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ORIGINAL ARTICLE

Metal based SOD mimetic therapeutic agents: Synthesis, characterization and biochemical studies of metal complexes

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Abstract Coordination compounds of Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) with the Schiff base obtained through the condensation of L^1 and L^2 (L^1 – obtained through the condensation of 4-aminoantipyrine with furfuraldehyde and L^2 – derived from 2-aminobenzothiazole and 3-nitrobenzaldehyde) were synthesized under reflux conditions. The newly formed complexes were characterized using elemental analysis, magnetic susceptibility, molar conductance, ^{1}H NMR, UV–Vis., IR and ESR techniques. Cyclic voltammogram of the complexes in DMSO solution at 300 K was recorded and their salient features were summarized. The X-band ESR spectrum of the copper complex in DMSO solution at 300 and 77 K was recorded. The in vitro biological screening of the investigated compounds was tested against the bacterial species, and fungal species by disc diffusion method. The antimicrobial activity of metal complexes was dependent on the microbial species tested, ligand and the metal salts used. A comparative study of inhibition values of Schiff bases and their complexes indicates that the complexes exhibit higher antimicrobial activity than the free ligands. The DNA binding studies were performed for the complexes using cyclic voltammetry and electronic absorption spectra. Superoxide dismutase activity of these complexes has also been examined.

1. Introduction

During the past few decades, considerable attention has been paid to the chemistry of metal complexes of Schiff bases containing nitrogen and other donor atoms (Djebbar et al., 1997; Bhattacharya et al., 1998; He et al., 1999; Wu et al., 2001; Chatterjee et al., 2004; Minu et al., 2004). This may be attributed to their stability, biological activity (Liu et al., 1996) and potential applications in many fields such as oxidation catalysis (Djebbar et al., 1998), electrochemistry (Hamada, 1997) etc. Schiff base complexes showed photochromism and thermochromism in the solid state by proton transfer from the (O) to the imine (N) atoms in the ligand molecule (Abd El-Wahab, 2007).

2-Aminobenzothiazole is found as structural unit in antioxidants, anti-inflammatory, herbicides, antibiotics, thermo-

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plastic polymers, flavouring, and odour agents and in the luciferine responsible for the bioluminenescence of fire flies (Ramalingam et al., 2004; Zitouni et al., 2004; Neelakandan et al., 2008). The coordinating property of 4-aminoantipyrine has been modified to give a flexible ligand system, formed by condensation with a variety of reagents like aldehydes, ketones, thiosemicarbazides carbazides, etc. (Tudor Rosu et al., 2010). The Schiff base of 4-aminoantipyrine and its complexes has a variety of applications in biological, clinical, analytical and pharmacological areas (Hitoshi et al., 1997; Punniyamurthy et al., 1995; Trivedi et al., 1992). Mixed ligand complexes of transition metals containing ligands with N, S or N, S, O donors are known to exhibit interesting stereochemical, electrochemical and electronic properties (De Sousa et al., 1992; Prabhakaran et al., 2005).

The metal complex-DNA interactions have received much importance for the development of new metal-based chemotherapeutic drugs (Afrati et al., 2009). Transition metal complexes have shown considerable attention as catalysts due to their DNA binding and cleavage properties (Saglam et al., 2002; Ji et al., 2001; Dupureur et al., 1997; Mesmaeker et al., 1996; Norden et al., 1996; Khairul et al., 2009; Khan et al., 2010) and ability to probe electron-transfer reactions involving metalloproteins (Schoumacker et al., 2003). These metal complexes can interact non-covalently with nucleic acids by intercalation, groove binding or external electrostatic binding (Metcalfe et al., 2003; Deshpande et al., 2009; Zeglis et al., 2007; Sastri et al., 2004). Among the above interactions, the intercalative interaction of ligands with the DNA is very interesting since many anti-cancer drugs and antibiotics show their biological activities through DNA intercalation (Mariappan et al., 2005, 2011). Several mixed ligand complexes have been found to exhibit SOD mimetic activities which are related to the flexibilities of the geometric transformations around the metal centres. Furthermore, low molecular weight compounds with superoxide dismutase mimetic activity have potential use as antioxidant pharmaceuticals in the treatment or prevention of several diseases related with the overproduction of an undesired $O_2\cdot^-$. In particular, some mixed ligand complexes with SOD mimetic activity have demonstrated to possess anti-inflammatory activity, and anticarcinogenic and antimutagenic effects (Oberley, 2005; Mitrunen et al., 2001).

With this considerations in mind, in this paper we have discussed the synthesis, characterization, DNA binding, biological studies including SOD activity of mixed ligand complexes containing a Schiff base L^1 and L^2 (L^1 – obtained through the condensation of 4-aminoantipyrine with furfuraldehyde and L^2 – derived from 2-aminobenzothiazole and 3-nitrobenzaldehyde) tuning up of biochemical potentials.

2. Materials and methods

2.1. Materials

All chemicals used in the present work viz, 4-aminoantipyrine, furfuraldehyde, 2-aminobenzothiazole, 3-nitrobenzaldehyde, Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) chlorides were of analytical reagent grade (Merck). The solvents used in the synthesis of the ligands and metal complexes were distilled before use. Calf thymus DNA was purchased from Genie Biolab, Bangalore, India.

