Bioorganic & Medicinal Chemistry 16 (2008) 9616–9625

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

2,2′-Bipyridinebutyldithiocarbamatoplatinum(II) and palladium(II) complexes: Synthesis, characterization, cytotoxicity, and rich DNA-binding studies

Hassan Mansouri-Torshizi [a,*], Mahboube I-Moghaddam [a], Adeleh Divsalar [b], Ali-Akbar Saboury [b]

[a] Department of Chemistry, University of Sistan & Baluchestan, Daneshgah Street, Zahedan, Iran
[b] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

ARTICLE INFO

Article history:
Received 17 June 2008
Revised 2 August 2008
Accepted 5 August 2008
Available online 9 August 2008

Keywords:
DNA binding
Cytotoxicity
Platinum complex
Palladium complex

ABSTRACT

Butyldithiocarbamate sodium salt (Bu-dtcNa) and its two complexes, [M(bpy)(Bu-dtc)]NO_3 (M = Pt(II) or Pd(II) and bpy = 2,2′-bipyridine), have been synthesized and characterized on the basis of elemental analysis, molar conductivities, IR, ^{1}H NMR, and UV–vis spectra. In these complexes, the dithiocarbamato ligand coordinates to Pt(II) or Pd(II) center as bidentate with two sulfur atoms. These complexes show 50% cytotoxic concentration (Cc_{50}) values against chronic myelogenous leukemia cell line, K562, much lower than that of cisplatin. The interaction of these complexes with calf thymus DNA was extensively investigated by a variety of spectroscopic techniques. These studies showed that both complexes presumably intercalate in DNA. UV–vis studies imply that they cooperatively bind with DNA and unexpectedly denature the DNA at very low concentrations (~100 μL). Palladium complex breaks the DNA into two unequal fragments and binds stronger to the lighter fragment than to the heavier one. In the interaction studies between the Pt(II) and Pd(II) complexes with 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.

1. Introduction

Despite the clinical anti-cancer utility of cisplatin, carboplatin, oxaliplatin, nedaplatin, iproplatin, and several other complexes in clinical trials, there is a continued interest in the design of new platinum complexes that shows anti-tumor activities equivalent or better than these agents.[1–4] Cisplatin is an important cytotoxic drug used in the treatment of a variety of human cancers.[5] It has some dose limiting toxic side effects such as neurotoxicity, ototoxicity, and tissue toxicity.[6] Also, there is less nausea, nephrotoxicity, gastrointestinal, and bone marrow toxicity.[7,8] Moreover, resistance after continued treatment by several mechanisms is witnessed.[9] Many efforts have been made to reduce the toxicity of platinum anti-cancer complexes. One of them is using a variety of sulfur-containing ligands as detoxicant agents against metal-containing drugs.[10] It has been observed that the use of diethyldithiocarbamate in combination with cisplatin has protected a variety of animal species from renal, gastrointestinal and bone marrow toxicity, induced by cisplatin.[11] This may be due to strong binding of platinum with dithiocarbamate, which prevents or at least limits the reaction with other sulfur-containing renal proteins.[10] That is why the chelating sulfur atoms of dithiocarbamates to the platinum or palladium moiety selectively inhibits the nucleophilic attack of enzyme-thiol to the metal center and protects normal tissue without inhibiting the anti-cancer activities of metal dithiocarbamate complexes.[12]

Recently, palladium(II) complexes have attracted significant attention due to their biological activities.[2] Moreover, palladium complexes having chelating ligands such as N–N-diamines,[13] O–S-donor,[14] N–S-amino-thioether,[15] diaminoacids,[16] dicarboxylic acids,[17] and the most important dithiocarbamates[12,18] are the recent advances of palladium(II) complexes. It has been assumed that chelating ligands not only impose the cis coordination of the palladium(II) complexes but also prevent the formation variety of aquo, hydroxo, or even polynuclear species which may be formed from dichloro complexes at physiological pH and ionic strength. Moreover, such palladium(II) complexes can serve as good models for the understanding of more inert platinum(II) anti-cancer drugs.[19] Also, it has been suggested by Saddler et al.[20] that the palladium complexes may be useful for the treatment of tumors of the gastrointestinal region where cisplatin fails.

It has been generally accepted that the ultimate target for platinum and palladium anti-cancer agents is likely to be DNA. But the reaction mechanism of cisplatin by which it induces cell death is still to be verified. However, cisplatin inhibits the biosynthesis of DNA and evidences of its in vivo activity at the transcription level had been reported.[21] Thus, the cytotoxicity of platinum-based drugs is perhaps due to the combined effects of the various lesions caused by these agents.[22,23]

* Corresponding author. Tel.: +98 541 2449807; fax: +98 541 2446565.
E-mail address: hmtorshizi@hamoon.usb.ac.ir (H. Mansouri-Torshizi).

0968-0896/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2008.08.021

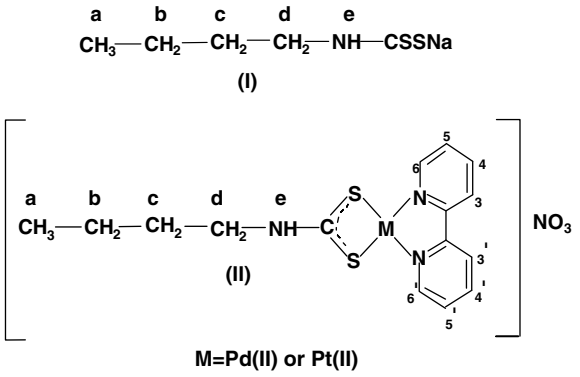
Keeping the above criteria in mind and considering the point that the lack of water solubility of some of platinum or palladium anti-tumor species explains their limited bioavailability and low in vivo activity,[24] we synthesized and characterized the two water soluble and structurally related platinum(II) and palladium(II) dithiocarbamate complexes. Presence of 2,2′-bipyridine as a carrier ligand in the structure of these compounds which has primary effect on their anti-tumor properties[25] make them to have (i) a UV–vis absorption band in a region where DNA does not show absorption and can be monitored in the processes of interaction studies with DNA and (ii) provide a planar moiety to the complexes which can intercalate in DNA. Fluorescence titration, electronic absorption titration, and CD spectroscopy were all employed to study the interaction of prepared compounds with calf thymus DNA. In these interaction studies, binding parameters, thermodynamic parameters, and the types of bindings between these agents and DNA are also described. Information obtained from these studies will be helpful to understand the mechanisms of the interaction between the dithiocarbamate complexes and nucleic acids. These interaction mechanisms must be quite different from that of cisplatin.

2. Results and discussion

2.1. Characterization of compounds

2.1.1. Infrared spectra

The IR spectra of the above-mentioned ligand and complexes have been recorded in the range 4000 to 400 cm^{-1} as KBr pellets. Two important bands are of interest: The Bu-dtcNa ligand showed a strong absorption at 1480 cm^{-1}. This band, which is assigned to the N–CSS stretching mode,[26–28] appears at 1525 and 1535 cm^{-1} in the IR spectra of $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ and $[Pd(bpy)(Bu\text{-}dtc)]NO_3$, respectively. These data suggested that the N–CSS bond order is between a single bond (ν = 1250–1350 cm^{-1}) and a double bond (ν = 1640–1690 cm^{-1}).[29,30] As evident from IR spectral data of the free dithiocarbamate ligand and the corresponding complexes, the ν(N–CSS) mode has shifted to higher frequencies upon coordination. This indicates that the nitrogen–carbon double bond character has increased due to the electron delocalization toward the platinum or palladium centers. Thus, the above butyldithiocarbamate ligand coordinates to Pt(II) or Pd(II) through sulfur atoms. The second interesting feature in the IR spectra of the Bu-dtcNa ligand and its metal complexes is the presence of a single strong band at 923 cm^{-1} for Bu-dtcNa and at 1021 and 1026 cm^{-1} for $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ and $[Pd(bpy)(Bu\text{-}dtc)]NO_3$, respectively. This band is attributed to ν(SCS) mode[31] which is indicative of symmetrical bonding of dithiocarbamate ligand, acting in a bidentate mode in our complexes (Scheme 1). Otherwise a doublet would be expected in the 1000 ± 70 cm^{-1} region, which indicates an asymmetrically bonded ligand or a monodentate bound ligand.[32]



Scheme 1. Proposed structures and nmr numbering schemes of (I) Bu-dtcNa, (II) $[M(bpy)(Bu\text{-}dtc)]NO_3$.

