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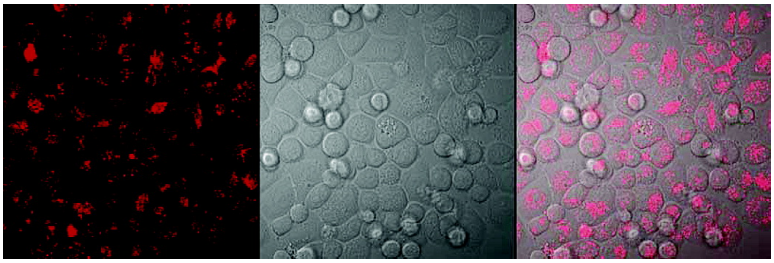

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In Vitro Anticancer Activities and Optical Imaging of Novel Intercalative Non-Cisplatin Conjugates

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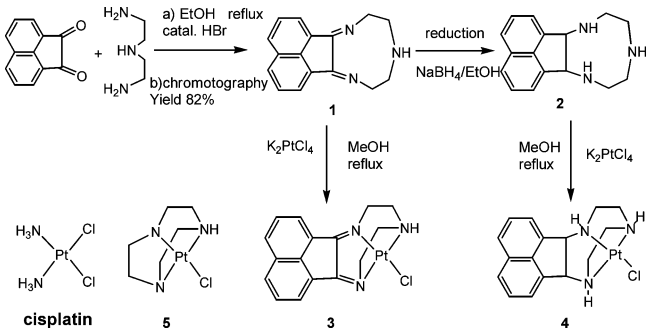
The first π-conjugated macrocyclic diimine and triaza DNA-binding intercalators and their platinum(II) conjugates have been synthesized by direct Schiff base cyclocondensation. The in vitro anticancer activities of compounds **3**, **4**, and **5** were tested on five cancer cell lines: MCF-7, A549, P388, A2780, and A2780cisR. Ovarian tumors were included specifically to evaluate the new conjugates' ability to circumvent A2780cisR resistance. Antitumor effects of the newly conjugated compounds were compared to those of cisplatin. The data clearly indicate that improved drug efficiencies are achieved as a result of the intercalative moieties. The luminescent probe that was integrated in complexes **8**−**10** made it possible to monitor drug penetration using optical imaging. Enhanced targeting of tumor nuclei by the study compounds was confirmed by confocal microscopy. This paper describes a new class of platinum-based antitumorals differing from cisplatin in several critical aspects with the potential for significantly improving clinical outcomes in cancer patients.

Introduction

Platinum-based drugs, for example, cisplatin, are widely used in the treatment of various types of primary solid tumors and metastases.[1] However, cisplatin's unacceptable toxicity levels and the development of drug resistance have led to the creation of a large number of cisplatin analogues.[2] Research on the intercalative platinum(II)-based complexes is a fruitful arena for the development of new therapeutic agents.[3−5] The intercalative ligands that were investigated include such agents as naphthalimides,[6] acridine,[7] and anthraquinone.[8] Structural modifications of DNA induced by these specific adducts include bending and unwinding the duplex.[9] The resultant activity from structural modification is greater than that produced by simply binding the individual components. Of importance to the present research is the potential for intercalator-carried platinum agents to achieve site-specific DNA recognition[11] thus becoming effective therapeutic agents. We speculated that integrating a π-conjugated moiety into a polyaza macrocyclic ligands can result in a new class of intercalators, which would potentially enhance the DNA-binding effect in the corresponding platinum complexes.

In this paper, we report the first examples of a π-conjugated macrocyclic diimine **1** and a triaza **2** ligand and the synthesis of three new square-planar platinum(II) complexes carrying only one exchangeable chloride ligand (Scheme 1). Discovery of the presence of structurally novel Pt(II)-containing pharmacophores is significant because they should effectively prevent any cross-linking within the duplex DNA. A primary focus of this project was to explore the biological effects of introducing an intercalative ligand to a platinum(II)-based drug. Enhancement of in vitro anticancer activities was observed in a series of five cancer cell lines, namely, MCF-7 (human breast), A549 (human lung), P388 (murine leukemia), A2780 (cisplatin-sensitive human ovarian), and A2780cisR (cisplatin-resistant human ovarian). The drug-resistant ovarian cell line was tested to determine the ability of the designed molecule to circumvent cisplatin resistance.

