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Synthesis and Characterization and Binding of Amine-Palladium(II) Complexes and Their Interaction with DNA

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Three new planaraminepalladium(II) complexes of the form *trans*-$PdCl_2L_2$, code named TH5, TH6 and TH7, where L = 3-hydroxypyridine, 2-hydroxypyridine and 4-hydroxypyridine, respectively have been synthesized and characterized by elemental analyses and IR, mass and ^1H NMR spectral studies. The nature of interaction of the compounds with salmon sperm DNA and pBR322 plasmid DNA has also been investigated. The compounds differ in their molar conductivity values, ability to cause DNA damage and changes in DNA conformation. Solution of TH7 in 1:1 mixture of DMF and water has the highest molar conductivity value whereas that of TH5 has the lowest value indicating that the compounds differ in degree of dissociation into ions. TH5 is found to cause the greatest damage to pBR322 plasmid DNA and is also most effective in preventing BamH1 digestion. Both the DNA damage and prevention of BamH1 digestion are believed to be a consequence of binding of the compounds with DNA. The results illustrate structure-activity relationship.

Key Words: *Cis*-platin, Palladium(II), 2,3,4-Hydroxypyridine, DNA damage, Anticancer activity, Gel electrophoresis, Molar conductivity.

INTRODUCTION

Although *cis*-platin is a widely used anticancer drug[1,2], it has a number of side-effects including neurotoxicity, nephrotoxicity, ototoxicity, vomiting and hair-loss[3–8]. Also, cancer cells develop resistance to the continued use of cisplatin. In an attempt to reduce toxicity and widen the spectrum of activity, many cisplatin analogues have been prepared by varying the nature of the leaving groups and the carrier ligands. However, it is found that all cisplatin analogues generally have a similar spectrum of activity and develop similar resistance[9].

Currently attention is given to rule breaker platinum and other complexes of other metals with the aim of widening spectrum of activity and reducing toxicity. Two such classes of compounds are the *trans*-planaramineplatinum(II) complexes and the polynuclear platinum complexes[10] that contain two or more platinum units linked together by diaminoalkane chains[11]. A notable example of polynuclear complexes is BBR3464 that consists of three *trans*-platinum units joined together by two 1,6-diaminohexane chains. BBR3464 has been found to circumvent the inherent or acquired cisplatin-resistance *in vitro* and *in vivo* in a panel of human

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adult tumour models[12–15]. It was in phase II stage of clinical trial[16] before it was stopped because of high organ toxicity. Only the two terminal platinum centres in BBR3464 undergo covalent binding (mainly interstand) with DNA whereas the central platinum unit undergoes only noncovalent interactions such as hydrogen bonding and electrostatic interactions[17–19]. Based on the hypothesis that the replacement of the central platinum unit with other suitable metal units may not significantly alter the covalent interactions of the terminal platinum units (although it may have a subtle effect on the noncovalent interactions), a number of Pt-Pd-Pt have been designed, some of which have shown significant antitumour activity[20]. Since the Pt-Pd-Pt compounds are found to be less active than BBR3464, the therapeutic window of the compounds would be greater than BBR3464. It has been hypothesized that the introduction of one or more bulky ligands (such as planaramine) attached to one or more of the metal atoms is likely to increase steric hindrance causing a reduction in reactivity and possibly also in activity. However, some reduction in activity may not be undesirable since it will widen the therapeutic window. It may be noted that because of very high activity of BBR3464, its therapeutic window is extremely narrow. With the aim of preparing such multicentred complexes where one or more of the ammine ligands bonded to the central centre and/or the terminal metal centres are replaced by planaramine ligands, we have synthesized three palladium compounds of the type *trans*-PdL_2Cl_2 code named TH5, TH6 and TH7 where L stands for 3-hydroxypyridine, 2-hydroxypyridine, 4-hydroxypyridine, respectively (Fig. 1).

