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ORIGINAL PAPER

Synthesis, Characterization, DNA Binding Properties, Fluorescence Studies and Toxic Activity of Cobalt(III) and Ruthenium(II) Polypyridyl Complexes

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Abstract The new ligand 4-(isopropylbenzaldehyde)imidazo[4,5-*f*][1,10]phenanthroline (ippip) and its complexes $[Ru(phen)_2(ippip)]^{2+}$ (**1**), $[Co(phen)_2(ippip)]^{3+}$ (**2**), $[Ru(bpy)_2(ippip)]^{2+}$ (**3**), $[Co(bpy)_2(ippip)]^{3+}$ (**4**) (bpy=2,2-bipyridine) and (phen=1,10-phenanthroline) were synthesized and characterized by ES^+-MS, ^1H and ^{13}C NMR. The DNA binding properties of the four complexes were investigated by different spectrophotometric methods and viscosity measurements. The results suggest that complexes bind to calf thymus DNA (CT-DNA) through intercalation. When irradiated at 365 nm, the complexes promote the photocleavage of pBR322 DNA, and complex 1 cleaves DNA more effectively than 2, 3, 4 complexes under comparable experimental conditions. Furthermore, photocleavage studies reveal that singlet oxygen ($^1O_2$) plays a significant role in the photocleavage.

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Keywords Cobalt(III) and ruthenium(II) mixed-ligands · DNA-binding · Fluorescence studies · Photocleavage of plasmid DNA · Toxic studies

Introduction

Among the host of DNA-binding and cleaving agents reported so far, transition metal complexes are of relevance to the present work. Major interests in this regard concern the utility of metal complexes in the development of site specific cleavers, structural/spectroscopic probes, restriction enzymes, molecular photo switches and, of course, anti-tumor drugs. Metal complexes have been found to be particularly useful for the above mentioned purposes because of their potential to bind DNA through multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities. During the last decade, a number of transition-metal complexes have been utilized to probe nucleic acid structure [1, 2], and in the development of DNA-cleaving agents [3, 4], DNA photo probes [5, 6] DNA-molecular light switches [7, 8] and so forth. It has also been well documented that metal complexes can bind to DNA covalently as well non covalently [6, 9].

On the other hand, Barton and co-workers reported that a number of metal complexes containing aromatic diimine ligands [10] could act as photoluminescent metal-based probes for the study of DNA binding. These complexes have been reported to bind DNA through π–π* and/or electrostatic interactions and cause oxidative cleavage of DNA. Moreover, an oxidative cleavage of DNA on irradiation with visible light has gained considerable interest due to its potential application in photodynamic

therapy of cancer [11, 12]. Recently, several mixed ligand complexes of ruthenium(II) and cobalt(III) have also been reported [13–16]. In view of the above, we explore DNA binding and photo-induced cleavage activity of cobalt and ruthenium complexes.

Experimental

Materials

CT (calf thymus) DNA was purchased from Aldrich. The synthesis of 1,10 phenanthroline-5, 6-dione was prepared as per the procedure given in literature [17]. Interactions of the complexes with DNA were studied in tris buffer (5 mM Tris-HCl, 5 mM NaCl, p^H 7.0). DNA had a ratio of UV absorbance at 260 and 280 nm of about 1.90 indicating its purity [18]. The DNA concentration per nucleotide was determined by absorbance at 260 nm using the molar absorption coefficient (6,600 M^{-1} cm^{-1}) [19].

Synthesis of Ligands

(ippip) =4-(isopropyl benzaldehyde)imidazo[4,5-f][1, 10] phenanthroline

Phen-dione (0.53 g, 2.50 mmol), 4-isopropyl benzaldehyde (0.152 g, 1.2 mmol) and ammonium acetate (3.88 g, 50.00 mmol) and glacial acetic acid (15 mL) were refluxed together for 4 h as per Steck and Day [20], cooled thereafter to room temperature and diluted with water. Dropwise addition of liquor ammonia gave a yellow precipitate which was collected, washed with water, dried and purified by recrystallization. Formula: $C_{22}H_{18}N_4$, Anal. Calc. H 5.36, C 78.08, N 16.56 found: H 4.9, C 77.0, N 15.6 (yield=80%) Analytical data:ES^+-MS calculated: 338.15 found: 339. ^{1}H NMR (DMSO, TMS): 9.03 (d, 2H), 8.95 (d, 2H), 8.24 (d, 2H), , 7.21 (d, 1H), 7.03 (d, 1H), 6.95 (d, 1H), 2.51(d, 1H), 1.65 CH_3 peak.

