Synthesis, characterization and cytotoxic properties of platinum(II) complexes containing the nucleosides adenosine and cytidine

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ABSTRACT

Cytidine (cyt) and adenosine (ado) react with cis-[L2Pt(μ-OH)2(NO3)2] (L = PMe3, PPh3) in various solvents to give the nucleoside complexes cis-[L2Pt(cyt(H)-N=N)]2(NO3)2 (L = PMe3, 1), cis-[L2Pt(cyt(H)-N=N)]2[cyt(N=N)] NO3 (L = PPh3, 2), cis-[L2Pt(ado(H)-N=N)]2(NO3)2 (L = PMe3, 3) and cis-[L2Pt(ado(H)-N=N)]2(NO3)2 (L = PPh3, 4). When the condensation reaction is carried out in solution of nitriles (RCN, R = Me, Ph) the ammine derivatives cis-[[PPh3]2PtNH=C(R)(cyt−2H)]NO3 (R = Me, 5a; R = Ph, 5b) and cis-[[PPh3]2PtNH=C(R)(ado−2H)]NO3 (R = Me, 6a; R = Ph, 6b) are quantitatively formed. The coordination mode of these nucleosides, characterized in solution in multinuclear NMR spectroscopy and mass spectrometry, is similar to that previously observed for the nucleobases 1-methylcytosine (1-MeCy) and 9-methyladenine (9-MeAd). The cytotoxic properties of the new complexes, and those of the nucleobase analogs, cis-[[[PPh3]2PtNH=C(R)[1-MeCy(−2H)]]NO3] (R = Me, 7a; R = Ph, 7b) and cis-[[[PPh3]2PtNH=C(R)[9-MeAd(−2H)]]NO3] (R = Me, 8a; R = Ph, 8b) have been investigated in a wide panel of human cancer cells. Interestingly, whereas the Pt(II) nucleoside complexes (1–4) did not show appreciable cytotoxicity, the corresponding ammine derivatives (7a, 7b, 8a, 8b, 9b, and 6b) exhibited a significant in vitro antitumor activity.

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In this paper we report the synthesis and characterization of new nucleoside complexes along with an investigation on their cytotoxic properties against a panel of human cancer cell lines. The high antitumor activity exhibited by the species cis-[[[PPh3]2PtNH=C(Ph)](cyt−2H)]NO3 and cis-[[[PPh3]2PtNH=C(Ph)[ado−2H]]NO3], quantitatively formed when the condensation reaction between the nucleoside and cis-[[[PPh3]2Pt(μ-OH)2]2(NO3)2] is carried out in benzoniitrile, prompted us to include, in this biological investigation, some of the related ammine nucleobase adducts, previously described.

2. Experimental section

2.1. General methods

Adenosine, cytidine and all the solvents (MeOH, EtOH, PhCN, CH2CN, DMSO-d6) are Aldrich products. cis-[[[PPh3]2Pt(μ-OH)2]2(NO3)2] [20], cis-[[[PMe3]2Pt(μ-OH)2]2(NO3)2] [22], cis-[[[PPh3]2PtNH=C(Me)[1-MeCy(−2H)]]NO3] (7a) [20], cis-[[[PPh3]2PtNH=C(Me)[1-MeCy(−2H)]]NO3] (7b) [21], cis-[[[PPh3]2PtNH=C(Me)[9-MeAd(−2H)]]NO3] (8a) [20], cis-[[[PPh3]2PtNH=C(Me)[9-MeAd(−2H)]]NO3] (8b) [21] were synthesized as previously reported.

2.2. NMR and ESI mass spectroscopy

1H and 31P NMR experiments were recorded on a Bruker AVANCE 300 MHz (operating at 300.13, and 121.49 MHZ respectively) and 15N
NMR with a Bruker 400 AMX-WB spectrometer (operating at 40.6 MHz). The $^1$H chemical shifts were referenced to the residual impurity of the solvent and to TMS (tetramethylsilane). The external references were H$_2$PO$_4$ (85% w/w in D$_2$O) for $^31$P and CH$_3$NO$_2$ (in CDCl$_3$ at 50% w/w) for $^1$H. Inverse detected spectra were obtained through heteronuclear multiple bond correlation (HMBC) experiments, using parameters similar to those previously reported [23]. ESI-MS (electrospray ionization mass spectrometry) spectra were performed with a MSD SL trap mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) operating in positive ion mode from m/z 102 to 2200. A $5 \times 10^{-3}$ M solution was directly infused into the ion source at a flow rate of 10 $\mu$L min$^{-1}$ by a syringe pump.

