FULL PAPER

Dalton
www.rsc.org/dalton

Reaction of (*C*-(6-aminomethyl-pyridin-2-yl)methylamine)-chloroplatinum(II) with nucleosides and its biological activity

S. W. Annie Bligh,*[a] Alan Bashall,[a] Christelle Garrud,[a] Mary McPartlin,[a] Nick Wardle,[a] Ken White,[a] Subhash Padhye,[b] Vivek Barve[b] and Gopal Kundu[c]

[a] *Department of Health and Human Sciences, London Metropolitan University, 166-220 Holloway Road, London, UK N7 8DB. E-mail: a.bligh@londonmet.ac.uk*

[b] *Department of Chemistry, University of Pune, Pune, 411 007, India. E-mail: sbpadhye@chem.unipune.ernet.in*

[c] *National Centre for Cell Science, NCCS Complex, Pune, 411007, India*

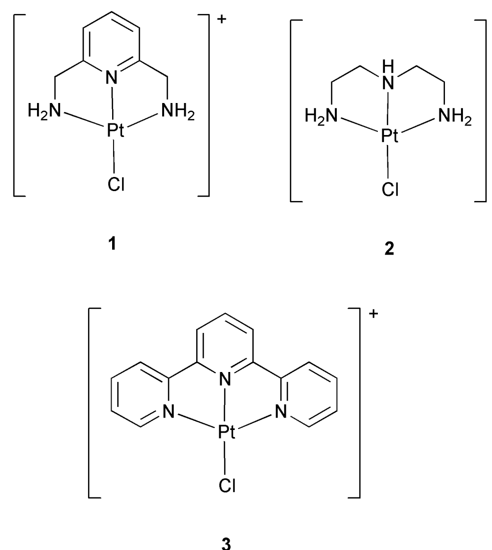
Received 10th September 2002, Accepted 19th November 2002
First published as an Advance Article on the web 16th December 2002

^{1}H NMR and LC-ESI-MS studies of the interaction between nucleosides (adenosine, cytidine, guanosine, uridine and thymidine) and a monocation platinum(II) complex [Pt(*C*-(6-aminomethylpyridin-2-yl)methylamine)Cl]$^+$ (**1**) shows it forms platinum(II) complexes with A, G and C at pH 6 and with T and U at pH 9. The X-ray structure of **1** as the hydrated chloride salt shows extensive hydrogen bonding. Amongst a series of related complexes **1** shows a strong interaction with DNA, demonstrated by inhibition of binding by the intercalator ethidium bromide, and moderate cytotoxicity. The interaction with nucleosides and the structural data suggest that **1** disrupts the DNA structure by coordinate covalent interaction and hydrogen bonding rather than simple intercalation.

Introduction

It has been widely accepted that DNA is the molecular target of cisplatin, *cis*-[Pt(NH_3)$_2$$Cl_2$]. Cisplatin reacts with nucleobases and forms d(ApG), d(GpG) and d(GpNpG) intrastrand crosslinks, and monofunctional adducts.[1] Formation of these species unwinds the DNA and bends it toward the major groove, blocking transcription and replication. The design of platinum anti-cancer drugs hence focusses mainly on the binding of the metal to DNA, and aims especially at the interaction with the N^7 of guanine.[2] However the interaction of platinum complexes with double helix DNA can also be non-covalent *via* intercalation and hydrogen bonding.[3] Intercalation of these complexes largely depends on their aromaticity and planarity.

