Synthesis, characterization, anti-microbial, DNA binding and cleavage studies of Schiff base metal complexes

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Abstract  A novel Schiff base ligand has been prepared by the condensation between butanedione monoxime with 3,3'-diaminobenzidine. The ligand and metal complexes have been characterized by elemental analysis, UV, IR, 1H NMR, conductivity measurements, EPR and magnetic studies. The molar conductance studies of Cu(II), Ni(II), Co(II) and Mn(II) complexes showed non-electrolyte in nature. The ligand acts as dibasic with two N4-tetradentate sites and can coordinate with two metal ions to form binuclear complexes. The spectroscopic data of metal complexes indicated that the metal ions are complexed with azomethine nitrogen and oxyimino nitrogen atoms. The binuclear metal complexes exhibit octahedral arrangements. DNA binding properties of copper(II) metal complex have been investigated by electronic absorption spectroscopy. Results suggest that the copper(II) complex bind to DNA via an intercalation binding mode. The nucleolytic cleavage activities of the ligand and their complexes were assayed on CT-DNA using gel electrophoresis in the presence and absence of H2O2. The ligand showed increased nuclease activity when administered as copper complex and copper(II) complex behave as efficient chemical nucleases with hydrogen peroxide activation. The anti-microbial activities and thermal studies have also been studied. In anti-microbial activity all complexes showed good anti-microbial activity higher than ligand against gram positive, gram negative bacteria and fungi.

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1. Introduction

In the recent and past years a large number of binuclear Schiff base metal complexes have been synthesized and characterized (Atakol et al., 2003). Schiff base ligand played central role in transition metal coordination chemistry (Shebl, 2008; Hobady and Smith, 1972). The tetra dentate Schiff base metal complexes used as metal enzymes (Cerchiaro and Ferreira, 2006), catalyst (Cozzi, 2004; Denmark et al., 2005), material chemistry (Huang et al., 2001), and biomimetic chemistry (Molenfeld et al., 2000). The binuclear complexes have greater cleaving efficiency than mononuclear complexes (Oliveira et al., 2005). The Schiff bases are able to inhibit the growth of several animal tumors, and some metals have shown good antitumor activity against animal tumors (Eudnenn and Mooney, 1970; Eudnenn and Dunn, 1972). The interest in preparation of new metal complexes gained the tendency of studying on the interaction of metal complexes with DNA for their applications in biotechnology and medicine. Although cisplatin and carboplatin are in use, there are several side effects of these chemotherapeutic drugs. Therefore, our main aim is to prepare the chemotherapeutic drugs without side effects or fewer side effects. Many transition metal complexes are known to bind to DNA via both covalent and non-covalent interactions. In covalent binding the labile ligand of the complexes is replaced by a nitrogen base of DNA. On the other hand, the non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of cationic metal complexes outside of the DNA helix, major or minor groove. DNA molecules are prone to be damaged under various conditions like interactions with some molecules. This damage may cause various pathological changes in living organisms, which is due to their possible application as new therapeutic agents and their photochemical properties which make them potential probes of DNA structure and conformation (Arturo et al., 2004; Maribel, 2003; Metcalfe and Thomas, 2003).

In this paper the novel complexes derived from butanedione monoxime with 3,3’-diaminobenzidine were synthesized and characterized by elemental analysis, molar conductance, UV, IR, NMR, EPR and magnetic studies. Thermal study has also been studied. The Schiff base ligand and its complexes were investigated for their DNA binding, cleavage, anti-bacterial and anti-fungal properties.

2. Experimental

2.1. Materials and reagents

All the chemicals used were of analytical reagent grade and the solvents were dried and distilled before use according to a standard procedure (Vogel, 1989). Butanedione monoxime and 3,3’-diaminobenzidine were purchased from Aldrich and were used as received.