Scheme 1. The Synthetic Chemistry of New Intercalative Macrocycles and the Corresponding Platinum(II) Complexes



Many biochemical and biophysical methods have been used in an effort to elucidate the critical governing dynamics in cytotoxic platinum complexes, especially cisplatin. It is generally well understood that platinum(II) complexes are nonfluorescent in either aqueous or alcohol solutions at room temperature. To date, fluorescent platinum complexes created under any conditions have been rarely reported.[12] The ability to dynamically visualize the intracellular penetration behavior of platinum(II) analogues required the development of nondestructive methods for coupling platinum(II) drugs

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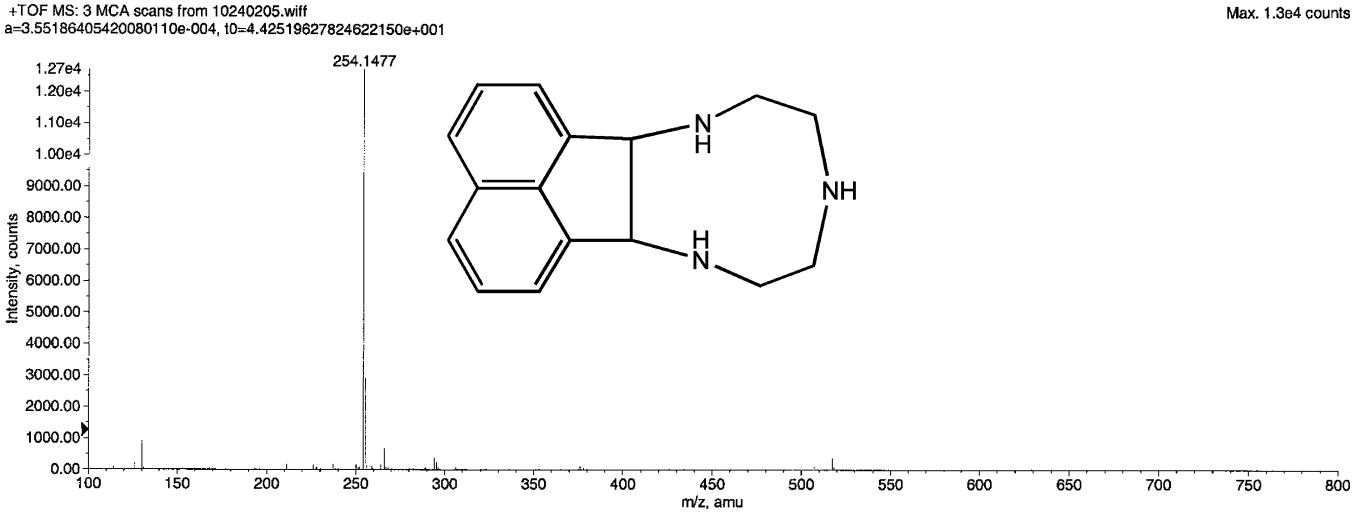

Figure 1. Electrospray ionization mass spectroscopy (ESI-MS) spectral data for compound **2**.

with luminescent dyes. In this study, novel bioconjugation techniques were developed permitting the creation of several fluorescent dye tagged intercalative non-cisplatin drugs. The value of this effort was subsequently confirmed by confocal cell imaging showing the synthetic drugs to be more effective in penetrating cell membranes than cisplatin.

Results and Discussion

Chemistry. The synthetic chemistry used in this study is summarized in Scheme 1. Compound **1** was prepared by an acid-catalyzed condensation between acenaphthenequinone and diethylenetriamine. The resulting reactant mixture was refluxed in EtOH under argon at 80 °C for 24 h. An excess quantity of ethyl ether was added to the cooled mixture producing a yellow-colored precipitate that was removed by filtration. The pure ligand was obtained by recrystallization from MeOH. Reduction of compound **1** with $NaBH_4$ in an EtOH solution produced a good yield of compound **2**, which was further purified by gel column chromatography ($MeOH/CH_2Cl_2$). Electrospray ionization mass spectroscopy (ESI-MS) spectral data for compound **2** indicated that the obtained synthetic ligands were of a high degree of purity (Figure 1). The platinum(II) conjugates [**L**Pt(II)-Cl]Cl (**L** = **1**, **2**, and 1,4,7-triazonane) were then prepared with high yield by direct reaction of the corresponding ligand using K_2PtCl_4 in a $MeOH/H_2O$ solvent mixture at 80 °C for 24 h.

^1H NMR, ^{13}C NMR, and ^{195}Pt NMR spectroscopy and elemental analyses were utilized to identify the structures of the target compounds. The structural characterization confirmed the formation of a 1:1 ligand−Pt(II) conjugate in a tridentate fashion. A critical structural difference between cisplatin and complexes **3**, **4**, and **5** emerged with respect not only to their stereo coordination but also to their acid−base chemistry. The cationic nature of the heterocyclic Pt(II) complexes is expected to exhibit high cell-membrane affinity and DNA-binding ability. These characteristics were in turn expected to reveal critical differences in biological behavior between the Pt(II) complexes and cisplatin.

