Studies on the Activity of Two *Trans*-Planaramine Platinum(II) Complexes Coded as DH4 and DH5 in Human Ovarian Tumour Models

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Abstract. *In this article, we report the synthesis and the in vitro activity of trans-bis(2-methylimidazole)dichloroplatinum(II) (coded as DH4) and trans-(ammino)(2,3-diaminopyridine) dichloroplatinum(II) (coded as DH5) in the human ovarian tumour models. DH4 is less active than cisplatin against the parental A2780 cell line but more active than cisplatin against the resistant A2780^{cisR} cell line, thus indicating that it is better able to overcome mechanisms of resistance operating in the A2780^{cisR} line. In contrast, DH5 is less active than cisplatin against all three cell lines. The higher activity of DH4 than cisplatin in the A2780^{cisR} cell line is in line with the associated higher platinum−DNA binding level. Whereas cisplatin binds with DNA, forming mainly intrastrand 1,2-Pt(GG) and 1,2-Pt(AG) adducts, DH4 and DH5 are expected to form more 1,2-interstrand Pt(GG) and monofunctional adducts. The results of interaction with pBR322 plasmid DNA combined with BamH1 digestion show that DH4 and DH5 are less able to prevent BamH1 digestion than cisplatin, indicating that cisplatin causes a greater conformational change in the DNA than do DH4 and DH5, although DH5 is more damaging to DNA. The difference in the activity of DH4 and DH5, with 2-methylimidazole and 2,3-diaminopyridine respectively as carrier ligands, can be seen to illustrate structure−activity relationships.*

Currently much attention is given to platinum compounds that bind with DNA differently from cisplatin, with the idea that the difference in nature of binding with DNA may translate into an altered spectrum of activity. We have

synthesized a number of *trans*-planaramine platinum(II) complexes with *in vitro* activity in ovarian tumour models, similar to or better than that of cisplatin (1, 2). We report here the synthesis, characterization, cytotoxic activity, cell uptake, and DNA binding of *trans*-dichlorobis(2-methylimidazol)platinum(II) (coded as DH4) and *trans*-dichloro(ammine)(2,3-diaminopyridine)platinum(II) (coded as DH5) (Figure 1).

Materials and Methods

Potassium tetrachloroplatinate (K2PtCl4), N,N-dimethylformamide (DMF), 2-methylimidazole and 2,3-diaminopyridine were obtained from Sigma Chemical Company, St. Louis, Mo, USA; hydrochloric acid was obtained from Asia Pacific Specialty Chemicals Ltd., (NSW, Australia); acetone and silver nitrate from Ajax Chemicals, (Auburn, NSW, Australia); silver acetate from Sigma Chemical Company, Germany and charcoal from Sigma Aldrich Chemical Company (Milwaukee, WI, USA); 200 mM L-glutamine, 5.6% sodium bicarbonate, foetal calf serum (FCS), RPMI-1640 from Trace Biosciences Pty Ltd. Australia; other chemicals were obtained from Sigma-Aldrich, Sydney, Australia. A2780, A2780^{CisR} and A2780^{ZD0473R} ovarian cancer cell lines were gifts from Ms. Mei Zhang, Royal Prince Alfred Hospital, Sydney, Australia. Stock solutions of platinum compounds (1 mM) were prepared in 1:1 DMF and milli Q water mixture.

Synthesis. Cisplatin: Cisplatin required for the synthesis of DH4 and DH5, and also used as a reference was prepared according to the modified Dhara method (3). Briefly, to one millimol of tetrachloroplatinate dissolved in 5 ml of milli-Q water was added potassium iodide (4.12 mmol) dissolved in 1 ml of mQ water. The mixture left on a shaking water bath, at 37˚C, for 5 min, produced K2PtI4 which then reacted with 2 mmol of aqueous ammonia at 37˚C for 1 h. The dark-yellow precipitate of *cis*-Pt(NH$_3$)$_2$I$_2$ was washed with ice-cold water and ethanol and air dried. Silver nitrate (2 mmol) and *cis*-Pt(NH3)2I2 (1 mmol) were mixed together followed by the addition of 4 ml of mQ water. The mixture was left on a shaking water-bath at 37˚C for 30 min to produce *cis*-Pt(NH$_3$)$_2$(NO$_3$)$_2$ and solid silver iodide. The mixture was centrifuged. The supernatant consisting of *cis*-Pt(NH$_3$)$_2$(NO$_3$)$_2$ in solution was collected. Potassium chloride (0.11 g, 1.5 mmol) was

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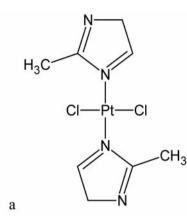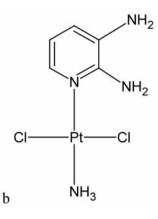
Figure 1. *Structures of DH4 (a) and DH5 (b).*

added to the filtrate. The mixture was left on a water bath at 37°C for 30 min for crystals of cisplatin to form.

