CHAPTER-5

Study of coordinative versatility of ligands with quinoxaline core
Section-A

Synthesis, structural elucidation, antimicrobial and DNA cleavage studies of transition metal complexes derived from N, O, O donor ligands with quinoxaline core.
Introduction

Over the past three decades, the extensive chemistry has surrounded the use of hydrazone ligands and their metal complexes which is attested by large number of publications and reviews [1-3]. One of the reasons to use the hydrazone ligand is their biological activity and synthetic flexibility. Another reason is, these ligands process multidentate sites, which means for most metals, several binding sites are occupied, leaving vacant sites for potential catalytic/ enzymatic activity. Also it has been recognized that many of these complexes may serve as models for biological species.

Hydrazones are a special group of compounds in the Schiff base family. They are characterized by the presence of >C=N-N<. The presence of two interlinked nitrogen atoms separate this from imines, oximes etc. Hydrazones are usually obtained by the condensation of hydrazides with aldehydes or ketones.

Hydrazones have extended their applications in various fields and hence become the interesting substance because of their pharmacological applications, like in the treatment of several diseases such as tuberculosis, leprosy and mental disorders. These activities are attributed to the formation of stable chelates with transition metals which catalyze the physiological process [4-11]. Metal complexes derived from the hydrazone derivatives also play a significant role in binding and cleavage of DNA. They also act as herbicides, insecticides, nematocides, plant growth regulators etc [5].

Compared to the simple hydrazones, or hydrazones have additional >C=O donor sites, which make them more flexible and versatile which can exist in keto or enol form.
This part of the work is mainly focused on the construction of ligands from quinoxaline, quinoline and coumarin derivatives and to study the coordination strategy and biological activity with the incorporation of the metal ion into it. The concerned literatures are surveyed and are presented in the following paragraph.

Konidaris et al [12] have proved the polymeric featuring of the 2,3-dioxyquinoxalinate (-2) ligand with the x-ray crystal structure. Goroufis et al [13] have synthesized cobalt complexes with 2-(2′ pyridyl) quinoxaline ligand, and they shown the six and five coordinated complexes with single crystal analysis. Ananth Lakshmi et al [14] have studied the biological activity of the 3d transition complexes with quinoxaline compound and shown the biambidentate property of it. The quinoxaline skeleton has been used as a synthetic intermediate for the preparation of numerous compounds with interesting biological properties. Due to their conformational rigidity and their wide range of properties, such heterocyclic systems play an essential role as scaffolds in biologically active compounds. Quinoxaline derivatives are one of the main classes of known antagonists of amino propanoic acid (AMPA). They also present in peptide antibiotics, including metal binding properties and also have the many biological activity such as, bacteriocides and insecticides. Complexes of quinoxaline derivatives with metals show the high ability to bind/cleave the DNA strand [15]. This may suggest that conjugation of biologically active peptides with the quinoxaline analogs can lead to new therapeutic agents possessing interesting anticancer properties. Similarly,
quinoline and coumarin derivatives represent the major class of heterocycles, and a number of preparations have been known since the late 1980s. These derivatives have been well known in medicinal chemistry, because of their wide occurrence in natural products [16] and drugs [17]. The quinoline skeleton is often used for the design of many synthetic compounds with diverse pharmacological properties. Dynemicin A and Streptonigrin are naturally occurring members of the class of antitumor antibiotics, whose syntheses are based on the utilization of preformed quinoline derivatives [18]. These derivatives have also known to possess a variety of biological activities such as antiplatelet, antidepressant, antiulcer, anticancer, anti-HIV, anticoagulant, antibacterial and antifungal activity [19, 20]. In the same way the coumarin derivatives also processes variety of pharmacological activity. Some literature survey on coumarin and quinoline derivative are presented here.

Kostova I. et al [21] have synthesized and characterized the Ce(III) complexes by using coumarin derivative. And they have investigated the cytotoxicity of the synthesized compounds. Complexes have found to be effective against the myeloid HL-60 cells. Creaven B.S. et al [22] have synthesized the Cu(II) complexes of coumarin derived Schiff bases and they investigated the anti-candida activity of the synthesized compounds.

Mostafa E.B. et al [23] have synthesized and characterized the transition metal complexes with quinoline derivatives. The synthesized compounds were carried out for antimicrobial study. They concluded that, the complexes have shown good results than the ligands. These results are stimulated us to
incorporate the transition metal ions namely, Cu(II), Co(II), Ni(II) and Zn(II) into the above said heterocyclic ligand moiety and evaluate their biological study.

Experimental

Synthesis of precursors

Preparation of 2-hydroxy-3-formylquinoline is given in chapter 3.

Preparation of 2-hydroxy-3-hydrazinoquinoxaline [24]

Preparation of 2-hydroxy-3-hydrazinoquinoxaline has been carried out in two steps as follows.

a. Preparation of 2,3-dihydroxyquinoxaline

Oxalic acid (5.04 g, 0.04 mole) and o-phenylene diamine (4.32 g, 0.04 mole) were dissolved in 30 cm\(^3\) of 4N HCl and the solution was heated to boiling until a crystalline precipitate is formed. The precipitate was washed with water and dissolved in 1N NaOH solution. The solution was decolorized using charcoal and filtered and later reprecipitated using dilute HCl to give pure white 2,3-dihydroxy quinoxaline. The precipitate was washed with water and dried at 100 °C. The prepared compound may also exist in the amido- imidol tautomeric form.

(Yield: 90 %, M. P.: above 250 °C)
DNA cleavage study

Schematic presentation of 2,3-dihydroxyquinoxaline.

\[
\begin{align*}
\text{Oxalic acid} & \quad \text{o-Phenelyne diamine} \quad 4 \text{N HCl} \\
& \quad \text{2,3-Dihydroxyquinoxaline}
\end{align*}
\]

Tautomeric form of the compound 2, 3-dihydroxyquinoxaline

b. Preparation of 2-hydroxy-3-hydrazinoqumoxaline

Hydrazine hydrate (5.06 g, 4.91 ml, 0.1 mole) was added drop wise into 150 cm\(^3\) of water containing 2,3-dihydroxyquinoxaline (3.24 g, 0.02 mole), and then the mixture was refluxed for 3 h. The yellow colored 2-hydroxy-3-hydrazinoquinoxaline was obtained. The precipitate was washed with water and dried at 100 °C.

(Yield : 90 %, M.P.: above 250 °C)

Schematic representation of the compound 2-hydroxy-3-hydrazinoquinoxaline
Preparation of 3-acetylcoumarin [25]

Salicylaldehyde (12.21 g, 10.52 ml, 0.1 mol) and ethylacetoacetate (33.41 g, 33.57 ml, 0.1 mol) were taken in a flask and cooled in ice bath. To this cold mixture 1 ml of piperidine was added with shaking. The mixture was left overnight when a yellow solid mass was separated. The lumps were broken in ethanol and filtered. The solid mass was washed with cold ethyl alcohol and dried to give of 3-acetylcoumarin. Recrystallisation from hot ethyl alcohol yielded silky needles.

