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New Coordination Modes of L-Ascorbic Acid and Dehydro-L-ascorbic Acid as Dianionic Chelating Ligand for Platinum

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A variety of coordination modes of L-ascorbic acid as an anionic bidentate ligand has been exploited to prepare platinum(II) complexes **1**–**7** that contain phosphanes or *R*,*R*-dach (1*R*,2*R*-diaminocyclohexane) as neutral ligands in which O2, O3, O5, O6 and C2 act as anionic donating functionalities. An alternative synthetic route to known O2,O3 complexes is proposed, and their solubility in water has been enhanced by introducing PTA (1,3,5-triaza-7-phosphaadamantane) as a neutral ligand. A new coordination mode of ascorbic acid (O2 and O3 protected) as an O5,O6-diolate chelating ligand has been characterised in solution by NMR spectroscopy and in the solid state by X-ray crystallography. The first example of a platinum complex that contains dehydroascorbic acid, **7**, has also been prepared and its X-ray crystal structure has been determined. The antiproliferative activity in vitro of complexes **1**–**7** has been tested, and the best values were obtained for the DHA complex **7**, which was found to be more active than cisplatin on both a cisplatin-sensitive and a cisplatin-resistant cell line.

Introduction

The lack of efficacy of chemotherapies for brain tumours arises mainly because of the difficulty in crossing the blood-brain barrier. This is a cause of major frustration, but it also represents a great challenge in the search of new approaches for delivering drugs inside the brain.[1]

Among the strategies for the delivery of drugs to the central nervous system (CNS), there is now a wide interest in the so-called "trojan horse tactic", which is performed by attaching an active drug molecule to a vector that accesses a specific transport mechanism.

An example of this strategy was reported by Manfredini et al. who illustrated the effectiveness of the anticonvulsant drug nipecotic acid in inhibiting inducted convulsions when conjugated to ascorbic acid, by exploiting ascorbate specific transporters.[2]

The interaction of transition metals with L-ascorbic acid, a natural, low-cost molecule, has been extensively studied,[3] mainly because ascorbic acid is widely used as a nontoxic reducing agent. A few coordination modes to platinum(II) have been described, and the potential anticancer activity of the resulting complexes has been mentioned,[4] as well as the action of ascorbic acid as a cellular reducing agent of anticancer Pt^{IV} complexes.[5]

Nevertheless, the above-mentioned discovery of the ability of ascorbic acid to act as a carrier for drugs across the brain-blood barrier made us reconsider its binding ability to metal ions with known medicinal activity with the perspective of developing metal-containing drugs that are able to take advantage of ascorbic acid specific transporters (carrier-mediated transport). Moreover, also the oxidised form, dehydroascorbic acid (DHA), can be taken into consideration as a carrier; in fact, it was reported that DHA crosses the blood-brain barrier through the glucose transporters GLUT1 and GLUT2.[6]

The application of this approach to platinum-based drugs would be of paramount importance because it is known that cisplatin, one of the most successful anticancer drugs in clinical use, is unable to pass the brain-blood barrier, and it is therefore inactive against brain tumours;[7] for this reason, we present in this paper a complete picture of the coordination chemistry of ascorbic acid to platinum and the first example of a DHA–metal complex.

Results and Discussion

Following the most successful pattern of efficacious platinum anticancer drugs, i.e. two neutral and two anionic ligands in a *cis* disposition, we have considered several possibilities of introducing L-ascorbic acid and its oxidised form, DHA, as a dianionic bidentate ligand on platinum.

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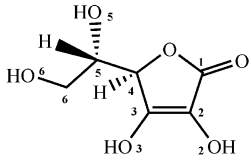
All the reported complexes were obtained from Pt precursors bearing amines or phosphanes as neutral ancillary ligands, in addition to easily replaceable anionic ligands.



L-ascorbic acid

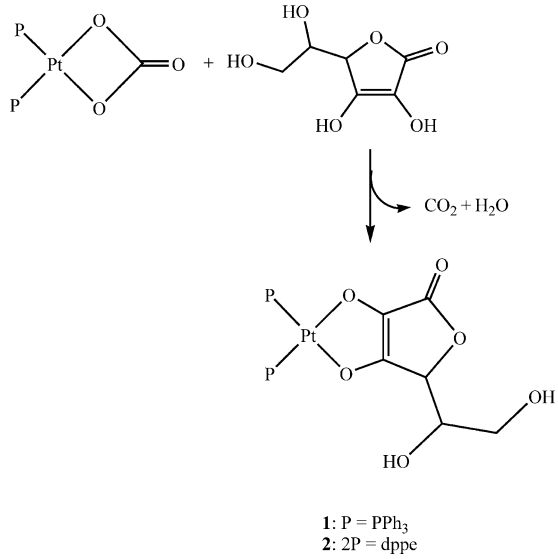
We describe here three coordination modes of L-ascorbic acid as a dianionic chelating ligand: (i) through deprotonated atoms O2 and O3, (ii) through deprotonated atoms O5 and C2, (iii) through deprotonated atoms O5 and O6 (this requires the protection of the atoms O2 and O3).

O2,O3 Coordination – A New Route to Known Complexes

The coordination of an alcoholic oxygen atom (hard donor) to platinum (soft acid) is thermodynamically disfavoured and occurs exclusively when it is supported by an additional driving force like the precipitation of an insoluble side product or the development of a volatile side product.

When ascorbic acid reacts with a platinum precursor under such conditions, the reacting groups are selectively O2–H and O3–H, because the ene–diolic system makes them more nucleophilic than other groups in the molecule. The formation of O2,O3-coordinated platinum complexes has already been obtained through the reaction of phosphane–nitrato precursors[4] or through the reaction of a dihydroxy Me_3PPt complex[8] and ascorbic acid. We obtained O2,O3-coordinated ascorbate–platinum complexes (Scheme 1), with better yields and with an easier work up procedure, by treating ascorbic acid in dichloromethane with Pt–carbonate precursors, which are known to react with vicinal diols, as described by Andrews.[9]

The complex [Pt(O2,O3-asc)(PPh_3)$_2$] (**1**) was recovered after solvent evaporation as the only product, and it was characterised by NMR spectroscopy. The ^{31}P NMR spectrum in $CDCl_3$ shows two doublets with satellites assigned to the two nonequivalent phosphanes that are *trans* to the two oxygen atoms at δ = 9.7 and 6.9 ppm with $^2J_{P,P}$ = 20 Hz. The coupling constants $^1J_{Pt,P}$ of 3490 Hz and 3776 Hz, respectively, are typical of a PPh_3 group *trans* to an oxygen atom.[4] By comparison with the data of complex **5** (see below), we assign the larger Pt–P coupling constant to a P atom *trans* to O3. The IR spectrum shows a broad signal between 3500 and 3100 cm^{-1}, which can be assigned to the hydroxy groups O5–H and O6–H. The ^{1}H NMR spectrum in $CDCl_3$ shows a doublet at δ = 4.30 ppm for the proton 4-H, while the multiplets at δ = 3.60 and 3.30 ppm are assigned to the proton 5-H and to the two diastereotopic protons 6-H. The chemical shifts of these signals are very similar to the corresponding signals in free ascorbic acid, because they belong to protons that are located far away from the coordination site.



