Synthesis and spectroscopic studies on the Schiff base ligand derived from condensation of 2-furaldehyde and 3,3′-diaminobenzidene, L and its complexes with Co(II), Ni(II), Cu(II) and Zn(II): Comparative DNA binding studies of L and its Cu(II) and Zn(II) complexes

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ABSTRACT

The Schiff base ligand, N,N′-bis-(2-furancarboxaldimine)-3,3′-diaminobenzidene (L) obtained by condensation of 2-furaldehyde and 3,3′-diaminobenzidene, was used to synthesize the mononuclear complexes of the type, [M(L)(NO3)2] [M = Co(II), Ni(II), Cu(II) and Zn(II)]. The newly synthesized ligand, (L) and its complexes have been characterized on the basis of the results of the elemental analysis, molar conductance, magnetic susceptibility measurements and spectroscopic studies viz, FT-IR, 1H and 13C NMR, mass, UV–vis and EPR, EPR, UV–vis and magnetic moment data revealed a square planar geometry for the complexes with distortion in Cu(II) complex and conductivity data show a 1:2 electrolytic nature of the complexes. Absorption and fluorescence spectroscopic studies support that Schiff base ligand, L and its Cu(II) and Zn(II) complex exhibit significant binding to calf thymus DNA. The highest binding affinity in case of L may be due to the more open structure as compared to the metal coordinated complexes.

1. Introduction

Active and well-defined Schiff base ligands are considered as ‘privileged ligands’ because they are easily prepared by condensation of aldehydes or ketones with amines and are able to stabilize different metals in various oxidation states [1]. Schiff base complexes are extensively studied due to synthetic flexibility, selectivity and sensitivity towards variety of metal ions [2]. Schiff bases have application towards, degradation of organic compounds [3], radiopharmaceuticals [4] and as corrosion inhibitors in especially acidic environments for various alloys and metals like steel, aluminium and copper [5]. Complexes of transition and non-transition metals with Schiff base ligands are promising materials for optoelectronic applications due to their outstanding photo- and electroluminescent properties, and the ease of synthesis that readily allows structural modification for optimization of material properties [6].

Bidentate and tetradentate Schiff base ligands involving N,O donor sites possess many advantages such as facile approach for synthesis, relative tolerance, readily adjusted ancillary ligands, and tunable steric and electronic coordination environments on the metal centre. In view of above, tetradentate N2O2 ligands and their transition metal complexes, act as catalyst [7]. Various transition and inner transition metals complexes with bi-, tri- and tetradentate Schiff bases containing nitrogen and oxygen donor atoms play important role in biological systems and represent interesting models for metalloenzymes, which efficiently catalyze the reduction of dinitrogen and dioxygen [8].

Di- and tetra-Schiff base precursors based on 3,3′-diaminobenzidene have been reported in literature [9,10]. Bolos et al. have reported biological studies of Cu(II) coordination compounds with Schiff bases of polyamines with heterocyclic aldehydes as ligands [11,12]. Polyamines and heterocyclic aldehydes (2-thiophene-carboxaldehyde and 2-furaldehyde) were selected, the former due to its coordinative, conformational and physicochemical properties, while the latter in order to mimic biological systems and mechanisms in the process of drug design. The five- or six-membered chelate ring Schiff bases stabilize, in thermodynamic terms of entropy, the compounds synthesized [13].

In view of aforesaid importance of Schiff bases and their complexes, we have synthesized a novel Schiff base ligand, N,N′-bis-(2-furancarboxaldimine)-3,3′-diaminobenzidene derived from condensation reaction of 3,3′-diaminobenzidene with 2-furaldehyde and its complexes with Co(II), Ni(II), Cu(II), Zn(II) and their DNA binding studies.
2. Experimental

2.1. Materials and instrumentation

The metal salts, M(NO₃)₂·xH₂O [M = Co, Ni, Zn, x = 6; Cu, x = 3] (all E. Merck) were commercially pure samples. The chemicals 2-furaldehyde and 3,3′-diaminobenzidene (both Acros) were used as received. Solvent acetonitrile (AR) was used without further purification. The DMSO was dried before use by standard procedure in order to avoid the effect of water on conductivity [14]. Deionized water was used in synthesis. Highly polymerized calf thymus DNA sodium salt (7% Na content) was purchased from Sigma Chemical Co. Other chemicals were of reagent grade and used without further purification. The stock solution of 12.5 mM DNA/phosphate calf thymus DNA was prepared by dissolving 0.5% (w/w) in 0.1 M sodium phosphate buffer (pH 7.40) at 310 K for 24 h with occasional stirring to ensure homogeneity of solution. The absorption ratio of DNA was sufficiently free from protein. The stock solution of N,N′-bis-(2-furancarboxaldimine)-3,3′-diaminobenzidene and its Cu and Zn complexes with 5 mg/ml concentration were prepared. The elemental analysis was obtained from micro-analytical laboratory of CDRI, Lucknow using PerkinElmer 2400 CHN Elemental Analyzer. The FT-IR spectra (4000–400 cm⁻¹) were recorded as KBr pellets on Shimadzu 8201 TC spectrophotometer. ¹H and ¹³C NMR spectra in DMSO-d₆ at room temperature were recorded using Bruker Avance II 400 NMR spectrometer. The DART-MS of the Schiff base ligand was recorded on JEOL-AccuTOF JMS-T100LC Mass spectrometer having a DART source. The given sample was subjected as such in front of DART source. Dry helium was used with 4 LPM flow rate for ionization at 3500 °C. EPR spectrum at room temperature was recorded on Varian E-112 X-band EPR spectrometer using TCNE as standard at RSIC, IIT, Bombay, India. The electronic spectra at room temperature were recorded with Varian Cary 5E spectrophotometer in 1200–185 nm range using DMSO as solvent. The electrical conductivities of 10⁻³ M solution in DMSO were obtained on a Systronic type 302 conductivity bridge equilibrated at 25 ± 0.1°C. The magnetic susceptibility measurements were carried out using Faraday balance at room temperature. Fluorescence measurements were performed on a spectrophotometer Model RF-5301PC (Shimadzu, Japan) equipped with a 150 W Xenon lamp and a slit width of 3 nm. A 1.00 cm quartz cell was used for measurements. For the determination of binding parameters, 50 μM of complex solution was taken in a quartz cell and increasing amounts of ct DNA solution was titrated. Fluorescence spectra were recorded at 310K temperatures in the range of 740–880 nm upon excitation at 280 (λₑm = 772 nm). The UV measurements of calf thymus DNA were recorded on a Shimadzu double beam spectrophotometer model-UV1700 using a cuvette of 1 cm path length. Absorbance value of DNA in the absence and presence of compound was fixed at 0.1 mM, while the compound was added in increasing concentration.

2.2. Synthesis of N,N′-bis-(2-furancarboxaldimine)-3,3′-diaminobenzidene (L)

A hot solution of 3,3′-diaminobenzidene (1 mmol, 0.214 g) in 1:1 acetonitrile to water mixture (25 ml) was slowly added to a solution of 2-furaldehyde (2 mmol, 0.167 ml) dissolved in acetonitrile (5 ml). The reaction mixture was magnetically stirred for 24 h at room temperature leading to the isolation of yellow solid product which was washed with water and acetonitrile and dried in vacuum over anhydrous calcium chloride.