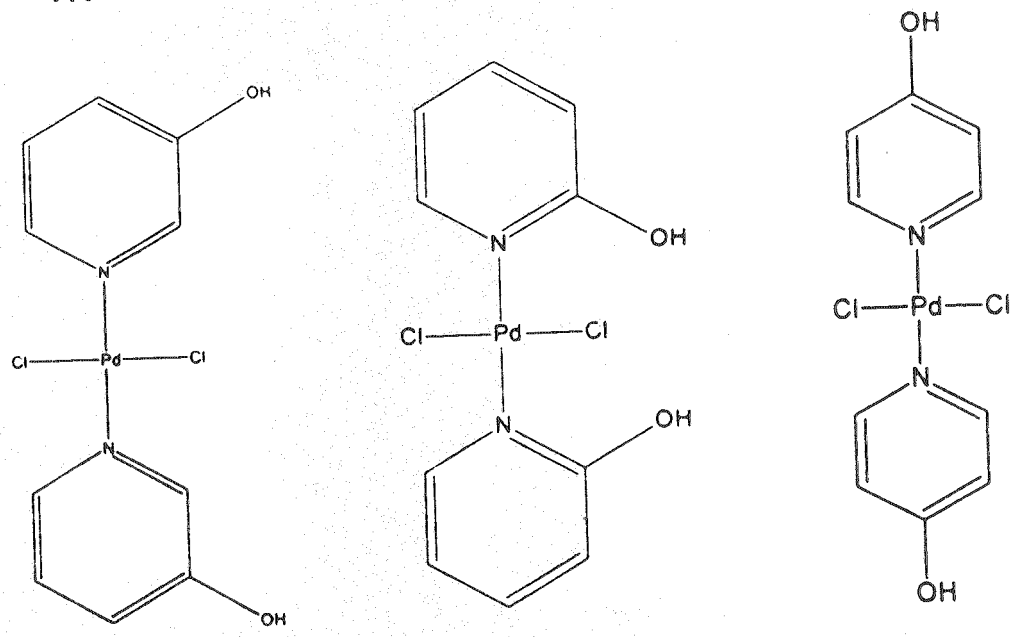


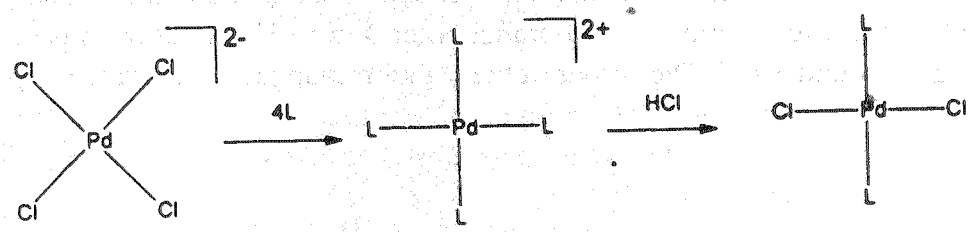
Fig. 1. Structures of ligands

The compounds would serve as the starting materials for the synthesis of aforementioned multicentred complexes. Before the multicentred complexes are prepared and investigated for antitumour activity and nature of binding with DNA, it is thought that the nature of interaction of the mononuclear *trans*-planaraminepalladiu(II) complexes with DNA should be investigated first. The

information would be useful in the understanding of the nature of interaction of multicentred complexes with DNA especially if they break down into dinuclear and mononuclear species in the biological system.

In this paper, the synthesis and spectral characterization of three *trans*-planaraminepalladium(II) complexes of the type *trans*-PdL_2Cl_2 where L stands for 2-hydroxypyridine, 3-hydroxypyridine, 4-hydroxypyridine and the results of their interaction with pBR322 plasmid and salmon sperm DNA are reported..

Trans-(3-hydroxypyridine)dichloropalladium(II) [TH5], *trans*-(2-hydroxypyridine)dichloropalladium(II) [TH6], and *trans*-(4-hydroxypyridine)dichloropalladium(II) [TH7], have been synthesized from potassium tetrachloropalladate ($K_2[PdCl_4]$) according to modified Kauffman method[19] as shown in Fig. 2.



L = 2-hydroxypyridine, 3-hydroxypyridine or 4-hydroxypyridine

Fig. 2. Schematic representation of the general procedure used for the synthesis of TH5, TH6 and TH7

EXPERIMENTAL

Potassium tetrachloropalladate(II) ($K_2[PdCl_4]$) and N,N-dimethylformamide (DMF), 2-hydroxypyridine, 3-hydroxypyridine and 4-hydroxypyridine were obtained from Sigma Chemical Company, St. Louise USA; hydrochloric acid and methanol were obtained from Asia Pacific Speciality Chemicals Ltd., NSW, Australia.

C, H, N and Cl were determined using the facility at the Australian National University. Palladium 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 400 cm^{-1}. To obtain mass spectra, solutions of TH5, TH6 and TH7, made in 10% of DMF and 90% methanol, were sprayed into a Finnigan LCQ ion trap mass spectrometer in which fragmentation was produced by electrospray ionization (ESI). ^1H NMR spectra of YH9, YH10, YH11 and YH12 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).

Synthesis of the complexes

[*trans*-Bis-(3-hydroxypyridine)dichloropalladium(II)] (TH5): 1 mmol of potassium tetrachloropalladate (0.326 g) was dissolved in 7.5 mL of milliQ (mQ) water and 0.25 mL of concentrated hydrochloric acid was added. The solution was heated to 50°C. 10 mmol of 3-hydroxypyridine (0.951 g), dissolved in 2 mL of DMF, was slowly added to the potassium tetrachloropalladate solution. The solu-

tion was kept on stirring at around 50°C. White precipitate appeared after about 30 min when the volume was reduced to about 5 mL. 40 mL of 6 M hydrochloric acid was added which caused the formation of some yellow precipitate. The mixture was heated at 50°C with reflux for 5 h following which the flask was brought to room temperature and then kept on ice for 1 h. Yellow precipitate of TH5 was collected at the pump, washed with mQ water and ethanol and air-dried. The weight of the final product was 0.220 g giving a yield of about 59%.

[*trans*-Bis-(2-hydroxypyridine)dichloropalladium(II)] (TH6): 1 mmol of potassium tetrachloropalladate (0.326 g) was dissolved in 7.5 mL of mQ water and 0.25 mL of concentrated hydrochloric acid was added. The solution was heated to 45°C. 10 mmol of 2-hydroxypyridine (0.951 g), dissolved in 2 mL of DMF, was slowly added to the potassium tetrachloropalladate solution. The colour turned to red after 1 min, then to orange after 4 min. The solution was kept on stirring at around 30°C. The colour of the solution changed from orange to yellow after about 25 min when the volume was reduced to 5 mL. 40 mL of 6 M hydrochloric acid was added. The mixture was heated at 50°C with reflux for 5 h. The flask was cooled to room temperature and then kept on ice for 1 h. The yellow precipitate of TH6 was collected at the pump, washed with mQ water and ethanol and air-dried. The weight of the final product was 0.262 g giving a yield of about 71%.

