© *Proc. Indian natn. Sci. Acad.*, **52**, A, No. 4, 1986, pp. 924–930
Printed in India.

STRUCTURE AND ANTITUMOR ACTIVITY OF SOME AMINO ACID DERIVATIVES OF 2,2′-BIPYRIDINE-PLATINUM(II) AND THEIR INTERACTION WITH CALF THYMUS DNA*

RENU MITAL *and* T S SRIVASTAVA

Department of Chemistry, Indian Institute of Technology, Bombay-400076, India

(*Received 7 March 1986*)

The structures of [Pt(bipy) (AA)] Cl complexes (where bipy is 2,2′-bipyridine and AA is anion of glycine, alanine or leucine) have been established by ^1H NMR and infrared spectroscopy. These complexes have been tested against P-388 lymphocytic leukemic cells. Glycine complex shows low activity whereas alanine and leucine complexes show comparable activity as compared to *cis*-[$Pt(NH_3)_2 Cl_2$]. DNA is the target biomolecule of these complexes and, therefore, these binding studies with calf thymus DNA have been carried out. The binding mode of these complexes to DNA seems to be through hydrogen bonding.

Key Words : *cis* **Platinum Complex; Anticancer Activity; Drug-DNA Binding**

INTRODUCTION

Cis-[$Pt(NH_3)_2 Cl_2$] (*cis*-DDP) was successfully used against mice with transplanted tumors of Sarcoma 180 and Leukemia L1210.[1] It was also found active against a wide range of tumors in animal and in man[2] whereas *trans*-[$Pt(NH_3)_2 Cl_2$] was inactive. Further, a wide range of platinum complexes have been tested in which the ligands have been systematically varied. Active complexes always involve a *cis* geometry with a pair of good leaving groups, and they are neutral species.[3] These are square planar complexes of the type *cis*-[PtA_2X_2], where A_2 has two monodentate (NH_3) or one bidentate ($H_2NCH_2CH_2NH_2$) amino group as carrier ligand and X_2 has two monodentate (Cl^-) or one bidentate anionic ligand ($^-OOCCH_2COO^-$) as leaving group. The carrier ligands are neutral and strongly bonded, and leaving groups are weakly-bonded anionic ligands.

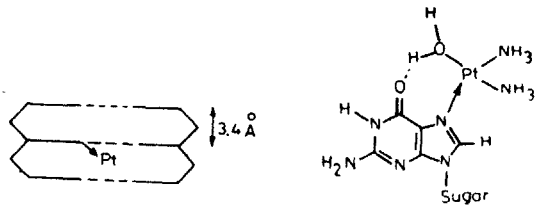
Cis-DDP inhibits selectively and irreversibly the synthesis of DNA *in vitro* and *vivo* and, therefore, it is directly involved in the DNA replication.[4,5] In fact, the aquated species of *cis*-DDP, namely *cis*-[$Pt(NH_3)_2 (H_2O)_2]^{2+}$ reacts with DNA inside the cell by forming cross links between base pairs of DNA. For this type of cross-linking to occur the centres must be about 3.4Å apart. The most probable modes of binding between DNA bases and two *cis*-positions (3.3Å on platinum are

*Presented at the INSA Golden Jubilee Symposium on *Recent Trends in Inorganic Chemistry* held in Calcutta in December 1985.

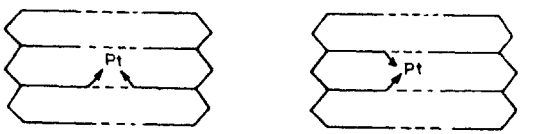
shown in Fig. 1(*a*) and (*b*). Platinum can bind mono-functionally to ond base as shown in Fig. 1(*a*), Alternatively, it can bind to two bases on one strand (intrastrand cross-linking) or between two bases on different strands (interstrand crosslinking) (See Fig. 1(*b*)). The interstrand crosslinking is the most accepted mode of binding of the *cis*-DDP to DNA.

Cis-DDP has considerable side effects on most patients.[2] They suffer nausea, diarrhoea, decrease in haemoglobin levels and kidney damage. Kidney toxicity is very serious, which can now be dealt with by giving volume of fluids (and diuretics) during treatment or by use of drug combinations. In order to improve upon *cis*-DDP, the principal requirements for new and better drugs should have (*a*) low toxicity as compared to *cis*-DDP, (*b*) equal or better activity as compared to *cis*-DDP, (*c*) suitable solubility in tissue fluids, and (*d*) target to DNA molecule (binding mode may preferably be different than *cis*-DDP).

