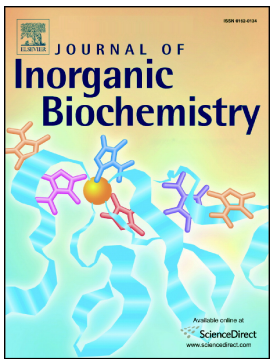

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Journal of Inorganic Biochemistry

DNA interaction of a fluorescent, cytotoxic pyridinimino platinum(II) complex

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Abstract New pyridinimino complexes of platinum(II) $[PtCl_2(N^N\text{-}R)]$ (N^N= 2-pyridylmethanimino, R =$-(CH_2)_2O(CH_2)_2OH$, $-(CH_2)_2O(CH_2)_2OCH_2Pyr$), Pyr = pyren-1-yl) have been prepared. They are characterized by a dioxygenated alkyl side chain and, in one case, by a fluorescent terminal 1-pyrenyl residue. The complexes were characterized by elemental analysis, IR, ^1H-, ^{13}C- and ^{195}Pt NMR spectroscopies. For $[PtCl_2(N^N\text{-}(CH_2)_2O(CH_2)_2OH]$ the molecular structure was determined by single crystal X-ray diffraction. The complexes are soluble and stable in DMSO/H_2O (80/20, v/v). The pyrenyl terminated compound was tested as antiproliferative agent against selected human cancer

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cell lines. Comparable cytotoxic effect was obtained on human ovarian carcinoma A-2780 and A-2780cis cells, thus suggesting a certain ability to circumvent cisplatin resistance. The interaction of this complex with DNA was investigated by linear flow dichroism and by spectrophotometric (absorbance and fluorescence) titrations. Both techniques enlightened the presence of a complex mode of interaction with DNA, involving both groove binding and intercalation.

Keywords: pyridinimino platinum(II) complexes; antiproliferative properties; DNA interaction; groove binding; intercalation

Introduction

Cisplatin anticancer properties were discovered serendipitously in the Sixties[1] and the complex was accepted by FDA as a chemotherapeutic drug at the end of the Seventies.[2] It is nowadays well known that cisplatin main target is DNA.[3][4] After crossing the cellular membrane, the complex can be activated by hydrolysis, affording positively charged species that react with DNA coordinating sites and modify the bio macromolecule structure. This causes various grades of DNA damages that initiate a process that leads to cellular death. Despite the still wide use of cisplatin in the treatment of cancer, two main problems remain unsolved and strongly limit the drug efficacy: cisplatin is scarcely tolerated by treated patients and it is inactive toward resistant cancer cell lines. Resistance is a multifactorial event generally ascribed also to the ability developed by a cell to repair DNA damages, or to side reactions occurring before the drug reaches its target. For this reason, many efforts are ongoing in the attempt to prepare more effective compounds changing the ligand nature. In particular, steric hindrance on the metal can be enhanced as in picoplatin,[5][6] to slow down side reactions, while additional functional groups can be added to favor synergistic effects. Over the last fifteen years, the pyridinimino scaffold

(Figure 1) has been exploited,[7,8,9,10,11,12,13,14,15] using a steric hindrance on the metal center similar to picoplatin and changing the side residue R nature through an easy synthetic route, the reaction of pyridine-2-carboxaldehyde with the suitable amine. The reported complexes have side residues R, decorated by different functional groups, from simple alkyl-[9,15] or aryl[8,13,14] substituents, to modified carbohydrates,[12] with antiproliferative profiles against selected cancer cells, changing with the nature of R. Mechanisms of action have not always been elucidated, but the reported data seem to indicate a correlation between the observed cytotoxicity and the side chain flexibility. As a matter of fact, a relatively low ($IC_{50} \geq 20$ μM) cytotoxicity can be observed in systems where side functional groups are connected to the pyridinimino scaffold through short and rigid spacers,[10,12,13] while structural analogues characterized by longer spacers proved to be more active.[10,16]

Figure 1

Herein the preparation and the antiproliferative activity of a Pt(II) pyridinimino complex, bearing a fluorescent pyrenyl side substituent is described. A flexible, dioxygenated alkyl chain has been used as a spacer to link the pyrenyl group to the pyridinimino scaffold. The interactions of the fluorescent complex with DNA were investigated, by both linear flow dichroism and spectroscopic (UV-vis and fluorescence) titrations.

Experimental

General

All operations were carried out under dry dinitrogen, if not otherwise stated. Solvents were purified according to reported methods[17] and stored over dry molecular sieves. 2-(2-aminoethoxy)ethanol, 2-pyridinecarboxaldehyde, 1-pyrenecarboxaldehyde (Sigma-

Aldrich) were used without further purification. *Cis*-[$PtCl_2(DMSO)_2$] was prepared according to a reported procedure.[18]

^1H-, ^{13}C- and ^{195}Pt NMR spectra were recorded on a Bruker "Avance DRX 400" spectrometer, in $CDCl_3$ solution, if not otherwise stated. *Chemical shifts* (δ ppm) are referred to tetramethylsilane (^1H- and ^{13}C-) and H_2PtCl_6 in D_2O solution (^{195}Pt-, external standard). When non deuterated solvents were used, a sealed capillary containing C_6D_6 was used to lock the instrument. Following abbreviations were used to describe signals: s (singlet), d (doublet), t (triplet), m (multiplet), br (broad).