(Yield 80%, M.P.: 120°C)

Schematic representation of preparation of 3-acetylcoumarin

![Schematic](image)

Preparation of the ligands

Preparation of ligand L\(^1\)H

To a hot ethanolic solution of 2-hydroxy-3-hydrazinoquinoxaline (1.76 g, 0.01 mol), ethanolic solution of 2-hydroxy-3-formylquinoline (1.73 g, 0.01 mol) was added drop wise with constant stirring. There after the reaction mixture was refluxed for about 3-4 hours on water bath. The resulting solid product was separated out which was filtered and washed with ethanol and dried.

(M.P.: 263-265 °C, Yield 74%)
Preparation of ligand \( \text{L}^2 \)

A solution of 3-acetylcoumarin (1.88 g, 0.01 mol) was added drop wise to a hot ethanolic solution of 2-hydroxy-3-hydrazinoquinoxaline (1.76 g, 0.01 mol). Then the reaction mixture was refluxed for about 3-4 hours on water bath. The resulting solid product was separated out which was filtered and washed with ethanol and dried.

(M.P.: 250-253 °C, Yield 71 %)

(i) 2-Hydroxy-3-hydrazinoquinoxaline

(ii) 2-Hydroxy3-formylquinoline

(iii) 3-Acetylcoumarin

(Scheme-1)

Synthesis of the complexes

An ethanolic solution of metal(II) chloride \{CoCl\(_2\)6H\(_2\)O (0.475 g, 0.002 mole), NiCl\(_2\)6H\(_2\)O (0.475 g, 0.002 mole), CuCl\(_2\)2H\(_2\)O (0.341 g, 0.002 mole) and ZnCl\(_2\) (0.272 g, 0.002 mole)\} was added with stirring to an ethanolic solution
(100 ml) of the ligand \{L^1H (0.662 g, 0.002 mole), L^2 (0.692 g, 0.002 mole)\} and refluxed at water bath temperature for 3-4 h. So obtained solid complex was separated by filtration under suction, washed with hot ethanol and dried in \textit{vacuum}.

\textbf{Biochemistry}

Methodology for anti-biogram analysis against bacteria and fungi, also DNA cleavage experiment including DNA isolation, agarose gel electrophoresis is given in chapter 3.

\textbf{Result and discussion}

Complexes obtained in the present study were of non-hygroscopic and in the form of amorphous solids. Analytical, magnetic and conductivity data for the complexes are given in Table-1. Complexes are insoluble in water, ethanol, methanol and completely soluble in DMF, DMSO and acetonitrile. The stoichiometry of the complexes were found to be (M:L) 1:1. Melting points of all the complexes are found to be above 300 °C and yield of the complexes are about 60-65 %.

\textbf{IR spectral studies}

The important IR assignments of the free ligands and their metal complexes are given in Table 2. The strong band located at 1683 cm\(^{-1}\) in ligand \(L^1H\) and \(L^2\) is assigned to the carbonyl stretching frequency of the quinoxaline moiety, and medium intensity band observed around 3152-3225 cm\(^{-1}\) can be assigned to the \(-NH\) vibrations of the quinoxaline ring system. The presence of quinoxaline
carbonyl and -NH band in the spectra of both the ligands explore the amido form of the quinoxaline moiety. Upon complexation carbonyl stretching frequency of the quinoxaline moiety shifted to lower region, exhibiting the involvement of carbonyl group in the coordination [26]. The -NH band of quinoxaline ring remained unaffected upon complexation, confirms its non-involvement in the coordination and existence of amido form of the quinoxaline moiety in the complexation, which is also supported by the ^1H NMR spectra. The strong and sharp bands observed at 1615 and 1609 cm$^{-1}$ are assigned to the $\nu$(C=N) stretching mode of the azomethine group of ligands L$^1$H and L$^2$ respectively. The $\nu$(C=N) group of ligands (L$^1$H and L$^2$) have shown negative shift in the spectra of all the complexes, indicating their involvement in the coordination with metal ions [27]. The band observed at 1719 cm$^{-1}$ in the ligand L$^2$ is assigned to lactonic carbonyl of coumarin moiety. This band shows shift towards low frequency side confirming its involvement in the coordination. The ligand (L$^1$H) also display the band at 3431 cm$^{-1}$ which is assigned to $\nu$(OH) of quinoline molecule. The presence of water molecules in the complexes leads to the broad band at 3468–3410 cm$^{-1}$. Due to the presence of coordinated/crystal held water molecule in the complexes, the involvement of -OH of quinoline moiety in coordination to the metal ion is not recognized by the IR spectral study. However, the coordination through -OH of quinoline moiety is unambiguously confirmed by NMR spectra of the Zn(II) complex. The low frequency band at 515-464 cm$^{-1}$ is assigned to $\nu$(M-N) (spectra 1-4).
The $^1$H NMR spectra of the ligands ($L^1H$ and $L^2$) and their Zn(II) complexes (C4 and C8) were recorded in DMSO-d$_6$ solvent over the range of 0-16 ppm. The peak observed at 8.6 ppm, is assignable to the azomethine proton of the ligand $L^1H$, shifts to down field indicating the coordination of the azomethine nitrogen to the metal ion [28]. The peaks resonating at 12.0 ppm in the ligand $L^1H$ is attributed to the -OH of the quinoline moiety. In the zinc complex (C4) resonance arising from quinoline -OH proton disappears, which confirms the involvement of -OH in coordination via deprotonation. In case of ligand $L^2$ methyl protons resonate at 2-3 ppm. The peak corresponding to ring –NH of quinoxaline resonates at 11.5 and 10.8 ppm in the ligands $L^1H$ and $L^2$ respectively. Upon complex formation with Zn(II) metal ion above said peaks remained unchanged supporting the non-involvement of the quinoxaline ring –NH in coordination and the amido form of the ligand. In addition to this, a set of multiplet observed in the range 6.93 – 8.11 ppm are ascribed to the aromatic protons. The conclusions drawn from these studies provide further support to the mode of coordination shown by IR spectral studies (spectra 5-6).

**Molar conductivity measurements**

Molar conductivities of the complexes were measured in DMF solution with 10$^{-3}$ M concentration. All these complexes show molar conductance value in the range 6.9-12.21 mho cm$^2$ mol$^{-1}$ Table-2. These low conductance values of the complexes indicate the non-electrolytic nature [29].
Electronic spectral studies

Electronic spectra of the ligands $L_1^1H$, $L_2^2$ and their complexes (C1 to C8) were recorded in DMF solvent. Compared to electronic spectra of the free ligands, significant changes in the wavelengths of absorption maxima were observed in the complexes. The free ligands ($L_1^1H$ and $L_2^2$) exhibit a strong absorption bands at 297 and 336 nm which are due to the $\pi-\pi^*$ and $n-\pi^*$ transitions respectively. The band due to $n-\pi^*$ transition of both the ligands have shown red shift in all the complexes is the evidence for the ligation of the azomethine group to the metal ion. The electronic spectra of the cobalt and nickel (C1 and C2) complexes are virtually identical. Two new bands at 430 and 445 nm are observed in these complexes, which are due to ligand to metal charge transfer (LMCT) transitions. In the spectra of the copper complexes (C3 and C7) the broad band is observed at 583 and 564 nm which is assigned to the d-d transition of the metal complexes. In case of Zn complexes (C4 and C8) the band observed around 410 nm is assigned to the charge transfer transition (spectra 7-10).

