Pure & Appl. Chem., Vol. 60, No. 8, pp. 1271–1278, 1988.
Printed in Great Britain.

Structure, activity and mode of action of antitumor platinum compounds

Tang Wenxia, Qu Yun, Dai Anbang

Coordination Chemistry Institute of Nanjing University, P.R.China

Abstract - Quantitative relationship beween activity and structure of antitumor platinum complexes was studied by CNDO/2 method. On the basis of the obtained regression results, some new platinum complexes with methyl substituted aliphatic and cycloaliphatic amines were synthesized and screened, and a new complex [Pt(TMCPDA)(Cl-Ac)$_2$] possessing high activity as cisplatin but with much lower toxicity was found. The interaction of cisplatin with the constituents of DNA was studied by ^{13}C NMR and CNDO/2 method. The reaction mechanism of cisplatin was discussed.

INTRODUCTION

Cisplatin is an effective agent in the treatment of several human cancers. Its clinical effectiveness is limited, however, by dose toxicities such as renal failure, nausea and vomiting. Ever since the early 1970's efforts have been underway to develop additional platinum complexes that would possess higher antitumor activity and lower toxicity, to discover the relationship between activity and structure of the compounds and to elucidate the molecular mechanism of cisplatin. In recent years, antitumor platinum chemistry achieved a rapid development. Some new second generation platinum analogues such as [Pt(isopr)$_2$Cl$_2$(OH)$_2$] [Pt(NH$_3$)$_2$(1, 1-CBDCA)] and diaminocyclohexane analogues, which have similar activity with cisplatin but lower toxicity than the parent compound, were found and have been put in clinical trials. The relationship between activity and structure of antitumor platinum complexes has become comparatively clear, although extensive quantitative study of the relationship remains to be desired. There is not enough theoretical ground to predict and design a new antitumor Pt complex of high activity and low toxicity. With regard to the action mechanism of cisplatin, there is currently considerable evidence that DNA is the principal intracellular target of cisplatin in vivo. The intrastrand cross-linking formed between cisplatin and DNA through N_7,N_7 atoms of two neighbouring guanines, the chelation formed through binding of cisplatin with N_7,O_6 atoms of guanine in DNA sequence, and the interstrand cross-linking mechanism have been proposed. The results obtained recently by high frequency ^{1}H NMR study of oligonucleotide-cisplatin adducts and enzymatic digestion studies show that Pt(NH$_3$)$_2$ unit has a strong preference for chelation to two N_7 atoms of neighbouring guanines, and induce a distortion of DNA duplex, which might be quite similar to that of a kink or of a bent B DNA, but does not preclude duplex formation with a complementary strand in the case of decamer which has the G-G dimer situated in the center (refs. 1,2,3). The action mechanism of cisplatin has not yet been elucidated clearly.

In this paper we summarize recent results obtained in our laboratory including (1) the quantitative relationship between activity and structure of platinum complexes, (2) design, preparation and activity of some new antitumor compounds on the basis of quantitative relationship found and (3) action mode of cisplatin with DNA.

QUANTITATIVE RELATIONSHIP BETWEEN ACTIVITY AND STRUCTURE OF ANTITUMOR Pt COMPOUNDS

In order to find and design new antitumor platinum compounds with higher activity and lower toxicity, the study of quantitative relationship between activity and structure of platinum compounds is important. Webb (ref. 4) and Dimogro (ref. 5) have made some study on this problem, but their conclusions can scarcely be relied upon to predict more active compounds. We have studied by CNDO/2-SCF method the electronic structure of about 40 platinum complexes of cis-[PtAm$_2$Cl$_2$], whose activity against ADJ/PC6 in mice of each is well known, and cis-[PtAm$_2$X$_2$], activity of which against S-180 in mice is also known. Complexes are taken to be square planar, with Pt-Cl bond distance of 2.309 Å as in PtCl$_2$ and Pt-N bond distance of 2.052 Å as in Pt(NH$_3$)$_4^{2+}$. Substituent coordinates are determined using standard bond parameters from Pople (ref. 6). The torsional angle of R-NH$_2$ group about its bond with Pt in cis-[PtAm$_2$Cl$_2$] has been optimized. For the bidentate amino ligand, the geometries chosen comprise the known bonding characteristics of the moieties involved as closely as possible. In addition, for [PtAm$_2$X$_2$] complexes with different acidate groups the Pt-X bond distance and

the geometries of acidate groups were selected from crystal structure data of related compounds. Electronic indices such as charges on Pt,X,N atoms, acidate and amine moieties, overlap population of Pt-Cl,Pt-N bonds, and ΔE,ΔQ for 21 complexes of $[PtAm_2Cl_2]$ and 23 complexes of $[PtAm_2X_2]$ were obtained and are shown in Table 1 and 2, and used as independent variables in the following regressions. The variables used are known as lethal dose (LD_{50}),