2.2. Physical measurements (apparatus and experimental condition)

C, H and N contents were determined by Perkin Elmer CHN 2400 elemental analyzer, and IR Spectra were recorded in the range 4000 cm⁻¹–100 cm⁻¹ with a Bruker IFS66V in KBr and polyethylene medium for all complexes. The molar conductance of the complexes in DMF (10⁻³ M) solution was measured at 27 ± 3 °C with an Elico model conductivity meter. UV–visible spectra were recorded in DMF with Perkin–Elmer Lambda 35 spectrophotometer in the range of 200–800 nm. ¹H NMR spectra were recorded on Bruker 300 Hz spectrophotometer using DMSO d₆ as solvent. Chemical shifts are reported in ppm relative to tetramethylsilane, using the solvent signal as internal reference. EPR spectra were recorded at room temperature on JEOL JESTE100 ESR spectrometer. The spectrometer was operated at X-band (8–12 GHz) with microwave power of 1 mW. The room temperature magnetic moments were measured on a PAR vibrating sample magnetometer (Model-155). The TGA and DTA curves of the complexes were recorded on NETZSCH-STA 409PC thermal analyzer in heating rate of 10 K/min with the range of 50 °C to 900 °C.

2.3. Anti-microbial activity

The Schiff base ligand and its complexes were investigated for anti-bacterial and anti-fungal against Staphylococcus aureus and Streptococcus pyogenes as gram positive bacteria and Escherichia coli and Klebsiella pneumoniae as Gram-negative and the fungi Fusarium oxysporum and Aspergillus fumigatus using disc-agar diffusion method. All complexes exhibit anti-bacterial and anti-fungal activities against these organisms and are found to be more effective than the free ligand.

The anti-microbial activity was carried out at Progen Lab at Salem, Tamilnadu (India). The standard disc-agar diffusion method (Gross and De vay, 1977) was followed to determine the activity of the synthesized compounds against the sensitive organism S. aureus and S. pyogenes as gram positive bacteria and Escherichia coli and K. pneumoniae as Gram-negative and the fungi F. oxysporum and A. fumigatus. The antibiotic chloramphenicol was used as standard reference in the case of Gram-negative bacteria, tetracycline was used as standard reference in case of Gram-positive bacteria and clotrimazole was used as standard anti-fungal reference. The tested compounds were dissolved in DMF (which have no inhibition activity), to get concentration of 100 µg/mL. The test was performed on medium potato dextrose agar contains infusion of 200 g potatoes, 6 g dextrose and 15 g agar (William and Stephen, 1989). Uniform size filter paper disks (three disks per compound) were impregnated by equal volume from the specific concentration of dissolved tested compounds and carefully placed on incubated agar surface. After incubation for 36 h at 27 °C in the case of bacteria and for 48 h at 24 °C in the case of fungus, inhibition of the organism which evidenced by clear zone surround each disk was measured and used to calculate mean of inhibition zones.

2.4. DNA binding studies

The DNA binding experiments were performed in Tris–HCl/NaCl buffer (50 mM Tris–HCl/1 mM NaCl buffer, pH 7.5) using DMF (dimethylformamide) solution (10%) of the metal complexes. The concentration of calf-thymus (CT) DNA was determined from the absorption intensity at 265 nm with a ε value (Li et al., 2010) of 6600 M⁻¹ cm⁻¹. Absorption titration experiments were made using different concentrations of CT-DNA [40, 60, 80 µM], keeping the concentration of the complexes constant, with due correction for the absorbance of...
the CT-DNA itself. Samples were equilibrated before recording each spectrum.

2.5. DNA cleavage study

The DNA cleavage activity of the Schiff base metal complexes was monitored by agarose gel electrophoresis on CT-DNA. The tests were performed under aerobic conditions with H$_2$O$_2$ as an oxidant. Each reaction mixture contained 30 µM of CT-DNA, 40 µM of each complex in DMSO and 50 µM of hydrogen peroxide in 50 mM Tris–HCl buffer (pH 7.2). The reaction was incubated at 37 °C for 2 h. After incubation, 1 µL of loading buffer (bromophenol blue in H$_2$O) was added to each tube and the mixed samples were loaded on 1% agarose gel. The electrophoresis was carried out for 2 h at 50 V in Tris–acetic acid–EDTA buffer (pH 8.3). After electrophoresis, the gel was stained with 1 µg/cm$^3$ ethidium bromide (EB) for 30 min prior to being photographed under UV light.