DH4: Although synthesis of DH4 was reported earlier (4), no work on the activity of the compound has been performed. Briefly the method of synthesis was as follows: 1 mmol (0.415 g) of potassium tetrachloroplatinate was dissolved in 4 ml of mQ water to which 0.25 ml of concentrated hydrochloric acid were added. The solution was heated to 50°C. 10 mmol (0.82 g) of 2-methylimidazole, dissolved in 2 ml of DMF, were slowly added to the solution of potassium tetracholoroplatinate. The temperature of the solution was increased to 65°C and maintained at that temperature for 1 h while the solution was stirred. The volume of the solution was reduced to about 5 ml, following which 40 ml of 6 M hydrochloric acid were added and the mixture was heated under reflux for 24 h at 90°C. The mixture was cooled to room temperature, resulting in the formation of dark-purple precipitate of DH4, which was collected by filtration, giving a yield of 35%.

DH5: From the reaction of 1 mmol (0.3 g) of cisplatin with 2 mmol (0.218 g) of 2,3-diaminopyridine, synthesis of 1 mmol of *trans*-dichloro(ammino)(2,3-diaminopyridine)platinum(II) was attempted, giving a yield of 45%. The method of synthesis was as follows: Cisplatin (0.300 g, 1 mmol) was added to 8 ml of milli-Q water. 2,3-Diaminopyridine (0.218 g, 2 mmol), dissolved in 2 ml of DMF, was added to the cisplatin suspension. The mixture was heated under reflux for 1 h at 70°C to cause 2,3-diaminopyridine ligand to replace chloro-ligands. The mixture was cooled to room temperature to stop the reaction. Concentrated hydrochloric acid (1.22 ml, 12.2 mmol) was added to the mixture, followed by heating to 70°C under reflux, to produce dark-black crystals of DH5. The crystals of DH5 formed were collected by filtration, washed with ice-cold water and ice-cold ethanol, and air dried, giving a yield of 45%.

Characterization. Microanalyses: Pt was determined by graphite furnace atomic absorption spectroscopy (AAS) using a Varian Spectra-20 Atomic Absorption Spectrophotometer. C, H, N, and Cl were determined at the Australian National University.

DH4: Calc.% C: 22.4; H: 2.4, N: 13.1; Cl: 16.6; Pt: 45.6; Obs.%: C: 22.3±0.4, H: 2.8±0.4, N: 13.1±0.4, Cl: 16.4±0.4, Pt: 45.2±1.0. DH5: Calc.% C: 19.3; H: 2.6, N: 14.3; Cl: 18.1; Pt: 49.8; Obs.%: C: 19.6±0.4, H: 2.3±0.4, N: 14.5±0.4, Cl: 18.5±0.4, Pt: 50.2±1.0.

Molar Conductivity: The limiting molar conductivity value (in $ohm^{-1}cm^2mol^{-1}$) at zero concentration (Λ_0) for DH4, DH5 and cisplatin are 254, 470 and 304 respectively, indicating that the degree of ionization is slightly less than that of cisplatin, but that of DH5 is much greater than that of cisplatin.

Spectral studies: Infrared, mass and 1H (NMR) spectra were used to aid in the structural characterization of DH4 and DH5.

IR (KBr). IR spectrum was collected using a Bruker IFS66 spectrometer equipped with a Spectra-Tech Diffuse Reflectance Accessory (DRA) (Bruker Australia Pty Ltd Bruker Sydney NSW Australia), an air-cooled DTGS detector and a KBr beam splitter. Spectrum was recorded at a resolution of 4 cm^{-1}, with the addition of 128 scans and a Blackman-Harris 3-term apodization function was applied. The peaks listed below can be seen to provide support for the suggested structures of DH4 and DH5.

DH4: 3367.4 and 3121.8.4 cm^{-1}: N-H stretch; 1621.5 and 1426.3 cm^{-1}: C=N stretch; 1283.7 cm^{-1}: imidazole ring stretch; 539.4 cm-1: Pt–N stretch.

