Synthesis and Characterisation of 2:1 Guanosine and Inosine Derivatives of Aromatic Diamine Platinum(II) Complexes

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The six platinum complexes of formula [Pt(LL)-(NuO)$_2$]Cl$_2$, where LL is o-phenylenediamine, 4,5-dimethyl-o-phenylenediamine or 2,2'-bipyridyl and NuO is a nucleoside such as guanosine or inosine, have been prepared by interacting an appropriate dichlorodiamine-platinum(II) with an appropriate nucleoside in excess. The resulting complexes have been characterized by chemical analysis and by ultraviolet-visible, infrared and ^{1}H NMR spectroscopy. The ^{1}H NMR study has established the binding of guanosine and inosine to platinum through N$_7$. The dicationic nature of the complexes has been established by conductivity measurements which show them to be 1:2 electrolytes.

Introduction

Cis-[Pt(NH$_3$)$_2$Cl$_2$] (cisplatin) is an anticancer agent which is now comparable to organic drugs such as adriamycin and fluorouracil [1, 2]. The selective killing of tumor cells is believed to be due to the attack of guanine and cytosine rich regions of DNA, producing damage which is repairable by normal cells. Many compounds of cisplatin with nucleosides, particularly guanosine, have been described [3–9] as models of cisplatin–DNA interaction. The *cis*-Pt(NH$_3$)$_2$ moiety can give complexes such as *cis*-[Pt(NH$_3$)$_2$(guanosine)$_2$]Cl$_2$ in the presence of excess nucleoside. In these complexes the guanosine molecules coordinate *via* N7 [3–7]. The *cis*-Pt(NH$_3$)$_2$ moiety can also give monoguanosinato platinum complexes such as *cis*-[Pt(NH$_3$)$_2$(guanosine-H)]NO$_3$, where the guanosine anion is bonded through N(7)–O(6) chelation [8, 9].

Recently 1:2 complexes of cisplatin with guanosine, inosine and xanthosine have shown a low but significant degree of anticancer activity and have definitely lower toxicity and higher solubility than cisplatin [7]. The aromatic diamine platinum(II) complexes such as [Pt(OPDA)Cl$_2$] and [Pt(DMOPDA)Cl$_2$] show higher anticancer activity [10, 11] and [Pt(bipy)Cl$_2$] shows lower anticancer activity [12] than cisplatin. The difference in anticancer activity between these complexes suggests that they bind differently to DNA. This may be reflected in different modes of binding in 1:2 complexes of cisplatin, [Pt(OPDA)Cl$_2$], [Pt(DMOPDA)Cl$_2$] and [Pt(bipy)Cl$_2$] with guanosine. Therefore, we report here the synthesis and characterization of 1:2 complexes of [Pt(OPDA)Cl$_2$], [Pt(DMOPDA)Cl$_2$] and [Pt(bipy)Cl$_2$] with guanosine and inosine.

Experimental

Commercially available guanosine (Guo) of SISCO, India, Inosine (Ino) of Sigma, U.S.A., *o*-phenylenediamine (OPDA), 4,5-dimethyl-*o*-phenylenediamine-(DMOPDA) and 2,2'-bipyridine(bipy) of B.D.H., U.K., were used without further purification. Potassium tetrachloroplatinate(II), K$_2$PtCl$_4$, was purchased from Strem Chemical Co., U.S.A.

Synthesis of Platinum Complexes

The reported procedures were followed to synthesize [Pt(OPDA)Cl$_2$ [13], [Pt(DMOPDA)Cl$_2$] [13] and [Pt(bipy)Cl$_2$] [14].

[Pt(bipy)(Guo)$_2$]Cl$_2$

An aqueous suspension of [Pt(bipy)Cl$_2$] and guanosine of 50 ml in molar ratio of 1:2.5 was stirred at 60–70 °C for 4–6 hours. The resulting light yellow solution was concentrated to 10 ml and kept overnight in the refrigerator. The unreacted guanosine was removed by filtration. The filtrate was further concentrated to 3–4 ml and cooled. The unreacted guanosine was again filtered. The filtrate was mixed with acetone to precipitate the desired compound.

