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Palladium(II) 4,5-Diphenylimidazole Cyclometalated Complexes: DNA Interaction

Félix Zamora,[1] Víctor M. González,[2] José M. Pérez,[2] José R. Masaguer,[1] Carlos Alonso[2] and Carmen Navarro-Ranninger[1]*

[1] Departamento de Química Inorgánica, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain

[2] Centro de Biología Molecular 'Severo Ochoa', Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain

In this paper, we show the synthesis of palladium(II) 4,5-phenylimidazole cyclometalated complexes. They have been characterized by IR, ^{1}H- and ^{13}C-NMR spectroscopy. The cyclometalated dimer compound 2 [Pd($C_{15}H_{11}N_2$)(μ-OAc)]$_2$ and the cyclometalated monomer compound 5 [PdBr(SEt_2)($C_{15}H_{11}N_2$)], having OAc and Br as leaving groups, interact with DNA, modifying its secondary structure (as measured by T_m and CD), without modifying its tertiary structure (as determined by measurement of the electrophoretic mobility in agarose gels). The monomeric compound 5 seems to be the one that induces the highest alterations in DNA secondary structure since it strongly modifies the CD spectrum of the DNA. Melting data of drug–DNA complexes suggest that, at low drug concentration, the 4,5-Imd ligand intercalates between the base pairs in the DNA molecule, increasing the T_m, while at high drug concentrations the palladium(II) centers destabilize the double helix, producing a lowering in T_m values. These results indicate that complexes containing planar structures might selectively bind to DNA that is not supercoiled, and that therefore it only has a secondary structure. © 1997 by John Wiley & Sons, Ltd.

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* Correspondence to: Carmen Navarro-Ranninger.

INTRODUCTION

It is generally accepted that the antitumor activity of the antineoplastic drug *cis*-diamminedichloroplatinum(II) [*cis*-DDP, $Pd(NH_3)_2Cl_2$] is due to the production of bifunctional lesions on DNA which result from the crosslinking of two adjacent guanines in the same DNA strand.[1] By biological testing of *cis*-DDP analogs, a series of structure–activity relationships has been empirically established for platinum compounds. Thus, particular neutral molecules within a *cis*-platinum environment and having chloride as the leaving group and primary amines as the inert group seem to be the most active drugs.[2] UV, CD and gel electrophoresis determinations indicated that all of these metallic compounds modify the secondary and tertiary structure of the DNA.

In recent years the established structure–activity relationship present in classical platinum antitumor agents[3] has been violated by the synthesis of some new classes of complexes. Thus, antitumor compounds such as $[Pt(NH_3)_2AmCl]^+$ (where Am is a heterocyclic amine based on pyridine, pyrimidine, purine or piperidine substituents),[4] and platinum complexes containing other intercalator ligands such as anthraquinones and ethidium bromide,[5] induce monofunctional or intercalative binding on DNA. It has also been found, on the other hand, that some orthopalladated complexes may bind to DNA by means of intercalative or monofunctional covalent interactions. Of particular interest are cyclopalladated complexes derived from several aromatic and aliphatic amines. Some of these compounds exhibit cytotoxic effects against some tumor cells.[6] We have also shown that palladium(II) and platinum(II) benzoylbenzylidenamine complexes bind to DNA in a different way from *cis*-DDP, and that they have

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remarkable *in vitro* antitumor activity.[7,8] These results suggest that cyclometalated complexes (containing planar structures) and particular coordination complexes containing planar ligands may produce an intercalative lession on DNA, inducing a strong cytotoxic effect.

In the present paper, we report the synthesis and structural characterization of palladium(II) 4,5-diphenylimidazole cyclometalated complexes. The T_m and CD data indicate that these complexes alter the secondary structure of the DNA without modifying its tertiary structure as measured by gel electrophoresis

EXPERIMENTAL

The solvents were purified and dried by standard methods.[9] Palladium chloride was purchased from Johnson–Matthey and palladium(II) acetate and 4,5-phenylimidazole (4,5-Imd) were purchased from Aldrich Chemie.

