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DNA-Binding and Thermodynamic Parameters, Structure and Cytotoxicity of Newly Designed Platinum(II) and Palladium(II) Anti-Tumor Complexes

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The complexes [Pd(bpy)(Hex-dtc)]NO_3 and [Pt(bpy)(Hex-dtc)]NO_3 (bpy is 2,2'-bipyridine and Hex-dtc is hexyldithiocarbamato ligands) were synthesized and characterized by elemental analysis and spectroscopic studies. The cytotoxicity assay of the complexes has been performed on chronic myelogenous leukemia cell line, K562, at micromolar concentration. Both complexes showed cytotoxic activity far better than that of cisplatin under the same experimental conditions. The binding parameters of the complexes with calf thymus DNA (CT-DNA) was investigated using UV-visible and fluorescence techniques. They show the ability of cooperatively intercalating in CT-DNA. Gel filtration studies demonstrated that platinum complex could cleave the DNA. In the interaction studies between the Pd(II) and Pt(II) complexes with CT-DNA, several binding and thermodynamic parameters have been determined, which may provide deeper insights into the mechanism of action of these types of complexes with nucleic acids.

Key Words : DNA binding and thermodynamic parameters, Anti-tumor activity, Pd(II) and Pt(II) complexes

Introduction

Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biology.[1,2] Therefore, the changing of DNA replication and the inhibiting growth of the tumor cells, which is the basis of designing new and more efficient antitumor drugs and their effectiveness depend on the mode and affinity of the binding.[3,4] Generally, there are three main kinds of binding models for small molecules with DNA, i.e., (I) intercalation, (II) groove binding, and (III) external electrostatic binding.[5-7]

The main goal of cancer therapy is to attain the maximum therapeutic damage to tumor cells with the minimum concentration of the drug. There have been a number of reports highlighting the use of transition metal complexes as anticancer agents.[8-10] Current metal-based drugs research is moving towards the development of the new agents able to improve effectiveness and reduce the severe side effects of *cis*-diamminedichloroplatinum(II) (cisplatin) and its analogues that are still the most widely used anticancer therapeutic.[11,12] Geometry and complex forming processes of palladium(II) are very similar to those of platinum(II), it was speculated that palladium complexes may also have anti-tumor activities and serve as good models for the understanding of more inert platinum(II) anticancer drugs.[11,13-15] Therefore, the search continuse for the new potent palladium(II) and platinum(II) complexes possessing high antitumor activity and lack of toxicity and cross-resistance.[16] It has been reported the Pd(II) and Pt(II) complexes with different sulfur containing ligands such as cysteine, cystamine, penicilamine, methionine, thiourea, thiosulphate and dithiocarbamate derivatives could overcome some toxicity of cisplatin or its analougos.[17,18]

The present work is an extension of previously studied platinum(II) and palladium(II) complexes of 2,2'-bipyridine and dithiocarbamate derivatives with potentially interesting biological activities.[17-19] It describes the synthesis and spectroscopic properties of two newly designed antitumor active palladium(II) and platinum(II) complexes with dithiocarbamate. In this work, we described the DNA binding and thermodynamic parameters as well as evaluation of binding modes of the complexes. The antitumor activity *in vitro* of complexes was investigated against leukemia cell line K562 and compared with the activity of cisplatin.

Experimental Section

Materials and Methods. Palladium(II) chloride anhydrous, sodium chloride, NaOH and KBr were obtained from Fluka (Switzerland). Hexylamine, $AgNO_3$ and carbon disulfide were purchased from Aldrich (England). Potassium tetrachloroplatinate, 2,2'-bipyridine, Sephadex G-25 and Tris-HCl buffer were obtained from Merck (Germany). Highly polymerized calf thymus DNA sodium salt and ethidium bromide (EB) were obtained from Sigma Chemical Co. (USA) and used as received. Other chemicals used were of analytical reagent or higher purity grade. Solvents used were of reagent grade and purified before use by the standard methods and the doubly distilled water was used all along. [Pt(bpy)Cl_2] and [Pd(bpy)Cl_2] were prepared by the procedures described in the literature.[20] All the experiments

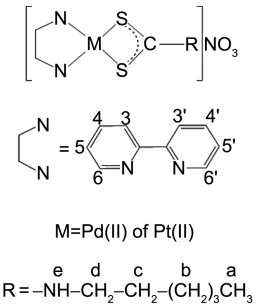
involving interaction of the complexes with CT-DNA were performed in Tris-HCl buffer (20 mM) of pH 7.0 medium containing 20 mM NaCl. Sodium salt was used to adjust the ionic strength of the solution.[21] A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm more than 1.8, indicating that CT-DNA was sufficiently free from protein.[22] The concentration of CT-DNA was determined spectrophotometrically using a molar absorptivity of 6600 M^{-1} cm^{-1} (260 nm).[23] The stock solution of Pd(II) and Pt(II) complexes (0.1 mM and 0.5 mM, respectively) were made in the above-mentioned Tris-HCl buffer by gentle stirring and heating at 308 K, while that of CT-DNA (4 mg/mL) at 241 K until homogenous and used for about a week only.

^{1}H NMR spectra were recorded on a Brucker DRX-500 Avance spectrometer at 500 MHz in DMSO-d_6 using tetramethylsilane as internal reference. Infrared spectra of the metal complexes were recorded on a $J_{AS.CO}$- 460 Plus FT-IR spectrophotometer in the range of 4000 to 400 cm^{-1} in KBr pellets. Microchemical analysis of carbon, hydrogen, and nitrogen for the complexes was carried out on Herause CHNO-RAPID elemental analyzer. Electronic absorption spectra of the title metal complexes were measured on a $J_{AS.CO}$ UV/vis-7850 recording spectrophotometer. Fluorescence intensity measurements were carried out on a Hitachi spectrofluorimeter model MPF-4. Conductivity measurements of the above platinum and palladium complexes were carried out on a Systronics conductivity bridge 305, using a conductivity cell of cell constant 1.0. Melting points were measured on a Unimelt capillary melting point apparatus and were reported uncorrectedly. The following spectrometric measurements were all performed in a quartz cuvette of 1 cm path length.

Preparation of Ligand and Metal Complexes.

