SYNTHESIS, CHARACTERIZATION, ELECTROCHEMISTRY AND BIOLOGICAL ACTIVITY OF SOME METAL COMPLEXES OF LIGAND HAVING BENZO[B]THIOPHENE AND COUMARIN MOIETIES (HL7).
6.1. INTRODUCTION

Schiff bases are a special class of ligands with a variety of donor atoms exhibiting interesting coordination modes towards various metals [1-3]. The azomethine linkage in Schiff bases is responsible for the biological activities such as antitumor, antibacterial, antifungal and herbicidal activities [4]. The benzothiophene moiety represents an important pharmacophore/toxophore in drug chemistry and agrochemistry [5-7]. Benzothiophene was isolated in 1902, in coal tar. In technical naphthalene, it is the major sulphur containing impurity [8-10]. Many benzothiophene derivatives have been reported to possess anti-microbial [11-13], local anesthetic [14], anti-inflammatory [15, 16], analgesic [17], antiviral [18], anti-leukotriene [19] activities and act as inhibitor of lipoxygenase [20], sPLA 2 inhibitors [21].

Undoubtedly, the major driving force behind advances in the chemistry of benzothiophene is their biological applications, namely pharmaceuticals or agrochemicals. Within the last two decades, benzothiophene has increasingly been recognized as a pharmacophore that offers advantages including superior chemical and pharmacological stability, low intrinsic toxicity [22-25] and, most importantly, a rich chemistry that enables medicinal chemists to explore molecular diversity in a rapid fashion. Lot of work related to benzothiophene has emerged with the development and introduction of two new chemical entities (NCE) containing this heterocycles as pharmaceuticals in recent years. Zileutin (i) a 5-lipoxygenase inhibitor has been approved and is on the market for anti-inflammatory indications. Sertaconale (ii) has recently been introduced to the market as a broad spectrum antifungal reagent.

![Chemical structures](image)

Coumarin derivatives are considered to be important class of compounds due to their various applications in the biological fields. [26-28]. A large number of coumarin
derivatives were found to affect the formation and scavenging of reactive oxygen species (ROS), and reactive nitrogen species (RNS), displaying tissue protective antioxidant properties [29]. Among them, hydroxycoumarins are effective metal chelators, free radical scavengers and powerful chain breaking antioxidants. Further, 8-substituted-4-methyl-7-hydroxy coumarin has exhibited good complexing ability and has shown anticoagulant and plant growth regulating properties [30]. An increasing interest in antioxidant, particularly in those intended to prevent the mischievous effects caused by the free radicals in the human body is attracting one. The free radicals are also believed to be associated with carcinogenesis, mutagenesis, arthritis, diabetes, inflammation, cancer and genotoxicity due to the oxidative stress which arises as a result of imbalance between free radical generations [31]. The antioxidant activity of metal complex is found to be induced by both the identity of the metal and the ligands bound to it [32]. The interaction of transition metal complexes with DNA has been studied extensively for their usage as probes for DNA structure and their potential application in chemotherapy. Very recently Cu(II) complexes have been reported to be active as in DNA strand scissors [33, 34]. In view of these findings, we found it is interesting to synthesize Schiff base ligand containing both benzo[b]thiophene and coumarin moieties viz. 3-chloro-N'-(7-hydroxy-4-methyl-2-oxo-2H-chromen-8-yl)methylene)benzo[b]thiophene-2-carbohydrazide (HL7) and its Cu(II), Co(II), Ni(II) and Zn(II) complexes and study their pharmacological activities like antimicrobial, antioxidant and DNA cleavage activities.

![Image](image_url)
6.2. EXPERIMENTAL

Details of the synthesis of Schiff base ligand $\text{HL}_7$ and its complexes have already discussed in the chapter II.

6.3. RESULT AND DISCUSSION

Characterization and biological evaluation of the ligand 3-chloro-$\text{N}'$-((7-hydroxy-4-methyl-2-oxo-2$H$-chromen-8-yl)methylene)benzo[b]thiophene-2-carbohydrazide ($\text{HL}_7$) and its Cu(II), Co(II), Ni(II) and Zn(II) complexes are discussed below.

6.3.1. Characterization of ligand 3-chloro-$\text{N}'$-((7-hydroxy-4-methyl-2-oxo-2$H$-chromen-8-yl)methylene)benzo[b]thiophene-2-carbohydrazide ($\text{HL}_7$)

The analytical data of the ligand $\text{HL}_7$ is given in the Table 6.1. The IR spectrum of the ligand $\text{HL}_7$ is depicted in Fig. 6.1. In the IR spectrum of ligand $\text{HL}_7$ (Fig. 6.1), absorption due to phenolic OH displayed band at 3420 cm$^{-1}$, absorption due to NH of CONH function have displayed band at 3232 cm$^{-1}$. Two sharp peaks observed at 1726 and 1692 cm$^{-1}$ are due to lactone carbonyl function of coumarin and amide carbonyl function attached to 2-position of benzo[b]thiophene moiety respectively of ligand $\text{HL}_7$. Absorption bands due to azomethine and phenolic C-O functions of the ligand $\text{HL}_7$ have appeared at 1663 and 1252 cm$^{-1}$ respectively.