Elemental analyses were carried out on a Perkin–Elmer elemental analyzer 240B. The IR spectra in the 4000–200 cm^{-1} region were recorded as polyethylene and KBr pellets on a Perkin-Elmer 1650 spectrophotometer. The NMR spectra were obtained on $CDCl_3$ (TMS as internal reference) and DMSO-d_6 (undeuterated residual DMSO as reference) and recorded on a Bruker AMX-300 spectrometer. The NMR spectra were assigned by chemical shift, in ppm, and were assisted with HMQC[10–12] (^{1}H-detected heteronuclear multiple-quantum coherence), HMBC[13] (heteronuclear multiple-bond connectivity) and COSY-45 when necessary. The MS spectra were recorded on a WG AutoSpec, in L-SIMS conditions (3-nitrobenzyl alcohol as matrix).

Synthesis of $[PdCl_2(C_{15}H_{12}N_2)_2]$ (1)

To a suspension of $PdCl_2$ (1 mmol) in 15 ml of methanol a solution of 4,5-Imd (2 mmol) in 5 ml of methanol was added. After stirring for 30 days at 20 °C the precipitate was filtered off, washed with methanol and dried under vacuum (71.0% yield). M.p. 280–282 °C, dec. Analysis: Found: C, 58.64; H, 3.73; N, 9.03. Calcd for $PdCl_2C_{30}H_{24}N_4$: C, 58.32; H, 3.92; N, 9.07%.

Synthesis of $[Pd(C_{15}H_{11}N_2)(\mu\text{-}(OAc)]_2$ (2)

Palladium(II) acetate (1 mmol) was dissolved in 2 ml of glacial acetic acid under nitrogen. 4,5-Imd (1 mmol) in 5 ml of glacial acetic acid was added and the solution stirred at 60 °C for 6 h. The solvent was removed under vacuum and the residue was extracted with 100 ml CH_2Cl_2/H_2O (1:1), three times. The combined extracts were dried with Na_2SO_4, filtered and concentrated on a rotary evaporator. Yellow crystals were obtained by slow evaporation. The crystals were filtered off, washed with hexane and dried under vacuum (86.3% yield). M.p. 268–270 °C, dec. Analysis: Found: C, 53.15; H, 3.49; N, 7.30. Calcd for $Pd_2O_4C_{34}H_{28}N_4$: C, 53.03; H, 3.64; N, 7.28%.

Synthesis of $[Pd(C_{15}H_{11}N_2)(\mu\text{-}Cl)]_2$ (3)

To a stirred solution of complex **2** (0.5 mmol) in CH_2Cl_2 (10 ml) an excess of a saturated solution of sodium chloride in water was added. After stirring for three days the precipitate formed was filtered off, washed with water and acetone, and dried under vacuum (43.7% yield). M.p. >300 °C. Analysis: Found: C, 49.84; H, 2.95; N, 7.62. Calcd for $Pd_2Cl_2C_{30}H_{22}N_4$: C, 49.89; H, 3.07; N, 7.76%.

Synthesis of $[Pd(C_{15}H_{11}N_2)(\mu\text{-}Br)]_2$ (4)

To a stirred solution of complex **2** (0.5 mmol) in CH_2Cl_2 (10 ml) an excess of a saturated solution of lithium bromide in water was added. After stirring for three days, the yellow precipitate formed was filtered off, washed with water and acetone, and dried under vacuum (74.3% yield). M.p. >300 °C. Analysis: Found: C, 44.34; H, 2.69; N, 6.81. Calcd for $Pd_2Br_2C_{30}H_{22}N_4$: C, 44.42; H, 2.73; N, 6.91%.

Synthesis of $[PdBr(SEt_2)(C_{15}H_{11}N_2)]$ (5)

To a CH_2Cl_2 suspension of complex **4**, SEt_2 (2 mmol) was added. The yellow solution formed was filtered. The addition of diethyl ether resulted in a precipitate, which was filtered off, washed with diethyl ether and dried under vacuum (yield 98.2%). M.P. 198–200 °C. Analysis: Found: C, 45.86; H, 4.17; N, 5.69. Calcd for $PdBrC_{19}H_{22}N_2S$: C, 45.94; H, 4.21; N, 5.64%.

