cis-[$PtCl_2$(4,7-H-5-methyl-7-oxo[1,2,4]triazolo[1,5-*a*]pyrimidine)$_2$]: A Sterically Restrictive New Cisplatin Analogue. Reaction Kinetics with Model Nucleobases, DNA Interaction Studies, Antitumor Activity, and Structure−Activity Relationships

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The formation and isolation of the antitumor drug cisplatin analogue *cis*-[$PtCl_2$(Hmtpo-N^3)$_2$]·-$2H_2O$ (**1**) (where Hmtpo = 4,7-H-5-methyl-7-oxo[1,2,4]triazolo[1,5-*a*]pyrimidine) by reaction of Hmtpo with $K_2[PtCl_4]$ in HCl (0.5 N) is reported. This complex crystallizes in the monoclinic space group $P2_1/c$ with unit cell dimensions $a = 15.215(2)$ Å, $b = 9.629(1)$ Å, $c = 13.115(3)$ Å, $\beta = 97.40(2)°$, and $Z = 4$. The molecular structure shows that Pt is in an almost square planar environment, PtN_2Cl_2, which has a cis configuration. The Hmtpo ligands show a head to head orientation in the solid state and nonrestricted rotation about the Pt−N bonds in solution. The reactivity of the complex to model nucleobases 9-ethylguanine (9-EtGH) and 1-methylcytosine (1-MeC) has been investigated by ^{1}H NMR spectroscopy at 45 °C in aqueous media. The results show that **1** reacts slowly with 9-EtGH ($t_{1/2} \approx 5$ days) by displacement of Cl^-, producing *cis*-[Pt(mtpo-N^3)$_2$(9-EtGH-N^7)$_2$], which is similar to the major cross-link adduct of cisplatin with DNA. However, **1** gives no reaction with 1-MeC. This appears to be due to the lesser reactivity of 1-MeC and to competition between the cross-link reaction and dimerization of **1** to [Pt_2(μ-mtpo-N^3,N^4)$_4$]. Circular dichroism studies of DNA in the presence of **1** show that the platinum complex reacts efficiently after 48 h at a optimum ratio of 0.25 Pt atom/mol of DNA nucleotide. These results and those obtained from reaction of **1** with 9-EtGH suggest that the platinum compound binds the N7 atoms of two guanines of the same strand, forming intrastrand cross-linked adducts. Chelation of DNA bases by **1** causes important conformational changes, bringing the guanines close together. The anticancer activity of complex **1** has been tested against the human cancer cell lines MCF-7 breast carcinoma and A121 ovarian carcinoma. Results indicate a moderate antitumor activity against breast carcinoma and a marked and selective cytotoxic effect against ovarian carcinoma.

Introduction

Rosenberg's discovery of the antitumor properties of *cis*-$PtCl_2(NH_3)_2$ (cisplatin)[1] and its subsequent clinical success has prompted numerous studies of Pt compounds with nucleobases. The interest of nucleobases arises from the widespread belief that DNA is the molecular target of this drug. Thus, as a result of intensive research efforts over the past several years, there is now a fairly detailed understanding of how these complexes bind to DNA,[2] the structural modifications promoted in this molecule,[3] and how platinum binding changes the specificity of some DNA−protein interactions.[4] The use of cisplatin, however, is limited by toxic side effects and lack of activity against tumors with natural or acquired resistance to this drug.[2b] Although some new platinum complexes have very promising biological properties,[5,6] there is still need to synthesize platinum complexes with novel ligands and to test them for antitumor activity, in the hope of overcoming the above-mentioned limitations. Consequently, a call has been made[3] for the design of new platinum complexes with cross-linking abilities as antitumor drug candidates. Concretely, the major adducts formed between DNA and cisplatin facilitate the necessary conformational switch for binding DNA to high mobility group domain containing proteins.[2b] This type of interaction is considered critical for antitumor activity, and therefore, it would be interesting to modify ligands on new platinum complexes in order to stabilize further the conformational changes induced by drug binding. Moreover, reactions of platinum complexes are usually viewed as kinetically controlled processes.[7] Steric hindrance of the nonparticipant ligands can slow reaction kinetics 6-fold,[8] also restricting nucleobase rotation[9] in the resulting cross-link adduct. These facts may explain the generally lower antitumor activity of platinum compounds with bulky amines and cisplatin selectivity toward guanine residues.[2b]

We present the results of the synthesis and structural characterization of the novel compound *cis*-[$PtCl_2$(Hmtpo-N^3)$_2$] (**1**) (where Hmtpo = 4,7-H-5-methyl-7-oxo[1,2,4]-triazolo[1,5-*a*]pyrimidine). Additionally, the reactivity