IR spectra were recorded at 298 K by an FT-IR Perkin Elmer "Spectrum One" spectrometer, equipped with ATR accessory. The position of absorption bands were expressed in cm^{-1} and the following abbreviations were used to indicate the bands relative intensities: w (weak), m (medium), s (strong), vs (very strong), sh (shoulder), br (broad).

Elemental analyses were expressed as C, H, N % content and were carried out with an Elementar "vario MICRO cube" instrument.

When necessary, products were purified by flash chromatography (column diameter 2.5 cm); 60 Å silica gel (40-63 μm pore size) was used as stationary phase.

As for the DNA binding studies, stock solutions of calf thymus DNA were done by dissolving the sodium salt (Sigma-Aldrich) in water, sonicated (producing short fragments of ca. 500 base pairs) and standardized spectrophotometrically (ε = 13200 M^{-1} cm^{-1} at 260 nm, I = 0.10 M, pH = 7.0).[19] Sodium cacodylate $(CH_3)_2AsO_2Na$ 2.5 mM is the pH = 7.0 buffer. UV-Vis absorption spectra were recorded with a double ray Shimazdu "UV-2450" spectrophotometer. Measurements were carried out using 1.0 cm quartz cuvettes and the temperature was controlled by a ± 0.1 °C precision thermostat. Spectrofluorimetric measurements were carried out with a Perkin Elmer "LS55" spectrofluorimeter, equipped with a pulsed xenon lamp. Quartz cuvettes (1.0 cm) were used and the temperature was

controlled by a ± 0.1 °C precision thermostat. Solutions were prepared in ultrapure water (Sartorius "Arium pro®" purification system). In these experiments C_D and C_P will be used to indicate respectively the total complex (D, dye) molar concentration and the total DNA (P, polynucleotide) molar concentration in base pairs.

Linear dichroism (LD) measurements were performed on a Jasco "J500A" circular dichroism spectropolarimeter, converted for LD, and equipped with an IBM PC and a Jasco J interface. Linear dichroism was defined as:

$$LD(\lambda) = A//(\lambda) - A\perp(\lambda)$$

where A// and A⊥ correspond to the absorbances of the sample when polarized light was oriented parallel or perpendicular to the flow direction, respectively. The orientation was produced by a device designed by Wada and Kozawa[20] at a shear gradient of 500–700 rpm. Salmon testes DNA was purchased from Sigma-Aldrich. The concentration was determined by using (see above) an extinction coefficient of 13200 $M^{-1}cm^{-1}$ at 260 nm for DNA molar concentration in base pairs.

Volumes of concentrated solutions in DMSO of the examined compounds were added to a solution of salmon testes DNA (1.0×10^{-3} M) in milliQ water. Each spectrum was accumulated twice and recorded at room temperature at [drug]/[DNA] = C_D/C_P = 0, 0.04, 0.08 and 0.16.

Synthesis of ligands

For the synthesis of compounds **1-5**, [21,[22] see Supplementary Information.

Synthesis of platinum(II) complexes. General procedure.[23]

A suspension of $[PtCl_2(DMSO)_2]$ (162 – 440 mg), in CH_3NO_2 (10 mL) was treated with the suitable ligand (ligand/Pt = 1 in moles), at 25 °C, under vigorous stirring. A dark orange solution formed and, after 1-12 h, the complete conversion was checked by ^{1}H NMR. As

indicated below, the products precipitated out of the mixture spontaneously or upon addition of a mixture of solvents, were filtered, washed with Et_2O and dried under vacuum.

*Dichloro-[N-{2-[2'-(hydroxyethoxy)]ethyl}-pyridyl-2-methanimino]platinum(II), [$PtCl_2$(**1**)]* (**6**).

Orange solid (76 % yield). Found: C 25.3, H 3.5, N 5.5 %. $C_{10}H_{14}Cl_2N_2O_2Pt{\cdot}H_2O$ requires: C 25.1, H 3.4, N 5.8 %. IR: 3479m, 3084m, 3041m, 2935m, 2903m, 2866m, 1593w, 1562w 1475m, 1432m, 1133s, 1125s, 1070s, 1034s, 1016s, 895m, 883m, 773vs. ^{1}H NMR: 9.38 (d, 1H, $^3J_{H\text{-}Pt}$ = 37 Hz, NC*H*CH); 9.11 (s, 1H, $^3J_{H\text{-}Pt}$ = 94 Hz, C*H*NCH$_2$); 8.17 (d, 1H, H_{Py}); 7.93 (m, 1H, H_{Py}); 8.40 (m, 1H, H_{Py}); 4.60 (s, 1H, O*H*); 3.83-4.17 (2 m, 4H, OCH_2CH_2OH); 3.47 (m, 4H, CHNCH_2CH_2). ^{13}C{^{1}H} NMR:172.4, 163.2, 149.5, 141.3, 129.6, 129.0, 72.6, 68.3, 60.5, 59.0. ^{195}Pt{^{1}H} NMR: -2358. Crystals of **6** obtained from ethanol were suitable for the determination of the structure by single crystal x-ray diffraction.