The $^1$H NMR spectrum of the ligand $\text{HL}_7$ (Fig. 6.2) displayed two singlets at 12.56 and 9.19 ppm due to the proton of phenolic OH and amide NH. The signal due to proton of azomethine function has appeared at 8.20 ppm in ligand $\text{HL}_7$. The aromatic protons have resonated as multiplets in the region 6.99 - 7.99 ppm (m, 6H, ArH), proton due to $=\text{CH}$ of coumarin moiety appeared as a singlet at 6.27 ppm and three protons of methyl group attached to 4-position of coumarin moiety have resonated as distinct singlet at 2.42 ppm respectively in the ligand $\text{HL}_7$.

The ESI mass spectrum of ligand $\text{HL}_7$ (Fig. 6.3) showed molecular ion peak due to $M^+$ at m/z 412, 414 (51%, 12%) which is equivalent to its molecular weight. This on simultaneous loss of methyl radical, acetyl radical and a molecule of carbon monoxide gave a peak recorded at m/z 344, 346 (18%, 5%). Further, this on simultaneous loss of
chloride radical, two hydrogen radicals and hydroxyl radical gave a fragment ion peak at 290 (7%). The molecular ion peak due to $M^+$ at m/z 412, 414 (51%, 12%) has also underwent fragmentation by another route by simultaneous loss of chloride radical and methyl radical giving a fragment ion peak recorded at m/z 362 (28%). This on further loss of carbon dioxide molecule gave a fragment ion peak recorded at m/z 318 (100%) which is also a base peak. This fragment ion on simultaneous loss of acetylene radical, two hydrogen radicals and hydroxyl radical gave a fragment ion peak recorded at m/z 274 (82%). This on further loss of carbon monoxide molecule gave a fragment ion peak recorded at m/z 246 (8%). This fragmentation pattern is in consistency with its structure (Scheme 6.1).

Fig. 6.1: IR spectrum of ligand HL₇
Fig. 6.2: $^1$H NMR spectrum of ligand HL$_7$

Fig. 6.3: ESI mass spectrum of ligand HL$_7$
Scheme 6.1: Mass fragmentation of ligand HL₁₋₇.
6.3.2. Characterization of Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligand HL₇

1. Nature, Stoichiometry and molar conductivity

All the synthesized metal complexes are coloured solids, amorphous and non-hygroscopic in nature and possess high melting points (>300 °C). The complexes are insoluble in water and common organic solvents but are soluble in DMF and DMSO. Elemental analysis and analytical data of the complexes suggest that the metal to ligand ratio of the complexes is 1:1 stoichiometry of the type [M(L)(Cl)(H₂O)₂] for Cu(II) complex and 1:2 stoichiometry of the type [M(L)₂] for Co(II), Ni(II) and Zn(II) complexes of ligand HL₇, where L stands for deprotonated ligand. The molar conductance values are too low to account for any dissociation of the complexes in DMF (22.51 – 41.74 ohm⁻¹cm²mole⁻¹), indicating their non-electrolytic nature (Table 6.1) [35].

2. Infrared spectra of Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligand HL₇

The important IR bands of the ligand HL₇ and its metal complexes are represented in Table 6.2. The typical IR spectra and far IR spectra of Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligand HL₇ are depicted on Fig. 6.4 and 6.5, 6.6 and 6.7, 6.8 and 6.9 and 6.10 and 6.11 respectively.

The absence of absorption band due to phenolic OH group at 3420 cm⁻¹ in the IR spectra of Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligand HL₇ indicates the formation of bond between metal ion and phenolic oxygen atom via deprotonation. This is further confirmed by the increase in absorption frequency of phenolic C-O which appeared in the region 1270 - 1295 cm⁻¹ in the metal complexes of ligand HL₇ under the present study. The absorption band due to NH of CONH function of the above metal complexes of ligand HL₇ have displayed band in the region 3229 - 3234 cm⁻¹, which have appeared at about the same region as in the case of ligand, thus confirming the non-involvement of NH of CONH function in coordination with the metal ions. The absorption band due to lactone carbonyl function in all the metal complexes has appeared at about the same region 1724 - 1728 cm⁻¹ as that of the ligand HL₇, there by indicating the non-involvement of lactone carbonyl oxygen in coordination with metal ions. The absorption frequency of amide carbonyl and azomethine functions which have appeared at 1692 and 1663 cm⁻¹ in case of ligand HL₇, has been shifted to lower frequency by 11 -
19 and 44 - 51 cm\(^{-1}\) in the complexes and appeared in the region 1681 - 1673 and 1619 - 1612 cm\(^{-1}\) indicating the involvement of oxygen atom of carbonyl function as such without undergoing any enolization [36] and nitrogen atom of azomethine [37] function in complexation with the metal ions. This is further confirmed by the appearance of new bands in the region 545 - 516 and 489 - 443 cm\(^{-1}\) due to M-O and M-N stretching vibrations [38] in all the complexes of ligand HL\(_7\). The appearance of new band in the region 270 cm\(^{-1}\) in Cu(II) complex of ligand is due to M-Cl band. The broad band due to co-ordinated water molecule appeared at 3430 cm\(^{-1}\) in the case of Cu(II) complex of ligand HL\(_7\).

