Synthesis, Structure, Biological Activity, and DNA Binding of Platinum(II) Complexes of the Type *trans*-[$PtCl_2(NH_3)L$] (L = Planar Nitrogen Base). Effect of L and Cis/Trans Isomerism on Sequence Specificity and Unwinding Properties Observed in Globally Platinated DNA

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In order to establish fundamental structural requirements for the antitumor activation of the *trans*-platinum geometry, complexes of the general formulas [$PtCl_2(NH_3)L$] (L = planar N donor) have been synthesized. The trans isomers, *trans*-[$PtCl_2(NH_3)$(quinoline)] (**3**), *trans*-[$PtCl_2(NH_3)$(thiazole)] (**5**), *trans*-[$PtCl_2(NH_3)$(benzothiazole)] (**7**), and *trans*-[$PtCl_2(NH_3)$(isoquinoline)] (**8**) and the cis isomers *cis*-[$PtCl_2(NH_3)$(quinoline)] (**4**) and *cis*-[$PtCl_2(NH_3)$-(thiazole)] (**6**) were characterized by ^{1}H NMR and analytical data. In addition, the crystal structures of **3**, **5**, **7**, and **8** were determined: **3**, monoclinic, $P2_1/c$, with $a = 8.414(1)$ Å, $b = 12.373(3)$ Å, $c = 21.266(3)$ Å, $\beta = 96.78(1)°$, $V = 2198.3(6)$ Å^3, and $Z = 8$; **5**, monoclinic, $P2_1/n$, with $a = 8.815(4)$ Å, $b = 19.917(8)$ Å, $c = 14.498(5)$ Å, $\beta = 103.30(3)°$, $V = 2477(2)$ Å^3, and $Z = 12$; **7**, monoclinic, $P2_1/c$, with $a = 8.150(4)$ Å, $b = 23.196(9)$ Å, $c = 11.297(7)$ Å, $\beta = 90.94(4)°$, $V = 2135.3(2)$ Å^3, and $Z = 8$; **8**, monoclinic, $C2/c$, with $a = 19.043(4)$ Å, $b = 8.570(2)$ Å, $c = 29.127(6)$ Å, $\beta\_ = 111.59(2)°$, $V = 4420(2)$ Å^3, and $Z = 16$. In all cases, the Pt coordination plane and L are mutually twisted with angles between planes of 50−68°. Bulky quinoline in **3** produces intramolecular steric strain as evidenced by a short, nonbonding Pt···$H8_{quin}$ contact of 2.77 Å and concomitantly distorted Pt−N_{quin}−C bond angles. The trans complexes **3**, **5**, **7**, and **8** showed a significantly higher cytotoxicity in cisplatin-sensitive L1210 leukemia than *trans*-[$PtCl_2(NH_3)_2$] (**2**), with **3** and **5** being as potent as the corresponding cis isomers **4** and **6**. In addition, the presence of the planar ligand greatly enhanced the activity of all of the compounds in cells resistant to cisplatin, *cis*-[$PtCl_2(NH_3)_2$] (**1**). Complex geometry and L play an important role in the binding of **1**−**7** to DNA. For synthetic poly(dG)·poly(dC) and poly(dG-dC)·poly(dG-dC) the order of binding affinities (r_b, drug-to-nucleotide ratio) was **2** > **1** > **6** > **5** > **4** > **7** > **3** and **5** > **6** > **7** > **3** > **2** > **1** > **4**, respectively. Furthermore, **3** and **7**, carrying large planar ligands, were remarkably effective at unwinding negatively supercoiled, closed circular pUC19 DNA ($\phi = 15°$ and 17°, respectively). The consequences of structural effects caused by L on target DNA with respect to possible biological consequences are discussed.

Introduction

Cisplatin (*cis*-DDP), *cis*-[$PtCl_2(NH_3)_2$] (**1**, Chart 1), has received worldwide acceptance as a clinical drug for the treatment of various neoplastic diseases, including testicular and ovarian cancers,[1] while its congener transplatin (*trans*-DDP), *trans*-[$PtCl_2(NH_3)_2$] (**2**, Chart 1), was found to be therapeutically inactive.[2] This observation, which is considered a paradigm for the structure−activity relationships (SAR) of Pt-based antitumor compounds,[2] has dominated drug development in this field for more than two decades. While the geometry requirements expressed in the SAR certainly apply to complexes that contain NH_3 or "simple" amines as nonleaving groups, they are no longer valid for compounds where platinum carries planar, N-heterocyclic amines as nonreplaceable ligands. We have shown previously that the formal replacement of one or both NH_3 ligands in *trans*-[$PtCl_2(NH_3)_2$] (**2**) with planar amines such as pyridine (py), thiazole (tz), or quinoline (quin) (e.g., in *trans*-[$PtCl_2(py)_2$], *trans*-[$PtCl_2(tz)_2$], and *trans*-[$PtCl_2(NH_3)$(quin)][3,4] (**3**, Chart 1)) greatly enhances the cytotoxicity of the trans geometry. Such nonclassical *trans*-platinum antitumor complexes show an altered spectrum of biological activity in vitro compared to clinical cisplatin, including increased cytotoxicity in cisplatin-resistant tumor cells.[5]

In order to understand the factors that contribute to the unique biological properties of complexes of the general formula *trans*-[$PtCl_2(NH_3)L$] (L = planar amine), initial model studies have been performed that address both the overall reactivity of

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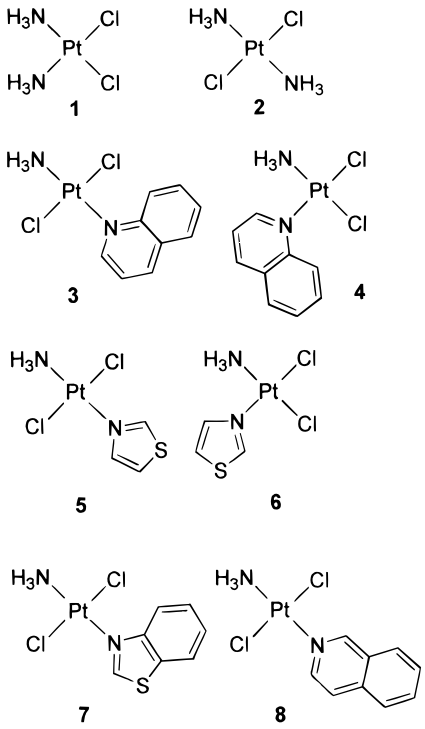
Chart 1



platinum in reactions with biologically relevant nucleophiles and the specific array of adducts formed with target DNA. The bulky, planar ligand in *trans*-[$PtCl_2(NH_3)$(quin)] (**3**) slows associative substitution reactions on platinum. Chloride displacement in **3** by guanine-N7,[6] the preferred DNA binding site for platinum(II),[7] and amino acid (methionine) sulfur,[8] a kinetically favored target,[9] occurs at rates that are ca. 2.5 times slower than for the classical DDP isomer **2**. The reduced reactivity toward biological sulfur, which counteracts unwanted intracellular detoxification (one source of acquired drug resistance of certain tumors against cisplatin[10]) and toxic side effects[11] of the drug, is considered beneficial to the biological activity of "thiophilic" platinum(II) drugs. Toward this objective, a cisplatin analogue, *cis*-[$PtCl_2(NH_3)$(2-methylpyridine)], has been developed, which is currently undergoing phase I clinical trials.[12]

