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## Articles

# Novel Soluble Cationic *trans*-Diaminedichloroplatinum(II) Complexes that Are Active against Cisplatin Resistant Ovarian Cancer Cell Lines

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Positively charged, water soluble *cis*/*trans*-[$PtCl_2$(piperazine)(Am1)] (where Am1 = $NH_3$, *n*-butylamine, isopropylamine, 4-picoline, piperidine, and piperazine) has significant cytotoxic activity against cisplatin resistant ovarian cancer cells. The charged complexes are taken up by cancer cells much more rapidly than cisplatin and bind to cellular DNA and to calf thymus DNA much faster than cisplatin or transplatin. The platinum−piperazine complexes bind proteins (ubiquitin and myoglobin) very slowly as compared to cisplatin and to their neutral piperidine analogues. Altogether, the results reported here suggest that combination of positively charged ligands with a *trans*-Pt(II)$Cl_2$ center may lead to the discovery of platinum complexes that are able to circumvent cisplatin resistance.

## Introduction

The discovery that cisplatin, but not *trans*-diaminedichloroplatinum(II) (transplatin; see Figure 1), is effective in the treatment of testicular, ovarian, head, and neck carcinomas prompted medicinal chemists to search for novel platinum-based drugs with improved therapeutic profiles.[1] Many compounds were prepared in attempts to overcome the limited solubility of the drug, its toxic side effects, its lack of activity against major forms of cancer (such as breast and colon cancers), and especially the inherent and acquired resistance to the drug.[2] The first comprehensive report on the structure−activity relationship (SAR) of platinum complexes directed the efforts of the medicinal chemists toward the design and preparation of neutral complexes with two inert ligands in the cis orientation and two semilabile leaving groups.[3]

In 1989, two exceptions to the classic SAR were reported proving that the electroneutrality of the complex and the cis orientation of the inert ligands are not a prerequisite for antitumor activity of divalent platinum complexes. Hollis et al. reported that positively charged triaminemonochloroplatinum(II) complexes of the general formula $[Pt(NH_3)_2(Am)Cl]^+$ (where Am = planar heterocyclic amine; see Figure 1d for one example) possess antitumor activity.[4,5] These complexes were neither neutral nor did they possess the classic diaminedichloro coordination sphere. Farrell et al. were the first to demonstrate that transplatin complexes could give rise to compounds that have significant cytotoxicity and thus paved the way for future studies on *trans*-Pt complexes as antitumor agents. They first reported that transplatin complexes with planar amine ligands (see Figure 1e for an example) have antitumor activity superior to transplatin especially in cisplatin resistant cell lines.[6] These were the first examples in a series of platinum-based drugs that defy the classic SAR. Since then, several "nonclassical" platinum complexes possessing antitumor activity have been reported.

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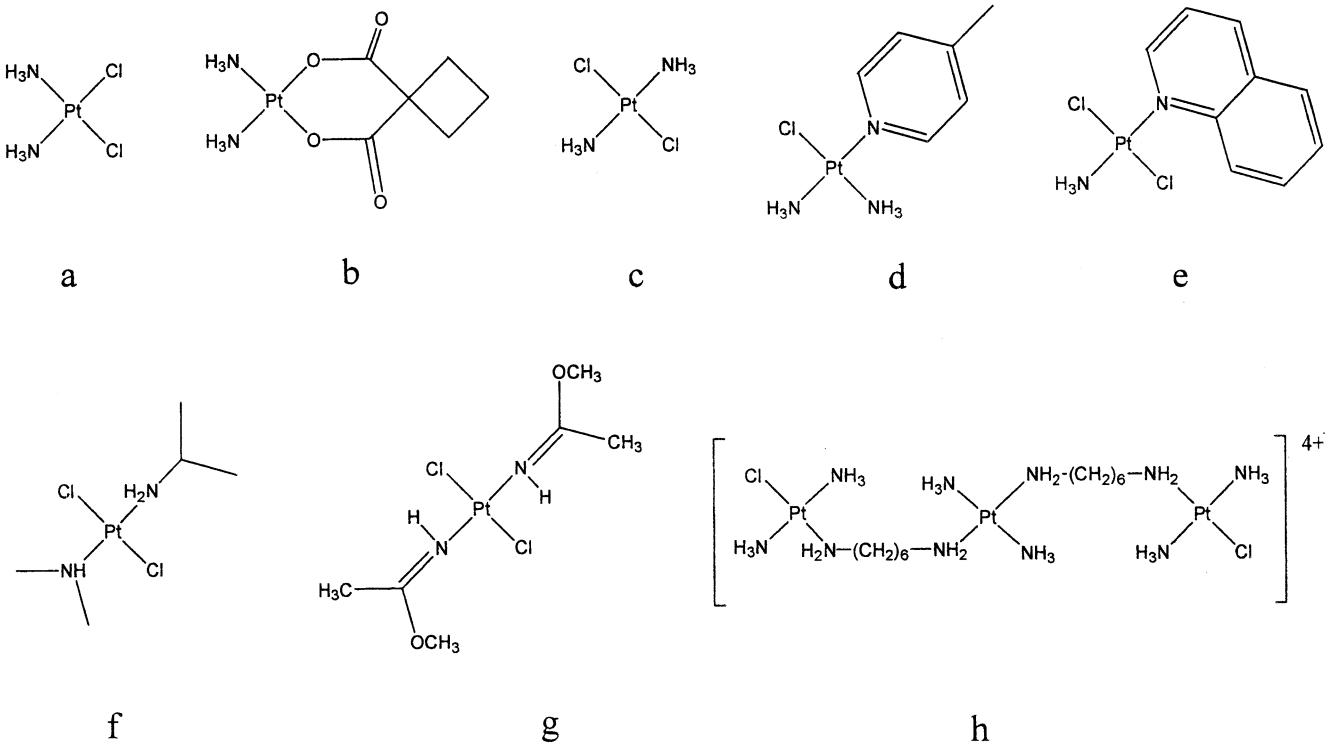