Platinum–triamine mono-cationic complexes such as [Pt(dien)Cl]$^+$ **2**, [Pt(terpy)Cl]$^+$ **3**, *cis*-[Pt(NH_3)$_2$(4-Br-pyridine)Cl]$^+$ and [Pt(NH_3)$_3$Cl]$^+$ all interact with DNA.[4] The complexes without an aromatic ligand, such as the dien complex **2** and *cis*-[Pt(NH_3)$_2$$Cl_2$], bind coordinate covalently to the bases with disruption of the DNA duplex. However only those with aromatic ligands such as the terpy complex **3** and *cis*-[Pt(NH_3)$_2$(4-Br-pyridine)Cl]$^+$ also show binding for intercalation sites on DNA as well as forming coordinate covalent Pt–DNA species.[4] The binding sites between **2** and nucleosides (A, C or U) have been studied by proton NMR spectroscopy.[5] The results indicate the binding sites at the nucleobases are at N^1 and N^7 for A forming a diplatinated complex, at N^3 for C, and that there is no reaction for U. Similar studies for reactions of the terpy complex **3** with ribo- and deoxyribo-adenosine (A and dA) show the binding sites are both at N^1 and N^6.[6] In the present work, a mono-cationic and mono-functional Pt(II) complex [Pt(bampy)Cl]$^+$ **1** has been synthesized and structurally characterised as its chloride monohydrate. The interaction of complex **1** with nucleosides A, C, G, U and T has been studied by proton NMR to identify potential binding sites at the nucleobases. The hydrolysis and substitution reaction of complex **1** over a period of time at different pH has been monitored by LC-MS. The DNA binding of the platinum complex **1**, together with those of **2** and **3** for comparison, has been examined and the toxicities of these complexes against MCF-7 breast cancer epithelial cells are compared in this study.



Experimental

Materials

Nucleosides, adenosine (A), guanosine (G), cytidine (C), uridine (U) and thymidine (T), were obtained from Sigma and used as received. [Pt(terpy)Cl]Cl was purchased from Fluka. *C*-(6-aminomethylpyridin-2-yl)-methylamine[7] (bampy), *cis*-[Pt(DMSO)$_2$$Cl_2$],[8] [Pt(COD)$Cl_2$][9] and [Pt(dien)Cl]Cl[10] were prepared as described in the literature. [Pt(bampy)Cl]Cl was prepared *via* the reaction of the ligand bampy with *cis*-[Pt(DMSO)$_2$$Cl_2$] in methanol or [Pt(COD)Cl_2] in water.

[Pt(bampy)Cl]Cl from *cis*-[Pt(DMSO)$_2$Cl$_2$]

A solution of bampy (0.112 g, 0.82 mmol) in methanol (10 cm^3) was added to a stirring suspension of *cis*-[Pt(DMSO)$_2$$Cl_2$] (0.34 g, 0.82 mmol) in MeOH (20 cm^3) and the mixture was refluxed with stirring for 2 h. A yellow residue was filtered, that corresponds to [Pt(bampy)Cl][Pt(DMSO)Cl_3]. IR ν_{max}/cm^{-1}: 3250, 3200 (NH), 3110, 3010, 2920 (C–H), 1600, 1580, 1480, 1420 (C=

DOI: 10.1039/b208790a

This journal is © The Royal Society of Chemistry 2003

N, C=C), 1132 (S–O), 1020. Found: C, 14.7; H, 2.3; N, 5.6. Calc. for $C_9H_{17}N_3Cl_4OSPt_2$: C, 14.4; H, 2.3; N, 5.6%. The filtrate was concentrated to 5 cm^3 and a yellow solid was filtered off. It was washed with CH_2Cl_2 and diethyl ether then dried in a desiccator. Yield: 0.15 g, 33%. [Pt(bampy)Cl]Cl, IR ν_{max}/cm^{-1} : 3220, 3129 (N–H); 3050, 2920 (C–H); 1598 (N–H); 1580, 1480, 1420 (C=N), 1260, 1230 (C–N). ^{1}H NMR (250 MHz, d_6-DMSO): δ 8.13 (t, 1H, py, $^3J_{1,2}$ = 8.0 Hz), 7.48 (d, 2H, py), 6.30 (s, 4H, NH_2), 4.42 (d, 4H, CH_2). ^{13}C NMR (62.9 MHz, D_2O): δ 163.6 (py, J_{Pt-C} = 41.5 Hz), 142.0 (py), 122.4 (py, J_{Pt-C} = 17.6 Hz), 58.1 (CH_2). Found: C, 20.9; H, 2.6; N, 10.5. Calc. for $C_7H_{11}N_3Cl_2Pt$: C, 20.9; H, 2.7; N, 10.4%.

