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Platinum(II) and Palladium(II) complexes with 1,10-phenanthroline and pyrrolidinedithiocarbamato ligands: synthesis, DNA-binding and anti-tumor activity in leukemia K562 cell lines

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Abstract

Two newly-created water-soluble complexes of palladium(II)/platinum(II)-dithiocarbamate, [Pd/Pt(phen)(pyr-dtc)]NO_3 (phen = 1,10-phenanthroline and pyr-dtc = pyrrolidinedithiocarbamate) were synthesized. Also, their chemical characteristics are reported in the current research paper. In these complexes, the dithiocarbamato ligand coordinates to Pt(II) or Pd(II) center with two sulfur atoms as bidentate. They were examined for their cytotoxic properties against chronic myelocytic leukemia K562 cell lines. These complexes showed 50% cytotoxic concentration (Cc_{50}) values much lower than those of cisplatin. The interactions of the two complexes with calf thymus DNA (CT-DNA) were investigated using UV-vis absorption, fluorescence emission, ethidium bromide displacement and gel filtration techniques. The results suggest that these complexes cooperatively bind with and denature the DNA at low concentrations and interact with CT-DNA in the intercalation mode. Gel filtration studies indicate that the binding of complexes with DNA is strong enough not to break readily.

Keywords: Pt(II)/Pd(II) complexes; cytotoxicity; DNA binding; thermodynamic/binding parameters

1. Introduction

Current interests in inorganic antitumor agents are concerned with the improvement of anti-cancer properties of cisplatin and carboplatin by appropriate changes in either leaving groups or amine groups which show efficient DNA binding properties and drug metabolism under physiological conditions (Kostova, 2006; Reedijk, 2003). This is due to the fact that a small change in ligand substituent may influence the biological activity of the compound under investigation. About 3000 platinum complexes have been tested for their antitumor properties in the last 40 years (Desoize & Madoulet, 2002). However, reports on the palladium analogous are scanty. Concerning metallic ion, owing to the similar coordination modes and chemical properties of palladium (II) and platinum (II), they both adopt dsp^2 orbital hybridization, forming square planar complexes. So, the structure and activity of palladium complexes have drawn broad attraction (Blight, Van Noortwyk, Wisner, & Jennings, 2005; Mota et al., 2005). Sometimes the activity of palladium complexes has the advantage over platinum complexes (R Mital, Shah, Srivastava, & Bhattacharya, 1992; Nikolis, Methenitis, & Pneumatikakis, 2003; Nikolis, Methenitis, Pneumatikakis, & Fiallo, 2002; Quiroga, Pérez, López-Solera, et al., 1998; Quiroga, Pérez, Montero, et al., 1998; Quiroga et al., 1999).

Preparation and studies of antitumor metal complexes containing chelating N-N and S-S donor ligands are the most recent advances in the realm of platinum and palladium compounds which have higher activity and reduced toxicity as compared with cisplatin (Frezza et al., 2010; Komeda & Casini, 2012; Renu Mital, Jain, & Srivastava, 1989; Montagner, Marzano, & Gandin, 2011). With respect to N-N donor, 1, 10-phenanthroline is a potentially intercalating ligand when bound with coordination squar of palladium as coplanar. The interest in S-S donor ligands has probably initiated

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from detoxicant properties of sulfur-containing ligands against heavy metal intoxication (Makedonas & Mitsopoulou, 2006). An example can be the use of sodium diethyldithiocarbamate (DDTC) in the treatment of patients with acute poisoning of nickel carbonyl, arsenic, and thallium (Pasini et al., 1994).

The reaction between a primary or secondary amines and carbon disulfide in basic media yields dithiocarbamate ligands. They can form a large number of complexes with transition metals by coordinating through sulfur atoms. The major benefit of utilizing the small bite-angle of dithicarbamato moiety, as a stabilizing chelating ligand, is its unique property to remain intact under a variety of reaction conditions (Coffey, Forster, & Hogarth, 1996). The interest in dithiocarbamate complexes of platinum increased after it was reported that dialkyl dithiocarbamate reduces its nephrotoxicity when co-administered with cisplatin (Manav, Mishra, & Kaushik, 2006). Moreover, it has been observed that the biological activity of certain organic compounds are enhanced through complex formation with metal ions (Abdullah & Salh, 2010), and metal coordination to sulfur induces, possibly, the formation of a drug reserve in the cell (Flora, Mittal, & Mehta, 2008) and reduces renal damages (Appleton, Connor, Hall, & Prenzler, 1989; Battin & Brumaghim, 2009; Burgeson & Kostic, 1991; Ercal, Gurer-Orhan, & Aykin-Burns, 2001; Faraglia et al., 2001; Matesanz et al., 1999; Ries & Klastersky, 1986). In our previous studies, the mixed ligand complexes $[M(S_2CNEt_2)(L)]NO_3$ (M= Pd or Pt, L= 2,2'-bipyridine or 1,10-phenanthroline and S_2CNEt_2=diethyldithiocarbamate) were found to be active towards P_{388} leukemia cells (Mansouri-Torshizi, Saeidifar, Divsalar, & Saboury, 2010). In this line, various palladium(II) and platinum(II) complexes containing both dithiocarbamate and α-diimine moieties have been reported (Mansouri-Torshizi, Eslami-Moghadam, Divsalar, & Saboury, 2011; Mansouri-Torshizi, Mahboube, Divsalar, & Saboury, 2008; Hassan Mansouri-Torshizi et al., 2010). They brought about interesting results when tested against leukemia cell line K_{562} and showed Cc_{50} values much lower than those of cisplatin. Thus, we thought to continue with our ongoing interest. Herein, we have chosen a bioactive pyrrolidindithiocarbamate ligand whose structure not only resembles a great number of antibacterial, antiviral, antifungal and insecticidal agents, but can also be used as inhibitor of cisplatin-induced nephrotoxicity (Ling, Allen, Vickery, & Hambley, 2000). Furthermore, the chelate property of 1, 10-phenanthroline and dithiocarbamate ligands in the structure of title complexes may compensate the labiality of Pd (II) as compared with Pt (II) analogous complexes of monodantate ligands.