Synthesis of Complexes

$[Ru(phen)_2(ippip)(ClO_4)_2.\ 3.5H_2O$

A mixture of $[Ru(phen)_2Cl_2]\cdot 2H_2O$ (0.531 g, 1.0 mmol), ippip (0.489 g,1.0 mmol) and ethanol (15 mL) was thoroughly deoxygenated. The purple mixture was heated for 8 h at 120 °C under N_2 atmosphere. When the solution finally turned red, it was cooled to room temperature and an equal volume of saturated aqueous sodium perchlorate solution was added under vigorous stirring. The red solid was collected and washed with small amounts of water, ethanol, and diethyl ether, then dried under vacuum, (yield= 60%). Analytical data for $RuC_{46}H_{39}N_8Cl_2O_{12.}$ ES^+-MS cal: 1,060 found: 1,059. IR: 1,479 (C=C), 1,598 (C=N), 721 (Co–N (L)), 623 cm^{-1} (Co–N(ippip)). ^{1}H NMR (DMSO, TMS): 9.09 (d, 6 H), 8.74 (d, 6 H), 8.50 (d, 4H), 8.28 (s, 4H), 8.13 (t, 4H), 8.02(t, 2H), 7.99 (d, 2H), 7.53 (d, 1H), 7.35(d, 1H), 3.61 (d, 1H), 1.8 CH_3 peak. ^{13}C NMR (200 MHz, DMSO, 298 K, major peaks) δ: 155.71, 155.38, 151.56, 151.30, 143.61, 143.42, 131.34, 127.32, 127.07, 126.87, 33.44, 23.64.

$[Co(phen)_2(ippip)](ClO_4)_3.\ 4H_2O$

A mixture of $[Co(phen)_2Br_2]$Br.3H2O (0.531 g,1.0 mmol), ippip (0.489 g,1.5 mmol) in ethanol (20 cm^3) was refluxed for 4 h to give a yellow solution. After filtration, the complex was precipitated by addition of a saturated ethanolic solution of $NaClO_4$. The complex was filtered and further dried under vacuum before recrystallization (Me_2CO-Et_2O). (Yield=75%) Analytical data for $CoC_{45}H_{38}N_8Cl_3O_{16}$. ES^+-MS calc:1,128 found:1,126. IR: 1,541 (C=C), 1,430 (C=N), 714 (Co–N (L)), 624 cm^{-1} (Co–N(ippip)). ^{1}H NMR (DMSO, TMS):δ 9.58(d, 6 H), 9.43(d, 6 H), 8.76 (d, 4H), 8.59 (s, 4H), 8.34 (t, 4H), 7.9(t, 2H), 7.74 (d, 2H), 7.55,(d,1H), 7.20,(d,1H), 3.8(d,1H), 1.6 CH_3 peak.

$[Ru(bpy)_2ippip)(ClO_4)_2.\ 3\ H_2O$

This complex was synthesized as described above complex, (yield=63%), analytical data for $RuC_{42}H_{34}N_8Cl_2O_{11}$. ES^+-MS calc: 1,004 found: 1,002. IR: 1,601 (C=C), 1,448 (C=N), 724 (Co–N (L)), 626 cm^{-1} (Co–N(ippip)). ^{1}H NMR ((DMSO, TMS), 300 MHz, TMS):

8.34 (d, 2H), 8.23(t, 2H), 8.16 (d, 2H), 8.10 (t, 3H), 8.0 (d, 2H), 7.971(m, 2H), 7.97(q, 4H), 7.93(d, 2H) 7.85(d, 2H), 7.7471(d, 2H), 7.5(d, 2H), 7.4(d, 1H), 7.34(d, 1H), 3.01(d, 1H), 1.5 CH_3 peak. ^{13}C NMR (200 MHz, DMSO, 298 K, major peaks) δ:152.87, 152.69, 150.91, 150.91, 150.165, 147.30, 147.22, 145.37, 136.86, 130.51, 128.13, 127.16, 126.75, 126.40, 126.10, 33.47, 23.77.

$[Co(bpy)_2(ippip)](ClO_4)_3.\ 2H_2O$

This complex was synthesized as described above complex, with a mixture of $[Co(bpy)_2Br_2]Br.3H_2O$ and ippip (0.564 g, 1.42 mmol), (yield=60%), analytical data for $CoC_{42}H_{34}N_8Cl_3O_{14}$. ES^+-MS cal: 1,042 found: 1,040. IR: 1,560 (C=C), 1,448 (C=N), 718 (Co–N (L)), 622 cm^{-1} (Co–N(ippip)). ^{1}H NMR, (DMSO, TMS):δ 9.46(d, 2H), 9.44(d, 4H), 9.40(d, 2H), 9.1(m,4H), 8.72(d, 2H), 8.62(t, 4H), 8.42 (t, 2H), 8.15(m, 4H), 7.65(d, 2H), 7.5(d, 1H), 7.38(d, 1H), 3.1(d, 1H), 1.5CH_3 peak. (Molecular structure of complexes Fig. 1).