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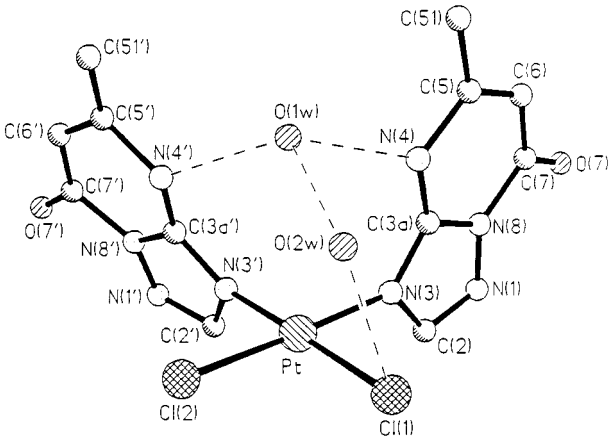
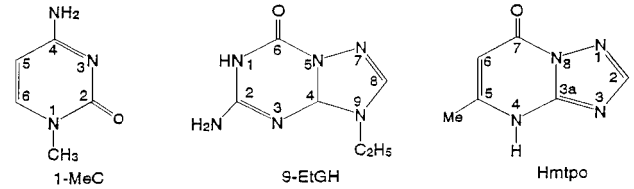


Figure 1. Molecular structure and atomic numbering scheme for *cis*-[$PtCl_2$(Hmtpo-*N3*)$_2$]·$2H_2O$ (**1**).

Scheme 1



of **1** with model nucleobases (9-ethylguanine (9-EtGH) and 1-methylcytosine (1-MeC), see Scheme 1), circular dichroism (CD) studies of its interaction with calf-thymus DNA, and in vitro antitumor activity against two different human cancer cell lines also have been studied. In conclusion, compound **1** may be considered an analogue of the anticancer drug cisplatin. The significant cytotoxicity of this complex against ovarian carcinoma and the CD effects observed are related to the slow kinetics of the reaction between **1** and model nucleobase 9-EtGH and the restrictive orientation of the Hmtpo moieties in the complex.

Results and Discussion

Structural Characterization of *cis*-[$PtCl_2$(Hmtpo-*N*^{3})$_2$]·$2H_2O$ (1). The reaction of Hmtpo with $K_2[PtCl_4]$ in HCl (0.5 M) yields *cis*-[$PtCl_2$(Hmtpo-*N*^{3})$_2$]·$2H_2O$. The dehydration process starts at low temperature, as indicated by its TG diagram (35−180 °C). Its easy dehydration is reflected by the X-ray results, which fit a dihydrate compound, and explain the poor quality of the single crystals. Once dehydrated, the compound is thermally stable up to 220 °C, when pyrolytic decomposition starts.

X-ray Structure. The structure of **1** consists of discrete *cis*-[$PtCl_2$(Hmtpo-*N*^{3})$_2$] molecules and two crystallization water molecules. A view of the molecule is shown in Figure 1, and a selection of bond distances and angles are given in Table 1. Pt is located in an almost square planar environment made up of two Hmtpo ligands bonded through N3 and two chlorine atoms. The PtN_2Cl_2 square has a cis configuration, which is consistent with the preparation method. The most interesting feature of this structure is the head to head orientation exhibited by the Hmtpo moieties (dihedral angle of 79.8°). The Pt−N and Pt−Cl bond distances agree with values found for other analogous platinum complexes.[10,11] The poor quality of the structural data, however, does not allow a detailed description of Hmtpo geometry. The hydrogen bond interactions O1w···O2w, 2.78(5); O2w···Cl1, 3.12(4); N4···O1w, 2.74(3); N4′···O1w, 2.86(4) Å appear to be responsible for the head to head conformation of the molecule (see Figure 1). Thus, the modification of the hydrogen-bonding pattern after chlorine substitution by NH_3 groups changes the ligand orientation to head to tail.[12]

Table 1. Selected Bond Distances and Angles for *cis*-[$PtCl_2$(Hmtpo)$_2$]·$2H_2O$ (**1**)

	Distances, Å		
Pt−Cl1	2.278(6)	Pt−N3	2.00(2)
Pt−Cl2	2.274(8)	Pt−N3′	1.98(2)
	Angles, deg		
Cl1−Pt−Cl2	90.2(3)	Cl2−Pt−N3	177.3(4)
Cl1−Pt−N3	91.7(4)	Cl2−Pt−N3′	89.7(7)
Cl1−Pt−N3′	178.5(6)	N3−Pt−N3′	88.4(7)
Pt−N3−C3a	129(1)	Pt−N3′−C3a′	130(2)
	Hydrogen Bonds, Å		
O1w···O2w	2.78(5)	O2w···Cl1	3.12(4)
N4···O1w	2.74(3)	N4′···O1w	2.86(4)

The head to head atropisomers in platinum complexes with heterocyclic ligands similar to the purine nucleobases are difficult to isolate and have been found to exist in only a few crystal structures of *cis*-[$Pt(NH_3)_2$(9-EtGH)$_2$][13] and *cis*-[$Pt(NH_3)_2$(9-EtGH)(9-methyladenine)],[14] the normal feature being a head to tail[9b] orientation. In DNA, the platinum compounds cross-link adjacent purine residues in a head to head conformation, these atropisomers being considered adequate for modeling platinum−DNA interactions.