The general consensus is that Pd(II) and Pt(II) complexes acquire their antitumor activity through adducts which are formed with DNA (Manav, Mishra, & Kaushik, 2004). Therefore, to confirm the mode of binding of the above-mentioned complexes to calf thymus DNA, extensive interaction studies of both complexes with CT-DNA were presented. In these interaction studies, several bindings and thermodynamic parameters have been described which may shed on the interaction mechanisms of these types of complexes with DNA of cell and possible side effects of these agents. From the other side, since free DNA is not the relevant instance of DNA *In vivo*, both complexes have been tested against chronic myelogenous leukemia K562 cell line.

2. Experimental

2.1. Materials and Physical Measurements

Potassium tetrachloroplatinate, 2,2'-bipyridine, highly polymerized calf thymus DNA sodium salt and Tris-HCl buffer were purchased from Merck (Germany). Palladium(II) chloride anhydrous was bought from Fluka (Switzerland). Pyrrolidine, carbon disulfide and ethidium bromide (EBr) were obtained from Aldrich (England). $[Pt(phen)Br_2]$ and $[Pd(phen)Br_2]$ were prepared based on what is mentioned in the literature (Palocsay & Rund, 1969). Other used chemicals were of analytical reagent or higher purity grade. Solvents were purified prior to be used by the standard procedures (AI, 1989).

The melting points of the compounds were determined on a Unimelt capillary melting point apparatus. Carbon, hydrogen and nitrogen were analyzed on a Herause CHNO-RAPID elemental analyzer. Infrared spectra (4000–400 cm^{-1}) were determined with KBr disks on a J_{ASCO}-460 plus FT-IR spectrophotometer. UV-vis spectra were recorded on a J_{ASCO} UV/VIS-7850 recording spectrophotometer. ^1H NMR spectra were measured on a Brucker DRX-500 Avance spectrometer at 500 MHz, using TMS as the internal reference in DMSO-d_6. The fluorescence spectra were carried out on a Varian spectrofluorimeter model Cary Eclipes. Conductivity measurements of the above platinum and palladium complexes were carried out on a Systronics Conductivity Bridge 305, using a conductivity cell with constant 1.0 and double distilled water was used as solvent.

2.2. Synthesis of Ligand and Metal Complexes

2.2.1. Synthesis of pyr-dtcNa

This ligand was prepared by a modified literature method (Menezes et al., 2005): A solution of sodium hydroxide (4.00 g, 0.1 mol) in 15 mL double distilled water was added to a stirred solution of pyrrolidine (8.3 mL 0.1 mol) in 100 mL acetone-water (1:1.5 v/v) mixture. The reaction mixture was placed in an ice bath and an excess of carbon disulfide (15 mL) was added slowly with constant stirring. The resulting yellowish solution was stirred in a closed vessel at 0 °C for 3 h and at room temperature for 5 h. It was then filtered and subsequently the mixture was evaporated at 35–40 °C to complete dryness. Recrystallization was carried out by stirring the crude product in 20 mL water at 25 °C and filtering out the small solid residue. After three days, diffusion of acetone into this filtrate gave needle shaped crystals. The crystals were isolated by filtration, washed with 15 mL acetone, and dried at 40 °C. Yield: 7.94 g (47%) with a melting point of 142-143 °C. Anal. Calcd. for $C_5H_8NS_2Na$ (169): C, 35.50; H, 4.73; N, 8.28% Found: C, 35.61; H, 4.71; N, 8.37%. Solid state FT-IR spectroscopy of the above ligand shows three characteristic stretching bands at 1422, 997 and 632 cm^{-1} assigned to ν(N-CSS), $\nu(CSS)_{as}$ and $\nu(CSS)_s$ modes respectively (Menezes et al., 2005). ^{1}H NMR (500 MHz, DMSO-d_6, ppm, sb= singlet broad): 1.78 (sb, 4H, H-b), 3.60 (sb, 4H, H-a) (see Fig. 1).

2.2.2. Synthesis of [Pt(phen)(pyr-dtc)]NO_3

[Pt(phen)Br_2] (0.267 g, 0.5 mmol) was suspended in 100 mL acetone-water (2:1 v/v) mixture, and (0.17 g, 1 mmol) of $AgNO_3$ was added to it with constant stirring. This reaction mixture was heated by stirring in darkness for 6 h at 60 °C and then for 16 h at room temperature (30°C). The AgCl precipitate was filtered through Whatman 42 filter paper. A solution of 0.085g, 0.5mmol pyrrolidinedithiocarbamate sodium salt in 15 mL water was slowly added to the clear yellow filtrate containing [Pt(phen)$(H_2O)_2$]$(NO_3)_2$. Stirring continued at ~ 50 °C for another 6 h and then filtered. The clear yellowish orange filtrate was evaporated at 35–40 °C to complete dryness. The precipitate obtained was stirred with 15 mL acetone to obtain a fine powder. The acetone was decanted and the process was repeated with the second portion of acetone to remove most of the impurities. The orange powder so obtained was dissolved in 10 mL methanol/acetonitrile (1:1 v/v) mixture and filtered. Diffusion of ether into this filtrate gave fine yellow crystals after 24 h. The crystals were isolated by filtration, washed with 15 mL acetone, and dried at 40°C. Yield: 0.131 g (45%) and decomposed at 294°C. Anal. Calcd. for $C_{17}H_{16}N_4O_3S_2Pt$ (583): C, 34.99; H, 2.74; N, 9.61%. Found: C, 35.00; H, 2.73; N, 9.59%. Solid state FT-IR spectroscopy of the above complex showed three distinguishing stretching bands at 1538, 851 and 713 cm^{-1} assigned to ν(N-CSS), $\nu(CSS)_{as}$ and $\nu(CSS)_s$ modes respectively (Manohar, Ramalingam, Bocelli, & Righi, 2001; Ronconi et al., 2005). The sharp band at 1375 cm^{-1} is assigned to uncoordinated NO_3^- ion (Nakamoto, 1986). ^{1}H NMR (500 MHz, DMSO-d_6, ppm, sb = singlet broad): 2.11 (sb, 4H, H-b), 3.69 (sb,4H, H-a), 8.14 (sb, 2H, H-3,8), 8.35 (sb, 2H, H-5,6), 8.69 (sb, 2H, H-4,7), 9.06 (sb, 2H, H-2,9). Electronic spectra exhibit four bands. The bands at 223 (logε = 3.09), 249 (logε = 3.11), 283 (logε =3.43) and 345 nm (logε =2.56) may be assigned to intraligand $\pi\rightarrow\pi^*$ and n→π* transitions of phenanthroline ligand as well as CSS^- group (Renu Mital et al., 1989). Molar conductance measurement for the complex is 109 Ω^{-1} mol^{-1} cm^2 indicating 1:1 electrolytes (Peters, Hayes, & Hieftje, 1974) (see Fig. 1).

