Research Article

DNA Binding Studies, Light Switch, Photocleavage, *In Vitro* Cytotoxicity, Antibacterial and Docking Studies of Ru(II)/Co(III) Complexes

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ABSTRACT

To explore the therapeutic potential of Ru(II)/Co(III) complexes, of the type $[Co(dmb)_2IIP](ClO_4)_3$ (1) and $[Ru(dmb)_2(IIP)](ClO_4)_2$ (2), (IIP = 2-(1H-Indol-3-yl)-1H- imidazo[4,5-f][1,10]phenanthroline) were synthesized and thoroughly characterized. *In vitro* DNA binding studies of two complexes were carried out employing UV–vis titrations, fluorescence, thermal denaturation and viscosity measurements which revealed that the complexes 1 and 2 bind to CT DNA preferably via groove binding. Upon irradiation at 365nm, the two complexes were found to promote the cleavage of plasmid pBR322 DNA from super coiled form I to nicked form II. Further in the presence of Co^{2+}, the emission of DNA–Ru(II)/Co(III) complexes can be quenched. And when EDTA was added, the emission was recovered. The experimental results show that all two complexes exhibited the ''on–off–on" properties of molecular ''light switch". The binding events were further validated by molecular docking studies. Complex 2 shows higher activity than complex 1 against the selected tumor cell lines. Additionally, the complexes were evaluated for antibacterial activity against *Escherichia coli* and *Staphylococcus aurous* and 2 was found to be most effective against Gram-positive bacteria.

Keywords: Antibacterial, DNA-binding, Docking, *in vitro* Cytotoxicity, Light switch, Photocleavage, Ru(II)/Co(III)complexes.

INTRODUCTION

Transition metal complexes are being explored with great interest in nucleic acid and peptide chemistry since they find use in a wide range of applications, such as diagnostic and therapeutic agents.[1–5] Compounds capable of cleaving DNA upon light irradiation are used in the photodynamic therapy of cancers (PDT), which involves a non-invasive treatment of tumors using photoactive drugs.[6,7] Photoactive pro-drugs capable of releasing a cytotoxic drug only at the irradiated site can increase specificity and thereby minimize toxicity to the surrounding healthy cells. Porphyrin based compounds, like photofrin and their analogues, have found clinical applications in PDT.[8,9] However, due to hepatotoxicity, there is an immediate need to search for new non-porphyrinic organic and inorganic compounds which overcome this defect.[10] Transition metal complexes with polypyridyl ligands are known to exhibit photo induced DNA cleavage. Some metal polypyridyl complexes, such as $[Ru(phen)_2dppz]^{2+}$, $[Ru(bpy)_2dppz]^{2+}$, $[Ru(bpy)_2dpq]^{2+}$, $[Co(phen)_2H_2biim]^{3+}$ and $[Co(bpy)_2\ H_2biim]^{3+}$ etc., are known to be photocleavers of DNA.[11,12]

Among them, Ru(II) polypyridyl complexes are extensively studied owing to their rich photo-physical, photochemical and redox properties. These studies have been extended to other transition metals such as Fe(III), Co(III), Ni(II), Zn(II), Cu(II), Rh(II) and Pt(II), but to a lesser extent. Among the less studied systems, cobalt(III) polypyridyl complexes also have interesting photo-physical properties and cleave DNA photolytic ally.

In this article, the comparative study on the interaction of the title complex with calf thymus DNA (CT-DNA) have been investigated by UV–visible (UV–vis) spectroscopy, fluorescence spectroscopy, viscosity, as well as thermal denaturation. Also, the antitumor activities of the complex have been evaluated by MTT method (MTT=(3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyl-tetrazolium bromide). We hope the results to be of value in understanding the mechanism of the interactions of metal complexes with nucleic acids, and should be useful in the development of nucleic acid molecular probes and new therapeutic reagents.

MATERIALS AND SOLUTIONS

Most of the reagents were of analytical grade and were used as supplied. 1, 10-phenanthrolinemonohydrate was purchased from Merck (India). Calf-thymus(CT) DNA, 2-(1H-Indol-3-yl)-1H- imidazo[4,5-f][1,10]phenanthroline) and 4,4′-dimethyl- 2,2′-bipyridine were obtained from Sigma. The pBR-322 DNA (Fermentas) was used as received. All other common chemicals and solvents were procured from locally available sources. The super-coiled pBR322, DNA was obtained from Fermentas life sciences. Calf-thymus DNA (CT-DNA), sodium chloride (NaCl), thiazole orange and ethidium bromide were purchased from Sigma Chemical Co. (India). All the solvents were purified before use as per standard procedures. Deionized, double-distilled water was used for preparing various buffers. Solutions of calf thymus DNA (CT-DNA) in 50 mM NaCl–5 mM Tris–HCl (pH 7.2) gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free of protein.[13] The concentration of DNA was determined spectrophoto metrically using a molar absorptivity of 6600 M^{-1} cm^{-1} (260 nm).[14]

Synthesis of ligand and complexes

1,10-Phenanthroline-5,6-dione [15] , 2-(1H-Indol-3-yl)-1H-imidazo[4,5-f][1,10] phenanthroline)[16], $[Co(dmb)_2Br_2]Br.3H_2O$ [17] and $[Ru(dmb)_2Cl_2]^{2+}$ [18] was synthesized by the reported procedure. Figure 1 shows Synthesis and structure of the complexes.

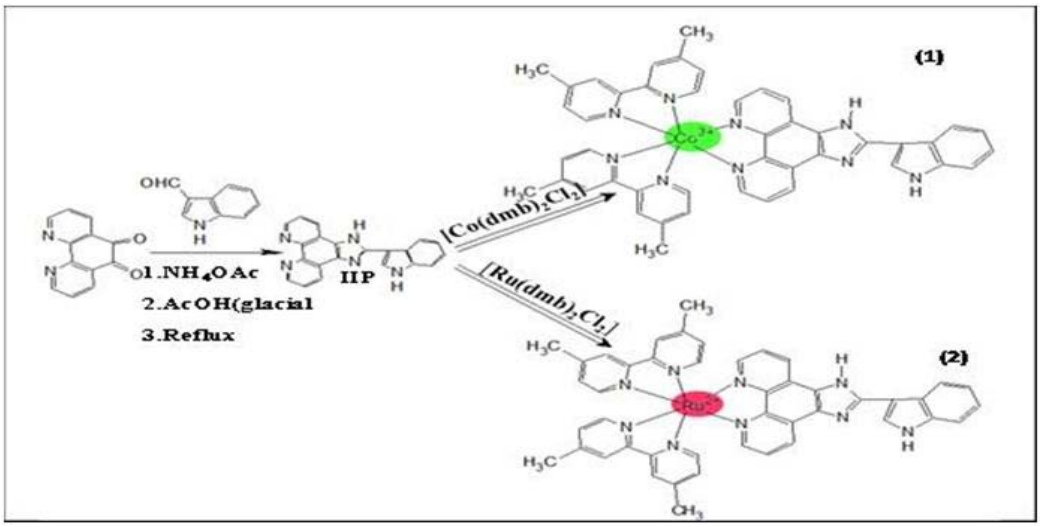

Figure 1: Synthesis and structure of the Ru(II)/ Co(III) complexes

Synthesis of $[Co(dmb)_2IIP](ClO_4)_3.\ 2H_2O$ (1)