[Pt(bipy) (*en*)]$^{2+}$ (*en* is ethylenediamine) and related complexes bind to DNA by intercalation,[6] and, therefore, we have recently reported the synthesis and characterzation of similar complexes of amino acids with 2,2′-bipyridineplatinum(II).[7] The latter complexes have also been tested against P-388 lymphocytic leukemic cells and their target biomolecule is DNA.[8] These complexes also meet most of the above requirements of new drugs. Here we report the binding studies of [Pt(bipy)(AA)] Cl to DNA.



(a) Binding to one base



(b) Binding to two bases-crosslinking,
Interstrand (left) and intrastrand (right)

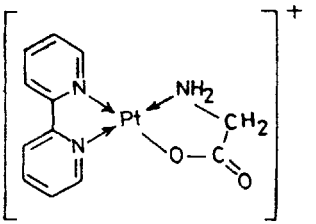
FIG 1 **Binding of *cis*-DDP to DNA**

Experimental

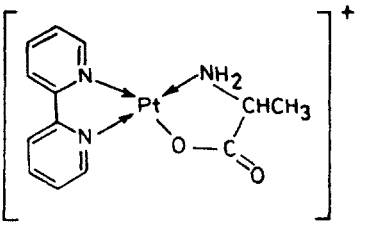
Pt(bipy) (AA)] Cl were synthesized by methods as described earlier.[7] The interacions of platinum complexes with DNA have been studied by difference absorption spectroscopy and other physical methods used are described earlier.[9]

Results and Discussion

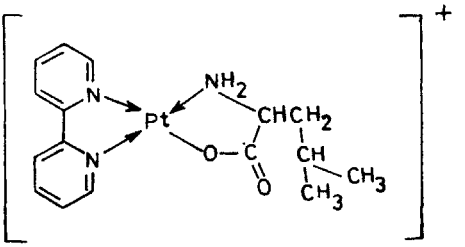
The ^1H NMR spectra of [Pt(bipy) (AA)] Cl, where AA is anion of glycine, alanine or leucine, have been recorded in D_2O. The H-6,6′ protons of 2,2′-bipyridine in the amino acid derivatives of platinum(II) are shifted to higher fields as compared to [Pt(bipy) Cl_2],[7] and these can be explained in terms of stronger bonding of amino acids than chloride ions to platinum.[7,10] The platinum complexes of glycine, alanine and leucine show downfield shifts of CH or CH_2 fragments as compared to free amino acids in zwitterion forms. This can be interpreted in terms of stronger binding of chelated amino acids to platinum.[11] The large values of[3] $J_{Pt-H(a)}$ (30Hz for glycine complex, 24Hz for alanine complex and 29Hz for leucine complex) of $195_{Pt-N-C-H}$ in these complexes confirm that the nitrogen is bonded to platinum by chelated amino acids. The above complexes give a $\nu(COO^-)$ band in the infrared region between 1660–1670cm^{-1} in dimethyl sulphoxide solution and this further supports the binding of coordinated $-COO^-$ group. Thus, the structures of these complexes are square planar and are as shown in I, II and III.



I



II



III

The above platinum complexes were screened for antitumor activity against P-388 lymphocytic leukemic cells. The 50 per cent inhibitory dose ($I.D._{50}$) values

of above complexes are given in Table I. The alanine and leucine complexes have $1.D._{50}$ values comparable to *cis*-DDP whereas the glycine complex has shown $1.D._{50}$ values much greater than *cis*-DDP. The procedure for growth inhibition studies of amino acid complexes established that the DNA is the target biomolecule for these complexes.[8] Therefore, the binding of platinum complexes to calf thymus DNA at *p*H 7.4 using buffer of 10 mM *Tris*-HCl, containing 0.01 M NaCl has been studied by difference absorption spectral technique.[12] The difference absorption spectra of [Pt(bipy) (Gly)] Cl and DNA between 280 to 380nm show two absorption minima at 305 and 317nm and an absorption maximum at 325nm. An isosbestic point was observed at 322nm. Similar difference spectra of alanine and leucine complexes with DNA are also observed.

TABLE I

50 per cent inhibitory dose ($1.D._{50}$) *of some platinum(II) complexes of amino acids*

Compound	$1.D._{50}$ (μg/ml)
Cis-DDP	5
[Pt(bipy) (Gly)] Cl	>20
[Pt(bipy) (Ala)] Cl	7
]Pt(bipy) (Leu)] Cl	8.5