DH5: 3307 and 3121.8.4 cm^{-1}: N-H stretch; 1623.5 and 1584.1 cm^{-1}: C=N and C=C stretch; 1222.4 cm^{-1}: ring stretch; 449.4 cm-1: Pt–N stretch.

Mass: The mass spectrum of DH3 was obtained using a Finnigan LCQ ion trap mass spectrometer (Thermo Scientific Massachusetts USA) in which fragmentation was produced by electrospray ionization (EIS).

MH4: (EIS-MS) (milli Q water) (m/z: M=428). The peak at m/z=427 corresponds to (M–H)).

MH5: (EIS-MS) (milli Q water) (m/z: M=392). The peak at m/z=316 corresponds to (Pt $(C_5H_7N_3)(NH_3)$–5H).

1H-NMR. 1H-NMR spectra of DH4 and DH5 dissolved in DMSO were recorded in a Bruker DPX400 spectrometer (Bruker Australia Pty Ltd Sydney NSW Australia) using a 5mm high precision Wilmad NMR tube.

DH4: 1H-NMR DMSO δ ppm: 12.42 (d, due to CH); 11.34 (d, due to CH); 6.66 (s, due to CH_3); 5.70 (s, due to NH); 4.63 (s, due to NH-Pt); 2.50 (due to DMSO).

DH5: 1H-NMR DMSO δ ppm: 7.95 (s, due to CH ortho); 7.10 (t, due to CH meta); 3.33 (d, due to CH para); 2.80 (s, due to NH_2); 2.70 (s, due to NH_2); 2.40 (s, due to NH_3); 2.50 (t, due to DMSO).

Cytotoxicity assays. The cell killing effect of DH4 and DH5 in human ovarian A2780, A2780^{cisR}, and A2780^{ZD0473R} cancer cell lines was determined using 3-(Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-terazolium bromide (MTT) reduction assay (5, 6), where cisplatin was used as a reference. Briefly, between 4,500 to 5,000 cells were seeded into the wells of the flat-bottomed 96-well culture plate in 10% FCS/RPMI-1640 culture medium. The plate was incubated for 24 h at 37°C in a humidified atmosphere to allow for cells to attach. Platinum complexes dissolved first in a minimum volume of DMF were diluted to the required concentrations with milli-Q water and filtered to sterilise. Serial dilutions ranging from 0.0064 μM to 0.8 μM in 10% FCS/RPMI 1640 medium were prepared and added to equal volumes of cell culture in quadruplicate wells following which the cells were incubated under normal growth conditions for 72 h, followed by the addition of MTT. Four hours after the addition of MTT, the yellow formazan crystals produced were dissolved in 150 μl of DMSO and the absorbance was measured at 570 nm.

Platinum accumulation and platinum−DNA binding. DH4, DH5 and cisplatin (at 50 μM final concentration) were added to culture plates containing exponentially growing A2780, A2780^{cisR} and A2780^{ZD0473R} cells in 10 ml 10% FCS/RPMI 1640 culture medium (cell density of 1×10^6 cells ml^{-1}) (7). The cells containing the drugs were incubated for periods of 2 h, 4 h and 24 h, at the end of which cell monolayers were trypsinized and cell suspension (5 ml) was transferred to centrifuge tubes and spun at 3,500 rpm for 2 min at 4°C. Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and cell pellets were stored at −20°C until assayed. At least three independent experiments were performed. For the determination of drug accumulation, cell pellets were suspended in 0.5 ml of 1% triton-X, held on ice then sonicated (5 min). Total intracellular platinum contents were determined by graphite furnace AAS.

For the determination of the level of platinum−DNA binding, the modified method of Bowtell (8) was used. Briefly, high molecular weight DNA was isolated from cell pellets using JETQUICK Blood DNA Spin Kit/50. The cell pellets were re-suspended in PBS to a final volume of 200 μl and mixed with 10 μl of RNase A, then incubated for 4 min at 37°C. 25 μl Proteinase K and 200 μl buffer K1 (containing guanidine hydrochloride and a detergent) were added, followed by incubation for 10 min at 70°C. Absolute ethanol (200 μl) was added and mixed thoroughly. The samples were centrifuged for 1 min at 10,600 rpm through the silica membrane using JETQUICK micro-spin columns. The columns containing the samples were washed with 500 μl of buffer KX (containing high-salt buffer to remove residual contamination), then centrifuged for 1 min at 10,600 rpm. The columns were washed again with 500 μl buffer K2, low-salt buffer, and centrifuged for 1 min at 10,600 rpm. The columns were centrifuged again for 2 min at the full speed (13,000 rpm) to further clear the silica membrane of the residual liquid. The purified DNA in the column was eluted from the membrane with 200 μl of 10 mM Tris-HCl buffer (pH 8.5). The DNA content was determined using UV spectrophotometry at 260 nm (Varian Cary 1E UV-Visible spectrometer with Varian Cary Temperature Controll). Platinum contents were determined by graphite furnace AAS. A$_{260}$/A$_{280}$ ratios were found to be between 1.75 and 1.8 ensuring high purity of the DNA.