IR Spectroscopy. The IR spectrum of **1** shows only small changes if compared to free Hmtpo. The only appreciable changes above 600 cm^{-1} are the appearance of a broad band centered at 3490 cm^{-1}, assigned to ν(O−H) of hydration water, and the up frequency shift (ca. 20 cm^{-1}) in the ν(C═O) band. Finally, in the far-IR appears a broad band assigned to ν(Pt−Cl) centered at 330 cm^{-1}. This band splits in two (335, 320 cm^{-1}) when the complex is dehydrated by heating at 195 °C. These absorptions were assigned, respectively, to A_1 and B_2 ν(Pt−Cl) modes.

^{1}H NMR Spectroscopy. The ^{1}H NMR spectrum of **1** in DMSO-d_6 is indicative of complex formation. ^{1}H NMR resonances of H4, H2, and CH_3 appear, respectively, 0.55, 0.44, and 0.09 ppm downfield-shifted with respect to free Hmtpo, while H6 appears unaltered. The large shift in the H2 signal is due to the proximity of this proton to platinum coordinated through N3. On the other hand, the shift in the H4 signal is a result of an increase in the N4−H4 acid character after platinum binding.[2a] The presence of only one set of signals in the ^{1}H NMR spectrum suggests the free rotation of the Hmtpo ligands about the Pt−N3 bond in the NMR time scale. Restricted rotation about the Pt−N3 bond would result in the presence of eight peaks related to the head to head and head to tail species.

If **1** is allowed to stand for a long period of time in DMSO (about 3 days), Hmtpo ligands are displaced totally, because of the strong *trans*-labilizing effect of the sulfur ligands. A similar result is obtained when **1** is treated with an excess of thiourea,[15] which is in agreement with its cis configuration.

Reactivity of *cis*-[$PtCl_2$(Hmtpo-*N*^{3})$_2$]·$2H_2O$ toward Model Nucleobases. *cis*-[$PtCl_2$(Hmtpo-*N*^{3})$_2$]·-

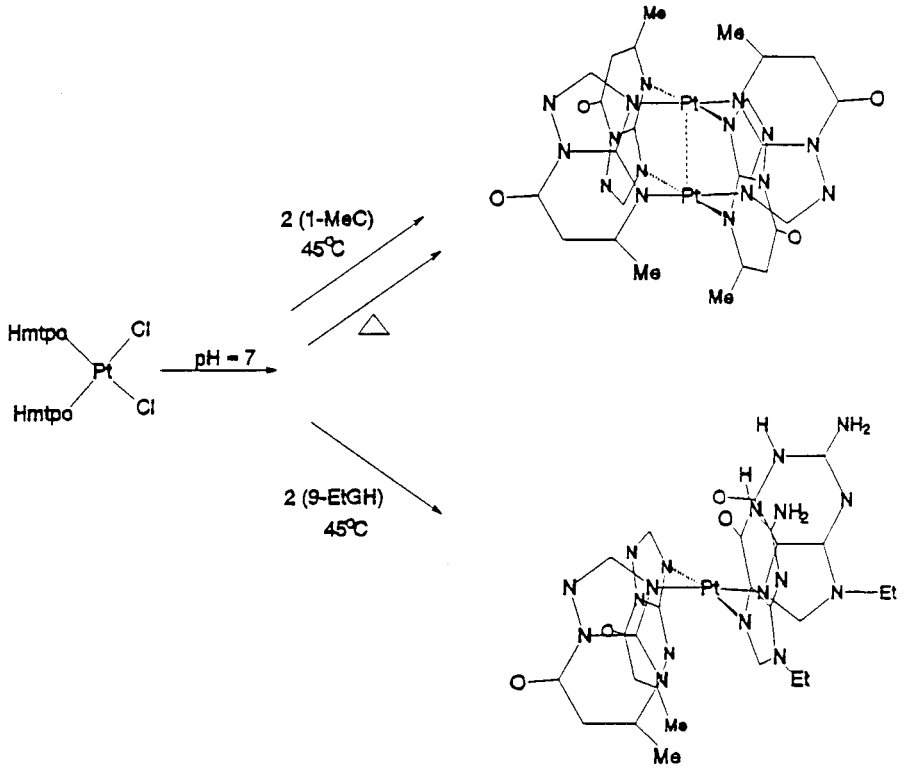


Figure 2. Reactivity of **1** toward model nucleobases 9-EtGH and 1-MeC.

$2H_2O$ is characterized by its high solubility in neutral aqueous media, due to deprotonation of Hmtpo in this pH range of (pK_a = 6.3). This feature makes this complex suitable for testing as a chemotherapeutic agent. Thus, we assayed its reactivity toward model nucleobases 9-EtGH and 1-MeC as models of the interaction of **1** with its possible molecular target, DNA. In the presence of 2 mol of 9-EtGH, the chlorine ligands are slowly substituted (see Figure 2), yielding the compound *cis*-[Pt(mtpo-N^3)$_2$(9-EtGH-N^3)$_2$] in which the guanine residues are bound to Pt at the N7 position.[2b] The presence of only one set of signals, in the ^{1}H NMR spectrum of the cross-link adduct (Figure 3), appears to be consistent with a fixed structure in solution.[16] The kinetics of the reaction are, however, too slow ($t_{1/2} \approx 5$ days, that is about 20 times slower than the reaction between cisplatin and DNA[17]) to be considered of biological relevance. On the other hand, **1** gives no reaction with 1-MeC and dimerization of Pt compound occurs instead. The lack of reactivity of **1** with 1-MeC is attributed to the steric hindrance of the NH_2 groups and presence of carbonyl groups that impede the binding of compound **1** to the N3 atoms. It appears that the lower reactivity of 1-MeC, together with competition of the dimerization reaction of **1** to Pt$_2$(mtpo-N^3,N^3)$_4$,[18] are responsible for the complex inertness toward the model nucleobase 1-MeC.