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TABLE I. Chemical Analyses, Color and Conductivity Data of $[Pt(LL)(Nuo)_2]Cl_2$ Complexes.

Complex	Color	Found			Calculated			Conductivity of 10^{-3} *M* water solutions $cm^2\ ohm^{-1}\ mol^{-1}$
		%C	%H	%N	%C	%H	%N	
$[Pt(OPDA)(Guo)_2]Cl_2 \cdot 4H_2O$	Violet-brown	29.84	4.45	16.00	30.83	3.75	16.60	190
$[Pt(OPDA)(Ino)_2]Cl_2 \cdot 2H_2O$	brown	33.14	3.86	15.23	32.98	3.38	14.80	275
$[Pt(DMOPDA)(Guo)_2]Cl_2 \cdot 4H_2O$	Yellowish-brown	31.57	4.80	15.88	32.30	4.04	16.15	230
$[Pt(DMOPDA)(Ino)_2]Cl_2 \cdot 2H_2O$	Brown	34.93	3.76	14.10	34.50	3.70	14.37	287
$[Pt(bipy)(Guo)_2]Cl_2 \cdot 4H_2O$	Yellow	33.58	5.03	15.30	33.96	3.58	15.85	223
$[Pt(bipy)(Ino)_2]Cl_2 \cdot 2H_2O$	Yellow	37.00	4.37	13.54	36.21	3.22	14.08	263

The complex was filtered, washed with acetone and dried in a vacuum at room temperature.

$[Pt(bipy)(Ino)_2]Cl_2$

$[Pt(bipy)Cl_2]$ (126.6 mg, 0.3 mmol) was suspended in 50 ml of water and inosine (160.8 mg, 0.6 mmol) dissolved in 10 ml of water was added to this suspension. The resulting mixture was stirred for 4–6 hours at 60–70 °C. The resulting clear yellow solution was concentrated to 10 ml and acetone was added to it, to obtain the desired compound. The yellow precipitate obtained was filtered, washed several times with acetone and dried in a vacuum at room temperature.

Other complexes of guanosine and inosine such as $[Pt(OPDA)(Guo)_2]Cl_2$, $[Pt(OPDA)(Ino)_2]Cl_2$, $[Pt(DMOPDA)(Guo)_2]Cl_2$ and $[Pt(DMOPDA)(Ino)_2]Cl_2$ were prepared by following the procedures used for $[Pt(bipy)(Guo)_2]Cl_2$ and $[Pt(bipy)(Ino)_2]Cl_2$ respectively.

Chemical analyses of carbon, hydrogen and nitrogen for the platinum complexes were carried out by the Microanalytical Laboratory of I.I.T., Bombay, India.

Physical Measurements

Physical measurements were carried out as described elsewhere [15]. The Pye Unichem SP-2000 infrared spectrophotometer was used for recording infrared spectra of these complexes in the range 4000 to 200 cm^{-1}.

Results and Discussion

Six platinum(II) complexes of formula $[Pt(LL)(Nuo)_2]Cl_2$, where LL = *o*-phenylenediamine, 4,5-dimethyl-*o*-phenylenediamine and 2,2′-bipyridine and Nuo = guanosine and inosine, have been prepared. These complexes are highly soluble in water. Their chemical analysis along with their colour and conductivity data are given in Table I. The conductivity data of these complexes suggest that they are 1:2 electrolytes in water [16].

The infrared spectra of these platinum complexes show the disappearance of ν(M–Cl) bands which are present in $[Pt(bipy)Cl_2]$ at 348 (shoulder) and 337 cm^{-1} [17] and in $[Pt(OPDA)Cl_2]$ and $[Pt(DMOPDA)Cl_2]$ at 324 and 319 cm^{-1} [13]. This indicates that nucleosides have replaced both chloride ions from the first coordination sphere of platinum. The presence of ν(C=O) at 1690–1700 cm^{-1} in these platinum complexes suggests that the C=O group of these complexes is not involved in binding to platinum. The nucleoside seems to be bonded to platinum through N_7 only.

