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ORIGINAL PAPER

Synthesis, Characterization, DNA Binding, DNA Cleavage and Antimicrobial Studies of Schiff Base Ligand and its Metal Complexes

Padmaja Mendu · C. Gyana Kumari · Rajesh Ragi

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Abstract A series of Cu(II) , Ni(II) , Co(II) , Mn(II) and Zn(II) complexes have been synthesized from the Schiff base ligand L. The Schiff base ligand 4-chloro-2–((4-oxo-4H-chromen-3yl) methylene amino) benzoic acid (L) has been synthesized by the reaction between chromone-3-carbaldehyde and 4-chloro-2-amino benzoic acid. The nature of bonding and geometry of the transition metal complexes as well as ligand L have been deduced from elemental analysis, FT-IR, UV–vis, ^{1}H NMR, ^{13}C NMR, ESR spectral studies, mass, magnetic susceptibility and molar conductance measurements. The complexes are found to have ML_2 composition and are neutral in DMSO. Based on elemental, conductance and spectral studies, six-coordinated geometry was assigned for these complexes. The ligand L acts as tridentate and coordinates through nitrogen atom of azomethine group, hydroxyl of the carboxyl group and oxygen atom of keto group of γ-pyrone ring. The interaction of Cu(II) complex with CT- DNA was carried out by UV–vis, fluorescence titrations and viscosity measurements. The complex binds to DNA through intercalative binding mode. The nuclease activity of the above metal complexes shows that Cu(II) and Co(II) complexes cleave DNA through redox chemistry. The biological activity of the ligand and its complexes have been studied on four bacteria *E.coli, B.subtilis, pseudomonas* and *Edwardella* and two fungi *penicillium* and *trichoderma* by well disc and fusion method and found that the metal complexes are more active than the free Schiff base ligand.

Keywords Chromone-3-carbaldehyde · DNA binding · Fluorescence · Viscosity · DNA cleavage · Biological activity

Introduction

In recent years, there has been interest in the studies of metal complexes both in solid and solution state due to their vast applications in different fields of biology and chemistry [1–6]. Although there are number of complexing and chelating ligands, Schiff bases have special interest due to their industrial and biological applications [7–15]. Earlier work reported that some drugs showed increased activity when administrated as metal complexes rather than as organic compound [16–18].

Chromones are a group of naturally and widely distributed compounds that are ubiquitous in nature, especially in the plant kingdom [19]. These compounds have attracted a great deal of attention and many synthesized chromone derivatives have been widely studied due to their biological activities including antimicobacterial, antifungal, anticonvulsant, antimicrobial and mushroom tyrosinase inhibition activities [20]. These derivatives also serve as intermediates to many products of fine chemical industries such as pharmaceuticals, agrochemicals, dyestuffs [21]. It should be especially noted that developing flavonoids as anticancer agents has interested medicinal chemists for many years. 4-Oxo-4H-chromene-3-carbaldehydes and their Schiff bases derivatives have attracted considerable interest in human colon cancer [22] and as potent topoisomerase inhibitor anticancer agents [23].

Chromones have a wide range of biological activities including tyrosine and protein kinase C inhibitors, antiallergic, antiviral, antitublin, antihypertensive as well being active at benzoazepine receptors, lipoxygenase, and cyclooxygenase

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P. Mendu (✉)
Department of chemistry, CMR institute of technology, Kandlakoya, Medchal, Hyderabad, Telangana 501401, India
e-mail: menduupadmaja@gmail.com

C. G. Kumari · R. Ragi
Department of Chemistry, Osmania University, Hyderabad, Andhra Pradesh 500 007, India

and modulating P-glycoprotein-mediated multidrug resistance [24, 25]. Chromone derivatives are essential for the synthesis of many important oxygen heterocycles such as pyrazoles and xanthones [26].

The Schiff base derived from chromone-3-carbaldehyde and 4-chloro-2-amino benzoic acid is having important biological applications. Hence, we have synthesized the transition metal complexes with this ligand and we have carried characterization by various spectroscopic techniques, mass, elemental analysis and also studied DNA binding, DNA cleavage, antibacterial and antifungal activities.

Experimental

Chemicals

Chromone-3-carbaldehyde and 4-chloro-2-amino benzoic acid were purchased from Sigma-Aldrich chemicals and remaining all chemicals and solvents were purchased from commercial sources and used as such without purification.