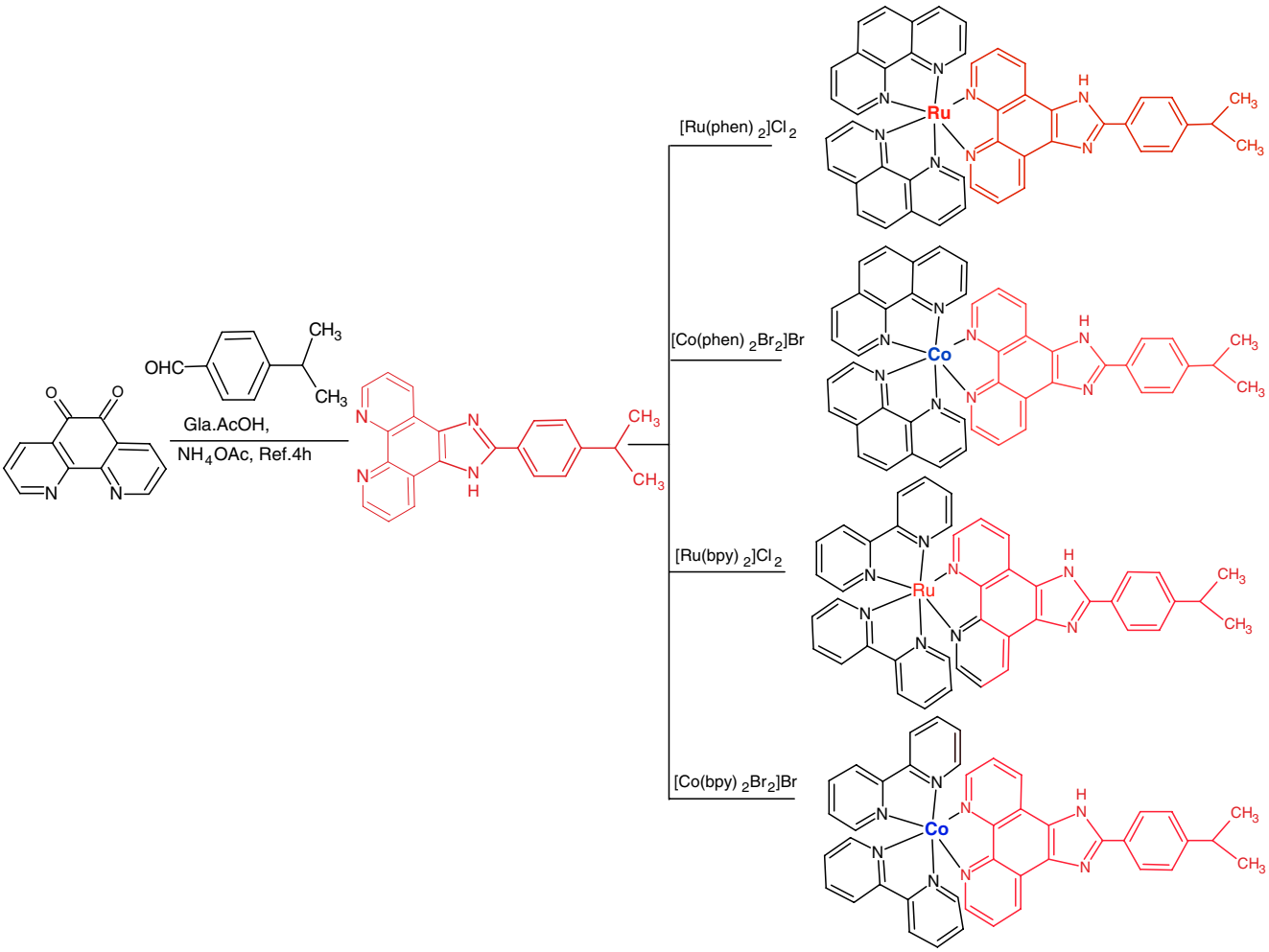

Fig. 1 Molecular structure of complexes

Physical Measurements

UV-Visible spectra were recorded in an *Elico Bio*-spectrophotometer model *BL198*. IR spectra were obtained in KBr phase in a *Perkin-Elmer FTIR-1605* spectrophotometer. NMR spectra were measured with a *Varian XL-400* MHz spectrometer with DMSO as solvent at room temperature and TMS as the internal standard. ES^{+}-MS mass spectra were recorded. Microanalyses (C, H, N) were carried out with a *Perkin-Elmer 240* elemental analyzer. Fluorescence measurements were performed in a *HitachiF 4500* spectrofluorimeter.

Spectrophotometric titrations were carried out at room temperature to determine the binding affinity of complexes to DNA. For this, the reference cuvette contained tris-buffer (pH 7.0, 3.0 ml). The complex (30 μm in buffer, 3.0 ml) was taken in another sample cuvette. Spectra were recorded in the range of 200–600 nm. During the titration small aliquots of buffered DNA (1–10 μl, ~5 to 10 mM in NP) were added to both solutions mixed for ~5 min and then the spectra were recorded. Titration was continued as above till there was no further change in absorption indicating saturation of binding. Change in concentration of complex due to dilution was negligible in view of the small volumes of DNA solution added. The above procedure was repeated minimum of three times.

Fluorescence emission titration experiments were performed at a fixed metal complex concentration (10 μM) to which DNA (1–160 μM) was added in a volume of (10–100 μL). Excitation wavelength was kept constant, emission was recorded. Fluorescence emission enhancement was based on comparison of emission intensity at 558 nm of complexes in the absence and presence of CT DNA.

Dialysis experiment were conducted at room temperature with 3 cm^3 of CT-DNA(120 μM) sealed in a dialysis bag and 6 cm^3 of complexes(20 μM) outside the bag with the solution stirring for 24 h.