A mixture of cis-$[Co(dmb)_2Br_2]Br.3H_2O$ (0.74 g, 1.0 mmol) and IIP (0.501 g, 1.5 mmol) in ethanol (20 ml) was refluxed for about 4 h. Then cooled to room temperature and the solution was filtered, and the complex precipitated by addition of a saturated ethanolic solution of $NaClO_4$, the light yellow color solid ,dried under vacuum and recrystallizaed from acetone-ether, (yield, 77%), analytical data for $CoC_{45}H_{37}N_9Cl_3O_{14}$, calculated: C 71.61 N 16.70 H 3.87 found: C 69.2 N 16.01 H.3.23. LCMS in DMSO M/Z:1092 found:1091. IR: 1446 (C=C), 1486 (C=N), 530 (Co–N (dmb)), 626cm^{-1} (Co–N(IIP)). ^{1}H-NMR (DMSO-d_6, δ ppm): 8.82(d, 6H), 8.62(d, 2H), 8.09(m, 6H), 7.60 (d, 4H), 7.52 (m, 2H), 7.40, (s, 1H), 7.20(s, 1H), 2.55(s,6H), 2.45 (s,6H). ^{13}C-NMR (100 MHz, DMSO-d_6, 298K, δ ppm, major peaks): 153.5, 152.01, 146.9, 143.90, 142.22, 135.53, 132.05, 129.82, 126.5, 120.11.

Synthesis of $[Ru(dmb)_2IIP](ClO_4)_2.\ 2H_2O$ (2)

A mixture of *cis*-$[Ru(dmb)_2(Cl)_2]\cdot 2H_2O$ (0.10g,0.16mmol) and (IIP) (0.076 g, 0.16 mmol) in ethanol (30 mL) was refluxed under nitrogen for 8h to give a clear red solution. After cooling, the clear solution was filtered. The filtrate was treated with a saturated solution of $NaClO_4$ and a red precipitate was obtained. The solid was collected washed with small amounts of water, ethanol, and diethyl ether, then dried under vacuum, yield (62%), and analytical data for $RuC_{45}H_{37}N_9Cl_2O_{10}$:found: C 67.83,H 3.67, N 15.82 Calcd (%) C: 67.52; H: 3.72; N: 14.75 LCMS in DMSO M/Z: 1033. IR: 1482 (C=C), 1621 (C=N), 747 (Ru–N (dmb)), 629 cm^{-1} (Ru–N(IIP). ^{1}H-NMR (DMSO- d_6, 400 MH_Z δ ppm): 9.10 (d, 2H), 8.95 (d, 4H), 8.80 (d, 2H), 8.70 (d, 2H), 8.53 (d, 2H), 8.30(s, 1H),8.00 (d,2H), 7.95 (d, 2H), 7.65 (d, 2H), 7.15 (d, 2H) 2.50(s,6H), 2.42(s,6H). ^{13}C-NMR (100 MHz, DMSO-d_6, 298K, δ ppm, major peaks): 153.18, 153.04, 147.89, 137.23, 131.00, 128.53, 126.95, 126.72, 126.25, 123.01, 121.73, 121.04, 112.95.

Physical measurements

Infrared spectra were recorded with a Perkin–Elmer FTIR 1605 spectrometer as KBr disks. ^{1}H NMR spectra were collected with a Bruker 400MHz spectrometer with DMSO-d_6 as a solvent at room temperature and TMS as the internal standard. Microanalyses (C, N and H) were performed with a Perkin Elmer 240 elemental analyzer. UV–Visible spectra were recorded on an Elico Bio-spectrophotometer model BL198. Emission spectra were carried out with Elico Bio-spectro fluorimeter mode SL174.

All experiments dealing with the interaction of Ru(II) and Co(III) complexes with calf thymus DNA (ct-DNA) were carried out in 5 mM Tris–HCl buffer (pH 7.1, 50 mM NaCl). Steady-state emission quenching experiments were carried out in Tris–HCl buffer by using $[Fe(CN)_6]^{4-}$ as the quencher. The experiments of DNA thermal denaturation were performed on UV-visible spectrophotometer in a buffer consisting of 1.5mM Na_2HPO_4, 0.5 mM NaH_2PO_4 and 0.25 mM Na_2EDTA.

Viscosity experiments were carried on Ostwald viscometer, immersed in thermostatted water-bath maintained at 30 ± 0.1°C· DNA samples approximately 200 base pairs in average length were prepared by sonication in order to minimize complexities arising from DNA flexibility.[19] Data were presented as $(\eta/\ \eta_0)^{1/3}$ versus concentration of [Ru or Co]/[DNA], where η is viscosity of DNA in the presence of complex, and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t > 100 seconds) corrected for the flow time of buffer alone (t_0),

$\eta = (t'-t_0)/t_0$.[20]

Thermal DNA denaturation experiments were carried out with a Perkin–Elmer Lambda 850 spectro photometer equipped with a Peltier temperature-control programmer (±0.1 °C). The temperature of the solution was increased from 50 to 95 °C at a rate of 1 °C min^{-1} and the absorbance at 260 nm was continuously monitored for solutions of CT-DNA (42 µM) in the absence and presence of the Ru(II) complex (20 µM). The data were presented as $(A - A_0)/(A_f - A_0)$ vs. the temperature, where A_f, A_0 are the final, initial absorbance, A is the absorbance at any given concentration of DNA added at 260 nm, respectively.

For the gel electrophoresis experiment, super-coiled pBR322 DNA (100 µM) was treated with the complexes in the buffer (50 mM Tris–HCl, 18 mM NaCl, pH 7.2), and the solution was then irradiated at room temperature with a UV lamp (365 nm, 10W). The samples were analyzed by electrophoresis for 1.5 h at 80 V on a 1% agarose gel in Tris–HCl buffer. The gel was stained with 1µg/mL ethidium bromide and photographed.

Cytotoxicity assay *in vitro*

Standard 3-(4,5-dimethylthiazole)-2,5-diphenyltetra azolium bromide (MTT) assay procedures were used.[21] Cells were placed in 96-well microassay culture plates (8 x 10^3 cells per well) and grown overnight at 37 °C in a 5% CO_2 incubator. Compounds tested were then added to the wells to achieve final concentrations ranging from 10^{-6} to 10^{-4} M. Control wells were prepared by addition of culture medium (100 mL). The culture medium and doxorubicin were used as negative and positive control, respectively. The plates were incubated at 37 °C in a 5% CO_2 incubator for 48 h. Upon completion of the incubation, stock MTT dye solution (20 mL, 5 mg mL^{-1}) was added to each well. After 4 h incubation, buffer (100 mL) containing N,N-dimethylformamide (50%) and sodium dodecyl sulfate (20%) was added to solubilize the MTT formazan. The optical density of each well was then measured on a micro plate spectrophotometer at a wavelength of 490 nm. The IC_{50} values were determined by plotting the percentage viability versus concentration on a logarithmic graph and reading off the concentration at which 50% of cells remain viable relative to the control. Each experiment was repeated at least three times to get the mean values. Four different tumor cell lines were the subjects of this study HeLa (ATCC No. CCL-2) derived from human cervical cancer cells, A549 (ATCC No. CCL-185) derived from human alveolar adenocarcinoma epithelial cells, MDA-MB-231 (ATCC No. HTB-26), and MCF7 (ATCC No. HTB-22) derived from human breast adenocarcinoma cells.