Interaction with DNA. CD spectra of free CT-DNA and those obtained in the presence of compound **1** with concentrations and conditions described in the Experimental Section are observed in Figure 4. As seen, the positive band registered at 275 nm shows an enhancement that corresponds to low r_b values, ranging between 0.02 and 0.25, the maximum increase being registered when r_b is 0.25. Higher r_b values produce a successive decrease in the positive band (lowest hypochromicity

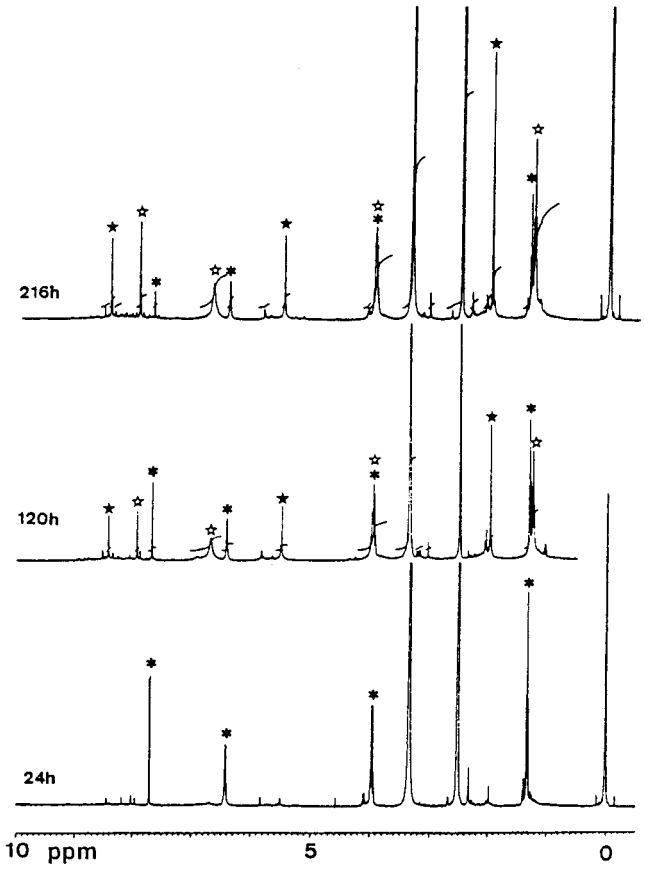


Figure 3. Evolution of the cross-link reaction between **1** and model nucleobase 9-EtGH. Free 9-EtGH (*) and 9-EtGH (☆) and mtpo (★) signals in the cross-link adduct. The ^{1}H NMR signal for the guanine residues (H8, δ = 7.96; NH_2, δ = 6.70 ppm) are consistent with the 9-ethylguanine bound through N7.[2a] mtpo signals appear as follow: H2, δ = 8.46; H6, δ = 5.51; CH_3, δ = 1.98 ppm.

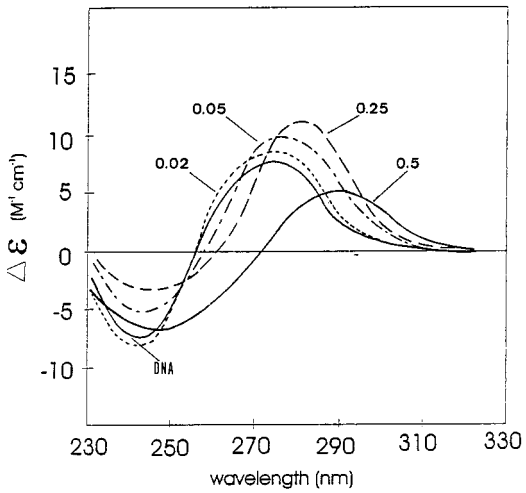


Figure 4. Circular dichroism spectra of calf-thymus DNA before and after reaction with complex **1** at the platinum/DNA ratios (r_b) given in the Figure. r_b indicates mol of Pt present per mol of nucleotide.