Magnetic studies

The magnetic moment values of the complexes are listed in Table-1. The $\mu_{\text{eff}}$ values of the cobalt(II) complexes (C1 and C5) are found to be in the range 5.08-5.22 BM indicating octahedral geometry [30]. The experimental values obtained for the magnetic moment of nickel(II) complexes (C2 and C6) are 2.95 and 3.4 BM respectively, which lie in the range expected for octahedral geometry. The copper(II) complexes (C3 and C7) show a normal magnetic
moment of 1.73 and 1.87 BM observed for d⁹ system with one unpaired electron.

**EPR spectral studies**

The X-band EPR spectra of Cu(II) complexes (C3 and C7) were recorded at room temperature. The complexes C3 and C7 exhibit a broad isotropic signal at 2.08 and 2.07 respectively, with no hyperfine splitting and half field absorption. The spectra of this type have been noted earlier for complexes bearing large organic ligand substituents [31] (spectrum 11).

**FAB mass studies**

FAB mass spectral data for C3 and C7 complexes clearly suggested the monomeric nature of the complex. The molecular ion peak for complexes, C3 and C7, were observed at m/z= 486 and 494 respectively. The spectra show some prominent peaks corresponding to the various molecular ion fragments. The results from mass spectra convince the monomeric nature of the complexes (spectra 12-13). Proposed structures of the complexes are presented in Figure-1.

**Thermal analysis**

The thermal stability of the complexes were investigated using TG-DTA experiments in temperature range 40-900 °C with heating rate of 10 °C / min. The complex C3 underwent decomposition in 3 distinct steps to give copper oxide. In the first step complex decomposed very slowly up to 180 °C and weight loss during this step corresponds to loss of one lattice and two...
coordinated water molecule. Further the decomposition is continued up to 250 °C, where the weight loss corresponds to the loss of chloride as HCl. From this temperature onwards the complex underwent rapid weight loss in single step up to temperature 600 °C. During this step, the weight loss corresponds to complete decomposition of organic moiety with the formation of cupric oxide, which is indicated by the plateau.

In the same way the complex C6 underwent decomposition very slowly up to 200 °C and the weight loss corresponds to loss of one coordinated water molecule. Further the complex underwent rapid weight loss in successive steps up to temperature 400 °C. During this step the weight loss corresponds to complete decomposition of organic moiety with the formation of nickel oxide, which is indicated by the plateau (Graph-1).

Cyclic Voltammetry study

The redox properties of ligands and their complexes were studied in the potential range from -1.0 to 1.0V with the scan rate of 0.5, 1.0, 1.5 V. In the CV profile only copper complexes have shown the redox property and remaining compounds were found to be inert in the above mentioned scan range. Cyclic voltammogram of copper complexes (C3 and C7) shows an quasi-reversible peak for the couple Cu(II)/Cu(III) at $E_{pa} = 0.681$ V, 0.189 V with the direct cathodic peak for Cu(III)/Cu(II) at $E_{pc} = 0.351$ V, 0.592 V respectively. Also the ($\Delta E_p$) peak separation value is found to be greater than 59 mV at scan rate 1.0 V/s. $\Delta E_p$ value of complex increases with increase in the scan rate but remained in the quasi-reversible system. The ratio $I_{pa}/I_{pc}$ is
close to unity. From these data, it can be concluded that this redox couple is a quasi-reversible one-electron transfer process [32] (spectra 14).

Biochemistry

The ligands and their complexes C1-C8 were screened for their antibacterial (Escherichia coli and Pseudomonas aeruginosa) and antifungal studies (Aspergillus niger and Cladosporium). The data are shown in Table 3. The compounds which show the promising antibacterial and antifungal activity were selected for minimum inhibitory concentration (MIC) studies, which are determined by assaying at 250 µg concentrations along with standards at the same concentrations. Even at the MIC level the complexes C2, C3, C7 and C8 were found to be more toxic against bacteria and fungi than the Schiff base ligands (Table-4). This enhancement in the activity can be explained on the basis of chelation theory [33]. Such a chelation could enhance the lipophilic character of the central metal atom. However, it may be noted that chelation is not the only criterion for antibacterial activity, but it is an intricate blend of several contributing factors such as nature of the metal ion, nature of the ligand, coordinating sites, geometry of the complex, concentration, hydrophilic nature [34]. By observing the higher activity of the compounds against Escherichia coli bacteria were chosen for the DNA cleavage study.

DNA Cleavage study

The cleavage efficiency of the complexes was compared to that of the control. Control experiments using DNA alone do not show any significant cleavage
even after a longer exposure time. But the increase in intensities of the bands was observed for the metal bound DNA, such intercalation will lead to change the confirmation of the DNA. The different DNA cleavage efficiency of the complexes may be due to the different binding affinity of the complexes to DNA [35-37]. In the present investigation, the nickel (C2) and copper (C3) complexes have shown increase in the intensity and tailing compared to remaining compounds which illustrates the higher binding ability of the said complexes with Escherichia coli DNA (Chart 1).

![Figure-1 Tentative structures of the complexes (C1-C8)](chart1)

**Conclusion**

The complexes synthesized from the ligands L\(^1\)H and L\(^2\) are well characterized by spectro-analytical techniques. The tentative structure drawn from these data are given in Figure-1. The ligands coordinate to the metal ion in O, O, N donor fashion. The redox behavior of copper complexes (C3 and C7) show a quasi-
reversible with one electron transfer process. All the complexes are found to be monomeric with (1:1) ligand to metal ratio. The prepared complexes were screened for their antibacterial and antifungal study. The complexes are found to be more toxic than ligands. Again by using these complexes DNA cleavage experiment was carried out by the gel electrophoresis method. Cleavage of DNA is not been observed but, on the basis of change in the intensity of the band binding can be predicted.
### Table 1 Analytical, conductivity and magnetic data for the ligands and their complexes