Docking studies

The DNA crystal structure of the DNA decamer (GGTAGCGATGG) (PDB ID: 1y9h), was downloaded from the protein data bank. Gold 3.0.1 (Genetic Optimization for Ligand Docking) program, [22, 23] which is based on Genetic Algorithms used for docking studies. This method allows partial flexibility of the hydroxyl groups of the respective DNA molecule and full flexibility of the ligand. In general, the docking parameters of Gold 3.0.1 were kept to their default values. The results were visualized using the Silver Descriptor 1.1 Viewer package. In order to evaluate the GOLD scoring function, all water molecules were removed from the DNA molecules. The function fitted was Gold Score:

Fitness = S(hb – ext) + 1.3750 x S(vdw- ext) + Sint

where S(hb- ext) is the DNA-ligand hydrogen bond score, S(vdw- ext) is the DNA-ligand van der Waals score, Sint is the score from intramolecular ligand interactions.[24]

***In vitro* DNA-binding studies**

It is a well-known fact that DNA is the primary pharmacological target of many antitumor compounds, and hence, the interaction between DNA and metal complexes is of paramount importance in understanding the mechanism of binding. Thus, the modes of binding of complexes 1 and 2 to CT DNA were studied with the aid of different techniques.

Electronic absorption titration

The absorption spectra of complex 1 and 2 in the absence and presence of CT-DNA (at a constant concentration of complexes) are given in Figure 2. In the presence of DNA, the absorption bands of **1** and **2** at about 318, 451 nm exhibited hypochromism of about 10.7% and 13.2% and bathochromism of about 5 and 8 nm, respectively. The spectroscopic changes suggest that the complexes have stronger interaction with DNA. Based on these observations we presume that there are some interactions between the complexes and the base pairs of DNA. In order to compare quantitatively the binding strength of the three complexes, the intrinsic binding constants K_b of the three complexes with CT-DNA were obtained by monitoring the changes of absorbance in the MLCT band with increasing concentration of DNA using the following equation [25] through a plot of $[DNA]/[\varepsilon_a - \varepsilon_f]$ vs [DNA].

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

where [DNA] is the concentration of DNA in base pairs, the apparent absorption co-efficients ε_a, ε_f and ε_b correspond to $A_{obsd/[Ru(II)or\ Co(III)]}$, the extinction co-efficients for the free Ru(II) or Co(III) complex, extinction co-efficients of complex in presence of DNA and the extinction co-efficients for the Ru(II) or Co(III) complex in the fully bound form, respectively. In plots $[DNA]/(\varepsilon_a - \varepsilon_f)$vs[DNA],*K* is given by the ratio of slope to intercept.

The intrinsic binding constants Kb of complexes **1** and **2** were 5.28 ×10^4 M^{-1}, and 1.08×10^5 M^{-1} respectively. The results indicate that the binding strength of complex **2** is stronger than that of **1**. Such a small change in λ max is more in keeping with groove binding, leading to small perturbations. The Kb value obtained here is lower than that reported for classical intercalator for ethidium

bromide and $[Ru(phen)_2DPPZ]$ whose binding constants have been found to be $1.6x10^7$ M^{-1}) [26,27]. The observed binding constant is more in keeping with the groove binding with DNA, as observed in the literature [28,29] These values are smaller than those of $[Ru(phen)_2dppca]^{2+}$ (3.4×10^5) ,$[Ru(bpy)_2dppca]^{2+}$ (2.1×10^5), $[Ru(dmb)_2dppca]^{2+}$ (1.2×10^5) [30], complex1 smaller than $[Co(bpy)_2apip]^3$ (7.3 x 10^4) [31] and greater than $[Ru(bpy)_2DMPIP]^{2+}$ (2.1 ± 0.1 x 10^4) , and $[Ru(dmb)_2DMPIP]^{2+}$ (1.7 ± 0.1 x 10^4 . [32] For ancillary dmb, substitution on the 4- 4-positions of the dmb may cause severe steric constraints near the core of Co(III)/Ru(II) when the complex intercalates into the DNA base pairs. The methyl groups may come into close proximity of base pairs at the intercalation sites. These steric clashes then prevent the complexes **1** and **2** from intercalating effectively, which causes a diminution of the intrinsic constant. Such clashes would not be present with the ancillary bpy ligand.

Luminescence titration studies

In the absence of DNA, complexes **1** and **2** emit weak luminescence in Tris buffer at room temperature, with a maximum appearing at 603 and 609 nm respectively. However, on addition of CT–DNA, the emission intensities of complexes **1** and **2** increase slightly Figure 3 (a). The emission intensities of complexes, **1** and **2** increase to around 1.26 and 1.58 times larger than the original, respectively. This implies that both complexes can interact with DNA and be protected by DNA. Comparing **1** with **2**, it has been found that complex **2** can be protected by DNA more effectively, which is due to the stronger binding affinity of the complex with DNA. Differential luminescence quenching was also utilized in monitoring DNA binding. A highly negatively charged quencher is expected to be repelled by the negatively charged phosphate backbone, and therefore a DNA-bound cationic molecule should be readily quenched. Figure 3 (b) shows the steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher. In the absence of DNA, the two complexes were efficiently quenched by $[Fe(CN)_6]^{4-}$, but when bound to DNA the complexes were protected from the quencher. This may be explained by repulsion between the highly negatively charged $[Fe(CN)_6]^{4-}$ and the DNA polyanion backbone which hinders access of $[Fe(CN)_6]^{4-}$ to the DNA-bound complexes. The quenching studies indicate that the DNA-binding abilities of the complex 2 greater than 1. Steady-state emission quenching experiments using $[Fe(CN)_6]^{4-}$ as quencher were also used to observe the binding of the complexes with CT-DNA. The Stern–Volmer quenching constant (Ksv) can be determined by using Stern–Volmer equation, [33]

$I_0/I = 1 + Ksv\,[Q]$

Where I_0 and I are the intensities of the fluorophore in the absence and presence of quencher respectively, Q is the concentration of the quencher, and Ksv is a linear Stern– Volmer quenching constant. In the quenching plot of I_0/I versus [Q], Ksv is given by the slope. Figure 3 (b) shows the Stern–Volmer plots for both the free complex in solution and the complex in the presence of increasing amounts of DNA. All the complexes show linear Stern–Volmer plots. The Ksv values for the complexes in the absence of DNA were 152 and 186 complexes (1) and (2) respectively. In the presence of DNA, the Ksv values were 26 and 17 for complexes (1) and (2) respectively. Hence, the Ksv values are smaller in the presence of DNA. At high concentration of DNA (1:200; Ru^{2+}:DNA), the plots have essentially zero slope, indicating that the bound species is inaccessible to quencher.

