Synthesis and Antitumor Activity of N-Substituted Iminodiacetato(1,1-bis[aminomethyl]cyclohexane)platinum(II) Complexes

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Abstract

A number of water-soluble N-substituted iminodiacetato(1,1-bis[aminomethyl]cyclohexane)platinum-(II) complexes have been synthesized, and their mode of coordination characterized by elemental analysis and infrared data. Preliminary *in vitro* screening tests for antitumor activity of these complexes against L1210 murine leukemia cells were performed. The results indicate that these complexes have an acceptable *in vitro* cytotoxicity against L1210 leukemia.

Introduction

In recent years there has been a great deal of effort devoted to developing analogs of the widely used antitumor drug *cis*-dichlorodiammineplatinum-(II) (*cis*-DDP)‡. These analogs are less toxic than *cis*-DDP, yet retain high antitumor activity [1]. We have been investigating several series of platinum carboxylate complexes as potential antitumor agents [2]. During the course of this work we have investigated various N-substituted iminodiacetatoplatinum(II) complexes. We report here the synthesis, characterization and *in vitro* antitumor activity of a series of such complexes containing the ligand 1,1-bis(aminomethyl)cyclohexane (CMA).

Experimental

$R-N(CH_2COOH)_2$ (R = Me, Bz, or HO-Et), $R'-NH_2$ (R' = Et, ^{n}Pr or ^{n}Bu) and silver sulfate were obtained from Aldrich Chemical Co. (Milwaukee, Wis.) and were used without further purification. Chloroacetic acid, barium hydroxide and barium chloride were purchased from Fisher Scientific Co. (Houston, Tex.). K_2PtCl_4 was purchased from Johnson Matthey (Seabrook, N.H.). Elemental analyses were performed by Robertson Laboratories (Madison, N.J.). Infrared spectra were recorded as KBr pellets using a Perkin-Elmer 1330 infrared spectrophotometer. NMR spectra were obtained on a Bruker WM 250/PFT spectrometer. Mass spectra were recorded using a Finnigan MAT 4610.

Synthesis of 1,1-Bis(aminomethyl)cyclohexane (CMA)

1,1-Cyclohexanedicarboxylic acid was prepared according to the method described by Vogel [3]. The dicarboxylic acid was converted to 1,1-cyclohexanediamide in the following manner. 1,1-Cyclohexanedicarboxylic acid (25 g, 0.15 mmol) and thionyl chloride (75 ml, 1.0 mmol) were heated under reflux for 2 h. The solution was concentrated to 20 ml under reduced pressure and was added drop-wise to cold concentrated NH_4OH. The resulting white precipitate was filtered, washed with water and dried at 120 °C. 1,1-Cyclohexanediamide was obtained as an off-white solid (21 g, 85%), m.p. 253–7 °C. ^{1}H NMR spectrum (DMSO-d_6), δ in ppm: 7.00 (exchangeable s, 2H), 6.90 (exchangeable s, 2H), 1.42–1.12 (m, 10H); mass spectrum *m/e* (relative intensity): 171 (14.4, *m*+1), 127 (69.2), 115 (100), 98 (45.3), 59 (41.8), 55 (55.2).

1,1-Cyclohexanediamide was converted to 1,1-bis(aminomethyl)cyclohexane as follows. 1,1-Cyclohexanediamide (15.98 g, 0.094 mmol) was added in portions at 5 °C to a slurry of lithium aluminum hydride (28.6 g, 0.75 mmol) in 300 ml of freshly distilled *N*-ethylmorpholine. The mixture was allowed to warm slowly to room temperature and was then refluxed for 36 h. The reaction mixture was cooled to 5 °C and was quenched with 20% aqueous KOH. After heating under reflux for an additional 2 h, the mixture was cooled and the liquid portion was decanted. The solid portion was extracted under reflux with an additional 300 ml of *N*-ethylmorpholine. Again, the mixture was allowed to cool and the liquid portion was decanted. The combined liquid layers were evaporated on a rotary evaporator. The

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‡Abbreviations used: *cis*-DDP = *cis*-dichlorodiammineplatinum(II); CMA = 1,1-bis(aminomethyl)cyclohexane; N-R-IDA = $R-N(CH_2COO^-)_2$; Me = methyl, Et = ethyl; ^{n}Pr = n-propyl; ^{n}Bu = n-butyl; Bz = benzyl; HO-Et = $-CH_2CH_2-OH$.

