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Synthesis and stereochemical characterisation of platinum(II) complexes with the antiviral agents penciclovir and famciclovir

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Abstract

The synthesis and the stereochemical characterisation of platinum complexes containing one molecule of antiviral drug, penciclovir or famciclovir (L), and different sets of ancillary ligands ($Cl_x(NH_3)_{3-x}$, $x=1$ or 2, and *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine, pmdien) are reported. Penciclovir is a guanosine analogue, while famciclovir is a prodrug of penciclovir lacking the oxygen in position 6 of the purine ring. The investigation has allowed comparison of structural features of platinum derivatives with different bulk of the carrier ligand(s) and of the purines. NMR experiments (particularly diagnostic are the H8 and H6 chemical shifts of the purine) indicate that in compounds with non-bulky carrier ligands ($Cl_x(NH_3)_{3-x}$) the purine is free to rotate about the Pt–N7 bond. In contrast, in complexes with bulky carrier ligand (pmdien) there is restricted rotation about the Pt–N7 bond and the purine is constrained in a "quasi orthogonal" position with respect to the platinum coordination plane. Because of the slow rotation for $[Pt(pmdien)(L)]^{2+}$ two rotamers are observed in solution differing for the relative positions of the six-membered ring of the purine and the central *N*-methyl of pmdien with respect to the platinum coordination plane (on the same side or on opposite sides for *endo* and *exo* rotamers, respectively). Penciclovir, having an oxygen atom in position 6 of the purine ring, favours the *exo* over the *endo* rotamer while famciclovir, having just a hydrogen atom in position 6, favours the *endo* over the *exo* rotamer. The change in rotamer preference suggests that intramolecular interactions involving mostly the substituent in position 6 of the purine and the terminal *N*-methyls of pmdien have opposite character for the two antiviral ligands. Biological tests have confirmed that cationic platinum species of formula *cis*-$[PtCl(NH_3)_2(L)]^+$ can have cytotoxicity towards tumour cells greater than corresponding compounds of formula *cis*-$[PtCl_2(NH_3)(L)]$.

Keywords: Penciclovir; Famciclovir; Platinum; Antitumour agents; Antiviral agents

1. Introduction

Cisplatin is an effective anticancer drug widely used in the treatment of several human carcinomas [1–4]. The mechanism of anticancer activity involves formation of platinum–DNA adducts that are capable of inhibiting DNA and RNA synthesis [5–16] and of inducing programmed cell death [17,18]. *Cisplatin* binds preferentially to the N7 position of purine residues; the initially formed monofunctional adduct subsequently closes to a bifunctional adduct by linking a second nucleobase which can be either of the same strand or of the opposite strand [19]. There is general consensus that the antitumour efficacy of *cisplatin* is associated with the formation of DNA 1,2-intrastrand d(GpG) or d(ApG) cross-links [5–16].

Even though *cisplatin* and related drugs (carboplatin, nedaplatin, oxaliplatin) are among the most successful antitumour compounds developed in recent years [20,21], they display limited activity against some very common tumours, such as breast and colon carcinomas. Moreover, a variety of adverse effects and the acquired resistance, which develops in patients receiving *cisplatin*

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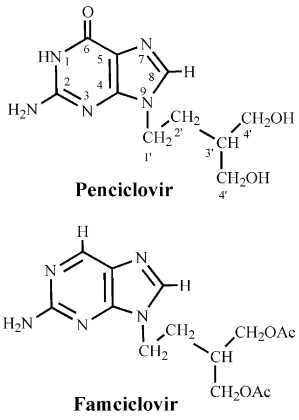
or carboplatin chemotherapy, greatly limit the potency of platinum drugs. The mechanisms underlying tumour resistance to *cisplatin* are known to be multifactorial and include decreased drug transport, increased cellular detoxification due to increased glutathione and metallothionein concentrations, changes in DNA repair efficiency, increased cell tolerance to DNA modifications, and alterations in the apoptotic cell death pathways [22–26].

These limitations have fostered the search for new platinum based drugs that would display improved therapeutic properties. Exploration of new structural motives of platinum complexes resulted in the discovery of various new classes of platinum antitumour drugs. Complexes with general formula *cis*-$[PtCl(NH_3)_2(L)]^+$ (with L = pyrimidine or purine derivative) have demonstrated activity in preclinical tumour screens, suggesting that also monofunctional DNA lesions (only one leaving chloride present in the substrate) might determine a cytotoxic effect [27,28]. In the case in which the L nucleoside itself is endowed with antiviral activity, also the platinum compound exhibits antiviral activity and in many instances results to be less toxic to normal cells than either *cisplatin* or the free antiviral nucleoside itself [29–32].

Several studies were performed in recent years on complexes with antiviral agents having purine structure [33–38]. Among others, the complex *cis*-$[PtCl(NH_3)_2(N7\text{-Acv})]^+$ (Acv = 9-(2-hydroxyethoxymethyl)guanine, a potent antiviral agent named acyclovir) revealed a particularly interesting antitumour activity. It was found to be as effective as *cisplatin* when equitoxic doses were administered in vivo to P388 leukaemia-bearing mice. Importantly, it was also active against a *cisplatin*-resistant subline of P388 leukaemia. The complex maintained also the antiviral activity of the parent drug acyclovir, though showing a minor efficacy on a molar basis [31,32].

