Antifungal studies of 1,7-bis(2,5-dimethyl phenyl) hepta-1,6-diene-3,5-dione (L₂) and its Zn(II) & Vanadyl complexes

For all the tested compounds they show maximum antifungal activity at a higher concentration of 500μg/ml. It is observed that antifungal nature increases with the concentration of the compounds. The ligand 1a exhibited a zone of inhibition of 10mm against Penicillium where as its activity against Alternaria and Aspergillus is comparable ie zone of inhibition produced is 8.5 and 8 mm respectively at higher conc. The ligand 1b exhibited more antifungal activity against Alternaria with a zone of inhibition of 15mm. Comparing the ligands, 1b had shown more antifungal activity towards all fungi species than 1a. Comparing Zn(II) and VO(IV) complexes of both ligands, it was observed that vanadyl complexes exhibited more antifungal activity. The Zn(II) complexes gave inhibitory activity against fungal cultures which was only slightly greater than the ligands. But the VO(IV) complexes of both ligands especially of 1b had appreciable antifungal activity against all fungal cultures. It produced a maximum zone of inhibition of 19mm against Penicillium which is comparable with the zone of inhibition produced by the std.drug (21mm).
CHAPTER-II

SYNTHESIS, CHARACTERISATION AND BIOCHEMICAL ACTIVITIES OF 1,7-DI(THIOPHEN-2-YL)HEPTA-1,6-DIENE-3,5-DIONE AND 1,7-BIS(3-METHYL THIOPHEN-2-YL)HEPTA-1,6-DIENE-3,5-DIONE AND THEIR TRANSITION METAL CHELATES WITH Cu (II), Zn(II), Ni(II) & OXOVANADIUM(IV)
SECTION-1

SYNTHESIS AND CHARACTERISATION OF 1, 7-DI (THIOPHEN-2-YL)HEPTA-1,6-DIENE-3,5-DIONE AND 1,7-BIS (3-METHYL THIOPHEN 2-YL) HEPTA-1,6-DIENE-3,5-DIONE

2.1.1 Synthesis of 1,7-dithiophenyl heptanoids

This chapter deals with the synthesis and characterization of two curcuminoid analogues with heterocyclic ring (thiophenyl ring). The compounds synthesized are 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b). They were prepared by the condensation of heterocyclic aldehydes (thiophene-2-carboxaldehyde and 3-methyl thiophene-2-carboxaldehyde) with acetylacetone-boric oxide complex in ethyl acetate medium in presence of tributyl borate and n-butyl amine. The reaction usually leads to α,β-unsaturated 1,3-diketones with heterocyclic rings attached to it. Studies on ligands with a heteroaryl ring system instead of a phenyl ring in the unsaturated diketone moiety has not been much. The products 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) were purified by column chromatography over silica gel (60 – 120 mesh) using 4:1 (v/v) chloroform:acetone mixture as the eluent and recrystallised twice from hot benzene to get pure crystalline material. The product formation can be represented in a schematic way (Scheme 2.1.1).
The compounds 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) are black in colour, crystalline in nature and obtained in good yield nearly 75%. They show sharp melting point and are soluble in organic solvents like ethylacetate, acetone, ethanol, chloroform etc. The aldehydes used for synthesis, structures of the ligands prepared, its systematic name and yield are given in Table 2.1.1.

Table 2.1.1  Synthetic details of ligands

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aldehyde used for Synthesis</th>
<th>Structure of Ligands</th>
<th>Systematic name</th>
<th>Yield/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>Thiophene - 2-aldehyde</td>
<td><img src="image" alt="Structure of 2a" /></td>
<td>1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione</td>
<td>75</td>
</tr>
<tr>
<td>2b</td>
<td>3-methyl-thiophene-2-aldehyde</td>
<td><img src="image" alt="Structure of 2b" /></td>
<td>1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione</td>
<td>70</td>
</tr>
</tbody>
</table>
The observed C, H percentage and molecular weight determination (Table 2.1.2) together with mass spectral data of the compounds clearly suggest the formation of bis-condensation product in which two equivalent of aldehyde condensed with one equivalent of acetyl acetone as shown in Scheme 2.1.1.

Table 2.1.2 Analytical & UV spectral data of 1,7–dithiophenyl heptanoids

<table>
<thead>
<tr>
<th>Compounds</th>
<th>M.P.(°C)</th>
<th>Elemental analysis (%)</th>
<th>Molecular weight</th>
<th>UV λmax (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C Found/(Calculated) H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>114</td>
<td>61.63(62.5) 3.84(4.16)</td>
<td>287(288)</td>
<td>233, 331</td>
</tr>
<tr>
<td>2b</td>
<td>143</td>
<td>63.75(64.55) 4.47(5.06)</td>
<td>314(316)</td>
<td>247, 341</td>
</tr>
</tbody>
</table>

2.1.2. Characterisation of 1,7–dithiophenyl heptanoids

The 1,7–dithiophenyl heptanoids 2a & 2b synthesized were characterized by various spectral techniques like UV, IR, $^1$HNMR, $^{13}$C NMR and Mass spectral techniques. The spectral techniques used are discussed below.

UV spectra

The UV spectra of the compounds are characterized by the presence of two absorption maxima (Table 2.1.2) the low energy band and high energy band in the spectra corresponds to n→π* and π→π* transitions respectively which are present in 1,3- diketones. The π→π* transitions are generally intense while n→π* transitions are weak. The value at 230-290nm are due to π→π* transition and at 330-370nm are due to n→π* transitions. The ligand 1a showed two broad bands at 233 and 331nm respectively due to π→π* and n→π* transitions. The ligand 1b gave two bands at 247 and 341nm respectively due to π→π* and n→π* transitions and has shown a bathochromic shift.
The UV spectra of 2a is given in Fig.2.1.1.