2.2. Instrumentation

The elemental analysis was performed using Elementar Vario EL III Carlo Erba 1108. The amount of metal present in the metal complexes was estimated using ammonium oxalate method (Vogel 1978). IR spectra of the Schiff base ligands and their metal complexes were recorded on Perkin–Elmer FT-IR 783 Spectrophotometer in 4000–300 cm^{-1} range using KBr disc. ^1H NMR spectra were recorded on a Bruker Avance Dry 300 MHz FT-NMR Spectrometer in DMSO with TMS as the internal reference. The FAB mass spectrum of the Schiff base ligands and their complexes were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. ESR spectra of the mixed ligand copper complex was recorded on a Varian E 112 EPR Spectrometer in DMSO solution both at room temperature (300 K) and at liquid nitrogen temperature (77 K) using TCNE (tetracyanoethylene) as the g marker. Electronic absorption spectra of the Schiff base ligands and their mixed ligand complexes were recorded in DMSO using a Systronics 2201 double beam UV–Vis., spectrophotometer. Molar conductance of the metal complexes was measured in DMSO solution using a coronation digital conductivity metre. The magnetic susceptibility values were calculated using the relation $\mu_{eff} = 2.83(\chi_m T)^{1/2}$. The diamagnetic corrections were made by Pascal's constant and $Hg[Co(SCN)_4]$ was used as a calibrant. Electrochemical experiments were performed on a CHI 604D electrochemical analysis system with a three-electrode system consisting a glassy carbon working electrode, Pt wire auxiliary electrode and Ag/AgCl reference electrode. Tetrabutylammoniumperchlorate (TBAP) was used as the supporting electrolyte. All solutions were purged with N_2 for 30 min prior to each set of experiments. Calf thymus DNA was purchased from Bangalore Genei (India). Tris–HCl buffer solution used for binding studies was prepared using double distilled water.

3. Preparation

3.1. Preparation of ligands

The Schiff base ligands (L^1, L^2) were prepared according to earlier reports, with slight modifications (Ismail et al., 1997; Vicini et al., 2003)

L^1: The ligand (L^1) was prepared as reported previously.

L^2: The Schiff base (L^2) was prepared by the condensation of 2-aminobenzothiazole (1 mM) and 3-nitrobenzaldehyde (1 mM) dissolved in 30 mL absolute EtOH, in the presence of few drops of piperidine. The resulting mixture was stirred well, refluxed for 7 h. The mixture was concentrated to its half volume then cooled. Petroleum ether was added to the reaction mixture dropwise until a product began to precipitate. The formed product was filtered off, washed several times with EtOH, recrystallized from EtOH.

3.2. Preparation of metal complexes

An ethanolic solution of metal (M = $FeCl_3.6H_2O$, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$ and $ZnCl_2$) (1 mM) chlorides was stirred with an ethanolic solution of ligand(s) (L^1/L^2) (1 mM) and the resultant mixture was refluxed for ca. 6–8 h.

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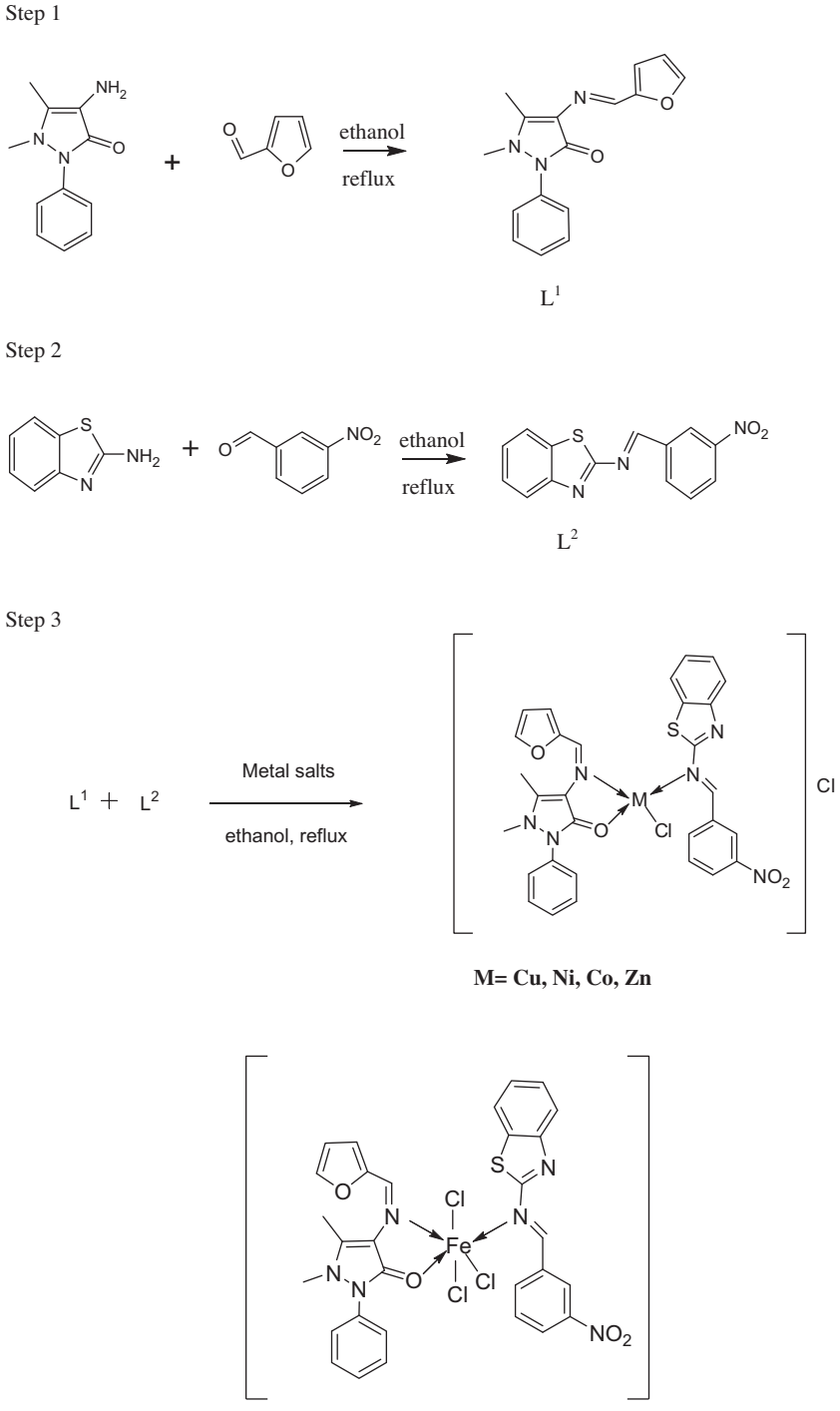