2.2.3. Synthesis of [Pd(phen)(pyr-dtc)]NO_3

The complex was prepared by a similar method to that of [Pt(phen)(pyr-dtc)]NO_3. Yield: 0.116 g (47%) and decomposed at 274-277 °C. Anal. Calcd. for $C_{17}H_{16}N_4O_3S_2Pd$ (494): C, 41.29; H, 3.24; N, 11.34%. Found: C, 41.30; H, 3.25; N, 11.35%. Solid state FT-IR spectroscopy of the above complex shows three distinctive stretching bands at 1532, 852 and 717 cm^{-1} assigned to ν(N-CSS), $\nu(CSS)_{as}$ and $\nu(CSS)_s$ modes respectively (Manohar et al., 2001; Ronconi et al., 2005). The sharp band at 1343 cm^{-1} is assigned to uncoordinated NO_3^- ion (Nakamoto, 1986). ^{1}H NMR (500 MHz, DMSO-d_6, ppm, sb = singlet broad): 2.06 (sb, 4H, H-b), 3.66 (sb, 4H, H-a), 8.04 (sb, 2H, H-3,8), 8.27 (sb, 2H, H-5,6), 8.59 (sb, 2H, H-4,7), 8.97 (sb, 2H, H-2,9). Electronic spectra exhibit four bands. The bands at 220 (logε = 3.29), 245 (logε = 3.39), 272 (logε = 3.34) and 338 nm (logε = 2.33) may be assigned to intraligand $\pi\rightarrow\pi^*$ and n→π* transitions of phenanthroline ligand as well as CSS^- group (Renu Mital et al., 1989). Molar conductance measurement for the complex is 121 Ω^{-1} mol^{-1} cm^2 indicating 1:1 electrolytes (Peters et al., 1974) (see Fig. 1).

2.3. Cytotoxic Studies

The procedure for cytotoxic studies of the [Pt(phen)(pyr-dtc)]NO_3 and [Pd(phen)(pyr-dtc)]NO_3 was similar to the one reported earlier

(Divsalar, Saboury, Yousefi, Moosavi-Movahedi, & Mansoori-Torshizi, 2007). Here, also 1×10^4 cells per ml of chronic myelogenous leukemia K562 cell line were used in Tris-HCl buffer solution of PH = 7.0.

2.4. DNA-denaturation

Absorption spectroscopy is one of the most useful techniques to study the binding of any drug to CT-DNA. The application of UV absorption method to the study of denaturation of CT-DNA with [Pt(phen)(pyr-dtc)]NO_3 and [Pd(phen)(pyr-dtc)]NO_3 complexes was similar to previously reported studies (Islami-Moghaddam, Mansouri-Torshizi, Divsalar, & Saboury, 2009; Mansouri-Torshizi, Saeidifar, Divsalar, Saboury, & Shahraki, 2010). In these experiments, the concentration of each metal complex at midpoint of transition, $[L]_{1/2}$, was determined. Besides, thermodynamic parameters such as: $\Delta G^{\circ}_{H_2O}$, i.e. conformational stability of CT-DNA in the absence of metal complex; $\Delta H^{\circ}_{H_2O}$, i.e. the heat needed for CT-DNA denaturation in the absence of metal complex; $\Delta S^{\circ}_{H_2O}$ that is the entropy of CT-DNA denaturation by metal complex as well as m, measure of the metal complex ability to denature CT-DNA were found using Pace method (Greene & Pace, 1974; Mansouri-Torshizi). All measurements were performed in 20 mM Tris-HCl buffer (pH = 7.0) at 300 and 310 K.

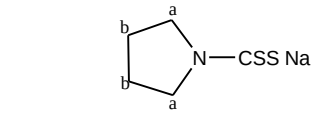
2.5. Electronic Absorption Titration

Electronic absorption spectroscopy is employed to determine the binding parameters (n, K, g) of metal complexes with DNA as reported earlier (Mansuri-Torshizi, Srivastava, Parekh, & Chitnis, 1992; A. Saboury, 2006). Where n is Hill coefficient, g is the number of binding sites per 1000 nucleotides of DNA and K is apparent binding constant. Also, the other thermodynamic binding parameters: molar Gibbs free energy of binding (ΔG°_b), molar enthalpy of binding (ΔH°_b) and molar entropy of binding (ΔS°_b) were determined according to reported method (Mansouri-Torshizi).

2.6. Fluorescence measurements

The fluorescence of ethidium bromide (EBr) is greatly enhanced in its intercalation between the base pairs of DNA (Krishna, Kumar, Khan, Rawal, & Ganesh, 1998). Ethidium bromide displacement assay was performed as reported in the literature (Pérez-Flores, Ruiz-Chica, Delcros, Sánchez-Jiménez, & Ramírez, 2008). At first, DNA (60 µM) was added to 2 µM aqueous ethidium bromide solution in 2 mL Tris-HCl buffer of pH = 7.0 and maximum quantum yield for ethidium bromide was achieved at 471 nm, so this wavelength as was selected as excitation radiation for all samples at different temperatures (300 and 310 K) in the range of 540–700 nm. Different concentrations of the Pd(II) or Pt(II) complex were added to this solution (containing ethidium bromide and CT-DNA). Measurements were done by applying a 1-cm path length fluorescence cuvette.

