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SYNTHESIS, CHARACTERISATION, DNA INTERACTION AND CLEAVAGE ACTIVITY OF A NOVEL SERIES OF ISOMERIC PYRIDYL-TETRAZOLE COBALT (II) COMPLEXES

B. UMAMAHESWARA RAO*[1], V. KRISHNA[2] AND G. NAGESWARA RAO[1]

*[*1]Department of Inorganic and Analytical Chemistry, Andhra University,Visakhapatnam, India.*
[2]Department of Chemistry, Malla Reddy Engineering College (Autonomous), Hyderabad, India

ABSTRACT

A novel series of Co(II) complexes of the type $Co(L^1)Cl_2$ (1), $Co(L^2)Cl_2$ (2), $Co(L^3)Cl_2$ (3), $Co(L^4)Cl_2$ (4) were prepared with biologically active isomeric pyridyl-tetrazole ligands such as N,N-dimethyl-2-[5-(pyridin-2-yl)-1H-tetrazol-1-yl]ethanamine (L^1), N,N-dimethyl-2-[5-(pyridin-2-yl)-2H-tetrazol-1-yl]ethanamine (L^2), 2-[5-(pyridin-2-yl)-1H-tetrazol-1-yl]ethanol (L^3), 2-[5-(pyridin-2-yl)-2H-tetrazol-2-yl]ethanol (L^4) and $CoCl_2.H_2O$. in 1:1 metal ligand ratio. These complexes were characterised by elemental analysis, UV-Vis, IR, ^1H, ^{13}CNMR and mass spectral studies. EPR spectra of 1-4 cobalt complexes are characteristic of square planar geometry, DNA binding studies were carried by UV-Vis absorption, viscosity and thermal denature studies revealed that each of these complexes are avid binders of calf thymus DNA. The nucleolytic cleavage activities of complexes were carried on double stranded pBR322 circular plasmid DNA by using a gel electrophoresis experiment under various conditions, where cleavage of DNA takes place by oxidative free radical mechanism ($^{\cdot}OH$)

KEYWORDS: Pyridyl-tetrazole derivatives; Cobalt; DNA binding interaction; cleavage.

*Corresponding author

B. UMAMAHESWARA RAO
Department of Inorganic and Analytical Chemistry, Andhra University,Visakhapatnam,

INTRODUCTION

Biologically active Isomeric pyridyl-tetrazole derivatives have been under great investigations as part of inorganic chemistry. Polyazole rings are versatile ligands[1] for coordinating transition metals, therefore synthesis of transition metal complexes containing polyazole rings, particularly tetrazoles and their derivatives have given enormous significance, due to their practical applications[2–4]. There is an increasing interest of tetrazole derivatives for the development of ''click'' chemistry which was reported by Sharpless and co-workers[5]. Conversely, tetrazole-based compounds have made known special functionalities with interesting structures[6]. Tetrazole derivatives have found applications in therapeutics as antihypertensive agents[7], antibiotics[8] and drugs for AIDS treatment[9]. Even though many tetrazole containing derivatives are available in the literature, there is always an increasing demand for the development of novel and effective tetrazole containing therapeutic agents. In continuation of our ongoing research on DNA binding and cleavage activities of transition metal complexes[10], we herein reported the synthesis, DNA binding, DNA cleavage of Co(II) complexes which are obtained by the reaction of pyridyl-tetrazole derivatives which contain pendant arms like ethyl -OH or -$N(CH_3)_2$ group.

EXPERIMENTAL

1 Physical measurement

Chemicals were purchased from Sigma-Aldrich and metals used in the preparation of the complexes are of reagent grade. The solvents used in the synthesis of the ligands and metal complexes were distilled before use. All other chemicals were of AR grade and were used without further purification. Agarose, used in gel electrophoresis, was purchased from Sigma–Aldrich, Calf thymus DNA and plasmid pBR322 were purchased from Genie Bio labs, Bangalore, India. The elemental analyses were performed using a Perkin–Elmer 2400 CHNS elemental analyzer. Magnetic moments were determined in the polycrystalline state on a PAR model-155 vibrating sample magnetometer operating at field strength of 2–8 kG. High purity Ni metal (saturation moment 55 emu/g) was used as standard. The electronic spectra were recorded in DMF with a Schimadzu UV-1800 spectrophotometer. FT-IR spectra were recorded in the range 4000–500 cm^{-1} with a Bruker IFS 66V in KBr and polyethylene medium.

