Synthesis, Characterization and Cytotoxic Studies of Diamine and Diimine Palladium(II) Complexes of Diethyldithiocarbamate and Binding of these and Analogous Platinum(II) Complexes with DNA

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Abstract

Three palladium(II) complexes of 2,2′-bipyridine (bipy), 1,10-phenanthroline (phen) and 1,2-diaminocyclohexane (dach) with diethyldithiocarbamate (ddtc) have been synthesized and characterized. These complexes have been screened *in vitro* for antitumor activity against P 388 lymphocytic leukemic cells and they show ID_{50} values lower than cisplatin. These and analogous platinum(II) complexes have been studied for their binding with calf thymus DNA. The mode of binding of the complexes to DNA has been characterized by ultraviolet difference and fluorescence spectroscopy. The complexes exhibit cooperative binding to DNA.

Introduction

cis-Diamminedichloroplatinum(II) (cisplatin) is an important cytotoxic drug used in the treatment of a variety of human cancers, such as testicular cancer, ovarian and bladder cancer, osteogenic sarcoma, head and neck cancer, endometrial and cervical cancer and non-small cell lung cancer [1, 2]. Due to the dose limiting toxic side effects there is a clinically important need to develop procedures that enhance the therapeutic efficacy of cisplatin. In response to this need one approach has used diethyldithiocarbamate (ddtc) in combination with cisplatin. This has protected a variety of animal species from renal, gastrointestinal and bone marrow toxicity, induced by cisplatin [3–8]. Recently it has been suggested that a 1:1 platinum–ddtc complex may be responsible for the above protective effect of ddtc [9], and two α-diimine platinum(II) complexes of ddtc have also shown good antitumor activity when tested against P 388 lymphocytic leukemic cells [10]. In this paper we report the synthesis, characterization and cytotoxicity of diamine and α-diimine palladium(II) complexes of ddtc and the binding study of these and analogous platinum(II) complexes with calf thymus DNA.

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Experimental

Sodium diethyldithiocarbamate (Naddtc) was bought from Fluka (Switzerland). Palladium chloride was from Loba (India). Other chemicals were of analytical reagent grade and obtained as described previously [11]. Solvents used were purified by standard procedures [12].

Synthesis of [Pd(bipy)(ddtc)]$NO_3 \cdot H_2O$

[Pd(bipy)Cl_2] (0.3 mmol) was suspended in 20 ml of distilled water and 5.94 ml of 0.1 M $AgNO_3$ (0.59 mmol) was added with constant stirring. This mixture was stirred for 6 h at 60 °C in dark and for 16 h at room temperature. The AgCl precipitate formed was removed by centrifugation and the supernatant filtered through Whatman filter paper no. 42 to remove any AgCl precipitate left. This filtrate was mixed with Naddtc (0.3 mmol) and the reaction mixture further stirred for 24 h at 45 °C in the dark. The solution was centrifuged to remove the white turbidity. The clear solution was concentrated to 15 ml on a water bath at 40 °C and then kept in a vacuum desiccator for further concentration. The yellow precipitate obtained was filtered and washed with cold distilled water. The complex was recrystallized twice from distilled water and dried in a vacuum desiccator over anhydrous calcium chloride.

The other complexes [Pd(phen)(ddtc)]$NO_3 \cdot H_2O$ and [Pd(dach)(ddtc)]NO_3 were prepared by following the above procedure, except that [Pd(bipy)Cl_2] was replaced by [Pd(phen)Cl_2] (0.3 mmol) and [Pd(dach)Cl_2] (0.3 mmol) respectively. The purity of these complexes was confirmed by elemental analyses.

Synthesis of the complexes [Pt(bipy)(ddtc)]-$NO_3 \cdot H_2O$ and [Pt(phen)(ddtc)]$NO_3 \cdot H_2O$ followed reported procedures [10].

Cytotoxic Studies

The method followed has been reported previously [13].

Binding Studies

Ultraviolet difference absorption spectroscopy and fluorescence spectroscopy were used to

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characterize the mode of interaction of the above complexes with DNA. The procedures followed have been reported earlier [11].

