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Synthesis and Antitumor Activity of Platinum Complexes of Protonated Diamines

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Abstract

Complexes of the formula *cis*-[Pt(HN^{+}~N)(L)Cl_2], where (HN^{+}~N) are the protonated diamines including 3-aminoquinuclidine, N-aminopiperidine, piperazine, N-methylpiperazine, 1,1,4-trimethylpiperazine, and N-methyl-1,4-diazabicyclo[2,2,2]octane (N-methyldabco) and L = SCN^-, NO_2^-, Br^-, and F^-, were synthesized from the protonated diamine complexes, [Pt(HN^{+}~N)Cl_3]. The antitumor activities of the complexes were evaluated *in vitro* against L1210 murine leukemia cells, and ID_{50} values for the L-substituted complexes were compared to values of the parent complexes. In each case it was found that replacement of a chloride ion by SCN^-, NO_2^-, Br^-, or F^-, either reduced or completely eliminated antitumor activity. This effect is explained in terms of the *trans*-directing ability of the ligand, L, compared to chloride. The NO_2-substituted complex of 3-aminoquinuclidine was tested *in vivo* and found to exhibit little or no antitumor activity.

Introduction

Since the discovery of the antitumor activity of *cis*-dichlorodiammine platinum(II), many platinum complexes have been explored as potential antitumor drugs [1]. Platinum complexes of protonated diamines (Scheme 1) have been synthesized and shown to exhibit antitumor activity against L1210 leukemia both *in vitro* and *in vivo* [2, 3].

The cytostatic activity of these and other platinum complexes is presumed to depend upon the rates of substitution reactions at platinum which result in replacement of anionic ligands from the metal complex by water ligands (aquation) [4]. The rates of ligand substitution in platinum complexes depend on labilizing effects of the ligand trans to the leaving group, termed the *trans*-effect [5]. The ligands believed to be substituted in the platinum-protonated diamine system are a pair of *cis*-chloride ions; the *trans*-directing ligands are the protonated diamine and the chloride ion *cis* to it. We postulated that if the nature of the *trans*-directing group (formerly Cl) was systematically varied to ligands both higher and lower in the *trans*-effect series, that this would affect the kinetics of substitution at platinum and therefore alter the antitumor activity of the complex. Here we report the results of synthesis and antitumor activity evaluation studies of a series of protonated diamine complexes in which the chloride ion *cis* to the diamine has been substituted by a variety of different *trans*-directors.

Experimental

All diamines and silver fluoride were purchased from Aldrich Chemical Co., and used without further purification. Other silver salts were purchased from Fisher Scientific Co. K_2PtCl_4 was purchased from Matthey Bishop, Inc., and used as received. Elemental analyses were performed by Robertson Laboratory, Florham Park, N.J. Infrared spectra of the complexes (as KBr pellets) were measured using a Nicolet 6000 Fourier Transform spectrometer.

Synthesis of [Pt(HN~N)Cl_3] Complexes

Platinum complexes of the protonated diamines, 3-aminoquinuclidine, N-aminopiperidine, piperazine, N-methylpiperazine, 1,1,4-trimethylpiperazine, and N-methyldabco were synthesized using the method of Brown *et al.* [2].

Synthesis of cis-[Pt(NH~N)(L)Cl_2] Complexes

All the complexes listed in Table I were prepared by the following general method.

The appropriate [Pt(NH~N)Cl_3] complex (1 mmol) was suspended in 20 ml of water and the silver salt of the anion, AgL (1 mmol), was added. The mixture was stirred at room temperature for 12 h in the dark. The resulting solution was filtered through celite to remove AgCl, and the filtrate was concentrated. A yellow solid precipitate was obtained and washed with ethanol and then acetone and dried *in vacuo*. Yield: 60%.

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TABLE I. Elemental Analysis and Infrared Spectra for *cis*-[Pt($HN^{+}\sim N$)(L)(Cl_2) Complexes.

($HN^{+}\sim N$)	(L)	% Observed (% Calculated)			
		C	H	N	Pt–Cl
3-aminoquinuclidine	NO_2	19.13(19.12)	3.51(3.41)	9.18(9.56)	322,328
	Br	16.28(16.22)	2.87(2.89)	5.20(5.40)	318,325
N-aminopiperidine	SCN	15.15(15.18)	2.94(2.84)	9.78(9.62)	306,321
	Br	10.22(10.48)	2.24(2.70)	4.66(5.29)	319,325
	F	16.54(16.63)	3.52(3.13)	7.55(7.29)	325,333
Piperazine	NO_2	14.40(12.00)	2.50(3.00)	10.10(10.52)	
N-methylpiperazine	SCN	16.91(17.02)	2.40(2.84)	10.56(9.93)	320,333
	NO_2	14.60(14.60)	2.94(2.92)	9.89(10.22)	319,331
	Br	13.26(13.48)	3.00(2.72)		315,331
	F	15.29(15.63)	2.92(3.13)		320,331
1,1,4-trimethylpiperazine	NO_2	19.03(19.00)	4.11(4.10)	9.17(9.50)	
N-methyldabco	NO_2	19.13(19.12)	3.51(3.41)	9.18(9.56)	