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>Elemental analysis (%) found/calculated</th>
<th>Molar conductance in Ω cm² mol⁻¹</th>
<th>Magnetic moment in μmB.M.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>L'H</td>
<td>(C₈H₁₄N₄O₃)</td>
<td>65.11/65.25</td>
<td>3.73/3.92</td>
<td>21.03/21.14</td>
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<tr>
<td>C1</td>
<td>[Co(C₈H₁₄N₄O₃)Cl₂H₂O] H₂O</td>
<td>44.04/44.15</td>
<td>3.51/3.76</td>
<td>14.48/14.63</td>
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<tr>
<td>C2</td>
<td>[Ni(C₈H₁₄N₄O₃)Cl₂H₂O] H₂O</td>
<td>44.98/45.17</td>
<td>3.59/3.76</td>
<td>14.55/14.64</td>
</tr>
<tr>
<td>C3</td>
<td>[Cu(C₈H₁₄N₄O₃)Cl₂H₂O] H₂O</td>
<td>44.62/44.72</td>
<td>3.65/3.72</td>
<td>14.27/14.49</td>
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<td>C4</td>
<td>[Zn(C₈H₁₄N₄O₃)Cl₂H₂O] H₂O</td>
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<td>3.43/3.71</td>
<td>14.21/14.43</td>
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<td>(C₈H₁₄N₃O₂)</td>
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<td>3.87/4.04</td>
<td>15.95/16.18</td>
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<td>46.03/46.25</td>
<td>3.09/3.24</td>
<td>11.15/11.36</td>
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<td>3.11/3.24</td>
<td>11.08/11.36</td>
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<td>C7</td>
<td>[Cu(C₈H₁₄N₃O₂)Cl₂]</td>
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<td>C8</td>
<td>[Zn(C₈H₁₄N₃O₂)Cl₂] H₂O</td>
<td>45.55/45.73</td>
<td>2.97/3.20</td>
<td>11.09/11.23</td>
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Table 2: IR spectral data of the ligands and their complexes in cm$^{-1}$

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<th>Compound</th>
<th>$v$(C=N) (azomethine)</th>
<th>$v$(C=O) (quinonoid)</th>
<th>$v$(C=O) (lactone)</th>
<th>$v$(O-H)</th>
<th>$v$(M-N)</th>
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<tr>
<td>L$^1$H</td>
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<td>--</td>
<td>3431</td>
<td>--</td>
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<td>Pseudomonas aeruginosa</td>
<td>% inhibition</td>
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<td>Gentamycin</td>
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Table 4 Antibacterial and antifungal studies of compounds in MIC level (250μg)

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<th>Pseudomonas aeruginosa</th>
<th>% inhibition</th>
<th>Zone of inhibition (cm)</th>
<th>Aspergillus Niger</th>
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Spectrum 1 IR spectrum of ligand L'H

Spectrum 2 IR spectrum of complex C3
Spectrum 3 IR spectrum of ligand L²

Spectrum 4 IR spectrum of complex C5
Spectrum 5 $^1$H NMR spectrum of ligand $L^1$H

Spectrum 6 $^1$H NMR spectrum of ligand $L^2$
Chapter-5A Spectra

Spectrum 7 UV-Visible spectrum of ligand L\textsuperscript{1}H

Spectrum 8 UV-Visible spectrum of complex C3

Spectrum 9 UV-Visible spectrum of ligand L\textsuperscript{2}

Spectrum 10 UV-Visible spectrum of complex C8

Spectrum 11 EPR spectrum of complex C3
Spectrum 12 FAB Mass spectrum of complex C3

Spectrum 13 FAB Mass spectrum of complex C7
Graph-1 TG-DTA thermogram of complex C3

Spectrum 14 Cyclic Voltammogram of complex C7
Chart 1  Agarose gel showing the results of electrophoresis of *Escherichia coli* - DNA with the Schiff base ligand L¹H and its complexes (C1-C4) [M-Standard Molecular weight Marker; C- *Escherichia coli* Control DNA of *Escherichia coli*; 1. ligand (L¹H) 7. Cobalt (C1), 8. Nickel (C2), 9. Copper(C3) and 10. Zinc (C4)]
References


Section-B

Synthesis, structural elucidation, antimicrobial and DNA cleavage studies of transition metal complexes derived from S, N, O donor ligands with quinoxaline core.
Introduction

The sulfur, nitrogen, oxygen containing heterocyclic hydrazone ligands are constitutes one of the most important places in coordination chemistry [1]. The formation of variety of metal complexes from these ligands speaks for their spectacular progress in coordination and bioinorganic chemistry. These hydrazones also have outstanding potential for inhibiting the growth of various pathogenic microorganisms. This property has been exploited in pharmacological interest, which provides the insight into the new drug system. The study reveals that the active sites of many metalloenzymes contains the SNO donor chelates of hydrazones [2-3]. The compounds which contain nitrogen and oxygen have a many pharmaceutical importance. Sulfur is also a ubiquitous element in nature both in inorganic and organic sulfur compounds. The presence of sulfur at the active site of various enzymes and the biochemical importance of the thiol and disulfide groups has prompted extensive research in organic synthesis which can mimic the natural products. Consequently, many drug molecules have been developed from sulfur containing naturally occurring compounds. The therapeutic use of sulfur initiated in the 17th century, when “brimstone” was used for toothache [4]. The medicinal properties of organo sulfur compounds however were only discovered accidentally in the latter part of the 19th century. During the development of aromatic chemistry for the dye industry, it was noted that some of these sulfur containing compounds have shown antibacterial properties. This gave rise to revolutionary development of sulfur chemistry and ultimately to an increased understanding of its role in physiological mechanisms.
Furthermore, sulfur containing ligands and their metal complexes are active catalysts in a number of homogeneous reactions. They are active against influenza, protozoa, ulcer and certain kinds of tumours [5] and also associated with many medicinal properties. The coordination chemistry of sulfur donor ligands are of immense interest as, these compounds mimic the cystein sulfur coordination in metalloenzymes. It is well known from the literature that compounds containing >C=S moiety have a strong ability to form metal complexes and exhibit wide range of biological activity. Furthermore these compounds show electronic and structural properties of the active sites as in blue copper proteins involving S and N-coordination. In addition to this, the SNO donor atoms containing hydrazone metal complexes have found to be DNA intercalating agents. In the present decade the aspects of bioinorganic chemistry is mainly focused on designing of metal complexes that are capable of binding and/or cleaving DNA, is related to their utility in the design and development of new drug molecules. Studying the interaction model and the mechanism of transition metal complexes with DNA and exploring the application of metal complexes in antineoplastic medication, molecular biology and bio-engineering field have become hotspots in recent years. Metal complexes which interact with DNA can induce the breakage of DNA strands, which may leads to the inhibition of its further replication through which its function may alter. In the case of cancer genes, after DNA strands are cleaved, the DNA double strands break, the replication ability of cancer gene is destroyed. In the literature we can find numerous examples of metal complexes derived from the S, N and O donor ligands.
Ayman K. et al [6] have prepared the Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes of substituted isatin thiosemicarbazone. The authors have proposed the octahedral geometry structure for both the complexes with SNO donor atoms.

Sengupta et al [7a] have synthesized the Ru(II) complexes of SNO donor ligands using salicyldehyde and o-hydroxyacetophenone 4-phenyl-thiosemicarbazone. The authors have established the octahedral geometry for the prepared complex with single crystal analysis. Wang L.F. et al [7b] have synthesized the transition metal complexes of SNO donor ligands with thiosemicarbazone derived from 3-acetyllumbelliferone. The authors have proposed the octahedral geometry structure. And they performed the antitumor activity against HL-60 human leukemia cells. Author concluded the metal complexes have shown good activity against tumor cells than the ligand.