Light switch on/off

Figure 4 gives the emission spectra of DNA–$[Ru(dmb)_2IIP]^{2+}$ in the absence and presence of Co^{2+}. From Figure 4, it could be seen that after binding to DNA(switch on), the emission of DNA–$[Ru(dmb)_2IIP]$ can be quenched by Cobalt(II) ion, thus turning the light switch off.[34,35] The addition of 1 mM Co^{2+} to 5 µM complex bound to 250 µM DNA results in the loss of almost 98% of the luminescence due to the formation of Co^{2+} $[Ru(dmb)_2IIP]$ heterometallic complex. In order to further provide additional evidence for the quenching originated the formation of heterometallic complex, the emission spectra of $[Ru(dmb)_2IIP]$ without DNA in the absence and presence of Co^{2+} are measured Figure 4. Similar quenching of luminescence is observed. However, the emission can be recovered in the presence of 1.2 mM EDTA Figure 4, thus turning the light switch on. This is because Co^{2+} was removed by EDTA, and Co^{2+}–$[Ru(dmb)_2IIP]^{2+}$ heterometallic complex cannot be formed. The present results should be of value in further developing luminescence DNA probe.

Salt titration

Reverse salt titrations of **1** and **2** bound to DNA were performed, and the results are shown in Figure 5. The binding constant at each titration point was then calculated, and a plot of $\log[K_b]$ vs $\log[Na^+]$ was constructed. From polyelectrolyte theory, the slope of this graph provides an estimate of $SK=(\delta \log[K_b]/ \delta \log[Na^+])= Z\psi$, where Z is the charge of the metal complex and ψ is 0.88 for DNA. [37-39] Figure 5 shows the decrease of K_b of **1** and **2** as the concentration of Na^+ is increased. As expected, the plot becomes nonlinear at ionic strengths greater than 0.1 M.[36, 37]

The slopes of the lines in Figure 5 are being -1.03, and -1.23 for **1** and **2** complexes respectively. The value of complex **1** and **2** are less than the theoretically expected values of $Z\psi$ ($2\times0.88=1.76$). Such lower values could arise from coupled anion release or from change in complex or DNA hydration upon binding. The knowledge of $Z\psi$ allows for a quantitative estimation of the nonelectrostatic contribution to the DNA binding constant for these complexes.

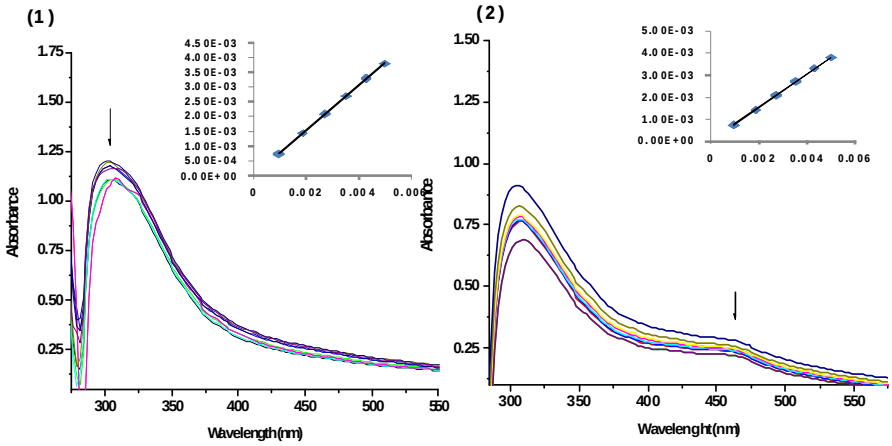

Figure 2: Absorption spectra of $[Co(dmb)_2(IIP)](ClO_4)_3$ (1) and $[Ru(dmb)_2(IIP)](ClO_4)_2$ (2) in tris-HCl buffer upon addition of CT-DNA at room temperature in the presence of [complex]= 20 µM. Arrow shows the absorbance changes upon increasing DNA concentrations. Insert plots of [DNA]/(Σa–Σf) *vs* [DNA] for the titration of Ru(II)/ Co(III) complex.

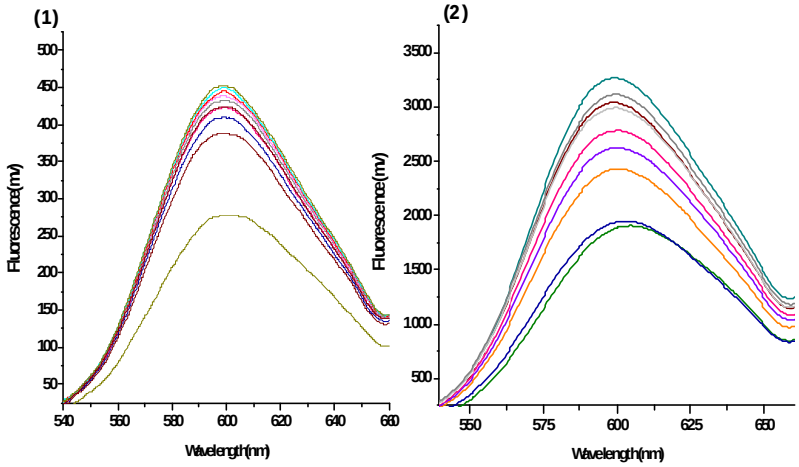

Figure 3 (a): Emission spectra of complexes $[Co(dmb)_2(IIP)](ClO_4)_3$ (1) and $[Ru(dmb)_2(IIP)](ClO_4)_2$ (2) in Tris-HCl buffer at 25°C upon addition of CT DNA, [Ru(II)/ Co(III)] = 20µM, [DNA] = 0-120µM. Arrow shows the intensity change upon increasing DNA concentrations.

Viscosity measurements

Further clarification of the interactions between the Ru(II) complexes and DNA was carried out by viscosity measurements. It is popularly accepted that a partial and/or nonclassical intercalation of ligand could bend (or kink) the DNA helix, reduces its effective length and, concomitantly, its viscosity; A classical intercalation of a ligand into DNA is known to cause a significant increase in the viscosity of a DNA solution due to an increase in the separation of the base pairs at the intercalation site and, hence, an increase in the overall DNA molecular length.[38]

For complexes 1 and 2, the viscosity of DNA increases greatly with increasing concentration of complex, which is similar to that of the proven intercalator EtBr.[39] Both complexes change the relative viscosity of DNA in a manner consistent with binding by the intercalation mode. The viscosity of DNA increases with the increase of concentration of EtBr. So these two complexes increase the DNA helix length. On the basis of the viscosity results, the complexes bind with DNA through the intercalation mode.

