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Preparation, characterization and cytotoxic activity of new compounds *trans*-[$PtCl_2NH_3$(3-(hydroxymethyl)-pyridine)] and *trans*-[$PtCl_2NH_3$(4-(hydroxymethyl)-pyridine)]

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Abstract

We report the synthesis, characterization and cytotoxic assays of new *trans*-platinum compounds, *trans*-[$PtCl_2NH_3$(3-(hydroxymethyl)-pyridine)] and *trans*-[$PtCl_2NH_3$(4-(hydroxymethyl)-pyridine)]. In the present work, we found that the replacement of the ammine ligand in ''classical'' transplatinum with the two new ligands does not increase the cytotoxic activity, maybe because these complexes do not produce a stability of the intrastrand cross-links in DNA.

Keywords: *Trans*-platinum complexes; Cytotoxic activity; NMR; (hydroxymethyl)-Pyridine

1. Introduction

Since the discovery of the anticancer properties of cisplatin, *cis*-diamminedichloroplatinum (II), it has been generally accepted, via structure–activity relationships, that the *cis* configuration of the two leaving groups is essential for antitumor activity of Pt(II) compounds [1,2]. However, this antitumor agent exhibits some important dose-limiting toxicities, mainly nephrotoxicity, neurotoxicity, and cytotoxicity [3]. Acquired resistance to cisplatin is also a problem. Current platinum based drug research is moving towards the development of new agents which can overcome the problem of acquired resistance to cisplatin in order to be active against a wider range of cancer types [4,5]. For decades, platinum complexes with *trans* geometry were not considered as potential drugs because transplatin is a non-active isomer. While it is generally thought that the presence of two good leaving groups in the *cis* configuration is necessary for antitumor activity in Pt(II) complexes, several exceptions have recently been reported [6,7]. These exceptions are the following: (i) *trans*-$PtCl_2(L)(L')$ complexes with planar heterocyclic ligands as inert group [6], (ii) polynuclear platinum complexes with bridging diamine linkers of the composition [{*trans*-$PtCl(NH_3)_2\}_2\{\mu$-$NH_2(CH_2)_n$-$NH_2\}]^{2+}$ ($n = 2$–6) [8], (iii) *trans*-$PtCl_2$ complexes with an imino ether as the inert group [7,9] and (iv) *trans* amino complexes like *trans*-[$PtCl_2$(dimethylamine)(isopropylamine)] are able to circumvent *cis*-DDP resistance in tumor cells transformed by *ras* oncogenes (Pam212-*ras*), as well as to kill these cells by apoptosis [10]. These results motivated us to search new *trans* platinum complexes by combining aliphatic, aromatic amines and the NH_3. In this paper, we report the synthesis, characterization and cytotoxic studies in cisplatin resistant cells of *trans*-[$PtCl_2(NH_3)$(L)] complexes, where L is 3-(hydroxymethyl)-pyridine and 4-(hydroxymethyl)-pyridine.

2. Results and discussion

2.1. Synthesis and characterization of the new trans complexes

The reaction between *cis*-[$PtCl_2(NH_3)_2$] and an excess amount of the ligands 3-(hydroxymethyl)-pyridine and

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Table 1
IC_{50} mean values obtained for complexes **1**, **2**, *cis*- and *trans*-DDP against A2780, A2780cisR, CH1 and CH1cisR cell lines for a drug-treatment period of 24 h

	A2780	A2780cisR	CH1	CH1cisR
Complex **1**	>100	>100	>100	>100
Complex **2**	>100	>100	>100	84 ± 3
cis-DDP	3.6 ± 0.4	58 ± 4	6 ± 1	23 ± 3
trans-DDP	110 ± 8	>300	>300	>300

4-(hydroxymethyl)-pyridine, respectively, in water at 85 °C, affords the tetramine complex. Subsequent addition of hydrochloric acid afforded the corresponding *trans* complexes [11]. Those complexes were characterized by elemental analyses, infrared spectra, and ^{1}H, ^{13}C and ^{195}Pt NMR spectra in D_2O as solvent. The microanalytical data are consistent with the empirical formula $C_6H_{10}ON_2PtCl_2$. The assignment of the *trans* geometry is supported by IR spectral data (a single band at 338 and 339 cm^{-1} in compounds with 3-(hydroxymethyl)-pyridine and 4-(hydroxymethyl)-pyridine, respectively) which suggest *trans*-chloride ligands [12]. The IR spectra also show bands at 423 and 440 cm^{-1}, respectively, assigned to *v* asymmetric Pt–N.

