3.1 Introduction

The coordination chemistry of semicarbazones appeared to be very interesting from the point of view of both the number of metals forming complexes with them and the diversity of the ligand system themselves. Ligands derived from substituted semicarbazones have played an important part in revealing the preferred coordination geometries of metal complexes. The two semicarbazones employed for the present investigation are

1. Benzaldehyde-\(N(4)\)-phenylsemicarbazone (HL\(^1\))

2. Acetone-\(N(4)\)-phenylsemicarbazone (HL\(^2\))

This chapter describes the synthesis and characterization of the two \(N(4)\)-phenylsemicarbazones.

The structure and the numbering scheme of the two ligands are given in Fig. 3.1.
3.2 Experimental

3.2.1 Materials

\(N(4)\)-phenylsemicarbazide (Sigma-Aldrich), benzaldehyde (Merck) and acetone (Merck) were of analar grade and used as received. Methanol (Merck) was used without further purification.

3.2.2 Synthesis of the benzaldehyde-\(N(4)\)-phenylsemicarbazone (HL\(^1\))

The compound (HL\(^1\)) was prepared in methanol solution by the condensation of benzaldehyde and \(N(4)\)-phenylsemicarbazide in acid medium (Scheme 3.1). A methanolic solution (30 ml) of \(N(4)\)-phenylsemicarbazide (0.151 g, 1 mmol) was added to a solution of benzaldehyde (1 mmol) in methanol and the reaction mixture was refluxed for 3 h on a water bath. To this few drops of dil. acetic acid were also added to change the pH. On slow evaporation colorless crystals of the semicarbazone were separated out. It was recrystallized from methanol (yield: 75%) and the melting point was found to be 176 °C. These crystals were filtered, washed with ether and dried over \(P_4O_{10}\) in vacuo.
3.2.3 Synthesis of the acetone-\(N(4)\)-phenylsemicarbazone (HL\(^2\))

The compound (HL\(^2\)) was prepared in methanol solution by the condensation of acetone and \(N(4)\)-phenylsemicarbazide in acid medium (Scheme 3.2). A methanolic solution (30 ml) of \(N(4)\)-phenylsemicarbazide (0.151 g, 1 mmol) was added to a solution of acetone (1 mmol) in methanol and the reaction mixture was refluxed for 3 h on a water bath. To this few drops of dil. acetic acid were also added to change the pH. On slow evaporation colorless crystals of the semicarbazone were separated out. It was recrystallized from methanol (yield: 80\%) and the melting point is found to be 152 °C. These crystals were filtered, washed with ether and dried over \(P_4O_{10}\) in vacuo.

3.2.4 Physical measurements

Elemental analyses were carried out using a Vario EL-III CHNS analyzer. Infrared spectra were recorded on a Thermo Nicolet, Avator 370 spectrometer in the range 4000-400 cm\(^{-1}\) using KBr pellets. Electronic spectra were recorded in
methanol on a Shimadzu UV-2450 UV-Vis. spectrophotometer. The $^1$H and $^{13}$C NMR spectra were recorded using Bruker DRX-500 MHz NMR spectrometer, with CDCl$_3$ as solvent and TMS as the standard.

### 3.2.5 X-ray crystallography

Single crystals of compound HL$^2$ suitable for X-ray diffraction analysis were grown from the methanol solution by slow evaporation at room temperature in air and were found to be monoclinic. The crystallographic data and structure refinement parameters are presented in Table 3.1. The data were collected using a Bruker AXS Kappa Apex2 CCD diffractometer, with graphite-monochromated Mo Kα ($\lambda = 0.71073$ Å) radiation. The unit cell dimensions and intensity data were measured at 293 K. The program SAINT / XPREP was used for data reduction and APEX2 / SAINT for cell refinement (Bruker, 2004). The structure was solved using SIR92 (Altomare et al., 1993) and refinement was carried out by full-matrix least squares on $F^2$ using SHELXL-97 (Sheldrick, 2008). All non-hydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms with the exception of those on nitrogen atoms were geometrically fixed and refined using a riding model. The hydrogen atoms on nitrogen atoms were located from the difference Fourier maps and refined isotropically. Molecular graphics employed were DIAMOND Version 3.1f (Brandenburg, 2008) and PLATON (Spek, 2009).