**Figure 1.** Platinum anticancer agents (a) cisplatin, (b) carboplatin, (c) transplatin (not active), (d) *cis*-$Pt(NH_3)_2$(4-pic)Cl, (e) *trans*-$PtCl_2(NH_3)$(quin), (f) *trans*-$PtCl_2$(IPA)(dimethylamine), (g) *trans*-$PtCl_2(EE)_2$, and (h) BBR3464.

Two such examples are the positively charged dinuclear and trinuclear platinum complexes (see Figure 1h), and the trans EE iminoether complexes (see Figure 1g).[7−9]

There are several classes of mononuclear transplatin complexes having antitumor activity: *trans*-$PtCl_2$ complexes with planar aromatic amine ligands, *trans*-$PtCl_2$ with mixed aliphatic amines, and *trans*-$PtCl_2(NH_3)$-(cyclohexylamine) and *trans*-$PtCl_2$ complexes with EE iminoether ligands.[10,11] The antitumor activity and biological and pharmacological properties of these complexes have been studied, and one interesting fact that emerges from these studies is that trans complexes often exhibit higher activity than their cis analogues in cisplatin resistant cell lines.[12] The reduced cross-resistance with cisplatin could be due to the fact that the spectrum of adducts that trans compounds form with cellular DNA is different than that formed by cisplatin.

The cationic charges of the platinum complexes prepared by Hollis and by Farrell resulted from the substitution of one of the anionic chloride ligands by a neutral ligand and the charges of the complexes reside on the metal center. These complexes do not have the "classic" diaminedichloro coordination sphere. In this paper, we describe the synthesis, characterization, cytotoxicity studies, preliminary DNA, and protein binding properties of a novel class of water soluble cationic transplatin complexes with piperazine (pz) ligands.