**Fig.2.1.1**  UV spectrum of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a)

**IR spectra**

Importance of IR spectra in establishing the keto-enol tautomers of β-diketones has been well established. The spectra mainly gives idea about the nature of the carbonyl group present, whether free or in the hydrogen bonded form. Presence of α,β - unsaturation in the compound can be very well established using IR spectroscopy. The IR spectral datas of compounds 2a & 2b are given in (Table 2.1.3).
### Table 2.1.3  IR spectral data (cm$^{-1}$) of 1,7- dithiophenyl heptanoids

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Probable IR assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>2b</td>
</tr>
<tr>
<td>1641</td>
<td>1652</td>
</tr>
<tr>
<td>1585</td>
<td>1591</td>
</tr>
<tr>
<td>1546</td>
<td>1534</td>
</tr>
<tr>
<td>1517</td>
<td>1521</td>
</tr>
<tr>
<td>1411</td>
<td>1410</td>
</tr>
<tr>
<td>1145,1037</td>
<td>1179,1023</td>
</tr>
<tr>
<td>972</td>
<td>961</td>
</tr>
</tbody>
</table>

The values corresponding to $\nu$(C=O) are 1641 and 1652 cm$^{-1}$ for 2a & 2b respectively. Usually free carbonyl group gives stretching frequency at $\sim$ 1710 cm$^{-1}$. There is no peak in that region indicating that the C=O group is not in the keto form, instead it is in the enolic form due to peaks in the range $\sim$ 1650 cm$^{-1}$. The shift is a result of internal hydrogen bonding. Resonance also contributes to the lowering of carbonyl frequency in the enol form. Thus the observed position and intensity of these bands indicate that the compound exists in strong intra molecular hydrogen bonded enolic form. A weak, broad O-H stretch is observed for the enol form at 3200-2400 cm$^{-1}$. There are a number of medium intensity vibrations observed in the region 1550-1600 cm$^{-1}$ due to various $\nu$(C=C) vibrations of the thiophenyl group. Other IR peaks due to $\nu$(C-C) alkenyl, $\nu_{as}$(C-C-C) chelate ring, $\nu_s$(C-C-C) chelate ring & $\beta$ (C-H) chelate ring are present in the spectra. The IR spectra of these compounds are also characterized by the trans $\nu$(CH=CH) vibrations occurring at 972 & 961 cm$^{-1}$ respectively for 2a & 2b. The IR spectra of 2a & 2b are given below. IR spectrum of 2a is given in Fig.2.1.2 and 2b in Fig.2.1.3.
Fig. 2.1.2. IR spectrum of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a)

Fig. 2.1.3. IR spectrum of 1,7-di(3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione (2b)
**1H NMR spectra**

The different types of protons have characteristic values of chemical shifts in 1H NMR spectra. The numerical value in ppm of the chemical shift for a proton gives a clue regarding the type of proton originating the signal. The 1,7-dithiophenyl heptanoids show specific peaks corresponding to enolic, methine, alkenyl and thiophenyl protons (Table 2.1.4). Compounds 2a & 2b displayed a one proton singlet at ~ 16ppm assignable to strong intramolecularly hydrogen bonded enolic proton. Another one proton singlet at ~ 5.9 ppm corresponds to the strong intramolecularly hydrogen bonded methine proton. The spectral data suggests the structure given below.

![Enolic structure of 1,7-dithiophenyl heptanoids](image)

**Table 2.1.4 Characteristic 1H NMR spectral data of 1,7-dithiophenyl heptanoids**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Chemical shifts (δ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enolic</td>
</tr>
<tr>
<td>2a</td>
<td>16.1</td>
</tr>
<tr>
<td>2b</td>
<td>16.15</td>
</tr>
</tbody>
</table>

Other signals appearing in the 1H NMR spectra are that of the thiophenyl protons present in the range δ 7.0 – 7.3 ppm and alkenyl protons in the range of 6.7 – 7.9 ppm. The methyl group on the thiophenyl ring of compound 2b showed an additional peak at 2.4 ppm as expected. The 1H NMR spectra of 2b is given in Fig.2.1.4.
Fig. 2.1.4  $^1$H NMR spectrum of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione
\( ^{13} \text{C} \text{ NMR spectra} \)

The \( ^{13} \text{C} \) NMR spectral data of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) are given in Table 2.1.5 & 2.1.6.

**Structure representing different carbon atoms in 2a.**

![Structure representing different carbon atoms in 2a.](image)

**Table 2.1.5 \( ^{13} \text{C} \) NMR spectral data of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (chemical shift in ppm)**

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2,C2'</th>
<th>C3,C3'</th>
<th>C4,C4'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>106.80</td>
<td>190.99</td>
<td>140.26</td>
<td>129.32</td>
</tr>
<tr>
<td></td>
<td>143.2</td>
<td>133.67</td>
<td>131.73</td>
<td>127.45</td>
</tr>
</tbody>
</table>

In \( ^{13} \text{C} \) spectra, nearly every non-equivalent carbon atom in an organic molecule gives rise to a peak with a different chemical shift. It helps in the identification of chemically and magnetically distinct carbons. The peak corresponding to C1 (methine) is present at a position ~ at 107ppm in 2a & ~101ppm in 2b. Usually CH\(_2\) carbon which is flanked between two carbonyl groups appears at a position nearer to 55ppm. Instead, there is a possibility of keto-enol tautomerism which makes the methine to become an alkenyl carbon. Thus there is a downward shift in the peak of C1.
The C2 carbon of carbonyl appears at a position at~ 190ppm in 2a & ~183ppm in 2b. In $^{13}$C NMR, the carbons of carbonyl groups have the largest chemical shifts. The alkenyl carbon of both 2a & 2b are present at a position nearer to the thiophenyl ring system. They are seen at 140,138ppm(C3) & 129,126ppm (C4) of compounds 2a and 2b respectively. The thiophenyl carbon atoms are present between 124 – 143 ppm. The carbon (C5) which is attached to the alkenyl carbon atom is downfielded and present at a position 143ppm and 135ppm respectively. The $^{13}$C NMR spectrum of 2b is given in Fig.2.1.5. In the $^{13}$C NMR spectrum of 2b the carbon to which the methyl group is attached is shifted to 134.92ppm. Also in 2b the methyl group is present at a position ~ 20ppm.

Structure representing different carbon atoms in 2b.

Table 2.1.6 $^{13}$C NMR spectral data of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (chemical shift in ppm)

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C2,C2'</th>
<th>C3,C3'</th>
<th>C4,C4'</th>
<th>C5,C5'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>101.82</td>
<td>183.50</td>
<td>138.35</td>
<td>126.78</td>
<td>135.72</td>
</tr>
<tr>
<td>C6,C6'</td>
<td></td>
<td>C7,C7'</td>
<td>C8,C8'</td>
<td>C9,C9'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>134.92</td>
<td>130.8</td>
<td>124.85</td>
<td>20.98, 19.33</td>
<td></td>
</tr>
</tbody>
</table>
Mass Spectra

In mass spectrum, ions are detected as a function of their m/z (mass to charge) ratio. The value of m/z at which the molecular ion appears on the mass spectrum helps to find the molecular weight of the compound. The molecular ion undergoes fragmentation producing a series of molecular fragments called fragment ions. These ions appear at m/z values corresponding to their individual masses. The mass spectrum of 2a & 2b are represented in Fig.2.1.6 &2.1.7 respectively. The mass spectrum of 2a shows a molecular ion (M+2) peak at 290 and a base peak (most intense peak) at 109 which is due to fragment F in Scheme 2.1.2. Removal of different groups from the molecular ion, according to the scheme, leads to smaller fragments which can be easily identified in the spectrum and are given in Table 2.1.7. Mass peaks due to the elimination of small groups like S, O, OH, CO, C₂H₂, CH₂=CH=CH=O, CH₂=CH, C₂H₂O etc. are also present in the spectra. In the mass spectrum of 2a several small fragments are observed apart from the fragmental pattern described in Scheme 2.1.2. A strong peak is observed at 246 due to the removal of a sulphur and CH group. Removal of additional CH groups leads to the peaks at 229, 217 and 206(G).

