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Studies on the Synthesis and Characterization, Binding with DNA and Activity of *cis*-Bis{imidazo(1,2-α)-pyridine}dichloroplatinum(II)

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A *cis*-planaramineplatinum(II) complex: *cis*-bis{imidazo(1,2-α)pyridine}dichloroplatinum(II) (AH6) has been prepared and characterized based on elemental analyses, IR, Raman, mass and ^{1}H NMR spectral measurements. The interactions of the compounds with salmon sperm and pBR322 plasmid DNAs have been investigated and the activity of the compound against ovarian cancer cell lines: A2780, A2780$^{cis\text{-}R}$ and A2780^{ZD0473R} has been determined. The compound is believed to form mainly monofunctional N7(G) and bifunctional intrastrand N7(G)N7(G) adducts with DNA, causing a local distortion of DNA as a result of which gel mobility of the DNA changes. The compound is found to be less active than cisplatin against the three ovarian cancer cell lines. This is believed to be due to its reduced ability to bind with DNA because of a greater steric hindrance introduced by two bulkier planaramine ligands.

Key Words: **Cisplatin, Transplatin, Imidazo(1,2-α)pyridine, Gel electrophoresis, pBR322 plasmid DNA, Anticancer activity.**

INTRODUCTION

Although cisplatin is highly effective against testicular and ovarian cancers and has proved beneficial in the treatment of head and neck, lung and bladder cancers[1, 2], it has two major drawbacks namely severe tissue toxicity and a limited spectrum of activity[3–7]. In an attempt to reduce toxicity and widen the spectrum of activity thousands of cisplatin analogues have been prepared and tested by varying the nature of the labile ligands (also called the leaving groups) and non-labile ligands (also called the carrier ligands). However, it is found that all cisplatin analogues generally have a similar spectrum of activity and develop similar resistance[8]. By manipulating the structure of the leaving groups it has been possible to reduce toxicity (*e.g.*, substitution of the more stable cyclobutanedicarboxylate for the two chlorides led to the development of

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carboplatin which produces substantially less nausea, vomiting and neurotoxicity but causes more of myelosuppression), but it has not been possible to prevent cross resistance. It was hypothesized that the modification of the carrier ligands would change the spectrum of activity and indeed oxaliplatin which has 1,2-diaminocyclohexane as the carrier ligands has been found to be active against colorectal cancer[9–11] whilst cisplatin is not.

Currently attention is given to rule breaker platinum compounds with the idea that because of different nature of interaction with DNA, tumour active compounds with a different spectrum of activity may result. The reason why transplatin is toxic rather than being anticancer active is believed to be associated with its higher reactivity than cisplatin. It is therefore thought that the introduction of sterically hindered planar ligands may reduce the reactivity of the *trans*-complexes sufficiently so as to result into tumour-active compounds and because of a different nature of binding with DNA, the compounds may have a spectrum of activity different from that of cisplatin[9]. One such class of compound is *trans*-planaramineplatinum(II) complexes. In general, *trans*-platinum complexes with bulky planar ligands are found to be active in both murine and human cisplatin-resistant tumour cell lines[12]. It has been found that *cis*-planaramineplatinum(II) complexes could also be anticancer active. For example, ZD0D473 has been found to show significant antitumour activity against a number of cancer cell lines[13].

Recently, Huq *et al.*[14, 15] prepared a number of *trans*-planaramineplatinum(II) complexes of the form: *trans*-$PtCl_2NH_3L$ (L = 2-hydroxypyridine, 3-hydroxypyridine, imidazo[1,2-α]pyridine) which have shown significant anticancer activity. One of the compounds is twice as active as cisplatin against $A2780^{cis\text{-}R}$ cell line. The variations in activity of the compounds and conformational changes induced in pBR322 plasmid DNA illustrate structure-activity relationship. Since some *cis*-planaramineplatinum(II) complexes have also been found to be tumour active, it was thought appropriate to study the *cis*-isomers of the above complexes. Recently, we reported on the synthesis, DNA binding and activity of two *cis*-planaramineplatinum(II) complexes of the forms: *cis*-PtL_2Cl_2 and cis-$PtL(NH_3)Cl_2$ where L = 3-hydroxypyridine and imidazo(1,2-α)pyridine respectively[16]. In this paper, we report on the synthesis, spectral characterization and nature of interaction with pBR322 plasmid DNA of a *cis*-planaramine platinum(II) complex of the form: cis-PtL_2Cl_2, where L = imidazo(1,2-α)pyridine type (Fig. 1).

