pad. Ethanol was removed using a rotary evaporator, yielding 10 g of a brownish yellow crystal mass. 5.00 g (15.4 mmol) of the crude product was recrystallized from 150 mL of methanol. A fraction of a golden yellow, microcrystalline **15** was obtained after the solution was kept at 4 °C for 12 h. Yield: 3.30 g (66%); mp 196 °C (dec). UV-Vis (MeOH): 395 (9143), 413 (12357), 436 (9964). <sup>1</sup>H NMR (MeOH-*d*<sub>4</sub>): δ 2.98 (3H, s), 3.03 (3H, s), 4.18 (2H, t), 4.38 (2H, br m), 7.32 (2H, t), 7.64 (2H, t), 7.82 (2H, d), 8.40 (2H, d). Anal. (C<sub>18</sub>H<sub>20</sub>N<sub>4</sub>S) C, H, S, N: calcd, 17.27; found, 16.71.

The corresponding acridinium salts were generated by adding 14 mL of a 1 M solution of the appropriate acid to 5.00 g (15.4 mmol) of crude **15** in 150 mL of methanol. **15a** and **15b** precipitated spontaneously as bright yellow needles, which were filtered off, washed with small amounts of diethyl ether, and dried in a vacuum.

**15a.** Yield: 3.90 g (66%); mp 230 °C (dec). <sup>1</sup>H NMR (MeOH-*d*<sub>4</sub>): δ 3.06 (3H, s), 3.09 (3H, s), 4.51 (4H, m), 7.55 (2H, t), 7.78 (2H, d), 7.96 (2H, t), 8.69 (2H, d). Anal. (C<sub>18</sub>H<sub>21</sub>N<sub>5</sub>O<sub>3</sub>S) C, H, N, S.

**15b.** Yield: 3.51 g (63%); mp 240 °C (dec). <sup>1</sup>H NMR (MeOH-*d*<sub>4</sub>): δ 3.03 (3H, s), 3.10 (3H, s), 4.51 (4H, m), 7.55 (2H, t), 7.79 (2H, d), 7.96 (2H, t), 8.70 (2H, d). Anal. (C<sub>18</sub>H<sub>21</sub>N<sub>4</sub>ClS) C, H, N, S.

[PtCl(en)(C<sub>18</sub>H<sub>21</sub>N<sub>4</sub>S)](NO<sub>3</sub>)<sub>2</sub>·MeOH (**16**). A mixture of 0.652 g (2.00 mmol) of [PtCl<sub>2</sub>(en)] and 0.338 g (2.00 mmol) of AgNO<sub>3</sub> in 10 mL of anhydrous DMF was stirred at room temperature in the dark for 14 h. Precipitated AgCl was filtered off through a Celite pad, 0.740 g (1.91 mmol) of **15a** was added to the filtrate, and the suspension was stirred for 3 h in the dark. The solution was evaporated to dryness in a vacuum at 30 °C yielding a yellow residue, which was dissolved in 1.8 L of dry methanol. Activated carbon was added, and the solution was stirred for 15 min. Carbon was filtered off, and the solution was concentrated to a final volume of 150 mL. Crude **16** was obtained as a bright yellow solid after the solution was stored for 24 h at 4 °C. The crude batch was recrystallized from hot methanol. The solution was stored in the refrigerator for 48 h to afford **16** as a microcrystalline yellow solid, which was dried at 60 °C in a vacuum for 4 h. Yield: 0.850 g (58%). <sup>1</sup>H NMR (D<sub>2</sub>O): δ 2.62 (4H, s, broad base due to unresolved Pt satellites), 2.87 (3H, s), 3.08 (3H, s), 3.37 (3H, s), 4.38 (2H, m), 4.42, (2H, m), 7.57 (2H, d), 7.62 (2H, t), 7.94 (2H, t), 8.21 (2H, d). <sup>195</sup>Pt NMR (DMF-*d*<sub>7</sub>): δ -2873. Anal. (C<sub>21</sub>H<sub>33</sub>N<sub>8</sub>ClO<sub>7</sub>PtS) C, H, N, Cl, S.

**Cytotoxicity.** Cytotoxicity assays were carried out as described previously.<sup>23</sup> Solutions of the drugs in saline were prepared immediately before the incubations from 0.100 mM stocks, which were protected from light and stored at -20 °C. IC<sub>50</sub> data (drug concentration at which colony growth was inhibited by 50%) were calculated as a percentage of control cells from logarithmic plots of drug concentration versus colony counts. IC<sub>50</sub> values are averages of two individual experiments, with each incubation performed in quadruplicate.