Preparation of Hexyldithiocarbamate Sodium Salt (Hex-dtcNa): The preparation of this ligand was carried out using our previous procedure,[18] except that butylamine was replaced by hexylamine (13.2 mL, 100 mmol). The yield was 7.5 g (78%) with melting point of 138 ºC. Anal. Calcd. For $C_7H_{14}NS_2Na$ (199): C, 42.19; H, 7.03; N, 7.03%. Found: C, 42.13; H, 7.00; N, 7.04%. Solid-state FT-IR spectroscopy of the above ligand shows three main characteristic bands at 1491, 983 and 3185 cm^{-1} assigned to ν (N-CSS), ν (SCS) and ν (N-H) modes respectively.[18] ^{1}H NMR (500 MHz, DMSO-d_6, ppm, m = multiplet and sb = singlet broad): 0.84 (m, 3H, H-a), 1.24 (m, 6H, H-b), 1.41 (m, 2H, H-c), 3.32 (m, 2H, H-d) and 7.98 (sb, -NH-) (Scheme 1).

Preparation of [Pd(bpy)(Hex-dtc)]NO_3: This complex was synthesized following our previous procedure,[18] except that Bu-dtcNa was replaced by Hex-dtcNa. Yield (67%) and decomposes at 180 ºC. Analysis calculated for $C_{17}H_{22}N_4O_3S_2Pd$ (500): C, 40.80; H, 4.40; N, 11.20%. Found: C, 40.65; H, 4.39; N, 11.00%. Solid-state FT-IR spectroscopy of the complex shows three main characteristic stretching bands at 1551, 1026 and 2985 cm^{-1} assigned to ν (N-SS), ν (SCS) and ν (N-H) modes respectively.[15,24] The sharp band at 1384 cm^{-1} is assigned to uncoordinated NO_3^- ion.[25] ^{1}H NMR (500 MHz, DMSO-d_6, ppm, sb = singlet broad, d = doublet, t = triplet and m = multiplet):[26] 0.83 (m, 3H, H-a), 1.23 (m, 6H, H-b), 1.54 (m, 2H, H-c), 3.40 (m, 2H, H-d), 11.21 (sb, 1H, H-e), 7.71 (t, 2H, H-4,4'), 8.22 (t, 2H, H-5,5'), 8.32 (d, 2H, H-3,3'), 8.54 (d, 2H, H-6,6') (Scheme 1). Molar conductance of the complex is 92 Ω^{-1} mol^{-1} cm^2 indicating 1:1 electrolytes.[27] Electronic spectra exhibit four bands. The bands at 319 (ε = 2.13) and 308 (ε = 2.06) are assigned to MLCT and the other bands at 249 (ε = 5.71) and 201 (ε = 3.57) may be assigned to first and second intraligand π-π* transition of 2,2'-bipyridine ligand as well as -CSS^- group of dithiocarbamate ligand.[14]



Scheme 1. Proposed structures and proton nmr numbering schemes of [Pd(bpy)(Hex-dtc)]NO_3 and [Pt(bpy)(Hex-dtc)]NO_3.

Preparation of [Pt(bpy)(Hex-dtc)]NO_3: This complex was prepared by a similar method to that of [Pd(bpy)(Hex-dtc)]NO_3, except [Pt(bpy)Cl_2] was used in place of [Pd(bpy)Cl_2]. The yield was 77% and the product decomposes at 193 ºC. Analysis calculated for $C_{17}H_{22}N_4O_3S_2Pt$ (589): C, 34.63; H, 3.74; N, 9.51%. found: C, 34.61; H, 3.75; N, 9.50%. solid state FT-IR spectroscopy of the above complex shows three characteristic stretching bands at 1575, 1030 and 2950 cm^{-1} assigned to ν (N-CSS), ν (SCS) and ν (N-H) modes respectively.[15,24] The sharp band at 1385 cm^{-1} is assigned to uncoordinated NO_3^- ion.[25] ^{1}H NMR (500 MHz, DMSO-d_6, ppm, Sb = singlet broad, t = triplet, d = doublet and m = multiplet:[26] 0.87 (t, 3H, H-a), 1.31 (m, 6H, H-b), 1.64 (t, 2H, H-c), 3.53 (t, 2H, H-d), 11.59 (sb, 1H, H-e), 7.78 (t, 2H, H-4,4'), 8.47 (t, 2H, H-5,5'), 8.6 (d, 2H, H-3,3'), 8.73 (d, 2H, H-6,6') (Scheme 1). Molar conductance of the complex is 99.44 Ω^{-1} mol^{-1} cm^2 indicating 1:1 electrolytes.[27] Electronic spectra exhibit five bands. The band at 366 (ε = 3.78) is assigned to MLCT and the other bands may be due to first, second, and higher internal π-π* transitions of 2,2'-bipyridine and dithiocarbate ligands.[14]

Cytotoxic Studies. Cell proliferation was evaluated by using a system based on the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT] which is reduced by living cells to yield a soluble formazan product that can be assayed colorimetrically.[28-30] The MTT assay is dependent on the cleavage and conversion of the soluble yellowish MTT to the insoluble purple formazan by active mitochondrial dehydrogenase of living cells. The human cancer cell line K562 was grown in an incubator at 310 K in a humidified atmosphere consisting of 5% CO_2 and 95% air, being maintained in a PRMI medium supplemented with 10% heat-inactivated fetal calf serum

and 2 mM L-glutamine, streptomycin and penicillin (5 μg/mL). The harvested chronic myelogenous leukemia cells were seeded at a density 2.0×10^4 cells/ml into 96-well plate with varying concentrations of the sterilized drugs (0-250 μM) and incubated for 24 hours. At the end of four hours incubations 25 μL of MTT solution (5 mg/mL in PBS) was added to each well containing, fresh and cultured medium.[31] The insoluble formazan produced was then dissolved in solution containing 10% SDS and 50% DMF (under dark condition for 2 h at 310 K), and optical density (OD) was read against reagent blank with multi well scanning spectrophotometer (ELISA reader, Model Expert 96, Asys Hitchech, Austria) at a wavelength of 570 nm. Absorbance is a function of concentration of converted dye. The OD value of study groups was divided by the OD value of untreated control and presented as percentage of control (as 100%). Results were analyzed for statistical significance using two-tailed Student's t-test. Changes were considered significant at $p < 0.05$.

