Synthesis and Antitumour Activity of a New Trinuclear Platinum Compound [{*cis*-$PtCl(NH_3)_2\mu$ {*trans*-$Pt(3\text{-hydroxypyridine})_2H_2N(CH_2)_5NH_2)_2$}] Cl_4 in Human Ovarian Cancer Cells

SHAHNAZ A. HAMAD[1], PHILIP BEALE[2], JUN QING YU[1] and FAZLUL HUQ[1]

[1]*Discipline of Biomedical Science, The University of Sydney, Lidcombe, NSW, Australia;*
[2]*Sydney Cancer Centre, Sydney, NSW, Australia;*
[3]*School of Chemistry, The University of Sydney, Sydney, NSW, Australia*

Abstract. *A trinuclear platinum compound with a cis-geometry for the terminal metal centers coded as QH5 has been synthesized and investigated for activity against the human ovarian A2780, A2780^{cisR} and A2780^{ZD0473R} cancer cell lines. Cellular accumulation of platinum, level of platinum–DNA binding and nature of interaction of the compound with pBR322 plasmid DNA have also been determined. QH5 is found to be more active than cisplatin against all the three cell lines and to have much lower resistant factors than cisplatin. The compound is 2.5-times more active than cisplatin against the A2780^{cisR} cell line and 11.5-times more active than cisplatin against A2780^{ZD0473R} cell line. When the activity of QH5 in A2780 cell line is compared with its activity in the A2780^{ZD0473R} cell line, it is found to be 2.4-times more active against the resistant A2780^{ZD0473R} cancer cell line than the parent A2780 cell line, thus indicating that it has been able to overcome mechanisms of resistance operating in the A2780^{ZD0473R} cell lines. The higher activity of QH5 as compared to cisplatin is found to be associated with higher platinum accumulation at all time points and high level of platinum-DNA binding at 24 h in all the three human ovarian cancer cell lines. Provided QH5 has the right toxicity profile and its in vitro activity is complemented with sufficient activity in vivo, the compound may have the potential for development as a novel platinum–based anticancer drug targeted to ovarian cancer.*

Correspondence to: Fazlul Huq, Discipline of Biomedical Science, School of Medical Sciences, Sydney Medical School, The University of Sydney, Cumberland Campus C42, 75 East Street, Lidcombe, NSW 1825, Australia. Tel: +61 293519522, fax: +61 293519520, e-mail: fazlul.huq@sydney.edu.au

Key Words: Ovarian cancer, A2780, cisplatin, drug resistance, pBR322 plasmid DNA, BamH1 digestion, interstrand adducts.

Currently great attention is given to platinum compounds that are structurally and functionally different from cisplatin with the idea that the difference in nature of interaction with the DNA may result in an altered spectrum of activity (1). One such class of compounds is multi-nuclear platinum complexes that are often found to be much more active than cisplatin in both cisplatin-responsive and cisplatin-resistant tumor models. An example is BBR3464 that is significantly more cytotoxic than cisplatin and has been found to retain activity against cisplatin-resistant cell lines *in vitro* as well *in vivo* (2). The compound showed a complete lack of cross-resistance in cisplatin-resistant cells (3, 4). *In vivo*, BBR3464 also showed very promising anti-tumor activity, independent of p53 level, in a range of human tumor xenografts (5-7). The drug underwent Phase II clinical trials in cisplatin-resistant and refractory cancers (6, 8) but met with the problems of toxicity. Huq and co-workers have also designed a number of multi-centered platinums including DH6Cl, DH7Cl, TH1 and CH25 that have been found to be significantly more active than cisplatin in a number of ovarian tumor models (1, 9-12). The overall charge, the linker flexibility and the hydrogen-bonding capability are thought to be related to the improved cytotoxic and anti-tumor properties of multi-nuclear platinum compounds (13-15). One of the problems of multinuclear platinum compounds with *trans*-geometry for the terminal metal centres is that the compounds are expected to break-down significantly inside the cell. This is because of the *trans*-labilizing effect due to the chloride ligands. The breakdown of trinuclear cations will serve to reduce the number of long-range adducts with DNA and may cause toxicity problems. This is because the degradation products are expected to bind with cellular thiols such as glutathione that plays key role in de-toxication of reactive oxygen and reactive nitrogen species. In contrast, trinuclear platinum complexes with a *cis*-geometry for the terminal metal centers are expected to