TABLE 1. Electronic structures of complexes $[PtAm_2Cl_2]$

No.	Am	q_{Pt}	$-q_{Cl}$	$-q_N$	q_{Am}	ΔE	Q_{Pt-Cl}	Q_{Pt-N}	ΔQ	N_H
1	Methylamine	0.5825	0.8666	0.2121	0.2841	0.3426	0.4574	0.3103	0.1474	4
2	2-Methyl-propylamine	o.5920	0.8996	0.2428	0.3076	0.3080	0.4545	0.3180	0.1366	4
3	Tetromethyleneimine	0.6349	0.9234	0.2200	0.2885	0.3270	0.4408	0.3183	0.1225	2
4	OPHENDA*	0.5847	0.8752	0.2481	0.2905	0.2305	0.4575	0.3056	0.1519	4
5	4-Methyl-OPHENDA	0.5893	0.8910	0.2555	0.3017	0.2210	0.4562	0.3091	0.1471	4
6	4,5-Dimethyl-OPHENDA	0.5943	0.9100	0.2644	0.3157	0.2045	0.4547	0.3133	0.1414	4
7	4-Carboxy-OPHENDA	0.5917	0.9074	0.2715	0.3157	0.1997	0.4548	0.3135	0.1413	4
8	Cyclopropylamine	0.6037	0.9058	0.1986	0.3021	0.3292	0.4537	0.3114	0.1423	4
9	Cyclopentylamine	0.6172	0.9304	0.2463	0.3132	0.3092	0.4471	0.3204	0.1267	4
10	Pentamethyleneimine	0.6131	0.8984	0.2282	0.2853	0.3168	0.4468	0.3127	0.1341	2
11	Isopropylamine	0.5984	0.9094	0.2372	0.3110	0.3226	0.4506	0.3149	0.1358	4
12	Tert-butylamine	0.5928	0.9098	0.2413	0.3170	0.3063	0.4504	0.3083	0.1422	4
13	n-Ethylamine	0.5854	0.8796	0.2264	0.2942	0.3304	0.4562	0.3130	0.1433	4
14	n-Propylamine	0.5851	0.8790	0.2292	0.2939	0.3216	0.4563	0.3127	0.1436	4
15	n-Butylamine	0.5891	0.8852	0.2272	0.2961	0.3128	0.4557	0.3132	0.1425	4
16	Ammine	0.5791	0.8561	0.2466	0.2770	0.3739	0.4599	0.3150	0.1449	6
17	Dimethyleneimine	0.6364	0.8722	0.1598	0.2358	0.3483	0.4516	0.2931	0.1585	2
18	Cyclohexanamine	0.6026	0.9338	0.2558	0.3312	0.2867	0.4482	0.3204	0.1278	4
19	Ethylenediamine	0.5888	0.8382	0.2366	0.2494	0.3006	0.4630	0.2828	0.1802	4
20	Cyclobutylamine	0.6093	0.9344	0.2633	0.3251	0.2871	0.4498	0.3230	0.1268	4
21	1,2-Diaminocyclohexane	0.5949	0.8708	0.2381	0.2859	0.2558	0.4581	0.3056	0.1525	4

* OPHENDA: o-phenylenediamine

q_{Pt},q_{Cl},q_N,q_{Am} are electronic charge on corresponding atoms and moiety; ΔE, difference energy between LUMO and HOMO; Q_{Pt-Cl},Q_{Pt-N}, overlap population on corresponding bond of Pt-Cl and Pt-N; $\Delta Q \equiv Q_{Pt-Cl} - Q_{Pt-N}$; N_H, number of protons N atoms of amine.

TABLE 2. Electronic structures of complexes $[PtAm_2X_2]$

No.	Complexes	q_{Pt}	$-q_B$	$-q_N$	$-q_X$	q_{Am}	Q_{Pt-Cl}	Q_{Pt-N}	ΔQ	ΔE
	Ammine									
1	Cl	0.5791	0.4280	0.2466	0.8561	0.2770	0.4599	0.3150	0.1449	0.3739
2	Br	0.5714	0.4187	0.2478	0.8374	0.2660	0.5684	0.3143	0.2541	0.3300
3	I	0.5587	0.3931	0.2422	0.7863	0.2276	0.6231	0.3054	0.3177	0.2542
4	Ox	0.4536	0.3862	0.2279	0.7192	0.2656	0.4414	0.3051	0.1363	0.2605
5	Mal	0.4606	0.3371	0.2300	0.7237	0.2637	0.4096	0.3044	0.1052	0.2756
6	OHmal	0.4582	0.3283	0.2314	0.7223	0.2641	0.4071	0.3059	0.1012	0.2426
7	Memal	0.5422	0.3604	0.2444	0.8210	0.2788	0.4194	0.3099	0.1095	0.2540
8	Etmal	0.5353	0.3529	0.2439	0.8145	0.2792	0.4143	0.3100	0.1043	0.2610
9	(1,1-CBDCA)	0.6743	0.3631	0.2582	0.9991	0.3248	0.4131	0.3234	0.0897	0.2773
10	SCN	0.6023	0.3480	0.2524	0.8832	0.2809	0.5077	0.3192	0.1885	0.2093
	Methylamine									
11	Cl	0.5825	0.4333	0.2121	0.8666	0.2841	0.4574	0.3103	0.1471	0.3426
12	Br	0.5774	0.4233	0.2140	0.8466	0.2692	0.5649	0.3095	0.2554	0.3112
13	I	0.5686	0.3935	0.2122	0.7870	0.2184	0.6174	0.3022	0.3152	0.2451
14	Ox	0.4659	0.3868	0.1944	0.7329	0.2670	0.4399	0.3012	0.1387	0.2252
	Ethylenediamine									
15	Cl	0.5888	0.4191	0.2366	0.8382	0.2494	0.4630	0.2828	0.1802	0.3006
16	Br	0.5808	0.4105	0.2380	0.8210	0.2403	0.5759	0.2822	0.2937	0.2738
17	I	0.5722	0.3869	0.2331	0.7738	0.2015	0.6378	0.2770	0.3608	0.2191
18	Mal	0.4876	0.3965	0.2773	0.7726	0.2849	0.4314	0.2967	0.1347	0.1814
19	Memal	0.5431	0.3866	0.2838	0.8391	0.2961	0.4194	0.3015	0.1179	0.1597
20	Etmal	0.5046	0.3953	0.2778	0.7969	0.2923	0.4284	0.2984	0.1300	0.1802
21	SCN	0.8096	0.6175	0.2502	1.0427	0.2331	0.4868	0.2754	0.2114	0.2220
	1,2-Diaminocyclohexane									
22	Cl	0.5849	0.4354	0.2381	0.8708	0.2859	0.4581	0.3056	0.1525	0.2558
23	Mal	0.7295	0.2580	0.1779	0.2291	-0.5004	0.3593	0.2991	0.0602	0.1647