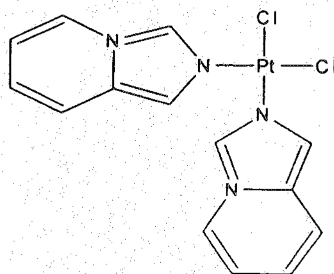


Fig. 1. Structure of AH6

EXPERIMENTAL

Potassium tetrachloroplatinate ($K_2[PtCl_4]$), cisplatin, transplatin, N,N-dimethylformamide [DMF] [C_3H_7NO] and imidazo(1,2-α)pyridine were obtained from Sigma Aldrich Chemical Company, Milwaukee, USA; acetone [$(CH_3)_2CO$] and silver nitrate [$AgNO_3$] were obtained from Ajax Chemicals, Auburn, NSW, Australia; methanol [CH_3OH] and ethanol [C_2H_5OH] were obtained from Merck Pty. Limited Kilsyth, VIC, Australia. pBR322 plasmid DNA was obtained from ICN Biochemicals, Ohio, USA.

Syntheses

cis-Bis-{imidazo(1,2-α)pyridine}(ammine)dichloroplatinum(II) (AH6) has been synthesized according to modified method of Dhara[17] as described below.

415 mg (1 mmol) of K_2PtCl_4] was dissolved in 10 mL of mQ water to which was added 2 g (about 12 mmol) of KI. 200 μL (2 mmol) of imidazo(1,2-α)pyridine was added with stirring to the mixture that was kept in ice. The colour of the precipitate changed from yellow to brown immediately. The mixture was kept in ice and stirred for 5 h following which it was left standing at room temperature for 12 h. The yellow precipitate of *cis*-Pt{imidazo(1,2-α)pyridine}$_2I_2$ was collected by filtration, washed with ice-cold water and ethanol and air-dried. The mass of the precipitate was 613 mg (0.9 mmol). The precipitate was suspended in 5 mL of mQ water to which was added 301 mg (1.77 mmol) of $AgNO_3$ with stirring in the dark. The mixture was kept in ice and stirring was continued for 24 h. The mixture was centrifuged to collect the yellow supernatant to which was added 140 mg (1.88 mmol) of KCl with stirring at room temperature. The yellow precipitate of *cis*-{imidazo(1,2-α)pyridine}$_2$dichloroplatinum(II) formed immediately. The mixture was kept in ice and stirred for 24 h. The precipitate was collected by filtration, washed with ice-cold water and ethanol and air-dried. The weight of the final product was 250 mg (0.48 mmol) corresponding to 48% yield.

Characterization

C, H, N and Cl were determined by using the facility at the Australian National University. Platinum was determined by graphite furnace atomic absorption spectroscopy (AAS) using the Varian Spectra-20 atomic absorption spectrophotometer. Infrared spectra were collected using a Bruker IFS66 spectrometer equipped with a Spectra-Tech diffuse reflectance accessory (DRA), an air-cooled DTGS detector, a KBr beamsplitter with a spectral range of 4000–650 cm^{-1}. Spectra were recorded at a resolution of 4 cm^{-1}, with the co-addition of 128 scans and a Blackman-Harris 3-term apodisation function was applied. Prior to analysis the samples were mixed and lightly ground with finely ground spectroscopic grade KBr. The spectra were then manipulated using the Kubelka-Munk mathematical function in the OPUS software to convert the spectra from reflectance into absorbance. Raman spectra were collected using a Bruker RFS100 Raman spectrometer equipped with an air cooled Nd:YAG laser emitting at a wavelength of 1064 nm and a liquid nitrogen cooled germanium detector with an extended spectral band range of 3500 to 50 cm^{-1}. 1800 sampling geometry was employed.

Spectra were recorded at a resolution of 4 cm^{-1}, with the co-addition of 100 scans at a laser power of 0.065 mW. To obtain mass spectra, solution of AH6, made in 10% DMF and 90% methanol, was sprayed into a Finnigan LCQ ion trap mass spectrometer in which fragmentation was produced by high energy electron beam bombardment. ^{1}H NMR spectrum of AH6 was recorded in dimethylsulfoxide-d_6 (DMSO-d_6) solution in a Bruker AVANCE DPX 400 spectrometer. Spectra were referenced to internal solvent residues and were recorded at 300 K (11 K).

