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Studies on the Synthesis and Characterization, and Binding with DNA of Two *Cis*-Planaramineplatinum(II) Complexes of the Forms: *Cis*-PtL_2Cl_2 and *Cis*-$PtL(NH_3)Cl_2$ and Where L=3-Hydroxypyridine and Imidazo (1,2-α) Pyridine

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ABSTRACT: Two *cis*-planaramineplatinum(II) complexes: *cis*-bis(3-hydroxypyridine)dichloro platinum(II) (code named AH4) and *cis*-{imidazo(1,2-α) pyridine}(ammine)dichloroplatinum(II) (code named AH5) have prepared and characterised 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. The compounds are 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. Both the compounds are less active than cisplatin against ovarian cancer cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$. The compounds are found to cause some prevention of BamH1 digestion which is believed to be due to the formation of intrastrand bifunctional Pt(GG) adducts. The compounds are also found to cause damage to pBR322 plasmid DNA, AH4 being more damaging than AH5, possibly due to greater stacking interaction involving planaramines.

Keywords: Cisplatin; transplatin; 3-hydroxypyridine; imidazo(1,2-α)pyridine; gel electrophoresis; pBR322 plasmid DNA; anticancer activity

Introduction

Although cisplatin is a widely used anticancer drug that 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 severe tissue toxicity and a limited spectrum of activity [3-7] and it is found that cisplatin analogues generally have a similar spectrum of activity and develop similar resistance [8-11].

Currently platinum compounds with markedly different structures are considered with the idea these compounds may have distinctly different types of interaction with DNA that may translate into a different spectrum of anticancer activity. 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*-planaramine platinum(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

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to show significant antitumour activity against a number of cancer cell lines [13].

In our laboratory a number of *trans*-planaramineplatinum(II) complexes of the form: *trans*-$PtCl_2NH_3L$ (L= 2-hydroxypyridine, 3-hydroxypyridine, imidazo[1,2-α]pyridine) have been prepared which have shown significant anticancer activity [14, 15]. One of the compounds is twice as active as cisplatin against $A2780^{cisR}$ 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. In this paper, we report on the synthesis, spectral characterisation and nature of interaction with pBR322 plasmid DNA and salmon sperm DNA of two *cis*-planaramineplatinum(II) complexes of the forms: *cis*-PtL_2Cl_2 and cis-$PtL(NH_3)Cl_2$ and where L= 3-hydroxypyridine and imidazo(1,2-a)pyridine respectively (Figure 1).

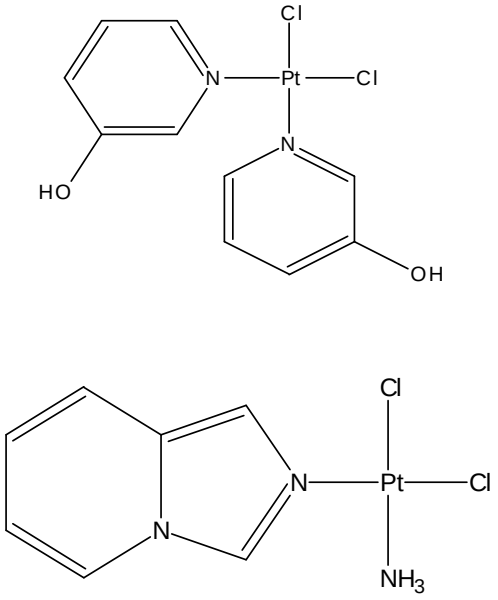

Figure 1: Structures of AH4 and AH5

Experimental

Materials

Potassium tetrachloroplatinate ($K_2[PtCl_4]$), N,N-dimethylformamide [DMF] [C_3H_7NO], 3-hydroxypyridine, 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. Salmon sperm DNA was obtained from Sigma-Aldrich NSW Australia and pBR322 plasmid DNA was obtained from ICN Biochemicals Ohio USA.

Syntheses

cis-planaramineplatinum(II) complexes: cis-bis (3-hydroxypyridine) dichloroplatinum(II) (code named AH4) and *cis*-{imidazo(1,2-α) pyridine} (ammine) dichloroplatinum(II) (code named AH5) have been synthesized according to modified Dhara [16] method.