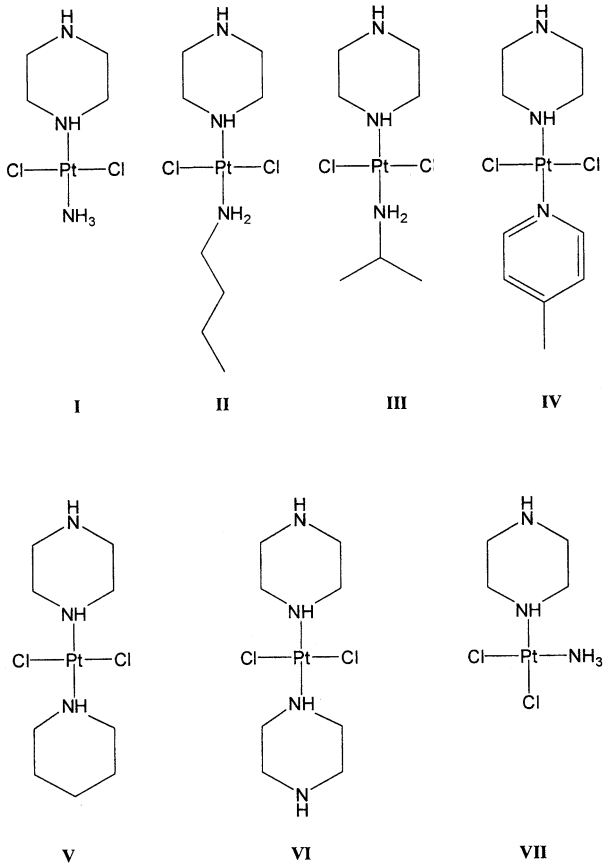


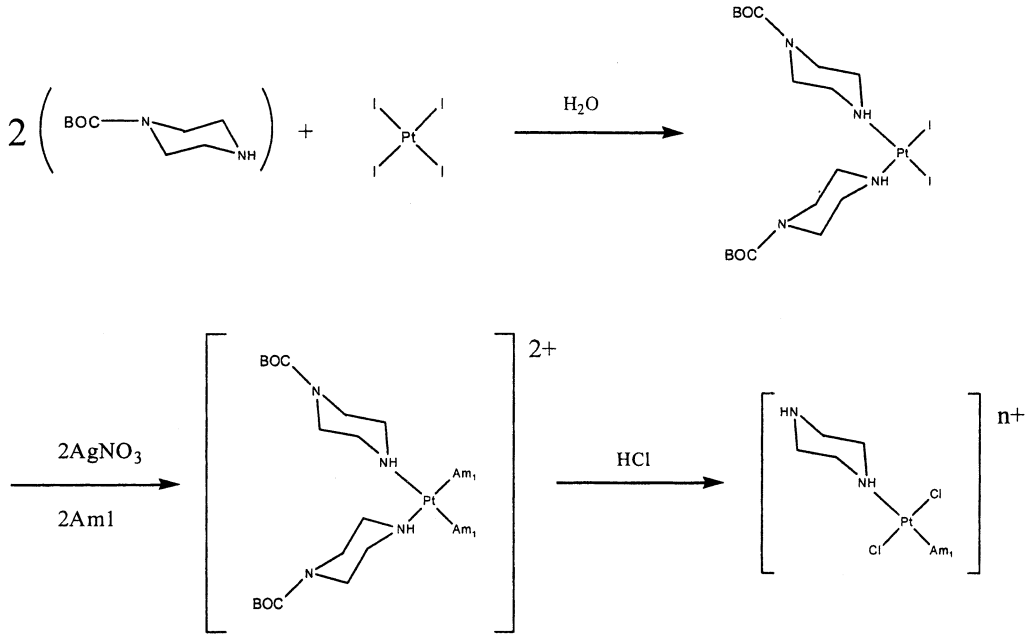

**Figure 2.** Pz complexes used in this study.

## Results

**Synthesis of the Platinum Complexes.** We prepared six *trans*-$[PtCl_2(pz)(Am1)]$ (where Am1 = $NH_3$, *n*-butylamine (NBA), isopropylamine (IPA), 4-picoline (4-pic), piperidine (pip), and pz) and for comparison *cis*-$[PtCl_2(pz)(NH_3)]$. The structures of these complexes are depicted in Figure 2.

Pz can act as a monodentate ligand, a chelating ligand, or a bridging ligand.[13] To ensure that the pz will

**Scheme 1**




act as a monodentate ligand, during the initial synthetic steps, we used pz where one of the amines was protected with the acid labile *tert*-butyloxycarbonyl (BOC). The trans complexes, both symmetric and nonsymmetric, were prepared by a modification of standard methods (see Scheme 1). Initially, the *cis*-$PtI_2$(BOC-pz)$_2$ was prepared and characterized by $^{195}$Pt NMR (dimethylformamide (DMF)) ($\delta = -3286$ ppm) and its purity was verified by elemental analysis. *cis*-$PtI_2$(BOC-pz)$_2$ was reacted in DMF with 2 equiv of $AgNO_3$ and with 2 equiv of Am1 (where Am1 = $NH_3$, NBA, IPA, 4-pic, pip, and BOC-pz) to yield *cis*-[Pt(BOC-pz)$_2$(Am1)$_2$]$^{2+}$ from which the [*trans*-$PtCl_2$(pz)(Am1)]$^{n+}$ ($n = 2$ for Am1 = BOC-pz and $n = 1$ for all the rest) was obtained following treatment with HCl. All of the compounds were synthesized in reasonable yield (70−85%) and were characterized by $^{195}$Pt NMR, and their purity was ascertained by elemental analysis.

The *cis*-$PtCl_2(NH_3)$(pz) was prepared by reacting [$Pt(NH_3)Cl_3$]$^-$ with 1 equiv of BOC-pz in a 1:1 mixture of acetone:water. After a 7 day reaction at room temperature, $^{195}$Pt NMR indicated the formation of the desired product ($\delta = -2187$ ppm) concurrent with the total disappearance of the starting material. The BOC was cleaved using HCl in ethanol, and the hydrochloride salt, *cis*-[$PtCl_2(NH_3)$(pz)]·HCl, precipitated and was collected by filtration.

**Solubility.** The low solubility of the neutral diaminedichloroplatinum(II) compounds that results in poor bioavailability was one of the reasons for the design and synthesis of the positively charged complexes of the general formula [$PtCl_2$(Am1)(pz)]·HCl. Compounds **I**−**VII** are significantly more soluble at neutral pH than their neutral counterparts having solubilities in the range of 20 mM as compared with 6.3 mM for cisplatin and 0.8 mM for transplatin.