Interaction with pBR322 plasmid DNA. Interaction between DH4, DH5 and cisplatin with pBR322 plasmid DNA combined with BamH1 digestion, was carried out using gel electrophoresis

Table I. *(IC$_{50}$) values and resistance factors (RF) for DH4, DH5 and cisplatin, as applied to the human ovarian A2780, A2780^{cisR} and A2780^{ZD0473R} cancer cell lines.*

Compound	IC$_{50}$ (μM) ±SD			IC$_{50}$ (μM) ±SD	
	A2780	A2780^{cisR}	RF	A2780^{ZD0473R}	RF
DH4	2.09± 0.62	3.40±0.24	1.5	3.69±0.62	5.9
DH5	9.39± 0.10	11.69±0.17	9.4	7.54±0.62	0.8
Cisplatin	0.33±0.02	4.41±0.32	13	3.24±0.38	9.8

following a method described by Stellwagen (8). BamH1 is a type II restriction endonuclease that binds at the recognition sequence 5'-GGATCC-3' and chops these sequences just after the 5'-guanine on each strand (9). pBR322 plasmid DNA contains a single restriction site for BamH1 that converts the supercoiled form I and singly-nicked circular form II to linear form III DNA. Exactly 1.5 μl of supplied pBR322 plasmid DNA, in solution, was added to the solutions of DH4, DH5 and cisplatin at different concentrations ranging from 5 μM to 30 μM.

The DNA blank was prepared by adding 18.5 μl milli Q water to 1.5 μl of pBR322 plasmid DNA. The samples and the DNA blank were incubated for 4 h on a shaking water bath at 37°C and then subjected to BamH1 (10 units μl^{-1}) digestion. To each 20 μl of the incubated drug−DNA mixture was added 2 μL of 10× digestion buffer SB first and then 0.1 μL BamH1 (1 unit). The mixtures were left in a shaking water bath at 37°C for another 1 h, at the end of which the reaction was terminated by rapid cooling. Four microlitres of ethidium bromide were added to each sample before loading onto the gel. The gel was photographed using Eastman Kodak Company, Molecular Imaging Systems.

Results

Cytotoxicity. Table I lists the (IC$_{50}$) values and resistance factors (RF) for DH4, DH5 and cisplatin applying to the human ovarian A2780, A2780^{cisR} and A2780^{ZD0473R} cancer cell lines. The IC50 value is defined as the drug concentration required for 50% cell kill and the resistance factor (RF) is defined as the ratio of the IC$_{50}$ value of the resistant cell line over that of the parent cell line.

The results show that both DH4 and DH5 are less active than cisplatin against A2780 and A2780^{ZD0473R} cell lines. However, DH4 is more active than cisplatin against the resistant A2780^{cisR} cell line.

Platinum accumulation and platinum−DNA binding. Table II gave the total intracellular platinum levels (expressed as nanomoles platinum per 2×10^6 cells) found in the A2780, A2780^{cisR} and A2780^{ZD0473R} cell lines after their exposure to 50 μM concentrations of DH4, DH5 and cisplatin for 2, 4 and 24 h. Table III gave the levels of platinum−DNA binding (nmol platinum per 2×10^6 cells) in the A2780, A2780^{cisR} and

Table II. *Pt accumulation in A2780, A2780^{cisR} and A2780^{ZD0473R} cell lines in 2, 4 and 24 h as applied to DH4, DH5 and cisplatin.*