q_B,q_X are electronic charge on donated atom of acidate groups and on acidate group respectively.

inhibition dose (ID_{90}) and therapeutic index (TI) for complexes [$PtAm_2Cl_2$] against ADJ/PC6 (ref. 7) and known as toxic level (TL) and activity index (T/C) for [$PtAm_2X_2$] against S-180 (ref. 8) in mice.

Three set of regressions are obtained for cis-[$PtAm_2Cl_2$] against ADJ/PC6 in mice as shown in the following:

A. Lethal Dose (LD_{50})

$$p(LD_{50}) = 14.7384 + 12.0736q_X + 5.9897q_N + 0.2985N_H \quad \ldots (1)$$
$$R = 0.76,\ S = 0.43,\ F(3,17) = 7.86$$

B. Inhibition Dose (ID_{90})

$$p(ID_{90}) = -94.2087 + 41.9454q_{Pt} - 14.9199q_X + 7.7977q_N + 131.7526Q_{Pt-Cl} + 0.6751N_H \quad \ldots (2)$$
$$R = 0.71,\ S = 0.57,\ F(5,15) = 3.12$$

C. Therapeutic Index (TI)

$$lg(TI) = -30.3900 + 34.5077q_{Pt} - 9.4685q_X + 0.6080N_H \quad \ldots (3)$$
$$R = 0.74,\ S = 0.51,\ F(3,17) = 7.03$$

The significance level for each of the three regressions is more than 99%. Here R is the correlation coefficient; S, the standard deviation; and F, the variance ratio. All the regression results are better than those obtained by Webb. Eqns. (1), (2) and (3) indicate that higher the positive charge on Pt atom and the negative charge on chloro atom and lower the overlap population on Pt-Cl bond would result in higher drug activity and therapeutic index, and lower drug toxicity. The eqns. also indicate more hydrogen atoms in amino group, would mean higher activity and therapeutic index of the compound, although higher toxicity. It is seen in Table 1 the analogues of cisplatin with alicyclic and the methyl substituted aliphatic amines have higher positive charge on Pt atom and higher negative charge on chloro atom (ref. 9).

Two sets of regression are obtained for cis-[$PtAm_2X_2$] against S-180 in mice as shown in the following:

E. Toxic Level (TL)

$$p(TL) = 4.7921 - 1.2888q_{Pt} - 2.2855q_B + 1.0447q_X + 0.9669Q_{Pt-B} - 5.3158Q_{Pt-N} + 3.9787\Delta E \quad \ldots (4)$$
$$R = 0.88,\ S = 0.17,\ F(6,16) = 9.05$$

F. Inhibition (T/C)

$$lg(T/C) = 3.2758 + 8.2755q_N - 0.7640q_X + 2.5201\Delta Q - 3.5125\Delta E \quad \ldots (5)$$
$$R = 0.70,\ S = 0.32,\ F(4,18) = 4.4$$

The significance level for each of the above two regressions is also more than 99%. Eqn. (4) shows that the toxicity of complexes is dependent mainly on the quantities of Q_{Pt-N}, ΔE and q_B. Eqn. (5) confirms dependence of the inhibition of complexes on q_N, ΔE and ΔQ. It is noticeable that Q_{Pt-B}, Q_{Pt-N} and E appear in both the p(TL) and lg(T/C) regressions. As decreasing Q_{Pt-B} and increasing Q_{Pt-N} would decrease the toxicity and increase the activity, and increasing ΔE would increase the toxicity and decrease the activity, so a compound possessing lower toxicity and higher activity against S-180 must have lower Q_{Pt-B}, higher Q_{Pt-N} and medium ΔE values. In addition, higher the charge on N atom, higher would be the activity of the compound. From Table 2 it is clear that 6-9, 18-20 and 23 compounds with malonato and substituted malonato groups have higher charge on N atom, lower Q_{Pt-B}, higher Q_{Pt-N} and medium E values (ref. 10). All the above results show that regressions with $p(LD_{50})$, $p(ID_{90})$ and lg(TI) for cis-[$PtAm_2Cl_2$] of different amino groups against ADJ/PC6 and regressions with p(TL) and lg(T/C) for cis-[$PtAm_2X_2$] of different acidate and amino groups against S-180 as dependent variables have a high correlation and are highly significant. These findings might therefore be used as basis to predict platinum compounds with higher activity and lower toxicity.