Molar Conductivity: The molar conductivity values were determined for AH6 as well as for cisplatin and transplatin, using PW9506 digital conductivity meter available at the School of Chemistry, University of Sydney. The compounds were dissolved in a 1 : 1 mixture of DMF and water. The measurements were done at concentrations 1, 0.5, 0.2 and 0.05 mM. The molar conductivity (Λ_m) was calculated as $\Lambda_m = k/c$ where k is the conductivity and c is the concentration[18]. The molar conductivity values were plotted against concentration and the curve was extrapolated to zero concentration to give the limiting value.

Interaction with pBR322 plasmid DNA

Interaction between AH6 and pBR322 plasmid DNA with that for cisplatin was studied by agarose gel electrophoresis. The method used was a modification of that described by Stellwagen[19]. Solutions of pBR322 plasmid DNA (at concentration 0.5 μg mL^{-1}) were incubated with increasing concentrations of compounds ranging from 5 to 50 μM in a shaking water bath at 37°C for 4 h. 16 μL aliquot of drug-DNA mixtures containing 0.6 μg of DNA was loaded on to the 1% gel and electrophoresis was carried under TAB buffer for 2 h at 5 V cm^{-1}. At the end of electrophoresis, the gel was stained in the same buffer containing ethidium bromide (0.5 mg mL^{-1}). The gel was visualized under UV light using the Bio-Rad Trans illuminator IEC 1010. The illuminated gel was photographed with a Polaroid camera (a red filter and Polaroid type of film was used).

BamH1 digestion: BamH1 is known to recognize the sequence G/GATCC and hydrolyse the phosphodiester bond between adjacent guanine sites[20]. pBR322 contains a single restriction site for BamH1[21] which converts pBR322 plasmid DNA from supercoiled form I and singly nicked circular form II to linear form III DNA. In this experiment, the same set of drug-DNA mixtures as that described previously was first incubated for 4 h in a shaking water bath at 37°C and then subjected to BamH1 (10 units μL^{-1}) digestion. To each 20 μL of incubated drug-DNA mixtures were added 3 μL of 10x digestion buffer SB followed by the addition of 0.2 μL BamH1 (2 units). The mixtures were left in a shaking water bath at 37°C for 1 h at the end of which the reaction was terminated by rapid cooling. The gel was subsequently stained with ethidium bromide, visualized by UV light, then a photograph of the gel was taken as described previously.

Cytotoxicity

Cytotoxicity of AH6 against human ovarian cancer cell lines, A2780, A2780$^{cis\text{-}R}$ and A2780^{ZD0473R} was determined using MTT growth inhibition assay[22,23]. cisplatin was used as the reference. Briefly, between 5000 to 9000 cells were seeded

into the wells of the flat-bottomed 96-well culture plate in 10% FCS/RPMI 1640 culture medium. The plate was then incubated for 24 h at 37°C in a humidified atmosphere to allow them to attach. Platinum complexes were first dissolved in a minimum volume of DMF, then diluted to the required concentrations by adding mQ water and finally filtered to sterilize. A serial fivefold dilutions of the drugs ranging from 0.02 μM to 62.5 μM in 10% FCS/RPMI 1640 medium were prepared and added to equal volumes of cell culture in quadruplicate wells, then left to incubate under normal growth conditions for 72 h. The inhibition of the cell growth was determined using the MTT assay[22]. 4 h after the addition of MTT (50 μL per well of 1 mg mL^{-1} MTT solution), the cells were dissolved in 150 mL of DMSO and read with a plate reader (Bio-Rad Model 3550 Microplate Reader). The IC_{50} values were obtained from the results of quadruplicate determinations of at least three independent experiments.

RESULTS AND DISCUSSION

Characterization

Anal. (%) Calcd: C, 33.48; H, 2.41; N, 11.15; Pt, 38.84; Cl, 14.12; Found: C, 32.70 ± 0.40; H, 2.27 ± 0.40; N, 11.10 ± 0.40; Pt, 38.72 ± 1.00; Cl, 14.22 ± 0.30.

Molar Conductivity: Fig. 2 gives a plot of molar conductivity of AH6 with those for cisplatin and transplatin.