| Fragment | Ligand | Mass pattern | M+/(M+1)/(M+2) ion | A | B | C | D | E | F | G | H |
|----------|--------|--------------|---------------------|---|---|---|---|---|---|---|---|---|
| 2a       | 290    | 152          | 137                 | 99 | 109| 83 | 109| 206| 184 |
| 2b       | 317    | 166          | 151                 | 111| 123| 103| 122| 218| 193 |

*The alphabets corresponds to the fragments given in Scheme 2.1.2
Fig. 2.1.5 $^{13}$C NMR spectrum of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione
The mass spectrum for 2b shows a much less intense molecular ion (M+1) peak at 317. The fragment peak at 151 due to B(Ar-\text{CH}=\text{CH}-\text{C}=\text{O})^+ is the most intense in the mass spectrum. The next intense peak is observed at m/z 111 which is due to fragment C(Ar-CH^+). All other peaks in the spectrum can be explained from the fragmentation pattern given in scheme. Removal of 3-methyl thiophenyl group, CH₂, C₂H₂O, C₂H₂, CH₂=C=O, CH=C=O etc. give rise to different fragment ions. A strong peak is observed at 275 which is due to the removal of one S and CH group. Again removal of the second S and CH group leads to the peak at 233. The mass spectrum of 2b is given in Fig. 2.1.7.

![Mass spectrum of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione](image)
Thermogravimetric analysis

Thermogravimetric studies of 1,7-diheteroaryl heptanoids (Table 2.1.8) give some important results regarding the structure of molecules. The thermogram of 2a shows a one-stage decomposition pattern as shown in Fig.2.1.8. The compound is stable up to a temperature of 210°C and then decomposition begins slowly with a sharp drop in the mass upto a
temperature of 427°C. The peak temperature in DTG is found at 347.5°C. There appears a shoulder in the DTG curve at a temperature of 266°C. The sample was heated up to a temperature of 740°C and the remaining portion was found to be an aryl group.

Table 2.1.8  Thermogravimetric studies of 1,7-diheteroaryl heptanoids

<table>
<thead>
<tr>
<th>Compound (mol. mass)</th>
<th>Temp. range in TG (°C)</th>
<th>Peak Temp. (°C)</th>
<th>Mass loss %</th>
<th>Pyrolysis %</th>
<th>Final product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TG</td>
<td>Theoretical</td>
<td></td>
</tr>
<tr>
<td>2a (288)</td>
<td>210 - 427</td>
<td>347.5</td>
<td>59.39</td>
<td>60.63</td>
<td>70.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thiophenyl</td>
</tr>
<tr>
<td>2b (316)</td>
<td>236 - 469</td>
<td>365</td>
<td>56.35</td>
<td>57.13</td>
<td>73.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3-methyl Thiophenyl</td>
</tr>
</tbody>
</table>

Fig. 2.1.8  Thermogravimetric studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione
Fig. 2.1.9  Thermogram of 1,7-di(3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione
SECTION-II

SYNTHESIS AND CHARACTERISATION OF TRANSITION METAL CHELATES OF 1, 7-DITHIOPHENYL HEPTANOIDS & METHYL SUBSTITUTED DERIVATIVE

2.2.1 Synthesis of metal complexes of 1,7-di(thiophen-2-yl)hepta-1,6 diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6 diene-3,5-dione (2b)

Copper(II), Zinc(II), Nickel(II) and Oxovanadium(IV) complexes of 1,7-di(thiophen-2-yl)hepta-1,6 diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6 diene-3,5-dione (2b) were synthesized by the following general method.

To a refluxing solution of the diketone (0.002 mol) in methanol (25 ml), a methanolic solution of metal salt (0.001 mol) was added and the reaction mixture was refluxed for nearly 2 hrs and cooled to room temperature. The precipitated complex was filtered, washed with 1:1 methanol:water mixture and recrystallised from hot methanol.

Preparation of Cu(II) complex of the ligands

The Cu(II) complexes were prepared by adding a methanolic solution of copper(II) acetate (25 ml, 0.001 mol) to a solution of 2a & 2b (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1 methanol:water mixture and recrystallised from hot methanol.

Preparation of Zn(II) complex of the ligands

The Zn(II) complexes were prepared by adding a methanolic solution of zinc acetate (25 ml, 0.001 mol) to a solution of 2a & 2b (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature.
Precipitated complex was filtered, washed with 1:1 methanol:water mixture and recrystallised from hot methanol.

**Preparation of Ni(II) complex of the ligands**

The Ni(II) complexes were prepared by adding a methanolic solution of nickel(II) acetate (25 ml, 0.001 mol) to a solution of 2a & 2b (25 ml, 0.002 mol) in methanol and repeating the above procedure.

**Preparation of Oxovanadium(IV) complex of the ligands**

The VO(IV) complexes were prepared by adding a methanolic solution of vanadium (IV) oxide sulphate (25 ml, 0.001 mol) to a solution of 2a & 2b (25 ml, 0.002 mol) in methanol and refluxed gently for 2 h. After reducing the volume to half, the solution was cooled to room temperature. The precipitated complex was filtered, washed with 1:1 methanol:water mixture and recrystallised from hot methanol.

The reaction involved in the formation of complexes is represented below in Scheme 2.2.1.
2.2.2 Characterisation of metal complexes of methyl substituted 1,7-dithiophenyl heptanoids

Transition metal chelates (Cu, Zn, Ni, Vanadyl) of ligands 2a & 2b were characterized using physical, analytical and spectral data. The spectral techniques used in characterization include UV, IR, NMR and Mass spectral analysis. Elemental analysis (C, H and metal percentages), physical data and UV, IR spectral data of metal complexes of 2a are given in Table 2.2.1 and metal complexes of 2b are given in Table 2.2.2 respectively. The data given below suggest a ML₂ stochiometry for all complexes prepared.