Bifunctional cross-linking agents, such as dichloroplatinum(II) species, will form monofunctional adducts on DNA, which subsequently transform into bifunctional DNA adducts (either intrastrand or interstrand). Incubations of **3** with calf thymus DNA for 48 h show that ca. 34% of all covalent DNA−platinum adducts remain monofunctional.[13] Conformational changes in globally platinated DNA caused by **3**[13] are to some extent suggestive of those caused by the 1,2 intrastrand (GG) cross-link, the most frequently formed and commonly believed cytotoxic lesion of cisplatin.[14,15] Molecular mechanics calculations[13] suggest that a combination of monofunctional platination of N7 and stacking of quinoline with adjacent 5′-nucleobases—i.e., partial intercalation—can mimic the major *cis*-DDP adduct and may produce similar biological effects. Furthermore, it has been demonstrated that the replacement of an ammine ligand in **2** with quinoline increases the rate of bifunctional interstrand adduct formation. This has been explained with a changed cross-link specificity[13] of the *trans*-platinum geometry from purine−pyrimidine (GC, *trans*-DDP-like[16]) to purine−purine (GG, *cis*-DDP-like[17]). For **3**, both monofunctional adducts and readily formed bifunctional interstrand adducts may be considered potential cytotoxic lesions. A further reason for study of the structural factors dictating DNA binding of species such as in *trans*-[$PtCl_2(NH_3)$(quin)] is the observation that, in cells, DNA−protein associated strand breaks, reminiscent of topoisomerase inhibitors, are produced.[5] Model studies on *trans*-[PtCl(9-EtGua)(NH_3)(quinoline)]$^+$ (representing the monofunctional DNA binding step) showed a kinetic preference for methionine over 5′-GMP binding,[8] indicating that DNA−protein rather than DNA−DNA cross-links may be induced under suitable conditions in vivo.

Given the unusual biological and biophysical properties of **3**, we extended our study to ligands other than quinoline, to establish the generality of the above findings. The present work describes the synthesis and solid state structures of four members of this class of cytotoxic compounds with L = quinoline (quin), thiazole (tz), isoquinoline (isoquin), and benzothiazole (btz). The binding affinity to and conformational changes caused in double-stranded DNA have been monitored. Critical differences emerge between the classical DDP isomers and [$PtCl_2(NH_3)$L] but also between the different geometric isomers of the latter structure, with respect to sequence specificity and the degree of DNA unwinding.

Experimental Section

Synthesis and Characterization. The starting complexes *cis*-[$PtCl_2(NH_3)_2$][18] (**1**) and K[$PtCl_3(NH_3)$][19] and the complex *cis*-[$PtCl_2(NH_3)$(quinoline)][4] (**4**) were synthesized by the published procedures. *trans*-[$PtCl_2(NH_3)_2$] (**2**) was from Alfa Products, Ward Hill, MA. All other chemicals and solvents were purchased from common vendors and used as supplied. All syntheses used distilled water as solvent.

^{1}H NMR spectra of DMF-d_7 solutions of **5**−**8** were recorded at 300 MHz on a General Electric QE 300 instrument at 294 K. Chemical shifts (δ, ppm) are referenced to TMS. Elemental analyses were performed by Robertson Microlit Laboratories, Madison, NJ.

***trans*-[$PtCl_2(NH_3)$(quinoline)][4] (3). Alternative, Improved Method.** A mixture of 10 g (0.033 mol) of **1** and 9.73 mL (0.83 mol) of quinoline in 250 mL of water was stirred for 1 h at 90−100 °C. The light-yellow solution was treated with 1 g of activated carbon and stirred for 10 min without heating. After the solution was passed through a Celite pad (while hot), 50 mL of concentrated HCl was added to the filtrate, and heating was continued for 6 h. Precipitation of poorly water soluble **3** started after ca. 2 h. After the mixture was stored at 4 °C for 12 h, the precipitate was filtered off, washed with hot water, ethanol, and

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Table 1. Crystal Data for *trans*-[$PtCl_2(NH_3)$(quin)] (**3**), *trans*-[$PtCl_2(NH_3)$(tz)] (**5**), *trans*-[$PtCl_2(NH_3)$(btz)] (**7**), and *trans*-[$PtCl_2(NH_3)$(isoquin)] (**8**)

	3	**5**	**7**	**8**
space group	$P2_1/c$	$P2_1/n$	$P2_1/c$	$C2/c$
a, Å	8.414(1)	8.815(4)	8.150(4)	19.043(4)
b, Å	12.373(3)	19.917(8)	23.196(9)	8.570(2)
c, Å	21.266(3)	14.498(5)	11.297(7)	29.127(6)
β, deg	96.78(1)	103.30(3)	90.94(4)	111.59(2)
V, Å^3	2198.3(6)	2477(2)	2135.3(2)	4420(2)
fw	412.18	368.15	418.21	412.18
D_{calcd}, g cm^{-3}	2.491	2.962	2.602	2.478
empirical formula	$C_9H_{10}Cl_2N_2Pt$	$C_3H_6Cl_2N_2PtS$	$C_7H_8Cl_2N_2PtS$	$C_9H_{10}Cl_2N_2Pt$
Z	8	12	8	16
abs coeff, cm^{-1}	133.45	179.88	134.85	132.25
temp, °C	21	21	21	21
λ, Å	0.710 69	0.710 69	0.710 69	0.710 69
R (F_o)[a]	0.034	0.049	0.028	0.026
R_w[b]	0.036	0.057	0.025	0.032

[a] $R = \sum(||F_o| - |F_c||)/\sum(|F_o|)$. [b] $R_w = [\sum w(||F_o| - |F_c||)^2/\sum w(|F_o|)^2]^{1/2}$.

diethyl ether, and finally dried in vacuo. Yield: 8.90 g (65%) of a light-yellow, microcrystalline solid. ^{1}H NMR results were in accordance with published data.[4] Anal. Calcd for $C_9H_{10}N_2Cl_2Pt$: C, 26.23; H, 2.44; N, 6.80; Cl, 17.20. Found: C, 26.14; H, 2.27; N, 6.83; Cl, 17.06.

***trans*-[$PtCl_2(NH_3)$(thiazole)] (5).** A mixture of 1.5 g (5 mmol) of **1** and 0.78 mL (11 mmol) of thiazole in 30 mL of water was heated at 60−70 °C with stirring until a pale yellow solution had formed. Activated carbon was added, and the solution was filtered through Celite. After addition of 6.25 mL of concentrated HCl to the filtrate, heating was continued for 4 h. The solution was cooled to room temperature, concentrated to about half the volume under reduced pressure, and cooled in an ice bath for 30 min. Crude **5** precipitated as a greenish-yellow solid, which was recrystallized from a minimum quantity of boiling water, washed with ethanol and diethyl ether, and dried at 50−60 °C in vacuo. Yield: 1.29 g (70%) of pale yellow, compact prisms. ^{1}H NMR: δ 4.22 (br s, 3 H), 7.97 (m, 1 H), 8.26 (m, 1 H), 9.60 (m, 1 H). Anal. Calcd for $C_3H_6N_2Cl_2PtS$: C, 9.79; H, 1.64; N, 7.61; Cl, 19.26. Found: C, 9.93; H, 1.51; N, 7.61; Cl, 19.25.