Synthesis of the Schiff Base Ligand (L)

The Schiff base ligand is prepared by condensation of chromone-3-carbaldehyde (1.74 g, 0.01 M) and 4-chloro anthranilic acid (1.82 g, 0.01 M) in absolute ethanol (25 ml), by adding traces of glacial acetic acid the mixture was refluxed for 3 h with continuous stirring. The excess of ethanol was distilled off and the mixture solution was poured on crushed ice. Then the yellow color solid was separated out. The compound is collected by filtration, washed several times with double distilled water and dilute ethanol, recrystallized from hot ethanol and dried in a vaccume desiccator. The melting point of the resulting Schiff base ligand (Fig. 1) is 217 °C. The yield is 94 %.

Synthesis of Metal Complexes

The Schiff base ligand L (0.01 M) is dissolved in hot methanol and hot methanol solution of corresponding salts (0.005 M) MX_2 [where M=Cu(II), Ni(II), Co(II), Mn(II) and Zn(II), X= chloride/nitrate/acetate] were mixed together and refluxed with constant stirring for 2–3 h at refluxing temperature. On cooling colored solids were precipitated out. The products were filtered, washed with cold methanol, dried in air and in desiccator over anhydrous $CaCl_2$ and stored in an airtight sample bottle. All the compounds are colored and are stable to air and moisture.

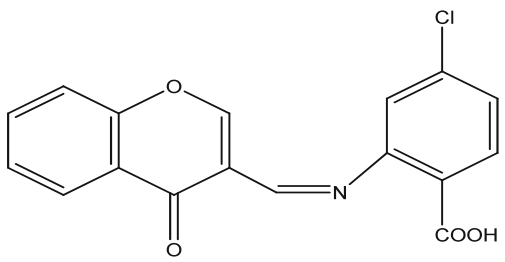

Fig. 1 Proposed structure of Schiff base ligand

Instruments Used

The percentage composition of C, H and N of complexes and ligand L were determined using micro analytical methods on Perkin Elmer 240C (USA) elemental analyzer. FT-IR spectra of the ligand and its complexes were recorded by using KBr pellets in the range 4000–400 cm^{-1} on Perkin Elmer Infra red model 337. The UV-Visible spectra of the Schiff base ligand and its metal complexes were carried out in DMSO using a Shimadzu UV-1601 spectrophotometer. ^{1}H NMR spectrum of the ligand was recorded at 200 MHz and 300 MHz on Varian Gemini Unity Spectrometer using TMS as internal standard. ^{13}C NMR spectra of the ligand was recorded at 100.6 MHz on Varian Gemini Spectrometer. The mass spectra of the compounds were recorded by ESI technique on VG AUTOSPEC mass spectrometer. The X-band ESR spectra was recorded with a EPR VARIAN-E-112 at RT. TGA and DTA analysis of complex were carried on Mettler Toledo Star system in the temperature range 0-1000 °C. The heating rates were controlled by 10 °C min^{-1}. Magnetic measurements were carried out on a Gouy balance model 7550 using $Hg[Co(NCS)_4]$ as standard. The conductivity measurements were carried out in DMSO (10^{-3} M) using Digisum Electronic Digital conductivity meter, 0.01 M KCl solution is used for calibration. Melting points of the ligand and decomposition temperature of complexes were determined on Polmen instrument (model no.MP-96). Molecular modelings of the compounds were carried out using Chem Office software.

DNA Binding Studies

Electronic Absorption Titration

In order to affirm quantitatively the affinity of the compounds binding to DNA, the intrinsic binding constants K_b of the copper complex is obtained by the electronic absorption spectroscopic method. The complex was dissolved in a mixture solvent of 1 % DMSO and 99 % Tris–HCl buffer (5 mM Tris–HCl; 50 mM NaCl, pH 7.1). Absorption titration experiments were performed in absence and presence of DNA. In these titration experiments fixed concentration of the complex (10 μM) was titrated with increasing amounts of DNA over a range of 10 μM to 100 μM. The reference solution was the corresponding Tris–HCl buffer solution. While measuring the absorption spectra, equal amounts of DNA was added to both

the compound solution and the reference solution to eliminate the absorbance of DNA itself. Each sample solution was scanned in the range 250–450 nm. The binding constant for the interaction of complexes with DNA was obtained from absorption titration data. The binding constant of the complexes (K_b) were calculated by using the following Eq. (1).

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

Where ε_a is the apparent extinction coefficient was obtained by calculating A_{obs} / [compound], the terms ε_f and ε_b correspond to the extinction coefficients of free metal complex and the extinction coefficient of the metal complex in the fully bound form respectively. From the plot of [DNA] / (ε_a- ε_f) Vs. [DNA] will give a slope ε_b- ε_f and an intercept 1 / K_b is the ratio of the slope and the intercept.