2.3. Synthesis of metal complexes

A solution of hydrated metal nitrate (0.085 mmol) in acetonitrile (10 ml) was added slowly to a hot solution of L (0.085 mmol) in acetonitrile (60 ml). The resulting solution was stirred under reflux for 6 h resulting in the isolation of solid product. The product thus formed was filtered, washed with acetonitrile and dried in vacuum over anhydrous calcium chloride.

2.4. Binding data analysis of L and its Cu and Zn complex

To elaborate the fluorescence quenching mechanism the Stern–Volmer equation (1) was used for data analysis [15].

\[
F_0/F = 1 + K_{SV}[Q]
\]

where \(F_0\) and \(F\) are the steady-state fluorescence intensities in the absence and presence of quencher, respectively. \(K_{SV}\) the Stern–Volmer quenching constant and \([Q]\) is the concentration of quencher (DNA). The \(K_{SV}\) for L and its Cu and Zn complex were calculated to be 1.79 × 10⁴, 1.42 × 10⁴ and 1.36 × 10⁴LM⁻¹, respectively. A higher \(K_{SV}\) value of L as compared to its Cu(II) and Zn(II) complexes suggests its stronger quenching ability. Further implicates its higher binding affinity toward the DNA then its complexes. The linearity of the \(F_0/F\) versus \([Q]\) (Stern–Volmer) plots for DNA-L and its Cu(II) and Zn(II) complexes (Fig. 1) depicts that the quenching may be static or dynamic, since the characteristic Stern–Volmer plot of combined quenching (both static and dynamic) is an upward curvature. When ligand molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the equation [16]:

\[
\log \left[ \frac{F_0 - F}{F} \right] = \log K + n\log[Q]
\]

where \(K\) and \(n\) are the binding constant and the number of binding sites, respectively. Thus, a plot of \(\log(F_0 - F)/F\) versus \(\log[Q]\) (Fig. 2) can be used to determine \(K\) as well as \(n\). The binding constant and the number of binding sites for L and its Cu and Zn complex were found to be \(K = 2.75 \pm 0.22 \times 10^4\) M⁻¹; \(n = 1.07, K = 0.56 \pm 0.41 \times 10^4\) M⁻¹; \(n = 0.91\) and \(K = 0.91 \pm 0.34 \times 10^4\) M⁻¹; \(n = 0.94\) respectively. The results suggest the compounds exhibit different degree of affinity toward the DNA molecule. The highest binding affinity in case of L may be due to the more open structure as compared to the metal coordinated complexes.
3. Results and discussion

Schiff base ligand, L was synthesized by condensation of 3,3′-diaminobenzidine and 2-furaldehyde in 1:2 molar ratio dissolved in water and acetonitrile. The complexes of type, [M(L)](NO₃)₂ were synthesized by the reaction of L and metal salt in 1:1 molar ratio in acetonitrile (Scheme 1). All complexes were stable at room temperature and dissolve in DMSO. The molar conductivity data for 1 mM solutions of complexes (Table 1) suggest that complexes have 1:2 electrolytic nature. The analytical data along with some physical properties of Schiff base ligand and its complexes are summarized in Table 1. The formation of Schiff base ligand L and its complexes and bonding modes were inferred from characteristic band positions in FT-IR spectra and resonance signals in ¹H NMR and ¹³C NMR spectra corresponding to coordinated Schiff base moiety. The geometry around Co(II), Ni(II), Cu(II) ions in the complexes were deduced from the positions of absorption bands observed in the UV–vis spectra and magnetic moment values. The binding parameters were found to be $K = 2.75 ± 0.22 \times 10^4 \text{ M}^{-1}$; $n = 1.07$, $K = 0.56 ± 0.41 \times 10^4 \text{ M}^{-1}$; $n = 0.91$ and $K = 0.91 ± 0.34 \times 10^4 \text{ M}^{-1}$; $n = 0.94$ for L and its Cu(II) and Zn(II) complexes, respectively. These parameters suggested that the Schiff base ligand L and its Cu and Zn complexes have good binding affinity toward DNA molecule.

3.1. IR spectra

The prominent bands observed in the IR spectra of the Schiff base ligand (L) and its complexes are listed in Table 2. The IR spectra of complexes were compared with that of free ligand in order to determine the coordination sites. Spectra of ligand as well as its complexes showed absence of band at 1735 cm⁻¹ due to $\nu$(=O) stretching frequency [17]. The appearance of bands in the region 3150–3450 cm⁻¹ in the spectrum of ligand, L corre-
sponded to N−H stretching frequency of the uncondensed 1–2 amino
of diamino benzidine [18]. However, the position of these
groups remained unchanged in the complexes indicating that –NH2
groups were not involved in coordination to metal ion. The band
at 1621 cm$^{-1}$ in the free ligand was assigned to υ(N−O) stretching
[19]. However in the complexes this band was shifted to higher
(2−27 cm$^{-1}$) or lower (5 cm$^{-1}$) wavenumbers indicating the par-
ticipation of azomethine nitrogen in the coordination to metal ion
[20]. A medium intensity band due to υ(−C−O−C) stretching vibra-
tion of furan appeared at 1247 cm$^{-1}$ in the ligand L [21]. This band
disappeared in Zn(II) complex while shifted to lower frequency at
1231 cm$^{-1}$, 1230 cm$^{-1}$ and 1236 cm$^{-1}$ in Co(II), Ni(II) and Cu(II)
metal complexes, respectively suggesting a coordination through oxy-
gen of furanyl moiety [22]. Appearance of new bands in the spec-
tro of complexes in the regions 552–596 cm$^{-1}$ and 424–468 cm$^{-1}$
were assigned to υ(M−O) and υ(M−N) stretching vibration [21]. The bands
appearing in the region 1480–1440 cm$^{-1}$, 1100–1064 cm$^{-1}$
and 805–740 cm$^{-1}$ were usual modes of phenyl ring vibration. In
all complexes a very strong band corresponding to υ(N−O) of free
nitrate anion was observed around 1383 cm$^{-1}$ [23].

3.2. $^{1}$H NMR and $^{13}$C NMR spectra

The $^{1}$H NMR spectra of the ligand L and its Zn(II) complex shown
in Figs. 3 and 4. The spectrum of the ligand shows a singlet at
8.5 ppm [24] assigned to azomethine protons (2H) which undergo
upfield shift in the Zn(II) complex and appears at 7.98 ppm [21]
suggesting the coordination of azomethine nitrogen to Zn(II) ion.
The upfield shift in the chemical shift values of aromatic protons as
well as furan ring protons in complex 5.69–7.74 ppm (m,12H,6Ar
The upfield shift in the chemical shift values of aromatic protons as
suggesting the coordination of azomethine nitrogen to Zn(II) ion.
upfield shift in the Zn(II) complex and appears at 7.98 ppm [21]
in Figs. 3 and 4. The spectrum of the ligand shows a singlet at
all complexes a very strong band corresponding to
moiety [9] in the ligand which remain unchanged in the1HN M R
spectral data were consistent with $^{1}$H NMR spectral
The resonance signals
at 168 ppm may reason-
ably be assigned to azomethine carbon [25]. The resonance signals
observed in the region 119–141.8 ppm were assigned to phenyl and
furanyl moiety of the ligand [25,26]. These signals showed down-
field shift (1–2 ppm) in the Zn(II) complex invoking coordination of
the ligand to Zn(II) ion through its azomethine N and furan O.