[*trans*-Bis-(4-hydroxypyridine)dichloropalladium(II)] (TH7): 1 mmol of potassium tetrachloropalladate (0.326 g) was dissolved in 7.5 mL of mQ water and 0.25 mL of concentrated hydrochloric acid was added. The solution was heated to 45°C. 10 mmol of 4-hydroxypyridine, dissolved in 2 mL of mQ water, was slowly added to the potassium tetrachloropalladate solution. The colour turned to yellow that was followed by the formation of yellow precipitate. The solution was stirred for about 25 min with the temperature kept at 45°C, at the end of which the volume was reduced to about 10 mL. 40 mL of 6 M hydrochloric acid was added and stirring continued at room temperature for 8 h. The flask was cooled to room temperature and then kept on ice for 1 h. The yellow precipitate of TH6 was collected at the pump, washed with mQ water and ethanol and air-dried. The weight of the final product was 0.295 g giving a yield of about 80%.

Molar Conductivity

The conductivity values of solutions of TH5, TH6 and TH7 in 1 : 1 mixture of DMF and water at concentrations ranging from 0.025 mM to 0.4 mM were measured using PW9506 digital conductivity meter at 25°C. The molar conductivity (Λ) was calculated as ($\Lambda = k/c$) where k is the conductivity and c is the concentration[21]. The molar conductivity values obtained were then plotted against concentration to determine the limiting values at zero concentration.

Interaction with DNA

Interaction between TH5, TH6 and TH7 and pBR322 plasmid and ssDNAs was studied by agarose gel electrophoresis. The method used was a modification of that described by Stellwagen[22]. Solutions of pBR322 plasmid DNA and ssDNA (at concentration 0.5 mg mL^{-1} in the case of pBR322 plasmid DNA and 1.5 mg mL^{-1}

in the case of ssDNA) were incubated with increasing concentrations of compounds ranging from 30–80 μM in a shaking water bath at 37°C for 4H. 16 μL aliquots of drug-DNA mixtures containing 0.6 μg of DNA were loaded on to the 1% gel and electrophoresis was carried out under TAE buffer for 2.3 h at 2.67 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 a type II restriction endonuclease that is known to recognize the sequence G/GATCC and hydrolyse the phosphodiester bond between adjacent guanine[22]. pBR322 contains a single restriction site for BamH1[22] 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-pBR322 plasmid 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 was added 2 μL of 10x digestion buffer SB followed by the addition of 0.3 μL BamH1 (3 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. 16 μL aliquots of drug-DNA mixtures containing 0.6 μg of DNA were loaded on to the 1% gel and electrophoresis was carried under TAE buffer for 2.3 h at 2.67 V cm^{-1}. The gel was subsequently stained with ethidium bromide, visualized by UV light, then a photograph of the gel was taken as described previously.

RESULTS AND DISCUSSION

Table-1 gives the elemental compositions of TH5, TH6 and TH7.

TABLE-1
COMPOSITION OF TH5, TH6 AND TH7

	TH5		TH6		TH7	
	Calculated (%)	Found (%)	Calculated (%)	Found (%)	Calculated (%)	Found (%)
C	32.8	32.7 ± 0.4	32.8	32.8 ± 0.4	32.8	32.8 ± 0.4
H	2.7	2.9 ± 0.4	2.7	2.8 ± 0.4	2.7	2.9 ± 0.4
N	7.6	7.7 ± 0.4	7.6	7.6 ± 0.4	7.62	7.6 ± 0.4
Cl	19.3	19.4 ± 0.4	19.3	19.4 ± 0.4	19.3	19.3 ± 0.4
Pd	29.0	29.1 ± 1.2	29.0	29.1 ± 1.2	29.0	29.0 ± 1.2

The observed purity of each of the compounds is greater than 99%. As stated earlier, the compounds were synthesized from cisplatin utilizing the difference in the *trans* effect of halide and amine ligands in platinum(II) and palladium(II) complexes[19].

Spectral Analyses

The major peaks observed in IR spectra of TH5, TH6 and TH7 are listed in Table-2. The major peaks observed in the mass and ^1H NMR spectra of TH5, TH6 and TH7 are listed in Table-3. Most of the peak assignments are based on published spectra of the ligands and metal complexes of pyridine, substituted pyridines[23–25].