In this paper we report on the synthesis and stereochemical characterisation of platinum compounds with two nucleoside analogues in use as antiviral drugs: penciclovir (9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine, Pen) and famciclovir (2-amino-9-(4-acetoxy-3-acetoxymethylbut-1-yl)purine, Fam) (Scheme 1).

Pen is used in the treatment of herpes simplex and herpes zoster infections [39]. Inside the cell it undergoes enzymatic phosphorilation to give Pen-triphosphate which competes with dGTP in the duplication of viral DNA; once incorporated it precludes further growing of the transcribed viral DNA strand [40]. Fam is a prodrug of Pen used in oral treatment of herpes zoster infections [41,42]. It is converted into the active Pen by cellular enzymes which remove the acetyl groups and oxidise the carbon atom in position 6 by introducing an oxygen atom [43]. Hence this investigation has also allowed



Scheme 1.

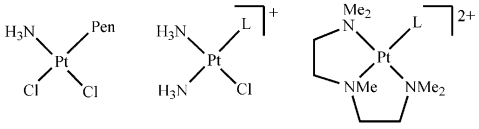
comparison of structural features of platinum derivatives with two purines, both coordinating through N7, but one having an oxygen in position 6 (Pen), and the other just an hydrogen (Fam).

2. Results and discussion

Five compounds will be described and discussed: *cis*-$[PtCl_2(NH_3)(N7\text{-Pen})]$ (**1**), *cis*-$[PtCl(NH_3)_2(N7\text{-Pen})](NO_3)$ (**2**·NO_3), *cis*-$[PtCl(NH_3)_2(N7\text{-Fam})](NO_3)$ (**3**·NO_3), [Pt(pmdien)-(*N7*-Pen)]$(NO_3)_2$ (**4**·$2NO_3$), and [Pt(pmdien)(*N7*-Fam)]$(NO_3)_2$ (**5**·$2NO_3$). All complexes considered in this investigation contain one molecule of purine per platinum but differ for the set of ancillary ligands (Scheme 2).

2.1. Complexes with small steric demand of ancillary ligands ($Cl_x(NH_3)_{3-x}$, x = 1 or 2)

The preparation of *cis*-$[PtCl_2(NH_3)(N7\text{-Pen})]$ (**1**) was facilitated by its low solubility in water in which the starting materials ($K[PtCl_3(NH_3)]$ and penciclovir) are soluble. The N7 coordination of Pen was clearly demonstrated by the presence, in the aromatic region, of only one signal at 8.32 ppm (spectrum taken in D_2O, Table 1) which is to be assigned to the H8 proton. The downfield shift of 0.49 ppm with respect to the free ligand is a clear indication of platinum binding to N7 [44,45]. The aliphatic protons of the *N9*-alkyl substitu-



1 **2/3** **4/5**

L = Pen (**2** and **4**) or Fam (**3** and **5**)

Scheme 2.

Table 1
^{1}H NMR data for *cis*-[$PtCl_2(NH_3)$(*N7*-Pen)] (**1**), *cis*-[$PtCl(NH_3)_2$(*N7*-Pen)](NO_3) (**2**·NO_3), *cis*-[$PtCl(NH_3)_2$(*N7*-Fam)](NO_3) (**3**·NO_3), [Pt(pmdien)(*N7*-Pen)](NO_3)$_2$ (**4**·2NO_3), and [Pt(pmdien)(*N7*-Fam)](NO_3)$_2$ (**5**·2NO_3)

Compound	H6	H8	central *N*-CH_3	*anti* *N*-CH_3	*syn* *N*-CH_3
[Pt(pmdien)(D_2O)]$^{2+}$			3.04	2.92	2.76
Pen		7.83			
Fam	8.60	8.13			
1		8.32			
2		8.27			
3	8.95	8.72			
4 *endo*		8.64	3.20	2.83	2.48
4 *exo*		8.74	3.12	2.74	2.58
5 *endo*	9.33	9.00	3.24	2.88	2.52
5 *exo*	9.10	9.04	3.17	2.82	2.58

Chemical shifts in ppm downfield from TSP (solvent: D_2O).

ent are slightly shifted downfield and the deshielding effect decreases as the distance from the aromatic system increases: differences between coordinated and free ligand are 0.09 ppm for C1′H_2, 0.06 ppm for C2′H_2, 0.04 ppm for C3′H and C4′H_2.

cis-[$PtCl(NH_3)_2$(*N7*-Pen)](NO_3) (**2**·NO_3) was prepared in water from *cis*-[$PtCl(NO_3)(NH_3)_2$] and penciclovir. While compound **1**, being neutral, was insoluble in water, compound **2**, being cationic, was soluble in water and could be isolated by evaporation of the solvent under vacuum. Again the chemical shift of the H8 signal (8.27 ppm), which was shifted 0.44 ppm downfield with respect to that of the free ligand (spectrum taken in D_2O), was diagnostic for the N7 coordination of penciclovir to platinum. Also in this case a slight downfield shift (which however is smaller than that observed in the previous compound) was observed for the protons of the *N9*-alkyl substituent as a consequence of the ligand complexation (0.05 ppm for C1′H_2, 0.03 ppm for C2′H_2, negligible downfield shift for C3′H and C4′H_2).

