Synthesis and Antitumor Activity of Organometallic *cis*-Dichloro(enamine)(amine) Platinum(II) Complexes≠

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Abstract

A series of organometallic enamine complexes of platinum(II) have been synthesized and their antitumor activity *in vitro* have been evaluated against L1210 murine leukemia cells. Preliminary results indicate that this class of complexes have good *in vitro* cytotoxicity against L1210 murine leukemia cells.

Introduction

Since the discovery of the antitumor activity of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) by Rosenberg *et al.* [1], DDP has become valuable in the treatment of several human malignant neoplasms [2–4]. Because of the relative narrow range of tumor sensitivity and the rather severe toxicities associated with the use of *cis*-DDP, a number of laboratories have attempted to synthesize new, more effective or less toxic platinum antitumor complexes. To date, the vast majority of complexes tested have been coordination complexes of the formula, *cis*-(PtAX), in which A represents either two monodentate amines or one bidentate amine and X represents either two monodentate or one bidentate anionic-leaving group.

There has recently been a growing interest in the synthesis of organometallic complexes as antitumor agents [5, 6]. At present, we are reporting on the antitumor activities of a series of organometallic enamine complexes of platinum(II) that represent a novel class of transition metal complexes with oncolytic activity. The general structure of these complexes is depicted in Table I in which platinum-(II) exists in a square planar configuration with *cis*-pair of chloride ions, an enamine coordinated through its nucleophilic carbon and an amine derived from the parent enamine.

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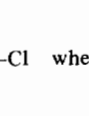
TABLE I. Structures of the *cis*-Dichloro(enamine)(amine) Platinum(II) Antitumor Complexes[a]

Complex	Enamine	Amine
1	1-pyrrolidino-1-cyclohexene	pyrrolidine
2	1-diethylamino-1-propene	diethylamine
3	3-diethylamino-2-pentene	diethylamine
4	1-pyrrolidino-1-butene	pyrrolidine
5	1-diisopropylamino-1-butene	diisopropylamine
6	1-diethylamino-1-butene	diethylamine
7	2-diethylamino-3-methyl-2-butene	diethylamine
8	1-diethylamino-1-propene	pyridine
9	1-pyrrolidino-1-butene	pyridine

[a]  where X = amine, Y = enamine.

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Experimental

Platinum Complex Synthesis

The actual synthesis and chemical characterization of these complexes have been published previously [7]. In brief, Ziese's dimer (1 eq) was suspended in 50 ml of dry distilled diethyl ether, under N_2. The appropriate enamine (4 eq) was then added and the Ziese's dimer dissolved immediately at room temperature with a resultant solution color change from orange to yellow. After an additional 15 to 20 min of stirring at room temperature, the solution was filtered and the filtrate was stored at 4 °C for 1 to 2 days. Yellow crystals were obtained with an overall yield of approximately 90%. Each complex was submitted for elemental analysis before biological testing.

Cytotoxicity Evaluation

L1210 murine leukemia cells are routinely cultured in McCoy's 5A medium supplemented with glutamine, penicillin, streptomycin, and 10% horse serum. When grown at 37 °C in a humidified atmosphere consisting of 90% air: 10% CO_2, these cells have a doubling time of approximately 15 h. To determine the cytotoxicity of the platinum complexes, 4 ml of cell suspension (10^5 cells/ml) were added to culture tubes in triplicate, and the test compound was added at final concentrations of 0.01, 0.1, 1 or 10 μg/ml. After 72 h of incubation, the cell concentration of nontreated control cultures and drug-treated cultures were determined using a Coulter counter (Coulter Electronics, Hialeah, FL). The ID_{50} value (defined as that concentration of drug required to inhibit cell growth by 50%) was calculated by extrapolation of line defined by drug concentration *versus* percent inhibition of growth.

TABLE II. Elemental Analysis of *cis*-Dichloro(enamine)-(amine) Platinum(II) Complexes

Complex	Elemental Analysis					
	Found (%)			Calculated (%)		
	C	H	N	C	H	N
1	33.05	5.41	5.75	33.50	5.34	5.75
2	28.81	5.87	5.80	29.27	5.76	6.20
3	33.28	4.33	5.98	33.26	4.26	5.97
4	31.04	5.27	5.92	31.10	5.00	6.08
5	36.54	7.29	4.73	36.92	6.73	5.38
6	31.07	6.09	5.83	30.97	6.02	6.02
7	32.51	6.70	6.11	32.57	6.56	6.13
8	31.51	4.16	5.57	31.51	4.30	6.13
9	33.30	4.40	5.81	33.19	4.47	5.96

