Synthesis and characterization of new transition metal complexes containing DNA intercalators of the acridine family

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The coordinating ability of two DNA intercalates of the acridine family, 4-hydroxyacridine (4-OH-Acrid) and acridine orange (AO), with the transition metal ions Ni(II), Pd(II) and Pt(II), are investigated. Octahedral complexes, [Ni(4-O-Acrid)$_2$(en)] (**1**), and [Ni(4-O-Acrid)$_2$(H$_2$O)$_2$] (**2**), are obtained when the 4-OH-Acrid ligand reacts with [Ni(Cl)$_2$(en)$_2$] and NiCl$_2$·6H$_2$O, respectively. Single crystal X-ray analysis of complex **1** has shown that two acridine molecules are bonded to nickel through their nitrogen and oxygen atoms in a chelate fashion. Moreover, when AO reacts with different Pd(II) and Pt(II) precursors, new triamine complexes of the general formula *cis*-[M(A)$_n$(Cl)(AO)]$^+$X$^-$ (M = Pd(II), Pt(II); A = en, NH$_3$; n = 1, 2; X$^-$ = BF$_4^-$, NO$_3^-$, PF$_6^-$) (**3**–**6**) are synthesized. Full characterization by IR, ^{1}H NMR and electrospray ionization mass spectrometry in solution has demonstrated the coordination of the AO ligand to the metal ion through the endocyclic nitrogen atom. The biological activity of these new acridine orange derivatives is conducted on complex *cis*-[Pt(NH$_3$)$_2$Cl(AO)][NO$_3$] (**5**). The cytotoxic activity of complex **5** is compared with that of cisplatin (*cis*-DDP) in DU145, A2780 and A2780-cp8 cancer cell lines and the results show that its activity is twice as effective as that of cisplatin in the two *cis*-DDP resistant cell lines DU145 and A2780-cp8, respectively.

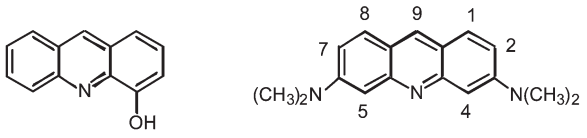
Introduction

Cisplatin (*cis*-diaminedichloroplatinum(II), *cis*-DDP) is a well-established antitumor agent effective especially against ovarian, testicular and head cancers.[1,2] *Cis*-DDP is believed to exert its cytotoxicity by coordinating bifunctionally to DNA through the N(7) atoms of two adjacent guanines on the same strand, arresting DNA replication.[3] Despite its clinical success, *cis*-DDP exhibits only a narrow spectrum of activity and has been associated with severe side effects and acquired resistance.[4] Cisplatin is often administered in combination with other intercalative drugs such as adriamycin and actinomycin.[5,6] The general idea behind modifying the cisplatin structure to derive platinum-DNA adducts of high cytotoxicity is to utilise a suitable carrier ligand to better "target" the platinum moiety to DNA.[7–9] Examples of DNA-targeted platinum drugs showing improved selectivity with respect to cisplatin-resistant cell lines include doxorubicin,[10,11] 9-anilinoacridine[12] and the more soluble acridinecarboxamide derivatives.[13] Moreover, a study of the antitumoral activity of platinum complexes tethered to variously coordinated anthraquinones has given promising results.[14]

Anticancer activity has also been observed in complexes of the general formula *cis*-[Pt(A)$_n$(Cl)(Am)]$^+$, where A is either NH$_3$ (n = 2) or en (n = 1) and Am is a heterocyclic amine ligand based on pyridine, pyrimidine, purina and piperidine.[15] Despite the lack of structural features characteristic of standard cisplatin analogues, the unanticipated activity of these complexes can be ascribed to the possibility that the aromatic ligand might intercalate between base pairs adjacent to the site of platinum coordination, thereby forming pseudo-bifunctional adducts which are capable of inhibiting replication.[16] In addition, the positive charge of the Pt(II) complexes may contribute to their binding with the negatively charged phosphate oxygen atoms of DNA through electrostatic interactions.[17] Acridine is an important aza-aromatic compound and its derivatives have been widely used in pharmacology.[18] Their polyaromatic nature facilitates insertion or intercalation between base pairs of double stranded DNA.[19] The driving force of intercalation is based on intermolecular interaction between the plane of base pairs of double stranded DNA and the aromatic plane of the polyaromatic intercalator.

On this basis Lippard and co-workers have synthesized and analyzed new Pt(II) adducts containing, as the Am ligand, intercalators of the acridine family. The intercalators used in their studies were 9-aminoacridine and 3,6-bis-(dimethylamino)acridine (acridine orange) quaternized at the endocyclic nitrogen atom.[20,21] None of these ligands coordinated the metal ion through the endocyclic nitrogen atom despite its greater basicity compared with the exocyclic one. In the case of 9-aminoacridines intercalation in DNA was found with the endocyclic nitrogen atom placed in the major groove and the exocyclic one placed in the minor groove.[22] Therefore coordination between the platinum and the endocyclic nitrogen atom could bring the metal in the major groove close to the nitrogen atom of an adjacent purine residue, allowing them to interact. Recently the endocyclic coordination to platinum in nitro-9-aminoacridines was shown to depend on both the position of the nitro group with respect to the endocyclic nitrogen atom and the charge of the metal complexes used as precursors.[23]

Metallointercalation has also been extended to three dimensions using octahedral complexes of Ru(II), Co(III), Os(II), Rh(III) and Ni(II), which, through their photophysical,



4-hydroxy-Acridine **Acridine orange**

4-OH-Acrid **AO**

Chart 1

photochemical and redox properties, offer the possibility of becoming sensitive spectroscopic and reactive DNA probes.[24] Octahedral complexes capable of being metallointercalators contain an extended aromatic heterocyclic ligand which can insert and stack between base pairs.