Viscosity was determined in an Ostwald viscometer at 30.0±0.1° in a thermostated water-bath. CT DNA (~200 base pairs) was prepared by sonication [21]. Data are

Table 1 Absorption spectrophotometric titration and thermal denaturation of DNA bound to complexes 1–4

Complexes	Excitation peaks	Emission peaks	UV/Vis peaks (nm)	Δλ	Absorption λ_{max} (nm)		TM °C	Hypochromism (%)
					Free	Bound		
DNA alone		–	260 nm	–	–		60	
1	274, 329, 383 nm	319, 358, 582 nm	298, 366, 446 nm	4	366	370	67	20%
2	358, 403, 451 nm	380, 359, 409 nm	297, 349 nm	3	297	300	66	19%
3	268, 318, 405 nm	360, 410, 591 nm	256, 288, 446 nm	3	446	449	64	17%
4	356, 452, 405 nm	370, 360, 409 nm	362, 302 nm	3	307	310	64	15%

presented as $(\eta/\eta_o)^{1/3}$ vs the concentration of complex bound to DNA, where ŋ is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were based on flow time of DNA-containing solution (t>100 s) corrected for flow time of buffer alone (t_o), $\eta=t-t_{o/}\ t_o$ [22].

Thermal denaturation studies were carried out with an *Elico Bio*-spectrophotometer model *BL198*, equipped with temperature-controlling programmer (±0.1 °C). The absorbance at 260 nm was continuously monitored for solutions of CT-DNA (100 μM) in tris-buffer in the absence and presence of the complex (10 μM), with temperature

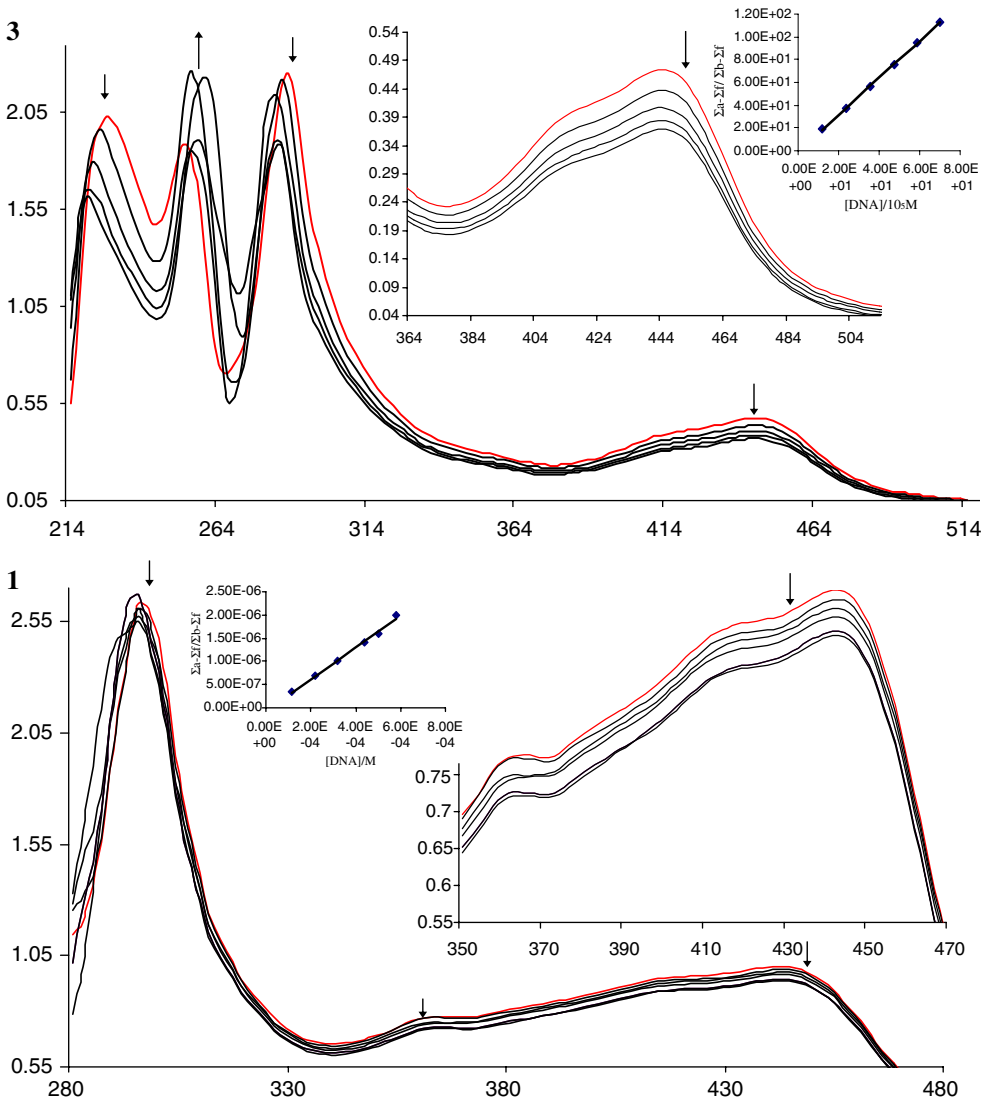

Fig. 2 Absorption spectra of complexes **3** and **1** in absence (*red*) and presence (*lower*) of DNA. [complex]=10 μM; [DNA]/complex]=0, 5, 10, 15. *Arrow* shows the absorbance changes upon increasing DNA concentrations. Insert plots of $[DNA]/[\varepsilon_a-\varepsilon_f]$ *vs* [DNA] for the titration of DNA with complexes; (•) experimental data points; solid lines, linear fitting of the data

increments of 1 °C, min^{-1}. Supercoiled pBR322 DNA and complex mixture was irradiated for 1 h at λ_{max}=365 nm. All samples were analyzed by electrophoresis for 2.5 h at 40 v/cm on a 0.8% agarose gel in buffer, pH 8.2, gel DNA was visualized by staining with 1 μg/ml ethidium bromide and then photographed under UV light.