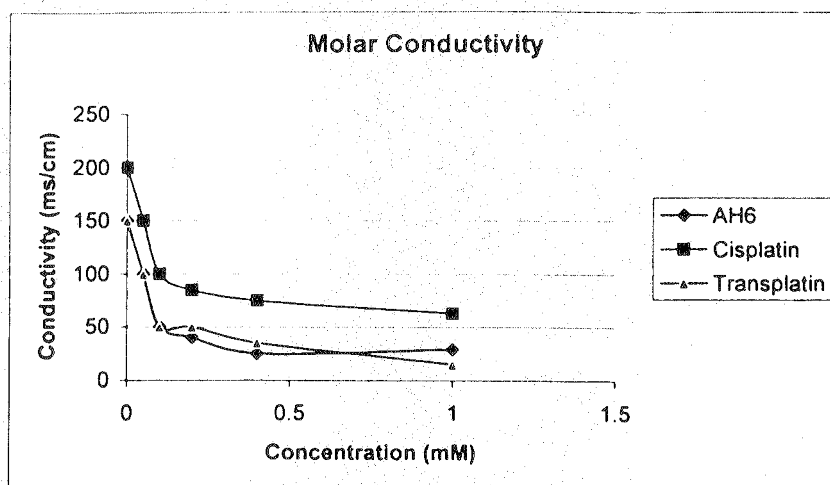


Fig. 2. Plot of molar conductivity against concentration as applied to AH6, cisplatin and transplatin

The limiting value of molar conductivity (in ohm^{-1} cm mol^{-1}) at zero concentration of AH6 was found to be 150 as against 200 for cisplatin and 150 for transplatin. This value is significantly lower than the expected value of about 280 for a 1 : 2 electrolyte, indicating that the degree of dissociation of the compound is of the order of 53.6. This result suggests that AH6 may be crossing the cell membrane by both passive diffusion and active transport. The lower molar conductivity value of AH6 as compared to that for cisplatin suggests that the degree of dissociation of the compound is less than that for cisplatin. This is in line with the presence of two bulkier planaramine ligands in AH6, that introduces

a greater steric hindrance. It is not however clear why transplatin is found to have a lower molar conductivity value than cisplatin. Since the degree of dissociation in biological fluids such as extracellular and intracellular fluids is expected to be different (possibly higher) than that in 1 : 1 mixture of DMF and water, it is not possible to draw any further conclusion from the results.

IR and Raman spectral analyses: The key bands observed in the IR and Raman spectra of AH4 and AH5 are given in Table-1.

TABLE-1
KEY IR RAMAN SPECTRAL BANDS OBSERVED IN AH6 (ν cm^{-1})

IR (cm^{-1})	Raman
3118 (s, NH stretch), 3043 (s, CH stretch), 1639 (w, NH bending), 1548 (w, C═C stretch), 1517 (m, ring stretch), 1473 (m, ring stretch), 1444 (m, ring stretch), 1234 (w, C—N stretch), 1163 (w, ring stretch), 1012 (w, CH in-plane bending), 749 (s, CH out-of-plane bending), 429 (m, Pt—N), 285 (s, Pt—Cl), 270 (s, Pt—Cl), 255 (s, Pt—N)	3078 (w, CH), 1638 (w, aromatic CH overtone), 1506 (m, ring stretch), 1443 (w, ring stretch), 1383 (w, CH), 1323 (m, NH), 1235 (w, NH), 1136 (w, ring stretch), 1015 (w, NH), 776 (m, CH), 639 (w, Pt—N), 580 (w, Pt—N), 334 (w, Pt—Cl), 120(s, Pt—N)

The infrared band at 3118 cm^{-1} is believed due to (N—H) stretching vibration. The band at 3043 cm^{-1} is believed to be due to (C—H) stretch. The band at 1548 cm^{-1} is due to (C═C) stretch. The band at 1517 cm^{-1} is due to ring stretch. The band at 1234 cm^{-1} is due to (C—N) bending vibration. The band at 1163 cm^{-1} is due to ring stretching vibration. The band at 1012 cm^{-1} is due to (C—H) in-plane bending of the hetereocyclic ring. The band at 749 cm^{-1} is believed to be due to (C—H) out-of-plane bending vibration. The band at 429 cm^{-1} is believed due to (Pt—N) stretching vibration. The bands at 285 and 270 cm^{-1} are due to (Pt—Cl) stretching vibrations. The band at 255 cm^{-1} is due to (Pt—N) stretching vibration.

The Raman spectral band at 3078 cm^{-1} is believed due to N—H stretching vibration. The band at 1638 cm^{-1} is due to N—H bending vibration whereas the bands at 1030 and 664 cm^{-1} are due to C—H in-plane bending of the hetereocyclic ring. The band at 872 cm^{-1} is due to N—H wagging. The band at 203 cm^{-1} is due to Pt—N. The band at 76 cm^{-1} is associated with the lattice mode.

Mass ^{1}H and NMR spectral analyses: The important peaks observed in the EIS mass and ^{1}H NMR spectra of AH6 are given in Table-2.