The aim of the present study is to give few more insight about the coordination between transition metal ions and 4-hydroxy-acridine (4-OH-Acrid) and acridine orange (AO),[25] two intercalators of the acridine family whose coordinating ability has been less explored.

While in both cases an endocyclic nitrogen atom is present, offering a potential strong binding site for metal coordination, 4-OH-Acrid has the potential of working both as a monodentate and bidentate (chelating) ligand. The coordinating ability of these ligands with the transition metal ions Ni(II), Pd(II) and Pt(II), starting with different metal precursor complexes, was tested with the generation of both octahedral and square planar metallointercalators.

Experimental

General details

4-Hydroxyacridine, acridine orange, $NiCl_2{\cdot}(H_2O)_6$ and $[Pd(en)Cl_2]$ were used without further purification. $[Ni(en)_2Cl_2]$,[26] $[Pt(en)Cl_2]$,[27] and *cis*-$[Pt(NH_3)_2Cl_2]$[28] were synthetized as reported in the literature. *cis*-DDP was dissolved in a 0.9% (w/v) NaCl solution, prepared fresh prior to use. The infrared spectra were recorded on a Spectrum One FT-IR PerkinElmer spectrometer in diffuse reflectance mode. The ^1H NMR spectra were recorded on a Bruker WH-300 spectrometer in $CDCl_3$ solution, with TMS as internal standard. Elemental analyses were performed with a PerkinElmer 2400 analyzer.

Preparations

[Ni(4-O-Acrid)$_2$(en)] (1). $[Ni(en)_2Cl_2]$ (30 mg, 0.12 mmol) was dissolved in water (3 mL) and a solution of 4-hydroxyacridine (46.86 mg, 0.24 mmol) and KOH (6.73 mg, 0.12 mmol) in a mixture water–ethanol (2 mL + 1 mL) was added. The mixture was stirred at room temperature for 3 h. The red solid was filtered, recrystallized from a mixture of chloroform–hexane and dried *in vacuo* (43.0 mg, 71%). Found: C, 66.38; H, 5.00; N, 10.85%. Required for $C_{28}H_{24}N_4NiO_2$ (507.23): C, 66.30; H, 4.77; N, 11.05%.†

[Ni(4-O-Acrid)$_2$(H$_2$O)$_2$] (2). $NiCl_2{\cdot}(H_2O)_6$ (30 mg, 0.13 mmol) was added to a solution of 4-hydroxyacridine (49.19 mg, 0.25 mmol) and piperidine (0.024 mL, 0.25 mmol) in water (25 mL). The resulting suspension was stirred at room temperature for 2 h. The orange solid was filtered, recrystallized from ethanol and dried in vacuum (31.5 mg, 50%).

ESI (+) MS: m/z 483.0939, $[C_{26}H_{21}N_2O_4Ni]^+$ (calculated mass 483.08, ppm error 18.56); m/z 465.09 $[C_{26}H_{19}N_2O_3Ni]^+$ (corresponding to $[Ni(4\text{-}O\text{-}Acrid)_2(H_2O)H]^+$); m/z 447.08, $[C_{26}H_{17}N_2O_2Ni]^+$ (corresponding to $[Ni(4\text{-}O\text{-}Acrid)_2H]^+$).

Found: C, 65.73; H, 4.15; N, 5.79%. Required for $C_{26}H_{20}N_2NiO_4$ (483.16): C, 65.63; H, 4.12; N, 5.80%.

[Pd(en)Cl(AO)][BF$_4$] (3). $[Pd(en)Cl_2]$ (30 mg, 0.13 mmol) and $Ag(BF_4)$ (24.52 mg, 0.13 mmol) were stirred in 10 mL of water, at room temperature, for 12 h. The resulting AgCl precipitate was removed by filtration and a solution of acridine orange (33.39 mg, 0.13 mmol) in water (3 mL) was added to the filtrate. After the mixture was stirred for 24 h, the resulting solid was filtered, recrystallized from ethanol and dried *in vacuo* (31.1 mg, 43.2%).

IR (KBr): ν 1074 cm^{-1} (BF_4). ESI (+) MS: m/z 484.0994, $[C_{19}H_{29}N_5OClPd]^+$ (calculated mass 484.10, ppm error −20.94); CID spectrum of $[M]^+$ (m/z 484.09): m/z 466.2, $[M–H_2O]^+$; m/z 441.87, $[M–C_2H_4N]^+$; m/z 423.88 $[M–C_2H_4N–H_2O]^+$; m/z 401.04, $[M–C_4H_8N_2]^+$; m/z 381.90 $[M–C_4H_8N_2–H_2O]^+$; m/z 363.01 $[M–C_4H_8N_2–H_2O–Cl]^+$; m/z 223.20 $[(AO–C_2H_4N)H]^+$.

Found: C, 40.93; H, 4.83; N, 11.52%. Required for $C_{19}H_{27}BClF_4N_5Pd$ (554.12): C, 41.18; H, 4.91; N, 11.64%.