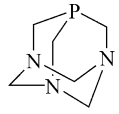
1: P = PPh_3
2: 2P = dppe

Scheme 1.

The complex [Pt(O2,O3-asc)(dppe)] [**2**, dppe = 1,2-bis(diphenylphosphanyl)ethane], obtained from [Pt(CO_3)(dppe)], shows two doublets at δ = 32.8 and 29.2 ppm with $^1J_{Pt,P}$ = 3364 and 3664 Hz, respectively, in its ^{31}P NMR spectrum. Further, in this case, the phosphorus atom *trans* to O3 is assigned by the value of the coupling constant $^1J_{Pt,P}$, which is 300 Hz larger than the value of the other coupling constant. The ^{1}H NMR spectrum in $CDCl_3$ is similar to that of **1**, with the exception of the presence of a multiplet at δ = 2.40 ppm, which is assigned to the CH_2 protons of dppe. In the IR spectrum, a broad band between 3500 cm^{-1} and 3100 cm^{-1} confirms the presence of OH at positions 5 and 6.

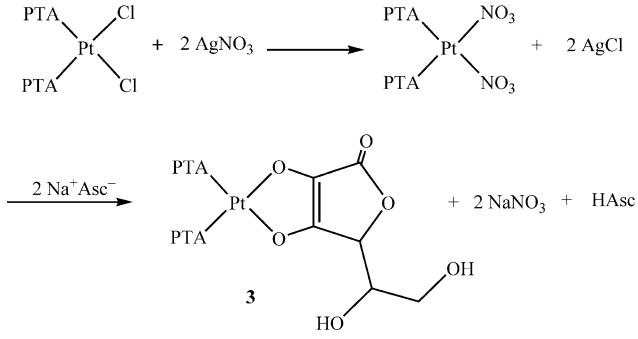
Complexes **1** and **2** are stable in the solid state and in organic solvent solutions. Isomerisation to the C2,O5 coordination has never been observed.

Complex **3** is another example of the O2,O3 coordination mode that we obtained, which contains the water-soluble phosphane PTA (triazaphosphaadamantane). There is an increasing interest in coordination compounds of this phosphane and in their use in medicinal chemistry because of their favourable properties like hydrophilicity.[10]



PTA = 1,3,5-triaza-7-phosphaadamantane

Complex **3** was prepared from the known precursor *cis*-[$PtCl_2(PTA)_2$],[11] subsequently converted in the nitrato species by treatment with silver nitrate in water. Sodium ascorbate was then added directly to the solution, and complex **3** was promptly formed, as confirmed by ^{31}P NMR analysis (Scheme 2).



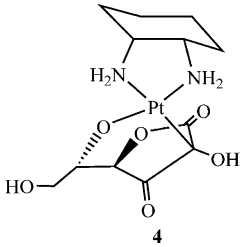
Scheme 2.

In the ^{31}P NMR spectrum of **3** in D_2O, two doublets with satellites at δ = –58.34 and –58.71 ppm with $^1J_{Pt,P}$ = 3302 and 3343 Hz, respectively, are observed. The ^1H NMR spectrum consists of a doublet at 4.42 for the proton 4-H with $^3J_{H,H}$ = 2 Hz, while the protons 5-H and 6-H give rise to a triplet of doublets at δ = 3.93 ppm with $^3J_{H,H}$ = 7 Hz and $^4J_{H,H}$ = 2 Hz and a doublet of doublets at δ = 3.65 ppm with $^3J_{H,H}$ = 10.5 Hz and $^4J_{H,H}$ = 2 Hz, respectively.

The aqueous solution of **3** does not show any sign of decomposition in 10 d, but it is reconverted to the dichlorido complex after 48 h in the presence of an excess (4 equiv.) of added chloride.

C2,O5 Coordination – Known Complex

It has been reported that amine–Pt precursors such as $[Pt(NH_3)_2(OH_2)_2]^{2+}$ or $[Pt(R,R\text{-dach})(OH_2)_2]^{2+}$ react in water with ascorbate giving C2,O5 coordination complexes.[4,12] This coordination mode seems to be precluded with phosphanic precursors, probably because of steric interactions.[4] On the contrary, any attempts to obtain the O2,O3 coordination with amine–Pt precursors invariably gave C2,O5 complexes. The complex [Pt(C2,O5-asc){*trans*-(*R*,*R*-dach)}] (**4**) was prepared as reported by Hollis.[13]



4

Although the solubility of **4** in water is less then 1 mg/mL, it is possible to acquire the ^1H NMR spectrum in D_2O, which is diagnostic for the coordination mode by careful comparison of the signals of protons 4-H, 5-H and 6-H in free, salified and coordinated ascorbic acid, as reported in Table 1. The ^1H NMR spectrum in D_2O does not reveal any sign of decomposition of complex **4** after 10 d, even when NaCl is added.

Table 1. ^1H NMR spectroscopic data in D_2O.

