Synthesis, Characterisation and Antiviral Activity of Platinum(II) Complexes with 1,10-Phenanthrolines and the Antiviral Agents Acyclovir and Penciclovir

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Four novel hybrid drugs $[Pt(acy)_2(Me_2phen)]I_2$ (**1**), $[Pt(pen)_2(Me_2phen)](NO_3)_2$ (**2**), $[Pt(acy)_2(phen)](NO_3)_2$ (**3**), and $[Pt(pen)_2(phen)](NO_3)_2$ (**4**) have been prepared by reaction of the antiviral guanine derivatives acyclovir (acy) and penciclovir (pen) with platinum complexes having 2,9-Me_2-1,10-phenanthroline (Me_2phen) and 1,10-phenanthroline (phen) as carrier ligands. Both the Me_2phen and phen carrier ligands are able to hinder rotation of the guanine bases about the Pt–N(7) bonds rendering the interconversion among rotamers very slow on the NMR time scale. A favourable dipole–dipole interaction between the *cis*-coordinated purine bases stabilises the *HT* rotamers, which are the only species detected in solution. The stability in a physiological medium and the anti-HIV properties in vitro of the complexes have been evaluated. In a 0.1 M solution of NaCl, the compounds with Me_2phen (**1** and **2**) were found to be very stable while the compounds with phen (**3** and **4**) underwent displacement of one purine base by a chloride ion. This rather unexpected result may have some relevance in connection with the stability of major DNA adducts of cisplatin in which two guanines are cross-linked by a $Pt(NH_3)_2$ moiety. Although none of the complexes showed anti-HIV activity, complexes **1** and **2**, bearing methyl substituents in the *ortho* positions of the phenanthroline, were much more cytotoxic than **3** and **4**. This is probably due to a greater stability of the former complexes in biological media caused by the presence of the two methyl groups wrapping the metal centre and inhibiting the nucleophilic attack of an incoming ligand at the platinum centre.

Introduction

The discovery of acyclovir [9-(2-hydroxyethoxymethyl)-guanine, acy] in 1977, the first drug with high selectivity, was the starting point of antiviral chemotherapy.[1] Acyclovir is a synthetic analogue of guanosine with a linear chain in place of the ring sugar, it inhibits selectively the replication of Herpes Simplex Virus (HSV) by interfering with DNA synthesis. After entering into the infected cell, acyclovir is phosphorylated by the virus-induced thymidine kinase (TK) to acyclovir-monophosphate (acyclo-GMP).[2] The rate of phosphorylation by mammalian TK is negligible and this makes acyclovir highly selective towards HSV. The drug is further phosphorylated by cellular enzymes to produce first acyclo-GDP and then acyclo-GTP. The latter is a selective inhibitor of the viral DNA polymerase because it competes with dGTP for incorporation into the growing DNA strand. The inclusion of acyclovir, containing a single hydroxyl group, stops the duplication process.

Acyclovir is the drug of choice for the treatment of HSV and Varicella Zoster Virus (VZV) infections. It is commonly used in the treatment of primary and recurrent genital herpes and mucocutaneous HSV and VZV infections in immunosuppressed patients.

Hundreds of synthetic analogues of acy have been synthesised, among these analogues are ganciclovir and penciclovir (pen). Ganciclovir shows higher activity towards cytomegalovirus (CMV) than acyclovir, while penciclovir shows a similar spectrum of activity as acyclovir, as well as activity against human hepatitis B virus.[3]

The coordination ability of acyclovir and its analogues has attracted the interest of many researchers in view of the fact that DNA polymerases contain Zn^{2+} and/or are activated by metal ions such as Mg^{2+}, Mn^{2+}, and Co^{2+}. Complexes of acyclovir with Cd^{II}, Co^{II}, and Cu^{II} have been synthesised and characterised and,[4] in some instances, their antiviral properties were also investigated.[5,6] The structural analogies of acyclovir with purine nucleotides have inspired the synthesis of complexes with platinum(II).[7–10] Anticancer platinum(II) drugs are known to crosslink DNA at adjacent purine bases, and bifunctional adducts of this type are believed to be responsible for anticancer activity.[11,12]

Amines have usually been used as carrier ligands for platinum drugs;[13] however, polycyclic aromatic amines, such as phenanthroline, in addition to being bidentate N-donor ligands, also have an intercalating ability towards double-helix DNA.[14] The binding to Calf Thymus DNA has been investigated for some phenanthroline complexes.[15]

A special case is that of the *ortho* disubstituted phenanthroline 2,9-Me_2-1,10-phenanthroline (Me_2phen, neocup-

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roine) which has long been investigated for its ability to increase the chemical reactivity of platinum(II) substrates of formula $[PtX_2(Me_2phen)]$ (X = halogen ligand).[16,17] Because of the steric interaction between the *ortho* substituents of the phenanthroline and the *cis* halogen ligands, $[PtX_2(Me_2phen)]$ substrates react readily with an extra ligand (L) to give the corresponding addition product $[PtX_2(L)(Me_2phen)]$. For L = a π-acceptor ligand (such as alkene, alkyne, and carbon monoxide) the addition product has a five-coordinate geometry; in contrast, for L = a σ-donor ligand (such as amine, phosphane, etc.) the platinum remains four-coordinate and the neocuproine acts as a monodentate ligand. With longer reaction times the displacement of one X and back chelation of Me_2phen is also observed.