***cis*-[$PtCl_2(NH_3)$(thiazole)] (6).** To a solution of 1.50 g (4.2 mmol) of K[$PtCl_3(NH_3)$] in 25 mL of water was added 0.30 mL (4.2 mmol) of thiazole, and the solution was stirred at room temperature for 12 h. A yellow precipitate formed after the mixture had been stored at 4 °C for 24 h, and the filtrate was filtered off and recrystallized from 25 mL of boiling water. The final product precipitated as a microcrystalline solid at 4 °C and was filtered off and washed with ice cold ethanol and diethyl ether and dried in the air. Yield: 0.68 g (42%). ^{1}H NMR: δ 4.48 (br s, 3 H), 8.02 (m, 1 H), 8.26 (m, 1 H), 9.61 (m, 1 H, $^3J(^1H-^{195}Pt) = 26$ Hz). Anal. Calcd for $C_3H_6N_2Cl_2PtS$: C, 9.79; H, 1.64; N, 7.61; Cl, 19.26. Found: C, 9.69; H, 1.50; N, 7.59; Cl, 19.32.

***trans*-[$PtCl_2(NH_3)$(benzothiazole)] (7).** A mixture of 6.0 g (20 mmol) of **1** and 5.45 mL (50 mmol) of benzothiazole in 400 mL of water was heated until all of the platinum complex was dissolved (ca. 30 min at 100 °C). During the reaction period a dark brown, oily residue had formed (probably due to the decomposition of the heterocyclic amine), which was removed by filtration through Celite after the hot solution was treated with 2 g of activated carbon for 20 min. Concentrated HCl (50 mL) was added to the filtrate, heating was continued for 3 h, and the mixture was finally refrigerated overnight to give a yellow precipitate. Thus obtained crude **7** was recrystallized from 800 mL of hot acetonitrile. The solution was concentrated to a final volume of 250 mL and stored at 4 °C overnight. Bright-yellow, compact needles of **7** formed, which were filtered off and dried in vacuo at 50−60 °C. Yield: 2.6 g (31%). ^{1}H NMR: δ 4.33 (br s, 3 H), 7.63 (m, 1 H), 7.76 (m, 1 H), 8.28 (m, 1 H), 8.93 (d, 1 H), 9.88 (s, 1 H). Anal. Calcd for $C_7H_8N_2Cl_2PtS$: C, 20.01; H, 1.92; N, 6.70; Cl, 16.95. Found: C, 20.36; H, 1.84; N, 6.65; Cl, 16.75.

***trans*-[$PtCl_2(NH_3)$(isoquinoline)] (8)** was synthesized analogously to the procedure for **3**. Yield: 6.12 g (89%), starting from 5.0 g (16 mmol) of **1**. ^{1}H NMR: δ 4.25 (br s, 3 H), 7.84 (m, 1 H), 7.95−8.01 (m, 2 H), 8.11 (m, 1 H), 8.35 (m, 1 H), 8.69 (m, 1 H), 9,68 (s, 1 H). Anal. Calcd for $C_9H_{10}N_2Cl_2Pt$: C, 26.23; H, 2.44; N, 6.80; Cl, 17.20. Found: C, 25.99; H, 2.61; N, 6.92; Cl, 17.53.

X-ray Structure Determination. Cell constants were determined by least-squares fits to the θ values of 25 independent reflections, measured and refined on an Enraf-Nonius CAD4-F diffractometer fitted with a graphite monochromator. The crystallographic data are summarized in Table 1. Data reduction and application of Lorentz, polarization, and absorption corrections (numerical **3**, **5**, and **8** or analytical **7**) were carried out using the Enraf-Nonius Structure Determination Package[20] (**3**, **5**, and **8**) or teXsan[21] (**7**). The structures were solved by direct methods using SHELXS-86[22] and refined using full-matrix least-squares methods with either SHELX-76[23] (**3**, **5**, and **8**) or teXsan (**7**). All of the hydrogen atoms were included at calculated sites with isotropic thermal parameters, and the non-hydrogen atoms were refined anisotropically.

The thiazole ligand in **5** is disordered by rotation about the Pt−N bond by approximately 180°, resulting in an overlap of C(12) and S(1) sites. The disorder is minor and has only a modest effect on bond lengths and angles. Neutral atom scattering factors were taken from Cromer and Waber.[24] Anomalous dispersion effects were included in F_c;[25] the values for $\Delta f'$ and $\Delta f''$ were those of Creagh and McAuley.[26] The values for the mass attenuation coefficients are those of Creagh and Hubbell.[27] All calculations were performed using SHELX-76 or the teXsan crystallographic software package of Molecular Structure Corporation, and plots were drawn using ORTEP.[28] Selected bond lengths and angles are given in Tables 2−5. Atomic coordinates, thermal parameters, bond lengths and angles, and details of least-squares planes have been deposited.

Platination of DNA. The poly(dG)·poly(dC) and poly(dG-dC)·poly(dG-dC) duplexes (Pharmacia Biotech, Piscataway, NJ) were incubated ($c_{DNA} = 0.05$ mg mL^{-1}) with **1**−**7** at varying concentrations of platinum for 48 h at 37 °C. The platinum content of the samples was determined

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Table 2. Selected Bond Lengths (Å) and Angles (deg) with Standard Deviations for *trans*-[$PtCl_2(NH_3)$(quin)] (**3**)

Bond Lengths			
Cl(1)−Pt(1)	2.303(3)	Cl(2)−Pt(1)	2.284(3)
N(1)−Pt(1)	2.015(8)	N(2)−Pt(1)	2.050(9)
Bond Angles			
Cl(2)−Pt(1)−Cl(1)	177.1(1)	N(1)−Pt(1)−Cl(1)	91.4(2)
N(1)−Pt(1)−Cl(2)	91.1(2)	N(2)−Pt(1)−Cl(1)	88.0(3)
N(2)−Pt(1)−Cl(2)	89.5(3)	N(2)−Pt(1)−N(1)	177.0(3)
C(1)−N(1)−Pt(1)	116.8(7)	C(9)−N(1)−Pt(1)	124.4(7)

Table 3. Selected Bond Lengths (Å) and Angles (deg) with Standard Deviations for *trans*-[$PtCl_2(NH_3)$(tz)] (**5**)