2 DNA binding and cleavage experiments

2.1 DNA binding experiment

All measurements with Calf Thymus DNA (CT DNA) were performed in buffer Tris–HCl 5 mM (pH 7.2), 50 mM NaCl. The UV absorbance ratio 260/280 was 1.8-1.9, indicating the DNA was sufficiently free of protein[11]. The concentration of CT DNA per nucleotide was determined from the absorption intensity at 260 nm with the known value of 6600 $M^{-1}cm^{-1}$. The absorption titrations were performed by adding increasing amounts of CT DNA to a solution of the complex at a fixed concentration contained in a quartz cell and recording the UV-Vis spectrum after each addition. The absorption of CT DNA was subtracted by adding the same amount of DNA to a blank. The data were then fitted to Eq. (1) to obtain the intrinsic binding constant, K_b[12]. $[DNA]/(\varepsilon a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$ where [DNA] is the molar concentration of CT-DNA, ε_a, ε_b and ε_f are apparent, free and bound metal complex extinction coefficients, respectively. DNA melting experiments were performed using a spectrophotometer connected to a thermostat. The absorbance of DNA (60 µM) at 25 – 80^0C in both the absence and presence of 10.5 µM of the complex was recorded at 260 nm. The melting temperature (Tm) was calculated by plotting the temperature vs relative absorption intensity (A/A°). Viscosity experiments were carried on an Ostwald viscometer, immersed in a thermo stated water-bath maintained at a constant temperature at 30.0 ± 0.1°C. CT DNA samples of approximately 0.5 mM were prepared by sonicating in order to minimize complexities arising from CT DNA flexibility[13] Flow time was measured with a digital stopwatch three times for each sample and an average flow time was calculated. Data were presented as $(\eta/\eta^0)^{1/3}$ vs the concentration of the cobalt complexes, where η is the viscosity of CT DNA solution in the presence of the complex, and η^0 is the viscosity of CT DNA

solution in the absence of the complex. Viscosity values were calculated after correcting the flow time of buffer alone (t^0), $\eta = (t - t^0) / t^0$ [14]. A DMF solution containing the metal complexes (250 µM) in a clean Eppendorf tube was treated with pBR322 plasmid DNA (3.3 µl of 150 µg/mL) in Tris-HCl buffer (0.10M, pH 8.0) containing NaCl (50 mM) in presence and absence of additives. The contents were incubated for 1 h at 37 ^{0}C and loaded onto a 1% agarose gel after mixing 5 µl of loading buffer (0.25% bromophenol blue + 25% xylene cyanol + 30% glycerol, sterilized). The electrophoresis was performed at a constant voltage (80 V) until the bromophenol blue had travelled through 75% of the gel. Subsequently, the gel was stained for 10 min by immersion in ethidium bromide solution. The gel was then distained for 10 min by keeping it in sterile distilled water. The plasmid bands were visualized by viewing the gel under a transilluminator and photographed. A control experiment was done in presences of hydroxyl radical scavenger DMSO, EDTA, DTT and singlet oxygen quencher azide ion (NaN_3).

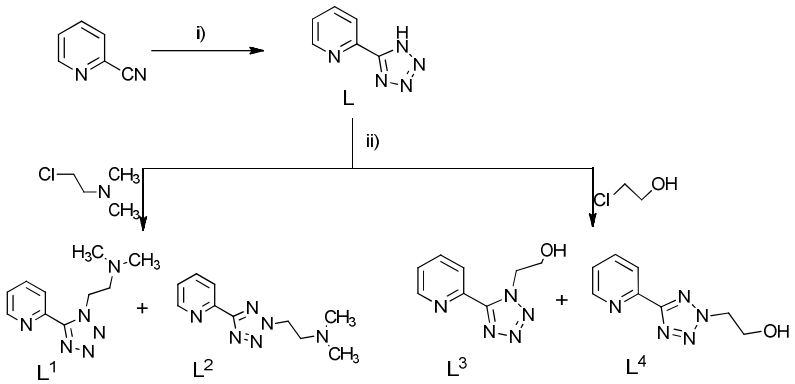
2.2. DNA Cleavage experiment

The DNA cleavage activity of the Co(II) complexes was studied by agarose gel electrophoresis method. pBR322 plasmid was cultured, isolated and used as DNA for the experiment. Test samples (1mg/ml) were prepared in DMF. 25 µg of the test samples was added to the isolated plasmid and incubated for 2h at 37°C. After incubation, 30 µl of plasmid DNA sample mixed with bromophenolbluedye(1:1) was loaded in to the electrophoresis chamber wells along with the control DNA, 5M FeSO4 (treated with DNA) and standard DNA marker containing TAE buffer(4.84g Trisbase, pH8.0, 0.5MEDTA/1L). Finally, it was loaded on to anagarosegel and electrophoreses at 50V constant voltage up to 30min. After the run, gel was removed and stained with 10.01µg/ml ethidium bromide and the image was taken in Versa doc (Biorad) imaging system. The results were compared with standard DNA marker. The same procedure was followed in the presence of H_2O_2 also.

