Anticancer Agents

Structure and Unique Interactions with DNA of a Cationic *Trans*-Platinum Complex with the Nonplanar Bicyclic Piperidinopiperidine Ligand**

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The ability of square-planar platinum(II) complexes to covalently bind to cellular DNA and distort its structure has had an overwhelming impact on the lives of many cancer patients worldwide. The anticancer drug cisplatin (Figure 1a), exerts its cytotoxic effect by binding covalently to two adjacent guanine residues on the same DNA strand (1,2-GpG cross-link), and the ensuing distortion of the DNA, triggers cellular processes that lead to the death of the cancer cell.[1,2] The two crucial properties of cisplatin that make it an efficient anticancer agent are its inertness that enables it to survive the onslaught of the plethora of platinophiles in the extra- and intracellular fluids on the way to the DNA, and its ability to distort the DNA.[3,4] Cisplatin is an extremely effective anticancer agent, whose clinical success is marred by the ability of tumors to acquire resistance to the drug. One approach to try and overcome the acquired resistance to cisplatin was to prepare platinum(II) complexes having *trans* geometry that are incapable of binding two adjacent guanines on the same strand, so they end up forming other lesions with the DNA and distort it differently than cisplatin. Transplatin itself (Figure 1b) is not cytotoxic, yet, several classes of *trans*-platinum complexes with planar heterocyclic amine ligands, bulky aliphatic amine ligands or iminoether ligands, or nonplanar heterocyclic amine ligands have displayed a variety of DNA binding properties as well as impressive cytotoxic properties.[5–11]

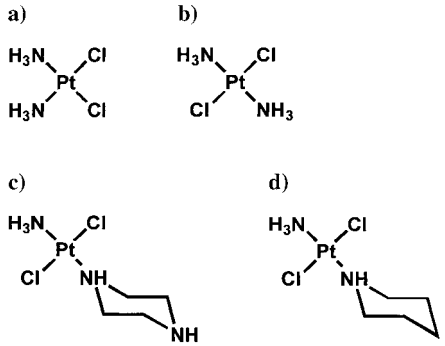

Figure 1. The structures of platinum complexes. a) cisplatin; b) transplatin; c) *trans*-$[PtCl_2(NH_3)(pz)]^+$; d) *trans*-$[PtCl_2(NH_3)(pip)]$.

We have recently reported on the preparation, cytotoxicity and the DNA-binding properties of *trans*-platinum complexes with piperazine (pz) and piperidine (pip) ligands (Figure 1c and d), which circumvent cisplatin resistance in human ovarian cancer cell lines.[12,13]

Piperazine was selected as a ligand because we wanted a soluble, cationic, *trans*-platinum complex that will not only covalently modify the DNA, but in addition, the ligand itself will interact with the DNA at a second site which is removed from the Pt^{II} modification site.

As a natural extension of this rationale, we have recently prepared the complex *trans*-$[PtCl_2(NH_3)(pip\text{-}pip)]\cdot HCl$ (**1**),[14] and now we report its X-ray crystal structure and some of its DNA-binding and pharmacological properties. X-ray quality crystals were obtained by slow evaporation from aqueous solution (Figure 2a).[15] The conformation of the two piperidine rings together with the molecular dimensions are depicted in Figure 2b. Structurally, we can divide the molecule into three parts: 1) the platinum(II) coordination sphere, 2) the first piperidine ring, and 3) the second piperidine ring. The platinum forms the usual square-planar coordination geometry with the Pt–Cl bond lengths around 2.30 Å and the Pt–N bonds around 2.06 Å.

In the solid state structure both piperidine rings adopt the chair conformation (Figure 2b). While the platinum can in

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Supporting information for this article (NMR spectra of **1**) is available on the WWW under http://www.angewandte.org or from the author.

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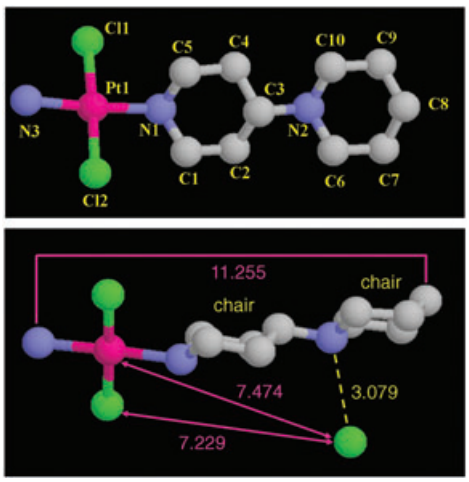

Figure 2. a) The X-ray crystal structure of **1**. b) A side view of the same structure showing that both piperidine rings are in the chair conformation and that N2 is hydrogen bonded to the chloride counterion (yellow dotted line). Depicted in pink are the distances between both ends of the complex (top) and between the platinum region and the hydrogen-bond acceptor (bottom).

principle, bind to either the equatorial or axial lone pair of N1, in this case the platinum is bound to the equatorial position, probably to minimize steric repulsions. N2 is protonated, conferring one positive charge to the complex, making it water soluble, and causing it to crystallize as a salt, with a Cl^- counterion (Figure 2b) that is hydrogen bonded to N2 (N···Cl distance of 3.079 Å). The positive charge is 6.44 Å from the metal center allowing the ligand to interact with hydrogen-bond acceptors at a distance of approximately 7 Å.