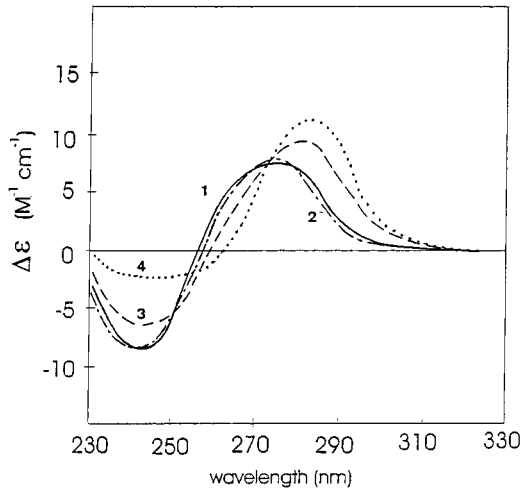


Figure 5. Circular dichroism spectra of free calf-thymus DNA (curve 1) and in the presence of complex **1** at different times of reaction as explained in text. All experiments were carried out at a constant r_b value of 0.25.

obtained at r_b 0.5) accompanied by significant bathochromic changes. It must be remembered that the saturation value of DNA with cisplatin is close to 0.5,[19] indicating that no further change would be detectable over this value. Only one isodichroic effect is observed at r_b values between 0.02 and 0.2, while no clear isodichroic effect is registered at higher r_b values.

Figure 5 shows the CD spectra of free CT-DNA (curve 1) in the presence of compound **1** at a constant r_b value of 0.25. No variation of the positive band at 275 nm is observed 1 h after mixing both solutions (curve 2). However, when the DNA−Pt complex solution is left overnight at room temperature, an important enhancement accompanied by a red-shifting of the positive band is detected (curve 3). After 48 h at rest, the effect increases: the ellipticity reaches a maximum of 11 M^{-1} cm^{-1} and the DNA band at 275 nm shifts to 282 nm (curve 4).

The results obtained in this study differ from those reported for CT-DNA−cisplatin CD studies, the major differences being the significant increase and red-shifting of the positive band observed in our case at an r_b value of 0.25. Concretely, Srivastava[20] observes a moderate hyperchromicity and red-shifting of the positive band at an r_b of 0.1. On the other hand, Macquet[21] reports similar results using salmon sperm DNA instead of CT-DNA. As in our experiments, both authors observe the decrease of the positive band at higher Pt/DNA ratios.

Base stacking is important in determining the strength of a CD band in DNA. In our case, the strength of the positive CD band after the interaction of compound **1**, indicates an effect similar to that observed when cisplatin is bound to DNA. Because this type of behavior is associated with the chelation of cisplatin complexes to different guanines of the same strand (intrastrand binding), we deduce that complex **1** forms a bifunctional chelate, which bends the duplex and alters the DNA structure by destacking the nucleobases.

Biological Studies. The effects produced by the addition of compound **1** at different concentrations to the cultured human cell lines MCF-7 breast carcinoma and A121 ovarian carcinoma are shown in Figure 6. Although low doses of **1** do not have significant effects on MCF-7 cell proliferation (Figure 6a), a dose of 41.5 mM causes a cell inhibition of 54.8% after 48 h of treatment. When the dose is doubled (83 mM), a moderate enhancement of cell inhibition is obtained. These results and the ID_{50} value (33.4 mM, 48 h) suggest moderate cytotoxicity of compound **1** against the MCF-7 cell line.

In A121 ovarian carcinoma (Figure 6b), a substantial increase in cytotoxic behavior occurred at doses as low as 8.3 mM drug. A dose of 16.6 mM produces 53% cell inhibition after 48 h of treatment, while the addition of 83 mM causes a considerably higher cytotoxicity (84% cell inhibition). These data and the corresponding ID_{50} value (14.3 mM, 48 h) indicate a high antitumor activity against human ovarian A121 cell line. Concretely, compound **1** is less active than cisplatin and more active than carboplatin[22] against ovarian tumor. Therefore, the new platinum drug studied here warrants further investigation into its in vivo antitumor activity against different types of tumors.

Structure−Activity Relationships. The chemical substitution of bonded NH_3 on cisplatin by two Hmtpo ligands influences the tumor-inhibiting activity of compound **1** in different ways. First, the bulky nature of the mtpo moieties causes the slow reaction between **1** and nucleobases. On the other hand, the steric conformation and characteristic electronic crowding of these aromatic nitrogen heterocyclic ligands modify the distances and angles of the molecular complex **1**.

Clearly, the nonbonding Cl···Cl distance (bite) for cisplatin, equal to 3.35 Å, corresponds to the distance between two appropriate DNA−base donor atoms in a way that enables cross-links to be built after dissociation of the chlorine atoms.[23] The bite between these leaving groups is directly correlated with the distance between adjacent coordination sites at the metal atom and another given pair of entrant ligands.[24]

In compound **1**, the less nucleophilic character of the Hmtpo ligands and the repulsive electronic interaction exerted by these ligands on bonded chlorine atoms induce a significant reduction of the Cl1−Pt−Cl2 angle (90.2° versus 91.9° for cisplatin) and a shortening of the