3.3. Electronic spectra and magnetic moments

The electronic spectrum of Co(II) complex showed two bands
at 17,055 cm$^{-1}$ and 14,960 cm$^{-1}$ assignable to $^{4}$A$_{2g}$ $→$ $^{4}$T$_{1g}$ (P)
and $^{4}$A$_{2g}$ $→$ $^{4}$T$_{1g}$ (F) transitions, respectively. However, a band
at 22,730 cm$^{-1}$ may be assigned to $π−π^*$ transition supporting a
square planar geometry [27]. The observed magnetic moment of
3.12 B.M. further corroborated the electronic spectral finding.
The electronic spectrum of Ni(II) complex exhibited three
absorption bands at 13,720 cm$^{-1}$, 21,207 cm$^{-1}$ and 25,070 cm$^{-1}$
which may be assigned to three spin allowed transitions,
$^{4}$A$_{1g}$ $→$ $^{4}$A$_{2g}$, $^{4}$A$_{1g}$ $→$ $^{4}$B$_{2g}$ and $^{4}$A$_{1g}$ $→$ $^{4}$E$_{g}$, respectively character-
istic of square planar geometry around Ni(II) ion. The diamagnetic
nature revealed by magnetic moment studies further confirm the
square planar environment around the Ni(II) ion [28].
However, the electronic spectrum of Cu(II) complex showed two bands at 17,827 and 22,275 cm$^{-1}$ assignable to $^{2}$B$_{1g}$ $→$ $^{2}$A$_{1g}$
and $^{2}$B$_{1g}$ $→$ $^{2}$E$_{g}$ transitions, respectively which corresponded to a
square planar geometry around Cu(II) ion [27]. The observed mag-
netic moment of 1.72 B.M. further supported the electronic results.

3.4. EPR spectra

EPR spectrum of Cu(II) complex (Fig. 5) shows two regions of
absorption centered at $g_{∥}$ and $g_{⊥}$ with well resolved nuclear hyper-
fine splitting of $g_{∥}$ signal due to copper nuclei in parallel orientation. There is no observable splitting of $g_{⊥}$ signal. The analysis of spec-
tum gives $g_{∥}$ = 2.23, $g_{⊥}$ = 2.05, $A_{∥}$ = 167 × 10$^{-4}$ cm$^{-1}$ which agree well with the values reported for distorted square planar geom-
metry around Cu(II) ion [29,30]. The spectrum shows the relation
$g_{∥}$ > $g_{⊥}$ > 2.0023 which is typical of axially symmetric d$^{9}$ Cu(II) hav-
ing one unpaired electron in $dx^2−y^2$ orbital [31]. The absence of signal corresponding to ($ΔM_s = ±2$) in the half field indicates the absence of any Cu–Cu interaction thus ruling out possibility of
dimeric structure. In axial symmetry, the $G_{∥}$ parameter defined as
$G_{∥}$ = $g_{∥}^2$−2/$g_{⊥}^2$−2, reflects the spin interaction between Cu(II) cen-

Table 1:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Analysis found (calcld)/%</th>
<th>Yield (%)</th>
<th>Color</th>
<th>mp (°C)</th>
<th>Molar conductivity (Ω$^{-1}$ cm$^{2}$ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>C$<em>2$H$</em>{18}$N$_6$O$_2$</td>
<td>69.05</td>
<td>4.96</td>
<td>15.43</td>
<td>50 Yellow</td>
</tr>
<tr>
<td></td>
<td>69.03 (4.89) (15.12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="NO$_3$">Co(L)</a>$_2$</td>
<td>48.99</td>
<td>3.42</td>
<td>15.37</td>
<td>&gt;300</td>
<td>120 Green</td>
</tr>
<tr>
<td></td>
<td>48.96 (3.38) (15.34)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="NO$_3$">Ni(L)</a>$_2$</td>
<td>47.97</td>
<td>3.00</td>
<td>15.20</td>
<td>&gt;300</td>
<td>113 Red brown</td>
</tr>
<tr>
<td></td>
<td>47.77 (3.28) (15.30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="NO$_3$">Cu(L)</a>$_2$</td>
<td>52.07</td>
<td>3.74</td>
<td>15.48</td>
<td>&gt;300</td>
<td>117 Dark brown</td>
</tr>
<tr>
<td></td>
<td>51.94 (3.85) (15.30)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><a href="NO$_3$">Zn(L)</a>$_2$</td>
<td>42.20</td>
<td>2.86</td>
<td>14.63</td>
<td>&gt;300</td>
<td>114 Light brown</td>
</tr>
<tr>
<td></td>
<td>42.37 (3.24) (15.01)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2:

IR spectral data of Schiff base ligand (L) and its metal complexes (cm$^{-1}$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>υ(C=O) (cm$^{-1}$)</th>
<th>υ(N−H) (cm$^{-1}$)</th>
<th>υ(C−O−C) (cm$^{-1}$)</th>
<th>υ(M−O) (cm$^{-1}$)</th>
<th>υ(M−N) (cm$^{-1}$)</th>
<th>υ(N−O) (cm$^{-1}$)</th>
<th>Phenyl ring vibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>1621</td>
<td>3474</td>
<td>1247</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1480, 1146, 753</td>
</tr>
<tr>
<td><a href="NO$_3$">Co(L)</a>$_2$</td>
<td>1616</td>
<td>3470</td>
<td>1231</td>
<td>585</td>
<td>430</td>
<td>1384</td>
<td>1461, 1069, 668</td>
</tr>
<tr>
<td><a href="NO$_3$">Ni(L)</a>$_2$</td>
<td>1648</td>
<td>3471</td>
<td>1228</td>
<td>591</td>
<td>434</td>
<td>1384</td>
<td>1420, 1073, 745</td>
</tr>
<tr>
<td><a href="NO$_3$">Cu(L)</a>$_2$</td>
<td>1646</td>
<td>3472</td>
<td>1236</td>
<td>590</td>
<td>436</td>
<td>1383</td>
<td>1440, 1078, 765</td>
</tr>
<tr>
<td><a href="NO$_3$">Zn(L)</a>$_2$</td>
<td>1623</td>
<td>3470</td>
<td>–</td>
<td>590</td>
<td>438</td>
<td>1383</td>
<td>1471, 1105, 750</td>
</tr>
</tbody>
</table>
ters in solid polycrystalline complexes. According to Hathaway and Billing [32], if $G > 4$, the spin-exchange interaction is negligible and if it is less than 4 considerable spin-exchange interaction prevails. In the present case this value comes out to be 4.6 which again indicates absence of Cu–Cu interaction, thus supporting proposed monomeric structure. Kivelson and Neiman have reported that $g_z$ values less than 2.3 indicates considerable covalent character of M–L bond and greater than 2.3 indicates ionic character.

Fig. 3. $^1$H NMR spectrum of the ligand.

Fig. 4. $^1$H NMR spectrum of the Zn(II) complex.
The present value comes out to be less than 2.3 which indicate considerable covalent character of M–L bond [33].

3.5. Mass spectroscopy

The mass spectrum of the ligand is given in Fig. 6. The observed molecular ion peak at m/z 371.17 of the ligand, L in the mass spectrum is consistent with the proposed molecular formula of the ligand.

