Platinum (II) Complexes with Stereochemically-defined Thiepane Dioxide Diamine Ligands as Anticancer Drugs

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Abstract. Platinum (II) complexes are accredited with biological activities. New complexes with thiepane dioxide diamine as ligands, characterized by defined stereochemical features, a flexible 7-membered thiepane moiety and by C₂ symmetry, were prepared. The complexes, related to the diamino cyclohexane family of platinum complexes, were soluble in dimethyl sulfoxide with the solvent substituting one chloride ion. These positively-charged complexes were tested against a human carcinoma cell line A431 and its cisplatin-resistant counterpart A431/Pt and were found to show: i) capability in bypassing cisplatin-resistance; ii) cytotoxicity comparable to that of oxaliplatin; iii) lower activity than cisplatin. In both cells lines, [PtCl(DACH)(DMSO)]⁺ was more cytotoxic than oxaliplatin. The best activity was shown by the platinum complexes with ligands which presented C₂ symmetry.

In the quest for cisplatin (CDDP) analogs with a better toxicity profile and an improved spectrum of activity, a large number of platinum (Pt) derivatives (several thousand) has been synthesized and investigated. The Pt complexes are widely used antitumor drugs. Furthermore, the relevance of CDDP was also recently verified in postoperative administration, since the rate of local and regional control was reported to increase significantly (1).

Among the Pt derivatives, the complexes containing the 1,2-diaminocyclohexane (DACH) ligand demonstrated anticancer activity in cell lines with acquired CDDP resistance (2) and the trans-1,2-diaminocyclohexane oxalate platinum (II) (oxaliplatin, L-OHP) appears to be the most effective (3). Since the molecule is chiral, the consensus is that the R,R isomer is generally more active than the S,S and R,S isomers (4). In particular, Kidani and co-workers suggested that the stereochemical conformation of the DACH ligand can affect interaction with DNA and cytotoxicity (5). Quite recently, similar conclusions were obtained after studying the effects of L-OHP on naked and intracellular DNA (6) and comparing molecular modeling of the intrastrand guanine-guanine DNA adducts produced by CDDP and L-OHP (7). This information underlines the particular interest of the DACH family of Pt compounds and suggests that other DACH-like Pt compounds could possess a new spectrum of activity. Hanessian and co-workers synthesized a series of analogs of L-OHP, either by adding DACH ligand stereochemically-defined hydroxy groups (8) or by substituting the DACH ligand with a 6-atom ring, such as the mono- and dihydroxy-diaminotetrahydropyran derivatives (9). The results they obtained indicated that the increased hydrophilicity of the Pt complexes improved their anticancer activity. Furthermore, the stereochemical disposition of the substituents on the ring seemed to affect the cytotoxic activity. The anticancer activity and the possibility of overcoming CDDP resistance with Pt complexes, with ligands having defined chiral centers in a larger and more flexible ring than DACH, were evaluated here. These ligands were characterized by two vicinal amino groups in a 7-atom ring derived from an oxidized thiepane with defined chiral centers. The synthesis of the complexes, their solution chemistry and in vitro cytotoxic activity in the A431 and its CDDP-resistant counterpart A431/Pt cell lines are reported.

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The synthesis of ligands and related platinum complexes.

Experimental protocols

A) Chemicals: Common chemical reagents were purchased from Sigma Chemical Co. (Milano, Italy); cisplatin (Platamine 10 mg) was a gift from Pharmacia & Upjohn S.p.A., Milano, Italy; oxaliplatin was provided by Dr. Martelli L. by Sanofi-Synthelabo S.p.A., Milano, Italy; 1R,2R(-)-1,2-diamino-2-chloroethane (DACH) was purchased from Aldrich. Fetal calf serum (FCS), L-glutamine, Trypsin-EDTA and RPMI-1640 medium were obtained from Bio Whittaker Italia S.r.l., Milano, Italy.

B) Physical measurements: Melting points were determined on a Büchi apparatus and are uncorrected. The 1H and 13C NMR spectra were recorded at 300 and 75 MHz respectively. The chemical shifts are in ppm downfield of tetramethylsilane (TMS) and signal multiplicities were established by DEPT experiments. Decoupling 1H NMR, if necessary, elucidated the signal assignments. Optical rotations were measured at 589 nm. Infrared spectra were recorded on a FT IR spectrophotometer. Mass spectra were recorded using electron impact at 70 eV, or electron spray ionization LRM (ESI). The analyses indicated by the symbols of elements were within ± 0.4% of the theoretical values.