Scheme 1 Schematic route for synthesis of Schiff base ligands and its metal complexes.

Then, the volume of the solution was reduced to one-third on a water bath. The solid complex precipitated was filtered, washed thoroughly with ethanol and dried *in vacuum*. The schematic route for the synthesis of Schiff base ligands and their metal complexes is given in Scheme 1.

3.3. DNA binding experiments

Interaction of the complex with calf thymus DNA has been studied by recording electronic absorption spectra. A solution of CT-DNA in 5 mM Tris–HCl/50 mM NaCl (pH 7.0) gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.8–1.9, indicating that the DNA is sufficiently free of proteins. A concentrated stock solution of DNA was prepared in 5 mM Tris–HCl/50 mM NaCl in water at pH 7.0 and the concentration of CT-DNA was determined per nucleotide by taking the absorption coefficient ($6600\ dm^3\ mol^{-1}\ cm^{-1}$) at 260 nm. Stock solutions are stored at 4 °C and were used after no more than 4 days. Doubly distilled water was used to prepare buffer solutions. Solutions were prepared by mixing the complex and CT-DNA in DMF medium. After the equilibrium reached ca. 5 min the spectra were recorded against an analogous blank

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solution containing the same concentration of DNA. UV spectral data were fitted into Eq. (1) to obtain the intrinsic binding constant (K_b)

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

where [DNA] is the concentration of DNA in base pairs, ε_a, ε_b and ε_f are apparent extinction coefficient (A_{obs}/[M]), the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M), respectively. A plot of [DNA]/ ($\varepsilon_a^- \varepsilon_f$) versus [DNA] gave a slope of 1/ (ε_b- ε_f) and *Y*-intercept equal to $1/K_b(\varepsilon_b$- $\varepsilon_f)$; K_b is the ratio of the intercept.

3.4. Antimicrobial activity

The in *vitro* evaluation of antimicrobial activity was carried out. The prepared compounds were tested against some fungi and bacteria to provide the minimum inhibitory concentration (MIC) for each compound. MIC is the lowest concentration of the solution to inhibit the growth of a test organism. The in vitro biological screening effects of the investigated compounds were tested against the bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Pseudomonas aeruginosa* and fungal species *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* by disc diffusion method. One day prior to the experiment, the bacterial and fungal cultures were inoculated in nutrient broth (inoculation medium) and incubated overnight at 37 °C. Inoculation medium containing 24 h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get uniform distribution. This solution was poured (25 ml in each dish) into petri dishes and then allowed to attain room temperature. Wells (6 mm in diameter) were cut in the agar plates using proper sterile tubes. Then, the wells were filled up to the surface of agar with 0.1 ml of the test compounds dissolved in DMSO (200 μg/ml). The plates were allowed to stand for an hour in order to facilitate the diffusion of the drug solution. Then the plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi and the diameter of the inhibition zones was measured. Minimum inhibitory concentrations (MICs) were detected by the serial dilution method. The lowest concentration (μg/ml) of compounds, which inhibits the growth of bacteria after 24 h incubation at 37 °C, and of fungi after 72 h incubation at 37 °C was taken as the MIC.

3.5. Superoxide dismutase (SOD) activity

In vitro SOD activity was measured using alkaline DMSO as a source of superoxide radical ion (O_2^-) and nitrobluetetrazolium (NBT) as O_2^- scavenger (Bhirud et al., 1990, 1991). In general, 400 μL of the sample to be assayed was added to a solution containing 2.1 mL of 0.2 M potassium phosphate buffer (pH 7.8) and 1 mL of 56 μM NBT. The tubes were kept in ice for 20 min and then 1.5 mL of alkaline DMSO solution was added while stirring. The absorbance was then monitored at 540 nm against a sample prepared under a similar condition except that NaOH was absent in DMSO. The measure of SOD activity, expressed as IC_{50} is the concentration of the substrate or complex which causes 50% inhibition of the reduction of NBT.