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**Supporting Information Available:** Detailed X-ray crystallographic data for **7**; synthetic procedures, including intermediates and compounds not included in the cytotoxicity studies; details of the  $pK_a$  measurements and clonogenicity assays; listing of analytical data for target compounds **7**, **15b**, and **16**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- Wong E.; Giandomenico, C. M. Current Status of Platinum-based Antitumor Drugs. *Chem. Rev.* **1999**, *99*, 2451-2466.
- Oliver, R. T. D. Testicular Cancer. *Curr. Opin. Oncol.* **2001**, *13*, 191-198.
- Singh, G.; Dorward, A.; Moorehead, R.; Sweet, S. Novel Mechanisms of Resistance to Cancer Chemotherapy. *Cancer J.* **1995**, *8*, 304-307.
- Ziegler, C. J.; Silverman, A. P.; Lippard, S. J. High-throughput Synthesis and Screening of Platinum Drug Candidates. *J. Biol. Inorg. Chem.* **2000**, *5*, 774-783.
- Jamieson, E. R.; Lippard, S. J. Structure, Recognition, and Processing of Cisplatin-DNA Adducts. *Chem. Rev.* **1999**, *99*, 2467-2498.
- Farrell, N. Nonclassical Platinum Antitumor Agents: Perspectives for Design and Development of New Drugs Complementary to Cisplatin. *Cancer Invest.* **1993**, *11*, 578-589.
- Adams, A.; Guss, J. M.; Collyer, C. A.; Denny, W. A.; Wakelin, L. P. G. Crystal Structure of the Topoisomerase II Poison 9-Amino-1-[N-(2-dimethylamino)ethyl]acridine-4-carboxamide Bound to the DNA Hexanucleotide d(CGTCAG)<sub>2</sub>. *Biochemistry* **1999**, *38*, 9221-9233.
- Kohn, K. W.; Orr, A.; O'Connor, P. M.; Guziec, L. J.; Guziec, F. S., Jr. Synthesis and DNA Sequence Selectivity of a Series of Mono- and Difunctional 9-Aminoacridine Nitrogen Mustards. *J. Med. Chem.* **1994**, *37*, 67-72.
- See, for example: Pérez, J. M.; López-Solera, I.; Montero, E. I.; Braña, M. F.; Alonso, C.; Robinson, S. P.; Navarro-Ranninger, C. Combined Effect of Platination and Intercalation upon DNA Binding of Novel Cytotoxic Pt-Bis(naphthalimide) Complexes. *J. Med. Chem.* **1999**, *42*, 5482-5486.
- Temple, M. D.; McFadyen, W. D.; Holmes, R. J.; Denny, W. A.; Murray, V. Interaction of Cisplatin and DNA-Targeted 9-Aminoacridine Platinum Complexes with DNA. *Biochemistry* **2000**, *39*, 5593-5599.
- Bierbach, U.; Roberts, J. D.; Farrell, N. Modification of Platinum-(II) Antitumor Complexes with Sulfur Ligands. 2. Reactivity and Nucleotide Binding Properties of Cationic Complexes of the Types [PtCl(diamine)(L)]NO<sub>3</sub> and {[PtCl(diamine)]<sub>2</sub>(L-L)}(NO<sub>3</sub>)<sub>2</sub> (L = Monofunctional Thiourea Derivative; L-L = Bifunctional Thiourea Derivative) in Relation to Their Cytotoxicity. *Inorg. Chem.* **1998**, *37*, 717-723.
- Sundquist, W. L.; Bancroft, D. P.; Lippard, S. J. Synthesis, Characterization, and Biological Activity of *cis*-Diammineplatinum(II) Complexes of the DNA Intercalators 9-Aminoacridine and Chloroquine. *J. Am. Chem. Soc.* **1990**, *112*, 1590-1596.
- Xu, D.; Prasad, K.; Repic, O.; Blacklock, T. J. Ethyl Trifluoroacetate: A Powerful Reagent for Differentiating Amino Groups. *Tetrahedron Lett.* **1995**, *36*, 7357-7360.
- Okeya, S.; Fujiwara, Y.; Kawashima, S.; Hayashi, Y.; Isobe, K.; Nakamura, Y.; Shimomura, H.; Kushi, Y. Novel Bis(triphenylphosphine)platinum(II) Complexes Containing a Thiourea or a 1,3-Diethylthiourea Dianion as an S,N-Chelating Ligand. *Chem. Lett.* **1992**, 1823-1826.
- Brader, M. L.; Ainscough, E. W.; Baker, E. N.; Brodie, A. M. Copper(II) Promoted Desulfurization of N-Phenylthiourea. The Synthesis and X-ray Structure of [Cu(bipy)(pc)<sub>2</sub>]<sub>2</sub> (bipy = 2,2'-Bipyridine, pc = Phenylcyanamide). *Polyhedron* **1989**, *8*, 2219-2221.
- Neidle, S. *DNA Structure and Recognition*; IRL Press: Oxford, UK, 1994; pp 74-83.
- Avdeef, A.; Comer, J. E. A.; Thomson, S. J. pH-Metric log P. 3. Glass Electrode Calibration in Methanol-Water, Applied to  $pK_a$  Determination of Water-Insoluble Substances. *Anal. Chem.* **1993**, *65*, 42-49.
- IC<sub>50</sub> values for cisplatin in the 2008 and C13\* ovarian cell lines used in this study were 3.1 and 37 μM, respectively (Chaney, S. G., personal communication).
- Jekunen, A. P.; Hom, D. K.; Alcaraz, J. E.; Eastman, A.; Howell, S. B. Cellular Pharmacology of Dichloro(ethylenediamine)-platinum(II) in Cisplatin-Sensitive and Resistant Human Ovarian Carcinoma Cells. *Cancer Res.* **1994**, *54*, 2680-2687.

- (20) Mann, S. C.; Andrews, P. A.; Howell, S. B. Modulation of *cis*-Diamminedichloro-platinum(II) Accumulation and Sensitivity by Forskolin and 3-Isobutyl-1-methylxanthine in Sensitive and Resistant Human Ovarian Carcinoma Cells. *Int. J. Cancer* **1991**, *48*, 866–872.
- (21) Raulins, N. R. Acridines. In *The Chemistry of Heterocyclic Compounds*, 2nd ed.; Vol. 9, Acridines; Acheson, R. M., Ed.; Wiley: New York, 1973; pp 9–108 and literature cited therein.
- (22) Dhara, S. C. A Rapid Method for the Synthesis of *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>-Cl<sub>2</sub>]. *Indian J. Chem.* **1970**, *8*, 193–194.
- (23) Cartee, L.; Kucera, G. L. Gemcitabine Induces Programmed Cell Death and Activates Protein Kinase C in BG-1 Human Ovarian Cancer Cells. *Cancer Chemother. Pharmacol.* **1998**, *41*, 403–412.

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