**Cytotoxicity.** In contrast to the inactive *trans*-[$PtCl_2(NH_3)_2$], trans complexes where at least one of the amine ligands was substituted with bulky aromatic or aliphatic amines are endowed with antitumor properties especially against cisplatin resistant cell lines. Thus, we decided to test the cytotoxic activity of compounds **I**−**VII** against three pairs of cisplatin sensitive and resistant cancer cell lines (A2780/A2780cisR, 41M/41McisR, and CH1/CH1cisR), which have been described elsewhere.[14] These pairs of cell lines were selected on the basis of encompassing all of the known major mechanisms of resistance to cisplatin: 41McisR being resistant primarily through reduced drug transport,[15] CH1cisR through enhanced DNA repair/tolerance,[16] and A2780cisR through a combination of decreased uptake, enhanced DNA repair/tolerance, and elevated reduced glutathione (GSH) levels.[17]

The compounds were incubated for 24 h with the above-mentioned ovarian tumor cell lines, and the cell survival in compound-treated cultures was evaluated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) method as previously reported.[18] The results of the $IC_{50}$ studies are shown in Table 1.

Four of the pz complexes studied (**II**−**IV** and **VII**) are more potent than cisplatin against A2780cisR cells, and two more are only slightly less active than cisplatin. Compound **II** is also more potent than cisplatin against the other two resistant cell lines CH1cisR and 41McisR.

**DNA Binding.** The binding rates of cisplatin, *trans*-[$PtCl_2$(4-pic)(pz)]·HCl, and *trans*-[$PtCl_2$(4-pic)(pip)] with calf thymus DNA (CT DNA) were measured. Solutions of double-helical CT DNA at a concentration of 0.032 mg/mL were incubated with *trans*-[$PtCl_2$(4-pic)(pip)] and *trans*-[$PtCl_2$(4-pic)(pz)] complexes at the value of $r_i$ of 0.05 in 10 mM $NaClO_4$ at 37 °C ($r_i$ is defined as the molar ratio of free platinum complex to nucleotide phosphates at the onset of incubation with DNA). At various time intervals, an aliquot of the reaction mixture was withdrawn and assayed by differential pulse polarography (DPP) for platinum not bound to DNA. The amount of platinum bound to DNA ($r_b$) was calculated by subtracting the amount of free (unbound) platinum from the total amount of platinum present in the reaction. No changes in the pH of the reaction

**Table 1.** $IC_{50}$ Mean Values (± SD) Obtained for Compounds **I**−**IV**[a]

| | | A2780 | A2780cisR | CH1 | CH1cisR | 41M | 41McisR |
|---|---|---|---|---|---|---|---|
| **I** | *trans*-$PtCl_2(NH_3)(pz)$ | 5 ± 1 | 44 ± 4 (8.8) | 12 ± 3 | 34 ± 4 (2.8) | 52 ± 5 | 155 ± 12 (3.0) |
| $PtCl_2$ | *trans*-PtCl2(NBA)(pz) | 16 ± 2 | 28 ± 2 (1.8) | 17 ± 2 | 19 ± 3 (1.1) | 32 ± 5 | 48 ± 3 (1.5) |
| **III** | *trans*-$PtCl_2$(IPA)(pz) | 14 ± 1 | 30 ± 2 (2.1) | 10 ± 1 | 50 ± 3 (5.0) | 38 ± 3 | 122 ± 8 (3.2) |
| **IV** | *trans*-$PtCl_2$(4-pic)(pz) | 10 ± 3 | 24 ± 3 (2.4) | 16 ± 2 | 42 ± 3 (2.6) | 45 ± 3 | 147 ± 10 (3.3) |
| $PtCl_2$ | *trans*-PtCl2(pip)(pz) | 18 ± 2 | 64 ± 5 (3.6) | 22 ± 3 | 85 ± 7 (3.9) | 37 ± 4 | 118 ± 9 (3.2) |
| **VI** | *trans*-$PtCl_2$(pz)(pz) | 17 ± 3 | 43 ± 3 (2.5) | 26 ± 2 | 53 ± 3 (2.0) | 43 ± 3 | 153 ± 10 (3.6) |
| **VII** | *cis*-$PtCl_2(NH_3)(pz)$ | 10 ± 1 | 25 ± 2 (2.5) | 28 ± 2 | 56 ± 3 (2.0) | 46 ± 3 | 112 ± 12 (2.4) |
| | *trans*-$PtCl_2(NH_3)_2$ | >200 | >200 | >200 | >200 | >200 | >200 |
| | *cis*-$PtCl_2(NH_3)_2$ | 2.2 ± 0.6 | 38 ± 3 (17.3) | 6 ± 1 | 23 ± 3 (3.8) | 26 ± 2 | 107 ± 8 (4.1) |

[a] The numbers in parentheses are the RF ($IC_{50}$ resistant/$IC_{50}$ sensitive).