[Pt(en)Cl(AO)][BF$_4$] (4). $[Pt(en)Cl_2]$ (30 mg, 0.09 mmol) and $Ag(BF_4)$ (17.47 mg, 0.09 mmol) was stirred in 3 mL of water, at room temperature, for 12 h. The resulting AgCl precipitate was removed by filtration and a solution of acridine orange (23.87 mg, 0.09 mmol) in water (3 mL) was added to the filtrate. After the mixture was stirred for 24 h, the solid formed was filtered, recrystallized from ethanol and dried in vacuum (29.8 mg, 52%).

IR (KBr): ν 1074 cm^{-1} (BF_4). ESI (+) MS: m/z 573.2088, $[C_{19}H_{29}N_5OClPt]^+$ (calculated mass 573.17, ppm error 67.16), m/z 520.84, $[C_{19}H_{28}N_5Pt]^+$ (corresponding to $[Pt(AO)(en)H]^+$); m/z 478.83, $[C_{17}H_{22}N_3OPt]^+$ (corresponding to $[Pt(AO)(H_2O)H]^+$); m/z 460.78 $[C_{17}H_{20}N_3Pt]^+$ (corresponding to $[Pt(AO)H]^+$); m/z 266.19 $[C_{17}H_{20}N_3]^+$ (corresponding to $[AOH]^+$). Found: C, 36.06; H, 4.00; N, 10.06%. Required for $C_{19}H_{27}BClF_4N_5Pt$ (642.81): C, 35.50; H, 4.23; N, 10.89%.

***Cis*-[Pt(NH$_3$)$_2$Cl(AO)][NO$_3$] (5).** *Cis*-DDP (70 mg, 0.23 mmol) and $AgNO_3$ (39.60 mg, 0.23 mmol) was stirred in 10 mL of water, at room temperature, for 16 h. After the AgCl was filtered, a solution of acridine orange (61.74 mg, 0.23 mmol) in water (3 mL) was added to the filtrate and the mixture was stirred for 24 h. The resulting orange solid was filtered, recrystallized from ethyl acetate and dried *in vacuo* (92.2 mg, 68%).

^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ 8.38 (s, 1H, H_9), 7.68 (d, 2H, $H_{1,8}$), 7.23 (d, 2 H, $H_{4,5}$), 7.03 (dd, 2H, $H_{2,7}$), 3.24 (s, 12H, CH_3). IR (KBr): ν 1384 cm^{-1} (NO_3). ESI (+) MS: m/z 565.17 $[C_{17}H_{29}N_5O_2ClPt]^+$ (calculated mass 565.16, ppm error 17.70)]; CID spectrum of $[M]^+$ (m/z 565.17): m/z 547.98, $[M–NH_3]^+$; m/z 530.94, $[M–2NH_3]^+$; m/z 511.99, $[M–2NH_3–H_2O]^+$; m/z 266.2 $[AOH]^+$. Found: C, 30.15; H, 3.89; N, 14.89%. Required for $C_{17}H_{25}ClN_6O_3Pt$ $2H_2O$ (628.0): C, 32.51; H, 4.65; N, 13.38%.

***Cis*-[Pt(NH$_3$)$_2$Cl(AO)][PF$_6$] (6).** *cis*-DDP (0.05 g, 0.17 mmol) and $AgPF_6$ (41.96 mg, 0.17 mmol) was stirred in 3 mL of water for 12 h. The resulting AgCl was filtered off and a solution of acridine orange (43.99 mg, 0.17 mmol) in water (3 mL) was added to the filtrate. After the mixture was stirred for 72 h, the resulting orange solid was filtered, recrystallized from ethyl acetate and dried in vacuum (63.4 mg, 55%).

IR (KBr): ν 851 cm^{-1} (PF_6); ESI (+) MS: m/z 565.17 $[C_{17}H_{29}N_5O_2ClPt]^+$ (calculated mass 565.16, ppm error 17.70). CID spectrum of $[M]^+$ (m/z 565.17): m/z 547.98, $[M–NH_3]^+$; m/z 530.93, $[M–2NH_3]^+$; m/z 511.99, $[M–2NH_3–$

† CCDC reference number 207089. See http://www.rsc.org/suppdata/nj/b3/b304288j for crystallographic data in CIF or other electronic format.

$H_2O]^+$; m/z 266.20 $[AOH]^+$. Found: C, 31.03; H, 4.24; N, 10.14%. Required for $C_{17}H_{25}ClF_6N_5PPt$ (674.93): C, 30.25; H, 3.73; N, 10.38%.

Molecular mechanics

Universal force field (UFF)[29] was employed as implemented in the *Cerius* modelling package.[30] All calculations were performed on a Silicon Graphics RS 4000 Indigo workstation and carried out with atomic charges. The nickel, palladium and platinum centres were taken as force-field atom type M4+2. Initial trials on model systems were found to give reasonable agreement with the CSD-derived results noted below without main changes in the force field used for the calculations.

X-ray crystallography

Crystal data for **1**: $C_{28}H_{24}N_4O_2Ni$, $M = 507.2$, monoclinic, $a = 18.180(2)$, $b = 12.346(3)$, $c = 10.680(2)$ Å, $\beta = 108.95(2)°$, $U = 2267.3(8)$ Å^3, $T = 298$ K, space group $C2/c$, $Z = 4$, μ(Mo Kα) = 8.91 cm^{-1}, 2133 reflections measured, 2013 reflections unique (R(int) = 0.032), 156 variable parameters, $R_1 = 0.0386$ ($I > 2\sigma(I)$), $wR_2 = 0.0977$, GOF(F^2) = 0.945.