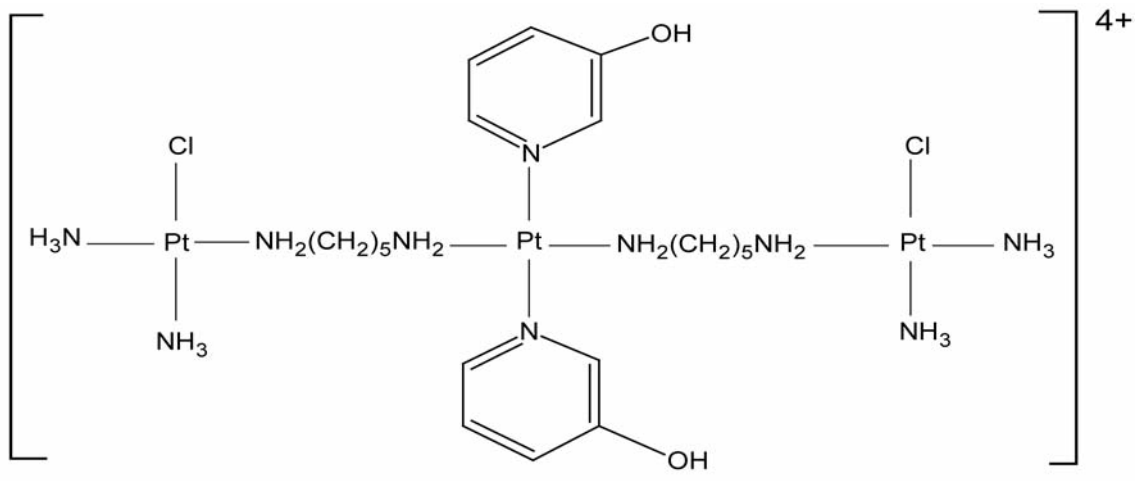

Figure 1. *Structure of the tetrapositive cation of QH5 – the balancing chloride anions are not shown.*

be less subjected to breakdown and hence may produce a greater number of long-range inter- and intrastrand adducts with DNA. This means that the toxicity problems may be less pronounced with the trinuclear platinums with a *cis*-geometry for the terminal metal centres. The compounds may also differ in the spectrum of activity from the corresponding complexes with a *trans*-geometry for the terminal metal centres. In this article we report on the synthesis, characterization, and activity of [{*cis*-$PtCl(NH_3)_2$ μ-{*trans*-$Pt(3\text{-hydroxypyridine})_2(H_2N(CH_2)_5NH_2)_2$}]$Cl_4$ (coded as QH5). Figure 1 gives the structure of QH5.

Materials and Methods

Chemicals. 3-hydroxypyridine (Sigma Chemical Company St. Louis, MO, USA); *N, N*-dimethylformamide [DMF], dimethyl sulfoxide (DMSO), 1,-diaminohexane dihydrochloride, sodium hydroxide, silver nitrate, concentrated hydrochloric acid, potassiumtetrachlo-roplatinate(II) (K_2PtCl_4), ethanol, methanol, diethyl ether (Ajax chemicals Auburn NSW Australia); concentrated ammonia solution, triethylamine, dichloromethane and 28% ammonia solution (Asia Pacific Specialty Chemicals Ltd Auckland New Zealand); pBR322 plasmid DNA (ICN Biomedicals, Ohio, USA); Foetal calf serum, 5 × RPMI 1640, 200 mM L-glutamine, and 5.6% sodium bicarbonate (Trace Biosciences Pty Ltd, Australia); commercially available JETQUICK Blood DNA Spin Kit/50 (Astral Scientific, Sydney, Australia).

Synthesis. CH1, denoting [*trans*-$PtCl_2(3\text{-hydroxypyridine})_2$ required for the synthesis of QH1, was prepared according to previously published method [14]. Yellow-orange solid; Formula: $C_5H_8N_2Cl_2OPt$; purity: 99.8%; Yield: 68.0%
Composition: Calc.%: C, 26.3; H, 2.2; N, 6.2; Cl, 15.3; Pt, 42.8 Obs. %: C, 26.4±0.4; H, 2.0±0.4; N, 6.2±0.4; Cl, 15.3±0.4; Pt, 43.1±1.2.