These results stimulated us to construct the SNO donor ligand system with the aid of precursors namely, quinoxaline, quinoline and coumarin derivatives. Quinoxaline derivatives are a very important class of nitrogen-heterocycles and have been widely used as pharmaceuticals [8, 9] and electrical/photochemical materials [10-12] and they can serve as backbone for the construction of multidentate ligands [13, 14]. Quinoline, being the backbone for the most of the natural products, is used for the design of many synthetic compounds having diverse pharmacological applications [15, 16]. On the other hand coumarin derivatives are well known precursors for the design of many organic ligands and complexes with multiple applications [17]. By considering all these
parameters a series of later first row transition metal complexes have been prepared with SNO donor system and the ligational behavior of ligands, the structural features of complexes, the electrochemical behavior and antibiogram analysis of compounds are investigated.

Experimental

Synthesis of precursors

Preparation of the precursor namely 2-hydroxy-3-formylquinoline is given in chapter 3, and preparation of 3-acetylcoumarin is presented in section A of chapter- 5.

Preparation of 2,3-dimercaptoquinoxaline

Preparation of the above said compound involves three steps. The first step preparation namely 2,3-dihydroxyquinoxaline is given in section A of chapter 5.

Preparation of 2,3-dichloroquinoxaline [18]

2,3-Dihydroxyquinoxaline was heated above 140 °C for drying using an oil bath. 1.62 g (0.1 mol) of 2,3-dihydroxyquinoxaline was ground together with phosphorous pentachloride (3.55 g, 0.2 mol) and the entire mixture was placed in 250 ml round bottom flask fitted with a water cooled condenser. The mixture was heated to ~200 °C in an oil bath and held at this temperature for two hours. At the end of this time, a yellow solid has formed. It was allowed to cool and excess phosphorous pentachloride was removed by intimately mixing the solid with 200 g of crushed ice. After allowing the mixture to reach room temperature, the product was filtered, washed with cold water and dried.
(Yield: 92 %, M.P.: 176-179 °C)

Schematic representation of the 2, 3-dichloroquinoxaline

![Schematic representation of 2, 3-dichloroquinoxaline](image)

Preparation of 2,3-dimercaptoquinoxaline [18]

A solution of 2,3-dichloroquinoxaline (4 g, 0.05 mol) and thiourea (8 g, 0.10 mol) in 100 ml of absolute alcohol was refluxed for two hours. The solution was concentrated to a small volume, diluted with 250 ml of water, made alkaline by the addition of 25 g sodium hydroxide, and then refluxed for one and half hour. On acidifying with acetic acid, a brownish-orange product formed which was separated by filtration, washed with water and dried. The prepared 2,3-dimercapto quinoxaline may exist in thiol –thione tautomeric form.

(Yield: 96.4 %)

Schematic representation of 2,3-dimercaptoquinoxaline

![Schematic representation of 2,3-dimercaptoquinoxaline](image)
2,3-dimercaptoquinoxaline exist in thione-thiole tautomeric form

\[
\text{H} \quad \text{C} \quad \text{S} \\
\text{N} \quad \text{H} \quad \text{S} \\
\text{S} \quad \text{N} \quad \text{H} \\
\text{C} \quad \text{C} \quad \text{S}
\]

**Preparation of 2-mercapto-3-hydrazinoquinoxaline**

To a hot ethanolic solution of 2,3-dimercaptoquinoxaline (0.97 g, 0.005 mol), hydrazine hydrate (1.26 g, 1.22 ml, 0.025 mol) was added, which was refluxed for about 3-4 hours on a water bath. The resulting shining dark yellow color solid product was filtered in hot condition and thoroughly washed with ethanol and air dried (Yield: 80 %)

**Schematic presentation of 2-mercapto-3-hydrazinoquinoxalines**

\[
\begin{align*}
\text{2,3-Dimercaptoquinoxaline} & \quad + \quad \text{NH}_2\text{NH}_2\text{H}_2\text{O} \\
\text{EtOH} & \quad \text{Reflux} \\
\text{2-Mercapto-3-hydrazinoquinoxaline} & \quad \text{Hydrazine monohydrate}
\end{align*}
\]

**Preparation of the ligands**

**Preparation of ligand L\(^1\)**

To a hot ethanolic solution of 2-mercapto-3-hydrazinoquinoxaline (0.96 g, 0.005 mol), ethanolic solution of 2-hydroxy-3-formylquinoline (0.86 g, 0.005 mol) was added with stirring. And the reaction mixture was refluxed for about 3-4 hours on a water bath. The resulting solid product was filtered in hot condition and air dried.
Preparation of ligand $L^2$

To a hot ethanolic solution of 2-mercapto-3-hydrazinoquinoxaline (0.96 g, 0.005 mol), ethanolic solution of 3-acetylcoumarin (0.94 g, 0.005 mol) was added. And then reaction mixture was refluxed for about 3-4 hours on a water bath. The resulting solid product was filtered and dried.

The schematic presentation of the ligands $L^1$ and $L^2$

(Scheme 1)

Synthesis of the complexes

The appropriate amount of the ligands ($L^1$ (0.694 g, 0.002 mol) and $L^2$ (0.724 g, 0.002 mol) were taken in 30-40 ml of hot ethanol. To this, hot ethanolic solution of metal chloride {CoCl$_2$·6H$_2$O (0.475 g, 0.002 mol), NiCl$_2$·6H$_2$O (0.475 g, 0.002 mol), CuCl$_2$·2H$_2$O (0.341 g, 0.002 mol), ZnCl$_2$ (0.272 g, 0.002 mol)}
was added drop wise with stirring at 60-65 °C. After complete addition of metal salt solution, the reaction mixture was stirred for another 30-40 minutes at the same temperature and refluxed for 3-4 hours on water bath. The isolated complexes were filtered in hot condition, washed with hot aqueous ethanol and dried.

Biochemistry

Methodology for anti-biogram analysis against bacteria and fungi, also DNA cleavage experiment including DNA isolation, agarose gel electrophoresis is given in chapter 3.

Result and discussion

Complexes obtained in the present study were non-hygroscopic and in the form of amorphous solids. Analytical, magnetic and conductivity data for the complexes are given in Table-1. All the complexes are insoluble in water, ethanol, methanol but they are completely soluble in DMF, DMSO and acetonitrile. The stoichiometry of the complexes for ligand L¹ were found to be (M:L) 1:1, and 1:2 in case of the ligand L². Melting points of all the complexes are found to be above 300 °C and yield of the complexes are about 60-67%.