[Pt(bampy)Cl]Cl from [Pt(COD)Cl_2]

[Pt(COD)Cl_2] (0.10 g, 0.77 mmol) was added to a stirring solution of bampy (0.105 g, 0.77 mmol) in water (20 cm^3). The mixture was warmed at 40–50 °C. After 15 min all the solid was dissolved and a red–orange clear solution was obtained which was cooled at room temperature and then filtered to remove unreacted [Pt(COD)Cl_2]. Water was then removed under reduced pressure leaving a yellow solid that was collected and washed thoroughly with diethyl ether and air-dried. Yield: 0.22 g, 71%. ^{1}H NMR (250 MHz, D_2O): δ 8.1 (t, 1H, py), 7.45 (t, 2H, py), 6.4 (s, 4H, NH_2), 4.43 (t, 4H, CH_2). Found: C, 20.8; H, 2.6; N, 10.4. Calc. for $C_7H_{11}N_3Cl_2Pt$: C, 20.9; H, 2.7; N, 10.4%.

X-Ray crystallography

Suitable pale yellow crystals for structural determination studies were obtained from recrystallisation of the chloride of **1** in methanol–water for structural determination studies. X-Ray intensity data were collected on a Siemens *P*4 diffractometer, and absorption corrections using psi-scans were applied to the data.[11]

Crystal data and refinement. Crystal data for [Pt(bampy)-Cl]Cl.H_2O: $C_7H_{13}C_{12}N_3OPt$, M = 421.19, triclinic, a = 7.0737(19), b = 8.7288(6), c = 9.8096(12) Å, α = 73.420(9), β = 81.566(6), γ = 78.215(11)°, U = 565.75(17) Å^3, T = 223(2) K, space group $P\bar{1}$ (no. 2), Z = 2, μ(Mo-Kα) = 12.845 mm^{-1}, 2334 reflections measured, 1949 unique (R_{int} = 0.0171) which were used in all calculations. The final $wR(F^2)$ was 0.0748 (all data).

The positions of the non-hydrogen atoms were located by direct methods and refinement was based on F^2.[11] The two water hydrogen atoms were located directly and were included in structure factor calculations without refinement, the displacement parameters being set equal to $1.2U_{eq}$ of the oxygen atom. The remaining hydrogen atoms were placed in idealised positions with displacement parameters set equal $1.2U_{eq}$ (or $1.5U_{eq}$ for methyl groups) of the parent carbon atoms. In the final cycles of full-matrix least-squares refinement the non-hydrogen atoms were assigned anisotropic displacement parameters.

CCDC reference number 177857.

See http://www.rsc.org/suppdata/dt/b2/b208790a/ for crystallographic data in CIF or other electronic format.

^{1}H NMR studies of reactions of 1 and nucleosides

Solutions of **1** (0.004 g, 10 µmol) and the nucleoside (A, G, C, T or U; 10 µmol) in D_2O (600 µl) containing sodium phosphate buffer pH 6 (20 mM, 10 µl) were incubated for 3 d. The experiments were carried out in NMR tubes at 310 K and the ^{1}H NMR spectra were recorded at 250 MHz on a Bruker AM250 spectrometer using TSP-d_4 as internal reference.

LC-ESI-MS studies of reactions of 1 and nucleosides

Stock solution of **1** (20 mM) in deionised water was added to an equal volume of nucleoside (A, G, C, T or U; 20 mM in water). A series of the above solutions were prepared at various pH values (2–10) using 0.1 M NaOH and 0.1 M HCl. The solutions were monitored for complex formation by LC-MS performed on a Waters Alliance HT System fitted with the 2790 Separations Module and the Micromass LCT mass spectrometry detector. Hydrolysis of **1** in water at pH 6 was also monitored regularly for 7 d.

DNA binding studies

Binding to DNA was assessed by reaction of complex with linearised plasmid DNA of about 3000 base pairs length, prepared by digesting pSP72 (Promega Ltd), carrying a 500 bp fragment of the β-globin gene, with EcoRI. The preparation was treated with phenol–$CHCl_3$, precipitated and washed with 70% EtOH and dissolved in 10 mM Hepes buffer pH 7.4 to a concentration of 0.5 µg μl^{-1}, equivalent to 1.5 mM in nucleotides. Platinum complexes were dissolved in DMSO and used in a range of r_b 0.1–1.2 in 5 mM Hepes pH 7.0. Reactions were carried out at 37 °C for 24 h and analysed by agarose electrophoresis followed by staining with ethidium bromide, EtdBr, (10 µg ml^{-1}). Images of gels were taken under UV light and collected and analysed with UVItech imaging equipment and software (UVItech Ltd., Cambridge, UK).