2.1.2. ^{1}H NMR spectra

The ^{1}H NMR spectral data of butyldithiocarbamate ligand and its corresponding 2,2′-bipyridine platinum(II) and palladium(II) complexes in DMSO-d_6 are summarized in Table 1. The proposed structure and NMR numbering scheme are given in Scheme 1. In the ^{1}H NMR spectrum of Bu-dtcNa, the triplet observed at δ 0.82, three multiplets at δ 1.21, δ *1.39,* and δ *3.31,* and a broad singlet at δ *8.09* are assigned to H-a, H-b, H-c, H-d, and H-e protons, respectively (see Scheme 1 and Table 1). The signal due to the H-e protons in the spectra of $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ and $[Pd(bpy)(Bu\text{-}dtc)]NO_3$ complexes appears at δ 11.50 and δ 11.51, respectively. The downfield shift of 3.41 ppm in $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ and 3.42 ppm in $[Pd(bpy)(Bu\text{-}dtc)]NO_3$ as compared to the free ligand indicates the bidentate coordination of Bu-dtcNa ligand to Pt(II) and Pd(II), and is consistent with the IR spectral data (vide supra).

The coordination of the dithiocarbamate ligand to platinum or palladium invariably produces a downfield shift in the position of its proton signals relative to those of the free dithiocarbamate ligand. The extent of this downfield shift decreases regularly with distance from the coordination site.[33] This is due to the electron donation by $-CSS^-$ group to palladium or platinum. However, the signals corresponding to the H-6,6′ (8.67 ppm) and H-5,5′ (7.72 ppm) protons of the 2,2′-bipyridine moiety in $[Pt(bpy)Cl_2]$ are invariably shifted upfield in the dithiocarbamate complexes (see Table 1).[12] This may be explained in terms of back donation of electron density from platinum or palladium to 2,2′-bipyridine ligand as well as stronger binding of dithiocarbamate to Pt(II) or Pd(II) relative to the chloride anion in $[Pt(bpy)Cl_2]$ or $[Pd(bpy)Cl_2]$. The protons of 2,2′-bipyridine in $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ appear as four multiplets centered at 8.67, 8.46, 8.42, and 7.72 ppm, which are assigned to H-6,6′, H-4,4′, H-3,3′, and H-5,5′ protons, respectively. Similar assignments have been done for protons of 2,2′-bipyridine in the analogous complex $[Pd(bpy)(Bu\text{-}dtc)]NO_3$ (see Scheme 1 and Table 1). Thus, based on the spectroscopic data, the structures, which are shown in Figure 1(II) have been assigned to these two complexes which are also in accord with observed molar conductance values of 101 and 96 $cm^2\,\Omega^{-1}\,cm^{-1}$ for $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ and $[Pd(bpy)(Bu\text{-}dtc)]NO_3$, respectively, as

Table 1
^{1}H NMR spectral data of Bu-NH-CSSNa ligand and $[M(bpy)(Bu\text{-}NH\text{-}CSS)]NO_3$ complexes in deuterated DMSO

Compound	Dithiocarbamate protons					2,2′-Bipyridine protons			
	δH-a	δH-b	δH-c	δH-d	δH-e	δH-6,6′	δH-4,4′	δH-3,3′	δH-5,5′
Bu-dtcNa	0.82[a](t)[b]	1.21(m)	1.39(m)	3.31(m)	8.09(sb)	–	–	–	–
$[Pt(bpy)(Bu\text{-}dtc)]NO_3$	0.91(t)	1.34(m)	1.61(m)	3.46(m)	11.50(sb)	8.67(m)	8.46(m)	8.42(m)	7.72(m)
$[Pd(bpy)(Bu\text{-}dtc)]NO_3$	0.86(m)	1.29(m)	1.55(m)	3.37(m)	11.51(sb)	8.50(m)	8.25(m)	8.06(m)	7.64(m)

[a] Chemical shifts in ppm.
[b] s, d, t, q, m, and sb are singlet, doublet, triplet, quartet, multiplet, and singlet broad, respectively.

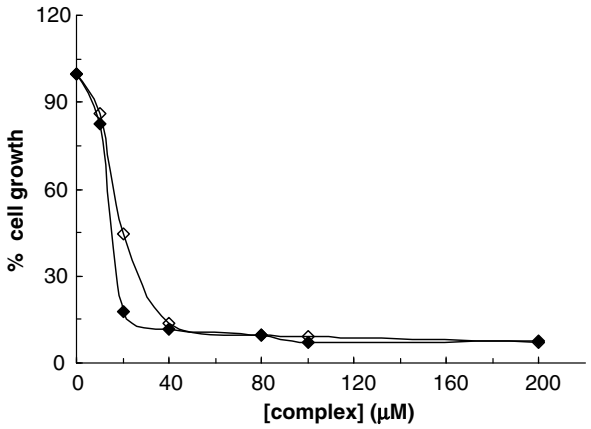


Figure 1. The growth suppression activity of the Pd(II) complex (◇) and Pt(II) complex (♦) on K562 cell line was assessed using MTT assay as described in material and methods. The tumor cells were incubated with varying concentrations of the complexes for 24 h.

1:1 electrolyte.[34] Further support for the proposed structures comes from the ratio of the integrated areas under the peaks of 2,2′-bipyridine and dithiocarbamate protons being 8:10 for $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ and $[Pd(bpy)(Bu\text{-}dtc)]NO_3$ complexes. Finally, no changes were observed in the ^1H NMR spectra of the above complexes dissolved in DMSO-d_6 and recorded after 24 h, suggesting no dissociation of dithiocarbamate anions.

2.1.3. Electronic spectra

The maxima absorption bands in electronic absorption spectra of above platinum(II) and palladium(II) complexes in distilled water with their extinction coefficients[18] are given in Table 2 and assigned as follows: band I in the platinum complex is assigned to MLCT because it is blue shifted by 10–15 nm on going from acetone to water.[35] In this complex, band I may be due to charge transfer from platinum to π^* of 2,2′-bipyridine ligand. However, band I may as well be due to the charge transfer from platinum to π^* of dithiocarbamate.[36] However, this type of charge transfer transition is not observed in 2,2′-bipyridine analogous complexes reported earlier,[12] because it may be hidden in much stronger charge transfer transition from platinum to π^* of 2,2′-bipyridine ligand. Bands II, III, IV, and V in platinum complex may be due to first, second, and higher internal $\pi \rightarrow \pi^*$ transitions of 2,2′-bipyridine ligand.[12,35] Bands III, IV, and V in the platinum complex have also overlapping components of $\pi \rightarrow \pi^*$ transitions of dithiocarbamate ligand. The above electronic absorption data suggest that these complexes have square planar configuration.[37] In palladium complex, bands II and III show blue shifts by 13–14 nm, respectively, on going from less polar chloroform to more polar dimethylsulfoxide. Therefore, these bands can tentatively be assigned to charge transfer from palladium to 2,2′-bipyridine ligand. Bands IV and V are assigned to first and higher internal $\pi \rightarrow \pi^*$ transition of 2,2′-bipyridine.[35] Also, bands III, IV, and V have overlapping components of $\pi \rightarrow \pi^*$ transitions of dithiocarbamate ligand.

2.2. Cytotoxicity measurements of the Pd(II) and Pt(II) complexes

The prepared platinum and palladium complexes were tested in vitro against human cancer cell line K562.[38] In this study, various concentrations of Pd(II) and Pt(II) complexes ranging from 0 to 250 μM of stock solutions were used to culture the tumor cell lines for 24 h (Fig. 1). The 50% cytotoxic concentration (Cc_{50}) of each compound was determined 14.3 and 18 μM for Pt(II) and Pd(II) complexes, respectively (see Fig. 1). As shown in Figure 1, cell growing after 24 h was significantly reduced in the presence of various concentrations of the two compounds. Based on this figure, it is also clear that the Pd(II) and Pt(II) complexes produced a dose–response suppression on growing of K562 leukemia cell lines. Furthermore, the Cc_{50} cytotoxicity values of the complexes were compared to that found for anti-cancer agents used nowadays, that is, cisplatin under the same experimental conditions. This value (154 μM) is much higher as compared to those achieved for the metal complexes reported in this article.