3. Synthesis of ligands.

3.1. Ligands

The preparation of L is carried as per literature[15a] M.p. 221–223°C. ^{1}H NMR (DMSO-d_6): 8.56 (d, 1H, J = 7.9 Hz, pyr-H), 8.0 (d, 1H, J = 7.8 Hz, pyr-H), 7.79 (t, 1H, J = 7.8 Hz, pyr-H), 7.26 (t, 1H, J = 7.9 Hz, pyr-H), 7.1 (s, 1H, tetrazole-H) ppm.



Scheme-1. Synthetic route for ligands L^1-L^4, reaction conditions (i) NaN_3, LiCl, NH_4Cl, DMF, reflux 10 h; (ii) K_2CO_3, acetonitrile, reflux 24 h.

3.1. a. N,N-Dimethyl-[2-(5-pyridin-2-yl-tetrazol-1-yl)-ethyl]-amine (L^1-L^2)

To the compound L (1.0 gm, 6.8 mmol) dissolved in acetonitrile (30 mL) was added potassium carbonate (4.6 gm, 34 mmol). The resulting solution was refluxed for 30 min and to hot solution was added 2-chloroethyl-N, N-dimethylamine (3.1 gm, 22 mmol). The reaction mixture was then stirred at reflux temperature for a further 24 h. After cooling, the solvent was removed under reduced pressure to afford a white precipitate, which was purified by column chromatography on silica gel (Ethyl acetate: Hexane by the ratio of 20:80) to give isomers L^1 and L^2. L^1: white brown solid (0.55 g, yield 26%). M.p. 72–76°C.

^{1}H NMR ($CDCl_3$, 300 MHz) : δ 8.71 (d, 1H, J = 4.5 Hz, pyr-H), 8.37 (d, 1H, J = 8.1 Hz, pyr-H), 7.91 (dt, 1H, J = 7.8, 1.8 Hz, pyr-H), 7.45 (dd, 1H, J = 7.5, 5.1 Hz, pyr-H), 5.12 (t, 2H, J = 6.9 Hz, CH_2N), 2.88 (t, 2H, J = 6.9 Hz, CH_2), 2.29 (s, 6H, $N(CH_3)_2$) ppm. ^{13}C-NMR ($CDCl_3$, 60 MHz): δ 150.45 (CN_4), 148.53, 144.66, 137.16, 123.15, 122.24, 48.24 (CH_2N_4), 43.56 (CH_2N-$(CH_3)_2$), 35.34 ppm. L^2: white brown solid (0.55 g, yield 26%). M.p. 66-68^{o}C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.65 (d, 1H, J = 4.5 Hz, pyr-H), 8.28 (d, 1H, J = 8.1 Hz, pyr-H), 7.87 (dt, 1H, J = 7.8, 1.8 Hz, pyr-H), 7.34 (t, 1H, J = 7.5, 5.1 Hz, pyr-H), 5.06 (t, 2H, J = 6.9 Hz, CH_2N), 2.82 (t, 2H, J = 6.9 Hz, CH_2), 2.27 (s, 6H, $N(CH_3)_2$) ppm. ^{13}C-NMR ($CDCl_3$, 60 MHz): δ 151.35 (CN4), 149.44, 145.36, 138.08, 124.64, 122.12, 49.54 (CH_2N4), 44.34 (CH_2N-$(CH_3)_2$), 35.68, ppm.

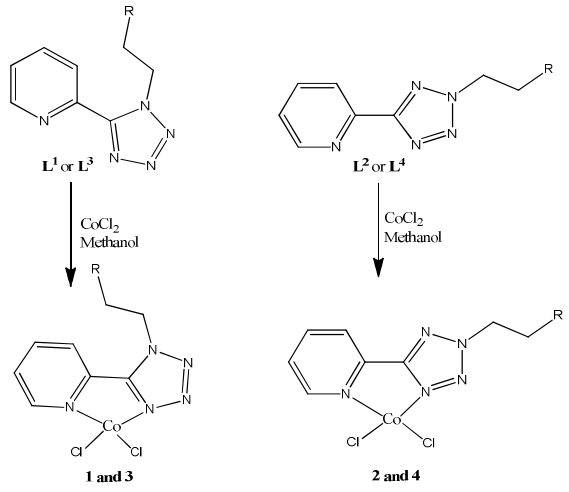
3.1. b. Synthesis of 2-[5-(pyridin-2-yl)-1H-tetrazol-1-yl] ethanol (L^3-L^4)