The electronic absorption maxima (λ_{max}) of the platinum complexes with their extinction coefficients (ϵ_{max}) are given in Table II. The spectrum of $[Pt(OPDA)Cl_2]$ shows five bands. These bands can be tentatively assigned following the band assignments of aqueous $PtCl_4^{2-}$ [18]. The band at 17.06 kK is assigned to a singlet → triplet transition. The three bands between 21 to 30.5 kK are assigned to d–d transitions. The band at 36.36 kK is tentatively assigned to a charge transfer transition. In the $[Pt(OPDA)(Guo)_2]Cl_2$, the singlet → triplet transition is observed at 17.92 kK. The two bands between 28 and 33.5 kK are assigned to d–d transitions. The band at 35.84 kK is assigned to a π–π^* transition of the guanosine base and the band at 39.22 kK is assigned to a π–π^* transition of *o*-phenylenediamine.

In the $[Pt(OPDA)(Ino)_2]^{2+}$, the singlet → triplet transition is observed at 17.61 kK. The three d–d transitions are observed between 28 and 33.5 kK. The transition at 40.16 kK is assigned to a π–π^* transition of *o*-phenylenediamine. The bands of $[Pt(DMOPDA)Cl_2]$, $[Pt(DMOPDA)(Guo)_2]^{2+}$ and

TABLE II. Electronic Absorption Spectra of $[Pt(LL)(Nuo)_2]Cl_2$ Complexes.

Complex	λ_{max} in kK[a]				
$[Pt(OPDA)Cl_2]$[b]	17.06,	21.46,	25.25,	30.30,	36.36
$[Pt(OPDA)(Guo)_2]Cl_2 \cdot 4H_2O$[c]	17.92, (0.40)[d]	19.61, (0.43)	28.82, (0.67)	35.84, (2.15)	39.22 (2.67)
$[Pt(OPDA)(Ino)_2]Cl_2 \cdot 2H_2O$[c]	17.61, (0.20)		28.82, (0.33)	33.33, (0.49)	40.16 (2.24)
$[Pt(DMOPDA)Cl_2]$[b]	17.86,	22.22,	25.00,	36.23	
$[Pt(DMOPDA)(Guo)_2]Cl_2 \cdot 4H_2O$[c]	16.95, (0.06)	25.00, (0.32)	29.41, (0.42)	35.71, (1.88)	39.22 (2.50)
$[Pt(DMOPDA)(Ino)_2]Cl_2 \cdot 2H_2O$[c]	17.36, (0.06)	22.22, (0.26)	28.99, (0.42)	40.49 (2.50)	
$[Pt(bipy)Cl_2]$[c]	26.31,	30.96,	32.15,	37.45,	39.68
$[Pt(bipy)(Guo)_2]Cl_2 \cdot 4H_2O$[c]	31.35, (2.07)	32.47, (1.95)	40.65 (4.64)		
$[Pt(bipy)(Ino)_2]Cl_2 \cdot 2H_2O$[c]	29.15, (0.20)	31.55, (1.33)	32.59, (1.14)	40.49 (3.78)	

[a]kK is 1×10^3 cm^{-1}. [b]Dimethyl formamide is used as solvent. [c]Water (double distilled) is used as solvent. [d]Extinction coefficients in 1 mol^{-1} $cm^{-1} \times 10^{-4}$ are given in parentheses.

TABLE III. ^1H NMR Spectral Data of $[Pt(LL)(Nuo)_2]^{2+}$ Complexes.

Compound	LL protons		Nuo protons		
	δ[a] benzene protons or $\delta_{H\text{-}6,6'}$	δ dimethyl protons or $\delta_{H_{5,5'}}$	δ_{H_8}	δ_{H_2}	$\delta_{H_1'}$
Guanosine[b]			7.99		5.75
Inosine[b]			8.34	8.21	6.09
$[Pt(OPDA)Cl_2]$[c]	7.34				
$[Pt(OPDA)(Guo)_2]^{2+}$[b]	7.64		8.64		6.16
$[Pt(OPDA)(Ino)_2]^{2+}$[b]	7.58		9.04	8.48	6.28
$[Pt(DMOPDA)Cl_2]$[c]	7.08	2.27			
$[Pt(DMOPDA)(Guo)_2]^{2+}$[b]	7.34	2.36	8.63		6.10
$[Pt(DMOPDA)(Ino)_2]^{2+}$[b]	7.30	2.34	9.02	8.42	6.22
$[Pt(bipy)Cl_2]$[c]	9.52	7.86			
$[Pt(bipy)(Guo)_2]^{2+}$[b]	9.18	7.70	8.80		6.10
$[Pt(bipy)(Ino)_2]^{2+}$[b]	9.24	7.68	8.48	8.30	6.28