C) Synthesis of ligands: (4S,5S)-4,5-diaminothiepane-1,1-dioxide (4) was synthesized using a previously reported procedure (14).

D) Synthesis of complexes: The new Pt complexes were synthesized using the procedure previously adopted by Hanessian and Wang (8). 

![Figure 1. Synthesis of ligands and related platinum complexes.](image-url)
were synthesized starting from 4 (14), 5 and 6, respectively, in 60-70% yield. The compounds were fully characterized by several spectroscopic techniques. The $^{13}$C NMR data in (DMSO-d$_6$) of Pt4, $\delta$ were: 62.7, 51.1, 25.6, of Pt5: $\delta$: 77.2, 67.0, 62.1, 58.9 and of Pt6: $\delta$: 78.3, 77.8, 68.5, 66.8, 61.9, 60.3, 58.9, 59.0. However, for the latter two compounds, after 2 h, a definitive spectrum showing a 2-fold number of signals was revealed, which was due to the substitution of one chlorine atom by DMSO-d$_6$.

(PtCl$_2$(DACH)) was prepared according to the procedure previously reported (8). Finally, all the cationic complexes were prepared from the PtdiamineCl$_2$ precursor by the same procedure: DMSO (0.25 mL) was added to PtdiamineCl$_2$ (2 mg) and the mixture was left to react overnight. After addition of 0.75 mL of saline, this solution was used to prepare, by serial dilution, the solution used for cytotoxicity.

**E) Cell culture:** For cytotoxicity studies, a human cervical squamous cell carcinoma cell line (A431) and its CDDP-resistant counterpart (A431/Pt) were used. These cells were a gift from Dr. P. Perego and PtCl$_2$(DACH) was prepared. All these cationic complexes, with a chloride ion substituted by DMSO molecule.


<table>
<thead>
<tr>
<th>Cell lines Pt4$^b$</th>
<th>Pt5$^b$</th>
<th>Pt6$^b$</th>
<th>(PtCl$_2$(DACH))$^b$</th>
<th>CDDP$^c$</th>
<th>L-OHP$^c$</th>
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<tbody>
<tr>
<td>A431</td>
<td>179.11±3.91</td>
<td>160.30±4.72</td>
<td>205.51±10.52</td>
<td>100.81±1.71</td>
<td>41.31±7.01</td>
</tr>
<tr>
<td>A431/Pt</td>
<td>167.11±1.41 (0.93)</td>
<td>188.22±2.12 (1.17)</td>
<td>240.71±9.80 (1.17)</td>
<td>108.41±4.11 (1.08)</td>
<td>114.51±15.0 (2.77)</td>
</tr>
</tbody>
</table>

$^a$IC$_{50}$ is defined as the concentration of drug required to inhibit cell growth by 50% compared to the control. The experiments were performed in triplicate. Values in parenthesis indicate the ratio of the IC$_{50}$ of the CDDP-resistant cell line and the IC$_{50}$ of the parental sensitive cell line (resistance factor-RF).

$^b$as complexes with chloride ion substituted by DMSO molecule.


**Results**

The effect of the aqueous solution containing 0.2% DMSO v/v on the cell cultures was initially examined. Under these conditions, the cell lines showed no statistically significant difference in doubling-time, morphology or viability, when compared to the control cultures. The *in vitro* cytotoxic properties of the complexes were evaluated against sensitive (A431) and CDDP-resistant (A431/Pt) human cervical carcinoma cell lines. The cytotoxicity of CDDP and L-OHP were also checked against the same cell lines (L. Martelli, data from Ph.D., thesis, to be published). The complexes were dissolved in DMSO and diluted by serial dilution in saline to the appropriate final concentration to be used in the biological assays. By this procedure, the ([PtCl(diamm)DMSO]$^+$ cation (diamm = 4, 5, 6 and DACH) was prepared. All these cationic complexes, with a chloride ion substituted by DMSO, are termed "complexes". The IC$_{50}$ values (mM) of the "complexes" compared with those of CDDP and L-OHP are summarized in Table I. The results indicated that all the complexes were able to circumvent CDDP resistance. Furthermore, they presented the same order of efficacy of L-OHP. When compared with CDDP, their cytotoxic activity was 2.5- to 5-fold reduced in the sensitive A431 cell line, while in the CDDP-resistant counterpart the cytotoxicity was reduced by a maximum of 2.5-fold. It is noteworthy that the DACH complex [PtdiamineCl$_2$(DMSO)]$^+$ was 1.8-fold more active than the neutral L-OHP in both cell lines. It would have been interesting to extend this comparison to Pt4, Pt5 and Pt6, but their insolubility in water prevented us from obtaining these results. Finally, in comparison with thiepane dioxide complexes, the DACH complex showed a better activity (1.5- to 2-fold).
Discussion