Results and Discussion

Three ddtc derivatives of palladium(II) with 2,2′-bipyridine, 1,10-phenanthroline and 1,2-diaminocyclohexane have been prepared. They have been characterized by elemental analyses, conductivity measurements and spectroscopic techniques such as infrared, electronic absorption and ^{1}H NMR spectroscopy. The conductivity of the above complexes has been done in conductivity water. The molar conductance values are 123.7, 127.7 and 112.9 cm^2 ohm^{-1} mol^{-1}, indicating they are 1:1 electrolytes in water [14]. The electronic absorption spectra of these complexes are given in Table 1. The band 1 in the 1,10-phenanthroline complex is assigned to a charge-transfer transition because the band shifts to a higher energy by about 15 nm from solvent dimethylformamide to water [15]. This band is due to the charge-transfer transition from the palladium(II) d orbital to the π^* orbital of the diimine ligand. The bands 2, 3, 4 and 6 are due to first, second and higher intraligand $\pi-\pi^*$ transitions of diimine ligands, because these bands shift to a lower energy by 5 to 6 nm from solvent dimethylformamide to water. The bands 3, 4 and 6 in α-diimine complexes have overlapping components of $\pi-\pi^*$ transitions of ddtc ligand. The bands 3, 4, 5 and 6 in the dach complex are due to the first, second and other higher $\pi-\pi^*$ transitions of ddtc [16, 17].

In the infrared spectra of these complexes, the presence of a ν(C–N) band in the region of 1525 to 1550 cm^{-1} and a medium intensity ν(C–S) band in the region of 1070 to 1095 cm^{-1} show that ddtc is bound to palladium(II) as a bidentate ligand. This bidentate coordination is further confirmed by observing a ν(Pt–S) band in the region of 380 to 390 cm^{-1} [18]. The ν(N–H) band in the dach complex appears as a sharp doublet in the region of 3120 to 3240 cm^{-1}. This suggests the bidentate coordination of dach to palladium(II) [19].

The ^{1}H NMR spectra of the above complexes have been recorded in D_2O in the range of 0 to 10 ppm. The chemical shifts of important protons are given in Table 2. The structures and the numbering schemes of these protons are shown in Fig. 1. In the ^{1}H NMR spectrum of [Pd(bipy)(ddtc)]$^+$ complex a quartet is obtained at 3.37 ppm and is assigned to the methylene protons of ddtc ligand. It shows an upfield shift of 0.67 ppm in the complex as

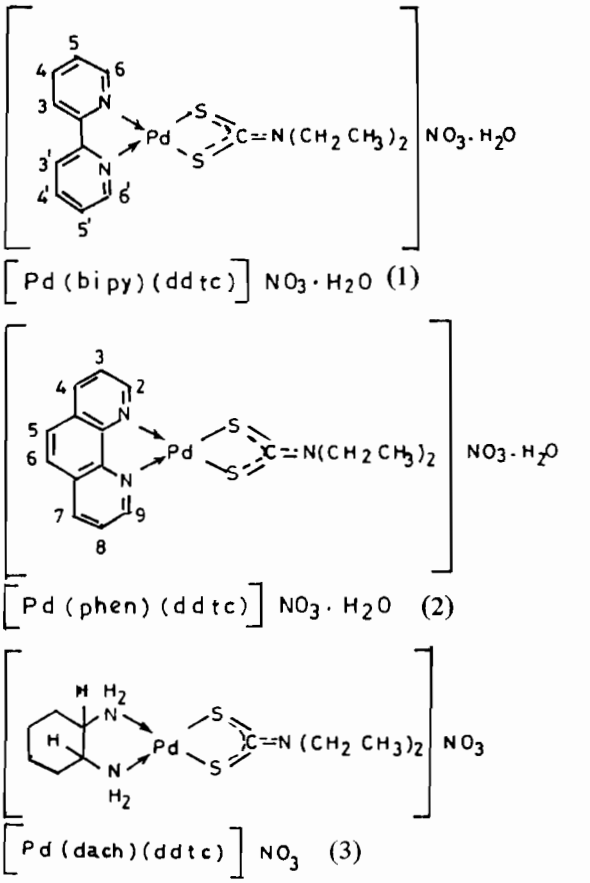

Fig. 1. Structures and numbering schemes of the compl

TABLE 1. Electronic absorption spectra of the complexes

Complexes	Band maxima (nm)					
	Band 1	Band 2	Band 3	Band 4	Band 5	Band 6
[Pd(bipy)(ddtc)]$NO_3 \cdot H_2O$		313 (1.41)	304 (1.45)	247 (3.99)		201 (3.69)
[Pd(phen)(ddtc)]$NO_3 \cdot H_2O$	360 (0.17)[a]	340 (0.24)	273 (2.38)	245 (2.69)		210 (3.99)
[Pd(dach)(ddtc)]NO_3			310 (0.08)	280 (0.28)	249 (0.81)	206 (3.11)

[a]Extinction coefficients in 1 mol^{-1} cm^{-1} × 10^{-4} are given in parentheses.