The ^{1}H and ^{13}C NMR data confirm the structures of the complexes proposed by IR data. The interchange of the N–H signal for the ammonia group should be expected in D_2O solvent, but interestingly the proton signal corresponding to the ammonia group is anyway observed at 3.83 ppm in D_2O for both compounds.

The ^{13}C NMR data for 3-(hydroxymethyl)-pyridine consists of six different signals while four signals are observed for the 4-(hydroxymethyl)-pyridine complex spectra. These differences in the NMR spectra are due to the presence of an AA′BB′ pattern in the 4-(hydroxymethyl)-pyridine ligand while the 3-(hydroxymethyl)-pyridine ligand does not present this pattern.

2.2. Cytotoxic activity of the synthesized trans-Pt(II) compounds

We have tested the cytotoxic activity of *trans*-[$PtCl_2NH_3$(3-(hydroxymethyl)-pyridine)] (**1**) and *trans*-[$PtCl_2NH_3$(4-(hydroxymethyl)-pyridine)] (**2**), *cis*- and *trans*-DDP against the two cell lines A2780/A2780cisR and CH1/CH1cisR after a treatment period of 24 h. Table 1 shows that the new complexes in these cell lines do not show cytotoxic activity. Only the *trans*-[$PtCl_2NH_3$(4-(hydroxymethyl)-pyridine)] (complex **2**) has a IC_{50} value lower than 100 μM.

3. Conclusions

It has been demonstrated that transplatin does not form double-helical DNA stable intrastrand cross-links and this property of transplatin was also related to its clinical inefficiency [13]. In the present work it is shown that the replacement of the ammine ligand in "classical" transplatinum by 3-(hydroxymethyl)-pyridine or 4-(hydroxymethyl)-pyridine does not increase the cytotoxicity activity. The results offer additional information for strategies based on the activation of *trans* geometry in order to circumvent the resistance to cisplatin using chemical modification of the transplatinum structure. But not all modification in transplatin structures are useful, as these complexes do not produce an increase in the cytotoxic activity.

4. Experimental

Infrared spectra were recorded in Nujol mulls on CsI windows and KBr pellets in the 4000–200 cm^{-1} range with a Perkin–Elmer Model 283 Spectrophotometer. NMR spectra were recorded on a Bruker AMX-300 (300 MHz) spectrometer in D_2O solution. Elemental analysis was performed on a Perkin–Elmer 2400 Series II microanalyzer.

4.1. Synthesis and characterization

The complex *cis*-[$PtCl_2(NH_3)_2$] was obtained by the Kauffman's method with variations [14]. A suspension of *cis*-[$PtCl_2(NH_3)_2$] (0.498 mmol, 150 mg) in water (3 ml) was treated with four equivalent of 3-(hydroxymethyl)-pyridine (1.99 mmol, 217 mg) or two equivalent of 4-(hydroxymethyl)-pyridine (0.996 mmol, 108 mg). The mixture was stirred and heated at 85 °C during 48 h. A clear pale yellow solution was obtained. After the solution was allowed to cool at room temperature, hydrochloric acid (12 M, 0.5 ml) was added and the solution heated to reflux for 48 h at 85 °C. After cooling at room temperature, the water was removed under reduced pressure. EtOH was added and the *trans*-[$PtCl_2(NH_3)$(L)] was dissolved. The solution was filtered to remove the insoluble impurities. The solvent is removed under reduced pressure and hot water was added. A yellow solid was isolated cooling down the water solution.