QH5 was prepared using the step-up method of synthesis following a method similar to that used for the synthesis of TH1 (11). Briefly, cisplatin (0.5 mmol, 0.15 g) was dissolved in 10 mL of DMF to which 0.495 mmol of silver nitrate (0.0849 g) were added. The mixture was stirred at room temperature for 24 h in the dark followed by centrifugation at 4,800 rpm for 30 min at 21˚C to remove the precipitate of AgCl. The supernatant containing *cis*-$Pt(NH_3)_2Cl(DMF)$ in-solution was collected and kept at −16˚C. A suspension of CH1 (0.25 mmol, 0.114 g) in 10 mL of DMF was gently heated with stirring at 60˚C for 20 min. 0.5 mmol of 1,5-diaminopentane dihydrochloride (0.0875 g, 0.5 mmol) was dissolved in 4 mL of DMF. The diamine solution was stirred for a further 15 min. It was then added to the CH1 suspension drop-wise with stirring within 30 min of its preparation followed by the addition of 0.5 mL of 1 M NaOH with stirring at 70˚C. Stirring was continued at 60˚C for about 1 h, then at 50˚C for 4 h to result into a light yellow solution with some brown precipitate. 200 μL of 1 M NaOH was added to the solution and the mixture was stirred for further 5 min at 50˚C. Cisplatin supernatant (0.25 mmol) was added to the yellow solution with stirring. The temperature of the solution was increased to 70˚C. The mixture was stirred for further 5 min at the same temperature. Then, a further 200 μL of 1 M NaOH were added to the mixture followed by the addition of a further 0.25 mmol of cisplatin supernatant. The mixture was left standing while being stirred at 60˚C for 50 min and then for 15 days at room temperature. The volume of the filtrate was reduced to 4 mL using a vacuum concentrator consisting of Javac DD150 Double stage High Vacuum Pump Savant RVT 4104 Refrigerated Vapor Trap and Savant Speed Vac 110 Concentrator. About 20 mL of dichloromethane was added to the concentrated solution. The mixture was left standing at 5˚C for 6 h. The light yellow precipitate produced was collected by filtration at the pump, washed in succession with ice-cold water and ethanol. It was then air-dried. The steps in synthesis are shown in Figure 2. Attempts were made to increase the purity of the compounds by repeated dissolution in DMF followed by precipitation with dichloromethane. However, the best results were obtained when crude products were repeatedly washed with 95 % ethanol:5 % water mixture.

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

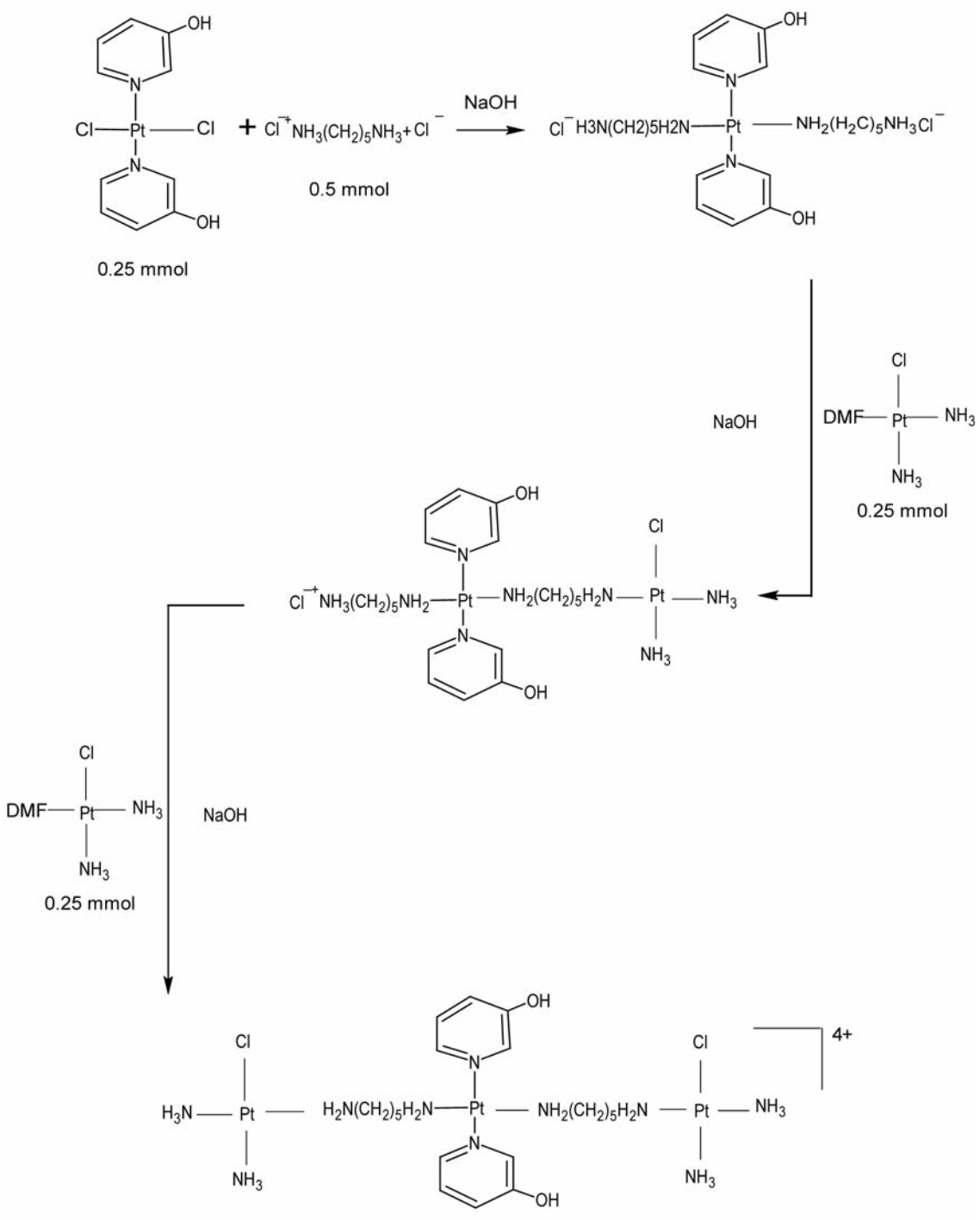

Figure 2. *Schema for the synthesis of QH5*.