Cell line	Compound	Platinum accumulation (nmol Pt per 2×10^6 cells) at		
		2 h	4 h	24 h
A2780	DH4	0.23±0.02	1.15±0.24	3.16±0.28
	DH5	0.32±0.06	1.00±0.14	1.66±0.23
	Cisplatin	0.50±0.03	0.99±0.10	1.22±0.02
A2780^{cisR}	DH4	0.66±0.02	0.78±0.06	1.06±0.11
	DH5	0.34±0.02	0.99±0.11	1.25±0.06
	Cisplatin	0.31±0.03	0.84±0.09	1.17±0.15
A2780^{ZD0473R}	DH4	0.51±0.05	1.18±0.10	1.53±0.04
	DH5	0.24±0.02	0.74±0.05	1.15±0.05
	Cisplatin	0.32±0.02	1.19±0.15	1.85±0.22

Table III. *Levels of platinum−DNA binding (mol platinum per 2×10^6 cells) in A2780, A2780^{cisR} and A2780^{ZD0473R} cell lines in 2, 4 and 24 h from DH4, DH5 and cisplatin*

Cell line	Compound	2 h	4 h	24 h
A2780	DH4	14.1±2.1	19.8±8.1	29.7±4.1
	DH5	16.5±1.4	25.6±7.9	31.2±5.2
	Cisplatin	4.8±0.9	12.9±1.6	17.4±0.4
A2780^{cisR}	DH4	5.6±1.7	6.1±1.0	7.0±2.4
	DH5	16.5±1.4	25.6±7.9	31.2±5.2
	Cisplatin	3.51±0.81	5.02±1.1	7.93±1.3
A2780^{ZD0473R}	DH4	9.5±0.4	15.3±7.3	22.8±2.7
	DH5	19.7±5.2	26.5±4.0	35.6±4.9
	Cisplatin	4.1±1.5	6.0±0.5	9.0±3.2

A2780^{ZD0473R} cell lines at 2, 4 and 24 h from DH4, DH5 and cisplatin. In the A2780 cell line, DH4 and DH5 produced higher cellular platinum accumulations after 24 h whereas cisplatin had the higher values at 2 h and 4 h. In the A2780^{cisR} and cell line, DH4 produced the highest cellular accumulations of platinum at 2 h, DH5 did so at 4 h and 24 h. In the A2780^{ZD0473R} cell line, DH4 produced highest cellular accumulations of platinum at 2 h whereas cisplatin did so at 24 h. Both DH4 and DH5 produced higher levels of platinum−DNA binding than cisplatin at all the time points in the cell lines A2780 and A2780^{ZD0473R} even though the compounds were less active than cisplatin against A2780 and A2780^{ZD0473R} cell lines. In the A2780^{cisR} cell line, DH5 was found to be associated with the highest platinum−DNA binding levels at all time points.

pBR322 plasmid DNA. When pBR322 plasmid DNA interacted with increasing concentrations of compounds followed by BamH1 digestion, for DH4 three bands corresponding to forms I, II and III for concentrations ranging from 5 μM to 20 μM and no clear band at higher concentrations (Figure 2) were seen, whereas for DH5, three bands corresponding to forms I, II and III were observed for concentrations ranging from 5 μM to 30 μM and for cisplatin, two bands corresponding to forms I and II were observed for concentrations ranging from 5 μM to 30 μM. Table IV lists the observed bands.
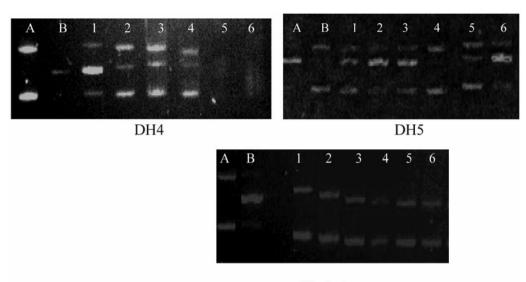
Discussion

Structures. The structures of DH4 and DH5 could not be confirmed by single-crystal x-ray diffractometry as no suitable single crystal was available. However, the results of elemental analyses and spectral studies (described earlier) can be seen to provide support for the suggested structure of the compounds (Figure 1).

Activity. Both DH4 and DH5 are less active than cisplatin against A2780 and A2780^{ZD0473R} cell lines but DH4 is more active than cisplatin against the resistant A2780^{cisR} cell line. The lower RFs for DH4 and DH5 is due to the fact that they form mainly interstrand bi-functional adducts with the DNA, unlike cisplatin, which forms mainly intrastrand bi-functional adducts (10).