*Dichloro-[N-{2-[2'-(pyren-1''-ylmethoxy)ethoxy]ethyl}-pyridyl-2-methanimino]platinum(II), [$PtCl_2$(**5**)]* (**7**)

EtOH/Et_2O (1/5 v/v) was added at 0 °C, under vigorous stirring. Orange solid (87 %). Found: C 46.3; H 3.6; N 3.9 %. $C_{27}H_{24}Cl_2N_2O_2Pt{\cdot}H_2O$ requires: C 46.6; H 3.8; N 4.0 %. IR: 3040m, 2861m, 1595m-br, 1560w, 1474m, 1446m, 1344m, 1305m, 1263w, 1251w, 1236w, 1218w, 1180w, 1125s, 1113s, 1094s, 1033m, 914m, 891w, 877w, 843vs, 838vs, 759vs, 708vs. ^{1}H NMR: 9.05 (d, 1H, $^3J_{HH}$ = 4.9 Hz, $^3J_{HPt}$ = 34.6 Hz, -CHC*H*NPt-); 8.24 (m, 4H); 8.09 (m, 5H); 7.97 (d, 1H, $^3J_{HH}$ = 7.3 Hz); 6.74 (m, 1H); 6.64 (m, 1H); 5.67 (d, 1H, $^3J_{HH}$ = 7.3 Hz); 5.14 (s, 2H, -OCH_2Ar); 4.26 (m, 2H, -CHNCH_2-); 3.95 (m, 2H, -NCH$_2$CH_2-); 3.83 (m, 2H, -OCH_2CH$_2$O-); 3.76 (m, 2H, -OCH$_2$CH_2O-). ^{13}C{^{1}H} NMR: 169.2; 156.1, 149.0, 137.9, 131.3, 131.1, 130.6, 129.4, 128.1, 127.7, 127.3 (2C), 126.9,

126.4 (2C), 125.8, 125.7, 125.5, 124.8, 124.5 (2C), 123.5; 72.0, 70.2, 69.7, 67.9, 59.1. ^{195}Pt {^{1}H} NMR: - 2355.

Solubility and stability of platinum(II) complexes.

Complexes **6-7** are not soluble in water nor ethanol. They are all well soluble in DMSO and in DMSO/water mixtures (DMSO/water = 80/20 v/v). Their stability was checked by ^{1}H NMR. In a typical experiment, an NMR test tube was charged with a 2×10^{-2} M solution of the complex in DMSO/H_2O (80/20 v/v) and ^{1}H NMR spectrum was registered at 30 °C. Temperature was gradually increased to 70 °C and ^{1}H NMR spectra were registered at 40, 50 and 70 °C, showing no significant modification of the signals pattern. The set of ^{1}H NMR measurements was repeated after 48h.

Inhibition growth assay

HeLa (human cervix adenocarcinoma cells) were grown in Nutrient Mixture F-12 [HAM] (Sigma Chemical Co.); A2780 (human ovarian carcinoma wild type) and A2780cis (human ovarian carcinoma cisplatin-resistant) were grown in RPMI 1640 (Sigma Chemical Co.). MSTO-211H (human mesothelioma cells) were grown in RPMI 1640 (Sigma Chemical Co.), Hepes (2.38 g/L), sodium piruvate (0.11 g/L) and glucose (2.5 g/L). 1.5g/L $NaHCO_3$, 10 % heat-inactivated fetal calf serum (Invitrogen), 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (Sigma Chemical Co.) were added to the media. The cells were cultured at 37 °C in a moist atmosphere of 5 vol.% carbon dioxide in air. The cells ($2.5\text{-}3\cdot10^{4}$), were seeded into each well of a 24 well cell culture plate. After incubation for 24 h, various concentrations of the test agents were added to the complete medium and incubated for a further 72 h. Stock solutions of new complexes were made in DMSO at 20 mM concentration and then diluted with complete medium in such a way that

the final amount of solvent in each well did not exceed 0.5 vol.%. Cisplatin was dissolved in 0.9 wt.% NaCl. A Trypan blue assay was performed to determine cell viability. Cytotoxicity data were expressed as GI_{50} values, i.e., the concentration of the test agent inducing 50 % reduction in cell number compared with control cultures.

X-Ray study

Crystals of **6** were selected at room temperature (296 K), glued to glass fibers and analyzed with a Bruker "Smart Breeze" CCD diffractometer. Table 1 summarizes the lattice parameters and the space group. Intensity data were collected in the range of 2θ angles reported in Table 1. After correction for Lorentz and polarization effects and for absorption, the structure solution was obtained using the direct methods contained in SHELXS program.[24] The final reliability factors of the refinement procedure, done using SHELXL program,[25] are listed in Table 1. Other control calculations were performed with the programs contained in the WINGX suite.[26]

Supplementary crystallographic data for compound **6** have been deposited with the Cambridge Crystallographic Data Centre (see Tab. 1 for deposition number). These data can be obtained free of charge by quoting the present paper.