2.3. Synthesis of the complexes

2.3.1. cis-[$\text{PMe}_3\text{Pt}\{\text{cyd}(-H)\}N^2N^5\text{PCH}_3\text{NO}_3]$ (1)

A mixture of cis-[$\text{PMe}_3\text{Pt}{\text{µ-OH}}]$$_2$NO$_3$ (238 mg, 0.279 mmol) and cytidine (136 mg, 0.56 mmol) was dissolved in 10 mL of DMF and the solution was heated at 75 °C for 10 days. Addition of Et$_2$O afforded a yellow oil which, treated several times with Et$_2$O, became a pale yellow solid. The solid was washed again with Et$_2$O and dried under vacuum (yield 88%). El. An. Calc. per C$_{13}$H$_{13}$O$_6$Pt: Pt: 21.55; C: 41.84; H: 5.84; N: 9.52. Found: Pt: 21.58; C: 41.64; H: 5.92; N: 9.44. $^{31}$P{$_1$H} NMR in DMSO-$d_6$: AB multiplet at $\delta$ (ppm) = 29.91 ($^1$J$_{PP} = 3117$ Hz) with $^3$J$_{PP} = 24.5$ Hz; AB multiplet at $\delta$ (ppm) = -28.25 ($^1$J$_{PP} = 3252$ Hz) and -29.91 ($^1$J$_{PP} = 3117$ Hz) with $^3$J$_{PP} = 24.5$ Hz. Relative intensities 60/40 Hz. $^1$H NMR in D$_2$O (6): 7.56 and 7.55 (d, doublet), 1H, H6, $^3$J$_{HH} = 7.68$ Hz; 6.67 and 6.62 (s, singlet), 1H, NH; 6.29 (d, 1H, H5, $^3$J$_{HH} = 7.68$ Hz); 5.88 and 5.76 (d, 1H, H1'; $^3$J$_{HH} = 5.11$ Hz); 4.29 and 4.23 (1H, H2'); 4.11 (1H, H4'); 4.07 (1H, H3'); 3.82 (2H, H5'); 1.78 (d, 3H, PCH$_3$, $^3$J$_{PP} = 10.99$ Hz). $^{31}$P{$^1$H} NMR in DMF (D$_2$O insert): AB multiplet at $\delta$ (ppm) = -27.01 ($^1$J$_{PP} = 3126$ Hz) and -28.31 ($^1$J$_{PP} = 3234$ Hz) with $^3$J$_{PP} = 24.9$ Hz; AB multiplet at $\delta$ (ppm) = -27.14 ($^1$J$_{PP} = 3126$ Hz) and -28.58 ($^1$J$_{PP} = 3234$ Hz) with $^3$J$_{PP} = 24.9$ Hz. Relative intensities 60/40 Hz. 

2.3.2. cis-[$\text{PPh}_3\text{Pt}{\text{µ-OH}}]$$_2$NO$_3$ (2)

cis-[$\text{PPh}_3\text{Pt}{\text{µ-OH}}]$$_2$NO$_3$ (230.5 mg, 0.144 mmol) and cytidine (77.1 mg, 0.286 mmol) were suspended in a mixture of CH$_2$Cl$_2$ and MeOH (3 + 3 mL). After 30 min of stirring, a pale yellow solution was obtained. After 24 h the addition of 30 mL of Et$_2$O afforded a yellow oil which, after being isolated and washed several times with

2.3.3. cis-[$\text{PMe}_3\text{Pt}{\text{µ-OH}}]$$_2$NO$_3$ (3)