cis-bis(3-hydroxypyridine) dichloro platinum (II) (AH4): One mmol (415.0 mg) of $K_2[PtCl_4]$ was dissolved in 10 mL of mQ water to which was added 2 g (about 12 mmol) of KI. 192 mg (2 mmol) of 3-hydroxypyridine, dissolved in 5 mL of mQ water by sonification, was added with stirring to the mixture that was kept in ice. The mixture was stirred at room temperature for about 24 h. The yellow precipitate of *cis*-$Pt(3\text{-hydroxypyridine})_2I_2$ was collected by filtration, washed with ice cold water and ethanol, and air-dried. The mass of the precipitate was 300 mg (0.47 mmol). The precipitate was suspended in 2 mL of mQ water to which was added 158 mg (0.93 mmol) of $AgNO_3$ with stirring in the dark at room temperature. Stirring was continued for 24 h. The mixture was centrifuged to collect the yellow supernatant to which was added 74 mg (99 mmol) of KCl with stirring at room temperature. Stirring was continued for 1 h. The yellow precipitate of *cis*-$Pt(3\text{-hydroxypyridine})_2Cl_2$ was collected by filtration, washed with ice cold water and air-dried. The weight of the final product was 127 mg (0.3 mmol) corresponding to 30% yield. It was characterized by elemental analyses, IR, Raman, mass and ^1H NMR spectral studies.

cis-{imidazo(1,2 –α)pyridine}(ammine) dichloro platinum (II) (AH5): One mmol (415.0 mg) of $K_2[PtCl_4]$ was dissolved in 10 mL of mQ water to which was added 2 g (about 12 mmol) of KI. The mixture was stirred at room temperature for 5 min. One mmol (0.14 mL) of 1:1 aqueous solution of ammonia was added with stirring to the mixture that was kept in ice. Stirring was continued for about 30 min. The yellow precipitate of $Pt(NH_3)I_3$ formed immediately after the addition of ammonia solution. One mmol (100 mL) of imidazo (1,2-a) pyridine was added with stirring to the mixture. The colour of the precipitate changed from yellow to brown immediately. The mixture was kept in ice and stirred for further 30

min. The brown precipitate of *cis*-Pt{imidazo(1,2-a)pyridine}$(NH_3)I_2$ was collected by filtration, washed with ice-cold water and air-dried. The mass of the precipitate was 500 mg (0.9 mmol). The precipitate was suspended in 5 mL of mQ water to which was added 290 mg (1.71 mmol) of $AgNO_3$ with stirring in the dark at room temperature. Stirring was continued for 3 h. The mixture was centrifuged to collect the golden-yellow supernatant to which was added 130 mg (1.74 mmol) of KCl with stirring at room temperature. The yellow precipitate of *cis*-{imidazo (1,2 –α) pyridine} (ammine) dichloroplatinum(II) formed immediately. The mixture was stirred at room temperature for 2 h. The precipitate was collected by filtration, washed with mQ water and air-dried. The weight of the final product was 152 mg (0.4 mmol) corresponding to 40% yield. It was characterized by elemental analyses, IR, Raman, mass and ^{1}H NMR spectral studies.

Characterization

C, H, N and Cl were determined out using the facility at the Australian National University. Carlo Erba 1106 automatic analyzer was used for the determination of C, H and N contents and Cl was determined by titration with standardized mercuric nitrate. Platinum was determined by graphite furnace atomic absorption spectroscopy (AAS) using the Varian Spectraa-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 to 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 OPUSä 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}. 180° 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 AH4 and AH5, made in 10% DMF and 90% methanol, were sprayed into a Finnigan LCQ ion trap mass spectrometer in which fragmentation was produced by a high energy electron beam bombardment. ^{1}H NMR spectra of AH4 and AH5 were 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 (±1 K).

Interaction with pBR322 plasmid DNA and salmon sperm DNA (ssDNA)

Interaction of AH4, AH5 and cisplatin with pBR322 plasmid DNA and ssDNA was studied by agarose gel electrophoresis. The method used was a modification of that described by Stellwagen [18]. Solutions of pBR322 plasmid DNA (at concentration 0.5 mg mL^{-1}) were incubated with increasing concentrations of compounds ranging from 5 mM to 50 mM in a shaking water bath at 37°C for 4 h. 16 mL aliquots of drug-DNA mixtures containing 1.5 mg of DNA was loaded onto the 1% gel and electrophoresis was carried under TAE buffer for 2 h at 5 V cm^{-1}. In the case of ssDNA, 2 mL of ssDNA (1 mg mL^{-1}) was used to provide 2 mg of the DNA. At the end of electrophoresis, the gel was stained in the same buffer containing ethidium bromide (0.5 mg mL^{-1}). The gel was visualised 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 [19]. pBR322 contains a single restriction site for BamH1 [20] which converts pBR322 plasmid DNA from supercoiled form I and singly nicked circular form II to linear form III DNA. In this experiment, a 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 mL^{-}) digestion. To each 20 mL of incubated drug-DNA mixtures was added 3 mL of 10x digestion buffer SB followed by the addition of 0.2 mL 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, visualised by UV light then a photograph of the gel was taken as described previously.

Cytotoxicity

Cytotoxicity of the compounds against human ovarian cancer cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$ was

determined using MTT growth inhibition assay [21, 22]. Briefly, cells between 5000 and 9000 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 mM to 62.5mM 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 [21]. 4 h after the addition of MTT (50 mL 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

Characterisation

The compositions of AH4 and AH5 are given in Table 1.