QH5: $C_{20}H_{50}Cl_6N_{10}O_2Pt_3$; Formula Weight=1260.63, Percentage yield=70%

Calc.%: C: 19.1; H: 4.0; N: 11.1; Cl: 16.9; Pt: 46.4 Obs. %: C: 18.8±0.4; H: 3.8±0.4; N: 10.8±0.4; Cl: 17.2±0.4; Pt: 46.6±1. Limiting molar conductivity=392.0 ohm^{-1}cm^{2}mol^{-1}.

Spectral studies. IR (KBr) :3396, 3272.2, 2963.0, 2777.6, 1607.1, 1582.6, 1466.7, 1301.3, 1220.2, 1108.5, 1020.8, 806.6, 696.3, 512.3, 444.0; MS (ESI) m/z 1302.71=[Pt_3(3-hydroxypyridine)$_2$($NH_2(CH_2)_5NH2)_3(NH_3)_3Cl_2$+3Cl+3H], 791=[$Pt_2(NH_2(CH_2)_5NH_2)$(3-hydroxypyridine)$Cl_5$+Cl+8H], 702.51=[$Pt_2(NH_3)_4(NH_2(CH_2)5NH_2)$

Cl2+2Cl], 621.57=[$Pt_2(NH_3)_5(NH_2(CH_2)5NH_2)Cl$+9H], 522.91=[$Pt(NH_2(CH_2)_5NH_2)_2$(3-hydroxypyridine)Cl – 7H], 402=[Pt(3-hydroxypyridine)(pyridine)Cl – 3H], 386=[$Pt(pyridine)_2$ – 3H], 335=[$Pt(NH_3)_4Cl_2$+H]; ^{1}H NMR (400.2 MHz, [D6] DMSO] δ=8.5 ppm: due to CH ortho; δ=7.5 ppm: due to CH meta; δ=7.4 ppm: due to CH para; δ=4.2: due to NH_2-Pt; δ=2.9 ppm: due to NH_2-Pt; δ=1.8 ppm: due to CH2; δ=1.5 ppm: due to CH_2.

Cytotoxicity assays. Cytotoxicity of QH5 against human ovarian A2780, A2780^{cisR}, and A2780^{ZD0473R} cancer cell lines was determined using the MTT (3-(Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-terazolium bromide) reduction assay (16). Approximately 5,000 to 9,000 cells seeded into the wells of flat-bottomed 96-well culture plates in 10% FCS/RPMI 1640 culture medium were incubated for 24 h at 37˚C in a humidified atmosphere to allow cells to attach. Platinum complexes dissolved first in a minimum volume of DMF were diluted to the required concentrations with mQ 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. The plates containing cells and drugs were left to incubate under normal growth conditions for 72 h. The inhibition of cell growth was determined using the MTT assay (16). Four hours after the addition of MTT (50 μL per a mg mL^{-1} MTT), the yellow formazan crystals produced from the reduction of MTT were dissolved in 150 μL of DMSO and the absorbance of the resulting solution was read with Bio-Rad Model 3550 Microplate Reader set at 570 nm.

Cellular platinum accumulation and platinum-DNA binding. The method used for the determination of cellular accumulation of platinum and the level of platinum-DNA binding was a modification of the method described by Di Blasi *et al*. (17). In short, QH5 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=1×10^6 cells ml^{-1}). 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. Ice-cold phosphate-buffered saline (PBS) was used to wash the cells twice and the pellets were stored at –20˚C until assayed. At least three independent experiments were performed. For the determination of drug accumulation in the cells, cell pellets were suspended in 0.5 mL 1% triton-X, held on ice then sonicated (5 min). Total intracellular platinum contents were determined by graphite furnace atomic absorption spectroscopy using the Varian SpectrAA-240 graphite furnace AAS.

For the determination of the level of platinum-DNA binding, the modified method of Bowtell (18) was used. Briefly, high-molecular weight DNA was isolated from cell pellets using JETQUICK Blood DNA Spin Kit/50. Cell pellets were resuspended 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 to the mixture followed by incubation for 10 min at 70˚C. Absolute ethanol (200 μL) was added and mixed thoroughly to prevent any precipitation of nucleic acids due to high local alcohol concentrations. 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 to change the high-salt conditions on the silica membrane to low-salt, 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 from the residual liquid. The receivers were changed and the purified DNA in the column was eluted from the membrane with 200 μL of 10 mM Tris-HCl buffer (pH 8.5). DNA content was determined using UV spectrophotometry set at 260 nm (Varian Cary 1E UV-Visible spectrometer with Varian Cary Temperature Controller). Platinum levels were determined by graphite furnace AAS. A_{260}/A_{280} ratios were found to be between 1.75 and 1.8 for all samples ensuring high purity of the DNA.