cis-[$\text{PMe}_3\text{Pt}{\text{µ-OH}}]$$_2$NO$_3$ (229.1 mg, 0.269 mmol) and adenosine (143.5 mg, 0.537 mmol) were dissolved in 10 mL of H$_2$O. After 4 days, the solution was dried under vacuum and the solid recrystallized from MeOH and Et$_2$O. Obtained 308 mg (yield 85%). El. An. Calc. per C$_{15}$H$_{15}$O$_{10}$Pt: Pt: 21.55; C: 41.84; H: 5.84; N: 9.52. Found: Pt: 21.58; C: 41.64; H: 5.92; N: 9.44. $^{31}$P{$_1$H} NMR in DMSO-$d_6$: AB multiplet at $\delta$ (ppm) = -30.78 ($^1$J$_{PP} = 3080$ Hz) and -31.31 ($^1$J$_{PP} = 3238$ Hz) with $^3$J$_{PP} = 25.9$ Hz; $^1$H NMR in D$_2$O (6): 8.16 (s, 1H, H8); 8.14 (s, 1H, H2); 6.34 (s, 1H, NH); 5.77 (1H, H1'); 4.24 (1H, H2'); 4.10 (1H, H3'); 4.50 (1H, H4'); 3.68 (2H, H5'); 1.89 (d, 3H, PCH$_3$, $^3$J$_{PP} = 10.8$ Hz); 1.43 (d, 3H, PCH$_3$, $^3$J$_{PP} = 10.3$ Hz). $^{31}$P NMR in DMSO-$d_6$: 8.36 and 8.35 (s, 1H, H8); 8.16 and 8.14 (s, 1H, H2); 6.81 and 6.78 (s, 1H, NH); 5.71 (1H, H1'); 5.41 e 5.38 (1H, H1'); 5.19 (1H, H4'); 5.03 (1H, OH5'); 4.37 (1H, H2'); 4.06 (1H, H3'); 3.86 (2H, H4'); 3.54 (2H, H5'); 1.87 (d, 3H, PCH$_3$, $^3$J$_{PP} = 11.1$ Hz); 1.44 (d, 3H, PCH$_3$, $^3$J$_{PP} = 10.4$ Hz).

2.3.4. cis-[$\text{PPh}_3\text{Pt}{\text{µ-OH}}]$$_2$NO$_3$ (4)

cis-[$\text{PPh}_3\text{Pt}{\text{µ-OH}}]$$_2$NO$_3$ (230.5 mg, 0.144 mmol) and adenosine (77.1 mg, 0.286 mmol) were suspended in a mixture of CH$_2$Cl$_2$ and MeOH (3 + 3 mL). After 30 min of stirring, a pale yellow solution was obtained. After 24 h the addition of 30 mL of Et$_2$O afforded a yellow oil which, after being isolated and washed several times with

Scheme 1. Representation of cytidine (cyt) and adenosine (ado).

(cyt) and adenosine (ado).
2.3.7. cis-[(PPh3)2PtHN=C(Me){Ado(H3)]NO3 (6a)

This complex has been synthesized in the same way of 5a starting from cis-[(PPh3)2Pt(μ-OH)2(NO3)2] (0.08 mg, 0.032 mmol) and adenine (16 mg, 0.06 mmol). The reaction mixture was stirred for 5 days at r.t., obtaining 48 mg (yield 73%) of product.

El. An. Calc. for C36H34N5O10Pt: % C: 55.85; H: 3.82; N: 5.07. Found: % C: 55.82; H: 3.81; N: 5.05.

2.4. Experiments with human cell lines

Pt(II) nucleosides (1-4) as well as the corresponding Pt(II) ammine nucleoside (5b, 6b) and nucleobase (7a, 7b, 8a, and 8b) derivatives were dissolved in DMSO just before the experiment and a calculated amount of drug solution was added to the growth medium. The final solvent concentration was 0.5%, which had no discernible effect on cell killing. Cisplatin was dissolved in purified water just before the experiment. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and cisplatin were obtained from Sigma Chemical Co, St.Louis, USA.

2.4.1. Cell cultures

Human breast (MCF-7) carcinoma cell line along with melanoma (A375) cell line were obtained by American Type Culture Collection (ATCC, Rockville, MD) in 2008 and its cisplatin resistant variant, C13*, are human ovarian cancer cell lines, and they were kindly provided by Prof. G. Marveretti (Dept. of Biomedical Science of Modena University, Italy).

A431 and A431/Pt are sensitive and resistant human cervical carcinoma cells, respectively; kindly provided by Prof. F. Zunino (Division of Experimental Oncology B, Istituto Nazionale dei Tumori, Milan, Italy).

LoVo human colon-carcinoma cell line and its derivative multidrug-resistant sub-line (LoVo MDR) were kindly provided by Prof. F. Majone (Dept. of Biology of Padova University, Italy). Cell lines were maintained in the logarithmic phase at 37 °C in a 5% carbon dioxide atmosphere using the following culture media containing 10% fetal calf serum (Euroclone, Milan, Italy), antibiotics (50 units·mL−1 penicillin and 50 μg·mL−1 streptomycin) and 2 mM L-glutamine; i) RPMI-1640 medium (Euroclone) for MCF-7, 2008, C13* cells; ii) F-12 HAM’S medium (Euroclone) for A375, A431/Pt cells and LoVo MDR culture medium; iii) F-12 HAM’S supplemented with 0.1 μg·mL−1 doxorubicin.