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TABLE I. Elemental Analyses of [Pt(N-R–IDA)(CMA)]·xH_2O Complexes

R	X	Observed (%)			Calculated (%)		
		C	H	N	C	H	N
Me	2	30.47	5.40	7.89	30.11	5.64	8.11
Et	3	30.95	5.48	7.26	30.54	6.04	7.63
^{n}Pr	3	31.55	5.66	6.73	31.91	6.25	7.44
^{n}Bu	2	34.76	6.06	6.99	34.28	6.29	7.49
Bz	2	39.06	5.24	6.15	38.37	5.59	7.06
HO–Et	3	30.06	5.85	7.43	29.67	5.87	7.41

residue was dissolved in diethyl ether, dried over anhydrous sodium sulfate, and filtered. After evaporation of the diethyl ether, the product was purified by fractional distillation under vacuum (82–3 °C, 3 mmHg). In this way, 8.90 g (67%) of pure 1,1-bis(aminomethyl)cyclohexane was obtained. ^{1}H NMR spectrum ($CDCl_3$), δ in ppm: 2.62 (s, 4H), 1.45 (m, 6H), 1.29 (m, 4H), 1.09 (exchangeable s, 4H); mass spectrum *m/e* (relative intensity): 143 (55.9), 125 (53.5), 85 (77.5), 84 (100).

Synthesis of [Pt(SO$_4$)(OH$_2$)(CMA)]

[$PtCl_2$(CMA)] was prepared by mixing K_2PtCl_4 with one equivalent of 1,1-bis(aminomethyl)cyclohexane (CMA) in water. The [$PtCl_2$(CMA)] that precipitated was filtered, washed with water and dried *in vacuo*. [$PtCl_2$(CMA)] was then stirred in an aqueous solution of silver sulfate (1 eq) for 12 h in a darkened flask. The silver chloride was removed by filtration and the filtrate was evaporated under reduced pressure to give [Pt(SO_4)(OH_2)(CMA)].

Synthesis of [Pt(N-Me–IDA)(CMA)]·H$_2$O

A solution of barium *N*-methyliminodiacetic acid was prepared by mixing *N*-methyliminodiacetic acid with one equivalent of barium hydroxide in water. To this solution was added one equivalent of [Pt(SO_4)(OH_2)(CMA)]. After stirring for 30 min, the barium sulfate was filtered and the solvent was evaporated under reduced pressure. The residue was dissolved in methanol and filtered. Evaporation of the solvent gave [Pt(N-Me–IDA)(CMA)]·$2H_2O$ in an 80% yield.

The other [Pt(N-R–IDA)(CMA)] complexes were prepared in a similar manner. Where R = Et, ^{n}Pr or ^{n}Bu, the Ba(N-R–IDA) salt was prepared following the method of Stein *et al.* [4].

Cytotoxicity Evaluation

L1210 murine leukemia cells are routinely cultured in McCoy's SA 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 the cell suspension (10^5 cells/ml) was added to culture tubes in triplicate, and the test complex was added at final concentrations of either 0.01, 0.1, 1 or 10 μg/ml. After 72 h of incubation, the cell concentrations of non-treated control cultures and drug-treated cultures were determined using a Coulter counter (Coulter Electronics, Hialeah, Fla.). The IC_{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.

Results and Discussion

Reaction of [Pt(SO_4)(OH_2)(CMA)] with Ba(N-R–IDA) (R = Me, Et, ^{n}Pr, ^{n}Bu, Bz or HO–Et) leads to the formation of the water-soluble complexes [Pt(N-R–IDA)(CMA)]:

$$[Pt(SO_4)(OH_2)(CMA)] + Ba(N\text{-}R\text{–}IDA) \longrightarrow$$

$$\longrightarrow [Pt(N\text{-}R\text{–}IDA)(CMA)] + BaSO_4\downarrow$$

Filtration of the barium sulfate followed by evaporation of the solvent yields [Pt(N-R–IDA)(CMA)] as a crystalline solid. These compounds can be purified by dissolution in methanol followed by filtration and evaporation of the methanol. The stoichiometry of one N-R–IDA ligand per platinum atom has been confirmed by elemental analysis (Table I).