3. \(^1\)H NMR spectra of Zn(II) complex of ligand HL\(_7\)

The \(^1\)H NMR data of the ligand HL\(_7\) and its Zn(II) complex are presented in Table 6.3. The typical \(^1\)H NMR spectrum of Zn(II) complex of ligand HL\(_7\) is depicted in Fig. 6.12.

In the case of Zn(II) complex, the absence of signal due to proton of phenolic OH group confirms the involvement of bonding of the phenolic oxygen atom to metal ion via deprotonation. The signals appeared at 9.25 ppm, 8.25 ppm, 7.04 - 8.04 ppm, 6.32 ppm and 2.47 ppm are due to amide NH proton, azomethine proton, aromatic protons, \(=\)CH proton of coumarin moiety and three protons of methyl group attached to 4-position of coumarin moiety respectively. When compared to the \(^1\)H NMR spectra of ligand HL\(_7\) and its Zn(II) complex, all the signals due to protons of ligand have been shifted towards down field strength confirming the complexation of Zn(II) ion with the ligand. Thus the \(^1\)H NMR data supports the structures of ligand and its complex.
Table 6.1: Physical, analytical and magnetic susceptibility data of ligand HL and its complexes.

<table>
<thead>
<tr>
<th>Ligand/complexes</th>
<th>Molecular formula</th>
<th>Mol.Wt (g)</th>
<th>M.p. °C (yield %)</th>
<th>Elemental analysis (%)</th>
<th>Mag. Moment (B.M)</th>
<th>Molar cond (µM ohm⁻¹ cm² mol⁻¹)</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL Ligand</td>
<td>C₂₀H₁₃N₂O₄SCl</td>
<td>412</td>
<td>298-299 (70)</td>
<td>M 58.25 C 3.15 H 6.79 N 8.49</td>
<td>-</td>
<td>-</td>
<td>Yellow</td>
</tr>
<tr>
<td>7a</td>
<td>[Cu(L₇)(Cl)(H₂O)₂]</td>
<td>545.54</td>
<td>&gt;300 (68)</td>
<td>M 11.64 C 43.99 H 2.93 N 5.13</td>
<td>12.83</td>
<td>1.75</td>
<td>Green</td>
</tr>
<tr>
<td>7b</td>
<td>[Co(L₇)₂]</td>
<td>880.93</td>
<td>&gt;300 (58)</td>
<td>M 6.68 C 54.48 H 2.72 N 14.53</td>
<td>7.94</td>
<td>4.71</td>
<td>Brown</td>
</tr>
<tr>
<td>7c</td>
<td>[Ni(L₇)₂]</td>
<td>880.69</td>
<td>&gt;300 (56)</td>
<td>M 6.66 C 54.50 H 2.72 N 14.53</td>
<td>7.94</td>
<td>2.85</td>
<td>Green</td>
</tr>
<tr>
<td>7d</td>
<td>[Zn(L₇)₂]</td>
<td>887.40</td>
<td>&gt;300 (55)</td>
<td>M 7.36 C 54.09 H 2.70 N 14.42</td>
<td>7.88</td>
<td>-</td>
<td>Orange</td>
</tr>
</tbody>
</table>
Table 6.2: IR spectral data (cm$^{-1}$) of ligand HL$_7$ and its complexes.

<table>
<thead>
<tr>
<th>Ligand/complexes</th>
<th>$\nu$$_{H_2O}$ (Phenolic)</th>
<th>$\nu$$_{OH}$ (amide)</th>
<th>$\nu$$_{NH}$ (Lactone)</th>
<th>$\nu$$_{C=O}$ (carbonyl)</th>
<th>$\nu$$_{HC=N}$ (azomethine)</th>
<th>$\nu$$_{C-O}$ (phenolic)</th>
<th>$\nu$$_{M-O}$</th>
<th>$\nu$$_{M-N}$</th>
<th>$\nu$$_{M-Cl}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL$_7$ Ligand</td>
<td>-</td>
<td>3420</td>
<td>3232</td>
<td>1726</td>
<td>1692</td>
<td>1663</td>
<td>1252</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7a [Cu(L$_7$)(Cl)(H$_2$O)$_2$]</td>
<td>3430   -</td>
<td>3234</td>
<td>1728</td>
<td>1673</td>
<td>1614</td>
<td>1291</td>
<td>545</td>
<td>484</td>
<td>270</td>
</tr>
<tr>
<td>7b [Co(L$_7$)$_2$]</td>
<td>-</td>
<td>-</td>
<td>3230</td>
<td>1724</td>
<td>1680</td>
<td>1618</td>
<td>1270</td>
<td>542</td>
<td>477</td>
</tr>
<tr>
<td>7c [Ni(L$_7$)$_2$]</td>
<td>-</td>
<td>-</td>
<td>3229</td>
<td>1724</td>
<td>1680</td>
<td>1619</td>
<td>1273</td>
<td>516</td>
<td>489</td>
</tr>
<tr>
<td>7d [Zn(L$_7$)$_2$]</td>
<td>-</td>
<td>-</td>
<td>3231</td>
<td>1727</td>
<td>1681</td>
<td>1612</td>
<td>1295</td>
<td>544</td>
<td>443</td>
</tr>
</tbody>
</table>