The above procedure is followed with 2-chloroethanol (2.0 gm, 24 mmol) to give L^3 and L^4. L^3: White solid (0.35 g, yield 17%). M.p. 56–58 ^{o}C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.70 (d, 1H, J = 4.8 Hz, pyr-H), 8.37 (d, 1H, J = 7.8 Hz, pyr-H), 7.96 (dt, 1H, J = 7.8, 1.5 Hz, pyr-H), 7.53 (dd, 1H, J = 6.9, 5.1 Hz, pyr-H), 5.08-5.05(m, 2H, CH_2N), 4.21-4.18 (m, 3H, CH_2O, OH). ^{13}C NMR ($CDCl_3$, 60 MHz): δ 152.15 (CN_4), 149.73, 144.39, 138.14, 125.79, 124.35, 59.42 (CH2O), 51.39 ppm (CH_2N).

L^4: White solid (0.35 g, yield 17%). M.p. 54–56 ^{o}C. ^{1}H- NMR ($CDCl_3$, 300 MHz): δ 8.79 (d, 1H, J = 4.2 Hz, pyr-H), 8.25 (d, 1H, J = 7.8 Hz, pyr-H), 7.88 (t, 1H, J = 6.6 Hz, pyr-H), 7.42 (dd, 1H, J = 7.5, 4.8 Hz, pyr-H), 4.87 (t, 2H, J = 4.8 Hz, CH_2N), 4.28 (t, 2H, J = 5.1 Hz, CH_2O) ppm. ^{13}C NMR ($CDCl_3$): δ 154.77 (CN_4), 150.24, 145.72, 138.81, 124.81, 123.33, 60.52 (CH_2O), 51.43 ppm (CH_2N).

3.2 Synthesis of complexes

The appropriate ligand (L^1-L^4) (1.36 mmol) was dissolved in methanol (30 mL) and added to a $COCl_2.H_2O$ (1.36 mmol) in methanol solution (10 mL). The resulting pale red to red coloured solutions were then heated to reflux for 2-3 h; the solution was left over night at room temperature and filtered to collect respective precipitate.



Where R= -OH or -$N(CH_3)_2$

Scheme 2. Synthesis of 1N and 2N –substituted cobalt(II) tetrazole complexes (1- 4)

3. RESULTS AND DISCUSSION

Synthesis of 2-(2H-tetrazol-5-yl) pyridine (L) and its derivatives L^1-L^4 were carried out with small modification that described in the literature[15b], which is shown in Scheme 1 A signal at 154.9 ppm in the ^{13}C -NMR spectrum of compound L indicated the formation of a 1,5-disubstituted tetrazole and the presence of a signal at 2220 cm^{-1} in the IR spectrum indicated an azide bond (N-N or N=N band)[16]. The ^{1}H NMR spectrum of L, showed the expected signals for the pyridine ring, while the NH proton on the tetrazole has appeared as a broad signal at 7.1 ppm. Ligand (L) on treatment with N,N-Dimethyl-[2-(5-pyridin-2-yl-tetrazol-1-yl)-ethyl]-amine.HCl and 2-chloroethanol in basic medium afford regio iosmers L^1, L^2, L^3 and L^4 by alkylation at either the 1-N or 2-N positions (Scheme 1). The structures for the isomers are readily assigned by their ^{13}C NMR and ^{1}H -NMR spectra. The

chemical shift values for the quaternary Carbon of tetrazole ring appeared in range ~ 150.1 - 154.7 ppm in the 1-N- and 2-N-isomers respectively. The 1-N isomer gave the signal at 150.45 (L^1) and 152.15 (L^3) ppm respectively; while the 2-N isomer gave the signal at 151.35(L^2) and 154.77(L^4) ppm respectively. The ^{1}H NMR spectra of L^1-L^4 showed separately four signals corresponding to pyridyl protons and two triplets for the methylene groups. a singlet is observed in the spectra of L^1 and L^2 at 2.29 and 2.27 ppm which indicated the presence of six protons of $–N(CH_3)_2$ groups respectively. The methylene protons of L^1-L^4 beside the tetrazole ring appeared at δ 5.12, 5.06, 5.08 and 4.87 ppm respectively. Presence of –OH in L^3 and L^4 is confirmed by I.R spectra with broad peak at 3350-3200 cm^{-1}. The ligands (L^1-L^4) are treated with $CoCl_2.2H_2O$ salt, in methanol at reflux temperature under N_2 atmosphere for 2-3 h. All reactions were carried out using a 1:1 metal: ligand stoichiometry ratio to give corresponding complexes (1-4) in Scheme 2. Physical, analytical and magnetic data of cobalt(II) complexes are shown in Table 1. Elemental analysis of the obtained complexes showed that these (1-4) are in 1:1 composition. All Cobalt complexes have magnetic moments value in range 4.12 to 4.58 BM, slightly higher than the spin only values (3.98 μ_{eff}) expected for a d^7 system with three unpaired electron[17] in Co(II) complexes.