Cell proliferation studies on MCF-7 breast cancer epithelial cells

Exponentially growing cells were trypsinized, counted and plated in a 6 well-plate at the density 1×10^5 cells $well^{-1}$ in 2 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, and 2 mM glutamine in a humified atmosphere of 5% CO_2 and 95% air at 37 °C. After two days of incubation when the cells were in the exponentially growth phase, a test compound dissolved in DMSO (final concentration not exceeding 1%) was added.[12] The control wells received similar additions with solvent vehicle alone. After 72 h exposure period the exponentially growing cells were counted by using a haemocytometer using the Trypan blue exclusion method[13] to quantify cell viability.

Results and discussion

Synthesis and characterisation of 1

The reaction of *cis*-[Pt(DMSO)$_2$$Cl_2$] with bampy in methanol gave a yellow mixture after the solution was heated for 1 h. A yellow solid initially isolated was characterised as [**1**][Pt-(DMSO)Cl_3] by elemental analysis and infrared spectroscopy. The presence of a strong peak at 1105 cm^{-1} provides evidence of ν(S=O) with bonding of DMSO to platinum through the sulfur atom. The same anion [Pt(DMSO)Cl_3]$^-$ was previously observed during the reaction of terpy with *cis*-[Pt(DMSO)$_2$-Cl_2].[10] The subsequent work up produced the chloride salt of **1** with a yield of 33%. In order to improve the yield of this complex, another method was employed using [Pt(COD)Cl_2] (COD = 1,5-cyclooctadiene) as starting material. A pure product was easily obtained in 30 min by reacting equimolar amounts of [Pt(COD)Cl_2] and bampy in water at 40–50 °C. The ease of the reaction is probably due to the high *trans*-labilizing effect of the coordinated diolefin and its stability when monocoordinated to the metal ion. The yield obtained with this procedure, 71%, is superior to that obtained when *cis*-[Pt-(DMSO)$_2$$Cl_2$] was used as starting material.

^{1}H NMR spectrum of **1** exhibits shifts of aromatic resonances as well as –CH_2– of the ligand, strongly indicating that bampy is coordinated to the platinum metal ion. All the peaks obtained from the ^{1}H NMR in d_6-DMSO shifted downfield upon coordination to 8.13 and 7.48 ppm from 7.69 and 7.26 ppm for H^{para} and H^{meta} of the pyridine ring, respectively. The methylene protons also shifted downfield giving a $\Delta\delta$ = 0.7 ppm. Mass spectrometry results are consistent with 1 : 1 metal to

Table 1 Selected bond lengths (Å) and angles (°) for [Pt(bampy)Cl]Cl·H_2O

Pt(1)–Cl(1)	2.308(2)	Pt(1)–N(1)	1.936(5)
Pt(1)–N(2)	2.033(6)	Pt(1)–N(3)	2.040(6)
N(1)–C(2)	1.381(8)	N(1)–C(6)	1.313(9)
N(2)–C(7)	1.475(8)	N(3)–C(8)	1.497(8)
C(2)–C(7)	1.508(9)	C(6)–C(8)	1.524(9)
C(2)–C(3)	1.373(10)	C(5)–C(6)	1.373(9)
C(3)–C(4)	1.370(11)	C(4)–C(5)	1.405(10)
O(1w)–H(1W)	1.12	O(2W)–H(2W)	0.96
Cl(1)–Pt(1)–N(1)	179.4(2)	N(2)–Pt(1)–N(3)	164.5(2)
Cl(1)–Pt(1)–N(2)	97.4(2)	Cl(1)–Pt(1)–N(3)	98.0(2)
N(1)–Pt(1)–N(2)	83.2(2)	N(1)–Pt(1)–N(3)	81.4(2)
Pt(1)–N(1)–C(2)	116.6(5)	Pt(1)–N(1)–C(6)	120.7(4)
Pt(1)–N(2)–C(7)	110.4(4)	Pt(1)–N(3)–C(8)	109.5(4)
N(1)–C(2)–C(7)	114.5(5)	N(1)–C(6)–C(8)	112.9(6)
N(2)–C(7)–C(2)	110.6(5)	N(3)–C(8)–C(6)	110.4(5)

ligand complexation and the base peak corresponds to the complex ion $[1]^+$, which confirmed the presence of the chloride atom coordinated to the metal.