Table 6.3: $^1$H NMR data of ligand HL$_7$ and its Zn(II) complex

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$^1$H NMR data (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL$_7$ Ligand</td>
<td>12.56 (s, 1H, phenolic OH), 9.19 (s, 1H, CONH), 8.20 (s, 1H, HC=N), 6.99-7.99 (m, 6H, ArH), 6.27 (s, 1H, =CH) and 2.42 (s, 3H, coumarin CH$_3$).</td>
</tr>
<tr>
<td>7d [Zn(L$_7$)$_2$]</td>
<td>9.25 (s, 1H, CONH), 8.25 (s, 1H, HC=N), 7.04-8.04 (m, 6H, ArH), 6.32 (s, 1H, =CH) and 2.47 (s, 3H, coumarin CH$_3$).</td>
</tr>
</tbody>
</table>
Fig. 6.4: IR spectrum of Cu(II) complex of ligand HL\textsubscript{7}

Fig. 6.5: Far-IR spectrum of Cu(II) complex of ligand HL\textsubscript{7}
Fig. 6.6: IR spectrum of Co(II) complex of ligand HL₇

Fig. 6.7: Far-IR spectrum of Co(II) complex of ligand HL₇
Fig. 6.8: IR spectrum of Ni(II) complex of ligand HL$_7$

Fig. 6.9: Far-IR spectrum of Ni(II) complex of ligand HL$_7$
Fig. 6.10: IR spectrum of Zn(II) complex of ligand HL$_7$

Fig. 6.11: Far-IR spectrum of Zn(II) complex of ligand HL$_7$
Fig. 6.12: $^1$H NMR spectrum of Zn(II) complex of ligand HL$_7$

4. ESI-Mass spectra of Cu(II) and Zn(II) complexes of ligand HL$_7$

Mass spectral data confirms the structure of the complexes as indicated by the peaks corresponding to their molecular mass. Cu(II) and Zn(II) complexes have been studied for their mass spectral studies.

The mass spectrum of Cu(II) complex (7a) (Fig. 6.13) shows molecular ion peak due to $M^+ + 1$ at m/z 546, 548, 550 (100%, 46%, 11%) which is a base peak. The molecular ion on simultaneous loss of carbon dioxide molecule, acetylene molecule and three hydrogen radicals gave a fragment ion peak recorded at m/z 473, 475, 477 (17%, 9%, 2.5%). This fragment ion, has undergone fragmentation by simultaneous expulsion of two water molecule, methyl radical and four hydrogen radicals to give a fragment ion peak recorded at m/z 418, 420, 422 (36%, 12%, 3%). This fragmentation pattern (Scheme 6.2) is in consistency with its structure of Cu(II) complex.
The mass spectrum of Zn(II) complex (7d) (Fig. 6.14) showed molecular ion peak due to $\text{M}^+ + 1$ at m/z 888, 889 (5%, 1.2%). This on loss of hydrogen radical gave the fragment ion peak recorded at m/z 887, 889 (9%, 2.4%) which is equivalent to its molecular weight. This on simultaneous loss of two carbon dioxide molecule, two C$_3$H$_3$ radicals, two C$_4$H$_3$ radicals, C$_8$H$_3$SCl radical and a hydrogen radical gave a peak recorded at m/z 451, 453 (24%, 8%). Further, this on simultaneous loss of a chloride radical, carbon monoxide molecule and C$_4$H$_2$ molecule gave a fragment ion peak at m/z 338 (9%). This fragment ion on further expulsion of C$_4$H$_2$S molecule, C$_2$O molecule and a hydrogen radical gave a fragment ion peak recorded at m/z 215 (100%) which is also a base peak. This fragmentation pattern is in consistency with its structure (Scheme 6.3).

Fig. 6.13: ESI mass spectrum of Cu(II) complex of ligand HL$_7$
Fig. 6.14: ESI mass spectrum of Zn(II) complex of ligand HL_7

Scheme 6.2: Mass fragmentation of Cu(II) complex (7a).
Scheme 6.3: Mass fragmentation of Zn(II) complex (7d).
5. Electronic spectra of Cu(II), Co(II) and Ni(II) complexes of ligand HL₇

The formation of metal complexes was also confirmed by electronic spectra. The electronic absorption spectra of Cu(II), Co(II) and Ni(II) complexes of ligand HL₇ were recorded in distilled DMF (10⁻³ M) at room temperature. The band positions of absorption maxima assignments are listed in Table 6.4. On the basis of electronic spectral data of the Cu(II), Co(II) and Ni(II) complexes, the ligand field parameters such as Dq, B', β, β%, L.F.S.E and ν₂/ν₁ ratio were calculated and recorded in the Table 6.4.

Cu(II) complex (7a)

The Cu(II) complex of ligand HL₇ displayed single broad band with low intensity in the region 13976-18143 cm⁻¹ (7a). The broad band is assigned due to three transitions \(^2\)B₁g → \(^2\)Eg, \(^2\)B₁g → \(^2\)B₂g and \(^2\)B₁g → \(^2\)A₁g, which are of similar in energy and give rise to only single broad absorption band and the broadness of the band is due to dynamic Jahn-Teller distortion. Based on these data the Cu(II) complex has distorted octahedral geometry [39].