Pursuing our interest in the synthesis of hybrid drugs combining in one molecule the features of *cis*-amine platinum antitumor drugs and antiviral nucleotide analogues,[7−10] we have carried out the synthesis of platinum complexes containing a bidentate phenanthroline and two antiviral purine bases in the coordination sphere. The stereochemistry of the new compounds, as well as preliminary data on their antiviral activity, are reported in the present work.

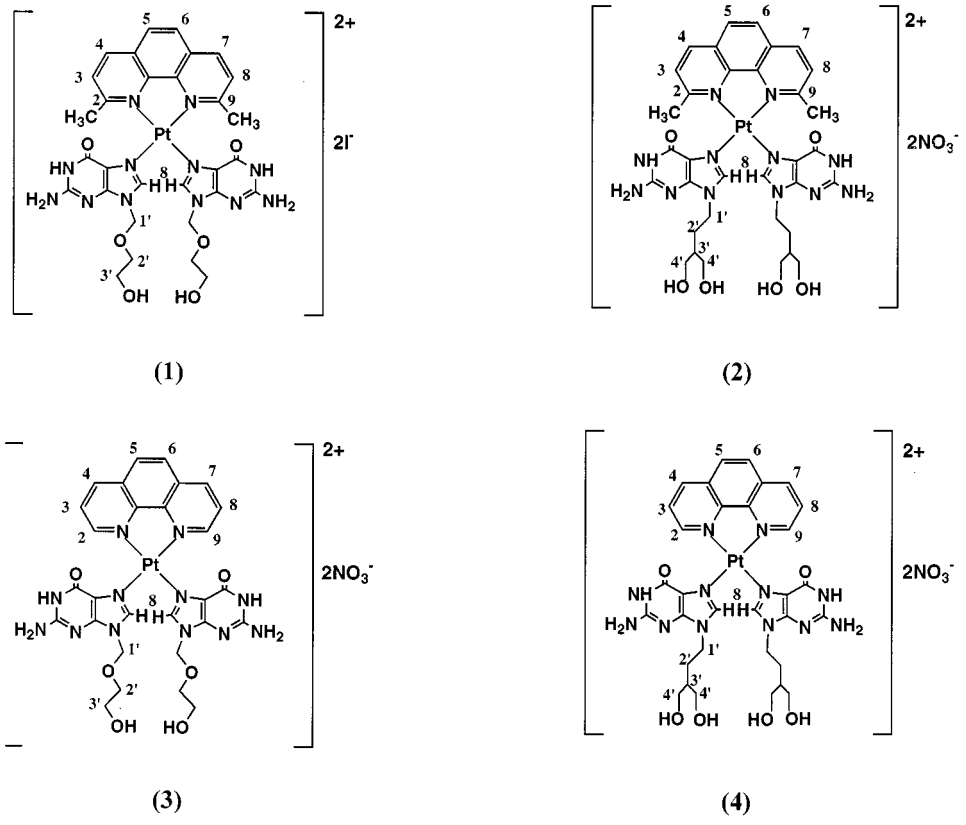
Results and Discussion

In a recent paper[8] we investigated the antiviral and anticancer properties of *cis*-$[PtCl(acy)(NH_3)_2](NO_3)$ in comparison to those of acyclovir and cisplatin. The platinum-acyclovir complex maintained the antiviral activity of acyclovir, although it showed a minor efficacy on a molar basis (ID_{50}= 7.85 and 1.02 μM for *cis*-$[PtCl(acy)(NH_3)_2](NO_3)$ and acyclovir, respectively). However, the novel hybrid drug, although less toxic towards tumour cells than cisplatin, showed similar activity when equitoxic doses were administered in vivo to P388 leukaemia-bearing mice (% T/C = 209 and 211 for *cis*-$[PtCl(acy)(NH_3)_2](NO_3)$ and cisplatin, respectively). *cis*-$[PtCl(acy)(NH_3)_2](NO_3)$ was also active against a cisplatin-resistant subline of the P388 leukaemia, thus indicating that the mechanism of action could be different from that of cisplatin.

On this basis we directed our attention to the synthesis of platinum compounds containing antiviral purinic bases and a phenanthroline as ligands (Scheme 1), with the aim of improving the antitumoral and/or the antiviral properties by taking advantage of the intercalating ability of phenanthrolines.

Synthesis of the Compounds

The preparation of the complexes was straightforward. The diiodoplatinum complex $[PtI_2(Me_2phen)]$ is a rather reactive substrate due to the steric clash between the *ortho* methyl substituents of the phenanthroline and the *cis* iodo ligands, which strongly destabilises the square-planar geometry.[16,17] It reacts smoothly with acyclovir (solvent CH_2Cl_2/CH_3OH, 1:1 v/v) to give substitution of the iodo ligands and formation of the cationic species



Scheme 1. Schematic drawing of compounds **1**−**4**

$[PtI(acy)(Me_2phen)]^+$ and $[Pt(acy)_2(Me_2phen)]^{2+}$. The separation of the two reaction products was accomplished by taking advantage of their different solubilities in water. The divalent salt $[Pt(acy)_2(Me_2phen)]I_2$ (**1**) is very soluble in water while the monovalent salt $[PtI(acy)(Me_2phen)]I$ (**5**) is sparingly soluble in water but rather soluble in methanol.

