Synthesis, characterisation, activities, cell uptake and DNA binding of a trinuclear complex: [{trans-PtCl(NH3)}2μ-{trans-Pd(NH3)(2-hydroxypyridine)-(H2N(CH2)6NH2)2]Cl4

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Laboratory note

Synthesis, characterisation, activities, cell uptake and DNA binding of a trinuclear complex: [{*trans*-PtCl(NH_3)}$_2$μ-{*trans*-Pd(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4

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Abstract

The trinuclear complex: [{*trans*-PtCl(NH3)}$_2$μ-{*trans*-Pd(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4 (code named CH25) has been synthesized and characterized. The activity of the compound against human ovarian cancer cell lines: A2780, A2780 cisR and A2780 ZD0473R, cell up take, level of binding with DNA and nature of its interaction with pBR322 plasmid DNA have been determined. The compound is found to exhibit significant anticancer activity against the cell lines–about 45 times as active as cisplatin against A2780 cell line, about 76 times as active as cisplatin against A2780^{cisR} cell line and about seven times as active as cisplatin against A2780cell line. The higher activity of CH25 suggests that the compound is able to overcome multiple mechanisms of resistance operating in A2780^{cisR} and A2780^{ZD0473R} cell lines. The compound is believed to form a range of interstrand GG adducts with duplex DNA that induces global changes in the DNA conformation, unlike cisplatin and ZD0473 [also known as AMD473 and JM473: *cis*-(2-methylpyridine)(ammine)dichloroplatinum(II)] that form mainly intrastrand adducts that induces a local kink in a DNA strand. The increasing prevention of BamH1 digestion of form I and form II pBR322 plasmid DNA with the increase in concentration of the compound is believed to be due to interstrand binding that brings about global changes in DNA conformation.

Keywords: Platinum; Palladium; Anticancer activity; Cell culture; DNA binding; Gel electrophoresis

1. Introduction

Polynuclear platinum complexes [1] that contain two or more platinum ions linked together by diaminoalkane chains [2] have shown activity in both cisplatin-responsive and cisplatin-resistant cancer cell lines. Only the two terminal platinum ions in polynuclear complexes such as BBR3464 undergo covalent binding (mainly interstrand) with DNA whereas the central platinum ion undergoes only noncovalent interactions such as hydrogen bonding and electrostatic interactions [3–5]. Based on the idea that the changes in central metal ion, may not significantly alter covalent binding of the terminal metal ions with DNA, Huq et al. (Cheng [6]) prepared a number of tumour active PtPdPt trinuclear complexes of the form [{*trans*-PtCl(NH_3)$_2$}$_2$μ-{*trans*-Pd(NH_3)$_2$(H_2-N$(CH_2)_N NH_2$}$_2$]Cl_4 code named DH4Cl, DH5Cl, DH6Cl and DH7Cl where $N = 4, 5, 6$. It was also suggested that the presence of one or two planaramine ligands bonded to the central metal ion could introduce additional types of noncovalent interactions such as stacking interaction with nucleobases in DNA that may influence the level and spectrum of activity of the compounds. Indeed the compound [{*trans*-PtCl(NH_3)}$_2$μ-{*trans*-Pt(NH_3)(2-hydroxypyri-

Abbreviations: AAS, atomic absorption spectrophotometry; CH25, [*trans*-PtCl(NH_3)}$_2$μ{*trans*-Pd(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4; Cisplatin, *cis*-dichlorodiammineplatinum(II); DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDTA, ethylene diamine tetraacetic acid; mQ water, milli Q water; MTT, 3-(4,5-dimethyl2thiazolyl)2,5-diphenyl2Htetrazolium bromide; PBS, phosphate buffered saline; ssDNA, Salmon sperm DNA; 1 × TAE buffer, 0.05 M Tris base + 0.05 M glacial acetic acid + 1 mM EDTA pH = 8.0; Tris, Trizma base; ZD0473 (also known as JM473 and AMD473), *cis*-(2-methylpyridine)(ammine)dichloroplatinum (II).

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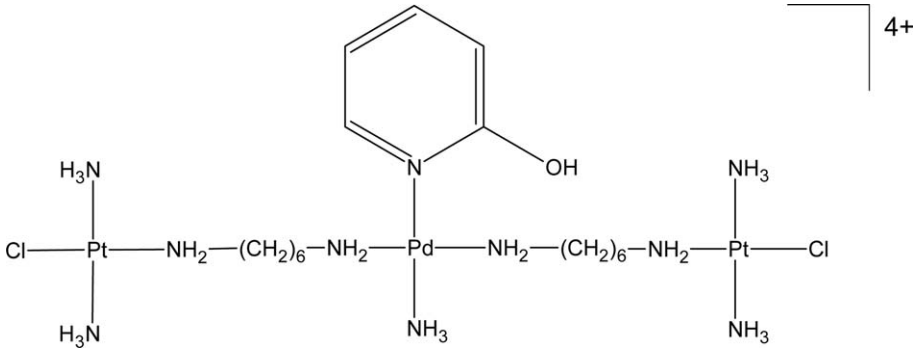


