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In vitro antitumour activity of two isomeric cyclopalladiated compounds derived from benzoylbenzylidenimines

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The DNA thermal stabilizing effect and antitumour properties of two diastereoisomeric cyclopalladiated compounds, $Pd_2[4\text{-}CH_3O\text{–}C_6H_4N{=}C(COC_6H_5)C_6H_4]_2(\mu\text{-}OAc)_2$ (I and II), derived from benzoylbenzylideneimines have been studied. The atropisomers containing two acetate-bridged PdL_2 units have a folded structure in boat form. The results show that both complexes interact with the DNA double helix but that compound II stabilizes the DNA more than compound I. It was also observed that the *in vitro* antiproliferative activity of compound II against colon (CX-1) and lung (LX-1) human tumour cells is higher than that of compound I. It is probable that the higher reactivity of compound II relative to compound I is due to the specific orientation of the benzoyl group with respect to the CO–CN chiral bond.

Keywords: Palladium, cyclometallated compounds, antitumour

INTRODUCTION

Following the introduction and subsequent development of clinically important platinum complexes[1] for the treatment of certain forms of cancer, intensive work has been directed to the synthesis of other compounds containing Platinum Group metals such as palladium and rhodium.[2,3] In general the finding that most of the palladium complexes synthesized so far have little or no antitumour activity relative to platinum complexes, has been attributed to the high lability of the palladium(II) atoms. Whereas the platinum atoms of platinated complexes maintain their structural integrity *in vivo* for a period long enough to reach their cellular targets as PtL_2, the palladium atoms of palladiated compounds not only undergo rapid hydrolysis but also substitution reactions and *cis–trans* isomerizations. Thus, it is likely that the first step in the development of potential antitumour palladium drugs should be directed to maintain the structural integrity of the palladium atoms as PdL_2 inside the cells during treatment. As has been previously shown, the use of carboxylate chelates and/or 1,2-diamino cyclohexane as ligands may substantially improve the efficacy of palladiated complexes.[4–8] It is probable that the combined analysis of the kinetic, thermodynamic, structural and pharmacological properties of metallic complexes could determine the structure–activity relationship of these drugs. Until now, only a small number of studies have addressed the importance of chirality to DNA reactivity.[9–14]

In the present paper we report the DNA reactivity and *in vitro* antiproliferative activity of two cyclometallated palladium compounds which differ in chirality. The rationale for the synthesis of these compounds was based on the hypothesis that the lability of the palladium(II) atom would be reduced by increasing the stability of the electrophilic group and that the difference in chirality could influence its reactivity with the DNA. These two compounds are atropisomers derived from benzoylbenzylidenimines containing two acetate-bridged PdL_2 units of formula $(AcOPdL)_2$ with a folded structure in boat form, where L is *N*-(4-methoxyphenyl)-1-benzoylbenzylidenimine. It was observed that both isomers interact with the DNA double helix and that they inhibit the proliferation of colon (CX-1) and lung (LX-1) human tumour cells. Our data suggest that the chirality of the isomers may play an important role in the reactivity of palladiated compounds

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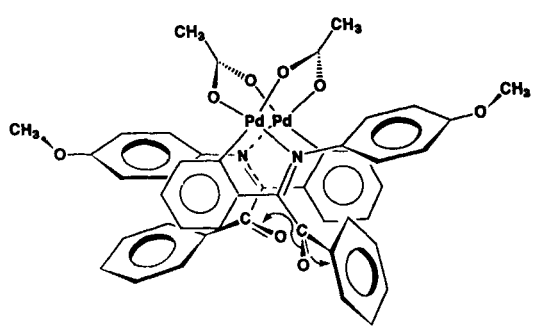

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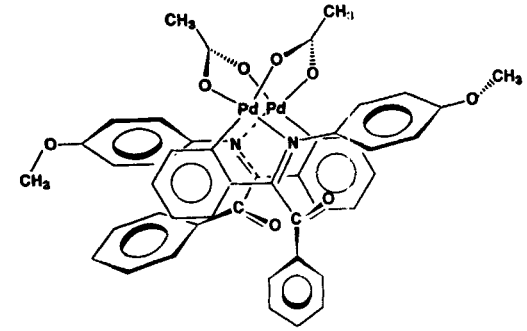

II

Figure 1 Spatial differences between complexes **I** and **II**.

and that this particular characteristic could be responsible for the specific pattern of activity of compounds **I** and **II** (Fig. 1).