<table>
<thead>
<tr>
<th>Metal chelate</th>
<th>M.P. (°C)</th>
<th>Elemental analysis (%)</th>
<th>UV λmax (nm)</th>
<th>Characteristic IR stretching bands (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Found/calculated</td>
<td></td>
<td>(C=O) (C-C) (M-O)</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>139</td>
<td>56.47 (57.25)</td>
<td>9.99 (10.01)</td>
<td>234, 336</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.45 (4.00)</td>
<td></td>
<td>1614, 1531</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>141</td>
<td>56.89 (58.01)</td>
<td>9.27 (10.90)</td>
<td>233, 337</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.47 (4.20)</td>
<td></td>
<td>1583, 1520</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>150</td>
<td>56.30 (56.91)</td>
<td>11.22 (11.90)</td>
<td>235, 337</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.44 (3.61)</td>
<td></td>
<td>1616, 1532</td>
</tr>
<tr>
<td>VO(IV)</td>
<td>173</td>
<td>56.16 (57.30)</td>
<td>7.94 (8.05)</td>
<td>236, 334</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.43 (3.52)</td>
<td></td>
<td>1592, 1528</td>
</tr>
</tbody>
</table>
Table 2.2.2 Analytical and UV, IR spectral data of metal complexes of 2b

<table>
<thead>
<tr>
<th>Metal chelates</th>
<th>M.P. (°C)</th>
<th>Elemental analysis (%)</th>
<th>UV λmax nm</th>
<th>Characteristic IR stretching bands (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Found/(calculated)</td>
<td></td>
<td>(C=O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
<td>Metal</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>189</td>
<td>58.05 (58.83)</td>
<td>4.25 (4.32)</td>
<td>9.00 (9.15)</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>192</td>
<td>58.55 (59.24)</td>
<td>4.20 (4.35)</td>
<td>8.05 (8.52)</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>194</td>
<td>58.50 (58.67)</td>
<td>4.25 (4.31)</td>
<td>9.01 (9.40)</td>
</tr>
<tr>
<td>VO(IV)</td>
<td>196</td>
<td>57.75 (58.54)</td>
<td>4.26 (4.31)</td>
<td>7.03 (7.30)</td>
</tr>
</tbody>
</table>

2.2.3. Characterization by various spectral techniques

UV spectra

The spectra of metal complexes also show two UV transitions, the $\pi \rightarrow \pi^*$ transition & $n \rightarrow \pi^*$ transition. The UV absorption bands of the ligands were almost unaffected by complexation with metal ions. The spectra of complexes closely resembles the spectra of respective ligands. So there is no much change in the structure due to complex formation. The $n \rightarrow \pi^*$ transition of the dicarbonyl chromophore of the free ligand showed a slight red shift indicating involvement of the dicarbonyl moiety in chelate formation. For comparison, the UV spectra of ligand 2a and its Zn(II) complex are shown in Fig.2.2.1
Fig. 2.2.1 UV spectra of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and its Zn(II) complex

IR spectra

Instead of the intramolecularly hydrogen bonded carbonyl band at 1641 cm\(^{-1}\) for ligand 2a and the band at 1652 cm\(^{-1}\) for ligand 2b, a strong band assignable to the stretching of the coordinated carbonyl moiety appears in the region 1585 - 1620 cm\(^{-1}\) in the spectra of all metal complexes. Involvement of the carbonyl group in coordination is further supported by the observation of two medium intensity bands in the region of 400 – 490 cm\(^{-1}\) due to \(\nu_{M-O}\) vibrations (Metal-Oxygen). The replacement of enolic proton by a metal ion is also evident from the absence of the broad band in the region of 2600 - 3500 cm\(^{-1}\) present in the ligand. All these support the formation of metal complexes. There is no change in the nature of alkenyl carbon due to metal complexation. The IR spectra of Cu(II) complex of 2a is depicted in Fig. 2.2.2 and the IR spectra of VO(IV) complex of 2b is depicted in Fig. 2.2.3.
Fig.2.2.2  IR spectra of Cu(II) complex of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a)

Fig.2.2.3  IR spectra of VO(IV) complex of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (2b)
$^1$H NMR spectra

A characteristic feature of $^1$H NMR spectra of metal complexes is the absence of singlet signal at $\delta \sim 16$ ppm which suggests the replacement of enolic proton in the ligand by metal atom in metal complexes. The phenyl and alkenyl protons are not altered much since they are not involved in metal complexation. However, the observed downfield shift of the methine proton signals is consistent with decreased electron density around the central atom of the pseudo aromatic metal chelate ring. Thus the spectra of ligand and complexes are much similar except those of enolic proton. The $^1$H NMR spectra of Zn(II) complex of ligand 2b is given in Fig 2.2.4.
Mass spectra

In their mass spectra, all the complexes showed relatively intense peaks at m/z corresponding to ML₂ stoichiometry, where M is metal and L is ligand. Mass spectral fragments are another important tool in elucidating the structure of metal complexes.

In all the cases, [ML₂]+ ion peak, the molecular ion peak is found. The mass spectral analysis shows that stepwise removal of aryl groups is a characteristic feature of all the complexes. Smaller molecules like O, OH, CH etc. are also eliminated. Peaks due to [ML₂]+, L+(fragment F in Table) and fragments of L+ are also detected in the spectrum. It was found that some fragments rearrange to form stable cyclic species as shown in the Scheme. The fragmental patterns of the metal chelates 2a & 2b can be identified from Scheme 2.2.2, which is given below. The fragment F given below in the table corresponds to that of the ligand peak. Mass spectrum of VO(IV) complex of 2b is given in Fig.2.2.5.

<table>
<thead>
<tr>
<th>Fragments</th>
<th>Metal chelates</th>
<th>M+/ (M+1)/ (M+2) ion</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Pattern</td>
<td>Cu(II)</td>
<td>637</td>
<td>471</td>
<td>350</td>
<td>121</td>
<td>303</td>
<td>184</td>
<td>289</td>
</tr>
<tr>
<td></td>
<td>Zn(II)</td>
<td>639</td>
<td>473</td>
<td>352</td>
<td>121</td>
<td>305</td>
<td>184</td>
<td>289</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>633</td>
<td>467</td>
<td>346</td>
<td>121</td>
<td>301</td>
<td>180</td>
<td>289</td>
</tr>
<tr>
<td></td>
<td>VO(IV)</td>
<td>641</td>
<td>475</td>
<td>354</td>
<td>121</td>
<td>309</td>
<td>188</td>
<td>289</td>
</tr>
</tbody>
</table>

*The alphabets correspond to the fragments given in Scheme 2.2.2
<table>
<thead>
<tr>
<th>Fragments</th>
<th>Metal chelates</th>
<th>M+/ (M+1)/ (M+2) Ion</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Pattern</td>
<td>Cu(II)</td>
<td>694</td>
<td>500</td>
<td>379</td>
<td>121</td>
<td>306</td>
<td>184</td>
<td>317</td>
</tr>
<tr>
<td></td>
<td>Zn(II)</td>
<td>696</td>
<td>501</td>
<td>381</td>
<td>121</td>
<td>308</td>
<td>187</td>
<td>317</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>689</td>
<td>495</td>
<td>374</td>
<td>121</td>
<td>301</td>
<td>180</td>
<td>317</td>
</tr>
<tr>
<td></td>
<td>VO(IV)</td>
<td>697</td>
<td>503</td>
<td>382</td>
<td>121</td>
<td>309</td>
<td>188</td>
<td>317</td>
</tr>
</tbody>
</table>

