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Contrasting Chemistry of *cis*- and *trans*-Platinum(II) Diamine Anticancer Compounds: Hydrolysis Studies of Picoline Complexes

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cis-[$PtCl_2(NH_3)$(2-picoline)] (AMD473) is currently on clinical trials as an anticancer drug. The trans isomer, AMD443 (**1**), is also cytotoxic in a variety of cancer cell lines. The X-ray crystal structure of the trans isomer (**1**) shows that the pyridine ring is tilted by 69° with respect to the platinum square-plane in contrast to the cis isomer in which it is almost perpendicular (103°). In the 3-picoline (**2**) and 4-picoline (**3**) trans isomers, the ring is tilted by 58°/60° (2 molecules/unit cell) and by 56°, respectively. Hydrolysis may be an important step in the intracellular activation and anticancer mechanism of action of these complexes. The first hydrolysis step is relatively fast even at 277 K, with rate constants (determined by ^1H,^{15}N NMR) of $k_1 = 2.6 \times 10^{-5}$ s^{-1}, 12.7×10^{-5} s^{-1}, and 5.2×10^{-5} s^{-1} ($I = 0.1$ M) for formation of the monoaqua complexes of **1**–**3**, respectively. Although the hydrolysis of **3** is slower than **2**, it is hydrolyzed to a greater extent. No formation of the diaqua complex was observed for any of the three complexes at 277 K, and it accounts for <3% of the platinum species at 310 K. In general the extent of hydrolysis of the trans complexes is much less than for their cis analogues. The pK_a values for the monoaqua adducts of **1**–**3** were determined to be 5.55, 5.35, and 5.39, respectively, suggesting that they would exist largely as the monohydroxo complex at physiological pH. The pK_a values for the diaqua adducts were determined to be 4.03 and 7.01 for **1**, 3.97 and 6.78 for **2**, and 3.94 and 6.88 for **3**, the first pK_a being >1 unit lower than for related cis complexes.

Introduction

Cisplatin is now a widely used clinical drug for the treatment of various neoplastic diseases, including testicular and ovarian cancers, while its congener transplatin is therapeutically inactive.[1] This observation, which is considered a paradigm for the structure−activity relationships (SARs) of platinum-based antitumor compounds, has dominated development in this field for more than 2 decades. The SARs have aided the design of new drugs and guided attempts to overcome some of the drawbacks associated with cisplatin, such as nausea, neurotoxicity, nephrotoxicity, and ototoxicity, as well as drug resistance. These SARs led to a second generation of platinum drugs, all of which are analogues of cisplatin containing two cis primary or secondary amine groups and two anionic leaving groups. The most successful of these is carboplatin, which has less severe side effects than cisplatin.[2,3] However, many of the second-generation platinum drugs appear to be cross-resistant with cisplatin.[4]

Much recent research work has been aimed at the discovery of platinum complexes which are not cross-resistant with cisplatin, have fewer toxic side effects, are active against a broader range of types of cancer, and can be administered orally. In 1989, two exceptions to the classic SAR were reported showing that the overall charge on the complex and the cis orientation of the N-bound ligands are not a prerequisite for antitumor activity of Pt^{II} complexes. Hollis et al.[5] reported that positively charged triaminemonochloroplatinum(II) complexes of the general formula [$Pt(NH_3)_2$(Am)Cl]$^+$ (where Am = planar heterocyclic amine) possess antitumor activity. These complexes were neither neutral nor did they possess two cis weakly bound leaving groups. Subsequently, Farrell et al.[6] reported that trans platinum complexes with planar amine ligands have antitu-

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Table 1. Cytotoxicity of Trans Pt^{II} Complexes in L1210 and Cisplatin-Resistant (L1210R) Leukemia Cells

	IC_{50} (μM)[b]	
complex[a]	L1210	L1210(R)
cis-[$PtCl_2(py)_2$]	4.4	3.3
trans-[$PtCl_2(py)_2$]	1.2	1.1
cis-[$PtCl_2(tz)_2$]	2.8	7.3
trans-[$PtCl_2(tz)_2$]	1.6	7.4
cis-[$PtCl_2(NH_3)$(quin)][c]	0.5	2.8
trans-[$PtCl_2(NH_3)$(quin)][c]	0.5	1.4
cis-[$PtCl_2(NH_3)$(tz)][c]	3.2	3.1
trans-[$PtCl_2(NH_3)$(tz)][c]	4.2	15.0
cis-[$PtCl_2(NH_3)_2$]	0.3	9.2
trans-[$PtCl_2(NH_3)_2$]	15.7	22.0

[a] py = pyridine, tz = thiazole, and quin = quinoline. [b,c] IC_{50} values were determined from studies of percent growth inhibition after 72 h[b] or 96 h[c] of drug exposure (data from refs 7 and 8).

mor activity superior to transplatin, especially in cisplatin-resistant cell lines. These were the first examples of platinum-based anticancer agents that defy the classic SARs.

The presence of planar ligands in *trans*-[$PtCl_2(L)_2$] (L = pyridine, thiazole) and *trans*-[$PtCl_2(NH_3)(L)$] (L = quinoline) complexes greatly enhances the cytotoxicity of such species, with respect to their corresponding cis isomers and also to transplatin.[7] The cytotoxicity is further marked by high activity toward both sensitive and cisplatin-resistant murine leukemia (L1210) cells (Table 1).[7,8] Thus, the activity is 1 order of magnitude greater than that of transplatin. DNA interstrand cross-linking is enhanced relative to transplatin, and conformational changes such as unwinding are accentuated in the presence of the planar ligand. The sequence specificity is also altered, with the trans complexes exhibiting a higher affinity for alternating purine−pyrimidine (GC) runs.[9]

Unfortunately, high antitumor activity of such trans complexes has not yet been obtained in vivo. Nevertheless, the observations on the enhanced cytotoxicity are important mechanistically, because they demonstrate that there is no restriction, per se, to development of active antitumor trans platinum complexes. The mechanistic importance of these findings is also emphasized by the fact that the vast majority of studies examining the differences between cis- and transplatin have been carried out in tissue culture or in cell-free systems. Factors such as water solubility and pharmacokinetic considerations (e.g. partition coefficient, drug metabolism, and tissue distribution) may all contribute to in vivo deactivation of cytotoxic agents.

Two major chemical differences between NH_3 and a planar amine are evident: steric hindrance by the appropriately positioned H atoms of the ring system reduces chemical reactivity, and surface and stacking interactions become more pronounced over the H-bonding associated with the NH_3 groups. These structural features suggest simple chemical solutions to the design of sequence-specific agents as well as using steric effects to prevent metabolic deactivation.