Thermal denaturation studies

DNA melting experiments are useful to establish the extent of intercalation because the intercalation of the complex into DNA base pairs causes stabilization of the base stacking and, therefore, raises the melting temperature of double-stranded DNA.[40]

It is well known that when the temperature in solution increases, the double-stranded DNA gradually dissociates to single strands, and generates a hyperchromic effect on the absorption spectra of DNA base pairs (λ = 260 nm). In the absence of complexes, the thermal denaturation carried out for DNA gave a T_m of 62.00 ± 0.5 °C under our experimental conditions. The observed melting temperatures in the presence of complexes 1 and 2 were 67.19 ± 0.5 and 68.92 ± 0.5 °C, respectively. The increases in T_m of the two complexes (the ΔT_m is 5.19 and 6.92 °C for 1 and 2) are comparable to those observed for Ru(II)

complexes [41- 43] and lend strong support for intercalation into the helix of DNA. The experimental results also indicate that complex 2 exhibits larger DNA-binding affinity than complex 1 does.

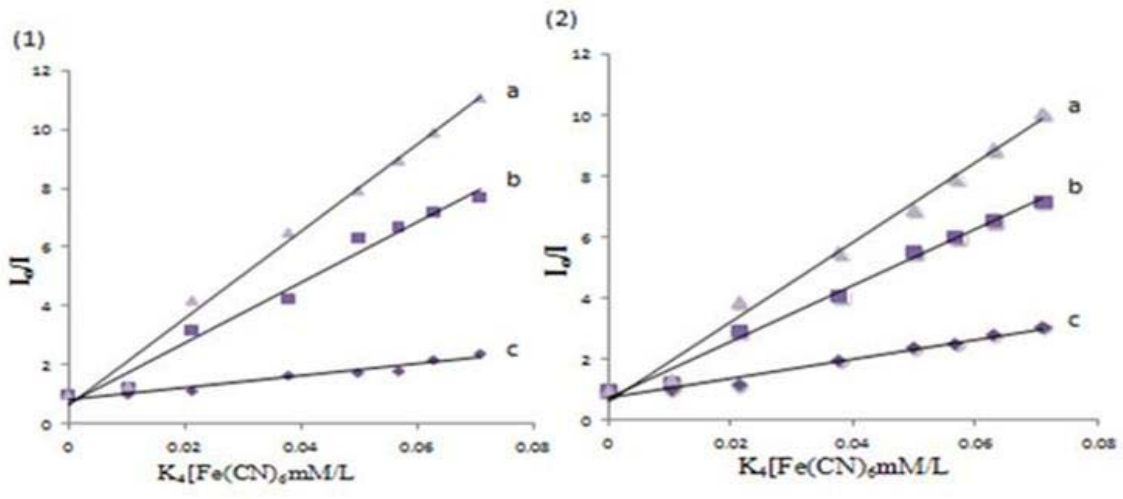

Figure 3 (b): Emission quenching of $[Co(dmb)_2(IIP)](ClO_4)_3$ (1) and $[Ru(dmb)_2(IIP)](ClO_4)_2$ (2) with $K_4[Fe(CN)_6]$ in the absence (a) and presence (b) and excess of DNA (c) [Ru/Co] = 20 µM.

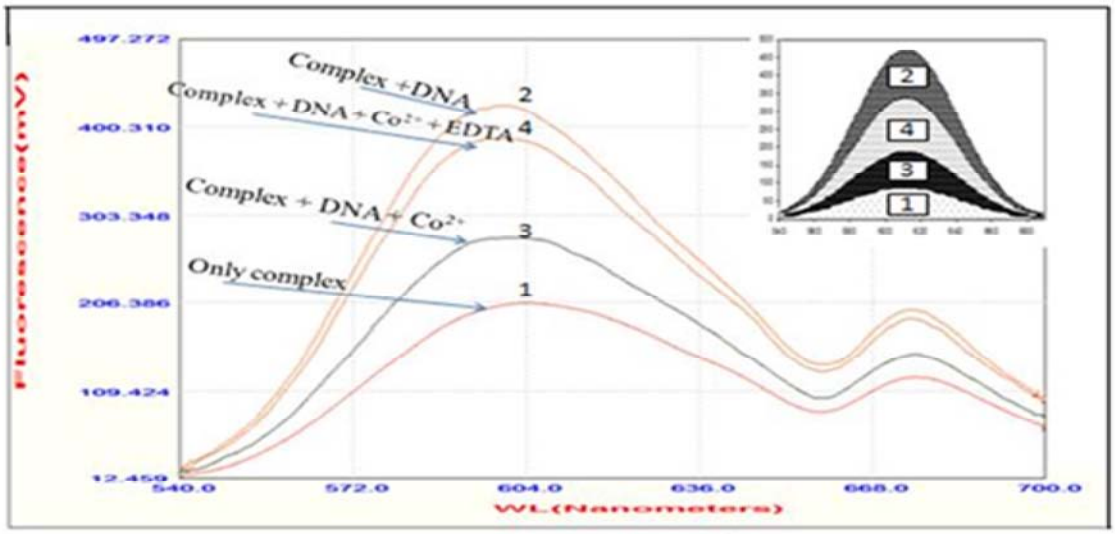

Figure 4: $[Ru(dmb)_2IIP]^{2+}$ in tris buffer(1), complex + DNA (2)(switch on), complex + DNA + Co^{2+} (3)(switch off) , and complex +DNA +Co^{2+} EDTA(4)

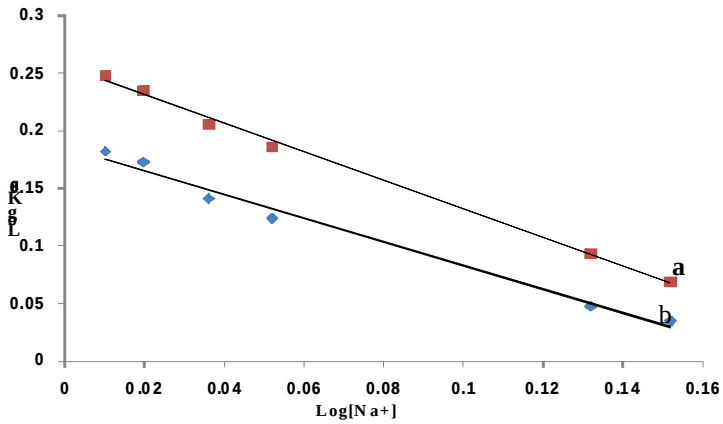

Figure 5: Salt dependence of the equilibrium binding constants for DNA binding of complexes (a) $[Ru(dmb)_2(IIP)](ClO_4)_2$ and (b) $[Co(dmb)_2(IIP)](ClO_4)_3$. The lines indicates the slope of the linear square fit to the data as (a) -1.23 (b) -1.03