2.6. Synthesis of ligand and its complexes

3,3′-Diamino benzidine (1 mM) in 10 mL of methanol, butanedione monoxime (4 mM) in 20 mL of methanol were mixed and heated at reflux for 2 h at 80 °C. The resulting dark red color solution was allowed to cool. The dark red color product was obtained and dried in desiccator using silica gel as drying agent. The metal complexes were prepared by reacting metal chlorides (2 mM) and ligand (1 mM) in acetonitrile were mixed and refluxed for about 2 h at 90 °C. The resulting product was filtered and dried in desiccator using silica gel (Fig. 1).

3. Results and discussion

The general characteristic properties of the ligand and its metal complexes are shown in Table 1. All the complexes are insoluble in common solvents such as water, benzene, chloroform,
dichloromethane, etc., they are soluble in DMF and DMSO. So the single crystal could not be obtained.

3.1. Molar conductivity measurements

The results of the elemental analysis are in good agreement with the calculated values. The metal contents of the complexes were determined according to literature methods (Jeffery et al., 1989). The electrolytic nature of the complexes is measured in DMF at 10^{-3} M. The molar conductivity \( \lambda_m \) lies between 8 and 13 \( \Omega^{-1}\) cm\(^2\) mol\(^{-1}\). This result shows that the complexes were non-electrolyte in nature, and anions were coordinated inside the coordination sphere (Tabl et al., 2008).

3.2. IR spectral studies

The IR spectra of the complexes are compared with those of the free ligands in order to determine the coordination sites that may involve in chelation. The position and or the intensities of these peaks are expected to be changed upon chelation. The IR spectra of metal complexes and ligand were recorded in the range of 100–4000 cm\(^{-1}\). The IR spectra of metal complexes exhibit absorption at 442, 542, 657 nm which are assigned to 4T1g \( \rightarrow \) 4A2g, 4T2g (F) \( \rightarrow \) 4A2g(F), 4T1g (F) \( \rightarrow \) 4T2g transitions, respectively, corresponding to cobalt(II) octahedral complex (Lever, 1984; Sathyaranayana, 2001).

3.3. Electronic spectral studies

Electronic spectra of all the complexes were recorded in DMF medium. In electronic spectra of metal complexes the wide range of bands are due to transition of \(-\text{CH} = \text{N}\) to 900 cm\(^{-1}\) and two oximino groups with the metal ions. The broad band in the range of 3400–3414 cm\(^{-1}\) is assigned to the stretching frequency of \(\text{OH}/\text{H}_2\text{O}\) molecules. The weak band around 460–480 cm\(^{-1}\) could be assigned to the stretching frequency of M–N bands for metal complexes. The bands at 270–280 cm\(^{-1}\) are due to the M–Cl (Murphy et al., 1987).

3.4. \(^1\)H NMR spectra

The structure of ligand was confirmed by \(^1\)H NMR spectra. The \(^1\)H NMR spectrum of DMSO d\(_6\) solution of oxime ligand shows a well resolved signal as expected. The spectrum of oxime ligand shows singlet at 1.95 ppm and singlet at 2.68 ppm corresponding to CH\(_3\)-1 and CH\(_3\)-4.

3.5. ESR and magnetic studies

ESR measurement has been made for copper complex using powder sample at room temperature, which could provide only value of \(g_{\text{iso}}\) (Fig. 3). The \(g_{\text{iso}}\) value of the complex is 2.071. The value of \(g_{\text{iso}}\) shows that the copper(II) complex is in octahedral environment. The magnetic moments of Cu(II), Ni(II), Co(II), Mn(II) are 1.95, 2.97, 4.87 and 5.94 B.M., respectively. The values are almost equal spin only value. This indicates that the two metal centers are equivalent and there is no interaction between the two metal centers. The pairing of electron is prevented by greater distance between the two metal centers (Venkatachalam et al., 2008).

3.6. Thermal studies

The thermal stability of the metal complexes was studied by TGA/DTA. The thermo gravimetric analysis was carried out in temperature range 50 °C to 900 °C. The sample were placed in platinum crucible and Al\(_2\)O\(_3\) was used as reference material. Heating was performed under N\(_2\) atmosphere. In copper(II) complex (Fig. 4) one endothermic peak was observed at 120 °C and one exothermic peak was observed at 200 °C which