We performed an NMR spectroscopic analysis of this complex, and assigned the ^1H and ^{13}C resonance signals (see Supporting Information). The most interesting feature was the NOESY that appears in Figure 3. In phase sensitive NOESY, cross peaks that have the same phase as the diagonal peaks are not NOE[14] peaks but exchange peaks. In the spectrum of **1**, the phases of all the cross peaks of the first piperidine ring (protons 1–3) are opposite to those of the

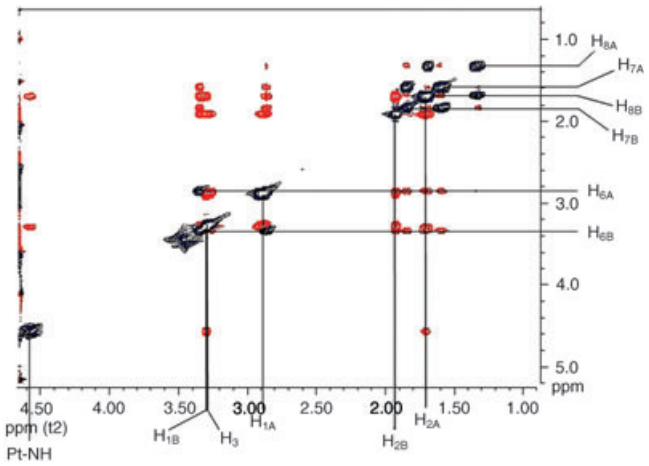

Figure 3. The NOESY spectrum of **1** showing negative contours in black and positive contours in red. For details see text.

diagonal peaks, while all the cross peaks originating from the second piperidine ring (protons 6–8) have the same phase as the diagonal peaks. This result indicates that the cross peaks generated by the protons of the second piperidine ring are exchange peaks and that the second ring is fluxional and its conformation is rapidly changing on the NMR time scale.

The $t_{1/2}$ of the covalent binding of cisplatin and transplatin to DNA is 120 min, and the rate-determining step is the aquation of the first chloride ligand to form the cationic $[PtCl(H_2O)(NH_3)_2]^+$.[16] The classic kinetic studies with square-planar Pt^{II} complexes teach that substitution reactions proceed by an associative mechanism involving a trigonal-bipyramidal intermediate, and that a dramatic decrease of substitution rate occurs for complexes with sterically hindered ligands.[17] Thus, it seems reasonable to expect that the steric hindrance of the bulky bicyclic pip-pip ligand will cause compound **1** to bind to DNA significantly slower than cisplatin or transplatin. The $t_{1/2}$ of the covalent binding of **1** to calf thymus DNA (CT-DNA) is 11 min,[18] indicating extremely rapid binding to DNA.

We studied the rate of conversion of *trans*-$[PtCl_2(NH_3)$-(pip-pip)$]^+$ into *trans*-$[PtCl(H_2O)(NH_3)$(pip-pip)$]^{2+}$ by UV spectroscopy and by ^{195}Pt NMR spectroscopy. The UV spectra of 0.1 mM of **1** (Figure 4) and of *trans*-$[PtCl(H_2O)(NH_3)$(pip-pip)$]^{2+}$ (Figure 4) were recorded and the value of A_{275}/A_{250} was determined (2.3 for **1** and 1.0 for *trans*-$[PtCl(H_2O)$-(NH_3)(pip-pip)$]^{2+}$). Over two hours at 37 °C, no change in the

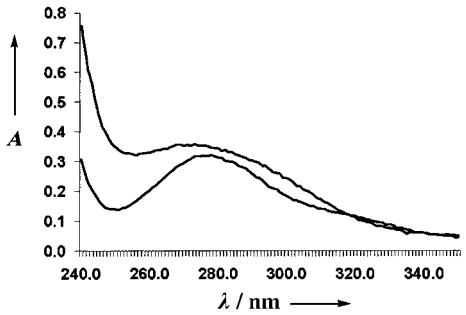

Figure 4. The UV spectra of **1** (lower trace) and *trans*-$[PtCl(H_2O)(NH_3)$-(pip-pip)$]^{2+}$ (upper trace).