	4-H	5-H	6-H
Complex **4**	4.27 ppm (s)	4.08 ppm (t) $J_{H,H}$ = 6.5 Hz	3.42 ppm (d) $^3J_{H,H}$ = 6.5 Hz
Ascorbic acid	$^3J_{H,H}$ = 1.5 Hz 4.90 ppm (d) $^3J_{H,H}$ = 1.5 Hz	$^3J_{H,H}$ = 6.4 Hz; 3.78 ppm (td) $^3J_{H,H}$ = 6.4 Hz; $^3J_{H,H}$ = 1.5 Hz	$^3J_{H,H}$ = 6.4 Hz 3.72 ppm (d) $^3J_{H,H}$ = 6.4 Hz
Sodium ascorbate	4.41 ppm (d) $^3J_{H,H}$ = 1.6 Hz	3.90 ppm (td) $^3J_{H,H}$ = 7.5 Hz; $^3J_{H,H}$ = 1.6 Hz	3.73 ppm (d) $^3J_{H,H}$ = 7.5 Hz

The infrared spectrum of **4** presents a broad band between 3550 and 2860 cm^{-1} assigned to the O2–H, O6–H and NH_2 stretching modes, a strong signal at 1716 cm^{-1} assigned to the C=O stretching mode and two signals at 1630 and 1590 cm^{-1} for the C2=C3 stretching mode.

O5,O6 Coordination of Protected Ascorbic Acid – A New Coordination Mode

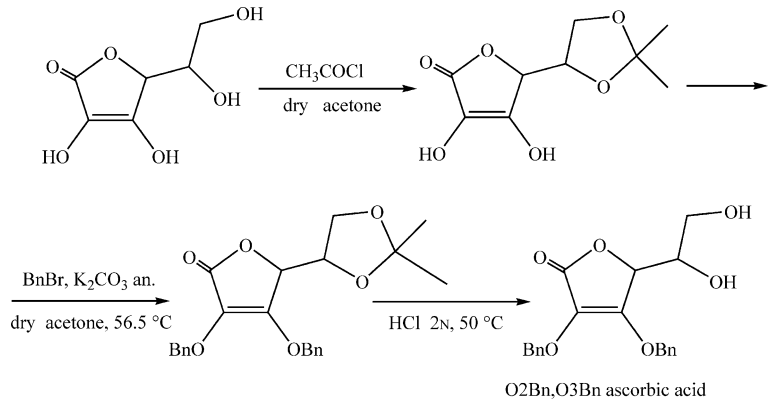
From the information on the interaction between L-ascorbic acid and its specific transporter through the blood-brain barrier, the integrity of the ene–diolic side seems to be crucial.[14] It is therefore of interest to drive the coordination of Pt elsewhere, for example, towards the diolic O5 and O6 atoms.

The coordination of the oxygen atoms of O5–H and O6–H of ascorbic acid to a metal has never been reported before. Because of the more acidic character of O2–H and O3–H, it is clear that protection of the groups is required in order to avoid their coordination with high specificity as shown above.

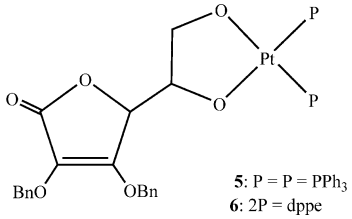
All the steps for the protection of O2–H and O3–H with benzyl groups have been reported previously[15] and are described in Scheme 3.

The reaction between O2Bn,O3Bn ascorbic acid and the carbonate precursor $[Pt(CO_3)(PPh_3)_2]$ gave complex **5**. Also in this case, the ^{31}P NMR spectrum in acetone shows two close signals with satellites at δ = 13.7 ppm ($^1J_{Pt,P}$ = 3512 Hz) and 12.8 ppm ($^1J_{Pt,P}$ = 3372 Hz) coupled each other with $^2J_{P,P}$ = 21.4 Hz. The NMR spectroscopic observation over a long period indicates that **5** is unchanged for 20 d in acetone, while it is rapidly converted to the dichloride complex *cis*-$[PtCl_2(PPh_3)_2]$ in the presence of added Cl^-. Comparison of the ^{31}P NMR spectroscopic data of **5** (O5,O6 complex) and **2** (O2,O3 complex) shows that, although the patterns appear to be very similar, it is possible to distinguish between the two coordination modes by careful analysis of the ^{31}P NMR spectroscopic data.

The analogue dppe complex **6** was obtained in the same way and characterised by NMR spectroscopy. The ^{31}P NMR spectrum in [D_6]acetone shows two doublets with satellites at δ = 29.7 and 29.4 ppm with $^1J_{Pt,P}$ = 3280 and 3229 Hz, respectively, and $^2J_{P,P}$ = 9.0 Hz. The ^1H NMR spectrum of **6** in [D_6]acetone shows four signals at 5.39, 5.22, 4.77 and 4.72 ppm assigned to the CH_2Ph protons, a



Scheme 3.



multiplet at δ = 4.44, 4.14, 3.89 and 3.66 ppm (for 4-H, 5-H, 6-H and 6′-H, respectively) together with the signal assigned to the CH_2 of dppe at δ = 2.80 ppm.

The recrystallisation of crude **6** from acetone and diethyl ether gave crystals suitable for X-ray structure determination (Figure 1). This is the first reported X-ray crystal structure for an O5,O6-coordinated ascorbic acid derivative. In Table 2 a selection of bond lengths and angles for **6** is reported.

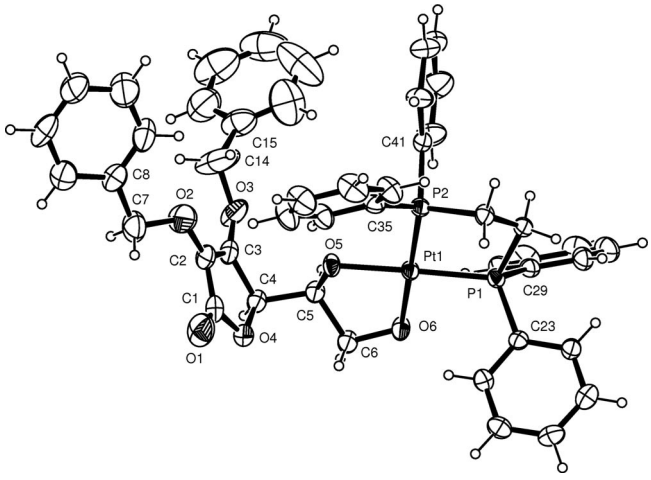


Figure 1. ORTEP[16] view of complex **6**. The thermal ellipsoids are at 30% level of probability.

The structure of complex **6** shows that the coordination around platinum is a slightly distorted square planar – the Pt1 atom is displaced from the mean plane passing through the basal atoms P1, P2, O5 and O6 by 0.0024(2) Å. The C1–C2–C3–C4–O4 ring is essentially planar and forms an angle of 85.7(2)° with the coordination plane. The two coordination rings Pt1–O5–C5–C6–O6 and Pt1–P1–C21–C22–P2 exhibit twisted ($^3T_4$) and envelope (E_4) conformations, respectively.