Co(II) complex (7b)

The electronic spectra of Co(II) complex of ligand HL₇ showed two absorption bands observed at 15732 and 21237 cm⁻¹ (7b) due to two transitions \(^4\)T₁g (F) → \(^4\)A₂g (F) (ν₂) and \(^4\)T₁g (F) → \(^4\)T₂g (P) (ν₃). These data are in good agreement with the reported values [40] for octahedral geometry. The lowest band, ν₁ was not observed due to the limited range of the instrument, it is calculated using the band fitting procedure suggested by Underhill and Billing [41]. These transitions suggest octahedral geometry for both the Co(II) complexes.

The calculated B' value is less than the free ion value (B'\(_{\text{free ion}}\) = 971 cm⁻¹) for Co(II) complex (7b) which is an indication of orbital overlapping and delocalization of d-orbital. The nephelauxetic parameter which is calculated by using formula, \(\beta = B_{\text{complex}} / B'_{\text{free ion}}\) Co(II) complex was found to be less than unity, suggesting a considerable cobalt-ligand bond character (Table 6.4).
The ligand field stabilization energy (LFSE) is calculated [42] for the relation, 
\[ \text{LFSE} = 6 \times \frac{\text{Dq}}{350} \text{ K.Cal/mol} \] which is well within the range for octahedral Co(II) complexes [43-45].

**Ni(II) complex (7c)**

The Ni(II) complex under present investigation exhibited two bands each in the region 14138 and 23184 cm\(^{-1}\) (7c). These bands are assigned to \( ^3A_{2g} (F) \rightarrow ^3T_{1g} (F) \) (\( \nu_2 \)) and \( ^3A_{2g} (F) \rightarrow ^3T_{1g}(P) \) (\( \nu_3 \)) transitions in an octahedral environment [46]. The band \( \nu_1 \) was calculated by using a band fitting procedure [41].

For the present Ni(II) complex (7c), the Table 6.4 showed the ligand field parameter such as Dq, B’, \( \beta \), \( \beta^\circ \) %, \( \nu_2/\nu_1 \) and LFSE. Racah parameter B’ is 731 cm\(^{-1}\) for Ni(II) complex, which is less than the free ion value of 1040 cm\(^{-1}\) indicating the covalent character of the complex. Moreover, the ratio \( \nu_2/\nu_1 \) was found to be 1.61 which is well within the range of 1.40-1.69 and is indicative of octahedral geometry of Ni(II) complex.

**Table 6.4:** Electronic spectral data and ligand field parameters of the Cu(II), Co(II) and Ni(II) complexes in DMF (10\(^{-3}\)M) solution.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Transitions in cm(^{-1})</th>
<th>Dq (cm(^{-1}))</th>
<th>B’ (cm(^{-1}))</th>
<th>( \beta )</th>
<th>( \beta^\circ ) %</th>
<th>( \nu_2/\nu_1 )</th>
<th>LFSE (k cal.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Cu(L}_7\text{)}(\text{Cl})(\text{H}_2\text{O})_2])</td>
<td>13976 - 18143</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>27.529</td>
</tr>
<tr>
<td>([\text{Co(L}_7\text{)}_2])</td>
<td>7303</td>
<td>15732</td>
<td>21237</td>
<td>842.9</td>
<td>983</td>
<td>0.945</td>
<td>5.48</td>
</tr>
<tr>
<td>([\text{Ni(L}_7\text{)}_2])</td>
<td>8780</td>
<td>14138</td>
<td>23184</td>
<td>878</td>
<td>731</td>
<td>0.844</td>
<td>29.71</td>
</tr>
</tbody>
</table>

*calculated values.

**6. Magnetic susceptibility data of Cu(II), Co(II) and Ni(II) complexes of ligand HL\(_7\)**

The room temperature magnetic measurements were obtained for paramagnetic Cu(II), Co(II) and Ni(II) complex of ligand HL\(_7\) and are listed in Table 6.1. The observed magnetic moment for Cu(II) complex is 1.75 BM (7a) which attributes to one unpaired electron with slight orbital contribution to the spin only value of 1.73 BM and the absence of spin-spin interactions in the complex accounting for the possibility of a distorted octahedral geometry [47]. In octahedral Co(II) complex the ground state is \(^4T_{1g}\) and the orbital contribution to the singlet state lowers the magnetic moment values for the
various Co(II) complexes which are in the range 4.12 - 4.70 and 4.70 - 5.20 BM for tetrahedral and octahedral complexes respectively [48]. In the present study the observed magnetic moment value for Co(II) complex is 4.71 BM (7b) indicates octahedral geometry for Co(II) complex. For Ni(II) complex the observed magnetic moment value are 2.85 BM (7c) which is well within the expected range for Ni(II) complex with octahedral geometry i.e. 2.83-3.50 BM [49].

7. ESR spectral studies of Cu(II) complex of ligand HL

The ESR spectra of the Cu(II) complex in a polycrystalline state were recorded at room temperature to elucidate the geometry and degree of covalency of the metal-ligand bond or environment around the metal ion.