2.3. DNA-binding studies

2.3.1. Absorbance titration experiments

A fixed amount of each metal complex (0.05 mL of 0.1 mmol/L stock) was titrated with increasing concentration of DNA (0.2–0.6 mL for Pt(II) system and 0.02–0.2 mL for Pd(II) system of 0.1 mmol/L stocks) in total volume of 2 mL at 27 °C and 37 °C, separately. The values of ΔA_{max}, change in the absorbance when all

Table 2
Electronic absorption bands and molar conductance values of $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ and $[Pd(bpy)(Bu\text{-}dtc)]NO_3$ complexes in water

Compound	Band maxima in nm					Molar conductance of 1×10^{-4} solution ($cm^2\ \Omega^{-1}\ mol^{-1}$)
	Band I	Band II	Band III	Band IV	Band V	
$[Pt(bpy)(Bu\text{-}dtc)]NO_3$	364(3.15)	321(6.74)[a]	310(6.34)	283(1.22)	208(2.42)	101
$[Pd(bpy)(Bu\text{-}dtc)]NO_3$	—	317(2.19)	307(1.96)	248(4.71)	203(2.76)	96

[a] Extinction coefficients in $L\ mol^{-1}\ cm^{-1} \times 10^{-4}$ are given in parentheses.

Table 3
Values of ΔA_{max} and binding parameters in the Hill equation for interaction between Pt(II) and Pd(II) complexes and DNA in 10 mmol/L Tris–HCl buffer and pH 7.0

Compound	Temperature (°C)	ΔA_{max}[a]	g[b]	K[c] $(mol/L)^{-1}$	n[d]	Error[e]
$[Pt(bpy)(Bu\text{-}dtc)]NO_3$	27	0.102	8	0.057	3.14	0.012
	37	0.222	8	0.036	2.75	0.008
$[Pd(bpy)(Bu\text{-}dtc)]NO_3$	27	0.047	8	0.317	1.48	0.005
	37	0.033	8	0.401	1.79	0.008

[a] Change in the absorbance when all the binding sites on 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 $\bar{v}$.

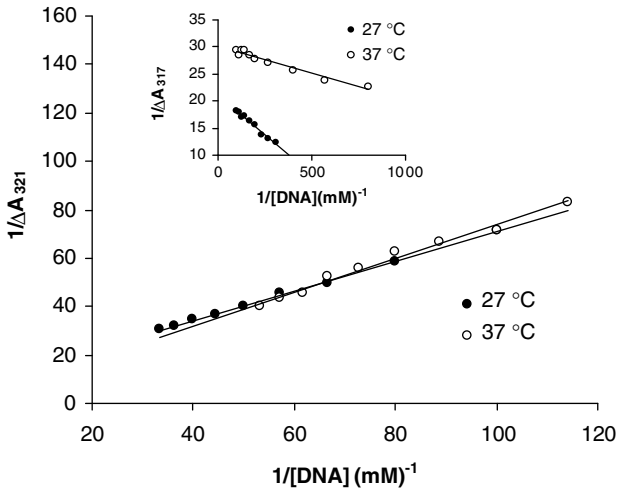


Figure 2. The changes in the absorbance of fixed amount of each metal complex in the interaction with varying amount of DNA at 27 and 37 °C. The linear plot of the reciprocal of ΔA versus the reciprocal of [DNA] for [Pt(bpy)(Bu-dtc)]NO_3. Inset, for [Pd(bpy)(Bu-dtc)]NO_3.

binding sites on DNA were occupied by metal complex, are given in Table 3 and Figure 2. These values were used to calculate the concentration of metal complex bound to DNA, $[L]_b$, and the concentration of free metal complex, $[L]_f$ and $\bar{\nu}$, the ratio of the concentration of bound metal complex to total [DNA] in the next experiment, that is, titration of fixed amount of DNA (0.05 mL of 0.1 mmol/L stock) with varying amounts of each metal complex (0.1–0.18 mL for Pt(II) system and 0.025–0.25 mL for Pd(II) system of 0.1 mmol/L stock) in total volume of 2 mL at 27 and 37 °C, separately. Using these data ($\bar{\nu}$ and $[L]_f$), the Scatchard plots were constructed for the interaction of each metal complex at two temperatures of 27 and 37 °C. The Scatchard plots are shown in Figure 3 for [Pt(bpy)(Bu-dtc)]NO_3 and the inset for [Pd(bpy)(Bu-dtc)]NO_3. These plots are curvilinear concave downwards, suggesting cooperative binding.[39] To obtain the binding parameters, the above experimental data ($[L]_f$, $\bar{\nu}$) were substituted in Hill equation $[\bar{\nu} = g(K[L]_f)^n/(1 + (K[L]_f)^n)]$ to get a series of equation with unknown parameters n, K, and g. Using Eureka software, the theoretical values of these parameters could be deduced. The results are tabulated in Table 3, which are comparable with those of 2,2′-bipyridine-platinum and -palladium complexes of dithiocarbamate as reported earlier.[12] The maximum errors between experimental and theoretical values of $\bar{\nu}$ are also shown in Table 3 which are quite low. The K, apparent binding constant in the interaction of [Pd(bpy)(Bu-dtc)]NO_3 with DNA is higher than that of [Pt(bpy)(Bu-dtc)]NO_3 with DNA (see Table 3). This indicates that the interaction affinity of Pd(II) complex to DNA is more than Pt(II) complex. Because palladium complexes are about 10^5 times more labile than their platinum analogs.[20] In addition, the data in Table 3 show that the values of n, the Hill coefficient (as a criterion of cooperativity), for palladium complex are higher than that of platinum analog. Similar trends are observed in the results of cytotoxic studies of these two compounds. Knowing the experimental (dots) and theoretical (lines) values of $\bar{\nu}$ in the Scatchard plots and superimposibility of them on each other, these values of $\bar{\nu}$ were plotted versus the values of Ln $[L]_f$. The results are sigmoidal curves and are shown in Figure 4 at 27 and 37 °C. These plots indicate positive cooperative binding at both temperatures for both complexes. Finding the area under the above plots of binding isotherms and using Wyman-Jons equation,[16] we can calculate the K_{app} and $\Delta G°_b$ at 27 and 37 °C for each particular ν and also $\Delta H°_b$. Plots of the values of $\Delta H°_b$ versus the values of $[L]_f$ are shown in Figure 5 for [Pt(bpy)(Bu-dtc)]NO_3 and the inset for [Pd(bpy)(Bu-dtc)]NO_3 at 27 °C. Deflections are observed in both plots. These deflections indicate 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.

2.3.2. Analysis of denaturation data and evaluation of thermodynamic parameters

The above platinum(II) and palladium(II) complexes can denature DNA. In this experiment, the sample cell was filled with 1.8 mL DNA (0.1 mmol/L of Pt system and 0.04 mmol/L of Pd system). In these concentrations, the absorption of DNA is around 0.8 and 0.4, respectively. However, reference cell is only filled with 1.8 mL Tris–HCl buffer. Both cells were set separately at constant temperature of 27 or 37 °C, and then 25 μL solution of metal complex (1.4 mmol/L) was added to each cell. After 3 min, the absorption was recorded at 258 nm for DNA and at 640 nm to eliminate the interference of turbidity. Addition of metal complex to both

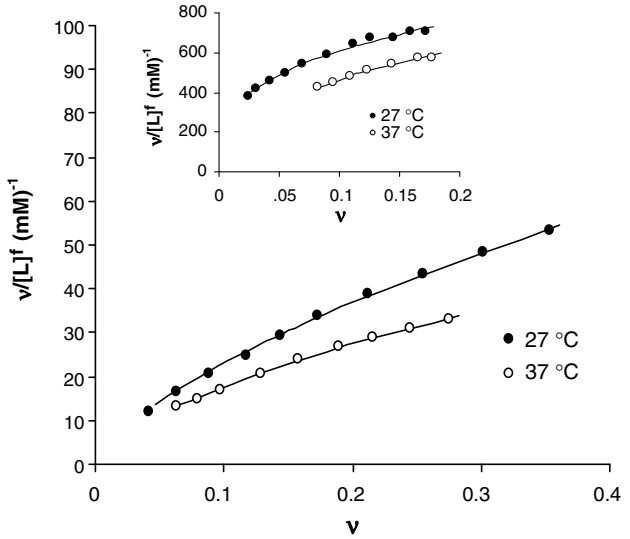


Figure 3. Scatchard plots for binding of [Pt(bpy)(Bu-dtc)]NO_3 with DNA. The inset is Scatchard plots for binding of [Pd(bpy)(Bu-dtc)]NO_3 with DNA.