Table 1
Physical, analytical and magnetic data of the pyridyl-tetrazole cobalt(II) complexes

S.No	Complex	Colour	Elemental analysis Calculated (found)				μ_{eff} (BM)
			% C	%H	%N	% Co	
1	$Co(L^1)Cl_2$	Red	29.93 (29.43)	2.83 (2.80)	21.82 21.22)	18.36 (18.28)	4.08
2	$Co(L^2)Cl_2$	Pale Red	29.93 (29.44)	2.83 (2.78)	21.82 21.26)	18.36 (18.30)	4.10
3	$Co(L^3)Cl_2$	Red	34.50 (33.98)	4.05 (3.96)	24.14 (23.74)	16.93 (17.96)	4.46
4	$Co(L^4)Cl_2$	Pale Red	34.50 (34.00)	4.05 (3.94)	24.14 (23.78)	16.93 (17.99)	4.50

The ESI mass spectra of cobalt complexes are used to compare the stoichiometric composition. The molecular ion peaks (M^+) for cobalt complexes were observed at m/z = 319.5, 347.0, 319.5 and 347.1 for complexes (1-4). Suggesting the stoichiometry of cobalt: ligand as 1: 1 [$Co(L)Cl_2$] respectively. Elemental analysis values are in close agreement with the values calculated from molecular formula assigned to these complexes, which is supported further by the ESI-mass studies of representative complexes. The electronic spectral data of the cobalt complexes recorded in DMF are shown in Table 2. Electronic spectra of cobalt complexes (1-4) showed bands in the region 19610-19896 cm^{-1} which are due to the metal to ligand charge transfer transition (n – π*). While single broad band observed in the region 16220-16580 cm^{-1} is assigned to d-d transition. The electronic spectra of these complexes display weak d–d bands in the low intensity 16118–16474 cm^{-1} region are assigned to $^4T_{1g} \longrightarrow {}^4A_{2g}$ electronic transition, these assignments suggest a square planar geometry for the Co(II) complexes [18, 19].

Table 2
Electronic spectral data (cm^{-1}) of the cobalt(II) complexes

Complex	MLCT	d-d (cm^{-1})
1	19874	16342
2	19610	16220
3	19896	16580
4	19756	16542

The I.R. spectral data of the metal complexes are given in Table 3. I.R spectra of ligands (L^3 and L^4) show a broad band in range 3350-3100 cm^{-1} corresponding to –OH, and peaks at 1650-1500 cm^{-1} and two similar peaks around 1150-900 cm^{-1} corresponds to tetrazole group[20]. The formation of coordination bonds between tetrazole ring and Co(II) is confirmed by observing the IR frequencies of tetrazole ring. The ligands L^1-L^4 showed characteristic absorption bands (IR) at 1630-1570 cm^{-1} which are shifted to lower frequencies in all cobalt complexes. Additional peaks around 1340-1200 cm^{-1} and 600-400 cm^{-1} are appeared due to the coordination of pyridine ring with Co(II) atom.

Table 3
Selective I.R. bands cm^{-1}, of Co(II) complexes

Vibration	L^1	1	L^2	2	L^3	3	L^4	4
-OH	-	-	-	-	3355	3134	3325	3126
C=N (tetrazole)	1614	1575	1606	1568	1633	1595	1626	1592
C=C (tetrazole)	1498	1416	1486	1408	1523	1400	1508	1398
Py / M-Py	1433	1298	1434	1294	1454	1334	1443	1324

The solid state EPR spectra of cobalt(II) complexes 1-4 were recorded in the X-band region at room temperature (25 ^{0}C) and the data are summarized in Table 4. Complexes 1-4, exhibit $g_{\parallel}$ values of 6.64 - 6.51 and $g_{\perp}$ values of 3.05- 2.94, respectively. From the observed values of complexes 1-4, it is clear that $g_{\parallel} > g_{\perp} > 6.00$ which suggest the fact that the unpaired electron lies predominantly in the $d_{X^2-Y^2}$ orbital; characteristic of square planar geometry in cobalt(II) complexes. The geometric parameter G, which is a measure of the exchange interaction between the cobalt centers in the polycrystalline compound, is calculated using the equation: $G = (g_{\parallel} - 2.0023)/ (g_{\perp} - 2.0023)$ According to Hathaway and Tomlinson [21], if G > 4.0, considerable exchange interaction is negligible because the local tetragonal axes are aligned parallel or slightly misaligned. If G < 4.0, exchange is considerable and the local tetragonal axes are misaligned. The observed G values of complexes 1-4 (3.05-2.94, respectively) suggest that there is no exchange interaction in the cobalt(II) complexes.