TABLE 2. ^{1}H NMR spectral data of the complexes

Complexes	ddtc protons		2,2'-Bipyridine protons		1,10-Phenanthroline protons				Different diamine protons
	$-CH_3$	$-CH_2$	H-6,6'	H-5,5'	H-2	H-9	H-3	H-8	H-a
$[Pd(bipy)(ddtc)]NO_3 \cdot H_2O$	1.10(t)[a] (+0.14)	3.37(q) (+0.67)	7.77	7.09					
$[Pd(phen)(ddtc)]NO_3 \cdot H_2O$	1.09(t) (0.15)	3.35 (−0.69)			8.87(d)	8.52(d)	7.06	7.04	
$[Pd(dach)(ddtc)]NO_3$	1.22(t)	3.70 (−0.34)							2.45(m)

[a]Chemical shift δ in ppm; d, t, q and m in parentheses are the doublet, triplet, quartet and multiplet respectively.

compared to its value in sodium diethyldithiocarbamate. This suggests the bonding of ddtc to palladium(II) through both sulphur atoms. The triplet obtained at 1.10 ppm is due to the methyl protons of ddtc. The bipy protons have been observed at 7.77, 7.68, 7.26 and 7.09 ppm, which are assigned to $H_{6,6'}$, $H_{3,3'}$, $H_{4,4'}$ and $H_{5,5'}$ protons respectively. The integrated areas for the bipy and ddtc protons are in the ratio 4:5, so structure **1** as given in Fig. 1 is assigned to the complex.

In the ^{1}H NMR spectra of the $[Pd(phen)(ddtc)]^+$ complex, the methylene protons of ddtc show a quartet at 3.35 ppm, which is 0.69 ppm upfield as compared to that of the methylene protons of sodium diethyldithiocarbamate, suggesting coordination of this ligand to palladium(II) through both sulphur atoms. The methyl protons of the complex are obtained at 1.09 ppm. The signals obtained at 8.87, 8.52, 7.85, 7.16 and 7.05 ppm are due to the H_2, H_9, $H_{4,7}$, $H_{5,6}$ and $H_{3,8}$ protons of phen respectively. Thus structure 2 as in Fig. 1 has been assigned to the complex. The integration areas for the phen and ddtc protons are in the ratio of 4:5, further supporting the above structure.

The ^{1}H NMR spectrum of the $[Pd(dach)(ddtc)]^+$ complex shows the methylene and methyl protons of ddtc as a quartet and triplet at 3.70 and 1.22 ppm respectively. The protons of dach are observed as sets of multiplets centered at 2.45, 1.97, 1.75 and 1.69 ppm respectively. The integration areas are in the ratio of 1:1, indicating 1:1 stoichiometry for the dach and ddtc ligands. Thus the complex has been assigned structure **3** as given in Fig. 1.

Cytotoxicity Studies

The *in vitro* cytotoxicity of the above palladium(II) complexes of ddtc in P 388 lymphocytic leukemic cells has been investigated. The ID_{50} value is 1.60 μM for the $[Pd(bipy)(ddtc)]^+$ and 1.50 μM for the $[Pd(phen)(ddtc)]^+$. Thus, they have ID_{50} values lower than that of cisplatin (16.6 μM). The ID_{50} value for the $[Pd(dach)(ddtc)]^+$ complex is greater than 88 μM. The higher cytotoxicity of the bipy and phen complexes is related to the flat aromatic planar structures of the α-diimines and more hydrophobic nature of the complexes [10]. These complexes are expected to be less nephrotoxic, since both α-diimine and ddtc ligands bind strongly to palladium(II). They cannot therefore be replaced by sulphydryl groups of cysteines in proteins of kidney tubules. They are also expected to bind to DNA by a mechanism different from cisplatin.