The cell line HL-60 (human myeloid leukemia) was obtained from the National Centre for Cellular Sciences (NCCS), Pune, India. HL-60 cells were cultured in RPMI 1640 media supplemented with 10% (*v*/*v*) heat inactivated fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin. HL-60 cell lines were maintained in culture at 37 °C in an atmosphere of 5% CO_2.

Spectroscopic Characterization

The IR spectral data for the complexes exhibit bands at 1,458 cm^{-1} and at 1,578–1,590 cm^{-1} due to C=C and C=N vibrations of the ring respectively. Bands were present around 587 cm^{-1} and 570 cm^{-1} corresponding to Co–N (bpy) or Co–N of (phen). In the ^{1}H-NMR spectra of the complexes the peaks due to various protons of ligand shifted downfield compared to the free ligand suggesting complexation. As expected, the signal for the ligand appeared in the range around 7 to 9.8 ppm and the 6 hydrogens of the methyl group peaks appeared at 1.5–1.8 ppm

Results and Discussion

DNA-binding Studies

The absorption titrations of Ru(II) & Co(III) complexes in buffer were performed by using a fixed concentration of ruthenium or cobalt complexes concentration to which increments of the DNA stock solution(conc 4.5×10^{-4}) were added. Ruthenium (or) cobalt-DNA solutions were allowed to incubate for 5 min before the absorption spectra was recorded. The intrinsic binding constants K_b of complexes to DNA were derived as follows [23].

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/(K_b(\varepsilon_b - \varepsilon_f))$$

where [DNA] is the concentration of CT-DNA in base pairs, the apparent absorption coefficients ε_a, ε_f, and ε_b correspond to A_{obsd}/[Ru](or)[Co], the absorbance for the free complex, and the absorbance for the complex in fully bound form, respectively. K_b is given by the ratio of slope to intercept.

Absorption Titration

The absorption spectra of 1, 2, 3 and 4 complexes shows three or two well resolved bands in the range of 200–600 nm. The bands below 300 nm are attributed to intraligand (IL) π–π* transitions, the band at 354 nm is attributed to the π–π* transition and the lowest energy bands at 446, 448, 349 and 362 nm for complexes 1, 2, 3 and 4 are assigned to the metal-to-ligand charge transfer (MLCT) transitions (Table 1) by comparison with the spectra of other polypyridyl Ru(II) complexes [24–27]. The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques. The complex binding with DNA through intercalation usually results in hypochromism and bathochromism. The interaction of these complexes with CT-DNA was monitored by the absorption titration method. In the presence of increasing CT-DNA, both complexes 1, 3 showed a strong decrease in intensity (hypochromicity) and a small red shift Fig. 2. The change in the absorbance of the MLCT band with increasing amounts of CT-DNA was used to derive the intrinsic binding constants (K_b). The values of K_b derived for 1, 2, 3, and 4 complexes are $2.1\pm0.2\times10^5$, $1.9\pm0.2\times10^5$, $8.5\pm0.2\times10^4$ and $7.32\pm0.2\times10^4$ respectively. The different DNA-binding properties of complexes 1, 2, 3, and 4 are due to the different ancillary ligand. On going

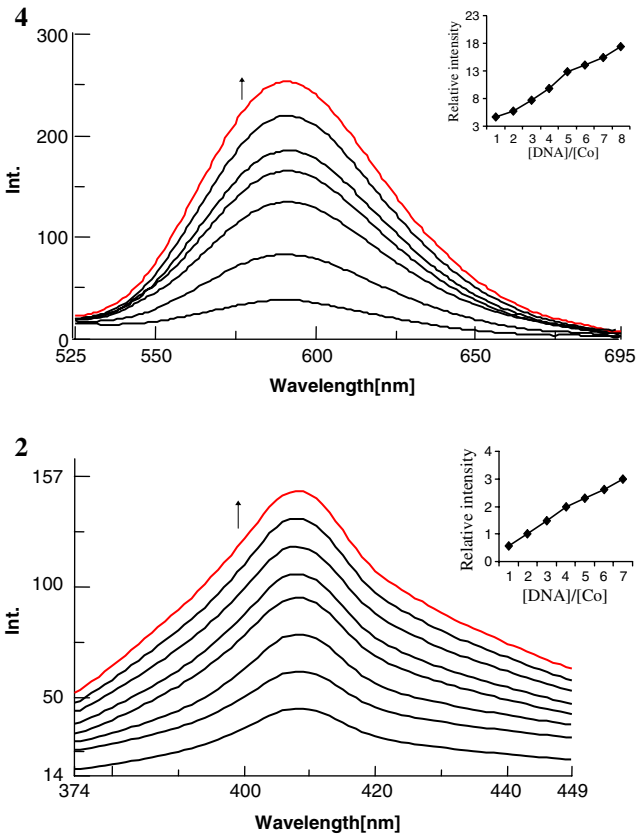

Fig. 3 Fluorescence emission spectra of complexes **2** and **4** in tris buffer in the presence of CT DNA, where [cobalt or ruthenium]= 20 μM, [DNA]/Co=0, 5, 10, 15, 20 etc. The *arrows* indicate the intensity changes upon increasing concentration. Insert: plots of relative integrated emission intensity versus [DNA]/[Co]

