Synthesis, Characterization and Antitumor Activity of a Series of N-Substituted Iminodiacetato(diammine)platinum(II) Complexes

ABDUL R. KHOKHAR*, GREGG J. LUMETTA, ROBERT A. NEWMAN and SHERYL L. DORAN

Department of Medical Oncology, The University of Texas M.D. Anderson Hospital and Tumor Institute at Houston, Houston, Tex. 77030, U.S.A.

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Abstract

A series of water-soluble N-substituted iminodiacetato(diammine)platinum(II) complexes [Pt(N–R–IDA)$(NH_3)_2$]† have been synthesized and characterized by measurement of physical properties (conductivity and pH) and by various spectroscopic techniques (infrared, ^1H and $^{13}C\{^1H\}$ nuclear magnetic resonance). The iminodiacetate ligand is coordinated to platinum through an O,N linkage. The results obtained suggest that these complexes are relatively stable for more than 24 h in aqueous solution. Preliminary *in vitro* and *in vivo* screening tests for antitumor activity of these complexes against L1210 murine leukemia were performed. Many of the complexes had acceptable *in vitro* cytotoxicity, but none displayed a significant level of *in vivo* antitumor efficacy.

Introduction

The compound *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) is currently used in the treatment of various forms of cancer [1]. In recent years a great deal of effort has been devoted to developing other platinum antitumor drugs that are less toxic than *cis*-DDP and that yet retain antitumor activity [2]. We have been investigating a series of platinum(II) complexes containing ligands of the type N–R$(CH_2COO^-)_2$, which we refer to as iminodiacetate (IDA) ligands. Many of these water-soluble compounds display a high degree of antitumor activity and are less nephrotoxic than *cis*-DDP [3].

In this paper we report the synthesis and spectroscopic characterization of a series of [Pt(N–R–IDA)$(NH_3)_2$] complexes. Many of these complexes have been shown to display significant antitumor activity *in vitro* against the L1210 leukemia cell line.

Experimental

N–R$(CH_2COOH)_2$ (R = H, Me, Ph, Bz or $-CH_2CH_2OH$), R′–NH_2 (R′ = Et, ^nPr, ^iPr, ^nBu, ^sBu, ^iBu, ^tBu, or ^nAm), potassium iodide 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 of the platinum complexes were performed by Robertson Laboratories (Madison, N.J.). Infrared spectra were recorded as KBr pellets in the range 250–4000 cm^{-1} using a Beckman 250MX spectrophotometer. NMR spectra were obtained on an IBM NR200/AF spectrometer.

Biological Studies

Each complex was assayed for cytotoxicity *in vitro* against an L1210 leukemia cell line sensitive to *cis*-DDP (L1210/0). Cells were maintained in McCoy's 5A medium supplemented with 10% horse serum (L1210/0), glutamine and antibiotics. For testing purposes, 4 ml of cells (10^5 cells/ml) was added to culture tubes and the test compound was then added to achieve final drug concentrations of 0.01 to 10 g/ml. The cell concentration of control and drug-treated cultures was then determined 72 h later using a Coulter Counter, model ZB (Coulter Electronics, Hialeah, Fla.). The percentage of growth inhibition was calculated and the IC_{50} value (*i.e.* that drug concentration producing a 50% inhibition of cell growth) was determined by linear regression analysis.

To assess the *in vivo* oncolytic activity of the complexes, BDF_1 mice were inoculated i.p. with 10^6 L1210/0 cells. Drug administration was begun approximately 24 h later (day 1) as a single i.p. injection, or as a series of i.p. injections on days 1, 5

*Author to whom correspondence should be addressed.

†Abbreviations used: N–R–IDA = N–R(iminodiacetate); Me = methyl; Et = ethyl; ^nPr = n-propyl; ^iPr = isopropyl; ^nBu = n-butyl; ^sBu = sec-butyl; ^iBu = isobutyl; ^tBu = t-butyl; ^nAm = n-amyl; Ph = phenyl; Bz = benzyl; HO–Et = CH_2CH_2OH; i.p. = intraperitoneally.