The fluorescence intensities of the Pd(II) and Pt(II) complexes have been checked at the highest denaturant concentration at 471 nm excitation wavelength, and the emission intensities of these compounds were found to be very small and negligible.



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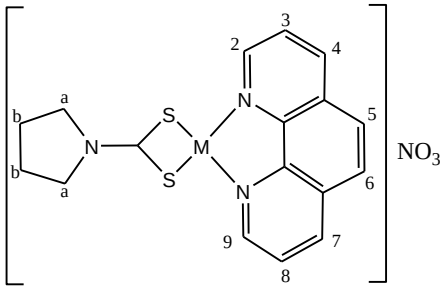

II

M = Pt(II) or Pd(II)

Fig. 1. Proposed structures and nmr numbering scheme of (I) pyr-dtcNa and (II) [M(phen)(pyr-dtc)]NO_3

3. Results and Discussion

3.1. Cytotoxic Measurement of the Metal Complexes

[Pt/Pd(phen)(pyr-dtc)]NO_3 complexes were screened for their antitumor activities against K562 chronic myelogenous leukemia cells (Divsalar et al., 2007). These cells were maintained in RPMI 1640 medium supplemented with 10% FCS in a humid incubator (310 K and 5% CO_2). The cells were then grown in RPMI medium supplemented with L-glutamine (2 mM), streptomycin and penicillin (5 g/mL), and 10% heat inactivated fetal calf serum, at 310 K under a 5% CO_2/95% air atmosphere. In this study, the harvested cells were seeded into 96-well plate (1×10^4 cell/mL) with various concentrations of metal complexes ranging from 0 to 0.25 mM and incubated for 24 h

(Freimoser, Jakob, Aebi, & Tuor, 1999). The 50% cytotoxic concentrations (Cc_{50}) of the Pt(II) and Pd(II) complexes were determined to be 67 and 46 µM, respectively (Fig. 2 for Pt(II) complex and the inset for Pd(II) complex).

As shown in Fig. 2, cell growth after 24 h was significantly reduced in the presence of various concentrations of the metal complexes. Furthermore, Cc_{50} value of cisplatin was determined under the same experimental conditions. This value (0.154 mM) is much higher than the Cc_{50} values of two complexes discussed above. However, the Cc_{50} values of these complexes are comparable with those of our analogous Pt(II) and Pd(II) dithiocarbamate complexes reported earlier (Islami-Moghaddam et al., 2009; Mansouri-Torshizi et al., 2008; Hassan Mansouri-Torshizi et al., 2010). The procedure for growth inhibition studies of the metal complexes established that the DNA cell is the most probable target biomolecule for these complexes (Islami-Moghaddam et al., 2009).

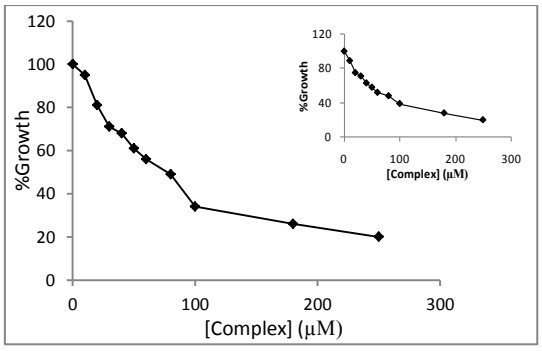

Fig. 2. The growth suppression activity of [Pt(phen)(pyr-dtc)]NO_3 on K562 cell line (Insert for [Pd(phen)(pyr-dtc)]NO_3). The tumor cells were incubated with varying concentrations of the complexes for 24 h

3.2. Denaturation of CT-DNA and evaluation of thermodynamic parameters

Denaturation of CT-DNA in the presence of increasing concentration of the above mentioned Pt(II) and Pd(II) complexes were conducted using our previous method (Mansouri-Torshizi et al., 2008). These experiments were carried out separately at two temperatures of 300 and 310 K in Tris–HCl buffer medium. The absorbance was monitored at 260 nm for either CT-DNA or mixtures of DNA with complexes at 300 and 310 K. Also, the absorbance of DNA and mixture of DNA-complexes were measured at 640 nm to eliminate the interference of turbidity. The profiles of denaturation of CT-DNA by [Pt(phen)(pyr-dtc)]NO_3 and [Pd(phen)(pyr-dtc)]NO_3 are shown in Fig. 3.

As Fig. 3 shows, the concentration of metal complexes in the midpoint of transition, $[L]_{1/2}$, for Pt(II) complex at 300 K is 0.146 and at 310 K is 0.143 mmol/L and for Pd(II) complex at 300 K is 0.106 and at 310 K is 0.097 mmol/L. One of the most important observations made in this research is the low values of $[L]_{1/2}$ for [Pd(phen)(pyr-dtc)]NO_3 complex. This means that the complex can denature CT-DNA at low concentration and if it is used as anticancer agent, very low doses will be needed, which may have fewer side effects.