[a]δ is chemical shift in ppm. [b]D_2O is used as a solvent. [c]DMSO-d_6 is used as a solvent.

$[Pt(DMOPDA)(Ino)_2]^{2+}$ can be assigned following assignments of bands of corresponding platinum derivatives of *o*-phenylenediamine.

The bands of $[Pt(bipy)(Guo)_2]^{2+}$ and $[Pt(bipy)(Ino)_2]^{2+}$ can be assigned following band assignments of $[Pt(bipy)Cl_2]$ [19]. The two bands between 31–32 kK, and 40–41 kK in nucleoside derivatives are assigned to first and second internal π–π^* type transitions respectively of 2,2′-bipyridine. In the inosine derivative, the band observed at 29.15 kK is assigned to charge transfer from the platinum orbital to the π-antibonding orbital of 2,2′-bipyridine.

The ^1H NMR spectral data of platinum nucleoside complexes are given in Table III. The data include only important proton chemical shifts (δ ppm) of aromatic diamines and nucleosides. These shifts have been compared with the chemical shifts of free nucleosides and $[Pt(LL)Cl_2]$ complexes. The coupling constants of ^{195}Pt (natural abundance

of 34%) with protons of guanosine and inosine in platinum nucleoside complexes could not be measured because of the overlap of protons of nucleosides with the protons of aromatic diamines. However, the large chemical shifts of protons of guanosine and inosine have been used to determine the binding sites in these platinum nucleoside complexes [3], and therefore these only are discussed below.

The H_8 proton of guanosine and the H_8 and H_2 protons of inosine in $[Pt(OPDA)(Guo)_2]^{2+}$, $[Pt(DMOPDA)(Guo)_2]^{2+}$, $[Pt(OPDA)(Ino)_2]^{2+}$ and $[Pt(DMOPDA)(Ino)_2]^{2+}$ are shifted downfield compared with free guanosine or inosine. The H_8 proton shifts are sufficiently large and suggest that the binding site of guanosine and inosine is N_7. The marginal downfield chemical shifts of H_1' proton in the platinum nucleoside complexes compared to their values in free nucleosides also support the binding site of guanosine and inosine as N_7 [3]. The aromatic protons of diamines in nucleoside complexes as compared to $[Pt(OPDA)Cl_2]$ and $[Pt(DMOPDA)Cl_2]$ are also shifted downfield. The dimethyl protons of 4,5-dimethyl-*o*-phenylenediamine are also shifted marginally downfield in the nucleoside complexes. This may be due to a change of conformation of aromatic diamines on formation of nucleoside derivatives.

The $H_{6,6'}$ and $H_{5,5'}$ protons of 2,2'-bipyridine in $[Pt(bipy)Guo)_2]^{2+}$ and $[Pt(bipy)(Ino)_2]^{2+}$ are shifted upfield compared with $[Pt(bipy)Cl_2]$. The considerably larger upfield shifts of $H_{6,6'}$ protons than $H_{5,5'}$ protons can be explained in terms of stronger binding of nucleosides than chloride ions in their nucleoside derivatives [15]. The downfield shifts of the H_8 proton of guanosine and the H_8 and H_2 protons of inosine in their nucleoside derivatives indicate that these nucleosides are bonded to platinum through N_7 [3].

Six aromatic diamine (LL) platinum(II) complexes such as $[Pt(LL)(Guo)_2]^{2+}$ and $[Pt(LL)(Ino)]^{2+}$ have been prepared. Guanosine and inosine bind to platinum through N_7 in these nucleoside complexes.

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