2.4.2. Cytotoxicity assays

MTT test. The growth inhibitory effect towards tumor cell lines was evaluated by means of MTT (tetrazolium salt reduction) assay [24,25]. Briefly, 3–8·103 cells/well, dependent upon the growth characteristics of the cell line, were seeded in 96-well microplates in growth medium (100 μL) and then incubated at 37 °C in a 5% carbon dioxide atmosphere. After 24 h, the medium was removed and replaced with a fresh one containing the compound to be studied at the appropriate concentration. Triplicate cultures were established for each treatment. After 72 h, each well was treated with 10 μL of a 5 mg·mL−1 MTT saline solution, and following 5 h of incubation, 100 μL of an SDS solution in HCl 0.01 M was added. After overnight incubation, the inhibition of cell growth induced by the tested complexes was detected by measuring the absorbance of each well at 570 nm using a Bio-Rad 680 microplate reader. Mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted vs. drug concentration. IC50 values represent the drug concentrations that reduced the mean absorbance at 570 nm to 50% of those in the untreated control wells.
3. Results and discussion

3.1. Syntheses of the nucleoside complexes

As previously shown for the model nucleobase 1-MeCy [18], cytidine reacts with the dinuclear hydroxo complex cis-\([\text{PMe}_3\text{Pt}(\mu-\text{OH})_2(\text{NO}_3)_2]\), to give the trinuclear species cis-\([\text{PMe}_3\text{Pt}(\mu-\text{cyt}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]\), 1, in which the N(4)-deprotonated nucleoside binds two metal centers through the N3,N4 atoms (Scheme 2).

The condensation reaction, carried out in DMF solution was monitored by \(^{31}\text{P}\) NMR spectroscopy showing that, at room temperature, a complex mixture of products is formed. At 70 °C, in a few days, it converts quantitatively into the trinuclear species cis-\([\text{PMe}_3\text{Pt}(\mu-\text{cyt}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_3]\), 12+.

The nature of the phosphine ligands plays an important role on the nuclearity of the nucleoside adducts. In fact, reaction of cytidine with cis-\([\text{PPh}_3\text{Pt}(\mu-\text{OH})_2(\text{NO}_3)_2]\) gives only the mononuclear bis-adduct cis-\([\text{PPh}_3\text{Pt}(\text{cyt}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]\), 2, containing two molecules of nucleoside, one neutral N3-coordinated, and one N4-deprotonated, coordinated at the same metal center (Scheme 2).

Similarly, adenosine reacts with cis-\([\text{PPh}_3\text{Pt}(\mu-\text{OH})_2(\text{NO}_3)_2]\) giving the mononuclear species cis-\([\text{PPh}_3\text{Pt}(\text{ado}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]\), 4, in which the N(6)-deprotonated nucleoside binds the metal center through the N6,N7 atoms (Scheme 2). On the contrary, cis-\([\text{PMe}_3\text{Pt}(\mu-\text{ado}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]\), 3, in which the adenosinate ions act as bridging ligands, binding two metal centers through the N1 and N6 atoms.

The reaction of the hydroxo complex with the bulkier \text{PPh}_3 ligands, was carried out in DMF or CH\(_2\)Cl\(_2\)/MeOH solution and monitored by \(^{31}\text{P}\) NMR spectroscopy. In both cases, complex 4 was formed in high yield (>80%). The \(^1\text{H}\) and \(^{31}\text{P}\) analysis of the isolated solid indicates the formation of a single species whose proton resonances were attributed analyzing the COSY (\(^1\text{H}-^1\text{H}\) Correlation NMR Spectroscopy) spectrum (reported in Fig. S1 of the Supplementary Material).

\[\text{Scheme 2. Pathway reaction for the nucleosides Pt(II) complexes 1–4.}\]