TABLE-2
PROMINENT IR (ν cm^{-1}) SPECTRAL BANDS OBSERVED IN TH5, TH6 AND TH7

	IR ν(cm^{-1})
TH5	3368 ν(w, OH str.), 3276 ν(s, NH str.), 3253 ν(w, OH str.), 3070 ν(w, CH str.), 1653 ν(w, C=C and C=N str.), 1585 ν(w, C=N str.), 1576 ν(m, pyridine ring str.), 1482 ν(s, pyridine str.), 1345 ν(w, OH bend.), 1278 ν(m, OH bend.), 1221 ν(b, CO str.), 662 ν(w, CH out of plane), 451 (m, out of plane ring C=C bend.), 345 ν(s, PdCl str.), 329 ν(w, PdCl str.), 282 ν(m, PdN str.), 276 (m, PdCl str.), 255 ν(w, PdN str.)
TH6	3244 ν(w, O—H str.), 3215 ν(w, N—H str.), 3156 (s, OH str.), 3109 ν(n, CH str.), 3099 ν(s, CH str.), 3007 ν(w, C—H str.), 1610 ν(w, C=C and C=N str.), 1584 ν(w, CN str.), 1456 ν(w, ring str.), 1161 ν(n, CO str.), 1122 ν(w, C—O str.), 496 ν(w, out of plane ring), 459 ν(w, ring out of plane), 279 ν(s, PdN str.), 339 ν(w, PdCl str.), 333 ν(w, PdCl str.), 261 ν(m, PdN str.)
TH7	3453 ν(w, OH str.), 3408 ν(w, NH str.), 3350 ν(b, OH str.), 3322 ν(m, OH str.), 3291 ν(s NH str.), 3257 ν(w, NH str.), 3216 ν(w, NH str.), 1642 ν(w, C=C and C=N str.), 1589 ν(m, C=N str.), 1507 ν(s, ring str.), 1454 ν(s, ring str.), 1359 ν(w, OH bend.), 1309 ν(m, CH in plane bend.), 1290 ν(w, CO str.), 1062 ν(m, CH in plane bend.), 572 ν(w, OH bend.), 528 ν(w, ring out of plane bend.), 305 ν(w, PdCl str.), 253 ν(s, PdN str.)

TH5: The weak bands at 3368 and 3276 cm^{-1} are believed to be due to OH stretching vibrations. The band at 3276 cm^{-1} is believed to be due to NH stretching. The band at 3070 cm^{-1} is due to CH stretching vibration. The band at 1653 cm^{-1} may be due to ν(C=C) and ν(C=N) stretching vibrations. The band at 1585 cm^{-1} is due to ν(C=N) stretching vibration. The bands at 1576 and 1482 cm^{-1} are due to pyridine ring stretching vibrations. The bands at 1345 and 1278 cm^{-1} are due to OH bending vibrations. The band at 1221 cm^{-1} is due to CO stretch. The band at 662 cm^{-1} is due to CH out of plane vibration. The band at 451 cm^{-1} is due to out of plane ring ν(C=C) bending. The bands at 345, 329 and 276 cm^{-1} are due to Pd—Cl stretching vibrations and those at 282 and 255 cm^{-1} are due to Pd—N (pyridine) stretching vibrations.

TH6: The weak band at 3244 cm^{-1} is believed to be due to O—H stretching vibration, the band at 3215 cm^{-1} is believed to be due to N—H stretching and bending vibration. The band at 3109, 3099, 3007 cm^{-1} is due to C—H stretching vibrations. The band at 1610 cm^{-1} is due to ν(C=C) and ν(C=N) stretching vibrations. The band at 1584 cm^{-1} is due to ν(C—N) stretching vibration. The band at 1456 cm^{-1} is due to ring stretch. The bands at 1161 and 1122 cm^{-1} are

due to CO stretching vibrations. The band at 496 cm^{-1} is due to ring out of plane bending vibrations. The bands at 279 and 261 cm^{-1} are due to Pd—N (ring) stretching vibrations. The bands at 339 and 333 cm^{-1} are due to Pd—Cl stretching vibrations.

TH7: The bands at 3453, 3350 and 3322 cm^{-1} are due to OH stretch and those at 3408, 3291 and 3257 cm^{-1} are due to NH stretch. The band at 1642 cm^{-1} may be due to ν(C=C) and ν(C=N) stretching vibrations. The strong bands at 1507 and 1454 cm^{-1} are due to ring stretching vibrations. The weak band at 1359 cm^{-1} is due to OH bending vibration. The medium band at 1309 cm^{-1} is due to CH in-plane bending vibration and that at 1290 cm^{-1} is due to CO stretch. The band at 1062 cm^{-1} is due to CH in-plane bending vibration. The band at 572 cm^{-1} is due to OH bending vibration and that at 528 cm^{-1} is due to ring out of plane bending vibration. The band at 305 cm^{-1} is due to Pd—Cl stretch and that at 253 cm^{-1} is due to Pd—N (pyridine) stretch.

Mass and ^{1}H NMR spectral analyses

The prominent peaks observed in the EIS mass and ^{1}H NMR spectra of TH5, TH6 and TH7 are given in Table-3. The numbers in parentheses indicate the percentage intensity of the peak.

TABLE-3
PROMINENT PEAKS OBSERVED IN THE ESI MASS AND ^{1}H NMR SPECTRA OF TH5, TH6 AND TH7. THE NUMBRS IN PARENTHESES INDICATE THE PERCENTAGE INTENSITY OF THE PEAK

	ESI Mass	^{1}H NMR
TH5	m/z: M$^+$ = 368 (20); (M—3H) = 365 (35); (M—2H—Cl) = 331 (28); (M—2Cl—H) = 295 (33).	^{1}H NMR DMSO δ ppm: 10.7 (s, due to OH); 8.28 (s, due to CH ortho); 8.20 (d, due to CH ortho); 7.35 (q, due to CH meta); 7.31 (d, due to CH para); 3.3 (s, due to water); 2.75 (due to NH—Pd); 2.5 (s, due to DMSO)
TH6	m/z: M = 368 (39); (M—4H—Cl) = 329 (100); (M—H—2Cl) = 295 (70)	^{1}H NMR DMSO δ ppm: 11.54 (br s, due to OH); 7.40 (d, due to CH ortho); 7.35 (d, due to CH meta); 6.25 (q, due to CH meta); 6.15 (q, due to CH para); 3.4 (br s, due to water); 2.5 (s, due to DMSO)
TH7	m/z: M = 368 (35); (M—2H—O) = 351 (28); (M—3H) = 365 (50); (M—Cl) = 333 (52); (M—H—2Cl) = 295 (60)	^{1}H NMR DMSO δ ppm: 11.70 (br s, due to OH); 8.30 (d, due to CH ortho); 6.90 (d, due to CH meta); 3.35 (br s, due to water); 2.5 (s, due to DMSO)

TH5

Mass spectrum: The mass spectrum of TH5 has a large peak with m/z = 367 corresponding to (3-hydroxypyridine)$_2$ Cl_2, the peak at m/z 365 corresponding to (M—3H), that at m/z 331 corresponding to (M—2H—Cl), that at m/z 295 corresponding to (M—2Cl—H).