Fluorescence Titration

To understand the interaction pattern of the complexes with DNA more clearly, fluorescence titration method was used. Fluorescence measurements were carried out by keeping the concentration of complex constant (15 μM) and varied concentrations of CT DNA from 10 μM to 100 μM. An excitation wavelength 340 nm was used.

Viscosity Measurements

Viscosity measurements were carried out using an Ostwald viscometer maintained at a constant temperature at 28.0± 0.1 °C in a thermostatic bath. Flow time was measured with a digital stopwatch and each sample was measured five times for which an average flow time was calculated. Data were presented as $(\eta/\eta^{o})^{1/3}$ versus the ratio of the concentration of the complex and CT-DNA, where η is the viscosity of DNA in the presence of complex and η^{o} is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solution corrected for the flow time of buffer alone (t_o) η=t - t_o.

DNA Cleavage

A DMSO solution containing the metal complexes (15 μM) in a clean Eppendorf tube was treated with pUC19 plasmid DNA (3.3 μl of 150 μg/ml) in Tris–HCl buffer (0.10 M, pH 8.0) containing NaCl (50 mM) in presence and absence of additives. The contents were incubated for 1 h at 37 °C and loaded onto a 1 % agarose gel after mixing 5 μl of loading buffer (0.25 % bromophenol blue). 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 ethidiumbromide solution. The gel was then destained for 10 min by keeping it in sterile distilled water. The plasmid bands were visualized by viewing the gel under a transilluminator and photographed.

Biological Activity

Antibacterial Activity

The antibacterial activity of Schiff base ligand and its transition metal complexes were studied against four bacteria, *E.coli, B.subtilis*, *pseudomonas* and *Edwardella.* Each of the compound is dissolved in DMSO at a concentration1mg/ml was prepared. Paper discs of Whatmann filter paper No.1 are used after sterilization. The paper discs were saturated with 10 μl of the compound dissolved in DMSO solution and were placed in Petri dishes containing nutrient agar media inoculated with the above-mentioned bacteria separately. The inhibition zone was measured in millimeters after 24 h incubation at 37 °C.

Antifungal Activity

The complexes were screened for their antifungal activity against fungi viz. *Pencillium* and *Trichoderma*. These fungal species were isolated from the infected parts of the host plants i.e. potato dextrose agar. The cultures of the fungi were purified by single spore isolation technique. A concentration of 1 mg/ml of each metal complex compound in DMSO solution was prepared for testing against spore germination of each fungus. Filter paper discs of 5 mm in size, prepared by using Whatman filter paper no. 1 (sterilized in an autoclave) were saturated with 10 μl of the metal complex compounds dissolved in DMSO solution or DMSO as negative control. The fungal culture plates were inoculated and incubated at 25±2 °C for 48 h. The plates were then observed and the diameters of the inhibition zones (in mm) were measured and tabulated.

Results and Discussion

The analytical and physical data of Schiff base ligand and its metal complexes are given in Table 1. The data shows that the ligand L forms a 1:2 (M : L) complexes with all the metal ions. The prepared complexes are found to have the formulae $[ML_2]$ where L is Schiff base ligand, it coordinates as a uninegative ligand.

C, H, and N analysis of Schiff base ligand and its complexes are found in good agreement with the expected values.

Table 1 Analytical and physical data of Schiff base ligand L and its complexes

Compound	Empirical formula	Molecular weight	Color	Yield (%)	Melting Point in °C	Molar Conductance (Ohm^{-1} cm^2 mol^{-1})
Schiff base ligand (L)	$C_{17}H_{10}NO_4Cl$	328	yellow	90	216	–
Cu(II) complex	$[CuC_{34}H_{18}N_2O_8Cl_2]$	717	Dark green	71	>250	20.4
Ni(II) complex	$[NiC_{34}H_{18}N_2O_8Cl_2]$	712	brown	68	>250	14.4
Co(II) complex	$[CoC_{34}H_{18}N_2O_8Cl_2]$	712.2	green	75	>250	12.2
Mn(II) complex	$[MnC_{34}H_{18}N_2O_8Cl_2]$	708.3	greenish yellow	61	>250	25.7
Zn(II) complex	$[ZnC_{34}H_{18}N_2O_8Cl_2]$	718.8	Light yellow	60	>250	12.8

Molar Conductance

Molar conductance of the complexes were measured in DMSO at a concentration of 0.001 M. The observed conductance values indicating that the complexes are non-electrolyte.

IR Spectra and Mode of Bonding

The FT-IR spectral data of few important functional groups of the Schiff base ligand and its transition metal chelates are presented in Table 2.