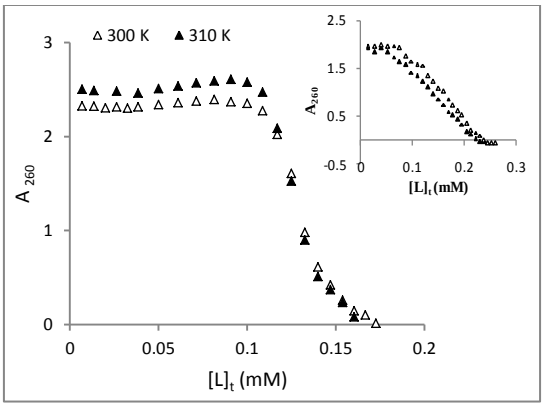

Fig. 3. The changes of absorbance of CT-DNA (λ_{max} = 260 nm due to increasing the total concentration of [Pt(phen)(pyr-dtc)]NO_3 and insert, [Pd(phen)(pyr-dtc)]NO_3, $[L]_t$, at constant temperature of 300 K and 310 K)

Using the DNA denaturation plots (Fig. 3) and Pace method (Greene & Pace, 1974), the value of K, i.e. unfolding equilibrium constant, and $\Delta G^{\circ}_{(H_2O)}$, i.e. unfolding free energy change of DNA at two temperatures of 300 and at 310 K in the presence of Pt(II) and Pd(II) complexes have been calculated. A straight line is observed when the values of $\Delta G^{\circ}_{(H_2O)}$ are plotted against the concentration of each metal complex in the transition region at 300 and 310 K. These plots are shown in Fig. 4. The values of m which is the slope of these plots (a measure of the metal complex ability to denature DNA) and those of the intercept on ordinate $\Delta G^{\circ}_{(H_2O)}$ (conformational stability of DNA in the absence of metal complex) are summarized in Table 1. The values of m for Pd(II) complex are higher than those of Pt(II) complex which indicate the higher ability of Pd(II) to denature DNA. These m values are similar to those of Pd(II) complex as well as surfactant, reported earlier (Saboury, Bordbar, & Moosavi-Movahedi, 1996). As we know, the higher the values of $\Delta G^{\circ}_{(H_2O)}$, the larger the conformational stability of DNA. However, the values of $\Delta G^{\circ}_{(H_2O)}$ (Table 1) decreases as the temperature for both complexes rise. This is as expected because in general, the decrease in $\Delta G^{\circ}_{(H_2O)}$ value is the main reason for the reduction of DNA stability. Another

important thermodynamic parameter is molar enthalpy of DNA denaturation in the absence of Pt(II) and Pd(II) complexes, $\Delta H^{\circ}_{(H_2O)}$. To find this, we calculated the molar enthalpy of DNA denaturation in the presence of each metal complex, $\Delta H^{\circ}_{conformation}$ or $\Delta H^{\circ}_{denaturation}$, in the range of two temperatures using Gibbs–Helmholtz equation, $\Delta H^{\circ} = [(\Delta G^{\circ}_{(T1)}/T_1) - (\Delta G^{\circ}_{(T2)}/T_2)]/(1/T_1 - 1/T_2)$, (Atkins, 1998). When plotting the values of these enthalpies versus the concentration of each metal complex, straight lines will be obtained which are shown in Fig. 5 for [Pt(phen)(pyr-dtc)]NO_3 and the inset for [Pd(phen)(pyr-dtc)]NO_3. Intrapolation of these lines (intercepting on ordinate, i.e. absence of metal complex) gives the values of $\Delta H^{\circ}_{(H_2O)}$ (Table 1). These plots show that in the range of 300-310 K the change in the enthalpies in the presence of Pt(II) and Pd(II) complexes are ascending. These observations indicate that when the concentration of both complexes is increaswd, the stability of DNA increases.

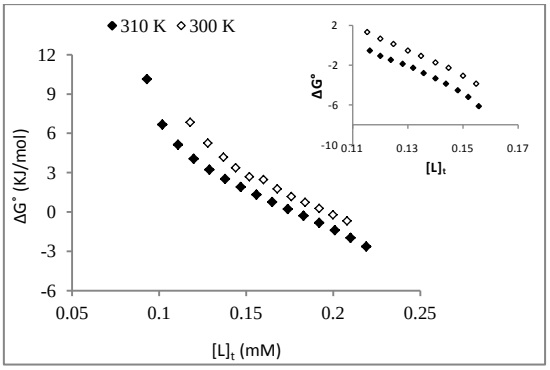

Fig. 4. The molar Gibbs free energies plots of unfolding of CT-DNA in the presence of [Pt(phen)(pyr-dtc)]NO_3. (Insert: in the presence of [Pd(phen)(pyr-dtc)]NO_3.)

Moreover, the entropy of DNA unfolded by Pt(II) and Pd(II) complexes, $\Delta S^{\circ}_{(H_2O)}$ have been calculated by means of equation $\Delta G = \Delta H - T\Delta S$ and the data are given in Table 1. These data show that increasing temperature does not change the values of entropies. This might be due to proximity of the temperature range. Also, the metal-DNA complex is more disordered than that of native DNA, because the entropy changes are positive and the extent disorder in Pd(II)-DNA complex is more than Pt(II)-DNA complex (see Table 1). These thermodynamic parameters are compared favorably well with those of Platinum(II) and palladium (II) complexes of 1,10-phenanthroline and dithiocarbamates as reported earlier (H Mansouri-Torshizi et al., 2010; Hassan Mansouri-Torshizi et al., 2010).

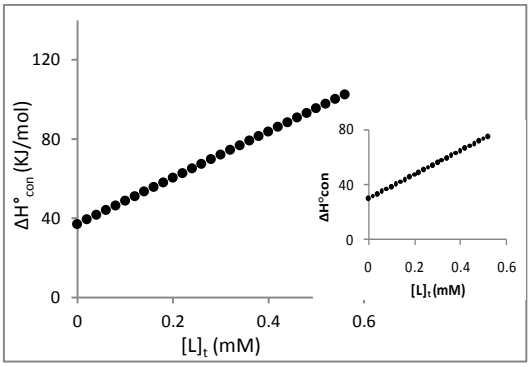

Fig. 5. Plots of the molar enthalpies of CT-DNA denaturation in the interaction with [Pt(phen)(pyr-dtc)]NO_3. (Insert for [Pd(phen)(pyr-dtc)]NO_3)