Bond Lengths			
Cl(11)−Pt(1)	2.295(4)	Cl(12)−Pt(1)	2.284(4)
N(11)−Pt(1)	1.989(11)	N(12)−Pt(1)	2.047(11)
Bond Angles			
Cl(12)−Pt(1)−Cl(11)	178.4(1)	N(11)−Pt(1)−Cl(11)	93.6(3)
N(11)−Pt(1)−Cl(12)	88.1(3)	N(12)−Pt(1)−Cl(11)	88.6(4)
N(12)−Pt(1)−Cl(12)	89.8(4)	N(12)−Pt(1)−N(11)	176.8(4)
C(11)−N(11)−Pt(1)	122(2)	C(13)−N(11)−Pt(1)	126(2)

Table 4. Selected Bond Lengths (Å) and Angles (deg) with Standard Deviations for *trans*-[$PtCl_2(NH_3)$(btz)] (**7**)

Bond Lengths			
Pt(1)−Cl(1)	2.288(2)	Pt(1)−Cl(2)	2.291(2)
Pt(1)−N(1)	1.997(6)	Pt(1)−N(2)	2.006(6)
Bond Angles			
Cl(1)−Pt(1)−Cl(2)	176.77(7)	Cl(1)−Pt(1)−N(1)	90.2(2)
Cl(1)−Pt(1)−N(2)	88.9(2)	Cl(2)−Pt(1)−N(1)	92.9(2)
Cl(2)−Pt(1)−N(2)	88.0(2)	N(1)−Pt(1)−N(2)	178.5(3)
Pt(1)−N(1)−C(1)	123.2(5)	Pt(1)−N(1)−C(7)	125.8(5)

Table 5. Selected Bond Lengths (Å) and Angles (deg) with Standard Deviations for *trans*-[$PtCl_2(NH_3)$(isoquin)] (**8**)

Bond Lengths			
Cl(1)−Pt(1)	2.289(2)	Cl(2)−Pt(1)	2.296(2)
N(1)−Pt(1)	2.023(5)	N(2)−Pt(1)	2.027(5)
Bond Angles			
Cl(2)−Pt(1)−Cl(1)	177.7(1)	N(1)−Pt(1)−Cl(1)	92.3(2)
N(1)−Pt(1)−Cl(2)	89.6(2)	N(2)−Pt(1)−Cl(1)	88.0(2)
N(2)−Pt(1)−Cl(2)	90.0(2)	N(2)−Pt(1)−N(1)	179.4(2)
C(1)−N(1)−Pt(1)	120.1(4)	C(2)−N(1)−Pt(1)	120.4(4)

by flameless atomic absorption spectroscopy (FAAS), and r_b values (bound drug-to-nucleotide ratio) were calculated as described earlier.[29] All procedures were performed according to experimental conditions[30] that have been developed for DNA binding studies on *cis*- and *trans*-[$PtCl_2$(pyridine)$_2$].

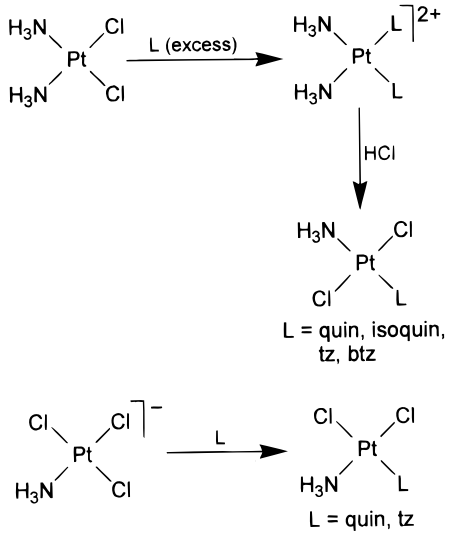
Unwinding of Negatively Supercoiled DNA. Unwinding of closed circular, supercoiled pUC19 plasmid DNA (2686 bp, Gibco BRL, Grand Island, NY) by **1**−**7** was monitored by an agarose gel mobility shift assay,[31] using the experimental setup that has been described for an analogous study on *cis*- and *trans*-[$PtCl_2$(pyridine)$_2$].[30]

Cytotoxicity. In vitro cytotoxicity data stated in this paper were determined for 96-h incubations at 37 °C using a standard assay described previously.[32] Stock solutions of the platinum complexes in HPLC grade DMF were diluted by serial dilution with saline to a final concentration of 0.5% in DMF.

Results

Synthesis and Structural Characterization. The mixed ammine/planar amine complexes of the type *trans*-[$PtCl_2$-(NH_3)L] (**3**, **5**, **7**, and **8**, see Chart 1) were synthesized according to a method shown in Scheme 1. The procedure involves direct substitution of both chloro ligands in **1** by planar amine (L), which produces the intermediate *cis*-$[Pt(NH_3)_2L_2]^{2+}$. The *trans*-dichloro species are formed in the second reaction step under strongly acidic conditions in the presence of chloride. The first am(m)ine ligand is labilized at low pH and subsequently substituted by chloride, which directs the second chloro ligand into the trans position, due to the relative order of trans effects, i.e., $Cl^- >$ am(m)ine.[33] Direct substitution of chloride by L at elevated temperatures proved to be the method of choice for sterically demanding quinoline. Yields were dramatically higher than for the previously described method (65% vs 23%), which involved prior abstraction of chloride in **1** by $AgNO_3$ and reaction of the diaqua form with quinoline at room temperature.[4] Similarly, the synthesis of the cis analogues of **3** and **5**, i.e., **4** and **6** (Chart 1, Scheme 1), utilized the mutual labilization of the trans-oriented chloro ligands in $[PtCl_3(NH_3)]^-$ for the stereospecific introduction of L.

Scheme 1



^{1}H NMR and analytical data (see Experimental Section) for **5**−**8** are consistent with the structures depicted in Chart 1. For **3**, **5**, **7**, and **8**, a single-crystal X-ray structure determination was carried out. The structures consist in all cases of two or three independent neutral complex molecules (Figure 1a−d). In all structures, weak intermolecular hydrogen bonds are observed between the chloro and ammine ligands. The geometry about each of the Pt atoms is close to square planar with out-of-plane deviations less than 0.05 Å. Bond lengths (for structural parameters of **3**, **5**, **7**, and **8**; see Tables 2−5) to the chloro ligands are in the usual range observed for divalent platinum.[33] Pt−N bond lengths are similar for the ammine and planar ligands with the average of those to the heterocyclic nitrogen (2.02 Å) being marginally shorter than those to NH_3 (2.04 Å). Angles between the coordination planes and the planes through the aromatic ligands range from 50.8° (in **5**) to 67.9° (in **3**).