Fig. 1. Structure of CH25: [{*trans*-PtCl(NH_3)}$_2\mu$-{*trans*-Pd(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4 (code named CH25)—only the tetrapositive ion is shown, the balancing anions (4 Cl) are not included.

dine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4 (code named CH9) has been found to show significant activity in a number of human ovarian cancer cell lines [6]. In this paper, we describe the synthesis, characterization, activity against human ovarian cancer cell lines, cell uptake, nature and level of binding with DNA of the PtPdPt analogue of CH9 namely [{*trans*-PtCl(NH_3)}$_2\mu$-{*trans*-Pd(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4 (code named CH25) (Fig. 1). The compound is found to exhibit significant anticancer activity against ovarian cancer cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$—about 45 times as active as cisplatin against $A2780^{ZD0473R}$ cell line, about 76 times as active as cisplatin against $A2780^{cisR}$ cell line and about seven times as active as cisplatin against A2780 cell line. We also report on the synthesis and characterization of the compound: *trans*-(2-hydroxypyridine)(ammine)dichloropalladium(II) that corresponds to its central metal ion.

2. Materials and methods

2.1. Materials

Potassium tetrachloroplatinate $K_2[PtCl_4]$, potassium tetrachlorpalladate $K_2[PdCl_4]$, *N*,*N*-dimethylformamide [DMF] [$(CH_3)_2NCHO$], 2-hydroxypyridine and 1,6-diaminohexane were obtained from Sigma Chemical Company St. Louise USA; acetone [$(CH_3)_2CO$] and silver nitrate ($AgNO_3$) were obtained from Ajax Chemicals Auburn NSW Australia; methanol [CH_3OH], ethanol [C_2H_5OH], dichloromethane [CH_2Cl_2] were obtained from Merck Pty. Limited Kilsyth VIC Australia. pBR322 plasmid DNA was purchased from ICN Biomedicals, Ohio, USA. Foetal calf serum, 5 × RPMI 1640, 200 mM L-glutamine and 5.6% sodium bicarbonate were obtained from Trace Biosciences Pty Ltd, Australia. Other reagents were obtained from SigmaAldrich Pty Ltd, NSW, Australia. Commercially available JETQUICK Blood DNA Spin Kit/50 used to isolate high molecular weight DNA from cell pellet was obtained from Astral Scientific, Australia.

2.2. Synthesis

CH25 was synthesized using step-up method of synthesis starting with *trans*-(2-hydroxypyridine)(ammine)dichloropalladium(II) as shown in Scheme 1 according to a method similar to that used for the synthesis of CH9 [6].

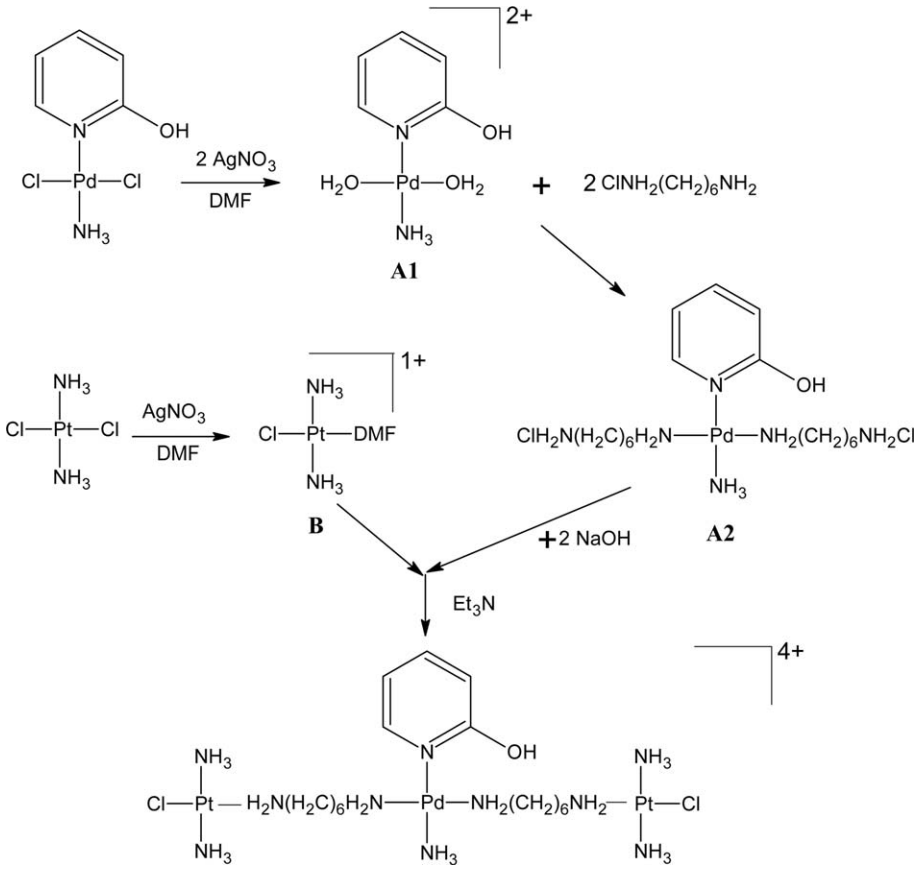
First, *trans*-(2-hydroxypyridine)(ammine)dichloropalladium (II) was prepared from the reaction between potassium tetrachloropalladate(II) and 2-hydroxypyridine, as described previously [16]. Then, CH25 was prepared from reaction between *trans*-(2-hydroxypyridine)(ammine)dichloropalladium(II), 1,6-diaminohexane and transplatin.