TABLE-2
IMPORTANT PEAKS OBSERVED IN THE ESI MASS AND ^{1}H NMR SPECTRA OF AH6

	ESI Mass	^{1}H NMR
AH6 MM = 502.3	EIS-MS (DMF) (M-4H) = 498 (0.60); (M-2Cl) = (M-Cl + imidazole (1,2-α) pyridine) = 584.9 (1.00))	^{1}H NMR DMSO δ ppm: 8.84 (d, due to CH); 8.37 (t, due to CH); 8.26 (d, due to CH); 8.21 (s, due to CH), 8.0 (d, due to CH), 7.27 (d, due to CH), 3.67 (d, water), 3.10 (quintet), 2.93 (quartet, DMSO)

The mass spectrum of AH6 has a peak with m/z = 498 that is believed to be due to (M—4H). The peak with m/z = 431 corresponds to (M—2Cl). It can be seen that the mass spectral data of AH6 provide support for the suggested structure of the compound.

The resonance at δ = 8.84 ppm is due to H attached to the ninth carbon and that at δ = 8.37 ppm is due to H attached to the eighth carbon. The resonance at δ = 8.26 ppm is due to H attached to the seventh carbon. The resonance at 8.21 ppm is due to H attached to the sixth carbon. The resonance at 8.0 ppm is due to H attached to the third carbon and that at 7.27 ppm is due to H attached to the second carbon. The resonance at δ = 3.67 ppm is due to water and that at δ = 2.93 ppm is due to DMSO. The resonance at δ = 3.10 ppm could not be identified. It is not clear why the resonances due to water and DMSO are shifted slightly from the expected values of 3.40 and 2.60 ppm.

Finally, it can be seen that the IR, Raman, mass and ^{1}H NMR spectra of AH6 provide support for the suggested structures of the compound.

Interaction with pBR322 plasmid DNA

Fig. 3 gives the electrophoretograms applying to the interaction of pBR322 plasmid DNA with cisplatin, transplatin and AH6 at concentrations ranging from 1 to 10 μM in the case of cisplatin and transplatin and 5 to 50 μM in the case of AH6.

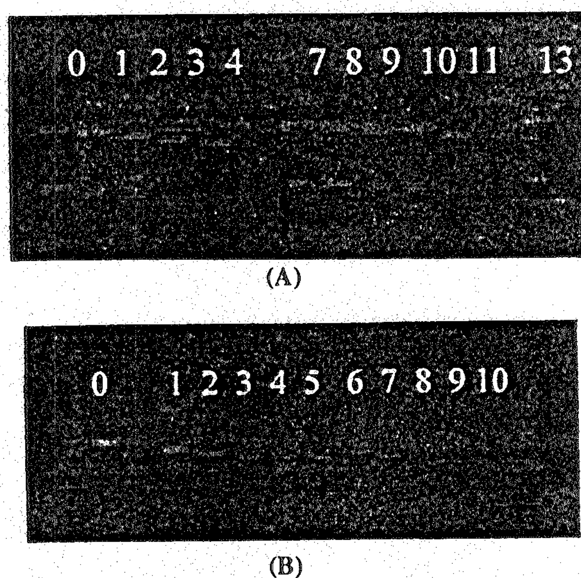


Fig. 3. (A) Lanes: Interaction of pBR322 plasmid DNA with increasing concentrations of cisplatin [lanes 0–4: (1) 1 μM; (2) 2.5 μM; (3) 5 μM; (4) 10 μM] and transplatin [lanes 7–13: (7) 0 μM; (8) 1 μM; (9) 2.5 μM; (10) 5 μM; (11) 10 μM, (13) 0 μM] ranging from 1 to 10 μM. (B) Interaction of pBR322 plasmid DNA with increasing concentrations of AH6 ranging from 1 μM to 50 μM in the interaction with pBR322 plasmid DNA: lane 0: untreated DNA and lanes 1–10: DNA interacted with increasing concentrations of compounds: [(1) 5 μM; (2) 10 μM; (3) 15 μM; (4) 20 μM; (5) 25 μM; (6) 30 μM; (7) 35 μM; (8) 40 μM; (9) 45 μM; (10) 50 μM]

As the concentration of AH6 is increased, there is an initial increase in the mobility of the form I DNA band to reach almost a constant value at higher concentrations of the compound. There is also a gradual decrease in the intensity

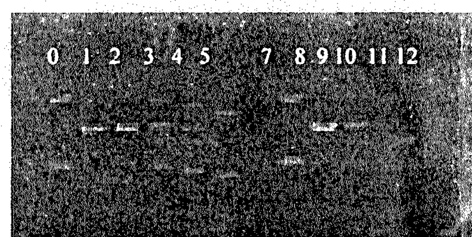
of the form I band with the increase in concentration of AH6. The change in mobility of the form I pBR322 plasmid DNA band is due to change in DNA conformation brought by covalent binding of AH6 with the DNA.