DNA Binding Experiments. The stock solutions of Pd(II) and Pt(II) complexes as well as CT-DNA were made in the above mentioned Tris-HCl buffer of pH 7.0 medium containing 20 mM sodium chloride. All the experiments involving interaction studies also were carried out in the same buffer.[32,33] The metal complex solutions, with and without CT-DNA were incubated at 300 K and 310 K. Then, the spectrophotometric readings at λ_{max} (nm) of the complexes where DNA has no absorption were measured. Using trial and error, the incubation time for solutions of DNA-metal complexes at 300 K and 310 K was found to be 6 h. No further changes were observed in the absorbance reading after longer incubation.

Electronic Absorption Studies. It is well known that electronic absorption spectroscopy is an effective method for the binding studies of metal complexes with CT-DNA.[34-36] In this experiment, the sample cell was filled with 1.8 mL DNA of 0.165 mM for Pd(II) system and 0.213 mM for Pt(II) system. In these concentrations, the absorption of DNA is around 1.09 and 1.41, respectively. However, reference cell is only filled with 1.8 mL Tris-HCl buffer. Both cells were set separately at constant temperature of 300 K or 310 K, and then 20 μL from Pd(II) (0.1 mM) or 50 μL from Pt(II) (0.5 mM) was added to each cell. After 3 min, the absorption was recorded at 260 nm for DNA and at 640 nm to eliminate the interference of turbidity. Addition of metal complex to both cells was continued until no further changes in the absorption readings were observed.

Absorption spectral titration experiment was performed by keeping the concentration of the drugs constant (75 μL and 100 μL from 0.1 mM stock of Pd(II) complex and 90 μL and 95 μL from 0.5 mM stock of Pt(II) complex at 300 K and 310 K, respectively) while varying the CT-DNA concentrations (30-130 μL and 30-120 μL from 1.15 mM stock solution of DNA for Pd(II) complex and 28-62 μL and 25-39 μL from 0.8 mM stock solution of DNA for Pt(II) complex AT 300 K and 310 K, respectively) in total volume of 2 mL.

In the interaction of the metal complexes with CT DNA, the procedures followed to determine thermodynamic parameters: molar Gibbs free energy of binding $\Delta G^{o}_{(H_2O)}$, molar enthalpy of binding $\Delta H^{o}_{(H_2O)}$, and molar entropy of binding $\Delta G^{o}_{(H_2O)}$ were similar to what was reported earlier.[37] m, measure of the metal complex ability to destabilize CT-DNA were found out using Pace method.[10,38] Also, the other binding parameters: *n*, *K*, and *g*, where *n* is Hill coefficient, *g* is the number of binding sites per 1000 nucleotides of DNA, and *K* is apparent binding constant, were determined according to reported method.[39] All measurements were performed separately at 300 K and 310 K and repeating three times for these complexes.

For the gel filtration experiments, CT-DNA (68.4 μM and 26.65 μM for pd(II) and Pt(II) complexes, respectively) was interacted with pd(II) and Pt(II) complexes (10 μM and 48.49 μM, respectively) for 6 h at 300 K in Tris-HCl buffer. Each DNA-metal complex solution was then passed through a Sephadex G-25 column equilibrated with the same buffer. The elusion of the column fractions of 2.0 mL was monitored at 315 nm for Pd(II) complex and 321 nm for Pt(II) complex and 260 nm for both systems.

Flurescence Measurments. For florescence experiment, CT-DNA was pretreated with EB for 30 min. To this pretreated CT-DNA solution various amount of Pd(II) or Pt(II) complex were added and their effection on the emission intensity was measured at 300 K temperature. Moreover, fluorescence intensity of each metal complex at the highest denaturant concentration at 471 nm excitation wavelength has been checked, and the emission intensities of the two compounds in the range of 540-700 nm was very small and negligible.

Results and Discussion

The compounds correspond to the composition Hex-dtcNa, $[Pd(bpy)(Hex\text{-}dtc)]NO_3$ and $[Pt(bpy)(Hex\text{-}dtc)]NO_3$, where bpy=2,2'-bipyridine and Hex-dtc=hexyldithiocarbamato ligands were prepared and characterizad by: chemical analysis, conductance measurements, ultraviolet-visible, infrared and ^{1}H-NMR spectroscopic methods. These analytical data of the complexes are given in experimental section and the proposed structure in Scheme 1. Cytotoxicity and detailed calf thymus DNA-binding studies of these water-soluble complexes have been studied:

Cytotoxic Activity. The prepared complexes were tested for their *in vitro* cytotoxic activity by MTT assay. IC_{50} values were estimated for chronic myelogenous leukemia cell line, K562. Figure 1 reveals the effect on cell growth after a treatment period of 24 hours. The Ic_{50} value of the Pd(II) complex is 80 μM and the Pt(II) complex is 20 μM. Among the compounds studied here, both complexes exhibit cytotoxic activity and Pt(II) complex demonstrates more active than Pd(II) complex. Thus, both may be potential antitumor agent. Furthermore, Ic_{50} value of cisplatin under the same experimental conditions was determined. This value (154 μM) is much higher as compared to the Ic_{50} values of

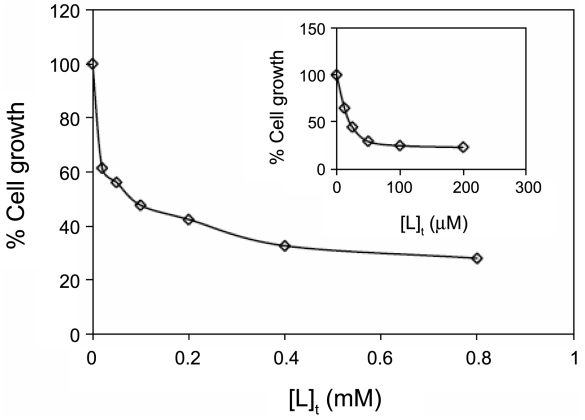


Figure 1. The growth suppression activity of the Pd(II) complex and the inset for Pt(II) complex on K562 cell line was assessed using MTT assay as described in material and methods. The tumor cells were incubated with varying concentrations of the complexes for 24 h.