*The alphabets corresponds to the fragments given in Scheme 2.2.2*
Fig 2.2.5 Mass spectrum of VO(IV) complex of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5 dione
In the mass spectrum of VO(IV) complex of 1,7-bis (3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione a less intense M+2 peak is observed at 699. The peak at 646 is due to the removal of Vanadium from the molecular ion. The intense peak at 613 is due to the removal of Vanadyl and one oxygen from molecular ion. The peak at 503 is due to the fragment ion formed by the removal of 2 Ar groups from molecular ion (Ar=3-methylthiophenyl). The peak due to the ligand is observed at 317. The peaks at 275 and 233 are due to fragments of ligand and are observed in the spectrum of ligand. The peak at 275 is due to the removal of one S and CH group from ligand and the base peak at 233 is due to the removal of the second S and CH group.
SECTION-III

CYTOTOXIC AND ANTITUMOUR STUDIES OF 1,7–DITHIOPHENYL HEPTANOIDS AND THEIR TRANSITION METAL CHELATES

This section deals with the cytotoxic and antitumour activities of 1,7-diheteroaryl heptanoids namely 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) and their metal chelates Cu(II),Zn(II),Ni(II) &VO(IV). In vitro cytotoxic activity against DLA and EAC cell lines were studied. The invivo antitumour activity was determined by using DLA cell line induced solid tumour and EAC cell line induced ascites tumour model in mice and its comparison with a std. anticancer drug cyclophosphamide.

2.3.1. In vitro cytotoxic activity:

Short term cytotoxic activity of compounds 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) and their metal chelates Cu(II),Zn(II),Ni(II) &VO(IV) were assayed by determining the percentage viability of DLA and EAC cells using Trypan blue dye exclusion technique (Moldeus et al., 1978).

2.3.2. In vitro Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their metal complexes [Cu(II),Zn(II),Ni(II) &VO(IV)] towards EAC cells

The curcuminoid analogue 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their metal complexes were used for in vitro cytotoxicity study towards EAC cells. All the test compounds were prepared in different concentrations namely 10, 20, 50, 100, 200 μg/ml. The cytotoxic nature of the compounds were determined in terms of % cell death produced by them. The results of the study is given in Table 2.3.1 and represented diagrammatically in Fig.2.3.1.
**Table 2.3.1.** *In vitro* Cytotoxic studies of 1,7-di(thiophen-2-yI)hepta-1,6-diene-3,5-dione (HL₁) and their metal complexes towards EAC

<table>
<thead>
<tr>
<th>Drug Con. μg/ml</th>
<th>% Cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL₁</td>
<td>Cu(L₁)₂</td>
</tr>
<tr>
<td>200</td>
<td>40</td>
</tr>
<tr>
<td>100</td>
<td>28</td>
</tr>
<tr>
<td>50</td>
<td>14</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

**Fig. 2.3.1.** *In vitro* Cytotoxic studies of 1,7-di(thiophen-2-yI)hepta-1,6-diene-3,5-dione (HL₁) and their metal complexes towards EAC

The ligand 2a produced 40% cell death towards EAC cell lines at a concentration of 200μg/ml. At lower concentration the activity of the compound is negligible. All the metal
complexes produced greater % of cell death. They were quite active even at lower concentrations. As concentration increases the % of cell death increases. All the metal complexes showed marked cytotoxic activity. The % cell death produced by Cu(II), Zn(II), Ni(II) and VO(IV) complexes at 200μg/ml are 92, 84, 75 & 82 % respectively. The Cu(II) complex of ligand was very effective in producing a cell death of 92% indicating its potent cytotoxic nature. The Zn(II) and VO(IV) complexes showed comparable cytotoxic activity nearly 80% which is twice that of the ligand. The Ni(II) complexes possessed minimum activity among complexes, but even then it could produce 75% cell death. The results indicate that metal chelation enhance cytotoxicity of compounds considerably.

2.3.3 In vitro Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their metal complexes [Cu(II), Zn(II), Ni(II) & VO(IV)] towards DLA cells

The % cell death were also calculated with DLA cell lines. The results of the study in terms of % cell death is represented in Table 2.3.2 and diagrammatically in Fig. 2.3.2.

Table 2.3.2 In vitro Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (HL₁) and their metal complexes towards DLA

<table>
<thead>
<tr>
<th>Drug Con. µg/ml</th>
<th>% Cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HL₁</td>
</tr>
<tr>
<td>200</td>
<td>38</td>
</tr>
<tr>
<td>100</td>
<td>23</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>
Fig. 2.3.2 *In vitro* Cytotoxic studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (HL1) and their metal complexes towards DLA

The results of the study indicate that the activity of compound 2a and metal complexes towards DLA cells follows a similar pattern as that with EAC cells. The ligand as well as the metal complexes showed a slight decrease in activity when compared with their activities towards EAC cells. But all the metal complexes showed enhanced activity when compared with the ligand. The compound 2a produced 38% cell death whereas its Cu(II) complex produced 89% cell death. The activity of metal complexes followed the order Cu(II)>Zn(II)>VO(IV)>Ni(II) and the % cell death produced by them were 89, 80, 79 and 73 respectively. The values show that all the metal complexes possess significant cytotoxic nature.
2.3.4. *In vitro* Cytotoxic studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(2b) and their metal complexes [Cu(II), Zn(II), Ni(II) & VO(IV)] towards EAC cells

*In vitro* Cytotoxic studies were done using 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(2b) and their metal complexes towards EAC cell lines. The observations are given in Table 2.3.3 and graphically in Fig. 2.3.3. The ligand 2b which has a methyl group on the thiophenyl ring as compared to ligand 2a gave lesser % of cell death with EAC cells. All metal complexes possessed more cytotoxic activity than ligands. But comparing with the metal complexes of 2a, the metal complexes of 2b produced lesser % of cell death. The % cell death produced by Cu(II) complex of 2b was 80% and it is less active than Cu(II) complex of 2a. Comparing the ligand and metal complexes, the complexes were twice more active than the ligand.