The geometry of the quinoline ligand in **3** results in a close contact of 2.77 Å between its H8 atom and the metal center. This interaction leads to a distortion of the geometry about the N(quinoline)-donor atom which causes an opening of the Pt−N−C angle in the direction of H8 to ca. 124° with that on the

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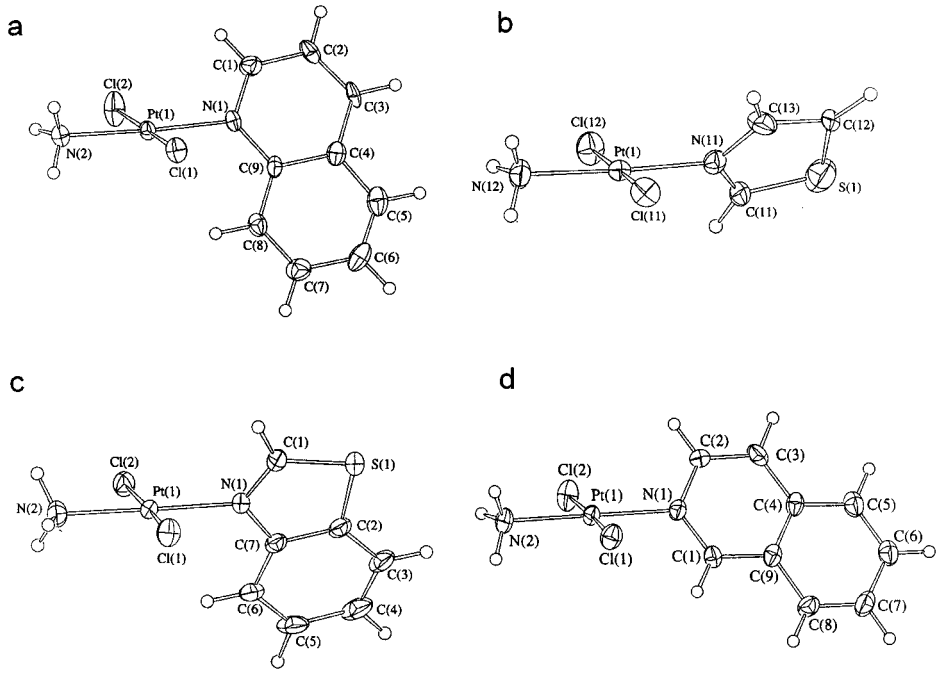

Figure 1. Molecular structures of (a) *trans*-[$PtCl_2(NH_3)$(quin)] (**3**), (b) *trans*-[$PtCl_2(NH_3)$(tz)] (**5**), (c) *trans*-[$PtCl_2(NH_3)$(btz)] (**7**), and (d) *trans*-[$PtCl_2(NH_3)$(isoquin)] (**8**) in the crystal (view of one independent molecule in all cases); ellipsoids are drawn at the 30% probability level.

other side being closed to 117°.[34] Characteristically, for isoquinoline in **8** the equivalent angles are both between 120.0° and 120.7°. The geometry of benzothiazole in **7** is similar to that of quinoline, but because of the five-membered ring no close Pt···H contact results (Pt···H > 3.0 Å). The Pt−N−C angles about the N donors of thiazole and benzothiazole are unequal, ranging from 120° to 124° on the side adjacent to the S atom and from 126° to 129° on the other. In this case, however, the inequality is probably a consequence of the asymmetry in the five-membered ring that results from the presence of the S atom.

Cytotoxicity. The cytotoxicity of the *trans*-platinum complexes and their cis counterparts, where available, were studied in murine L1210 leukemia cells, Table 6. In cisplatin-sensitive cells (L1210/0), the *trans*-[$PtCl_2(NH_3)$L] geometry with L = quin (**3**), tz (**5**), btz (**7**), and isoquin (**8**) shows remarkable toxicity which is significantly higher than for classical *trans*-DDP (**2**). The order of effectiveness in cell-growth inhibition was found to be **3** > **7** > **8** > **5**. The quinoline complex **3** shows an ID_{50} value (micromolar drug concentration necessary to inhibit cell division by 50%) that is indeed of the same magnitude as that observed for *cis*-DDP (**1**). In cisplatin-resistant cells (L1210/DDP), **3** and **7** are considerably more active than the two DDP isomers (**1** and **2**). **5**, however, only moderately active in L1210/0, exhibits poor activity in L1210/DDP. Interestingly, here the order of cytotoxicities, **3** > **7** > **5**, proves to be the same as in the sensitive cell line.

The cis complexes **4** and **6** are also both active in sensitive and resistant cells. While **4** appears to be less cytotoxic than its congener **3** in L1210/DDP, the thiazole-based complex **6** gave an ID_{50} value that is significantly lower than for the trans compound **5**. Characteristically, **6** exhibits the lowest resistance factor in the series of compounds tested. In summary, the results confirm the generality of the biological effects of planar ligand substitution in *trans*-platinum complexes. The results with **4** and **6** further suggest that planar ligands other than substituted pyridine may also produce antitumor activity profiles similar to that of *cis*-[$PtCl_2(NH_3)$(2-methylpyridine)].[12]

Table 6. Cytotoxicity of *cis*- and *trans*-[$PtCl_2(NH_3)$L] in L1210 Leukemia Cells[a]

			ID_{50}, μM	
L	isomer	compd no.	L1210/0	L1210/DDP
NH_3	cis	**1**	0.33	9.22 (28)[b]
	trans	**2**	15.7	22.0 (1.40)
quin	trans	**3**	0.51	1.35 (2.65)
	cis	**4**	0.48	2.75 (5.73)
tz	trans	**5**	4.20	15.0 (3.57)
	cis	**6**	3.20	3.10 (0.97)
btz	trans	**7**	1.20	2.10 (1.75)
isoquin	trans	**8**	2.50	nd[c]

[a] Data for **1**−**4** are from ref 4; L1210/0 is the cisplatin-sensitive and L1210/DDP the cisplatin-resistant cell line. [b] Value in parentheses is the resistance factor, ID_{50}(L1210/DDP)/ID_{50}(L1210/0). [c] Not determined.

Platination of DNA. Incubations (48 h) at 37 °C of **1**−**7** with the synthetic duplex polynucleotides poly(dG)·poly(dC) (homopolymer) and poly(dG-dC)·poly(dG-dC) (alternating copolymer) at varying drug concentrations (20−200 μM) were carried out to study both the overall binding affinity to double-stranded DNA and the sequence specificity of the drugs. The amount of platinum covalently bound to both polymers, represented by the drug-to-nucleotide ratio, r_b, is illustrated as a function of the initial doses of platinum complex in Figure 2. The classical complexes, *cis*- and *trans*-DDP (**1**, **2**), had the highest affinity to poly(dG)·poly(dC), with r_b values of 0.272 and 0.305 at the highest dose, respectively (Figure 2a). Under analogous conditions, both compounds show a significantly reduced affinity to poly(dG-dC)·poly(dG-dC), as indicated by

(34) Despite the short H8···Pt distance, no through-space $^1J(^1H-^{195}Pt)$ coupling is observed in ^1H NMR spectra of **3** (see also: Albinati, A.; Pregosin, P. S.; Wombacher, F. *Inorg. Chem.* **1990**, *29*, 1812). However, H8 in quinoline experiences a pronounced downfield shift on binding to the metal: $\delta_{H8} > \delta_{H2}$ in **3**; $\delta_{H8} < \delta_{H2}$ in free quinoline. This chemical shift anomaly is probably caused by H8 being exposed to an electronically anisotropic environment along the tetragonal axis of the square-planar d^8 low-spin complex. Characteristically, for the analogous platinum(IV) complex, *trans*,*trans*,*trans*-[$PtCl_4(NH_3)$(quin)], the order of H2 and H8 chemical shifts was found to be "normal" (Bierbach, U.; Roat, R. M.; Hambley, T. W.; Farrell, N. Manuscript in preparation).