The synthesis of $[Pt(pen)_2(Me_2phen)](NO_3)_2$ (**2**) was carried out using DMF as solvent because of the insolubility of penciclovir in more common solvents. The starting $[PtI_2(Me_2phen)]$ substrate was first converted into the solvato species $[Pt(DMF)_2(Me_2phen)]^{2+}$ by reaction with a stoichiometric amount of $AgNO_3$. The solvato species reacts smoothly with a stoichiometric amount of penciclovir (ratio 1:2) to give complex **2**.

The preparation of $[Pt(acy)_2(phen)](NO_3)_2$ (**3**) and $[Pt(pen)_2(phen)](NO_3)_2$ (**4**) was carried out using the same procedure as described for **2**. The use of DMF as solvent was caused by the insolubility of the starting $[PtI_2(phen)]$ complex in common solvents.

Stereochemical Investigations

Complex 1

All complexes were characterised by elemental analysis, IR and NMR spectroscopy. A bathochromic shift of the stretching frequency of the C=O group in position 6 of the purine ring (1697 cm^{-1} in complex **1** and 1717 cm^{-1} in free acyclovir) is indicative of coordination of the guanine base to platinum through N(7).[18,19]

The ^1H NMR spectrum of **1** in D_2O shows a single set of signals (Figure 1, Table 1) which is in agreement with a symmetrical complex. The phenanthroline is symmetrical with chemical shifts at $\delta = 2.35$ for the methyls in positions 2 and 9 and at $\delta = 8.69$ ($J_{H\text{-}H} = 8.4$ Hz), 8.11, and 7.71 ($J_{H\text{-}H} = 8.4$ Hz) for protons 4/7, 5/6, and 3/8, respectively. Also, the two acyclovir ligands give a single set of resonances. The chemical shift of the H(8) protons at $\delta = 8.53$ is also indicative of N(7) coordination of the purine bases to platinum ($\delta = 7.91$ for free acyclovir).[13,20]

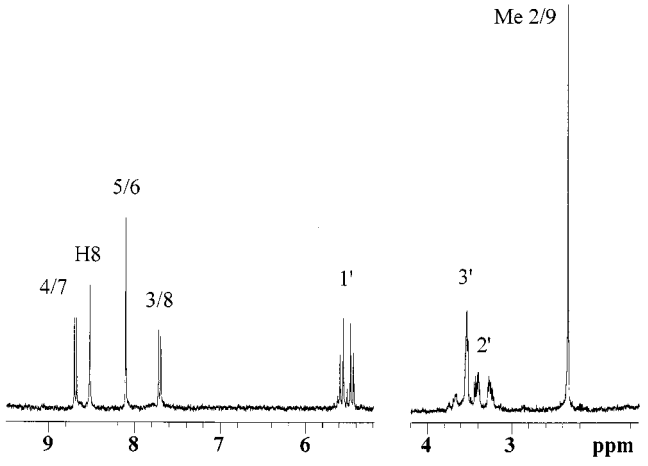


Figure 1. Sections of the ^1H NMR spectrum in D_2O of complex **1**

For $Pt(A)G_2$ complexes (where A is a C_2-symmetrical bidentate ligand and G a guanine derivative) different rotamers are possible: one *HH* conformer (Head-to-Head) with the H(8) of the two G's on the same side of the platinum coordination plane, and two *HT* conformers (Head-to-Tail) with the H(8) on opposite sides of the coordination plane (Scheme 2).[21,22] The two *HT* conformers differ in their chirality (Λ or Δ). The relation between the trace of the helix [a line connecting the O(6) atoms of the two guanines] and the axis of the helix (the perpendicular to the coordination plane passing through the platinum centre) is described by the index and thumb of the left hand in the Λ conformer and of the right hand in the Δ conformer. In the case of nonchiral G ligands all rotamers will be symmetrical (C_s and C_2 symmetry for the *HH* and *HT* rotamers, respectively); moreover the two *HT* rotamers will constitute a couple of enantiomers and will be identical from an NMR point of view.

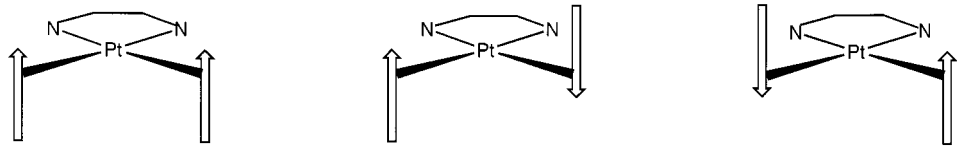
From previous work[23–27] it is well-known that in $Pt(A)G_2$ complexes the rate of interconversion between rotamers [which requires rotation of the nucleobases around the Pt–N(7) bonds] depends upon the steric bulk of the carrier ligand (A). This interconversion is always fast, with respect to the NMR time scale, for primary amines, but can become very slow (half-lives of hours) for secondary and tertiary amines with a rigid structure. Me_2phen is to be ascribed to this second class of ligands since the steric bulk created by the methyls in positions 2 and 9 of the phenanthroline is expected to hinder the rotation of the two acyclovir molecules about the Pt–N(7) bonds, therefore the observation of only one set of signals was quite surprising. A single set of signals would mean either a free rotation about the Pt–N(7) bonds or slow rotation and a predominance of either the *HH* or the *HT* conformers. To answer this question a similar complex containing guanosine, instead of acyclovir, was prepared.[28] The ribose rings present in the guanosine ligands are chiral and thus render the two *HT* rotamers inequivalent (although they remain C_2-symmetrical) and the *HH* rotamer nonsymmetrical (C_1 symmetry). Assuming a slow interconversion different signals should be observed for the different rotamers. The ^1H NMR spectrum of $[Pt(guo)_2(Me_2phen)]Cl_2$ in D_2O showed, in the region of H(8), two very strong and rather shielded signals (at $\delta = 8.38$ and 8.35), and two very weak and rather deshielded signals (at $\delta = 8.86$ and 8.88). Literature data indicate that the *HT* orientation is stabilised with respect to the *HH* orientation by a better dipole–dipole interaction between the two guanine bases; this must be the case also for the $[Pt(guo)_2(Me_2phen)]Cl_2$ complex. Therefore the two strong signals can be assigned to the H(8) protons of the two *HT* rotamers (ΔHT and ΛHT), while the two very weak signals can be assigned to the single, nonsymmetrical, *HH* conformer. Big changes in the isomer distribution are not expected for substitution of the ribose ring of guanosine by the 2-hydroxyethoxymethyl chain of acyclovir, therefore we can expect that the preferred isomer for **1** has an *HT* conformation. The value of the H(8) chemical shift (at rather high field) supports the above conclusion.