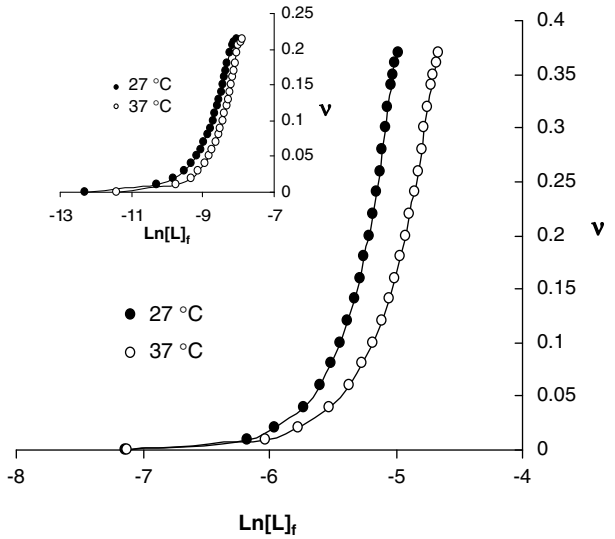


Figure 4. Binding isotherm plots for [Pt(bpy)(Bu-dtc)]NO_3 in the interaction with DNA. Inset, for [Pd(bpy)(Bu-dtc)]NO_3.

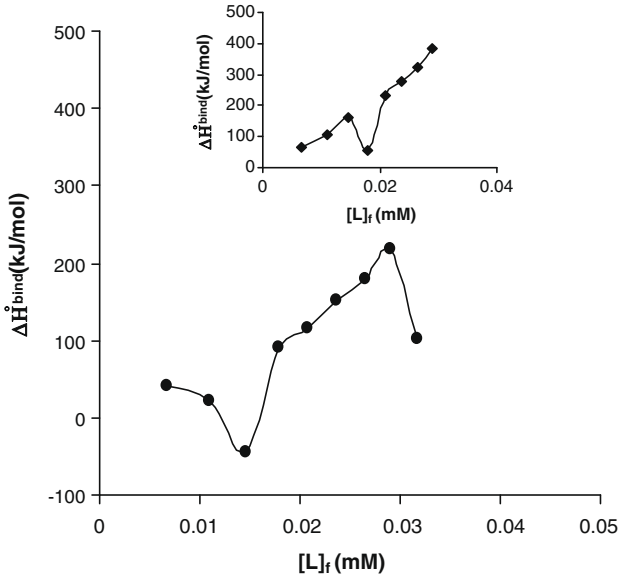


Figure 5. Molar enthalpies of binding in the interaction between DNA and [Pt(bpy)(Bu-dtc)]NO_3 (inset, Pd(bpy)(Bu-dtc)]NO_3) versus free concentrations of complexes at pH 7.0 and 27 °C.

cells was continued until no further changes in the absorption readings were observed. The profiles of denaturation of DNA by [Pt(bpy)(Bu-dtc)]NO_3 and [Pd(bpy)(Bu-dtc)]NO_3 are shown in Figure 6 at the two temperatures of 27 and 37 °C. The concentration of metal complexes in the midpoint of transition, $[L]_{1/2}$, for Pt(II) complex at 27 °C is 0.145 and at 37 °C is 0.137 mmol/L and for Pd(II) complex at 27 °C is 0.093 and at 37 °C is 0.085 mmol/L. The improving effect of the temperature, leading to the decrease in $[L]_{1/2}$ from 0.145 to 0.137 for Pt(II) system and from 0.093 to 0.085 for Pd(II) system, indicates that the increase in the temperature lowers the stability of the DNA against denaturation caused by these complexes. The important observation of this work is the low values of $[L]_{1/2}$ for these complexes,[40–42] that is, both complexes (in particular 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.

Furthermore, some thermodynamic parameters found in the process of DNA denaturation are discussed as follows: Using the DNA denaturation plots given in Figure 6 and Pace method,[43] the values of K, unfolding equilibrium constant and $\Delta G°$, unfolding free energy of DNA at two temperatures of 27 and 37 °C in the presence of [Pt(bpy)(Bu-dtc)]NO_3 and [Pd(bpy)(Bu-dtc)]NO_3 have been calculated. A straight line is obtained when the values of $\Delta G°$ are plotted versus the concentrations of each metal complex in the transition region at 27 and 37 °C. These plots are shown in Figure 7 for Pt(II) and the inset for Pd(II) systems. The m, slope of these plots (a measure of the metal complex ability to denature DNA) and the intercept on ordinate, $\Delta G°_{(H_2O)}$, (conformational stability of DNA in the absence of metal complex) are summarized in Table 4. The values of m for Pd(II) complex are higher than that of Pt(II) complex, which indicate the higher ability of Pd(II) to denature DNA. These m values are similar to that of Pd(II) complex as well as surfactant reported earlier.[16] As we know, the higher the value of $\Delta G°$, the larger the conformational stability of DNA. However, the values of $\Delta G°$ (see Table 4) are decreased by increasing the temperature for both complexes. This is as expected because in general, most of the macromolecules are less stable at higher temperature.[41] Another important thermodynamic parameter found is the molar enthalpy of DNA denaturation in the absence of metal complexes, that is, $\Delta H°_{(H_2O)}$. For this, we calculated the molar enthalpy of DNA denaturation in the presence of each metal complex, $\Delta H_{conformation}$ or $\Delta H°_{denaturation}$, in the range of the two temperatures using Gibbs–Helmholtz equation.[44] On plotting the values of these enthalpies versus the concentrations of each metal complex, straight lines will be obtained which are shown in Figure 8 for [Pt(bpy)(Bu-dtc)]NO_3 and the inset for [Pd(bpy)(Bu-dtc)]NO_3. Intrapolation of these lines (intercept on ordinate i.e., absence of metal complex) gives the values of $\Delta H°_{(H_2O)}$ (see Table 4). These plots show that in the range of 27–37 °C the changes in the enthalpies in the presence of Pd(II) complex is descending, while that of Pt(II) is ascending. These observations indicate that by increasing the concentration of Pd(II) complex, the stability of DNA is decreased while in the case of Pt(II) the opposite trend is observed which may be due to higher tendency of interaction of Pd(II) than Pt(II) complexes with DNA. In addition, the entropy ($\Delta S°_{(H_2O)}$) of DNA unfolding by Pt(II) and Pd(II) complexes have been calculated using equation $\Delta G = \Delta H - T\Delta S$, and the data are given in Table 4. These data show that the metal–DNA complex is more disordered

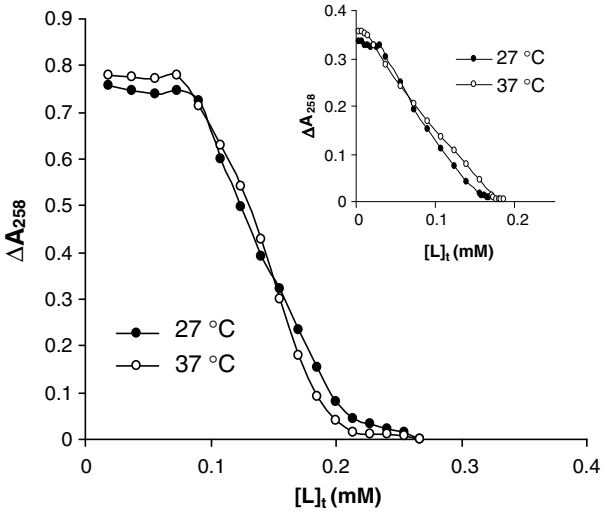


Figure 6. The changes of absorbance of DNA at λ_{max} = 258 nm due to increasing the total concentration of [Pt(bpy)(Bu-dtc)]NO_3 and the inset, [Pd(bpy)(Bu-dtc)]NO_3, $[L]_t$, at constant temperature of 27 and 37 °C.

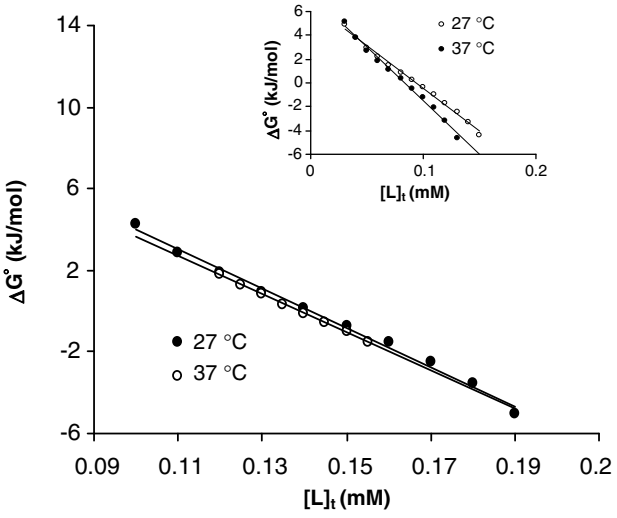


Figure 7. The molar Gibbs free energies plots of unfolding ($\Delta G°$ vs $[L]_t$) of DNA in the presence of [Pt(bpy)(Bu-dtc)]NO_3. Inset, in the presence of [Pd(bpy)(Bu-dtc)]NO_3.