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and 9. Drugs were dissolved in sterile double-distilled water. The efficacy of the given drug treatment schedule was determined by the following formula:

$$T/C\,(\%) = \frac{\text{mean survival of treated mice}}{\text{mean survival of control mice}} \times 100.$$

Preparation of [Pt(N–Me–IDA)(NH$_3$)$_2$]

An aqueous solution of $[Pt(SO_4)(NH_3)_2]$ was prepared in the following manner. A filtered aqueous solution of potassium tetrachloroplatinate (0.415 g, 1.00 mmol) was added to an aqueous solution of potassium iodide (0.665 g, 4.00 mmol). The solution was stirred (approx. 15 min) until the deep yellow coloration of PtI_4^{2-} was observed. Concentrated ammonium hydroxide (0.15 ml, 2.22 mmol) was added and the solution was stirred. The resulting precipitate $[PtI_2(NH_3)_2]$ was collected by filtration after stirring for several hours. The $[PtI_2(NH_3)_2]$ was then mixed with an equimolar amount of silver sulfate in water for 12 h. The resulting silver iodide was removed by filtration, giving the aqueous solution of $[Pt(SO_4)(NH_3)_2]$ (100% conversion to the sulfatoplatinum(II) complex was assumed for subsequent reactions).

An aqueous solution of Ba(N–Me–IDA) was prepared by dissolving *N*-methyliminodiacetic acid (0.147 g, 1.00 mmol) and $Ba(OH)_2 \cdot 8H_2O$ (0.316 g, 1.00 mmol) in 10 ml of water. This solution was added to the aqueous solution of $[Pt(SO_4)(NH_3)_2]$ (1.00 mmol). After stirring for 30 min, the mixture was filtered and the solution was evaporated to dryness on a rotary evaporator. The resulting residue was dissolved in water and was filtered through a fine-mesh sintered-glass funnel that had been packed with Celite. The filtrate was evaporated under reduced pressure, then the solid product was dried *in vacuo*. $[Pt(N–Me–IDA)(NH_3)_2]$ was obtained as an off-white solid in 60% (0.25 g) yield. The product was purified by ion-exchange chromatography, with an aqueous solution of the product being passed through an Amberlite–MB (Sigma Chemical Co., St. Louis, Mo.) ion-exchange column.

Preparation of other [Pt(N–R–IDA)(NH$_3$)$_2$] Complexes

The complexes where R = H, Ph, Bz or HO–Et were prepared in the same way as described above for $[Pt(N–Me–IDA)(NH_3)_2]$. For the complexes in which R = Et, ^{n}Pr, ^{i}Pr, ^{n}Bu, ^{s}Bu, ^{i}Bu, ^{t}Bu, or ^{n}Am, the barium salts of the ligands were not prepared *in situ* as described above. Rather, they were prepared from $R–NH_2$, chloroacetic acid and $BaCl_2$ following the procedure outlined by Stein *et al.* [4]. The rest of the procedure for the preparation of these complexes was analogous to that discussed above.

Results and Discussion

Synthesis and Characterization of Complexes

When an aqueous solution of barium *N*-methyliminodiacetate is mixed with an aqueous solution of $[Pt(SO_4)(NH_3)_2]$, immediate precipitation of barium sulfate occurs. The precipitate can be filtered and the solution evaporated to dryness to give $[Pt(N–Me–IDA)(NH_3)_2]$ as a solid (eqn. (1)).

$$[Pt(SO_4)(NH_3)_2] + Ba(N–Me–IDA) \longrightarrow [Pt(N–Me–IDA)(NH_3)_2] + BaSO_4 \quad (1)$$

A number of other $[Pt(N–R–IDA)(NH_3)_2]$ complexes can be prepared in an analogous manner (see Experimental Section). These complexes can be purified by passing an aqueous solution of the complex through an Amberlite-MB ion-exchange column, followed by evaporation to dryness. The iminodiacetato(diammine)platinum(II) complexes obtained in this manner usually crystallize with some degree of hydration, as evidenced by broad O–H stretching bands (around 3400 cm^{-1}) in their infrared spectra, and by elemental analyses (Table I).

