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Synthesis and antitumor activity of a series of (aminoethylpyrrolidine) platinum complexes*

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Summary. A series of aminoethylpyrrolidine-platinum complexes were synthesized and partially characterized for chemical structure. The leaving groups in this series of complexes were varied in an attempt to identify cytotoxic, water-soluble aminoethylpyrrolidine-platinum complexes. The cytotoxic activity was tested in vitro against L1210 cells sensitive to *cis*-diamminedichloroplatinum(II) (L1210/0), L1210 cells resistant to *cis*-diamminedichloroplatinum(II) (L1210/DDP), and L1210 cells resistant to 1,2-diaminocyclohexane platinum (L1210/DACH). The complexes were also tested for in vivo antitumor activity against L1210/0 cells injected i.p. The results of these studies indicate that the aminoethylpyrrolidine-platinum complexes have moderate antitumor activity but are cross-resistant in L1210/DDP cells. The degree of antitumor activity was dependent on the characteristic leaving group of a given complex.

Introduction

Since the original observation in 1969 that *cis*-diamminedichloroplatinum(II), or cisplatin, had significant antitumor activity [9], its role in the treatment of selected human tumors has markedly grown. Indeed, cisplatin has had dramatic effects on testicular, ovarian, and head and neck tumors [2, 5]. Unfortunately, it has been shown to have a variety of significant host toxicities, including myelosuppression, nephrotoxicity, neurotoxicity, ototoxicity, nausea, and vomiting [7, 10]. Because of these toxicities and a relatively narrow spectrum of sensitive tumor types, many attempts have been made to synthesize other platinum-based complexes in hopes of improving the therapeutic benefits of such complexes.

The vast majority of such synthesized complexes consist of symmetrical primary, secondary, or tertiary amine substituents as the inert ligand. In our search for novel platinum-based anticancer agents, we recently prepared and tested a series of nonsymmetrical heterocyclic systems for antitumor activity, using aminoethylpyrrolidine (AEP) as the inert ligand. The synthesis, chemistry, and biological activity of these complexes are discussed.

Materials and methods

Aminoethylpyrrolidine (AEP) and all anionic ligands were purchased from Aldrich Chemical Co., Milwaukee, Wis. K_2PtCl_4 was obtained from Matthey Bishop, Inc. The basic procedure used for the synthesis of aminoethylpyrrolidine-platinum(II) complexes is described in the scheme below.

$$\begin{aligned} &AEP + K_2PtCl_4 &&\rightarrow (AEP)PtCl_2 + 2KCl \\ &(AEP)PtCl_2 + Ag_2SO_4 &&\rightarrow (AEP)PtSO_4 + 2AgCl \\ &(AEP)PtSO_4 + BaX &&\rightarrow (AEP)PtX + BaSO_4 \end{aligned}$$

Briefly, a solution of K_2PtCl_4 was mixed with an equimolar amount of AEP in water and allowed to react at room temperature for 6–8 h with constant stirring. The water-insoluble $(AEP)PtCl_2$ was collected by filtration and washed successively with water, ethanol, and acetone. After drying in vacuo, the $(AEP)PtCl_2$ was stirred at room temperature with an equimolar amount of Ag_2SO_4 in water for 24 h in a dark environment. The water-soluble $(AEP)PtSO_4$ was separated from the AgCl precipitate by filtration and the filtrate was evaporated to dryness using a rotary evaporator. The product was vacuum-dried over P_2O_5. The dried $(AEP)PtSO_4$ was dissolved in water, and an appropriate amount of the barium salt of a specific organic ligand was added. After being stirred for 30 min at room temperature, the $BaSO_4$ precipitate was removed by filtration and the filtrate was evaporated to dryness at 45° C under reduced pressure. The solid was then recrystallized from methanol and the product was dried in vacuo.

A modification of the synthetic procedure was required to obtain the complex tetrachloro(aminoethylpyrrolidine)platinum(IV). Briefly, a heated suspension of dichloro(aminoethylpyrrolidine)platinum(II) was treated with 30% H_2O_2 and conc. HCl for 30 min, during which time a vigorous effervescence occurred. When the effervescence ceased, the product was then obtained by cooling the solution and filtering the yellow precipitate, which was then washed with water, and purified from acetone prior to drying over P_2O_5 in vacuo.

Chemical characterization of Pt complexes. All six complexes were subjected to elemental analysis carried out by Integral Microanalytical Laboratories, Inc., Raleigh, NC.