2.3. Characterization

C, H, N and Cl were determined using the facility at the Australian National University. Palladium and platinum were 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 4000–650 cm^{-1}. 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 3500–50 cm^{-1}. To obtain mass spectra, solution of CH25, made in 10% DMF and 90% methanol, were sprayed into a Finnigan LCQ ion trap mass spectrometer. Attempt was made to obtain ^1H NMR spectrum of CH25 dissolved in dimethyl sulfoxided6 (DMSOd6) using Bruker AVANCE DPX 400 spectrometer [with spectra referenced to internal solvent residues and recorded at 300 K (± 1 K)]. After prolonged sonication for a week only a small amount of the compound was found to dissolve forming a light orange solution. It is believed that when attempt was made to dissolve the compound in DMSOd6 with prolonged sonication, the compound to some extent broke down with DMSO possibly replacing the planar-amine ligands. The compound did not dissolve in DMF.

2.4. Interaction with pBR322 plasmid DNA

Interaction of CH25 and cisplatin with pBR322 plasmid DNA was studied by agarose gel electrophoresis [7]. Solutions of pBR322 plasmid DNA (at concentration 0.5 mg ml^{-1}) were



Scheme 1. Steps in the synthesis of CH25.

incubated with increasing concentrations of compounds ranging from 1.25 μM to 15 μM in a shaking water bath at 37 °C for 4 h. 16 μl aliquots of drug-DNA mixtures containing 0.6 μg of DNA was loaded onto the 1% gel and electrophoresis was carried under TAE buffer (0.05 M Tris base + 0.05 M glacial acetic acid + 1 mM EDTA, pH = 8.0) for 2 h at 5 V cm^{-1}. At the end of electrophoresis, the gel was stained in the same buffer containing ethidium bromide (0.5 mg ml^{-1}). The gel was visualised under UV light using the Bio-Rad Trans illuminator IEC 1010.

2.5. BamH1 digestion

pBR322 that contains a single restriction site for BamH1 [8] converts pBR322 plasmid DNA from supercoiled form I and singly nicked circular form II to linear form III DNA. 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 μl^{-1}) digestion. To each 20 μl of incubated drug-DNA mixtures were added 3 μl of 10 × digestion buffer SB followed by the addition of 0.2 μl BamH1 (2 units). The mixtures were left in a shaking water bath at 37 °C for 1 h at the end of which the reaction was terminated by rapid cooling. The gel was subsequently stained with ethidium bromide, visualised under UV light then a photograph of the gel was taken.

2.6. Cytotoxicity assays

The activity of CH25 and cisplatin again human ovarian cancer cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$ were determined based on MTT reduction assay following a procedure that was described previously [6]. Briefly, the ovarian cancer cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$ grown and maintained in logarithmic phase were treated with solutions of CH25 and cisplatin at concentrations ranging from: 0.004 to 16 μM in the case of CH25 and 0.05 to 10 μM in the case of cisplatin and then left to incubate under normal growth conditions for 72 h [9]. The inhibition of the cell growth was determined using the MTT reduction assay [10]. The IC_{50} values were obtained from the results of quadruplicate determinations of at least three independent experiments.

2.7. Drug uptake and binding with DNA

Platinum complexes (at 50 μM final concentration) were added to culture plates containing exponentially growing A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$ cells. The cells containing the drugs were incubated for 2, 4 and 24 h at the end of which cell monolayers were trypsinized and cell suspensions was transferred to centrifuge tube and spun at 3500 rpm for 2 min at 4 °C. The cells were washed twice with ice-cold phosphate-buffered saline (PBS) and the pellets were stored at 20 °C until assayed.

2.7.1. Drug accumulation in cells

Following drug incubation the cell pellets were suspended in 0.5 ml 1% triton-X, held on ice then sonicated. Total intracellular platinum contents were determined by graphite furnace AAS [11].