^{1}H NMR spectrum: The resonance at $\delta = 10.7$ ppm is believed to be due to OH proton. The resonance at $\delta = 8.28$ ppm is due to H attached to the second carbon. That at $\delta = 8.2$ ppm is due to H attached to the sixth carbon. The resonance at $\delta = 7.35$ ppm is due to H attached to the fifth carbon and that at $\delta = 7.31$ ppm is due to H attached to the fourth carbon. The resonance at $\delta = 3.3$ ppm is due to water. The resonance at $\delta = 2.5$ ppm is due to DMSO.

TH6

Mass spectrum: The mass spectrum of TH6 has a large peak with m/z = 368 corresponding to (2-hydroxypyridine)$_2$Cl$_2$, the peak at m/z 329 corresponding to (M—4H—Cl), that at m/z 295 corresponding to (M—H—2Cl).

^{1}H NMR spectrum: The resonance at $\delta = 11.54$ ppm is believed to be due to OH proton. The resonance at $\delta = 7.40$ ppm is due to ortho CH proton and that at $\delta = 7.35$ ppm is due to CH proton adjacent to the carbon to which OH group is attached. The resonance at $\delta = 6.25$ ppm is due to CH proton at the para position. The resonance at $\delta = 6.15$ ppm is due to CH proton at the second meta position. The resonance at $\delta = 3.4$ ppm is due to water. The resonance at $\delta = 2.78$ ppm is due to NH bonded to Pd. The resonance at $\delta = 2.5$ ppm is due to DMSO.

TH7

Mass spectrum: The mass spectrum of TH7 has a large peak with m/z = 368 corresponding to (2-hydroxypyridine)$_2$Cl$_2$, the peak at m/z 351 corresponding to (M—2H—O), that at m/z 295 corresponding to (M—H—2Cl), that at m/z 265 corresponding to (M—3H), that at m/z 333 corresponding to (M—Cl).

^{1}H NMR spectrum: The resonance at $\delta = 11.7$ ppm is believed to be due to OH proton. The resonance at $\delta = 8.30$ ppm is believed to be due to CH protons at the ortho position. The resonance at $\delta = 6.90$ ppm is due to CH protons at the meta position. The resonance at $\delta = 3.35$ ppm is due to water and that at $\delta = 2.50$ ppm is due to DMSO.

Molar conductivity values

The limiting values of molar conductivity (in ohm^{-1} mol^{-1}) at zero concentration of TH5, TH6 and TH7 at 298 K were found to be 32, 64 and 48, respectively. The values of TH5 and TH6 are significantly lower than the expected values of 280 for 1 : 2 electrolyte whereas that for TH7 is much higher[26]. The results suggest that the degrees of dissociation in the case of TH5, TH6 and TH7 are respectively about 11, 23 and 17%.

Interaction with salmon sperm DNA (ssDNA): Fig. 3 gives the interaction of salmon sperm DNA in TAE buffer at pH 7.6 ranging from 30 to 100 μM. Lane B on both sides applies to untreated ssDNA as control, lanes 1 to 8 apply to ssDNA interacted with increasing concentrations of the compounds (lane 1: 30 μM, lane 2: 40 μM, lane 3: 50 μM, lane 4: 60 μM, lane 5: 70 μM, lane 6: 80 μM, lane 7: 90 μM and lane 8: 100 μM).

Only a single band was observed in both untreated and reacted salmon sperm DNA. In general, as the concentration of the compound was increased the intensity of the band was found to decrease. The decrease in intensity was most prominent in the case of TH6 indicating that the compound caused greatest damage to DNA. All the three compounds TH5, TH6 and TH7 are likely to form monofunctional and interstrand bifunctional adducts with DNA and hence are likely to cause similar