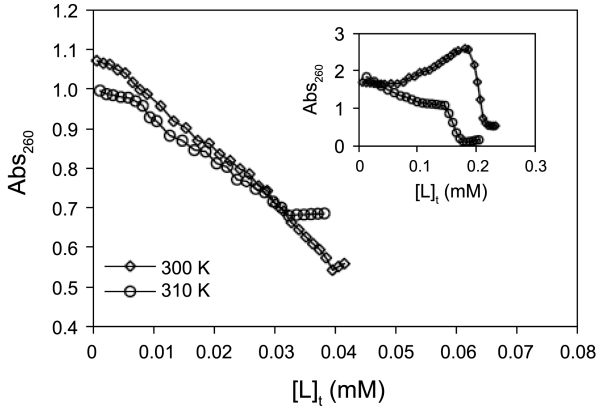


Figure 2. The changes of absorbance of CT-DNA at λ_{max} = 260 nm due to increasing the total concentration of [Pd(bpy)(Hex-dtc)]NO_3 and inset for [Pt(bpy)(Hex-dtc)]NO_3, $[L]_t$, at constant temperatures of 300 K and 310 K.

the above two complexes. However, the Ic_{50} values of these complexes are comparable with those of our analogous Pd(II) and Pt(II) dithiocarbamate complexes reported earlier.[14,18,19,24]

Denaturation of CT-DNA by the Pt(II) and Pd(II) Complexes. The profiles of denaturation of DNA by [Pd(bpy)(Hex-dtc)]NO_3 are shown in Figure 2, the inset is denaturation of CT-DNA by [Pt(bpy)(Hex-dtc)]NO_3 at the two temperatures of 300 K and 310 K. The concentration of metal complexes in the midpoint of transition, $[L]_{1/2}$, for Pd(II) complex at 300 K is 0.025 mM and at 310 K is 0.024 mM and for Pt(II) complex at 300 K is 0.199 mM and at 310 K is 0.163 mM. The improving effect of the temperature, leading to the decrease in $[L]_{1/2}$ from 0.199 to 0.163 for Pt(II) system, indicates that the increase in the temperature lowers the stability of the DNA against denaturation caused by this complex. The important observation of this work is the low values of $[L]_{1/2}$ for these complexes,[21,40] that is, both complexes (especially Pd(II) complex) can denature DNA at very low concentrations. Thus, if these complexes are used as anti-tumor agents, low doses will be needed, this may have fewer side effects. It is noticeable that, absorbance of DNA at 260 nm should increase in presence of increasing amount of each metal complex (denaturant agent). The decrease in the absorbance at 260 nm with increase of amount of metal complexes added to CT-DNA may be due to: (i) a possibility that interaction between CT-DNA and the metal complexes cause the double helix of CT-DNA to become more straight leading to stacking. This stacking may cause conformational changes leading to a sort of denaturation, or (ii) each strand after denaturation get associated in a more stacked structure and (iii) metal complexes slip into the helix and mask the hydrophobic bases leading to a decrease in absorbance.

In the interaction of the metal complexes with CT-DNA were found some thermodynamic parameters. Using the CT-DNA denaturation plots (Fig. 2) and Pace method,[39] the value of K, i.e. unfolding equilibrium constant, and ΔG^o, i.e. unfolding free energy of CT-DNA at two temperatures of 300 K and at 310 K in the presence of [Pt/Pd(bpy)(Hex-dtc)]NO_3 have been calculated. In this method, Pace had assumed two-state mechanism, nature and denature, and then calculated unfolding free energy of DNA i.e. (ΔG^o) by using Eqs. (1) and (2):

$$K = \frac{A_N - A_{obs}}{A_{obs} - A_D} \quad (1)$$

$$\Delta G^o = -RT\,Ln\,K \quad (2)$$

Where A_{obs} is absorbance readings in transition region, A_N and A_D are absorbance readings of nature and denatured conformation of DNA, respectively. A straight line is observed when the values of ΔG^o are plotted against the concentrations of each metal complex in the transition region at 300 K and at 310 K. These plots are shown in Figure 3 for Pd(II) and the inset for Pt(II) systems. The equation for these lines can be written as follow:[41]

$$\Delta G^o = \Delta G^o_{(H_2O)} - m[\text{complex}] \quad (3)$$

Here the values of $\Delta G^o_{(H_2O)}$ for each curve is measured

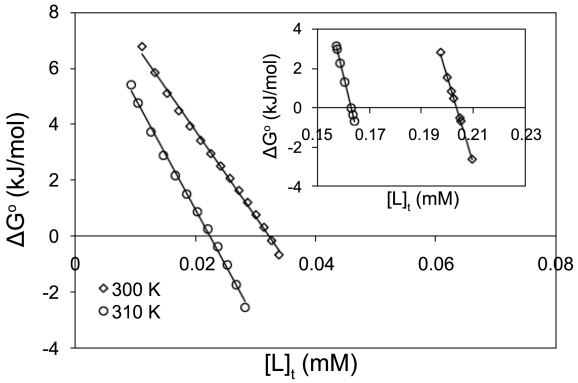


Figure 3. The molar Gibbs free energies of unfolding (ΔG^o *vs* $[L]_t$) of CT-DNA in the presence of [Pd(bpy)(Hex-dtc)]NO_3 and inset for [Pt(bpy)(Hex-dtc)]NO_3.