**Table 2.3.3. In vitro Cytotoxic studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-Dione (HL₂) and their metal complexes towards EAC**

<table>
<thead>
<tr>
<th>Drug Con.</th>
<th>% Cell death</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg/ml</td>
<td>HL₂</td>
</tr>
<tr>
<td>200</td>
<td>37</td>
</tr>
<tr>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>
2.3.3 *In vitro* Cytotoxic studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (HL$_2$) and their metal complexes towards EAC

The results of the study are given in Table 2.3.4 and represented graphically in Fig. 2.3.4. The ligand 2b and its metal complexes were not as effective as ligand 2a and its metal complexes in its activity against DLA cells. All the results show that little activity was found with 10µg/ml. Also it is found that the ligand and complexes show maximum activity towards EAC cells than DLA. Even though all the metals are divalent better results are observed for Cu(II).
Table 2.3.4. *In vitro* Cytotoxic studies of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (HL$_2$) and their metal complexes towards DLA

<table>
<thead>
<tr>
<th>Drug Concentration (µg/ml)</th>
<th>% Cell Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HL$_2$</td>
</tr>
<tr>
<td>200</td>
<td>34</td>
</tr>
<tr>
<td>100</td>
<td>21</td>
</tr>
<tr>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 2.3.4. *In vitro* Cytotoxic studies of 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (HL$_2$) and their metal complexes towards DLA
Moderate results of cytotoxic activity was found with 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) and their metal complexes towards DLA. Here, even though the values were doubled with metal chelation, comparable results are obtained with all the three metals except Cu.

**Conclusion**

A comparative study of the complexes of the ligands 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) shows that ligand 2a and its metal complexes gives good results than that of 2b both towards EAC & DLA. So, out of the two hetero ligands, 2a, the unsubstituted thiophene ligand and its complexes are more active than methyl substituted ones in the in vitro studies conducted.
**IN VIVO ANTITUMOUR STUDIES OF 1,7-DITHIOPHENYL HEPTANOIDS AND THEIR Cu(II) METAL COMPLEXES**

The effect of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione(2a) and 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(2b) and their Cu(II) complexes on the survival rate of ascites tumour bearing animals were studied. Swiss Albino mice (male, 6-8 weeks old) weighing 28-30g were divided into 14 groups of five animals each. Viable EAC cells in 0.1 ml of PBS were injected into the peritoneal cavity. **Group 1**, Control: Oral administration of 0.1 ml of distilled water/animal without drug treatment. **Group 2**, Standard: Cyclophosphamide 25mg/kg body weight. **Group 3-5**: Ligand, 1,7-di(thiophen-2-yl)-1,6-heptadiene-3,5-dione(2a) with concentrations 20μg/ml, 10μg/ml and 5μg/ml was given as drug. **Group 6-8**: Cu(II) complex of 2a as drug with concentrations 20μg/ml, 10μg/ml & 5μg/ml respectively. **Group 9-11**: Ligand 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione(2b) with concentrations 20μg/ml, 10μg/ml and 5μg/ml was given as drug. **Group 12-14**: Cu(II) complex of 2b as drug with concentrations 20μg/ml, 10μg/ml & 5μg/ml respectively.

**2.3.6 Effect of 1,7-di(thiophen-2-yl)-1,6-heptadiene-3,5-dione(2a) and the Cu(II) complex on ascites tumour**

All the test compounds were injected intraperitoneally and their effect in reducing ascites tumour development in mice were studied. The no. of days survived by the control group, the animals given standard drug, and the animals treated with test compounds and their % increase in life span is found and the results are presented in Table 2.3.5.
Table 2.3.5 Effect of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and its Cu(II) complex on ascites tumour reduction

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Concentration (μg/ml)</th>
<th>No. of animals with tumour</th>
<th>No. of days Survived</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td></td>
<td>5/5</td>
<td>17.3±1.10</td>
<td></td>
</tr>
<tr>
<td>2. Standard drug</td>
<td></td>
<td>5/5</td>
<td>30.6±0.489</td>
<td>76.87</td>
</tr>
<tr>
<td>3. L1</td>
<td>20</td>
<td>5/5</td>
<td>26.8±2.9</td>
<td>54.9</td>
</tr>
<tr>
<td>4. L1</td>
<td>10</td>
<td>5/5</td>
<td>26.4±3.6</td>
<td>52.21</td>
</tr>
<tr>
<td>5. L1</td>
<td>5</td>
<td>5/5</td>
<td>24.4±3.26</td>
<td>41.04</td>
</tr>
<tr>
<td>6. Cu(L1)2</td>
<td>20</td>
<td>5/5</td>
<td>30.2±1.04</td>
<td>74.61</td>
</tr>
<tr>
<td>7. Cu(L1)2</td>
<td>10</td>
<td>5/5</td>
<td>30.1±1.16</td>
<td>73.98</td>
</tr>
<tr>
<td>8. Cu(L1)2</td>
<td>5</td>
<td>5/5</td>
<td>28.8±1.78</td>
<td>66.50</td>
</tr>
</tbody>
</table>

The treatment with test compounds namely 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and its Cu(II) complex in different concentrations namely 5,10,20μg/ml increased the average life span of tumour bearing animals. The no. of days survived by control group is 17.3±1.1 where as for standard drug cyclophosphamide it is 30.6±0.489. At 5,10,20μg/ml concentrations, the ligand 2a increased the survival rate of animals by 24.4±3.26, 26.4±3.6, 26.8±2.9 days respectively. The ligand produced 54% ILS at a concentration of 20μg/ml. But the Cu(II) complex could produce an increase in life span of 74%, 73% and 66% at 5,10,20μg/ml concentrations respectively. The Cu(II) complex has significantly increased the life span of ascites tumour bearing animals. The % ILS due to Cu(II) complex is comparable to the results obtained with standard drug.
Fig. 2.3.5 The % ILS with different conc. of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and its Cu(II) complex

2.3.7 Effect of 1,7-bis(3-methyl thiophen-2-yl)-1,6-heptadiene-3,5-dione(2b) and the Cu(II) complex on ascites tumour

All the test compounds were injected intraperitoneally and their effect in reducing ascites tumour development in mice were studied. The no. of days survived by the control group, the animals given standard drug, and the animals treated with test compounds and their % increase in life span is found and the results are presented in Table 2.3.6 and in Fig. 2.3.6.
Table 2.3.6 Effect of 1,7- bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (L2) and its Cu(II) complex on ascites tumour reduction

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Concentration μg/ml</th>
<th>No.of animals with tumour</th>
<th>No. of days Survived</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td></td>
<td>5/5</td>
<td>17.3±1.10</td>
<td></td>
</tr>
<tr>
<td>2. Standard drug</td>
<td></td>
<td>5/5</td>
<td>30.6±0.489</td>
<td>76.87</td>
</tr>
<tr>
<td>3. L₂</td>
<td>20</td>
<td>5/5</td>
<td>25.0±3.7</td>
<td>44.17</td>
</tr>
<tr>
<td>4. L₂</td>
<td>10</td>
<td>5/5</td>
<td>24.4±3.26</td>
<td>41</td>
</tr>
<tr>
<td>5. L₂</td>
<td>5</td>
<td>5/5</td>
<td>21.0±2.09</td>
<td>21.38</td>
</tr>
<tr>
<td>6. Cu(L₂)₂</td>
<td>20</td>
<td>5/5</td>
<td>29.2±1.04</td>
<td>68.81</td>
</tr>
<tr>
<td>7. Cu(L₂)₂</td>
<td>10</td>
<td>5/5</td>
<td>28.8±1.16</td>
<td>66.58</td>
</tr>
<tr>
<td>8. Cu(L₂)₂</td>
<td>5</td>
<td>5/5</td>
<td>27.0±1.78</td>
<td>56.06</td>
</tr>
</tbody>
</table>