Table 4
EPR spectral assignments for complexes 1-4 at room temperature

Complex	$g_{\parallel}$	$g_{\perp}$	g_{av}	G
1	6.58	3.02	4.80	4.50
2	6.64	3.05	4.84	4.43
3	6.51	2.94	4.72	4.81
4	6.56	2.95	4.75	4.82

DNA Binding Studies.-

The binding interactions of 1-4 complexes with CT- DNA were monitored by UV–Vis spectroscopy. The absorption spectra of the cobalt complexes are compared with and without CT DNA at 400-450 nm. Electronic absorption spectral data upon addition of CT-DNA and binding constants of these complexes are given in Table 5. Typical absorption spectrum of complex 3 is shown in Fig. 1. In the presence of increasing amounts of CT-DNA, the UV–Visible absorption spectra of complexes 1–4 showed increase in absorbance exhibiting bathochromic shift (1.8-3.2 nm) with hyperchromism (10-12%) with respect to control (0µl DNA). The change in absorbance values with increasing amount of CT DNA were used to evaluate the intrinsic binding constant K_b.

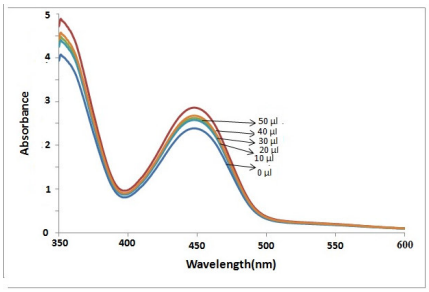


Figure 1

Absorption spectra of complex 3 in the absence and in the presence of increasing concentration of CT-DNA; bottom spectrum is recorded in the absence of DNA and above spectra on addition of 10µl DNA each time.

It is evident from Table 5, that all the complexes bind with DNA with high affinities and, the estimated binding constants are in the range 5.2- 6.9 x 10^5 M^{-1}. The binding order of the complexes is 1 > 2 < 3 > 4. The K_b value of 1-4 complexes are comparable with the reported values for redox active cobalt(II) pyridine-based tetrazolo[1,5-a]pyrimidine complexes[22]. The intrinsic binding constant for cis platin, which shows a hyperchromic shift, after subsequent addition of CT-DNA is reported as 3.20 x 10^4 M^{-1}. The binding nature of present complexes is significant due to π-stacking interaction of planar pyridyl-tetrazole rings. Binding of complexes to CT-DNA by external contact (electrostatic or groove binding) brings about bathochromic shift with hyperchromism absorption intensity[23]. A strong binding constant for 1, may be attributed to greater symmetric nature and electrostatical interaction with calf thymus[24].

Table 5

Effect of CT DNA on the absorption and binding constant of cobalt(II) complexes

Complex	λ_{max} /nm		Δλ/nm	H%	K_b / M^{-1}
	Free	Bound			
1	434.8	437.2	2.4	11.4	6.9 x 10^{-5}
2	438.1	439.9	1.8	10.3	5.8 x 10^{-5}
3	434.6	437.8	3.2	11.6	6.4 x 10^{-5}
4	438.3	440.3	2.0	10.8	5.2 x 10^{-5}

H% = Hyperchromism; Δλ = Bathochromic shift.

3.2.1. Thermal Denaturation Studies

The transition temperature from helix to coil can be determined by monitoring the absorbance of the DNA bases at 260 nm. The thermal melting studies were performed at [DNA] / [complex] = 25. The Tm values were determined by monitoring the absorbance of DNA at 260 nm as a function of temperature. The melting point of free CT-DNA was 60.1^{0}C under the employed experimental conditions. Under the same set of conditions, addition of Co(II) complexes of 1, 2, 3 and 4 show increased Tm (± 0.5 -1.0 ^{0}C) by 4, 3, 5 and 3^{o}C, respectively. This increase of the Tm values of CT-DNA in the presence of the complexes indicated that pyridyl-tetrazole Co(II) complexes stabilize the double helix of DNA.