Table 2. Selected bond lengths [Å] and angles [°] for complex **6**.

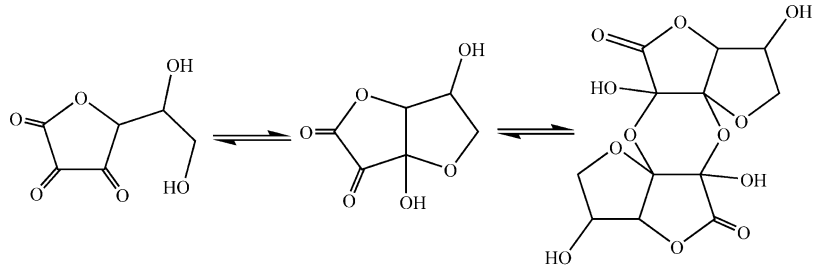
Bond lengths			
Pt1–P1	2.222(1)	Pt1–P2	2.220(1)
Pt1–O5	2.028(3)	Pt1–O6	2.028(3)
C5–O5	1.410(5)	C6–O6	1.412(6)
C5–C6	1.533(7)	C5–C4	1.524(7)
C4–C3	1.484(7)	C4–O4	1.421(7)
C1–O4	1.366(8)	C1–O1	1.221(10)
C1–C2	1.436(10)	C2–C3	1.330(9)
C2–O2	1.387(8)	C3–O3	1.335(8)
Angles			
P1–Pt1–P2	85.8(1)	P1–Pt1–O5	177.5(1)
P1–Pt1–O6	94.3(1)	P2–Pt1–O5	96.6(1)
P2–Pt1–O6	179.6(1)	O5–Pt1–O6	83.3(1)
Pt1–O5–C5	108.9(2)	Pt1–O6–C6	109.0(3)
O5–C5–C6	108.0(3)	O6–C6–C5	108.7(4)

In order to exploit the ene–diolic system of ascorbic acid with respect to the interaction with its specific transporter, it is necessary to deprotect the O2 and O3 atoms in complexes **5** and **6** after coordination to platinum.

Unfortunately, every attempt to remove the benzylic groups with known procedures induced decomposition of the complex. In particular, the reductive route with H_2/Pd produced a mixture of several Pt-containing species, probably Pt-hydride. The alternative debenzylation with BCl_3 at –78 °C[17] did not also work, because the oxygen donors are replaced by chloride ions in the complex as a result of the HCl formed in situ, and only *cis*-[$PtCl_2(PPh_3)_2$] was identified in the reaction mixture.

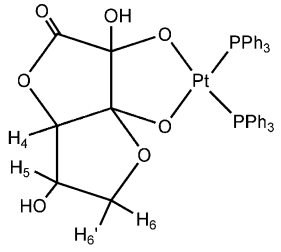
Coordination of Dehydro-L-ascorbic Acid

The commercially available DHA is a crystalline solid and is available in the monomeric form (a) or the dimeric form (c), whereas in solution, there is an equilibrium between the monomeric diketonic form (a), the monomeric hemiacetalic form (b) and the dimeric form (c). The ratio between the three forms depends on the solvent and on the preparation procedure (Scheme 4).[18]



Scheme 4.

The reaction of DHA with $[Pt(CO_3)(PPh_3)_2]$ was performed because we wanted to check whether the major reactivity of O6–H with Pt would have shifted the equilibrium towards the monomeric form (a) to give the complex in which O5 and O6 are bonded to Pt. Unexpectedly, the reaction gave a single product **7**, which corresponds to the coordination of form (b) in addition to one molecule of water.



7

The ^{31}P NMR spectrum of **7** in $CDCl_3$ shows two doublets with satellites at δ = 12.1 ppm ($^1J_{Pt,P}$ = 3617 Hz, $^2J_{P,P}$ = 24 Hz) and 8.3 ppm ($^1J_{Pt,P}$ = 3553 Hz, $^2J_{P,P}$ = 24 Hz). The ^1H NMR spectrum in $CDCl_3$ presents a singlet at δ = 4.72 ppm for the proton 4-H, a doublet at δ = 4.20 ppm ($^2J_{H,H}$ = 10 Hz) for the proton 5-H and two doublets at δ = 3.95 and 3.90 ppm for the diastereotopic protons 6-H and 6′-H.

Complex **7** is stable in various solvents (in CH_2Cl_2, $CHCl_3$ and DMSO no decomposition was detected by ^{31}P NMR spectroscopy after 3 d). In DMSO in the presence of NBu_4Cl (3 equiv.), only traces of *cis*-$[PtCl_2(PPh_3)_2]$ were detected by ^{31}P NMR spectroscopy after 18 h. Complex **7** was recrystallised from acetone and ether to give crystals suitable for X-ray diffraction. An ORTEP projection of the molecule is shown in Figure 2. Table 3 reports a selection of bond lengths and angles for **7**.

The projection presented in Figure 2 indicates that the Pt has an almost distorted square-planar coordination geometry, and the Pt1 atom is displaced from the mean plane passing through the basal atoms P1, P2, O2′ and O3′ by 0.0089(2) Å. The conformation of the coordination ring Pt1–O2′–C2–C3–O3′ is twisted ($^4T_5$), and the other two five-membered rings, C1–C2–C3–C4–O4 and C3–C4–C5–C6–O3, assume twisted ($^3T_2$) and envelope (E_5) conformations, respectively. The O2–H2 and O5–H5 hydroxy groups give rise to intermolecular hydrogen bonds whose parameters are reported in Table 4.

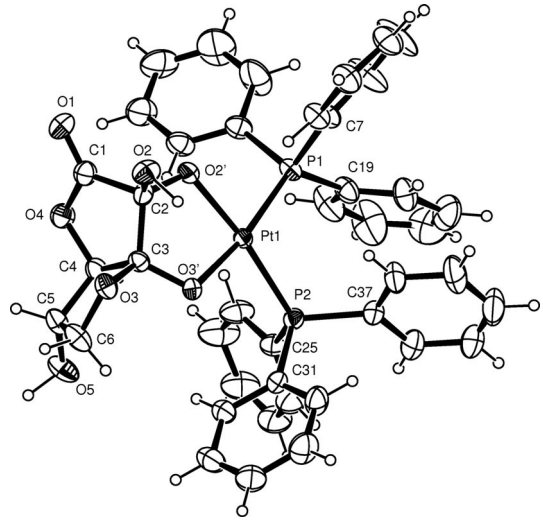


Figure 2. ORTEP view of complex **7**. The thermal ellipsoids are at 30% level of probability.