IR spectral studies

The pertinent IR absorption bands of both L¹ and L² ligands and their metal complexes, along with their assignments are summarized in Table-2. The ligands L¹ and L² show sharp bands at 1608 and 1596 cm⁻¹ respectively which are assigned to v(C=N). This band experiences a shift towards low frequency
side in case of (C1-C4) complexes and towards high frequency side in case of (C5-C8) complexes due to the coordination of azomethine nitrogen to the metal ion [19]. The bands observed at 1660 and 1725 cm\(^{-1}\) are attributed to the (>C=O) of quinoline [20] and coumarin [17] respectively. The absence of a band at around 2500 cm\(^{-1}\) attributable to v(S-H) in both the ligands rules out the possibility of thione-thiol tautomerism and suggest the stable thione form of the ligand. The coordination of the quinoline carbonyl is evidenced by the negative shift observed for v(C=O) in complexes. The v(C=O) of coumarin remain unaltered upon complexation suggesting the non-involvement of carbonyl group in the coordination. The v(C=S) in complexes (C1-C8), experiences negative shift and fall in intensity due to the thione sulphur coordination [21]. Which is further supported by the presence of the v(N-H) band in the spectra of the complexes and the \(^1\)H NMR study. The bands at 3300-3400 cm\(^{-1}\) indicate the presence of coordinated/crystal held water molecule. The low frequency non-ligand bands in the 480 cm\(^{-1}\) and 415 cm\(^{-1}\) region are assigned to v(M-N) and v(M-S) respectively (spectra 1-4).

\(^1\)H NMR spectral studies

The \(^1\)H NMR spectra of the ligands and their Zn(II) complexes were recorded in DMSO-d\(_6\) solvent over the range of 0-16 ppm. The peak at 8.62 ppm exhibited by the ligand L\(^1\) is assigned to azomethine proton, upon complexation with zinc metal ion it experiences the down field shift, which confirms the coordination of the azomethine nitrogen atom to the metal ion [22]. The ring – NH of quinoxaline moeity are resonated at 11.62 and 10.64 ppm in ligands L\(^1\)
and \( L^2 \) respectively, where as ring –NH of quinoline in case of ligand \( L^1 \) observed at 12.02 ppm. A multiplet at 6.93-7.91 ppm is assigned to aromatic protons of the ligands. Similarly zinc complexes of ligands (\( L^1 \) and \( L^2 \)) have not shown much variation which is attributable to a variation in electron density and steric constraints brought about in the compounds upon complexation [23] (spectra 5-6).

**Molar conductivity measurements**

The molar conductance values of the complexes measured at room temperature in DMSO solution at \( 10^{-3} \) mol dm\(^{-3} \) concentration fall in the range 6.9 to 15.4 ohm\(^{-1} \) cm\(^2\) mol\(^{-1} \) (Table-1), which are in agreement with non-electrolytic nature of the complexes [24].

**Electronic spectral studies**

The electronic spectra of the ligands and their complexes were measured in DMF. The free ligands exhibit strong absorption bands around 275-292 nm (\( \varepsilon \sim 15000 \) 1 cm\(^{-1}\) mol\(^{-1}\)) due to the intra-ligand \( \pi-\pi^* \) transitions. These bands remain almost unchanged in the spectra of complexes. The peaks around 350-370 nm (\( \varepsilon \sim 12000 \) 1 cm\(^{-1}\) mol\(^{-1}\)) are attributed to the \( n-\pi^* \) transition associated with azomethine linkage. Red shift of these absorptions upon complexation indicates the coordination of azomethine nitrogen to the metal ion [25-26]. In the electronic spectra of cobalt (C1 and C5) complexes the bands observed around 465-434 nm (\( \varepsilon \sim 20000 \) 1 cm\(^{-1}\) mol\(^{-1}\)) are assigned for ligand to metal charge transfer (LMCT) transitions. A broad distinct peak observed around 550 nm (\( \varepsilon \)
SNO donor chelates

~ 200 l cm\(^{-1}\) mol\(^{-1}\)) assignable to \(^4T_{1g} \rightarrow ^4A_{2g}\) transition suggest the octahedral structure for the complexes [27]. Both the nickel complexes (C2 and C6) have shown the LMCT transitions around 423 nm (\(\varepsilon \approx 20000\) l cm\(^{-1}\) mol\(^{-1}\)). In addition to this, they exhibit d-d transitions around 600 nm (\(\varepsilon \approx 150\) l cm\(^{-1}\) mol\(^{-1}\)) [28] assigned to \(^3A_{2g} \rightarrow ^3T_{1g}\) transition indicating the octahedral geometry.

The electronic spectra of copper complexes exhibit a broad intense peak at 445 nm (\(\varepsilon \approx 25000\) l cm\(^{-1}\) mol\(^{-1}\)) assignable to the S→ Cu(II) ligand to metal charge transfer transition. Along with this, the complex (C3) exhibit a broad peak around 565 nm (\(\varepsilon \approx 100\) l cm\(^{-1}\) mol\(^{-1}\)) with low energy shoulder which is assigned for the square pyramidal geometry [29] of the complex. The d-d transition observed at 565 nm in complex (C7) evidences the octahedral geometry. Generally Cu(II) ions can adopt square-planar, square-pyramidal, trigonal-bipyramidal, octahedral and tetrahedral geometries, which, except for the first, are generally distorted from the idealized structures. The d-d spectra shown by these coordination geometries are distinctive only in the case of the tetrahedral environment where the absorptions occur at much lower energies and generally show, well separated absorption peaks. In case of all other distorted geometries, spectrum shows closely spaced absorption. Hence it is difficult to predict the accurate structure for the complexes only on the basis of electronic spectral analysis. Zinc(II) complexes (C4 and C8) show strong absorption band around 370-390 nm, which is accounted for charge transfer transition (spectra 7-10).
Magnetic studies

The magnetic moment values of the synthesized metal complexes are given in Table-1. The magnetic moments of the Co(II) complexes (C1 and C5) were found to be 5.22 and 5.18 BM. at room temperature. These results agree with the reported values for high-spin octahedral cobalt(II) complexes [30, 31]. In the nickel (C2 and C6) complexes the magnetic moment values are observed to be 2.95 and 3.2 BM respectively indicating the spin free octahedral configuration [32], which is in consistent with two unpaired electrons. The effective magnetic moment of copper (C3 and C7) complexes is found to be 1.73 and 1.98 BM which is reliable with the presence of one unpaired electron. The higher magnetic moment values of these complexes can be rationalized in terms of the orbital contribution of donor atoms towards the spin only value [30, 31].

EPR spectral studies

The solid state X-band EPR spectra of copper complexes C3 and C7 exhibit isotropic intense broad signals with $g_{iso}$ values 2.06 and 2.08 respectively, with no hyperfine splitting is observed. These types of spectra were reported earlier for the complexes bearing large organic ligand substituents having considerable covalent character for metal-ligand bonds [33] (spectrum 11).

FAB mass studies

FAB mass spectral studies provide supporting evidence for the proposed constitutions of the complexes. The two copper complexes C3 and C7, show
the molecular ion peaks at m/z value 486 and 882 that corresponds to the formula weight of \([\text{CuL}^1\text{Cl}_2]\) and \([\text{Cu}(\text{L}^2)_2\text{Cl}_2]\)H₂O respectively. Along with this, spectra show some prominent peaks corresponding to the various molecular ion fragments (spectra 12-13). The tentative structures of the complexes are presented in Figure-1.

