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Cytotoxic Profile and Peculiar Reactivity with Biomolecules of a Novel "Rule-Breaker" Iodidoplatinum(II) Complex

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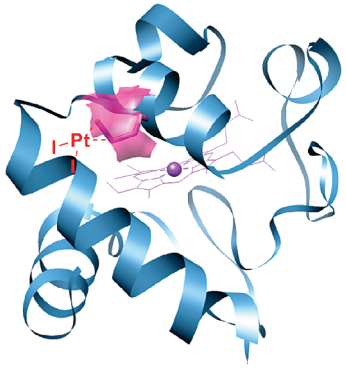
ABSTRACT Novel and surprising biological properties were disclosed for the platinum(II) complex *cis*-diiodidodiisopropylamineplatinum(II). Remarkably, this new platinum(II) complex manifests pronounced antiproliferative properties in vitro, in some cases superior to those of cisplatin. A peculiar reactivity with the model protein cytochrome *c* was indeed highlighted based on the loss of amine ligands and retention of iodides.

KEYWORDS Iodidoplatinum complex, cytotoxicity, DNA and protein interaction



Cisplatin (CDDP) has been the most active drug for the treatment of ovarian cancer for the last four decades, showing patients with platinum-resistant ovarian tumors very poor prognosis. Therefore, there is now great interest in the design of innovative platinum(II) compounds that might exhibit chemical and biological profiles substantially different from CDDP, displaying a wider spectrum of anticancer activities and overcoming platinum resistance. Much attention was devoted to platinum(II) compounds that contradict canonical structure−activity relationships (an active platinum antitumor complex should have square-planar geometry and should contain two labile leaving groups in cis and two inert amine ligands in the nonleaving group positions)[1,2] and yet show a favorable biological profile. Indeed, a number of "rule-breakers" platinum complexes, including polynuclear platinum compounds,[3] platinum(IV) complexes,[4,5] monofunctional platinum(II) complexes,[6,7] and Pt compounds with *trans* stereochemistry,[8−11] were developed in the past decade that turned out to display promising and unexpected biological and pharmacological activities.

Recent research work carried out in our laboratories is exploiting the synthesis of platinum(II) complexes where chlorido ligands are replaced by bulkier halides such as iodido. It was long believed that iodido analogues of classical anticancer platinum(II) compounds should be poor pharmacological agents, their inactivity being ascribed to the greater stability and lower reactivity of Pt−I bonds as compared to Pt−Cl bonds in aqueous solution.[12−14] Moreover, early studies on *cis*-[$PtI_2(NH_3)_2$] reported it to be inactive as an anticancer agent in an animal model.[15] These considerations greatly hampered the further development of iodidoplatinum complexes as experimental anticancer agents. However, a few subsequent studies highlighted a considerable and unexpected reactivity for iodido Pt(II) and Pt(IV) complexes[16] toward important biomolecular targets (e.g., serum albumin and glutathione).[17,18] These results have prompted us to reconsider in more depth iodidoplatinum complexes as a source of new anticancer agents. Within this frame, we report here on the antiproliferative activity of the platinum(II) complex *cis*-diiodido diisopropylamine platinum(II) **1** (Chart 1) and on its reactivity with representative biomolecules in vitro.

Complex **1** was prepared according to an established synthetic procedure, and the resulting product was characterized through standard techniques (see the Supporting Information). Afterward, the antiproliferative activity of **1** was evaluated against a panel of representative human tumor cell lines including A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon), using the SRB assay.[19] The experimental GI_{50} values are summarized in Table 1 and compared to those of CDDP after 48 h of treatment at 37 °C. Notably, for all of the selected cell lines, **1** was found to be more active than CDDP. The GI_{50} values were in the range 0.26−2.6 μM, with the ovarian cancer cell line A2780 being the most sensitive to the new compound. In this particular context, complex **1** was 5- and 11-fold more active than CDDP in the more resistant cancer cell lines T-47D and WiDr, respectively. These results may point to relevant differences in the respective mechanisms of action.

To shed some light on the reactivity and the mode of action of this promising diiodidoplatinum(II) complex, we investigated its interactions with a few representative

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Chart 1. Schematic Representation of the *cis*-Diiodidodiisopropylamineplatinum(II) **1** Studied in This Work

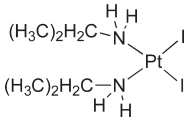

Table 1. Antiproliferative Activity (GI_{50}, μM) of **1** in Comparison to CDDP in Human Solid Tumor Cells[a]

cell line	1	CDDP
A2780	0.26 (±0.05)	1.9 (±0.6)
HBL-100	1.1 (±0.5)	1.9 (±0.2)
HeLa	1.9 (±0.1)	2.0 (±0.3)
SW1573	1.4 (±0.7)	3.4 (±0.7)
T-47D	2.6 (±0.8)	15 (±2.3)
WiDr	2.3 (±0.4)	26 (±5.3)

[a] Data were collected after 48 h of exposure to the drugs. Values are given in μM ± standard deviations and are means of 3−5 experiments.