The preparation of *cis*-[$PtCl(NH_3)_2$(*N7*-Fam)](NO_3) (**3**·NO_3) was similar to that of **2**·NO_3. Upon coordination to platinum, both aromatic protons of Fam are shifted downfield (Table 1). The shifts (0.59 ppm for H8 and 0.35 ppm for H6) are indicative of N7 coordination. The coordination to the metal also has an effect on the chemical shifts of the aliphatic protons of the *N9*-chain. The C1′H_2 protons undergo a little downfield shift (0.04 ppm), while the other protons undergo upfield shifts of 0.05, 0.21, and 0.09 ppm for C2′H_2, C3′H, and C4′H_2, respectively.

2.2. Reactivity of compounds *1–3* in DMSO

The *cis* geometry of compounds **1**–**3** is dictated by the reactivity of the starting substrates. In the case of compound **1** the starting substrate is [$PtCl_3(NH_3)$]$^-$; the chloride *trans* to NH_3 is less reactive than the two chlorides *cis* to NH_3 because of the lower *trans*-effect of the ammine as compared to that of a chloro ligand; as a consequence the Pen ligand displaces one of the two chlorides *cis* to NH_3 leading to a reaction product having *cis* geometry. In the case of compounds **2** and **3** the starting substrate is *cis*-[$PtCl_2(NH_3)_2$] which has been activated by removal of one chloride with silver nitrate; whatever the displaced chloride is, the reaction product will have two *cis* ammine ligands, one chloro, and one purine ligand.

Compounds **1** and **2** undergo in DMSO solution a solvation pattern (Fig. 1) which is in full agreement with the prediction of Kurnakow's test describing the different reactivity of thiourea towards *cis*- and *trans*-[$PtCl_2(NH_3)_2$] [46].

In the case of *cis*-[$PtCl_2(NH_3)_2$] the displacement of the ligands by thiourea takes place in a one step process since both pairs of *trans* ligands contain one chloro and one ammine ligand. In contrast, in the case of *trans*-[$PtCl_2(NH_3)_2$] the displacement of the coordinated ligands by thiourea takes place in two steps. This is because the two pairs of *trans* ligands are different, one contains two chloro ligands (both exerting strong *trans*-labilizing effects), while the second pair contains two ammine ligands (both exerting very weak *trans*-labilizing effects).

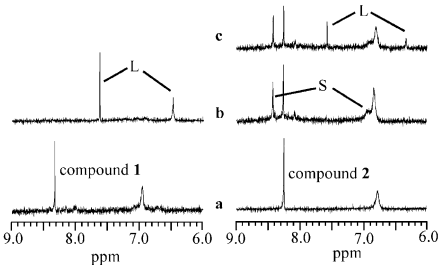


Fig. 1. ^{1}H NMR spectra in DMSO-d_6 of *cis*-[$PtCl_2(NH_3)$(*N7*-Pen)] (**1**) and *cis*-[$PtCl(NH_3)_2$(*N7*-Pen)](NO_3) (**2**·NO_3) taken at different times: (**a**) just after dissolution; (**b**) after 24 h; (**c**) after 48 h. Only the region of H8 (lower field signal) and C2NH_2 (higher field signal) is shown. **S** indicates the intermediate species observed in the case of **2** and **L** indicates the free penciclovir ligand.

In the case of *cis*-[$PtCl_2(NH_3)$(*N7*-Pen)] (**1**) the solvolysis in DMSO leads, in a single step, to release of Pen (Fig. 1).

In this complex there are two pairs of *trans*-ligands and each pair contains one chloro and one *N*-donor ligand (either penciclovir or ammine), therefore the situation is analogous to that of the reaction of thiourea with *cis*-[$PtCl_2(NH_3)_2$].

In the case of *cis*-[$PtCl(NH_3)_2$(*N7*-Pen)](NO_3) (**2**·NO_3) in DMSO, a two-step process is observed (Fig. 1). In the first step a new compound is formed still containing a coordinated Pen ligand (presence, in the NMR spectrum, of H8 and NH_2 resonances of Pen close to those of the starting complex). In the second step the Pen ligand is released as indicated by the appearance in the NMR spectrum of the signals of free Pen. Complex **2** contains two pairs of *trans*-ligands; one pair comprises one chloro and one ammine ligand, the second pair one Pen and one ammine ligand. The former pair of *trans*-ligands, containing one *trans*-labilizing chloride, will be displaced first. The latter pair of *trans*-ligands, none of which having significant *trans*-labilizing effect, will be displaced in a later step. Therefore the overall behaviour resembles that observed in the reaction of thiourea with *trans*-[$PtCl_2(NH_3)_2$].

The behaviour of compound **3** was completely similar to that of compound **2**.