3.3. UV-vis spectral studies and determination of binding parameters

Various concentrations of CT-DNA were added to the fixed amount of each metal complex in Tris-HCl buffer of pH 7.0. Change in the absorbance of Pt(II) and Pd(II) complexes, ΔA, was calculated by subtracting the absorbance reading of each DNA-metal complex solution from absorbance reading of free metal complex solution at 309 nm and 338 nm. The maximum ΔA (ΔA_{max}), i.e. changes in the absorbance when all binding sites on DNA were occupied by each metal complex (Fig. 6) is 0.090 and 0.085 for [Pt(phen)(pyr-dtc)]NO_3 and 0.246 and 0.230 for [Pd(phen)(pyr-dtc)]NO_3 at 300 and 310 K, respectively. These values of ΔA_{max} were used to calculate the concentration of metal complexes bound to DNA, $[L]_b$, and the concentration of free metal complex, $[L]_f$, and ν, the ratio of the concentration of bound metal complex to total concentration of DNA in the next experiment (Mansouri-Torshizi et al., 2008) i.e., titration of fixed amount of DNA with varying concentration of Pt(II) or Pd(II) complexes. Using these data (ν and $[L]_f$), the Scatchard plots (Barrow, 1988) were constructed for the interaction of each metal complex at two temperatures of 300 and 310 K (Fig. 7). Figure 7 shows Scatchard plots, concerned with curvilinear concave downwards, suggesting cooperative binding for both complexes (A. Saboury, 2006). Similar cooperation in binding of analogous complexes with DNA has been observed as well (Hassan Mansouri-Torshizi et al., 2010).

Table 1. Thermodynamic parameters and values of $L_{1/2}$ of CT-DNA denaturation by palladium (II) and platinum(II) complexes

Compound	T (K)	$L_{1/2}$	[a]m (kJ/mol)(mmol/L)$^{-1}$	[b]$\Delta G^{\circ}_{(H_2O)}$ (kJ/molK)	[c]$\Delta H^{\circ}_{(H_2O)}$ (kJ/mol)	[d]$\Delta S^{\circ}_{(H_2O)}$ (kJ/mol)
[Pt(phen)(py-dtc)]NO_3	300	0.146	77.38	14.99	36.13	0.068
	310	0.143	83.28	14.90		0.068
[Pd(phen)(py-dtc)]NO_3	300	0.106	125.7	15.85	36.98	0.070
	310	0.097	133.8	15.14		0.070

[a]Measure of the metal complex ability to denature 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

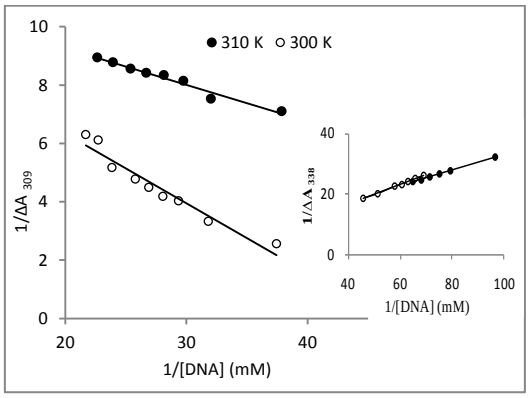

Fig. 6. The changes in the absorbance, ΔA, of fixed amount of metal complexes in the interaction with varying amount of CT-DNA. The linear plot of the reciprocal of ΔA vs the reciprocal of [DNA] for [Pt(phen)(pyr-dtc)]NO_3. Insert for [Pd(phen)(pyr-dtc)]NO_3

When substituting these data (ν and $[L]_f$) in Hill equation, $\nu = g(K[L]_f)^n/(1+(K[L]_f)^n$, we get a series of equations with unknown binding parameters n, K and g. Using Eureka software (James, Smith, & Wolford, 1985), the theoretical values of these parameters have been deduced (see Table 2). The maximum errors between experimental and theoretical values of ν are also shown in Table 2, which are quite low. The K_{app}, apparent binding constant in the interaction of [Pd(phen)(pyr-dtc)]NO_3 with DNA is higher than that of [Pt(phen)(pyr-dtc)]NO_3 with DNA (see Table 2). This indicates that the interaction tendency of Pd(II) complex to DNA is more than that of Pt(II) complex. This is due to the fact that palladium complexes are about 10^5 times more labile than their platinum analogs (Sadler et al., 1984). In addition, the data in Table 2 show that the values of n, the Hill coefficient (as a criterion of cooperation), for palladium complex are higher than those of platinum analog. Similar trends are observed in the results of cytotoxic studies of these two compounds. Figure 7 also illustrates the experimental values of ν, obtained from Scatchard (lines), and the theoretical values of ν from Hill equation (dots) and their superimposability on each other.

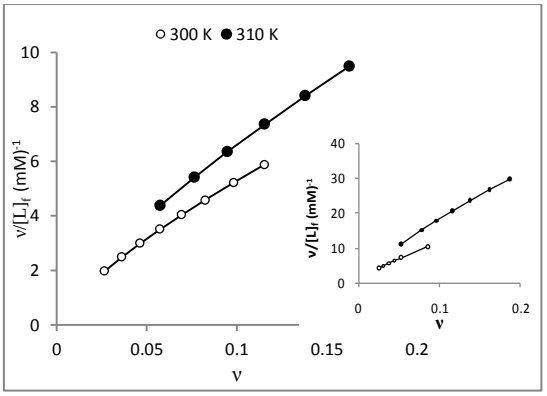

Fig. 7. Scatchard plots for binding of [Pt(phen)(pyr-dtc)]NO_3 with CT-DNA. The insert is Scatchard plots for binding of [Pd(phen)(pyr-dtc)]NO_3 with DNA

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

Compound	T (K)	[a]ΔA_{max}	[b]g	[c]K×10^3 (mol/L)$^{-1}$	[d]n
[Pt(phen)(py-dtc)]NO_3	300	0.090	5	17.19	3.51
	310	0.085	5	24.94	3.98
[Pd(phen)(py-dtc)]NO_3	300	0.246	5	39.85	3.77
	310	0.230	5	73.23	4.42

[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 cooperation)

