CHAPTER-3

Characterization of various substituted phenyl hydrazino butanoate derivatives as ligands [L-1 to L-12]

- Elemental Analysis
- Infrared Spectroscopy
- Nuclear Magnetic Resonance Spectroscopy
- Liquid chromatography–mass spectrometry
- Results and Discussion
Chapter-3

Characterization of Various substituted phenyl hydrazino butanoate Ligands [L-1 to L-12].

The present chapter deals with the characterization of various phenyl hydrazino butanoate ligands (i.e. L-1 to L-12) derivatives described in Chapter 2 by

(I) Elemental analysis
(II) Infrared spectral studies and
(III) NMR and Mass spectral studies
(IV) LC-MS

The general remarks for ligands L-1 to L-12 are:

• The Melting points (°C) of all the compounds were measured by capillary method. All the mp are uncorrected.
• The yields of all compounds reported are of crystallized. All solvents used were distilled and dried. The purity of the compounds was checked by TLC. Column chromatography was performed on silica gel (60-120 mesh).

3.1 ELEMENTAL ANALYSIS.

All the ligands synthesized and described in chapter 2 were analyzed by their elemental contents. The C, H and N elements of all the samples were measured by Elemental analyzer Thermofinigan flash1101 EA (Italy).

The C, H, and N contents of all L-1 to L-12 derivatives are shown in Table 3.1. The data are consistent with the predicted structures of ligands.
### Table 3.1 Characterization of Ligands L-1 to L-12.

<table>
<thead>
<tr>
<th>Ligand No.</th>
<th>Molecular Formula</th>
<th>Mol. Wt gm/Mole</th>
<th>Yield %</th>
<th>Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cal.</td>
</tr>
<tr>
<td>L-1</td>
<td>C₈H₁₆N₂O₂</td>
<td>220</td>
<td>75</td>
<td>65.45</td>
</tr>
<tr>
<td>L-2</td>
<td>C₈H₁₄N₂O₄</td>
<td>265</td>
<td>77</td>
<td>54.33</td>
</tr>
<tr>
<td>L-3</td>
<td>C₈H₁₄N₂O₄</td>
<td>310</td>
<td>78</td>
<td>46.45</td>
</tr>
<tr>
<td>L-4</td>
<td>C₈H₁₄N₂O₂</td>
<td>206</td>
<td>76</td>
<td>64.07</td>
</tr>
<tr>
<td>L-5</td>
<td>C₈H₁₃N₂O₄</td>
<td>251</td>
<td>78</td>
<td>52.58</td>
</tr>
<tr>
<td>L-6</td>
<td>C₈H₁₃N₂O₄</td>
<td>296</td>
<td>79</td>
<td>44.59</td>
</tr>
<tr>
<td>L-7</td>
<td>C₈H₁₃N₂O₂</td>
<td>282</td>
<td>75</td>
<td>72.34</td>
</tr>
<tr>
<td>L-8</td>
<td>C₈H₁₃N₂O₄</td>
<td>327</td>
<td>82</td>
<td>62.38</td>
</tr>
<tr>
<td>L-9</td>
<td>C₈H₁₃N₂O₄</td>
<td>372</td>
<td>84</td>
<td>54.83</td>
</tr>
<tr>
<td>L-10</td>
<td>C₈H₁₃N₂O₂</td>
<td>234</td>
<td>78</td>
<td>66.66</td>
</tr>
<tr>
<td>L-11</td>
<td>C₈H₁₃N₂O₄</td>
<td>279</td>
<td>80</td>
<td>55.91</td>
</tr>
<tr>
<td>L-12</td>
<td>C₈H₁₃N₂O₄</td>
<td>324</td>
<td>84</td>
<td>48.14</td>
</tr>
</tbody>
</table>
3.2 INFRARED SPECTROSCOPY:

The atoms of a molecular behave as if they were connected by flexible springing, rather than by rigid bond resembling the connectors of a ball and stick model. Their component parts can oscillate in different vibrational modes, designed by such terms as rocking, scissoring, twisting, wagging and symmetrical and asymmetrical stretching. When infra red radiation is passed through a sample of a given compound, its molecules can absorb radiation of the energy (and frequency) needed to bring about transitions between vibration of ground states and vibration of excited states.