The IR spectrum of the Schiff base ligand show a very strong absorption band at 1691 cm^{-1}, which is characteristic of the υ(C=O) of γ-pyrone ring. In the spectra of analyzed complexes, this absorption band has been shifted to lower region by around 40 cm^{-1}, indicating the coordination of the Schiff base ligand through the oxygen atom present in the 4-position of γ-pyrone ring [27].

The IR spectrum of free Schiff base ligand show another strong absorption band at 1605 cm^{-1} assigned for the υ(C=N) of azomethine group. In the spectra of analyzed complexes, this absorption band have been shifted to lower region by about 10–20 cm^{-1}, which confirms the coordination of azomethine group through nitrogen atom [28].

In addition to the above, the IR spectra of metal chelates shows absorption bands at 529–546 cm^{-1} and 432–447 cm^{-1} are due to (M-O) and υ(M-N) respectively [29]. These bands are absent in the spectrum of free Schiff base ligand.

The ν(OH)(carboxylic acid) and ν(C=O)(carboxylic acid) stretching vibrations are observed around 3400 and 1640 cm^{-1}, respectively for the ligand. The participation of the carboxylate oxygen atom in the complexes formation was evidenced from the disappearance of ν(OH) and ν(C=O) stretching vibrations in the spectra of the complexes. In the same way, the coordination of carboxylic group as carboxylate ion through oxygen atom is evidenced by the appearance of $\nu_{sym}(COO^-)$ and $\nu_{asym}(COO^-)$ vibrations in the spectra of the complexes in the regions around 1380 and 1560 cm^{-1}, respectively [30].

^{1}H NMR / ^{13}CNMR Spectra

The ^{1}H NMR spectrum of the Schiff base ligand is recorded in DMSO-d_6. In the ^{1}HNMR spectra of Schiff base ligand a peak at 10.26 δ is assigned for the proton of carboxylic acid of Schiff base and a singlet at 8.93 δ assignable for proton of azomethine group.

The ^{13}C NMR spectrum of the Schiff base ligand is recorded in DMSO-d_6. In the ^{13}C NMR spectra of Schiff base ligand a peak at 188.4 δ is assigned for the carbon of chromone carbonyl, a peak at 168.8 is assigned for the carbon of carboxylic group and a peak at 163.4 is assigned for azomethine carbon.

Mass Spectra of the Compounds

The mass spectral data of Schiff base ligand and its metal chelates are given in Table 3. Mass spectra of the ligand and its metal chelates show molecular ion peaks, which are in good agreement with the expected values. The mass spectrum

Table 2 Characteristic IR stretching bands of Schiff base ligand and its metal complexes in cm^{-1}

Compound	$\upsilon_{C=O}$(γ-pyrone ring)	$\upsilon_{C=N}$ (azomethine)	$\upsilon_{as(COO^-)}$(carboxylate)	$\upsilon_{s(COO^-)}$(carboxylate)	υ_{M-O}	υ_{M-N}
$C_{17}H_{10}NO_4Cl$	1691	1605	–	–	–	–
$[CuC_{34}H_{18}N_2O_8Cl_2]$	1643	1594	1570	1383	529	447
$[NiC_{34}H_{18}N_2O_8Cl_2]$	1655	1588	1560	1384	543	442
$[CoC_{34}H_{18}N_2O_8Cl_2]$	1656	1588	1536	1388	545	437
$[MnC_{34}H_{18}N_2O_8Cl_2]$	1654	1587	1529	1384	546	438
$[ZnC_{34}H_{18}N_2O_8Cl_2]$	1652	1585	1535	1383	529	432

Table 3 Mass spectral data of Schiff base ligand and its metal complexes

Compound	Calculated mass m/Z	Obtained mass m/Z	Peak assigned
$C_{17}H_{10}NO_4Cl$	327.6	328	L+H
$[CuC_{34}H_{18}N_2O_8Cl_2]$	717	719	M+2
$[NiC_{34}H_{18}N_2O_8Cl_2]$	712	712	M
$[CoC_{34}H_{18}N_2O_8Cl_2]$	712.2	714	M+2
$[MnC_{34}H_{18}N_2O_8Cl_2]$	708.3	709	M+2
$[ZnC_{34}H_{18}N_2O_8Cl_2]$	718	720	M+2

of ligand L gives a peak at 328 m/Z, which is assigned for [L+H] peak. Cu, Co, Mn and Zn complexes gives molecular ion peak at 719, 714, 709, 720 m/Z respectively, which are assigned as [M+2] peak. The mass spectrum of Ni (II) complex gives a peak at 712 m/Z and is assigned for [M] peak.