Table 1

Results and Discussion

Synthesis

Chelating pyridinimino ligands were obtained by condensation of 2-pyridinecarboxaldehyde with the suitable primary amines in refluxing ethanol. While 2-(2-aminoethoxy)ethanol is commercially available, 2-[2'-(pyren-1''-ylmethoxy)ethoxy]ethylamine (**4**) was synthesized from 1-pyrenecarboxaldehyde, according to the steps reported in Scheme 1 and purified by gradient flash chromatography.

Scheme 1

The condensation of amines with 2-pyridinecarboxaldehyde afforded the pure chelating pyridinimino ligands.

The following reaction between the iminopyridine chelating ligands and *cis*-$[PtCl_2(DMSO)_2]$ afforded the corresponding platinum(II) dichloro complexes (Scheme 2), with very good yields (69-87%).

Scheme 2

Complex **6** was crystallized from a very diluted 80/20 EtOH/Et2O v/v solution and its structure was determined by single crystal X-ray diffraction (Figure 2). The coordination geometry around platinum is square planar, with bond lengths and angles in good agreement with those reported for similar compounds.[11,15,27,28,29,30] The main contributions to the crystal packing are given by π-π stacking interactions acting in the a direction and to O–H···Cl interactions (O(2)···Cl(2') 3.354 Å) acting between molecules organized in head-to-tail rows.

Figure 2

In order to investigate the antiproliferative properties and the interaction with DNA of the prepared compounds, their solubility was checked. Complexes **6** and **7** are not water soluble, slightly soluble in ethanol, but well soluble in DMSO/water mixtures. Since it is known that the presence of water can enhance platinum complexes reactivity towards

DMSO,[31] the stability of the platinum derivatives in DMSO/water mixtures was checked by ^{1}H NMR spectroscopy. Representatively, a fresh 2×10^{-2} M solution of complex **7** in d^6-DMSO/D_2O (80/20 v/v) was prepared into an NMR tube and its ^{1}H NMR spectrum was registered at 30 °C. Spectra were recorded also at 40, 50 and 70 °C. While no new signals attributable to hydrolysis/substitution products appeared with heating, small ($0.01 \leq \Delta\delta \leq 0.10$ ppm) signal displacements were observed throughout the spectra, well ascribable to temperature changes.[32] Spectra at 30 °C, repeated soon after the heating cycle and after 48 h, were superimposable with the ^{1}H NMR spectrum of the freshly prepared solution (Fig. S1), proving the stability of the complex in 20 mM DMSO/H_2O solutions, the usual concentration of the stock solutions used for biological tests.

The stability of complex **7** was checked spectroscopically (UV) in more diluted solutions, for concentrations similar to those used for biological studies (Fig. S2). Stock DMSO solutions of complex **7** were diluted (final **7** concentration = 1.8×10^{-5} M) with an aqueous solution of sodium cacodilate ([NaCac] = 2.5 mM, pH 7.0), with DMSO total amount < 1%. In order to overcome precipitation and/or aggregation phenomena, the working solutions were heated (50 °C) and then cooled (25 °C). UV spectra (Figure S2A) were registered at 25 °C on the freshly prepared solution (curve a), at 50 °C (curve b) and at 25 °C after the thermal treatment (curve c). In curve c the spectrum profile is much more definite, with peaks shape and baseline resembling those obtained for an analogous solution of **7** in chloroform (Figure S2B), where the complex is well soluble and stable. The heating procedure, followed by a spectrophotometric check, was repeated for all working solutions of **7**, before titrations.

DNA binding tests

The possible interaction of **7** with calf thymus DNA was analysed by means of spectrophotometric and spectrofluorometric titrations. Note that the pyrene fluorophore both enables the use of these optical techniques and favours the presence of hydrophobic π-π interactions with DNA base pairs, i.e. an intercalation binding mode which could have pharmacological significance. The titrations were done by adding increasing amounts of DNA directly into the cuvette containing the complex solution and were repeated at different temperatures. Fig. 3 shows an example of spectrophotometric titration (for others see Fig. S3) and enlightens the presence of hypochromic and bathochromic effects which confirm that a strong binding does indeed take place. The presence of a not-well defined isosbestic point would suggest the presence of multiple equilibria.

Figure 3

The binding process can be better inspected by plots of binding isotherms ($\Delta A/C_D$ vs. C_P, Figure S4) and by using equation (1) to try to evaluate the binding constant between P and D.

$$C_D/\Delta A = 1/\Delta\varepsilon + 1/\Delta\varepsilon K \times 1/[P] \quad (1)$$

Equation (1) needs an iterative calculation for [P] (the concentration of free DNA base pairs) which is first set as equal to C_P and then evaluated using i-th step K estimates. However, in the present case, no convergence is reached indicating quantitative binding (1:1 stoichiometry, see Fig. S4).