Dark red crystals of **1** were crystallized at room temperature from a chloroform/hexane mixture.

Data collection was carried out at room temperature on a Siemens $R3m/v$ diffractometer using graphite-monochromated Mo Kα ($\lambda = 0.71073$ Å) radiation. The data were corrected for Lorentz and polarization effects. Absorption correction was applied (XABS2).[31] Since it was not possible to distinguish between the Cc and $C2/c$ space groups from the analysis of the systematic absences, attempts to solve the structure in both acentric and centric systems were made. The best solution was found in the $C2/c$ space group. The structure solution and full-matrix least-squares refinements based on F^2 were performed with XS and XL routines in the SHELXTL-NT program package. All non-hydrogen atoms were refined anisotropically, with nickel atom lies on a mirror plane. Hydrogen atoms were included as idealized atoms riding on the respective nitrogen and carbon atoms, with N–H and C–H bond lengths appropriate to the atom hybridization.

Electrospray ionization mass spectrometry

ESI experiments were carried out on a hybrid Q-Star *Pulsar-i* (PE-SCIEX) mass spectrometer equipped with an ionspray ionization source. Complexes **2**–**6** were dissolved in a solution of water–methanol (98 + 2) and analyzed by direct infusion (5 μL min^{-1}) at the optimum ion spray voltage of 4800 V. Complex **5** was also been analyzed by direct infusion of diluting mother solution (2 μL to 1 mL of water).

MS–MS experiments were performed in the collision cell q on the isotopically pure peak of the selected precursor ions by keeping the CE at 20 eV and unit resolution, and scanning the TOF analyser. All acquisitions were averaged over 60 scans at 8000 TOF resolution.

Cytotoxicity

The human prostatic carcinoma cell line, DU145, and the human ovarian carcinoma cell line A2780 and its cisplatin-resistant subline A2780-cp8,[32] were cultured in RPMI-1640 medium supplemented with fetal calf serum (10%), gentamycin (0.1%) and L-glutamine (1%) at 37 °C in a 5% CO_2–95% air atmosphere in humidified incubator. After harvesting in the logarithmic growth phase, cells were seeded in six-well plates and treated with varying doses of complex **5** and *cis*-DDP for 1 h. The medium was then removed and cells were rinsed in phosphate-buffered saline (PBS) and incubated in fresh medium for an additional 48 and 72 h at 37 °C in 5% CO_2 humidified atmosphere. The cells were then trypsinized and counted in a particle counter (Coulter Counter, Coulter Electronics, Luton, Bedfordshire, UK). The percentage of adherent viable cells was determined through trypan blue dye exclusion. Each experimental sample was run in triplicate. The results were expressed as the total number of adherent cells in treated samples compared with untreated (control) samples. *In vitro* drug activity was expressed in terms of the concentration which inhibited cell proliferation by 50% (IC_{50}).

Cell cycle and apoptosis analysis

Cells treated as described above, were trypsinized, harvested, and washed once in PBS and then fixed in 80% ethanol. After 1 h of incubation at 4 °C cells were stained with propidium iodide (PI, 50 μg mL^{-1}, Sigma) in PBS containing Rnase-A (75 kU mL^{-1}, Sigma) and analyzed for DNA content. The analysis of cell cycle and apoptosis was performed, using a FACScan flow cytometer (Becton Dickinson, USA) and the Cell Quest software package (Becton Dickinson).

Results and discussion

Chemical studies of octahedral complexes

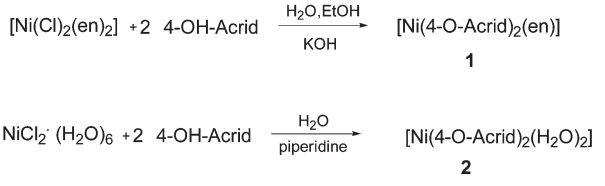
In order to obtain octahedral metallointercalators Ni(II) hexa-coordinated precursors dichloro-bis-ethylenediamine nichel(II), $[Ni(Cl)_2(en)_2]$, and $NiCl_2{\cdot}6H_2O$, were used in reaction with two equivalents of 4-hydroxyacridine ligand (Scheme 1).

The reaction between $[Ni(Cl)_2(en)_2]$ and two equivalents of 4-hydroxyacridine was conducted in a basic aqueous solution to deprotonate the acridine ligand, which then acts as a chelating ligand, coordinating with both the nitrogen and the oxygen atoms. A new Ni(II) complex of the general formula [Ni(4-O-acrid)$_2$(en)], **1**, was synthesized, in which two chlorine and one ethylenediamine ligands were substituted by two acridine ligands. Single crystal X-ray analysis confirmed the stoichiometry initially postulated from the analytical data. A view of the structure of complex **1** is shown in Fig. 1. Selected bond distances and angles are reported in Table 1.

The coordination about Ni, which is situated on the C_2 axis, is distorted octahedron . Two acridine molecules are bonded to nickel through their nitrogen and oxygen atoms in a chelate fashion. The Ni–N(1) bond distance is found to be 0.098 Å longer than Ni–O(1) and both distance values as well as the bite angle of 79.31(8)° are in agreement with those observed in 8-quinolinate Ni(II) octahedral complexes searching NiNOCC five membered chelated rings in the CSD (Cambridge Structural Database, version 5.23, April 2002). The two chelated acridine molecules are not coplanar, as demonstrated by the dihedral angle of 92.2° between the two five-membered rings. The coordination sphere is completed by one ethylenediamine molecule.