Magnetic Moment and Electronic Absorption Spectra

The electronic absorption spectral data and magnetic moment values of Schiff base ligand and its transition metal chelates are given in Table 4. The electronic spectra of Schiff base ligand shows strong absorption bands at 30,487 cm^{-1} and 36,630 cm^{-1}, which are attributed to n→Π* and Π→Π* transitions respectively [31].

The observed magnetic momentum value of Cu(II) complex is 1.90 BM, falls within the range observed for octahedral geometry. Further, the electronic spectra of Cu(II) complex shows one broad peak at 13,315 cm^{-1} due to transition between $^2E_g \rightarrow {}^2T_{2g}$ indicating octahedral geometry [32].

The Ni(II) complex exhibits peaks at 11148, 17035 and 20964 cm^{-1} attributed to the $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ transitions respectively, suggests octahedral geometry. The magnetic moment value of Ni(II) complex is found to be 3.11 BM, falls within the range of 2.8–3.5 BM for octahedral complexes, suggesting octahedral geometry [33].

The magnetic moment of Co(II) complex has been found to be 4.94 B.M, and it lies within the range expected for octahedral geometry. The electronic spectrum of Co(II) complex shows three peaks at 11876, 14771 and 16,207 cm^{-1} corresponding to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(F)$, suggesting octahedral geometry [34].

The Mn(II) complex shows three absorption peaks at 14,347 cm^{-1} expected for $^6A_{1g} \rightarrow {}^4T_{1g}(S)$, at 17,331 cm^{-1} corresponding to $^6A_{1g} \rightarrow {}^4T_{2g}(G)$, suggesting octahedral geometry [35]. Further, the octahedral geometry is proposed based on magnetic moment. The magnetic moment of the complex is found to be 5.81 BM, falls within the range expected for octahedral geometry.

The electronic spectrum of Zn(II) complex show one peak at 23,640 cm^{-1} due to charge transfer from ligand to metal. The observed magnetic moment value for this complex is zero, indicating diamagnetic nature of the complex. On the basis of analytical, conductance and spectral data, octahedral geometry is assigned to zinc complex.

ESR Spectral Studies

ESR spectra of Cu(II) complex was recorded at room temperature in the polycrystalline state, on X- band at frequency of 9.1 GHZ under the magnetic field strength of 3000G. The spectrum show one intense absorption in the high field and is isotropic due to tumbling of the molecule. The g values of the complex are $g_{\|}$ (2.397)>$g_{\perp}$ (2.056) >2.0023, indicating that the unpaired electron in the ground state of Cu(II) is predominantly in d_{x2-y2}. The spin-orbit coupling constant, λ value (−342 cm^{-1}) is less than the free Cu(II) ion (−832 cm^{-1}) which supports covalent character between metal and ligand.

Table 4 Electronic absorption spectral data and magnetic moment values of Schiff base ligand and its metal complexes

Compound	Absorption (υ) in cm^{-1}	Transition	Mag. Moment μ (B.M.)	Geometry
$C_{17}H_{10}NO_4Cl$	26525 40160	n→Π* Π→Π*	–	–
$[CuC_{34}H_{18}N_2O_8Cl_2]$	13315	$^2E_g \rightarrow {}^2T_{2g}$	1.90	Octahedral
$[NiC_{34}H_{18}N_2O_8Cl_2]$	11148 17035 20964	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$	3.11	Octahedral
$[CoC_{34}H_{18}N_2O_8Cl_2]$	11876 14771 16207	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	4.97	Octahedral
$[MnC_{34}H_{18}N_2O_8Cl_2]$	14347 17331	$^6A_{1g} \rightarrow {}^4T_{1g}(S)$ $^6A_{1g} \rightarrow {}^4T_{2g}(G)$	5.81	Octahedral
$[ZnC_{34}H_{18}N_2O_8Cl_2]$	23640	CT	dia	Octahedral