The X-band ESR spectra of Cu(II) complex [Cu(L)_7(Cl)(H_2O)_2] (7a) (Fig. 6.15) has been recorded at a frequency of 9.1 GHz with a field set of 3000 G. The spin Hamiltonian parameters for the Cu(II) complex is used to derive the ground state. In octahedral geometry the g-tensor parameter with g ⊥ > g || > 2.0023, the unpaired electron lies in the d^2 orbital and g || > g ⊥ > 2.0023, the unpaired electron lies in the d_x^2-y^2 orbital in ground state [50]. In the present study the observed measurements for Cu(II) complex [Cu(L)_7(Cl)(H_2O)_2] (7a), g || (2.128) > g ⊥ (2.032) > 2.0027 indicating that the complex is axially symmetric and copper site has a d_x^2-y^2 ground state characteristic of octahedral geometry [51]. The g || value is an important function for indicating the metal-ligand bond character, for covalent character g || < 2.3 and for ionic g || > 2.3 respectively [52]. In the present Cu(II) complex the g || values were less than 2.3, indicating an appreciable covalent character for the metal–ligand bond. The geometric parameter (G), which is the measure of extent of exchange interaction and is calculated by using g-tensor values by the expression G = g || - 2 / g ⊥ - 2. According to Hathaway [53], if the G value is less than 4, the exchange interaction between the copper centers is noticed whereas if its value is greater than 4, the exchange interaction is negligible. The calculated G-value for the present Cu(II) complex is 4.028 (7a) indicate that the exchange coupling effects are not operative in the present complexes.
8. Thermal studies

The thermal stabilities were investigated for Cu(II), Co(II), Ni(II) and Zn(II) complexes of ligand H\textsubscript{L}\textsubscript{7} as a function of temperature. The proposed stepwise thermal degradation of the complexes with respect to temperature and the formation of respective metal oxides are depicted in Table 6.5.

TG curve of Cu(II) complex (7a) (Fig. 6.16) showed that the complex is stable up to 268 °C and no weight loss occurs before this temperature. The first stage of decomposition represents weight loss of two coordinated water molecules at 269 °C with practical weight loss of 7.13% (Cald. 6.59%). The resultant complex underwent second stage of degradation and gave break at 368 °C with a practical weight loss of 13.43% (Cald. 13.73%), which corresponds to the loss of two chlorine atoms. Further the complex underwent third stage of decomposition and gave a break at 395 °C with a weight loss of 56.92% (Cald. 56.65%) due to loss of one C\textsubscript{9}H\textsubscript{5}ON\textsubscript{2}S moiety, carbon dioxide molecule and a methyl group. Thereafter, the compound showed decomposition in a gradual manner rather than with the sharp decomposition up to 738 °C and onwards.
due to the loss of the remaining organic moiety. The weight of the residue corresponds to two moles of cupric oxide.

The thermogram of Co(II) complex (7b) (Fig. 6.17) showed the first stage of decomposition at 363 °C with practical weight loss of 31.53% (Cald. 31.35%), which corresponds to weight loss due to C\(_{11}\)H\(_7\)O\(_2\) moiety, carbon dioxide molecules and two chlorine atoms. Further the complex underwent decomposition and gave a break at 439 °C with a practical weight loss of 57.99% (Cald. 58.73%), corresponds to weight loss of two C\(_9\)H\(_5\)SON moieties. Thereafter the complex showed gradual decomposition up to 648 °C with a weight loss of remaining organic moiety, the weight of the residue corresponds to two moles of cobalt oxide.

In the thermogram of the Ni(II) complex (7c) (Fig. 6.18), the first stage of decomposition represents the weight loss due to two carbon dioxide molecules and two chlorine atoms at 370 °C with a practical weight loss of 17.18% (Cald. 17.94%). The resultant complex underwent further degradation and gave break at 442 °C with a practical weight loss of 56.43% (Cald. 56.45%), which corresponds to the decomposition of two C\(_9\)H\(_5\)N\(_2\)OS moieties and two methyl groups. Thereafter, the compound showed a gradual decomposition up to 738 °C with a weight loss of remaining organic moiety. The weight of the residue corresponds to two moles of nickel oxide.

In case of Zn(II) complex (7d) (Fig. 6.19) the first stage of decomposition occurs at 357 °C with practical weight loss of 13.43% (Cald. 13.29%), which represents the loss due to two carbon dioxide molecules and two methyl groups. Further the resultant complex underwent second stage of decomposition and gave a break at 397 °C with practical weight loss of 22.48% (Cald. 21.70%), due to loss of one C\(_8\)H\(_4\)SCl moiety. The third stage of decomposition occurred at 495 °C with practical weight loss of 50.96% (Cald. 50.96%), due to loss of one C\(_{10}\)H\(_6\)N\(_2\)SOCl, CONH\(_2\) moieties and C≡N group. Thereafter, the compound showed a gradual decomposition up to 736 °C with the weight loss of the remaining organic moiety. The weight of the residue corresponds to two moles of zinc oxide. The percentage metal content in all the complexes as done by elemental analysis agrees well with the thermal studies.
Table 6.5: Thermal data of the complexes of ligand HL$_7$.