2.7.2. Drug–DNA binding

Following drug incubation high molecular weight DNA was isolated from cell pellet using JETQUICK Blood DNA Spin Kit/50 [12]. The cell pellets were resuspended in PBS to a final volume of 200 μl and mixed with 10 μl of RNase A incubated for 4 min at 37 °C. 25 μl Proteinase K and 200 μl Buffer K1 (containing guanidine hydrochloride and a detergent) were then added to the mixture followed by incubation for 10 min at 70 °C. 200 μl of absolute ethanol 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 microspin column. The columns containing the samples were then washed with 500 μl buffer KX (containing high-salt buffer to remove residual contaminations) and centrifuged for 1 min at 10,600 rpm, again washed with 500 μl buffer K2 (containing 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. To further clear the silica membrane from residual liquid, the sample columns were centrifuged again for 2 min at full speed (13,000 rpm). The column 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). Platinum contents were determined by graphite furnace AAS. A260/A280 ratios were found to be between 1.75 and 1.8 for all samples indicating high purity of the DNA [13].

3. Results and discussion

3.1. Characterisation

3.1.1. Elemental composition

CH25 formula: $C_{17}H_{52}N_{10}Cl_6OPdPt_2$

Molar mass: 1070.18 g mol^{-1}.

The composition of CH25 was: Calcd. C: 21.3, H: 5.0, Cl: 6.6; N: 13.1, Pt: 36.5, Pd: 9.9.

Obs. C: 21.6 ± 0.4; H: 4.9 ± 0.4; Cl: 6.4 ± 0.3; N: 13.0 ± 0.4; Pt: Obs. 36.0 ± 1.2; Pd: 10.2 ± 1.2.

The yield was 30.0%.

It can be seen that the observed values for the elements C, H, N, Cl, Pt, and Pd agree with the calculated values within the limits of the measurement.

3.1.2. IR, Raman and mass and spectral analyses

3.1.2.1. IR. CH25

Although the IR spectrum alone of a complex molecule like CH25 cannot provide conclusive evidence for its structure, it can be seen that the peaks observed are in agreement with the suggested structure. In particular, it indicates the presence of groups such as CH, NH and 'aromatic ring', and Pt-N and Pd-N bonds. The band at 2858 cm^{-1} is due to aliphatic C-H stretching vibration. The band at 1303 cm is believed to be due to N-H bending vibrations whereas that at 1288 cm^{-1} is due to C-H bending vibration. The band at 1197 cm^{-1} is due to ring C-O stretching vibration. The band at 1126 cm^{-1} is believed to be due to C-H bending vibration found in o-di-substituted ring. The band at 863 cm^{-1} is believed to be due to CN stretching vibration in $–CH_2-NH_2$ chain. The band at 729 cm^{-1} is due to C-H bending vibration associated with o-di-substituted ring. The band at 518 cm^{-1} is due to Pt-N and Pd-N stretching vibrations. The band at 412 cm^{-1} is associated with the vibration of the pyridine ring.

3.1.2.2. Raman. The band at 2989 cm^{-1} is due O-H stretching vibration whereas that at 2900 cm^{-1} is believed to be due to N-H stretching vibration. The band at 2850 cm^{-1} is due to C-H stretching vibration of an $A-CH_2-NH_2-A$ chain. The band at 1435 cm^{-1} is believed to be due to C-H bending vibration in odi-substituted ring. The band at 1037 cm^{-1} is due to CH_2 wagging. The band at 534 cm^{-1} is due to Pt-N and Pd-N stretching vibrations. The band at 102 cm^{-1} is due to Pt-N and Pd-N bending vibrations.

3.1.2.3. Mass. The mass spectrum of CH25 like that of CH9 appears to be complex perhaps because of the nature of the compound. It is an ionic compound composed of a long polyvalent cation and four balancing anions. A part of the polyvalent cation is flexible and can be broken easily whereas another part is rigid. More importantly, the compound was found to be practically insoluble in DMF and DMSO. What was sprayed onto the mass spectrometer was not the molecular species. Thus it is not surprising that the mass spectrum of the CH25 does not have the molecular peak. The peaks observed are described as follows. The peak observed in the mass spectrum of CH25 with $m/z = 380$ may be due to $CH_3CH_2NH2Pd(2\text{-hydroxypyridine})(NH_3)(NH_2(CH_2)_6NH_3)$ produced from the fragmentation of CH25. The peak with $m/z = 362$ may be due to $CH_2NH_2Pd(2\text{-hydroxypyridine})(NH_3)(NH_2CH_2)$ that is believed to be produced again from the fragmentation of CH25. The peak with $m/z = 323$ may be due to $Cl(NH_3)_2Pt(NH_2(CH_2)_2CH_3)$ and that at 288 may be due to $(NH_3)_2Pt(NH_2(CH_2)_3)$. The peak with $m/z = 221$ may be due to $Pd\{NH_2(CH_2)_6\}NH_3$ or Pd (2-hydroxypyridine)(NH_3) + 3H. The peak with $m/z = 117$ may be due to $NH_2(CH_2)_6NH_2$ or (2-hydroxypyridine + H). The peak with $m/z = 100$ may be due to $(CH_2)_6NH_2$.