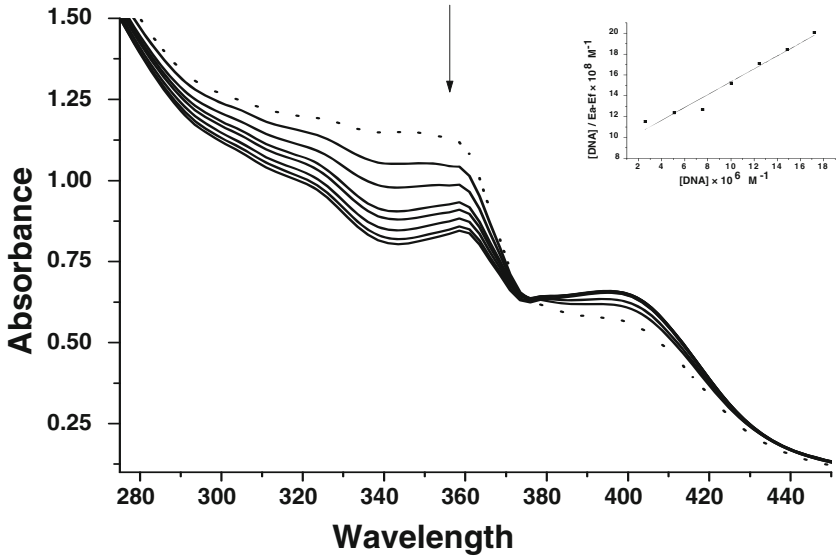

Fig. 2 Electronic spectra of copper complex (10 μM), in the presence of increasing amounts of CT-DNA, [DNA]=10–100 μM. Arrow shows the absorbance changes upon increasing DNA concentration; Inset: Linear plots for the calculation of intrinsic binding constant K_b

The value of exchange interaction term G, estimated from the following expression is 4.4.

$$G = g_{||}{}^{-2.0023}/g_{\perp}{}^{-2.0023}$$

If G>4.0, the local tetragonal axes are aligned parallel or only slightly misaligned. If G<4.0, significant exchange coupling is present and misalignment is appreciable. The observed value for the exchange interaction term G suggests that the complex has regular octahedral geometry.

DNA Binding Studies

Electronic Absorption Titration

The interaction of the copper complex with CT DNA was investigated using absorption spectra. The absorption spectra of the complex in the absence and presence of CT-DNA (at a constant concentration of complexes) are given in Fig. 2. As the concentration of DNA is increased the LMCT transition bands of complex exhibited hypochromism. These spectral characteristics may suggest that there are some interactions between the complex and CT DNA. The intrinsic binding constant K_b of complex with DNA was determined from the decay of the absorbance monitored for complex. The intrinsic binding constant K_b of the complex with CT-DNA was determined from the Eq. (1) [36].

Quenching Studies

Ethidium bromide (EB) emits intense fluorescence light in the presence of DNA, due to strong intercalation between the adjacent DNA base pairs. It was previously reported that the enhanced fluorescence can be quenched by the addition of a second molecule [37, 38]. The extent of fluorescence of EB bound to DNA is used to determine the extent of binding between the second molecule and DNA.

The emission spectra of EB bound to DNA in the absence and in the presence of Cu(II) complex is given in Fig. 3. The

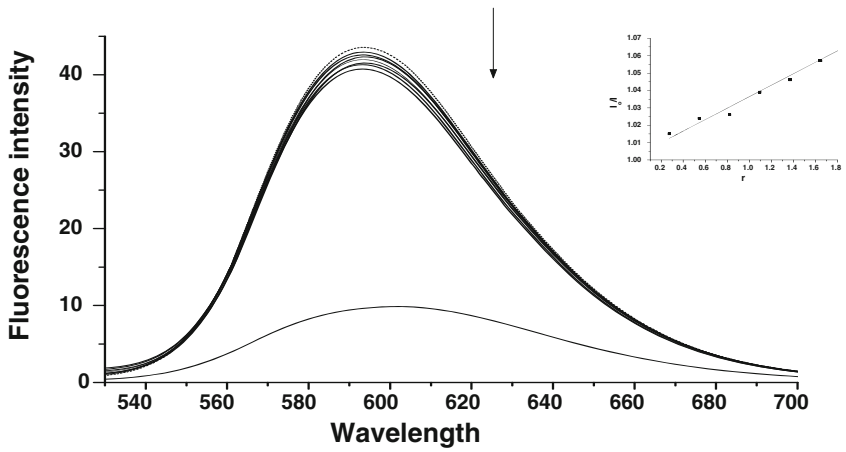

Fig. 3 Emission spectra of EB bound to DNA in the absence and presence of complex (10 μM); Inset: Stern-Volmer quenching curve