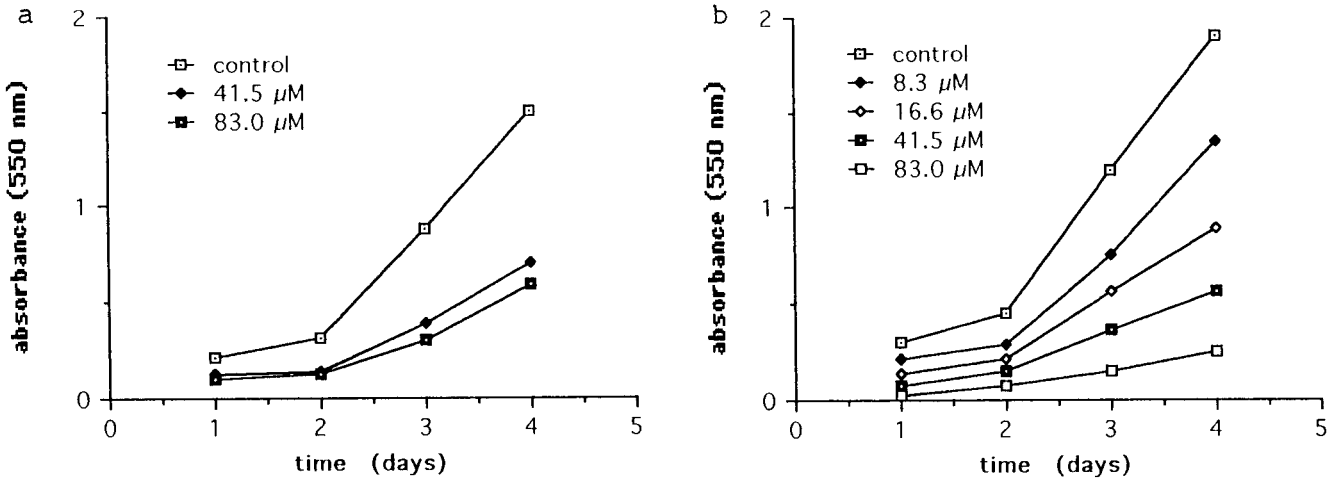


Figure 6. (a) Cytotoxic effect (tumor cell growth inhibition) produced by complex **1** on the human cell line MCF-7 breast carcinoma at the drug concentrations given in the Figure. (b) Cytotoxic effect (tumor cell growth inhibition) produced by complex **1** on the human cell line A121 ovarian carcinoma at the drug concentrations given in the figure.

Pt−Cl distances (2.28 Å versus 2.33 Å for cisplatin). However, the bite value for compound **1** (3.22 Å) is in the range 3.20−3.50 Å which seems to be adequate for the formation of DNA−Pt cross-links. Therefore, the *cis*-dichlorometal moiety is apparently of critical significance for the mechanism of action. In compound **1**, the reduction of the Pt−Cl distances and Cl1−Pt−Cl2 angle impede the formation of the bifunctional chelate bond responsible for its antitumor activity.

Experimental Section

Reagents and Chemicals. 4,7-H-5-methyl-7-oxo[1,2,4]-triazolo[1,5-*a*]pyrimidine (Hmtpo) was purchased from Aldrich Chemical Co. and used as received. Calf-thymus DNA (CT-DNA)(<1% protein) was purchased from Sigma Chemical Co. Other chemical reagents and solvents were supplied by commercial sources. All experiments were performed in air.

Preparation of *cis*-[$PtCl_2$(Hmtpo-N^3)$_2$]·$2H_2O$ (1). A warm solution of Hmtpo (4.8 mmol in ca. 20 mL of HCl (0.5 M) was mixed with another solution containing $K_2[PtCl_4]$ (2.4 mmol in ca. 20 mL of HCl (0.5 M)). The resulting red solution produced an abundant precipitate of yellow single crystals of **1**, suitable for X-ray studies 2 days later. The crystals were rinsed with water and air-dried. However, the crystals changed with time due to their partial dehydration. Yield: 93%. Elemental analysis data of single crystals of the complex nearly fits the description as dihydrate in accordance with the X-ray results. Anal. Calcd for $C_{12}H_{16}N_8O_4Cl_2Pt$: C, 23.91; H, 2.74; N, 18.60; Pt, 32.45. Found: C, 23.19; H, 2.72; N, 18.15; Pt, 31.50. IR (cm^{-1}): 320 s, 335 s, 475 m, 535 s, 660 m, 840 m, 1140 s, 1180 s, 1410 s, 1580 vs, 1645 vs, 1720 vs, 3100 br, 3490 br. ^{1}H NMR in DMSO-d_6 (δ, ppm): 13.7 (s, 1H, H4), 8.64 (s, 1H, H2), 5.83 (s, 1H, H6), 2.43 (s, 3H, CH_3).