<table>
<thead>
<tr>
<th>Complex</th>
<th>Color</th>
<th>Molecular weight</th>
<th>Yield (%)</th>
<th>M.P. (°C)</th>
<th>C (%) Found(Cal)</th>
<th>H (%) Found(Cal)</th>
<th>N (%) Found(Cal)</th>
<th>Metal (%) Found(Cal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>Dark red</td>
<td>546</td>
<td>80</td>
<td>215</td>
<td>60.9(61.5)</td>
<td>6.2(6.2)</td>
<td>20.1(20.5)</td>
<td>-</td>
</tr>
<tr>
<td>[Cu(L)(Cl)(_2)]</td>
<td>Deep green</td>
<td>815</td>
<td>86</td>
<td>345</td>
<td>41.0(41.2)</td>
<td>4.0(4.2)</td>
<td>13.7(13.7)</td>
<td>15.2(15.6)</td>
</tr>
<tr>
<td>[Ni(L)(Cl)(_2)]</td>
<td>Yellowish green</td>
<td>805</td>
<td>78</td>
<td>320</td>
<td>41.5(41.7)</td>
<td>4.0(4.1)</td>
<td>13.5(14.0)</td>
<td>14.6(14.6)</td>
</tr>
<tr>
<td>[Co(L)(Cl)(_2)]</td>
<td>Dark pink</td>
<td>806</td>
<td>84</td>
<td>330</td>
<td>41.7(41.7)</td>
<td>4.1(4.2)</td>
<td>13.4(13.9)</td>
<td>14.1(14.6)</td>
</tr>
<tr>
<td>[Mn(L)(Cl)(_2)]</td>
<td>Pink</td>
<td>798</td>
<td>75</td>
<td>315</td>
<td>41.9(42.1)</td>
<td>4.0(4.3)</td>
<td>13.8(14.0)</td>
<td>13.0(13.8)</td>
</tr>
</tbody>
</table>
Figure 2  $^1$H NMR spectra of ligand.

Figure 3  ESR spectrum of $\text{[Cu}_2(\text{L})\text{Cl}_4] \text{ complex at room temperature (frequency 9.445 GHz).}$
is assigned to the loss of four chloride and four hydroxyl ions 27.14(25.77)%. Another exothermic peak was observed at 490 °C which is attributed to the loss of 8CH$_3$CN groups 40.12(40.24)%. The decomposition is not completed after 900 °C. In nickel complex (Fig. 5) one endothermic peak was observed at 165 °C which is assigned to the loss of chloride and hydroxyl ions 26(26.09)%. One exothermic was observed at 250 °C and another at 300 °C which is attributed to the loss of 8CH$_3$CN groups 41.00(40.75)%. After 900 °C the decomposition is not completed.

3.7. Anti-microbial studies

Biological activity of the ligand and a series of its metal complexes [Cu(II), Ni(II), Co(II) and Mn(II)] were screened for anti-bacterial activity against *S. aureus* and *S. pyogenes*.

![Figure 4](image1.png)  
**Figure 4** TGA/TG studies of [Cu$_2$(L)Cl$_4$] complex.

![Figure 5](image2.png)  
**Figure 5** TGA/TG studies of [Ni$_2$(L)Cl$_4$] complex.
as Gram-positive bacteria and *E. coli* and *K. pneumoniae* as Gram-negative and the fungi *F. oxysporum* and *A. fumigatus* by using disc-agar diffusion method (Figs. 6–8). The remarkable activity of Schiff base ligands may arise from the presence of four imine and four oxyimino groups which impart in elucidating the mechanism of transformation reaction in biological systems. The results indicate that the complexes show more activity and the ligand have less activity against

**Figure 6** Anti-bacterial studies (Gram-negative) of Schiff base ligand and its metal complexes. 1. *E. coli*. 2. *K. pneumoniae*. 
Standard = chloramphenicol inhibition zone in mm, concentration 100 μg/mL.

**Figure 7** Anti-bacterial studies (Gram-positive) of Schiff base ligand and its metal complexes. 1. *S. aureus*. 2. *S. pyogenes*. 
Standard = tetracycline inhibition zone in mm, concentration 100 μg/mL.

**Figure 8** Anti-fungal studies of Schiff base ligand and its metal complexes. 1. *F. oxysporum*. 2. *A. fumigatus*. Standard = clotrimazole inhibition zone in mm, concentration 100 μg/mL.
same microorganisms under identical experimental conditions (Table 2). This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy’s chelation theory (Tweedy, 1964). Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane. The copper(II) complex shows higher anti-fungal activity than other complexes and Co(II) complex shows higher anti-microbial activity than other complexes. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells (Mehmet Sonmez et al., 2010; Kurtomg lu et al., 2006).