Additional information about the conformational rigidity of complex **1** could be gained from examination of the

Table 1. ^{1}H NMR Shifts (ppm) of the complexes (numbering of the protons as in Scheme 1)[a]

Compound	Solvent	H(4/7)	H(5/6)	H(3/8)	H/Me(2/9)	H(8)	C(1′)H$_2$	C(2′)H$_2$	C(3′)H$_{1/2}$	C(4′)H$_2$
Acyclovir	D_2O	–	–	–	–	7.91 s	5.50 s	3.67 m		–
1	D_2O	8.69 d (8.4)	8.11 s	7.71 d (8.4)	2.35 s	8.53 s	5.52 m	3.36 m	3.55 m	–
	CD_3OD	8.77 d (8.1)	8.18 s	7.78 d (8.1)	2.35 s	8.57 s	5.51 s	3.54 m		–
5	CD_3OD	8.71 d (8.1), 8.62 d (8.1)	8.07 s	7.82 d (8.1), 7.67 d (8.1)	3.34 s, 2.25 s	8.66 s	5.59 s	3.66 m		–
3	D_2O	8.28 dd (8.3, 4.4)	8.23 s	7.91 dd (8.3, 4.4)	8.95 dd (8.3, 4.4)	8.71 s	5.62 dd	3.63 m		–
Penciclovir	D_2O	–	–	–	–	7.85 s	4.18 m	1.88 m	1.68 m	3.62 m
2	D_2O	8.64 d (8.1)	8.04 s	7.69 d (8.1)	2.32 s	8.37 s	4.19 m	1.74 m	1.30 m	3.47 m
4	D_2O	8.27 d (8.2, 5.4)	8.01 s	7.89 dd (8.2, 5.4)	8.79 d (8.2)	8.60 s	4.31 m	1.88 m	1.48 m	3.54 m

[a] $J_{H\text{-}H}$ (Hz) are given in parentheses.



Head-to-*Head* Δ *Head*-to-*Tail* Λ *Head*-to-*Tail*

Scheme 2. Representations of the *HH* (*Head*-to-*Head*) and *HT* (*Head*-to-*Tail)* orientations of the two guanine bases (empty arrows) with respect to the platinum/N4 coordination plane

NMR signals belonging to the 2-hydroxyethoxymethyl chains. The assignment of the multiplet centred at δ = 5.52 to the C(1′)H$_2$ protons was straightforward. Quite more difficult was the assignment of the multiplets at δ = 3.36 and 3.55 to the C(2′)H$_2$ and C(3′)H$_2$ protons, respectively. These two methylenes give coincident signals (δ = 3.67) in free acyclovir. A two dimensional ^{13}C,^{1}H HETCOR experiment was diagnostic for their assignment (an expansion of the aliphatic region of the two dimensional ^{13}C,^{1}H spectrum is shown in Figure 2).

On the basis of characteristic values of ^{13}C chemical shifts for similar organic chains (e.g. diethylene glycol), the cross peak A (at δ = 61 on the ^{13}C axis) is assigned to C(3′)H$_2$ and the two cross peaks B (at δ = 72 on the ^{13}C axis) are assigned to C(2′)H$_2$. It is worth noting that both methylene groups bound to the ether oxygen, C(1′)H$_2$ and C(2′)H$_2$, exhibit a marked diastereotopic splitting of the CH$_2$ protons. Such a splitting is absent in the uncoordinated acyclovir and is indicative of the formation of a chiral centre in the complex. The platinum becomes a chiral centre only in the *HT* rotamers, therefore the observation of such a diastereotopic splitting also lends support to the *HT* conformation being by far the most abundant for compound **1**. Furthermore, the large value of the diastereotopic splitting indicates that the rate of interconversion between *HT* enantiomers is rather slow.