Anticancer Activities. As noted in the Introduction, numerous non-cisplatin compounds containing an intercalative fragment have been investigated by others. These compounds display significant levels of in vitro antitumoral activity against several human cancer cell lines. For the present research, compounds **3**, **4**, and **5** were chosen for cancer cell line screening. Initially one of the breast cancer cell lines, MCF-7 was tested with final drug concentrations of 0.1, 1.0, 10, 50, and 100 μM, respectively. Cisplatin served as the control in each test with the results shown in Figure 2 and presented as the percentage of cell growth inhibition. Compound **4** consistently displayed the highest drug activity across the full range of the concentrations being tested. Interestingly, complexes **3** and **5** proved to be cytotoxic at micromolar concentrations. Compared with cisplatin, all of the synthetic complexes displayed an enhanced level of biological activity. A similar pattern of cytotoxicity was observed for the A549 and P388 cell lines; however complex **5** appears to be only moderately active. For the three nonovarian cell lines, the percentage of cell growth inhibition (Figure 2, A−C) at the drug concentration of 10 μM was shown to be in the order cisplatin < **5** < **3** < **4**, indicating that the integration of the intercalator to a cyclic polyaza improved the drug efficiency.

In contrast to cisplatin, complexes **3**, **4**, and **5** possess just one available chloride binding site. Given that these were extreme cases, we decided to test their cytotoxicity and bioactivity against a pair of ovarian cell lines. One (A2780) is cisplatin-sensitive and the other (A2780cisR) is cisplatin-resistant based on decreased uptake and enhanced DNA repair, as described by others.[13] Cell line A2780cisR was chosen based on its known mechanisms of resistance to cisplatin that include decreased drug uptake, enhanced DNA repair, and highly elevated glutathione levels. Glutathione is mediated by cystine as well as glutamate, which decreases glutathione but does so competitively with cystine uptake.[14] The compounds were incubated for 24 h with the ovarian tumor cell line. The cytotoxicity results, IC_{50}, of the complexes are summarized in Table 1. Complexes **3** and **4** display enhanced cytotoxicity as compared with cisplatin; however complex **5** was found to be approximately 2 times less cytotocic than cisplatin. The cytotoxicity for cisplatin is much lower in the resistant cell line and the resistant factor, RF, was found to be 15.0. However, the RF value for all the synthetic complexes are much smaller than that for cisplatin, indicating that com-

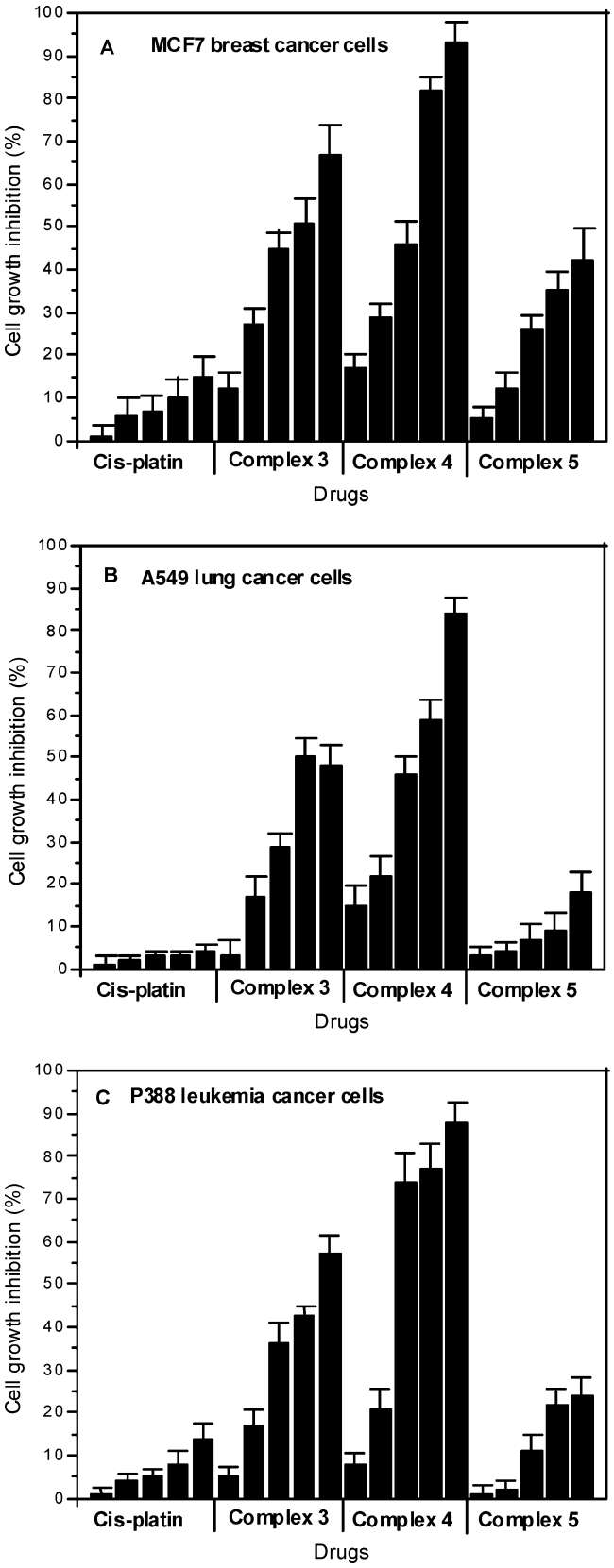

Figure 2. Anticancer activities of complexes **3**−**5** against breast, lung, and leukemia cancer cell lines. The cells were treated with increasing concentrations of 0.1, 1.0, 10, 25, and 100 μM for each complex from left to right.

plexes **3**, **4**, and **5** are more potent than cisplatin against A2780cisR cells.