**Table 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(P_a (J_{ppm}))</th>
<th>(P_b (J_{ppm}))</th>
<th>(J_{PPt})</th>
<th>H2/H5</th>
<th>H8/H6</th>
<th>NH6/NH4</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-([\text{PMe}_3\text{Pt}(\text{cyd}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]) 1</td>
<td>−27.06 (3266)</td>
<td>−28.59 (3097)</td>
<td>20.4</td>
<td>5.97</td>
<td>7.49</td>
<td>7.27</td>
</tr>
<tr>
<td>cis-([\text{PMe}_3\text{Pt}(\text{MeCy}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]) [18]</td>
<td>−27.77 (3221)</td>
<td>−29.14 (3135)</td>
<td>24.4</td>
<td>6.05</td>
<td>7.44</td>
<td>6.46</td>
</tr>
<tr>
<td>cis-([\text{PPh}_3\text{Pt}(\text{cyd}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{cyd}L^1)^3(\text{NO}_3)_2]) 2</td>
<td>12.14 (3190)</td>
<td>0.36 (3615)</td>
<td>20.4</td>
<td>5.30</td>
<td>7.22</td>
<td>8.58</td>
</tr>
<tr>
<td>cis-([\text{PPh}_3\text{Pt}(\text{MeCy}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{MeCy}L^1)^3(\text{NO}_3)_2]) [19]</td>
<td>12.11 (3190)</td>
<td>0.41 (3615)</td>
<td>20.6</td>
<td>5.09</td>
<td>7.49</td>
<td>8.55</td>
</tr>
<tr>
<td>cis-([\text{PPh}_3\text{Pt}(\text{MeCy}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{MeCy}L^1)^3(\text{NO}_3)_2]) [19]</td>
<td>12.34 (3241)</td>
<td>0.21 (3452)</td>
<td>20.0</td>
<td>5.25</td>
<td>6.66</td>
<td>10.63</td>
</tr>
<tr>
<td>cis-([\text{PMe}_3\text{Pt}(\text{ado}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]) 3</td>
<td>−29.81 (3025)</td>
<td>−30.48 (3233)</td>
<td>26.4</td>
<td>8.36</td>
<td>8.16</td>
<td>6.81</td>
</tr>
<tr>
<td>cis-([\text{PMe}_3\text{Pt}(\text{MeAd}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]) [23]</td>
<td>−29.83 (3025)</td>
<td>−38.38 (3323)</td>
<td>26.6</td>
<td>8.35</td>
<td>8.14</td>
<td>6.78</td>
</tr>
<tr>
<td>cis-([\text{PPh}_3\text{Pt}(\text{ado}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]) 4</td>
<td>−30.33 (3004)</td>
<td>−30.65 (3236)</td>
<td>26.1</td>
<td>8.11</td>
<td>8.02</td>
<td>6.58</td>
</tr>
<tr>
<td>cis-([\text{PPh}_3\text{Pt}(\text{MeAd}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]) [26]</td>
<td>8.74 (3874)</td>
<td>5.64 (3131)</td>
<td>19.9</td>
<td>8.05</td>
<td>6.76</td>
<td>5.20</td>
</tr>
<tr>
<td>cis-([\text{PPh}_3\text{Pt}(\text{MeAd}(-\text{H}),\text{N}^3\text{N}^4)^3(\text{NO}_3)_2]) [26]</td>
<td>9.20 (3850)</td>
<td>5.57 (3162)</td>
<td>20.0</td>
<td>8.03</td>
<td>6.62</td>
<td>4.51</td>
</tr>
</tbody>
</table>
whereas the binding mode of the nucleoside was established by $^{15}$N-1H and $^{31}$P-1H HMBC experiments in CDCl$_3$ (Figs. 1 and 2). The proton resonances at $\delta$ 5.43 and 5.83 ppm, attributable to the H1' and H8 adenosine protons, respectively, correlate with the $^{15}$N nucleus at $\delta$ –200.0 ppm, assigned to N(9). The H8 proton further correlates also with N(7) at $\delta$ –191.4 ppm. The proton resonance at $\delta$ 8.13 ppm, assigned to H2, correlates with the $^{15}$N resonances at $\delta$ –160.9 (N1) and at –135.2 ppm N(3). Finally, the N(6)H proton at $\delta$ 4.64 ppm (Figure S2 Supplementary Material) correlates with N(6) at $\delta$ –242.1 ppm and exhibits the expected couplings $^{15}$J$_{HN}$ = 84 and $^{15}$J$_{HP}$ = 88 Hz.

The $^{31}$P NMR spectrum exhibits an AB multiplet, with sharp $^{195}$Pt satellites, at $\delta$ 8.26 ($^{1}J_{PM}$ = 3846 Hz) and 6.58 ($^{3}J_{PM}$ = 3124 Hz). As shown in Fig. 2, the first resonance correlates with the adenine H8 proton at $\delta$ 5.83 and both of them show correlation with the N(6)H proton at $\delta$ 4.64 (partially overlapped with the ribose H2' multiplet, at 300 MHz). These results led to the conclusion that the nucleoside is chelated to the metal through the N6 and N7 atoms, as previously found for the nucleobase 9-methyadenine [26]. Selected $^{31}$P and $^{1}$H NMR data of the nucleosides and the corresponding model nucleobase are collected in Table 1. It is noteworthy that the replacement of the methyl group with the ribose does not change significantly the spectroscopic parameters of these nucleobase/nucleoside adducts.