### 3.3 Results and discussion

The elemental analyses data are in good agreement with the stoichiometry of benzaldehyde-N(4)-phenylsemicarbazone and acetone-N(4)-phenylsemicarbazone. The analytical data of ligands are presented in Table 3.2. The semicarbazones, can exist in keto or enol form. However, the IR spectra of HL$^1$ and HL$^2$ indicate that in solid state they remain in keto form. The structure of one of the ligands, HL$^2$ has been confirmed by single crystal X-ray diffraction studies.
Table 3.1
Crystal data and structure refinement parameters of HL$^2$

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HL$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C$<em>{10}$H$</em>{12}$N$_3$O</td>
</tr>
<tr>
<td>Formula weight (M)</td>
<td>191.23</td>
</tr>
<tr>
<td>Temperature (T) (K)</td>
<td>293 (2)</td>
</tr>
<tr>
<td>Wavelength (Mo Kα) (Å)</td>
<td>0.71073</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>$P2_1/n$</td>
</tr>
<tr>
<td>Lattice constants</td>
<td></td>
</tr>
<tr>
<td>$a$ (Å)</td>
<td>13.3696 (7)</td>
</tr>
<tr>
<td>$b$ (Å)</td>
<td>5.4201 (3)</td>
</tr>
<tr>
<td>$c$ (Å)</td>
<td>15.8946 (8)</td>
</tr>
<tr>
<td>$\alpha$ (°)</td>
<td>90.00</td>
</tr>
<tr>
<td>$\beta$ (°)</td>
<td>114.651 (2)</td>
</tr>
<tr>
<td>$\gamma$ (°)</td>
<td>90.00</td>
</tr>
<tr>
<td>Volume $V$ (Å$^3$)</td>
<td>1046.83 (10)</td>
</tr>
<tr>
<td>$Z$</td>
<td>4</td>
</tr>
<tr>
<td>$D_{\text{calc}}$ ($\rho$) (Mg/m$^3$)</td>
<td>1.213</td>
</tr>
<tr>
<td>Absorption Coefficient, $\mu$ (mm$^{-1}$)</td>
<td>0.082</td>
</tr>
<tr>
<td>$F_{(000)}$</td>
<td>408</td>
</tr>
<tr>
<td>Crystal size (mm)</td>
<td>0.30 x 0.20 x 0.20</td>
</tr>
<tr>
<td>Colour, nature</td>
<td>Colorless, block</td>
</tr>
<tr>
<td>$\theta$ Range for data collection (°)</td>
<td>1.68 - 27.53</td>
</tr>
<tr>
<td>Limiting indices</td>
<td>-17 ≤ $h$ ≤ 17</td>
</tr>
<tr>
<td></td>
<td>-5 ≤ $k$ ≤ 7</td>
</tr>
<tr>
<td></td>
<td>-20 ≤ $l$ ≤ 20</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>20274</td>
</tr>
<tr>
<td>Independent reflections ($R_{\text{int}}$)</td>
<td>2395 (0.0229)</td>
</tr>
<tr>
<td>Observed reflections ($</td>
<td>I</td>
</tr>
<tr>
<td>Completeness to $\theta$</td>
<td>27.53° (99.6%)</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Maximum and minimum transmission</td>
<td>0.936 and 0.901</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least squares on $F^2$</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
<td>2395/0/138</td>
</tr>
<tr>
<td>Goodness-of-fit on $F^2$</td>
<td>1.025</td>
</tr>
<tr>
<td>Final $R$ indices ($</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>$wR_2 = 0.1151$</td>
</tr>
<tr>
<td>$R$ indices (all data)</td>
<td>$R_I = 0.0761,$</td>
</tr>
<tr>
<td></td>
<td>$wR_2 = 0.1477$</td>
</tr>
<tr>
<td>Largest difference peak and hole (e Å$^{-3}$)</td>
<td>0.139 and -0.177</td>
</tr>
</tbody>
</table>

$$wR_2 = \frac{\Sigma w(F_o^2 - F_c^2)^2 / \Sigma w(F_o^2)^2}{1/2}$$
$$R_I = \frac{\Sigma |F_o| - |F_c|} {\Sigma |F_o|}$$
Table 3.2
Analytical and physical data of HL\textsuperscript{1} and HL\textsuperscript{2}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Color</th>
<th>Empirical Formula</th>
<th>Composition % found (calc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>HL\textsuperscript{1}</td>
<td>Colorless</td>
<td>C\textsubscript{14}H\textsubscript{13}N\textsubscript{3}O</td>
<td>70.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(70.27)</td>
</tr>
<tr>
<td>HL\textsuperscript{2}</td>
<td>Colorless</td>
<td>C\textsubscript{10}H\textsubscript{13}N\textsubscript{3}O</td>
<td>62.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(62.80)</td>
</tr>
</tbody>
</table>