Biological assays

Formation of drug–DNA complexes

Stock solutions of each compound (1 mg/ml) were stored in the dark at room temperature until use. Drug–DNA complexes were formed by

addition to DNA (calf thymus DNA, purchased from Sigma) of aliquots of each of the compounds at different concentrations in 0.02 × SSPE (1 × SSPE = 180 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA, pH 7.0). The amount of drug added to the DNA solution was expressed as r_i (the input molar ratio of Pd to nucleotides). The mixture was incubated at 37 °C for various periods of time as indicated below.

Compounds **1**, **3** and **4** could not be studied because of their low solubility in water.

Ultraviolet spectral data

UV measurements of the drug–DNA complexes (20 mg/ml DNA, $r_i = 0.05$, 0.10 and 0.25) were carried out at 25 °C by differential spectrophotometry in a Shimadzu PR-1 spectrophotometer. The T_m of native DNA and compound **2**–DNA and compound **5**–DNA complexes (20 mg/ml) were recorded at 260 nm by differential spectrophotometry at an increase rate of 1 °C/min from 45 °C to 95 °C in a Beckman Acta cIII spectrophotometer attached to a temperature programmer. The maximum value of the hyperchromicity in control DNA at 95 °C was 33%. The data represent the mean values of three independent determinations.

Circular dichroism (CD) spectroscopy

The CD spectra were measured in a 1-cm rectangular quartz cell in a JASCO J-600 spectropolarimeter attached to a temperature programmer using a computer for spectral subtraction and noise reduction. The CD analysis was done at 20 °C. Each sample was scanned twice in a range of wavelengths between 220 and 310 nm. The CD spectra generated represent the mean of three independent scans. The data are expressed as mean residue molecular ellipticity [Θ] in units of degree cm^{-1} $dmol^{-1}$.

Gel electrophoresis of drug–pUC8 complexes

pUC8 DNA aliquots (50 mg/ml) were incubated with the drugs in a buffer solution containing 50 mM NaCl, 10 mM Tris–HCl, pH 7·4, and 0.1 mM EDTA at $r_i = 0.1$ and 0.25. Incubations were performed in the dark at 37 °C. Aliquots of 20 ml of the drug–DNA complexes containing 1 mg of DNA were subjected to 1.5% agarose gel electrophoresis for 16 h at 25 V in 40 mM Tris–acetate and 2 mM EDTA, pH 8.0, buffer. The DNA was stained with ethidium bromide (0.5 mg/ml). The gels were photographed with an MP-4 Polaroid camera using a 665 Polaroid film and an orange filter.

RESULTS AND DISCUSSION

Synthesis and characterization of the palladium(II) complexes

A schematic representation of the routes used for the synthesis of the compounds is given in Fig. 1.

The reaction of $PdCl_2$ with 4,5-Imd in MeOH led to the formation of a *trans*-palladium(II) coordination complex **1**. The IR spectrum of complex **1** shows two bands at 308 and 342 cm^{-1} assignable[14–16] to ν_{as}(Pd–N) and ν_{as}(Pd–Cl), respectively, as an indication of a *trans* geometry. Due to the low solubility of this complex it was not possible to record ^1H and ^{13}C NMR spectra.

The reaction between $Pd(OAc)_2$ and 4,5-Imd at 60 °C in HOAc, under nitrogen, for 6 h led to the formation of the yellow complex **2**. This complex is poorly soluble in common organic solvents such as $CHCl_3$, CH_2Cl_2 or acetone. The IR spectrum of complex **2** shows the presence of two strong bands as 1413 and 1566 cm^{-1} which are typical of bridging acetate[17] suggesting, thus, that the complex is a dimer of formula $[LPd(OAc)]_2$.