Binding Studies

Increasing concentrations of calf thymus DNA cause a change in the absorption spectra of the $[Pt(bipy)(ddtc)]^+$ and $[Pd(bipy)(ddtc)]^+$ complexes, as shown in Fig. 2(a) and (b) respectively. This

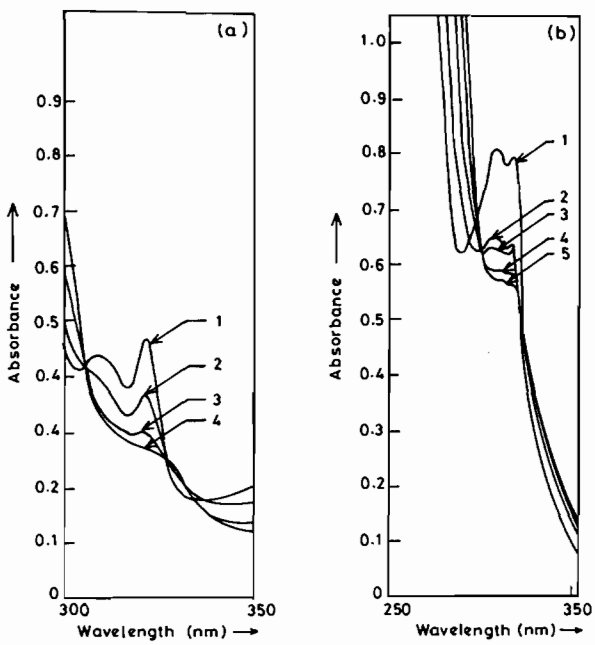

Fig. 2. Effect of calf thymus DNA on absorption spectra of (a) $[Pt(bipy)(ddtc)]^+$ and (b) $[Pd(bipy)(ddtc)]^+$. [DNA] concentration increases in the order 0, 0.06, 0.16, 0.31 mM for lines 1–4 in (a) and 0, 0.08, 0.15, 0.23, 0.31 mM for lines 1–5 in (b) respectively.

decrease is monitored at 321 nm and no clear isosbestic points are observed at 327 and 335 nm, indicating the presence of more than one bound and free forms of the metal complex. Figure 3(a) gives the Scatchard plot obtained for the [Pt(bipy)(ddtc)]$^+$ complex to DNA, and is not amenable to simple Scatchard analysis [20]. The plot shows a characteristic humped appearance of a cooperative binding process, which is explained in terms of the cooperative model of McGhee and Von Hippel [21]. From equation 15 of this model we get $\omega > 1$, indicating cooperativity. In addition, a sigmoidal plot for r versus c (data not shown), also indicates positive cooperativity. Here the intercept on the ordinate is $K_{(o)}$ the intrinsic association constant for an isolated potential binding site and has a value of $1.8 \pm 0.2 \times 10^3$ M^{-1}. The intercept on the abscissa is $1/n$, which is the number of nucleotides occluded by the binding of one molecule of the metal complex and has a value of 7.14. This indicates occlusion of approximately four base pairs along the DNA helix. This is less than the length of the DNA helix covered by actinomycin-D [22], which covers six base pairs, but more than other intercalators like EthBr or proflavin, where one molecule is bound for every two

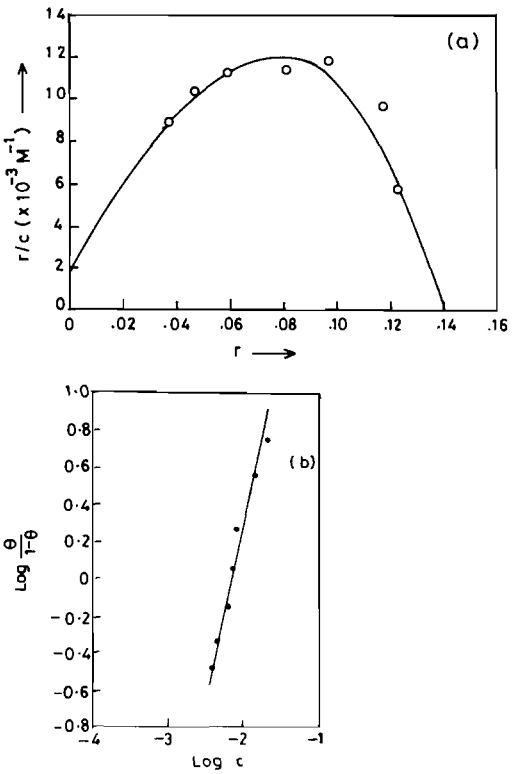

Fig. 3. (a) Scatchard plot for the binding of [Pt(bipy)(ddtc)]$^+$ to DNA. (b) Hill plot for the binding of [Pt(bipy)(ddtc)]$^+$ to DNA. θ is the fractional saturation of DNA with metal complex assuming 0.14 binding sites/mol of DNA. The solid lines are best fit of experimental data.