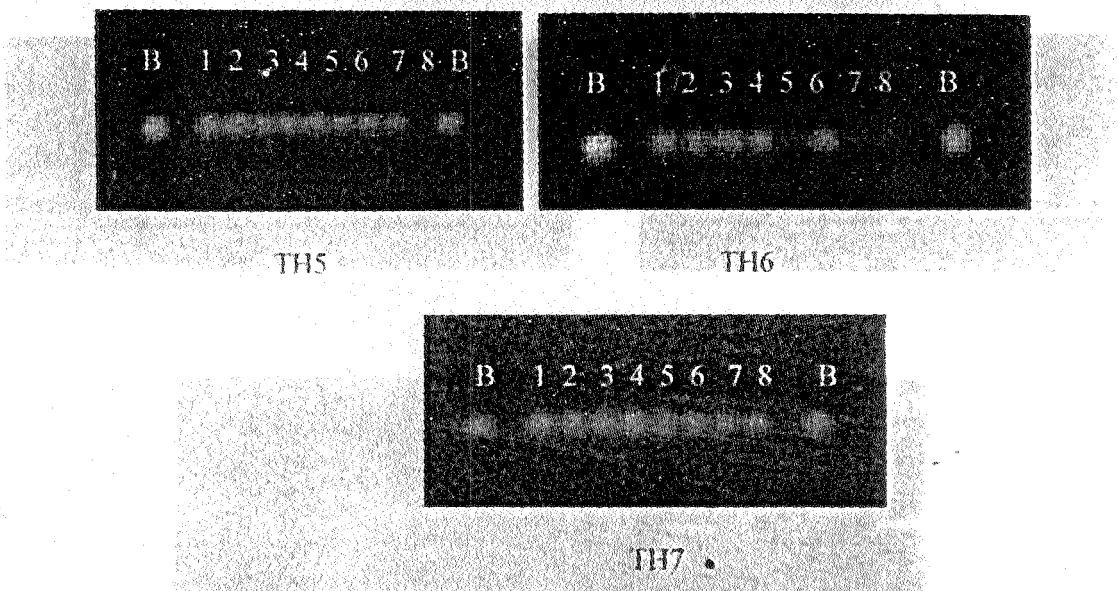


Fig. 3. Interaction between TH6, TH7 and TH8 and salmon sperm DNA: lane B: blank DNA, lanes 1 to 8: ssDNA interacted with increasing concentrations of the compounds (lane 1: 30 μM, lane 2: 40 μM, lane 3: 50 μM, lane 4: 60 μM, lane 5: 70 μM, lane 6: 80 μM, lane 7: 90 μM and lane 8: 100 μM).

types of conformational changes in DNA. Damage to DNA caused by the TH6 at high concentrations is believed to be due to binding of the compound with nucleobases in DNA where both covalent and non-covalent interactions may be involved. These results are different from those of the corresponding platinum complexes in which the greatest decrease in intensity with the increase in concentration was observed for the compound containing two 3-hydroxypyridine ligands in the *trans*-configuration rather than the compound containing two 2-hydroxypyridine ligands in the *trans*-configuration (as in TH6)[27]. It is difficult to explain whether the differences in results are a consequence in reactivity of platinum and palladium complexes.

pBR322 plasmid DNA: Fig. 4 gives the electrophoretograms applying to the interaction of pBR322 plasmid DNA with increasing concentrations of TH5, TH6 and TH7. Lane B applies to untreated pBR322 plasmid DNA to serve as a control; lanes 1 to 8 apply to plasmid DNA interacted with increasing concentrations of the compounds (lane 1: 30 μM, lane 2: 35 μM, lane 3: 40 μM, lane 4: 50 μM, lane 5: 55 μM, lane 6: 60 μM, lane 7: 70 μM and lane 8: 80 μM).

Initially pBR322 plasmid DNA showed a prominent supercoiled form I band and a weak singly nicked form II band. When it was interacted with increasing concentrations of compounds, changes in both mobility and intensity of the bands were observed. As the concentrations of TH5 and TH6 were increased, the mobility of both form I and II bands increased but at different rates such that the separation between the bands decreased. Form II band became less distinct for concentrations of TH5 ≥ 35 μM. In the case of TH6 and also in the case of TH7, the increase in mobility of the form I band was not found to be gradual—at some concentrations, there was a slight decrease in mobility. For TH7, essentially only form I band was observed for all concentrations of the compound.

The intensity of form I and form II bands was found to be very high at the lowest concentration of TH5 namely 30 μM whereas at the highest concentration (namely 80 μM), the intensity of the form II band was found to be higher than that of form I.