The ^1H and ^{13}C NMR parameters were assigned on the basic of HMBC, HMQC and COSY experiments. The ^1H parameters were confirmed by selective proton decoupling. The ^1H NMR spectrum of compound **2** shows (Table 1) a characteristic shielding in the aromatic signals due to the 'open book' structure of the complex. The signal at 1.90 ppm assignable to the methyl protons of the acetate group is indicative of an *anti* geometry. The signal at 11.94 ppm is assigned to the NH proton since it disappears when D_2O is added.

The ^{13}C NMR signals of compound **2** show the effects of cyclopalladation on chemical shifts (Table 2). Thus, the quaternary carbon at 149.7 ppm assigned to C1 undergoes a large deshielding due to Pd–C back-bonding since an increase in the order of the M–C bond increases the deshielding term in Poeple's equation.[18] The deshielding shown in the signals corresponding to C1′ and C3′, adjacent to the Pd–N bond, can be attributed to Pd–N coordination which would indicate a delocalization of the lone pair at the pyrrolic N on the aromatic ring π-system, causing a large charge-density decrease at the *ortho* position.[19] The signal at 142.2 ppm, assigned to the C2 carbon undergoes a significant

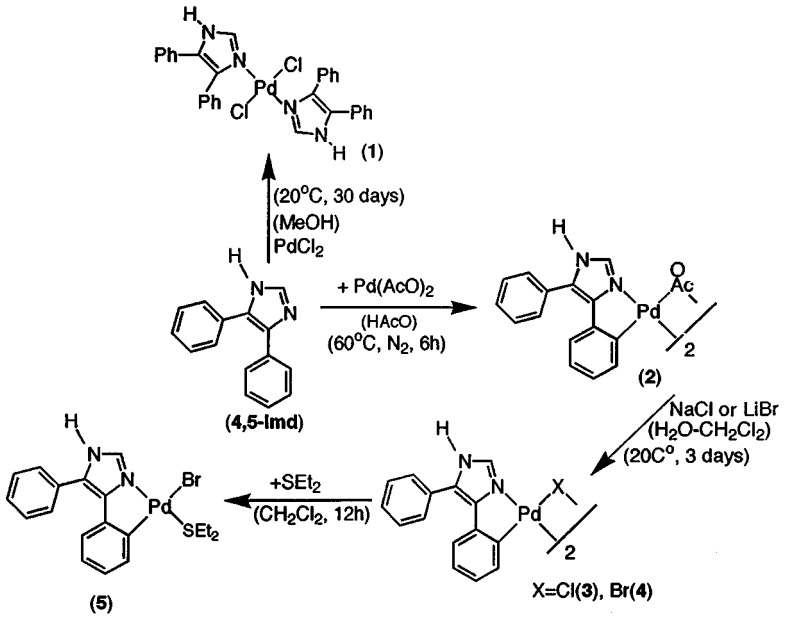
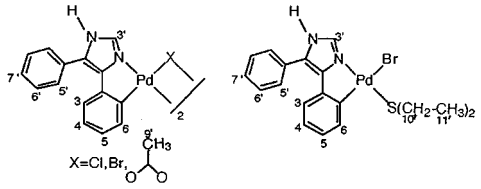


Figure 1 Scheme of synthetic routes for the compounds **1**–**5**.

deshielding. C3 also shows a large variation in the chemical shift resulting from cyclopalladation, attributed to changes in conformation. The upfield shift of C4, unaffected by steric interactions, indicates the existence of some metal–ligand back-bonding.[20]

The mass spectrum shows peaks at *m/z* 769.7 and 710.9, assigned to the molecular ion [M^+] and to [$M^+ - OAc$], respectively. The attempts to obtain the cyclopalladated chloro-bridged com-

Table 1 ^{1}H NMR parameters (δ, ppm)