As anticipated, adenosine reacts with cis-[(PMe$_3$)$_2$Pt(μ-OH)$_2$ (NO$_3$)$_2$], to give the dinuclear species cis-[(PMe$_3$)$_2$Pt(μ-ado(−H), N$^\prime$N$^\prime$)](NO$_3$)$_2$, 3, that has been isolated as a pure compound performing the condensation reaction in water (see Section 2). The coordination mode of the nucleoside was established by comparison of the multinuclear NMR data with those of the nucleobase analog cis-[(PMe$_3$)$_2$Pt(μ-9-MeAd(−H), N$^\prime$N$^\prime$)](NO$_3$)$_2$ [23], (Table 1) and cis-[(PMe$_3$)$_2$Pt(μ-9-EtAd(−H), N$^\prime$N$^\prime$)](NO$_3$)$_2$ [27]. For this last adduct the N1,N6-coordination of the nucleobase was confirmed by X-ray analysis [27]. The dinuclear species for 3 is characterized by the presence of two AB multiplets in the $^{31}$P NMR spectrum, as well as of distinct resonances for the nucleobase H2, H8 and N(6)H protons (see Table 1), in line with the existence of two conformers resulting in the relative orientation, syn and anti, of the ribose groups with respect to the coordination plane of the metal.

The reaction, monitored via $^{31}$P NMR spectroscopy, in DMSO, leads initially to a mixture of products that slowly rearranges quantitatively into the thermodynamically stable species 3. As an example, the $^{31}$P NMR spectrum of the mixture containing cis-[(PMe$_3$)$_2$Pt(μ-OH)$_2$ (NO$_3$)$_2$] and ado (molar ratio 1:2) in DMSO-d$_6$ is shown in Fig. 3.

The spectrum obtained immediately after the dissolution of the reagents shows still a small amount of the reacting hydroxo complex (singlet at $\delta$ –23.9 ppm), in agreement with a relatively slow condensation reaction at ambient conditions. The main product (Fig. 3a) is characterized by an AB multiplet at $\delta$ –26.64 ($^{1}J_{PM}$ = 3285 Hz) and –28.13 ($^{3}J_{PM}$ = 3111 Hz; $^{3}J_{PH}$ = 25.2 Hz), tentatively attributed to mononuclear N6,N7-chelate analogous to 4 or its linkage isomer cis-[(PMe$_3$)$_2$Pt(ado(−H),N$^\prime$N$^\prime$)](NO$_3$)$_2$. In a few days at 27 °C, these signals are completely replaced by two AB multiplets, having relative intensities ca. 45:55, at higher field (Fig. 3b), attributable to the isolated dinuclear species 3. The spectrum does not change after 4 days at 75 °C, suggesting a high stability of the dimeric structure of this adduct.

3.2. Syntheses of the amidine complexes

When the reaction of cis-[(PPh$_3$)$_2$Pt(μ-OH)$_2$ (NO$_3$)$_2$] with the nucleosides cyt and ado is carried out in solution of nitriles (RCN, R = Me, Ph) the amidine derivatives cis-[(PPh$_3$)$_2$PtNH=C(R)(cyt−2H)]NO$_3$ (R = Me, 5a; R = Ph, 5b) and cis-[(PPh$_3$)$_2$PtNH=C(R)(ado−2H)]NO$_3$ (R = Me, 6a; R = Ph, 6b), depicted in Scheme 3, are formed.

The formal insertion of a nitrile molecule into a Pt-nitrogen bond of the cytidine that occurs at room temperature in a few hours, is
quantitative (by $^{31}\text{P}$ NMR) and no intermediates are detectable. On the contrary, in the case of adenosine the initial product is the nucleoside adduct 4, which slowly reacts with the solvent to give the amidines 6a,b.