2.3. Complexes with sterically demanding pmdien ligand (*4* and *5*)

In the ^1H NMR spectrum of the starting [Pt(pmdien)(D_2O)]$^{2+}$ substrate, three singlets are observed at 3.04, 2.92 and 2.76 ppm with intensity ratios of 1:2:2 (Table 1). The singlet at the lowest field belongs to the central *N*-methyl. The intermediate singlet belongs to the two "quasi axial" *N*-methyls which are on the side opposite to that of the central *N*-methyl with respect to the platinum coordination plane (*anti* methyls). Finally, the singlet at the highest field belongs to the two "quasi equatorial" *N*-methyls which are on the same side of the central *N*-methyl with respect to the platinum coordination plane (*syn* methyls) [33]. The NMR signals of the methylene protons also indicate that the puckering of the chelate rings is fixed and the ligand structure rather rigid [33].

The reaction of the aqua complex [Pt(pmdien)(D_2O)]$^{2+}$ with Pen to afford [Pt(pmdien)(*N7*-Pen)]$^{2+}$ (**4**) was carried on in D_2O at pD 6.9 and 24 °C and is rather slow. After 1 week six singlets were observed in the region of *N*-methyls (Fig. 2). On the basis of the intensity ratios, it was possible to identify two sets of three signals each: set **A** (3.20, 2.83 and 2.48 ppm) and set **B** (3.12, 2.74 and 2.58 ppm).

Within each set, the three signals had intensity ratios of 1:2:2, and were assigned, in the given order, to the central, to the *anti* and to the *syn* methyls. The intensity ratio between sets **A** and **B** was 2:3. It can be noted that corresponding signals, in the two sets, differ by less than 0.10 ppm, indicating that species leading to sets **A** and **B** must be structurally very similar. Two singlets were present in the region of the aromatic protons, at 8.64 and 8.74 ppm, and were assigned to H8 of penciclovir. The intensity ratio of 2:3 between the latter two signals, corresponds to that observed between sets **A** and **B**, and suggests that the more shielded and less intense peak (**A**′ in Fig. 2) is associated with set **A**, while the less shielded and more intense peak (**B**′ in Fig. 2) is associated with set **B**.

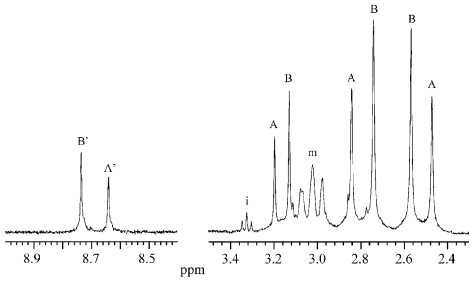
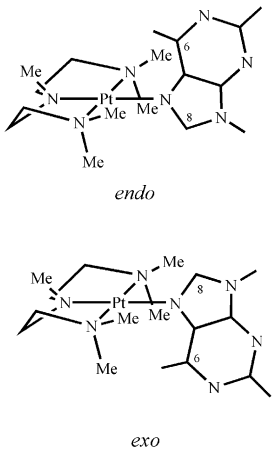


Fig. 2. ^1H NMR spectrum in D_2O of [Pt(pmdien)(*N7*-Pen)](NO_3)$_2$ (**4**·$2NO_3$). The two sets of resonances are labelled **A** and **B**, respectively; **i** stands for impurity; the triplet labelled **m** belongs to methylene protons of the pmdien moiety.

On the basis of the experimental data we can conclude that two species are formed in comparable yields. These could be either two different compounds, or two conformers of the same compound. Penciclovir can only bind to one site of Pt(pmdien), therefore different compounds can only stem either from a change in the platinum/ligand ratio (e.g. one or two platinum units per Pen molecule) or from a change in the donor atom of penciclovir (being the platinum/Pen ratio 1:1). The observation that **A** and **B** are always formed in the same ratio using either an excess or a substoichiometric amount of Pen ligand, excludes the possibility of different platinum-to-ligand ratios (an excess of Pen should decrease the possibility of formation of a di-nuclear species). Moreover, the observation that the two H8 signals are shifted by a similar amount (0.81 and 0.91 ppm) with respect to the free ligand rules out the possibility of coordination of penciclovir by different donor atoms. Therefore, it must be concluded that the two sets of signals belong to two conformers of the same species having formula [Pt(pmdien)(*N7*-Pen)]$^{2+}$. It is most likely that the two conformers are rotamers differing in the orientation of the purine base with respect to the pmdien ligand (Scheme 3).

The six-membered ring of the purine and the central *N*-methyl of pmdien can be either on the same side (*endo* rotamer) or on opposite sides (*exo* rotamer) of the platinum coordination plane [33].



Scheme 3.

2-D-NOESY experiments were carried out in order to assign the two sets of signals to either rotamer (Fig. 3).