In order to establish the neutrality of the complexes and check their stability in solution, conductivity measurements were performed. Molar conductivities were recorded for 1.0 mmolar aqueous solutions of the complexes immediately after the solutions were prepared and also after standing at room temperature for 24 h. The results are displayed in Table II. It can be seen that the molar conductivity values for all the complexes fall well below the 118–131 cm^2 eq^{-1} Ω^{-1} range expected for a 1:1 electrolyte [5]. Thus, the $[Pt(N–R–IDA)(NH_3)_2]$ complexes are neutral in solution. Furthermore, the molar conductivity shows little change after the

TABLE I. Elemental Analyses for $[Pt(N–R–IDA)(NH_3)_2] \cdot xH_2O$ Complexes

R	*x*	Found(calc.) (%)		
		C	H	N
H	0	13.29(13.33)	3.48(3.08)	11.54(11.66)
Me	2	14.54(14.63)	3.72(4.18)	10.21(10.24)
Et	1	18.10(17.73)	4.49(4.22)	9.81(10.34)
^{n}Pr	1	19.67(20.00)	4.20(4.56)	9.71(10.00)
^{i}Pr	1	19.72(20.00)	4.03(4.56)	9.58(10.00)
^{n}Bu	1	22.52(22.12)	4.67(4.87)	9.63(9.67)
^{s}Bu	2	21.67(21.24)	4.98(5.15)	8.83(9.29)
^{i}Bu	0	23.87(23.08)	5.20(4.60)	8.08(10.09)
^{t}Bu	2	21.41(21.24)	5.07(5.12)	9.24(9.29)
^{n}Am	1	24.06(24.11)	4.86(5.17)	9.51(9.37)
Ph	0	27.49(27.52)	3.73(3.44)	9.24(9.63)
Bz	0	28.99(29.33)	4.19(3.78)	9.11(9.33)
HO–Et	2	16.47(16.36)	4.36(4.35)	9.34(9.54)

TABLE II. Molar Conductivity Data[a] for [Pt(N–R–IDA)-$(NH_3)_2$] Complexes

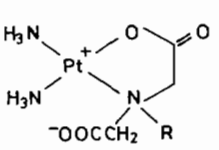
R	Λ_M (t = 0)	Λ_M (t = 24 h)
H	26.7	26.6
Me	53.9	48.8
Et	18.8	18.7
^{n}Pr	24.8	21.8
^{i}Pr	46.8	50.5
^{n}Bu	60.1	61.5
^{s}Bu	39.2	50.6
^{i}Bu	nd[b]	nd
^{t}Bu	58.3	55.7
^{n}Am	8.6	13.8
Ph	nd	nd
Bz	31.3	25.7
HO–Et	27.8	28.6

[a]Complexes were dissolved in water at a final concentration of 1 mM and molar conductivity measurements were made immediately after dissolution (t = 0) then again after a 24 h incubation at room temperature (t = 24). Molar conductivity values are given in units of $cm^2\ eq^{-1}\ \Omega^{-1}$. [b]nd = not determined.

solutions have kept at ambient temperature for 24 h, suggesting that the complexes are not susceptible to hydrolysis.

The pH of a 0.1 M solution of [Pt(N–Me–IDA)-$(NH_3)_2$] was measured over a 72 h period. The pH essentially remained constant over this period, at an average pH value of 5.88. The pH for such a solution would be expected to be neutral. The slightly acidic pH measured may be due to a small degree of hydrolysis, which might also explain the somewhat high molar conductivity value obtained for this complex (Table II), although no evidence for such a hydrolysis reaction is observed in the ^{1}H NMR spectrum of [Pt(N–Me–IDA)$(NH_3)_2$] (see below).