Table 1
Composition of AH4 and AH5

	AH4		*AH5*	
	Calculated %	*Observed %*	*Calculated %*	*Observed %*
C	26.3	26.5±0.4	21.0	21.1±0.4
H	2.2	2.3± 0.4	2.3	2.2±0.4
N	6.1	6.2±0.4	10.5	10.3±0.4
Cl	15.5	15.3±0.3	17.7	17.6±0.3
Pt	42.8	41.9±1.0	48.6	48.9±1.0

IR and Raman spectral analyses

The prominent bands observed in the IR and Raman spectra of AH4 and AH5 are given in Table 2.

Table 2
Prominent IR and Raman Spectral Bands Observed in AH4 and AH5 (ν? cm^{-1})

	IR (cm^{-1})	*Raman*
AH4	3309 (s, OH), 3066 (w d, CH), 1595 (s, C=C), 1496 (m, C=N), 1446 (s, CH bending), 1281 (s, pyridine), 1213 (m, CO stretch), 888 (m, CH), 844 (m, pyridine ring), 796 (s, CH), 687 (s, CH), 584 (m, ring stretch), 546 (m, ring stretch)	3087 (w, CH), 1030 (s, CH in-plane bending), 203 (w, Pt-N), 76 (s, lattice)
AH5	3260 (m br, NH), 3214 (m br, NH), 3141 (m br, CH), 1638 (m, ring stretch), 1511 (s, ring stretch), 1321 (s, ring), 1232 (w, CN stretch), 1162 (w br, CN stretch), 757 (s, CH), 749 (m, CH), 445 (m, Pt-N), 426 (s, Pt-N)	3090 (w, CH), 1638 (w, ring), 1512 (m, ring), 1321 (m, NH), 1235 (w, NH), 1136 (w, NH), 1015 (w, CH), 771 (w, CH), 639 (w, CH), 569 (w, Pt-N), 328 (m, Pt-Cl), 101 (Pt-N)

The letters 's', 'm', 'w', and 'br' stand for 'strong', 'medium', 'weak' and 'broad' respectively.

AH4

IR: The broad band at 3309 cm^{-1} is believed due to O-H stretching vibration. The band at 3066 cm^{-1} is believed to be due to C-H stretch. The band at 1595 at cm^{-1} is due to C = C stretching vibration whereas that 1496 at cm^{-1} is due to C = N stretching vibration. The band at 1446 cm^{-1} is believed to be due to CH bending vibration. The band at 1281 cm^{-1} is due to pyridine ring stretch. The band at 1213 cm^{-1} is due to C-O stretching vibration. The band at 888 cm^{-1} is believed due to C-H wagging. The bands at 796 and 687 cm^{-1} are due to C-H out of plane bending vibrations. The bands at 584 and 546 cm^{-1} are due to ring stretching vibrations.

Raman: The band at 3078 cm^{-1} is believed due to C-H stretching vibration. The band at at 1030 cm^{-1} is due to C-H in plane bending of the heterocyclic ring. The band at 203 cm^{-1} is due to Pt-N(3-hydroxypyridine). The band at 76 cm^{-1} is associated with the lattice mode.

AH5

IR: The bands at 3260 and 3214 cm^{-1} are believed to be due to N-H stretching vibrations. The band at 3141 cm^{-1} is believed to be due to C-H stretching vibration. The bands at 1638, 1511 and 1321 cm^{-1} are believed to be due to ring stretch. The bands at 1232 and 1162 cm^{-1} are believed to be due to C-N stretching vibrations. The bands at 757 and 749 cm^{-1} are believed to be due to C-H out-of-plane bending vibrations. The bands at 445 and 426 cm^{-1} are believed to be due to Pt-N(NH_3) stretching vibrations.

Raman: The band at 3090 cm^{-1} is believed to be due to C-H stretching vibration. The bands at 1638 and 1512 cm^{-1} are due to ring stretching vibrations. The bands at 1321, 1235 and 1136 cm^{-1} are believed to be due to N-H bending vibrations. The band at 1015 cm^{-1} is due to C-H in-plane bending of the heterocyclic ring. The bands at 771 and 639 cm^{-1} are due to C-H out-of-plane bending vibrations. The band at 569 cm^{-1} is due to Pt-N(NH_3) stretching vibration. The band at 328 cm^{-1} is due to Pt-Cl stretching vibration. The band at 101 cm^{-1} is believed to be due to Pt-N(NH_3) bending vibration.

Mass and ^1H NMR spectral analyses

The prominent peaks observed in the ESI mass and ^{1}H NMR spectra of AH4 and AH5 are given in Table 3.