mixture containing DNA and platinum compounds were measured within 48 h after mixing DNA with the platinum complex. The amount of *trans*-[$PtCl_2$(4-pic)(pip)] or *trans*-[$PtCl_2$(4-pic)(pz)] bound to DNA increased with time. In these binding reactions, the time at which the binding reached 50% ($t_{50\%}$) was 260 and 12 min for *trans*-[$PtCl_2$(4-pic)(pip)] and *trans*-[$PtCl_2$(4-pic)(pz)], respectively. The value of $t_{50\%}$ for the reaction of cisplatin or transplatin with DNA under identical conditions was ~120 min. This comparison indicates that the binding of *trans*-[$PtCl_2$(4-pic)(pz)] to natural double-helical DNA is considerably faster than the binding of cisplatin or transplatin very likely due to the higher charge whereas the binding of the neutral *trans*-[$PtCl_2$(4-pic)(pip)] is slower. These results agree with the results of cellular uptake studies and cellular DNA binding in which the pz complexes showed higher cell uptake and higher DNA binding than cisplatin, transplatin, and the neutral pipridine analogue.[19]

**Pt−Protein Binding.** We conducted a mass spectrometric study in which we examined the reactivity of complexes **I**−**IV** toward two model proteins, ubiquitin ($M_W$ = 8565) and horse heart myoglobin ($M_W$ = 16 951). These proteins were chosen because both are well-characterized and have accessible platinum binding sites. The 1:1 reactions between the platinum complexes and the proteins were carried out at 1−2 mM concentrations, in 10 mM phosphate buffer, pH 6.4, at 37 °C. Protein binding profiles were measured directly on the reaction mixtures by electrospray ionization mass spectrometry (ESIMS). Unlike cisplatin that forms four different adducts with Ub, the pz complexes form one major adduct corresponding to monofunctional adduct PtCl(pz)(Am1)(Ub).

Rather than present the detailed binding kinetics of all of the pz complexes to the proteins, we chose to report a comparison between the binding profiles of a typical pz complex {*trans*-[$PtCl_2(NH_3)(pz)$]·HCl} with ubiquitin and the binding profiles of cisplatin, transplatin, and the uncharged analogue, *trans*-[$PtCl_2(NH_3)$(pip)]. These results are depicted in Figure 3. The charged pz complex clearly binds to ubiquitin and myoglobin (data not shown) more slowly and forms a lower percentage of adducts as compared with cisplatin, transplatin, and its neutral analogue *trans*-[$PtCl_2(NH_3)$(pip)].

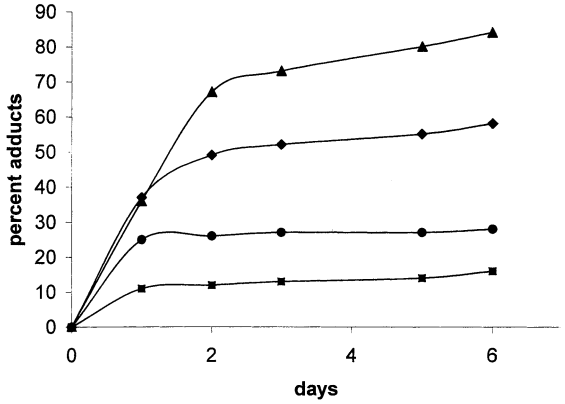



**Figure 3.** Binding curves of ubiquitin to (a) cisplatin (●), (b) transplatin (◆), (c) *trans*-[$PtCl_2(NH_3)$(pip)] (▲), and (d) *trans*-[$PtCl_2(NH_3)$(pz)]·HCl (■). Reactions were carried out at 1−2 mM concentrations, in 10 mM phosphate buffer, pH 6.4, 37 °C.

## Discussion

Our goal was to design and prepare water soluble platinum complexes able to react rapidly with DNA and capable of forming DNA adducts different from those formed by cisplatin and transplatin. Because the anticancer activity and DNA binding patterns of the different novel trans complexes are largely determined by the amine ligands, we set out to find an amine ligand that will address the points mentioned above. We chose the monodentate pz ligand for several reasons: (i) the positive charge would ensure adequate aqueous solubility and rapid interaction with the polyanionic DNA, (ii) it is a nonplanar heterocyclic amine ligand that is flexible and has a hydrogen bond donor that can interact with the DNA to hopefully form new types of lesions, and (iii) it is bulky enough to affect the kinetics and the cytotoxicity as do the planar ligands that were used by Farrell[20] or the 2-picoline in ZD0473 or the bulky aliphatic amines used by Navarro-Ranninger and Perez.[21] To the best of our knowledge, there are no reports on monodentate platinum−pz complexes possessing antitumor activity.