3.3.1 Infrared spectra

The tentative assignments of important infrared bands of HL\textsuperscript{1} and HL\textsuperscript{2} are presented in Table 3.3 and the spectra of the ligands are shown in Fig. 3.2 and Fig. 3.3. The medium band at 3357 cm\textsuperscript{-1} and a strong band at 3375 cm\textsuperscript{-1} is assigned to ν(\textsuperscript{4}NH) vibrations of HL\textsuperscript{1} and HL\textsuperscript{2} respectively. Medium bands observed at 2917 cm\textsuperscript{-1} for HL\textsuperscript{1} and 3196 cm\textsuperscript{-1} for HL\textsuperscript{2} due to ν(CZNH) vibrations indicate that the ligands exist in the keto form in the solid state. The ν(C=O) bands are present at 1708 cm\textsuperscript{-1} and 1682 cm\textsuperscript{-1} for HL\textsuperscript{1} and HL\textsuperscript{2} respectively. The band at 1600 cm\textsuperscript{-1} and 1591 cm\textsuperscript{-1}, for HL\textsuperscript{1} and HL\textsuperscript{2} respectively is assigned to ν(C=N). These values are in agreement with earlier reports of N(4)-substituted semicarbazones (Reena \textit{et al.}, 2008b). The infrared spectral bands of HL\textsuperscript{1} and HL\textsuperscript{2} observed at 1032 cm\textsuperscript{-1} and 1129 cm\textsuperscript{-1} correspond to ν(N–N).

Table 3.3
Selected infrared spectral assignments (cm\textsuperscript{-1}) of HL\textsuperscript{1} and HL\textsuperscript{2}

<table>
<thead>
<tr>
<th>Compound</th>
<th>ν(C=O)</th>
<th>ν(C=N)</th>
<th>ν(\textsuperscript{4}NH)</th>
<th>ν(\textsuperscript{4}NH)</th>
<th>ν(N–N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL\textsuperscript{1}</td>
<td>1708</td>
<td>1600</td>
<td>2917</td>
<td>3357</td>
<td>1032</td>
</tr>
<tr>
<td>HL\textsuperscript{2}</td>
<td>1682</td>
<td>1591</td>
<td>3196</td>
<td>3375</td>
<td>1129</td>
</tr>
</tbody>
</table>
Fig. 3.2
Infrared spectrum of HL\(^1\)

Fig. 3.3
Infrared spectrum of HL\(^2\)
3.3.2 Electronic spectra

The tentative assignments of the significant electronic spectral bands of ligands are presented in Table 3.4. The spectra of HL\textsuperscript{1} and HL\textsuperscript{2} are shown in Fig. 3.4 and Fig. 3.5 respectively. The electronic spectra of HL\textsuperscript{1} and HL\textsuperscript{2} in methanol solution show the following absorptions. The observed band at 41150 cm\textsuperscript{-1} for both HL\textsuperscript{1} and HL\textsuperscript{2} are assigned to the $\pi\rightarrow\pi^*$ transition of the phenyl ring and imine function of the semicarbazone moiety. The $n\rightarrow\pi^*$ transitions of the azomethine group and imine function of the semicarbazone moiety are observed at 34070 cm\textsuperscript{-1} for HL\textsuperscript{1} and 33960 cm\textsuperscript{-1} for HL\textsuperscript{2}.