Biocojugation and Confocal Imaging. It is generally accepted that the therapeutic activity of metallo-

Table 1. IC_{50} Mean Values (±SD) Obtained for Complexes **3**−**5**

complexes	A2780	A2780cisR	RF[a]
cisplatin	2.4 ± 0.4	36 ± 2.0	15.0
3	1.4 ± 0.1	5.0 ± 0.2	3.6
4	1.2 ± 0.2	3.6 ± 0.1	2.2
5	5.0 ± 0.3	43 ± 2.5	8.5

[a] RF = IC_{50}(resistant)/IC_{50}(sensitive).

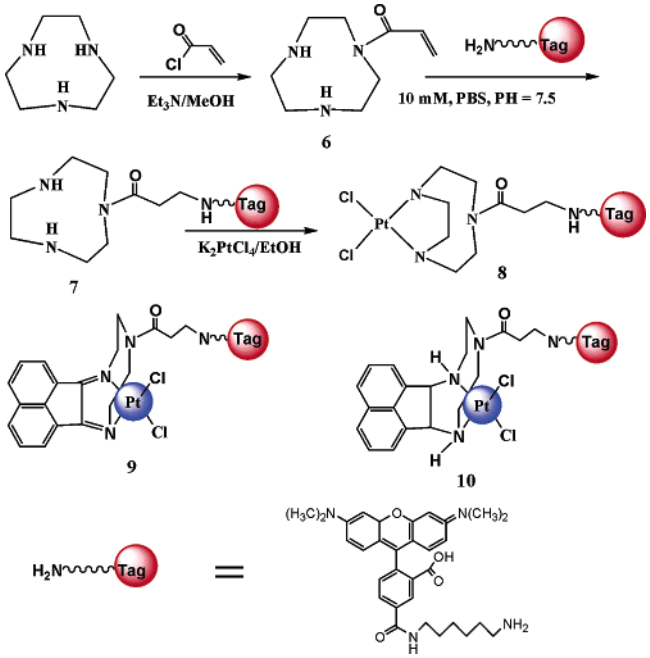

Figure 3. The biocojugation strategy for complexes **6**−**10**.

anticancer drugs, such as cisplatin and its derivatives, is based on their interaction with DNA. Although this postulate is generally accepted, it is based primarily on direct observations of the interaction between DNA and drug molecules outside living cells. Clearly it is important to understand the means by which platinum drugs could penetrate the cell membrane and whether they in fact penetrate the cell nuclei. To investigate these questions, the triaza macrocyclic molecules were conjugated with the dye probe rhodamine by means of a novel bioconjugation strategy (Figure 3). This was followed by reacting these labeled molecules with K_2PtCl_4. The resulting complexes are stable in a 0.01 mM phosphate-buffered saline solution at pH = 7.4 for 24 h, allowing for performance and completion of confocal imaging. The luminescent-tagged drug molecules are well suited for mapping their spatial and temporal distribution. The results of treating SK-BR-3 cells (human breast cancer cell line) with complexes **8**, **9**, and **10** for 30 min as tracked by confocal imaging are shown in Figure 4.

Complex **8** showed minimal translocation into the cells and little of the complex attached to the cell membranes. The uptake of the intercalative diimine complex **9** was comparatively higher with internalized luminescent complexes found both on the inner cellular membrane and in the perinuclear zone. Complex **10** proved to be the most efficient of all the complexes in demonstrating the strongest red cell imaging. A significant fraction of the complex was identified inside the cell nuclei with additional portions seen surrounding the perinuclear region. The drugs observed in the cytoplasm

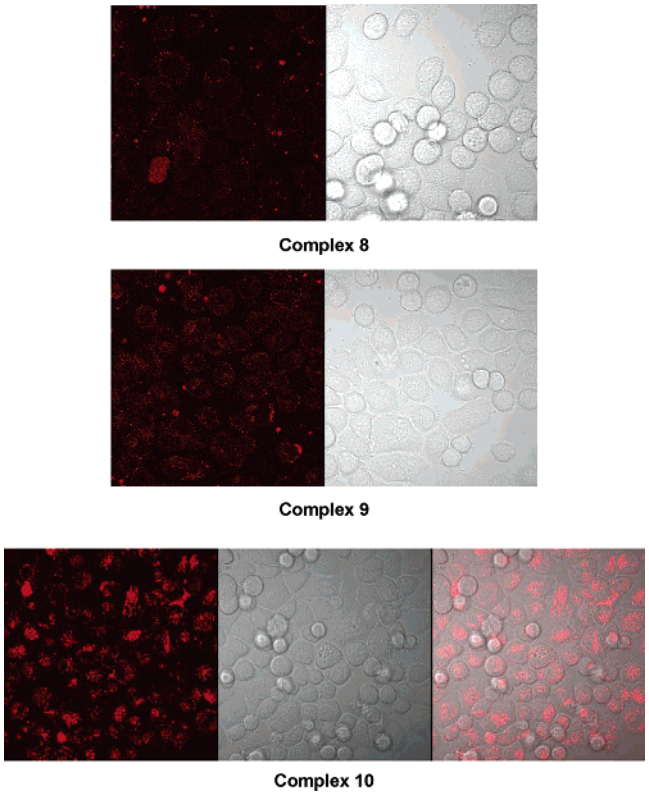

Figure 4. Confocal cellular imaging of a human breast cancer cell line (SK-BR-3) treated by the drug candidates **8**, **9**, and **10**. The intercalative polyaza complex, **10**, and **9** shown enhanced levels of penetration compared with the nonintercalative counterpart.