AH6 can form monofunctional Pt(G) or Pt(A) adducts and bifunctional intrastrand Pt(GG) and Pt(AG) adducts with the DNA. When AH6 binds covalently with DNA, the two imidazo(1,2-α)pyridine ligands in *cis*-configuration can undergo non-covalent interactions, *e.g.*, stacking interaction with nucleo bases in DNA. The decrease in intensity of the form I band at higher concentrations of AH6 indicates the occurrence of DNA damage possibly brought about by stacking interaction between planaramine ligands in AH6 and nucleobases in DNA. The presence of only a small amount of AH6 is found to be sufficient to greatly increase the intensity of the form II band indicating that in the presence of the compound some of form I DNA is changed to form II DNA due to nicking of a phosphodiester bond in one strand of the DNA. As this happens, the singly nicked DNA changes to relaxed circular form which has a lower mobility than the supercoiled form I. The changes in pBR322 plasmid DNA induced by increasing concentrations of AH6 are found to be qualitatively similar to those introduced by cisplatin except that for cisplatin the changes occur at much lower concentrations (whereas concentrations used for AH6 were 1–50 μM, those used for cisplatin were 1–10 μM). The results indicate that cisplatin binds with DNA much more readily than AH6. It will be seen later in the paper that antitumour activity of AH6 against ovarian cancer cell lines A2780, $A2780^{cis\text{-}R}$ and $A2780^{ZD0473R}$ is less than that for cisplatin. Since antitumour activity of platinum-based drugs is believed to be associated with their binding with DNA, the weaker binding of AH6 with DNA as compared to that for cisplatin provides an explanation as to why AH6 is less active than cisplatin. Changes in pBR322 plasmid DNA brought by transplatin are found to be different from those brought by cisplatin and AH6—there is a less marked change in both mobility and intensity. It should be noted that whereas cisplatin and AH6 can form monofunctional and intrastrand bifunctional adducts, transplatin can form monofunctional and interstrand bifunctional adducts. Changes in DNA conformation induced by intrastrand bifunctional adducts are different from those induced by interstrand bifunctional adducts; the latter would cause more of a global change in DNA conformation.

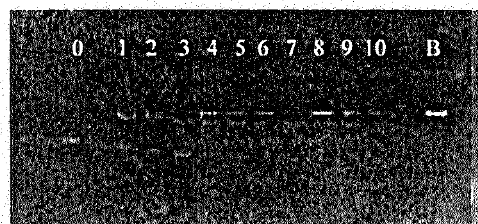
BamH1 digestion: Fig. 4 shows the electrophoretograms for the incubated mixtures of pBR322 plasmid DNA and varying concentrations of cisplatin and transplatin ranging from 0 to 10 μM and of AH6 ranging from 0 to 5 μM, followed by BamH1 digestion.

The untreated and undigested pBR322 plasmid DNA used was found to be a mixture of supercoiled form I and singly nicked form II. In the absence of platinum compounds, the digested DNA gave only the linear form III band indicating that all of the pBR322 plasmid DNA was doubly nicked by BamH1 at the specific GG site.

A mixture of forms I, II and III bands was observed for all concentrations of cisplatin with forms I and II bands fainter at 1 μM cisplatin and form III band being faintest at 10 μM cisplatin. The results (Table-3) indicate increasing prevention of BamH1 digestion with the increase in concentration of cisplatin.