An understanding of the aqueous chemistry of diamine Pt^{II} complexes is crucial for establishing their mechanism of action. Once dissolved in water, the labile chloride ions of cisplatin are slowly replaced by water molecules in a stepwise manner: first forming monoaqua species and then further hydrolysis to form diaqua species.[10] The relative amounts of all these hydrolysis species vary as a function of pH and chloride concentration. Hydrolysis is likely to be more extensive inside the cells where the Cl^- concentration is much lower (4−23 mM) than outside cells (~100 mM).[11] Hydrolysis is the rate-limiting step in the reaction of cisplatin with DNA. Since water is a far better leaving group than chloride and hydroxide,[12] it is important to determine the pK_a values of the aqua adducts.

Although both the kinetics and thermodynamics of hydrolysis reactions of cisplatin and related *cis*-Pt^{II} amine complexes are well understood, surprisingly little is known about analogous reactions of trans complexes. For transplatin the first aquation step is rapid, whereas the second aquation step is very slow.[13] This is due to the stabilizing effect of the entering trans oxygen ligand. Moreover, it has been postulated that since transplatin is more reactive than the cis isomer, undesired side reactions occurring on its way to the pharmacological target are likely to contribute, at least in part, to the lack of anticancer activity. Sterically demanding ligands, like imino ethers in *trans*-[$PtCl_2${(*E*)-HN=C(OMe)Me}$_2$] (*trans*-EE) and substituted pyridine ligands in *cis*-[$PtCl_2(NH_3)$(2-pic)], could in principle reduce the axial accessibility of the platinum center and slow the hydrolysis reaction and the subsequent substitution of the aqua ligand by biologically relevant substrates.[14]

Here we have studied three trans isomers of *cis*-[$PtCl_2(NH_3)$(2-pic)] (2-pic = 2-methylpyridine), AMD473, a recently reported anticancer complex currently in phase II clinical trials. AMD473 is active against cisplatin-resistant cell lines and against an acquired cisplatin-resistant subline of a human ovarian carcinoma xenograph, by injection and oral administration.[15,16] It has shown significantly reduced cross-resistance to cisplatin in a panel of three cell lines with known acquired platinum drug resistance mechanisms: reduced accumulation; increased cytoplasmic detoxification by cellular thiols; increased DNA repair/tolerance of platinum−DNA adducts.[15,16] The toxicity of AMD473 is also greatly reduced, with no renal toxicity observed. The trans isomers [$PtCl_2(NH_3)$(2-pic)] (**1**), [$PtCl_2(NH_3)$(3-pic)] (**2**), and [$PtCl_2(NH_3)$(4-pic)] (**3**) have been labeled with ^{15}N, and 2D [^1H,^{15}N] HSQC spectroscopy was used to compare their hydrolysis behavior and to determine the pK_a values for the

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mono- and diaqua complexes. The data reveal notable differences between the chemistry of these trans complexes (**1**−**3**) and that of *cis*-[$PtCl_2(NH_3)$(2-pic)] and cisplatin.

Experimental Section

Materials. 2-, 3-, and 4-Picoline and $^{15}NH_4Cl$ (>98% ^{15}N) were purchased from Aldrich, and $^{15}NH_4OH$ (>98% ^{15}N) was purchased from Isotec. *cis*-[$PtCl_2(NH_3)_2$] and *cis*-[$PtCl_2(^{15}NH_3)_2$] were prepared according to a reported procedure.[17] Complexes **2** and **3** were prepared by a general procedure described in the literature for natural abundance, mixed-ligand ammine/amine Pt^{II} complexes.[18]

Preparation of *trans*-[$PtCl_2(NH_3)$(2-pic)] (AMD443) (1). *cis*-[$PtCl_2$(2-pic)$_2$] (0.392 g, 0.866 mmol), prepared by literature methods,[19] was dissolved in deionized water (7 mL), and the solution was heated at reflux; then NH_4OH (2.77 mL, 2.5 M) was added to give a yellow suspension, which was heated at reflux and stirred overnight. This produced a clear solution, which was filtered and then rotary evaporated. The *cis*-[$Pt(NH_3)_2$(2-pic)$_2$]Cl_2 intermediate was dissolved in water (2 mL), and the solution was heated and stirred under reflux. HCl (289 μL, 11.98 M) was added to the solution, and the mixture allowed to stir under reflux for 5 days under nitrogen, during which time it was necessary to add additional aliquots of water. The mixture was then placed on an ice bath to induce precipitation. The yellow product was then filtered off, washed with ice-cold water, and dried in vacuo overnight. The product was recrystallized from 0.1 M HCl, giving small yellow crystals. Yield: 258.5 mg, 79%. Anal. Calcd for $C_6H_{10}Cl_2N_2Pt$: C, 19.16; H, 2.68; N, 7.45. Found: C, 18.87; H, 2.54; N, 7.11. ^1H NMR (acetone-d_6): δ = 8.81 (d, J = 5.7 Hz, 1H, H-6), 7.78 (t, 1H, H-4), 7.43 (d, J = 7.9 Hz, 1H, H-3), 7.27 (t, 1H, H-5), 3.69 (broad, NH_3), 3.14 (s, 3H, CH_3). MS: m/z 399.3 corresponding to [$PtCl_2(NH_3)$(2-pic)]Na^+. *trans*-[$PtCl_2(^{15}NH_3)$(2-pic)] (^{15}N-**1**) was prepared by the same method using $^{15}NH_4OH$.

Preparation of *trans*-[$PtCl_2(NH_3)$(3-pic)] (2). Cisplatin (0.233 g, 0.775 mmol) was suspended in 23 mL of water, which had been bubbled with argon, and 2 mol equiv of 3-picoline (151 μL, 1.55 mmol) was added. The mixture was stirred at 363 K for 3 h, allowed to cool to room temperature, and filtered to remove any traces of precipitate, and then concentrated HCl (1.6 mL) was added to the clear solution. The acidic mixture was refluxed under nitrogen overnight. The resulting solution was bright yellow and was placed on ice for several hours to induce precipitation. The yellow precipitate was filtered off, washed with ice-cold water, followed by diethyl ether, and dried in vacuo overnight. Yield: 188.3 mg, 64.6%. Anal. Calcd for $C_6H_{10}Cl_2N_2Pt$: C, 19.16; H, 2.68; N, 7.45. Found: C, 19.30; H, 2.63; N, 7.39. ^1H NMR (acetone-d_6): δ = 8.66 (s, 1H, H-2), 8.64 (d, J = 5.49 Hz, 1H, H-6), 7.78 (d, J = 7.68 Hz, 1H, H-4), 7.32 (t, 1H, H-5), 3.73 (broad, NH_3), 2.37 (s, 3H, CH_3). MS: m/z = 399.0 corresponding to [$PtCl_2(NH_3)$(3-pic)]Na^+. *trans*-[$PtCl_2(^{15}NH_3)$(3-pic)] (^{15}N-**2**) was prepared by the same method using ^{15}N-cisplatin as the starting material.