3.1.2.4. ^1H NMR. As stated earlier, only a small amount of the compound dissolved in DMSO after prolonged sonication. It is quite likely that the compound could have partially decomposed e.g. DMSO might have replaced 2-hydroxypyridine ligand. The resonance at 7.960 ppm is believed to ring CH at the ortho position that at 7.356 ppm is due to ring CH at the para position, those at 4.375 and 3.950 ppm are believed to be due to NH_2 protons, and those at 1.354 ppm and 1.316 ppm are believed to be due to CH2 protons of the diaminoalkyl chains.

3.2. Gel electrophoresis

3.2.1. Interaction between pBR322 plasmid DNA and compounds (CH25 and cisplatin)

Fig. 2 gives the electrophoretograms applying to the interaction of pBR322 plasmid DNA with increasing concentrations of CH25 and cisplatin ranging from 0 to 10 μM, for 4 h at 37 °C—(a) and (b) apply to CH25 without and with BamH1 digestion, respectively, (c) and (d) apply to cisplatin without and with BamH1 digestion, respectively. In the case of BamH1 digestion, the highest concentration of CH25 used was 7.5 μM as no DNA band was observed at this and higher concentrations.

As pBR322 plasmid DNA which was initially found to be mixture of super-coiled form I and singly-nicked form II, was allowed to interact with CH25 (Fig. 2a), mobility of form I band decreased whereas that of form II band remained essentially unchanged so that the two bands came closer together. At high concentrations of CH25, the intensity of both bands decreased such that no DNA band was observed at 7.5 μM and higher concentrations. When pBR322 plasmid was allowed to interact with increasing concentrations of cisplatin (Fig. 2c), mobility of both form I and form II bands increased essentially at the same rates so that bands remained parallel at all concentrations of the compound. There was a slight decrease in the intensity of the form I band with the increase in concentration of cisplatin. The change in mobility of plasmid DNA bands as a result of interaction with CH25 is believed to be due to the formation of a range of interstrand GG adducts (causing a global change in DNA conformation) [14] whereas that in the case of cisplatin is believed to be due to the formation of mainly monofunctional Pt(G) and intrastrand bifunctional Pt(GG) adducts (the latter causing a local distortion of DNA). The decrease in intensity at higher concentrations indicates the occurrence of DNA damage caused by the binding of the compounds. When CH25 binds covalently with DNA, 2-hydroxypyridine ligand bonded to the central metal ion (Pd^{2+}) may undergo stacking interaction with nucleobases in DNA. It is believed that this may play a significant role in DNA damage. The Pt-Pt-Pt analogue of CH25 namely CH9 was also found to be more damaging to pBR322 plasmid DNA than cisplatin [6].