Crystal structure of 1

The Pt(II) complex cation $[Pt(bampy)Cl]^+$ (**1**) has the expected distorted square-planar structure which can be seen in Fig. 1

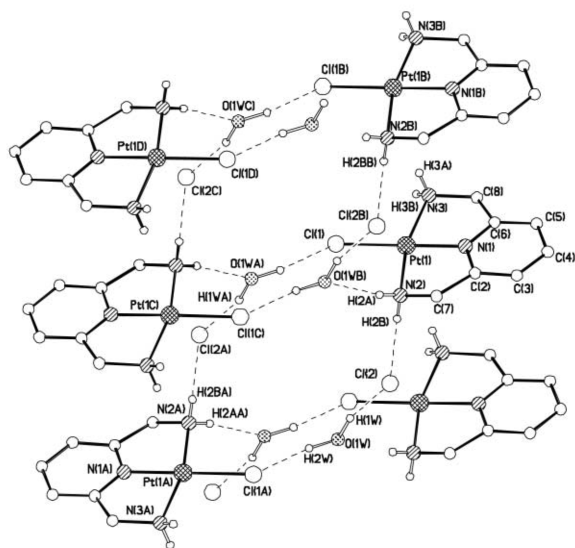


Fig. 1 The H-bonded double chain in the solid state structure of [Pt(bampy)Cl]Cl·H_2O, linked by the chloride counter-ion and the solvating water molecule.

where the overall hydrogen-bonded structure of the crystalline material [Pt(bampy)Cl]Cl·H_2O is illustrated. Selected bond lengths and angles for the cation are listed in Table 1. The main deviations from ideal square-planar coordination at Pt(II) may be attributed to the small bites of the bampy ligand resulting in a mean *cis*-N–Pt–N angle of 83.2(2)° and a *trans*-N–Pt–N angle of 164.5(2), considerably less than the respective idealised values of 90 and 180°.

The atoms of the coordination sphere are co-planar to within 0.008 Å [deviations Pt(1) 0.006, Cl(1) 0.003, N(1) 0.006, N(2) −0.008, N(3) 0.008 Å], but there are significant deviations from the overall best plane through the non-hydrogen atoms of the Pt(bampy) unit [maximum deviations N(2) 0.239, N(3) −0.231, C(7) −0.162, C(8) 0.153 Å]. However in considering the potential of the cation **1** for intercalation there is a marked difference from the structures of Pt(II) complexes of fully planar aromatic ligands such as terpy. In **1** the planar region of the complex is limited to the pyridyl aromatic ring with the hydrogen atoms of the CH_2 and NH_2 units lying well out of this plane. It may be envisaged that the pyridyl unit will fit between pairs of bases of DNA with the terminal NH_2 groups protruding and available for hydrogen bonding. The potential for hydrogen bonding by this unit is illustrated by the overall structure of the crystals. The chloride salt of the cation **1** crystallises as a monohydrate with an extensive hydrogen bonded network in the solid, in which the cations do not link directly to each other but *via* H_2O and Cl^- bridges (Fig. 1). The hydrogen bonding parameters are summarised in Table 2.

NMR studies of reactions of 1 and nucleosides

Reactions of **1** and nucleoside (A, G, C, T or U; 20 mM) in a 1 : 1 ratio in D_2O phosphate buffer (pH 6) at 310 K were followed by ^1H NMR spectroscopy (Table 3). No change of chemical shifts was observed from the pyridine protons of **1** and aromatic protons of T and U in the reactions of **1** and nucleosides (T and U) which indicated no complex was formed even after 5 days incubation at pH 6. Two new sets of H^2, H^8 and $H^{1'}$ signals of A and one new set of pyridine proton signals of **1** were observed when **1** was reacted with A. NMR assignments were made with reference to a similar study on **2** and adenosine.[14] Since only two new sets of doublets of $H^{1'}$ were observed for the newly formed complexes, this strongly indicated two monoplatinated adenosine complexes, $[(bampy)Pt–(N^1\text{-}A)]^{2+}$ and $[(bampy)Pt–(N^7\text{-}A)]^{2+}$, were formed. The downfield shifts of H^8 and $H^{1'}$ of G and H^6, H^5 and $H^{1'}$ of C in the reactions of **1** with G and C confirm the platinum is bound to G *via* N^7 and C *via* N^3, similar to the results reported on the reactions of **3** with G and dC.[6] Interestingly, only the monoplatinated guanosine complex $[Pt(bampy)(G)]^{2+}$ was observed when **1** (80 mM) was reacted with a four nucleosides mixture (A, G, C, and U; 20 mM each) in an NMR tube at pH 6, 310 K. The results of the competition experiment shows that **1** has a relatively higher affinity for G than the other nucleosides. In view of its ability binding to both G and A indicates the potential of **1** as an anti-cancer compound.