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The infrared (IR) spectra of three complexes as KBr pellets were measured over the 4000-250 cm^{-1} region using a Nicolet FT-IR-6000 spectrophotometer.

Biological testing. Each complex was assayed for in vitro cytotoxicity against an L1210 murine leukemia cell line either sensitive (L1210/0) or resistant to cisplatin (L1210/DDP) or to dichloro-1,2-diaminocyclohexane platinum (L1210/DACH). The cell lines were routinely maintained in McCoy's 5A medium supplemented with glutamine, antibiotics, and either 10% horse serum (L1210/0, L1210/DACH) or 10% fetal bovine serum (L1210/DDP). For testing purposes, 4 ml cells (10^5 cells/ml) were treated with the test complex (final concentration: 0.01, 0.1, 1, or 10 μg/ml), and after a 72-h incubation in a humidified atmosphere of 90% air:10% CO_2, the cell concentrations of control and test cultures were determined using a Coulter counter model Zf (Coulter Electronics, Hialeah, Fla). When grown under these conditions, L1210 cells have a doubling time of approximately 12 h (L1210/0) or 18 h (L1210/DDP), and control cultures of both cell lines are in exponential growth after 72 h incubation.

To assess the in vivo antitumor activity of the (AEP)Pt complexes, male BDF_1 mice (weighing 18–20 g) purchased from Jackson Laboratories, Bar Harbor, Me, were inoculated i.p. with 10^6 viable L1210/0 leukemic ascites cells (day 0). The animals were then given a single i.p. injection of the test compound the next day (day 1) and were observed daily for survival and signs of toxicity. Deaths occurring on or before day 7 (appproximately 1.5 days less than the mean survival time of nontreated tumor-bearing mice) were considered to be related to drug toxicity. The efficacy of the test complexes was calculated using the following formula:

$$\%\ T/C = \frac{\text{mean survival time treated}}{\text{mean survival time control}} \times 100.$$

Results and discussion

A series of complexes with nitrogen heterocyclic ligands, including pyridylmethyl-platinum(II), having chlorides as the leaving groups have been synthesized and tested by Brunner et al. [1]. These complexes had in vitro cytotoxicity against a hormone-independent human mammary carcinoma cell line (MDA-MB) but were water-insoluble and significantly less potent than cisplantin. The series of complexes reported in the present study is related to those discussed by Brunner et al. except that (a) our complexes had aminoethylpyrrolidine as the inert ligand, and (b) we modified the anionic ligand in such a way as to enhance the water solubility of these complexes.

Each product was submitted for elemental analysis prior to biological testing; as shown in Table 1, the observed C, H, N, and Cl values are in close approximation to the calculated values. Infrared (IR) analysis of compounds 3 and 4 (Table 2) provide strong evidence that the anionic ligands are coordinated to platinum through the carboxylate functions. Furthermore, IR analysis of

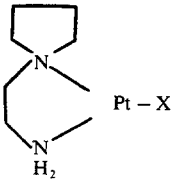
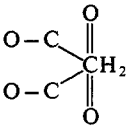
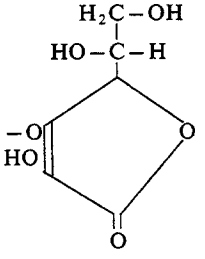


Fig. 1 Structural formula

Table 1. Elemental analysis of the (aminoethylpyrrolidine) platinum complexes

Compound no.	Compound	Elemental analysis % observe (% calculated)				
		X	C	H	N	Cl
1	dichloride	Cl	19.20 (19.00)	3.70 (3.70)	7.60 (7.40)	18.50 (18.70)
2	sulfate	SO_4	16.62 (16.62)	3.67 (3.93)	6.44 (6.48)	N.D.
3	oxalate	O–C=O O–C=O	23.29 (23.13)	3.78 (3.85)	6.47 (6.74)	N.D.
4	malonate		24.51 (24.65)	4.03 (4.33)	6.56 (6.39)	N.D.
5	*bis*-ascorbate		31.89 (31.74)	4.43 (3.80)	4.13 (4.17)	N.D.
6	tetrachloride	Cl[a]	15.90 (15.96)	3.34 (3.10)	6.10 (6.20)	31.48 (30.98)

[a] For compound 6, platinum was in the Pt(IV) state
[b] N.D., Analytical assay was not done