Table 4
Thermodynamic parameters of DNA denaturation by platinum (II) and palladium (II) complexes

Compound	Temperature (°C)	m[a] (kJ/mol))mmol/L)$^{-1}$	$\Delta G^{\circ}_{(H_2O)}$[b] (kJ/molK)	$\Delta H^{\circ}_{(H_2O)}$[c] (kJ/mol)	$\Delta S^{\circ}_{(H_2O)}$[d] (kJ/mol)
$[Pt(bpy)(Bu\text{-}dtc)]NO_3$	27	96.1	13.61	5.89	~0
	37	93.4	12.98		
$[Pd(bpy)(Bu\text{-}dtc)]NO_3$	27	72.12	7.47	29.36	0.073
	37	89.9	6.74		

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

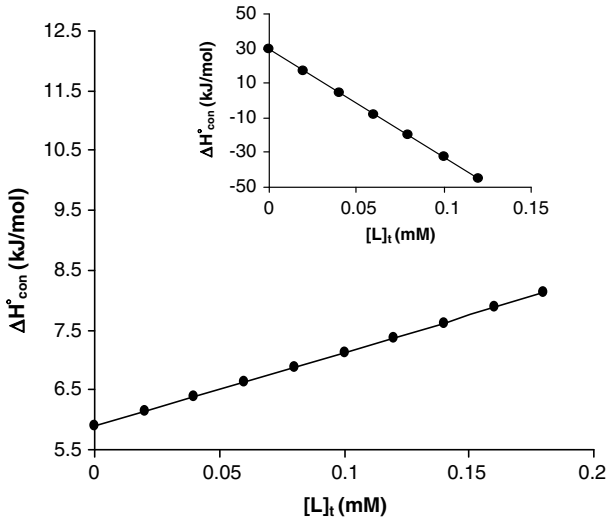


Figure 8. Plots of the molar enthalpies of DNA denaturation in the interaction with $[Pt(bpy)(Bu\text{-}dtc)]NO_3$ and the inset with $[Pd(bpy)(Bu\text{-}dtc)]NO_3$ complexes in the range of 27–37 °C.

than that of native DNA, because the entropy changes are positive and the extent of disorder in Pd(II)–DNA complex is more than that of Pt(II)–DNA complex (see Table 4). This again shows that ability of palladium complex in the denaturation of DNA is more than that of the platinum complex.

2.3.3. Evaluation of binding modes

The solution of each interacted DNA–metal complex was passed through a Sephadex G-25 column equilibrated with Tris–HCl buffer. Elution was done with buffer and each fraction of the column was monitored spectrophotometrically at 321 and 258 nm for Pt(II)–DNA system and 317 and 258 nm for Pd(II)–DNA system. The gel chromatograms obtained from these experiments are given in Figure 9A and B. 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 bindings with DNA are strong enough and do not break readily. This type of binding of metal complexes to DNA is strictly true for kinetically inert platinum(II)–DNA complex. However, in the case of palladium(II)–DNA system, for both aforementioned wavelengths two peaks were observed none of which were resolved. This indicates that in the presence of palladium(II) complex, DNA partially breaks into two fragments, one with higher and the other one with lower molecular weight. Surprisingly, the amount of palladium(II) metal complex bound to the fragment with lower molecular weight is higher than the amount bound to the fragment with higher molecular weight as indicated from the absorption readings at 317 nm. To confirm the breaking of DNA by this metal complex, a solution of DNA alone was passed through the same column and each eluted fraction of 2 mL was monitored at 258 nm. The gel chromatogram obtained is shown in Figure 9C. This indicates that the highly polymerized calf thymus DNA has fragments with approximately similar molecular weights.

The binding between DNA and metal complexes was also studied by precipitating the DNA from the interacted DNA–metal complexes with absolute ethanol. In this experiment, the precipitated DNA was separated out and washed several times with the alcohol. This precipitate was redissolved in Tris–HCl buffer, and the solution was monitored spectrophotometrically for DNA at 258 nm, Pt(II) complex at 321 nm, and Pd(II) complex at 317 nm. The presence of DNA–metal complex in this solution, as observed by spectral method, suggests that the bonding of these metal complexes with DNA is strong enough and it does not break readily, and it is responsible for stabilizing the above DNA–metal complexes. Because, the interaction between DNA and involved metal complex was very weak, the DNA should be precipitated separately and all free metal complexes would remain in the supernatant.[45]

2.3.4. Intercalation studies using fluorescence method

Fluorescence titration of solutions containing the DNA and ethidium bromide with Pt(II) or Pd(II) complexes has been investigated. It is known that the fluorescence intensity of ethidium bromide grows when it goes from a polar to a nonpolar medium due to the lowering of the inter-system crossing lifetimes.[46] Ethidium bromide is a dye whose fluorescence is quenched in water, but strongly enhanced when it intercalates within DNA duplexes.[47] The fluorescence emission spectra of the intercalated ethidium with increasing concentrations of Pd(II) and Pt(II) complexes are shown in Figure 10. Figure 10 shows a significant reduction of the ethidium emission intensity by adding different concentrations of Pd(II) and Pt(II) complexes (a: 0.00, b: 0.05, c: 0.1, and d: 0.15 mM) at 27 °C. Similar observations were made when the experiments were carried out at 37 °C. It can then be concluded that the fluorescence intensity of DNA-intercalated ethidium is quenched, when ethidium is removed from the duplexes by the action of Pd(II) and Pt(II) complexes and it is released into buffer. These results suggest that the above metal complexes presumably intercalate into DNA through the planar 2,2′-bipyridine ligand present in their structures. Similar observations have been made in the interaction of $[Pt(bpy)(ddtc)]NO_3$, where ddtc is diethyldithiocarbamate, with calf thymus DNA.[12]

2.3.5. Circular dichroism

The CD spectral technique is useful in monitoring the conformational variations of DNA in solution. Thus, CD spectra of calf thymus DNA incubated with platinum or palladium complexes at several ratios were recorded.[38] The observed CD spectrum of DNA consists of a positive band at 265 nm due to base stacking and a negative band at 247 nm due to helicity, which are characteristics of calf thymus DNA (Fig. 11a). When the complexes are free in the solution, they do not have any CD spectrum; whereas they have an induced CD spectrum as interact with DNA. When our