Spectrofluorometric titrations were done similarly to spectrophotometric experiments and confirmed a fluorescence emission variation occurring upon DNA binding (Figure S5).

Very interestingly, the amplitude of the binding isotherm turns out to be a function of temperature, being it high and negative at 10.1°C, very small at 26.6°C and high and positive at 44.8°C (Fig. 4A). Under highly diluted conditions (Fig. 4B and S6) a biphasic trend is visible.

Figure 4

This finding confirms the presence of two different, overlapping and very strong, binding modes as suggested by the non-perfect UV-vis isosbestic point. The data analysis according to equation (1) (where signal change ΔA is simply replaced by ΔF) renders again quantitative binding with the exception of the binding mode related to signal decrease at 10.1°C ($K = 2.4\times10^6\ M^{-1}$) and that related to fluorescence increase at 44.8.°C ($K = 3.2\times10^6\ M^{-1}$). Therefore, the first phase, occurring in the presence of dye excess conditions and producing fluorescence emission decrease, is related to a binding constant that increases with temperature ($\Delta H > 0$): these features are indicative of external/groove binding. On the contrary, the second phase, occurring in the presence of DNA excess conditions and producing fluorescence emission increase, is related to a binding constant that decreases upon temperature increase ($\Delta H < 0$): these features are indicative of intercalation.

On the whole, it can be concluded that the affinity of **7** for DNA is very high, likely due to the presence of two flexibly linked aromatic moieties. The possible binding modes are both groove and intercalation, the prevailing one being dependent on temperature and reagents concentrations. This dual behaviour was already found for other small molecule /DNA

systems.[19,33,34,35] Literature data also confirm that pyrene derivatives can either bind in the DNA minor groove producing a fluorescence emission decrease,[36] or intercalate producing a fluorescence emission increase.[37]

DNA interaction geometry

To clarify the geometry of complexation between **7** and DNA, linear flow dichroism (LD) experiments were performed. The LD spectra of aqueous solutions of DNA alone (trace a) and in the presence of complex **7** at different [**7**]/[DNA] ratios (traces b-d) are shown in Figure 5.

The LD spectrum of the macromolecule shows, as expected, a strong negative signal at 260 nm, due to the contribution of purine and pyrimidine base pairs (trace a). Following the addition of **7** (traces b-d) a concentration-dependent induced dichroic signal appears at higher wavelengths (300-450 nm), which is a spectral region where no contribution from DNA base pairs can be detected and only the chromophore of the added complex can absorb. Since small molecules cannot be oriented in the flow field, the occurrence of this latter signal is indicative that **7** forms a molecular complex with the macromolecule and becomes oriented.

Figure 5

Furthermore, the sign of this induced dichroic signal depends on the orientation of the bound chromophore, relative to the axis of the DNA helix. In particular, for planar aromatic scaffolds, it is possible to assume that the strong π-π* transitions are generally polarised in the plane of the chromophore. Then, a negative signal is ascribable to a parallel orientation to the plane of DNA bases, which is an intercalative mode of binding,

where the aromatic plane of the drug and DNA bases are essentially coplanar. Otherwise, for compounds bound in the groove of DNA, an induced dichroic signal of positive sign is expected.[38] In the spectra of the DNA solution incubated with **7** (traces b-d) the induced signal shows both positive and negative contribution suggesting a heterogeneity of binding. This behaviour can be explained by the presence of drug molecules that both intercalate between base pairs and accommodate along the groove.

Cytotoxicity studies

The antiproliferative activity of **7** was assayed on four human tumor cell lines and the obtained results, expressed as GI_{50} values, i.e. the concentration (μM) of test complex able to induce a 50% reduction in cell number in comparison with the control culture, were shown in Table 2. The well-known drug cisplatin was taken into account as reference.

Table 2

The obtained results indicate the ability of **7** to induce a significant antiproliferative effect on most of the human tumor cell lines taken into consideration, with GI_{50} values in the micromolar range. Notwithstanding these values are higher with respect to those calculated for the reference drug, indicating a lower efficacy on cells, the capacity of **7** to exert a comparable cytotoxicity on both resistant (A2780cis) and sensitive (A2780wt) ovarian carcinoma cells, suggests for this chemical scaffold the ability to overcome the resistance phenomenon and renders it worthy for further development.