The in vitro cytotoxicity results (IC_{50}) indicated that the complexes were able to bypass CDDP resistance in the A431/Pt cell line. Furthermore, their cytotoxic activity in this line was comparable to that of L-OHP and was reduced to a maximum of 2-fold that of CDDP. In the sensitive A431 cell line, these complexes were 2.5- to 5-fold less active than CDDP, while they presented the same cytotoxicity as that of L-OHP. When comparing the IC_{50} of L-OHP with that of [PtCl(DACH)(DMSO)]^{+} in both the cell lines, it appears that the charged compound doubled its activity. It has been reported that the CDDP positively-charged aqueous species in human head and neck carcinoma UMSSC10b cells was characterized by doubled cytotoxicity and a 3.2-fold greater accumulation than the parental neutral species (21). Similarly, the total Pt levels on DNA were 1.9-fold higher when 2008 human ovarian carcinoma cells were exposed to pre-aquated CDDP in CT-deficient medium compared to CDDP in regular medium (22). These results, which derived from a direct comparison between the charged and the corresponding neutral complex, seemed to suggest a correlation among positive charge, accumulation and the cytotoxic activity of the drug. This observation has been confirmed in some previous reports. Interactions between phospholipids and CDDP are specific for negatively-charged phospholipids and take place at low chloride ion concentrations (23). This indicates that the aquated positively-charged CDDP was involved, for instance, with phosphatidic acid and phosphatidyl serine (23).

A great effort has been made to define the mechanisms by which CDDP crosses the plasma membrane (24). The available evidence shows that this drug enters cells by diffusion through the lipid bilayer, whereas carrier-mediated transport and endocytic mechanisms have not been confirmed (24, 25). The interactions with elements of the plasma membrane could favor the access of positively-charged Pt species over that of the neutral ones. Similar conclusions were obtained in multidrug-resistant systems. In this case, the observed passive diffusion process of drug accumulation seemed to be modulated by the protonated lipophilic drug to interact, distort and cross the lipid molecular packing (26). Studies on the effect of temperature, ATP depletion and sulfhydryl group blockade did not provide evidence for the uptake of aqueous CDDP species via a channel or a transporting agent (21). However, this cannot exclude the fact that substantially different mechanisms of cellular accumulation may operate for positively-charged and neutral species. Moreover, Pt drug-phospholipid interactions may have important consequences in cell processes, since negatively-charged phospholipids play crucial roles, for instance in signal transduction (27), cell proliferation and apoptosis (28).

Interesting information is obtained on comparing the relevance of the stereochemistry on the IC_{50} of the different complexes. The results obtained on the A431 and A431/Pt cell lines indicated that the best cytotoxic activity was shown in the presence of cyclic ligands with C_{2} symmetry. When this symmetry was not present, as in 6, the IC_{50} was at the highest value in both the cell lines. How this particular arrangement affects the pharmacological activity is not clear, but it is intriguing to note that the C_{2} symmetric cyclic structure of the thiepane dioxides is one of the requirements for their HIV-1 protease inhibition activity (11).

In conclusion, our study shows that the ligands with a bigger and more flexible ring than DACH do not improve the cytotoxicity of the Pt complexes in the sensitive and in the CDDP-resistant counterpart A431 cell lines. Nevertheless, the activity of the cationic species, obtained by the substitution of the chloride ion by a DMSO molecule in Pt dichloro complexes, was 2-fold higher than that of the neutral species. Positively-charged Pt complexes could promote possible interactions with the negatively-charged phospholipids of the membranes, either by favoring access of the charged species to the interior of the cell or by playing a crucial role in signal transduction and/or apoptosis. Moreover, the diamine thiepane dioxide Pt complexes, when acting as anticancer drugs, underline the importance of ligand C_{2} symmetry. This specific requirement could suggest that the enzymes are additional biomolecular targets for these Pt drugs. We are currently focusing on these features and testing the complexes in different cell lines.

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References