All of the [Pt(N–R–IDA)$(NH_3)_2$] complexes have been characterized by infrared spectroscopy, and in the case of R = H, Me, Et or ^{n}Pr by proton NMR spectroscopy. The infrared spectroscopic data are presented in Table III, and the proton NMR data appear in Table IV. The spectroscopic data are consistent with structure **A**. The infrared spectra display two bands (not resolved in some cases) in the carbonyl region. These bands correspond to two different types of carbonyl groups, that is, the carbonyl group that is coordinated to the platinum center and that which is uncoordinated. A similar situation is seen in the region 1300–1400 cm^{-1},



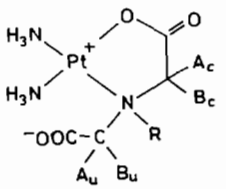
A

TABLE III. Infrared Data[a] for [Pt(N–R–IDA)$(NH_3)_2$] Complexes

R	C=O[b]	C=O[c]	C–O[c]	C–O[b]
H	1620[d]		1391	1321
Me	1652	1610	1390	1320
Et	1675	1602	1415	1305
^{n}Pr	1645	1605	1395	1320
^{i}Pr	1625[d]		1398	1330
^{n}Bu	1671	1619	1390	1322
^{s}Bu	1660	1630	1390	1336
^{t}Bu	1645[d]		1400	1330
^{n}Am	1675	1600	1380	1335
Bz	1680[d]		1420	1320
HO–Et	1650	1610	1408	1321

[a]All spectra were recorded as KBr pellets. Band positions are reported in cm^{-1}. [b]Coordinated carboxylate group. [c]Uncoordinated carboxylate group. [d]For those complexes in which the two carboxylate peaks are not resolved.

TABLE IV. Proton NMR data[a, b] for [Pt(N–R–IDA)-$(NH_3)_2$] Complexes



R	H	Me	Et
δA_c	3.89	3.77	3.88
δB_c	3.34	3.69	3.61
$J(A_cB_c)$	18.8	16.2	16.6
δA_u	3.56	3.43	3.46
δB_u	3.35	3.29	3.40
$J(A_uB_u)$	16.2	15.9	15.9
δA_α	–[c]	2.83[d]	3.07
δB_α			2.90
$J(A_\alpha B_\alpha)$			14.0
δA_β			1.31
$J(A_\beta A_\alpha) = J(A_\beta B_\alpha)$			7.2

[a]The notation used in the table is defined as follows:

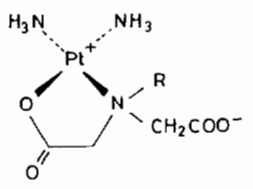
	R		
	H	$-CH_3$	$-CH_2CH_3$
Designation	α	α	α β

For the various AB quartet patterns, A_cB_c = methylene group incorporated into chelate ring; A_uB_u = methylene group of uncoordinated end. Other ABs designated as α or β as indicated above. [b]All spectra were recorded at 200 MHz for D_2O solutions. Chemical shifts (δ (ppm)) are referenced to the HDO peak at 4.67 ppm. All coupling constants are given in Hz. [c]Imino proton not observed because of exchange with solvent. [d]^3J(Pt, H) = 29.9 Hz.

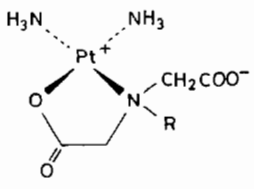
where the C–O single bond stretch for the coordinated carboxylate group is observed, as well as the symmetric C–O stretch for the uncoordinated carboxylate group see (Table III).