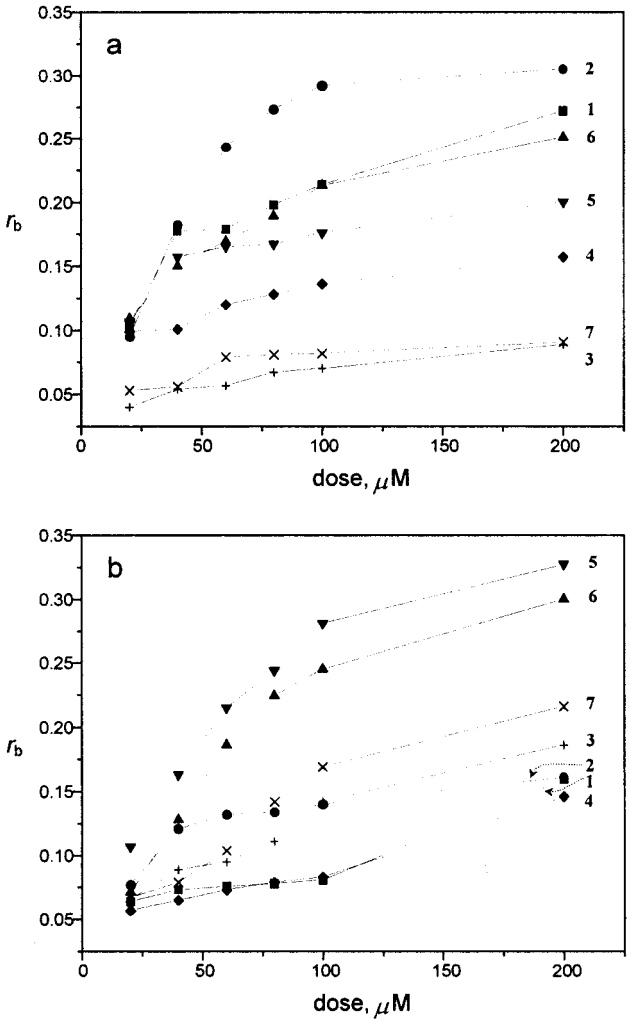

Figure 2. Degree of platination of double-stranded DNA as a function of concentration (dose) for incubations of **1**−**7** with (a) poly(dG)·poly(dC) and (b) poly(dG-dC)·poly(dG-dC).

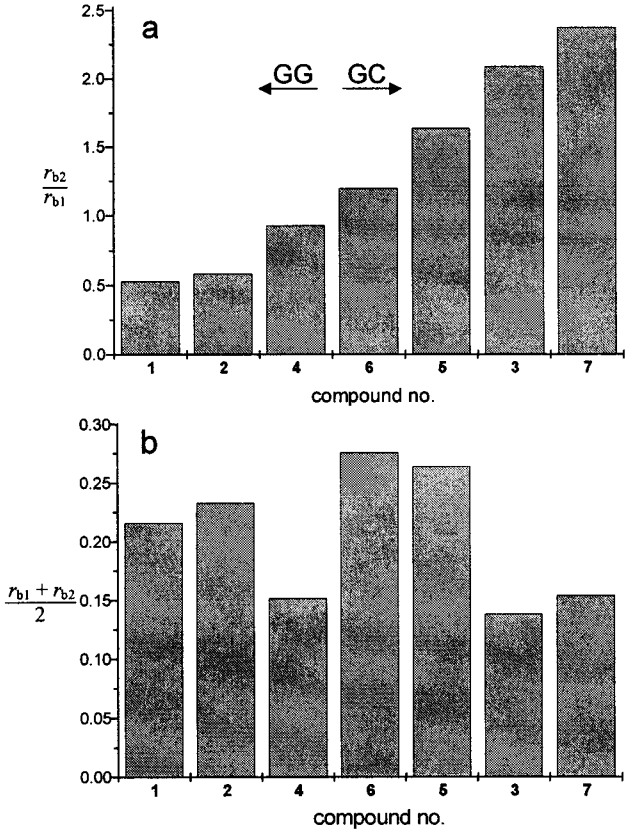

Figure 3. Bar diagrams demonstrating (a) the sequence specificity and (b) global DNA binding affinity of **1**−**7**. The arrows in panel a indicate increasing binding affinity of platinum to poly(dG)·poly(dC) ("GG") and poly(dG-dC)·poly(dG-dC) ("GC"), respectively. All ratios were calculated for r_b values at the maximum platinum dose of 200 μM.

r_b values of 0.159 (**1**) and 0.161 (**2**) (Figure 2b). For **1**, these findings are in agreement with an earlier study[35] by Leng et al. on competitive platinum binding to the above duplex polynucleotides. At low molar drug concentrations these authors found a 3:1 distribution of covalently bound platinum between poly(dG)·poly(dC) and poly(dG-dC)·poly(dG-dC).

In the series $[PtCl_2(NH_3)L]$, complexes that carry the planar ligand of least steric demand (tz ≪ btz < quin, see solid state structures) show the highest affinity to poly(dG)·poly(dC). The order of r_b values found for the cis and trans isomers are **6** > **4** and **5** ≫ **7** > **3**, respectively. For the pairs of geometric isomers, in turn, the cis form shows the higher affinity (**6** > **5**; **4** > **3**). Binding of **3** and **7** to poly(dG)·poly(dC) appears to be complete ("saturated") at relatively low platinum-to-nucleotide ratios, which is in accordance with the results for platination of pUC19 plasmid DNA by structurally related *trans*-$[PtCl_2(py)_2]$.[30]

Dose-dependent r_b values for poly(dG-dC)·poly(dG-dC) (Figure 2b) indicate a higher binding affinity for *cis*- and *trans*-$[PtCl_2(NH_3)L]$ (except for **4**, for which the value is slightly lower), compared with the situation for poly(dG)·poly(dC). Interestingly, the geometry preference is reversed for each pair of isomers, i.e., the binding affinity observed for the trans isomers is higher than for the cis isomers (**5** > **6**; **3** > **4**). As mentioned previously, the r_b values are evidently affected by the size of the planar ligands, which in the present series leads to a significantly reduced degree of drug binding for the quinoline- and benzothiazole-based compounds (**3**, **4**, and **7**) compared to *trans*- and *cis*-$[PtCl_2(NH_3)(tz)]$ (**5**, **6**). The classical ammine complexes **1** and **2**, together with *cis*-$[PtCl_2(NH_3)(quin)]$ (**4**), exhibit the lowest affinity to the alternating purine−pyrimidine sequence.