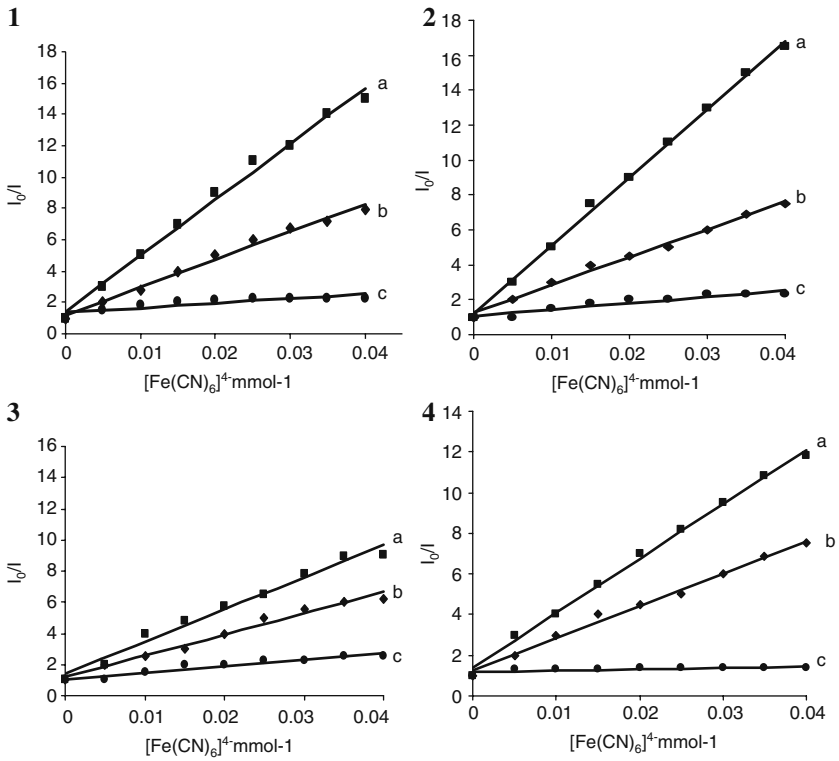

Fig. 4 Emission quenching curves of complexes (**1**), (**2**), (**3**), and (**4**) in absence of DNA (**a**) presence of DNA (1:20) (**b**), excess of DNA (1:200 μM) (**c**)

from bpy to phen, the plane area and hydrophobicity increases, and leading to a greater DNA-binding affinity for complex 1. The intrinsic binding constant K_b of four complexes with DNA is in the order 1>2>3>4.

Fluorescence Quenching

Complexes 1, 2, 3 and 4 exhibit fluorescence in tris buffer at ambient temperature with λ_{max} at 582, 408, 405 and 409 nm, addition of CT-DNA, the fluorescence emission intensities of 1, 2, 3 and 4 increased by a factor of 4.2, 3.2, 2.1 and 1.2 respectively. This indicates that the strong intercalation with DNA and the consequent restriction of mobility of the complexes at the binding site has the effect of enhancing, emission intensity Fig. 3. As per the procedures described [21], fluorescence quenching experiments were performed at ambient temperature with $K_4[Fe(CN)_6]^{4-}$ as the quenching agent. From the data for all complexes shown in Fig. 4, maximal quenching of fluorescence of DNA bound complex is seen with complex 1 and this effect decreases as one goes from complex 1 to complex 4.

Viscosity Measurements

Since Intercalation result in enhanced DNA viscosity [21, 22], viscosity of DNA in presence of complexes 1–4, have been determined as shown in Fig. 5. All complexes lead to increased relative viscosity of DNA. Based on relative magnitude of viscosity binding affinity of complexes to DNA follows the order 1>2>3>4 in agreement with the conclusion that these complexes intercalate between the base pairs of DNA.

Thermal Denaturation

To examine the effects of complex binding on thermal denaturation, all complexes were allowed to interact with DNA according to the conditions detailed and were heated

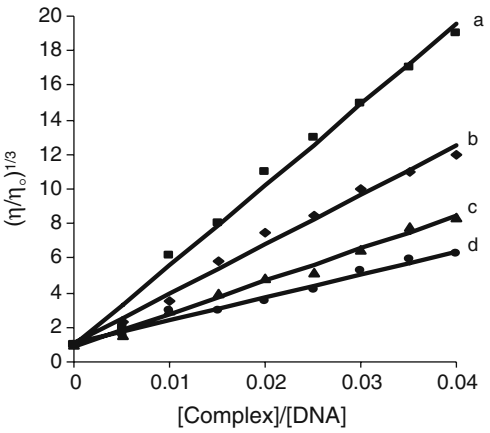

Fig. 5 Effect of increasing amount of complexes on the relative viscosities of CT DNA at 25±0.1 °C complex -**1** (**a**), complex-**2** (**b**), complex -**3** (**c**) and complex -**4** (**d**)

from room temp up to 80 °C to obtain DNA melting curves from which T_m values were derived. Free un-complexed CT DNA used herein had a T_m=60 °C. T_m for each complex was determined as the difference between T_m of complex + DNA and T_m of DNA alone. On this basis T_m of complex increased by 4–7 °C, in the order 1>2>3>4 as shown by the data of Table 1. Thus the binding of complexes did enhance the stability of DNA and furthermore, strength of binding was greatest for complex 1, decreasing in the same order as indicated by other data of this study. (Table 1).

