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Structure–activity relationship of new *trans*-platinum(II) and (IV) complexes with cyclohexylamine. Interference with cell cycle progression and induction of cell death

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Abstract

The new *trans*-Pt complexes, derived from *trans*-[$PtCl_2$(amine)(dimethylamine)] and *trans*-[$PtCl_2(OH)_2$(amine)(dimethylamine)], were synthesized and characterized studying the structure–activity relationship and testing their antiproliferative activity. Their evaluation as cytotoxic agents towards different cancer and normal cell lines is presented. These compounds are active in a panel of tumor cell lines at low micromolar range. Compounds seems to be more active in tumoral than in normal primary human cell lines. Cytotoxic activity is closely related to the amine ligand. Cyclohexylamine ligand was the most active among the amine-ligands tested. Cytotoxic activity correlates with an increase in anexin V positive cells indicating an apoptotic effect of the compounds. Mechanistically, the antitumor activity correlates with a blockade of the cell cycle in S phase and a complete abolishment of G2/M checkpoint arrest suggesting physical interaction of compound with DNA inhibiting S phase transition.

Keywords: Antitumor drugs; Platinum anticancer drugs; Transplatin; Cytotoxic activity; Cell cycle

1. Introduction

The discovery by Rosenberg of the anticancer activity of cisplatin, *cis*-[$PtCl_2(NH_3)_2$], and *cis*-[$PtCl_4(NH_3)_2$] in the 1960s [1], precipitated the search for related complexes with similar or better activity [2–5]. It has been generally accepted as a paradigm of the biochemical pharmacology of platinum antitumor drugs that a *cis* configuration of the leaving groups is necessary for antitumor activity of platinum compounds [6]. However, cisplatin has two major drawbacks: severe toxicity that includes nephrotoxicity, neurotoxicity and ototoxicity, and the presence or acquisition of resistance to the drug [7]. Because enhanced removal of cisplatin–DNA adducts has been reported as one of main causes of cell resistance to cisplatin [8–10], there is general consensus that this particular resistance mechanism may be circumvented by platinum complexes that bind differently to DNA [11–13]. Certain *trans*-platinum complexes [14–21] exhibit similar or even higher antitumor activity than their *cis* counterparts [18–22]. We recently synthesized new *trans*-$PtCl_2$ complexes with an asymmetric set of aliphatic amines as non-leaving groups [23] and we reported that *trans*-[$PtCl_2$(dimethylamine)(isopropylamine)] circumvents cisplatin resistance in CH1cisR ovarian tumor cell lines endowed with mechanism of resistance due to enhanced DNA repair/tolerance [23]. Its corresponding Pt(IV) complex, *trans*-[$PtCl_2(OH)_2$(dimethylamine)(isopropylamine)] also is able to circumvent cisplatin resistance in the pairs of cell lines CH1-CH1cisR

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[23]. Besides, these compounds induce a higher apoptotic response than cisplatin in CH1cisR cells [23].

It is known that a simple modification in the structure of a certain compound could alter its DNA binding patterns, thus affecting its anticancer activity [5,24]. The cyclohexylamine ligand is a non-planar amine ligand that is flexible and bulky enough to affect the kinetics and cytotoxicity, as do the aliphatic amines used by us previously [23] or the non-planar heterocyclic amine ligands (4-picoline, piperidine and piperazine) that were used by Najajreh et al. [25]. On the other hand, Pt(II) complex: *cis*-[$PtCl_2(NH_3)$(cyclohexylamine)] is the major metabolite of JM-216 [26], [diacetatoamminedichloridocyclohexylamine-platinum(IV)], one of the few complexes to enter in clinical trials [27].

In order to broaden our knowledge on the cellular pharmacology of *trans*-platinum complexes we synthesized and characterized several *trans*-Pt(II), *trans*-[$PtCl_2$(dimethylamine)(amine′)], and *trans*-Pt(IV), *trans*-[$PtCl_2(OH)_2$(dimethylamine)(amine′)], where amine′ is cyclohexylamine or propylamine, with the aim of screening for potential antiproliferative and/or cytotoxic activities in different types of human cancer cells. The results obtained from cytotoxicity assays show that all these compounds were active in a panel of tumor cell lines at low micromolar range. Those compounds with cyclohexylamine as ligand showed higher antitumor activity. The cytotoxic activity of the new compounds has been compared in the case of CH1/CH1cisR cells lines with the activity of related Pt(II) and Pt(IV) complexes previously described [23].

It is well established that apoptosis is a mode of cell death believed to account for the most of the programmed cell death responsible for tissue modelling in vertebrate development [28,29]. It has been proposed that apoptosis might be an important and ubiquitous mode of cell death for cells treated with chemotherapeutic drugs [23]. Morphological changes associated with apoptosis include chromatin condensation, fragmentation of the nucleus and packaging of cell remnants into apoptotic bodies, which are then rapidly removed by macrophages. It is well known, on the other hand, that the success of platinum-based chemotherapy might be due in part to apoptosis induction. It has been found that cisplatin kills CH1 human ovarian carcinoma cells by apoptosis [30].