Biological Studies

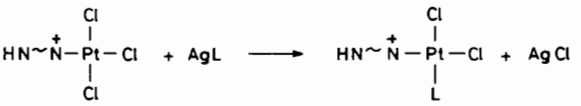
L1210 cells were cultured in McCoy's 5A medium supplemented with 10% horse serum, glutamine, and antibiotics. Aqueous solutions of the test compound were added to cultures of L1210 leukemia cells (at an initial concentration of approximately 5×10^4 cells/ml) at final concentrations of 10.0, 1.0, 0.1, and 0.01 μg/ml. Each drug concentration was tested in triplicate, and after 72 h of drug exposure in a 95% air: 5% CO_2 atmosphere at 37 °C, cell concentrations of control and drug-tested cultures were determined using an electronic cell counter (Coulter Counter Model ZF). From comparison of the cell growth of the drug-treated cultures and the control, the ID_{50} (dose required to inhibit cell growth by 50%) was determined.

To evaluate the activity against L1210 *in vivo*, L1210 cells (1×10^6 suspended in 0.1 ml of physiological saline solution) were inoculated intraperitoneally into BDF_1 mice, obtained from Jackson Laboratories, Bar Harbor, Maine, (weighing 20–22 g) and intraperitoneal drug treatment initiated approximately 24 h after tumor inoculation. Drugs were dissolved or suspended in 0.3% hydroxypropyl cellulose in saline ('Klucel' obtained from the National Cancer Institute, NIH, Bethesda, Md.). All animals were housed in central animal facilities having controlled temperature, relative humidity, and photoperiods.

Results and Discussion

Chemical Characterization

The general reaction for synthesis of *cis*-[Pt($HN^{+}\sim N$)(L)Cl_2] complexes is given in Scheme 1.



Scheme 1.

The *cis*-[Pt($HN^{+}\sim N$)Cl_3] complexes react with AgL, where L = SCN, NO_2, Br of F, in water in the dark at room temperature to give the [Pt($NH^{+}\sim N$)(L)Cl_2] complexes.

The formulae of the resulting complexes were established through elemental analysis. The *cis*-geometry of the complexes was determined through infrared spectroscopy studies by the presence of two platinum–chlorine stretching bands in the low frequency region of the spectrum. Analytical and IR data are given in Table I.

Biological Studies

All complexes were dissolved in sterile double distilled water and added to L1210 murine leukemia cells. After 72 h of continuous exposure to the drug, cell concentrations of control and drug-treated cultures were determined. ID_{50} values (50% inhibitory doses) for the compounds against L1210 leukemia cells are given in Table II. A complex is considered to possess acceptable *in vitro* antitumor activity if its ID_{50} value is <10 μg/ml. The relatively poor *in vitro* cytotoxicity of these complexes may be a function of their low water solubility. However, solubilitics among the complexes did not vary significantly, so relative activities of the complexes may be compared.

From the ID_{50} values it seems that substitution of the chloride ion with ligands higher in the *trans*-effect series (more labilizing: SCN^-, NO_2^-, Br^-)

TABLE II. Cytotoxicity of *cis*-[Pt(HN̂~N)(L)Cl_2] Complexes Against L1210 Cells *in vitro*.

(HN̂~N)	(L)	ID_{50} (μg/ml)
3-aminoquinuclidine	NO_2	6
	Br	6
	Cl	2
N-aminopiperidine	SCN	>10
	Br	>10
	Cl	4.2
	F	2.7
Piperazine	NO_2	>10
N-methylpiperazine	SCN	>10
	NO_2	>10
	Br	>10
	Cl	9
	F	>10
1,1,4-trimethylpiperazine	NO_2	>10
N-methyldabco	NO_2	>10

results in diminished activity of the complexes. In the case of the F-substituted systems, the activity remains essentially the same in the N-aminopiperidine system, but is lessened in the N-methylpiperazine system. In the F-substituted systems, because F is a lower *trans*-director (less labilizing) than Cl^-, the leaving groups would likely be one chloride and the floride ion, as opposed to two chloride ions as in the other systems studied. Because the activity of the F-substituted N-aminopiperidine complex cannot be considered to be better than that of the parent complex, we conclude that no improvement of antitumor activity is attained by F-substitution.

These *in vitro* data suggest that changes in the rates of ligand substitution by water molecules in the cell (aquation), affected by varying *trans*-directing ligands, lead to diminished antitumor activity. This can be related to the hypothesis that the differing rates of aquation affect the ability of the aquated moiety to bind to DNA, which is believed to be the origin of antitumor activity [6].

Thus it can be stated that in the platinum complexes of 3-aminoquinuclidine, N-aminopiperidine, piperazine, N-methylpiperazine, 1,1,4-trimethylpiperazine, and N-methyldabco, a chloride ion in the *trans*-directing position, L, appears to provide the optimal *trans*-labilizing influence to result in ligand substitution rates which give maximum antitumor activity *in vitro*.

Acknowledgements

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