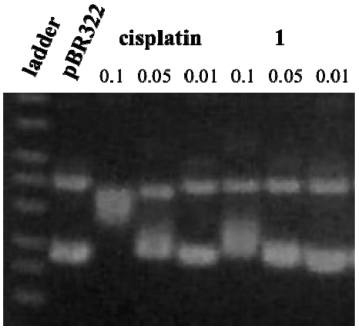

Figure 1. Gel electrophoresis of pBR322 plasmid DNA treated with different concentrations of **1** or CDDP (r = 0.1, 0.05, and 0.01; r = metal complex:DNA base pairs) after 24 h of incubation at 37 °C.

biomolecules such as plasmid DNA and cytochrome *c* (cyt *c*). The reactivity of **1** with pBR322 plasmid DNA was analyzed by gel electrophoresis according to established procedures, using CDDP as a reference.[20] Figure 1 shows the results obtained on pBR322 samples treated with increasing amounts of **1** (the applied metal/DNA base pair molar ratios were r = 0.1, 0.05, and 0.01) and incubated for 24 h at 37 °C. Notably, **1**, at variance with CDDP, altered the mobility of the supercoiled form of pBR322 only at the highest tested concentration (r = 0.1). This observation is suggestive of a reduced reactivity with DNA as compared to CDDP, indicating that nucleic acids are not the only or the primary pharmacological target for this platinum complex.

Afterward, we analyzed the interactions of **1** with cyt *c*, used here as a general model for globular proteins, relying on an established electrospray ionization−mass spectrometry (ESI-MS) approach.[21−27] Cyt *c* was incubated with a 3-fold molar excess of **1** in water (pH 5−6) or in 25 mM tetramethyl ammonium acetate buffer (pH 7.4), and the sample was analyzed by ESI-MS at different time intervals over 48 h at 37 °C. Representative ESI-MS spectra for **1** are shown in

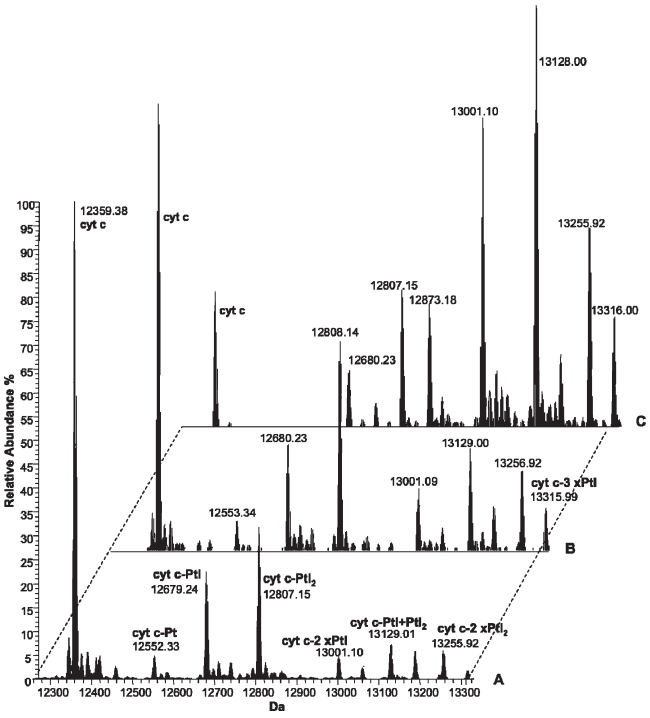

Figure 2. Deconvoluted ESI-MS of cyt *c* treated with **1** (metal:protein ratio = 3:1) in buffer TMeAmAc (pH 7.4) recorded after 1 (A), 6 (B), and 24 h (C) of incubation at 37 °C.

Table 2. Main Peaks Present in the Deconvoluted ESI Mass Spectra of **1**-Treated Cyt *c* in Buffer TMeAmAc (pH 7.4) Recorded at Different Times over 24 h of Incubation at 37 °C

Da	cyt *c*−metal adduct
12552	cyt *c*−Pt
12679	cyt *c*−PtI
12807	cyt *c*−PtI_2
13001	cyt *c*−2x[PtI]
13129	cyt *c*−[PtI] + [PtI_2]
13256	cyt *c*−2x[PtI_2]

Figure 2, while the overall peak assignment is shown in Table 2.