A better understanding of mechanism of drug-induced cytotoxicity is important since it would help in the design of more effective chemotherapeutic agents, and to study the mechanism through these compounds might be acting we analysed the status of the cell cycle on non-apoptotic cells treated with compounds.

These compounds induce cytotoxicity with a complete abolishment of G2/M checkpoint arrest and features of apoptosis such as an increased of anexin V positive cells.

We think that these results may lead to the discovery of more specific and less toxic drugs for cancer chemotherapy.

2. Experimental section

The infrared spectra were recorded in Nujol mulls and KBr pellets in the 4000–200 cm^{-1} range using a Perkin–Elmer model 283 spectrophotometer. NMR spectra were recorded on a Bruker AMX-3000 spectrometer. ^1H NMR spectra were determined with respect to TMS as internal standard. ^{195}Pt NMR spectra were calibrated using Na_2PtCl_6 as an external reference at 0 ppm. The C, H, and N analyses were carried out with a Perkin–Elmer 2400 microanalyzer and were within ±0.4% of calculated values. All solvents were purified by standard methods prior to use. Platinum(II) salts were a gift from Johnson Matthey. The amine ligands were purchased from Aldrich.

trans-[$PtCl_2$(cyclohexylamine)(dimethylamine)] **1**. A suspension of *cis*-[$PtCl_2$(dimethylamine)$_2$] (50 mg, 0.14 mmol) in water (1 ml) was treated with cyclohexylamine (0.064 ml, 0.562 mmol). The mixture was stirring at 70 °C over 1 h and the solution obtained was filtered, washed with water and dried under reduced pressure. The solid obtained was dissolved in water (1.5 ml), treated with hydrochloric acid (137 μl, 1.5 mmol) and heated at 70 °C overnight. After cooling in an ice bath, the product was collected by filtration and washed with water. Yield 65%. Anal. Calcd for $C_8H_{20}Cl_2N_2Pt$: C, 23.42; H, 4.91; N, 6.83. Found: C, 23.45; H, 4.87; N, 6.86. IR: ν(N–H) = 3220, 3131 cm^{-1}, ν(Pt–N) = 453 cm^{-1}, ν(Pt–Cl) = 334 cm^{-1}. ^{195}Pt NMR ($CDCl_3$): $\delta = -2204$ ppm.

trans-[$PtCl_2$(dimethylamine)(propylamine)] **2**. Compound **2** was synthesized as indicated for compound **1**. Yield 40%. Anal. Calcd for $C_5H_{16}Cl_2N_2Pt$: C, 16.26; H, 4.37; N, 7.59. Found: C, 16.22; H, 4.35; N, 7.57. IR: ν(N–H) = 3226, 3148 cm^{-1}, ν(Pt–N) = 465 cm^{-1}, ν(Pt–Cl) = 332 cm^{-1}. ^{195}Pt NMR ($CDCl_3$): $\delta = -2209$ ppm.

trans-[$PtCl_2(OH)_2$(cyclohexylamine)(dimethylamine)] **3**. Complex **1** (39 mg, 0.095 mmol) was suspended in water (0.8 ml) and hydrogen peroxide (35%, 0.121 ml) added. The mixture was stirred and heated at 70 °C during 4 h in the darkness. After cooling, the product was collected by filtration as a pale yellow solid, washed with cool water and chloroform, and dried under reduced pressure. Yield 40%. Anal. Calcd for $C_8H_{22}Cl_2N_2O_2Pt$: C, 21.63; H, 4.99; N, 6.30. Found: C, 21.67; H, 4.96; N, 6.24. IR: ν(O–H) = 3557 cm^{-1}, ν(Pt–OH) = 553, 536 cm^{-1}, ν(Pt–N) = 458 cm^{-1}, ν(Pt–Cl) = 347 cm^{-1}. ^{195}Pt NMR (CD_3OD): $\delta = 654.8$ ppm.

trans-[$PtCl_2(OH)_2$(dimethylamine)(propylamine)] **4**. Hydrogen peroxide solution (35%, 0.086 ml, 0.999 mmol) was added to a suspension of complex **2** (74 mg, 0.199 mmol) in water (1.5 ml). The mixture was stirred at 70 °C overnight in the dark. After cooling the solution was filtered and the mother liquor was evaporated under reduced pressure. The yellow precipitate was washed with ethyl ether. Yield 54%. Anal. Calcd for $C_5H_{18}Cl_2N_2O_2Pt$: C, 14.88; H, 4.50; N, 6.94. Found: C, 14.89; H, 4.45; N, 6.84. IR: ν(O–H) = 3554 cm^{-1}, ν(N–H) = 3282 cm^{-1},