MATERIALS AND METHODS

The atropisomeric cyclopalladiated complexes **I** and **II** (Fig. 1) resulting from the reaction of palladium(II) acetate with *N*-(4-methoxyphenyl)-1-benzoylbenzylidenimine used in this study are identical to those previously reported.[15]

Gel electrophoresis of Pd-benzoylbenzylidenimine–DNA complexes

pUC8 plasmid DNA was isolated from the JM83 strain of *E. coli* according to the alkaline lysis method.[16] Stocks of compounds **I** and **II** were prepared by dissolving the drugs in dimethyl sulphoxide (DMSO) to reach a concentration of 10^{-4} M. DNA aliquots (100 μg cm^{-3}) were incubated in the presence of the drugs in a medium containing 50 mM-NaCl, 10 mM-Tris-HCl and 0.1 mM-EDTA, pH 7.4, at different palladium/nucleotide molar ratios. Incubations were carried out in the dark at 37 °C for 24 h. Aliquots (20 μl) of drug–DNA complexes each containing 1 μg of DNA were subjected to agarose gel electrophoresis (1.5% agarose) for 16 h at 1 V cm in 40 mM-Tris acetate, pH 8.0, containing 12 mM-EDTA. Gel staining was performed in the same buffer containing ethidium bromide (0.5 mg cm^{-3}). Gels were photographed with a MP-4 Polaroid camera on 665 Polaroid film using an orange filter.

Melting of drug–DNA complexes

Aliquots of compounds **I** and **II** at a concentration of 10^{-4} M were added to the DNA (calf thymus DNA; Sigma) in 0.02 × SSPE buffer (SSDE = 180 mM-NaCl, 10 mM-NaH_2PO_4, 1 mM-EDTA, pH 7.0). The amount of each compound added to the DNA solution was expressed as $r_i = 0.1$ (the input molar ratio of Pd to nucleotides). Drug–DNA complexes were formed by incubation of DNA (20 μg cm^{-3}) with compounds **I** and **II** for 15 min, 1 h, 5 h, 16 h, 24 h and 48 h at 37 °C in the dark. Melting profiles were recorded at 260 nm by differential spectrophotometry and at an increase rate of 1 °C min^{-1} from 45 °C to 95 °C (Beckman Acta eIII attached to a temperature programmer). The maximum value of hyperchromicity in control DNA at 95 °C was 32%.

Interactions with carcinoma cells

The Crystal Violet assay performed to determine quantitatively the antiproliferative effect of compounds **I** and **II** was carried out according to the method described by Flick and Gifford.[17] Test cells were plated in 96 flat-bottom microtitre plates at a density of (2–3) × 10^3 cells per well and incubated under standard culture conditions (RPMI 1640 with 10% fetal calf serum and 1% non-essential amino-acids) at 37 °C and 5% CO_2 for one day. The cells were then exposed to several concentrations of the test compound. Control cells were incubated in the medium alone. After a further incubation period of 72 h the culture medium was removed and 50 μl of a Crystal Violet staining solution was added to each well. After a staining period of 20 min the staining solution was removed and the plates were washed vigorously with water until all unbound dye was

removed. The remaining insoluble dye crystals were dissolved in 100 µl of a solution containing 50% ethanol and 0.1% acetic acid to each well. The absorbance of each well was determined at 540 nm in an ELISA plate reader (Titertec Multiscan; Flow Lab., Meckenheim, Germany).

RESULTS AND DISCUSSION

Effect of palladium-cyclometallated compounds on plasmid pUC8 DNA

The cyclopalladiated compounds **I** and **II** of formula $Pd_2[4\text{-}CH_3O—C_6H_4N{=}C(COC_6H_5)C_6H_4]_2(\mu\text{-}OAc)_2$, derived from benzoylbenzylidenimines, are atropisomers exhibiting a non-planar open-book shape differing only in the dihedral angle formed between the $C_6H_5—C{=}O$ and the $C_6H_5—C{=}N$ planes[15] (Fig. 1).

In an attempt to analyse the interaction of these isomeric palladium-cyclometallated compounds with DNA, we have determined the effect of the binding of these compounds on the electrophoretic mobility of the ccc (covalently closed circular) and oc (open circular) forms of the DNA of pUC8 plasmid. The electrophoretic mobility pattern of the ccc and oc forms of pUC8 DNA incubated with compounds **I** and **II** at palladium/nucleotide ratios (r_i) from 0.05 to 0.5 are shown in Fig. 2. It may be observed that the electrophoretic mobility of the ccc forms decreases with increasing palladium/nucleotide ratios and that compound **II** seems to induce a larger conformational change on the DNA than compound **I**. On the contrary, we observed that the electrophoretic mobility of the oc forms of the DNA was not altered by any of these compounds. As has been previously reported[18,19] for the interaction of Pt(II) centres with the DNA of supercoiled plasmids, it is probable that the alteration of the electrophoretic mobility of the ccc forms attributable to the binding of compounds **I** and **II** to pUC8 DNA may be due to the formation of local microloops which would uncoil the double helix. The data show, however, that the Pd(II)–DNA adducts resulting from the interaction of compounds **I** and **II** with the oc form of pUC8 should be different from the Pt(II)–DNA adducts of *cis*-DDP:DNA since compounds **I** and **II** do not alter the mobility of the oc forms, whereas *cis*-DDP compounds increase the mobility of these forms.[20]