The 200 MHz proton NMR spectrum of [Pt(N–Me–IDA)$(NH_3)_2$] in D_2O displays two AB patterns, which are assigned to the protons of the two different methylene groups. The methylene protons incorporated into the chelate ring appear as an AB pattern slightly downfield from the resonances due to the uncoordinated methylene group, as indicated in Table IV. The methyl protons appear as a singlet at 2.83 ppm with platinum satellites (^3J(Pt, H) = 29.9 Hz). Furthermore, the 75.5 MHz $^{13}C\{^1H\}$ NMR spectrum of this compound exhibits two different peaks in the carbonyl region, the peak at 183.7 ppm being assigned to the coordinated carboxylate carbon and the peak at 172.3 ppm being assigned to that for the uncoordinated carboxylate group. Again, two different methylene groups are observed (65.3 and 64.2 ppm), and one methyl resonance (49.3 ppm). Appleton *et al.* have reported the formation of this compound during the reaction of $[Pt(OH_2)_2(NH_3)_2]^{2+}$ with H_2(N–Me–IDA) [6]. The spectral parameters they report are qualitatively similar to the ones that we have obtained, but the peak positions are different for our compound. This is probably due to the fact that Appleton *et al.* recorded their spectra at a pD of 3.5, whereas ours were recorded in neutral solution.

The structure depicted as A can exist as two stereoisomers: **B** and **C**. In the case of the [Pt(N–R–IDA)$(NH_3)_2$], the two stereoisomers are enantiomeric, thus giving identical peaks in the NMR spectrum. This contrasts to complexes of the type [Pt(N–R–IDA)(DACH)] (DACH = *cis*-, *trans-R,R*- or *trans-S,S*-1,2-diaminocyclohexane), where, because of the chirality of the DACH ligand, the two stereoisomers are diastereomeric. These latter compounds display resonances due to both **B** and **C** in their proton, carbon-13, and platinum-195 NMR spectra [7].



B



C

The proton NMR spectrum of [Pt(H–IDA)$(NH_3)_2$] in D_2O is similar to that for [Pt(N–Me–IDA)$(NH_3)_2$]. Two AB patterns are observed, one corresponding to the methylene group incorporated into the five-membered chelate ring, the other due to the methylene group of the uncoordinated end of the IDA ligand (Table IV). The imino proton is not observed because of exchange with the deuterated solvent.

The proton NMR spectrum of [Pt(N–Et–IDA)$(NH_3)_2$] is consistent with a structure of the type **A** (Table IV). Like [Pt(N–Me–IDA)$(NH_3)_2$], the two different methylene groups appear as AB quartets. Interestingly, the methylene group of the ethyl group does not appear as the usual simple quartet; rather, the two protons of this group are nonequivalent, resulting in a complex multiplet. The interpretation of the spectrum is simplified by homonuclear decoupling experiments. When the triplet resonance for the adjacent methyl group is irradiated, the multiplet collapses into a simple AB ($A\alpha B\alpha$) pattern, which can be analyzed in the usual way (Table IV). Because the two methylene protons A and B are magnetically nonequivalent, the methyl protons would be expected to appear as a doublet of doublets. In actuality, the methyl resonance appears as a 1:2:1 triplet, which indicates that the couplings between the methyl protons and A and B are equal ($J(A_\beta, A_\alpha) = J(A_\beta, B_\alpha) = 7.2$ Hz).

Because the methylene protons of the ethyl group (protons A_α and B_α) are nonequivalent, rotation about the N–C_α bond must be restricted. The reason for this is not entirely clear, but it is most probably due to some steric interaction. It should also be noted that because the methylene protons of the uncoordinated part of the IDA ligands (A_u and B_u) are nonequivalent, rotation about this N–C bond must also be restricted. This is most probably due to an interaction between the positively charged platinum center and the negatively charge carboxylate group.