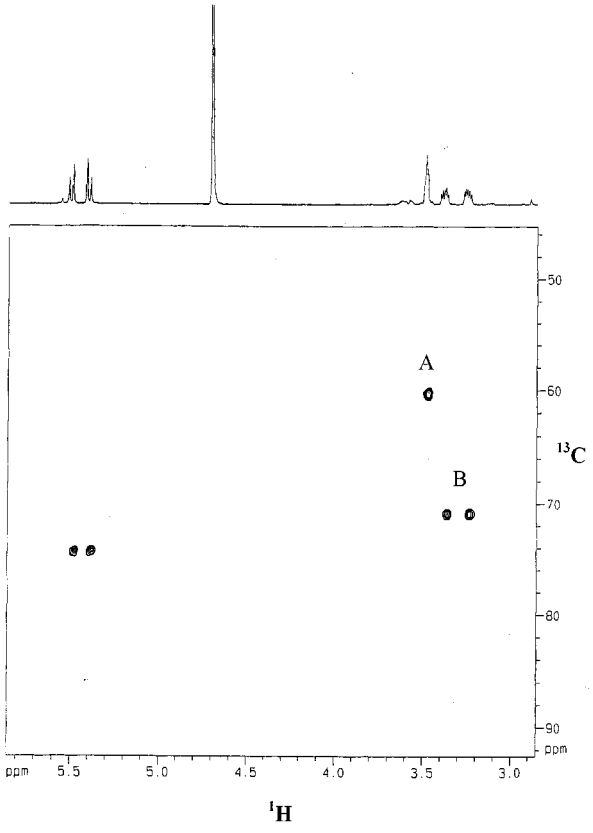


Figure 2. ^{1}H,^{13}C two dimensional (HETCOR) spectrum in D_2O of complex **1** in the region of the aliphatic protons; A = C(3′)H$_2$, B = C(2′)H$_2$

Ligand Exchange in Complex 1

Complex **1** is the only compound of the series which has been isolated with iodide as counterions. In CD_3OD (Figure 3, Table 1) compound **1** is in equilibrium with two new species: one of the new species is easily recognised as free acyclovir, the second species is characterised by having two singlets (at δ = 3.34 and 2.25) in the methyl region of the spectrum indicating that the two methyls of Me_2phen are inequivalent. Furthermore, integration of the signals indicates that the new complex contains only one molecule of

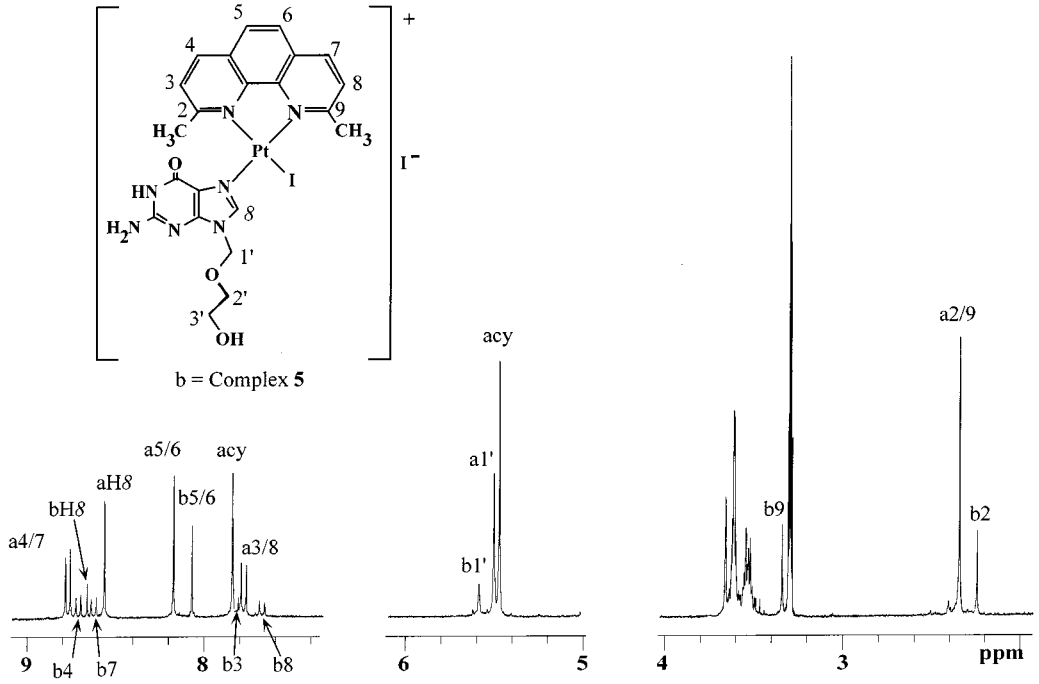


Figure 3. ^{1}H NMR spectrum in CD_3OD of complex **1**; a = complex **1**, b = complex **5**, acy = free acyclovir

acyclovir per phenanthroline and therefore must be formulated as [PtI(acy)(Me_2phen)]I (**5**) with a bidentate Me_2phen, one iodide, and one acyclovir residue in the coordination sphere of platinum.

To check this hypothesis complex **5** was prepared independently by using the same experimental conditions as for the preparation of complex **1** but adding only one equivalent of acyclovir per molecule of platinum substrate. The isolated complex **5**, fully characterised by elemental analysis and IR spectroscopy, exhibits a ^{1}H NMR spectrum in CD_3OD that is identical to that observed for the newly formed complex in the NMR spectrum of **1** in CD_3OD. Therefore we can conclude that in methanol the following equilibrium is established:

$$[Pt(Acy)_2(Me_2phen)]^{2+} + I^- \rightleftharpoons [PtI(Acy)(Me_2Phen)]^+ + Acy$$

The presence of the above equilibrium was also confirmed by addition of KI (five times the stoichiometric amount) to a solution of **1** in CD_3OD. The molar ratio **1**:**5** which was 1.75 before the addition of KI became 0.5 after the addition of KI clearly supporting the involvement of the iodide ion in the equilibrium of displacement of one acyclovir ligand from **1**.