3.6. Fluorescence measurements

3.6.1. DNA binding of L and its Cu(II) and Zn(II) complexes

The fluorescence spectroscopy provides insight of the changes taken place in the microenvironment of DNA molecule on ligand binding. The binding of these compounds with calf thymus DNA was studied by monitoring the changes in the intrinsic fluorescence of these compounds at varying DNA concentration. Fig. 7 shows the representative fluorescence emission spectra of the synthesized Schiff base ligand L upon excitation at 280 nm. The addition of DNA caused a gradual decrease in the fluorescence emission intensity of L and its Cu and Zn complexes with a conspicuous change in the emission signals. The spectra illustrates that a higher excess of DNA led to more effective quenching of the fluorophore molecule fluorescence. The quenching of the compound fluorescence clearly indicated that the binding of DNA to L and its Cu and Zn complex changed the microenvironment of fluorophore residue. The shift in the emission peak of synthesized molecule further depicts effective interaction at higher DNA concentration which is more prominent in case of L. The reduction in the intrinsic fluorescence of synthesized molecules upon interaction with DNA could be due to masking or burial of compound fluorophore upon interaction between the stacked bases with in the helix and/or surface binding at the reactive nucleophilic sites on the heterocyclic nitrogenous bases of DNA molecule.

3.6.2. Absorption spectroscopy

UV–vis absorption studies were performed to further ascertain the DNA binding to Schiff base ligand L and its Cu and Zn complex. The maximum absorbance of DNA was located around 260 nm. It is well established that the absorption peak of the double helical DNA occurs at this wavelength. The UV absorbance showed an increase with the increase in the L and its Cu and Zn complex concentration (Fig. 8A–C). Since Schiff base ligand L and its Cu and Zn complex does not show any peak in this region (Fig. 8A–C), hence the rise in the DNA absorbance is indicative of the interaction between DNA and L and its Cu(II) and Zn(II) complex. Schiff base ligand and its Cu and Zn complex exhibited hyperchromism but of varied degree. Slight bathochromic shift from 2 to 5 nm was observed. As hypochromism and hyperchromism are both the spectral features of DNA concerning of its double helix structure. Hypochromism means the DNA binding mode of the complex is electrostatic effect or interaction which can stabilize the DNA duplex [34,35], and hyperchromism signifies the breakage of the secondary structure of the DNA. So we primary speculate the complex interacting with the secondary structure with the calf thymus DNA resulting in its breakage and perturbation. After interaction with base pairs of DNA, the π→π* orbital of the bound ligand can couple with π orbital of the base pairs, due to decrease π→π* transition energy, which results in bathochromic shift [36]. The shift in the spectra of L suggests the more interference of the orbital by this molecule, which also corroborates with the high binding affinity of this Schiff base. The above changes are indicative of the conformational alteration of the DNA on, but of varied extent.
Fig. 8. Absorbance spectra of DNA in the absence and presence of different concentration of (A) L, (B) Cu(II) complex and (C) Zn(II) complex. DNA concentration was 0.10 mM (a). L, Cu(II) complex, Zn(II) complex concentration for DNA-compound system was at 12.5 μM (b), 25 μM (c) and 50 μM (d).

4. Conclusion

A novel tetradentate Schiff base ligand (L) containing N₂O₂ donor set and its corresponding Co(II), Ni(II), Cu(II) and Zn(II) complexes have been synthesized and spectrally characterized. Comparative DNA binding studies of L with its Cu(II) and Zn(II) complexes reveals that the Schiff base ligand exhibits highest binding affinity towards DNA molecule due to more open structure as compared to metal coordinated complexes.

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References

Synthesis, spectroscopic studies and crystal structure of the Schiff base ligand L derived from condensation of 2-thiophenecarboxaldehyde and 3,3′-diaminobenzididine and its complexes with Co(II), Ni(II), Cu(II), Cd(II) and Hg(II): Comparative DNA binding studies of L and its Co(II), Ni(II) and Cu(II) complexes

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1. Introduction

Schiff base ligands have been in chemistry catalogue for over 150 years [1]. Their instant and enduring popularity undoubtedly stem from the ease of their synthesis, bewildering versatility and wide ranging complexing ability once formed. Schiff base ligands with sulphur and nitrogen donor atoms in their structures act as good chelating agents for transition and non-transition metal ions [2–5]. The coordination of such compounds with metal ions, such as copper, nickel, iron, often enhance their activities [6], as has been reported for pathogenic fungi [7]. There is a continuing interest in metal complexes of Schiff bases. Because of the presence of hard nitrogen or oxygen and soft sulphur donor atoms in the backbone of these ligands, they readily coordinate with wide range of transition metal ions yielding stable and intensely coloured metal complexes, some of which have been shown to exhibit interesting physical and chemical properties [8]. The properties of Schiff base metal complexes stimulated much interest for their noteworthy contributions to single molecule-based magnetism, material science [9], catalysis of many reactions like carbonylation, hydroformylation, oxidation, reduction and epoxidation [10], their industrial applications [11] in the past two decades. Recently group 12 metal complexes, which contain a stable d10 electronic configuration, have received a lot of attention in the fields of inorganic chemistry, biochemistry and environmental chemistry [12]. There is substantial interest in the coordination chemistry of cadmium and mercury because of their toxic environmental effects. Therefore mobilization and immobilization of them in the environment, in organisms, and in some chemical processes have been found to depend significantly on complexation of these metal ions by coordination with nitrogen donor ligands [13].

The transition metal complexes that effectively bind and cleave DNA under physiological conditions have wide applications in nucleic acid chemistry as foot printing and sequence specific binding agents, for modelling the restriction enzymes in genomic research, and as new structural probes in diagnostic medicinal...
applications for the treatment of cancer [14–16]. Numerous studies have demonstrated that DNA is the primary intracellular target of anticancer drugs due to interaction between small molecules and DNA, which can cause DNA damage resulting in cell death [17,18]. Schiff bases have been shown as important anticancer drug candidates as they exhibit remarkable biological activities such as DNA damage, plasmid cleavage, protein cleavage and apoptosis [19–22]. Binuclear complexes of Schiff bases derived from 3,3’-diaminobenzidine have been reported to exhibit good DNA binding, DNA cleavage, SOD and antimicrobial activities [23,24].

In view of aforesaid importance of Schiff bases and their complexes, we report synthesis and characterization of novel Schiff base ligand, NN’-bis-(2-thiophenecarboxaldimine)-3,3’-diaminobenzidine (L) and its complexes with Co(II), Ni(II), Cu(II), Cd(II), Hg(II) and crystal structure of the ligand, L and comparative DNA binding studies of L and its Co(II), Ni(II) and Cu(II) complexes.