Instrumentation. Microanalysis of C, H, and N was performed with a Fisons-Instruments EA-1008 by the Instrumentation Center of the University of Granada. Infrared spectra were recorded in the 4000−180 cm^{-1} range on a Perkin-Elmer 983G spectrophotometer using KBr and polyethylene pellets. Pt and water content were determined thermogravimetrically at a heating rate of 20 K/min, using an atmosphere of pure air (100 mL/min), on a Mettler TA-3000 instrument equipped with a Mettler TG-50 thermobalance. ^{1}H NMR spectra were recorded in DMSO-d_6 and D_2O, respectively, with TMS and TSP as internal standards on Bruker AM-300 FT NMR and Bruker AC-200 FT NMR spectrometers, respectively, at the Instrumentation Center of the University of Granada and Department of Chemistry of University of Dortmund. Circular dichroism spectra were recorded on a JASCO 600 spectropolarimeter at the Instrumental Technical Service of the University of Sevilla, in the wavelength range 230−330 nm. Wavelength was calibrated with a solution of camphorsulfonic-d_{10} acid. All measurements were performed in a 1 cm circular thermostated quartz cell. Results are expressed as $D_e = e_L - e_R$ (M^{-1} cm^{-1}). Ultraviolet spectra were recorded using a JASCO V500 spectrophotometer. All pH measurements were made with a Crison 2022 pH meter.

Reactivity Studies. The reactions of **1** with 9-EtGH and 1-MeC were carried out in D_2O, at 45 ± 1 °C with model nucleobase and complex concentrations of 80 and 40 mmol/L, respectively. The pD was adjusted to 7.0 by adding 1 N NaOD. The course of the reaction was followed by ^{1}H NMR spectroscopy. Due to lack of solubility of both 9-EtGH and the resulting cross-link adduct, the solid produced during the reaction was periodically solubilized in DMSO-d_6 in order to record its ^{1}H NMR spectra. Attempts made to isolate the cross-link adduct between **1** and 9-ethylguanine have not yet been fully successful.

Stock solutions of DNA were prepared by carefully adding one or two DNA fibers to a 10 mM solution of $NaClO_4$. The resulting solution was gently stirred at 4 °C for 48 h and used without further purification. The extinction coefficient at 260 nm (6550 M^{-1} cm^{-1}) was used to determine the concentration in mononucleotide units.[25] Aqueous platinum solutions were made at room temperature and stored two weeks at pH 7.0 before using. Aliquots of DNA and **1** solutions were mixed and diluted to the desired concentration for platinum/DNA ratios (r_b) ranging from 0.02 to 0.5 mol of Pt per mol of nucleotide. DNA concentration for CD measurements was fixed at 0.012 mmol of nucleotide. Final pH was carefully adjusted to 7.0−7.2 with dilute hydrochloric acid or sodium hydroxide solution and the ionic strength was adjusted with $NaClO_4$ to $m = 0.1$ M. All solutions were incubated for 48 h at room temperature before CD measurements. This incubation time corresponds to the maximum CD effects observed at every r_b value.

X-ray Crystallography. Table 2 presents the relevant crystallographic data and details of refinement for compound **1**, 5366 unique reflections were measured on a Nonius CAD4 diffractometer. Data collection was poor because of the flatness (0.05 mm) of the crystal. The structure was determined by means of the heavy atom method using the Structure Determination Package (SDP)[26] and isotropically refined, with the exception of platinum and chlorine atoms which were refined anisotropically by means of the Shelxl-93[27] package. It was necessary to take the first ligand as a model of the second one, due to severe deformations which arise in this

Table 2. Crystallographic Data and Details of Refinement for *cis*-$[PtCl_2(Hmtpo)_2]\cdot 2H_2O$ (**1**)

formula	$C_{12}H_{16}N_8O_4Cl_2Pt$
fw, g/mol	602.3
crystal system	monoclinic
space group	$P2_1/c$
a, Å	15.215(2)
b, Å	9.629(1)
c, Å	13.115(3)
β, deg	97.40 (2)
V, Å^3	1905.4(5)
Z	4
crystal size, mm	0.05 × 0.25 × 0.25
d_{calc}, g/cm^3	2.10
λ(Mo Kα), Å	0.71073
$\mu_{MoK\alpha}$, cm^{-1}	80.3
F(000)	1152
scan	$\omega-2\Theta$
no. of reflcns meas	5362
no. of reflcns obs	2926 ($I > 4\sigma(I)$)
R^a	0.11 ($I > 4\sigma(I)$)^a
goodness-of-fit	1.21
largest feature in the final diff map, e·Å^{-3}	2.4
no. of parameters refined	125

^{a} $R = \sum||F_o| - |F_c||/\sum|F_o|$.

Table 3. Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients *B*(eq) for *cis*-$[PtCl_2(Hmtpo)_2]\cdot 2H_2O$ (**1**)