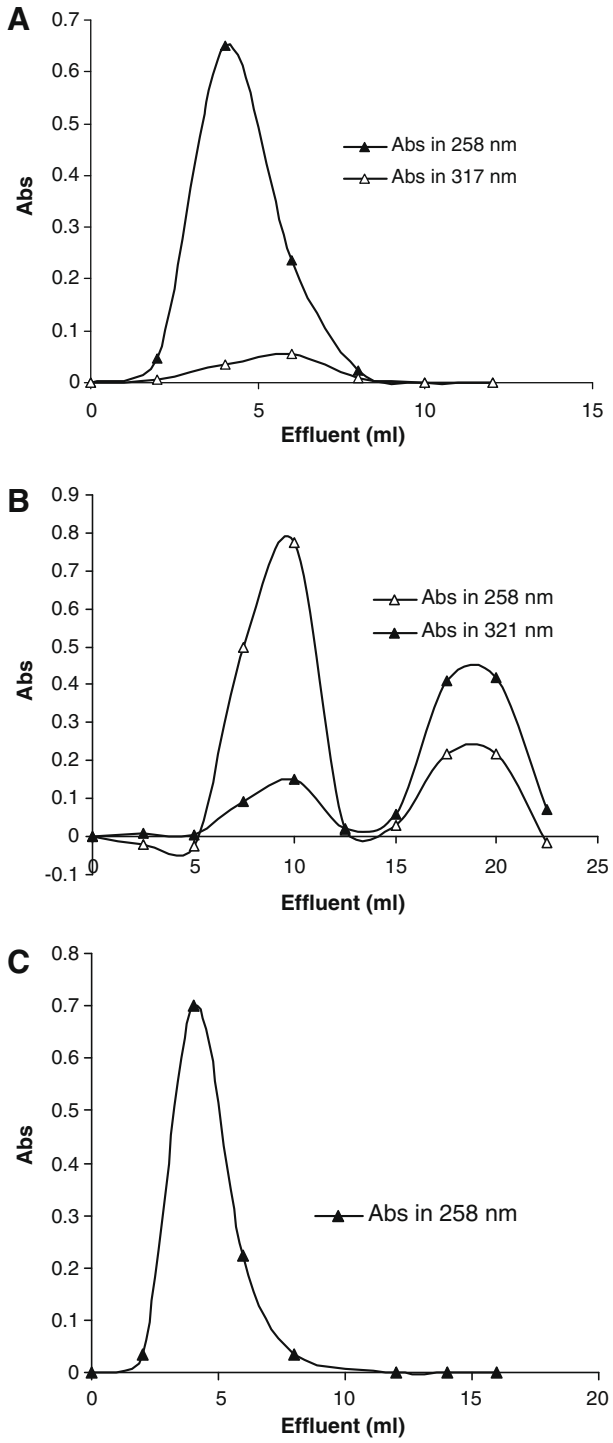


Figure 9. Gel chromatograms of (A) [Pt(bpy)(Bu-dtc)]NO_3–DNA complex, (B) [Pd(bpy)(Bu-dtc)]NO_3–DNA complex and (C) DNA alone, obtained on Sephadex G-25 column, equilibrated with 10 mmol/L Tris–HCl buffer of pH 7.0 in the presence of 0.01 mol/L sodium chloride.

compounds were incubated with DNA, the CD spectra displayed changes of both positive and negative bands (Fig. 11b and c). At concentrations lower than $[L]_{1/2}$ (see Section 2.3.2), the complexes may induce certain conformational changes in DNA as indicated by significant decrease in the intensities of positive and negative bands (Fig. 11b). However, at concentrations higher than $[L]_{1/2}$, the precipitate formed and spectra collapsed completely (Fig. 11c). Similar observations have been made in the interaction of crocin and crocetin with calf thymus DNA.[41] Moreover, in the case of Pd complex a further decrease in the positive and negative bands was observed at concentrations higher than $[L]_{1/2}$ (Fig. 11c, inset). This indicates that conformational changes in the structure of DNA in the presence of Pd complex must be different from that of Pt complex.

3. Conclusion

Two water soluble Pt(II) and Pd(II) complexes have been synthesized and characterized. They exhibited potent cytotoxic properties against chronic myelogenous leukemia cell line, K562. The cells showed different sensitivities to complexes. Cc_{50} values of Pt(II) complex are slightly lower than those of Pd(II) complex and both are much lower than those of cisplatin. Both complexes have been interacted with calf thymus DNA. They cooperatively bind to DNA presumably through intercalations. They unexpectedly denature DNA at very low concentrations. Determination of several binding and thermodynamic parameters has also been attempted. Palladium complex breaks the DNA into two unequal fragments and binds stronger to the lighter fragment than the heavier one.

4. Experimental

4.1. Materials and methods

Potassium tetrachloroplatinate, 2,2′-bipyridine, highly polymerized calf thymus DNA sodium salt and Tris–HCl buffer were obtained from Merck (Germany). Palladium(II) chloride anhydrous was bought from Fluka (Switzerland). Butylamine and carbon disulfide were purchased from Aldrich (England). [Pt(bpy)Cl_2] and [Pd(bpy)Cl_2] were prepared by the procedure described in the literature.[48] Solvents used were of reagent grade and purified before being used by the standard methods. Other chemicals used were of analytical reagent or of higher purity grade.

Infrared spectra of the metal complexes were recorded on a JASCO-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. ^{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. *Electronic absorption spectra* of the title metal complexes were measured on a JASCO UV/VIS-7850 recording spectrophotometer. *Melting points* were measured on a Unimelt capillary melting point apparatus and here reported uncorrectedly. *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. The doubly distilled water was used as solvent. *Fluorescence intensity measurements* were carried out on a Hitachi spectrofluorimeter model MPF-4. *Circular dichroism spectra* were recorded on an Aviv Spectropolarimeter model 215.

4.2. Synthesis of ligand and metal complexes

4.2.1. Synthesis of Bu-dtcNa

This ligand was synthesized by a modified literature method[49]: butylamine (5 mL, 50 mmol) and sodium hydroxide (2.0 g, 50 mmol) were added to 50 mL acetone and stirred for one hour at 0 °C in an ice bath. Carbon disulfide (5 mL, excess) was added dropwise while vigorous stirring. The curdy yellow solution so obtained was stirred for another 4 h at 0 °C and kept overnight at room temperature. It was then filtered and 100 mL diethylether was added to the filtrate and placed in refrigerator overnight.

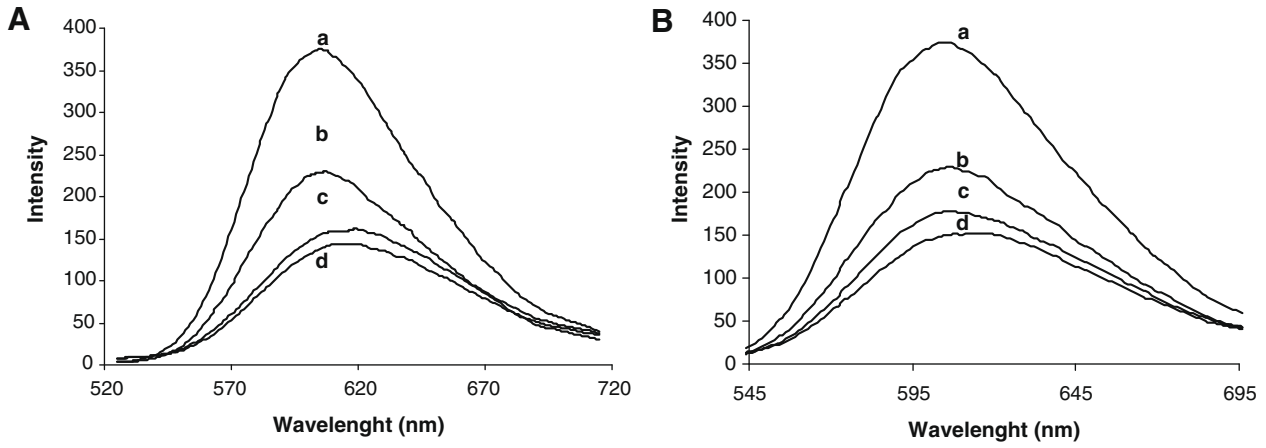


Figure 10. Fluorescence emission spectra of interacted ethidium–DNA in the absence (a) and presence of different concentrations of Pt(II) complex (A) and Pd(II) complex (B): 0.05 mM (b); 0.1 mM (c); 0.15 mM (d) at 27 °C.

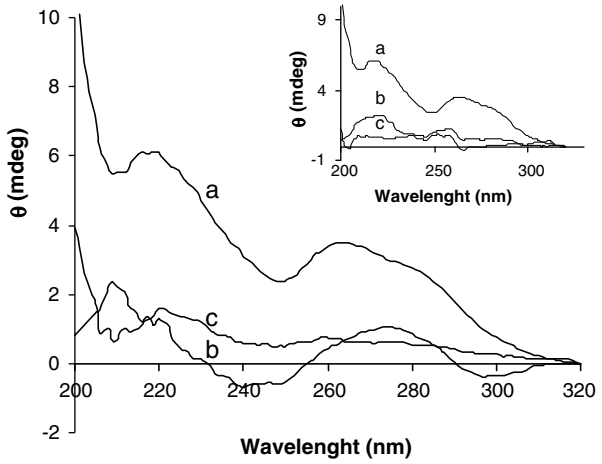


Figure 11. CD spectra of DNA adduct with compound. Plot for titration of DNA with the Pt(II) complex and inset for Pd(II) complex at 27 °C. (a) Free DNA (120 μM); (b) in the presence of 100 μM complex and (c) in the presence of 200 μM complex.

The resulting white precipitate of crude product was filtered off and vacuum dried. Recrystallization was carried out by stirring the crude product in 35 mL acetone and filtering the undissolved particles out. Thirty microliters dichloromethane was added to the filtrate and then left in a refrigerator overnight. The desired product was collected by filtration as white needle-like crystals and washed with small amount of dichloromethane and vacuum dried. Yield was 6.84 g (80%) with a melting point of 73 °C. Anal. Calcd for $C_5H_{10}NS_2Na$ (171.12): C, 35.09; H, 5.85; N, 8.19%. Found: C, 35.02; H, 5.84; N, 8.18%.