The products have been isolated, in good yield, as pale yellow solids and characterized by elemental analysis and NMR spectroscopy in CDCl$_3$. The $^1\text{H}$ and $^{31}\text{P}$ NMR data are very similar to those exhibited by the nucleobase analogs[20,21], while and forms reversibly a complex mixture of uncharacterized products {

In solution, complexes 5a, 6a could be associated with the high kinetic inertness of these molecules as assessed by mass and NMR studies. Among Pt(II) cytidine and cytosine complexes, acetamidine derivatives proved to be much more effective than those containing adenine. In particular, adenosine and adenine derivatives 6b, 8a and 8b showed mean IC$_{50}$ ($\mu$M) values of 24.87 (34.24–12.41), 21.68 (24.91–16.34) and 20.55 (24.73–10.47), respectively, which were about four times higher than that of cisplatin. Among Pt(II) cytidine and cytosine complexes, acetamidine nucleobase 7a showed the worst cytotoxic potency, eliciting a cancer cell growth inhibition roughly 2.5 times lower than cisplatin. Conversely, Pt(II) benzamidine nucleobase 7b distinguished itself as the most promising derivative, being able to decrease cell viability.

3.3. Cytotoxicity tests

Pt(II) nucleosides (1–4) proved to be completely ineffective over 8 cell lines; the negligible activity shown by 1–4 could be associated with the high kinetic inertness of these molecules as assessed by mass and NMR studies. Among Pt(II) amidine nucleoside and nucleobase complexes, cytosine derivatives proved to be much more effective than those containing adenine. In particular, adenosine and adenine derivatives 6b, 8a and 8b showed mean IC$_{50}$ ($\mu$M) values of 24.87 (34.24–12.41), 21.68 (24.91–16.34) and 20.55 (24.73–10.47), respectively, which were about four times higher than that of cisplatin (5.45 $\mu$M). Among Pt(II) cytidine and cytosine complexes, acetamidine nucleobase 7a showed the worst cytotoxic potency, eliciting a cancer cell growth inhibition roughly 2.5 times lower than cisplatin. Conversely, Pt(II) benzamidine nucleobase 7b distinguished itself as the most promising derivative, being able to decrease cell viability.

Table 2

<table>
<thead>
<tr>
<th>Complex</th>
<th>$^3\text{J}_{\text{NN}}$ (Hz)</th>
<th>$^4\text{J}_{\text{NN}}$ (Hz)</th>
<th>$^5\text{J}_{\text{NN}}$ (Hz)</th>
<th>H2/H5</th>
<th>H8/H6</th>
<th>NH</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-([PPh$_3$]$_2$PtHN=C(Me)[$\text{cyt}$ (−2H)])NO$_2$ 5a</td>
<td>8.47 (3468)</td>
<td>7.92 (3432)</td>
<td>25.0</td>
<td>6.00</td>
<td>7.95</td>
<td>5.50</td>
</tr>
<tr>
<td>cis-([PPh$_3$]$_2$PtHN=C(Ph)[$\text{cyt}$ (−2H)])NO$_2$ 5b</td>
<td>8.50 (3485)</td>
<td>7.86 (3450)</td>
<td>24.8</td>
<td>6.24</td>
<td>7.98</td>
<td>6.00</td>
</tr>
<tr>
<td>cis-([PPh$_3$]$_2$PtHN=C(Me)[$\text{ado}$ (−2H)])NO$_2$ 6a</td>
<td>11.10 (3220)</td>
<td>10.71 (3417)</td>
<td>23.8</td>
<td>8.02</td>
<td>7.14</td>
<td>6.35</td>
</tr>
<tr>
<td>cis-([PPh$_3$]$_2$PtHN=C(Ph)[$\text{ado}$ (−2H)])NO$_2$ 6b</td>
<td>11.30 (3256)</td>
<td>10.14 (3399)</td>
<td>24.3</td>
<td>8.06</td>
<td>7.14</td>
<td>6.24</td>
</tr>
<tr>
<td>cis-([PPh$_3$]$_2$PtHN=C(Me)[$\text{cyt}$ (−2H)])NO$_2$ 7a</td>
<td>8.99 (3477)</td>
<td>7.77 (3419)</td>
<td>24.8</td>
<td>6.02</td>
<td>7.20</td>
<td>5.72</td>
</tr>
<tr>
<td>cis-([PPh$_3$]$_2$PtHN=C(Ph)[$\text{cyt}$ (−2H)])NO$_2$ 7b</td>
<td>9.03 (3486)</td>
<td>7.77 (3417)</td>
<td>24.9</td>
<td>6.22</td>
<td>7.14</td>
<td>6.05</td>
</tr>
<tr>
<td>cis-([PPh$_3$]$_2$PtHN=C(Me)[$\text{ado}$ (−2H)])NO$_2$ 8a</td>
<td>11.33 (3385)</td>
<td>10.86 (3369)</td>
<td>24.7</td>
<td>8.11</td>
<td>7.81</td>
<td>5.44</td>
</tr>
<tr>
<td>cis-([PPh$_3$]$_2$PtHN=C(Ph)[$\text{ado}$ (−2H)])NO$_2$ 8b</td>
<td>10.95 (3263)</td>
<td>10.70 (3370)</td>
<td>24.5</td>
<td>8.28</td>
<td>7.95</td>
<td>6.32</td>
</tr>
</tbody>
</table>