	4,5-Imd[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
H3	7.45, m[c]	7.16, dd (7.5, 1.2), 1H	7.15, m, 1H	7.14, m, 1H	7.25, dd (2.1, 7.5), 1H
H4	7.23, m[c]	6.83, td (1.2, 7.5), 1H	6.90, m[c]	6.91, m[c]	6.91, m, 1H
H5	7.23, m[c]	6.74, td (1.2, 7.5), 1H	6.90, m[c]	6.91, m[c]	6.97, m, 1H
H6	(see H4)	7.53, m[c]	7.92, m, 1H	7.98, m, 1H	7.53, m, 1H
H3′	7.76, s, 1H	7.06, s-b, 1H	8.19, s-b, 1H	8.33, s-b, 1H	8.46, s-b, 1H
H5′	7.45, m[c]	7.86, m, 2H	7.55, m[c]	7.55, m[c]	7.49, m, 2H
H6′	7.23, m[c]	7.43, m, 2H	7.55, m[c]	7.55, m[c]	7.55, m[c]
H7′	7.23, m[c]	7.53, m[c]	7.55, m[c]	7.55, m[c]	7.55, m[c]
H9′		1.90, s, 3H			
H10′					3.17, s-a, 2H
H11′					1.47, t(7.4), 3H
NH	n.o.	11.94, s-b, 1H	13.32, s-b, 1H	13.30, s-b, 1H	9.30, s-b, 1H

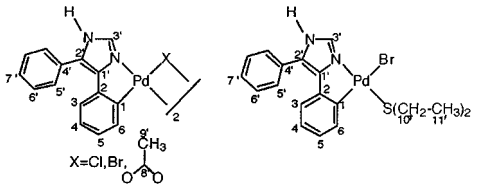
[a] DMSO-d_6. [b] $CDCl_3$. [c] Overlapped signal. Abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; b, broad. In parentheses: *J* (Hz).

plex **3** by direct reaction between 4,5-Imd and Li_2PdCl_4 or $PdCl_2$ by refluxing in MeOH was not successful. Complex **3** could only be obtained by an acetate–chloride exchange reaction of compound **2** with NaCl. Similarly, the reaction between compound **2** and LiBr results in the formation of the bromo-bridged complex **4**. The chloro- and bromo-bridged complexes are insoluble in most organic solvents, except DMSO and DMF.

The IR spectrum of compound **3** shows a band at 328 cm^{-1}, assigned to ν_{as}(Pd–N), as well as two bands at 270 and 334 cm^{-1}, corresponding to ν_{as}(Pd–Cl) *trans* to C and N atoms bonding to Pd, respectively[21]. The IR spectrum of compound **4** shows a band at 327 cm^{-1} assignable to ν_{as}(Pd–N).[14–16]

The ^{1}H NMR spectra of compounds **3** and **4** are very similar (Table 1). The only difference observed between them is the greater deshielding shown in the bromo-bridged complex by the H3′, H4, H5 and H6 protons. This fact could be attributed to decreasing Pd–C back-bonding, since bromo ligands are better π-acceptors than chloro atoms. This effect can also be observed in the ^{13}C NMR spectrum. The effect of cyclopalladation on the chemical shifts of the aromatic protons H3, H4, H5 and H6 agrees with those reported for other cyclopalladated compounds.[19] The ^{13}C NMR spectra of compounds **3** and **4** show (Table 2) similar shifts to those observed in the spectrum of complex **2**. The mass spectra of compounds **3** and **4** show the molecular ion at *m/z* 721.7 and 810.8 [M^+], respectively, as well as peaks at 686.8 and 730.9, assignable to [$M^+ - Cl$] and [$M^+ - Br$], respectively.

Table 2 ^{13}C NMR parameters (δ, ppm)



	4,5-Imd[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
C1	127.5	149.7	148.9	150.6	149.0
C2	129.5	142.2	140.4	140.4	140.9
C3	127.5	119.9	120.2	120.4	121.4
C4	128.5	124.9	125.9	126.1	121.8
C5	128.5	124.8	125.2	126.1	126.4
C6	128.5	134.9	132.4	132.3	133.2
C1′	129.5	142.9	136.6	138.1	136.8
C2′	129.5	129.3	125.1	125.4	124.6
C3′	135.6	141.8	141.0	141.4	142.6
C4′	129.5	133.6	138.0	138.1	136.8
C5′	127.5	129.8	128.9	129.2	129.0
C6′	128.5	127.9	128.9	129.2	129.0
C7′	128.5	128.8	128.9	129.2	129.0
C8′		172.0			
C9′		21.1			
C10′					32.4
C11′					13.6

[a] DMSO-d_6; [b] $CDCl_3$.