LC-ESI-MS Reaction studies

The distinctive isotopic pattern of platinum (% abundance of ^{194}Pt, ^{195}Pt, ^{196}Pt and ^{198}Pt is 32.9, 33.8, 25.3 and 7.2, respectively) has provided a useful means to identify platinum complexes formed in a reaction monitored by liquid chromatography electrospray ionisation mass spectrometry (LC-ESI-MS). The soft ionisation technique used allows whole complexes and hydrogen-bonded non-covalent complexes to be detected. A series of aqueous solutions of **1** (10 mM) at pH 2.1 to pH 9.6 was prepared and sampled regularly by LC-ESI-MS

Table 2 Hydrogen bonding parameters for [Pt(bampy)Cl]Cl·H_2O

D	H	A	D–H/Å	H ··· A/Å	D ··· A/Å	D–H ··· A/°	Symmetry operation for A
N(2)	H(2A)	O(1W)	0.91	1.97	2.87	172.3	$-1 + x, +y, +z$
N(2)	H(2B)	Cl(2)	0.91	2.28	3.81	173.9	
O(1W)	H(1W)	Cl(2)	1.12	1.97	3.05	159.9	
O(1W)	H(2W)	Cl(1)	0.96	2.33	3.22	155.0	$2 - x, 1 - y, 2 - z$
N3	H(3A)	Cl(1)	0.91	2.76	3.48	137.6	$1 - x, -y, 2 - z$
N3	H(3B)	Cl(2)	0.91	2.58	3.34	141.3	$2 - x, -y, 2 - z$

Table 3 Relevant ^1H chemical shifts [in ppm relative to TSP-d_4 in D_2O phosphate buffer (pD 5.5)] for **1** and its complexes formed upon mixing with nucleosides (A, G, C) in 1 : 1 ratio

Complex	H^{para}, py	H^{meta}, py	H^6	H^5	H^2	H^8	$H^{1'}$
1	8.11	7.44	—	—	—	—	—
1 + A	8.24	7.62	—	—	8.84	8.53	6.19 (binding to N^1)
			—	—	8.47	9.16	6.26 (binding to N^7)
					8.34	8.41	6.15 (adenosine)
1 + G	8.24	7.57	—	—	—	8.63	6.06
						8.07	5.99 (guanosine)
1 + C	8.22	7.59	8.02	6.02	—	—	6.29
			7.90	5.98	—	—	6.14 (cytidine)

for 14 days at 298 K. Apart from the peak at *m*/*z* 368.11 which was assigned to the mono-cation **1**, aqua complexes such as $[Pt(bampy)(H_2O)]^{2+}$ or hydrolysed species such as $[Pt(bampy)(OH)]^+$ were not observed. This can be explained by the concentration of chloride ions (10 mM) present which stabilizes the chloride complex. Hydrolysis of **2** was also reported to be negligible from [^1H,^{15}N] NMR studies.[15] However in the presence of nucleosides (**1** : nucleoside = 1 : 1) complex formation was observed within 24 h for some cases, *e.g.* guanosine at pH 6.

Reaction of **1** and adenosine studied by NMR revealed the presence of two mono-platinated species and was not conclusive about the formation of a diplatinated complex. However in LC-ESI-MS, a signal at *m*/*z* 233.2 with a lower intensity isotopic platinum pattern for the diplatinated complex ($[(bampy)Pt–(A)–Pt(bampy)]^{4+}$, *m*/*z* 233.3) was observed along with a peak at *m*/*z* 299.3 for the monoplatinated $[Pt(bampy)(A)]^{2+}$. Interestingly **2** has been reported to form two monoplatinated complexes with A,[5] and **3** forms a diplatinated species with A binding *via* N^1 and the deprotonated N^6 atoms.[6] The diplatinated species of **1** did not indicate that deprotonation had occurred at one of the nitrogen atoms. However deprotonated complexes $[Pt(bampy)(T) - H]^+$ and $[Pt(bampy)(U) - H]^+$ were formed when **1** was reacted with T and U, respectively, at pH 9 and no diplatinated species were observed in either case. The deprotonation of purine or pyrimidine platinum complexes can have a significant effect on the hydrogen bonding of the bases and hence the formation of complexes such as $[Pt(bampy)(T) - H]^+$ and $[Pt(bampy)(U) - H]^+$ may have an added advantage of preventing the base pairing of DNA or RNA.