Table I. *IC_{50} values and resistant factors (RF) for QH5 and cisplatin*

Compound	A2780	A2780^{cisR}	RF	A2780^{ZD0473R}	RF
QH5	0.43±0.06	0.70±0.05	1.62	0.18±0.02	0.42
Cisplatin	1.09±0.06	8.08±1.03	7.45	0.13±0.02	3.95

Interaction with pBR322 plasmid DNA. Interaction between QH5 and cisplatin with pBR322 plasmid DNA, with and without BamH1 digestion, was studied out using gel electrophoresis based on a method described by Stellwagen (19). The amount of DNA was kept constant while the concentrations of compounds varied. Exactly, 1.5 μl of supplied pBR322 plasmid DNA in-solution was added to solutions of the compounds at different concentrations ranging from 0.55 μM to 70 μM for QH1 and cisplatin. The DNA blank (B) was prepared by adding 18.5 μl mQ 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. The reaction was stopped by rapid cooling to 0˚C for 20 min. The samples were mixed with 1 μl of marker dye ethidium bromide. 16 μl of each sample was loaded onto 1% agarose gel made in TAE buffer. The gel was photographed using Eastman Kodak Company, Molecular Imaging Systems.

BamH1 restriction enzyme digestion. BamH1 is a type II restriction endonuclease that hydrolyses the phosphodiester bonds. It binds at the recognition sequence 5'-GGATCC-3', and chops these sequences just after the 5'-guanine on each strand (20). 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. The same identical set of drug-DNA mixtures as described previously, was first 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. 4 μl of ethidium bromide were added to each sample before loading onto the gel. The gel was photographed following a method described previously.

Results and Discussion

Activity against ovarian cancer cell lines. Table I lists the IC_{50} values and resistance factors (RF) for QH5 and cisplatin for the human ovarian A2780, A2780^{cisR} and A2780^{ZD0473R} cancer cell lines. Each IC_{50} value is defined as the drug

Table II. *Cellular accumulation of platinum from QH5 and cisplatin in A2780, A2780^{cisR} and A2780^{ZD0473R} cells at 2, 4 and 24 h.*

Cell line	Compound	Cellular platinum accumulation (nanomoles Pt per 2×10^6 cells)		
		2 h	4 h	24 h
A2780	QH5	0.80±0.07	1.07±0.07	3.35±0.43
	Cisplatin	0.26±0.03	0.45±0.05	1.18±0.08
A2780^{cisR}	QH5	1.54±0.17	2.37±0.18	2.13±0.25
	Cisplatin	0.34±0.02	0.69±0.05	0.69±0.05
A2780^{ZD0473R}	QH5	0.70±0.06	1.88±0.18	6.59±0.32
	Cisplatin	0.26±0.003	0.35±0.05	1.98±0.11

Table III. *Levels of platinum-DNA binding from QH5 and cisplatin in A2780, A2780^{cisR} and A2780^{ZD0473R} cells.*

Cell line	Compound	Levels of platinum-DNA binding (nanomoles Pt per mg of DNA)		
		2 h	4 h	24 h
A2780	QH5	8.71±0.36	9.26±0.08	25.52±2.42
	Cisplatin	15.58 ±1.30	15.58 ±1.30	21.54±1.18
A2780^{cisR}	QH5	9.44±0.77	6.44 ±0.07	15.26±2.85
	Cisplatin	17.85±0.06	12.34±0.49	7.15±1.54
A2780^{ZD0473R}	QH5	13.03±0.27	9.29±0.01	25.37±0.97
	Cisplatin	4.71±0.64	9.71±0.13	9.71±0.13

concentration required for 50% cell kill and resistance factor (RF) is defined as the ratio of the IC_{50} value in the resistant cell line over that in the parent cell line.

QH5 is found to be significantly more active than cisplatin against the three cell lines A2780, A2780cisR and A2780ZD0473R – 2.5 times more active against A2780 cell line, 11.5 more active against resistance cell lines A2780^{cisR} and 23-times more active against A2780^{ZD0473R}. In addition, QH5 has a much lower resistance factor than cisplatin (0.42 *vs*. 3.95) as applied to the cell lines A2780 and A2780^{ZD0473R}. The results indicate that QH5 has been better-able to overcome resistances operating in both A2780^{cisR} and A2780^{ZD0473R} cell lines. When the activity of QH5 is compared to analogous compound QH1 with 1,6-diaminohexane as the linker chain instead of 1,5-diaminopentane in QH5, it is found that QH5 is somewhat less active than QH1 (21) in line with the reported results that cytotoxic activity is maximum when the number of carbon atoms in the linker diamine chain is six (1).