Moreover, these values of ν were plotted versus the values of Ln $[L]_f$. The results are sigmoid curves at 300 and 310 K and are shown in Fig. 8. These plots indicate positive cooperative binding at both temperatures for both complexes. The area was found

under these plots of binding isotherms and by employing Wyman-Jones equation (Saboury, Shamsaei, Moosavi-Movahedi, & Mansuri-Torshizi, 1999), $\int \nu d\mathrm{Ln}[L]_f = \mathrm{Ln}[1 + K_{app}[L]_f^{\nu}$, ($[L]_f$ and K_{app} are concentration of free metal complex and apparent binding constant for each particular ν, respectively), the values of K_{app} can be calculated at the two temperatures 300 and 310 K for each particutical ν. Using the values of K_{app}, we can determine the corresponding values of molar Gibbs free energy of binding (ΔG°_b) from Eq. $\Delta G^{\circ}_b = -RT\ \mathrm{Ln}K_{app}$, molar enthalpy of binding (ΔH°_b) from Eq. $\mathrm{Ln}(K_{app}T_1/K_{app}T_2) = -\Delta H^{\circ}_b/R(1/T_2 - 1/T_1)$ and molar entropy of binding (ΔS°_b) from Eq. $\Delta G^{\circ}_b = \Delta H^{\circ}_b - T\Delta S^{\circ}_b$ (Islami-Moghaddam et al., 2009).

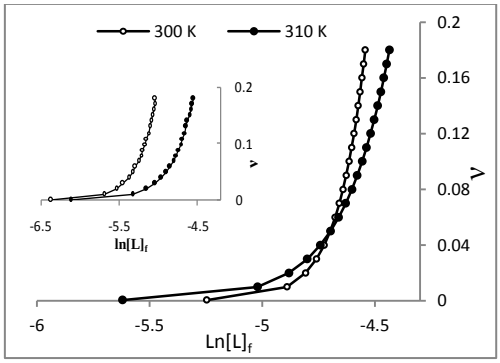

Fig. 8. Binding isotherm plots for $[Pt(phen)(pyr\text{-}dtc)]NO_3$ in the interaction with CT-DNA. Insert for $[Pd(phen)(pyr\text{-}dtc)]NO_3$

Plots of the values of ΔH°_b versus the values of $[L]_f$ are shown in Fig. 9 for $[Pt(phen)(pyr\text{-}dtc)]NO_3$ and the insert for $[Pd(phen)(pyr\text{-}dtc)]NO_3$ at 300 K. Deflections are observed in both plots indicating that at particular $[L]_f$ there is a sudden change in enthalpy of binding which may be due to binding of metal complex to macromolecule or macromolecule denaturation.

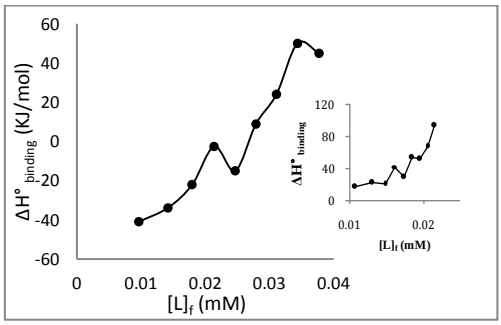

Fig. 9. Molar enthalpies of binding in the interaction between CT-DNA and $[Pt(phen)(pyr\text{-}dtc)]NO_3$ (Insert for $[Pd(phen)(pyr\text{-}dtc)]NO_3$) versus free concentrations of complexes at pH 7.0 and 300 K

3.4. Emission spectral studies

The evidence for Pt(II) and Pd(II) complexes binding to DNA via intercalation is witnessed through the emission quenching experiment. The experiments of DNA competitive binding with ethidiumbromide were carried out in the buffer by keeping [DNA]/[EBr] = 30 ([DNA] = 60 μM, [EtBr] = 2 μM) and varying the concentrations of the metal complexes (0-90 μM). The fluorescence emission spectra of EBr binding to CT-DNA in the absence and presence of the above complexes are shown in Fig. 10. From Fig. 10, it can be clearly understood that an appreciable reduction in the fluorescence intensity is observed on adding of Pd(II) or Pt(II) complex to CT-DNA pretreated with EBr, indicating the replacement of the EBr molecules accompanied by intercalation of the complexes (Gao, Liu, & Duan, 2007; Jain, Mital, Ray, Srivastava, & Bhattacharya, 1987). The extent of quenching fluorescence of EBr-CT-DNA can be used to determine the extent of binding between the complexes and CT-DNA, namely, the binding affinity of the complexes to CT-DNA.

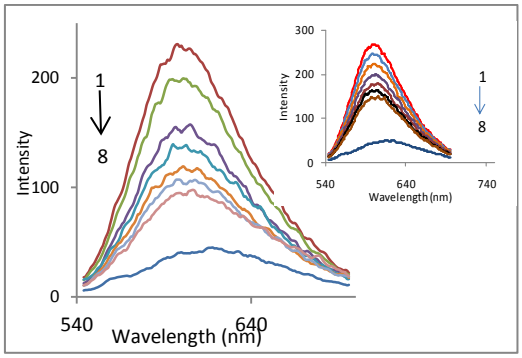

Fig. 10. Fluorescence emission spectra of interacted EBr-CT-DNA in the absence (1) and presence of different concentrations of $[Pt(phen)(pyr\text{-}dtc)]NO_3$: 15 μM (2), 30 μM (3), 45 μM (4), 60 μM (5), 75 μM (6), 90 μM (7), EBr alone (8) (Inset for $[Pd(phen)(pyr\text{-}dtc)]NO_3$)