2. Experimental

2.1. Materials and instrumentation

The metal salts, MCl₂·xH₂O [M = Co, Ni, x = 6; Cu, x = 3; Cd, x = 1; Hg, x = 0] (All Merck) were commercially pure samples. The chemicals 2-thiophenecarboxaldehyde and 3,3’-diaminobenzidine (both Acros) were used as received. Solvents acetonitrile, THF and methanol (AR) were used without further purification. Deionized water was used in synthesis. Highly polymerized calf-thymus DNA (Sigma Chemical Co. of reagent grade and used without further purification. The stock solution of 12.5 mM DNA phosphate calf thymus DNA was prepared by dissolving 0.5% (w/v) in 0.1 M sodium phosphate buffer (pH 7.40) at 310 K for 24 h with occasional stirring to ensure homogeneity of solution. The absorption ratio A₂₆₀/A₂₈₀ in the range 1.8–1.9 indicated that DNA was sufficiently free from protein. The stock solution of NN’-bis-(2-thiophenecarboxaldimine)-3,3’-diaminobenzidine and its Co(II), Ni(II) and Cu(II) complexes with 5 mg/ml concentration were prepared. The elemental analysis was obtained from micro-analytical laboratory of CDRI, Lucknow using PerkinElmer 2400 CHN Elemental Analyzer. The metal contents of the complexes were determined by EDTA titration after decomposing the complexes with mixture of perchloric, sulphuric and nitric acids [25]. FT-IR spectra (4000–400 cm⁻¹) were recorded as KBr pellets on Shimadzu 8201 PC spectrophotometer. 1H and 13C NMR spectra in DMSO-d₆ were recorded using Bruker Avance II 400 NMR spectrometer at room temperature. The mass spectrum of the Schiff base ligand was recorded on JEOL-AccuTOF-JMS-T100LC Mass spectrometer having a DART source. The given sample was subjected as such in front of DART source. Dry helium was used with 4 LPM flow rate for ionization at 3500 °C. Mass spectra of Co(II) and Cu(II) complex were recorded on JEOL GC MAT II GC–MS mass spectrometer. EPR spectrum at room temperature was recorded on Varian E-112 X-band EPR spectrometer using TCNE as standard at RSCIC, IIT, Bombay, India. The electronic spectra at room temperature were recorded with Varian Cary 5E spectrophotometer in 1200–185 nm range using DMSO as solvent at IIT Madras. The molar conductivities of 10⁻³ M solution in DMSO were obtained on a Systronic type 302 conductivity bridge equilibrated at 25 °C ± 0.01 °C. The magnetic susceptibility measurements were carried out using Faraday balance at room temperature. Fluorescence measurements were performed on a spectrophotometer Model RF-5301PC (Shimadzu, Japan) equipped with a 150 W Xenon lamp and a slit width of 5 nm. A 1.00 cm quartz cell was used for measurements. For the determination of binding parameters, 50 µM of DNA–EtBr (1:1) complex was taken in a quartz cell and increasing amounts of tested compounds were titrated. Fluorescence spectra were recorded at temperatures 310 K in the range of 510–670 nm upon excitation at 355 (λmax was 594 nm). The UV measurements of calf thymus DNA were recorded on a Shimadzu double beam spectrophotometer model-UV1700 using a cuvette of 1 cm path length. Absorbance value of DNA in the absence and presence of complexes were made in the range of 220–300 nm. DNA concentration was fixed at 0.1 mM, while the complexes were varied from 5 µM to 20 µM.

2.2. Synthesis of NN’-bis-(2-thiophenecarboxaldimine)-3,3’-diaminobenzidine (L)

A hot solution of 3,3’-diaminobenzidine (1 mmol, 0.214 g) in 1:1 water to acetonitrile mixture (25 ml) was slowly added to a solution of 2-thiophenecarboxaldehyde (2 mmol, 0.19 ml) dissolved in acetonitrile (5 ml). The reaction mixture was magnetically stirred for 24 h at room temperature leading to the isolation of orange solid product which was washed with water and acetonitrile and dried in vacuum over anhydrous calcium chloride. Orange colour crystals were obtained from slow evaporation of this solid product dissolved in 10 ml of 1:1 THF to acetonitrile mixture at room temperature over a period of a week.

2.3. Synthesis of metal complexes

A solution of hydrated metal chloride (0.85 mmol) in methanol (20 ml) was added slowly to a hot solution of L (0.85 mmol) dissolved in 20 ml of 1:1 THF to methanol mixture. The resulting solution was magnetically stirred for 24 h at room temperature resulting in the isolation of solid product. The product thus formed was filtered, washed with methanol and dried in vacuum over anhydrous calcium chloride.

2.4. Binding data analysis of L and its Co(II), Ni(II) and Cu(II) complex

To elaborate the fluorescence quenching mechanism the Stern–Volmer equation (1) was used for data analysis [26].

\[
\frac{F_0}{F} = 1 + K_{SV}[Q]
\]

(1)

where \(F_0\) and \(F\) are the steady-state fluorescence intensities in the absence and presence of quencher, respectively. \(K_{SV}\) is the Stern–Volmer quenching constant and \([Q]\) is the concentration of quencher (DNA). The \(K_{SV}\) for L and its Co(II), Ni(II) and Cu(II)complexes were found to be of the order of 10⁴ and among them highest is for Cu(II) complex (i.e. 25.7 ± 10⁴ L·mol⁻¹). A higher \(K_{SV}\) value of Cu(II) complex as compared to L and its Co(II) and Ni(II) complexes suggested its stronger quenching ability. Further implicate its higher binding affinity toward the DNA. The linearity of the \(F_0/F\) versus \([Q]\) (Stern–Volmer) plots for DNA–L and its Co(II), Ni(II) and Cu(II) complexes (Fig. 1) depicts that the quenching may be static or dynamic, since the characteristic Stern–Volmer plot of combined quenching (both static and dynamic) is an upward curvature. When ligand molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the equation [27]:

\[
\log \left[ \frac{F_0 - F}{F} \right] = \log K + n \log [Q]
\]

(2)

where \(K\) and \(n\) are the binding constant and the number of binding sites, respectively. Thus, a plot of \(\log(F_0 - F)/F\) versus \(\log [Q]\) (Fig. 2) can be used to determine \(K\) as well as \(n\). The values of \(K\) and \(n\) (Table 1), suggested the high binding affinity between the
complexes and the DNA, among which Cu(II) complex showed the highest binding.

2.5. X-ray crystallography

Slow evaporation of this solid product, N,N′-bis-(2-thiophenecarboxaldiamine)-3,3′-diaminobenzidine (L) which is dissolved in 10 ml of 1:1 THF to acetonitrile mixture at room temperature over a period of a week afforded orange colour cuboid crystals suitable for X-ray structure analysis. A crystal of the approximate dimension 0.31 mm × 0.28 mm × 0.23 mm was selected covered with paraffin oil. The oil “freezes” at reduced temperature and holds the crystal static in the X-ray beam then the crystal was cooled down to −100 °C ± 3 °C in a stream of nitrogen. Data was collected on Bruker SMART APEX with CCD area detector using graphite monochromatized Mo Kα-radiation (λ = 0.70930 Å). The quality factors R1 and wR2 are defined by the following formulas with m (number of reflections) and n (number of parameters):

\[ R = R_1 = \frac{\sum |F_o - |F_c| |}{\sum F_o} , \quad wR = \sqrt{\frac{\sum w(F_o - |F_c|)^2}{\sum wF_o^2}} \]

\[ wR_2 = \sqrt{\frac{\sum w(F_o - |F_c|)^2}{\sum w(F_o^2)^2}} , \quad \text{GOF} = \sqrt{\frac{\sum w(F_o - |F_c|)^2}{m - n}} \]

A least squares fit using the position of 30 selected reflections in a range of 2° < 2θ < 60° supplied correct unit cell dimensions. The crystal belong to the orthorhombic crystallographic space group Pca2₁ and Z = 4 with cell parameters a = 8.6402(19) Å, b = 6.3315(14) Å, c = 35.613(8) Å and α = β = γ = 90°.