appear as a bright red spots, which suggests that the drug molecules integrate into the cells endocytotically.

In summary, we have developed novel non-cisplatin complexes designed to enhance the drug efficiency in human cancer cells and to circumvent cisplatin resistance in ovarian cancer cell lines. A new bioconjugation method has been developed to tag the drug molecules. Optical imaging has been employed to investigate the drug translocation mechanism of platinum(II). Therefore, further preparation of new derivatives with a variety of intercalative ligands and conjugation with other tumor targeting ligands should be of great interest and presents a promising direction in anticancer drug research.

Experimental Section

Synthesis of Compound 1. A solution of acenaphthylene-1,2-dione (1.0 mmol) in 50 mL of EtOH was added dropwise to a solution containing 1.0 mmol of diethylenetriamine in the present of catalytic amount of HBr (pH ≈ 3.5). Following 24 h of reflux, a large amount of yellow precipitate formed. Yield 46%. Anal. Calcd for $C_{16}H_{15}N_3$: C, 77.1; H, 6.1; N, 16.9. Found: C, 77.2; H, 6.0; N, 17.0%. ESI-MS calculated for $[C_{16}H_{15}N_3 + H]^+$ 250.13, found 250.12. ^{1}H NMR ($CDCl_3$): δ 2.91 (s, 4H, $-CH_2-$), 3.77 (s, 4H, $-CH_2-$), 7.65−8.00 (m, 6H, naphthalene).^{13}C NMR ($CDCl_3$): δ 49.2 ($-CH_2$-), 50.1($-CH_2-$), 126−139 (naphthalene), 166 ($-N{=}C-$).

Synthesis of Compound 2. A solution of compound **1** (0.10 mmol) in 50 mL of EtOH was added dropwise to a solution containing 0.4 mmol of $NaBH_4$ in EtOH. Reduction for 24 h at room temperature resulted in a colorless solution. The crude product was purified by gel column chromatography (MeOH/CH_2Cl_2 = 1:1). Yield 85%. Anal. Calcd for $C_{16}H_{19}N_3$: C, 75.9; H, 7.6; N, 16.6. Found: C, 75.8; H, 7.5; N, 16.4%. ESI-MS calculated for $[C_{16}H_{19}N_3+H]^+$ 254.15, found 254.15. ^{1}H NMR ($CDCl_3$): δ 2.52−2.66 (s, 8H, $-CH_2-$), 4.77(s, 2H, $-CH-NH-$), 7.05−7.48 (m, 6H, naphthalene).^{13}C NMR ($CDCl_3$): δ 48.2 ($-CH_2-$), 50.2($-CH_2-$), 68.2 ($-CH-NH-$), 126−136 (naphthalene).

Synthesis of Compound 3. A solution of compound **1** (0.02 mmol) in 10.0 mL of EtOH was added dropwise to an aqueous solution containing 0.02 mmol of K_2PtCl_4. Reduction for 2 h at room temperature resulted in the formation of a yellow-colored precipitate. Yield 65%. Anal. Calcd for $C_{16}H_{15}N_3PtCl_2$: C, 37.3; H, 2.9; N, 8.2. Found: C, 37.3; H, 3.0; N, 8.1%. ESI-MS calculated for $[C_{16}H_{15}N_3PtCl]^+$ 479.7, found 479.8. ^{1}H NMR (D_2O): δ 2.99 (s, 4H, $-CH_2-$), 3.82(s, 4H, $-CH_2-$), 7.85−8.20 (m, 6H, naphthalene).^{195}Pt NMR (DMF-d_7): δ −2278 ppm.

Synthesis of Compound 4. A solution of compound **2** (0.02 mmol) in 10.0 mL of EtOH was added dropwise to an aqueous solution containing 0.02 mmol of K_2PtCl_4. Reduction for 2 h at room temperature resulted in the formation of a yellow-colored precipitate. Yield 75%. Anal. Calcd for $C_{16}H_{19}N_3PtCl_2$: C, 36.9; H, 3.7; N, 8.1. Found: C, 37.1; H, 3.5; N, 8.0%. ESI-MS calculated for $[C_{16}H_{19}N_3PtCl]^+$ 483.7, found 483.9. ^{1}H NMR (D_2O): δ 2.72−2.86 (s, 8H, $-CH_2-$), 4.89(s, 2H, $-CH-NH-$), 7.15−7.68 (m, 6H, naphthalene).^{195}Pt NMR (DMF-d_7): δ −2177 ppm.