Table 3. Selected bond lengths [Å] and angles [°] for complex **7**.

Bond lengths			
Pt1–P1	2.255(1)	Pt1–P2	2.235(1)
Pt1–O2′	2.044(3)	Pt1–O3′	2.027(3)
C2–O2′	1.379(6)	C3–O3′	1.365(5)
C2–C3	1.537(7)	C2–O2	1.401(6)
C2–C1	1.534(7)	C3–O3	1.437(5)
C3–C4	1.562(6)	C4–O4	1.450(6)
C4–C5	1.518(7)	C1–O4	1.359(7)
C1–O1	1.196(6)	C5–C6	1.524(7)
C5–O5	1.412(7)	C6–O3	1.426(6)
Angles			
P1–Pt1–P2	99.9(1)	P1–Pt1–O2′	88.2(1)
P1–Pt1–O3′	170.7(1)	P2–Pt1–O2′	171.9(1)
P2–Pt1–O3′	89.4(1)	O2′–Pt1–O3′	82.6(1)
Pt1–O2′–C2	112.7(3)	Pt1–O3′–C3	107.1(2)
O2′–C2–C3	109.9(4)	O3′–C3–C2	113.8(4)

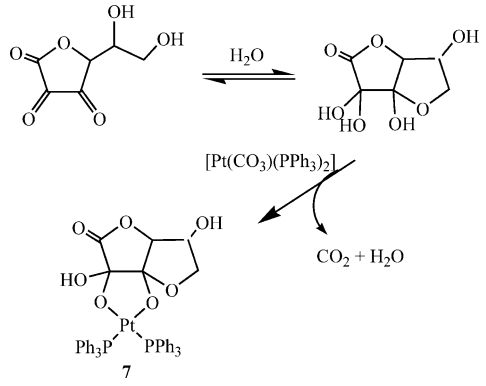
Table 4. Structural parameters of the intermolecular hydrogen bonds of complex **7**.

D–H···A	D–H [Å]	D···A [Å]	H···A [Å]	D–H···A [°]
O2–H2···O5^{i}	1.08(8)	2.924(6)	1.97(8)	145(6)
O5–H5···O3^{ii}	1.00(4)	2.808(5)	1.81(4)	175(3)

i: x – 1/2, $-y$ – 1/2, $-z$; ii: x + 1/2, $-y$ – 1/2, $-z$

Complex **7** is the first reported example of the coordination of the hemiketalic form of dehydroascorbic acid. This coordination locks this form in a very stable mode. This molecule could be of great interest for structural studies on the various forms of dehydroascorbic acid involved in the redox mechanism that makes ascorbic acid one of the most diffuse antioxidant in biological and synthetic systems.[19]

We propose a hypothesis for the formation of **7**, which is depicted in Scheme 5. Form (a) is in equilibrium with its hydrated hemiketalic form, which is intercepted by $[Pt(CO_3)(PPh_3)_2]$ to give complex **7**, with concomitant release of CO_2 and water. The formation of **7** is probably driven by the development of CO_2 and by the formation of three condensed five-membered rings. This reaction gives rise to two new chiral centres. Although several isomeric forms of **7** are possible, the reaction product always appears as a single species (NMR observation). The same product is obtained when the dimeric form of DHA (c) is used, but in this case, the process is much slower (10 d versus 3 h).



Scheme 5.

Cell Growth Inhibition of Complexes 1–7

In order to check if platinum complexes containing ascorbic acid are promising candidates for the development of anticancer drugs, we tested their antiproliferative activity in vitro on two human tumoural cell lines, the cisplatin-sensitive T2 and the cisplatin-resistant SKOV3 cell lines. The results of the cell growth inhibition obtained with complexes **1**–**7** for the cisplatin-sensitive T2 cell line, relative to cisplatin, are reported in Table 5 and Figure 3, and for the cisplatin-resistant SKOV3 cell line, in Table 6 and Figure 4.

Table 5. Percentage (±SD) of growth inhibition of T2 cells.

	50 μM	10 μM	2 μM
1	87.1	83.7	16.3
2	73.4	9.8	6.2
3	69.5	69.3	43.2
4	6.6	5.8	0.0
5	87.4	85.4	9.9
6	54.5	4.7	0.0
7	86.2	86.4	85.4
cisplatin	90.0	80.0	65.7

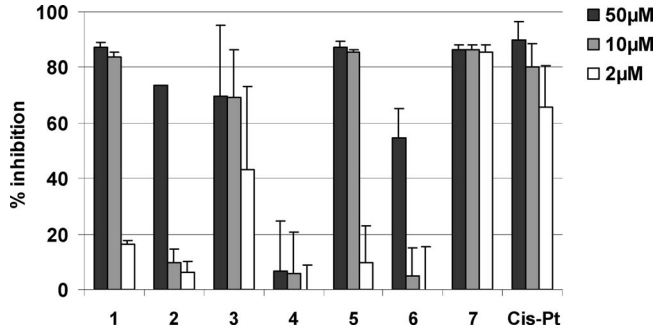


Figure 3. T2 Cell line growth inhibition.

Table 6. Percentage (±SD) of growth inhibition of SKOV3 cells.

	50 μM	10 μM	2 μM
1	81.7	6.7	0.4
2	15.5	3.3	0.4
3	10.9	6.2	4.6
4	3.6	3.3	1.2
5	86.2	17.4	2.1
6	35.5	11.5	4.1
7	84.3	73.7	18.1
cisplatin	20.9	13.7	8.1

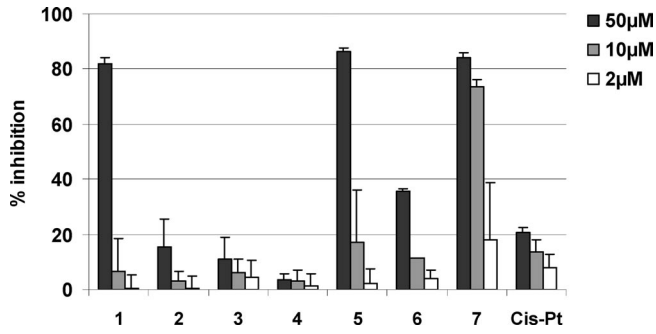


Figure 4. SKOV3 Cell line growth inhibition.