Table 1. Thermodynamic parameters of CT-DNA denaturation by palladium(II) and platinum(II) complexes

Compound	Temperature (K)	[a] m (kJ/mol)(mmol/L)$^{-1}$	[b] $\Delta G^{o}_{(H_2O)}$ (kJ/mol K)	[c] $\Delta H^{o}_{(H_2O)}$ (kJ/mol)	[d] $\Delta S^{o}_{(H_2O)}$ (kJ/mol)
[Pd(bpy)(Hex-dtc)]NO_3	300	308.56	9.95	40.46	0.102
	310	397.45	8.91		0.105
[Pt(bpy)(Hex-dtc)]NO_3	300	439.21	89.45	107.96	0.060
	310	544.92	88.75		0.062

[a]Measure of the metal complex ability to destabilize CT-DNA. [b]Conformational stability of CT-DNA in the absence of metal complex. [c]The heat needed for CT-DNA denaturation in the absence of metal complex. [d]The entropy of CT-DNA denaturation by metal complex.

from the intercept on ordinate of the plots and it is conformational stability of DNA in the absence of metal complex and the m (the slope of each curve in the same plots) is a measure of the metal complex ability to destabilize CT-DNA are summarized in Table 1. As we know, the higher the values of $\Delta G^{o}_{(H_2O)}$, the larger the conformational stability of CT-DNA. However, the values of $\Delta G^{o}_{(H_2O)}$ (Table 1) are decreased by rising the temperature. This is as expected because in general, the decrease in $\Delta G^{o}_{(H_2O)}$ value is the main reason for the decrease in DNA stability.[42] The values of m for the [Pt(bpy)(Hex-dtc)]NO_3 complex are much higher than those of [Pd(bpy)(Hex-dtc)]NO_3 complex. This indicate the higher ability of Pt(II) complex to decreased the stability of CT-DNA. These m values are similar to that of Pd(II) complexes as well as surfactant reported earlier.[14] Another important thermodynamic parameter found is molar enthalpy of CT-DNA denaturation in absence of Pd(II) and Pt (II) complexes, $\Delta G^{o}_{(H_2O)}$. To find this, we calculated the molar enthalpy of CT-DNA denaturation in the presence of each metal complex, $\Delta H^{o}_{conformation}$ or $\Delta H^{o}_{denaturation}$, in the range of two temperatures using Gibbs-Helmholtz equation.[43] On plotting the values of these enthalpies versus the concentration of each metal complex, straight line will be obtained which are shown in Figure 4 for Pd(II) and the inset for Pt(II) complexes. Intrapolation of these lines (intercept on ordinate, i.e., absence of metal complex) give the values of $\Delta H^{o}_{(H_2O)}$ (Table 1). Moreover, the entropy of CT-DNA unfolded by Pd(II) and Pt(II) complexes, $\Delta G^{o}_{(H_2O)}$ have been calculated by means of equation $\Delta G = \Delta H\text{-}T\Delta S$ and the data are given in Table 1.[44] These data show that the metal-DNA complex is more disordered than that of native CT-DNA, because the entropy changes are positive for Pd(II)- or Pt(II)-DNA complexes in the denaturation processes of CT-DNA (Table 1). The more negative values of $\Delta G^{o}_{(H_2O)}$ and the large positive enthalpic and entropic values with respect

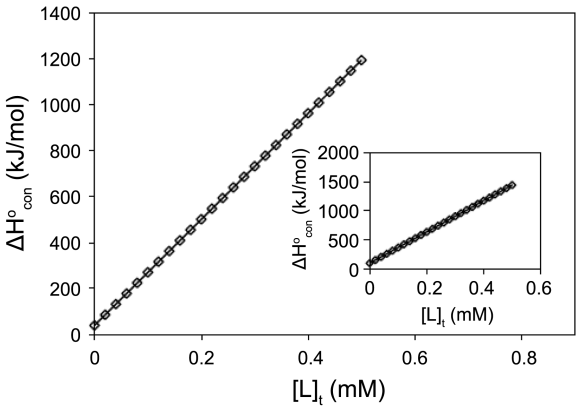


Figure 4. The molar enthalpies of DNA denaturation in the interaction with [Pd(bpy)(Hex-dtc)]NO_3 and inset for [Pt(bpy)(Hex-dtc)]NO_3 complexes in the range of 300 K to 310 K.

to total concentration of the Pd(II) and Pt(II) complexes suggest that the interactions between the two molecules are dominated by hydrophobic rather than electrostatic forces.[45] These thermodynamic parameters compare favorably well with those of Palladium (II) and platinum (II) complex as reported earlier.[14,15,19]

Absorption Spectral Titration Experiments. In this Part of Study Two Successive Experiments were Performed: **(i)** change in absorbance, A, was calculated by subtracting the absorbance reading of mixed solutions of each metal complex with various concentrations of CT-DNA, from absorbance reading of free metal complex. The values of ΔA_{max}, change in absorbance when all binding sites on CT-DNA were occupied by metal complex, are given in Table 2 and Figure 5 for Pd(II) and the inset for Pt(II) systems. These values were used to calculate the concentration of metal complex bound to CT-DNA, $[L]_b$, and the concentration of free metal complex, $[L]_f$ and ν, ratio of the

Table 2. Values of ΔA_{max} and binding parameters in the Hill equation for interaction between Pd(II) and Pt(II) complexes and CT-DNA in 20 mmol/L Tris-HCl buffer and pH 7.0

Compound	Temperature (K)	[a] ΔA_{max}	[b] g	[c] $K \times 10^3$ (M)$^{-1}$	[d] n	[e] Error
[Pd(bpy)(Hex-dtc)]NO_3	300	0.014	7	263.67	3.15	0.0518
	310	0.011		174.96	2.77	0.073
[Pt(bpy)(Hex-dtc)]NO_3	300	0.08	7	9.98	3.19	0.0078
	310	0.09		15.65	2.88	0.0087

[a]Change in the absorbance when all the binding sites on CT-DNA were occupied by metal complex. [b]The number of binding sites per 1000 nucleotides. [c]The apparent binding constant. [d]The Hill coefficient (as a criterion of cooperativity). [e]Maximum error between theoretical and experimental values of ν.