4. Results and discussion

The Schiff base ligands and their complexes have been synthesized and characterized by spectral and elemental data. The colour, analytical data, molar conductance and magnetic moment are given in Table 1. The elemental analyses of the Schiff base ligands and their complexes supported the present composition of the complexes. The molar conductance of all complexes was measured in DMSO using 10–3 M solutions at room temperature. The conductance (Table 1) values are fund to be in the range of 8–51 ($\Omega^{-1}cm^2\ mol^{-1}$), indicating that all complexes are 1:1 electrolytic in nature except iron complex (non electrolytic in nature) (Geary, 1971). All complexes are air stable. These complexes are found to be soluble in chloroform, dimethylformide, tetrahydrofuran and dimethylsulphoxide.

4.1. 4.1.^{1}H NMR Spectra

The ligand (L^1) shows the following signals and their assignments are given below: phenyl multiplet at 7.0–7.5 δ (5H), –CH═N at 7.8 (due to furfuryl moiety), –C–CH_3 at 2.2 δ, N–CH_3 at 1.5 δ. The ligand (L^2) shows the following signals and their assignments are given below: phenyl multiplet at 7.5–8.1 δ (8H), –CH═N at 7.9 δ (due to phenyl moiety). The azomethine proton (–CH═N) signal in the spectrum of zinc complex is shifted down field (7.6 and 7.7 ppm) compared to the free ligands, suggesting deshielding of the azomethine group due to the coordination with metal ion. All protons were found to be in similar regions.

4.2. IR spectra

In order to study the bonding mode of the Schiff base to the metal complexes, the IR spectra of the free ligands were compared with the spectrum of the complexes. In the ligand L^1, the absorption band at 1647 cm^{-1} may be assigned to ν(C═O). The absorption band is shifted to a lower wave number 20–32 cm^{-1} in the spectra of all complexes suggesting the involvement of the pyrazolone oxygen in chelation.

The strong absorption bands located at 1602 and 1629 cm^{-1} in the spectrum of the free ligands L^1 and L^2 are attributed to ν(–CH═N) vibrations. These bands are shifted (by ~10–40 cm^{-1}) towards lower frequencies in the spectra of all complexes, which clearly suggested that complexation has taken place through the nitrogen atom of the azomethine group.

In the case L^2, the bands at 1458 and 1155 cm^{-1} are characteristic of the ν(C–S) of the thiazole ring. This band remains at the same position in the spectra of the Schiff base as well as in the case of its complexes revealing that sulphur atom of the thiazole ring is not taking part in the coordinate bond formation with the metal ions.

The IR spectra of all complexes show another band in the region 374–378 cm^{-1}, which may be due to M–Cl stretching vibrations. Furthermore, the IR spectra of the Fe(III) show another band in the region 351 cm^{-1}, which may be due to ν(Fe–Cl) vibrations. The IR spectra of the metal complexes also showed some new bands in the region 478–453 and 447–410 cm^{-1} which may be assigned to ν(M–O) and ν(M–N)

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Table 1 Physical and analytical data of the synthesized complexes.

Compound	Colour	M. P	Yield (%)	Elemental analysis				Molar conductance ($\Omega^{-1}cm^2\ mol^{-1}$)	$\mu_{eff\ (BM)}$
				C	H	N	M		
L^1	Yellow	205	85	68.30 (68.2)	5.38 (5.37)	14.94 (14.92)	–	–	–
L^2	Yellow	227	81	59.36 (59.35)	3.20 (3.22)	14.84 (14.83)	–	–	–
$[CuL^1L^2Cl]Cl$	Dark Green	252	82	51.53 (51.54)	3.46 (3.47)	12.03 (12.01)	9.10 (9.08)	48	1.82
$[NiL^1L^2Cl]Cl$	Pale Blue	185	78	51.89 (51.90)	3.49 (3.50)	12.11 (12.13)	8.46 (8.48)	51	Dia
$[CoL^1L^2Cl]Cl$	Dark pink	198	82	51.87 (51.85)	3.49 (3.47)	12.11 (12.10)	8.49 (8.48)	32	4.1
$[FeL^1L^2(Cl)_3]$	Dark Brown	>240	80	49.56 (49.54)	3.33 (3.31)	11.57 (11.55)	7.69 (7.67)	8	5.8
$[ZnL^1L^2Cl]Cl$	Dark Yellow	225	84	51.39 (51.40)	3.45 (3.43)	11.99 (11.97)	9.34 (9.36)	37	Dia

Table 2 IR spectral data (cm^{-1}) for the free ligands and their metal complexes.

