In Vivo Properties of Some New *Cis*-Platinum Complexes Containing 7-Azaindole Ligands

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Toxicity data and some preliminary antitumor testing results are reported for the new complexes cis-$PtLX_2$ *(L = 1H-pyrrolo[2,3-b]pyridine, (7-azaindole); $X_2 = Cl_2$, I_2, oxalate). Although these complexes have the* cis-PtN_2X_2 *configuration recognised as desirable for effective antitumor behavior, no significant antitumor properties were observed against a number of primary screens; although interesting toxicity differences were observed between the three compounds.*

Introduction

Following Rosenberg's discovery of the antitumor properties of *cis*-diamminedichloroplatinum(II) [1–5] much effort has been devoted to attempts to understand structure–reactivity relationships in the general class of platinum complexes. This has taken the form of a search for new platinum drugs [6–9], and the possible mode of binding to *in vivo* systems [10, 11] including enzymes [12].

The strong relationship between the potency of platinum compounds and their chemical structure shows that the binding of those complexes which exhibit antitumor effects is not merely electrostatic in nature. Indeed, the variations in activity found between closely analogous compounds underlines the specificity of interaction. Examination of the basic chemical composition of nucleic acids shows that they offer a number of potential sites for strong covalent bond formation to platinum. Although the reactions of platinum complexes with purine and pyrimidine ribosides have been studied in depth few researchers have considered the interactions between platinum complexes and other, less common, nitrogen-containing heterocycles. The chemistry of the reactions between platinum compounds and heterocycles might be expected to shed some light upon aspects of the metal's biological action. In this paper we wish to report some *in vivo* properties of the new platinum(II) complexes of 1H-pyrrolo-[2,3-b]pyridine (7-azaindole): *cis*-bis(7-azaindole)dichloroplatinum(II), *cis*-bis(7-azaindole)diiodoplatinum(II) and *cis*-bis(7-azaindole)oxolatoplatinum(II). Their structures [13] are shown in Fig. 1.

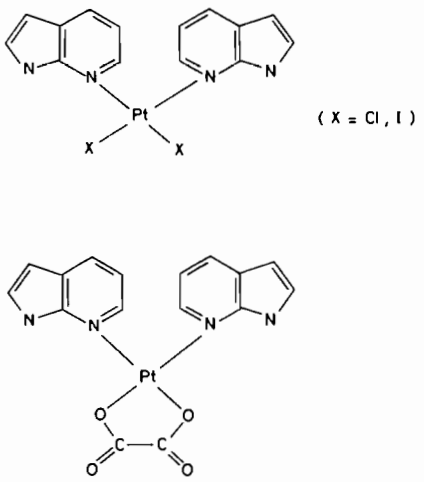

Fig. 1. Structure of the new complexes.

Results and Methods

[A] Cis-*Pt(7-azaindole)*$_2Cl_2$

Toxicity in the Rat

Method. *Cis*-Pt(7-azaindole)$_2Cl_2$ was suspended in arachis oil by mulling. The suspension was adminis-

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tered by i.p. injection to five groups of three, and one group of six, mixed sex Wistar rats. The weight range of the rats was 175–230 g at the time of injection. Doses varied between 50 and 300 mg/kg. The animals were weighed daily until the experiment was terminated 5 days after administration.

Results. Listed below are the number of survivors in each of the five groups of rats.

Dose (mg/kg)	50	75	100	200	300
Survivors	3/3	3/3	4/6	0/3	0/3

Post-mortem examination showed that the cause of death was acute peritonitis, combined with obstruction of the small intestine. For survivors, maximum weight loss occurred on the day following administration.

A log/probability plot was constructed which showed that for *cis*-Pt(7-azaindole)$_2$Cl$_2$ the LD_{50} = 110 mg/kg, and the LD_{10} = 96 mg/kg.

Activity of cis-*Pt(7-azaindole)$_2$Cl$_2$* versus *Yoshida Sarcoma*

Method. Fragments of Yoshida Sarcoma were implanted into the flanks of female Wistar rats. Only those animals where implantation was successful were selected.