The binding isotherm was constructed by treatment of data of difference between absorption of free and mixed solutions of varying concentrations of [Pt(bipy) (Gly)] Cl (4.8μM 48μM) and constant concentration of DNA (0.24mM) at 317nm (ΔA_{317nm}) according to Scatchard equation.[13,14] It shows a linear relationship as shown in Fig. 2(*a*). Similar Scatchard plot of alanine complex binding to DNA is obtained. On the other hand the Scatchard plot of leucine complex binding to DNA shows a biphasic nature and is given in Fig. 2(*b*). This indicates the involvement of at least two binding processes of leucine complex to DNA. From these Scatchard plots, the number of binding sites per 100 nucleotides (n) and the apparent association constant (K_{app}) are calculated, and these parameters are given in Table II. The interaction of [Pt(bipy) (*AA*)] Cl with polyadenylic acid (Poly[*A*]) and polyguanylic acid (Poly [*G*]) has also been studied. The K_{app} values of binding of [Pt(bipy) (*AA*)] Cl with Poly [*G*] are comparable to K_{app} values of binding of [Pt(bipy) (*AA*)] Cl with DNA. However, the [Pt(bipy) (*AA*)] Cl complexes do not bind to Poly [*A*]. In addition the [Pt(bipy) (*AA*))] Cl complexes also do not bind to nucleoside monophosphate and nucleoside triphosphate.

The K_{app} values of binding of [Pt(bipy) (*AA*) Cl complexes to DNA is comparable to K_{app} value of binding of *cis*-DDP to DNA.[15] This suggests that the [Pt(bipy) (*AA*)]Cl complexes may bind to DNA by coordinate covalent bonds. In order to check this type of binding in DNA-platinum complexes, the DNA-glycine complex in buffer of *p*H 7.4 was passed through a column of Sephadex-G25 equilibrated with buffer of *p*H 7.4. The column was eluated with buffer of *p*H 7.4.

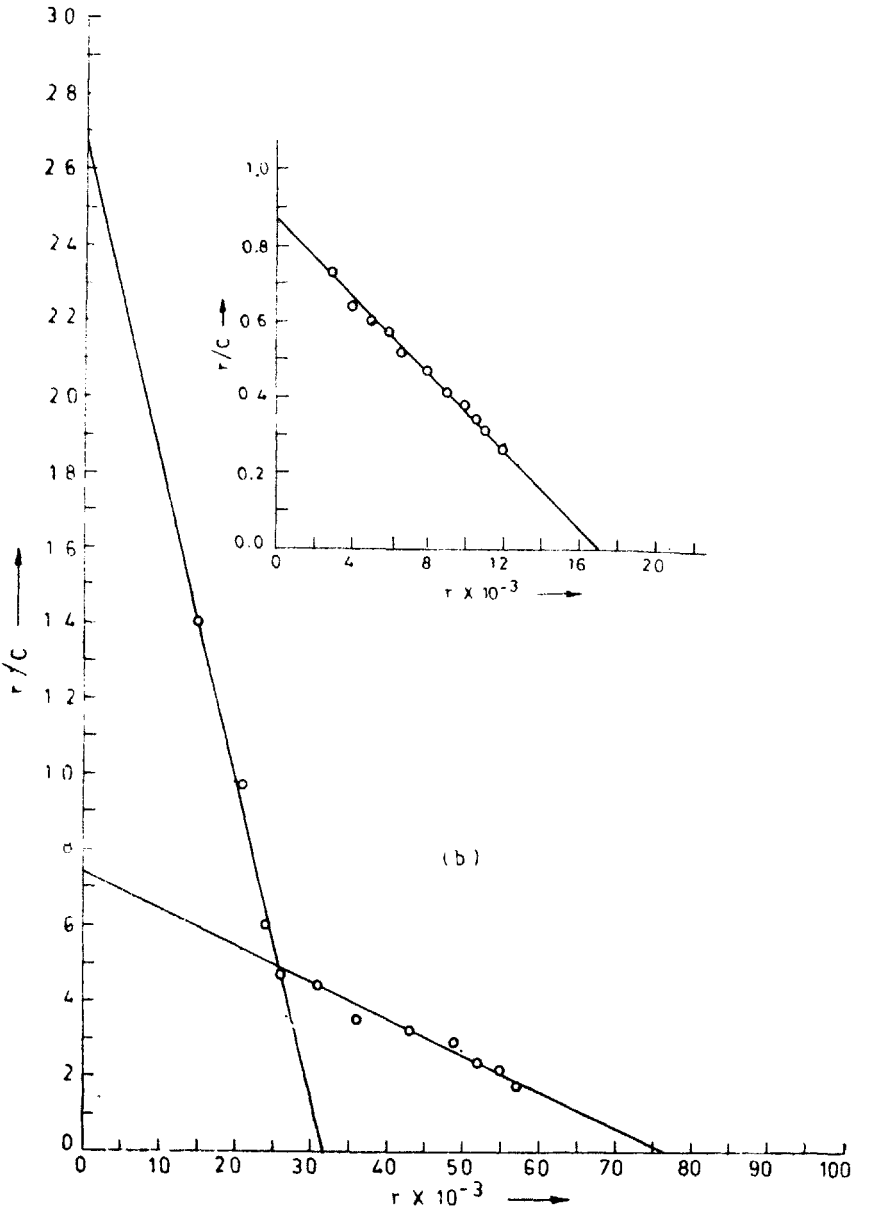


FIG 2 Scatchard plots of binding of (*a*) glycine complex to DNA and (*b*) leucine complex to DNA. *r* represents moles of bound platinum complex per mole of nucleotide residues of DNA, and *C* represents the corresponding molar concentration of unbound platinum complex.