DESIGNING, SYNTHESIS AND ACTIVITY OF SOME ANTITUMOR Pt COMPOUNDS

On basis of the obtainted regression results, some new platinum complexes of the type, cis-[$PtAm_2X_2$] and trans-[$PtAm_2X_2(OH)_2$] with methyl substituted aliphatic and cycloaliphatic

amines such as 2,3-dimethyl-2,3-butanediamine (DMBA) and 1,2,2'-trimethyl-1,3-cyclopentanediamine (TMCPDA) were synthesized and corresponding complexes with unsubstituted aliphatic and cycloaliphatic amine like 1,3-cyclopentanediamine (CPDA), 1,3-cyclohexanediamine (CHDA) and amantadine (AMT) were also synthesized for comparison (refs. 11-14). All compounds obtained were characterized by elemental analysis, infrared spectroscopic, thermogravimetric and differential thermal analysis. The animal tests were performed by Prof. Ji in Institute of Materia Medica, Chinese Academy of Medical Science. The activity of platinum compounds containing DMBA, TMCPDA and CPDA against L-1210 in DBA/2 mice is shown in Table 3 (refs. 11, 12). Data in Table 3 show that platinum compounds with methyl substituted amine have, in general, greater activity against L-1210 in DBA/2 than those of unsubstituted amine. In the dose range of 3-10 mg/kg all Pt^{2+} compounds of trimethyl-1,3-cyclopentanediamine have higher I.L.S% values, especially $[Pt(TMCPDA)Cl_2]$ and $[Pt(TMCPDA)(Cl\text{-}Ac)_2]$ have the highest values of greater than 124.4% and 117.3% respectively. At the dosage of 10-25 mg/kg I.L.S% of most Pt^{2} complexes are in the range of 30-50% and higher than that of the corresponding Pt complexes (ref. 11). Among all complexes of DMBDA tested, $[Pt(DMBDA)(Cl\text{-}Ac)_2]$ and $[Pt(DMBDA)(Cl\text{-}Ac)_2(OH)_2]$ possess the highest activity. Their I.L.S% are 107.6% and 104.2% respectively (ref. 12). In comparison with methyl substituted amine complexes Pt^{2+}, Pt^{4+} compounds of 1,3-cyclopentanediamine (Table 3) and amantadine possess lower activity against L-1210 in DBA/2 mice (refs. 14,15). With the exception of $[Pt(CPDA)Br_2]$ and $[Pt(AMT)_2(Cl\text{-}Ac)_2(OH)_2]$ whose I.L.S% are about 50%, all the rest complexes have only very low or no activity. The inhibition action of the compound of 1,3-cycloheptanediamine is rather strong, but the I.L.S% of $[Pt(CHDA)(Cl\text{-}Ac)_2]$, whose inhibition is stronger than that of the compound of 1,3-cycloheptanediamine, is about 96.5% and is lower than the corresponding compounds of 2,3-dimethyl-2,3-butanediamine and 1,2,2'-trimethyl-1,3-cyclopentanediamine (ref. 13). When $[Pt(TMCPDA)(Cl\text{-}Ac)_2]$ is compared with cisplatin (LD_{50} = 7.0mg/kg, LD_{50}/CD_{50} = 5.8 for the latter), the former has higher LD_{50} (13.0mg/kg) and chemotherapeutic index LD_{50}/CD_{50} (7.8) on S-180. In addition $[Pt(TMCPDA)(Cl\text{-}Ac)_2]$ has higher activity against S-180 (I.L.S% = 107.4) than the latter (I.L.S% = 94.9), but has lower activity against P388 (I.L.S% = 64.2), and both have similar activity against W-256 and Lewis Lung cancer at dosage of equal toxicity ($1/5LD_{50}$). Besides $[Pt(TMCPDA)(Cl\text{-}Ac)_2]$ scarcely has nephrotoxicity (ref. 16). The BUN, T-SH and Pr-SH in rat after single dose administration of $[Pt(TMCPDA)(Cl\text{-}Ac)_2]$ at LD_{50} dosage are similar to those of the control, only the serum creatinine value induced by the compound being somewhat higher, but much lower than the values induced by cisplatin at equal toxicity dosage (ref. 16).