**Thermal analysis**

Thermogravimetric analyses of complexes (C2 and C5) were studied in the temperature range of 40-900 °C. Nickel complex (C2) decomposes in two distinct steps to give nickel oxide. The complex is stable up to 110 °C indicating the absence of crystal held water. Weight loss due to one coordinated water molecule has been observed in the temperature range 120-230 °C. In next step of decomposition the weight loss is due to the ligand molecule is observed in the temperature around 450 °C. After this TG curve shows a plateau indicating formation of stable metal oxide.

The complex (C5) decomposes in two steps. The first step of decomposition is observed in the temperature range 95-98 °C with weight loss corresponds to the loss of one lattice celled water molecule. In the second step of the decomposition weight loss observed around temperature range 475-674 °C corresponds to the ligand moiety. Finally, no weight loss is observed beyond 700 °C, possibly due to the formation of the stable metal oxide. The results obtained from TG studies are well agreed with theoretical calculation (Graph-1).
Cyclic Voltammetry study

Cyclic voltammetry is the most versatile electro-analytical technique used for the study of electrochemical properties of metallo-organic species. The important parameters of cyclic voltammogram are the magnitudes of the peak current ($I_{pa}$, $I_{pc}$) and peak potential ($E_{pa}$, $E_{pc}$). The electrochemical properties of the ligands ($L^1$ and $L^2$) and complexes (C1–C8) were studied in the potential range 1.2 to -1.6 V. Except C3 and C7 complexes, all the remaining compounds are found to be electrochemically inert in the mentioned potential range. The cyclic voltammogram of the C3 and C7 complexes show a well defined redox process corresponding to the Cu(II)/Cu(I) couple. The complex C3 exhibits quasi-reversible process with cathodic peak at $E_{pc} = -0.51$ V corresponding to Cu(II)$\rightarrow$Cu(I) and the anodic peak for Cu(I)$\rightarrow$Cu(II) at $E_{pa} = 0.55$ V. In case of C7 $E_{pa}$ and $E_{pc}$ are found to be at 0.47 and 0.38 V respectively. In both cases (C3 and C7) the value of $\Delta E_p$, separation between the anodic and cathodic peak potentials, is greater than 59 mV indicating the quasi-reversible nature of the redox process [34]. The dependency of peak potentials on the scan rates and value (~1) for $I_{pa}$/ $I_{pc}$ (ratio of oxidative to reductive peak currents) indicate the simple one-electron redox process [35] (spectra 14).

Biochemistry

Antimicrobial activity

For in vitro antimicrobial activity, the compounds were tested against the bacteria Escherichia coli, Pseudomonas aeruginosa and fungi Aspergillus
The percentage zone of inhibition values at active dosage and MIC level of the compounds against the growth of microorganisms are summarized in Table 3–5. The values indicate that complexes C2, C3, C7 and C8 have higher antimicrobial activity than the free ligands. Such increased activity of the complexes can be explained on the basis of Overtone's concept [36] and Tweedy's chelation theory [37]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only the lipid-soluble materials due to which liposolubility is an important factor, 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 \( \pi \)-electrons over the whole chelate and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganism. 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. Furthermore, the mode of action of the compound may involve the formation of a hydrogen bond through the azomethine group with the active center of cell constituents, resulting in interference with the normal cell process [38].

**DNA cleavage study**

DNA cleavage reactions are generally targeted towards its basic constituents, viz. heterocyclic base, sugar and phosphate. While the reactions targeted to the
phosphodiester linkage proceed via hydrolytic cleavage pathways leading to the formation of fragments that could be related through enzymatic processes, such DNA cleavages carried out by metal ions. Suitably designed metal complexes can induce several changes in DNA conformation after the complex has been bound. Metal complexes which could induce DNA deformations, such as bending, local denaturation (overwinding and underwinding), intercalation, micro loop formation and subsequent DNA shortening leading to decrease in molecular weight of DNA. The cleavage efficiency of the complexes was compared to that of the control is due to their efficient DNA binding ability. Control experiments using DNA alone do not show any significant cleavage even after a longer exposure time. But the increase in intensities of the bands was observed for the metal bound DNA, such intercalation will lead to change the confirmation of the DNA. In the present study, the DNA gel electrophoresis experiment was conducted at 35 °C using the synthesized compounds. The complexes C2 and C3 have shown fair intensities compared to the remaining complexes and ligand which may be due to the binding of the complexes with Escherichia coli DNA (Chart 1).
Conclusion

In this chapter, we have presented the synthesis and structural investigations of sulfur containing hydrazone ligands and their transition metal(II) complexes. The structure of the ligands and their metal complexes were confirmed by various spectral and elemental techniques. The ligand to metal (L:M) stoichiometry is found to be 1:1 and 2:1 in case of L¹ and L² respectively. The spectroscopic study reveals that, the ligands coordinate to the metal ion in thione form rather than thiol form. Also these techniques give the evidence for the non involvement of lactonic oxygen (C5-C8). All the complexes are found
SNO donor chelates to have octahedral geometry except \([\text{CuL}^1\text{Cl}_2]\), which is square pyramidal in nature. Only the copper complexes are found to be electrochemically active in the applied potential range. It needs further detailed and extended studies to understand the mechanism involved in the electron transfer reactions and to check the utility of these compounds as pharmaceuticals. These compounds can be used as structural and functional models for the various metallobiosites. However the detailed studies are required to analyze the utility of these compounds as biomimetics. The increase in antibiogram activity is observed mainly in case of C2, C3, C7 and C8 compounds. Increase in intensity of the bands is observed in DNA cleavage study by gel electrophoresis method, which is due to the binding of complexes to DNA.
Table 1 Analytical, conductivity and magnetic data for the ligands and their complexes