From the present data, information about two important aspects of platinum−DNA binding can be deduced: The geometric isomerism of the complexes and the presence and nature of the planar ligand (L) dictate both the *overall reactivity* and *sequence specificity* of square-planar platinum in reactions with double-stranded DNA. Relative binding preferences for poly(dG-dC)·poly(dG-dC) and poly(dG)·poly(dC) can be estimated for a given drug concentration from the ratio of the corresponding r_b values. The bar diagram in Figure 3a arranges compounds **1**−**7** in their order of increasing r_{b2}/r_{b1}, the magnitude of which reflects a binding preference for either alternating GC or GG. Considering the numerical values for r_{b2}/r_{b1}, the compounds studied can be grouped as follows:

compound	r_{b2}/r_{b1}	sequence preference
classical DDP (**1**, **2**)	<1	GG
cis-$[PtCl_2(NH_3)L]$ (**4**, **6**)	≈1	low selectivity
trans-$[PtCl_2NH_3)L]$ (**3**, **5**, **7**)	>1	GC

For complexes of the trans series, the presence of the planar ligand produces a pronounced sequence specificity, although the overall affinity to DNA is very dependent on the size of the

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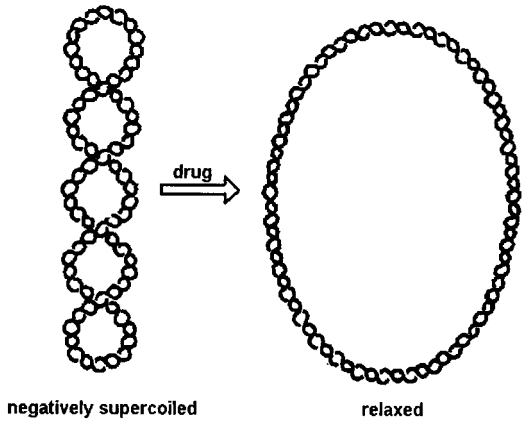

Figure 4. Schematic presentation of the unwinding of a supercoiled plasmid induced by drug binding. The term supercoiling describes the state of native, B-form DNA in its closed circular (cc) form being coiled on itself. Left-handed twisting of right-handed, double-stranded DNA along the helical axis untwists DNA. The strain produced by this process is partly compensated by the formation of supercoils (left). Untwisting of negatively supercoiled DNA along the helical axis by covalently binding drug or intercalator reduces and finally completely eliminates superhelical turns (writhe), giving totally relaxed DNA (right). Quantitation of drug-mediated unwinding (untwisting) using gel electrophoresis exploits the reduced mobility of the relaxed, less compact form of the plasmid.

planar ligand (**5** > **7** > **3**, Figure 2). Interestingly, the thiazole complexes **5** and **6** have the highest overall binding affinity, based on average r_b values (arithmetic means of r_{b1} and r_{b2} at a drug dose of 200 μM, Figure 3b). On the other hand, the trans isomers **3** and **7**, which exhibit a pronounced preference for the alternating GC sequence, show a relatively low level of DNA binding (Figure 3b).

Unwinding of DNA. Unwinding of closed circular, supercoiled plasmid DNA, quantified by electrophoretic mobility shift assays, is a useful tool for studying the effects of intercalating and covalently binding molecules on DNA topology.[36] A short description of the supercoiling phenomenon and how this can be exploited as a probe for conformational changes in DNA is given in the caption of Figure 4. Although no information can be deduced from such experiments about the exact structure of the adducts formed by platinum complexes on (random-sequence) DNA, the degree of duplex unwinding has been shown to be indicative of a characteristic binding mode. For platinum(II) complexes that associate with DNA in a covalent manner or through a combination of covalent binding and intercalation of planar moieties tethered to the complex, the unwinding angle per adduct (ϕ) was found to correlate with the mode of Pt−DNA interaction ("structure-unwinding relationships"):[31] Platinum complexes that bind to DNA in a monofunctional manner, e.g., in $[PtCl(NH_3)_3]Cl$ ($\phi = 6°$),[31] produce little unwinding. In contrast, the drug cisplatin (**1**) and its trans isomer **2**, which induce bifunctional adducts (vide infra) in DNA, cause local duplex unwinding of 13°[37] and 9−10°,[31] respectively. Unwinding was found to be even more pronounced in cases where covalent platinum binding is accompanied by intercalation of a Pt-bound, well-positioned planar group, such as ethidium (the classical intercalating dye), resulting in ϕ values of 13−23°.[31]

In the present study, *trans*-[$PtCl_2(NH_3)$(quin)] (**3**) and *trans*-[$PtCl_2(NH_3)$(btz)] (**7**) were remarkably efficient at unwinding negatively supercoiled pUC19 plasmid DNA, giving unwinding angles of 15° and 17°, respectively (Table 7). In contrast, unwinding per *trans*-[$PtCl_2(NH_3)$(tz)] (**5**)−DNA adduct (ϕ = 7°) was much less effective. In other words, complete removal of superhelical turns that leads to the open circular, relaxed form (Figure 4) by **3** and **7** occurs at relatively low r_b values, whereas a much higher amount of covalently bound platinum is necessary in the case of **5** to produce the same effect. A slightly higher degree of DNA unwinding has been established recently for **3** binding to the 2464-bp plasmid pSP73 (ϕ = 17°).[13] For compounds of the type *trans*-[$PtCl_2(NH_3)L$], ϕ obviously correlates with the size of the planar ligand (L). Both **3** and **7** induce relaxation of the supercoiled plasmid more efficiently than the classical DDP isomers **1** and **2**. Thus, by analogy with the above intercalator-linked complexes,[31] it may be suggested that the degree of unwinding by **3** and **7** is the result of base stacking and covalent binding. *trans*-[$PtCl_2(py)_2$], although carrying sterically less demanding pyridine, affects DNA topology to a similar extent[30] (Table 7) as the quinoline- and benzothiazole-containing complexes **3** and **7**. This may be due to an increased stacking contribution of *two* bases to the total helical unwinding.

Table 7. Unwinding of Plasmid DNA by Platinum Complexes

compd	r_b(c)[a]	unwinding angle (deg)
cis-[$PtCl_2(NH_3)_2$] (**1**)	0.043	13[b]
trans-[$PtCl_2(NH_3)_2$] (**2**)	0.053	10
trans-[$PtCl_2(NH_3)$(quin)] (**3**)	0.037	15
trans-[$PtCl_2(NH_3)$(tz)] (**5**)	0.075	7
trans-[$PtCl_2(NH_3)$(btz)] (**7**)	0.033	17
cis-[$PtCl_2(py)_2$]	0.156	4[c]
trans-[$PtCl_2(py)_2$]	0.033	17[c]

[a] The drug-to-nucleotide ratio (r_b), where the bands of the supercoiled plasmid and its nicked, totally relaxed form comigrate on the agarose gel (coalescence). [b] Assumed value, see ref 37. [c] Reference 30.