Table 3
Prominent Peaks Observed in the ESI Mass and ^{1}H NMR Spectra of AH4 and AH5

	ESI Mass	*^1H NMR*
AH4 MW= 456.2	ESI-MS (DMF) ((M – 3H) = 453 (0.27); (M – 2Cl – H) = 384.3 (0.88)	^{1}H NMR DMSO δ ppm: 10.8 (s, due to OH); 8.40 (d, *J* = 2.4, impurity); 8.30 (s, 1H); 8.15 (d, *J* = 5.6, 1H); 7.36 (d, *J* = 0.9, 1H); 7.34 (q, *J* = 1.0, 1H)
AH 5MW= 401.2	EIS-MS (DMF) (M – 2Cl – NH_3+ imidazo(1,2-a) pyridine)= 431 (0.63); (M–2Cl–NH_3–H + imidazo (1,2-α) pyridine) = 430 (0.81); (M – 2Cl – 2H + NH_3 = 345 (0.45); (M – NH_3–H+imidazo (1,2-α) pyridine) = 498 (1.00); ($PtCl_2(NH_3)$ = 284 (0.25)	^{1}H NMR DMSO? δ ppm: 8.67 (d, *J* = 0.8, 1H); 8.65 (d, *J* = 0.8, 1H); 8.22 (d, *J* = 0.8, 1H); 8.20 (d, *J* = 0.8, 1H); 8.09 (q, *J* = 2, 1H); 7.83 (q, *J* = 1.9, 1H); 7.57 (d, *J* = 0.8, impurity); 7.53 (d, *J* = 1.2, impurity); 4.20 (s, NH-Pt); 3.95 (s, NH-Pt)

Mass

AH4: The mass spectrum of AH4 has a peak with m/z = 453 that is believed to be due to (M–3H). The peak with m/z = 384 corresponds to (M–2Cl–H). It can be seen that the mass spectral data of AH4 provide support for the suggested structure of the compound. It is not clear why molecular peak is missing. Perhaps indicates the breakdown of the molecule.

AH5: The mass spectrum of AH5 has a peak with m/z = 431 that is believed to be due to (M–2Cl–NH_3+ imidazo (1,2-α) pyridine) and that at 430 is due to (M–2Cl–NH_3 – H + imidazo (1,2-α) pyridine) The peak with m/z = 345 corresponds to (M – 2Cl – 2H + NH_3), that with m/z = 498 corresponds to (M – NH_3 – 3H + imidazo(1,2-α)pyridine). The peak with m/z = 284 corresponds to $PtCl_2(NH_3)_2$. It appears that most of the peaks in the mass spectrum of AH5 are due to species formed in situ from the joining of the fragments produced from AH5. The absence of molecular peak indicates the breakdown of the molecule.

^{1}H NMR

AH4: The numbering scheme for 3-hydroxypyridine is given in Figure 2.

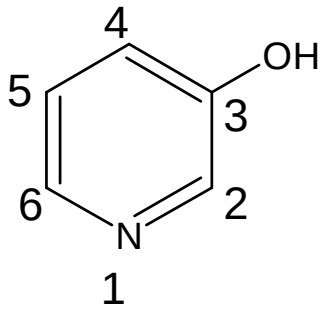

Figure 2: Numbering scheme of 3-hydroxypyridine

The resonance at δ = 10.80 ppm is due to OH proton, that at δ = 8.30 ppm is due to proton attached to C2. The resonance at d = 8.30 ppm is due to proton attached to C4, that at δ= 8.15 ppm is due to proton attached to C6 and that 7.34 is believed to be due proton attached to C5. The small resonance at δ = 8.40 ppm is believed to be due to some unidentified impurity. The resonance at δ = 3.32 ppm is due to water and that at δ = 2.50 ppm is due to DMSO.

AH5

The numbering scheme for the ligand: imidazo(1,2-a)pyridine is shown in Figure 3.

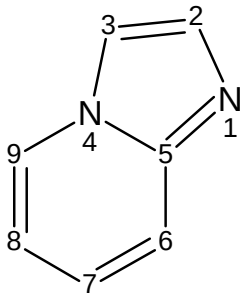

Figure 3: Numbering scheme for imidazo(1,2-α) pyridine

The resonance at δ ? = 8.67 ppm is due to H attached to C9 and that at δ = 8.65 ppm is due to H attached to C2. The resonance at δ = 8.22 ppm is due to H attached to C3. The resonance at 8.20 ppm is due to H attached to C6. The resonance at 8.09 ppm is due to H attached to C8 and that at 7.83 ppm is due to H attached to C7. The resonances at 4.20 and 3.95 ppm are due to NH bonded to Pt. The resonance at δ = 3.32 ppm is due to water and that at δ = 2.51 ppm is due to DMSO. The resonances at δ = 7.57 and 7.53 ppm are believed to be due to impurity. Finally, it can be seen that the IR, Raman, mass and ^{1}H NMR spectra of AH4 and AH5 provide support for the suggested structures of the compounds.