The reaction carried out to obtain monomeric complexes with SEt_2 as Lewis base led to the synthesis of the bromo monomeric complex **5**. This compound was obtained only when compound **4** reacted with SEt_2. Therefore, the Pd–Br bond appears to be more reactive than the Pd–Cl bond. The IR spectrum of **5** shows a band at 320 cm^{-1} corresponding to a ν_{as}(Pd–N).[14–16] The ^{1}H NMR spectrum of complex **5** shows (Table 1) unique signals for each proton of the 4,5-Imd. Therefore, this indicates that SEt_2 is *trans* to the nitrogen atom as a consequence of the high *trans* effect of the carbon atom. The ^{1}H and ^{13}C NMR spectra of the **4** dimeric and **5** monomeric compounds are similar (Table 2). However, the ^{1}H NMR spectra of **4** and **5** are different to that observed for the folded acetate-bridged compound **2** due to the unfolded structures of compounds **4** and **5**.

The elemental analyses together with the IR, ^{1}H and ^{13}C NMR spectroscopic data of the synthesized products indicate that have the following formulas: $[PdCl_2(C_{15}H_{12}N_2)_2]$ (compound **1**), $[Pd(C_{15}H_{11}N_2)(\mu\text{-OAc})]_2$ (compound **2**), $[Pd(C_{15}H_{11}N_2)(\mu\text{-Cl})]_2$ (compound **3**), $[Pd(C_{15}H_{11}N_2)(\mu\text{-Br})]_2$ (compound **4**) and $[PdBr(SEt_2)(C_{15}H_{11}N_2)]$, (compound **5**).

Analysis of drug–DNA interactions

Effect on DNA secondary structure

Ultraviolet absorption of drug–DNA complexes

The first indication that the binding of compounds **2** and **5** alters the conformation of the DNA was obtained by the change in the chromicity of the drug–DNA complexes relative to control DNA (Table 3). At an r_i of 0.25, compounds **2**, **5** and 4,5-Imd ligand induce a strong hyperchromic effect upon binding to DNA (34.1%, 37.5% and 27.7%, respectively).

Melting behavior of the drug–DNA complexes

Figure 2 shows that compounds **2** and **5** induce

Table 3 UV data ($\lambda = 260$ nm) of ligand and compounds **2** and **5**

Compound	r_i	O.D.$_{260\ nm}$	H (%)
CT DNA[a]	—	0.378	—
4,5-Imd	0.25	0.483	27.76
	0.05	0.469	24.07
2	0.10	0.466	23.28
	0.25	0.507	34.13
	0.05	0.379	n.d.
5	0.10	0.439	16.14
	0.25	0.520	37.57

[a] Calf thymus DNA.

Table 4 CD of ligand and compounds **2** and **5** [Θ (°C cm^2 dmol$^{-1} \times 10^3$)λ(nm)]

Compound	r_1	Θ_{max}	λ_{max}	Θ_{min}	λ_{min}
CT DNA	—	8.59	282	−11.10	241
4,5-Imd	0.25	8.59	282	−11.10	241
	0.05	7.39	278	−9.98	246
2	0.10	7.82	278	−9.64	242
	0.25	7.59	279	−10.26	245
	0.05	5.12	280	−9.79	242
5	0.10	4.21	279	−8.55	243
	0.25	2.75	280	−6.99	245

an increase in T_m values upon thermal denaturation of DNA. The highest increase in T_m was detected at the lowest r_i assayed ($r_i = 0.05$) since the T_m varied from 61 °C in control DNA to 64.5 °C in both compound–DNA complexes. That the increase in T_m is higher at $r_i = 0.05$ than at $r_i = 0.10$ may be explained by assuming that the 4,5-Imd ligand intercalates within DNA while at high concentrations the palladium(II) center creates 'kinks' on the DNA, destabilizing the double helix. Compounds **2** and **5** induced a lower stabilizing effect on DNA when complexes were formed at $r_i = 0.10$ and 0.25.