Discussion

The purpose of the present study was to establish structure−activity relationships for an extended series of nonclassical platinum antitumor complexes of the type *trans*-[$PtCl_2(NH_3)L$] (L = planar ligand) and to contribute to an understanding of their molecular mode of action. Previous model studies have shown that the simple replacement of one ammine ligand in **2** with quinoline profoundly affects the overall affinity of platinum to DNA constituents. Both the hydrolytic inertness of the Pt−Cl bonds in **3** (the source of which is not fully understood) and the bulkiness of the planar ligand slow associative substitution reactions on the metal center.[6] The latter steric effect is in agreement with the observation that the planar ligands tend to adopt a conformation in the solid state (vide infra) and in solution[6] that is unfavorable for a nucleophilic attack on platinum along the tetragonal axis. Therefore, the relatively low binding affinity of **3**, **4**, and **7** to poly(dG-dC)·poly(dG-dC) and poly(dG)·poly(dC) (Figure 3b) may reflect the reduced reactivity of platinum toward nucleic acid nitrogen (guanine-N7). In contrast, for **5** and **6**, which carry sterically less demanding thiazole, higher r_b values are observed for covalent platinum binding. While the bulkiness of L and the down-modulation of the substitution rates of platinum may—to some extent—be correlated with the degree of platination (r_b), this effect by itself cannot explain the distinct preference of **3**, **5**, and **7** for the alternating GC sequence.

The classical DDP isomers bind to DNA in a two-step mechanism: *cis*-[$PtCl_2(NH_3)_2$] (**1**) and *trans*-[$PtCl_2(NH_3)_2$] (**2**) form monofunctional adducts that subsequently close to bifunc-

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tional adducts, involving nucleobases on either the same (*intrastrand*) or opposite (*interstrand*) strand(s). The trend in binding affinities for the above isomers in the present study, GG > GC, is consistent with the array of cross-links formed on double-stranded DNA: cisplatin, **1**, predominantly forms intrastrand cross-links between neighboring (1,2) purine bases (GG with lesser amounts of AG[14]). Interstrand cross-links (1,2 GG[17]) are also formed by **1** but with a much lower frequency. This is in accordance with **1** preferentially binding to poly(dG)·poly(dC). On the other hand, **2** lacks the geometric requirements to form a 1,2 intrastrand adduct and has been shown to produce long-lived monofunctional adducts that slowly transform into interstrand cross-links between guanine and *complementary* cytosine (GC).[16] Therefore, the binding preference of **2** cannot be explained with the types of DNA adducts formed but may be controlled by parameters such as electrostatic effects. Oligo(dG)·oligo(dC) runs on DNA constitute sequences of highest negative electrostatic potential.[38] The DNA binding of the DDP isomers (their positively charged hydrolysis products) may be considered an electrostatically driven process, which directs the drugs to the major groove regions of highest electron density.

We have shown recently[13] that *trans*-[$PtCl_2(NH_3)$(quin)] (**3**) forms interstrand cross-links on DNA with a higher rate and frequency than its congener *trans*-DDP (**2**). Interestingly, in DNA containing the central sequence d(TCGCT)/d(AGCGA), **3** preferentially induces bifunctional adducts involving guanine bases (5′→5′, G*) on opposite strands:[39]

```
5′-TCG*CT-3′
3′-AGCG*A-5′
```

Interstrand cross-links constitute a major portion of DNA adducts formed by **3** (a similar situation may be expected for **5** and **7**). Thus, we suggest that these adducts significantly contribute to the high affinity of **3**, **5**, and **7** to poly(dG-dC)·poly(dG-dC) (Figure 3a). In contrast, the reduced degree of platination in the alternating sequence by the analogous cis complexes **4** and **6** may be attributed to the formation of a *cis*-DDP-like array of bifunctional adducts.

Intercalation of the planar ligand L in **3**−**7** into the DNA base stack may be considered an important binding mode that enhances DNA−drug association and thus a potential first binding step. A characteristic decrease in ethidium fluorescence that occurs upon global platination of DNA with **3**[13] suggests that quinoline interacts with base pairs adjacent to the covalent binding sites. This view is further corroborated by the degree of DNA unwinding (vide infra). N-Quarternization (N^+−R or N−metal) of planar bases greatly enhances horizontal base−nucleobase interactions.[40] Organic bisintercalators such as the antibiotic luzepeptin that contain quarternized quinoline as the intercalating moieties preferentially bind to alternating purine−pyrimidine sequences of duplex DNA.[41] Similarly, (partial) intercalation of the planar ligands in **3**−**7**, both as the first association step on DNA and in the final adducts, may be the driving force that ultimately changes the sequence specificity from purine−purine to purine−pyrimidine.

DNA unwinding angles produced by the *trans*-platinum complexes **3** and **7** and those reported for intercalator-linked complexes[31] of the type *cis*-[$Pt(NH_3)_2Cl$(intercalator)]$^{n+}$ are similar, which may reflect a common DNA binding mode. Both geometries form monofunctional adducts on DNA with the planar ligand oriented cis to the covalent binding site and well-positioned to induce stacking with neighboring nucleobases. Such "pseudobifunctional" binding has been put forward to explain the degree of local DNA unwinding for **3** and the above cationic complexes. Unlike ethidium intercalation,[38] which causes an extension of the double helix by ca. 3.4 Å and a local change of the helical twist of 26°, combined stacking of the (rigidly metal-linked) planar ligands and covalent platinum binding in *trans*-[$PtCl_2(NH_3)$L] is only feasible at the expense of bending of the double helix.[13] The reduced unwinding observed for the thiazole-based compound **5** compared to **3** and **7** suggests that planar ligand size is critical to the efficiency of changing the helical twist. In addition, there may be a significant contribution to conformational changes from nonintercalative interactions, such as the recently detected, bifunctional interstrand adducts and cross-links not yet identified.

In conclusion, the aim of this study was to establish the requirements for the antitumor activation of *trans*-platinum complexes by planar nitrogen donors (L) and to correlate structural aspects of the complexes with their DNA binding profiles. The rational design of new platinum drugs with clinical properties complementary to cisplatin was the major impetus for the present investigation. Second, the understanding of how the DNA binding modes may produce protein-associated strand breaks can be inferred from the DNA binding profiles. Complexes of the general formula *trans*-[$PtCl_2(NH_3)$L] exhibit DNA binding profiles (sequence specificity and conformational changes) that are distinctly different from those of *cis*- and *trans*-[$PtCl_2(NH_3)_2$]. Cytotoxic lesions different from those of cisplatin, produced by nonclassical *trans*-platinum on DNA, may enhance the activity in cisplatin-resistant cells by circumventing cisplatin-specific repair. Interestingly, in the series of trans complexes, those complexes which bind to alternating purine−pyrimidine with the highest specificity and untwist DNA most efficiently (**3**, **7** ≫ **5**) show the lowest resistance factors in L1210/DDP. It therefore may be suggested that the reduced cross-resistance of *trans*-[$PtCl_2(NH_3)$L] in L1210/DDP cells lies at the DNA level. Although it seems tempting to correlate the structure−affinity and structure−unwinding relationships delineated in this paper with the cytotoxicity of the complexes, one should bear in mind that factors such as changed cellular uptake and detoxification of platinum may contribute to the overall biological activity of the novel *trans*-platinum compounds.[42]

Acknowledgment. This work was supported by grants from The National Science Foundation and The American Cancer Society. We thank Dr. Yue Zou for technical advice in the unwinding experiments.

Supporting Information Available: Tables of crystallographic data, final positional parameters, anisotropic displacement coefficients, H-atom coordinates, bond lengths and angles, and least-squares planes for **3**, **5**, **7**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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