Preparation of *trans*-[$PtCl_2(NH_3)$(4-pic)] (3). Cisplatin (0.188 g, 0.627 mmol) was suspended in 19 mL of water, which had been bubbled with argon, and 2 mol equiv of 4-picoline (122 μL, 1.25 mmol) was added. The mixture was stirred at 363 K for 3 h, allowed to cool to room temperature, and filtered to remove any traces of precipitate, and then concentrated HCl (1.6 mL) was added to the clear solution. The acidic mixture was refluxed under nitrogen for 6 h. The resulting solution was bright yellow and was placed on ice for several hours to induce precipitation. The yellow precipitate was filtered off, washed with ice-cold water, followed by diethyl ether, and dried in vacuo overnight. Yield: 80.0 mg, 33.9%. Anal. Calcd for $C_6H_{10}Cl_2N_2Pt$: C, 19.16; H, 2.68; N, 7.45. Found: C, 19.35; H, 2.66; N, 8.42. ^1H NMR (acetone-d_6): δ = 8.66 (d, J = 6.3 Hz, 2H, H-2), 7.26 (d, J = 5.65 Hz, 2H, H-3), 3.70 (broad, NH_3), 2.41 (s, 3H, CH_3). MS: m/z = 399.1 corresponding to [$PtCl_2(NH_3)$(4-pic)]Na^+. *trans*-[$PtCl_2(^{15}NH_3)$(4-pic)] (^{15}N-**3**) was prepared by the same method using ^{15}N-cisplatin as the starting material.

X-ray Crystallography. Diffraction data were collected with Mo Kα radiation on a Bruker Smart Apex CCD diffractometer equipped with an Oxford Cryosystems low-temperature device operating at 150 K. Data were corrected for absorption using the SADABS[20] procedure. The structure of **1** was solved by direct methods (SHELXS),[21] while those of **2** and **3** were solved using Patterson methods (DIRDIF[22] and SHELXS, respectively). The structures were refined against F^2 using all data (SHELXL).[23] All non-H atoms were modeled with anisotropic displacement parameters, and H-atoms were placed in calculated positions. CH_3 and NH_3 groups were treated according to the Sheldrick rotating rigid group model.[23]

Crystal Data for 1. The sample was a pale-yellow needle of dimensions 1.00 × 0.14 × 0.14 mm^3; the crystals tended to split into smaller needles when cut: orthorhombic, space group *Pccn*; a = 10.5710(12), b = 22.826(3), c = 7.9743(9) Å; V = 1924.1(7) Å^3; Z = 8; D_{calc} = 2.597 Mg m^{-3}. The final conventional R-factor [R1, based on $|F|$ and 2018 data with $F > 4\sigma(F)$] was 0.0431, and wR2 (based on F^2 and all 2374 unique data to θ_{max} = 28.7°) was 0.0921. The final ΔF synthesis extremes were +2.32 and −1.82 e Å^{-3}.

Crystal Data for 2. The sample was a yellow block of dimensions 0.70 × 0.25 × 0.25 mm^3: monoclinic, space group $P2_1/c$; a = 8.5867(2), b = 22.8583(3), c = 9.8514(3) Å; V = 1930.00(13) Å^3; Z = 8 (there are two molecules in the asymmetric unit); D_{calc} = 2.589 Mg m^{-3}. The final conventional R1-factor (based on 4034 data) was 0.0386, and wR2 (based on 4576 unique data to θ_{max} = 28.6°) was 0.0865. The final ΔF synthesis extremes were +2.31 and −2.22 e Å^{-3}.

Crystal Data for 3. The sample was a pale-yellow needle of dimensions 1.06 × 0.08 × 0.04 mm^3: orthorhombic, space group *Pccn*; a = 11.5185(4), b = 22.3413(7), c = 7.6232(3) Å; V = 1961.74(12) Å^3; Z = 8; D_{calc} = 2.547 Mg m^{-3}. The final conventional R1-factor (based on 2193 data) was 0.0307, and wR2 (based on 2828 unique data to θ_{max} = 30.5°) was 0.0668. The final ΔF synthesis extremes were +1.70 and −0.81 e Å^{-3}.

NMR Spectroscopy. NMR spectra were recorded at 298 K, unless otherwise stated, on Bruker DMX500 (^1H 500.13 MHz) or Bruker AVA600 (^1H 599.81 MHz) spectrometers using 5 mm NMR tubes. ^1H NMR chemical shifts were referenced to TSP via dioxane (δ 3.76), and ^{15}N chemical shifts, to 1 M $^{15}NH_4Cl$ in 1.5 M HCl (external). Water suppression was achieved by presaturation. Spectra were processed using XWINNMR (version 3.5, Bruker U.K. Ltd).

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Table 2. X-ray Crystal Structure Data for Complexes **1**−**3**

param	1	2	3
empirical formula	$C_6H_{10}Cl_2N_2Pt$	$C_6H_{10}Cl_2N_2Pt$	$C_6H_{10}Cl_2N_2Pt$
M_r	376.15	376.15	376.15
space group	Pccn	$P2_1/c$	Pccn
a (Å)	10.5710 (12)	8.5867 (2)	11.5185 (4)
b (Å)	22.826 (3)	22.8583 (7)	22.3413 (7)
c (Å)	7.9743 (9)	9.8514 (3)	7.6232 (3)
β (deg)	90	93.506 (2)	90
V (Å^3)	1924.1 (4)	1929.99 (9)	1961.74 (12)
Z	8	8	8
λ (Å)	0.710 73	0.710 73	0.710 73
T (K)	150 (2)	150 (2)	150 (2)
ρ_{calcd} (g cm^{-3})	2.597	2.589	2.547
μ_{calcd} (mm^{-1})	15.080	15.034	14.791
R1 ($F_0 > 4\sigma(F_0)$)	0.0431	0.0386	0.0307
wR2 (all data)	0.0921	0.0865	0.0668

Table 3. Selected Bond Lengths (Å) and Angles (deg) for Complexes **1**−**3**

param	1	2	3
Pt−N(1)	2.028(7)	2.014(6)	2.014(4)
Pt −N(2)	2.037(8)	2.047(6)	2.043(4)
Pt−Cl(1)	2.313(2)	2.293(2)	2.2918(11)
Pt−Cl(2)	2.307(2)	2.292(3)	2.3002(12)
N(1)−Pt−N(2)	178.9(3)	176.9(3)	179.22(15)
N(1)−Pt−Cl(1)	90.1(2)	90.61(19)	90.95(11)
N(2)−Pt−Cl(1)	89.6(2)	88.35(19)	88.32(12)
N(1)−Pt−Cl(2)	92.0(2)	91.26(19)	90.63(11)
N(2)−Pt−Cl(2)	88.4(2)	89.8(2)	90.08(12)
Cl(2)−Pt−Cl(1)	177.48(8)	178.12(7)	177.40(5)

Sample Preparation. NMR samples for hydrolysis reactions were prepared by dissolving the platinum complex in an aliquot of DMF, followed by dilution with H_2O/D_2O to the required concentration. For pK_a determinations, the platinum complexes were reacted with 1.96 equiv of $AgNO_3$ (24 h, room temperature) and then filtered to remove AgCl. All samples were prepared in 90% H_2O/10% D_2O unless otherwise stated.