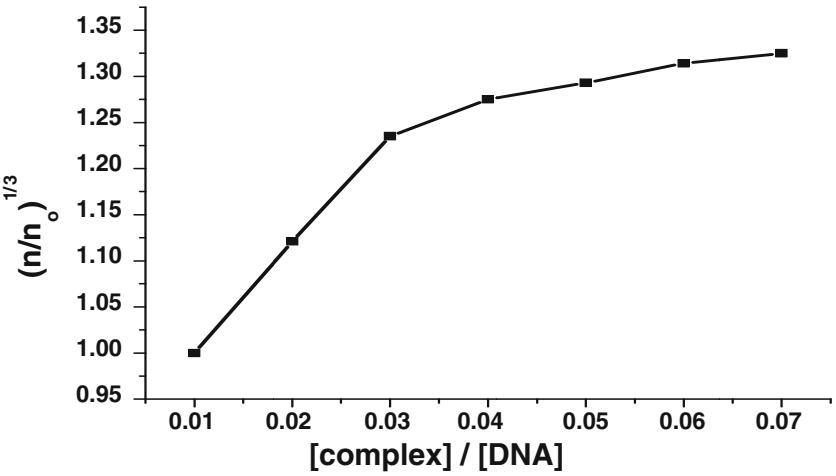

Fig. 4 Effect of increasing amount of Cu complex on the relative viscosity of CT-DNA at 28 °C±0.1, [DNA]=15 μM

addition of the complex to DNA pretreated with EB causes increase in emission intensity, indicating that the complex compete with EB in binding to DNA.

The classical Stern – Volmer equation is,

$$I_o/I = 1 + Kr$$

Where I_o and I are the fluorescence intensities in the absence and the presence of complex, respectively, K is a linear Stern-Volmer quenching constant dependent on the ration of r_{EB} (the ratio of the bound concentration of EB to the concentration of DNA) and r is the ratio of total concentration of complex to that of DNA.

The quenching plots illustrate that the quenching of EB bound to DNA by the copper complex is in good agreement with the linear Stern – Volmer equation, which proves that the complex bind to DNA. In the linear fit plot of I_o/I versus [complex] / [DNA], K is given by the ratio of the slope to the intercept. The K values for the complex is 0.31.

Viscosity Measurements

For further establishment of the interactions between the complex and DNA, viscosity measurements were carried out. Optical photo physical probes provide necessary, but not sufficient clues to support a binding model. The hydrodynamic measurements that are sensitive to length change (viscosity and sedimentation) are regarded as the least ambiguous and the most critical test of a binding in solution, in the absence of crystallographic structural data [39]. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, which leading to the increase of DNA viscosity. In contrast a partial and/or non-classical intercalation ligand could bend (or kink), then the DNA helix reduces its effective length and, concomitantly, its viscosity [40]. In the presence of complex, viscosity of DNA has been found to increase (Fig. 4). The increase in viscosity suggests that the complexes could bind to DNA by the intercalation binding mode.

DNA Cleavage Studies

Gel electrophoresis experiments using PUC19 DNA were performed with complexes in the presence and absence of H_2O_2 as an oxidant. The nuclease activity is greatly enhanced by the incorporation of metal ion in the respective copolymer; it is evident from Fig. 5 that the complexes 2 and 3 cleave DNA

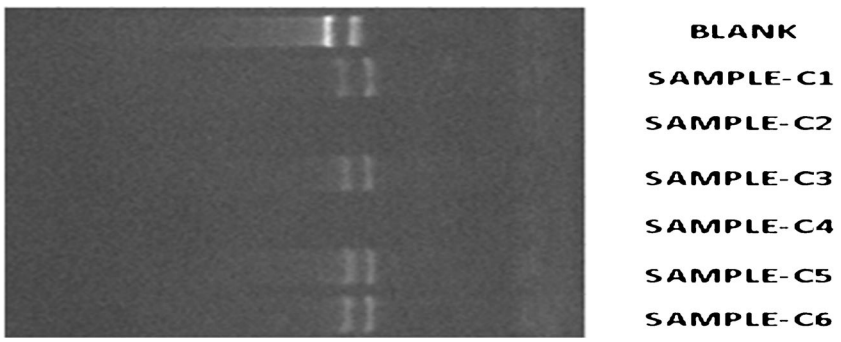

Fig. 5 Changes in the Agarose gel electrophoretic pattern of pUC19 plasmid DNA, induced by H_2O_2 and metal complexes: DNA alone (Blank); DNA+ligand+H_2O_2 (sample C1); DNA+Cu complex+H_2O_2 (sample C2); DNA+Ni complex+H_2O_2 (sample C3); DNA+Co complex+H_2O_2 (sample C4); DNA+Mn complex+H_2O_2 (sample C5); DNA+Zn complex+H_2O_2 (sample C6)