3.1.2. Viscosity measurement

Binding interactions of Co(II) complexes with CT-DNA were also investigated by viscosity measurements[25]. A classical intercalation model usually resulted in lengthening the DNA helix, as base pairs were separated to accommodate the binding ligand leading to the increase of DNA viscosity. As seen in Fig. 2, the viscosity of DNA increases with the increase in the ratio of complexes to DNA. This result further suggested an intercalative binding mode of the complex with DNA and in agreement with spectroscopic results, such as hyperchromism and bathochromism of complexes in the presence of DNA.

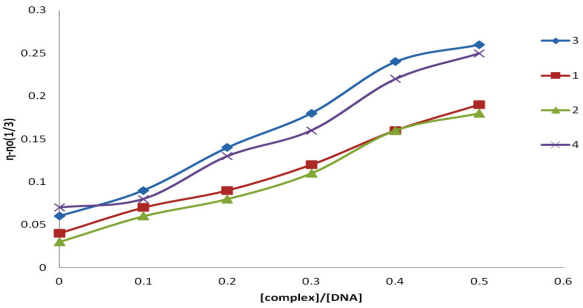


Figure 2

The effect of cobalt(II) complexes on the viscosity of DNA , Relative specific viscosity (R) Vs. [Complex]/[DNA].

3.1.3. DNA Cleavage Activity

The nuclease activity of 1-4 complexes has been investigated on pBR322 super coiled plasmid DNA by agarose gel electrophoresis in absence and presence of oxidant (H_2O_2). The gel electrophoresis diagram is given in Fig. 3; all even lanes are run in presence of oxidant, and all odd lanes are run in absence of oxidant (H_2O_2). The gel mainly contains complex 1 in lane 3 and 4, complex 2 in lane 5 and 6, complex 3 in lane 7 and 8 and complex 4 in lane 9 and 10, respectively. All complexes were in micro molar concentration for 120 min incubation period, in absence and presence of oxidant (H_2O_2) have shown significant cleavage activity at the physiological temperature and pH (7.2). From Fig. 3 is shown in Table 6. it is clear that all complexes show higher cleavage activity in presence of oxidant (H_2O_2, even Lanes) converting Form-I to Form-II and III. This may be attributed to the formation of hydroxyl free radicals. The production of a hydroxyl radical due to the reaction between the metal complex and oxidant may be explained as shown below.

$$(L)Co^{2+} + H_2O_2 \longrightarrow (L)Co^{3+} + OH + OH^{\cdot}$$

Table 6

Selected SC pBR322 DNA cleavage data of cobalt(II) complexes (Conc. 70.5 µM)

Lane No.	Reaction Conditions	Percentage of *		
		Form – I	From –II	From-III
1	DNA	96.3	3.7	ND
2	DNA + H_2O_2	94.4	5.6	ND
3	DNA + Complex 1	86.6	13.4	ND
4	DNA + Complex 1 + H_2O_2	46.3	41.1	6.8
5	DNA + Complex 2	82.3	17.7	ND
6	DNA + Complex 2 + H_2O_2	42.1	42.5	15.4
7	DNA + Complex 3	75.5	22.3	ND
8	DNA + Complex 3 + H_2O_2	ND	86.8	13.2
9	DNA + Complex 4	89.3	10.7	ND
10	DNA + Complex 4 + H_2O_2	ND	57.3	32.7

**** ~ ± 7- 10 % due to neglecting of discernible cleavage of pBR322***

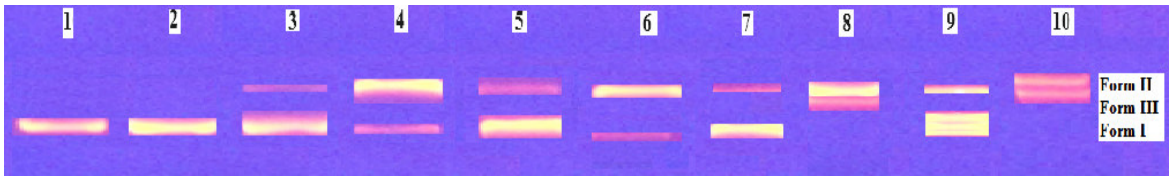


Figure 3

Agarose gel (1%) showing the results of electrophoresis of 1 µl of (0.10 µg/mL) pBR322 plasmid DNA, 2 µl of 0.1 M Tris-HCl (pH 8.0) buffer: 70.5 µM complex in DMF; 10 µM H_2O_2 were added, respectively, incubation at 37˚C(120 min): Lane 1: DNA (control); Lane 2: DNA + H_2O_2 (control); Lane 3: DNA + 1; Lane 4: DNA + 1 + H_2O_2; Lane 5: DNA + 2; Lane 6: DNA + 2 + H_2O_2; Lane 7: DNA + 3; Lane 8: DNA + 3 + H_2O_2; Lane 9: DNA + 4; Lane 10: DNA + 4 + H_2O_2.