Table 3.4

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\pi\rightarrow\pi^*$</th>
<th>$n\rightarrow\pi^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL\textsuperscript{1}</td>
<td>41150</td>
<td>34070</td>
</tr>
<tr>
<td>HL\textsuperscript{2}</td>
<td>41150</td>
<td>33960</td>
</tr>
</tbody>
</table>

Fig. 3.4
Electronic spectrum of HL\textsuperscript{1}
3.3.3 $^1$H NMR spectrum of HL$^1$

The $^1$H NMR spectrum of the compound HL$^1$ and its assignments are shown in Fig. 3.6 and the data of $^1$H NMR signals recorded in CDCl$_3$ are presented in Table 3.5. A sharp singlet, which integrates as one hydrogen at $\delta = 9.51$ ppm is assigned to the proton attached to the nitrogen atom N(2). Another singlet at $\delta = 7.85$ ppm is assigned to the proton attached to nitrogen atom N(4). The low field position of $-\text{N(4)H}$ can be attributed to the deshielding caused by phenyl group. This downfield shift is also explained by the H-bonding interaction with nitrogen atom N(1). Hydrogen bonding decreases the electron density around the proton and thus moves the proton absorption to a lower field. Absence of any coupling interaction by N(2)H and N(4)H protons due to lack of availability of protons on neighboring atoms render singlet peaks for the imine protons. The ligand does not show any peak attributable to $-\text{OH}$ proton indicating that it exists in the keto form.
Table 3.5
$^1$H NMR (CDCl$_3$) assignments of HL$^1$ and HL$^2$ (δ in ppm)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$^2$NH</th>
<th>$^3$NH</th>
<th>-CH=N-</th>
<th>$^1$CH$_3$</th>
<th>$^3$CH$_3$</th>
<th>Aromatic protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL$^1$</td>
<td>9.51</td>
<td>7.85</td>
<td>8.18</td>
<td>-</td>
<td>-</td>
<td>7.09-7.68</td>
</tr>
<tr>
<td>HL$^2$</td>
<td>8.21</td>
<td>8.04</td>
<td>-</td>
<td>2.03</td>
<td>1.90</td>
<td>7.02-7.53</td>
</tr>
</tbody>
</table>

Infrared spectral data are also in conformity with these observations. A signal at δ = 8.18 ppm is assigned to -CH=N- proton. The more downfield shift of -CH=N- can be attributed to the increased charge density on N(1) resulted by its hydrogen bonding to N(4)H and also due to electronic effect of the adjacent electronegative nitrogen. Aromatic protons of both phenyl rings appear with in the range 7.09-7.68 ppm with coupling constant 7 Hz.

Fig. 3.6
$^1$H NMR spectrum of HL$^1$. 

$\text{Fig. 3.6}
^1\text{H NMR spectrum of HL}^1$
3.3.4 $^1$H NMR spectrum of HL$^2$

The $^1$H NMR spectrum of the compound HL$^2$ along with the assignments are shown in Fig. 3.7 and $^1$H NMR data are presented in Table 3.5. The $^1$H NMR spectrum of HL$^2$, shows a singlet, integrating as one hydrogen, at $\delta = 8.21$ ppm which is assigned to the proton attached to the N(2)H. The N(4)H is observed as a singlet at $\delta = 8.04$ ppm. The low field position of N(4)H is attributed to the deshielding caused by phenyl group. This downfield shift is also explained by the intramolecular H-bonding interaction with nitrogen atom N(1). This is confirmed from the crystal structure.

![Fig. 3.7 $^1$H NMR spectrum of HL$^2$](image)
Hydrogen bonding decreases the electron density around the proton and thus moves the proton absorption to a lower field. Absence of any coupling interaction by N(2)H and N(4)H protons due to lack of availability of protons on neighboring atoms render singlet peaks for the imine protons. The ligand does not show any peak attributable to –OH proton indicating that it exists in the keto form. IR spectral data are also in confirmity with these observations. Two singlets at δ values 2.03 ppm and 1.90 ppm are attributed to the methyl protons which are chemically and magnetically equivalent. The resonances for the phenyl group appear as triplet at 7.02 ppm and 7.33 ppm for para and meta protons and as doublet at 7.53 ppm for ortho phenyl protons.