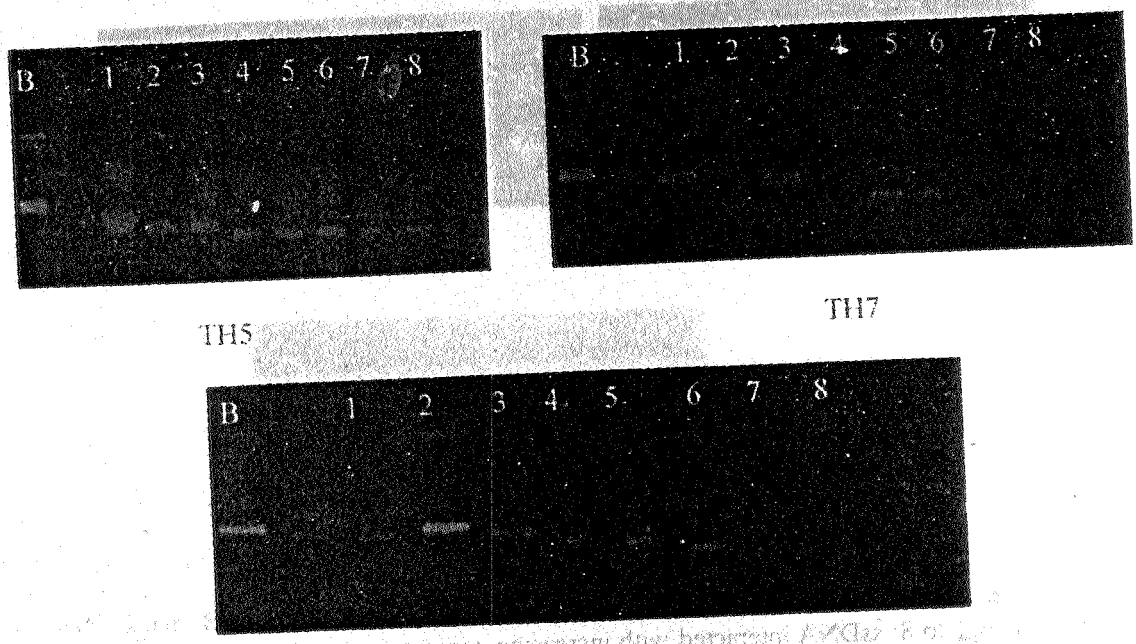


Fig. 4. Electrophoretograms applying to the interaction of pBR322 plasmid DNA with increasing concentrations of TH5, TH6 and TH7. Lane B applies to untreated pBR322 plasmid DNA, lanes 1 to 8 apply to plasmid DNA interacted with increasing concentrations of the compounds (lane 1: 30 μM, lane 2: 35 μM, lane 3: 40 μM, lane 4: 50 μM, lane 5: 55 μM, lane 6: 60 μM, lane 7: 70 μM and lane 8: 80 μM).

The increase in intensity of the bands at the lowest concentration is possibly due to an increase in DNA fluorescence whereas the decrease in intensity of the form I and increase in intensity of the form II band at the highest concentration is believed to be due to DNA damage. The change in mobility of form I DNA band with the increase in concentration of compounds is due to unwinding of the supercoiled form I DNA. As the negatively supercoiled DNA unwinds, its mobility should decrease to reach the minimum (when it is fully relaxed) and then increase when it becomes positively supercoiled[28]. When the DNA is fully relaxed forms I and II comigrate. Although in the present study also, the separation between form I and form II DNA bands was found to decrease with the increase in concentration of the compounds, the initial increase in mobility of the form I DNA band was rather unexpected. It is, however, believed to be an artefact of the gel conditions used.

The interaction of platinum drugs with DNA involves a number of stages including: aquation of the complex, pre-association with DNA, formation of monofunctional adducts with DNA, closure into bifunctional adducts, distortion of the DNA and recognition of the distortion[31]. TH5, TH6 and TH7 are believed to form monofunctional adducts with G. The monofunctional adducts may close to form initially bifunctional interstrand GC adducts that can evolve into interstrand GG adducts. When bifunctional interstrand adduct is formed, planaramine ligand (3-hydroxypyridine in the case of TH5, 2-hydroxypyridine in the case of TH6 and 4-hydroxypyridine in the cae of TH7) will be positioned along the helix axis so that they will push apart adjacent base pairs. Although TH5, TH6 and TH7 have similar structures (except that the planaramine ligand in TH5 is 3-hydroxypyridine, that in TH6 is 2-hydroxypyridine and that in TH7 is 4-hydroxypyridine), greater conformational change observed for TH6 than for TH5 and TH7, suggests that the three

ligands: 2-hydroxypyridine, 3-hydroxypyridine and 4-hydroxypyridine may differ in noncovalent interactions.

Whereas cisplatin forms mainly intrastrand bifunctional GG and AG adducts with duplex DNA[32] (and hence induces a local kink in a DNA strand), like transplatin TH5, TH6 and TH7 are more likely to form monofunctional adducts with guanine and bifunctional intrastrand GC and GG adducts[33]. The formation of bifunctional interstrand adduct (because of the mismatch between appropriate interstrand distance and that between the two *trans* 'arms' of platinum), brings the DNA strands closer together so that more global changes in DNA conformation are introduced. It was stated earlier that the positioning of the planaramine ligands along the helix adds to the distortion by pushing the neighbouring base pairs away.

BamH1 Digestion

As stated earlier, BamH1 is a restriction enzyme which recognizes the G/GATCC and hydrolyses phosphodiester bond between adjacent guanine sites[22]. pBR322 plasmid DNA has only one binding site for BamH1 that converts super-coiled form I pBR322 plasmid DNA and singly-nicked form II DNA to linear form III DNA. Platinum compounds are known to bind covalently with the nucleobases in DNA, as a result of which digestion by the restriction enzyme BamH1 may be prevented. Fig. 5 gives the electrophoretograms applying to incubated mixtures of pBR322 plasmid DNA and varying concentrations of TH5, TH6 and TH7 ranging from 5 to 40 μM that were digested with BamH1 for a period of 1 h at 37°C before they were subjected to electrophoresis.