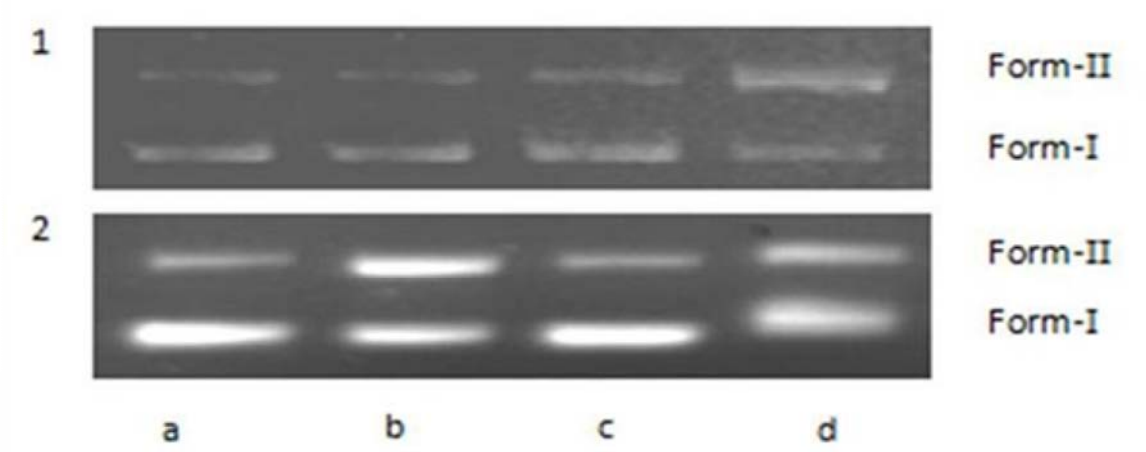

Figure 6: Photo activated cleavage of pBR 322 DNA in the presence of $[Co(dmb)_2(IIP)](ClO_4)$ (1) and $[Ru(dmb)_2(IIP)](ClO_4)_2$ (2) complexes, after irradiation at 365 nm. lanes a-b, addition of complexes 20, 40,60 and 80 M^{-1}

Photoactivated cleavage of pBR322 DNA by Ru(II)/Co(III) complexes

There has been considerable interest in DNA endonucleolytic cleavage reactions that are activated by metal ions.[44-47] The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subject to electrophoresis, relatively fast migration will be observed for the intact super coil form (Form I). If scission occurs on one strand (nicking), the super coil will relax to generate a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated.[48] Figure 6 shows gel electrophoresis separation of pBR322 DNA after incubation with the two Ru(II)/Co(III) complexes and irradiation at 365 nm. No DNA cleavage was observed for controls in which the complex was absent (lane 0), or incubation of the plasmid with either complex in dark (data not presented). With increasing concentration of complexes 1 and 2 (lanes a–d), the amount of Form I of pBR322 DNA diminish gradually, whereas Form II increases. At the concentration of 80 µM, complexes 1 and 2 can almost promote the complete conversion of DNA from Form I to Form II. The different DNA-cleavage efficiency of the two complexes was due to the different binding affinity of the complexes to DNA.

Molecular modeling studies

The design of molecules that can recognize specific sequences and structures of nucleic acids plays an important role both for understanding nucleic acid molecular recognition as well as for the development of new chemotherapeutic drugs. Molecular docking technique is an attractive scaffold to understand the Drug–DNA interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a small molecule into the binding site of the target specific region of the DNA mainly in a non-covalent fashion, [49] which can substantiate the spectroscopic results.

The calculation have been carried out for the docking of Ru(II)/Co(III) complex with the decamer DNA (GGTAGCGATGG) (PDB ID: 1y9h) to understand the nature of the interaction between Ru(II)/Co(III) and DNA. The theoretical results reveal groove binding for complexes with DNA. Both complexes bind to DNA by partial intercalation of the indole side of the planar IIP ligand. Ru(II)/Co(III) complex primarily interacts with the DNA via major groove and derives additional stabilization through a hydrogen bonding interaction between the H68 of IIP ligand and N2/N3 of Guanine at a distance of (2.63-2.72 Å). In complex 2, there is another H-bond between the H56 of IIP and O2 of Thymine at a distance of (2.350 Å). The H-bonding and Vander Waals interactions were listed in Table 1. The binding energies of these complexes with DNA have been found to vary with the intercalative depth of the complex and the value has been found -40.69 and -50.45 kcal mol^{-1} respectively. These results also support the binding studies, which also show that the DNA binding constant of both the complexes are nearly the same.

Cytotoxicity assay *in vitro*

The cytotoxicity in vitro assay for complexes was assessed using the method of MTT reduction. Doxorubicin was used as a positive control. After treatment of (HeLa derived from human cervical cancer cells, A549 derived from human alveolar adenocarcinoma epithelial cells, MDA-MB-231, and MCF7 derived from human breast adenocarcinoma cells), cell lines for 48 h with Ru(II)/Co(III) complex. The inhibitory percentage against growth of cancer cells was determined. The cytotoxicity of complexes was found to be concentration-dependent. The cell viability decreased with increasing the concentrations of complexes 1 and 2. The IC50 values were calculated and listed in Table 2. Comparing the IC50 values of complex 1 and 2, Complex 2 appeared to have higher cytotoxicity against all the selected cells than complex 1, but cytotoxicity of the two complexes was relatively low when compared with doxorubicin. The results obtained showed that the cytotoxicity for Ru(II)/Co(III) complex against the selected tumor cell lines is consistent with the DNA-binding affinity.

Table 1: The H –Bond Vander Waals interactions and scores for binding of Ru(II)/ Co(III) complexes to (PDB ID: 1y9h) DNA containing CG bases using docking calculations

Complex	H –Bond Donor-Acceptor	Bond Length (Å)	Vander Waals interactions (Complex – DNA)	Bond Length (Å)	Docking Score
$[Co(dmb)_2IIP]^{3+}$	H 68- DG4:N2	2.63	C52-DC5:OP1	2.55	-40.69
			C37- DC5:OP1	2.68	
			H80-DG24:H4	1.49	
			H79- DG24:H5	1.87	
			H68- DG4:H21	1.64	
			H57- DG4:H21	1.73	
$[Ru(dmb)_2IIP]^{2+}$	H68– DG4:N3	2.72	C49-DA6:OP1	2.32	-51.45
	H56-DT3:O2	2.35	H62-DG4:H21	1.18	
			C37-DA23:O3	2.47	
			H57-DA23:H2	1.88	
			C2-DG4:O4	2.47	
			N1-DG4:O4	2.13	
			N1-DG4:C4	2.72	

Table 2: Percentage cell viability of different cell lines and Antibacterial activity of Ru(II)/ Co(III) complexes

Complex	Cell lines				Bacterial Species	
	A549	MCF-7	HELA	MDA-MB-231	E.Coli	S. aureus
DMSO					Nil	Nil
$[Co(dmb)_2IIP]^{3+}$ (**1**)	22.70	48.08	18.34	35.80	19	15
$[Ru(dmb)_2IIP]^{2+}$ (**2**)	20.27	42.53	16.74	25.55	17	9
Doxorubicin	1.21	1.05	0.45	0.50		
Septomycine					22	14
Gentamycine					18	18

Zone of inhibition of diameter in (mm), at 20 µg/mL Ru(II)/ Co(III) complexes

Antimicrobial studies

The synthesized complexes were tested for their antimicrobial activity, Table 2.