It can be seen that complexes **1**, **3** and **5** show an antiproliferative activity on T2 that is similar to that of cisplatin at high and medium concentrations, whereas at 2 μM they are less active than the reference compound. Complexes **2**, **4** and **6** present low activity, probably because of their low hydrophilicity. Complex **7** appears much more active than all the others. This trend is maintained for the cisplatin-resistant cell line SKOV3, where **1** and **5** show some activity limited to the highest doses; complexes **2**, **3** and **4** show no activity and complex **7** has a remarkable activity at 50 and 10 μM.

Activity measurements at lower doses (Table 7), limited to the most-active complex **7** and cisplatin as reference compound, allowed us to estimate an IC_{50} value of 1.2 μM for both **7** and cisplatin on the T2 cell line, whereas on cisplatin-resistant SKOV3, we found a value of 6.9 μM for **7** and 86.2 μM for cisplatin. The subsequently estimated values for the resistance factors (ratio of the IC_{50} on resistant cells/IC_{50} on parental cells),[20] 5.75 for **7** and 71.8 for cisplatin, seem to support the hypothesis of a different mechanism of action for the two complexes.

Table 7. Percentage of growth inhibition of T2 and SKOV3 cells for **7** and cisplatin.

	50 μM	10 μM	2 μM	0.4 μM	0.08 μM	0.016 μM	IC_{50}
7 (T2)	86.2	86.4	85.4	0.0	0.0	0.0	1.2
Cisplatin (T2)	90.0	80.0	65.7	22.0	0.0	0.0	1.2
7 (SKOV3)	84.3	73.7	18.1	8.5	4.7	4.7	6.9
Cisplatin (SKOV3)	20.9	13.7	8.1	6.7	6.3	0.0	86.2

Conclusions

In this work, we have shown that ascorbic acid is a versatile ligand for platinum, where the O2, O3, O5, O6 and C2 atoms can act as anionic donating functionalities. Alternative synthetic routes to known complexes of L-ascorbic acid with platinum(II) have been proposed, and the hydrophilicity of O2,O3 complexes has been enhanced by introducing PTA as a neutral ligand (complex **3**). New modes of coordination of ascorbic acid and dehydroascorbic acid to platinum(II) have been characterised in solution by NMR spectroscopy and in the solid state by X-ray crystallography. The antiproliferative activity in vitro of complexes **1**–**7** was tested, and the best results were obtained for the DHA complex **7**, which was found to be more active than cisplatin towards both a cisplatin-sensitive and a cisplatin-resistant cell line.

Experimental Section

PTA [11] as well as the Pt complexes $[Pt(CO_3)(PPh_3)_2]$,[9] $[Pt(CO_3)(dppe)]$,[9] *cis*-$[PtCl_2(PTA)_2]$[11] and $[Pt\{trans\text{-}(R,R\text{-dach})\}I_2]$[13] were prepared as reported. All the other chemicals and solvents were used as purchased (reagent grade). Elemental analyses (C, H, N) were performed by using a Carlo Erba instrument model EA1110. FT-IR spectra were recorded with a Nicolet 510P FT-IR instrument (4000–300 cm^{-1}). NMR spectra were recorded with a Bruker AM spectrometer: 200 MHz (^1H NMR), 81.15 MHz (^{31}P NMR). Peak positions are relative to tetramethylsilane and were calibrated against the residual solvent resonance (^1H) and measured relative to external 85% H_3PO_4 with downfield values taken as positive (^{31}P).

Synthesis and Characterisation of Complexes 1–7

$[Pt(O2,O3\text{-asc})(PPh_3)_2]$ (1): The carbonate complex $[Pt(CO_3)(PPh_3)_2]$ (0.15 g, 0.19 mmol) was dissolved in dichloromethane saturated with water (25 mL), and L-ascorbic acid (0.034 g, 0.19 mmol) was added. The solution was left to stir whilst shielded from light for 18 h. It was then concentrated to dryness, and the solid residue was washed with diethyl ether to give **1** (0.14 g, 0.15 mmol, yield 81%). $C_{42}H_{36}O_6P_2Pt$ (894): calcd. C 56.37, H 4.03; found C 56.25, H 4.01. IR (KBr): $\tilde{\nu}$ = 3500–3100 (ν_{OH}), 1780–1630 ($\nu_{C=O}$ + $\nu_{C=C}$), 1480–1430 (aromatic $\nu_{C\cdots C}$) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, 25 °C): δ = 7.5–7 (m, 30 H, Ph), 4.30 (d, $^2J_{H,H}$ = 7.6 Hz, 1 H, 4-H), 3.60 (m, 1 H, 5-H), 3.30 (m, 2 H, 6-H) ppm. ^{31}P NMR (81.15 MHz, $CDCl_3$, 25 °C): δ = 9.7 ($^1J_{Pt,P}$ = 3490, $^2J_{P,P}$ = 20 Hz), 6.9 ($^1J_{Pt,P}$ = 3776, $^2J_{P,P}$ = 20 Hz) ppm.

$[Pt(dppe)(O2,O3\text{-asc})]$ (2): Complex **2** was prepared in the same way as complex **1** from the corresponding carbonate precursor in 75% yield. $C_{32}H_{30}O_6P_2Pt$ (768): calcd. C 50.04, H 3.94; found C 49.82, H 3.86 IR (KBr): $\tilde{\nu}$ = 3500–3100 (ν_{OH}), 1700 ($\nu_{C=O}$), 1620 ($\nu_{C=C}$), 1480–1430 (aromatic ν_{C-C}) cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, 25 °C): δ = 8.0–7.5 (m, 20 H, Ph), 4.49 (d, $^3J_{H,H}$ = 7.1 Hz, 1 H, 4-H), 3.71 (m, 3 H, 5-H, 6-H), 3.43 (m, 1 H, OH), 2.40 (m, 4 H, dppe) ppm. ^{31}P NMR (81.15 MHz, $CDCl_3$, 25 °C): δ = 32.8 ($^1J_{Pt,P}$ = 3364, $^2J_{P,P}$ = 9 Hz), 29.2 ($^1J_{Pt,P}$ = 3664, $^2J_{P,P}$ = 9 Hz) ppm.