Further studies to characterize the mode of binding of $[Pt(phen)(pyr\text{-}dtc)]NO_3$ and $[Pd(phen)(pyr\text{-}dtc)]NO_3$ complexes to CT-DNA have been carried out (Hassan Mansouri-Torshizi et al., 2010). The number of EBr molecules intercalated to CT-DNA in the presence of different concentrations of the Pt(II)/Pd(II) complexes was calculated using Scatchard analysis (Scatchard, 1949). In this experiment solutions of CT-DNA, EBr and metal complexes were prepared in Tris–HCl buffer of pH 7.0. In this medium, solutions of Pt(II)/Pd(II) complexes were interacted with CT-DNA by incubating them at 300 K for 3 h, appropriate amount of EBr was added and they were further incubated at room temperature for 2 h and finally processed for fluorescence spectral measurement. The fluorescence Scatchard plots obtained for binding of EBr to CT-DNA in the absence (○) and presence (▲, □ and ♦) of various concentrations of $[Pt(phen)(pyr\text{-}dtc)]NO_3$ (inset for $[Pd(phen)(pyr\text{-}dtc)]NO_3$) complex were shown in Fig. 11. This Fig. shows that Pt(II)/Pd(II) complexes inhibit competitively the EBr binding to CT-DNA (type-A behavior), where number of binding

sites g (intercept on the abscissa) remains constant and the slope of the graphs, which is K_{app} (apparent association constant) decreases with increasing the concentration of Pt(II)/Pd(II) complexes (Table 3). This implies that both complexes are intercalating in CT-DNA and thereby competing for intercalation sites occupied by EBr. Similar modes of binding have been seen for other planar aromatic compounds (Mansouri-Torshizi et al., 2011; Mansouri-Torshizi et al., 2008).

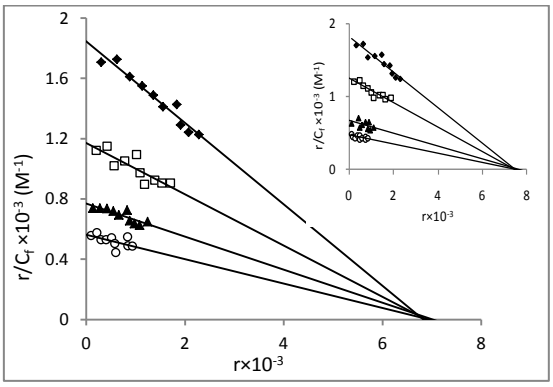

Fig. 11. Competition between [Pt(phen)(pyr-dtc)]NO_3 with ethidium bromide for the binding sites of DNA (The inset for [Pd(phen)(pyr-dtc)]NO_3). In curve no. 1(♦), Scatchard plot was obtained with calf thymus DNA alone. Its concentration was 60 µM. In curves no. 2(□), 3(▲), and 4(○), respectively, 15, 30 and 45 µM for Pt(II) complex and10, 20 and 30 µM, for Pd(II) complex were added, corresponding to molar ratio [complex]/[DNA] of 0.25, 0.5, and 0.75 for Pt(II) complex and 0.16, 0.33 and 0.5 for Pd(II) complex. Solutions were in 20 mM NaCl and 20 mM Tris-HCl (pH 7.0). Experiments were done at room temperature

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

Compound	[a]r_f	[b]$K\times10^5$ $(M)^{-1}$	[c]n
[Pt(phen)(py-dtc)]NO_3	0.00	0.242	0.0075
	0.25	0.167	
	0.5	0.088	
	0.75	0.62	
[Pd(phen)(py-dtc)]NO_3	0.00	0.270	0.0068
	0.16	0.170	
	0.33	0.109	
	0.5	0.08	

[a]Formal ratio of metal complex to nucleotide concentration
[b]Association constant
[c] Number of binding sites (n) per nucleotide

3.5. Binding of modes

The mode of binding between CT-DNA and the above Pd(II)/Pt(II) complexes were further investigated by gel filtration experiment. In this experiment, each metal complex was interacted with CT-DNA for 2h at 300 K in Tris-HCl buffer and then passed through a Sephadex G-25 column equilibrated with the same buffer. Elution was done with buffer and each fraction of the column was monitored spectrophotometrically at 309 nm and 260 nm for Pt(II)-DNA system and at 338 nm and 260 nm for Pd(II)-DNA system. The gel chromatograms obtained from these experiments are indicated in Fig. 12 for [Pt(phen)(pyr-dtc)]NO_3 and the inset for [Pd(phen)(pyr-dtc)]NO_3. These results show that the two peaks obtained at two wavelengths were not clearly resolved which indicate that metal complexes have not separated from DNA and their binding with DNA is strong enough that does not readily break (Hassan Mansouri-Torshizi et al., 2010).Because if the interaction was weak, the CT-DNA should have come out of the column separately and the peaks should have appeared in different places of the plots due to DNA and each of the metal complexes.

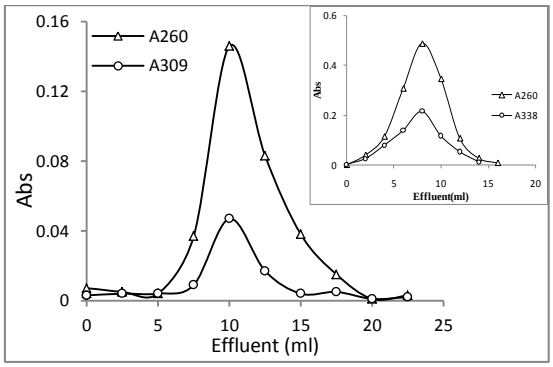

Fig. 12. Gel chromatograms of intercalated CT-DNA with [Pt(phen)(pyr-dtc)]NO_3 (Inset for [Pd(phen)(pyr-dtc)]NO_3). Obtained on Sephadex G-25 column, equilibrated with 20 mmol/L Tris-HCl buffer of pH 7.0 in the presence of 20 mmol/L sodium chloride

4. Conclusions

In the present study, two newly-created and water soluble compounds, [Pd/Pt(phen)(pyr-dtc)]NO_3, were synthesized and characterized by spectroscopic techniques, IR, ^{1}H NMR, UV-vis and elemental analysis. Different instrumental methods were used to investigate the interaction modes. The results support the notion that the complexes can bind to CT-DNA by intercalation. They have been found to be better cytotoxic agents than cisplatin against chronic myelocytic leukemia K562 cell lines. Also, they can denature CT-DNA at low concentration. Determinations of several bindings and thermodynamic parameters have also been attempted. Overall, experimental results indicate that interaction affinity of Pd(II) complex is more than that of its Pt(II) analogue.

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