4.2.2. Synthesis of [Pt(bpy)(Bu-dtc)]NO_3

[Pt(bpy)Cl_2] (0.422 g, 1 mmol) was suspended in 80 mL doubly distilled water, and (0.34 g, 2 mmol) of $AgNO_3$ was added to it with constant stirring. This reaction mixture was heated with stirring under dark 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. To the clear yellow filtrate containing [Pt(bpy)$(H_2O)_2$]$(NO_3)_2$, a solution of 0.171 g, 1 mmol Butyldithiocarbamate sodium salt in 15 mL water was slowly added. Stirring was continued at ~50 °C for another 6 h and 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 get 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 brown needle-shaped crystals after three days. The crystals were isolated by filtration, washed with 15 mL acetone, and dried at 40 °C. Yield: 0.330 g (59%) and decomposed at 235 °C. Anal. Calcd for $C_{15}H_{18}N_4O_3S_2Pt$ (561): C, 35.09; H, 3.21; N, 9.98%. Found: C, 35.03; H, 3.22; N, 10.00%.

4.2.3. Synthesis of [Pd(bpy)(Bu-dtc)]NO_3

This complex was prepared by a similar method to that of [Pt(bpy)(Bu-dtc)]NO_3. Yield: 0.273 g (58%) and decomposed at 170 °C. Anal. Calcd For $C_{15}H_{18}N_4O_3S_2Pd$ (472): C, 38.14; H, 3.81; N, 11.86%. Found: C, 37.99; H, 3.80; N, 11.80%.

4.3. Cytotoxic studies

4.3.1. Cell culture

In RPMI medium, chronic myelogenous leukemia cells were grown. This medium was supplemented with L-glutamine (2 mM), streptomycin and penicillin (5 μg/mL), and 10% heat-inactivated fetal calf serum, at 37 °C under a 5% CO_2/95% air atmosphere.

4.3.2. Cell proliferation assay

The above Pt(II) and Pd(II) complexes inhibit the growth of chronic myelogenous leukemia cell line, K562. This growth inhibition was measured by means of MTT assay. The cleavage and conversion of the soluble yellowish MTT to the insoluble purple formazan by active mitochondrial dehydrogenase of living cells has been used to develop an assay system alternative to other assays for measurement of cell proliferation. Harvested cells were seeded into 96-well plate (2×10^4 cell/mL) with varying concentrations of the sterilized drugs (0–250 μM) and incubated for 24 h. 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.[38] At the end, the insoluble formazan produced was dissolved in solution containing 10% SDS and 50% DMF (Left for 2 h at 37 °C in dark conditions), 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%).

4.3.3. Statistical analysis

Results were analyzed for statistical significance using two tailed Student's *t*-test. Changes were considered significant at $p < 0.05$.

4.4. Binding studies

Calf thymus DNA alters the absorption spectra of [Pt(bpy)(Bu-dtc)]NO_3 and [Pd(bpy)(Bu-dtc)]NO_3 complexes.[50,51] The stock solutions of Pt(II) and Pd(II) complexes (1.4 mmol/L) were made in the above-mentioned Tris–HCl buffer of pH 7.0 medium containing 10 mmol/L sodium chloride by gentle stirring and heating at 35 °C, while that of DNA (4 mg/mL) at 4 °C until homogenous. The metal complex solutions with and without DNA were incubated at 27 and 37 °C. Then, the spectrophotometric readings at λ_{max} (nm) of complexes, where DNA has no absorption, were measured. Using trial and error method, the incubation time for solutions of DNA–metal complexes at 27 and 37 °C was found to be 6.5 h. No further changes were observed in the absorbance reading after longer incubation. The concentration of DNA was found out based on determination of phosphate (P). Millimolar extinction coefficient of native DNA solution at E_{258} (ε_{258}) based on DNA P was 6.6×10^3.[52] Methods for DNA-binding studies of above complexes are described as follows.

4.4.1. Determination of binding parameters

In the interaction of the metal complexes with DNA, the procedures followed to determine *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 similar to what was reported earlier.[39] 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.[16]

4.4.2. DNA-denaturation studies

Denaturation of DNA was carried out by looking at the changes in the UV absorption spectrum of DNA solution in 258 nm upon addition of Pt(II) or Pd(II) complexes.[16,41] In these studies, the concentration of each metal complex at midpoint of transition, $[L]_{1/2}$, was determined. Also, thermodynamic parameters such as: $\Delta G^\circ_{(H_2O)}$, conformational stability of DNA in the absence of metal complex; $\Delta H^\circ_{(H_2O)}$, the heat needed for DNA denaturation in the absence of metal complex; $\Delta S^\circ_{(H_2O)}$, the entropy of DNA denaturation by metal complex as well as *m*, measure of the metal complex ability to denature DNA were found out using Pace method.[16,41,43]

4.4.3. Gel filtration

A Sephadex G-25 column was equilibrated with Tris–HCl buffer of pH 7.0 medium containing sodium chloride (10 mmol/L), and the solution of DNA–metal complex was passed through it. Elution was done with the same buffer and each fraction of the column (2.5 mL) was monitored spectrophotometrically at λ_{max} (nm) for platinum(II) and palladium(II) complexes and at 258 nm for DNA–metal complex, respectively.[53] Gel chromatograms are obtained by plotting of absorbance readings at the two wavelengths versus column fractions in the same plot. In this plot, the two peaks obtained may resolve or not. The former indicates that the DNA is separated from the metal complex and the binding of DNA to metal complex is weak and reversible. However, if the two peaks are not resolved, it indicates that the DNA is not separated from the metal complex and the binding is strong and irreversible.

4.4.4. Ethanol precipitation

Platinum(II) and palladium(II) complexes (0.5 mL from 5×10^{-4} mol/L stock) were interacted with DNA (50 μL of a solution containing 4 mg/mL) in 1–1.5 mL of the above-mentioned Tris–HCl buffer of pH 7.0. To this interacted solution, an excess of absolute ethanol was added. The precipitated DNA was separated and washed with alcohol. This precipitate was redissolved in the same buffer, and the solution was monitored spectrophotometrically for DNA at 258 nm and Pt(II) or Pd(II) complexes at their λ_{max} (nm).

4.4.5. Fluorescence measurements

The fluorescence of ethidium bromide (EthBr) is greatly enhanced on its intercalation between the base pairs of DNA.[46] Ethidium bromide displacement assay was performed as reported in the literature.[42] At first, DNA (60 μM) was added to 2 μM aqueous ethidium bromide solution and maximum quantum yield for ethidium bromide was achieved at 471 nm, so we selected this wavelength as excitation radiation for all samples at different temperatures (27 °C and 37 °C) in the range of 540–700 nm. To this solution (containing ethidium bromide and DNA), different concentrations of the Pd(II) or Pt(II) complex were added (0.05, 0.1 and 0.15 mM). Measurements were done by applying a 1-cm path length fluorescence cuvette. The fluorescence intensities of the Pd(II) and Pt(II) complexes at the highest denaturant concentration at 471 nm excitation wavelength have been checked, and the emission intensities of these compounds were very small and negligible.

4.4.6. CD spectropolarimetry

Circular dichroism spectra showed changes in the structure of DNA, which were monitored in the region (200–320 nm) using 1-cm path length cells.[46] The DNA concentration in the experiments was 120 μM. Induced CD spectra resulting from the interaction of the Pd(II) or Pt(II) complex (100 and 200 μM) with DNA at the temperatures of 27 °C and 37 °C were obtained by subtracting the CD spectrum of the native DNA and mixture of DNA–Pd(II) or –Pt(II) complex from the CD spectrum of the buffer and spectrum of buffer–Pd(II) or –Pt(II) complex solutions.

Acknowledgments

Financial assistance from the Research Council of University of Sistan and Baluchestan and of University of Tehran is gratefully acknowledged.