(A)



(B)

Fig. 4. (A) Electrophoretogram for the incubated mixtures of pBR322 plasmid DNA and varying concentrations of cisplatin [lanes 0–5: (0) untreated plasmid; (1) 0, (2) 1 μM; (3) 2.5 μM; (4) 5 μM; (5) 10 μM)] and transplatin [lanes 7–13: (7) untreated plasmid; (8) 0, (9) 1 μM; (10) 2.5 μM; (11) 5 μM; (12) 10 μM]. (B) Electrophoretogram for the incubated mixtures of pBR322 plasmid DNA and varying concentrations of AH6 [lanes 0–10: (0) untreated and undigested DNA, (1) 5 μM; (2) 10 μM; (3) 15 μM; (4) 20 μM; (5) 25 μM; (6) 30 μM; (7) 35 μM; (8) 40 μM; (9) 45 μM; (10) 50 μM] followed by digestion with BamH1. Lane B applies to untreated but digested pBR322 plasmid DNA

TABLE-3

BANDS OBSERVED IN THE INCUBATED MIXTURES OF pBR322 PLASMID DNA AND VARYING CONCENTRATIONS OF (A) CISPLATIN, TRANSPLATIN AND (B) AH6 AFTER THEIR DIGESTION WITH BamH1

(A)

Compound	0.00 μM	1.0 μM	2.5 μM	5.0 μM	10.0 μM
Cisplatin	III	I (faint), II (faint), III	I, II, III	I, II, III	I, II, III (faint)
Transplatin	III	I (faint), II (faint), III	I, II (faint), III (faint)	I, II, III	I, II, III

(B)

Compound	0 μM	5 μM	10 μM	15 μM	20 μM	25 μM	30 μM	35 μM	40 μM	45 μM	50 μM
AH6	II	I, II	I, II	I, II, III	I, II, III	I. II, III	I, III	I, III	I. II, III	II, III	II, III

This is due to conformational change in the DNA brought about by the covalent binding of cisplatin with the DNA (mainly intrastrand GG). A mixture of forms I, II and III was also observed for all concentrations of transplatin indicating that transplatin is able to prevent BamH1 digestion to some extent. The results suggest that formation of interstrand adducts (which would be true for transplatin) also brings about conformational change in DNA mainly due to mismatch between interbase distance and that between the two *trans* arms of transplatin. The progres-

sive decrease in intensity of the bands from lanes 9 to 13 appears to be an artefact as the band applying to untreated and undigested DNA (lane 13) could not be seen.

Cytotoxicity

Fig. 5 gives the per cent cell survival *vs.* concentration curves for cisplatin and AH6 as applied to the ovarian cancer cell lines: A2780, $A2780^{cis\text{-}R}$ and $A2780^{ZD0473R}$.

Table-4 gives the IC_{50} values of cisplatin and AH6 against the cell lines: A2780, $A2780^{cis\text{-}R}$ and $A2780^{ZD0473R}$.

TABLE-4
IC_{50} VALUE AND RESISTANCE FACTORS FOR CISPLATIN AND AH6

	A2780	$A2780^{cis\text{-}R}$	$IC_{50}A2780^{cis\text{-}R}$ $/IC_{50}A2780$	$A2780^{ZD0473R}$.
	IC_{50}	IC_{50}	RF	IC_{50}
Cisplatin	1.1 ± 10.5	2.3 ± 0.4	2.1	2.2 ± 0.6
AH6	2.6 ± 0.6	3.6 ± 0.5	1.4	3.8 ± 0.6

It can be seen that AH6 is significantly less active than cisplatin against the cell lines: A2780, $A2780^{cis\text{-}R}$ and $A2780^{ZD0473R}$. It should however be noted that the IC_{50} value for cisplatin found in the present study as applied to the cell line A2780 is significantly higher than the reported value[23]. This difference may be the result of some problems faced in growing A2780 cells in the present study. It is thus possible that the actual IC_{50} value of AH6 also could be significantly lower than the value found.

The other point to note is that although AH6 is less active than cisplatin, the decrease in activity of the compound in going from cisplatin-responsive cell line A2780 to the resistant cell lines $A2780^{cis\text{-}R}$ and $A2780^{ZD0473R}$ is less marked than that for cisplatin, The results suggest that at the level of its activity the compound has been able to overcome mechanisms of resistance operating in $A2780^{cis\text{-}R}$ and $A2780^{ZD0473R}$ cell lines. As stated earlier, like cisplatin AH6 is expected to form monofunctional and intrastrand bifunctional adducts with DNA. It is possible that the presence of two bulky planaramine ligands makes AH6 much less reactive than cisplatin. Also, AH6 may undergo intercalation with DNA or while binding covalently with nucleobases in DNA may undergo stacking interaction. Damage to pBR322 plasmid DNA caused by AH4\6 (discussed earlier) may in fact partly be due to intercalation.