A group of four rats received a single dose of a suspension of *cis*-Pt(7-azaindole)$_2$Cl$_2$ in arachis oil by i.p. injection. The administered dose was 50 mg/kg (*i.e.* 50% of LD_{10}). At daily intervals the dimensions of the tumors in the x, y and z directions were measured using callipers. The tumour was assumed to be an ellipsoid whose volume was determined by the formula $\pi/6\ (x \cdot y \cdot z)$. The experiment was ended four days after administration.

Results. Tumour volumes are listed below (Mean ± Standard Error of Mean).

Day	0	1	2	3	4
Volume/cm^3	8.09 ± 0.61	12.08 ± 0.27	13.02 ± 0.26	17.39 ± 0.96	20.87 ± 1.40

There was no sign of significant regression.

Activity of cis-*Pt(7-azaindole)$_2$Cl$_2$* versus *Osteogenic Sarcoma*

Method. Fragments of Osteogenic Sarcoma were implanted into the flanks of young female mice. After 21 days 15 animals, which showed successful implantation and similar tumour growth rates, were selected. At the time of drug administration the weight range of the mice was 25–31 g.

Cis-Pt(7-azaindole)$_2$Cl$_2$ was suspended in arachis oil by mulling. Three groups of four mice received 200, 400 and 600 mg/kg of *cis*-Pt(7-azaindole)$_2$Cl$_2$ i.p. injection. A control group of three mice received an equivalent amount of arachis oil. After seven days the mice were sacrificed, and the tumours dissected out and weighed.

Results. Tumour weights are listed below (Mean ± standard of error means).

Dose (mg/kg)	0	200	400	600
Tumour weight (g)	2.61 ± 0.71	2.35 ± 0.33	2.22 ± 0.20	2.61 ± 0.44

At the maximum dose tested there was no sign of significant tumour regression, nor were there any cases of acute toxicity.

Activity of cis-*Pt(7-azaindole)$_2$Cl$_2$* versus *ADJ/PC6A*

Method. Four groups of three female C$^-$ mice bearing ADJ/PC6A tumours were administered *cis*-Pt(7-azaindole)$_2$Cl$_2$, suspended in arachis oil by i.p. injection. The dose was given, 24 days after tumour implantation, at levels of 5, 25, 125, and 625 mg/kg. A group of ten mice bearing ADJ/PC6A tumours were used as control. After 10 days surviving mice were sacrificed, and tumour excised and weighed.

Results. For each group of mice, the number of survivors, the mean tumour weight, and the mean tumour weight as per cent of control are listed below:

Dose (mg/kg)	0	5	25	125	625
Survivors	10/10	3/3	3/3	3/3	0/3
Tumour weight (g)	11.2	9.2	10.3	9.4	
T/C (%)		85.5	91.8	83.7	

Inhibition of tumour growth was below the level normally considered significant.

Activity of cis-*Pt(7-azaindole)$_2$Cl$_2$* versus *P388 Lymphocytic leukemia*

Method. Female CDF$_1$ mice were inoculated i.p. with ascitic fluid containing 10^6 P388 lymphocytic leukemia cells. Three groups of six mice were given i.p. injections of *cis*-Pt(7-azaindole)$_2$Cl$_2$ in isotonic saline at doses of 100, 200 and 400 mg/kg. This dose was repeated after four days. A group of 33 mice were used as control.

Results. All mice lived beyond the fifth day when acute toxicity was evaluated. Median survival times for the treated and control groups are listed below:

Dose (mg/kg)	0	100	200	400
Median survival time (days)	11.1	12.1	12.3	12.3
T/C (%)		109	110	110

The increase in life span was below the level normally considered significant.

[B] Cis-*Pt(7-azaindole)$_2$I$_2$*

Toxicity of cis-*Pt(7-azaindole)$_2$I$_2$ in the Rat*

Method. Twenty-one female Wistar rats, weight range 180–235 g, were divided into five groups of three, and one group of six. The groups of three rats each received an i.p. injection of *cis*-Pt(7-azaindole)$_2$I$_2$ suspended in arachis oil. Doses given were 75, 100, 150, 300 and 400 mg/kg. The remaining group of six rats received 200 mg/kg of *cis*-Pt(7-azaindole)$_2$I$_2$. The animals were weighed daily for 35 days.

Results. There were no mortalities in any group throughout the course of the experiment. Maximum weight loss occurred between days 2–4.