Compound	$\nu_{C=O}$ (cm^{-1})	$\nu_{C=N}$ (furfuryl moiety) (cm^{-1})	$\nu_{C=N}$ (phenyl moiety) (cm^{-1})	ν_{M-O} (cm^{-1})	ν_{M-N} (cm^{-1})	ν_{M-Cl} (cm^{-1})
L^1	1647	1602	–	–	–	–
L^2	–	1629	–	–	–	–
$[CuL^1L^2Cl]Cl$	1634	1583	1576	457	410	374
$[NiL^1L^2Cl]Cl$	1653	1596	1584	474	442	377
$[CoL^1L^2Cl]Cl$	1650	1572	1565	453	429	376
$[FeL^1L^2(Cl)_3]$	1646	1579	1575	471	434	378
$[ZnL^1L^2Cl]\ Cl$	1638	1572	1569	478	447	375

Table 3 Electronic absorption spectral data of the complexes in DMSO solution.

Compound	Solvent	Absorption	Molar extinction coefficient, $cm^{-1}\ mol^{-1}$	Band assignment	Geometry
L^1	DMSO	31300	–	INCT	–
L^2	DMSO	28,996	–	INCT	–
$[CuL^1L^2Cl]Cl$	DMSO	25935, 20823	250 98	INCT $^2B_{1g} \rightarrow {}^2A_{1g}$	Square planar
$[NiL^1L^2Cl]Cl$	DMSO	20213, 24,662	125 107	$^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1B_{2g}$	Square planar
$[CoL^1L^2Cl]Cl$	DMSO	17648, 32751	132 155	$^1A_{1g} \rightarrow {}^1B_g$ INCT	Square planar
$[FeL^1L^2(Cl)_3]$	DMSO	18832, 19064	165 210	$^6A_{1g} \rightarrow {}^4T_{1g}(G)$ and $^6A_{1g} \rightarrow {}^4T_{1g}(G)$	Octahedral

bands, respectively. IR spectra of the ligands (L^1 and L^2) and metal complexes are presented in Table 2.

4.3. Electronic absorption spectra

The electronic absorption spectra of the ligands and their complexes were recorded in DMSO and presented in Table 3. In the present study, the copper complex exhibited bands at 25, 935 and 20,823 cm^{-1} which are assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transitions characteristic of square planar geometry with $d_{x^2y^2}$ ground state (Iskander et al., 2000; Reddy et al., 2000; Lever, 1984 Dutta et al., 1992). The magnetic moment value of 1.82 BM suggested the square-planar geometry around Cu(II) ion.

The absorption spectrum of the nickel complex shows two d-d bands at 20,213 and 24,662 cm^{-1} which are assigned as $^1A_{1g} \rightarrow {}^1B_{1g}$ and $^1A_{1g} \rightarrow {}^1B_{2g}$ transitions, indicating square planar geometry (Xu et al., 2003). This complex shows the diamagnetic behaviour suggesting the square planar environment around the Ni(II) ion.

The electronic spectrum of Fe(III) complex exhibits bands at 18,832 and 19,064 cm^{-1} which may be due to $^6A_{1g} \rightarrow {}^4T_{1g}(G)$ and $^6A_{1g} \rightarrow {}^4T_{1g}(G)$ transitions, respectively. These transitions are assigned for octahedral Fe(III) complexes. The electronic transitions together with the magnetic moment value of 5.8 BM suggested the octahedral geometry for the Fe(III) complex.

The spectrum for Co(II) complex shows d–d bands at 17,648 and 32,751 cm^{-1} may be assigned to $^1A_{1g} \rightarrow {}^1B_{1g}$ and $n–\pi^*$ respectively, in square planar stereochemistry. The magnetic moment value of 4.1 BM indicated the square planar geometry for the Co(II) ion.

Zn(II) is a d^{10} metal ion, no band is expected in the visible region and is also found as a diamagnetic complex, as expected. However, a strong band observed at 563 nm is assignable to the $L \rightarrow M$ charge transfer transition (Temel et al., 2002) which is compatible with this complex having a square planar geometry.

4.4. ESR spectra

ESR spectrum of the copper complex was recorded in DMSO at 300 and 77 K. The observed trend of $g_{||}(2.28) > g_{\perp}(2.05) > g_e(2.0023)$ describes the axial symmetry with the unpaired electron residing in the $d_{x^2y^2}$ orbital (Hathaway

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et al., 1970). The value of $g_{||} < 2.3$ in the present copper complex gives a clear indication of a covalent character of the metal–ligand bond and delocalization of the unpaired electron into the ligand.

Researchers reported a good correlation between f factor ($f = g_{||}/A_{||}$) and SOD like activity of copper complexes. The f value for CuZn SOD is 160 cm indicating a tetrahedral distortion from square planar geometry (f value is above 135 cm) and one of the features that enhances the catalytic activity of the enzyme. From the above EPR spectral data, the f values for copper complex is observed at 150 cm. Therefore, our synthesized copper complex exhibits appreciable square planar distortion that is expected to show high SOD like activity. The EPR parameters $g_{||}$, $g_{\perp}$, $A_{||}$ and the energies of d–d transition were used to evaluate the bonding parameters α^2, β^2, γ^2 which may be regarded as measures of the covalency of the in-plane σ bonds, in-plane π bonds and out-of-plane π bonds.