It was observed that the complexes studied for their antimicrobial activity inhibit the growth of E. coli and S. aureus cells. The activity has been found to be concentration dependent as the zone of inhibition increases with an increase in concentration of the complexes. Both complexes inhibit the growth of *E. coli* (MTCC 443) and *S. aureus* (MTCC 96). The potent antimicrobial activity of metal complexes could be attributed to the fact that metal complexes with labile ligands have long been known to undergo ligand-substitution reactions with biomolecular targets. This pronounced activity can be explained on the basis of Tweedy's chelation theory. [50]

There is a decrease in the polarity of the metal ion significantly after chelation, because of the partial sharing of its positive charge with the donor group and also due to p-electron delocalization on the whole chelate ring. The metal ion interaction is preferred with the lipids and polysaccharides which are the important constituents of the cell wall and membranes. Other components of cell wall viz., many phosphates, carbonyl and cysteinyl ligands which maintain the integrity of the membrane by acting as a diffusion barrier also provide suitable sites for binding. Furthermore, the reduction in polarity increases the lipophilic character of the chelates and an interaction between the metal ion and the lipid is favoured which may lead to the subsequent breakdown of the permeability barrier of the cell resulting in obstruction with the normal cell processes.

CONCLUSION

The following are the principal findings and conclusions of our present study. Ru(II) and Co(III) complexes with the same ancillary ligand were synthesized, characterized by NMR, IR, LC-MS, analytical and spectral methods and their biological potential as therapeutic agents for their possible use in the treatment of diseases viz. cancer and microbial was studied. The DNA binding capabilities of these two complexes were investigated by absorption, fluorescence spectroscopy, viscosity and thermal denaturation. The corroborative results of these experiments validate that all complexes 1and 2 bind to CT DNA by non-covalent interactions preferentially via external groove binding to the helix of DNA. The gel

electrophoresis assay demonstrated that the complexes 1 and 2 promote the cleavage ability of the pBR322 plasmid DNA. The two complexes were evaluated for antibacterial activity exhibiting specific activity against *Escherichia coli* and *Staphylococcus aureus*. Molecular docking studies further reveal that complex 1 binds to DNA in the major groove and such a binding is stabilized through hydrogen bonding between DNA base pairs and the metal complex. Authors hope that further investigations in this direction would contribute to understand the mechanism of action of organic ligands and their metal complexes with DNA as intracellular target and metal based drugs and may lead to explore new and effective metal based drug candidates for enlightening role of metal ions in the physio-chemical processes of life

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REFERENCES

1. Sigman DS, Mazumder A, Perrin D.M, Chemical nucleases, Chem. Rev., 93, 1993, 2295-2316.
2. Wolkenberg SE, Boger DL, Mechanisms of in situ activation for DNA-targeting antitumor agents, Chem. Rev., 102, 2002, 2477-2495.
3. Erkkila KE, Odom DT, Barton JK, Recognition and Reaction of Metallointercalators with DNA, Chem. Rev., 99, 1999, 2777.
4. Bruijnincx P.C.A, Sadler P.J, New trends for metal complexes with anticancer activity, Curr. Opin. Chem. Biol., 12, 2008, 197-206.
5. Ali H, Van Lier J.E., Metal Complexes as Photo- and Radio sensitizers, Chem. Rev, 99, 1999, 2379-2450.
6. Henderson B.W, Busch T.M, Vaughan L.A, Frawley N.P, Babich D, Sosa T.A, Zollo J.D, Dee A.S, Cooper M.T, Bellnier D.A, Greco W.R, Oseroff A.R, Photofrin photodynamic therapy can significantly deplete or preserve oxygenation in human basal cell carcinomas during treatment, depending on fluence rate, Cancer Res, 60, 2000, 525-529.
7. Bonnett R, Chemical Aspects of Photodynamic Therapy, Gordon & Breach, London, UK, 2000.
8. Ochsner M, Light scattering of human skin: A comparison between zinc(II)-phthalocyanine and photofrin II, J. Photochem. Photobiol, B Biol, 32, 1996, 3-9.
9. Wei WH, Wang Z, Mizuno T, Cortez C, Fu L, Sirisawad M, Naumovski L, Magda D, Sessler JL, New polyethyleneglycol-functionalized texaphyrins: Synthesis and in vitro biological studies, Dalton Trans., 2006, 1934-1942.
10. Moriwaki SI, Misawa J, Yoshinari Y, Yamada I, Takigawa M, Tokura Y, Analysis of photosensitivity in Japanese cancer-bearing patients receiving photodynamic therapy with porfimer sodium (Photofrin), Photodermatol. Photoimmunol. Photomed., 17, 2001, 241.
11. Friedman AE, Chambron JC, Sauvage JP, Turro NJ, Barton JK, Molecular 'Light Switch' for DNA: $Ru(bpy)_2(dppz)^{2+}$, J. Am. Chem. Soc., 112, 1990, 4960.
12. Indumathy R, Weyhermuller T, Nair BU, Biimidazole containing cobalt(III) mixed ligand complexes: Crystal structure and photonuclease activity, Dalton Trans., 39, 2010, 2087-2097.
13. Marmur J, A procedure for the isolation of DNA from microorganisms, J. Mol. Biol. 3, 1961, 208-218.
14. M.F. Reichmann, S.A. Rice, C.A. Thomas, P. Doty, A Further Examination of the Molecular Weight and Size of Desoxypentose Nucleic Acid. J Am Chem Soc, 76, 1954, 3047-3053.
15. Yamada M, Tanaka Y, Yoshimoto Y, Kuroda Shimao S, Synthesis and properties of diamino-substituted dipyrido [3, 2-a: 20, 30-c] phenazine, J. Bull. Chem. Soc. Jpn., 65, 1992, 1006–1011.
16. Tan L-F, Chao H, Li H, Liu Y-J, Sun B, Wei W, Ji L-N, Synthesis, characterization, DNA-binding and photocleavage studies of $[Ru(bpy)_2(PPIP)]^{2+}$ and $[Ru(phen)_2(PPIP)]^{2+}$, J. inog. Biochem., 99, 2005, 513–520.
17. Krotz AH, Kuo LY, Shields TP, Barton JK, DNA Recognition by Rhodium(III) Polyamine Intercalators: Considerations of Hydrogen Bonding and van der Waals Interactions, J. Am. Chem. Soc., 1993, 115, 3877.
18. Sullivan BP, Salmon DJ, Mayer TJ, Mixed Phosphine 2,2'-Bipyridine Complexes of Ruthenium, Inorg. Chem., 17, 1978, 3334-3341.
19. Satyanarayana S, Dabrowiak JC, Chaires JB, Tris (phenanthroline) ruthenium (II) enantiomer interactions with DNA: mode and specificity of binding, Biochemistry, 32, 1993, 2573–2584.
20. Liu JG, Ye BH, Li H, Zhen QX, Ji LN, Fu YH, Polypyridyl ruthenium(II) complexes containing intramolecular hydrogen-bond ligand: syntheses, characterization, and DNA-binding properties, J. Inorg. Biochem., 76, 1999, 265-271.
21. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods, 65, 1983, 55–63.
22. Jones G, Willet P, Glen RC, Leach AR, Taylor R, Development and Validation of a Genetic Algorithm for Flexible Docking, J. Mol. Biol., 267, 1997, 727–748.
23. Seng HL, Von ST, Tan KW, Jamil M M, Ng SW, Zaliha RN, Abd Rahman R, Caracelli I, Ng CH, Crystal structure, DNA binding studies, nucleolytic property and topoisomerase I inhibition of zinc complex with 1,10-phenanthroline and 3-methyl-picolinic acid, J. Biometals, 23, 2010, 99–118.
24. Jones G, Willet P, Glen RC, Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation, J. Mol. Biol, 245, 1995, 43–53.
25. Zhen QX, Ye BH, Zang QL, Liu JG, Li H, Ji LN, Wang L. Synthesis, characterization and DNA binding of ruthenium(II) complexes containing the atatp ligand, J. Inorg. Biochem., 78, 2000, 293–298.
26. Cory M, McKee DD, Kagan J, Henry DW, Miller JM, Design, synthesis, and DNA binding properties of bifunctional intercalators. Comparison of polymethylene and diphenyl ether chains connecting phenanthridine, J. Am. Chem. Soc., 107, 1985, 2528–2536.
27. Waring MJ, Complex formation between ethidium bromide and nucleic acids, J. Mol. Biol., 13, 1965, 269–282.
28. Xu ZH, Chen FJ, Xi PX, Liu XH, Zeng ZZ, Synthesis, characterization, and DNA binding properties of the cobalt(II) and nickel(II) complexes with salicylaldehyde 2-phenylquinoline-4-carboylhydrazone, J. Photochem. Photobio. A: Chem., 196, 2008, 77–83.
29. Vaidyanathan VG, Nair BU, Photooxidation of DNA by a cobalt(II) tridentate complex, J. Inorg. Biochem., 94, 2003, 121–126.
30. Praveen Y K, Shilpa M, Nagababu P, Rajender R M, Laxma R K, Nazar MG, Satyanarayana S, Study of DNA Light Switch Ru(II) Complexes : Synthesis, Characterization, Photocleavage and Antimicrobial Activity, J. Fluoresc., 22, 2012, 835.
31. Shilpa M, Nagababu P, Satyanarayana S, Studies on DNA-binding and plasmid-cleavage of cobalt (III) mixed ligand complexes, Main Group Chemistry, (8), 1, 2009, 33–45.
32. Vidhisha S, Laxma RK, Ashwini K, Praveen YK, Satyanarayana S, DNA interactions of ruthenium(II) complexes with a polypyridyl