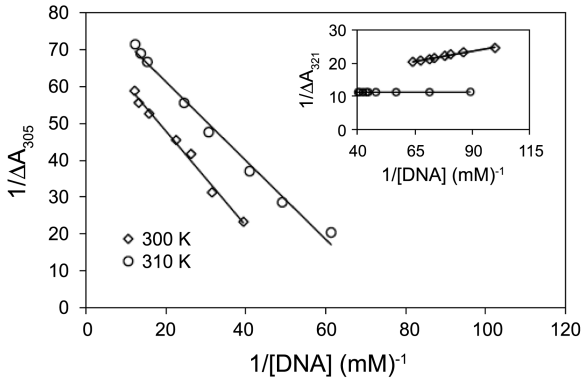


Figure 5. The changes in the absorbance of fixed amount of each metal complex in the interaction with varying amount of CT-DNA at 300 K and 310 K. The linear plot of the reciprocal of DA versus the reciprocal of [DNA] for [Pd(bpy)(Hex-dtc)]NO_3 and inset for [Pt(bpy)(Hex-dtc)]NO_3.

concentration of bound metal complex to total [DNA] in the next experiment: **(ii)** a fixed amount of CT-DNA (25 μL and 50 μL from 1.1 mM stock solution of CT-DNA for Pd(II) complex and 25 μL and 24 μL from 2.07 mM stock solution of CT-DNA for Pt(II) complex at 300 K and 310 K, respectively) was titrated with varying amount of each metal complex (30-80 μL and 60-150 μL from 0.1 mM stock solution of Pd(II) complex and 240-410 μL and 180-270 μL from 0.5 mM stock solution of Pt(II) complex at 300 K and 310 K, respectively) in total volume of 2 mL, separately. Using these data (v, $[L]_f$), the Scatchard plots[46] were constructed for the interaction of each metal complexes at the two temperatures 300 K and 310 K. The Scatchard plots are shown in Figure 6 for Pd(II) and the inset for Pt(II) systems. These plots are curvilinear concave downwards, suggesting cooperative binding.[47] To obtain the binding parameters, the above experimental data (v and $[L]_f$) were substituted in equation (1), i.e., Hill equation.[48]

$$\upsilon = g(K[L]_f)^n/(1 + (K[L]_f)^n) \quad (1)$$

Thus we get a series of equation with unknown parameters n, K and g. Using Eureka software, the theoretical values of these parameters could be deduced (Table 2). The values of n for two complexes will be more than 1, which means that the systems are cooperative thus, the binding at one site increases the affinity of others.[46] The apparent binding constants of two complexes obtained were 2.64×10^5 M^{-1} at 300 K and 1.75×10^5 M^{-1} at 310 K for Pd(II) complex and 9.98×10^3 M^{-1} at 300 K and 1.56×10^4 M^{-1} at 310 K for Pt(II) complex. These values are comparable to those of CT-DNA intercalators [Pd(dmphen)CO_3]·H_2O 1.6×10^4 M^{-1},[17] [Cu(GFL)(A^1)Cl]·$5H_2O$ 2.45×10^3 M^{-1},[49] [(bpy)$_2$Ru(*m*-bipp)Ru(bpy)$_2$](ClO_4)$_4$ 2.6×10^5 M^{-1}, [Ru(phen)$_2$(PPIP)]$_2$ 1.1×10^5 M^{-1} and [Ru(bpy)$_2$(PPIP)]$_2^{2+}$ 4.3×10^4 M^{-1} [50] and [Ru(dmb)$_2$(NMIP)]$_2$ 5.46×10^3 M^{-1}.[51] Obviously, these spectral characteristics suggest that the two complexes intercalatively bind to CT-DNA, involving a strong stacking interaction between the aromatic chromophore and the base pairs of the CT-DNA.[52] Moreover, higher values of apparent binding constant of Pd(II) may be because palladium complexes are about 10^5 times more labile than their platinum analogues.[53] The maximum errors between experimental and theoretical values of v are also shown in Table 2 that is quite low. Knowing the experimental (dots) and theoretical (lines) values of v in the Scatchard plots and superimpossibility of them on each other, these values of v were plotted versus the values of $\ln[L]_f$. The results are sigmoidal curves and are shown in Figure 7 for Pd(II) and the inset for Pt(II) systems at 300 K and 310 K. These plots indicate positive cooperative binding at both temperatures for the two complexes. Finding the area under the above plots of binding isotherms and using Wyman-Jons equation,[38] we can calculate the K_{app} and $\Delta G^o_{(H_2O)}$ at 300 K and 310 K for each particular v and also $\Delta H^o_{(H_2O)}$ (Table 1). Plots of the values of $\Delta H^o_{(H_2O)}$ versus the values of $[L]_f$ are shown in Figure 8 for Pd(II) and the inset for Pt(II) systems at 300 K. The changes are observed in both plots that may be due to binding of metal complexes to macromolecule or macromolecule denaturation. Similar observations can be seen in the literature where Pd(II) and Pt(II) complexes have been interacted with CT-DNA.[15,18]

Gel Filtration. Above results from the absorption spectroscopic studies show that the compounds in title binds to CT-DNA. Binding of these complexes with CT-DNA was also studied by gel filtration using Sephadex G-25. The solution

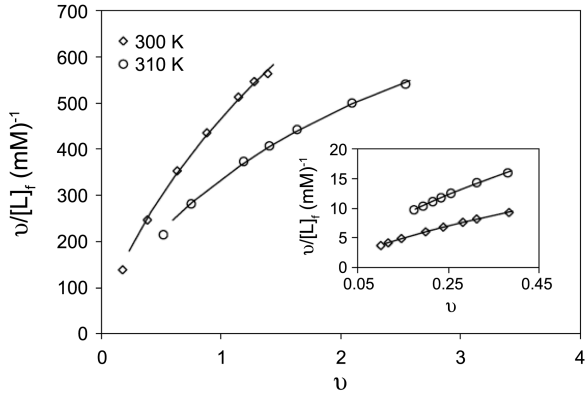


Figure 6. Scatchard plots for binding of [Pd(bpy)(Hex-dtc)]NO_3 and inset for [Pt(bpy)(Hex-dtc)]NO_3 to CT-DNA.

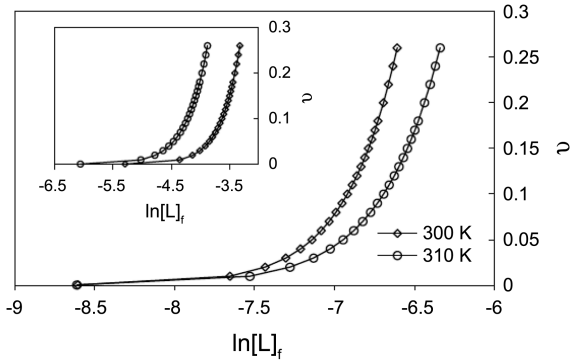


Figure 7. Binding isotherm plots for [Pd(bpy)(Hex-dtc)]NO_3 and inset for [Pt(bpy)(Hex-dtc)]NO_3 in the interaction with CT-DNA.