Interaction with pBR322 plasmid DNA and ssDNA

Figure 4 gives the electrophoretograms applying to the interaction of pBR322 plasmid DNA with cisplatin AH4 and AH5 at concentrations of compounds ranging from 1 μM to 50 μM in the case of pBR322 plasmid DNA and 1 μM to 500 μM in the case of ssDNA. Lanes 1, 2: only ssDNA or pBR322 plasmid DNA, lanes 4-12 apply to ssDNA or pBR322 plasmid DNA interacted with increasing concentrations of the compounds.

Interaction with pBR322 plasmid DNA

Untreated pBR322 plasmid DNA gave one band corresponding to supercoiled form I or two bands corresponding to supercoiled form I and singly-nicked form II. At [cisplatin] = 10 μM, two bands corresponding forms I and II were observed. As the concentration was further increased two bands came closer together so that essentially a single band was observed at [cisplatin] = 20 μM or greater. There was a sharp decrease in intensity of the band with the increase in concentration of cisplatin

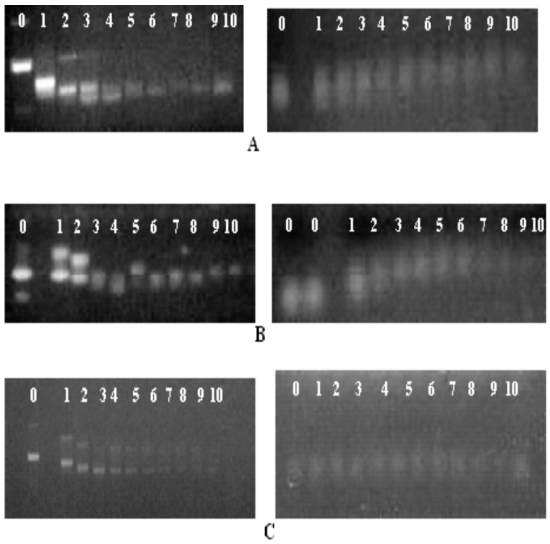

Figure 4: Interaction of pBR322 plasmid DNA and ssDNA with increasing concentrations of cisplatin (**A**), AH4 (**B**) and AH5 (**C**) ranging from: 5 μM to 50 μM in the interaction with pBR322 plasmid DNA (lane 0: 0, lane 1: 5 μM, lane 2: 10 μM, lane 3: 15 μM, lane 4: 20 μM, lane 5: 25 μM, lane 6: 30 μM, lane 7: 35 μM, lane 8: 40 μM, lane 9: 45 μM, lane 10: 50 μM) and 50 μM to 500 μM in the interaction of ssDNA (lane 0: 0, lane 1: 50 μM, lane 2: 100, lane 3: 150 μM, lane 4: 200 μM, lane 5: 250 μM, lane 6: 300 μM, lane 7: 350 μM, lane 8: 400 μM, lane 9: 450 μM, lane 10: 500 μM).

above 20 μM. The coalescing of the bands is due to conformational changes in supercoiled form I DNA as a result of covalent binding of the drug with DNA. The decrease in intensity of the bands with the increase in concentration of cisplatin indicates the occurrence of DNA damage.

When pBR322 plasmid DNA was interacted with increasing concentrations of AH4, two prominent bands corresponding forms I and II were observed at concentrations of AH4 ranging from 5 to 10 μM. A single was observed at [AH4] = 15 μM. At [AH4] = 20 μM, the observed band became more elongated with the front of the band slightly more prominent than the rear of the band. At the next higher concentration (25 μM), the tail of the band was found to be more prominent than the head of the band. At still higher concentrations, there was a sharp decrease in the intensity of the single band observed. The coalescing of the bands at [AH4] = 15 μM is believed to be due conformational changes brought about by the covalent binding of AH4 with pBR322 plasmid DNA. It is known that as platinum complexes bind to plasmid DNA, the conformation of supercoiled form I DNA can change from negatively supercoiled form I through relaxed circular form I to positively super coiled

form I DNA. The decrease in intensity of the band indicates the occurrence of DNA damage.

The plasmid DNA that was interacted with AH5 initially gave only one prominent band corresponding to supercoiled form I and faint band corresponding to form II. As the DNA was interacted with increasing concentrations of AH5, the intensity of the form II DNA band was found to increase at the expense of that for the form I DNA band. The decrease in intensity of the bands indicates the occurrence of DNA damage brought about by covalent binding of AH5 with the DNA. It should however be noted that AH4 appears to be more damaging to pBR322 plasmid DNA than AH5.