Cleavage Mechanism Studies-The nuclease cleavage mechanism of complex 3 investigated in the presence of EDTA (chelating agent) (1 x 10^{-6} M); DTT (reducing agent) (1 x 10^{-6} M); DMSO (hydroxyl radical scavengers) and NaN_3 (singlet oxygen quencher) (1 x 10^{-6} M) and data is shown in Table 7. In Fig.4 lane 1-2 are control and Lane 3-4 are run with cobalt complex 3 in absences and presence of H_2O_2. Cobalt Complex 3 cleaves DNA more efficiently in the presence of H_2O_2, which may be due to hydroxyl radical ($OH^{\cdot}$) reaction with DNA[26]. In Lane 5, presence of chelating agent EDTA diminishes the nuclease activity of cobalt complex 3, whereas in presence of reducing agent (DTT) in lane 6 the cleavage activity of cobalt complex 3 is enhanced. This may be due to formation of cobalt(II) complex by catalytic reduction. In Lane 7 in presence of DMSO and Lane 8 in presence of NaN_3, cobalt complex 3 show no much effect in cleavage activity confirming the absence of singlet oxygen involvement.

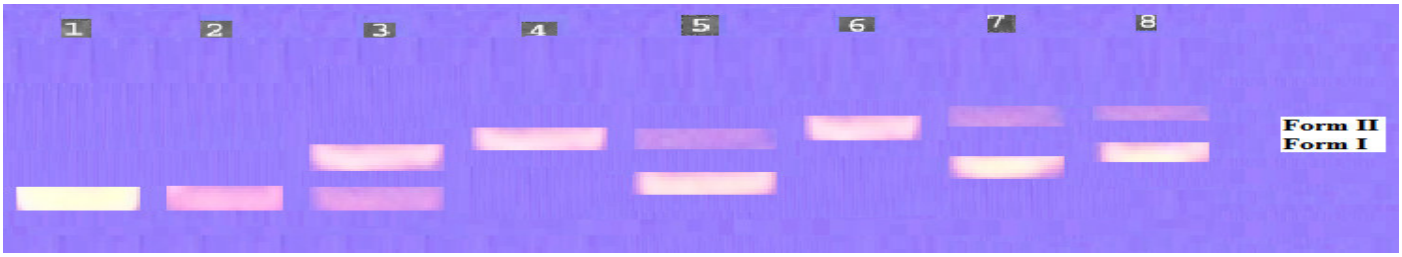


Figure 4

Lane-1: DNA Control; Lane-2: DNA + H_2O_2 (250 µM); Lane-3: DNA + 3; Lane-4: DNA + 3+ H_2O_2; Lane-5: DNA + 3+ EDTA; Lane-6: DNA + 3 + DTT; Lane-7: DNA + 3 + DMSO; Lane-8: DNA + 3+ NaN_3.

Table 7

Selected SC pBR322 DNA cleavage data of cobalt(II) complex 3 (Conc. 70.5 µM).

Lane No.	Reaction Conditions	Percentage of		
		Form – I	From –II	From-III
1	DNA	96.3	3.7	ND
2	DNA + H_2O_2 (10 µM)	93.4	6.6	ND
3	DNA + Complex 3	34.6	65.4	ND
4	DNA + Complex 3 + H_2O_2(10 µM)	4.3	92.7	ND
5	DNA + Complex 3 + EDTA(10 µM)	79.6	20.4	ND
6	DNA + Complex 3 + DTT(10 µM)	3.5	93.5	ND
7	DNA + Complex 3 + DMSO(0.5 mM)	36.5	63.5	ND
8	DNA + Complex 3 + NaN_3(10 µM)	38.2	61.8	ND

CONCLUSION

Pyridyl-tetrazole pendant arm containing -OH and $–N(CH_3)_2$, mononuclear cobalt(II) complexes of novel bidentate ligands have been synthesized and characterized. Physico-chemical and spectral studies reveal that mono nuclear complexes are distorted square planar geometry. All cobalt complexes are avid binder of CT-DNA. Complexes 1 and 3 show higher binding constant (K_b) may be due to more symmetric nature. In presence of H_2O_2 cobalt complexes cleave DNA more effectively which may be due to the reaction of hydroxyl radical with DNA. All the complexes cleave DNA via oxidative path. The highlights of the present investigations are: (i) Mononuclear complexes are synthesized with pyridyl-tetrazole pendant arm ligand. (ii) DNA binding and cleavage activities of the complexes are investigated. Binding constant values show these complexes are avid binder and cleave DNA through hydroxyl radical generation.

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