References and notes

1. Bierbach, U.; Sabat, M.; Farrell, N. *Inorg. Chem.* **2000**, *39*, 1882.
2. Alverdi, V.; Giovagnini, L.; Marzano, C.; Seraglia, R.; Beltio, F.; Sintran, S.; Graziani, R.; Fregona, D. J. *Inorg. Biochem.* **2004**, *98*, 1117.
3. Martinez, A.; Lorenzo, J.; Prieto, M. J.; Font-Bardia, M.; Solans, X.; Aviles, F. X.; Moreno, V. *Bioorg. Med. Chem.* **2007**, *15*, 969.
4. Kwon, Y. E.; Whang, K. J.; Park, Y. J.; Kim, K. H. *Bioorg. Med. Chem.* **2003**, *11*, 1669.
5. Rosenberg, B. In *Cisplatin, Chemistry and Biochemistry of a Leading Anticancer Drug*; Lippert, B., Ed.; Verlag Chemie, VCH: Basel, 1999; p 3.
6. Dorr, R. T. In *Platinum and other Metal Coordination Compounds in Cancer Chemotherapy*; Pinedo, H. M., Schornagel, J. H., Eds.; Plenum: New York, 1996; p 131.
7. Szucova, L.; Travnicek, Z.; Zatloukal, M.; Popa, I. *Bioorg. Med. Chem.* **2006**, *14*, 479.
8. Montana, A. M.; Bernal, F. J.; Lorenzo, J.; Farnos, C.; Batalla, C.; Prieto, M. J.; Moreno, V.; Aviles, F. X.; Mesas, J. M.; Alegre, M. T. *Bioorg. Med. Chem.* **2008**, *16*, 1721.
9. Woud, W. R. *Cancer Res.* **1987**, *47*, 6549.
10. Fregona, D.; Giovagnini, L.; Ronconi, L.; Marzano, C.; Trevisan, A.; Sitran, S.; Biondi, B.; Bordin, F. J. *Inorg. Biochem.* **2003**, *93*, 181.
11. Hidaka, S.; Tsuruoka, M.; Funakoshi, T.; Shimada, H.; Kiyozumi, M.; Kojima, S. *Ren. Fail.* **1994**, *16*, 337.
12. Mital, R.; Jain, N.; Srivastava, T. S. *Inorg. Chim. Acta* **1989**, *166*, 135.
13. Lomozik, L.; Odani, A.; Yamauchi, O. *Inorg. Chim. Acta* **1996**, *219*, 107.
14. Singh, R. V.; Fahmi, N.; Biyala, M. K. J. *Iran. Chem. Soc.* **2005**, *2*, 40.
15. Khan, B. T.; Bhatt, J.; Najmuddin, K.; Shamsuddin, S.; Annapoorna, K. J. *Inorg. Biochem.* **1991**, *44*, 55.

16. Mansouri-Torshizi, H.; Islami-Moghaddam, M.; Saboury, A. A. *Acta Biochim. Biophys. Sin.* **2003**, *35*(10), 886.
17. Mansouri-Torshizi, H.; Ghadimy, S.; Akbarzadeh, N. *Chem. Pharm. Bull.* **2001**, *49*(12), 1517.
18. Jain, N.; Srivastava, T. S. *Inorg. Chim. Acta* **1987**, *128*, 151.
19. Martin, R. B., In *Platinum, Gold and Other Chemotherapeutic Agents;* Lippard, S. J., Ed.; ACS Symposium Series No. 209, Washington, DC, 1993; p 231.
20. Hacker, M.P.; Douple, E.B.; Krakoff, I.H. *In Platinum Coordination Complexes in Cancer Chemotherapy;* Nijhoff, M.A.; Ed.; Boston, 1984; p. 267.
21. Roberts, J. J.; Pera, M. P. In *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S., Waring, M. J., Eds.; MacMillan Press: London, 1983; p 183.
22. Farrel, N. *Cancer Invest.* **1993**, *11*, 578.
23. Gay, M.; Montana, A. M.; Moreno, V.; Prieto, M. J.; Perez, J. M.; Alonso, C. *Bioorg. Med. Chem.* **2006**, *14*, 1565.
24. Farrel, N. *Met. Ions Biol. Syst.* **1996**, *32*, 603.
25. Tobe, M. L.; Kokhar, A. R. *J. Clin. Hematol. Oncol.* **1977**, *7*, 120.
26. Manohar, A.; Ramalingam, K.; Bocelli, G.; Righi, L. *Inorg. Chim. Acta* **2001**, *314*, 177.
27. Zheng, H.; Leung, W.-H.; Chim, J. L. C.; Lai, W.; Lam, C.-H.; Williams, I. D.; Wong, W.-T. *Inorg. Chim. Acta* **2000**, *306*, 184.
28. Ivanov, A. V.; Kritikos, M.; Antzutkin, O. N.; Forsling, W. *Inorg. Chim. Acta* **2001**, *321*, 63.
29. Unoura, K.; Abiko, Y.; Yamazaki, A.; Kato, Y.; Coomber, D. C.; Fallon, G. D.; Nakahara, K.; Bond, A. M. *Inorg. Chim. Acta* **2002**, *333*, 41.
30. Teruel, H.; Gorrin, Y. C.; Falvello, L. R. *Inorg. Chim. Acta* **2001**, *316*, 1.
31. Yoshida, T.; Yamasaki, K.; Sawada, S. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2908.
32. Ronconi, L.; Giovagnini, L.; Marzano, C.; Bettio, F.; Ganziani, R.; Pilloni, G.; Fregona, D. *Inorg. Chem.* **2005**, *44*, 1867.
33. Kumar, L.; Kandasamy, N. R.; Srivastava, T. S.; Amonkar, A. J.; Adwankar, M. K.; Chitnis, M. P. J. *Inorg. Biochem.* **1985**, *23*, 1.
34. Angelici, R. J., In *Synthesis Technique in Inorganic Chemistry*; Saunders, Ed.; Philadelphia, 1969; p 17.
35. Gidney, P. M.; Gillard, R. D.; Heaton, B. T. *J. Chem. Soc., Dalton Trans.* **1973**, 132.
36. Coucouvanis, D.. In *Progress in Inorganic Chemistry*; Lippard, S. J., Ed.; Willey: New York, 1970; Vol. 11, p 311.
37. Katsoulos, G. P.; Mannoussakis, G. F.; Tsipis, C. A. *Polyhedron* **1984**, *3*, 735.
38. Divsalar, A.; Saboury, A. A.; Mansouri-Torshizi, H.; Moosavi-Movahedi, A. A. *J. Biomol. Struct. Dyn.* **2007**, *25*(2), 173.
39. Saboury, A. A. J. *Iran. Chem. Soc.* **2006**, *3*, 1.
40. Xu, Z. H.; Chen, F. J.; Xi, P. X.; Liu, X. H.; Zeng, Z. Z. *J. Photochem. Photobiol., A Chem.* **2008**, *196*, 77.
41. Bathaie, S. Z.; Bolhasani, A.; Hoshyar, R.; Ranjbar, B.; Sabouni, F.; Moosavi-Movahedi, A. A. *DNA Cell Biol.* **2007**, *26*, 533.
42. Peres-Flores, L.; Ruiz-Chica, A. J.; Delcros, J. G.; Sanches, F. M.; Ramirez, F. *J. Spectrochim. Acta Part A* **2008**, *69*, 1089.
43. Greene, R. F.; Pace, C. N. *J. Biol. Chem.* **1974**, *249*, 5388.
44. Barrow, G. M. In *Physical Chemistry*, 5th ed.; McGraw-Hill: New York, 1988. Chapter 7.
45. Mansouri-Torshizi, H.; Srivastava, T. S.; Chavan, S. J.; Chitnis, M. P. J. *Inorg. Biochem.* **1992**, *48*, 63.
46. Krishna, A. G.; Kumar, D. V.; Khan, B. M.; Rawal, S. K.; Ganesh, K. N. *Biochim. Biophys. Acta* **1998**, *1381*, 104.
47. Butour, J. L.; Macquet, J. P. *Eur. J. Biochem.* **1977**, *78*, 455.
48. Palocsay, F. A.; Rund, J. V. *Inorg. Chem.* **1969**, *8*, 524.
49. Hadjikostas, C. C.; Katsoulos, G. A.; Shakhatreh, S. A. *Inorg. Chim. Acta* **1987**, *133*, 129.
50. Mital, R.; Ray, K. S.; Srivastava, T. S.; Bahattacharya, R. K. J. *Inorg. Biochem.* **1986**, *27*, 133.
51. Mital, R.; Srivastava, T. S. J. *Inorg. Chem.* **1990**, *40*, 111.
52. King, A. M. Q.; Nicholson, B. H. *Biochem. J.* **1969**, *114*, 679.
53. Mansouri-Torshizi, H.; Srivastava, T. S.; Parekh, H. K.; Chitnis, M. P. J. *Inorg. Biochem.* **1992**, *45*, 135.