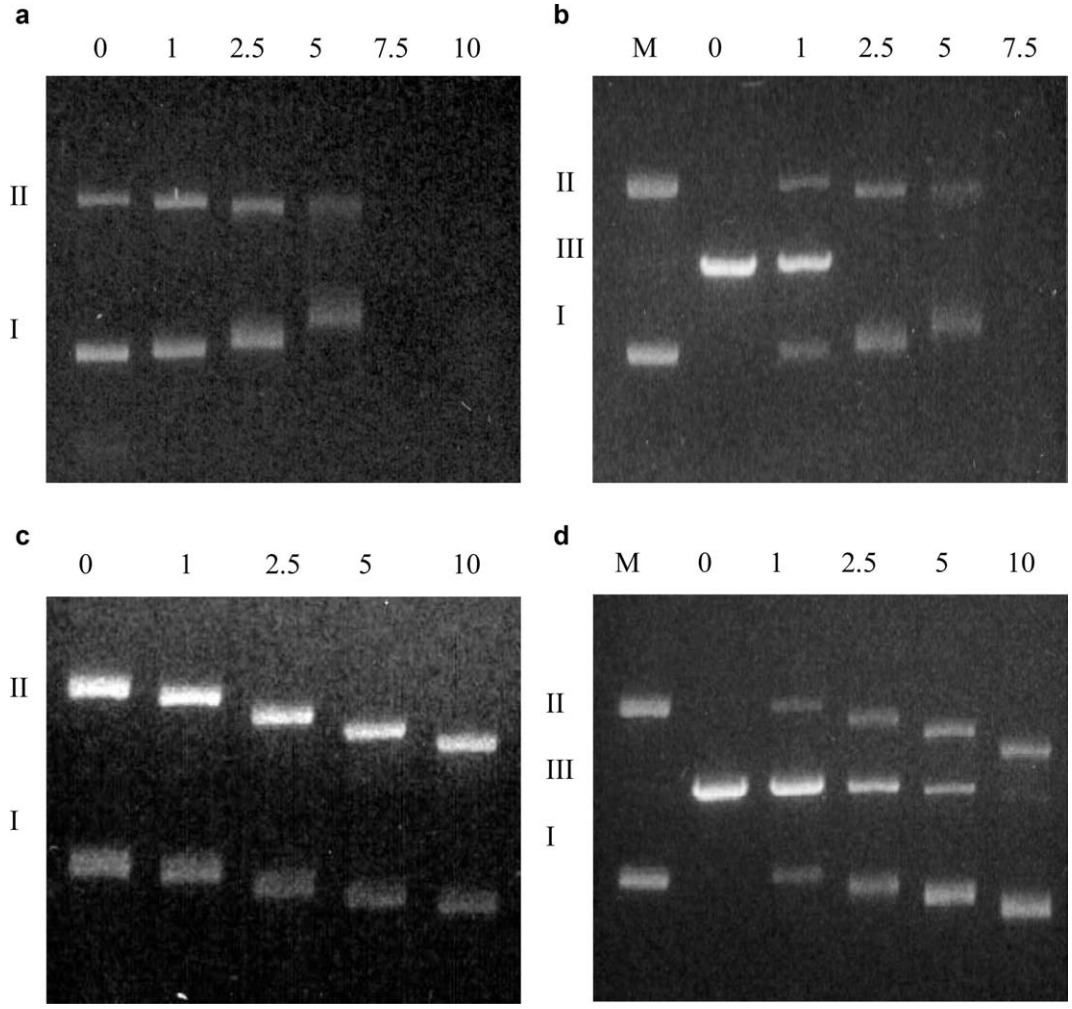


Fig. 2. (a) Interaction between pBR322 plasmid DNA and increasing concentrations of CH25, (b) interaction between pBR322 plasmid DNA and increasing concentrations of CH25 followed by BamH1 digestion, (c) interaction between pBR322 plasma DNA and increasing concentrations of cisplatin, (d) interaction between pBR322 plasmid DNA and increasing concentrations of cisplatin followed by BamH1 digestion.

3.2.2. BamH1 digestion

Fig. 2b and d give the electrophoretograms applying to the BamH1 digested incubated mixtures of pBR322 plasmid DNA and varying concentrations of CH25 and cisplatin (ranging from 0 μM to 7.5 μM in the case of CH25 and 0 to 10 μM in the case of cisplatin). Lane 1 applies to untreated and undigested pBR322 plasmid DNA, lane 2 applies to untreated but BamH1 digested pBR322 plasmid DNA, lanes 3 to 6 apply to pBR322 plasmid DNA interacted with increasing concentrations of compounds (3: 1.0 μM, 4: 2.5 μM, 5: 5 μM, 6: 7.5 μM for CH25 μM as against 10 μM for cisplatin).

When untreated pBR322 plasmid DNA was digested with BamH1, only form III band was observed whereas the unreacted and undigested pBR322 plasmid DNA gave forms I and II bands.

In the case of BamH1 digested incubated mixtures of pBR322 plasmid DNA and CH25 (Fig. 2b), forms I, II, III bands were observed at concentration of CH25 equal to 1.0 μM, and forms I and II bands were observed for concentrations ranging from 2.5 to 5 μM. In the case of cisplatin (Fig. 2d), forms I, II, III bands were observed for concentrations of cisplatin ranging from 1.0 to 5 μM, forms I and II bands were observed for concentration equal to 10 μM. Table 1 summarizes the above results. Whereas for the untreated pBR322 plasmid DNA BamH1 digestion at the specific GG site was not prevented, increasing concentrations of compounds increasingly prevented the digestion. Prevention of BamH1 digestion indicates change in DNA conformation. As stated earlier, CH25 is expected to form a range of interstrand GG adducts dictated by the sequence of nucleobases in the DNA whereas cisplatin is expected to form mainly intrastrand bifunctional GG adducts with DNA that cause a local pronounced bending of the DNA strand [14,15].

3.3. Anticancer activity

Table 2 gives the IC_{50} values and resistance factor of CH25 and cisplatin for the cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$. CH25 was found to be more active than cisplatin against all the three cell lines (about 45 in A2780, 78 times in $A2780^{cisR}$ and 7 times in $A2780^{ZD0473R}$). The high activity of CH25 as compared to BBR3464 and DH6Cl suggests that noncovalent interactions involving 2-hydroxypyridine ligand may be playing a significant role in enhancing activity of CH25. CH25 was also found to significantly more active than CH9 against the cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$. This is could be due to a greater cell uptake and greater drug–DNA binding.

3.3.1. Cell uptake and binding with DNA

3.3.1.1. Cell uptake. Since CH25 contains two platinum and one palladium ions, determination of both platinum and palladium levels should provide an equivalent measure of the drug uptake provided the trinuclear positive cation remained intact before entry into the cell. Based on Pt:Pd molar ratio, it was concluded that most of the trinuclear cation remained intact in Pt-Pd-Pt analogues of BBR3464 [6]. It is believed that the presence of bulky 2-hydroxypyridine ligand in CH25 (bonded to central Pd) would make the trinuclear cation more resistant to dissociation. Thus, in the present study only platinum was determined. Cellular uptake of platinum was calculated as nmol Pt per 2×10^6 cells. Fig. 3 gives the uptakes in 2, 4 and 24 h of CH25 and cisplatin in the human ovarian cell lines: A2780 and $A2780^{cisR}$ and $A2780^{ZD0473R}$.