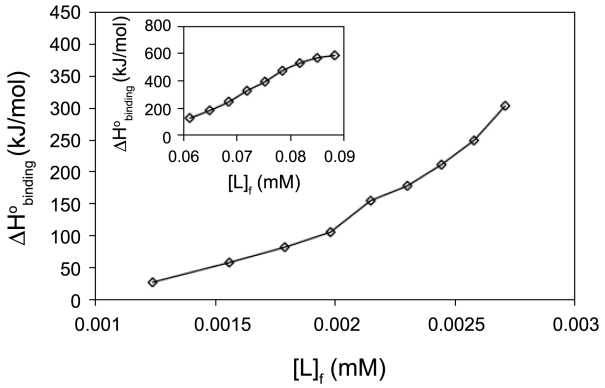


Figure 8. Molar enthalpies of binding in the interaction between CT-DNA and [Pd(bpy)(Hex-dtc)]NO_3 and inset for [Pt(bpy)(Hex-dtc)]NO_3 versus free concentrations of complexes at pH 7.0 and 300 K.

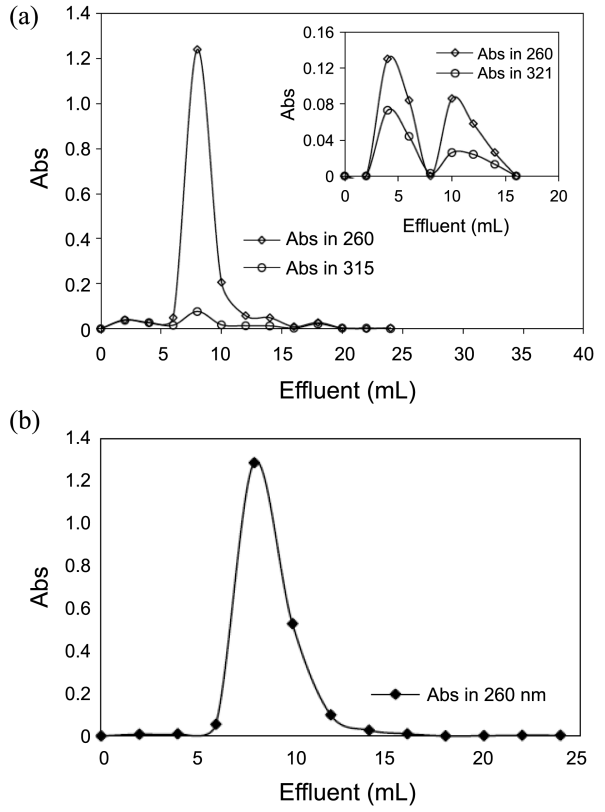


Figure 9. Gel chromatograms of [Pd(bpy)(Hex-dtc)]NO_3-DNA complex, inset for [Pt(bpy)(Hex-dtc)]NO_3-DNA complex (A) and free CT-DNA (B), obtained on Sephadex G-25 column.

of each interacted DNA-metal complex was passed through a Sephadex G-25 column. Elution was done with buffer and each fraction of the column was monitored spectrophotometrically at 315 nm and 260 nm for pd(II) system and at 321 nm and 260 nm for pt(II) system. The gel chromatograms obtained from plotting the values of absorbance versus the mL of effluents of each DNA-metal complex are shown in Figure 9 A for Pd(II) system and the inset for pt(II) system. This plot shows that the peak obtained for the two wavelengths are not resolved and suggests that the pd(II) complex has not separated from the CT-DNA. However, Pt(II) complex breaks the CT-DNA into two unequal fragments and binds to both. This clearly demonstrates that the binding between DNA and the metal complexes are not reversible under such circumstances, Because, if the interaction between DNA and metal complex was weak, the DNA should have come out of the column separately.[14,17,54] To confirm the breaking of CT-DNA by Pt(II) complex, a solution of CT-DNA alone was passed through the same column and each eluted fraction of 2 mL was monitored at 260 nm. The gel chromatogram obtained is shown in figure 9 B. This indicates that the CT-DNA has fragments with approximately similar molecular weights and Pt(II) complex should have been responsible to breaks it.

Fluorescence Studies. The results obtained from absorption titration and gel filtration experiments indicated that the Pd(II) and Pt(II) complexes could effectively bind to DNA. In order to confirm the binding mode and compare their binding affinities, EB displacement and Scatchard analysis experiments were carried out.[17,55] EB is a conjugate planar molecule. Its fluorescence intensity is very weak, but it is greatly increased when EB is specifically intercalated into the base pairs of double stranded DNA.[56] It has been reported that the enhanced fluorescence could be quenched, at least partially by the addition of a second molecule.[15,57] which could competed with EB to bind with DNA. This is a proof that the complexes intercalate to base pairs of DNA.[17,58,59]

Therefore, the binding of the compounds in title were evaluated using competitive binding studies involving EB. Figure 10 showed the emission spectra of DNA-EB system with increasing amounts of the Pd(II) and Pt(II) (inset) complexes. The addition of each complex to CT-DNA pretreated with EB cause appreciable reduction in the emission intensity, indicating the replacement of EB by the complexes.[1,17]

Further studies to characterize the mode of binding of Pd(II) and Pt(II) complexes to CT-DNA were carried

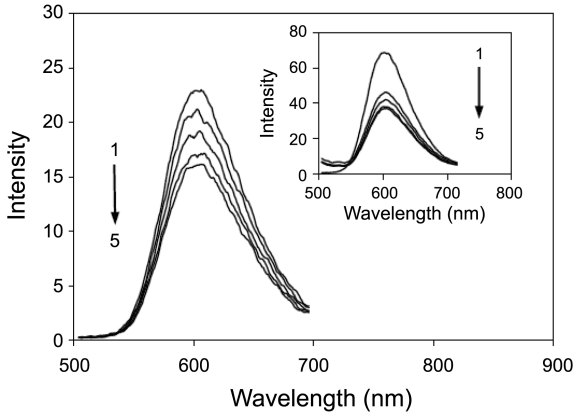


Figure 10. Fluorescence emission spectra of interacted EB-DNA in the absence (1) and presence of different concentrations of Pd(II) complex and inset for Pt(II) complex: 0.022 (2), 0.040 (3) and 0.085 (4) μM and 0.190 (2), 0.206 (3) and 0.216 (4) μM, respectively, at 300 K.