Like cisplatin, AH4 and AH5 are expected to form mainly monofunctional Pt(G) and bifunctional Pt(GG) adducts with DNA. However, AH4 and AH5 because of the presence of bulky planaramine ligands are expected to bind less strongly with DNA than cisplatin.

Interaction with ssDNA

When ssDNA was interacted with increasing concentrations of cisplatin, AH4 and AH5 ranging from 50.0 μM to 500 μM, there was a slight decrease in the mobility of the band in the case of cisplatin and AH4 (more in the case of AH4), but not in the case of AH5.

BamH1 digestion

Figure 5 shows the electrophoretograms for the BamH1 digested mixtures of pBR322 plasmid DNA after their treatment with the varying concentrations of AH4 and AH5 ranging from 0 to 50 μM.

In the absence of platinum compounds, only the linear form III band was observed, indicating that all of the pBR322 plasmid DNA was doubly nicked by BamH1 at the specific GG site. At lower concentrations of cisplatin (1-3.5 μM) (Figure 5 **A**), a mixture of forms I, II and III bands were observed. At concentrations of cisplatin ranging from 5.0 μM to 35.0 μM, a mixture of forms I and II were observed and at still higher concentrations only supercoiled form I band was observed. Also, as the concentration of cisplatin was increased above 5 μM, there was a progressive decrease in the intensity of the band indicating the occurrence of DNA damage due to covalent interaction of binding of the compound with DNA.

In the case of pBR322 plasmid DNA interacted with AH4 followed by BamH1 digestion, a mixture of forms I and II bands were observed for [AH4] ranging from 5

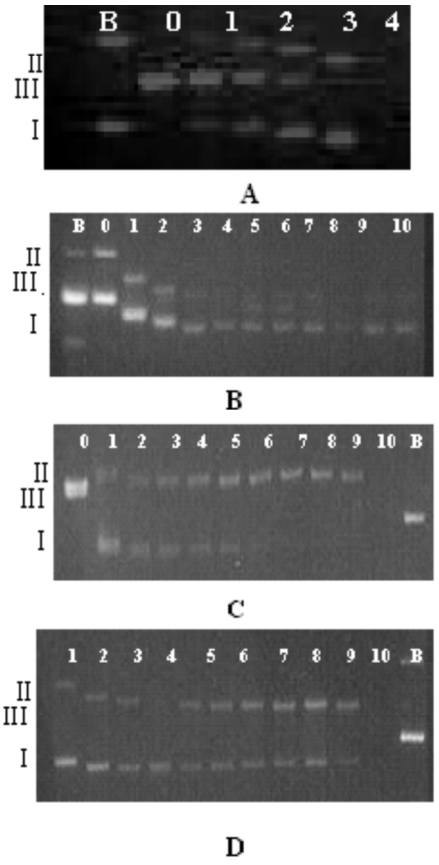

Figure 5: Electrophoretograms for the incubated mixtures of pBR322 plasmid DNA and varying concentrations of: cisplatin (**A** and **B**), AH4 (**C**), AH5 (**D**); **A**: (lanes 0-4: 1: 1 μM; 2: 2.5 μM; 3: 5 μM; 4: 10 μM.); **B**, **C** and **D**: (lane 0: 0, lane 1: 5 μM, lane 2: 10, lane 3: 15 μM, lane 4: 20 μM, lane 5: 25 μM, lane 6: 30 μM, lane 7: 35 μM, lane 8: 40 μM, lane 9: 45 μM, lane 10: 50 μM) followed by digestion with BamH1. Lane B applies to untreated and undigested pBR322 plasmid DNA.

μM to 25.0 μM and only form II band was observed for [AH4] = 30.0 μM to 45.0 μM. No band was observed at [AH4] = 50 μM. In the case of pBR322 plasmid DNA interacted with AH5 followed by BamH1 digestion, a mixture of forms I and II bands were observed for all concentrations of AH5 ranging from 5 μM to 50 μM. These results were summarised in Table 4.

The results indicate that AH4 is more damaging to DNA than either AH5 or cisplatin. That a mixture of forms I and II bands were observed at low concentrations of cisplatin, AH4 and AH5 (whereas in the absence of drug only form III band was observed), indicates that the three compounds are able to cause conformational change in supercoiled form I DNA so that BamH1 digestion is to some extent prevented. As cisplatin and its analogues form 1,2-intrastrand adducts such as

Pt(GG), a local bending of a DNA strand takes place so that recognition by BamH1 (which cuts the GG phosphodiester bond) is prevented. That only form II band was observed for [AH4] ranging from 30.0 mM to 45.0 mM and no band was observed at [AH4] = 50.0 mM, indicates that AH4 itself can cause DNA damage. It is believed that DNA damage due to AH4 may be mediated through non-covalent interactions such as stacking interactions between 3-hydroxypyridine ligands and DNA between the two planaramine ligands in the compound and nucleobases in DNA.