The higher cellular uptake of CH25 than cisplatin is in line with its higher activity in A2780 and $A2780^{cisR}$ and A2780 ZD0473R cell lines although cell uptake per se may not necessarily give an indication of the level of the anti-tumour activity. For CH25, platinum cell uptake in the resistant cell lines: $A2780^{cisR}$ and $A2780^{ZD0473R}$ were greater than that in the cisplatin-responsive cell line A2780. It was found that for both CH25 and cisplatin, the level of platinum–DNA binding was less in the cisplatin-resistant cell lines $A2780^{cisR}$ and $A2780^{ZD0473R}$ than in the cisplatin-responsive cell line A2780. It was also found that for CH25, the platinum uptake in A2780, A2780 and $A2780^{ZD0473R}$ cell lines was much greater than that for cisplatin. When the cell uptake of CH25 is compared with that of DH6Cl as applied to the cell lines A2780 and $A2780^{cisR}$, it is found that the uptake is greater for CH25 than DH6Cl, in line with the higher activity of CH25 [6].

3.3.1.2. Platinum binding with DNA. Fig. 4 gives the level of platinum binding (in nanomoles per milligram of DNA) in 2, 4 and 24 h in A2780 and $A2780^{cisR}$ and $A2780^{ZD0473R}$ cells as applied to CH25 and cisplatin. The level of platinum-DNA binding was greater for CH25 than cisplatin in all the cell lines, in line with its higher activity. The level of binding of CH25 with DNA was found to be maximum after 24 h as applied to cell lines: $A2780^{ZD0473R}$ and $A2780^{cisR}$ and after 4 h as applied

Table 1
Bands observed in the incubated mixtures of pBR322 plasmid DNA and varying concentrations of CH25 and cisplatin followed by BamH1 digestion

Drug	[Drug] in μM				
	0	1.0	2.5	5	7.5(10[a])
CH25	III	I, II, III	I, II	I, II	No band[b]
Cisplatin	III	I, II, III	I, II, III	I, II, III	I, II

[a] The number is the highest concentration of cisplatin;
[b] Total DNA damage.

Table 2
IC_{50} values and resistance factors (RF) for cisplatin and CH25 as applied cell linesA2780cisR $A2780^{cisR}$ and $A2780^{ZD0473R}$

	A2780 IC_{50} (μM)	$A2780^{cisR}$ IC_{50} (μM)	$\frac{IC_{50}A2780^{cisR}}{(RF)IC_{50}A2780}$	$A2780^{ZD0473R}$ IC_{50} (μM)	$\frac{IC_{50}A2780^{ZD0473R}}{(RF)IC_{50}A2780}$
Cisplatin	0.46 ± 0.10	4.96 ± 0.63	10.78	4.97 ± 0.94	10.80
CH25	0.0103 ± 0.0004	0.064 ± 0.001	6.21	0.76 ± 0.02	73.79

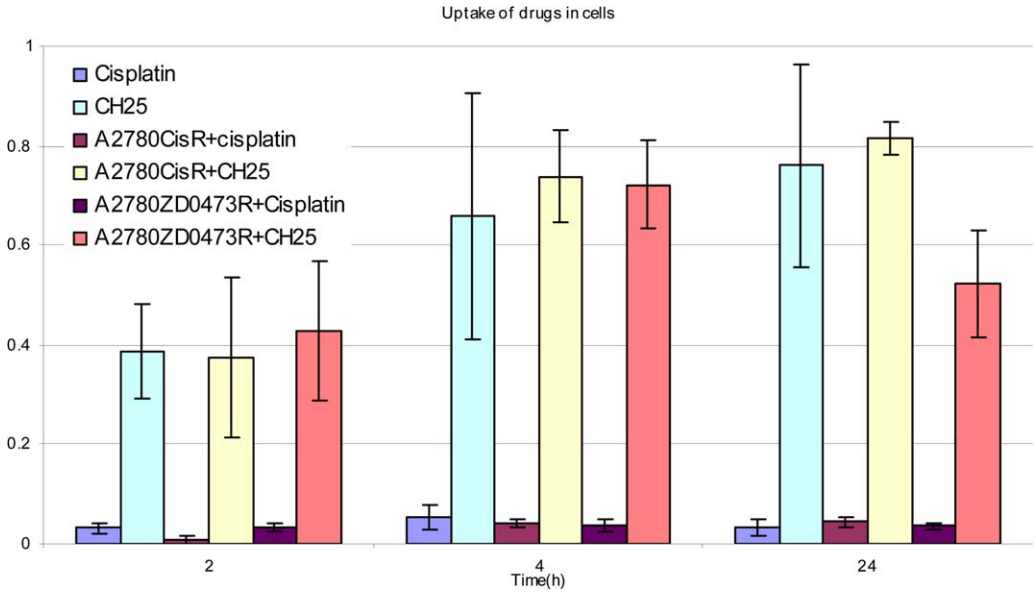


Fig. 3. Pt accumulation in A2780, A2780^{cisR} and A2780^{ZD0473R} cells as applied to cisplatin, transplatin and CH25 in 2, 4 and 24 h.