The formation of a number of metallodrug−protein adducts is already observed after 1 h of incubation (Figure 2, spectrum A). Remarkably and surprisingly, the resulting metal−protein adducts contain Pt or PtI or PtI_2 fragments, while complete loss of the amine ligands always occurs. Moreover, the presence of cyt *c* adducts with bound Pt(amine) fragment is not observed at all. Both mono and diplatinum cyt *c* adducts are formed bearing mixed PtI and PtI_2 fragments, indicating the presence of at least two Pt binding sites. Within monoplatinated adducts, the most abundant species is that containing a PtI_2 fragment; however, the species containing a PtI moiety is also detected in a quite appreciable amount. In Figure 3, the observed and theoretical spectra of 8^+ charged state are shown for the cyt *c*−PtI adduct. The obtained experimental data perfectly match theoretical expectations, therefore confirming our

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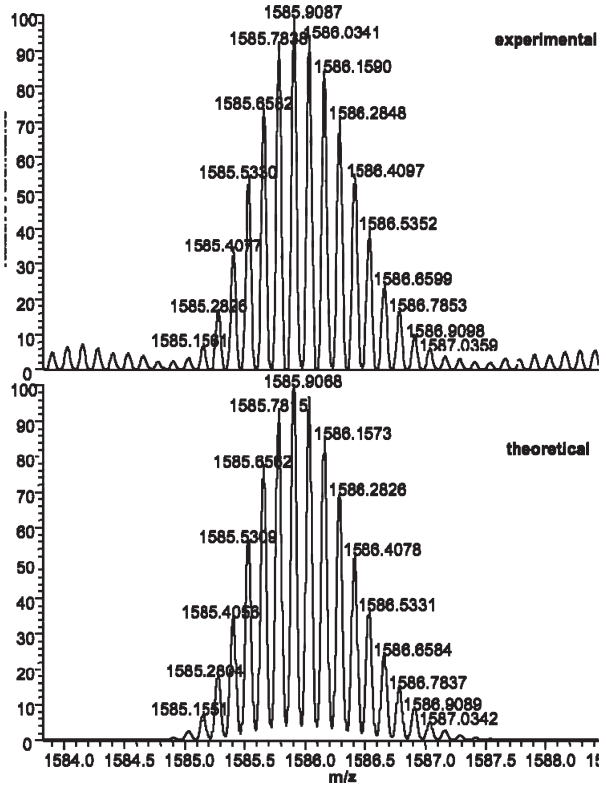

Figure 3. Comparison between the experimental and the theoretical spectra of 8^+ charge state of cyt *c* + $[PtI]^+$ fragment. Data were recorded with an Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA).

hypotheses on the chemical nature of the protein-bound fragment.

With time, the intensities of the peaks attributed to the diplatinated species increase progressively and become dominant over the monoplatinated ones. After 6 h (Figure 2, spectrum B), traces of triplatinated adducts of the type cyt *c*−$(PtI)_3$ were also observed. Overall, **1** showed a far higher reactivity than CDDP or carboplatin with cyt *c*,[27,28] and after 24 h, the platinated cyt *c* species dominated over the unbound protein in the ESI mass spectrum (Figure 2, spectrum C), with the relative intensities of the peaks remaining essentially the same thereafter.

In contrast, the reaction of the chlorido analogue of **1**, *cis*-dichloridodiisopropylamineplatinum(II), with cyt *c* resulted in the formation of platinum−cyt *c* adducts formally similar to those obtained in the case of CDDP,[27] that is, cyt *c*−$Pt(amine)_2$ adducts; however, this analogue showed a markedly reduced reactivity (see Figure S1 in the Supporting Information).

Thus, the above-reported ESI-MS results for the *cis* diiodidoPt complex **1** point out that the iodide ligands remain bound to the platinum center upon protein binding. Retention of idodido is a feature of great interest and novelty, in open contrast to common expectations.[29] Apparently, iodido produces a *trans* effect much greater than chlorido does, which strongly facilitates amine detachment. It follows that the classical reactivity pattern of PtL_2X_2 species is completely reversed upon Cl to I replacement. Labilization of the amine ligand is so relevant that cyt *c* platination afforded by **1** is far greater than cyt *c* platination produced by CDDP under similar conditions. These observations qualify **1** as an excellent example of a "rule-breaker" anticancer platinum complex.