Some decrease in the intensity of the form I band and the corresponding increase in that of the form II band with the increase in concentration of AH5 indicate that AH5 also has been able to cause some DNA damage. However, when we compare DNA damage due to AH4 with that due to AH5, it appears that AH4 is more damaging to DNA than AH5.

When we compare the prevention of BamH1 digestion by AH4 and AH5 with that by *trans*-$PtL(NH_3)Cl_2$ code named YH9, YH10, YH11 and YH12 where L = 2-hydroxypyridine, imidazole, 3-hydroxypyridine, and imidazol (1,2-α)pyridine respectively, it appears that AH4 and AH5 are less able to prevent BamH1 digestion (because of lesser conformational change in DNA) than the reported *trans*-planaramineplatinum(II) complexes [15]. Whereas cisplatin and *cis*-planaramineplatinum(II) complexes can form mainly monofunctional Pt(G) and intrastrand bifunctional 1,2-Pt(GG) adducts, *trans*-planaramineplatinum(II) complexes are likely to form mainly monofunctional Pt(G) and short-range interstrand bifunctional adducts such as Pt(GC) and Pt(GG). AH4 and AH5 are less likely to form intrastrand bifunctional Pt(GG) adducts than cisplatin because of the steric constrain imposed by bulky planaramine ligands (two in AH4 and one in AH5).

Cytotoxicity

Table 5 gives the IC_{50} values of cisplatin, AH4 and AH5 against the cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD047R}$. It can be seen that both AH4 and AH5 are significantly less active than cisplatin (considered more fully later in the section) [14, 15] against the cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$. However, when the results are compared with the previously reported values it is seen 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 [14, 23]. It may be noted that in this study there were some problems in growing A2780 cells. Thus it is possible that the A2780 viable cells even without any drug added to it did not represent a 'normal' healthy population so that the results in the present study underestimated the activity of all the drugs as applied to the cell line.

The other point to note is that although AH4 and AH5 are less active than cisplatin, the decrease in activity of the compounds in going from cisplatin-responsive cell line: A2780 to the resistant cell lines: $A2780^{cisR}$ and $A2780^{ZD0473R}$ is less marked than for cisplatin. The results suggest that at the level of their activity the compounds have been able to overcome mechanisms of resistance operating in $A2780^{cisR}$ and $A2780^{ZD0473R}$ cell lines. AH4 is found to be slightly more active in $A2780^{cisR}$ cell line than in A2780 cell line. As stated earlier, like cisplatin AH4 and AH5 are expected to form monofunctional and intrastrand bifunctional adducts with DNA. It is possible that due to the presence of one or two planaramine ligands, the reactivity of AH4 and AH5 is reduced as compared to cisplatin. Also, AH4 and AH5 may undergo intercalation with DNA.

Damage to pBR322 plasmid DNA caused by AH4 and AH5 (discussed earlier) may in fact at least partly be due to intercalation. When we compare the activity of AH4 and AH5 with that reported for YH9, YH10, YH11 and YH12 [15], it appears that AH5 is less active than its *trans*-counterpart (YH12) against the cell lines: $A2780^{cisR}$ and $A2780^{ZD0473R}$ but more active against the cell line A2780. The results suggest that AH5 is less able to overcome resistance in $A2780^{cisR}$ and $A2780^{ZD0473R}$ cell lines than YH12. This is not unexpected since (as noted earlier) the *cis*- and *trans*-planaramineplatinum(II)

Table 4

Bands observed in the in the incubated mixtures of pBR322 plasmid DNA and varying concentrations of AH4 and AH5 after their digestion with BamH1

Compound	*0.00μM*	*5.0μM*	*10.0μM*	*15.0μM*	*20.0μM*	*25.0μM*	*30.0μM*	*35.0μM*	*40.0μM*	*45.0μM*	*50.0μM*
Cisplatin*	III	I, II	I, II	I, II	I, II	I, II	I, II	I, II	I	I	I
AH4	III	I, II	I, II	I,II	I,II	I,II	II	II	II	II	No band
AH5	III	I, II	I, II	I, II	I, II	I, II	I, II	I, II	I, II	I, II	I, II

*At [Cisplatin] = 1.0 mM to 3.5 mM, a mixture of forms I, II and III bands are observed

complexes differ in the nature of bifunctional adducts that may be informed-whereas *cis*-isomers are more likely to form i1,2-intrastrand adducts the *trans*-isomers are more likely to form interstrand adducts. Whereas interstrand adducts are likely cause a global change in DNA conformation, 1,2-intrastrand adducts bend DNA sufficiently towards the major groove, exposing a shallow minor groove surface to which several proteins including high-mobility group (HMG) box proteins bind [24].