<table>
<thead>
<tr>
<th>Complex No.</th>
<th>Decomposition temp ($^\circ$C)</th>
<th>% Weight loss</th>
<th>% Metal oxide</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>269</td>
<td>7.13</td>
<td>6.59</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>368</td>
<td>13.43</td>
<td>13.73</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>395</td>
<td>56.92</td>
<td>56.65</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Upto 738</td>
<td>-</td>
<td>-</td>
<td>13.28</td>
</tr>
<tr>
<td>7b</td>
<td>363</td>
<td>31.53</td>
<td>32.35</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>439</td>
<td>57.99</td>
<td>58.73</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Upto 648</td>
<td>-</td>
<td>-</td>
<td>8.38</td>
</tr>
<tr>
<td>7c</td>
<td>370</td>
<td>17.18</td>
<td>17.94</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>442</td>
<td>56.43</td>
<td>56.45</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Upto 738</td>
<td>-</td>
<td>-</td>
<td>8.31</td>
</tr>
<tr>
<td>7d</td>
<td>357</td>
<td>13.43</td>
<td>13.29</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>397</td>
<td>22.48</td>
<td>21.70</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>495</td>
<td>50.96</td>
<td>50.96</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Upto 736</td>
<td>-</td>
<td>-</td>
<td>8.96</td>
</tr>
</tbody>
</table>
Fig. 6.16: TG curve of Cu(II) complex (7a) of ligand HL₇

Fig. 6.17: TG curve of Co(II) complex (7b) of ligand HL₇
Fig. 6.18: TG curve of Ni(II) complex (7c) of ligand HL\textsubscript{7}

Fig. 6.19: TG curve of Zn(II) complex (7d) of ligand HL\textsubscript{7}
9. Electrochemistry

Electrochemical methods offer the potential means to determine the number of electrons involved in the redox process. The electrode potential at which the complex undergoes oxidation or reduction can be rapidly located by cyclic voltammetry. The redox behavior of all the complexes were investigated in DMF (10^{-3} M) solution containing 0.05 M tetrabutylammonium perchlorate as a supporting electrolyte in an oxygen free condition using a glassy carbon working electrode by cyclic voltammetry, which is the most versatile electro analytical technique for the study of electroactive species. Only the Cu(II) complex exhibited redox properties.

The voltammogram of Cu(II) complex (7a) is shown in Fig. 6.20. The cyclic voltammogram of Cu(II) complex (7a) in DMF at a scan rate of 0.1 Vs^{-1} showed well defined redox process corresponding to the formation of Cu(II) / Cu(I) couple at (reduction peak) $E_{pc} = -0.018$ V and (oxidation peak) $E_{pa} = 0.066$ V versus Ag /AgCl. The peak separation of this couple was found to be quasi-reversible with $\Delta E_p = 0.066$ V and the ratio of anodic to cathodic peak height was less than one. The difference between forward and backward peak potential can provide a rough evaluation of the degree of the reversibility of one electron transfer reaction.

Thus, the analysis of cyclic voltammetric response to 0.1 Vs^{-1}, 0.15 Vs^{-1} and 0.2 Vs^{-1} scan rates gives the evidence for quasi-reversible one electron redox process. The ratio of anodic to cathodic peak height was less than one and peak current increases with the increase of square root of the scan rates, establishing diffusion controlled electrode process [54]. From the peak separation value $\Delta E_p$ and peak potential which increase with higher scan rates, one can suggest that the electrode process are consistent with the quasi-reversibility of Cu(II) / Cu(I) couple [55].
6.4. BIOLOGICAL ACTIVITY

6.4.1. In vitro antimicrobial activity

The synthesized ligand $\text{HL}_7$ and its metal complexes have been screened for their antimicrobial activity. The antibacterial activity of the synthesized compounds were tested for their effect on $\textit{E. coli}$, $\textit{S. typhi}$, $\textit{B. subtilis}$ and $\textit{S. aureus}$ bacterial strains and antifungal activity on $\textit{C. albicans}$, $\textit{C. oxysporum}$ and $\textit{A. niger}$ fungal strains. The minimum inhibitory concentration (MIC) values of the compounds against the respective strains are summarized in Table 6.6. The antimicrobial screening results of all the synthesized compounds exhibited antimicrobial properties and it is important to note that the metal complexes exhibited more inhibitory effect compared to their parent ligand and their metal chlorides. The enhanced antimicrobial activity of the complexes over the ligand and their respective metal chlorides can be explained on the basis of chelation theory [56, 57]. It is known that chelation enhances the ligand to act as more powerful and potent bactericidal agents by inhibiting the bacterial growth, thus zone of inhibition of metal complexes were found to be higher compared to its free ligand. The enhancement in the activity may be rationalized on the basis that ligands mainly possess azomethine ($\text{C = N}$) bond. More over in metal complex, the positive charge of the metal ion is partially shared with the hetero donor atoms (nitrogen and oxygen) present in the ligand, and there may be $\pi$ -electron delocalization over the whole chelating system [58,
Hence the increase in the lipophilic character of the metal chelates favors its permeation through the lipoid layer of the bacterial membranes and blocking of the metal binding sites in the enzymes of microorganisms. The other factor such as conductivity, solubility and bond length between the ligand and the metal ions also increases the activity.