The structure was solved by direct methods (SHELXS-97) [28]; refinement was done by full matrix least squares on F² using the SHELXL-97 program suite [28]. Due to relatively low calculated absorption coefficients and the cuboid shape of the crystal, absorption corrections were considered to be unnecessary. At the end the atomic coordinates of hydrogen atoms were calculated on the basis of an idealized geometry. Furthermore the riding model applied ensures that a change in the carbon position is automatically transferred to the adjacent hydrogen atoms. The individual isotropic U-values of these hydrogen atoms were adopted from the pertinent carbon or nitrogen atoms and increased by a factor of 1.2.

Finally the parameters of all heavier atoms were subjected to a last least-squares refinement to take into account the incorporation of hydrogen atoms.

3. Results and discussion

Schiff base ligand, L was synthesized by condensation of 3,3’-diaminobenzidine and 2-thiophenecarboxaldehyde in 1:2 molar ratio dissolved in 20 ml of 1:1 water and acetonitrile mixture. The complexes of type, [M₂L₂]Cl₄ synthesized by reaction of L and metal salt in 1:1 molar ratio dissolved in 25 ml of 1:1 THF and methanol mixture (Scheme 1). All complexes were stable at room temperature and dissolve in DMSO. The molar conductivity data for 1 mM solutions of complexes (Table 2) suggested that complexes have 1:2 electrolytic nature [29]. The analytical data along with some physical properties of Schiff base ligand and its complexes are summarized in Table 2. The formation of Schiff base ligand L and its complexes and bonding modes were inferred from characteristic band positions in FT-IR spectra and resonance signals in ¹H NMR and ¹³C NMR spectra corresponding to coordinated Schiff base moiety. The geometry of the complexes were deduced from the positions of absorption bands observed in the UV–vis spectra and magnetic moment values. The bonding parameters (Table 1) suggested that the Schiff base ligand L and its Co(II), Ni(II) and Cu(II) complexes have good binding affinity toward DNA molecule.

3.1. X-ray crystallographic analysis of the ligand

The ORTEP diagram of free Schiff base ligand is given in Fig. 3. C1–C’1 bond length, 1.488(3) Å is within the range of a single bond. Aromatic rings attached to azomethine double bonds in the Schiff base are coplanar (C4–N2–C7–C8 and C4′–N2′–C7′–C8′ torsion angles are 178.5(3)° and 179.1(2)°). The N2≡C7 and N2′≡C7′ imine bond lengths of 1.277(4) Å and 1.267(3) Å are typical of a double bond. No intermolecular contacts significantly shorter than Van der Waals distances were found. Details of crystal data and structural refinement are given in Table 3. Bond lengths and angles are given in Table 4.

![Fig. 1. Stern–Volmer plot for the binding of L and its Co(II), Ni(II) and Cu(II) complex with DNA–EBr.](image)

![Fig. 2. Comparative binding analysis plot of log(|F₀ – |F|) versus log[Q] for determining the binding parameters of L and its Co(II), Ni(II), Cu(II) complex with DNA.](image)

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>K (mol⁻¹)</th>
<th>n</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>0.90 x 10⁶</td>
<td>1.2</td>
<td>0.992</td>
</tr>
<tr>
<td>Co(II) complex</td>
<td>3.46 x 10⁶</td>
<td>1.3</td>
<td>0.989</td>
</tr>
<tr>
<td>Ni(II) complex</td>
<td>8.72 x 10⁷</td>
<td>1.6</td>
<td>0.997</td>
</tr>
<tr>
<td>Cu(II) complex</td>
<td>32.7 x 10⁷</td>
<td>1.7</td>
<td>0.993</td>
</tr>
</tbody>
</table>
Where \( M = \text{Co(II), Ni(II), Cu(II), Cd(II), Hg(II)} \).

\( L = \text{N,N'-bis-(2-thiophenecarboxaldimine)-3,3'-diaminobenzidene} \)

Scheme 1.

Fig. 3. ORTEP diagram.
Table 2  Analytical data, m/z, % yield, colour, melting point and molar conductivity of Schiff base ligand, L and its coordination complexes.

<table>
<thead>
<tr>
<th>Compound/formula</th>
<th>F.W. (m/z found)</th>
<th>Found (calcd) %</th>
<th>Yield%</th>
<th>Colour</th>
<th>M.P./molar conductivity (Ω⁻¹ cm² mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₂₂H₁₈N₄S₂</td>
<td>402.531</td>
<td>C 65.66</td>
<td>13.93</td>
<td>Orange</td>
<td>50/226–229/–</td>
</tr>
<tr>
<td>C₄H₅N₈S₄Cl₄</td>
<td>656.74</td>
<td>H 4.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[CoL₂]Cl₄</td>
<td>(403.12)</td>
<td>N 4.34</td>
<td>10.53</td>
<td></td>
<td>38/Pink &gt;300/112</td>
</tr>
<tr>
<td>C₁₈H₁₄Co₂N₈S₄Cl₄</td>
<td>(1064.89)</td>
<td>M 11.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Ni₂L₂]Cl₄</td>
<td>1064.25</td>
<td></td>
<td>10.54</td>
<td></td>
<td>44/Green &gt;300/113</td>
</tr>
<tr>
<td>C₁₈H₁₄Ni₂N₈S₄Cl₄</td>
<td>(1064.89)</td>
<td></td>
<td>11.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Cu₂L₂]Cl₄</td>
<td>1073.97</td>
<td></td>
<td>10.45</td>
<td></td>
<td>46/Green &gt;300/111</td>
</tr>
<tr>
<td>C₁₈H₃₆Cu₂N₈S₄Cl₄</td>
<td>(1073.98)</td>
<td></td>
<td>11.86</td>
<td></td>
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<tr>
<td>[Cd₂L₂]Cl₄</td>
<td>1171.69</td>
<td></td>
<td>9.58</td>
<td></td>
<td>42/Yellow &gt;300/98</td>
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<tr>
<td>C₁₈H₃₆Cd₂N₈S₄Cl₄</td>
<td>(1172.09)</td>
<td></td>
<td>19.20</td>
<td></td>
<td></td>
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<tr>
<td>[Hg₂L₂]Cl₄</td>
<td>1348.05</td>
<td></td>
<td>8.33</td>
<td></td>
<td>45/Dark Brown &gt;300/110</td>
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<tr>
<td>C₁₈H₃₆Hg₂N₈S₄Cl₄</td>
<td>(1349.02)</td>
<td></td>
<td>29.78</td>
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</table>

Table 3  Crystal data and structure refinement for L.