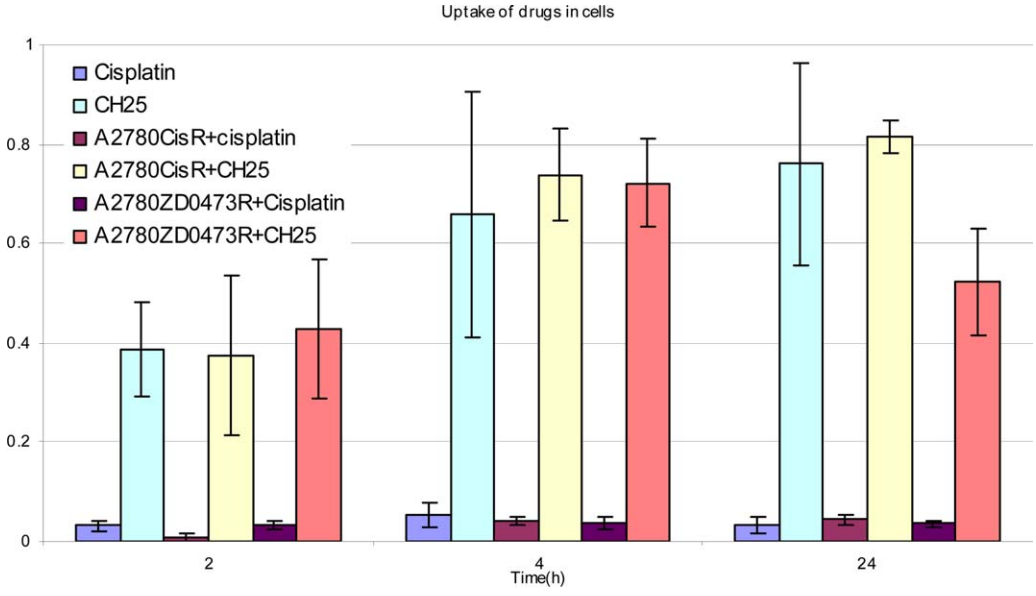


Fig. 4. Level of Pt–DNA binding in A2780, A2780^{cisR} and A2780^{ZD0473R} cells.

to cell line: A2780. The decreased level of binding of CH25 in A2780^{ZD0473R} cell line after 24 h than 4 h indicates continued DNA repair as a function of time. The lower level of the binding of the compound is in line with its lower activity against the cell line.

Much lower level of binding of cisplatin with DNA in A2780^{cisR} and A2780^{ZD0473R} cell lines as compared to that in A2780 cell line indicates decreased cell uptake and increased repair of cisplatin–DNA adducts. The bending induced in DNA by binding of cisplatin is recognized by high-mobility group domain (HMG) proteins, which is believed to be the pathway for processing and differential repair of cellular cisplatin–DNA adducts [16]. When long-range interstrand adducts are formed, DNA is not sufficiently bent to be recognized by such proteins [17–19]. The high charge on the multi-centred cation produced from CH25 would facilitate rapid binding with DNA. It has been suggested that the preassociation of multinuclear platinum complexes with polyanionic DNA will significantly affect the rate and site of platination because an increased local concentration will increase the probability of a covalent interaction at these sites. Also, pre-association may induce a local conformational change in the DNA that may influence binding at a specific site.

The levels of platinum-DNA binding of CH25 and DH6Cl as applied to the cell lines A2780 and A2780^{cisR} are similar although activity of CH25 is greater. This gives support to the idea that non-covalent interactions involving the planaramine ligand may be playing a key role in determining the activity of CH25. The higher activity of CH25 as compared to that for CH9 is believed to be due to a greater level of drug–DNA binding.

4. Conclusion

One new tumour active multi-centred metal complex has been synthesized and studied. The anticancer activity of the compound against ovarian cancer cell lines: A2780, $A2780^{cisR}$, $A2780^{ZD0473R}$ has been determined. The cell uptake and the extent of binding with DNA have also been determined. The nature of interaction with pBR322 plasmid DNA has also been determined. The gel electrophoresis results show that CH25 has been able to cause conformational changes in DNA and DNA damage at higher concentrations. This is believed to be due to covalent interstrand binding of the compound with DNA through the terminal metal centres. Like other multi-centred compounds [9], because of flexible nature, the compound would form a range of interstrand GG adducts dictated by the sequence of DNA bases. Noncovalent interactions are believed to play a significant role in DNA damage. For example, the planaramine ligand may be involved in stacking interaction with DNA bases. The compound displays significant anticancer activity against ovarian cancer cell lines much higher than that of cisplatin. CH25 is believed to form long-range interstrand adducts with DNA, causing long-range deformation of the DNA.

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