4.1.1. Trans-[$PtCl_2(NH_3)$(3-(hydroxymethyl)-pyridine)] (**1**)

Yield: 34%. *v*(Pt–Cl): 338 cm^{-1}, FAB-MS 392.15 *Anal*. Calc. for $C_6H_{10}ON_2PtCl_2$: C, 18.37; H, 2.57; N, 7.14. Found: C, 18.63; H, 2.73; N, 7.19%. ^{1}H NMR (300 MHz, D_2O, 25 °C) (ppm): 3.84 bs 3H (NH_3), 4.68 s 2H (CH_2OH), 7.39 m 1H (*meta*), 7.87 m 1H (*para*), 8.65 m 2H (*ortho*). ^{13}C NMR (300 MHz, D_2O, 25 °C) (ppm): 151.26 (C5), 150.77 (C4), 138.23 (C3), 137.29 (C2), 125.33 (C6), 59.82 (CH_2OH). ^{195}Pt NMR (300 MHz, D_2O, 25 °C) (ppm): −2028.5.

*4.1.2. Trans-[$PtCl_2(NH_3)$(4-(hydroxymethyl)-pyridine)] (**2**)*

Yield: 30%. *v*(Pt–Cl): 339 cm^{-1}, FAB-MS 392.15, *Anal*. Calc. for $C_6H_{10}ON_2PtCl_2$: C, 18.37; H, 2.57; N, 7.14. Found: C, 18.81; H, 2.06; N, 7.25%. ^1H NMR (300 MHz, D_2O, 25 °C) (ppm): 3.83 bs 3H (NH_3), 4.73 s 2H (CH_2OH), 7.44 d 2H (*meta*), 8.61 d 2H (*ortho*). ^{13}C NMR (300 MHz, D_2O, 25 °C) (ppm): 151.87 (C3 and C5), 153.19 (C4), 122.62 (C2 and C6), 60.88 (CH_2OH). ^{195}Pt NMR (300 MHz, D_2O, 25 °C) (ppm): −2028.

4.2. Biochemical probes

4.2.1. Biological reagents and drugs

Culture (100 mm) and micro well plates were obtained from Nunclon (Roskilde, Denmark). MTT was purchased from Sigma Co., FCS was supplied by Gibco-BRL, and ethanol was obtained from Merck. *Cis*-diamminedichloroplatinum (II), cisplatin or *cis*-DDP, was synthesized from K_2PtCl_4 supplied by Johnson Matthey. *cis*-DDP was dissolved in water. The *trans* platinum complexes were dissolved in water as 1 mg/ml solutions. These solutions were freshly prepared before use.

4.2.2. Cell lines and culture conditions

The pair of human ovarian tumor cell lines (A2780/A2780cisR) and human ovarian carcinoma cell lines (CH1/CH1cisR) were cultured in Dulbecco's modified Eagle's Medium (DMEM), supplemented with 10% FCS (foetal calf serum), 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in an atmosphere of 95% air and 5% CO_2.

4.2.3. Drugs cytotoxicity

Cell death was evaluated by using a system based on the tetrazolium compound MTT which is reduced by living cells to yield a soluble formazan product that can be detected colorimetrically [15]. Cells were plated in 96-well sterile plates at a density of 10^4 cells/well in 100 μl of medium and were incubated for 3–4 h. The compounds were added to final concentrations from 0 to 200 μM in a volume of 100 μl/well. Twenty-four hours later, 50 μl of a freshly diluted MTT solution (1/5 in culture medium) to a concentration of 1 mg/ml were pipetted into each well and the plate was incubated for 5 h at 37 °C in a humidified 5% CO_2 atmosphere. After the specified periods, the cell viability was evaluated by measurement of the absorbance at 520 nm, using a Whittaker Microplate Reader 2001. IC_{50} values (compound concentration that produces 50% of cell growth inhibition) were calculated from curves constructed by plotting (%) cell survival versus drug concentration (μM). All experiments were made in quadruplicate.

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