<table>
<thead>
<tr>
<th>Identification code</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C₂₂H₁₈N₄S₂</td>
</tr>
<tr>
<td>Formula weight</td>
<td>402.52</td>
</tr>
<tr>
<td>Temperature</td>
<td>297(2)K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system, space group</td>
<td>Orthorhombic, Pca2₁</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 6.6402(19) Å, α = 90°</td>
</tr>
<tr>
<td></td>
<td>b = 6.3315(14) Å, β = 90°</td>
</tr>
<tr>
<td></td>
<td>c = 35.613(8) Å, γ = 90°</td>
</tr>
<tr>
<td>Volume</td>
<td>1948.2(7) Å³</td>
</tr>
<tr>
<td>Z, Calculated density</td>
<td>4.1372 Mg/m³</td>
</tr>
<tr>
<td>Absorption coefficient</td>
<td>0.289 mm⁻¹</td>
</tr>
<tr>
<td>F(000)</td>
<td>840</td>
</tr>
<tr>
<td>Crystal size</td>
<td>0.31 mm × 0.28 mm × 0.23 mm</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>2.29–25.00°</td>
</tr>
<tr>
<td>Limiting indices</td>
<td>10 ≤ h ≤ 10, −6 ≤ k ≤ 7, −42 ≤ l ≤ 41</td>
</tr>
<tr>
<td>Reflections collected/unique</td>
<td>117013398 [R(int)] = 0.0227</td>
</tr>
<tr>
<td>Completeness to theta =</td>
<td>25.00, 99.6%</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.9352 and 0.9161</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
<td>33901/1261</td>
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<tr>
<td>Goodness-of-fit on F²</td>
<td>1.086</td>
</tr>
<tr>
<td>Final R indices</td>
<td>R₁ = 0.0420, wR₂ = 0.1141</td>
</tr>
<tr>
<td>Error indices (all data)</td>
<td>R₁ = 0.0427, wR₂ = 0.1149</td>
</tr>
<tr>
<td>Absolute structure parameter</td>
<td>0.0253</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.397 and -0.190 e Å⁻³</td>
</tr>
</tbody>
</table>

3.2. IR spectra

The prominent bands observed in the IR spectra of the Schiff base ligand (L) and its coordination complexes are listed in Table 5. In order to study the binding mode of Schiff base ligand to the metal ions in coordination complexes, the IR spectrum of the free ligand was compared with the spectra of the corresponding coordination complexes. The appearance of band in the region 3300–3450 cm⁻¹ in the spectrum of the ligand, L corresponded to N–H stretching frequency of the uncoordinated primary amino group of diaminobenzidine moiety. This band showed negative shift in the spectra of coordination complexes and appeared in the region 3000–3200 cm⁻¹ indicating the coordination of NH₂ groups to metal ions [30]. The band at 1612 cm⁻¹ in the free ligand was assigned to ν(C=N) vibration [31]. However, in the coordination complexes this band was shifted to higher (15–25 cm⁻¹) wavenumbers indicating the participation of azomethine nitrogen in coordination to metal ions [32,33]. The sharp band at 850 cm⁻¹ may be assigned to ν(C=S–C) vibration in the free ligand which undergoes a positive shift in the coordination complexes (Table 5) suggesting coordination through sulphur of thioephene moiety [33]. Appearance of new bands in the spectra of coordination complexes in the regions 466–528 cm⁻¹, 464–520 cm⁻¹ and 419–466 cm⁻¹ were assigned to ν(M–N) of azomethine group, ν(M–N) (of amino NH₂) and ν(M–S) vibrations [33,34, respectively. The bands appearing in the region 1480–1440 cm⁻¹, 1100–1064 cm⁻¹ and 805–740 cm⁻¹ were usual modes of phenyl ring vibrations.

3.3. ¹H NMR and ¹³C NMR spectra

The ¹H NMR spectrum of the ligand, L showed a singlet at 8.87 ppm [31] assigned to azomethine protons (2H) which undergo upfield shift in the Cd(II) and Hg(II) complexes and appeared at 7.97 ppm and 7.95 ppm, respectively, suggesting the coordination of azomethine nitrogen to Cd(II) and Hg(II) ions. The resonance signals at 6.61–7.88 ppm (m, 12H, 6Ar and 6 thiophene H) in the free ligand showed an upfield shift in the complexes and appeared at 5.69–7.74 ppm (m, 24H, 12Ar and 12 thiophene H) indicating the involvement of azomethine nitrogen and thiophene S in the coordination with Cd(II) and Hg(II) ions. The signal observed at 3.879 ppm for L, was assigned to NH₂ protons. This signal was found at 3.326 ppm and 3.33 ppm in Cd(II) and Hg(II) complexes. This indicated that the NH₂ groups is coordinated to Cd(II) and Hg(II) ions without proton displacement.

¹³C NMR spectral data were consistent with ¹H NMR spectral data. A strong signal appearing at 168 ppm and 169 ppm in Cd(II) and Hg(II) complex may reasonably be assigned to azomethine carbon [35]. The resonance signals observed in the region

Table 4  Bond lengths [Å] and angles [°] for L.

| S(1)–C(11) | 1.704(4) |
| S(1)–C(8)  | 1.718(3) |
| S(1)–C(8') | 1.700(3) |
| S(1)–C(11')| 1.720(4) |
| N(1)–C(3)  | 1.385(4) |
| N(2)–C(7)  | 1.277(4) |
| N(2)–C(4') | 1.412(4) |
| N(1')–C(3')| 1.385(4) |
| N(2')–C(7')| 1.267(3) |
| N(2')–C(4')| 1.429(3) |
| C(7)–C(8)  | 1.433(5) |
| C(7)–C(8') | 1.462(4) |
| C(11)–S(1)–C(8) | 91.87(18) |
| C(8)–S(1)–C(11) | 90.58(19) |
| C(3)–N(1)–H(1A) | 120.0 |
| C(3)–N(1)–H(1B) | 120.0 |
| C(7)–N(2)–C(4)  | 119.8(2) |
| C(3')–N(1')–H(1C) | 120.0 |
| C(3')–N(1')–H(1D) | 120.0 |
| C(7')–N(2')–C(4') | 118.5(2) |
| N(2')–C(7')–C(8') | 122.7(3) |
| N(2)–C(7)–C(8)  | 122.3(3) |
Table 5
IR spectral data of Schiff base ligand (L) and its coordination complexes (cm⁻¹).

<table>
<thead>
<tr>
<th>Compound</th>
<th>v(L=N)</th>
<th>v(N-H)</th>
<th>v(C=S-C)</th>
<th>v(M-N)</th>
<th>v(M-N) (NH₂)</th>
<th>v(M-S)</th>
<th>Phenyl ring vibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>1612</td>
<td>3336</td>
<td>850</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1480, 1146, 753</td>
</tr>
<tr>
<td>[Co₂L₂]Cl₄</td>
<td>1637</td>
<td>3103</td>
<td>858</td>
<td>517</td>
<td>490</td>
<td>423</td>
<td>1461, 1069, 668</td>
</tr>
<tr>
<td>[Ni₂L₂]Cl₄</td>
<td>1628</td>
<td>3159</td>
<td>858</td>
<td>527</td>
<td>520</td>
<td>463</td>
<td>1462, 1073, 745</td>
</tr>
<tr>
<td>[Cu₂L₂]Cl₄</td>
<td>1627</td>
<td>3154</td>
<td>858</td>
<td>519</td>
<td>470</td>
<td>438</td>
<td>1440, 1078, 765</td>
</tr>
<tr>
<td>[Cd₂L₂]Cl₄</td>
<td>1632</td>
<td>3078</td>
<td>858</td>
<td>528</td>
<td>487</td>
<td>459</td>
<td>1471, 1105, 750</td>
</tr>
<tr>
<td>[Hg₂L₂]Cl₄</td>
<td>1632</td>
<td>3083</td>
<td>858</td>
<td>517</td>
<td>491</td>
<td>452</td>
<td>1470, 1100, 756</td>
</tr>
</tbody>
</table>

119–1433 ppm were assigned to phenyl and thionyl moiety of the ligand [35,36]. These signals showed upfield shift (1–2 ppm) in the Cd(II) and Hg(II) complexes invoking coordination of the ligand to Cd(II) and Hg(II) ion through its azomethine N and thiophene S.