3.3.5 \(^{13}\)C NMR spectrum of HL\(^1\)

The \(^{13}\)C NMR spectrum provides direct information about the carbon skeleton of the semicarbazone ligand HL\(^1\). Assignment of different resonant peaks to respective carbon atoms are presented in Fig. 3.8. There are 9 unique carbon atoms in the molecule which give a total of 9 different peaks in the \(^{13}\)C NMR spectrum. The C(8) carbon atom resonance is observed farthest downfield at δ = 153.61 ppm resultant of the conjugative effect of the –N(1)–N(2)–C(O)–N(4)–semicarbazone skeleton. The protonated carbon atom at C(7) shows more downfield shift in the spectrum (δ = 141.78 ppm), due to an increase in electron density resulting from the presence of electronegative atom and π-electron delocalization on the C(7)=N(1) bond. In N(4) phenyl ring the C(9) carbon atom adjacent to more electronegative nitrogen atom N(4) are shifted further downfield to δ = 137.84 ppm when compared to the neighboring carbon atoms. The N(4) phenyl resonances are: C(9), 137.84 ppm; C(10) and C(14), 128.79 ppm; C(11) and C(13), 130.02 ppm; C(12), 129.02 ppm. Aromatic carbons of the benzaldehyde ring are observed C(1), 133.66 ppm; C(2) and C(6), 119.68 ppm; C(3) and C(5), 126.94 ppm; C(4), 123.54 ppm.
Fig. 3.8

$^{13}$C NMR spectrum of HL$^1$
3.3.6 $^{13}$C NMR spectrum of HL$^2$

The assignments made from $^{13}$C NMR spectrum of the ligand HL$^2$ are shown in Fig. 3.9. The $^{13}$C NMR spectrum provides direct information about the carbon skeleton of the molecule. There are 8 unique carbon atoms in the molecule, which give a total of 8 different peaks in the $^{13}$C NMR spectrum. The C(4) carbon atom resonance is observed farthest downfield at 153.69 ppm, resultant of the conjugative effect of the $\text{\textendash}N(1)\text{\textendash}N(2)\text{\textendash}C(O)\text{\textendash}N(4)$ semicarbazone skeleton. The non-protonated carbon atom at C(2) is shifted downfield in the spectrum ($\delta = 147.72$ ppm). The methyl carbon atoms are observed at $\delta = 25.28$ ppm and 16.52 ppm. The three different types of aromatic carbons on the N(4) phenyl ring are clearly distinguishable in the $^{13}$C NMR spectrum. The peak corresponding to the para positioned carbon atom C(8) is observed rather upfield when compared to its ortho [C(6) and C(10)] and meta [C(7) and C(9)] counterparts. The N(4) phenyl resonances are: C(6) and C(10), 119.36 ppm; C(7) and C(9), 128.90 ppm; C(8), 123.10 ppm; C(5), 138.25.

![Fig. 3.9 $^{13}$C NMR spectrum of HL$^2$](image-url)
3.3.7 Crystal structure of \(\text{HL}^2\)

The molecular structure of \(\text{HL}^2\) along with atom numbering scheme is shown in Fig. 3.10. Selected bond lengths and bond angles of \(\text{HL}^2\) are presented in Table 3.6. The compound crystallizes in a monoclinic lattice with the space group \(P2_1/n\). The molecule exists in the \(Z\) conformation with respect to the C(2)–N(1) bond. However, the O(1) and N(1) atoms are in \(E\) conformation with respect to the N(2)–C(4) bond and hence the semicarbazone moiety as a whole exists in the \(ZE\) conformation (Suni et al., 2007). A torsion angle of 179.43(18)° corresponding to the O(1)–C(4)–N(2)–N(1) moiety implies a \textit{trans} alignment of the keto carbonyl O(1) atom in \(\text{HL}^2\). The azomethine bond, C(2)–N(1) 1.270(2) Å is in conformity with a formal C=N double bond (1.28 Å) and the C(4)–O(1) bond distances of 1.220(2) Å, is very close to a formal C=O bond length 1.21 Å. It confirms the existence of the semicarbazone in the keto form in the solid state. The N(1)–N(2) [1.380(2) Å] and N(2)–C(4) [1.354(2) Å] bond distances in \(\text{HL}^2\) are intermediate between ideal values of corresponding single [N–N, 1.45 Å; C–N, 1.47 Å] and double bonds [N=N, 1.25 Å, C=N, 1.28 Å], giving evidence for an extended \(\pi\)-delocalization along the semicarbazone chain (Seena et al., 2008a).