Kinetic Analyses. The concentration/time data for each complex were computer-fitted to the following first-order rate equation:

$$A = C_0 + C_1 e^{-kt} \quad (1)$$

Here C_0 and C_1 are computer-fitted constants and A is the concentration corresponding to time t. All kinetic data were computer-fitted using the program Microcal Origin 7.5 to give the hydrolysis rate constant k_{H_2O} (k).

pH Measurements. The pH values of solutions were determined using a Corning 145 pH meter equipped with a micro combination electrode, calibrated with Aldrich buffer solutions at pH 4, 7, and 10. The pH was adjusted with dilute solutions of $HClO_4$ and NaOH. No correction has been made for deuterium isotope effects on the glass electrode.

pK_a Values. These were determined by fitting the NMR pH titration curves for samples in 90% H_2O/10% D_2O to the Henderson−Hasselbalch equation using the program Kaleidagraph (Synergy Software, Reading, PA).

Mass Spectrometry. Ion electrospray mass spectrometry was performed on a Platform II mass spectrometer (Micromass, Manchester, U.K.). The samples were infused at 8 μL/min and the ions produced in an atmospheric pressure ionization (API)/ESI ion source. The source temperature was 383 K, and the drying gas flow rate was 300 L/h. A potential of 3.5 kV was applied to the probe tip, and cone voltage gradients of 20−40 V over 200−1000 Da were used. Data acquisition was performed on a Mass Lynx (V2.5) Windows NT PC data system. All samples were prepared in water.

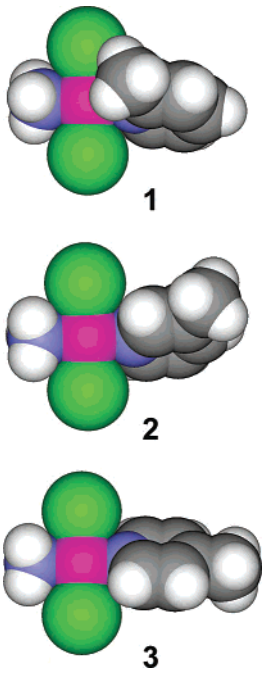

Figure 1. Space-filling models of the X-ray crystal structures of complexes **1**−**3** showing the steric hindrance caused by the 2-methyl group in complex **1**.

Results

X-ray Crystal Structures. X-ray crystallographic data and details of the refinement of the structures of the trans ammine/2-picoline, 3-picoline, and 4-picoline complexes (**1**−**3**, respectively) are listed in Table 2, and selected bond lengths and angles are given in Table 3. All complexes have a square-planar configuration with angles close to the ideal values of 90 and 180°, as shown in Figure 1. Pt−Cl bond lengths range from 2.292 to 2.313 Å and are close to the expected values. The Pt−N bond lengths of 2.014−2.047 Å are comparable to those of related structures. The most notable feature of the structures is the orientation of the picoline ring with respect to the platinum square plane. In complex **1**, the 2-picoline ligand is tilted by 69.2°, in complex **2** 3-picoline is tilted by 60.0 and 57.8° (2 molecules/unit cell), and for 4-picoline in **3** is tilted by only 56.2°. The tilt angle therefore decreases in the order 2-pic > 3-pic, 4-pic. In complex **1**, the 2-methyl group lies over the square plane ($H_3C\cdots Pt$: 3.207 Å), whereas in complexes **2** and **3** the

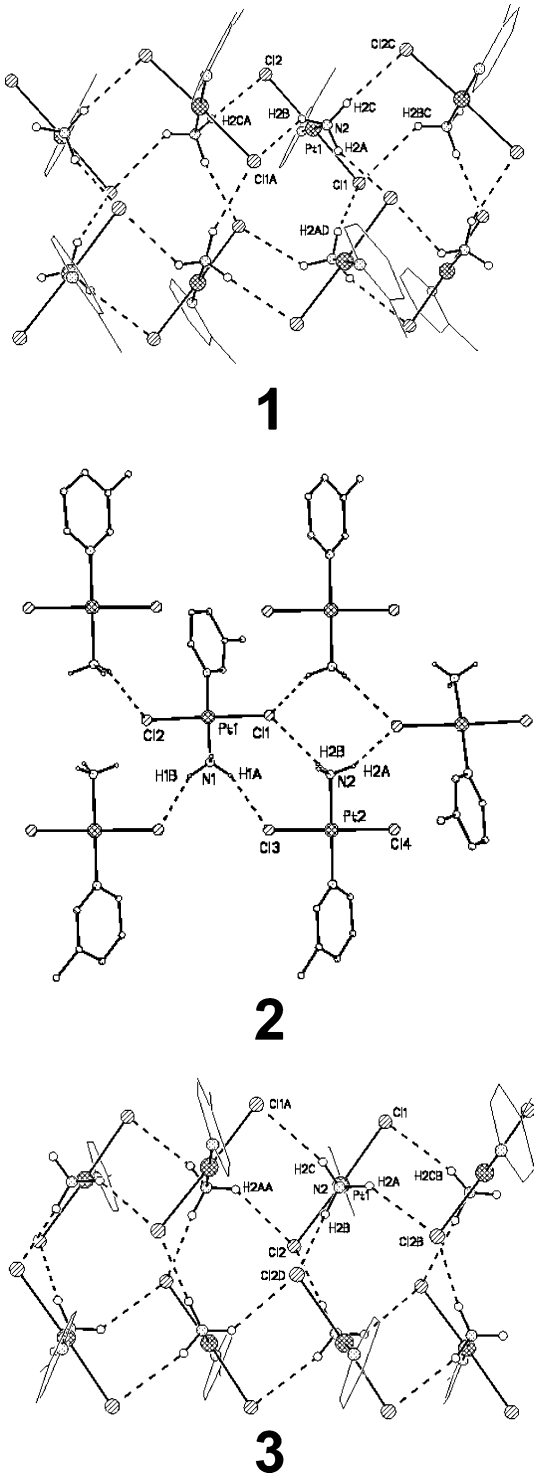

Figure 2. Intermolecular H-bonding in crystals of complexes **1**−**3**. N−H···Cl distances range from 2.46−2.85 Å for complex **1**, 2.49−2.55 Å for complex **2**, and 2.49−2.70 Å for complex **3**.

methyl group is much further away from Pt (H_3C···Pt: 5.452, 5.458 Å for **2** and 6.312 Å for **3**). The space-filling models in Figure 1 demonstrate that in complex **1** there is significantly more steric hindrance to an axial approach to Pt from above than in either complexes **2** or **3**.