The mice with EAC induced ascites tumour survived for a period of 17.3±1.10 days. The administration of standard drug cyclophosphamide increased the life span to 30.6±0.489 days. The %ILS produced by the ligand at 5,10,20 μg/ml are 21,41 and 44% respectively where as for Cu(II) complex at the same concentrations the %ILS are 56, 66, 68% respectively. All the compounds exhibited greater activity at higher concentrations. The Cu(II) complex was quite active even at lower concentration. The activity of the complex is comparable with the std.drug.

Comparing the ligands 2a and 2b and their Cu(II) complexes, the ligand 1,7- bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (L2) and its complex was not as effective as 2a and its complex in increasing the life span of animals. The Cu(II) complex of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) is found to be the most active compound in the in vivo cytotoxic study conducted and is very effective in increasing the life span of EAC induced ascites tumour bearing mice.
INVIVO CYTOTOXIC STUDY ON SOLID TUMOUR DEVELOPMENT

2.3.8 Effect of compounds on solid tumour development

DLA cells were injected subcutaneously on the right hind limb of mice to produce solid tumour. Swiss Albino mice were divided into six groups. Group 1: control (treated with DLA cells), Group 2: cyclophosphamide 10mg/kg b.wt. (reference drug) + DLA cells, Group 3: ligand 2a + DLA cells, Group 4: Cu(II) complex of 2a + DLA cells, Group 5: ligand 2b + DLA cells, Group 6: Cu(II) complex of 2b + DLA cells.

The ligands 2a and 2b and their copper complexes were used to find the effect on solid tumour development in mice. At 24 h after tumour inoculation, the test compounds (200μmol/Kg body weight) were injected for 10 consecutive days. The diameter of the tumour was measured using vernier calipers every third day for 1 month and tumor volume was calculated.

Table 2.3.7 Effect of Compounds on solid tumour

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Tumour volume on 31st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5.042 cm³</td>
</tr>
<tr>
<td>2a (L₁)</td>
<td>3.08 cm³</td>
</tr>
<tr>
<td>2b (L₂)</td>
<td>3.98 cm³</td>
</tr>
<tr>
<td>Cu (L₁)₂</td>
<td>2.25 cm³</td>
</tr>
<tr>
<td>Cu (L₂)₂</td>
<td>3.05 cm³</td>
</tr>
<tr>
<td>Std.group</td>
<td>1.982 cm³</td>
</tr>
</tbody>
</table>
All the compounds produced a significant reduction of solid tumour volume in mice. Compared to ligands, their respective Cu(II) chelates were more effective in bringing about reduction in solid tumour volume. The measured tumour volume was 5.042 cm$^3$ for the control group on the 31$^{st}$ day. The std.drug treated mice showed the reduced tumour volume 1.982 cm$^3$. The ligand 2a and 2b treated groups significantly decreased the tumour volume to 3.08 cm$^3$ and 3.98 cm$^3$ respectively. Comparing with that of the control group, the ligands produced a decrease in volume of 1.962 cm$^3$ and 1.062 cm$^3$ respectively. Among the ligands, 2a was more effective than 2b in reducing the tumour volume. The tumour volumes on day 31 for copper complexes of 2a and 2b were 2.25 cm$^3$ and 3.05 cm$^3$ respectively. The decrease in tumour volume was 2.792 cm$^3$ and 1.992 cm$^3$ respectively with respect to control group. The decrease in tumour volume for std.drug was 3.060 cm$^3$. The Cu(II) complex of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) had shown a pronounced effect in reducing tumour volume.
SECTION-IV

ANTIBACTERIAL STUDY OF 1,7-DITHIOPHENYL HEPTANOIDS AND THEIR Zn(II),Ni(II)&VO(IV)METAL COMPLEXES

Antibacterial studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-bis(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (2b) and their metal chelates Zn(II),Ni(II) & VO(IV)

Antibacterial screening of ligands and metal complexes were carried out by using Agar well diffusion method. Bacterial cultures included in the study are Escherichia Coli, Klebsiella Pneumoniae & Bacillus Subtilis. The test compounds showed varying degree of inhibition against different bacterial strains. All synthesized compounds have shown to be susceptible to excellent potency against the different bacterial strains. The results of the antibacterial activity of synthesized ligand with a heterocyclic ring attached to the unsaturated diketo moiety part and their complexes revealed that the ligands and their complexes possess comparable antibacterial activity to that of standard drug streptomycin. The activity is expressed in terms of diameter of zone of inhibition in mm. In all the cases metal complexes possess better antibacterial activity than that of ligands, which means that metal complexation enhance the activity.

2.4.1 Antibacterial studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their metal chelates Zn(II),Ni(II) & VO(IV)

The ligand 2a demonstrated comparable antibacterial activity against Bacillus Subtilis and Klebsiella Pneumoniae species by producing a zone of inhibition of 10 mm. The activity of the ligand was only half of the activity exhibited by the std. drug. All the complexes elicited inhibitory activities against all three bacterial strains and were more effective than ligand. The ligand 2a gave a zone of inhibition of 8.5 mm against E.coli species whereas its VO(IV) complex exhibited maximum inhibitory activity against E.coli species with a zone of
inhibition of 18mm which is comparable with the activity of streptomycin. The std. drug produced a zone of inhibition of 20 mm against all bacterial strains. The Zn(II) and Ni(II) complexes had shown a slight marginal increase in activity compared with the ligand. The Vanadyl complex was quite effective against all the three bacterial strains. The results of antibacterial study of 2a and its metal complexes are given in Table 2.4.1

Table 2.4.1 Antibacterial studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and their metal chelates Zn(II), Ni(II) & VO(IV)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Diameter of zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L₁</td>
</tr>
<tr>
<td>E Coli</td>
<td>8.5</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>10</td>
</tr>
<tr>
<td>Bacillus</td>
<td>10</td>
</tr>
<tr>
<td>Standard</td>
<td>20</td>
</tr>
</tbody>
</table>

**Fig. 2.4.1** Antibacterial studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and their metal chelates Zn(II), Ni(II) & VO(IV)
2.4.2 Antibacterial studies of 1,7-di(3-methyl thiophen-2-yl)hepta-1,6-diene-3,5-dione (2b) and their metal chelates Zn(II),Ni(II) & VO(IV)