UV spectrum of **1** could be detected indicating that no significant aquation occurred during this time. As an additional check, 5 mM aqueous solution of **1** was warmed to 37 °C in a 10-mm NMR tube and a spectrum was acquired every 5 min for 2 h. Over the two hour period the spectrum remained unchanged having a single resonance signal at $\delta = -2177$ ppm and no traces of *trans*-$[PtCl(H_2O)(NH_3)$(pip-pip)$]^{2+}$ at $\delta = -1895$ ppm. Thus, we think that the binding of **1** to the DNA occurs by an initial, rapid, electrostatically driven, "pre-association" of the complex with the double-stranded DNA (dsDNA) that positions the platinum center in the correct orientation for direct substitution of the Cl by the N7 of the purines. The $t_{1/2}$ values for the neutral *trans*-$[PtCl_2(NH_3)$(pip)$]$ (**2**) (113 min), and the cationic *trans*-$[PtCl_2(NH_3)$(pz)$]^+$ (**3**) (20 min), and *trans*-$[PtCl_2$(pz)(pip)$]^+$ (**4**) (22 min), coupled with the fact that no hydrolysis was observed in a 2 hour period for compounds **3** and **4**, support

the claim that electrostatics are the most important factor in determining the platination rate.

The *trans*-$[PtCl_2(pip)(pz)]^+$ ion can be considered the symmetric analogue of the *trans*-$[PtCl_2(NH_3)(pip\text{-}pip)]^+$ ion in the sense that they both have two nonplanar six membered rings and both have a cationic charge that is removed from the metal center. The nonsymmetric **1** binds DNA twice as fast as its symmetric analogue, *trans*-$[PtCl_2(pz)(pip)]^+$, and twice as fast as the less bulky cationic complex *trans*-$[PtCl_2(NH_3)(pz)]^+$. This situation may be due to either the location of the charge relative to the platinum center or to the flexibility of the second pip ring of **1**. Also, the binding of **1** stabilizes CT-DNA more than the symmetric analogue (raising the Tm by 14 °C for **1** and 7.9 °C for *trans*-$[PtCl_2(pz)(pip)]^+$ for $r_b = 0.05$; Tm is the melting temperature at which 50 % of the DNA double helix is denatured, r_b is the number of molecules of the platinum compound bound per nucleotide). Another feature of the unique interaction of **1** with the DNA is the 30° unwinding angle of dsDNA compared with 13° for cisplatin and 17° for the symmetric compound. The additional unwinding may be associated with the interaction of the pip-pip ligand with the duplex upon covalent binding of platinum.

The cytotoxicity of **1** was measured against three pairs of cisplatin sensitive and resistant cancer cell lines (A2780/A2780cisR, 41M/41McisR, and CH1/CH1cisR) that encompass all known resistance mechanisms to cisplatin.[19–22] Compound **1** is twice as potent as cisplatin in all three cisplatin-resistant cell lines (IC_{50} (compound concentration which induces 50 % cell death) values against A2780cisR, CH1cisR, and 41McisR are 24, 15, and 48 μM, respectively, for **1**, versus 38, 23, 107 μM for cisplatin) demonstrating the ability of this complex to circumvent all of the three known resistance mechanism to cisplatin. It is of note that the nonsymmetric 1 is 2–5 times more potent against the resistant cell lines than the symmetric analogue.

In summary, we described a new type of platinum complex that has a *trans* configuration, is asymmetric, has a singly charged nonplanar semifluxional bicyclic ligand, that binds extremely rapidly to DNA by direct substitution (not requiring initial aquation as does cisplatin), distorts DNA in a unique manner and circumvents all of the known cisplatin-resistance mechanism in human ovarian cancer cell lines.

Experimental Section

The compound **1** was prepared in a similar way to that described in ref. [11].

All NMR spectroscopy experiments were performed on an INOVA 500 MHz spectrometer using standard pulse sequences. The data were processed with VNMR or MestRe-C using cosine-squared apodization in both dimensions for the phase-sensitive experiment and unshifted sinbell for in both dimensions for the AV experiments.

The DNA binding studies have been performed as described.[12,13]

The cytotoxicities studies have been performed as previously described.[11]

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[14] Abbreviations: piperidinopiperidine (pip-pip), nuclear Overhauser effect (NOE).
[15] TEXSAN: Single Crystal Structure Analysis Software, Version 5.0, Molecular Structure Corp. The Woodlands, TX, **1989**. All crystallographic computing was done on a VAX 9000 computer at The Hebrew University of Jerusalem. CCDC-266822 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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