Strong intermolecular hydrogen bonds are involved in the crystal packing of all complexes (Figure 2). For complexes **1** and **3**, the three H atoms of the NH_3 ligand are H-bonded

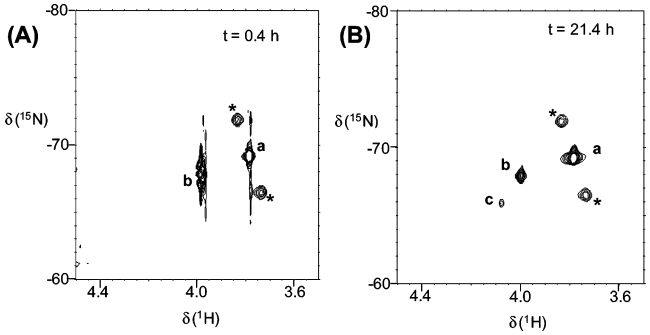

Figure 3. 2D [^{1}H,^{15}N] HSQC NMR spectra of a 5 mM aqueous solution of *trans*-[$PtCl_2$($^{15}NH_3$)(2-pic)] (**1**) at 310 K after (A) 0.4 h and (B) 21.4 h. Peak a is assigned to **1**, peak b to the monoaqua complex, and peak c to the diaqua complex; ∗ = ^{195}Pt satellites.

to three Cl ligands from neighboring molecules. Similarly for **1** and **3,** chains of complexes are connected by H-bonds and also interact with one another through interleaving π-stacks. Such intermolecular H-bonds are common in chloroplatinum(II) am(m)ine complexes. However, there appears to be an additional H-bonding interaction in complex **1** between the aromatic H5 proton of the 2-picoline ring and a Cl ligand (2.853 Å). For complex **2**, H-bonds of similar strength are formed but only to two Cl ligands, giving infinite chains composed of channels lined by Cl and NH_3 along the *c*-direction.

Hydrolysis of Complexes 1−3. The hydrolysis of $^{15}NH_3$-labeled **1**−**3** in aqueous solutions containing 0.1 M $NaClO_4$ was monitored by [^{1}H,^{15}N] 2D NMR for a period of over 20 h at 277 K. This low temperature was chosen because the rates were too fast at ambient temperature to allow determination by NMR. Initially for **1**, a single cross-peak was observed at δ = 3.63, −70.11, which was assigned to the dichloro complex **1**. After 2 h, a weak cross-peak was detected at δ = 3.69, −68.30, which was assigned to the monoaqua species. For trans complexes, the $^{15}NH_3$ ligand remains trans to the picoline N during substitution reactions in the cis position and only small changes in chemical shift are observed due to the cis effect. The peak for **1** decreased in intensity as the monoaqua peak increased in intensity over a period of 21 h. No peak assignable to a diaqua species was observed within the time the reaction was followed (48 h). At 310 K an additional peak at δ 4.07, −65.99 ppm assignable to the diaqua adduct was observed after 4 h, but its intensity was low and accounted for <3% of the Pt present in the solution after 21 h (Figure 3).

For the 3-picoline complex **2**, initially, two cross-peaks were observed. The most intense peak at δ = 3.64, −70.60 was assigned to the dichloro complex **2,** and the less intense peak at δ = 3.77, −69.21, to the monoaqua species. Again, no diaqua species was observed. The time dependence of the [^{1}H,^{15}N] 2D NMR spectrum was similar to that of complex **1**, except that equilibrium was reached more quickly (<7 h at 277 K).

The hydrolysis of the 4-picoline complex **3** followed a similar time dependence but, interestingly, reached equilibrium significantly more slowly than for the 3-picoline complex **2**. The chemical shifts for the dichloro complex

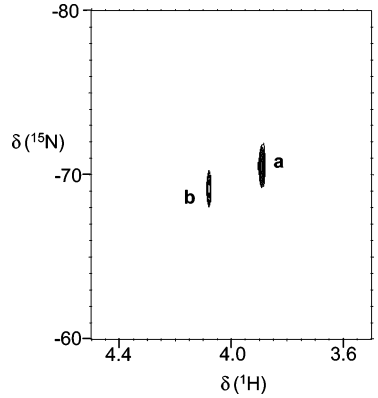

Figure 4. 2D [^{1}H,^{15}N] HSQC NMR spectrum of 5 mM aqueous solution of *trans*-[$PtCl_2(^{15}NH_3)$(4-pic)] (**3**) at 277 K after 21.3 h. Peak a is assigned to **3**, and peak b to the monoaqua complex.

and monoaqua species are similar to those of **1** and **2**, with the dichloro complex **3** having a cross-peak at $\delta = 3.63$, -70.60 and the monoaqua species at $\delta = 3.68$, -68.89 (Figure 4). No diaqua species was observed under these experimental conditions. It is noteworthy that the ^{1}H NMR peaks, in particular, tended to shift downfield throughout the course of the reaction. This is a consequence of a decrease in pH caused by deprotonation of coordinated water to produce the monohydroxo complex.

The time dependence of the concentrations of species detected during hydrolysis of complexes **1**−**3** is shown in Figure 5. The assignments of the peaks of the aqua complexes were confirmed by pH titrations (Figure 6), using aqua adducts of complexes **1**−**3** which had been generated by reactions with Ag^I to produce a mixture of monoaqua, diaqua, and dichloro species. These allowed the determination of pK_a value for each monoaqua complex as well as two pK_a values for each diaqua complex.

The NMR data allow the determination of the rates for monoaquation of complexes **1**−**3** at 277 K, and these are listed in Table 4. It is notable that the rate of monoaquation of the 3-picoline complex **2** is more than twice as fast as that of complex **3** and ca. five times as fast as complex **1**. However, the extent of hydrolysis of complex **2** was ca. 75% that of complexes **1** and **3**. An estimate of the half-lives for hydrolysis of complex **1** at 310 K is included in Table 6 and compared with related complexes. Half-lives for hydrolysis (310 K) and pK_a values (298 K) for complex **1** are shown in Scheme 1.

Discussion

cis-[$PtCl_2(NH_3)$(2-picoline)] is currently on clinical trial as an anticancer drug. Its trans isomer, complex **1**, shows in vitro cytotoxicity[24] even higher than that of cisplatin in a panel of human tumor cell lines (mean $IC_{50} = 3.5$ and 33.7 μM, respectively), whereas the trans 4-picoline complex **3**, tested by Kasparkova et al.,[25] exhibits a lower activity (mean $IC_{50} = 80.3$ μM), Table 5. The 3-picoline complex **2** has not yet been evaluated for its cytotoxic activity. Complex **1** overcomes cisplatin resistance in the resistant cell lines tested, but complex **3** is cross-resistant, implying that complex **1** may have a mechanism of action different from that for complex **3**. IC_{50} values for these and related compounds are listed in Table 5.[24−26] Interestingly, *trans*-[$PtCl_2(NH_3)$(pip)] (where pip = piperidine) and *trans*-$[PtCl_2(NH_3)(pz)]^+$ (where pz = piperazine) are less cytotoxic than both *trans*-[$PtCl_2$-(NH_3)(2-pic)] (**1**) or *trans*-[$PtCl_2(py)_2$] in the cell lines tested. Moreover, *trans*-[$PtCl_2(py)_2$] overcomes cisplatin resistance while *trans*-[$PtCl_2(NH_3)$(2-pic)] has a small average resistance factor of ca. 1.5.