Molecular orbital coefficients α^2 (covalent inplane σ-bonding), β^2 (covalent in- plane π- bonding) and γ^2 (out-plane π -bonding) were calculated using the following equations:

$$\alpha^2 = (A_{||}/0.036) + (g_{||} - 2.0027) + 3/7(g_{\perp} - 2.0023) + 0.04 \quad (2)$$

If the α^2 value is 0.5, it indicates a complete covalent bonding, while the value of $\alpha^2 = 1.0$ suggests a complete ionic bonding. The observed value of α^2 (0.75) indicates that the complex has some covalent character.

$$\beta^2 = (g_{||} - 2.0027)E/ - 8\lambda\alpha^2 \quad (3)$$

$$\gamma^2 = (g_{||} - 2.0027)E/ - 2\lambda\alpha^2 \quad (4)$$

The observed β^2 and γ^2 values of 1.29 and 0.78 indicate that there is an interaction in the out-of-plane π -bonding, whereas the in-plane π -bonding is predominantly ionic. Significant information about the nature of bonding in the Cu(II) complex can be derived from the relative magnitudes of $K_{||}$ and $K_{\perp}$.

$$K_{||} = \alpha^2\beta^2 \quad (5)$$

$$K_{\perp} = \alpha^2\gamma^2 \quad (6)$$

For the present complex, the observed order $K_{||}$ (0.96) > $K_{\perp}$(0.585) implies a greater contribution from out-of plane π -bonding than from in-plane π-bonding in metal–ligand π bonding.

The empirical factor $f = g_{//}/A_{//}$ cm^{-1} is an index of tetragonal distortion. Values of this factor may vary from 105 to 135 for small to extreme distortions in square planar complexes and it depends on the nature of the coordinated atoms (Pogni et al., 2000). The f values of complexes are found to be in the range 137–154, indicating significant distortion from planarity. These values are shown in Table 4.

4.5. Mass spectra

The FAB mass spectra of the Schiff base and its metal complexes are used to compare the stoichiometry compositions. The Schiff base ligands L^1 and L^2 show a molecular ion peak at $m/z = 282$ and 284. The molecular ion copper complex was observed at $m/z = 701$ which shows the stoichiometry of the complexes as supported by the FAB mass spectra of other complexes. Elemental analysis values are in close agreement with the values calculated from the molecular form of these complexes, which is further supported by the FAB-mass studies of representative complexes.

4.6. DNA binding studies

4.6.1. Electronic absorption spectroscopy

Electronic absorption spectroscopy is performed to determine the binding characteristics of the metal complex with DNA. In UV region, two intense bands absorbed at 370 nm are attributed to the ligand to metal charge transfer absorption and another at 265 nm which is assigned to the $\pi \rightarrow \pi^*$transition of aromatic chromophore. It has been reported that the intercalating ability of the complex depends on the planarity of ligands, the coordination geometry, ligand donor atom type and the metal ion type (Terenzi et al., 2009). Intercalative mode of binding results in hypochromism and red shift due to the strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The extent of red shift and hypochromism are found to correlate with the intercalative binding strength.

Metal complexes which bind non-intercalatively or electrostatically with DNA may result in hyperchromism. In copper(II) complex the decrease in the absorption intensity (hypochromism) with a slight red shift is due to the intercalative binding between DNA and metal complexes. The absorption bands of complex at 265 nm shifted to 259, 257, 255 nm, respectively, with increasing concentrations (40, 60, 80 μM).

4.6.2. Electro chemical studies

In the cyclic voltammetric (CV) study, mixed ligand complexes were performed in the presence and absence of CT DNA and are shown in Fig. 1. From the CV results, it could be found that mixed ligand complexes exhibited a pair of redox peaks for one electron transfer couple of Cu(II)/Cu(I) at the scan rate of +2 to −2 V. The ratio of oxidation peak current to reduction peak current (Ipa/Ipc) of 0.5 and the peak-to-peak separation (ΔEp) of 0.26 suggested the characteristic of the electrotransfer process, and this was fairly common for Cu(II)/Cu(I) couple because of the reorganization of the coordination sphere (Palaniandavar et al., 1995). After interaction with CT DNA, the value of ΔEp was decreased to 0.20 V, suggesting that the reversibility of the electron-transfer process of the copper complex changed better. Both the oxidation and the reduction peak potentials underwent positive shifts accompanied by the decreases of the redox peak currents. The electrochemical potential of the small molecules would shift positively when it intercalated into DNA double helix, and if it is bound to DNA by electrostatic interaction, the potential would shift in a negative direction (Carter et al., 1989). So, the positive shift

Table 4 ESR spectral data of the copper complex.