TABLE 3. Variation of activity of Pt compounds of DMBDA, TMCPDA and CPDA against L-1210 in DBA/2 mice

No.	Compounds	Dose mg/kg	No. of Animals initial/final	I.L.S%	p
1	$[Pt(DMBDA)Cl_2]$	10x1	8/0	34.4	<0.01
2	$[Pt(DMBDA)Br_2]$	10x1	8/0	50.5	<0.01
3	[Pt(DMBDA)mal]	10x1	8/0	49.0	<0.01
4	[Pt(DMBDA)ox]	25x1	8/0	33.3	<0.01
5	$[Pt(DMBDA)(Cl\text{-}Ac)_2]$	2.5x1	8/0	107.6	<0.01
6	$[Pt(DMBDA)Cl_2(OH)_2]$	20x1	8/0	57.1	<0.01
7	$[Pt(DMBDA)Br_2(OH)_2]$	20x1	8/0	14.6	
8	$[Pt(DMBDA)mal(OH)_2]$	20x1	8/0	12.2	
9	$[Pt(DMBDA)ox(OH)_2]$	50x1	8/0	34.3	<0.01
10	$[Pt(DMBDA)(Cl\text{-}Ac)_2(OH)_2]$	25x1	8/0	104.2	<0.01
11	$[Pt(TMCPDA)Cl_2]$	3x1	10/6	124.4	<0.01
12	$[Pt(TMCPDA)Br_2]$	3x1	10/5	98.4	<0.01
13	[Pt(TMCPDA)ox]	3x1	10/3	74.8	<0.01
14	[Pt(TMCPDA)mal]	10x1	10/1	45.7	<0.01
15	$[Pt(TMCPDA)(Cl\text{-}Ac)_2]$	3x1	10/7	117.3	<0.01
16	$[Pt(TMCPDA)SO_4H_2O]$	3x1	10/2	59.1	<0.01
17	$[Pt(TMCPDA)(NO_3)_2]$	3x1	10/3	84.3	<0.01
18	$[Pt(TMCPDA)Cl_2(OH)_2]$	5x1	10/0	21.3	0.05
19	$[Pt(CPDA)Cl_2]$	2x1	9/5	31.5	<0.05
20	$[Pt(CPDA)Br_2]$	4x1	10/2	45.1	<0.01
21	$[Pt(CPDA)(Cl\text{-}Ac)_2]$	2x1	10/2	27.2	<0.01
22	$[Pt(CPDA)(NO_3)_2]$	2x1	10/0	12.5	>0.05
23	$[Pt(CPDA)SO_4H_2O]$	2x1	10/1	23.5	<0.05
24	[Pt(CPDA)ox]	2x1	10/0	6.8	>0.05
25	[Pt(CPDA)mal]	10x1	10/3	34.0	<0.01
26	[Pt(CPDA)(CBDCA)]	20x1	10/2	25.9	<0.05
27	$[Pt(CPDA)Cl_2(OH)_2]$	5x1	10/1	16.7	<0.05
28	$[Pt(CPDA)(Cl\text{-}Ac)_2(OH)_2]$	2x1	10/1	9.3	<0.05

The electronic structures of some synthesized complexes were also studied by CNDO/2. The obtained data show, that compound [Pt(TMCPDA)(Cl-Ac)$_2$], which possess as higher activity and lower toxicity, does have higher positive charge on Pt atom, more nagative charge on X, lower overlap population on Pt-X, higher Q_{Pt-N} and medium E values just of electronic characters as required by a platinum drug with higher activity and lower toxicity as shown by the above structure-function relationship (eqns. 1-5). The study of pharmalogy and pharmacodynamics of compound [Pt(TMCPDA)(Cl-Ac)$_2$] is going on.

MODE OF ACTION OF CISPLATIN WITH DNA

In order to elucidate the antitumor action mechanism of cisplatin, we have undertaken studies on the interaction of cisplatin with the constituents of DNA by ^{13}C NMR and CNDO/2 method. The chemical shifts of proton-decoupled ^{13}C NMR spectra of interaction systems are shown in Table 4 (ref. 15).

TABLE 4. Chemical shifts of ^{13}C NMR of cisplatin-guanosine systems

No.	mole ratio	pH	C_6	C_2	C_4	C_8	C_5	C'_1	C'_4	C'_3	C'_2	C'_5	classification
1	Guo		90.3	87.3	84.6	69.1	50.3						
2	GuoH	11.5	100.36	94.17	84.32	69.83	51.61	21.46	19.04	6.74	4.35	-4.73	
3	Pt:Guo=1:2	5.5	90.20	88.07	83.98	73.38	47.79	22.22	19.09	7.57	3.63	-5.28	b
4	Pt:Guo=1:1	5.5	90.19	88.03	83.97	73.37*	47.79	22.22	19.05	7.51	3.58	-5.32	b
5	Pt:Guo=1:2	7	90.14	87.96	83.78	73.18	47.64	22.05	18.89	7.38	3.42	-5.47	b
				93.32	82.91	72.60	47.64	22.05	18.89	7.38	3.42	-5.47	b'
6	Pt:Guo=1:1	7	90.08	87.84	83.77	73.22	47.69	22.06	18.83	7.40	3.38	-5.50	b
			96.46	93.39	82.87	72.37	47.69	22.06	18.83	7.40	3.38	-5.50	b'

b, b': chemical shifts of differently bound ligand.
*: ^{195}Pt- $^{13}C_8$ coupling interaction with J = 40Hz.

pH 5.5 systems containing cisplatin and guanosine in 1:2 or 1:1 mole ratio yielded only a set of peaks which were different from those of the free ligand, and when they were compared, it was found that the resonances of C_8 and C_5 atoms of coordinated ligand were downfield shift 4.3 ppm and upfield shift 2.5 ppm respectively. Resonance change of other carbon atoms was less. Furthermore, it was found that the value J (^{195}Pt-^{13}C) = 40Hz. All these findings were in correspondence with ^{13}C NMR of cis-[Pt(NH$_3$)$_2$(Guo)$_2$](ClO$_4$)$_2$, and showed cis-[Pt(NH$_3$)$_2$(N$_7$-Guo)$_2$]$^{2+}$ in which guanosine bound to Pt by N_7 atom was the only species formed:

$$\text{cis-[Pt(NH}_3\text{)}_2\text{Cl}_2\text{]} + 2\text{Guo} \rightleftharpoons \text{cis-[Pt(NH}_3\text{)}_2\text{(N}_7\text{-Guo)}_2\text{]}^{2+} + 2\text{Cl}^-$$

Addition of 1 or 2 moles of guanosine per mole of cis-[Pt(NH$_3$)$_2$Cl$_2$] at pH 7 yielded ^{13}C NMR spectra with two sets of base peaks. The major one of them exhibited chemical shifts identical to those of cis-[Pt(NH$_3$)$_2$(N$_7$-Guo)$_2$]$^{2+}$, while in the second set the C_5,C_8,C_4 resonances were of the same values as those of cis-[Pt(NH$_3$)$_2$(N$_7$-Guo)$_2$]$^{2+}$, with addition of C_6,C_2 resonances being upfield shifts 3.9 ppm and 0.78 ppm respectively as compared with N_1 in the deprotonated guanosine. So the ^{13}C NMR spectra showed that besides cis-[Pt(NH$_3$)$_2$(N$_7$-Guo)$_2$]$^{2+}$, a minor new 1:1 complex, in which deprotonated guanosine bound to platinum not only by N_7 but also by N_1 atom, was formed. Considering the possibility of stereo arrangement and the formation of 1:1 complex in considerable amount in 1:1 mole ratio system but very little in 1:2 mole ratio system, the reaction was thought to proceed as follows:

$$n\text{cis-[Pt(NH}_3\text{)}_2\text{Cl}_2\text{]} + n\text{Guo} \rightleftharpoons \text{cis-[Pt(NH}_3\text{)}_2\text{(N}_7\text{,N}_1\text{-GuoH}_{-1}\text{)]}_n^{n+} + n\text{H}^+ + 2n\text{Cl}^-$$

The possible structure of the product is shown in Fig. 1. Addition of 2 moles of guanosine per mole of cis-[Pt(NH$_3$)$_2$(H$_2$O)$_2$](NO$_3$)$_2$ at pH 3 yielded ^{13}C NMR spectrum with a set of base peaks corresponding to that of cis-[Pt(NH$_3$)$_2$(N$_7$-Guo)$_2$]$^{2+}$, while addition of 1 mole of guanosine per mole of aqua platinum complex at the same pH yielded ^{13}C NMR spectra with two sets of base peaks, one exhibiting chemical shifts identical to that of cis-[Pt(NH$_3$)$_2$(N$_7$-Guo)$_2$]$^{2+}$, and the other to those of base peaks, in which the resonance of C_2,C_5,C_8 were of the same values as those of cis-[Pt(NH$_3$)$_2$(N$_7$-Guo)$_2$]$^{2+}$, but resonance of C_6 was downfield shift 5.7 ppm and possibly they belonged to bound ligand of cis-[Pt(NH$_3$)$_2$(N$_7$,O(C$_6$)-Guo)]$^{2+}$, wherein guanosine chelated platinum by N_7 and O(C_6) atoms (ref. 17). The formation of [Pt(NH$_3$)$_2$(N$_7$,N$_1$-GuoH$_{-1}$)]$_n^{n+}$·nNO$_3$ was further verified by its isolation and identification from 1:1 mole ratio reaction system of cis-[Pt(NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ and guanosine at pH = 7. It is known that for a long time the isolation of Pt-guanosine complex in 1:1 has been attempted, but no success achieved. We have tried to separate it by G-10,G-25 chromatography but also failed to get the pure compound. By ^{13}C NMR spectral analysis, we have found the main impurity in the

product obtained is $[Pt(NH_3)_2(N_7\text{-Guo})_2](NO_3)_2$ which can be separated out by partial crystallization and recrystallization at 0°C for the solubility of the compound with a 1:1 mole ratio of Pt and guanosine in water decreases markedly with decreasing temperature, but the solubility of $[Pt(NH_3)_2(N_7\text{-Guo})_2](NO_3)_2$ is rather little affected by temperature. The final powdery product was of light brown color. The results of elemental analyses, mole electrical conductivity and number mean molecular weight obtained by vapour pressure osmometer method are in good agrement with an octamer of $[\{Pt(NH_3)_2\}_8(N_7,N_1\text{-GuoH}_{-1})_7(OH)_2](NO_3)_7 8H_2O$ (ref. 18). In addition the ^{13}C NMR spectrum of the compound is identical with that of $[Pt(NH_3)_2(N_7,N_1\text{-GuoH}_{-1})]_n^{n+}$ obtained in the study on the interaction of cisplatin with guanosine in a neutral solution in mole ratio 1:1 or 1:2. The same species of $[Pt(NH_3)_2(N_7,N_1\text{-GuoH}_{-1})]_n^{n+}$ has been found by Reedijk two years later in the reaction system of cisplatin and 5'-GMP in 10:1 mole ratio and in presence of a excess of KCl at elevated temperatures (ref. 19).