The presence of a cross-peak between **A**′ (signal at 8.64 ppm of Pen H8) and the signal of *anti* methyls of set **A**, allowed to assign these resonances (**A** and **A**′) to the *endo* rotamer, in which the H8 of penciclovir and the *anti* *N*-methyls of pmdien are on the same side of the platinum coordination plane and close enough to give rise to a NOE crosspeak (Scheme 3). Similarly, the presence of a crosspeak between **B**′ and the *syn* *N*-methyls of set **B**, allows to assign **B** and **B**′ to the *exo* rotamer, in which the H8 and the *syn* *N*-methyls are on the same side with respect to the platinum coordination plane. The observation of different sets of signals for the two rotamers requires the rate of interconversion between them to be slow on the NMR time scale. It is conceivable that the four terminal *N*-methyl groups produce a steric crowding around the metal centre that the rotation of the purine base around the Pt–N7 bond becomes hindered. The steric clash between the terminal *N*-methyls of pmdien and the purine (particularly O6) reaches the highest value when penciclovir is dragged through the coordination plane. On this basis we expected a relief of steric interaction in the case of a purine ligand lacking a substituent in position 6. This is the case of famciclovir, therefore we investigated the reaction of $[Pt(pmdien)(D_2O)]^{2+}$ with famciclovir.

The reaction of $[Pt(pmdien)(D_2O)]^{2+}$ with Fam to afford $[Pt(pmdien)(N7\text{-Fam})]^{2+}$ (**5**) was also carried out in D_2O at pD 6.9 and 24 °C. After 1 week, it was possible to distinguish, in the region of aliphatic protons, two sets of three signals each: set **A** (3.24, 2.88, and 2.52 ppm) and set **B** (3.17, 2.82, and 2.58 ppm, Fig. 4).

The intensity ratio **A**:**B** was 2:1. Four singlets were observed in the region of aromatic protons, at 9.33, 9.10, 9.04, and 9.00 ppm. The assignment of the signals to H6 and H8 was based on the different behaviour of these protons under basic conditions. It is well known that under basic conditions the H8 protons of purine bases undergo exchange with deuterium of water solvent [47] with the consequence that the intensities of the corresponding ^{1}H NMR signals decrease with time. Therefore an aliquot of the D_2O solution of the compound under investigation was brought to pD 9.4; after 12 h at room temperature two signals (at 9.04 and 9.00 ppm) had disappeared and only two singlets (at 9.33 and 9.10 ppm) were still present. On this basis, the two signals persistent at basic pD were assigned to the H6 protons, while the two signals whose intensities decreased at high pD were assigned to the H8 protons. On the basis of the intensity ratios it was possible to associate the signals at 9.00 and 9.33 ppm (**A**′ and **A**″ in Fig. 4) to set **A** and the signals at 9.04 and 9.10 ppm (**B**′ and **B**″ in Fig. 4) to set **B**. The sets of signals **A**, **A**′, and **A**″ and **B**, **B**′, and **B**″ must belong, as in the case of Pen, to two conformers of the same compound having formula $[Pt(pmdien)(N7\text{-Fam})]^{2+}$. The values of the H8 chemical shifts and the intensity ratio between the two sets of signals, which was independent of platinum-to-ligand ratios, are in accord with the hypothesis of two conformers of the same compound.

In the case of the Fam ligand, in addition to the H8 proton, there is also the H6 proton which can be diagnostic for the assignment of the conformations to

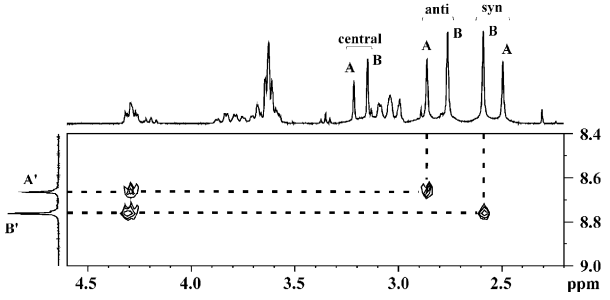


Fig. 3. 2-D-NOESY spectrum in D_2O of $[Pt(pmdien)(N7\text{-Pen})](NO_3)_2$ (**4**·2NO_3). The region of cross-peaks between H8 and C1′H_2 and *N*-methyls is shown. The intense cross-peaks between H8 and some of the terminal *N*-methyls allow the assignment of **A** and **A**′ to the *endo* rotamer and **B** and **B**′ to the *exo* rotamer.

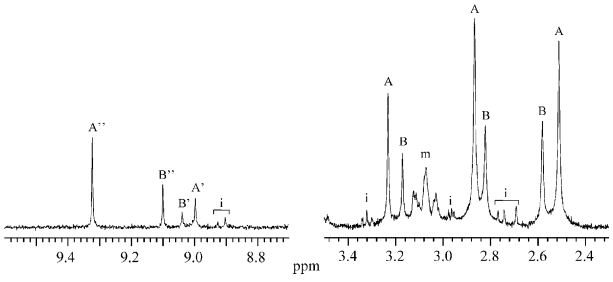


Fig. 4. ^{1}H NMR spectrum in D_2O of $[Pt(pmdien)(N7\text{-Fam})](NO_3)_2$ (**5**·2NO_3). The two sets of resonances are labelled **A** and **B**, respectively; **i** stands for impurity; **m** indicates a multiplet of methylene protons of pmdien ligand. The lower intensities of the H8 with respect to the H6 signals is due to H8 exchange with deuterium of the solvent.

the different rotamers. In particular, in addition to the crosspeak between **A′** (H8) and the *anti* *N*-methyls of **A**, there is also an intense cross peak between **A″** (H6) and the *syn* *N*-methyls of **A** and a weak crosspeak between **A″** and the central *N*-methyl of **A**. Therefore signals **A**, **A′**, and **A″** belong to the *endo* rotamer. Similarly, a crosspeak between **B′** (H8) and the *syn* *N*-methyls of **B** (rather weak because of the low intensity of the water exchangeable H8 signal) and an intense crosspeak between **B″** (H6) and the *anti* methyls of **B**, assign signals **B**, **B′**, and **B″** to the *exo* rotamer (Fig. 5).

Different trends are observed for the chemical shifts of the H8 and H6 protons of Fam. The H8 proton is slightly more shielded in the *endo* rotamer than in the *exo* rotamer. Vice versa H6 is more shielded in the *exo* rotamer than in the *endo* rotamer. This is the expected relationship since the H6 proton is on the opposite side of H8 with respect to the platinum coordination plane. Moreover it can be noted that the difference in chemical shifts between *endo* and *exo* rotamers is greater for H6 ($\Delta\delta = 0.23$ ppm) than for H8 ($\Delta\delta = 0.04$ ppm). This is in accord with the H6 proton protruding, more than the H8 proton, towards the ancillary ligand (pmdien) and sensing more deeply the asymmetry of this ligand with respect to the platinum coordination plane.

Some common features are observed in the NMR spectra of compounds **4** and **5**. *Syn* *N*-methyls are more shielded in the *endo* rotamer than in the *exo* rotamer, vice versa *anti* *N*-methyls are more shielded in the *exo* rotamer than in the *endo* rotamer. The central *N*-methyl is less shielded in the *endo* rotamer than in the *exo* rotamer. Therefore it appears that the purine shields the terminal *N*-methyls and deshields the central *N*-methyl, when the six-membered ring of the purine and the *N*-methyls are on the same side of the platinum coordination plane.

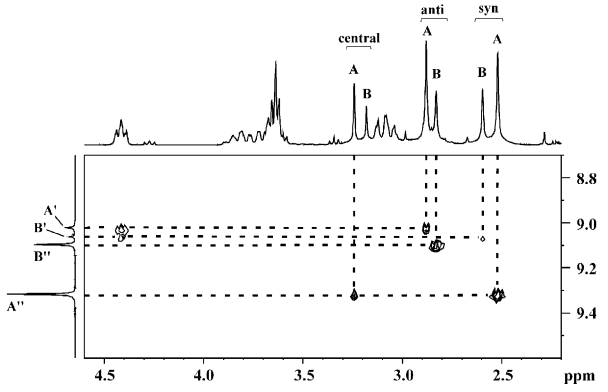


Fig. 5. 2-D-NOESY spectrum in D_2O of [Pt(pmdien)(*N7*-Fam)]$(NO_3)_2$ (**5**·$2NO_3$). The region of cross-peaks between H8/H6 and C1′H_2 and *N*-methyls is shown. The exchange of the H8 protons with deuterium causes decrease of the intensity of their signals in the monodimensional spectrum and of corresponding cross-peaks in the bidimensional spectrum, with respect to those of the H6 protons. The intense cross-peaks between H8 or H6 and some of the terminal *N*-methyls allow the assignment of **A**, **A′**, and **A″** to the *endo* rotamer and **B**, **B′**, and **B″** to the *exo* rotamer.

2.4. Antitumour activity

One of the aims of this investigation was to explore the antitumour and the antiviral activities of compounds combining in the same molecule features characteristic of antitumour *cisplatin* type complexes and antiviral nucleoside analogues. Within this research programme particularly interesting results were obtained with the complex *cis*-$[PtCl(NH_3)_2(N7\text{-Acv})]^+$, Acv being the potent antiviral drug acyclovir. This compound was as effective as *cisplatin* when equitoxic doses were administered in vivo to P388 leukaemia-bearing mice, moreover it was also active against a *cisplatin*-resistant subline of P388 leukaemia [31,32]. In the present investigation penciclovir was used in place of acyclovir and the investigation was restricted to compounds **1** and **2** differing for the overall charge of the complex (0 and +1, respectively) and the number of leaving chlorides (2 and 1, respectively). We have not considered compound **3** since famciclovir is a prodrug of penciclovir and it would require a preliminary metabolic activation. We have also not considered compounds **4** and **5** which are deprived of leaving groups which are essential for the drug to interact with DNA (an essential step for anticancer activity).

Preliminary cytotoxic effects on some human and murine tumour cell lines are reported in Table 2. Both compounds have lower toxicity than the reference compound *cisplatin*. Significant activity was found only against L1210/0 and, in the case of **2**, also FM3A/0 cells, while the compounds must be considered inactive towards Molt4/C8 and CEM/0 cells. Although these preliminary results indicate that the newly synthesised compounds are much less promising than the parent compound *cis*-$[PtCl(NH_3)_2(N7\text{-Acv})]^+$ previously investigated [31,32], nevertheless they confirm the peculiar behaviour of cationic complexes containing two *cis*-ammines and an aromatic base. Compounds of

Table 2
Cytotoxicity data for *cis*-$[PtCl_2(NH_3)(N7\text{-Pen})]$ (**1**) and *cis*-$[PtCl(NH_3)_2(N7\text{-Pen})](NO_3)$ (**2**·NO_3), and the reference compounds *cisplatin* and Pen

Compound	IC_{50} (μM)			
	L1210/0	FM3A/0	Molt4/C8	CEM/0
Cisplatin	0.33		1.70	
Pen	126	695	288	255
1	8.11	49.8	373	107
2	22.4	17.2	75.9	63.8

Four tumour cell lines were used: murine leukaemia cells (L1210/0), murine mammary carcinoma cells (FM3A/0), and human T-lymphocyte cells (Molt4/C8 and CEM/0).

this type can be endowed with significant antitumour activity, although not fulfilling two fundamental structure/activity relationships valid for *cisplatin* type complexes, that is presence of two leaving ligands and neutral charge of the compound [27,28].

3. Conclusions

The steric hindrance of the carrier ligands has been found to deeply influence the rotational freedom of a *N7*-coordinated purine base also in the case of monoadducts, like those presently investigated. Therefore in compounds with small steric demand of the ancillary ligands (**1**–**3**), the purine base is free to rotate about the Pt–N7 bond and only one set of signals, which is the average of different conformers, is observed. In contrast, in compounds with a sterically demanding ancillary ligand (**4** and **5**) distinct sets of resonances for the two rotamers are observed.

Comparison between the average chemical shifts (particularly those of H8 and H6) also allows some insight into the average canting of the nucleobase with respect to the coordination plane in the two types of complexes (**1**–**3** and **4**–**5**). For Pen ligand the average downfield shift of H8, as a consequence of platinum coordination at N7, is 0.47 for the first type of complexes (**1**–**3**) and 0.86 ppm for the second type of complexes (**4**–**5**). Similarly for the Fam ligand the downfield shift of H8 and H6 is, in the given order, 0.59 and 0.35 ppm for the first type of complexes and 0.89 and 0.62 ppm for the second type of complexes. The inductive effect of platinum in the two types of complexes is expected to be very similar, notwithstanding the set of donor atoms and the overall charge of the complexes is different [48]. The latter assumption is supported by the observation that the chemical shift of the H8 protons changes only by 0.05 ppm between compounds **1** and **2** although they have different set of donor atoms and different charge. Therefore we can assume that most of the difference in chemical shifts of H8 and H6 between complexes **1**–**3** and **4**–**5** stems from a different effect of the magnetic anisotropy of the platinum centre in the two cases. It is well documented that protons which come close to the axial site of a square-planar platinum(II) complex are deshielded with respect to protons which are closer to the equatorial coordination plane [49–52]. On this basis the greater downfield shift of H8 and H6 observed in compounds **4** and **5** can be interpreted as a consequence of the methyl substituents on the terminal nitrogen atoms of pmdien constraining the purine bases in a rigid position which is nearly orthogonal to the coordination plane. In contrast, the lower downfield shifts observed in compounds **1**–**3** can be interpreted as the purine ligands having an average orientation which is slant on the coordination plane. In compounds **1**–**3** (as a consequence of the small steric hindrance of the ancillary ligands) the purine is free to rotate about the Pt–N7 bond, however the residence time is expected to be greater for the conformer in which the plane of the purine is orthogonal to the coordination plane than for the conformer in which the purine is lying in the coordination plane. The average dihedral angle can be estimated to be of the order of 70°.

Another point of interest arising from the investigation of compounds **4** and **5** is the different ratio between *endo* and *exo* rotamers observed for penciclovir and famciclovir. In the former case (compound **4**) the *exo* rotamer is preferred over the *endo* rotamer by a factor of 3:2, while in the latter case (compound **5**) is the *endo* rotamer to be preferred over the *exo* rotamer by a factor of 2:1. The terminal *N*-methyls of pmdien which are *syn* to the central *N*-methyl are slightly more equatorial in character, while the *N*-methyls which are *anti* to the central *N*-methyl are slightly more axial in character. In compound **5** the interactions between H6 of famciclovir and the terminal *N*-methyls of pmdien (the interactions have to be considered essentially steric and repulsive in character) result in a definite preference for the *endo* rotamer (H6 on the same side of the more equatorial *N*-methyls with respect to the platinum coordination plane). If the interaction of O6 of penciclovir with terminal *N*-methyls were similar to those of H6 of famciclovir (steric in nature and repulsive) we would expect a similar rotamer preference also for compound **4**. However this is not the case, and, differently from famciclovir, penciclovir shows a small but definite preference for the *exo* rotamer.

Finally, cytotoxicity tests performed on compounds **1** and **2**, although not very exciting, have confirmed the peculiar behaviour of cationic compounds containing two *cis*-ammines and one aromatic base which can be more active than corresponding neutral compounds having only one ammine and one aromatic base in *cis* positions.