Complex	$g_{||}$	$g_{\perp}$	g_{iso}	$A_{||}$	$A_{\perp}$	$K_{||}$	$K_{\perp}$	α^2	β^2	γ^2	($f = g_{||}/ A_{||}$)cm
[CuL^1L^2Cl]Cl at 300 K	–	–	2.15	–	–	–	–	–	–	–	–
[CuL^1L^2Cl]Cl at 77 K	2.28	2.05	–	152	41	0.96	0.585	0.75	1.29	0.78	150

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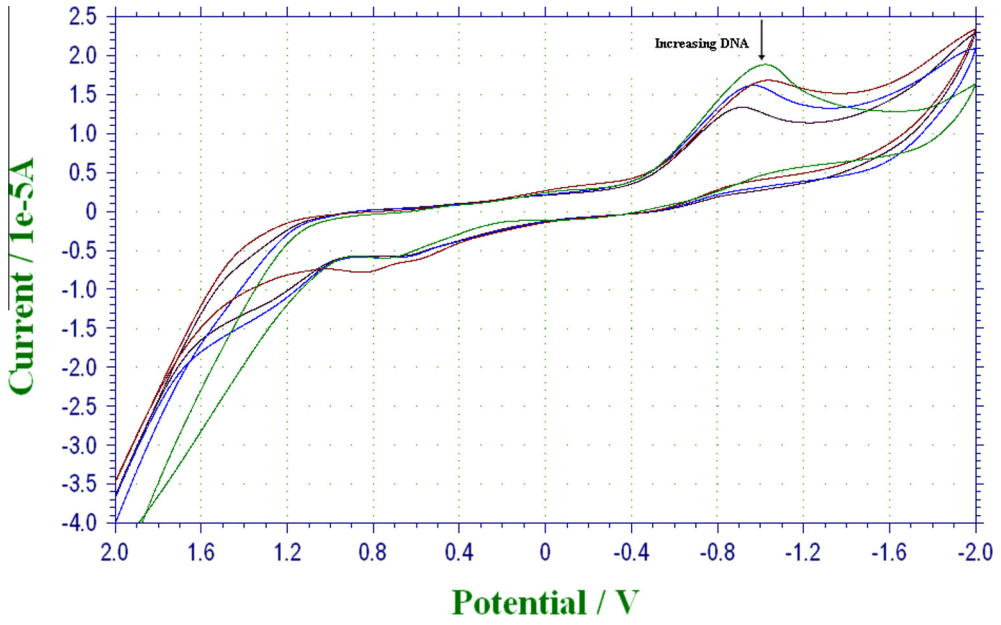

Figure 1 Cyclic voltammogram of $[CuL^1L^2Cl]Cl$ in buffer pH = 7.2 at 25 °C in the presence of increasing amount of DNA.

Table 5 Electrochemical parameters for the mixed ligand complexes on interaction with CT DNA.

Compound	Redox couple	[a]$E_{1/2}$(V)		[b]ΔEp(V)		Ip_a/Ip_c
		Free	Bound	Free	Bound	
$[CuL^1L^2Cl]Cl$	Cu(II) → Cu(I)	0.301	0.270	0.261	0.213	1.15
$[NiL^1L^2Cl]$ Cl	Ni(II) → Ni(I)	−0.331	−0.348	−0.435	−0.437	1.09
$[CoL^1L^2Cl]$ Cl	Co(III) → Co(II)	−0.242	−0.351	0.447	0.501	1.28
$[FeL^1L^2(Cl)_3]$	Fe (III) → Fe(II)	−0.218	−0.310	0.439	0.486	1.26
$[ZnL^1L^2Cl]$ Cl	Zn(II) → Zn(0)	−0.347	−0.353	0.338	−0.310	0.86

of the redox potentials implied that the present mixed ligand complex bind to DNA via an intercalation mode. The electrochemical parameters of mixed ligand complexes are shown in Table 5.

4.7. Superoxide dismutase (SOD) mimic activities

Transition metal complexes have received much attention in the development of superoxide dismutase (SOD) mimic activities for scavenging of the superoxide free radical ions ($O_2^{\cdot-}$) (Patel et al., 2006; 2007; 2009). The SOD activity for the complexes was tested using NBT assay. The SOD activities of these complexes have been compared with those of the well known native enzyme (Table 6) (Siddiqi et al., 2009, 2010, 2011; Sorenson, 1984). The IC_{50} values for Cu(II), Ni(II), Co(II), Fe(III) and Zn(II) complexes are found to be 98, 96, 87, 93, 85 μM respectively, indicating that the copper(II) complexes showed good activity. These values may be due to the strong field created by the Schiff base ligand which opposes the interaction of coordinated copper with $O_2^{\cdot-}$ radical. A greater interaction between superoxide ion and Cu(II) complex is induced due to the stronger axial bond, which results in an increased catalytic activity. In addition azomethine ligands containing electron withdrawing substituent stabilizes the Cu(I) complex formed during superoxide dismutation reaction which further reacts with superoxide ion to give hydrogen peroxide. The difference in reactivities of the synthesized complexes may be attributed to the coordination environment and the redox potential of the couple in metal complexes during the catalytic cycle. It has been reported that the redox potential of copper(II) ions is affected by the preset ligand system. The present Cu(II) complexes have a distorted square planar geometry encouraging a greater accessibility of the superoxide radical anion ($O_2^{\cdot-}$). Generally, metal-catalyzed superoxide dismutation is described by the following two equations:

$$Cu^{2+} + O_2^{\cdot-} \rightarrow Cu^{+} + O_2 \quad (7)$$

$$Cu^{+} + O_2^{\cdot-} + 2H^{+} \rightarrow Cu^{2+} + H_2O_2 \quad (8)$$

Table 6 IC_{50} values of some complexes and the native enzyme.