ligand: 2-(2, 5-dimethoxyphenyl)-1H-imidazo [4,5-f]1,10-phenanthroline, Transition Met. Chem., 35, 2010, 1027–1034.

33. Joseph R, Lakowicz GW, Quenching of fluorescence by oxygen. Probe for structural fluctuations in macromolecules, Biochemistry, 12, 1973, 4161–4170.

34. Liu XW, Shen YM, Lu JL, Chen YD, Li L, Zhang DS, Synthesis, DNA-binding and photocleavage of "light switch" complexes $[Ru(bpy)_2(pyip)]^{2+}$ and $[Ru(phen)_2(pyip)]^{2+}$, Spectrochemica Acta Part A Mol. Biomol. Spectrosc., 77, 2010, 522–527.

35. Chen M, Li H, Li Q, Xu Z, Luminescence properties of $[Ru(bpy)_2MDHIP]^{2+}$ modulated by the introduction of DNA, copper(II) ion and EDTA, Spectrochemica Acta Part A Mol. Biomol. Spectrosc., 75, 2010, 1566–1570.

36. Record M T, Anderson C F, Lohman T M, Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity, Q. ReV. Biophys, 86, 1978, 103–178.

37. Mudasir WK, Wahyuni ET, Yoshioka N, Inoue H, Salt-dependent binding of iron(II) mixed-ligand complexes containing 1,10-phenanthroline and dipyrido[3,2-a:2',3'-c] phenazine to calf thymus DNA, Biophys. Chem., 121, 2006, 44-50.

38. Satyanarayana S, Dabrowiak JC, Chaires JB, Tris (phenanthroline) ruthenium (II) enantiomer interactions with DNA: mode and specificity of binding, Biochemistry, 32, 1993, 2573-2584.

39. Zhang QL, Liu JG, Chao H, Xue GQ, Ji LN, DNA-binding and photocleavage studies of cobalt(III) polypyridyl complexes: $[Co(phen)_2IP]^{3+}$ and $[Co(phen)_2PIP]^{3+}$, J. Inoorg. Biochem., (1) 83, 2001, 49-55.

40. Kalouli ET, Katsaros N, The interaction of $[Ru(NH_3)_5Cl]^{2+}$ and $[Ru(NH_3)_6]^{3+}$ ions with DNA, J. Inorg. Biochem., 1989, 37, 271-282.

41. Tan LF, Liang XL, Liu XH, Synthesis, double stranded DNA-binding and photocleavage studies of a functionalized ruthenium(II) complex with 7,7' methylene dioxyphenyl dipyrido [3,2-a:2',3'-c]-phenazine, J. Inorg. Biochem., 103, 2009, 441-447.

42. Liu YJ, Zeng CH, Wu FH, Yao JH, He LX, Huang HL, Synthesis, characterization, photocleavage of DNA and cytotoxicity of ruthenium(II) mixed-ligand complexes J. Molecular Structure, J. Mol. Struct., 932, 2009, 105-111.

43. Chen LM, Liu J, Chen JC, Shi S, Tan CP, Zheng KC, Ji LN, Experimental and theoretical studies on the DNA-binding and spectral properties of water-soluble complex $[Ru(MeIm)_4(dpq)]^{2+}$, J. Mol. Struct., 881, 2008, 156-166.

44. Ji LN, Zou XH, Liu JG, Shape- and enantioselective interaction of Ru(II)/Co(III) polypyridyl complexes with DNA, Coord. Chem. Rev., (216–217), 2001, 513-536.

45. Sigman DS, Nuclease activity of 1,10-phenanthroline-copper ion, Acc. Chem. Res., (6) 19, 1986, 180-186.

46. Sitlani S, Long EC, Pyle AM, Barton JK, DNA Photocleavage by Phenanthrenequinone Diimine Complexes of Rhodium(III): Shape-Selective Recognition and Reaction, J. Am. Chem. Soc., 114, 1992, 2303.

47. Barton JK, Raphael AL, Photoactivated Stereospecific Cleavage of Double-Helical DNA by Cobalt(III) Complexes, J. Am. Chem. Soc., 106, 1984, 2466.

48. Subramanian R, Meares CF, Photosensitization of Cobalt blcomycin J. Am. Chem. Soc., 108, 1986, 6427.

49. Rohs R, Bloch I, Sklenar H, Shakked Z, Molecular flexibility in ab initio drug docking to DNA: binding-site and binding-mode transitions in all-atom Monte Carlo simulations, Nucl. Acids Res. 33, 2005, 7048–7057.

50. Ramesh R, Maheswaran S, Synthesis, spectra, dioxygen affinity and antifungal activity of Ru(III) Schiff base complexes, J. Inorg. Biochem., 96, 2003, 457–462.

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