4. Experimental

4.1. Starting materials

Penciclovir and famciclovir were supplied by the Institute of Chemistry of Ljubljana, Slovenia. The starting platinum substrates were prepared by literature methods: *cis*-$[PtCl_2(NH_3)_2]$ [53], $K[PtCl_3(NH_3)]$ [45], and $[Pt(NO_3)(pmdien)](NO_3)$ [54].

4.2. Preparation of compounds

4.2.1. *cis*-[PtCl(NO$_3$)(NH$_3$)$_2$]

cis-[PtCl$_2$(NH$_3$)$_2$] (0.100 g, 0.33 mmol) suspended in water (7 ml) was treated with silver nitrate (0.057 g, 0.33 mmol) at room temperature in the dark. The suspension was stirred for 12 h. The white solid (silver chloride) was removed by filtration of the mother liquor and the solution, evaporated to dryness, afforded *cis*-[PtCl(NO$_3$)(NH$_3$)$_2$] as crystalline solid (0.102 g, yield 95%). *Anal*. Calc. for $H_6ClN_3O_3Pt$: H, 1.85; N, 12.87. Found: H, 1.82; N, 12.85%.

4.2.2. *cis*-[PtCl$_2$(NH$_3$)(*N7*-Pen)] (**1**)

A water solution (10 ml) of K[PtCl$_3$(NH$_3$)] (0.150 g, 0.42 mmol) was treated with a stoichiometric amount of Pen (0.42 mmol). After 24 h stirring at room temperature, the yellow precipitate was separated from the mother liquor. Crystallisation from water/methanol (2:3, v/v) afforded *cis*-[PtCl$_2$(NH$_3$)(*N7*-Pen)] (0.135 g, yield 60%). *Anal*. Calc. for $C_{10}H_{18}Cl_2N_6O_3Pt$: C, 22.39; H, 3.38; N, 15.67. Found: C, 22.29; H, 3.40; N, 15.61%.

4.2.3. *cis*-[PtCl(NH$_3$)$_2$(*N7*-Pen)](NO$_3$) (**2**·NO$_3$)

A solution of *cis*-[PtCl(NO$_3$)(NH$_3$)$_2$] (0.108 g, 0.33 mmol) in water (10 ml) was treated with Pen (0.084 g, 0.33 mmol). After 24 h stirring at room temperature, the solvent was evaporated under vacuum and the resulting yellow solid, washed with ethanol and diethyl ether and dried, proved to be *cis*-[PtCl(NH$_3$)$_2$(*N7*-Pen)](NO$_3$) (0.115 g, yield 60%). *Anal*. Calc. for $C_{10}H_{21}ClN_8O_6Pt$: C, 20.71; H, 3.65; N, 19.33. Found: C, 20.68; H, 3.62; N, 19.25%.

4.2.4. *cis*-[PtCl(NH$_3$)$_2$(*N7*-Fam)](NO$_3$) (**3**·NO$_3$)

A solution of *cis*-[PtCl(NO$_3$)(NH$_3$)$_2$] (0.110 g, 0.34 mmol) in methanol (10 ml) was treated with Fam (0.080 g, 0.34 mmol). After 60 h stirring at room temperature, the solvent was evaporated under vacuum and the resulting yellow solid, washed several times with hot (60 °C) absolute ethanol and diethyl ether and dried, proved to be *cis*-[PtCl(NH$_3$)$_2$(*N7*-Fam)](NO$_3$) (0.124 g, yield 65%). *Anal*. Calc. for $C_{14}H_{25}ClN_8O_7Pt$: C, 25.94; H, 3.89; N, 17.30. Found: C, 25.91; H, 3.86; N, 17.28%.

4.2.5. [Pt(pmdien)(*N7*-L)](NO$_3$)$_2$, L = Pen (**4**·2NO$_3$) or Fam (**5**·2NO$_3$)

In a typical experiment [Pt(NO$_3$)(pmdien)](NO$_3$) (0.006 g, 0.012 mmol) in D_2O (0.5 ml) was treated with a stoichiometric amount of either Pen or Fam. The solution was kept at room temperature and the reaction was monitored by ^{1}H NMR. The pD (uncorrected) was maintained at about 6.9.

4.3. NMR spectroscopy

1-D and 2-D ^{1}H NMR spectra were obtained with a Bruker AVANCE DPX 300 MHz spectrometer at the temperature of 24 °C. For 1-D experiments the average sample concentration was 0.002 g of complex in 0.5 ml of solvent. A greater concentration (ca. 0.006 g of complex in 0.5 ml of solvent) was used in the case of 2-D experiments. When appropriate, the pD was adjusted to the required value by addition of DNO_3 or NaOD.

4.4. Antitumour activity assays

Antitumour activities against L1210/0 (murine leukaemia), FM3A/0 (murine mammary carcinoma), Molt4/C8, and CEM/0 (human T-lymphoblast) cell lines were measured as originally described for the mouse leukaemia/L1210 cell lines [55].

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