The ligand 2b was more active against E coli bacteria and produced a zone of inhibition with diameter 10.5mm compared with its activity against Bacillus Subtilis(8.5mm) and against Klebsiella Pneumoniae(9mm). The activity of metal complexes followed the order VO(IV)>Zn(II)>Ni(II). The vanadyl complex of ligand 2b had shown enhanced activity and produced a zone of inhibition of 17.5mm which is comparable with the diameter of zone of inhibition produced by the standard drug streptomycin ie 20mm. The vanadyl complex was active against Bacillus Subtilis and Klebsiella Pneumoniae and produced a zone of inhibition of 15mm and 13.5mm respectively. The Zn(II) complex exhibited moderate activity against all bacterial strains. The results of antibacterial study of 2b and its metal complexes are given in Table 2.4.2 and represented graphically in Fig. 2.4.2

Table 2.4.2 Antibacterial studies of 1,7-di(3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione (L2) and their metal chelates Zn(II),Ni(II) & VO(IV)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>L2 Zone of inhibition in mm</th>
<th>VO(L2)</th>
<th>Zn(L2)</th>
<th>Ni(L2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E Coli</td>
<td>10.5</td>
<td>17.5</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>9</td>
<td>13.5</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Bacillus</td>
<td>8.5</td>
<td>15</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Standard</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
Fig 2.4.2 Antibacterial studies of 1,7-di(3-methylthiophen-2-yl)hepta-1,6-diene-3,5-dione (L2) and their metal chelates Zn(II), Ni(II) & VO(IV)
SECTION-V

ANTIFUNGAL STUDY OF 1,7-DITHIOPHENYL HEPTANOIDS AND THEIR Zn(II) & VO(IV) METAL COMPLEXES

Antifungal Activity of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and 1,7-di(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) and their Zn(II) and VO(IV) complexes

The curcuminoid analogues with thiophenyl ring and their metal complexes namely Zn(II) & VO(IV) were studied for their antifungal activity against three fungal cultures namely Aspergillus Niger, Penicillium Chrysogenum and Alternaria Alternate. Kirby Baurer disc plate method was used to test the susceptibility of the fungi species to the test compounds. Different concentrations [100, 250, 500µg/ml] by dissolving in 2% DMSO solvent were used for all the test compounds and results were compared with the std.drug flucanazole. The antifungal activities are measured in terms of zone of inhibition in mm. The data of the study revealed that the synthesized 1,7-dithiophenyl heptanoids and their Zn(II) & VO(IV) complexes possess comparable antifungal activities to that of std.drug.

2.5.1 Antifungal Activity of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (2a) and their Zn(II) and VO(IV) complexes

The inhibition zone of the test compounds with the three fungi species in comparison to flucanazole (std.drug) is shown in Table 2.5.1
Table 2.5.1. Antifungal studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and its Zn(II) & Vanadyl complexes

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Diameter of zone of inhibition in mm</th>
<th>L1</th>
<th>Zn(L1)2</th>
<th>VO(L1)2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100µg</td>
<td>250µg</td>
<td>500µg</td>
<td>100µg</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>8</td>
<td>11</td>
<td>13.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Penicillium</td>
<td>9.5</td>
<td>11.5</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Alternaria</td>
<td>9</td>
<td>12.5</td>
<td>16</td>
<td>10</td>
</tr>
</tbody>
</table>

Fig. 2.5.1. Antifungal studies of 1,7-di(thiophen-2-yl)hepta-1,6-diene-3,5-dione (L1) and its Zn(II) & Vanadyl complexes

The ligand 2a exhibited moderate antifungal activity against all organisms at a concentration of 100µg/disc. The compound was found to be very active against all species in 500µg/disc concentration. The ligand showed maximum antifungal activity with Alternaria with a zone of
inhibition of 16 mm. The Zn(II) and VO(IV) complexes had shown significant activity as expected. It is observed that vanadyl complex of the ligand exhibited the most effective antifungal activity against all the three fungal cultures. The zone of inhibition produced by the VO(IV) complex is 18 mm, 17.5 mm and 18.5 mm against Aspergillus, Penicillium and Alternaria respectively. This is comparable with the zone of inhibition (21 mm) produced by the std. drug.

### 2.5.2 Antifungal Activity of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (2b) and their Zn(II) and VO(IV) complexes

The inhibitory effect of ligand and its metal complexes against the fungal cultures is represented in Table 2.5.2. For all the tested compounds they show maximum antifungal activity at a higher concentration of 500 μg/ml. It is observed that antifungal nature increases with the concentration of the compounds. The ligand 2b exhibited a zone of inhibition of 15.5 mm against Penicillium where as zone of inhibition produced is 16.5 and 14 mm against Alternaria and Aspergillus respectively at higher conc. The ligand 2b exhibited more antifungal activity against Alternaria. The Zn(II) complexes gave inhibitory activity against fungal cultures which was only slightly greater than the ligands. The VO(IV) complexes were quite effective against all fungi at all concentrations. The vanadyl complex of ligand 2b demonstrated promising antifungal activity producing a zone of inhibition of 19.5 mm with Alternaria species. It has been found to be a potent antifungal compound.

Comparing the ligands, 2b had shown a slight increase in antifungal activity towards all fungi species than 2a. The ligand 2b has a methyl group on the thiophenyl ring compared with 2a. Comparing Zn(II) and VO(IV) complexes of both ligands, it was observed that vanadyl complexes exhibited more antifungal activity. The VO(IV) complexes of both ligands especially of 2b had appreciable antifungal activity against all fungal cultures.
Table 2.5.2 Antifungal studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (L\(_2\)) and its Zn(II) & Vanadyl complexes

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Diameter of zone of inhibition in mm</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L(_2)</td>
<td>100µg</td>
<td>250µg</td>
<td>500µg</td>
<td>100µg</td>
<td>250µg</td>
<td>500µg</td>
<td>100µg</td>
<td>250µg</td>
<td>500µg</td>
<td></td>
</tr>
<tr>
<td>Aspergillus</td>
<td>8.5</td>
<td>11.5</td>
<td>14</td>
<td>9</td>
<td>11.5</td>
<td>15</td>
<td>11</td>
<td>13</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillium</td>
<td>10</td>
<td>12</td>
<td>15.5</td>
<td>11</td>
<td>14</td>
<td>16</td>
<td>14</td>
<td>17</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternaria</td>
<td>11</td>
<td>13</td>
<td>16.5</td>
<td>12</td>
<td>14</td>
<td>17</td>
<td>15</td>
<td>17.5</td>
<td>19.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2.5.2 Antifungal studies of 1,7-bis(3-methyl thiophen-2-yl) hepta-1,6-diene-3,5-dione (L\(_2\)) and its Zn(II) & Vanadyl complexes