<table>
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<th>No</th>
<th>Compounds</th>
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<th>Magnetic moment in μ₀ BM</th>
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<td>L¹</td>
<td>(C₁₉H₁₄N₄O₂S)</td>
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<td>19.93/20.17</td>
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<td>C¹</td>
<td>[Co(C₁₉H₁₃N₅O₂S)]Cl₂.H₂O</td>
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<td>[Ni(C₁₉H₁₃N₅O₂S)]Cl₂.H₂O</td>
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<td>12.31/12.85</td>
<td>7.19/7.34</td>
</tr>
<tr>
<td>C⁶</td>
<td>[Ni(C₁₈H₁₄N₄O₂S)]Cl₂.H₂O</td>
<td>52.14/52.37</td>
<td>12.24/12.86</td>
<td>7.15/7.35</td>
</tr>
<tr>
<td>C⁷</td>
<td>[Cu(C₁₈H₁₄N₄O₂S)]Cl₂.H₂O</td>
<td>51.90/52.08</td>
<td>12.17/12.79</td>
<td>7.15/7.31</td>
</tr>
<tr>
<td>C⁸</td>
<td>[Zn(C₁₈H₁₄N₄O₂S)]Cl₂.H₂O</td>
<td>51.61/51.97</td>
<td>12.05/12.76</td>
<td>6.89/7.29</td>
</tr>
</tbody>
</table>
### Table 2
IR spectral data of the ligands and their complexes in cm\(^{-1}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\nu(\text{C=N})) (azomethine)</th>
<th>(\nu(\text{C=O})) (quinoline)</th>
<th>(\nu(\text{C=O})) (lactonic)</th>
<th>(\nu(\text{H}_2\text{O}))</th>
<th>(\nu(\text{M-N}))</th>
<th>(\nu(\text{M-S}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(^1)</td>
<td>1608</td>
<td>1660</td>
<td>1255</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>C1</td>
<td>1556</td>
<td>1636</td>
<td>1228</td>
<td>--</td>
<td>3406</td>
<td>468</td>
</tr>
<tr>
<td>C2</td>
<td>1597</td>
<td>1638</td>
<td>1222</td>
<td>--</td>
<td>3385</td>
<td>472</td>
</tr>
<tr>
<td>C3</td>
<td>1545</td>
<td>1639</td>
<td>1220</td>
<td>--</td>
<td>3419</td>
<td>470</td>
</tr>
<tr>
<td>C4</td>
<td>1598</td>
<td>1638</td>
<td>1223</td>
<td>--</td>
<td>3401</td>
<td>469</td>
</tr>
<tr>
<td>L(^2)</td>
<td>1596</td>
<td>--</td>
<td>1246</td>
<td>1725</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>C5</td>
<td>1609</td>
<td>--</td>
<td>1236</td>
<td>1728</td>
<td>3432</td>
<td>480</td>
</tr>
<tr>
<td>C6</td>
<td>1609</td>
<td>--</td>
<td>1218</td>
<td>1729</td>
<td>3434</td>
<td>455</td>
</tr>
<tr>
<td>C7</td>
<td>1609</td>
<td>--</td>
<td>1230</td>
<td>1724</td>
<td>3430</td>
<td>465</td>
</tr>
<tr>
<td>C8</td>
<td>1608</td>
<td>--</td>
<td>1211</td>
<td>1728</td>
<td>3402</td>
<td>463</td>
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Table 3 Screening of compounds against bacteria and fungi

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Zone of inhibition in cm</th>
<th>% inhibition</th>
<th>Zone of inhibition in cm</th>
<th>% inhibition</th>
<th>Zone of inhibition in cm</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>3.2</td>
<td>100</td>
<td>2.9</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L(^1)</td>
<td>0.9</td>
<td>28.12</td>
<td>0.6</td>
<td>20.68</td>
<td>L(^1)</td>
<td>0</td>
</tr>
<tr>
<td>C1</td>
<td>0.7</td>
<td>21.87</td>
<td>0.4</td>
<td>13.79</td>
<td>C1</td>
<td>0.1</td>
</tr>
<tr>
<td>C2</td>
<td>1.7</td>
<td>53.12</td>
<td>1.1</td>
<td>37.93</td>
<td>C2</td>
<td>0.3</td>
</tr>
<tr>
<td>C3</td>
<td>2.8</td>
<td>87.5</td>
<td>1.6</td>
<td>55.17</td>
<td>C3</td>
<td>0.8</td>
</tr>
<tr>
<td>C4</td>
<td>1.0</td>
<td>31.25</td>
<td>1.0</td>
<td>34.48</td>
<td>C4</td>
<td>0</td>
</tr>
<tr>
<td>L(^2)</td>
<td>1.3</td>
<td>40.62</td>
<td>0.8</td>
<td>27.58</td>
<td>L(^2)</td>
<td>0.2</td>
</tr>
<tr>
<td>C5</td>
<td>0.2</td>
<td>6.2</td>
<td>0</td>
<td>0</td>
<td>C5</td>
<td>0.2</td>
</tr>
<tr>
<td>C6</td>
<td>0.4</td>
<td>12.5</td>
<td>0.1</td>
<td>3.44</td>
<td>C6</td>
<td>0.5</td>
</tr>
<tr>
<td>C7</td>
<td>0.3</td>
<td>9.37</td>
<td>1.3</td>
<td>44.82</td>
<td>C7</td>
<td>0.9</td>
</tr>
<tr>
<td>C8</td>
<td>0.5</td>
<td>15.62</td>
<td>0.3</td>
<td>10.3</td>
<td>C8</td>
<td>0.7</td>
</tr>
<tr>
<td>DMF</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>DMF</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 4 Antibacterial studies of compounds in MIC level (250µg)

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone of inhibition in cm</td>
<td>% inhibition</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>3.2</td>
<td>100</td>
</tr>
<tr>
<td>C2</td>
<td>1</td>
<td>31.25</td>
</tr>
<tr>
<td>C3</td>
<td>2.3</td>
<td>71.8</td>
</tr>
<tr>
<td>L²</td>
<td>0.7</td>
<td>21.87</td>
</tr>
<tr>
<td>C7</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>DMF</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 5 Antifungal studies of compounds in MIC level (250µg)

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Aspergillus niger</th>
<th>Cladosporium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zone of inhibition in cm</td>
<td>% inhibition</td>
</tr>
<tr>
<td>Flucanozole</td>
<td>1.2</td>
<td>100</td>
</tr>
<tr>
<td>C3</td>
<td>0.6</td>
<td>50</td>
</tr>
<tr>
<td>C7</td>
<td>0.8</td>
<td>66.6</td>
</tr>
<tr>
<td>C8</td>
<td>0.3</td>
<td>25</td>
</tr>
<tr>
<td>DMF</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Spectrum 1 IR spectrum of ligand L¹

Spectrum 2 IR spectrum of complex C¹
Spectrum 3 IR spectrum of ligand L^2

Spectrum 4 IR spectrum of complex C6
Spectrum 5 \(^1\)H NMR spectrum of complex C4

Spectrum 6 \(^1\)H NMR spectrum of complex C8
Chapter-5B Spectra

Spectrum 7 UV-Visible spectrum of ligand $L^1$

Spectrum 8 UV-Visible spectrum of complex $C^2$

Spectrum 9 UV-Visible spectrum of ligand $L^2$

Spectrum 10 UV-Visible spectrum of complex $C^7$

Spectrum 11 EPR spectrum of complex $C^7$
Spectrum 12 FAB Mass spectrum of complex C3

Spectrum 13 FAB Mass spectrum of complex C7
Spectrum 14 TG-DTA spectrum of complex C7

Spectrum 15 Cyclic Voltammogram of complex C3
Chart 1 The representative cleavage picture of L^1 and their complexes. [M-Standard Molecular weight Marker; C- Escherichia coli - Control DNA of Escherichia coli; (f) ligand (L^1) (g) Cobalt(C1), (h) Nickel(C2), (i) Copper(C3) and (j)
References:


