Syntheses, Anti-tumor Activities and Toxicities of Platinum(II) Complexes Containing Piperidine and Morpholine Derivatives

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Since the discovery of Rosenberg *et al.* that *cis*-$Pt(NH_3)_2Cl_2$ exhibits anti-tumor activity [1], numerous complexes with the general formula, *cis*-PtA_2X_2 (A = amine, X = halogen), have been synthesized and tested for anti-tumor activity. Various kinds of amines have been used as ligands and attempts have been made to find out the relation between the structure of the amine ligand and the anti-tumor activity [2]. One of the tendencies discovered was that when A was varied from cyclopropylamine to cyclooctylamine increasing the carbon ring size gradually, the anti-tumor activity was maximal at cyclohexylamine [3]. Cyclopentylamine also exhibits high activity but all other amines are only slightly active. The fact that the cyclohexyl ring is advantageous for anti-tumor activity is also exhibited by the markedly high activity of Pt(1,2-cyclohexanediamine)Cl_2 [4–7]. In this paper we report the syntheses, anti-tumor activities and acute toxicities of platinum complexes containing N-(2-aminoethyl) piperidine (aep) and N-(2-aminoethyl)morpholine (aem), Pt(aep)X_2 and Pt(aem)X_2 (X = halogen). These ligands are also one of the saturated six-membered ring compounds but the ring contains nitrogen (aep) and oxygen (aem), respectively.

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Experimental

As the complexes, Pt(aem)X_2, were prepared in a manner similar to that of Pt(aep)X_2, the preparations of Pt(aep)X_2 (X = Cl, Br, I) are given as examples. Infrared spectra (400–4000 cm^{-1}) in KBr pellets were recorded with a Hitachi 260-30 spectrometer and those of far infrared were recorded with a Hitachi EPI-L spectrometer. Platinum concentrations were measured with a Shimadzu atomic absorption spectrophotometer AA 640-13 equipped with a graphite furnace atomizer, Shimadzu GFA-3.

Synthesis of Pt(aep)I_2

Four grams of KI and 1 mmol of K_2PtCl_4 were dissolved in 20 ml of water, which resulted in a solution of 0.05 *M* in PtI_4^{2-} and 1 *M* in I^-. To the solution, 1 mmol of aep was added and a yellow precipitate of Pt(aep)I_2 immediately appeared. The precipitate was filtered, washed with water and ethanol, and then air-dried.

Syntheses of Pt(aep)Cl_2 and Pt(aep)Br_2

Two mmol of $AgNO_3$ was added to 1 mmol of Pt(aep)I_2 suspended in 20 ml of water. The suspension was stirred overnight in the dark and the resulting precipitate of AgI was removed by centrifugation and filtration. About 20 times equivalent of NaCl was added to the filtrate and Pt(aep)Cl_2

TABLE I. Elemental Analyses of Pt(aep)X_2 and Pt(aem)X_2.

Complex		%C	%H	%N	%X	%Pt
Pt(aep)Cl_2	found	21.19	4.14	7.26	18.02	50.1
	calcd	21.33	4.09	7.11	17.99	49.49
Pt(aep)Br_2	found	17.24	3.43	5.83	32.71	42.6
	calcd	17.40	3.34	5.80	33.08	40.38
Pt(aep)I_2	found	14.41	2.87	4.87	43.54	34.4
	calcd	14.57	2.79	4.85	43.98	33.80
Pt(aem)Cl_2	found	18.00	3.76	7.11	17.59	49.7
	calcd	18.19	3.56	7.07	17.90	49.24
Pt(aem)Br_2	found	14.73	3.04	5.75	32.69	38.9
	calcd	14.86	2.91	5.77	32.94	40.22
Pt(aem)I_2	found	12.30	2.48	4.80	43.55	33.8
	calcd	12.44	2.44	4.84	43.83	33.69

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TABLE II. Far Infrared Absorptions due to Pt–halogen Stretching Observed in Pt(aep)X_2 and Pt(aem)X_2.

Complex	Pt–X (cm^{-1})
Pt(aep)Cl_2	332 s, 316 vs
Pt(aep)Br_2	231 vs, 213 vs
Pt(aem)Cl_2	362 s, 320 vs
Pt(aem)Br_2	241 s, 213 vs

was precipitated. Pt(aep)Br_2 was obtained in a similar manner, where instead of NaCl, NaBr was added to the filtrate.

Procedure for Studies of Anti-tumor Activity

Complexes were suspended in water and administered i.p. to CDF_1 mice. The activity is expressed as T/C (%), the ratio of survival days for treated and untreated mice. Each of the treated and the untreated groups consisted of six mice and the tumor used was L1210.

Results and Discussion

Elemental analyses of the complexes synthesized are given in Table I. All the complexes are yellow and are insoluble in water and most organic solvents. The yields of the complexes are. Pt(aep)Cl_2 72.0%, Pt(aep)Br_2 53.2%, Pt(aep)I_2 96.0%, Pt(aem)-Cl_2 76.0%, Pt(aem)Br_2 66.1%, Pt(aem)I_2 96.4%. Although Pt(aep)Cl_2 and Pt(aem)Cl_2 can be synthesized directly from the reaction of K_2PtCl_4 and the amine ligands, the yields are markedly decreased to 25% with this procedure.

Infrared and far infrared spectra of each compound indicate two distinct absorption bands around 200 ~ 300 cm^{-1}, due to Pt–X stretching modes. These bands are tabulated in Table II. The fact that each compound shows two absorption bands due to Pt–X stretching confirms *cis* configuration of the two halogens. Absorptions of Pt–I stretching could not be measured, since they lie in a 170 ~ 180 cm^{-1} region.

The results of the studies on the anti-tumor activities are shown in Table III, as well as LD_{50}s. Pt-(aep)Cl_2 and Pt(aem)Cl_2 exhibit appreciably high activity, and the activity decreases as the ligand X goes from Cl to I in both amine series. Table III indicates that the toxicity also decreases as X changes from Cl to I.

TABLE III. Results of Preliminary Screening of Pt(aep)X_2 and Pt(aem)X_2 against L1210 and their LD_{50}s.

Complex	best dose (mg/kg)	T/C (%)	T/C (%)
Pt(aep)Cl_2	50	195	75
Pt(aep)Br_2	50	151	150
Pt(aep)I_2	25	127	100
Pt(aem)Cl_2	50	188	125
Pt(aem)Br_2	50	125	150
Pt(aem)I_2	50	117	200
cis-Pt$(NH_3)_2Cl_2$	12.5	278[8]	13[9]

The present result indicates that although the activities of Pt(aep)X_2 and Pt(aem)X_2 are slightly less than that of *cis*-Pt$(NH_3)_2Cl_2$, they are less toxic than the latter. This fact encourages a further survey of six-membered heteroring compounds as amine ligands in order to reduce the nephro toxicity, which is presently the most serious problem with anti-tumor platinum complexes.

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