TABLE III. *In Vitro* Cytotoxicity of *cis*-Dichloro(enamine)-(amine) Platinum(II) Complexes[a]

Complex	ID_{50} (μg/ml)
1	>10
2	3.5
3	>10
4	4.0
5	2.4
6	4.0
7	2.1
8	>10
9	>10

[a]Complexes were dissolved in sterile water and added to cultures of L1210 cells at final concentrations of 10, 1, 0.1, and 0.01 μg/ml. After 72 h of incubation, the cell concentration of control and drug-treated cultures were determined using a Coulter counter and the calculated ID_{50} value.

Results and Discussion

The specific complexes synthesized and evaluated for biological activity are shown in Table I. Before entry into the cytotoxicity assay, each complex was submitted for elemental analysis (Table II). All nine complexes were only marginally soluble in water (≦1 mg/ml), but were quite soluble in most organic solvents such as dimethyl sulfoxide or dimethylformamide.

To determine the cytotoxic potential of each complex, the test compound was dissolved in water at concentrations of 500, 50, 5 or 0.5 μg/ml, and 80 μl of solution were added to 4 ml of L1210 cell suspension (10^5 cells/ml). The ID_{50} values for all nine complexes are listed in Table III. It is generally believed that for a platinum complex to have potential as an antitumor agent, it should have an *in vitro* ID_{50} value ≦10 μg/ml [8]. As can be seen from Table III, several of our organometallic complexes had sufficient cytotoxicity activity to warrant further biological evaluation. One obstacle, that is common to many second-generation platinum complexes, and must be overcome before full-scale *in vivo* studies can be effectively performed is the relatively poor aqueous solubility of the platinum enamine complexes. This could be accomplished by replacement of the chloride ions by other anionic-leaving groups such as the carboxylate ions [9, 10] present in other water-soluble platinum antitumor complexes. Indeed, our research group has demonstrated that one can convert the dichloro-1,2-diaminocyclohexane platinum(II) complex which is virtually water-insoluble into a highly water-soluble state by substituting the chloride ions with either the ascorbate ion [11] or the iminodiacetate ion [12]. More important is that these water-soluble forms of 1,2-diaminocyclohexane

platinum(II) display excellent antitumor activity. A similar approach to enhanced water solubility of the platinum enamine complexes is currently under way in our laboratories.

In summary, this preliminary work has demonstrated the potential for platinum enamine complexes, the first organoplatinum complexes to exhibit antitumor activity for use in cancer chemotherapy.

References

1 B. Rosenberg, L. Van Camp, J. E. Trosko and V. H. Mansour, *Nature, 222,* 385 (1969).
2 G. Pouskouleli and P. Kourounakis, *Biochem. Exp. Biol., 16,* 191 (1980).
3 S. D. William and L. H. Einhorn, in A. W. Prestayko, S. T. Crooke and S. K. Carter (eds.), 'Cisplatin: Current Status and New Developments', Academic Press, New York, 1980, p. 323.
4 M. Rosenzweig, D. D. Von Hoff, M. Slavic and F. M. Muggia, *Ann. Intern. Med., 86,* 803 (1977).
5 P. Kopf-Maier and D. Krahl, *Chem. Biol. Interactions, 44,* 317 (1983).
6 P. Kopf-Maier and H. Kopf, in M. P. Hacker, E. B. Douple and I. H. Krakoff (eds.), 'Platinum Coordination Complexes in Cancer Chemotherapy', Martinus-Nijhoff Publishing, Boston, Mass., 1984, p. 279.
7 A. J. Kunin and D. B. Brown, *J. Organomet. Chem., 212,* C27 (1981).
8 R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep., 3,* 1 (1972).
9 Y. Kidani, K. Inagaki, M. Iigo, A. Hoshi and K. Yuretani, *J. Med. Chem., 21,* 1315 (1978).
10 J. P. Macquet, S. Cros and J. P. Armand, *Cancer Res., 44,* 3736 (1984).
11 R. C. Richmond, A. R. Khokhar, B. A. Teicher and E. B. Douple, *Radiat. Res., 99,* 609 (1984).
12 A. R. Khokhar, M. P. Hacker, J. J. McCormack, D. B. Brown and I. H. Krakoff, *Proceedings IUPHAR,* Ninth International Congress of Pharmacol., London, England: Macmillan, p. 1365 (1984).