Synthesis of Compound 5. A solution of 1,4,7-triazonane (0.02 mmol) in 10.0 mL of EtOH was added dropwise to an aqueous solution containing 0.02 mmol of K_2PtCl_4. Reduction for 2 h at room temperature resulted in the formation of a yellow-colored precipitate. Yield 50%. Anal. Calcd for $C_6H_{15}N_3PtCl_2$: C, 18.2; H, 3.8; N, 10.6. Found: C, 18.3; H, 3.9; N, 10.7%. ESI-MS calculated for $[C_6H_{15}N_3PtCl + H]^+$ 359.9, found 359.8. ^{1}H NMR (D_2O), δ 2.87 (s, 12H, $-CH_2-$). ^{195}Pt NMR (DMF-d_7): δ −2345 ppm.

Synthesis of Compound 6. A solution of 1,4,7-triazonane (0.02 mmol) in 10.0 mL of MeOH was added dropwise to a solution containing 0.02 mmol of acryloyl chloride in the presence of 0.02 mmol of NaOMe. The mixture was magnetically stirred for 8 h at room temperature and then was gently heated to 50 °C to complete the reaction. The solvent was treated with 50 mL of ethyl ether to give white precipitate and was then filtered. Yield 45%. Anal. Calcd for $C_9H_{17}N_3O$: C, 59.0; H, 9.4; N, 22.9. Found: C, 59.2; H, 9.5; N, 22.4%. ESI-MS calculated for $[C_9H_{17}N_3O + H]^+$ 184.1, found 184.2. ^{1}H NMR ($CDCl_3$), δ 2.62−3.11 (m, 12H, $-CH_2-$), 5.52−6.66 (m, 3H, $O{=}C-CH{=}CH_2$).^{13}C NMR ($CDCl_3$), δ 48.4−51.2 ($-CH_2-$), 128.9 ($-CH{=}CH_2$), 130.7 ($-CH{=}CH_2$), 165.2 ($-C{=}O$).

Synthesis of Compound 7. A solution of compound **6** (0.01 mmol) in 10.0 mL of MeOH was added dropwise to an aqueous solution (pH = 7.4, 100 mM PBS) containing 0.01 mmol of 5-(and 6)-carboxytetramethylrhodamine with a terminal amine group, which was modified by coupling with a hexane-1,6-diamine. The bioconjugation process completes in 8 h at 37 °C, and the final product is purified by a C-18 column. ESI-MS calculated for $[C_{40}H_{54}N_7O_5Cl + H]^+$ 749.2, found 749.1.

Synthesis of Compound 8. Compound **7** (1.0 mmol) in 10.0 mL of MeOH/H_2O was allowed to undergo reaction with an equivalent of K_2PtCl_4 in aqueous solution (PBS, pH = 7.4, 100 mM). The bioconjugation was complete in 8 h at 37 °C. ESI-MS calculated for $[C_{40}H_{54}N_7O_5PtCl_2]^+$ 978.7, found 978.8. ^{195}Pt NMR (DMF-d_7): δ −2872 ppm.

Complexes **9** and **10** were prepared and characterized by following similar procedures as described in the synthesis of complex **8** by replacement of 1,4,7-triazonane with compound **1** or **2**, and the formation of the final conjugated molecule was confirmed by ESI-MS and ^{195}Pt NMR.

Materials and Measurements. All chemical reagents and solvents were of analytical grade and obtained from the Sigma-Aldrich Chemical Co. Methanol and acetonitrile were dried over molecular sieves (4 Å) prior to use. Analysis for C, H, and N was carried out on a Perkin-Elmer analyzer, model 240. Positive ion ESI-MS spectra were recorded using LCQ electrospray mass spectrometry. The spectra were recorded over the mass range *m/z* 200−1000. The organic compounds were characterized by ^{1}H NMR and ^{13}C NMR on a Varian Unity

Inova 500 MHz. All of the platinum complexes that were synthesized were characterized by ^{195}Pt NMR spectroscopy. Data were collected on a Varian Unity Inova 500 MHz spectrometer equipped with a 5 mm switchable probe. ^{195}Pt chemical shifts were referenced to an external K_2PtCl_4 solution in D_2O, set at −1624 ppm.

In Vitro Anticancer Drug Screen. The cancer cell lines P388, A549, and MCF-7 were obtained from ATCC, Manassas, VA. The cells were grown as suspension cultures in RPMJ 1640 medium containing 15% fetal calf serum and 1% glutamine at 37 °C in an atmosphere humidified with 5% CO_2. The in vitro cytotoxicity was tested by following a NCI protocol for antitumor drug screen.[15] For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 μL at plating densities ranging from 5000 to 40 000 cells/well depending on the doubling time of individual cell lines. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition. Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin.

Following drug addition, the plates are incubated for an additional 48 h at 37 °C, 5% CO_2, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid, and the plates are air-dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm.