* Overlapped with the PPh$_3$ signals.
about 2 times more effectively than cisplatin (average IC50 of 3.22 μM and 5.45 μM for 7b and cisplatin, respectively). Pt(II) benzamidine nucleoside 5b, despite retaining an antiproliferative activity slightly lower than that of the relative Pt(II) nucleobase complexes 7a and 7b (average IC50 of 3.22 and 6.01 μM for 7b and 7a, respectively), has been found to possess a cytotoxic potency quite similar to that of the reference metallodrug. Among nucleobase amidine Pt(II) complexes, benzamidine derivatives proved to be more effective with respect to acetamidine analogs. This behavior may be related to the more lipophilic properties of the phenyl ring that could favor the crossing of the compound to the cell membrane. These data are in line with the results previously reported concerning trans-Pt(II) amidine complexes [28,29].

The in-house panel also included two cell lines that have been selected for their resistance to cisplatin (human ovarian adenocarcinoma C13* cells, human cervical carcinoma A431/Pt cells). Although cisplatin resistance is multifactorial, the main molecular mechanisms involved in Pt resistance of C13* and A431/Pt have been identified. In particular, in C13* cells' resistance is correlated to reduced cellular drug uptake, high cellular glutathione and thioredoxin reductase levels and enhanced repair of DNA damage [30,31]. In A431/Pt cells, resistance is due to a defect in drug uptake and to decreased levels of proteins involved in DNA mismatch repair (MSH2), which causes an increased tolerance to cisplatin-induced DNA damage [32]. By comparing the RF values (where RF is the resistance factor and is defined as the ratio between IC50 values calculated for the resistant and the parental cells), new Pt(II) amidine nucleoside and nucleobase complexes were found to be able to overcome cisplatin resistance. The resistance factors calculated for these derivatives were about 8 and 3 times lower than that of cisplatin on the 2008-C13* and A431-A431/Pt cell pairs, respectively.

Additionally, Pt(II) amidine complexes were tested against a multidrug resistant (MDR) colon carcinoma subline, LoVo MDR cells. It is well known that acquired MDR, whereby cells become refractory to multiple drugs, poses a most important challenge to the success of anticancer chemotherapy. The resistance of LoVo MDR cells to doxorubicin, a drug belonging to the MDR spectrum, is associated with an overexpression of the multi-specific drug transporters, such as, for example, the 170 kDa P-glycoprotein (P-gp) [33]. Although cisplatin is not a P-glycoprotein substrate; many multidrug resistance protein (MRP1, MRP2, MRP4) have been found involved in platinum complex transport and are responsible for its efflux/afflux from the cell [34–36]. Cytotoxicity assays testing Pt(II) amidine derivatives against this cell line pair showed a similar pattern of response across the parental and the resistant subline thus suggesting that these new Pt(II) complexes are not potential MDR substrates.

4. Conclusions

The main results of this study can be summarized as follows: a) the substitution of the methyl group in the model nucleobase 1-MeCy and 9-MeAd with the ribose group does not change significantly the reactivity pattern of these biomolecules toward the hydroxo complex cis-[L2Pt{(μ-OH)}2]2+. The less hindered phosphines stabilize polynuclear adducts in which the deprotonated nucleoside acts as bridging ligand. The di- and/or trimerization of the nucleoside adducts is prevented when the hydroxo reagent is stabilized by PPh3. b) A similar effect of the phosphate ligands is observed in the activation reactivity pattern of these biomolecules toward the hydroxo complex cis-[L2Pt{(μ-OH)}2]. The less hindered phosphines stabilize polynuclear adducts in which the deprotonated nucleoside acts as bridging ligand. The di- and/or trimerization of the nucleoside adducts is prevented when the hydroxo reagent is stabilized by PPh3. c) Among the isolated complexes, only the amidine derivatives exhibit a clear cytotoxic activity toward different human tumor cell lines. In particular, the benzamidine cis-[{(PPh3)2PtNH=C(Ph){1-MeCy(−2H)}[N6N7]}NO3]2− shows interesting properties, in terms of antiproliferative effects, even toward C13* cisplatin resistant cells and this important finding could be due to a different mechanism of action with respect to the reference metalloconjugate.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.jinorgbio.2011.03.009.

References