A question to be answered before concluding this section is why the above equilibrium is observed in methanol but not in water. A reasonable explanation for this is that methanol is less efficient than water in solvating charged species and keeping them well separated. A parameter directly related to this property is the dielectric constant, which is far bigger for water (78.5) than for methanol (32.6).

Complex 2

Significant IR data for complex **2** are the C=O stretching vibration at 1691 cm^{-1} and the NO_3^- stretching vibration at 1389 cm^{-1}. A complete assignment of the ^{1}H NMR spectroscopic data is given in Table 1. The discussion given for **1** applies entirely also to **2**.

Complex 3

Significant IR data for complex **3** are the C=O stretching vibration at 1693 cm^{-1} and the NO_3^- stretching vibration at 1384 cm^{-1}.

The ^{1}H NMR spectrum of complex **3** in D_2O is shown in Figure 4 and the chemical shift values are reported in Table 1.

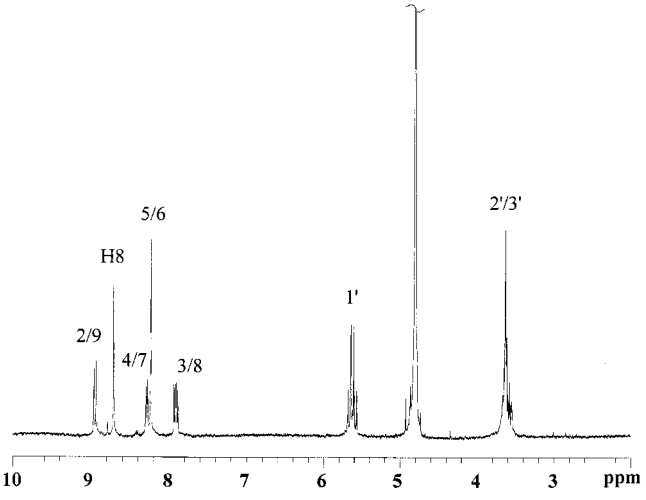


Figure 4. ^{1}H NMR spectrum in D_2O of complex **3**

As with **1**, a single set of signals was also observed for **3**. However, since the phen ligand does not contain bulky substituents in the *ortho* positions, the observation of a single set of resonances could stem from a fast interconversion (on the NMR time scale) of different rotamers (both *HH* and *HT*) and not from the presence of only the enantiomeric *HT* rotamers (as was the case for **1**). To answer this question the analogous compound containing guanosine instead of acyclovir ($[Pt(guo)_2(phen)](NO_3)_2$) was synthesised.[28] The presence of the chiral ribose renders the *HH* rotamer no longer C_s-symmetrical and causes the two *HT* rotamers, although still C_2-symmetrical, to be different from one another. The observation of two major sets of signals (belonging to the two *HT* rotamers) is clearly indicative of slow interconversion among rotamers, therefore we can conclude that the rate of interconversion among the rotamers of **3** is also slow on the NMR time scale and the single set of signals is assignable to the most favoured *HT* enantiomers. Also, the observation of a diastereotopic splitting for the $C(1')H_2$ protons, similar to that seen for complex **1**, supports the idea of a slow interconversion between the *HT* enantiomers of complex **3** in D_2O.

The steric bulk of phen is much smaller than that of Me_2phen (the deviations from the planar geometry caused by the steric interaction between the *cis* ligands is very large in platinum complexes with Me_2phen but is undetectable in platinum complexes with phen); however, the rigidity of the phen ligand does not permit any relief of the steric interaction which builds up when the rotating G comes across the *ortho* hydrogens of the phenanthroline.

Complex 4

Significant IR data for complex **4** are the C=O stretching vibration at 1692 cm^{-1}, and the NO_3^- stretching vibration at 1384 cm^{-1}. The complete assignment of the ^1H NMR spectroscopic data for **4** is given in Table 1. The discussion given for **3** applies also to **4**.

Stability of the Complexes in Physiological Medium

The observation that the iodide counter ion is capable of displacing the coordinated acyclovir from **1** prompted us to investigate the chemical stability of these compounds in physiological medium. Therefore **1**, **2**, **3**, and **4** were each dissolved in D_2O containing 0.1 M NaCl and ^1H NMR spectra were measured over a period of time.

Complexes **1** and **2** were found to be very stable over a period of two weeks. On the other hand, complexes **3** and **4** underwent ligand dissociation equilibria similar to that observed for **1** in methanol (entering of a chloro ligand and displacement of one purine base). After ten days the concentration of **3** and **4** was slightly less than 80% of the initial concentration.

This unexpected result (greater stability of the more sterically hindered compounds **1** and **2**) is probably a consequence of the *ortho* substituents of neocuproine shielding the metal centre and inhibiting the nucleophilic attack of an entering chloride upon the platinum atom.[29]

The displacement of a purine base by a chloride ion in saline solution may have some relevance in relation to the stability of the major DNA adduct of cisplatin in which two purines are cross-linked by a $Pt(NH_3)_2$ moiety.