Platinum accumulation. As noted earlier, no clear trend between activity and cellular accumulation of platinum was found. However, this may not be so surprising when we note that although entry into the cell precedes binding with the DNA, the level of cellular accumulation may not correspond to the level of the formation of critical platinum−DNA adducts. Indeed the results can be seen to illustrate that cellular accumulation of platinum *per se* may not be an indicator of the activity of compounds since the compounds may be de-activated by cellular thiols such as glutathione before binding with the DNA. The other point to note is that whereas cisplatin binds with nucleobases in DNA to form mainly bi-functional intrastrand 1,2-Pt(GG) and 1,2-Pt(AG) adducts that cause local bending of a DNA strand, DH3 would form mainly mono-functional Pt(G) and bi-functional interstrand Pt(GG) adducts, the latter causing more of a global change in the DNA conformation.

Platinum−DNA binding. Table III gives the levels of platinum−DNA binding in A2780, A2780^{cisR} and A2780^{ZD0473R} cell lines in 2, 4 and 24 h as applied to DH4, DH5 and cisplatin. DH4 and DH5 produced higher levels of platinum−DNA binding than cisplatin at all the time points in the A2780 and A2780^{ZD0473R} cell lines, even though the compounds are less active than cisplatin against A2780 and A2780^{ZD0473R} cell lines. Conversely, even though DH4 is more active than cisplatin against the A2780^{cisR} cell line, it is

Figure 2. *Electrophoretograms of the incubated mixtures of pBR322 plasmid DNA and different concentrations of DH4, DH5 and cisplatin followed by digestion with BamH1. Lane A: Untreated and undigested pBR322 plasmid DNA, lane B: untreated but digested pBR322 plasmid DNA, lanes 1 to 6: pBR322 plasmid DNA interacted with increasing concentrations of the compounds (lane 1: 5 μM, lane 2: 10 μM, lane 3: 15 μM, lane 4: 20 μM, lane 5: 25 μM, lane 6: 30 μM), followed by BamH1 digestion.*

associated with a lower level of platinum–DNA binding (after 24 h) than cisplatin in the cell line. The results can be seen to illustrate that even platinum–DNA binding *per se* may not necessarily be a true measure of the activity of platinum compounds. It should be noted that platinum–DNA binding is necessary but not sufficient to bring about apoptosis as the cell death is actually caused by downstream processes involving many proteins. It is hypothesized that the higher level of platinum–DNA binding from DH4 is due to the predominance of non-critical adducts such as mono-functional Pt(G) and DNA–protein crosslinks in A2780 and A2780^{ZD0473R} cell lines.

BamH1 digestion. As stated earlier, when the pBR322 plasmid DNA interacted with increasing concentrations of DH4 followed by BamH1 digestion, three bands corresponding to forms I, II and III were observed for concentrations ranging from 5 μM to 20 μM and no clear band was seen at higher concentrations. In the case of DH5, three bands corresponding to forms I, II and III were observed for concentrations ranging from 5 μM to 30 μM and for cisplatin, two bands corresponding to forms I and II were observed for concentrations ranging from 5 μM to 30 μM. The results indicate that the binding of DH4 and DH5 with pBR322 plasmid DNA was able to prevent partially BamH1 digestion of the DNA, indicating changes in DNA conformation brought about by covalent and non-covalent interactions between DH4, DH5 and cisplatin and nucleobases in the DNA. The absence of any clear DNA

Table IV. *Bands observed after BamH1 digestion of pBR322 plasmid DNA interacted with increasing concentrations of DH4, DH5 and cisplatin.*

Compound	Lane					
	1	2	3	4	5	6
DH4	I, II, III	I, II, III	I, II, III	I, II, III	--	--
DH5	I, II, III	I, II, III	I, II, III	I, II	I, II, III	I, II, III
Cisplatin	I, II	I, II	I, II	I, II	I, II	I, II

band in lanes 5 and 6 indicates that DH4 causes DNA damage at higher concentrations. In the case of cisplatin, bands for forms I and II were observed at all concentrations ranging from 5 μM to 30 μM. The results indicated that cisplatin was able to prevent BamH1 digestion to a greater extent than DH4 and DH5, indicative of greater conformational changes in the DNA.

Finally, two *trans*-planaramineplatinum(II) complexes have been synthesized and investigated for the activity against human ovarian tumour models and their nature of binding with pBR322 plasmid DNA. The lower activity of DH4 and DH5, as compared to cisplatin, suggests that the compounds may not offer any advantage over cisplatin as a potential anticancer drug.

Conflicts of Interest

Acknowledgements

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