Confocal Microscopy. Cells were plated in chambered coverglass wells (Nalgene Nunc) in full growth medium. Complexes were added 24 h later to a final concentration of 10 μM. Prior to microscopic evaluation, the cells were washed three times with phosphate-buffered saline. Confocal miscroscopy was performed in the Imaging Core Facility. All images were obtained under identical settings allowing for the comparison of fluorescence between samples.

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References

(1) (a) Wong, E.; Giandomenico, C. M. Current Status of Platinum-Based Antitumor Drugs. *Chem. Rev*. **1999**, *99*, 2451−2466. (b) Jamieson, E. R.; Lippard, S. J. Structure, Recognition, and Processing of Cisplatin-DNA Adducts. *Chem. Rev*. **1999**, *99*, 2467−2498.

(2) (a) Najajreh, Y. Perez, J. M.; Navarro-Ranninger, C.; Gibson, D. Novel soluble cationic trans-diaminedichloroplatinum(II) complexes that are active against cisplatin resistant ovarian cancer cell lines. *J. Med. Chem*. **2002**, *45*, 5189−5198. (b) Komeda, S.; Kalayda, G. V.; Lutz, M.; Spek, A. L.; Yamanaka, Y.; Sato, T.; Chikuma, M.; Reedijk, J. New isomeric azine-bridged dinuclear platinum(II) complexes circumvent cross-resistance to cisplatin. *J. Med. Chem*. **2003**, *46*, 1210−1219. (c) Zák, F.; Turánek, J.; Kroutil, A.; Sova, P.; Mistr, A.; Poulová, A.; Mikolin, P.; Zák, Z.; Kaná, A.; Záluská, D.; Nea, J.; Sindlerová, L.; Kozubík, A. Platinum(IV) complex with adamantylamine as nonleaving amine group: synthesis, characterization, and in vitro antitumor activity against a panel of cisplatin-resistant cancer cell lines. *J. Med Chem*. **2004**, *47*, 761−763.

(3) (a) Sundquist, W. I.; 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. (b) Culliname, C.; Wickham, G.; MecFayden, W. D.; Denny, W. A.; Palmer, B. D.; Philips, D. R. The use of bidirectional transcription footprinting to detect platinum DNA cross-links by acridine-tethered platinum diamine complexes and cisplatin. *Nucleic Acids Res.* **1993**, *21*, 393−400. (c) Martins, E. T.; Baruah, H.; Kramarczyk, J.; Saluta, G.; Day, C. S.; Kucera, G. L.; Bierbach, U. Design, Synthesis, and Biological Activity of a Novel Non-Cisplatin-type Platinum-Acridine Pharmacophore. *J. Med. Chem.* **2001**, *44*, 4492−4496.

(4) (a) Murray, V.; Motyka, H.; England, P. R.; Wickham, G.; Lee, H. H.; Denny, W. A.; Mcfayden, W. D. An investigation of the sequence-specific interaction of cis-diamminedichloroplatinum-(II) and four analogs, including two acridine-tethered complexes with DNA inside human cells. *Biochemistry* **1992**, *31*, 11812−11817. (b) Murray, V.; Motyka, H.; England, P. R.; Wickham, G.; Lee, H. H.; Denny, W. A.; Mcfayden, W. D. *J. Biol. Chem*. **1992**, *267*, 18805. (c) Budiman, M. E.; Alexander, R. W.; Bierbach, U. Unique base-step recognition by a platinum-acridinylthiourea conjugate leads to a DNA damage profile complementary to that of the anticancer drug cisplatin. *Biochemistry* **2004**, *43*, 8560−8567.

(5) (a) Mikata, Y.; Mogami, K.; Kato, M.; Okura, I.; Yano, S. Synthesis, characterization, interaction with DNA, and antitumor activity of a *cis*-Dichloroplatinum(II) complex linked to an intercalator via one methylene chain. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1083−1086. (b) Mikata, Y.; Yokoyama, M.; Mogami, K.; Kato, M.; Okura, I.; Chikira, M.; Yano, S. Intercalator-linked cisplatin: synthesis and antitumor activity of *cis*-dichloroplatinum(II) complexes connected to acridine and phenylquinolines by one methylene chain. *Inorg. Chim. Acta*. **1998**, *279*, 51−57. (c) Holmes, R. J.; McKeage, M. J.; Murray, V.; Denny, W. A.; McFayden, W. D. cis-Dichloroplatinum(II) complexes tethered to 9-aminoacridine-4-carboxamides: synthesis and action in resistant cell lines in vitro. *J. Inorg. Biochem.* **2001**, *85*, 209−217. (d) Todd, J. A.; Rendina, L. M. Synthesis and DNA-binding properties of cationic 2,2′:6′,2″-terpyridineplatinum(II) complexes containing 1,2- and 1,7-dicarba-*closo*-dodecaborane(12). *Inorg. Chem*. **2002**, *41*, 3331−3333. (e) Ackley, M. C.; Barry, C. G.; Mounce, A. M.; Farmer, M. C.; Spring, B. E.; Day, C. S.; Wright, M. W.; Berners-Price, S. J.; Hess, S. M.; Bierbach, U. Structure−activity relationships in platinum-acridinylthiourea conjugates: effect of the thiourea nonleaving group on drug stability, nucleobase affinity, and in vitro cytotoxicity. *J. Biol. Inorg. Chem.* **2004**, *9*, 453−461.