Biological Activity of Compounds 1−4

While the cytostatic activity of compounds **1**−**4** is still under investigation, preliminary results on their antiviral activity have already been obtained. So far the antiviral activity of the four hybrid drugs has been tested against the Human Immunodeficiency Virus (HIV) and more precisely against HIV-1(IIIB) and HIV-2(ROD) viruses in MT_4 cells. An automated in vitro tetrazolium-based colorimetric assay was used to screen the inhibitory activity of the drugs.[30] The results are reported in Table 2.

Table 2. Anti-HIV-1(IIIB) and HIV-2(ROD) activity and cytotoxicity properties of compounds **1**−**4**, free acyclovir, and penciclovir in MT_4 cells

Compound	HIV Strain	EC_{50} [a]	IC_{50}[b]	SI[c]
1	IIIB	>1.81	1.81	<1
	ROD	>2.47	2.47	<1
2	IIIB	>6.02	6.02	<1
	ROD	>11.8	11.8	<1
3	IIIB	>132	>132	–
	ROD	>132	>132	–
4	IIIB	>124	>124	–
	ROD	>124	>124	–
Acyclovir	IIIB	>555	>555	–
	ROD	>555	>555	–
Penciclovir	IIIB	>493	>493	–
	ROD	>493	>493	–

[a] EC_{50} is defined as the concentration of compound (μM) which inhibits 50% of the viral replication. – [b] IC_{50} is defined as the concentration of compound (μM) which reduces the viability of the treated cells to 50% of that of untreated cells. – [c] SI (Selective Index) = IC_{50}/EC_{50}.

In general, a drug is considered to be active against HIV-1 and HIV-2 viruses if the EC_{50} is ≤1 μM.[3,30,31] None of the tested compounds exhibited anti-HIV activity at subtoxic concentrations. It should be noted, however, that the free acyclovir and penciclovir are also deprived of anti-HIV activity. Therefore a better indication of a synergistic effect, if any, stemming from the coupling of antiviral nucleotides with cisplatin-like substrates will come from anti-HSV and antitumor assays which are in progress.

However, an interesting result stemming from these preliminary experiments is the cytotoxic activity against MT_4 cells, which is far greater for compounds **1** and **2** than for compounds **3** and **4**. The two couples of compounds have a similar charge (+2) and, presumably, similar cell permeability. Therefore, their different behaviour is to be ascribed to a different reactivity in the biological medium determined by the presence in **1** and **2** of two methyl substituents in the *ortho* positions of the phenanthroline.

Conclusions

Platinum(II) complexes containing a bidentate phenanthroline and the antiviral agents acyclovir and penciclovir

have been synthesised and characterised. Both the Me_2phen and the phen carrier ligands are able to hinder rotation of the guanine bases about the Pt−N(7) bond with the consequence that the interconversion between rotamers is very slow on the NMR time scale. This was expected for Me_2phen but was rather unexpected for phen and is a clear demonstration that in addition to the steric bulk the rigidity of the carrier ligand can also deeply influence the rate of interconversion among rotamers in $Pt(A)G_2$ complexes. The rigidity of the phen ligand does not allow any relief of the steric interaction that builds up when the rotating G comes across the *ortho* protons of phen.

The different behaviour in water and methanol of **1** indicates that the solvating ability and the dielectric constant of the solvent play an important role in the stabilisation of cationic platinum species in the presence of coordinating anions such as Cl^- or I^-. In this context, an unexpected result was the greater stability in water solution and in the presence of 0.1 M NaCl of complexes with neocuproine (**1** and **2**) with respect to those with phenanthroline (**3** and **4**). The higher stability of the former complexes is probably favoured by the shielding of the metal centre by the methyl substituents in positions 2 and 9 of the phenanthroline which prevents the chloride ions from entering into the coordination sphere of the metal.

Although complexes **1**−**4** are not active against HIV-1(IIIB) and HIV-2(ROD) viruses; nevertheless there are two important observations: i) the use of neocuproine (a phenanthroline with methyl substituents in positions 2 and 9) instead of unsubstituted phenanthroline can increase the cytotoxicity of the compounds by two order of magnitude, ii) the increase in cytotoxicity appears to be in relation to the greater stability in the biological medium of the neocuproine derivatives.

Experimental Section

Physical Measurements: Elemental analyses were performed on a Elemental Analyzer mod. 1106 Carlo Erba Instrument. IR Spectra were recorded on a spectrophotometer Perkin−Elmer Spectrum One using KBr as solid support for pellets. ^{1}H NMR spectra were recorded on a Bruker AM-WB 300 MHz instrument. Chemical shifts were referred to TMS by using the residual protic solvent peaks as internal references (δ = 4.80 for D_2O and δ = 3.30 for CD_3OD). ^{13}C,^{1}H HETCOR two dimensional experiments were performed on a DRX 500 MHz WB Avance Bruker Instrument with D_2O as solvent and internal CH_3OH as reference for ^{13}C (δ = 50.2).

Starting Materials: Commercial reagent grade chemicals, 2,9-Me_2-1,10-phenanthroline (Me_2phen, neocuproine), 1,10-phenanthroline (phen), guanosine (guo), DMSO and DMF, were used without further purification.

$[PtCl_2(DMSO)_2]$,[32] $[PtX_2(Me_2phen)]$ (X = Cl, I),[33] $[Pt(guo)_2(Me_2phen)]Cl_2$, $[Pt(guo)_2(phen)](NO_3)_2$,[28] acyclovir (acy) and penciclovir (pen)[34] were prepared by previously reported procedures.