As shown in Fig. 2, the supramolecular structure of complex **1** can be described in terms of both intermolecular hydrogen bonding and π-π interactions.

The hydrogen bonding interactions involve the oxygen atoms of the acridine ligands and the NH_2 fragment of the ethylenediamine molecules [O(1)···H(2a)* = 2.27 Å,



Scheme 1 Synthetic route to the octahedral complexes **1** and **2**.

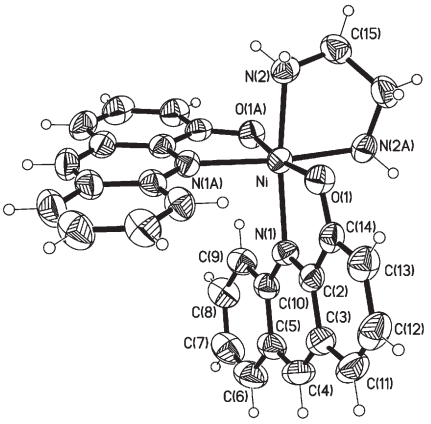


Fig. 1 Ortep drawing of the [Ni(4-O-Acrid)$_2$(en)], (**1**), complex molecule with the labelling scheme. The ellipsoids enclose 50% of probability.

O(1)···N(1)* = 3.168(3) Å, N(1)*–H(2a)*···O(1) = 156°, * = −x, −y, −z]. The second structural motif that characterises the crystal packing is the π-π interaction between aromatic rings which are not involved in the coordination of the acridine ligands. The shortest interplanar distance of 3.38 Å is found between the C(5)/C(10) phenyl ring and its centrosymmetrical one.

An analogous Ni(II) complex, [Ni(4-O-Acrid)$_2$(H$_2$O)$_2$] (**2**), was obtained by reacting NiCl$_2$·6H$_2$O with 4-hydroxyacridine in a basic aqueous solution using a weak aprotic base such as piperidine and working at pH 8.04 to avoid precipitation of Ni(OH)$_2$ (Scheme 1).

Complex **2** was characterized in an aqueous solution using high-resolution mass spectrometry. The results (see Experimental section) confirmed the chelation of two acridine molecules per metal ion, with the octahedral coordination sphere completed by two water molecules. The minimum energy structure, obtained performing MM calculations using UFF, confirm the coplanarity of the two chelated acridine ligands, with the two water molecules occupying the axial positions of the octahedral sphere (Fig. 3).

The Ni–OH$_2$ bond distances of 2.06 Å and the H$_2$O–Ni–OH$_2$ bond angle of 179° are in good agreement with values found in other octahedral Ni(II) aqua complexes which contain two coplanar N,O-chelated ligands (CSD).

Chemical studies of square-planar complexes

The coordinating capability of acridine orange was tested using Pd(II) and Pt(II) complexes. Cationic triamine complexes **3**–**6** of the general formula *cis*-[M(A)$_n$(Cl)(AO)]$^+$ (A = en, NH$_3$; n = 1, 2) were synthesized using dichloro-ethylenediamine Pd(II) and Pt(II) as well as *cis*-DPP as precursors.

The reactions, conducted in water solution, were performed in two steps: (i) synthesis of the intermediate reactive water derivatives; (ii) reaction in water solution with one equivalent of acridine orange (Scheme 2).

All the products isolated were ionic complexes as confirmed by the presence of the typical bands of the counter ions BF$_4^-$, NO$_3^-$, PF$_6^-$ at 1074, 1384 and 851 cm^{-1}, respectively, in the IR spectra. High resolution mass spectrometry confirmed the expected coordination sphere around the metal through the formation of mono-charged molecular ions, organic ligands and some ionic fragments, resulting from the direct and/or consecutive elimination of neutral ligands under ESI-MS condition. Moreover, the collision induced dissociation (CID) behaviour of these complexes characterized by a sequential loss of ligands has clarified the structure of the cations, even in solution: one acridine orange, one chloride and one diamine or two ammonia ligands per metal ion. It was only possible to carry out ^{1}H NMR analysis on complex **5** which was the most soluble of complexes **3**–**6**.

In the ^{1}H NMR spectrum of **5** the resonance attributable to the H^4 and H^5 protons is low-field shifted by about 0.5 ppm and appears as a doublet with respect to the singlet of the uncomplexed acridine orange. This observed shift can be attributed to the close interaction between the platinum centre and protons above and below the coordination plane,[23] confirming the coordination of AO to the platinum through the N atom. A slight downfield shift and a splitting into a doublet of doublets also occurs for the H^1 and H^8 protons as observed in the resonance of the corresponding protons in the ligand.