3.8. DNA binding studies

Electronic absorption spectroscopy is universally employed to determine the binding characteristics of metal complex with DNA. The absorption spectra of copper(II) complex in absence and presence of CT-DNA are shown in Fig. 9. In UV region two intense bands absorbed at 370 nm attributed to the ligand to metal charge transfer absorption and another at 265 nm which is assigned to the \( \pi-\pi^* \) 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 (Xu et al., 2003). Intercalative mode of binding usually 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 commonly found to correlate with the intercalative binding strength. But, metal complexes which bind non-intercalatively or electrostatically with DNA may result in hyperchromism or hypochromism. In general, the absorption spectra of metal complexes bound to DNA through intercalation exhibit significant hypochromism and red shift due to the strong \( \pi-\pi^* \) stacking interaction between the aromatic chromophore ligand of metal complex and the base pairs of DNA (Terenzi et al., 2009). In copper(II) complex the decrease in 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 red nearly 6, 8, 10 nm, respectively, with increasing concentrations (40, 60, 80 \( \mu \text{M} \)) (Colak et al., 2010).

3.9. DNA cleavage studies

Gel electrophoresis experiments were performed using CT-DNA with ligand, complexes in presence and absence of \( \text{H}_2\text{O}_2 \). Complexes exhibit cleavage ability at low concentration (40 \( \mu \text{M} \)). The ligand exhibits no significant activity in the presence of oxidant. The activity was much higher for the complexes in presence of \( \text{H}_2\text{O}_2 \). When calf-thymus DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coil form (Form I). If scission occurs on one strand (nicking), the super coil will relax to gener-

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-positive</td>
<td>Gram-negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ligand</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>[Cu2(L)Cl2]</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>[Ni2(L)Cl2]</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>[Co2(L)Cl2]</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>[Mn2(L)Cl2]</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>

Inhibition zone in mm, concentration 100 \( \mu \text{g/mL} \).
ate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated (Zhang et al., 2002). From Fig. 10 the complexes show more activity in the presence of oxidant which may be due to the reaction of hydroxyl radical with DNA. These hydroxyl free radicals participate in the oxidation of the deoxyribose moiety followed by hydroxyl cleavage of sugar phosphate backbone. The results of DNA cleavage studies have been shown in Fig. 10. All metal complexes were able to convert DNA (Form I) into open circular (Form II). The binuclear Cu(II) complex was found to be highly active in cleaving DNA in the presence of hydrogen peroxide. The H$_2$O$_2$ is coordinated to the copper ions of the complex, affording a peroxo-dicopper species. This coordinated peroxide ion attacks the DNA phosphate bond via a nucleophilic mechanism and hydrolyzes the P–O bond (Gao et al., 2000).

4. Conclusion

In the present study, novel Schiff base complexes were prepared and characterized by physico-chemical methods. The metal ions were complexed with nitrogen of the oxime and imine groups and presence of octahedral geometry around metal ions. The anti-bacterial and anti-fungal data given for the compounds presented in this paper allowed us to state that the metal complexes generally have better activity than ligands and less activity than standards. The DNA binding experiment results suggest that the interaction of the complex with DNA is by an intercalation mode. The DNA cleaving activities of metal complex with CT-DNA under aerobic conditions show more pronounced activity of Cu(II) complex in the presence of the oxidant.

References


Figure 10 Cleavage of CT-DNA (30 µM) by the metal complexes (40 µM) in the presence of reducing agent H$_2$O$_2$ 50 µM, in 50 mM of Tris–HCl buffer (pH 7.2). From left to right Lane 1. Control; Lane 2. DNA + Ligand + H$_2$O$_2$; Lane 3. DNA + copper(II) complex; Lane 4. DNA + copper(II) complex + H$_2$O$_2$; Lane 5. DNA + nickel(II) complex + H$_2$O$_2$; Lane 6. DNA + -cobalt(II) complex + H$_2$O$_2$; Lane 7. DNA + manganese(II) complex + H$_2$O$_2$. 
Synthesis, characterization, anti-microbial, DNA binding and cleavage studies