$[Pt(O2,O3\text{-asc})(PTA)_2]$ (3): A 0.2-M solution of *cis*-$[Pt(NO_3)_2(PTA)_2]$ was prepared from *cis*-$[PtCl_2(PTA)_2]$ (1.85 mmol, 1.07 g) and $AgNO_3$ (3.7 mmol, 0.62 g) in H_2O (20 mL). The mixture was left to stir for 90 min at 45 °C. AgCl was then filtered with filter paper, and sodium ascorbate (3.7 mmol, 0.73 g) was added to the filtrate whilst stirring under nitrogen. The mixture was left to stir for 24 h at room temperature. ^{31}P NMR inspection of a solution aliquot showed the complete formation of **5**. The pure product was precipitated with ethanol. Yield 84%. $C_{18}H_{30}N_6O_6P_2Pt\cdot 3H_2O$ (737): calcd. C 29.33, H 4.88, N 11.40; found C 29.29, H 4.87, N 11.37. IR (KBr): $\tilde{\nu}$ = 3300 (br. ν_{OH}), 1730 ($\nu_{C=O}$), 1641 ($\nu_{C=C}$) cm^{-1}. ^1H NMR (200 MHz, D_2O, 25 °C): δ = 4.50 (m, 12 H, NCH_2N), 4.42 (d, $^3J_{H,H}$ = 2 Hz, 1 H, 4-H), 4.29 (d, $^2J_{H,P}$ = 13 Hz, 12 H, NCH_2P), 3.93 (td, $^3J_{H,H}$ = 7, $^4J_{H,H}$ = 2 Hz, 1 H, 5-H), 3.65 (dd, $^3J_{H,H}$ = 10.5, $^4J_{H,H}$ = 2 Hz, 2 H, 6-H) ppm. ^{31}P NMR (81.15 MHz, D_2O, 25 °C): δ = –58.34 ($^1J_{Pt,P}$ = 3302, $^2J_{P,P}$ = 23.3 Hz), –58.71 ($^1J_{Pt,P}$ = 3343, $^2J_{P,P}$ = 23.3 Hz) ppm.

$[Pt(C2,O5\text{-asc})\{trans\text{-}(R,R\text{-dach})\}]$ (4): $[Pt\{trans\text{-}(R,R\text{-dach})\}I_2]$ (1.5 g, 2.6 mmol) was placed in water (13 mL), and a second solution containing $AgNO_3$ (0.88 g, 5.2 mmol) in H_2O (13.3 mL) was added. The mixture was left to stir for 90 min at 45 °C, and the precipitate of AgI was then removed by filtration with filter paper. The clear filtrate, containing the aqua-species $[Pt\{trans\text{-}(R,R\text{-dach})\}(OH_2)_2](NO_3)_2$, was degassed and kept under nitrogen, and then sodium ascorbate (1.02 g, 5.2 mmol) was added. The mixture was left to stir for 24 h at room temperature, and then at 4 °C overnight. Crystals slowly formed, which were collected, and filtered with filter paper. Yield 46%. $C_{12}H_{20}N_2O_6Pt\cdot 3H_2O$ (537): calcd. C 26.81, H 4.88, N 5.21; found C 26.45, H 4.75, N 5.27. IR (KBr): $\tilde{\nu}$ = 3550–2860 (ν_{OH}), 1716 ($\nu_{C=O}$), 1630 and 1590 ($\nu_{C=C}$) cm^{-1}. ^1H NMR (200 MHz, D_2O, 25 °C): δ = 4.27 (s, 1 H, 4-H), 4.08 (t, $^1J_{H,H}$ = 6.5 Hz, 1 H, 5-H), 3.42 (d, $^1J_{H,H}$ = 6.5 Hz, 2 H, 6-H), 2.32, 1.91, 1.50, 1.10 (m, 10 H, dach) ppm.

$[Pt(O5,O6\text{-}(O2,O3\text{dibenz})\text{-asc})(PPh_3)_2]$ (5): The carbonate complex $[Pt(CO_3)(PPh_3)_2]$ (0.15 g, 0.19 mmol) was dissolved in dichloromethane (25 mL). To this solution dibenzyl ascorbic acid (68 mg, 0.19 mmol) was added, and the mixture was left overnight whilst stirring. It was then concentrated to dryness, and the solid residue was washed with diethyl ether to give pure **5** (0.113 g, 0.12 mmol, 63% yield). $C_{56}H_{48}O_6P_2Pt$ (1073): calcd. C 62.62, H 4.47; found C 62.61, H 4.40. IR (nujol): $\tilde{\nu}$ = 1754 and 1667 ($\nu_{C=O}$) cm^{-1}. ^1H NMR (200 MHz, C_3D_6O, 25 °C): δ = 7.5–8.0 (m, 40 H, Ph), 5.25 (d, $^2J_{H,H}$ = 12 Hz,1 H, $PhCH_2$), 5.12 (d, $^2J_{H,H}$ = 12 Hz, 1 H, $PhCH_2$), 4.75 (d, $^2J_{H,H}$ = 11 Hz, 1 H, $PhCH_2$), 4.65 (d, $^2J_{H,H}$ = 11 Hz, 1 H, $PhCH_2$), 4.38 (d, $^3J_{H,H}$ = 11 Hz, 1 H, 4-H), 4.26 (m, 1 H, 5-H), 4.04 (m, 1 H, 6-H), 3.64 (m, 1 H, 6′-H) ppm. ^{31}P NMR (81.15 MHz, C_3H_6O, 25 °C): δ = 13.7 ($^1J_{Pt,P}$ = 3512, $^2J_{P,P}$ = 21.4 Hz), 12.8 ($^1J_{Pt,P}$ = 3372, $^2J_{P,P}$ = 21.4 Hz) ppm.