	x	*y*	*z*	*B*(eq)^a
Pt	0.19782(6)	0.21229(9)	0.34196(6)	4.94(2)
Cl1	0.1335(5)	0.3674(6)	0.2241(5)	6.8(2)
Cl2	0.3165(5)	0.194(1)	0.2537(5)	8.5(2)
N1	−0.0033(1)	0.178(2)	0.487(2)	6.0(4)*
C2	0.021(1)	0.144(2)	0.418(2)	5.7(5)*
N3	0.098(1)	0.229(2)	0.425(1)	4.3(4)*
C3a	0.089(1)	0.315(2)	0.502(1)	3.9(4)*
N4	0.144(1)	0.418(2)	0.539(1)	4.3(4)*
C5	0.117(1)	0.499(2)	0.620(2)	4.5(4)*
C51	0.179(2)	0.612(3)	0.654(2)	6.4(6)*
C6	0.039(1)	0.474(2)	0.655(2)	5.0(4)*
C7	−0.018(1)	0.359(2)	0.624(2)	4.6(4)*
O7	−0.084(1)	0.322(1)	0.655(1)	6.5(4)*
N8	0.010(1)	0.283(2)	0.538(1)	4.5(4)*
N1′	0.284(2)	−0.146(3)	0.504(3)	14(2)*
C2′	0.225(2)	−0.067(3)	0.443(3)	8.7(8)*
N3′	0.252(1)	0.074(2)	0.442(2)	6.8(5)*
C3a′	0.327(2)	0.079(3)	0.506(3)	8.7(8)*
N4′	0.379(2)	0.193(3)	0.527(3)	11.1(8)*
C5′	0.456(3)	0.171(4)	0.603(4)	14(2)*
C51′	0.508(3)	0.301(4)	0.616(5)	16(2)*
C6′	0.479(2)	0.040(4)	0.628(4)	13(2)*
C7′	0.429(4)	−0.083(4)	0.606(6)	23(3)*
O7′	0.442(3)	−0.205(4)	0.625(4)	21(2)*
N8′	0.345(2)	−0.056(3)	0.538(2)	11.1(8)*
O1w	0.311(2)	0.468(3)	0.490(2)	8.7(6)*
O2w	0.275(2)	0.593(4)	0.298(3)	14.2(8)*

^{a} $B(eq) = {}^4/_3\sum_i\sum_j\beta_{ij}\cdot a_i\cdot a_j$. Starred atoms were refined isotropically.

moiety. The final refinement gave an $R = 0.11$ for the 2926 reflections with $I > 4\sigma(I)$. Final atomic coordinates are given in Table 3.

Cytotoxicity Assay in Vitro. Tumor cell growth inhibition was determined against the human cell lines MCF-7 breast carcinoma and A121 ovarian carcinoma, obtained from the American Type Culture Collection (Rockville, MD). Cell lines were cultivated for a minimum of two passages after thawing prior to experimentation and grown in a standard culture medium containing RPMI 1640 supplemented with 10% fetal bovine serum and 2 mM glutamine. Cultures were incubated under conventional conditions (37 °C, 5% CO_2, 95% air, and 100% relative humidity) in a Heraeus incubator.

The effects of **1** on cellular proliferation were determined using MTT [3-(4,5-dimethylthyazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma) microculture colorimetric assay.[28] Briefly, cells were harvested with 0.125% w/v trypsin−0.02% EDTA (Bio-Whittaker) from exponential-phase maintenance cultures (T-25 cm^2 flasks; Nunc, Sweden) and centrifuged (100*g*, 5 min). After resuspension and counting (Coulter ZM cell counter), cells were dispensed within replicate 96-well culture plates (Nunc, Sweden) in 100 mL volumes. After 12 h incubation, the culture medium was discarded and replaced with 100 mL of fresh medium containing **1**. Culture plates were then incubated for different days before adding 10 mL of sterilized MTT solution (5 mg/mL in saline phosphate buffer, PBS) to each well. The tetrazolium/formazan reaction was allowed to proceed for 3 h at 37 °C, and then 100 mL of acid 2-propanol (40 mM HCl in 2-propanol) was added to all wells and mixed thoroughly to dissolve the dark blue formazan crystals. After 5 min at room temperature, plate absorbances were determined spectrophotometrically at a wavelength of 550 nm by an Antos 2000ELISA reader 1 h after the 2-propanol addition.

Conclusion

The slow substitution of the chlorine ligands by the appropriate nucleobase atoms is probably the consequence of the bulky nature of the mtpo ligands around the Pt(II) cation, which does not allow a faster nucleobase (DNA)−complex **1** reaction. It is obvious that the steric hindrance imposed by the triazolopyrimidine-type molecules significantly decreased the named kinetic reaction, although that does not prevent the further bonding of complex **1** to the DNA nucleobases. A reasonable hypothesis, deduced from our findings and previous results with cisplatin−DNA interactions, involves the formation of a cross-link between **1** and two adjacent guanine bases in a single DNA strand by bifunctional coordination of platinum. Moreover, our NMR kinetic experiments show that N7 atoms of the nucleobases are the primary binding site for platinum, as observed in other cisplatin complexes.[19,29] This coordination causes bending of the duplex, destacking of DNA nucleobases, and a significant alteration of CT-DNA.

The results of treatment of the tumor cell lines MDF-7 and A121 with the new Pt drug **1** confirm the cytotoxicity of this complex. Indeed, the antitumor behavior of **1** depends on the tumor nature: compound **1** is moderately active against breast carcinoma while its cytotoxic effect is pronounced and significant when ovarian tumor cells are treated. On one hand, increasing the drug concentration always increases the cytotoxic effect. Moreover, less drug concentration is required to elicit the same inhibitory effect in the most proliferative cell line. Both facts clearly show the antineoplastic character of **1**.

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