3.4. Electronic spectra and magnetic moments

The electronic spectrum of Schiff base ligand, L (Fig. 4a) exhibits band at 240 nm, 288 nm, 340 nm attributable to π-π* transitions of aromatic benzene ring and imino group and n-π* transitions of imino group [37,38]. In the complexes these bands are shifted to longer wavelengths (Table 6) as a consequence of coordination when binding with metal, confirming the formation of Schiff base metal complexes.

The electronic spectrum of Co(II) complex (Fig. 4b) exhibits two bands at 440 nm and 550 nm corresponding to 4T₁g(F) → 4T₂g(P), 4T₂g(F) → 4A₂g(F), transitions, respectively which is compatible with this complex having octahedral geometry [39]. The observed magnetic moment of 4.82 B.M. further corroborated the electronic spectral findings [40].

The electronic spectrum of Ni(II) complex (Fig. 4c) shows two bands at 510 nm and 650 nm assignable to 3A₂g(F) → 3T₁g(P), 3A₂g(F) → 3T₁g(F), transitions, respectively characteristic of octahedral geometry around Ni(II) ion [41]. The observed magnetic moment of 3.05 B.M. further confirmed the proposed geometry around the Ni(II) ion [42].

The electronic spectrum of Cu(II) complex (Fig. 4d) displays a broad band at 628 nm assignable to 2Eg → 2T₂g transition, characteristic of octahedral geometry around Cu(II) ion. The observed magnetic moment of 2.05 B.M. further supported the electronic result [43].

3.5. Mass spectroscopy

The mass spectra of Schiff base ligand L and its Co(II) and Cu(II) complexes exhibit molecular ion peak at m/z at 403.12, 1064.89, 1073.978 (Fig. 5a–c). The proposed molecular formula of these complexes was confirmed by comparing their molecular weights with m/z values (Table 2).

Fig. 4. Electronic spectra of L and its Co(II), Ni(II), Cu(II) complex (a–d).
3.6. EPR spectroscopy

The powder EPR spectrum of Cu(II) complex (Figure given in supplementary material) exhibit two signals at $g_\perp = 2.05$ and $g_\parallel = 2.22$. The shape of the spectrum is consistent with the octahedral geometry around each Cu(II) centre in the complex [44]. The G-parameter defined as $G = g_\parallel - 2/g_\perp - 2$, reflects the spin-interaction between Cu(II) centers in solid polycrystalline complexes. According to Hathaway and Billing [45], if $G > 4$, the spin-exchange interaction is negligible and if it is less than 4 con-
considerable spin-exchange interaction prevails. In the present case, $G$ value comes out to be 4.4, which indicated negligible exchange interaction between the metal ions. Kivelson and Neiman have reported that $g_r$ value less than 2.3 indicates considerable covalent character of M–L bond and greater than 2.3 indicates ionic character. The present value comes out to be less than 2.3 which indicate considerable covalent character of M–L bond [46].

### 3.7. Fluorescence measurements

#### 3.7.1. DNA binding of L and its Co(II), Ni(II) and Cu(II) complexes

The studies from several laboratories have established the binding of cationic metal complexes with DNA [20]. The interaction of ligand and its Co(II), Ni(II) and Cu(II) complexes were studied using calf thymus DNA as simple molecular model. Nucleic acid bound to some molecules generally exhibit marked changes in fluorescence properties and this phenomenon is used in the studies with DNA [47]. Ethidium bromide, a polycyclic aromatic dye, is the most widely used fluorescence probe for DNA structure. It binds to DNA by intercalation within the stacked bases [48]. It has been reported that the enhanced fluorescence of the EB–DNA complex can be quenched at least partially by addition of a second molecule and this could be used to assess the relative affinity of the molecule for DNA [49]. The emission spectra of EB bound to DNA in absence and presence of ligand and its complexes is given in Fig. 6. The addition of these molecules to DNA being complexed with EB causes appreciable reduction in emission intensity, indicating that both the ligand and its complexes compete with EB in binding to DNA. This experiment showed that relative strength of interaction between DNA and the tested molecules can be ordered as DNA–Cu(II) complex > DNA–Ni(II) complex > DNA–Co(II) complex > DNA–Ligand. On the basis of above-mentioned results, we suggested that the both the ligand and its complexes have ability to interact with DNA. However, the affinity for DNA is more in case of complexes as compared to the ligand. This difference in their DNA binding affinity could be attributed to the presence of an electron deficient centre in the charged complexes where an additional interaction between the complexes and phosphate rich DNA back bone may occur.

#### 3.8. Absorption spectroscopy

UV–vis absorption studies were performed to further ascertain the predicted binding trend of L and its Co(II), Ni(II), Cu(II) complexes with DNA. The UV absorbance showed an increase with the increase in L and its Co(II), Ni(II), Cu(II) complexes concentration (Fig. 7). Since the L and its Co(II), Ni(II), Cu(II) complexes do not show any peak in this region (Fig. 7), hence the rise in the DNA absorbance is indicative of the complex formation between DNA and the tested molecules. Cu(II) complex at 260 nm exhibited highest hyperchromism of 30% at 1:1 molar ratio. So we primarily speculate that complex interacting with the secondary structure of the calf thymus DNA resulting in its breakage and perturbation. After interaction with the base pairs of DNA, the $\pi$-$\pi^*$ orbital of the bound ligand can couple with the $\pi$ orbital of the base pairs, due to the decrease $\pi$-$\pi^*$ transition energy, which results in bathochromic shift [50]. The prominent shift in the spectra also suggests the tight complexation of synthesized molecule with DNA, which resulted in

![Representative fluorescence emission spectra of DNA–EtBr complex in the absence and presence of increasing amount of L. (a) 0, (b) 5, (c) 10, (d) 15, (e) 20, (f) 25, (g) 30, (h) 35, (i) 40 and (x) L alone; pH 7.4; $T = 298K$.](image)
the change in the absorption maxima of the DNA. Hence the overall results suggested that Co(II), Ni(II), Cu(II) complexes have higher binding affinity toward DNA than free Schiff base ligand.

4. Conclusion

A novel hexadentate Schiff base ligand, N,N′-bis-(2-thiophenecarboxaldehyde)-3,3′-diaminobenzidine (L) has been synthesized by condensing 3,3′-diaminobenzidine with 2-thiophenecarboxaldehyde. The complexes of the type [M2L2]Cl4 [M = Co(II), Ni(II), Cu(II), Cd(II) and Hg(II)] have also been synthesized. The ligand and its complexes were characterized by various physico-chemical methods and spectroscopic techniques. The crystal structure of ligand, L has also been reported. Comparative DNA binding studies of L with its Co(II), Ni(II) and Cu(II) complexes revealed that the complexes exhibited higher binding affinity toward DNA as compared to ligand. The Cu(II) complex showed the highest affinity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.saa.2011.05.077.

References