Conclusions

A *cis*-planaramineplatinum(II) complex of the form cis-PtL_2Cl_2 where L = imidazo(l,2-α)pyridine (code name AH6) has been synthesized and characterized by elemental analyses and spectral studies. The interaction of the compound with pBR322 plasmid DNA has been studied. The activity of the compound against ovarian cell lines A2780, $A2780^{cis\text{-}R}$ and $A2780^{ZD0473R}$ have also been determined where cisplatin has been used as the reference. Although both cisplatin and AH6 are expected to form monofunctional and intrastrand bifunctional adducts with DNA, the two compounds are found to differ in their ability to cause unwinding of supercoiled form I, pBR322 plasmid DNA and prevention of BamH1 digestion

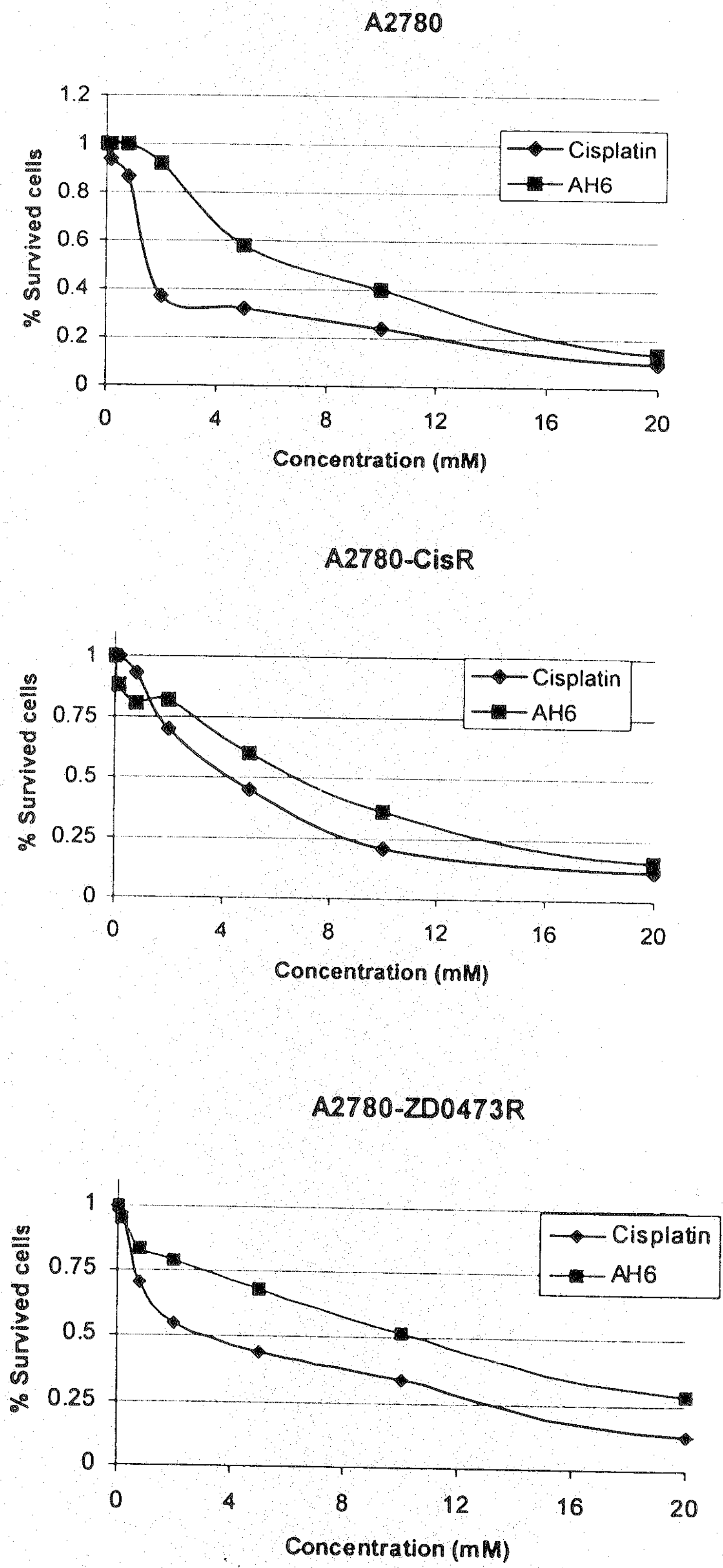


Fig. 5. Per cent cell survival *vs.* concentration curves for cisplatin and AH6 as applied to the ovarian cancer cell lines: A2780, $A2780^{cis\text{-}R}$ and $A2780^{ZD0473R}$

and induction of DNA damage. The lower activity of AH6 is believed to be due to its reduced ability to bind with DNA because of a greater steric constraint introduced by two bulkier planaramine ligands.

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