^{15}N-labeling of **1**−**3** and use of [^{1}H,^{15}N] NMR spectroscopy has allowed study of the speciation of these complexes in aqueous solution. The [^{15}N,^{1}H] NMR method has the advantage that the concentrations of aquated species can usually be measured directly at low (micromolar) concentrations, even when other species are present in the solution.

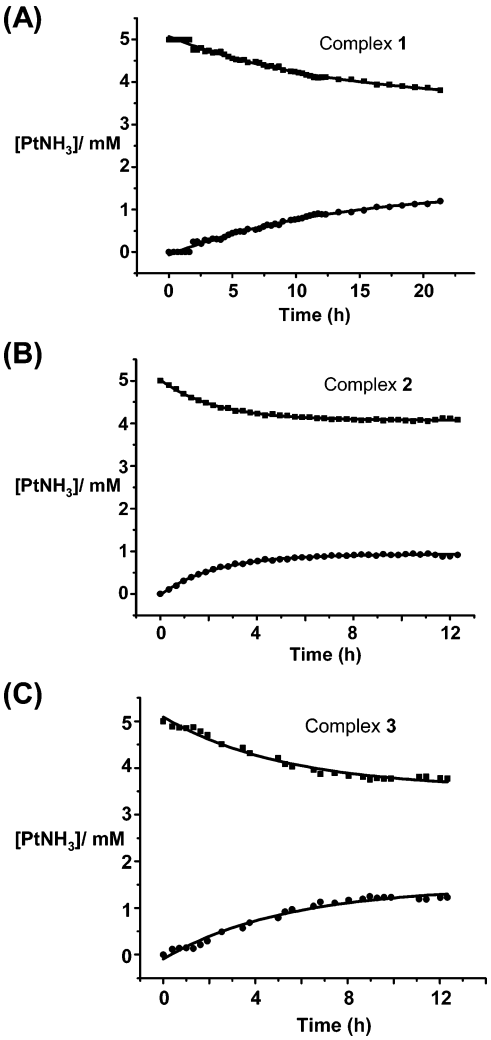

Figure 5. Time dependence of the concentrations of the dichloro complexes (A) **1**, (B) **2**, and (C) **3** and their aqua adducts during hydrolysis at 277 K. Concentrations are based on integration of 2D NMR cross-peaks. Labels: **1**−**3** (■); monoaqua complexes (●). The full lines represent computer fits giving the first-order rate constants listed in Table 4. Note the longer time scale for the hydrolysis of the 2-pic complex **1** in (A).

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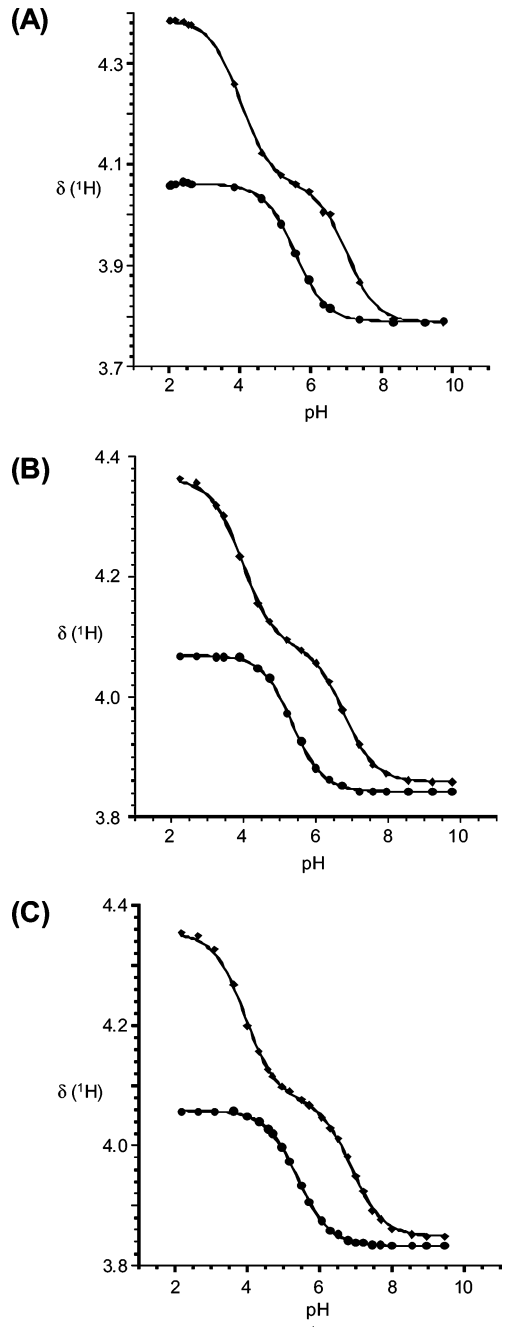

Figure 6. pH dependence of the ^{1}H NMR chemical shifts of NH_3 in the monoaqua and diaqua complexes (A) **1**, (B) **2**, and (C) **3**. The curves represent computer fits to the Henderson−Hasselbalch equation giving the pK_a values listed in Table 7. Labels: monoaqua complexes (●); diaqua complexes (◆).

Table 4. Rate Constants (k) and Equilibrium Constants (K) for the Hydrolysis Reactions of Complexes **1**−**3** and AMD473

complex	T (K)	k (10^{-5} s^{-1})[a]	K (10^{-4} M)[a]
1	277	2.6 ± 0.2	3.9 (3.7)[b]
2	277	12.7 ± 0.8	2.0
3	277	5.2 ± 0.3	4.0
AMD473[c]	310	3.2,[d] 2.2[e]	12.1,[d] 21.4[e]

[a] Based on concentrations derived from integration of 2D [^{1}H,^{15}N] NMR cross-peaks. [b] 310 K. [c] Data from ref 32. [d] Trans to 2-pic. [e] Trans to NH_3.

However, due to the extremely fast rate of the monoaquation step, rates were measured at lowered temperature (277 K). At 277 K, only peaks for the monoaqua species were detected, although a peak for the diaqua species was detected after ca. 4 h of hydrolysis of complex **1** at 310 K.