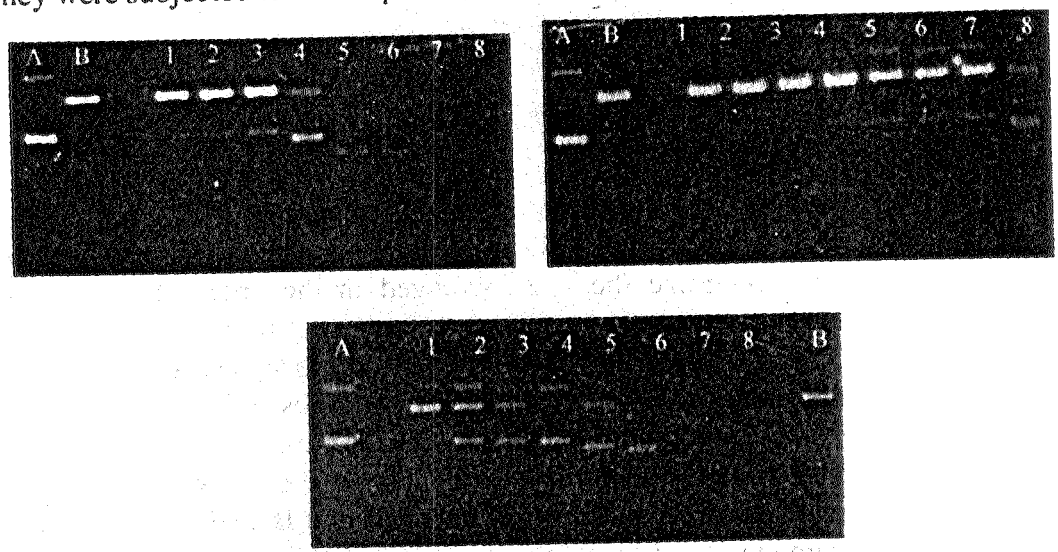


Fig. 5. Electrophoretograms applying to the incubated mixtures of pBR322 plasmid DNA and varying concentrations of compounds TH5, TH6 and TH7 followed by digestion with BamH1: Lane A applies to the untreated and undigested pBR322 plasmid DNA, Lane B applies to the untreated pBR322 plasmid DNA digested with BamH1, lanes 1 to 8 apply to pBR322 plasmid DNA interacted with increasing concentrations of the compound (5, 10, 15, 20, 25, 30, 35 and 40 μM respectively) followed by BamH1 digestion.

The untreated and undigested DNA gave one promiment form I band and a weak form II band (lane A's). DNA that was not treated with compounds but digested with BamH1 gave only form III band (lane B's). At lower concentrations of TH5 ranging from 5 to 20 μM, the DNA showed faint form I band, a faint form II band and a

prominent form III band (lanes 1 to 4). At higher concentrations of TH5 ranging from 25 to 40 μM, form I and form III bands could be seen (lanes 5 to 8). The presence of a streaking band covering the space between form I and form III bands and the absence of form II is believed to be due to DNA damage brought about by the compound.

In case of TH6, at the lowest concentration, namely 5 μM, a faint form II band and a prominent form III band were seen. As the concentration of TH6 was increased, the intensity of the form I and form II bands increased and that of form III band decreased.

At the lowest concentration of TH7, a prominent single form III band and a weak form II were observed. For concentrations of TH7 ranging from 10 μM to 20 μM, forms I, II and III bands could be seen. With the further increase in concentration of TH7, first form II and then all bands disappeared. This is believed to be due to DNA damage caused by its interaction with TH7. Table-4 summarizes the bands observed at different concentrations of compounds.

TABLE-4
BANDS OBSERVED IN ELECTROPHORETOGRAMS AFTER BamH1 DIGESTION

Compound	Concentration of compound (μM)								
	0	5	10	15	20	25	30	35	40
TH5	III	I, II, III	I, II, III	I, II, III	I, II, III	I, III	I. III	I, III	I, III
TH6	III	III	III	II, III	II, III	I, II, III	I, II, III	I, II, III	I, III
TH7	III	II, III	I, II, III	I, II, III	I, II, III	I, III	I, III	—	—

As stated earlier, BamH1 is a restriction enzyme that recognizes the G/GATCC and hydrolyses phosphodiester bond between adjacent guanine sites[16]. The prevention of BamH1 digestion means that DNA conformation is changed such that the enzyme is unable to recognize the site. Arranged in the order of increasing prevention of BamH1 digestion (from lowest to highest), the compounds were: TH6 < TH7 < TH5. Thus, within the concentration range 1 to 40 μM, TH6 was least efficient in preventing BamH1 digestion whilst TH5 was most efficient. It was also stated earlier that when bifunctional interstrand adducts are formed, DNA strands are brought closer together causing more of a global change in DNA conformation. Differential abilities of the compounds in the prevention of BamH1 digestion and induction of DNA damage illustrate structure-activity relationship. The determination of the activity of the compounds against cancer cell lines will provide further information. The concentrations required to result in equivalent prevention of BamH1 digestion by compounds in the present study are found to be significantly higher than the compounds of the form *trans*-$PtL(NH_3)Cl_2$ where L = 2-hydroxypyridine, imidazole, 3-hydroxypyridine and imidazo(1,2-α)pyridine (which contain only planaramine ligand whereas the compounds in the present study contain two planar amine ligands per molecule in the *trans*-geometry[34]. It is believed that because of a greater steric constraint caused by two planaramine ligands in the *trans*-geometry, at equivalent concentrations the compounds in the present study are less likely to form interstrand bifunctional adducts.

Conclusions

The compounds code named TH5, TH6 and TH7 have been characterized by elemental analyses and spectral studies including IR, mass and ^{1}H NMR. The nature of interaction of the compounds with pBR322 plasmid DNA and salmon sperm DNA have been investigated. It is found that the nature of the planaramine ligand may modulate both the chemical and biochemical properties of the compounds. For example, 3-hydroxypyridine bonded to platinum(II) in a *trans*-geometry is found to cause a greater unwinding than 2-hydroxypyridine or 4-hydroxypyridine. Induced DNA damage and changes in DNA conformation and molar conductivity values illustrate structure-activity relationship. Because of the decreased level of dissociation of TH5 and TH6, the rate of passive diffusion across the cell membrane is expected to be greater but not for TH7 for which the degree of dissociation is very high. If this were so, the cell uptake of TH5 and TH6 would be greater than that of TH7 and the level of drug-DNA binding could also be higher because of a lesser deactivation by cellular palladinophiles. Determination of activity of the compounds, cell uptake and level of platinum-DNA binding would provide further information on the matter.

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