Cellular platinum accumulation. Table II gives the total intracellular accumulation of platinum (expressed as nanomoles platinum per 2×10^6 cells) found in the cell lines A2780, A2780^{cisR} and A2780^{ZD0473R} after exposure to 50 μM concentrations of QH5 and cisplatin for 2, 4 and 24 h.

When platinum accumulations from QH5 are compared to those from cisplatin, it is found that QH5 is associated with higher levels of platinum accumulation at all stages of incubation (2, 4 and 24 h) in all the three human ovarian cancer cell lines, in line with higher compound activity.

DNA binding. Table III gives the levels of platinum-DNA binding expressed as nanomol of Pt per milligram of DNA for QH5 and cisplatin in the human ovarian A2780, A2780^{cisR} and A2780^{ZD0473R} cell lines.

It can be seen that the level of platinum-DNA binding for QH5 is higher than cisplatin in all cell lines at all the three time points. This is to be expected since the triplatinum cation of QH5 would be more readily attracted to the negatively-charged DNA than mono- and dipositively charged cations produced from cisplatin and its analogues (22).

Interaction with pBR322 plasmid DNA. When pBR322 plasmid DNA interacted with QH5 and cisplatin, generally two DNA bands corresponding to forms I and II were observed in both untreated and treated pBR322 plasmid DNA. As the concentration of the compounds was increased, the mobility of form I and form II pBR322 plasmid DNA increased in the case of QH5 and cisplatin but at different rates so that the separation between the bands decreased. In the case of QH5, a single coalesced band was observed in lane 6 corresponding to 17.5 μM concentration. In the case of cisplatin a faint coalesced band was observed in lane 7 corresponding 35 μM concentration (Figure 4).

The changes in mobility of the bands indicated changes in DNA conformation brought about by covalent binding of the compounds with the DNA (23). Increase in concentration of QH5 and cisplatin was also found to cause increasing damage to DNA indicated by a decrease in intensity of the bands.

BamH1 digestion. To provide further insight into changes in DNA confirmation, drug-DNA incubation followed by BamH1 digestion was used.

Figure 4 gives the electrophoretogram corresponding to the BamH1-digested incubated mixtures of pBR322 plasmid DNA and varying concentrations of QH5 and cisplatin.

Table IV gives a listing of the bands indicative of the incubated mixtures of pBR322 plasmid DNA and varying concentrations of QH5, and cisplatin followed by digestion with BamH1. Prevention of BamH1 digestion with increasing concentrations of QH1 and QH5, gives support to the idea that binding of the compounds with DNA induced global changes in its conformation.

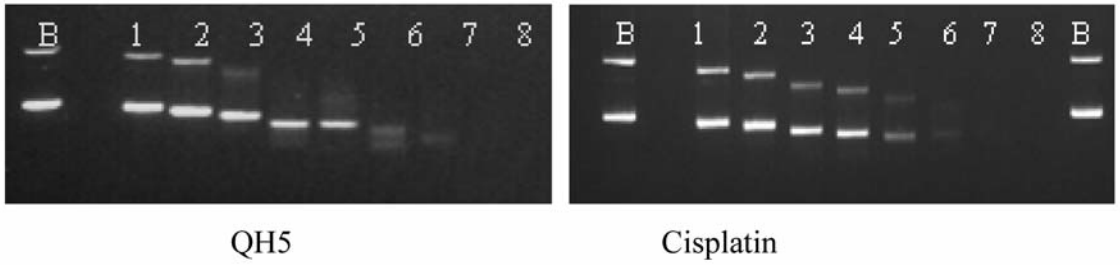

Figure 3. *Electrophotograms showing the interaction of pBR322 plasmid DNA with increasing concentrations of QH5 and cisplatin: lane B applies to untreated pBR322 plasmid DNA to serve as a control, lanes 1 to 8 apply to plasmid DNA interacted with increasing concentrations of QH5 and cisplatin. The concentrations are as follows: lane 1: 0.55 μM, lane 2: 1.09 μM, lane 3: 2.19 μM, lane 4: 4.38 μM, lane 5: 8.75 μM, lane 6: 17.50 μM, lane 7: 35 μM, and lane 8: 70 μM.*