S. no.	Complex	IC_{50} value (μM)
1	$[CuL^1L^2Cl]Cl$	98
2	$[NiL^1L2Cl]Cl$	96
3	$[CoL^1L^2Cl]Cl$	87
4	$[FeL^1L^2(Cl)_3]$	93
5	$[ZnL^1L^2Cl]Cl$	85
6	Cu / Zn SOD	0.04

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Table 7 Minimum inhibitory concentration of the synthesized compounds against growth of bacteria (μg/ml).

Compound	*E. coli*	*K. pneumonia*	*S. typhi*	*P. aeruginosa*	*S. aureus*
L^1	60	64	66	66	72
L^2	58	62	58	59	69
$CuCl_2.2H_2O$	82	85	87	91	88
$NiCl_2.6H2O$	66	74	81	85	83
$CoCl_2.6H_2O$	81	68	72	78	89
$FeCl_3.6H2O$	79	82	86	82	91
$ZnCl_2$	75	81	81	92	83
$[CuL^1L^2Cl]Cl$	51	54	52	61	62
$[NiL^1L^2Cl]Cl$	49	56	52	65	53
$[CoL^1L^2Cl]Cl$	51	55	61	63	51
$[FeL^1L^2(Cl)_3]$	43	58	62	59	52
$[ZnL^1L^2Cl]Cl$	50	42	55	63	51
Pencillin	12	17	8	15	5
Ampicillin	15	13	5	2	8
Vancomycin	8	16	14	10	12
Ofloxacin	11	14	8	8	17

Table 8 Minimum inhibitory concentration of the synthesized compounds against growth of fungi (μg/ml).

Compound	*A. niger*	*R .stolonifer*	*A. flavus*	*R. bataicola*	*C. albicans*
L^1	63	65	71	77	50
L^2	51	65	53	67	52
$CuCl_2.2H_2O$	94	81	82	81	89
$NiCl_2.6H2O$	86	75	71	72	82
$CoCl_2.6H_2O$	91	81	85	89	80
$FeCl_3.6H2O$	87	88	82	72	71
$ZnCl_2$	72	73	82	86	82
$[CuL^1L^2Cl]Cl$	29	34	33	33	27
$[NiL^1L^2Cl]$ Cl	23	29	32	28	23
$[CoL^1L^2Cl]$ Cl	18	22	31	21	17
$[FeL^1L^2(Cl)_3]$	19	21	32	28	24
$[ZnL^1L^2Cl]$ Cl	29	29	21	31	27
Nystatin	14	17	9	12	14
Kctoconazole	3	7	15	8	16
Clotrimazole	6	8	17	12	4

The results suggested that the superoxide scavenging properties and oxidative behaviour of mixed ligand complexes were identical to those of complexes supporting the above mechanism.

4.8. Antimicrobial activity

The *in vitro* biological screening effects of the investigated compounds were tested against the bacterial species and fungal species by the disc diffusion method. The minimum inhibitory concentration (MIC) values of the synthesized compounds are summarized in Tables 6 and 7. A comparative study of the metal salts, ligands and their complexes (MIC values) indicates that complexes exhibit higher antimicrobial activity than the free ligands. The enhanced activity of the complexes can be explained on the basis of Overtone's concept (Sabrina Belaida et al., 2008) and Tweedy's Chelation theory (Dharamaraj et al., 2001). According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid soluble materials which makes liposolubility as an important factor, and which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes (Tumer et al., 1999).

The antimicrobial activity of the metal salts was also investigated. It was found that the metal salts exhibit antimicrobial activity at the concentration range used to assay the activity of the complexes but can be toxic. The toxic nature was reduced by the incorporation of suitably substituted ligands instead of chloride ion in the metal salts. This increased lipophilicity enhances the permeation of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism and as a result microorganisms die. The increased activity of the complexes may also be explained on the basis of their high solubility, fitness of the particles, size of the metal ion and the presence of the bulkier organic moieties.

Furthermore, the mode of action of the compounds may involve the formation of hydrogen bonds through the azomethine(C═N) group of complexes with the active centres of cell constituents resulting in the interference with normal cell

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processes. The different lipophilic behaviour of the aromatic residues such as antipyrine, and furfuraldehyde is involved in the biological activity mechanisms. There are other factors which also increase the activity are solubility, conductivity and bond length between the metal and ligand (Mohamed et al., 2009; Mahajan et al., 2009; chohan et al., 2001).

5. Conclusions

We have synthesized novel mixed ligand complexes with 4-aminoantipyrine derivatives which were synthesized and characterized by elemental analysis, spectral studies (FT-IR, UV–Vis, ^{1}H NMR, ESR). Antimicrobial activity studies showed that the complexes have better biological activity as compared to free ligands. This activity may be due to increased lipophilicity of the complexes. The mixed ligand complexes showed the higher binding activity due to the presence of electron donating methyl group in the 4-aminoantipyrine. The DNA-binding properties of mixed ligand complexes have been studied by electronic absorption spectra and cyclic voltammetry. The observed results suggested that the complexes were in interaction with DNA through the intercalation binding mode. Among the metal complexes, copper complex showed a higher scavenging ability for oxygen radical. We hope that the preliminary studies have encouraged carrying out *in vivo* experiments and maybe act as potential chemotherapeutic agents. Table 8.

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