Modification of the melting properties of DNA upon binding of compounds I and II

Figure 3 shows that compounds **I** and **II** destabilize the DNA double helix in a way similar to that reported for *cis*-DDP and DNA interaction.[21] In fact, the T_m (melting temperature) of native calf thymus DNA was 58.5 °C while the T_m of compound **I**–DNA and compound **II**–DNA complexes formed within 15 min of incubation was 55.5 °C and 53.5 °C respectively. However, in contrast with *cis*-DDP, the destabilizing effect caused by compounds **I** and **II** is the highest in complexes formed during the first 15 min of incubation. The decrease in T_m induced by compounds **I** and **II** was higher than that induced by *cis*-DDP, even in complexes formed in 24–48 h (T_m = 56.5 °C). The decrease in T_m was drastically reduced when the period of incubation with the DNA increased. After 16–24 h of incubation the T_m of the DNA in drug–DNA compounds had a value of 57.5 °C, only 1 °C lower than the T_m of native DNA. Incubation for longer periods of time did not have any effect on the T_m of DNA. On the contrary, the T_m of the *cis*-DDP–DNA complexes decreased as the period of incubation of the drug with the DNA increased (T_m = 57.5 °C

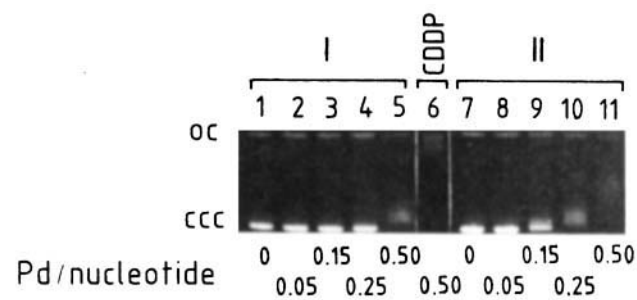

Figure 2 Changes in the electrophoretic mobility of the ccc (covalently closed circular) and oc (open circular) forms of pUC8 plasmid DNA after incubation with compounds **I** and **II**: controls, lanes 1 and 7; compound **I**, lanes 2, 3, 4 and 5; compound **II**, lanes 8, 9, 10 and 11; *cis*-DDP, lane 6.

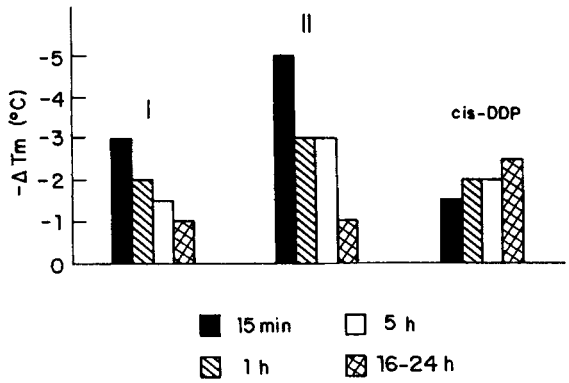

Figure 3 Changes in T_m of complex I–DNA, complex II–DNA and *cis*-DDP–DNA at $r_i = 0.25$. The period of complex formation was 15 min, 1 h, 5 h and 16–24 h.

for a 16–24 h incubation period). These results suggest, therefore, that although the *cis*-Pd(II) centres of the palladium-cyclometallated compounds may form bidentate palladium–DNA adducts similar to those formed by the *cis*-Pt(II) centres of *cis*-DDP,[22] they are displaced from the DNA as the period of incubation increases. The data indicate, moreover, that the reactivity of the atropisomer **II** against DNA is higher than that of atropisomer **I**. Since the overall structure of compounds **I** and **II** is similar, it is likely that the different degree of reactivity between compound **I** and **II** has to be explained by the specific orientation of one of the oxygen atoms of the benzoyl groups with respect to the chiral CO—CN. In compound **II**, one of the oxygen atoms would be more free to form weak interactions with DNA, favouring in addition the binding of *cis*-Pd(II) centres to the bases.

In vitro cytotoxicity of palladium-cyclometallated compounds

Since the synthesized palladium-cyclometallated compounds **I** and **II** induce DNA conformational changes, and DNA replication is a prerequisite for cell proliferation, we have carried out *in vitro* assays to measure the cytotoxic effect of these drugs. Table 1 indicates that the ID_{50} values obtained for these compounds against lung (LX-1) and colon (CX-1) human cancer cell lines are located in the μM range, suggesting that these drugs may have potential value as antitumour agents. The data indicate, moreover, that compound **II** shows higher specificity against lung and colon human cancer cells than compound **I**. It is likely that the higher DNA reactivity of compound **II** relative to compound **I** may be correlated with its higher specific cytotoxic activity.

Table 1 ID_{50} values (μM) obtained for atropisomeric cyclopalladiated compounds, $Pd_2[4\text{—}CH_3O\text{—}C_6H_4N{=}C(COC_6H_5)C_6H_4]_2(\mu\text{-}OAc)_2$, **I** and **II**, against colon (CX-1) and lung (LX-1) human tumour cells *in vitro*

Complex	Colon (CX-1)	Lung (LX-1)
I	29	24
II	22	14

CONCLUSIONS

The acetate-bridged palladium-cyclometallated compounds used in this study interact with plasmid DNA, destabilizing the double helix. The destabilization is dependent upon incubation time. It was observed, moreover, that these drugs inhibit *in vitro* the proliferation of colon and lung cells. The specific DNA reactivity of compound **II** may result from the particular orientation of the benzoyl group with respect to the CO—CN chiral bond.

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