Table 1 Selected bond distances (Å) and angles (°) for complex **1**

Ni–O(1)	2.0567(18)	Ni–N(1)	2.153(2)
Ni–N(2)	2.112(2)	O(1)–C(14)	1.302(3)
O(1)–Ni–O(1a)	172.02(11)	O(1)–Ni–N(1)	79.29(8)
O(1)–Ni–N(2)	89.32(8)	O(1)–Ni–N(1a)	106.41(8)
O(1)–Ni–N(2a)	84.71(8)	N(1)–Ni–N(1a)	91.93(12)
N(1)–Ni–N(2)	168.38(8)	N(1)–Ni–N(2a)	93.47(9)
N(2)–Ni–N(2a)	83.14(13)	Ni–O(1)–C(14)	114.45(17)

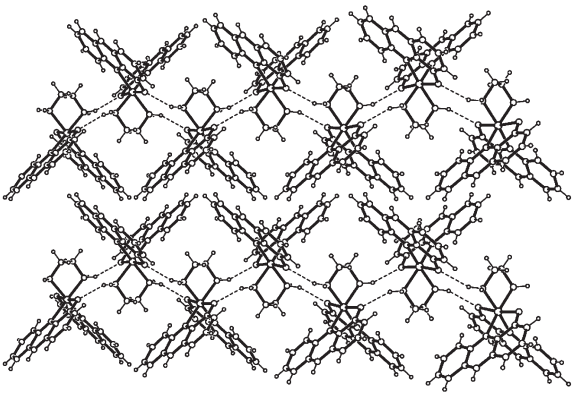


Fig. 2 Crystal packing of [Ni(4-O-Acrid)$_2$(en)] (**1**), viewed down the *a* axis.

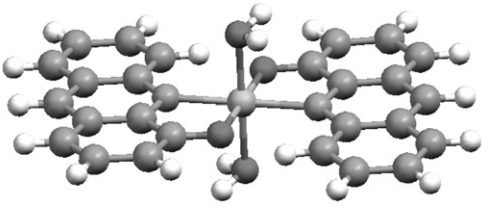
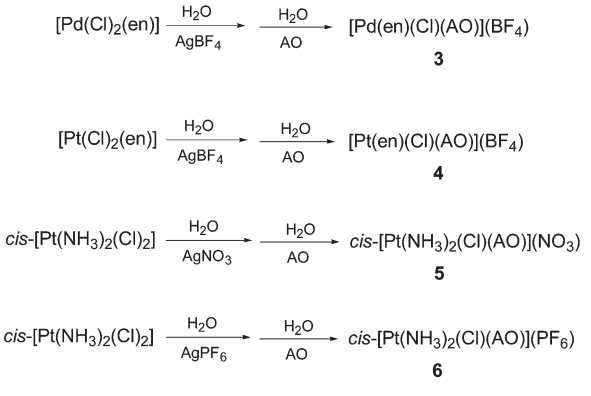


Fig. 3 Minimized structure of the [Ni(4-O-Acrid)$_2$(H$_2$O)$_2$] (**2**) complex.



Scheme 2 Synthetic route to the square-planar complexes **3**–**6**.

Moreover, the products **5** and **6**, which were obtained starting with cisplatin as precursor, are unique isomers, which most probably consist, in both cases, of the *cis* isomer. Figs. 4 (a) and 4 (b) show the minimum energy structures obtained performing MM calculations of the two cations $[Pt(en)(AO)Cl]^+$ and $[Pt(NH_3)_2(AO)Cl]^+$. The acridine orange ligand is coordinated to the metal centre *via* the nitrogen atom (bond distance $Pt–N_{acrid} = 2.06$ Å in both cases), with the mean plane nearly orthogonal to the mean coordination plane (mean torsion angle $Cl–Pt–N_{acrid}–C$ of 88° and 78°, respectively). This geometrical feature, as well as the bond distances and angles, is found to be in agreement with those of other ionic complexes of the type $[M(A)_n(Cl)(Am)]^+$ (A = en, NH_3; $n = 1, 2$) where Am is a DNA base N-bounded.[15,31,32]

Both complexes **5** and **6** are water soluble (**5** more so than **6**) and stability in water was assessed using ESI-MS (electrospray ionization mass spectrometry) experiments in positive ionization mode.[35] A typical mass spectrum obtained by direct infusion of a water solution containing complex **5** is shown in Fig. 5a. Clearly, the expected parent ion at 528 m/z is not present; in fact, the formation of $[C_{17}H_{29}N_5O_2ClPt]^+$ at 565.17 m/z indicates that the complex adds two molecules of water during the ionization processes.[36] Therefore, some ESI (+) experiments were performed by direct infusion of a diluted mother solution of **5** (Fig. 5b) and the initial addition of the first and the second water molecule with the rapid disappearance of the parent ion complex was observed. Positive product ion spectrum (CID) of $[C_{17}H_{29}N_5O_2ClPt]^+$ of **5** showed the typical rearrangement reaction,[37,38] which involves the loss of the small neutral ligands and the formation of the ionized AO (see Experimental section), providing further details about the coordination sphere.

Biological studies of *cis*-$[Pt(NH_3)_2Cl(AO)][NO_3]$ complex (5)

The biological activity of these new acridine orange derivatives was conducted with complex **5** which was the most water soluble of the **3**–**6** complexes.