When we compare the IC_{50} values of AH4 and AH5 in A2780 cell line, it is found that the value for AH5 (which has one planaramine ligand) is significantly lower than that for AH4 which has two planaramine ligands per molecule. However, for the resistant cell lines, the IC_{50} values for the compounds are comparable. Because the ligands: 3-hydroxypyridine and imidazo (1,2-α) pyridine are different in size (the former one has one ring whilst the latter has two fused rings) and presence of functional groups (eg whilst the former has a hydroxyl group the latter does not), the differences in activity of the compounds cannot be explained based on steric factors alone. It would be interesting to find out whether *cis*-(ammine) (3-hydroxypyridine) dichloroplatinum (II) (that would contain one 3-hydroxypyridine ligand) is more active or not than AH4 that contains two 3-hydroxypyridine ligands.

Table 5
IC_{50} value and Resistance Factors for Cisplatin, AH4 and AH5

	A2780	*A2780^{cisR}*	*IC_{50}A2780^{cisR} /IC_{50}A2780*	*A2780^{ZD0473R}*
	IC_{50}	*IC_{50}*	*RF*	*IC_{50}*
Cis-platin	1.6 ± 0.5	4.4 ± 0.4	2.8	3.4 ± 0.6
AH4	8.6 ± 0.6	7.6 ± 0.5	0.88	8.0 ± 0.6
AH5	3.8 ± 0.5	6.5 ± 0.7	1.7	10.0 ± 0.6

Further comment on the difference in activity of *cis*- and *trans*-isomers can be explained as follow.

As stated earlier, one of the two *cis*-planaramineplatinum (II) complexes in the present study (AH5) is found to be less active than its *trans*-counterpart *trans*-{imidazo(1,2-α)pyridine} (ammine) dichloroplatinum(II) [14, 15], the *trans*-isomer being about 2.2 times as active as the *cis*-isomer against A2780^{cisR} ovarian cell line. AH4 is found to be less active than its *trans*-counterpart [24] (the *trans*-isomer being 1.59 times more active) against A2780 cell line but marginally more active than its *trans*-counterpart (about 1.3 times more) against the cell line A2780^{cisR}. The results suggest that both AH4 and AH5 are better able to overcome resistance in A2780^{cisR} cell line than their *trans*-counterparts.

Since two isomers differ in the nature of bifunctional adducts that can be formed (intrastrand in the case of *cis*-isomer and interstrand in the case of *trans*-isomer), the results can be seen to provide to support to the idea that a possible mechanism of cisplatin resistance in A2780^{cisR} cell line is associated with repair of platinum-DNA lesions.

Conclusions

Two *cis*-planaramineplatinum(II) complexes of the form: *cis*-PtL_2Cl_2 and *cis*-$PtL(NH_3)Cl_2$, code named AH4 and AH5 for which L is 3-hydroxypyridine and imidazo (1,2-α) pyridine respectively have been synthesized and characterized by elemental analyses and spectral studies. The interaction of the compounds with pBR322 plasmid DNA and salmon sperm DNA has been studied. The activity of the compounds against the cell lines: A2780, A2780^{cisR} and A2780^{ZD0473R} have also been determined. The compounds are found to differ in their ability to cause unwinding of supercoiled form I pBR322 plasmid DNA and prevention of BamH1 digestion and DNA damage. AH4 is found to cause a greater damage to pBR322 plasmid DNA than AH5. Both the compounds are expected to bind with DNA to form predominantly intrastrand Pt(GG) adduct that causes local bending of a DNA strand towards the major groove, exposing a wide and shallow minor groove surface to which several classes of proteins including high-mobility group box proteins, transcription factors and other proteins (such as histone H1) that preferentially recognize 1,2-intrastrand crosslinks. The variations in activity of the compounds illustrate structure-activity relationship.

Abbreviations

AH4:*cis*-bis(3-hydroxypyridine)dichloroplatinum (II).
AH5: *cis*-{imidazo (1,2-α) pyridine} (ammino) dichloroplatinum(II).
ssDNA: Salmon sperm DNA.
Cisplatin: cis-dichlorodiamminplatinum(II).
1xTAE buffer: 0.05 M Tris base + 0.05 M glacial acetic acid + 1 mM EDTA, pH=8.0.
DMSO: Dimethyl sulfoxide.
AAS: Atomic absorption spectrophotometry. Adenine: A . Guanine: G . YH9: *trans*-(2-hydroxypyridine) (ammine) dichloroplatinum(II). YH10: *trans*-

(imidazole)(ammine)dichloro- platinum(II) .
YH11: *trans*-(3-hydroxypyridine)(ammine)dichloro-platinum(II) .
YH12: *trans*-(imidazo(1,2-α-pyridine))(ammine) dichloroplatinum (II).
HMG: high-mobility group.

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