Table 3.6

Selected bond lengths (Å) and bond angles (°) of \(\text{HL}^2\)

<table>
<thead>
<tr>
<th>Bond lengths</th>
<th>(\text{HL}^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(2)–N(1)</td>
<td>1.270(2)</td>
</tr>
<tr>
<td>C(4)–O(1)</td>
<td>1.220(2)</td>
</tr>
<tr>
<td>C(4)–N(3)</td>
<td>1.354(2)</td>
</tr>
<tr>
<td>C(4)–N(2)</td>
<td>1.354(2)</td>
</tr>
<tr>
<td>C(5)–N(3)</td>
<td>1.402(2)</td>
</tr>
<tr>
<td>N(1)–N(2)</td>
<td>1.380(2)</td>
</tr>
</tbody>
</table>

Conti........
### Bond angles

<table>
<thead>
<tr>
<th>Bond angles</th>
<th>HL$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(1)–C(2)–C(1)</td>
<td>116.90(16)</td>
</tr>
<tr>
<td>N(1)–C(2)–C(3)</td>
<td>125.59(16)</td>
</tr>
<tr>
<td>O(1)–C(4)–N(3)</td>
<td>123.80(16)</td>
</tr>
<tr>
<td>O(1)–C(4)–N(2)</td>
<td>121.18(15)</td>
</tr>
<tr>
<td>N(3)–C(4)–N(2)</td>
<td>115.02(15)</td>
</tr>
<tr>
<td>C(6)–C(5)–N(3)</td>
<td>116.88(15)</td>
</tr>
<tr>
<td>C(10)–C(5)–N(3)</td>
<td>123.95(15)</td>
</tr>
<tr>
<td>C(2)–N(1)–N(2)</td>
<td>118.58(14)</td>
</tr>
<tr>
<td>C(4)–N(2)–N(1)</td>
<td>120.17(14)</td>
</tr>
<tr>
<td>C(4)–N(3)–C(5)</td>
<td>128.26(15)</td>
</tr>
</tbody>
</table>

### Fig. 3.10

Structure of HL$^2$ showing the atom numbering scheme
The packing of the molecule in a unit cell along the \( b \) axis is given in Fig. 3.11. The assemblage of molecules in the respective manner in the unit cell is resulted by the H-bonding and C–H-\( \pi \) interactions as depicted in Table 3.7. The intramolecular hydrogen bonding interaction N(3)–H(3)–N(1), leads to the formation of one five membered ring comprising of atoms N(1), N(2), C(4), N(3), H(3) and one intermolecular hydrogen bonding interaction O(1)–H(2)–N(2) is also observed in Fig. 3.12. A weak intramolecular hydrogen bonding interaction is observed between C(10)–H(10A) and O(1) with an angle of 122°. The C–H-\( \pi \) interactions C(1)–H(1C) \( \rightarrow \) Cg(1) at a distance of 2.886 Å contribute stability to the unit cell packing.

### Table 3.7

**Interaction parameters of HL\(^2\)**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N(2)–H(2)–O(1)(^a)</td>
<td>0.86</td>
<td>2.03</td>
<td>2.870(2)</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>N(3)–H(3)–N(1)</td>
<td>0.86</td>
<td>2.20</td>
<td>2.620(2)</td>
<td>110</td>
<td></td>
</tr>
</tbody>
</table>

Equivalent position code :\( a = -x, 2-y, 2-z \)

<table>
<thead>
<tr>
<th>CH-( \pi ) interactions</th>
<th>X–H(I)–Cg(J)</th>
<th>H...Cg(Å)</th>
<th>X–H...Cg (°)</th>
<th>X...Cg(Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(1) –H(1C) ( \rightarrow ) Cg(1)(^b)</td>
<td>2.886</td>
<td>135.59</td>
<td>3.634(2)</td>
<td></td>
</tr>
</tbody>
</table>

Equivalent position code :\( b = \frac{1}{2}+x, 3/2-y, 1/2+z \)

Cg(1) = C(5),C(6),C(7),C(8),C(9),C(10)

D = donor, A = acceptor, Cg = centroid
Fig. 3.11
Unit cell packing diagram of HL$_2$ viewed along b axis

Fig. 3.12
Inter and intramolecular hydrogen bonding interactions of HL$_2$
(hydrogen atoms are omitted for clarity)
3.4 Antimicrobial studies