The complexes [Pt(N-R–IDA)(CMA)] have the general structure shown in Fig. 1. As is typical for platinum(II) complexes, the ligands form a square-planar arrangement around the metal center. Two of the four coordination sites are occupied by the

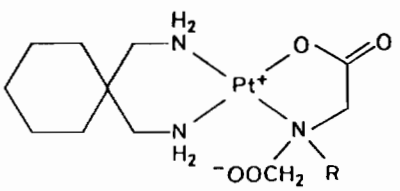

Fig. 1. Structural formula of [Pt(N-R–IDA)(CMA)] complexes, in which R = Me, Et, ^{n}Pr, ^{n}Bu and HO–Et.

TABLE II. Infrared Data[a] for [Pt(N-R–IDA)(CMA)] Complexes

R	ν(C=O)[b]	ν_a(C=O)[c]	ν_s(C–O)[c]	ν(C–O)[b]
Me	1662	1625	1400	1325
Et	1655(sh)	1630	1402	1324
^{n}Pr	1660(sh)	1615	1400	1330
^{n}Bu	1655(sh)	1625	1400	1324
Bz	1600	1630	1400	1325
HO–Et	nr[d]	1635	1407	1338

[a]All spectra were recorded as KBr pellets. Band positions are given in cm^{-1}; sh: shoulder. [b]Coordinated carboxylate group. [c]Uncoordinated carboxylate group. [d]nr = not resolved.

chelating diamine ligand (CMA), whereas the other two are occupied by the N-R–IDA ligand. The latter is bonded to the platinum center through one carboxylate oxygen and through the imino nitrogen atom. Such a structure is supported by spectroscopic data.

The infrared spectral data for the [Pt(N-R–IDA)(CMA)] complexes are shown in Table II. The infrared spectrum of [Pt(N-Me–IDA)(CMA)] consists of two very strong bands in the carbonyl region. The band at 1662 cm^{-1} is assigned to ν(C=O) of the coordinated carboxylate group; that at 1625 cm^{-1} is assigned as ν_a(C–O) of the uncoordinated carboxylate group. Similarly, two bands are observed in the region 1300–1410 cm^{-1}. The band at 1400 cm^{-1} is assigned to ν_s(C–O) of the uncoordinated carboxylate group and that at 1325 cm^{-1} is due to ν(C–O) of the coordinated carboxylate group. All the other complexes display similar patterns (Table II). These infrared data support the structure shown in Fig. 1.

Antitumor testing performed with these complexes was for *in vitro* cytotoxicity against L1210/0 cells (Table III). The IC_{50} values for the N-substituted iminodiacetato(CMA)platinum(II) complexes ranged from 3.4 to >10 *versus* 0.14 for *cis*-DDP. It is generally believed that for a platinum complex to have potential as an antitumor agent, it should have an *in vitro* IC_{50} value ≤ 10 μg/ml [5]. As can be seen from Table III, several of these complexes have sufficient cytotoxicity activity.

TABLE III. *In Vitro* Cytotoxicity of [Pt(N-R–IDA)(CMA)] Complexes[a]

R	IC_{50} (μg/ml)
Me	3.4
Et	5.0
^{n}Pr	4.5
Bz	5.0
HO–Et	>10
cis-DDP	0.14

[a]Complexes were dissolved in sterile water and added to cultures of L1210 cells at final concentrations of 0.01, 0.1, 1 and 10 μg/ml. After 72 h of incubation, cell concentration was measured using a Coulter counter and percentage growth inhibition was calculated. The IC_{50} values in these studies refer to the drug concentrations required to inhibit cell growth by 50%.

In summary, we have prepared a series of N-substituted iminodiacetato(CMA)platinum complexes and examined their cytotoxicity activity. The complexes exhibited reasonable *in vitro* cytotoxicity against L1210 leukemia cells.

Acknowledgement

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References

1 (a) A. W. Prestayko, S. T. Crooke and S. K. Carter (eds.), 'Cisplatin: Current Status and New Developments', Academic Press, New York, 1980, p. 383–392; (b) M. P. Hacker, E. B. Douple and I. H. Krakoff (eds.), 'Platinum Coordination Complexes in Cancer Chemotherapy', Martinus Nijhoff, Boston, 1984.
2 A. R. Khokhar, I. H. Krakoff, M. P. Hacker and J. J. McCormack, *Inorg. Chim. Acta, 108*, 63 (1985).
3 A. I. Vogel, *J. Chem. Soc.*, 1487 (1929).
4 A. Stein, H. P. Gregor and P. E. Spoerri, *J. Am. Chem. Soc., 77*, 191 (1955).
5 R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher and B. J. Abbott, *Cancer Chemother. Rep., 3*, 1 (1972).