The weaker basicity of 3-picoline appears to influence both the extent and rate of hydrolysis of complex **2** in comparison with complexes **1** and **3**. The pK_a value of 3-picoline is ca. 0.3 units lower than those of 2- or 4-picoline.[27] At equilibrium, the monoaqua species of complexes **1** and **3** account for only ca. 24% of all platinum species present and the monoaqua species of complex **2** accounts for only ca. 18% of all platinum species. The $t_{1/2}$ values for the first hydrolysis step at 277 K follow the order **1** (7.43 h) > **3** (3.68 h) > **2** (1.52 h). Since the steric hindrance due to the picoline ligand in complexes **2** and **3** is similar, it seems likely that the faster hydrolysis of complex **2** relative to complex **3** is due to electronic factors. A methyl substituent at the 2- and 4-positions (complexes **1** and **3**, respectively) increases electron density on the platinum center, making nucleophilic attack by the incoming water molecule less favorable. However, at the 3-position (complex **2**), the methyl substituent directs less electron density to the platinum center and thus promotes the hydrolysis reaction. Complex **1** is the slowest to hydrolyze due to both steric and electronic effects. The more sterically demanding 2-picoline ligand hinders axial approach of the incoming water molecule from above. Substitution reactions of square-planar Pt^{II} complexes (i.e. $PtL_2XY + Z \rightarrow PtL_2YZ + X$) usually occur through formation of a five-coordinate intermediate (PtL_2XYZ) in an associative process.[28] The use of a more sterically hindering nonleaving group (i.e. Y or L) compared to NH_3 raises the energy of the five-coordinate intermediate (i.e. increases the activation energy associated with entry of Z into the coordination sphere).

When hydrolysis of complex **1** was monitored at 310 K, approximate values of $t_{1/2}$ were determined as 0.9 h for the monoaquation and 70.2 h for the diaquation steps. These values compare well with $t_{1/2} = 0.92$ h and $t_{1/2} = 77.0$ h ($T = 298$ K) for mono- and diaquation, respectively, of *trans*-[$PtCl_2${*E*-HNC(OMe)Me}$_2$].[29] Farrell et al.[30] have obtained a $t_{1/2}$ value of 0.33 h for *trans*-[$PtCl_2(NH_3)$(4-pic)] (**3**) at $T = 298$ K. Table 6 lists $t_{1/2}$ values of various related compounds.[29−34] Only a few studies of transplatin hydrolysis have been reported, and there appears to be a large disparity between the reported half-lives. Aprile and Martin[33] reported a $t_{1/2}$ value of 1.96 h ($I = 0.318$ M, $T = 298$ K) for monoaquation of transplatin, whereas Miller et al.[34] calculated a $t_{1/2}$ value of 10.13 h ($I = 0.1$ M, $T = 298.2$ K) on the basis of the activation parameters, obtained from the temperature-dependent intercepts of plots of k_{obs} versus [Cl^-].

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Table 5. Mean IC_{50} Values for Trans Pt Compounds against Various Cell Lines[24−26]

complex[a]	IC_{50} (μM)[b] A2780	A2780(R)	CH1	CH1(R)	41M	41M(R)
cisplatin	2.2	38	6	23	26	107
transplatin	>200	>200	>200	>200	>200	>200
trans-$[PtCl_2(py)_2]$			1.6	1.7	2.2	2.0
trans-$[PtCl_2(NH_3)(pip)]$	5	20	15	94	27	150
trans-$[PtCl_2(NH_3)(pz)]^+$	5	44	12	34	52	155
trans-$[PtCl_2(NH_3)(2\text{-pic})]$ (**1**)	1.6	6	3.8	5.2	2.8	1.7
trans-$[PtCl_2(NH_3)(4\text{-pic})]$ (**3**)	7	80	22	154	32	187

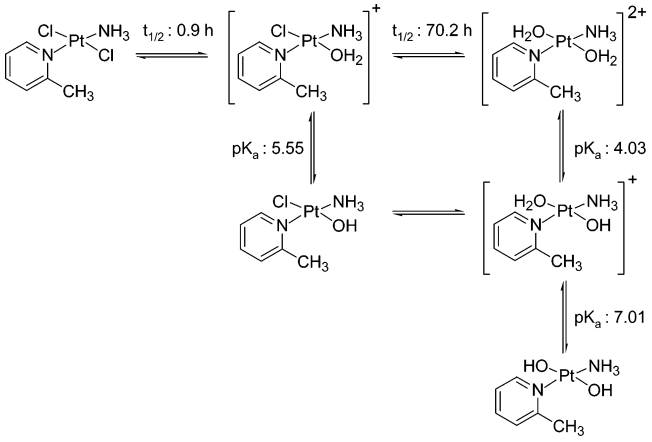
[a] py = pyridine, pip = piperidine, and pz = piperazine. [b] R = cisplatin-resistant cell line.

Table 6. Half-Lives [$t_{1/2}$ (h)] for Hydrolysis of Various Pt^{II} Complexes

complex	dichloro $t_{1/2}$	monoaqua $t_{1/2}$	ref
trans-$[PtCl_2(NH_3)(2\text{-pic})]$ (**1**)[a]	7.43 (0.9)[b]	(70.2)[b]	present work
trans-$[PtCl_2(NH_3)(3\text{-pic})]$ (**2**)[a]	1.52		present work
trans-$[PtCl_2(NH_3)(4\text{-pic})]$ (**3**)[a]	3.68		present work
transplatin[c]	1.96	3.85	33
transplatin[d]	10.13		34
trans-$[PtCl_2(NH_3)(4\text{-pic})]$	0.33		30
trans-$[PtCl_2\{EHNC(OMe)Me\}_2]$[c]	0.92	77.02	29
cis-$[PtCl_2(NH_3)(2\text{-pic})]$[b]	6.04, 8.71	2.64, 55.01	32
cis-$[PtCl_2(NH_3)(3\text{-pic})]$[b]	1.86, 4.31	2.47, 5.50	32
cisplatin[b]	1.9	2.1	31

[a] 277 K. [b] 310 K,. [c] 298 K. [d] 298.2 K, calculated from the activation parameters, which were determined from the temperature-dependent intercepts of plots of k_{obs} vs $[Cl^-]$.

Scheme 1. Half-Lives for Hydrolysis (310 K) of Complex **1** and pK_a Values (298 K) of Its Aqua Adducts



The equilibrium constants for monoaquation determined for complexes **1**−**3**, (2−4) × 10^{-4} M^{-1} (Table 4), are 1 order of magnitude less than reported values (298 K) for cisplatin[33−35] of (33−68) × 10^{-4} M^{-1}. The equilibrium constants reported for transplatin,[33,34] 3.2 × 10^{-4} and 6.2 × 10^{-4} M^{-1}, are also 1 order of magnitude smaller than those of cisplatin, and our values for complexes **1**−**3** fall within this range. Thus, the amount of (reactive) monoaqua species formed by the trans complexes at intracellular chloride concentrations (4−23 mM)[11] is likely to be <10%, much smaller than for the cis analogues (Table 4). A consequence of the higher stability of the trans chloro complexes is that little of the diaqua adduct is formed during the hydrolysis. It has been suggested for cisplatin that the diaqua complex plays a key role in the attack on DNA.[36]

[^{1}H,^{15}N] NMR allowed also the determination of the pK_a values of the aqua ligands in the monoaqua and diaqua species of complexes **1**−**3**. Movement of the methyl group from the 2- to the 3- to the 4-position has little affect on the pK_a values of the aqua ligands. A comparison of pK_a values for monoaqua adducts, Table 7,[37−42] shows those for complexes **1**−**3** are fairly typical for *trans*-{$Pt(NH_3)$(amine)}-type complexes (5.3−5.9) and significantly lower than those of cis complexes such as cisplatin and AMD473. Also, the first pK_a value for the trans diaqua complexes is ca. 1 unit lower than those for related cis complexes (Table 7).