Conclusions

A new pyridinimino platinum(II) complex has been prepared, bearing a terminal pyrene group linked to the chelating scaffold through a dioxygenated, flexible spacer (complex **7**). The antiproliferative activity of **7** was checked *in vitro* against both sensitive and resistant cancer cell lines. Complex **7** showed an interesting capacity to circumvent resistance towards A2780 cis cells, and its interaction with DNA was investigated by two independent spectroscopic techniques (UV-vis/fluorescence titrations and linear flow dichroism). A very strong and complex interaction with DNA was enlightened in both cases. In particular, collected data sets show that both groove binding and intercalation take place, with one interaction becoming more evident with respect to the other according to the involved species molar ratio and the temperature. The aromatic pyrene moiety is most likely responsible for intercalation through π-π interactions, common for this kind of residues, while groove binding could be ascribed to DNA-templated pyrene-pyrene interactions, favoured also by the metal containing residue and/or to the spacer. It has to be underlined that, due to the flexibility of the linking alkyl chain, cooperative effects cannot be excluded *a priori*, with two different interaction points of the same molecule acting at the same time. The collected data suggest that aryl-terminated pyridinimino complexes are a versatile class of antiproliferative complexes. Studies are in progress to investigate structural analogues of complex **7**.

Declaration of interests

None.

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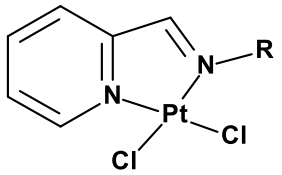


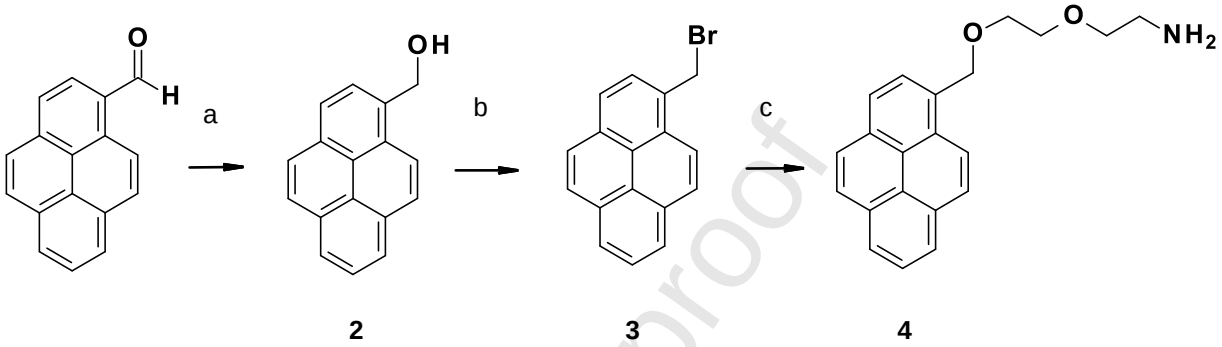
R = Alkyl, Aryl, modified carbohydrate...

Figure 1. Platinum(II) pyridinimino complexes

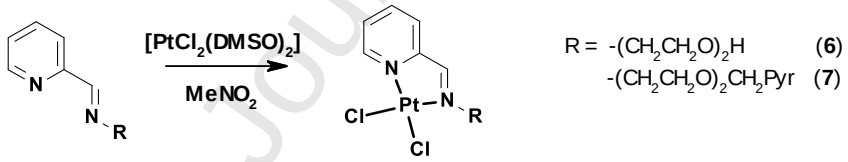
Table 1. Crystal data and structure refinement for complex **6.**

Compound	**6**
CCDC number	1938455
Empirical formula	$C_{10}H_{14}Cl_2N_2O_2Pt$
Formula weight	460.22
Crystal system	Triclinic
Space group	$P\ \bar{1}$
a (Å)	7.6622(2)
b (Å)	8.2251(3)
c (Å)	10.7771(3)
α (°)	84.6900(14)
β (°)	85.8650(12)
γ (°)	80.1320(12)
Volume (Å^3)	665.18(4)
Z	2
ρ_{calc} (g cm^{-1})	2.298
μ (mm^{-1})	10.940
F(000)	432
θ range (°)	2.7 to 36.0
Reflections collected	18913
Independent reflections	5603 [R_{int} = 0.0251]
Goodness-of-fit on F^2	0.993
Final R_1 [$I \geq 2\sigma(I)$]	0.0258
Final wR_2 [$I \geq 2\sigma(I)$]	0.0694

Final R_1 [all data]	0.0311
Final wR_2 [all data]	0.0724
Largest peak/hole (e Å^{-3})	2.155, –1.437



Scheme 1. Synthesis of amine **4**: a) $NaBH_4$, THF, 0 °C; b) PBr_3, THF, 0 °C; c) 1) $H_2N(CH_2CH_2O)_2H$, NaH, THF, 0 °C; 2) gradient flash chromatography



Scheme 2. Synthesis of complexes **6**-**7**.