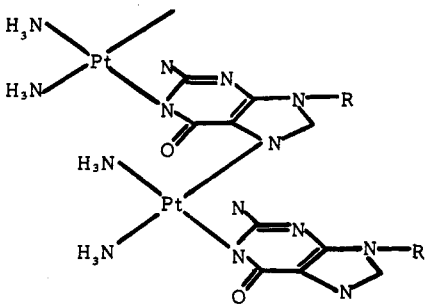
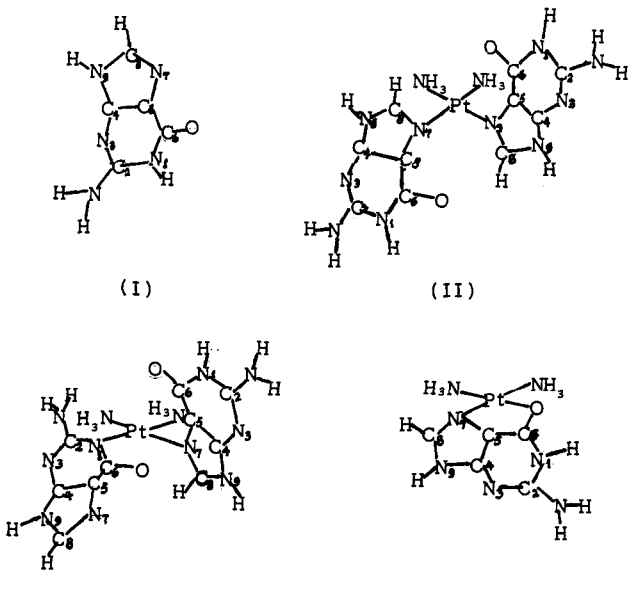


Fig. 1. Possible structure of $[Pt(NH_3)_2(N_7,N_1\text{-GuoH}_{-1})]_n^{n+}$

In order to estimate the bonding character of different coordinated species and the influence of platinum binding to different sites of guanine upon the electronic charge distribution in guanine melecule, the electronic structures of guanine and 3 model compounds of platinum differently coordinated with guanine has been studied by CNDO/2 method (Fig. 2). Structure I is guanine; II, $[Pt(NH_3)_2(N_7\text{-G})_2]^{2+}$ the model of intrastrand cross-linkage between cisplatin and DNA through N_7,N_7 atoms of two adjacent guanines in which the dihedral angle of two planes of the base molecules is 74°. III and IV are respectively the compounds in which bonding between cisplatin and guanines through N_7,N_1 atoms of two guanines and through N_7,O_6 atoms of the same guanine molecule — models of intrastrand cross-linkage between cisplatin and DNA through N_7,N_1 atoms of two adjacent guanines and of chelation mechanism, according to which cisplatin combined with O_6 and N_7 of guanine to from a chelate ring. The structure parameters of guanine and model compound II are taken from ref. 20, and the average of Pt-O, Pt-N bond distances and N-Pt-O, N-Pt-N bond angles, and the coordinates of guanine are adopted to construct the geometry of other model compounds.



(I) (II)

(III) (IV)

Fig. 2. Structures of guanine and model compounds of cisplatin and guanine

TABLE 5. Part of calcultion results of guanine and model compounds formed from cisplatin and guanine

Model compounds		I	II	III	IV
Electronic energy (a.u)		-403.5586	-1558.3674	-1559.8342	-700.0212
Total energy (a.u)		-99.0499	-248.0916	-248.4906	-149.3040
Overlap population (e)	$Pt-N(NH_3)$		0.3288	0.3203	0.3504
	$Pt-N_7$		0.4208	0.3875	0.3637
	$Pt-O(C_6)$				0.3749
	$Pt-N_1$			0.3964	
	$O-C_6$	0.9527	0.9391	0.8674	0.8164
	C_6-N_1	0.5557	0.5038	0.6668	0.5312
	C_6-N_7	0.8397	0.9547	0.9425	
	C_8-N_7	0.6902	0.6570	0.6712	
Charge on atom (e)	Pt		0.8676	0.5919	0.8798
	$N(NH_3)$		-0.3267	-0.2485	-0.3328
	N_7	-0.1765	-0.3466	-0.2677	-0.3958
	N_1	-0.2947	-0.2690	-0.3845	-0.2848
	$O(C_6)$	-0.2011	-0.2454	-0.2234	-0.3450
	$N(NH_2)$	-0.2983	-0.2479	-0.3072	-0.2427
	C_6	0.2585	0.2454	0.4349*	0.4214
				0.3249**	
	C_2	0.3851	0.5667	0.5150*	
				0.3643**	
Electronic configuration of Pt	s		0.4379	0.4905	0.4064
	p		0.4210	0.3826	0.3999
	d		8.2755	8.5349	8.3140

* charge on C_6,C_8 of guanine, whose N bound with Pt.
** charge on C_6,C_2 of guanine, whose N bound with Pt.