(6) Perez, J. M.; Lopez-Solera, I.; Momtero, E. I.; Brara, 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.

(7) (a) Gibson, D.; Gean, K. F.; Ben-Shoshan, R.; Ramu, A.; Ringel, I.; Katzhendler, J. Preparation, characterization, and anticancer activity of a series of cis-$PtCl_2$ complexes linked to anthraquinone intercalators, *J. Med. Chem*. **1991**, *34*, 414−420. (b) Gibson, D.; Binyamin, I.; Haj, M.; Ringel, I.; Ramu, A.; Katzhendler, J. Anthraquinone intercalators as carrier molecules for second-generation platinum anticancer drugs. *Eur. J. Med. Chem.* **1997**, *32*, 823−831.

(8) (a) Perez, J. M.; Lopez-Solera, I.; Momtero, E. I.; Brara, 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. (b) Perrin, L. C.; Prenzler, P. D.; Culliname, C.; Phillips, D. R.; McFayden, W. A. DNA targeted platinum complexes: synthesis, cytotoxicity and DNA interactions of *cis*-dichloroplatinum(II) complexes tethered to phenazine-1-carboxamides. *J. Inorg. Biochem*. **2000**, *81*, 111−117.

(9) (a) Baruah, H.; Rector, C. L.; Monnier, S. M.; Bierbach, U. Mechanism of action of non-cisplatin type DNA-targeted platinum anticancer agents: DNA interactions of novel acridinylthioureas and their platinum conjugates. *Biochem. Pharmacol.* **2002**, *64*, 191−200. (b) Baruah, H.; Bierbach, U. Unusual intercalation of acridin-9-ylthiourea into the 5′-GA/TC DNA base step from the minor groove: implications for the covalent DNA adduct profile of a novel platinum-intercalator conjugate. *Nucleic Acids Res*. **2003**, *31*, 4138−4146. (c) Baruah, H.; Day, C. S.; Wright, M. W.; Bierbach, U. Metal-intercalator-mediated self-association and one-dimensional aggregation in the structure of the excised major DNA adduct of a platinum-acridine agent. *J. Am. Chem. Soc*. **2004**, *126*, 4492−4493.

(10) Whittaker, J.; McFayden, W. D.; Wickham, G.; Wakelin, L. P. G.; Murray, V. The interaction of DNA-targeted platinum phenanthridinium complexes with DNA. *Nucleic Acids Res*. **1998**, *26*, 3933−3939.

(11) Temple, M. D.; McFayden, 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.

(12) (a) Criado, J. J.; Fernandez, E. R.; Manzano, J. L.; Alonso, A.; Barrena, S.; Medarde, M.; Pelaez, R.; Tabernero, M. D.; Orfao, A. Intrinsically fluorescent cytotoxic cisplatin analogues as DNA marker molecules. *Bioconjugate Chem*. **2005**, *16*, 275−282. (b) Molenaar, C.; Teuben, J. M.; Heerebrij, R. B.; Tanke, H. J.; Reedijk, J. *J. Biol. Inorg. Chem*. **2000**, *5*, 655−665.

(13) (a) Kelland, L. R.; Abel, G.; McKeage, M. J.; Jones, M.; Goddard, P. M.; Valenti, M.; Murrer, B. A.; Harrap, K. R. Preclinical antitumor evaluation of bis-acetato-amine-dichloro-cyclohexylamine platinum(IV): an orally active platinum drug. *Cancer Res.* **1993**, *53*, 2581−2586. (b) Pors, K.; Paniwnyk, Z.; Ruparelia, K. C.; Teesdale-Spittle, P. H.; Hartley, J. A.; Kelland, L. R.; Patterson, L. H. Synthesis and biological evaluation of novel chloroethylaminoanthraquinones with potent cytotoxic activity against cisplatin-resistant tumor cells. *J. Med. Chem*. **2004**, *47*, 1856−1859.

(14) Behrens, B. C.; Hamilton, T. C.; Masuda, H.; Grotzinger, K. R.; Whang-Peng, J.; Louie, K. G.; Knutsen, T.; McKoy, W. M.; Young, R. C. Ozols, R. F. Characterization of a cis-diaminedichloroplatinum (II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res.* **1987**, *47*, 414−418.

(15) (a) Alley, M. C.; Scudiero, D. A.; Monks, P. A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.* **1988**, *48*, 589−601, (b) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. The National Cancer Institute: Cancer Drug Discovery and Development Program. *Semin. Oncol.* **1992**, *19* (No. 6), 622-638, (c) Boyd, M. R.; Paull, K. D. Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. *Drug Dev. Res.* **1995**, *34*, 91−109.

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