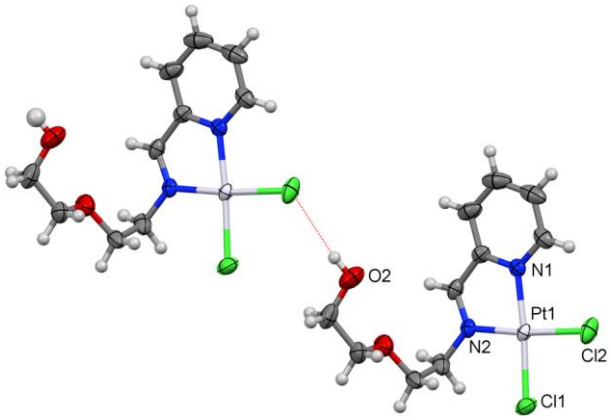


Figure 2. Molecular structure of **6**. Most significant bond lengths (Å): Pt(1)–N(2) 2.001(3); Pt(1)–N(1) 2.009(3); Pt(1)–Cl(1) 2.2782(12); Pt(1)–Cl(2) 2.2918(10) and angles (°): N(2)–Pt(1)–N(1) 80.37(13); N(2)–Pt(1)–Cl(1) 95.98(10); N(1)–Pt(1)–Cl(1) 175.30(9); N(2)–Pt(1)–Cl(2) 174.50(9); N(1)–Pt(1)–Cl(2) 94.40(10); Cl(1)–Pt(1)–Cl(2) 90.27(6).

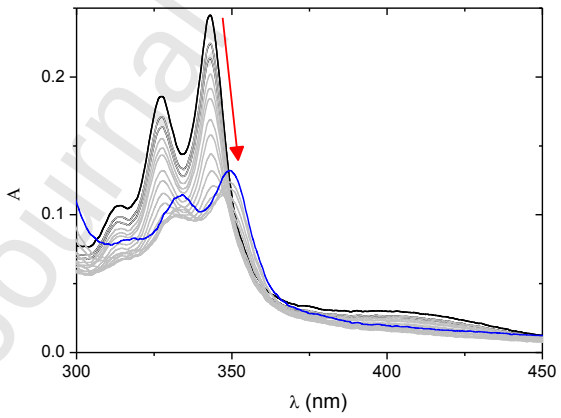


Figure 3. Spectrophotometric titration for the **7**/DNA system; C_D = 1.8×10^{-5} M, C_P from 0 M to 5.3×10^{-5} M (arrow follows increased DNA content), [NaCac] = 2.5 mM, pH 7.0, DMSO = 1%, T = 25.7 °C.

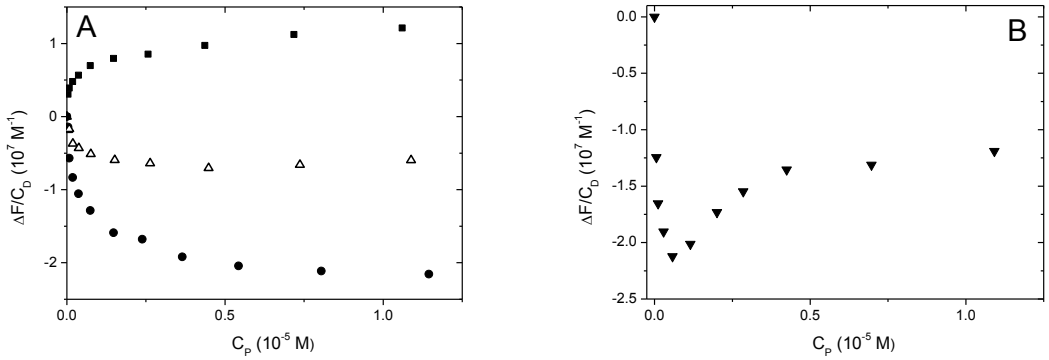


Figure 4. Spectrofluorometric binding isotherms for the **7**/DNA system, [NaCac] = 2.5 mM, pH 7.0, DMSO = 0.4%, λ_{ex} = 310 nm, λ_{em} = 378 nm; (A) $C_D = 7.1\times10^{-6}$ M, T = 10.1°C (circles), 26.6°C (open triangles), 44.8°C (squares), (B) $C_D = 1.6\times10^{-6}$ M, T = 21.6 °C.