Table 3. Main Peaks Present in the ESI Mass Spectrum of the N-ActMet Adducts with **1** (ipa = Isopropylamine)

m/z	N-ActMet−**1** adduct
385.018	N-ActMet−Pt
576.079	2x(N-ActMet)−Pt
703.992	2x(N-ActMet)−PtI
831.904	2x(N-ActMet)−PtI_2
890.978	2x(N-ActMet)−Pt(ipa)I_2

N-ActMet 192.069 Da

The unexpected reactivity of the *cis*-iodidoPt(II) complex with cyt *c*, leading to the release of the amine ligands with concomitant retention of the idodidos, was further investigated by NMR spectroscopy upon reacting **1** with *N*-acetyl-L-methionine (N-AcMet). Typically, N-AcMet has been used as a model for NMR studies of the interaction of metallodrugs with sulfur-containing amino acids.[30,31] When monitoring the reaction of **1** with an excess of N-AcMet, the starting complex is detected in solution without changes during the first few hours. After 6 h, an orange precipitate forms in the sample, therefore not allowing further NMR analysis at these conditions. The precipitate was recovered, dissolved in 1 % DMSO in water, and analyzed by NMR and ESI-MS. The obtained mass spectrum (Figure S2 in the Supporting Information) showed a series of peaks corresponding to adducts of **1** with N-AcMet that are assigned as reported in Table 3. Notably, metal−protein species containing PtI or PtI_2 fragments were observed in accordance with the above-mentioned cyt *c* adducts. Moreover, species in which one Pt is able to bridge two N-AcMet residues were also detected. These latter species are mainly due to the presence in solution of a single amino acid accessible for Pt binding and therefore more reactive than those buried in a folded protein. The NMR spectrum of the orange precipitate was studied in DMSO solution, and the coordination of the N-AcMet was assessed (experimental procedures in the Supporting Information). In particular, clear differences were observed in the ^1H and ^{13}C chemical shifts of the methyl group from the SCH_3 moiety, indicating platinum binding to the sulfur sites. The platinum shift at $\delta(^{195}Pt)$, −3752.8 ppm, is representative of sulfur coordination as well, and it shows a difference of 200 ppm as compared with the platinum complex **1**. Although real protein structural features cannot be mimicked by a single model amino acid, the general reactivity of **1** in the presence of sulfur binding sites could be assessed. The solvent does not seem to have relevance in this particular reactivity, as the interaction of the complex **1** with N-AcMet was performed using two different solvents, acetone and H_2O (1 % DMSO), with no changes in the nature of the final product.

The solution behavior of **1** was also studied by UV−visible spectrophotometry in aqueous solution. Representative spectra are reported in Figure 4. Two typical absorption

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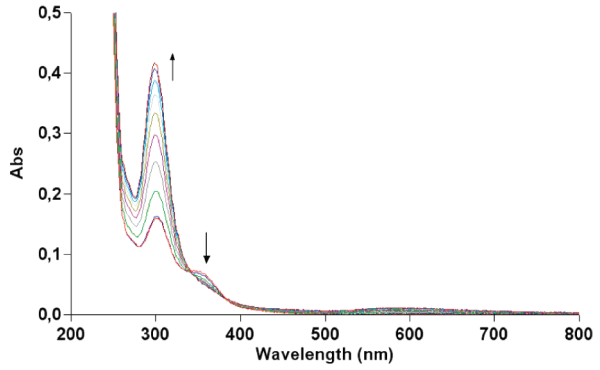

Figure 4. UV−visible spectra of **1** (10^{-4} M) in Milli-Q water recorded at different times over 24 h of incubation at 37 °C. The arrows indicate the evolution of the absorbance with time.

bands are observed in the spectra, which undergo major variations over 24 h at 37 °C: The absorption at 300 nm becomes more intense, while the absorption at 350 nm progressively decreases in intensity up to disappearance after 24 h. This spectral behavior suggests that progressive aquation of some of the Pt(II) ligands is taking place. NMR studies could not be performed in the same conditions because of the low NMR sensitivity at this concentration; therefore, more concentrated solutions of complex **1** (2.5 mM) in DMSO:H_2O (1:1) were analyzed over 24 h. Notably, after 1 h, free isopropylamine signals arise in the ^{1}H NMR spectra, which become important after 4 h, and minor speciation began to arise in the aliphatic region.

In conclusion, we have shown here that the novel diiodidoplatinum complex **1** manifests very important antiproliferative effects toward a number of human tumor cell lines. In spite of its lower reactivity with DNA, as compared to CDDP, it exhibits a greater and unconventional reactivity with cyt *c* characterized by the loss of amine ligands and retention of idodidos. In light of these results, we believe that it is worthwhile to expand the exploration of noncanonical iodidoplatinum(II) complexes as cytotoxic agents with an innovative mechanism of action.

SUPPORTING INFORMATION AVAILABLE

Experimental procedures, Figures S1 and S2, and references. This material is available free of charge via the Internet at http://pubs.acs.org.

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