In terms of the SAR, replacing one or both ammine ligands of transplatin with pz markedly increases the antitumor activity relative to transplatin indicating that the positively charged nonplanar amine ligand (pz) can activate the trans geometry. The most striking feature of these cytotoxicity studies is that all of the complexes (except for compound **V**) are at least as active as cisplatin against the A2780cisR cell line that is resistant through decreased drug accumulation, enhanced DNA repair/tolerance, and elevated GSH levels. Especially noticeable are the very low resistance factors (RF) of compound **II** *trans*-[$PtCl_2$(NBA)(pz)]·HCl against all three cell lines (RF < 2) indicating efficient circumvention of cisplatin resistance. Asymmetric complexes are often more active than their symmetric counterparts. In this case, there seems to be no great difference between the activity of the nonsymmetric singly charged compounds **I**−**V** and the symmetric doubly charged

complex *trans*-$PtCl_2(pz)_2$ (**VI**). They have similar antitumor activity and similar RF.

While it is known that some of the newly reported trans complexes are active against cisplatin resistant cell lines, it is interesting to note that the *cis*-$PtCl_2(NH_3)(pz)$ complex (**VII**) is quite active against A2780cisR cells. Substituting one amine ligand of cisplatin with pz resulted in a 4-fold reduction of the cytotoxic activity relative to cisplatin in the cisplatin sensitive cell lines. Interestingly, however, compound **VII** is superior to cisplatin and to most of the trans complexes (**I**−**VI**) in the cisplatin resistant A2780cisR cell line. The antitumor activity of the *cis*-pz complex is reminiscent of the properties of *cis*-[$PtCl_2(NH_3)$(2-picoline)], ZD0473, which was specifically designed to have reduced reactivity toward biological thiols and thioethers (proteins and peptides). This is considered beneficial since reaction of antitumor platinum agents with sulfur-containing biological nucleophiles is believed to be the main source of acquired resistance and of the toxic side effects of the drugs. To our knowledge, compounds **I**−**VII** are the first examples of diaminedichloroplatinum(II) compounds with a monodentate pz ligand that possess antitumor activity against cisplatin sensitive and resistant ovarian cancer cell lines.

Because the cytotoxicity of platinum drugs stems from the adducts that they form with DNA, a reasonable working hypothesis that guided many medicinal chemists is that it is desirable to design and prepare compounds that react slowly with proteins and other biological nucleophiles yet react reasonably quickly with DNA. We studied the cellular uptake of the pz complexes and their interactions with DNA and with two model proteins and compared them, under the identical conditions, with the same properties of cisplatin, transplatin, and the neutral pip analogues. Although cytotoxicity is associated with formation of platinum−DNA adducts, it seems that for these complexes there is no direct correlation between the levels of DNA platination and the in vitro cytotoxicity. Comparison of the DNA platination levels in both cell lines shows that the efficiency of DNA platination follows the order *trans*-$PtCl_2$(4-pic)(pz) ≫ *trans*-$PtCl_2$(4-pic)(pip) > cisplatin, while the relative cytotoxicities of these complexes are cisplatin > *trans*-$PtCl_2$(4-pic)(pip) > *trans*-$PtCl_2$(4-pic)(pz). DNA platination is a necessary condition for cytotoxic activity of platinum complexes but not a sufficient one. We are currently studying the DNA adducts of the platinum complexes of pip and pz in order to examine their nature, assess their ability to block replication and transcription, see whether they are recognized by high mobility group (HMG1) domain proteins, and whether the adducts are repaired by nuclear excision repair (NER). Only after obtaining those results will a clearer picture emerge.

Pt−protein adducts are important in defining the therapeutic profiles of the platinum drugs, and medicinal chemists are trying to design Pt complexes that will not react rapidly with proteins. We have been carrying out systematic studies of binding of platinum complexes to proteins in order to try and understand the basic principles underlying these interactions. We have shown that ESIMS is very powerful in studying the binding of platinum complexes to proteins.[22,23] The results show that replacing the neutral nonplanar pip ligand with the positively charged monocoordinated pz ligand has significant effects on the protein binding properties of the Pt complexes. The pz complexes bind to the proteins significantly slower than their pip analogues. This could be due to electrostatic interactions with the protein surface that position the Pt moiety in an unfavorable position for covalent binding or due to the formation of hydrogen bonds with the protein surface resulting in the same effect.

We have begun in vivo toxicity and efficacy studies of some of these compounds and only upon obtaining the in vivo efficacy study results will we know whether the reactivity of the trans conpounds is a major issue in the in vivo studies.

## Conclusions

In summary, we have prepared novel, positively charged, water soluble diaminedichloroplatinum(II) compounds able to circumvent cisplatin resistance in ovarian cancer cell lines. A positively charged complex, where the charge resides on the ligand, has shown remarkable cell uptake and DNA binding kinetics both toward cellular DNA and toward CT DNA. The pz complexes bind ubiquitin and myoglobin very slowly. These favorable findings have prompted us to carry out a broader study of platinum−pz complexes in the hope of finding candidates with favorable therapeutic profiles and to pursue in vivo studies with these compounds.