**Table 6.6: Minimum inhibitory concentration (MIC µg mL\(^{-1}\)) of ligand **\(\text{HL}_7\)** and its metal complexes.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bacteria</th>
<th></th>
<th></th>
<th></th>
<th>Fungi</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
<td>S. typhi</td>
<td>E. coli</td>
<td>S. aureus</td>
<td>A. niger</td>
<td>C. albicans</td>
<td>C. oxysporum</td>
<td></td>
</tr>
<tr>
<td>**HL}_7)</td>
<td>75</td>
<td>100</td>
<td>75</td>
<td>100</td>
<td>75</td>
<td>100</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>25</td>
<td>75</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>7b</td>
<td>25</td>
<td>75</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<td>50</td>
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</tr>
<tr>
<td>7c</td>
<td>25</td>
<td>75</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>75</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>7d</td>
<td>50</td>
<td>75</td>
<td>50</td>
<td>75</td>
<td>50</td>
<td>75</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>CuCl(_2).2H(_2)O</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>CoCl(_2).6H(_2)O</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>NiCl(_2).6HO</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>ZnCl(_2)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>Gentamycin</td>
<td>12.50</td>
<td>12.50</td>
<td>12.50</td>
<td>12.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.50</td>
<td>12.50</td>
<td>12.50</td>
<td></td>
</tr>
</tbody>
</table>

**6.4.2. DNA cleavage activity**

The ligand **\(\text{HL}_7\)** and its Cu(II), Co(II), Ni(II) and Zn(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method against Calf-thymus DNA (Cat. No- 105850) as a target molecule and the gel picture showing cleavage is depicted in Fig. 6.21.

DNA-cleavage studies are used to construct new and rational design of more efficient drugs that are targeted to DNA [60]. Treatment of complexes on DNA revealed that all the complexes have acted on DNA as there was molecular weight difference between the treated DNA samples and the control. The difference was observed in bands of lanes compared to the control Calf-thymus DNA. The results indicate the important role of nitrogen and oxygen atoms to the metal ions in these isolated DNA cleavage reactions. On the basis of the cleavage of DNA observed in case of ligand **\(\text{HL}_7\)** and its Cu(II), Co(II), Ni(II) and Zn(II) complexes, it can be concluded that all the compounds
under present study inhibited the growth of pathogenic organism by DNA cleavage as has been observed on the DNA cleavage of Calf-thymus DNA.

![Figure 6.21](image)

**Fig. 6.21:** DNA cleavage of calf-thymus DNA. M, standard molecular weight marker; C, control. Lane 7, 7a, 7b, 7c and 7d were treated DNA of calf-thymus DNA genome with respective compounds.

### 6.4.3. Antioxidant assay (DPPH free radical scavenging activity)

The free radical scavenging activity of ligand **HL**$_7$ and its metal complexes was done by DPPH method. Antioxidant activity of the test compounds was examined by measuring radical scavenging effect of DPPH radicals. The results of the free radical scavenging activity of the compounds at different concentrations are shown in Fig. 6.22. It was observed that the free radical scavenging activity of these compounds was concentration dependent. Among the examined compounds, ligand **HL**$_7$ and its Cu(II) and Co(II) complexes have exhibited good scavenging activity, whereas Ni(II) and Zn(II) complexes have showed moderate activity. The antioxidant activity of the ligand **HL**$_7$ was comparatively high when compared to all the metal complexes due the presence of phenolic OH group and the marked antioxidant activity of metal complexes is due to the coordination of metal with azomethine nitrogen, carbonyl oxygen of amide function attached to the 2-position of benzol[b]thiophene and phenolic oxygen of coumarin moiety via deprotonation. In case of the ligand **HL**$_7$ hydrogen of OH group donates a proton to the DPPH and converts itself into the stable free radical and in metal complexes the
hydrogen of azomethine is more acidic hence, hydrogen of azomethine could be easily donated to the DPPH free radical and convert itself into the stable free radical. Moreover, the acidic nature of hydrogen atom attached to azomethine nitrogen increases on complexation with metal ions. Hence that hydrogen atom becomes more liable.

![Graph showing antioxidant activity results of ligand HL and its complexes.](image)

**Fig. 6.22: Antioxidant activity results of ligand HL and its complexes.**

### 6.5. Conclusion

Based on the spectroscopic studies, the coordinating ability of the ligand has been proved in complexation reaction with Cu(II), Co(II), Ni(II) and Zn(II) ions. The newly synthesized ligand HL acts as an ONO donor tridentate chelate around the metallic ion. Metal ion is coordinated through oxygen atom of carbonyl function, azomethine nitrogen and phenolic oxygen atom via deprotonation. Cu(II), Co(II), Ni(II) and Zn(II) complexes possess octahedral geometries. Cu(II) complex (7a) of ligand HL exhibits one electron transfer quasi-reversible redox activity in the applied potential range. The antimicrobial activity results showed that all the complexes have exhibited higher activity when compared to its ligand HL. Ligand HL showed efficient antioxidant activity compared to their complexes. The electrophoretic studies indicated that Cu(II) and Co(II) complexes have good efficiency towards DNA cleavage. Based on the analytical data and spectral studies, proposed structure of all the complexes are depicted in Fig. 6.23.
Fig. 6.23: Proposed structure of the complexes.
REFERENCES