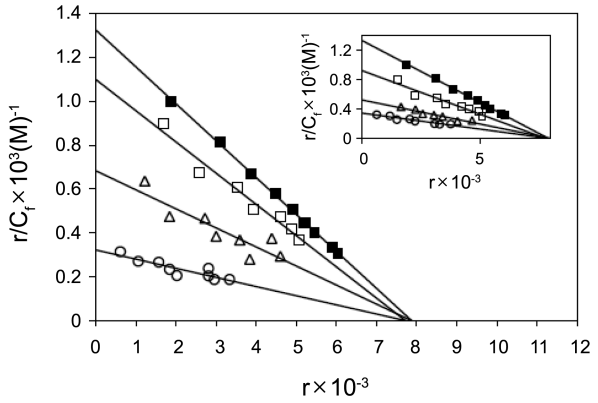


Figure 11. Competition between $[Pd(bpy)(Hex\text{-}dtc)]NO_3$ and inset for $[Pt(bpy)(Hex\text{-}dtc)]NO_3$ with EB for the binding sites of DNA (Scatchard plot). In curve no. 1, Scatchard's plot was obtained with calf thymus DNA alone. Its concentration was 60 μM. In curves nos. 2, 3 and 4 respectively, 22.2, 40.2 and 85.2 μM for $[Pd(bpy)(Hex\text{-}dtc)]NO_3$ complex and 190.2, 205.8 and 216 μM for $[Pt(bpy)(Hex\text{-}dtc)]NO_3$ complex, were added, corresponding to molar ratio [complex]/[DNA] of 0.37, 0.67 and 1.42 for $[Pd(bpy)(Hex\text{-}dtc)]NO_3$ complex and 3.17, 3.43 and 3.60 for $[Pt(bpy)(Hex\text{-}dtc)]NO_3$ complex. Solutions were in 20 mM NaCl, 20 mM Tris-HCl (pH 7.0). Experiments were done at room temperature.

out.[18,19,57] The number of EB molecules intercalated to CT-DNA in presence of different concentrations of the Pd(II) and Pt(II) complexes was calculated using Scatchard analysis.[49] In this experiment, the wavelengths of excitation and emission were set at 540 nm and 700 nm respectively. Both have 0.5 nm slit widths. Solutions of CT-DNA, EB and metal complexes were prepared in Tris-HCl buffer of pH 7.0. In this medium solutions of Pd(II) and Pt(II) complexes were interacted with CT-DNA by incubating them at 300 K and 310 K for 4 h, appropriate amount of EB was then added to them and further incubated at room temperature (300 K) for 2 h and finally processed for fluorescence spectral measurement. Saturation curves of fluorescence intensity for $[Pt/Pd(bpy)(Hex\text{-}dtc)]^+$-DNA systems at different r_f values (0.37, 0.67 and 1.42 for Pd(II) complex and 3.17, 3.43 and 3.60 for Pt(II) complex) were obtained in the presence of increased concentrations of EB (2, 4 to ..., 20 μM). The fluorescence Scatchard plots obtained for binding of EB to CT-DNA in absence (■) and presence (□, △, ○) of various concentrations of Pd(II) and Pt(II) (inset) complexes are shown in Figure 11. Consequently, it might be concluded that the Pd(II) and Pt(II) complexes inhibit competitively the EB binding to DNA (type-A behavior),[49] where number of intercalated molecules per 1000 nucleotide, (intercept on the abscissa) remain constant and the slope of the graphs that is K_{app} (apparent association constant) decrease in presence of increasing amount of complexes. The K_{app} values for the Pd(II) and Pt(II) complexes are listed in Table 3. These data suggested that the interaction of the Pd(II) complex with CT-DNA was stronger than that of Pt(II) complex, which were consistent with the above absorption spectral results. Compare their K_{app} values with those of other known DNA-intercalative complexes which possess analogical structure, $[Pd(Bu\text{-}dtc)(phen)]NO_3$ 0.45×10^5 M^{-1},[24] the Pd(II) and Pt(II) complexes in our paper have similar or stronger affinities with CT-DNA.

Table 3. Binding parameters for the effect of palladium and platinum complexes on the fluorescence of EBr in the presence of CT-DNA

Compound	$^a r_f$	$^b K \times 10^5$ $(M)^{-1}$	$^c n$
$[Pd/Pt(bpy)(Hex\text{-}dtc)]NO_3$	0.00	1.68	
	0.37	1.42	
$[Pd(bpy)(Hex\text{-}dtc)]NO_3$	0.67	0.87	
	1.42	0.42	0.0078
	3.17	1.19	
$[Pt(bpy)(Hex\text{-}dtc)]NO_3$	3.43	0.66	
	3.60	0.43	

Conclusions

2,2'-Bipyridine Pd(II) and Pt(II) complexes of hexyldithiocarbamate were prepared and characterized by analytical and spectral techniques. The absorption and fluorescence spectra studies on the interaction of the complexes with CT-DNA show that the complexes have the ability of interaction with CT-DNA. Determination of several binding and thermodynamic parameters has also been attempted. These water soluble complexes cooperatively bind CT-DNA and can denature CT-DNA at very low concentration (~250 μM for Pd(II) complex and ~297 μM for Pt(II) complex). Both complexes presumably intercalate in CT-DNA. The capability of cleavage of CT-DNA by the complexes was investigated by gel filtration, it indicates that only Pt(II) complex can break the CT-DNA into two unequal fragment and their binding with CT-DNA is strong enough not to break readily. These complexes have shown cytotoxic activity in chronic myelogenous leukemia cell line, K562, better than that of cisplatin. Thus the new synthesized palladium(II) and platinum(II) complexes may be potential anti-tumor agent due to its unique interaction mode with CT-DNA.

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