## Experimental Section

**Materials.** Potassium tetrachloroplatinate ($K_2PtCl_4$), 4-pic, pip diethylammonium chloride, *tert*-butyl 1-pz carboxylate, *cis*- and *trans*-DDP, ubiquitin, and horse heart myoglobin were all purchased from Sigma-Aldrich Israel Ltd. and were used without further purification.

**$^{195}$Pt NMR Spectroscopy.** 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. The platinum chemical shifts were measured relative to the external reference signal of $K_2PtCl_4$, set at −1624 ppm. A line broadening of 300 Hz was normally applied, and data were processed using the VNMR software.

**Synthesis. *trans*-[$PtCl_2(NH_3)(pz)$]·HCl (I).** *cis*-Diaminedichloroplatinum(II) (300 mg, 1 mmol) was dissolved in 30 mL of DMF, and 2 equiv (372.52 mg, 2 mmol) of *tert*-butyl 1-pz carboxylate and 2 equiv (339.76 mg, 2 mmol) of $AgNO_3$ were added simultaneously with stirring. Stirring continued in the dark for 24 h at room temperature. The precipitate was filtered off through a Celite sinter glass, and the filtrate was evaporated to dryness under reduced pressure. The resulting gum was dissolved in 30 mL of doubly distilled water (DDW), 2 mL of concentrated HCl was added, and the reaction mixture was stirred at room temperature for 24 h. The colored precipitates were removed, and the solution was heated to 85−90 °C for 60 min. After it was cooled to room temperature, the reaction mixture was filtered and the filtrate was chilled to 0 °C for 72 h. The yellow precipitate was filtered and washed with 10 mL of ice-cold DDW and 30 mL of diethyl ether. After it was dried, the yellow product (300 mg) was characterized as the hydrochloride salt of the desired *trans*-ammine-pz-dichloroplatinum(II) (**I**) complex. Yield 74%. Anal. calcd for $C_4H_{14}Cl_3N_3Pt·H_2O$: C, 11.34%; H, 3.81%; N, 9.92%. Found: C, 11.27%; H, 3.56%; N, 9.86%. $^{195}$Pt NMR ($\delta$, $H_2O$): −2177 ppm.

**General Procedure for Preparing *trans*-[$PtCl_2$(Am1)(pz)]·HCl, Am1 = NBA, IPA, 4-pic, pip, and pz. Synthesis of the Intermediate *cis*-[$PtI_2$(*tert*-butyl 1-pz carboxylate)$_2$].** Potassium tetrachloroplatinate (1 g, 2.4 mmol) was

dissolved in 40 mL of DDW, and 8 equiv (3.2 g, 19.27 mmol) of KI was added. The mixture was stirred at room temperature for 15 min. Two equivalents (0.9 g, 4.8 mmol) of *tert*-butyl 1-pz carboxylate was added, and the mixture was vigorously stirred for 1 h at room temperature. Throughout this period of time, the desired diiododiamineplatinum(II) precipitated. The yellow precipitate was collected by filtration, washed with 50 mL of DDW, and dried by suction. Yield 89%; $^{195}$Pt NMR (DMF): −3264 ppm.

*cis*-[$PtI_2$(*tert*-butyl 1-pz carboxylate)$_2$] (411 mg, 0.5 mmol) was dissolved in the dark in 15 mL of DMF, and 2 equiv (169.88 mg, 1 mmol) of $AgNO_3$ was added simultaneously with 2 equiv of the corresponding amine (98.83 $\mu$L of NBA, 85.17 $\mu$L of IPA, 97.4 $\mu$L of 4-pic, 99 $\mu$L of pip, or 186 mg of *tert*-butyl 1-pz carboxylate). Stirring continued in the dark for 24 h at room temperature. The precipitate was filtered off through a Celite sinter glass. The filtrate was evaporated to dryness under reduced pressure. The resulting gum was dissolved in 30 mL of DDW, 2 mL of concentrated hydrochloric acid was added, and the reaction mixture was stirred at room temperature for 24 h. The colored precipitates were removed, and the solution was heated to 85−90 °C for 60 min. After it was cooled to room temperature, the reaction mixture was filtered and the filtrate was chilled to 0 °C for 24 h. The yellow precipitate was filtered and washed with 20 mL of ice cooled DDW and 30 mL of diethyether. After it was dried, the yellow products were characterized as the hydrochloride salts of the desired transplatin complex.