Circular dichroism spectra of drug–DNA complexes

The CD spectra and the wavelength at which the maximum and minimum values of ellipticity [Θ] occur in control DNA and in DNA incubated with the compounds are shown in Table 4. The largest modifications of the CD spectrum relative to native DNA are detected in the compound **5**–DNA complex. The maximum value of ellipticity of the positive band in native DNA decreases from 8.59 [Θ] units to 7.39 [Θ] units in compound **2**–DNA (at $r_i = 0.05$) and to 2.75 [Θ] units in compound **5**–DNA (at $r_i = 0.25$) complexes. These changes in ellipticity values are accompanied by a bathochromic effect. The minimum value of the ellipticity of the negative band present in native DNA is also affected by binding of the drugs. Compound **2** slightly alters the minimum value of the ellipticity of the negative band, since from a value of −11.10 [Θ] units in control DNA, a value of −9.64 [Θ] units was observed in compound **2**–DNA complexes at $r_i = 0.10$. In compound **5**–DNA complexes the amplitude of the negative band decreases with the r_i reaching a value of −6.99 [Θ] units. The UV and CD data suggest, therefore, that compound **5** induces strong opening and rotation of the double helix.[22]

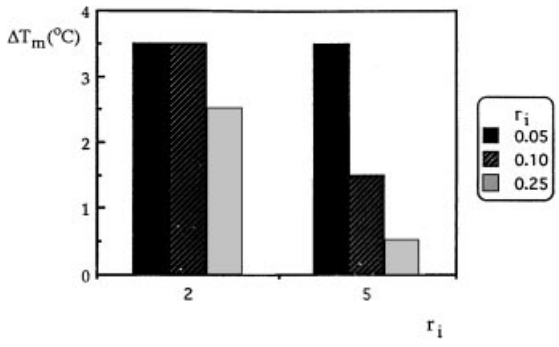


Figure 2 ΔT_m (°C) observed when DNA was incubated with compounds **2** and **5** at $r_i = 0.05$, 0.10 and 0.25.

Effect on DNA tertiary structure

Electrophoretic behavior of drug–DNA complexes

The effect of the binding of the drugs to DNA was determined by the ability of the compounds to alter the electrophoretic mobility of the covalently closed circular (ccc) and open circular (oc) forms of pUC8 plasmid. Figure 3 shows the mobility of native pUC8 plasmid DNA and of plasmid DNA incubated with the ligand, compounds **2**, **5** and *cis*-DPP at $r_i = 0.10$ and 0.25. It was observed that neither the 4,5-Imd ligand or compounds **2** and **5** alter the electrophoretic mobility of the ccc and oc forms of the DNA (lanes 2 to 7) but, as has been described[23] incubation of pUC8 DNA with *cis*-DDP at increasing r_i leads to a delay in the mobility of the ccc forms and to an increase in the mobility of the oc form (lanes 8 and 9). Altogether, our data indicate that while the compounds bind to

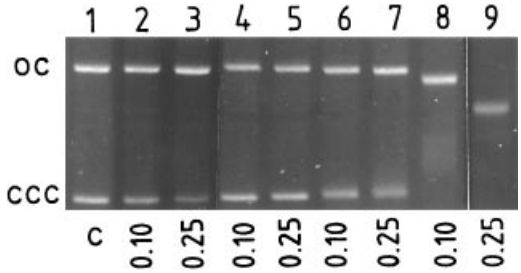


Figure 3 Changes in the electrophoretic mobility of pUC8 plasmid DNA after incubation with ligand, compounds **2** and **5**, and *cis*-DDP at $r_i = 0.10$ and 0.25: line 1, control pUC8 DNA; lines 2 and 3, 4,5-Imd; lines 4 and 5, compound **2**; line 6 and 7, compound **5**; lines 8 and 9, *cis*-DDP.

the helix they do not do so to the extent of altering its tertiary structure.

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