In recent years, there has been an increasing number of investigations of trans platinum complexes.[9,13,43] One of the most striking differences between cis and trans isomers apparent from such investigations is the variation in formation and reactivity of the diaqua species. However, Arpalahti[44] reported that, for cisplatin and transplatin, the second complexation step in the formation of the bis(inosine) adduct appears to be kinetically similar. In the case of multinuclear platinum am(m)ine complexes, which hydrolyze only to a small extent and yet have a high DNA affinity (rapid DNA binding), it has been suggested that formation of aqua species may not be a necessary step in DNA adduct formation of such compounds.[40] Moreover, the high cytotoxicity of the *trans*-$[PtCl_2(NH_3)$(planar amine)] class of compounds has been attributed to an alteration of the mode of DNA binding in comparison to cisplatin, resulting in an altered spectrum of antitumor activity and enhanced reactivity in cisplatin-resistant cell lines. For example, an intercalative manner of interaction of the quinoline or thiazole moiety with the duplex has been proposed for all or a significant fraction of the DNA adducts of *trans*-$[PtCl_2(NH_3)$(quin)] and *trans*-$[PtCl_2(NH_3)$-(tz)][45] (where quin = quinoline, tz = thiazole). Also, it has been reported that the mechanism of antitumor activity of

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Table 7. Comparison of pK_a Values for *trans*-(Diam(m)ine)platinum(II) Aqua Complexes and Aquated Cisplatin

complex	pK_a	ref
trans-[PtCl(OH_2)($^{15}NH_3$)(2-pic)]$^+$	5.55	present work
trans-[Pt(OH_2)$_2$($^{15}NH_3$)(2-pic)]$^{2+}$	4.03, 7.01	present work
trans-[PtCl(OH_2)($^{15}NH_3$)(3-pic)]$^+$	5.35	present work
trans-[Pt(OH_2)$_2$($^{15}NH_3$)(3-pic)]$^{2+}$	3.97, 6.78	present work
trans-[PtCl(OH_2)($^{15}NH_3$)(4-pic)]$^+$	5.39	present work
trans-[Pt(OH_2)$_2$($^{15}NH_3$)(4-pic)]$^{2+}$	3.94, 6.88	present work
trans-[PtCl(OH_2)($^{15}NH_3$)$_2$]$^+$	5.63	37
trans-[Pt(OH_2)$_2$($^{15}NH_3$)$_2$]$^{2+}$	4.35, 7.40	37
trans-[PtCl(OH_2)($^{15}NH_3$)(2-Me-(^{15}N)butylamine)]$^+$	5.90	38
trans-[Pt(OH_2)$_2$($^{15}NH_3$)(2-Me-(^{15}N)butylamine)]$^{2+}$	4.16, 7.17	38
trans-[PtCl(OH_2)(isopropylamine)((*S*)-2-methyl(^{15}N)butylamine)]$^+$	5.86	39
trans-[Pt(OH_2)$_2$(isopropylamine)((*S*)-2-methyl(^{15}N)butylamine)]$^{2+}$	4.21, 7.33	39
[{*trans*-PtCl($^{15}NH_3$)$_2$}{*trans*-Pt(OH_2)($^{15}NH_3$)$_2$}(μ-$^{15}NH_2(CH_2)_6{}^{15}NH_2$)]$^{3+}$	3.9	40
[{*trans*-Pt(OH_2)($^{15}NH_3$)$_2$}$_2$(μ-$^{15}NH_2(CH_2)_6{}^{15}NH_2$)]$^{4+}$	5.62[a]	40
trans-[PtCl(OH_2)($^{15}NH_3$)(c-$C_6H_{11}{}^{15}NH_2$)]$^+$	5.40	41
cis-[PtCl(OH_2)($^{15}NH_3$)(2-pic)]$^+$	6.13, 6.49	32
cis-[Pt(OH_2)$_2$($^{15}NH_3$)(2-pic)]$^{2+}$	5.22, 7.16	32
cis-[PtCl(OH_2)($^{15}NH_3$)(3-pic)]$^+$	5.98, 6.26	32
cis-[Pt(OH_2)$_2$($^{15}NH_3$)(3-pic)]$^{2+}$	5.07, 6.94	32
cis-[PtCl(OH_2)($^{15}NH_3$)$_2$]$^+$	6.41	42
cis-[Pt(OH_2)$_2$($^{15}NH_3$)$_2$]$^{2+}$	5.37, 7.21	42

[a] Average value.

trans-[$PtCl_2(NH_3)$(pip)] does not involve recognition of its DNA adduct by HMG domain proteins as a crucial step,[46] in contrast to the proposals for cisplatin and its analogues. Thus, further studies are warranted to develop the potential of such trans platinum anticancer complexes which act by a different mechanism and exhibit activity complementary to agents such as cisplatin.

Conclusions

^{1}H,^{15}N NMR studies of the aquation of the *trans*-ammine−picoline−dichloro complexes **1**−**3** (2-pic, 3-pic, and 4-pic, respectively) showed that the monoaquation step is relatively rapid ($t_{1/2}$ = 1.5−7.5 h at 277 K). However, aquation occurs only to a limited extent (18−24% hydrolysis, 5 mM Pt, 277 K), the equilibrium favoring the dichloro species, with little formation of the diaqua complex. Increased steric hindrance to an axial approach to Pt^{II} in the 2-picoline complex **1** was apparent in the X-ray crystal structure. This hindrance has the effect of destabilizing the expected trigonal bipyramidal transition state, an effect well-known in substitution reactions of square-planar Pt^{II} complexes. The low pK_a values of the monoaqua complexes **1**−**3** (5.3−5.6) implies that they will be largely in the less reactive hydroxo forms at physiological pH. The limited extent of hydrolysis (<10%) at intracellular chloride concentrations (ca. 4 and 23 mM in the nucleus and cytoplasm, respectively[11]) implies that reactions with nucleobases will be slow. This, together with the faster rate of hydrolysis, due to the higher trans effect of Cl^- compared with NH_3, is likely to have a major influence on the biological activity (including detoxification) of trans compared to cis complexes.

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Supporting Information Available: X-ray crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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