For the gel electrophoresis experiment, Supercoiled pBR322 DNA (0.1 l g) was treated with the Ru(II) & Co (III) complexes in 50 mM Tris–HCl, 18 mM NaCl buffer, and the solution was then irradiated at room temperature with a UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis for 1.5 h at 80 V on a 0.8% agarose gel in TAE buffer. The gel was stained with lg/ml-**1** ethidium bromide and photographed under UV light.

Photoactivated Cleavage of pBR322 DNA

There is substantial and continuing interest in DNA endonucleolytic cleavage reactions that are activated by metal ions [28, 29]. The cleavage reaction on plasmid DNA (pBR322 DNA) can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoil form (Form I). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower-moving open circular form (Form II). If both strands

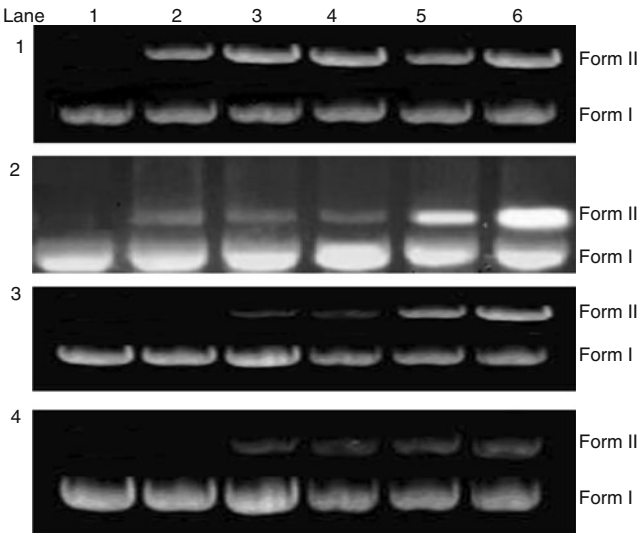

Fig. 6 Photocleavage of pBR 322 DNA, in absence and in the presence of complexes **1**, **2**, **3** and **4** light after 60 min irradiation at 365 nm. Lane 1 control plasmid DNA (untreated pBR 322), lanes 2 to 6 addition of complexes, in amounts of (0.01 M) 5, 10, 20, 30, 40 μl of complex concentraction

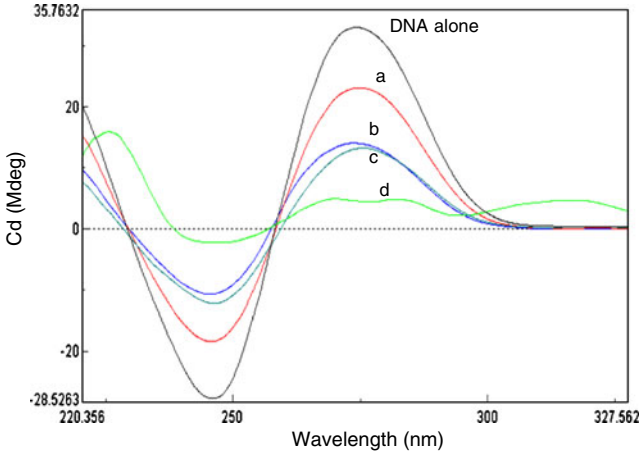

Fig. 7 Circular dichroism spectra of the dialysate of complexes **1** (**a**), **2** (**b**), **3** (**c**) and **4** (**a**) after 24 h dialysis against with CT DNA

are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated [30]. The cleavage effect upon irradiation of the plasmid pBR322 DNA in the presence of different concentrations of complexes 1, 2, 3, and 4 have been tested and shown in Fig. 6 Lane 1 DNA alone, lanes 2–6 DNA + complexes with increasing concentration of complex. Form II increases of and, Form I decreases gradually as the concentration of complex increase. The results suggest concentration-dependent single-strand cleavage of supercoiled Form I to the nicked Form II. Under comparable experimental conditions, complex 1 exhibits more effective DNA cleavage activity than complexes 2, 3, and 4. The different cleaving efficiency may be ascribed to the different binding affinity of complexes to DNA.

Enantioselective Binding

A difference in biological activities between the enantiomers of an optically active metal complex has been noted in many examples such as toxicity and drug efficiency [31, 32]. In order to understand such affects better at the level of molecular interaction. It is of therefore important to examine enantiomeric effects on the DNA binding of a metal complex. According to the insertion model proposed by Barton and coworkers [22, 33], the Δ enantiomer of the complex, a right-handed propeller-like structure, will display a greater affinity than the Λ enantiomer with the right-handed CT-DNA helix, due to the appropriate steric matching. This discrimination can be observed via equilibrium dialysis experiments and provide strong evidence in support of intercalation.