The proton NMR spectrum of [Pt(N–^{n}Pr–IDA)$(NH_3)_2$] is very complicated and the spectral parameters have not been worked out in detail. However, the spectrum is qualitatively similar to those discussed above, with AB patterns being observed for the coordinated and uncoordinated methylene groups ($\delta A_c = 3.94$, $\delta B_c = 3.61$ and $J(A_cB_c) = 16.7$ Hz; $\delta A_u = 3.47$, $\delta B_u = 3.39$ and $J(A_uB_u) = 16.0$ Hz). For the propyl group, the protons of the methylene group bound to the imino nitrogen are magnetically nonequivalent (A_α and B_α), as was the case with the analogous ethyl derivative. Furthermore, the other methylene group also displays nonequivalent protons (A_α and B_α). The methyl group appears as a 1:2:1 triplet.

Biological Studies

The initial antitumor testing performed with these complexes was *in vitro* cytotoxicity against L1210/0 cells (Table V). With the exceptions of [Pt(N–^{n}Pr–IDA)$(NH_3)_2$] and [Pt(N–^{n}Bu–IDA)$(NH_3)_2$], the IC_{50} values for the [Pt(N–R–IDA)$(NH_3)_2$] complexes ranged from 3.5 to 6.8 g/ml, indicating that all of the complexes had acceptable cytotoxicity *in vitro*. To evaluate the *in vivo* efficacy, the complexes

TABLE V. *In Vitro* Cytotoxicity of [Pt(N–R–IDA)$(NH_3)_2$] Complexes

R	IC_{50} (μg/ml)[a]
H	6.6
Me	4.6
Et	6.8
^{n}Pr	>10
^{i}Pr	4.8
^{n}Bu	>10
^{s}Bu	3.5
^{n}Am	5.9
Ph	5.5
Bz	4.6
HO–Et	6.4
cis-DDP	0.1

[a]The IC_{50} (*i.e.* that drug concentration causing a 50% inhibition of cell growth) was determined for each drug in L1210 murine leukemic cells as described in 'Experimental' section.

TABLE VI. *In Vivo* Antitumor Efficacy of [Pt(N–R–IDA)$(NH_3)_2$] Complexes[a]

R	Dose (mg/kg)	*T/C* (%)
Me	50	98
	25	103
	12.5	98
Et	50	113
	25	110
	12.5	117
^{n}Pr	50	130
	25	117
	12.5	130
^{t}Bu	50	115
	25	103
	12.5	98
^{n}Am	50	77
	25	98
	12.5	95
HO–Et	50	100
	25	103
	12.5	84
cis-DDP	10	156

[a]Drugs were administered as a single i.p. injection into mice 24 h following inoculation with L1210 leukemia cells as described in 'Experimental' section.

were administered as a single i.p. injection into BDF_1 mice that had been inoculated the previous day with 10^6 viable L1210/0 cells (Table VI). At the dosage used, none of the complexes exhibited significant antitumor activity. Selected complexes (R = Me, HO–Et, ^{t}Bu or ^{n}Am) were further evaluated for *in vivo* efficacy when administered on a multiple-dose schedule. As can be seen in Table VII, none of

TABLE VII. *In Vivo* Antitumor Efficacy of [Pt(N–R–IDA)$(NH_3)_2$] Complexes[a]

R	Dose (mg/kg)	*T/C* (%)
Me	25	106
	12.5	106
	6.25	104
^{t}Bu	25	125
	12.5	110
	6.25	125
^{n}Am	25	117
	12.5	111
	6.25	111
HO–Et	25	116
	12.5	109
	6.25	109
cis-DDP	5	220

[a]Drugs were administered to mice on days 1, 5 and 9 24 h following inoculation with L1210 leukemia cells as described in 'Experimental' section.

the complexes tested in this manner exhibited significant antitumor activity. We have concluded that, although these complexes are cytotoxic, they do not have significant antitumor activity in mice.

Summary

We have prepared a series of *N*-substituted iminodiacetato(diammine)platinum(II) complexes and examined their antitumor activity. These complexes exhibited modest cytotoxicity *in vitro* but no significant antitumor activity *in vivo*.

Acknowledgement

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