$[Pt(dppe)(O5,O6\text{-}(O2,O3\text{dibenz})\text{-asc})]$ (6): The preparation of complex **6** was carried out in the same manner as that for complex **5**. Yield 75%. $C_{46}H_{42}O_6P_2Pt$ (948): calcd. C 58.22, H 4.43; found C 58.21, H 4.45. IR (nujol): $\tilde{\nu}$ = 3500–2800 (ν_{OH}), 1754 ($\nu_{C=O}$), 1670 ($\nu_{C=C}$), 1480–1430 (ν_{C-C} aromatic) cm^{-1}. ^1H NMR (200 MHz, C_3D_6O, 25 °C): δ = 8.25 and 7.35 (2m, 30 H, Ph), 5.39 (d, $^2J_{H,H}$ = 12 Hz, 1 H, $PhCH_2$), 5.22 (d, $^2J_{H,H}$ = 12 Hz, 1 H, $PhCH_2$), 4.77

(d, $^2J_{H,H}$ = 11 Hz, 1 H, $PhCH_2$), 4.72 (d, $^2J_{H,H}$ = 11 Hz, 1 H, $PhCH_2$), 4.44 (m, 1 H, 4-H), 4.14 (m, 1 H, 5-H), 3.89 (m, 1 H, 6-H), 3.66 (m, 1 H, 6′-H), 2.80 (m, 4 H, dppe) ppm. ^{31}P NMR (81.15 MHz, C_3D_6O, 25 °C): δ = 29.7 ($^1J_{Pt,P}$ = 3280, $^2J_{P,P}$ = 9.0 Hz), 29.4 ($^1J_{Pt,P}$ = 3229 Hz, $^2J_{P,P}$ = 9.0 Hz) ppm.

[Pt(O2,O3-DHA)(PPh$_3$)$_2$] (7): The complex [Pt(CO_3)(PPh_3)$_2$] (0.1 g, 0.13 mmol) was dissolved in anhydrous dichloromethane (15 mL), and dehydroascorbic acid (22 mg, 0.13 mmol) was added. The reaction mixture was left to stir for 60 h and concentrated to dryness, and the solid residue recrystallised from acetone and ether (0.06 g, 0.067 mmol). Yield 52.5%. $C_{42}H_{36}O_7P_2Pt$ (910): calcd. C 55.44, H 3.96; found C 55.15, H 3.97. IR (nujol): $\tilde{\nu}$ = 3500–2800 (br., ν_{OH}), 1795 ($\nu_{C=O}$), 1680 ($\nu_{C=C}$), 1480 –1430 (ν_{C-C} aromatic) cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$, 25 °C): δ = 7.60–7.05 (m, 30 H, Ph), 4.72 (s, 1 H, 4-H), 4.20 (d, $2J_{H,H}$ = 10 Hz 1 H, 5-H), 3.95 (d, $2J_{H,H}$ = 10 Hz, 1 H, 6-H), 3.90 (d, $2J_{H,H}$ = 10 Hz, 1 H, 6′-H) ppm. ^{31}P NMR (81.15 MHz, $CDCl_3$, 25 °C): δ = 12.1 ($1J_{Pt,P}$ = 3617, $2J_{P,P}$ = 24 Hz), 8.3 ($^1J_{Pt,P}$ = 3553, $^2J_{P,P}$ = 24 Hz) ppm.

Crystal-Structure Determination: The crystal data of compounds **6** and **7** were collected at room temperature by using a Nonius Kappa CCD diffractometer with graphite monochromated Mo-K_α radiation. The data sets were integrated with the Denzo-SMN package[21] and corrected for Lorentz, polarisation and absorption effects (SORTAV[22]). The structures were solved by direct methods (SIR97[23]) and refined by using full-matrix least-squares with all non-hydrogen atoms refined anisotropically and with hydrogen atoms included on calculated positions, riding on their carrier atoms, except for the hydroxy hydrogen atoms of **7**, which were refined isotropically. All calculations were performed with SHELXL-97[24] and PARST[25] implemented in the WINGX[26] system of programs. The crystal data are given in Table 8. CCDC-655518 and -655519 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 8. Crystallographic data of complexes **6** and **7**.

Compound	**6**	**7**
Formula	$C_{46}H_{42}O_6P_2Pt$	$C_{42}H_{36}O_7P_2Pt$
M	947.83	909.74
Space group	$P2_12_12_1$	$P2_12_12_1$
Crystal system	orthorhombic	orthorhombic
a [Å]	10.2000(1)	10.7247(1)
b [Å]	18.9649(3)	17.7069(2)
c [Å]	21.8290(4)	19.8126(2)
U [Å^3]	4222.6(1)	3672.44(7)
Z	4	4
T [K]	295	295
D_c [gcm^{-3}]	1.491	1.606
F(000)	1896	1808
M(Mo-K_α) [cm^{-1}]	34.45	38.65
Measured reflections	29342	39562
Unique reflections	10157	9060
R_{int}	0.0502	0.0550
Observed reflections [$I \geq 2\sigma(I)$]	7777	7674
θ_{min}–θ_{max} [°]	2.34–28.00	3.62–28.00
hkl ranges	–11,13; –25,24; –28,28	–14,14; –23,23; –26,26
$R(F^2)$ (obs.reflections)	0.0356	0.0318
$wR(F^2)$ (all reflections)	0.0785	0.0673
No. variables	483	476
Goodness-of-fit	1.023	1.038
Flack parameter	–0.025(6)	–0.031(5)
$\Delta\rho_{max}$; $\Delta\rho_{min}$ [eÅ^{-3}]	1.93; –1.25	1.10; –1.27

Growth Inhibition Assays: Cell growth inhibition assays were carried out with the cisplatin-sensitive T2 human cell line and the cisplatin-resistant SKOV3 cell line. T2 is a cell hybrid obtained by the fusion of the human lynphoblastoid line 174 (B lynphocyte transformed by the Epstein-Barr virus) with the CEM human cancer line (leukaemia T), whereas SKOV3 is derived from a human ovarian tumour. The cells were seeded in triplicate in 96-well trays at a density of 50×10^3 in 50 μL of AIM-V medium for T2 and 25×10^3 in 50 μL of AIM-V medium for SKOV3. Stock solutions (10 mM) of the Pt^{II} complexes were made in DMSO and diluted in AIM-V medium to give final concentration of 2, 10 and 50 μM. Cisplatin was employed as a control for the cisplatin-sensitive T2 cell line and for the cisplatin-resistant SKOV3 cell line. Untreated cells were placed in every plate as a negative control. The cells were exposed to the compounds for 72 h, after which a 4,5-(dimethylthiozol-2-yl)2,5-diphenyltetrazolium bromide solution (25 μL, 12 mM) was added. After 2 h of incubation, 100 μL of lysing buffer (50% DMF + 20% SDS, pH 4.7) was added to convert 4,5-dimethylthiozol-2-yl-2,5-diphenyltetrazolium bromide into a brown formazane. After an additional 18 h, the solution absorbance, proportional to the number of live cells, was measured by spectrophotometry at 570 nm and converted to % of growth inhibition.[27]

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