DNA binding studies

The interaction of **1**–**3** and cisplatin with intercalation sites on double-stranded DNA was assessed by staining DNA with the intercalator ethidium bromide after treatment with the platinum complex, over a range of ratios of complex to the number of nucleotides in the DNA (r_b). The aromatic complexes **1** and **3** showed the strongest interaction, both being able to completely block ethidium bromide intercalation at an r_b value of 0.4 (Fig. 2). In contrast, cisplatin had no effect at that ratio and the dien complex **2** showed about 40% inhibition of ethidium bromide intercalation. This ability to interact with double stranded DNA intercalation sites does not correlate directly with cytotoxicity—notably cisplatin and **2** were more toxic (see below), but weaker in their interaction with DNA, than **1** or **3**.

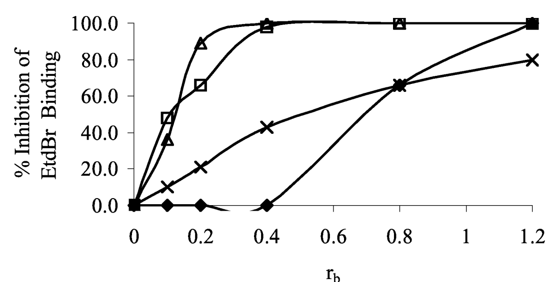


Fig. 2 Plot of percentage inhibition of ethidium bromide to DNA in the presence of cisplatin (◆), **1** (Δ), **2** (×) and **3** (□). Data are the means of three experiments, and the concentration of complex is expressed as the stoichiometric ratio (r_b) of complex to bases of DNA (75 μM).

In vitro cytotoxicity studies

The cytotoxicities of complexes **1**–**3** were examined against MCF-7 breast cancer epithelial cells and the IC_{50} values were estimated using the Trypan blue staining method.[13] Cisplatin was found to be the most active species against MCF-7 breast cancer epithelial cells, with the lowest IC_{50} value (5.6 μM), as shown in Fig. 3.

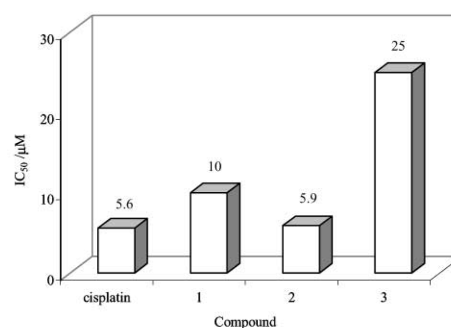


Fig. 3 The IC_{50} (μM) of cisplatin and complexes **1**–**3** against MCF-7 breast cancer epithelial cells.

For the series of triamine complexes the cytoxicity decreases progressively as more heterocyclic aromatic N-donors are present, *i.e.* the dien complex **2** (IC_{50} = 5.9 μM) is more active than **1** (IC_{50} = 10 μM) which is more active than the terpy complex **3** (IC_{50} = 25 μM). Since aquation of the chloro ligands in platinum complexes is found to precede their interactions with DNA, van Eldik and coworkers[16] have recently compared the substitution of aqua complexes of the same tridentate amine ligands by thiourea derivatives. They report that the rate of substitution is inversely proportional to the basicity of the aqua complex and not just that of the leaving or entering group. They also observed that it is possible to tune the pK_a of the aqua complexes through π-acceptor effects, which in turn may control the reactivities of the compounds. In Fig. 4 we compare the relationship between the pK_a and IC_{50} values of cisplatin and complexes **1**–**3**. The plot shows that higher the pK_a of the aqua

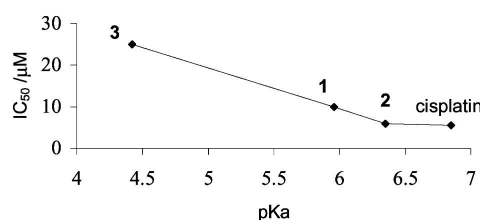


Fig. 4 Plot of IC_{50} (μM) against MCF-7 breast cancer epithelial cells *versus* the pK_a of the aqua complexes of cisplatin, **1**, **2** and **3**.

complex the higher is the toxicity against MCF-7 breast cancer epithelial cells. Cisplatin and the dien complex **2** have very similar cytotoxicity and they are more toxic than complexes **1** and **3**.