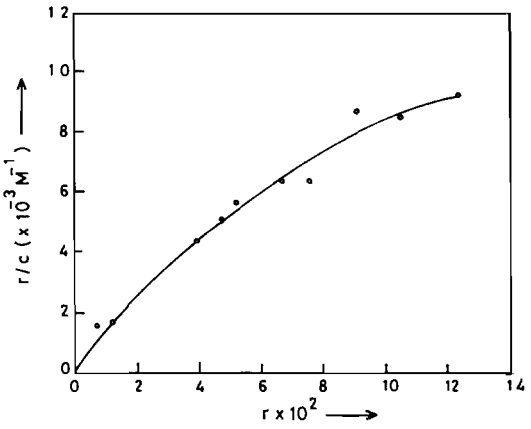

Fig. 4. Scatchard plot for the binding of [Pd(bipy)(ddtc)]$^+$ complex to calf thymus DNA.

or three base pairs [23]. So the interaction of the [Pt(bipy)(ddtc)]$^+$ complex with DNA can be considered to include intercalation, with moderate cooperativity arising from ligand–ligand interaction. This interacting site behaviour is more apparent from the Hill plot (Fig. 3(b)), which is linear [24]. The Hill co-efficient n_H calculated from the slope has a value of 1.86, indicating cooperativity in the binding of this complex to DNA.

For the analogous [Pd(bipy)(ddtc)]$^+$ complex, the Scatchard plot is given in Fig. 4, which is curvilinear concave downwards, suggesting cooperative binding. The intrinsic association constant to an isolated potential binding site $K_{(o)}$ has a value of 0.1×10^3 M^{-1}. However, no sensible estimate of the binding site size n can be made.

The large positive curvature in the Scatchard plots implies stabilization of the DNA structure by successively bound molecules of the metal complex, leading to a change in the conformation of DNA and hence facilitating occupation of a neighbouring site. The specificity of this binding, for single or double stranded regions of DNA, was studied by using heat denaturated DNA. There was no change in the interaction as compared to native DNA, indicating that the metal complexes bind with equal affinity. Similar behaviour was seen for the DNA–EthBr interaction, which was used as a control.

Other studies to determine the character of this interaction were done as described earlier [25]. When the DNA–metal complex was passed through a Sephadex G-25 column, DNA was seen to elute out separately, indicating that no covalent linkages had formed between the metal complexes and DNA. Increasing ionic strengths (6 mM $MgCl_2$ and 100 mM NaCl) reversed the binding completely, showing that electrostatic interactions as well as hydrogen bonding are involved in the binding. Further, the involvement of hydrogen bonding in the metal–DNA

complex was confirmed by precipitating the DNA from the metal–DNA complex with absolute ethanol. The metal complex is retained in the supernatant and only the DNA is precipitated. Thus hydrogen bond formation is involved in stabilizing the metal–DNA complex. Similar results were obtained for another series of platinum(II) complexes of 2,2′-bipyridine and amino acids [25]. To determine the specificity of binding of metal complexes to a particular base, studies were done with nucleosides and nucleoside -mono and -triphosphates. None of them showed any interaction with the metal complexes. But studies with poly G showed substantial interactions, whereas there was no interaction with either poly A or poly C. This implies specificity for guanine containing regions in polymeric double helical DNA, and supports intercalation as proposed above, because many known intercalators show GC base pair specificity.

Fluorescence Studies

Fluorescence Scatchard plots for the [Pt(bipy)(ddtc)]$^+$, [Pt(phen)(ddtc)]$^+$, [Pd(bipy)(ddtc)]$^+$ and [Pd(phen)(ddtc)]$^+$ complexes were obtained as reported earlier [11]. Saturation curves for the fluorescence intensity of a series of DNA–metal complexes, at increasing concentrations of the complex (r_f = 0 to 0.42) were obtained by addition of increasing concentrations of EthBr (2.4 to 24 μM). The fluorescence Scatchard plot obtained for the [Pt(bipy)(ddtc)]$^+$ complex is given in Fig. 5. At r_f = 0.07 and 0.21, there is competitive inhibition of EthBr binding (Type-A behaviour), where only the binding site remains constant [26]. Table 3 lists the values of K and n. The number of binding sites thus remains the same as obtained for DNA–EthBr complex, that is 0.22. This implies that the [Pt(bipy)(ddtc)]$^+$ complex is probably intercalating in DNA and thereby competing for intercalation sites occupied by EthBr.