Preparation of $[PtI_2(phen)]$: A suspension of $[PtCl_2(DMSO)_2]$ (300 mg, 0.71 mmol) in methanol was treated with a large excess of KI (581 mg, 3.5 mmol). After stirring for one day, the initially yellow suspension became an orange solution containing the dimeric complex $[Pt_2I_4(DMSO)_2]$. This solution was treated with phen (140 mg, 0.71 mmol) and left stirring for one hour during which time a yellow-orange precipitate was formed. The precipitate was collected by filtration of the mother solution, the filtrate was concentrated to half its volume by evaporation of the solvent under reduced pressure and kept at room temperature for a few days during which time another yellow precipitate was formed. The collected solid fractions were washed with methanol and dried under vacuum. Yield 424 mg (95%) based on the starting platinum complex. − $C_{12}H_8I_2N_2Pt$ (629.10): calcd. C 22.9, H 1.3, N 4.5; found C 22.6, H 1.3, N 4.4.

Preparation of $[Pt(acy)_2(Me_2phen)]I_2$ (1): A solution containing $[PtI_2(Me_2phen)]$ (102 mg, 0.155 mmol) dissolved in CH_2Cl_2/CH_3OH (200 mL; 1:1, v/v) was treated with acyclovir (70 mg, 0.31 mmol). After stirring for 2 hours, the initially orange solution became yellow. The solvent was evaporated under vacuum and the yellow residue was suspended in 100 mL of water and stirred overnight. The water solution was then filtered and taken to dryness by evaporation of the solvent under vacuum. The yellow residue proved to be the salt $[Pt(Me_2phen)(acy)_2]I_2$. Yield 178 mg (90%). − $C_{31}H_{36}Cl_2I_2N_{12}O_6Pt$ (**1**·CH_2Cl_2) (1192.5): calcd. C 30.1, H 3.0, N 13.2; found C 30.8, H 2.9, N 13.7.

Preparation of $[PtI(acy)(Me_2phen)]I$ (5): $[PtI_2(Me_2phen)]$ (82 mg, 0.125 mmol) was dissolved in CH_2Cl_2 (70 mL) and treated with a stoichiometric amount of acyclovir (28.4 mg, 0.126 mmol) previously dissolved in 70 mL of CH_3OH. The yellow solution was left stirring for one day and then concentrated to 50 mL by evaporation of the solvent under vacuum. Addition of diethyl ether (300 mL) caused the precipitation of a yellow solid, which was separated by filtration of the solution, carefully washed with ether, and dried under vacuum. Yield 109 mg (90%). − $C_{23}H_{25}Cl_2I_2N_7O_3Pt$ (**5**·CH_2Cl_2) (967.29): calcd. C 28.6, H 2.6, N 10.1; found C 28.1, H 2.4, N 10.2.

Preparation of $[Pt(pen)_2(Me_2phen)](NO_3)_2$ (2): A solution of $[PtCl_2(Me_2phen)]$ (149 mg, 0.31 mmol) in DMF (75 mL) was treated with $AgNO_3$ (109 mg, 0.628 mmol) previously dissolved in a minimum amount of water (1 mL). After stirring in the dark for 12 h at room temperature, the solution was filtered through celite to remove AgCl. The filtered yellow solution was treated with penciclovir (157 mg, 0.62 mmol) and stirred for 4 h. Addition of diethyl ether (300 mL) caused the formation of a creamy precipitate. The precipitate was collected by filtration of the solution, washed with acetone and ether, and dried under vacuum. The crude product was extracted with 100 mL of water, the solution was filtered and taken to dryness by evaporation of the solvent under vacuum. Yield 249 mg (75%). − $C_{34}H_{46}N_{14}O_{14}Pt$ (**2**·$2H_2O$) (1069.9): calcd. C 38.2, H 4.3, N 18.4; found C 38.1, H 4.3, N 18.4.

Preparation of $[Pt(acy)_2(phen)](NO_3)_2$ (3) and $[Pt(pen)_2(phen)](NO_3)_2$ (4): $[PtI_2(phen)]$ (150 mg, 0.24 mmol) was dissolved in DMF (80 mL) at 50 °C and treated with $AgNO_3$ (81.3 mg, 0.48 mmol). The solution was cooled to room temperature and stirred in the dark overnight. The solution was filtered through celite to remove the yellow AgI precipitate and treated with a stoichiometric amount of acyclovir (107 mg, 0.48 mmol) for complex **3** and penciclovir (120 mg, 0.48 mmol) for complex **4**. The solution was left stirring for 4 h and then treated with diethyl ether to induce the precipitation of the platinum complex. The solid was collected by filtration of the solution, washed with acetone and ether, and finally dried under vacuum. The crude product was extracted with

water (40 mL), the solution was filtered and taken to dryness under vacuum to give pure compounds **3** and **4**, respectively. Yields 189 mg (80%) and 200 mg (80%) for complex **3** and **4**, respectively. − $C_{28}H_{36}N_{14}O_{15}Pt$ (**3**·3H_2O) (1003.8): calcd. C 33.5, H 3.6, N 19.5; found C 33.2, H 3.2, N 18.9. − $C_{32}H_{44}N_{14}O_{15}Pt$ (**4**·3H_2O) (1059.3): calcd. C 36.3, H 4.2, N 18.5; found C 35.6, H 3.7, N 18.3.

Biological Tests: The antiviral assays were carried out according to the method reported in ref.[35]

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