A part of the results obtained by CNDO/2 calculation of model compounds of cisplatin and guanine are given in Table 5 (ref. 21). By comparing the models of the interastrand cross-linkage through N_7,N_7 atoms (II) and N_7,N_1 atoms(III), and of the chelation mechanism (IV) with each other, it is found that the overlap population of $Pt-N_7$ (0.4208e) of model II was the highest, those of $Pt-N_7$ (0.3875e) and $Pt-N_1$ (0.3964e) of model III mediate, and those of $Pt-N_7$ (0.3637e) and $Pt-O(C_6)$ (0.3749e) of model IV the lowest. These results indicated that formation of intrastrand cross-linkage between cisplatin and DNA through N_7,N_7 atoms was more possible than through N_7,N_1 atoms, and a chelation mechanism, according to which cisplatin combined with $O(C_6)$ and N_7 of guanine of DNA should be excluded. The results of calculation were in good agreement with those of spectrophotometric and ^{13}C NMR studies. It has also been found from results obtained in calculation of models II, III and IV and guanine (I) that combination of Pt with N_7,N_7 atoms of two guanines (model II) decreased the charge on N_1 and $N(NH_2)$ atoms from -0.2947e and -0.2983e to -0.2690e and -0.2479e respectively, increased the charge on $O(C_6)$ from -0.2011e to -0.2454e (Table 5) and weakened the hydrogen bonds $N_1H...N$ and $NH_2...O$ of G-C pair, but strengthened the hydrogen bond $O(C_6)-NH_2$. Therefore the intra-strand cross-linkage of cisplatin with DNA through N_7,N_7 atoms of two adjacent guanines on the same strand could not effectively prevent the formation of hydrogen bonds in G-C pair, this good agreement with the result, that the chelation of cisplatin with two N_7 atoms of neighbouring guanine in a decamer of oligonucleotide does not preclude duplex formation with a complementary strand obtained by Reedijk. The binding of cisplatin to N_7,N_1 atoms of guanine (model III) increased the charge on $O(C_6)$ and $N(NH_2)$ atoms from -0.2011e and -0.2983e to -0.2234e and -0.3072e respectively, therefore it is likely that the binding of Pt with N_1 of guanine will not weaken the ability of $O(C_6)$ and NH_2 to form the hydrogen bonds with corresponding groups of cytosine in base pairing, ignoring the steric hinderance of $Pt(NH_3)_2^{2+}$ moiety coordinating on N_1 atom of guanine.

In order to investigate further the influence of platinum binding to different sites of guanine upon base pairing between cytosine and differently coordinated guanine of DNA, ^{13}C NMR is resorted to study the hydrogen bonding capabilities of cytidine with free guanosine, guanosine combined with Pt through N_7 ($[Pt(NH_3)_2(N_7\text{-Guo})_2]^{2+}$) and through both N_7 and N_1 ($[Pt(NH_3)_2(N_7,N_1\text{-GuoH}_{-1})]_n^{n+}$). The interaction between the complex and cytidine is studied in D_2O. As indicated by obtained results the C_2,C_4 resonances of cytidine in the mixed system of $[Pt(NH_3)_2(N_7\text{-Guo})_2](NO_3)_2$ and cytidine in mole ratio 1:2 shifted to lower field 0.67 ppm and 0.39 ppm respectively as compared with the corresponding values of free cytidine. Quite different from the change of chemical shifts produced in Cyt—N_7-Guo system, the resonances of C_2,C_4 and almost all the rest of C atoms of cytidine in the water system of Cyt—N_7,N_1-Guo

retain the values of its free condition. These results indicate that the hydrogen bonds between cytidine and N_7 coordinated guanosine remain almost intact, but there is no hydrogen bond formation between cytidine and N_1 coordinated guanosine (ref. 22).

All above results imply that the binding of platinum to N_7 atom of neighboring guanines on the same strand of DNA only weakens the hydrogen bonds between G-C base pair, but does not rupture them. However when platinum is bonded to neighbouring guanine on the same strand of DNA through N_7,N_1 formation of hydrogen bonds and consequently of G-C base pair is all prevented. Therefore the intrastrand cross-linkage formed between cisplatin and DNA through N_7,N_1 atoms of two adjacent guanines is a possible mechanism of preventing replication of DNA by cisplatin. However the formation of intrastrand cross-linkage of two adjacent guanines through N_7,N_1 by cisplatin has not yet been found in the ^{1}H NMR study of oligonucleotide-cisplatin adducts and their enzymatic digestion studies. Possible reasons may be the mole ratio of cisplatin to oligonucleotide in the reaction systems is too low, and the rate of N_7,N_1 cross-linkage is comparatively lower than that of N_7,N_7 cross-linkage. The ^{1}H and ^{13}C NMR studies of the bonding of cisplatin with oligonucleotides in higher mole ratio are under way.

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