Cytotoxicity

The cytotoxic activity of complex **5** was compared with that of *cis*-DDP, in three human cancer cell lines including the DU145 human prostatic cancer cell line, which is inherently resistant to *cis*-DDP,[39] the A2780 *cis*-DDP-sensitive ovarian cancer cell line and A2780-cp8 sub-line, which manifests experimentally induced-resistance to *cis*-DDP.[40]

As shown in Table 2, A2780 cells were equally sensitive to both Pt-based drugs. On the other hand, the activity of

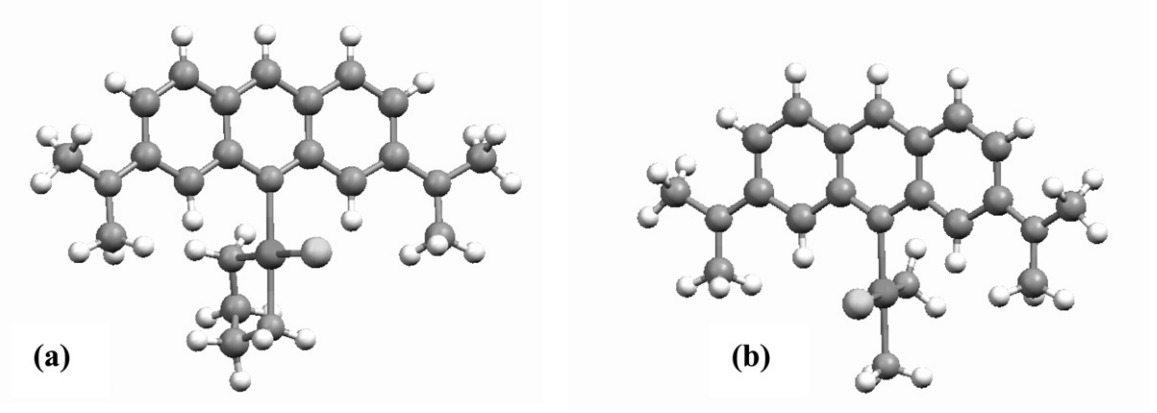


Fig. 4 Minimized structures of the (a) $[Pt(en)(AO)Cl]^+$ and (b) $[Pt(NH_3)_2(AO)Cl]^+$ cations of complexes **4** and **5**–**6**.

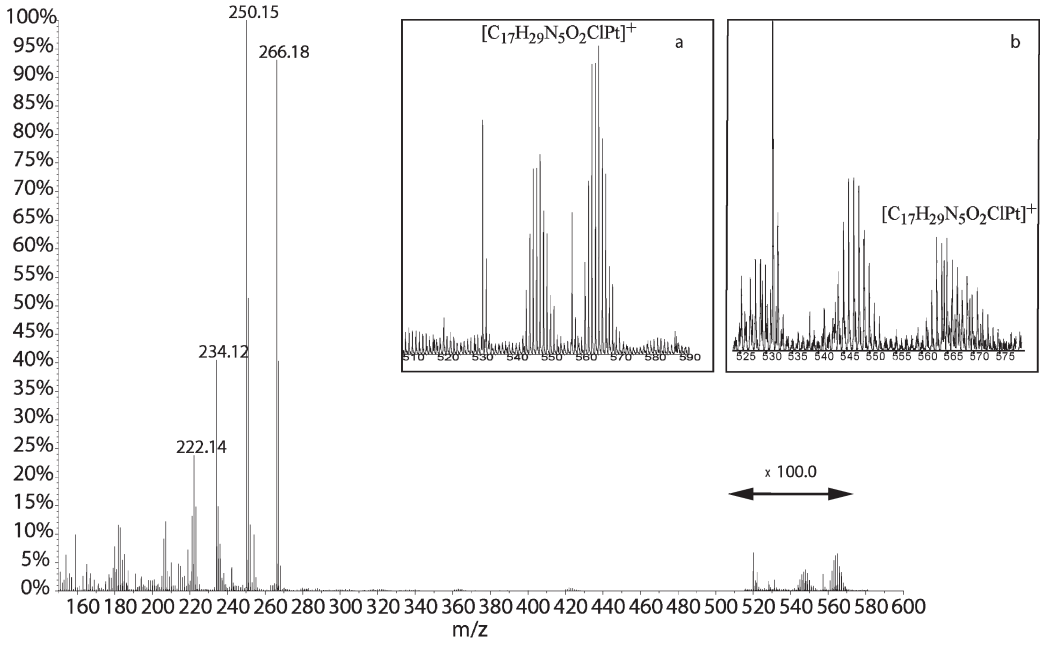


Fig. 5 ESI(+) spectrum of complex **5**; expanded region of molecular ion obtained by direct infusion (a) of a water solution containing **5**, (b) of diluted mother solution of **5**.

Table 2 Mean IC_{50} values obtained for *cis*-DDP and complex **5** against the three tumors cell lines

	$IC_{50}/\mu M \pm SD$, cell lines		
Complex	DU145	A2780	A2780-cp8
5	16 ± 0.4	3.4 ± 0.3	$31 + 0.5$ (9.1)[a]
Cis-DDP	33 ± 1.0	4.0 ± 0.5	60 ± 1.0 (15)

[a] Values in parentheses are resistance factors, $IC_{50\ resistant}/IC_{50\ sensitive}$.

complex **5** was found to be twice as effective as that of *cis*-DDP in the two *cis*-DDP resistant cell lines.

Specifically, in the A2780-cp8 cells the resistance factor was lowered from 15 to 9.1 when going from cisplatin to complex **5**. Overall, these results indicate that complex **5** is able to partially lower *cis*-DDP resistance. This enhanced cytotoxic activity has been observed in cell lines characterized by different mechanisms of *cis*-DDP resistance including decreased uptake, increased GSH (glutathione) level and DNA repair (as in the case of A2780-cp8)[41] and alteration in the apoptotic pathways, which compromise the ability to undergo *cis*-DDP induced apoptosis (as in the case of DU145).[42]

Cell cycle perturbation and apoptosis induction

DNA flow cytometric analysis was used to determine whether complex **5** enhanced *cis*-DDP cytotoxicity through perturbation of the cell cycle in these *cis*-DDP resistant cell lines.