Taken together the data suggest that, for the development effective platinum compounds against breast cancer, it is better to have ligands with lower π-acceptor properties and that using aromaticity to increase the ability of ligands to bind intercalation sites on DNA may not be advantageous.

Conclusion

We have shown that reactions of $[Pt(bampy)Cl]^{+}$ **1** with nucleosides can be readily monitored by proton NMR spectroscopy and ESI-MS. Detection of a diplatinated adenine complex was only made possible using the ESI-MS technique. The NMR studies revealed that the cation **1** binds to the same sites of the nucleosides as the terpy complex. The crystal structure of **1** showed the planarity of the molecule is restricted to the pyridyl unit and thus good intercalation cannot be the reason for its efficient inhibition of EtdBr binding to DNA. It appears that disruption due to the formation of coordinate covalent platinated complexes of **1** and/or extended H-bonding, induces its observed blocking of the binding of EtdBr. It is perhaps significant that the cytotoxicity studies of **1** against MCF-7 breast cancer epithelial cells show that although it is less toxic than cisplatin it is still comparable.

Acknowledgements

We thank the EPSRC for use of the Chemical Database Service at Daresbury, Erasmus exchange (C. G.), the Royal Society (S. W. A. B.) for an Industry Fellowship and the Royal Society of Chemistry (S. W. A. B.) Journal Grant for visiting University of Pune, India.

References

1 E. Jamieson and S. Lippard, *Chem. Rev.*, 1999, **99**, 2467; M. Kartalou and J. M. Essigmann, *Mutat. Res.*, 2001, **478**, 1.
2 T. W. Hambley, *Coord. Chem. Rev.*, 1997, **166**, 181.
3 W. I. Sundquist and S. J. Lippard, *Coord. Chem. Rev.*, 1990, **100**, 293.
4 M. Howe-Grant, K. C. Wu, W. R. Bauer and S. J. Lippard, *Biochemistry*, 1976, **15**, 4339; L. S. Hollis, A. R. Amundsen and E. W Stern, *J. Med. Chem.*, 1989, **32**, 128; G. Admiraal, M. Alink, C. Altona, F. J. Dijt, C. J. van Garderen, R. A. G. de Graaff and J. Reedijk, *J. Am. Chem. Soc.*, 1992, **114**, 930.
5 P-C Kong and T. Theophanides, *Inorg. Chem.*, 1974, **13**, 1981.
6 G. Lowe and T. Vilaivan, *J. Chem. Soc., Perkin Trans. 1*, 1996, 1499.
7 D. Chen, A. E. Martell and Y. Sun, *Inorg. Chem.*, 1989, **28**, 2647.
8 J. H. Price, A. N. Williamson, R. F. Schramm and B. B. Wayland, *Inorg. Chem.*, 1972, **11**, 1280.
9 J. X. McDermott, J. F. White and G. M. Whitesides, *J. Am. Chem. Soc.*, 1976, 6521.
10 G. Annibale, M. Brandolisio and B. Pitteri, *Polyhedron*, 1995, **451**, 1.
11 SHELTXL (PC version 5.03) Siemens Analytical Instruments Inc., Madison, WI, 1994.
12 C. Newton, *J. Steroid Biochem. Mol. Biol.*, 1995, **55**, 327.
13 M. Collins, D. Ewing, G. Mackenzie, E. Sinn, U. Sandbhor and S. Padhye, *Inorg. Chem. Commun.*, 2000, **3**, 453.
14 J. Arpalahti, K. D. Klika, R. Sillanp and R. Kiveks, *J. Chem. Soc., Dalton Trans.*, 1998, 1397.
15 Z. Guo, Y. Chen, E. Zang and P. J. Sadler, *J. Chem. Soc., Dalton Trans.*, 1997, 4107.
16 D. Jaganyi, A. Hofmann and R. van Eldik, *Angew. Chem., Int. Ed.*, 2001, **40**, 1680.