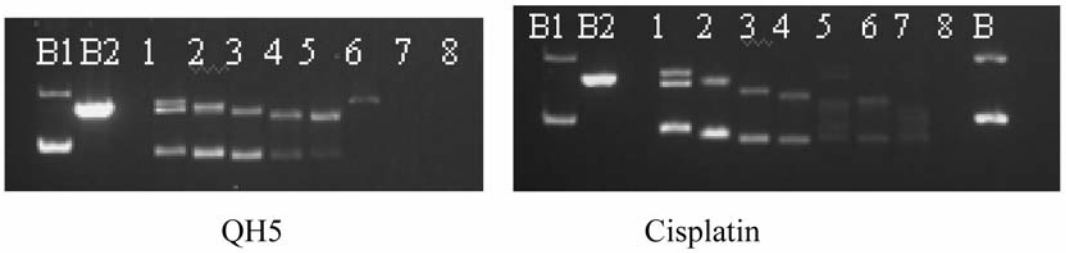

Figure 4. *Electrophoretograms applying to the incubated mixtures of pBR322 plasmid DNA and varying concentrations of compounds: QH5, and cisplatin followed by digestion with BamH1. Lane B1 corresponds to the untreated pBR322 plasmid DNA and undigested with BamH1, lane B_2 applies to untreated but digestion with BamH1. Lanes 1 to 8 apply to pBR322 plasmid DNA interacted with increasing concentrations of the compounds followed by BamH1 digestion. The concentrations for QH5 and cisplatin were as follows: lane 1: 0.55 μM, lane 2: 1.09 μM, lane 3: 2.19 μM, lane 4: 4.38 μM, lane 5: 8.75 μM, lane 6: 17.50 μM, lane 7: 35 μM, and lane 8: 70 μM.*

Table IV. *Bands observed after the incubated mixtures of QH5 and cisplatin with pBR322 plasmid DNA were digested with BamH1.*

Compound	Lane number							
	1	2	3	4	5	6	7	8
QH5	I, II, III	I, II	I, II	I, II	I, II	I	No band	No band
Cisplatin	I, II, III	I, II	I, II	I, II	I, II	I, II	I,II	No band

When the DNA interacted with increasing concentrations of QH5, followed by BamH1 digestion, three bands corresponding to forms I, II and III were observed at the lowest concentration of QH5 (0.55 μM), two bands corresponding to forms I and II were observed at concentrations ranging from 1.09 μM to 8.75 μM. At the next higher concentration of QH5 (17.50 μM) a very faint band could be seen and no DNA bands were observed at higher concentrations of the compound. In the case of cisplatin, three bands corresponding to forms I, II and III were observed at the lowest concentration of the compound namely 0.55 μM. Two bands corresponding to forms I and II were observed at concentrations ranging from 1.09 μM, to 4.38 μM. A much fainter band could be seen at concentrations ranging from 8.75 μM to 35.00 μM and no DNA band was found at the highest concentration of cisplatin. These results indicate that cisplatin is better-able to cause conformational changes in the DNA than QH5. Whereas trinuclear platinums can induce global changes in DNA conformation due to the formation of long-range interstrand and intrastrand GG adducts, cisplatin induces more of a local change in DNA conformation mainly due to formation of intrastrand bi-functional Pt(1,2-GG) and Pt(1,2-AG) adducts.

Finally, it should be noted that QH5 may have the potential for development as a novel platinum-based anticancer drug targeted against ovarian cancer provided it has the right toxicity profile and the *in vitro* activity of the compound is matched with sufficient *in vivo* activity.

Conflicts of Interest

Shahnaz A. Hamad, Philip Beale, Jun Qing Yu and Fazlul Huq declare that they have no financial and personal relationships with other people or organizations that could inappropriately influence their work.

Acknowledgements

This work was supported in part by the Biomedical Sciences Research Initiative Grant. Shahnaz A. Hamad is thankful to the Discipline of Biomedical Science for the award of a Ph.D completion scholarship.

References

1 Daghriri H, Huq F and Beale P: Studies on activities, cell up take and DNA binding of four multinuclear complexes of the form: [{*trans*-PtCl(NH_3)$_2$}2{μ-*trans*-Pd(NH_3)$_2$-($H_2N(CH_2)_nNH_2)_2$]Cl_4 where n=4 to 7. J. Inorg Biochem *98(11)*: 1722-1733, 2004.

2 Riccardi A, Meco D, Ferlini C, Servidei T, Carelli G, Segni G, Manzotti C and Riccardi R: *In vitro* and *in vivo* antitumor activity of the novel trinuclear platinum complex BBR 3464 in neuroblastoma. Cancer Chemother Pharmacol *47(6)*: 498-504, 2001.

3 Hausheer FH, Kanter P, Cao S, Haridas K, Seetharamulu P, Reddy D, Petluru P, Zhao M, Murali D, Saxe JD, Yao S, Martinez N, Zukowski A and Rustum YM: Modulation of platinum-induced toxicities and therapeutic index: mechanistic insights and first- and second-generation protecting agents. Seminars Oncol *25(5)*: 584-599, 1998.

4 Roberts JD, Peroutka J and Farrell N: Cellular pharmacology of polynuclear platinum anti-cancer agents. J Inorg. Biochem *77(1-2)*: 51-57, 1999.