Table 2. Infrared data for the (aminoethylpyrrolidine) platinum complexes

Compound no.	IR (cm^{-1})	
1	Pt–Cl	319, 332
2	SO_4	1130
3	C=O	1697, 1670, 1659
	C–O	1397
4	C=O	1670, 1650
	C–O	1370
5	*	
6	Pt–Cl	328, 334, 345

* There is no typical pattern for hydroxyl linkage

Table 3. In vitro cytotoxicity of the (aminoethylpyrrolidine) platinum complexes

Complex	ID^{50} (μg/ml)			RF1[a]	RF2[b]
	L1210/0	L1210/DDP	L1210/DACH		
1	0.3	5.0	0.33	16.7	1.1
2	0.5	4.2	3.5	8.4	7.0
3	0.7	8.9	3.0	12.7	4.3
4	0.5	4.7	>10.0	9.4	N.D.[c]
5	6.8	>10.0	>10.0	N.D.	N.D.
6	0.4	6.1	5.8	15.2.	14.5
DDP	0.2	3.5	0.29	17.5.	1.5

[a] RF1, Resistance factor calculated by $\frac{ID_{50}\,(L1210/DDP)}{ID_{50}\,(L1210/0)}$

[b] RF2, Resistance factor calculated by $\frac{ID_{50}\,(L1210/DACH)}{ID_{50}\,(L1210/0)}$

[c] N.D., Not determined because $ID_{50} > 10$ μg/ml

Table 4. In vivo efficacy of the (aminoethylpyrrolidine) platinum complexes

Compound no.	MED[a] (mg/kg)	%T/C
1	20	190
2	50	156
3	50	156
4	50	122
5	150	148
6	50	153
DDP	5	165

[a] Mice were inoculated i.p. with 10^6 L1210 cells (day 0) and were given a single i.p. injection of the test compound on the following day. The results represent the dose of the compound producing the greatest %T/C of all doses tested

compounds 1 and 6 indicate chloride-platinum coordination, and that of compound 2 supports a sulfate coordination site. Taken together, the elemental analysis and IR spectra suggest the structural formula depicted in Fig. 1.

Preliminary biological testing of these complexes was carried out against L1210/0, L1210/DDP, and L1210/DACH cells in vitro (Table 3). Of the complexes tested, all but complex 5 [*bis*-ascorbato-(aminoethylpyrrolidine)-platinum(II)] had cytotoxic potency approximately equivalent to DDP. We previously reported that another platinum complex containing ascorbate as the anionic ligand was also significantly less potent than cisplatin (DDP) [3]. The reason for this marked difference in potency as a result of anionic ligand modification is unknown. Recent results reported by Hollis et al. [4] suggest that the interaction between ascorbic acid and Pt ist extremely complex and may result in the production of several chemical species in solution. If only a specific species of this complex mixture were responsible for the cytotoxicity, the ID_{50} would be high.

Also shown in Table 3 are the ID_{50} values for these complexes against DDP- and DACH-resistant L1210 cells. All of the complexes were apparently cross-resistant to DDP, with resistance factor (RF) values between 8 and 17.5. This cross-resistance is not surprising, since others have found that (diaminoethyl)-platinum(II) complexes are cross-resistant to DDP. Similarly, complexes 2, 3, and 6 were cross-resistant to DACH in L1210/DACH cells. Surprisingly, complex 1, dichloro(aminoethylpyrrolidine)platinum(II), but not complex 6, tetrachloro(aminoethylpyrrolidine)platinum(IV), was non-cross-resistant to DACH. The reason for this difference in sensitivity is unknown.

To evaluate the in vivo efficacy of this series of complexes, BDF_1 mice were given a single i.p. injection of test complexes approximately 24 h after being inoculated i.p. with 10^6 L1210/0 ascites cells (Table 4). Complexes 1, 2, 3, 5, and 6 had a modest level of antitumor activity (%T/C > 140). Complex 5, which was the least potent complex for in vitro cytotoxicity, was also the least potent in the in vivo screen. A minor modification of the anionic ligand from oxalato to malonato resulted in a substantial loss of in vivo efficacy. Similar changes in anionic leaving groups have been achieved with other inert ligands such as 1,2-diaminocyclohexane without a concomitant loss in efficacy [6, 8].

Many discussions concerning the structural determinants for the anticancer activity of platinum complexes assert that a free amine group in the inert ligand is required for cytotoxicity. The complexes reported in the present study represent another example of $PtCl_2$ chelate complexes in which the N substituent is different from the $-NH_2$ moiety, suggesting that the assumed structural requirements for activity should be reconsidered.

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