For example, a C-H bond, that vibrates 90 trillion times a second, must absorb infrared radiation of just that frequency to jump to its first vibration excited state. This absorption of energy at various frequencies can be detected by an infrared spectrometer, which plots amount of infrared radiation transmitted through the sample as a function of the frequency (or wavelength) of the radiation. An infrared spectrum consists of comparatively broad absorption bands rather than sharp peaks such as those seen in NMR spectra. The bands are also usually “Inverted”-a deep valley rather than a peak represents strong absorption.

Infrared spectroscopy is extremely useful [1-5] in qualitative analysis. It can be used both to detect the presence of specific functional groups and other structural features from band positions and intensities and to establish the identity of an unknown compound with a known standard. The fingerprint region of the infrared spectrum, (1250-670 cm\(^{-1}\), 8-15 lym) is best for showing that two substance are identical, since the distinctive patterns found in this region are usually the characteristics of the whole molecule and not of isolated groups. Infrared spectra can also be used in establishing the purity of compounds, monitoring reaction rates, determining the structures of Chelate molecules, and carrying out theoretical studies of hydrogen bonding in other phenomena.
3.2.1 EXPERIMENTAL:

Infrared scanning for the synthesised ligands was made in the range 4000-600 cm\(^{-1}\) in KBr. AR grade KBr was used for this purpose. It was first fused, powdered and dried in vacuum. The absence of moisture in this dried KBr pellet was checked by scanning and IR spectra of purified KBr. Then the pellet of KBr with polymer was prepared as under.

A mixture of 4mg of pure dried sample and 1gm KBr powder was ground in a mini ball mill for about 10 minutes. The resulting mixture was placed on the disc and compressed at high pressure about 20,000 psi giving the transparent pellet. The IR spectrum of this transparent pellet was scanned on Nicollet FTIR 760 spectrophotometer.

3.2.2 RESULTS AND DISCUSSION.

The anticipated IR spectral frequencies of all the Various substituted phenyl hydrazino butanoate Ligands are given in Table 3.2. The infrared spectra of selected ligands are shown in Figures 3.1 to 3.4. The inspection of the infrared spectra of all the ligands reveals following.
Table – 3.2  Anticipated IR spectral features for Ligands L-1 to L-12.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>IR frequencies (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>-C=O- of ester</td>
<td>~1720 cm⁻¹</td>
</tr>
<tr>
<td>2.</td>
<td>=CH₂</td>
<td>~2920 cm⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~2850 cm⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~1430 cm⁻¹</td>
</tr>
<tr>
<td>3.</td>
<td>-CH₃</td>
<td>~2950 cm⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~2890 cm⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~1370 cm⁻¹</td>
</tr>
<tr>
<td>4.</td>
<td>-C=N-</td>
<td>~1590 cm⁻¹</td>
</tr>
<tr>
<td>5.</td>
<td>-NH</td>
<td>~3400 cm⁻¹</td>
</tr>
<tr>
<td>6.</td>
<td>-N-N-</td>
<td>~1060 cm⁻¹</td>
</tr>
<tr>
<td>7.</td>
<td>Aromatic</td>
<td>~ 1600, 1500, 3030 cm⁻¹</td>
</tr>
</tbody>
</table>

(a) The strong band at ~1720 cm⁻¹ and ~1590 cm⁻¹ in the spectra of ligand can be attributed to the unconjugated ketonic and azomethine linkage respectively.

(b) The bands of –N-N- stretching around ~1060 cm⁻¹ of the ligand is the diagnostic of hydrazone formation.

(c) The sharp band at ~3400 cm⁻¹ region shows the presence of N-H group.

(d) The weak band around 3030 cm⁻¹ might be due to aromatic C-H stretching vibrations.
(e) The other bands in the fingerprint region are appeared at their respective position. The bands around 1220 and 1020 cm$^{-1}$ are mainly due to C-N bending vibrations while the C=N stretching vibration features appear around 1690 and 1660 cm$^{-1}$. The weak bands due to out of plane deformation of 1, 2, 3 or 1,3 or 1,4-disubstituted benzene ring systems are appeared at 760, 860 and 810 cm$^{-1}$ respectively.