HL\textsuperscript{1} and HL\textsuperscript{2} were screened against five bacterial cultures viz., \textit{Escherichia coli} MTCC 585, \textit{Salmonella typhi} MTCC 734, \textit{Proteus vulgaris} MTCC 1771, \textit{Enterobacter aerogenes} MTCC 2990, \textit{Bacillus megaterium} MTCC 2248 and two fungal cultures viz., \textit{Aspergillus niger} MTCC 281 and \textit{Candida albicans} MTCC 3018. All the test micro-organisms were obtained from Microbial Type Culture Collection and Gene Bank (MTCC). The antimicrobial properties were determined by the standard disc diffusion method (Collins & Lyne, 1970). Test substances which produce a zone of inhibition of diameters 9 mm or more are regarded as positive, \textit{ie} having constructive antimicrobial activity, while in those cases where the diameter is below 9 mm, the bacteria are resistant to the sample tested and the sample is said to have no antimicrobial activity. In the present case, both benzaldehyde-N(4)-phenylsemicarbazone and acetone-N(4)-phenylsemicarbazone are found to be inactive against bacteria and fungi.

3.5 Cytotoxic activity

Cytotoxicities of HL\textsuperscript{1} and HL\textsuperscript{2} were determined using MTT assay (Supino, 1995) against MCF-7 breast cancer cell line. Cells were continuously exposed to test agent for 24 h, and their effect on cellular viability was evaluated. It was intended that the results from these studies would allow the identification of those compounds with cancer chemotherapeutic potential. Therefore, profiles of cell viability against compound concentration were established (Fig. 3.13) and were used to calculate the IC\textsubscript{50} values for each compound (Table 3.8). Semicarbazone ligands screened displayed a concentration dependent cytotoxic profile across the cell line studied here. The results show that IC\textsubscript{50} values are at 317.708 \textmu g/ml for HL\textsuperscript{1} and 407.311 \textmu g/ml for HL\textsuperscript{2}. However, this approach does not give direct information on the mechanism of action of the individual compound, but it could be reasonably concluded that these substances behave differently on the cells under study probably because they act on different mechanisms.
### Table 3.8

Percentage cytotoxicity of $\text{HL}^1$ and $\text{HL}^2$ with concentration

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% Cytotoxicity $\text{HL}^1$</th>
<th>% Cytotoxicity $\text{HL}^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>24.8</td>
<td>14.01</td>
</tr>
<tr>
<td>100</td>
<td>30.5</td>
<td>23.498</td>
</tr>
<tr>
<td>200</td>
<td>34.7</td>
<td>43.524</td>
</tr>
<tr>
<td>400</td>
<td>60.08</td>
<td>48.46</td>
</tr>
<tr>
<td>500</td>
<td>63.95</td>
<td>67.02</td>
</tr>
<tr>
<td>600</td>
<td>72.23</td>
<td>73.96</td>
</tr>
<tr>
<td>800</td>
<td>79.4</td>
<td>77.00</td>
</tr>
<tr>
<td>1000</td>
<td>80.9</td>
<td>79.7</td>
</tr>
</tbody>
</table>

![Figure 3.13](image)

**Fig. 3.13**

Percentage cytotoxicity of the ligands versus concentration
Conclusion

This chapter presents the synthesis and characterization of two semicarbazone ligands (HL\textsuperscript{1} and HL\textsuperscript{2}). Elemental analyses data are consistent with the empirical formula of ligands. The compounds are further characterized by infrared, electronic, \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectral studies. The IR spectral data of the ligands indicate that they exist in the keto form. The single crystal X-ray diffraction studies of HL\textsuperscript{2} is also presented. The antimicrobial studies show that the ligands do not have any antibacterial activity towards \textit{Escherichia coli} MTCC 585, \textit{Salmonella typhi} MTCC 734, \textit{Proteus vulgaris} MTCC 1771, \textit{Enterobacter aerogenes} MTCC 2990 and \textit{Bacillus megaterium} MTCC 2248 and do not have antifungal activity towards \textit{Aspergillus niger} MTCC 281 and \textit{Candida albicans} MTCC 3018. Cytotoxicities of HL\textsuperscript{1} and HL\textsuperscript{2} were determined using MTT assay against MCF-7 breast cancer cell line. The ligands show cytotoxic activity. Low IC\textsubscript{50} value of HL\textsuperscript{2} indicates that it has higher cytotoxic activity against the tumor cell line evaluated.