Two separate fractions of DNA and platinum glycine complexes respectively were obtained from the column in this order. This suggests that the platinum complexes bind to DNA by noncovalent interactions, namely ionic bonding, hydrophobic

TABLE II

Binding parameters of interaction [Pt(bipy) (AA)] Cl to calf thymus DNA at pH 7.4

Compound	K_{app} (M^{-1})	n
[Pt(bipy) (Gly)] Cl	5.2×10^4	1.7
[Pt(bipy) (Ala)] Cl	2.2×10^4	3.7
[Pt(bipy) (Leu)] Cl	9.7×10^4	7.7
	8.5×10^5	3.2

interaction, intercalation, hydrogen bonding or combination of two or more of these interactions.[16]

The ionic interaction between anionic phosphate groups of DNA and mono-cationic platinum(II) complexes is ruled out because there was no interaction between nucleoside monophosphate, nucleoside triphosphate or poly [*A*] and [Pt(bipy) (*AA*)] Cl complexes. The hydrophobic interaction between amino acid alkyl side chains of [Pt(bipy) (*AA*)] Cl complexes and DNA is also less likely because there is no increase in K_{app} values of DNA-amino acid complexes based on the increased side chains of amino acids. The intercalation of platinum complexes to DNA is also ruled out because there is no fluorescence change in DNA-ethidium complex in presence of platinum complexes.[15]

Magnesium ions (6mM) reverse the binding of glycine and leucine complexes to DNA by 100 per cent whereas they reverse only 85 per cent binding of alanine complex to DNA. These results suggest that the binding of these platinum complexes to DNA is through the hydrogen bonding. The DNA was also precipitated from DNA-platinum complexes by absolute alcohol[17] and the platinum complexes stay in solution. Thus, these observations further support the hydrogen bonding.[18]

The square planar antitumor active [Pt(bipy) (*AA*)] Cl complexes bind calf thymus DNA by hydrogen bonding possibly through guanine of DNA.

ACKNOWLEDGEMENT

The authors remain grateful to Dr R K Bhattacharya (BARC-Bombay) and Dr K S Ray for fruitful discussion. Renu Mital (J R F) is grateful to C S I R, New Delhi, for financial assistance.

REFERENCES

1. B Rosenberg L Van Camp J E Trosko and V H Mansour *Nature* (*London*) **222** (1969) 385
2. B Rosenberg *Metal Ions in Biological Systems* Vol II (*Ed* H Sigel) Marcel Dekker (1980) 127–196
3. M J Cleave P C Hydes B W Malerbi and D M Watkins *Biochim* **60** (1978) 835
4. J J Roberts and A J Thomas *Progr nucl Acid Res mol Biol* **22** (1979) 11
5. J J Roberts *Adv inorg Biochem* **3** (1981) 274
6. J K Barton and S J Lippard *Nuclic Acid-Metal Ion Interaction* Vol 1 (*Ed* T G Spiro) Wiley-Interscience New York (1980) 31–114

7. L Kumar N R Kandasamy and T S Srivastava *Inorg Chim Acta* **67** (1982) 139
8. L Kumar N R Kandasamy T S Srivastava A J Amonkar M K Adwankar and M P Chitnis *J inorg Biochem* **23** (1985) 1
9. R Mital K S Ray T S Srivastava and R K Bhattacharya *J inorg Biochem (In press)*
10. E Bielli P M Gidney R D Gillard and B T Heaton *J chem Soc Dalton Trans* (1974) 2133
11. L E Erickson J W McDonald J K Howrie and R P Clow *J Am chem Soc* **90** (1968) 6371
12. V S Aboobaker and R K Bhattacharya *Indian J Biochem Biophys* **14** (1977) 296
13. G Scatchard *Ann N Y Acad Sci* **51** (1949) 660
14. A M Q King and B H Nicholson *Biochem* **114** (1969) 679
15. J L Butour and J P Macquet *Eur J Biochem* **78** (1977) 455
16. W B Wood J H Wilson R M Benbow and L E Hood *Biochemistry : A Problem Approach* Benjamin Inc California (1974) 71-72, 465
17. V A Bloomfiled D M Crothers and I Tinaco Jr *Physical Chemistry of Nucleic Acids* Harper and Row New York (1974) 336
18. R V Hosur and A Pohorille *Int J Quant Chem* **20** (1981) 33