But as the concentration of metal complex is increased further, r_f = 0.42, non-competitive inhibition of EthBr binding is seen, with values of both K and n decreasing (see Table 3). Similar behaviour

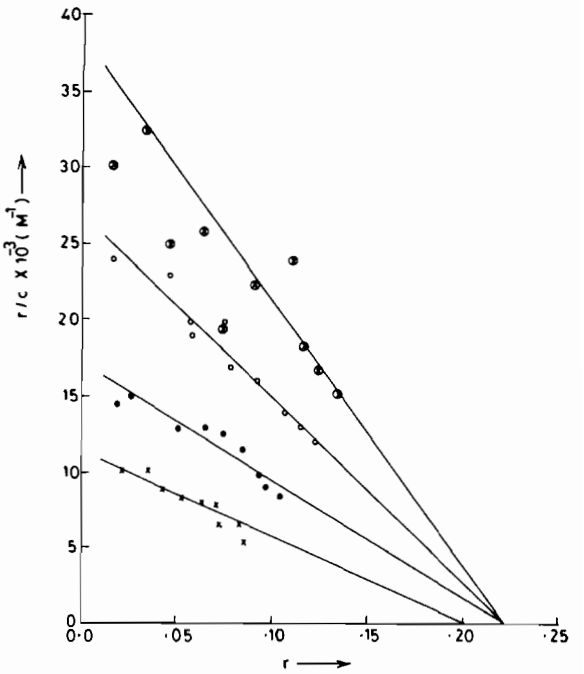

Fig. 5. Fluorescence Scatchard plots for the binding of EthBr (2.4 to 24 μM) to DNA (112 μM) in the presence of increasing concentrations of [Pt(bipy)(ddtc)]$^+$. r_f increases in the order 0 (⊗), 0.07 (○), 0.21 (●), and 0.42 (X).

is seen with high concentrations of the other metal complexes [Pt(phen)(ddtc)]$^+$, [Pd(bipy)(ddtc)]$^+$ and [Pd(phen)(ddtc)]$^+$. The values of the binding parameters are given in Table 3.

These studies, as well as the cooperative nature of the interaction seen in the UV difference spectroscopic studies, allows us to conclude that the ddtc metal complexes are intercalating in DNA, because of the planar 2,2′-bipyridine and 1,10-phenanthroline moieties. At low concentrations of the metal complex, the change in fluorescence intensity of EthBr binding is due to competition by the metal complex for the EthBr intercalating sites. But at higher binding ratios, this intercalation leads to disruption of the DNA structure and also

TABLE 3. Binding parameters for the effect of ddtc derivatives of diimine complexes of Pt(II) and Pd(II) on the fluorescence of EthBr in the presence of DNA

Complex	r_f[a]			
	0	0.07	0.21	0.42
[Pt(bipy)(ddtc)]$NO_3 \cdot H_2O$	1.72[b] (0.220)[c]	1.21 (0.220)	0.77 (0.220)	0.56 (0.220)
[Pd(bipy)(ddtc)]$NO_3 \cdot H_2O$	2.10 (0.200)			2.04 (0.138)
[Pt(phen)(ddtc)]$NO_3 \cdot H_2O$	2.10 (0.200)		1.16 (0.156)	
[Pd(phen)(ddtc)]$NO_3 \cdot H_2O$	2.10 (0.200)		1.56 (0.134)	1.14 (0.130)

[a]r_f = Formal ratio of metal complex to nucleotide concentration. [b]Association constant $K \times 10^{-5}$ (M^{-1}). [c]Number of binding sites (n) per nucleotide.

the regularity of base stacking. This reduces the EthBr intercalation sites, resulting in a decrease of fluorescence intensity. The ddtc moiety stays attached to the metal because of two strong M–S bonds. This may be involved in hydrogen bonding interactions with the bases of DNA, particularly guanine.

Another metal complex phenylthiolato(2,2′,2″-terpyridine)platinum(II) was studied by Wakelin *et al.*, and they showed intercalative binding to DNA at low binding ratios and an additional non-intercalated, externally bound form at higher binding ratios [27]. This binding behaviour agrees well with the one we have proposed for the above ddtc complexes.

Acknowledgements

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