5 Perego P, Caserini C, Gatti L, Carenini N, Romanelli S, Supino R, Colangelo D, Viano I, Leone R, Spinelli S, Pezzoni G, Manzotti C, Farrell N and Zunino F: A novel trinuclear platinum complex overcomes cisplatin resistance in an osteosarcoma cell system. Molecular Pharmacol *55(3)*: 528-534, 1999.

6 Pratesi G, Perego P, Polizzi D, Righetti SC, Supino R, Caserini C, Manzotti C, Giuliani FC, Pezzoni G, Tognella S, Spinelli S, Farrell N and Zunino F: A novel charged trinuclear platinum complex effective against cisplatin-resistant tumours: hypersensitivity of p53-mutant human tumour xenografts. Br J Cancer *80(12)*: 19122- 1999.

7 Kelland LR: Preclinical perspectives on platinum resistance. Drugs *59(Suppl 4)*: 1-8, 2000.

8 Farrell N: Polynuclear platinum drugs. Met Ions Biol Syst *42*: 251-296, 2004.

9 Cheng H, Huq F, Beale, P and Fisher K: Synthesis, characterisation, activities, cell uptake and DNA binding of the trinuclear complex: [{*trans*-PtCl(NH_3)}$_2$μ-{*trans*-Pt(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4. Eur J Med Chem *40(8)*: 772-781, 2005.

10 Cheng H, Huq F, Beale P and Fisher K: Synthesis, characterisation, activities, cell uptake and DNA binding of the trinuclear complex: [{*trans*-PtCl(NH_3)}2μ-{*trans*-Pd(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4, Eur J Med Chem *41*: 896-903, 2006.

11 Huq F, Tayyem H, Yu JQ, Beale P and Fisher K, Activity of a novel trinuclear platinum complex: [{*trans*-PtCl(NH_3)2}2μ-{*trans*-Pt(3-hydroxypyridine)$_2$($H_2N(CH_2)_6NH_2)_2$}]Cl_4 in ovarian cancer cell lines, ChemMedChem 10.1002/cmdc.200700204, 145-151, 2008.

12 Huq F, Tayyem H, Yu JQ, Beale P and Fisher K: Synthesis, activity and binding with DNA of [{*trans*-PtCl(NH_3)2}2μ-{*trans*-Pd(4-hydroxypyridine)$_2$($H_2N(CH_2)_6NH_2)_2$]Cl_4 (TH8). Med Chem *5*: 372-381, 2009.

13 Brabec V, Kasparkova J, Vrana O, Novakova O, Cox JW, Qu Y and Farrell N: DNA modifications by a novel bifunctional trinuclear platinum phase I anticancer agent. Biochemistry *38(21)*: 6781-6790, 1999.

14 Kloster MB, Hannis JC, Muddiman DC and Farrell N: Consequences of nucleic acid conformation on the binding of a trinuclear platinum drug. Biochemistry *38(45)*: 14731-14737, 1999.

15 Zehnulova J, Kasparkova J, Farrell N and Brabec V: Conformation, recognition by high mobility group domain proteins, and nucleotide excision repair of DNA intrastrand cross-links of novel antitumor trinuclear platinum complex BBR3464. J Biol Chem *276(25)*: 22191-22199, 2001.

16 Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods *65(1-2)*: 55-63, 1983.

17 Di Blasi P, Bernareggi A, Beggiolin G, Piazzoni L, Menta E and Formento ML: Cytotoxicity, cellular uptake and DNA binding of the novel trinuclear platinun complex BBR 3464 in sensitive and cisplatin resistant murine leukemia cells. Anticancer Res *18(4C)*: 3113-3117, 1998.

18 Bowtell DD: Rapid isolation of eukaryotic DNA. Analyt Biochem *162(2 SU)*: 463-465, 1987.

19 Stellwagen N: DNA gel electrophoresis. Nucleic acid electrophoresis. Springer: Berlin, New York, 1998.

20 Smith LA and Chirikjian JG: Purification and characterization of the sequence-specific endonuclease Bam HI. J Biol Chem *254(4)*: 1003-1006, 1979.

21 Hamad SA: New multicentred platinums with a *cis*-geometry for terminal metal centres. PhD Thesis, The University of Sydney: Sydney Australia, 2010.

22 Kabolizadeh P, Ryan J and Farrell N: Differences in the cellular response and signaling pathways of cisplatin and BBR3464 ([[*trans*-PtCl(NH_3)($_2$)]$_2$mu-(*trans*-Pt(NH_3)(2)($H_2N(CH_2)_(_6_)$-$NH_2)_2$)]$^{4+}$) influenced by copper homeostasis. Biochem Pharmacol *73(9)*: 1270-1279, 2007.

23 Lippard SJ: Metals in Medicine. *In*: Bioinorganic Chemistry, Bertini I, Gray HB, Lippard SJ and Valentine JS, Eds., University Science Books: Mill Valley, California pp. 505-583, 1994.

Received November 28, 2013
Revised January 8, 2014
Accepted January 10, 2014