***trans*-[$PtCl_2$(NBA)(pz)]·HCl (II).** Yield 67%; $^{195}$Pt NMR ($H_2O$): −2221 ppm.

***trans*-[$PtCl_2$(IPA)(pz)]·HCl (III).** Yield 71%; $^{195}$Pt NMR ($H_2O$): −2226 ppm.

***trans*-[$PtCl_2$(4-pic)(pz)]·HCl (IV).** Yield 61.2%. Anal. calcd for $C_{10}H_{18}Cl_3N_3Pt$: C, 24.99%; H, 3.56%; N, 8.74%. Found: C, 25.06%; H, 3.82%; N, 8.55%; $^{195}$Pt NMR ($H_2O$): −2086 ppm.

***trans*-[$PtCl_2$(pip)(pz)]·HCl (V).** Yield 56%; $^{195}$Pt NMR ($H_2O$): −2230 ppm.

***trans*-[$PtCl_2$(pz)(pz)]2HCl (VI).** Yield 83%; $^{195}$Pt NMR ($H_2O$): −2238 ppm.

***cis*-[$PtCl_2(NH_3)$(pz)]·HCl (VII).** A 300 mg (0.45 mmol) amount of tetraphenylphosphonium trichloromonoammineplatinum(II) was dissolved in 10 mL of a 1:1 acetone/DDW mixture. To the orange-colored solution 1 equiv (77.53 mg, 0.45 mmol) of *tert*-butyl 1-pz carboxylate was added. The mixture was stirred in a closed vessel at room temperature for 7 days. After the solution was evaporated to dryness under reduced pressure, the yellow solid was taken in 10 mL of absolute ethanol, 0.5 mL of concentrated hydrochloric acid was added, and the mixture was allowed to stand for overnight. The yellow precipitate was collected by filtration and washed with 10 mL of ethanol. Yield 58%; $^{195}$Pt NMR ($H_2O$): −2187 ppm.

***trans*-[$PtCl_2(NH_3)$(pip)] (VIII).** The synthesis of this compound is described in ref 19. **Cytotoxicity Studies. Biological Reagents.** MTT was purchased from Sigma. The platinum compounds were dissolved in 10 mM $NaClO_4$ in distilled water. Stock solutions of the compounds at concentrations of 1 mg/mL were freshly prepared before use.

**Cell Lines and Culture Conditions.** Cultures of pairs of cisplatin sensitive and resistant ovarian cancer cell lines (A2780/A2780cisR, 41M/41McisR, and CH1/CH1cisR normal) have been described elsewhere.[14] These pairs of cell lines were selected on the basis of encompassing all of the known major mechanisms of resistance to cisplatin: 41McisR being resistant primarily through reduced drug transport,[15] CH1cisR through enhanced DNA repair/tolerance,[16] and A2780cisR through a combination of decreased uptake, enhanced DNA repair/tolerance, and elevated GSH levels.[17]

**Drugs Cytotoxicity.** Cell survival in compound-treated cultures was evaluated by the MTT method as previously reported.[18] Platinum compounds were added to 96 microwell plates containing the cell cultures at final concentrations between 0 and 200 $\mu$M. After 24 h, cell survival was evaluated by measuring the absorbance at 520 nm, using a Whittaker microplate reader 2001. $IC_{50}$ values (compound concentration that produces 50% of cell killing) were calculated from curves constructed by plotting cell survival (%) vs compound concentration ($\mu$M). Experiments were carried out in quadruplicate.

**Binding to CT DNA.** CT or plasmid DNAs were incubated with the platinum complex in 10 mM $NaClO_4$ at 37 °C in the dark. After 48 h, the samples of plasmid DNA were precipitated by ethanol and redissolved in the medium required for subsequent biochemical or biophysical analysis whereas the samples of CT DNA were exhaustively dialyzed against such a medium. An aliquot of these samples was used to determine the value of $r_b$ ($r_b$ is defined as the number of molecules of the platinum compound bound per nucleotide residue) by flameless atomic absorption spectrophotometry (FAAS) or DPP.[24]

**Protein Binding Studies.** Platination reactions were carried out at 1−2 mM concentrations, in 10 mM phosphate buffer, pH 6.4, at 37 °C. Excess platinum was removed by ultrafiltration using Microcon YM-3 centrifugal filter devices at 4 °C and 12 000 rpm, prior to all adduct reactivity studies. Protein binding profiles by ESIMS were measured directly on the reaction mixtures following ZipTip (C18, Millipore) treatment. ESIMS was measured on a ThermoQuest Finnigan LCQ-Duo in the positive ion mode. In most cases, elution was in a mixture of 49:49:2 water:methanol:acetic acid at a flow rate of 15 $\mu$L/min. Samples of the platination reactions and adduct reactivity studies were diluted by a 100 fold prior to ESIMS analysis. Data were processed using ThermoQuest Finnigan's Xcalibur Biomass Calculation and Deconvolution software. To observe the mass spectra of the proteins in their native folded state, elution was carried out in 1% acetic acid in water, at a flow rate of 15 $\mu$L/min.

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