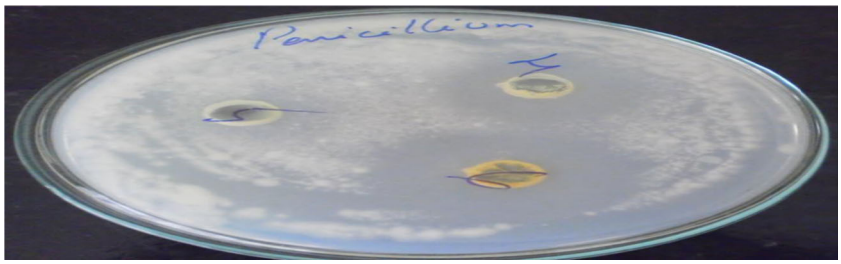

Fig. 6 Antifungal activity against *Penicillium*, where 6 is Co(II) complex and 4 is ZnnII) complex

more efficiently in the presence of oxidant, which may be due to the formation of hydroxyl free radicals. The production of hydroxyl free radical is due to the reaction between the metal complex and oxidant (Surendra Babu et al., 2007). These hydroxyl radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of the sugar phosphate backbone (Surendra Babu et al., 2007). The more pronounced nuclease activity in the metal complexes in the presence of oxidant may be due to the increased production of hydroxyl radicals. The cleavage efficiency was measured by determining the ability of the complex to convert the super coiled DNA to nicked (open circular) form or sheared form. As is evident from Fig. 5, there is a considerable increase in the intensity of bands for open circular form in the case of complexes 2 and 3. This suggests that Cu(II) and Co(II) complexes have nicking activity.

Biological Activity Studies

The antimicrobial activity of the Schiff base ligand and its metal complexes were tested against the four bacteria *E.coli, B.subtilis, Pseudomonas*, *Edwardella* and two fungi *penicillium* and *trichoderma* by the well disc and fusion method Fig. 6. The test solutions were prepared in DMSO at a concentration of 1 mg/ml. The zone of inhibition values were measured in millimeter after incubation at 37 °C for 24 h (bacteria) and 48 h (fungi). The antimicrobial results are given in Table 5.

The value in the above table indicates that the activity of the Schiff base ligand became more pronounced when coordinated with the metal ions. The presence of azomethine moiety and chelation effect with central metal enhances the antibacterial activities. This enhancement in antibacterial activity of these metal complexes can be explained based on the chelation theory.

When a metal ion is chelated with a ligand, its polarity will be reduced to a greater extent due to the overlap of ligand orbital and the partial sharing of the positive charge of the metal ion with donor groups. Furthermore, the chelation process increases the delocalization of the π-electrons over the whole chelate ring, which results in an increase in the lipophilicity of the metal complexes. Consequently, the metal complexes can easily penetrate into the lipid membranes and block the metal binding sites of enzymes of the microorganisms. These metal complexes also affect the respiration process of the cell and thus block the synthesis of proteins, which restrict further growth of the organism.

Conclutions

The Schiff base ligand 4-chloro-2–((4-oxo-4H-chromen-3yl) methylene amino) benzoic acid (L) and its five transition metal complexes have been synthesized. They have been characterized based on the analytical, spectral and magnetic measurements. The results of these investigations support the suggested structure of the metal complexes. All the metal ions are forming 1:2 (M:L) complexes and are neutral in DMSO. ESR spectra of copper complex suggest regular octahedron geometry. The interaction of Cu(II) complex with CT DNA was carried out by UV–vis, fluorescence titrations and viscosity measurements. The complex binds to DNA through intercaletive binding mode. The nuclease activity of the above metal complexes shows that Cu(II) and Co(II) complexes cleave DNA through redox chemistry. The biological activity of the ligand and its complexes have been studied on four

Table 5 Antimicrobial activity in centimeter (cm)

Compound	*E.coli*	*B.subtilis*	*Edwardella*	*Pseudomonas*	*Penicillium*	*Trichoderma*
$C_{17}H_{10}NO_4Cl$	+	+	+	+	+	+
$[CuC_{34}H_{18}N_2O_8Cl_2]$	+++	++	++	+	++	+
$[NiC_{34}H_{18}N_2O_8Cl_2]$	++	+	+	+	++	–
$[CoC_{34}H_{18}N_2O_8Cl_2]$	+++	++	++	+++	++	+++
$[MnC_{34}H_{18}N_2O_8Cl_2]$	+	+	+	++	–	–
$[ZnC_{34}H_{18}N_2O_8Cl_2]$	+++	+	+	++	++	++

bacteria *E.coli, B.subtilis, Pseudomonas*, *Edwardella* and two fungi *penicillium* and *trichoderma* by well disc and fusion method and found that the metal complexes are more active on microorganisms than the free Schiff base ligand.

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