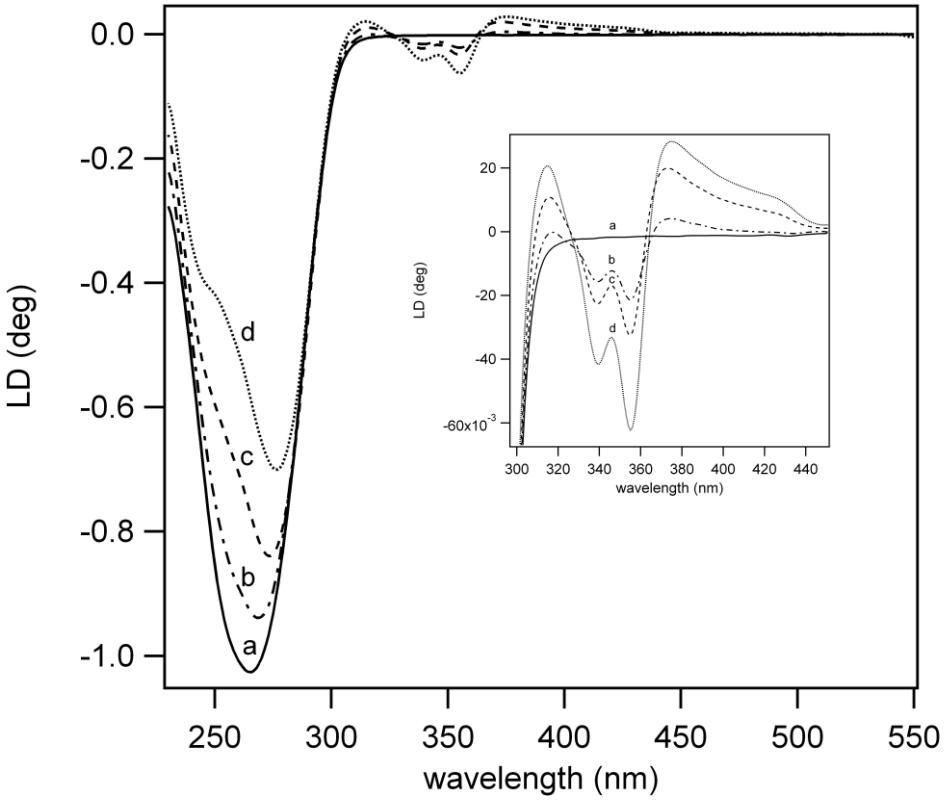


Figure 5: Linear flow dichroism (LD) spectra of **7** at different [drug]/[DNA] ratios: trace (a), 0; trace (b), 0.04; trace (c) 0.08 and trace (d) 0.16. [DNA]= $C_P = 1.0\times10^{-3}$ M.

Table 2. Human cell growth inhibition in the presence of **7** and cisplatin, taken as reference.[a]

Complex	**Cell Line GI_{50}[b] (μM)**			
	HeLa	**A2780wt**	**A2780cis**	**MSTO-211H**
7	>20	5.3±0.4	6.8±1.5	17.4±2.3
cisplatin	1.5±0.2	1.1±0.4	6.6±0.8	1.4±0.3

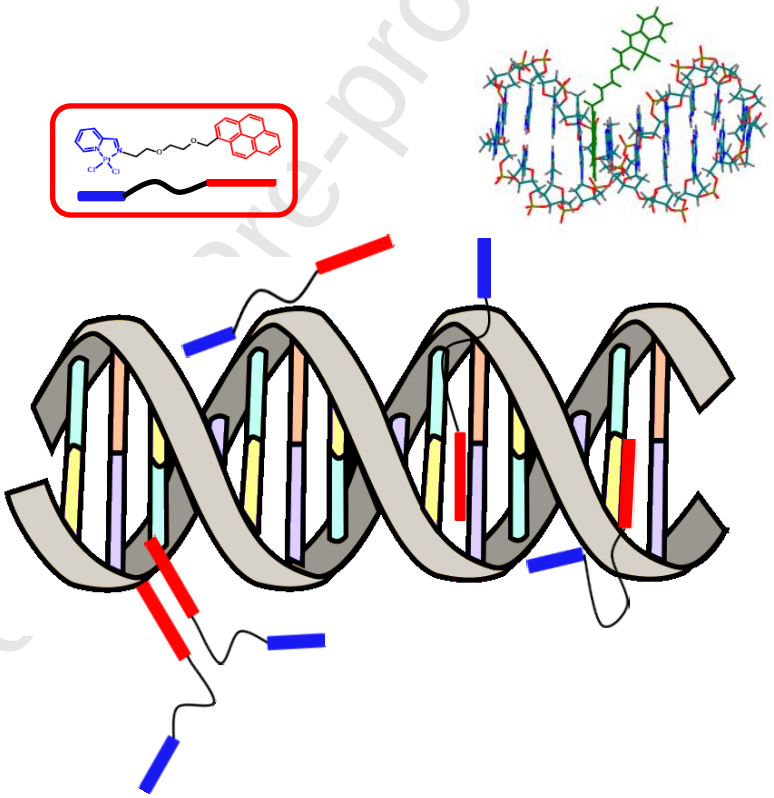
[a] Complex **6** was inactive towards the tested cell lines; [b] Values are the mean ± SD of at least three independent experiments.

GRAPHICAL ABSTRACT

DNA interaction of a fluorescent, cytotoxic pyridinimino platinum(II) complex.

Riccardo Bondi, Tarita Biver, Lisa Dalla Via, Federica Guarra, Mariafrancesca Hyeraci, Claudia Sissi, Luca Labella, Fabio Marchetti and Simona Samaritani

A versatile pyridinimino Pt(II) complex, bearing a pyrenyl-terminated side chain was synthesized. The complex circumvents cisplatin resistance towards A2780cis cells and is able to interact with DNA by both groove binding and intercalation.



Journal of Inorganic Biochemistry

DNA interaction of a fluorescent, cytotoxic pyridinimino platinum(II) complex

Highlights

- A fluorescently tagged, cytotoxic pyridinimino platinum(II) complex was prepared.
- The complex circumvents cisplatin resistance against A2780cis cancer cell line.
- Complex/DNA interaction was studied by independent spectroscopic techniques.
- DNA interaction takes place by both groove binding and intercalation.