The CD spectrum of CT DNA exhibits a positive band at 274 nm due to base stacking and a negative band at 243 nm due to the helicity of DNA. In the presence of complex an increase in the molar eliplicity values of the both positive and negative band of the CT DNA observed Fig. 7. It can

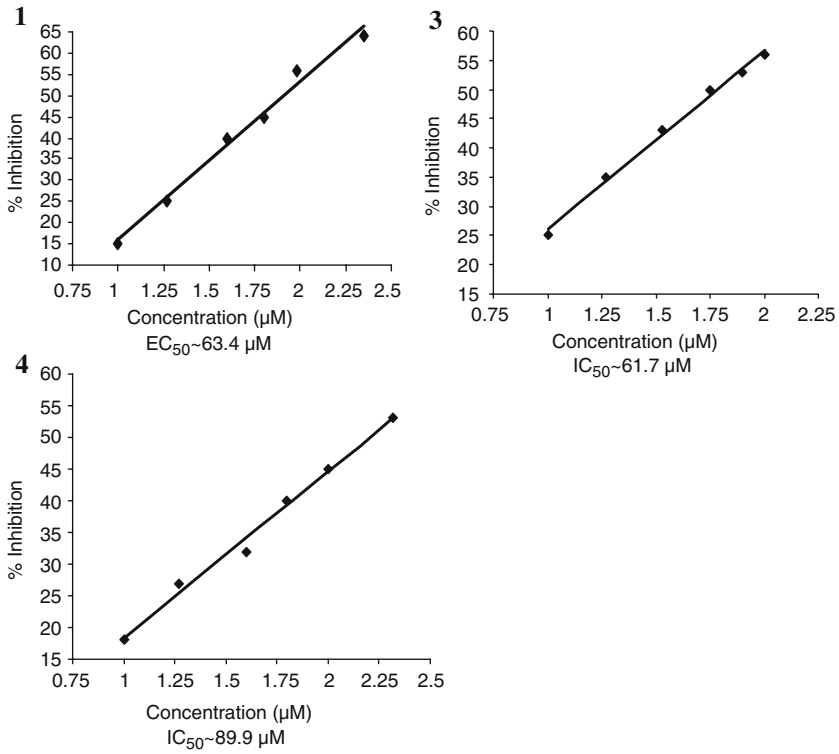

Fig. 8 Effects of complexes on the viability of HL-60 cells, following continuous incubation for 24 h, with increasing drug concentration (0.1–500 μM)

be concluded that the interaction of all the complexes towards CT-DNA follows the same order as the former experiments. Those significant changes indicate conformational changes and unwinding of DNA base pairs with destabilization of the DNA double helix, which is consistent with DNA intercalation mode suggested above.

Cytotoxicity Studies

Cell proliferation or viability was measured using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrasodium bromide] assay [34]. Cells were seeded in each well containing 100 μl medium at a final density of 2×10^4 cells/well, in 96 well microtiter plates at identical conditions. After overnight incubation, the cells were treated with different concentrations of testing ligand complexes (20–100 μg/ml in RPMI 1640 medium and filtered) in a final volume of 200 μl with five replicates each. After 24 h, 10 μl of MTT (5 mg/ml) was added to each well and the plate was incubated at 37 °C in the dark for 4 h. The formazan crystals were solubilized in DMSO (100 μl/well) and the reduction of MTT was quantified by absorbance at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFT max PRO 3.0). Effects of test compounds on cell viability were calculated using cells treated with DMSO as control. The data were subjected to linear regression analysis and the regression lines were plotted for the best straight line fit Fig. 8. The IC_{50} (inhibition of cell viability) concentrations were calculated using the respective regression equation.

Observations

The cytotoxic effects of test complexes were investigated on human leukemia cell lines. After 24 h of treatment, the number of live cells were measured by MTT assay and IC_{50} values for each cell line were determined (Table 2). It is evident from Table 1 that HL-60 cells were sensitive to

Table 2 CD data for the interaction of CT DNA and Cytotoxic data of complex 1 to 4

Complexes	Positive peak	Negative peak	Cytotoxic activity of test complexes(IC_{50} values in μM)
DNA alone	274 243 nm	−246 nm	
1	273, 242 nm	−244 nm	63.4 μM
2	275, 243 nm	−246.1 nm	No activity
3	275.2 243 nm	−247 nm	61.7 μM
4	276.2 242 nm	−246.8 nm	89.9 μM

complex 1 and 3, with an IC_{50} value of 61.7 and 63.4 μg/ml, as compared with complex 4. Complex 2 did not show any activity against the HL-60 cells and the decreasing order of activity among the test complexes was in the order 1>3>4 Fig. 8. The chemical structure of the mixed ligand complexes could be a factor for exhibiting differential anti-proliferative activities on the cancerous cell lines.

Conclusions

Four Ru(II) and Co(III) complexes of extended planar aromatic ligands have been isolated, characterized and their interaction with CT DNA examined. Absorption, Emission, thermal denaturation and viscosity experiments were performed and the result obtained suggests an intercalative mode of DNA binding for the four complexes. The planarity of the ligands plays an important role in dictating DNA binding affinity. Thus the complex 1 binds to DNA more strongly than remaining complexes binding, affinity follows the order 1>2>3>4.The present study demonstrates that the ancillary ligands with hydrogen bonding potential support the intercalative interaction of ligands with extended aromatic rings and enhances the DNA binding affinity.

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