Figure: 3.1 IR Spectrum of Ligand L-1
Figure: 3.2  IR Spectrum of Ligand L-4
Figure: 3.3 IR Spectrum of Ligand L-7
Figure: 3.4  IR Spectrum of Ligand L-10
3.3 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY:

Nuclear magnetic resonance (NMR) spectroscopy [6,7] is supplementary technique to IR spectroscopy to get details information about structure of organic compounds. Most widely studied nucleus is proton and then the technique is called NMR spectroscopy.

IR spectra give information about the functional group while NMR spectra provide information about the exact nature of proton and its environment. Thus this technique is more useful in the elucidation of an organic compound. IR spectra of isomers may appear same but their NMR spectra will markedly differ.

The phenomenon of nuclear magnetic resonance was first reported independently in 1946 by two groups of physicists: Block, Hansen and Packard at Stanford University detected a signal from the protons of water, and Purcell, Torrey and Pound at Harvard University observed a signal from the protons in paraffin wax. Block and Purcell were jointly awarded the Nobel Prize for physics in 1952 for this discovery. Since that time, the advances in NMR techniques leading to wide spread applications in various branches of science resulted in the Nobel Prize in chemistry in 1991. The applications of NMR in clinical, solid state and biophysical sciences are really marvelous.

The proton magnetic resonance (PMR) spectroscopy is the most important technique used for the characterization of organic compounds. It gives information about the different kinds of protons in the molecule. In other words it tells one about different kinds of environments of the hydrogen atoms in the molecule. PMR also gives information about the number of protons of each type and the ratio of different types of protons in the molecule.

It is well known that all nuclei carry a positive charge. In some nuclei this charge ‘spins’ on the nuclear axis, and this circulation of nuclear charge generates a magnetic dipole along the axis. Thus, the nucleus behaves like a tiny bar magnet. The angular momentum of the spinning charge is described in terms of spin number (I).
The magnitude of generated dipole is expressed in terms of nuclear magnetic moment (\(\mu\)).

The spinning nucleus of a hydrogen atom (\(^1\text{H}\) or proton) is the simplest and is commonly encountered in organic compounds. The hydrogen nucleus has a magnetic moment, \(\mu = 2.79268\) and its spin number (I) is \(\pm \frac{1}{2}\). Hence, in an applied external magnetic field, its magnetic moment may have two possible orientations.

The orientations in which the magnetic moment is aligned with the applied magnetic field is more stable (lower energy) than in which the magnetic moment is aligned against the field (high energy). The energy required for flipping the proton from its lower energy alignment to the higher energy alignment depends upon the difference in energy (\(\Delta E\)) between the two states and is equal to \(h\mu(\Delta E = h\mu)\).

In principle, the substance could be placed in a magnetic field of constant strength, and then the spectrum can be obtained in the same way as an infrared or an ultraviolet spectrum by passing radiation of steadily changing frequency through the substance and observing the frequency at which radiation is absorbed. In practice, however, it has been found to be more convenient to keep the radiation frequency constant and vary the strength of the magnetic field. At some value of the field strength the energy required to flip the proton matches the energy of the radiation, absorption occurs and a signal is obtained. Such a spectrum is called a nuclear magnetic resonance (NMR) spectrum.

Two types of NMR spectrometers are commonly encountered. They are:

a) Continuous wave (CW) NMR spectrometer
b) Fourier transform (FT) NMR spectrometer.

The CW-NMR spectrometer detects the resonance frequencies of nuclei in a sample placed in a magnetic field by sweeping the frequency of RF radiation through a given range and directly recording the intensity of absorption as a function of frequency. The spectrum is usually recorded and plotted simultaneously with a recorder synchronized to the frequency of the RF source.
In FT-NMR spectroscopy, the sample is subjected to a high power short duration pulse of RF radiation. This pulse of radiation contains a broad band of frequencies and causes all the spin-active nuclei to resonate all at once at their Larmor frequencies. Immediately following the pulse, the sample radiates a signal called free induction decay (FID), which is modulated by all the frequencies of the nuclei excited by the pulse. The signal detected as the nuclei return to equilibrium (intensity as a function of time) is recorded, digitized and stored as an array of numbers in a computer. Fourier transformation of the data affords a conventional (intensity as a function of frequency) representation of the spectrum.