Cell cycle analysis was performed on DU145 and A2780-cp8 cells 48 and 72 h after a 1 h exposure to equitoxic concentrations (IC_{50}) of complex **5** and *cis*-DDP. The kinetics of cell cycle distribution in untreated and drug-treated cells are shown in Table 3.

In A2780-cp8 cells, an S-phase cell accumulation was observed after 48 h treatment with complex **5** (32.2% *versus* 24% untreated cells and 28% *cis*-DDP treated cells). In addition apoptosis, evaluated as the sub-G1 peak (arrow in Fig. 6), was found in the 20% of A2780-cp8 cells treated with complex **5**.

After 72 hours of treatment, the S-phase accumulation of A2780-cp8 cells treated with complex **5** was constant, and a complete depletion of cells percentage in G2/M phase was recorded. Moreover, at this time point the presence of 33% apoptotic cells was observed, which indicates that the apoptosis induced by complex **5** in A2780cp8 is a consequence of S-phase cell accumulation. *Cis*-DDP treatment did not perturb the cell cycle at any time of recovery. In DU145 cells, 48 h incubation with complex **5** caused 54% of the cells to accumulate in the G2/M phase and 15% of the cells recovered were apoptotic. After 72 h of treatment a substantial increase in S

Table 3 Cell cycle perturbation induced by complex **5** and *cis*-DDP

	48 h			72 h		
	G1 (%)	S (%)	G2/M (%)	G1 (%)	S (%)	G2/M (%)
A2780-cp8 untreated	55	24	18	65	21	14
5[a]	46	32.2	21.8	70	27.2	2.4
Cis-DDP[a]	52	28	19.5	60	22	21.7
DU145 untreated	54	25	20	66	19	15
5[a]	25	21	54	3	43.3	54
Cis-DDP[a]	50.6	29.4	20	67	20	13

[a] Specific IC_{50}.

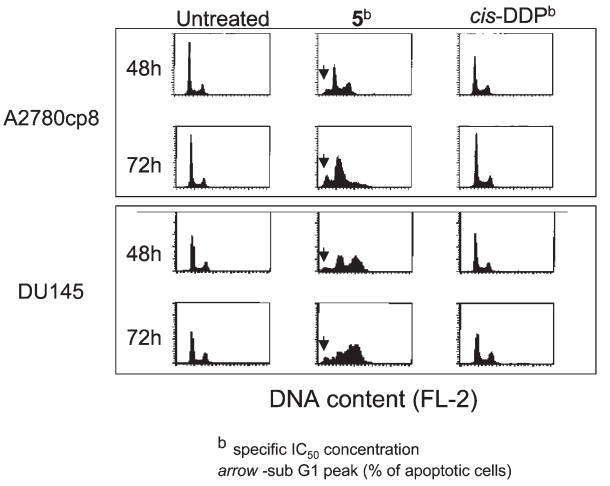


Fig. 6 Effect of complex **5** and *cis*-DDP on A2780-cp8 and DU145 cell cycle. Representative fluorescence-activated cell sorter analysis of DNA content (propridium iodide [PI] fluorescence).

phase fraction (43%) was observed as well as a persistent accumulation of cells in the G2/M compartment. In addition, at 72 h recovery, about 25% of apoptotic cells were estimated. *Cis*-DDP treatment failed to perturb the cell cycle as evaluated at both 48 h and 72 h recovery time.

These results show that in A2780-cp8 and DU145 cells, compared with *cis*-DDP, the higher cytotoxic activity observed for complex **5** could be attributed to the specific induction of cell cycle impairment and apoptosis. Consequently, complex **5** appears to activate pathways of cellular response for DNA damage different from those caused by *cis*-DDP in the same tumor models.

Conclusions

In conclusion, the present work has demonstrated the coordinating ability of 4-OH-Acrid and AO ligands with the transition metal ions Ni(II), Pd(II) and Pt(II), leading to the synthesis of new octahedral [(Ni(II)] and square-planar [Pd(II) and Pt(II)] complexes, potentially definable as metallointercalators. Between the two octahedral Ni(II) derivatives, complex **1**, [Ni(4-O-Acrid)$_2$(en)], is probably the best candidate to work as a metallointercalator. The presence of one molecule of ethylenediamine, together with two extended aromatic heterocyclic ligands, make this complex similar to other octahedral metal amine complexes whose capabilities of interaction with the DNA base pairs through both direct hydrogen bonding and/or specific van der Waals contacts has been already tested.[24] Therefore, deeper studies about the mode of interaction of these types of complex with DNA are under investigation. In the case of acridine orange ligand, the new triamine complexes **3**–**6** of the general formula *cis*-$[M(A)_n(Cl)(AO)]^+X^-$ (M = Pd(II), Pt(II); A = en, NH_3; n = 1, 2; X^- = BF_4^-, NO_3^-, PF_6^-) synthesized, fall in the general class of platinum triamine cations with potential antitumor activity.[15,16] The biological studies conducted on complex **5** have demonstrated the potential clinical utility of this type of derivative opening the way for further studies of DNA-binding able to clarify the structure–activity relationships of this class of acridine complexes.

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