Activity of Cis-*Pt(7-azaindole)$_2$I$_2$* versus *Yoshida Sarcoma*

Method. Fragments of Yoshida Sarcoma were implanted into the flanks of female Wistar rats. Only those animals where implantation was successful were selected.

Two groups of five rats were taken. Animal weights ranged from 195 to 270 g. One group received 200 mg/kg *cis*-Pt(7-azaindole)$_2$I$_2$ suspended in arachis oil by i.p. injection. The other group received 400 mg/kg. Tumor volume was assessed as previously described. The experiment was terminated three days after administration of the platinum complex.

Results. Tumour volumes are listed below. (Mean ± Standard Error of Mean).

	Dose (mg/kg)	
	200	400
Day		
0	5.25 ± 0.56	6.06 ± 0.27
1	10.41 ± 1.77	7.27 ± 0.71
2	13.31 ± 2.06	11.14 ± 0.11
3	20.32 ± 2.96	16.74 ± 0.70

There was no sign of significant regression at either dose level.

Activity of cis-*Pt(7-azaindole)$_2$I$_2$* versus *L1210 Lymphoid Leukemia*

Method. Male CDF$_1$ mice were innoculated i.p. with ascitic fluid containing 10^5 L1210 lymphoid leukemia cells. Four groups of six mice were given i.p. injections of *cis*-Pt(7-azaindole)$_2$I$_2$ in isotonic saline at doses of 25, 50, 100 and 200 mg/kg. This dose was repeated after four days. A group of 32 mice were used as control.

Results. All mice lived beyond the fifth day when acute toxicity was evaluated. Mean survival times for treated and control groups are listed below:

Dose (mg/kg)	0	25	50	100	200
Mean Survival time (days)	8.1	8.0	8.2	8.3	8.7
T/C (%)		98	101	102	107

Even at the highest dose level the increase in life span was less than normally considered significant.

[C] Cis-*Pt(7-azaindole)$_2$(C$_2$O$_4$)*

Activity of cis-*Pt(7-Azaindole)$_2$(C$_2$O$_4$)* versus *ADJ/PC6A*

Method. The method used was identical to that described for *cis*-Pt(7-azaindole)$_2$Cl$_2$.

Results. For each group of mice are listed the number of survivors, the mean tumour weight, and the mean tumour weight as percent of control.

Dose (mg/kg)	0	5	25	125	625
Survivors	10/10	3/3	3/3	3/3	3/3
Tumour weight (g)	11.2	13.2	10.8	12.3	10.2
T/C (%)		118.0	96.3	110.8	91.8

Discussion

Despite the fact that the complexes reported here have the *cis*-PtN$_2$X$_2$ geometry now recognized to be important in designing these antitumor drugs, no significant antitumor effects were observed in the screening systems used. Thus, whilst it may be expected that *cis*-platinum drugs are bound by heterocyclic nitrogen bases *in vivo* their antitumor properties may well be exhibited prior to such binding occurring. However, whilst none of the three

complexes exhibited significant antitumor behavior, other *in vivo* properties did show significant differences in the three compounds.

Cis-Pt(7-azaindole)$_2$Cl$_2$ was the most toxic of the analogues tested. This might reflect the relative ease with which displacement of coordinated chloride ion occurs to give highly reactive charged complex. In contrast, the iodide and oxalate complexes are expected to be relatively inert. Solubility differences may also be contributory factors.

The LD_{50} of *cis*-Pt(7-azaindole)$_2$Cl$_2$ in rats was 100 mg/kg. However, mice could tolerate much higher levels without fatality. The material had no significant effect against against Yoshida Sarcoma at a dose of 50 mg/kg, nor against Osteogenic Sarcoma at doses up to 600 mg/kg, ADJ/PC6A at doses up to 125 mg/kg, and P388 at doses up to 400 mg/kg.

The toxicity of *cis*-Pt(7-azaindole)$_2$I$_2$ in rats was not determined, no fatalities were caused by doses up to 400 mg/kg. The material had no significant anti-tumor effect against Yoshida Sarcoma at doses up to 400 mg/kg, or against L1210 Lymphoid Leukemia at doses up to 200 mg/kg.

Cis-Pt(7-azaindole)$_2$(C_2O_4) was also non-toxic at levels up to 625 mg/kg in mice. Neither did it cause any significant tumour inhibition.

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