The first step in running NMR spectrum is the complete dissociation of a requisite amount of the sample in the appropriate volume of a suitable NMR solvent. Commonly used solvents are: CCl₄, deuteron chloroform, deuteron DMSO, deuteron methanol, deuteron water, deuteron benzene, trifluoroacetic acid.

TMS is generally employed as internal standard for measuring the position of ¹H, ¹³C, and ²⁹Si in the NMR spectrum because it gives a single sharp peak, is chemically inert and miscible with a large range of solvents, being a highly volatile, can easily be removed if the sample has to be recovered, does not involve in intramolecular association with the sample.

3.3.1 INTERPRETATION OF THE NMR SPECTRA:

The following general procedure will form a useful initial approach to the interpretation of most spectra.

- By making table of the chemical shifts of all the groups of absorptions in the spectrum. In some cases it will not be possible to decide whether a particular group of absorptions arises from separate sets of nuclei, or from a part of one complex multiplet. In such cases it is probably best initially to include them under one group and to note the spread of chemical shift values.

- By measuring and recording the heights of the integration steps corresponding to each group of absorptions. With overlapping groups of protons it may not
be possible to measure these exactly, in which case a range should be noted. Work out possible proton ratios for the range of heights measured, by dividing by the lowest height and multiplying as appropriate to give integral values.

- By noting any obvious splitting of the absorptions in the table (e.g., doublet, triplet, etc.). For spectra which appear to show first-order splitting, the coupling constants of each multiplet should be determined by measuring the separation between adjacent peaks in the multiplet. Any other recognizable patterns which are not first order should be noted.

- By noting any additional information such as the effect of shaking with D$_2$O, use of shift reagent, etc.

- By considering both the relative intensities and the multiplicities of the absorptions attempt to determine which groups of protons are coupled together. The magnitude of the coupling constant may give indication of the nature of the proton involved.

- By relating the information to obtain other information available on the compound under considerations.

3.3.2 EXPERIMENTAL:

NMR spectra were recorded on Bruker NMR spectro-photometer. NMR chemical shifts are recorded in δ – value using TMS as an internal standard in CdCl$_3$/D$_6$-DMSO. Typical NMR spectra are shown in figures 3.5 to 3.7. The NMR data of all the ligands are covered in results and discussion.
3.3.3 RESULTS AND DISCUSSION:

The NMR spectra of all the ligands show the following common features, while individual having additional signals are given below:

Table 3.4 NMR Spectral data of Ligands L-1 to L-12

<table>
<thead>
<tr>
<th>L-1 to L-12</th>
<th>δ ppm 6.88 to 7.8</th>
<th>Multiplet, phenyl rings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>δ ppm 2.3 – 2.5</td>
<td>CH3-C=N-</td>
</tr>
<tr>
<td></td>
<td>δ ppm 3.35-3.77</td>
<td>CH2 bridge</td>
</tr>
<tr>
<td></td>
<td>δ ppm 11.1-11.35</td>
<td>-NH-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L-7 to L-9</th>
<th>δ ppm 3.7-3.8</th>
<th>-OCH2-Phenyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-10 to L-12</td>
<td>δ ppm 3.0 – 3.2</td>
<td>-CH-(CH3)2</td>
</tr>
</tbody>
</table>

On the basis of structure of known reactants and their reactive sites, the structures of all ligands are shown as in chapter-2. The structures are confirmed by NMR spectral data shown above and typical spectra shown in Figures: 3.5 to 3.7.
Figure: 3.5

NMR spectrum of ligand L-1
Figure: 3.6
NMR spectrum of ligand L-4
Figure: 3.7

NMR spectrum of ligand L-7
3.4 LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (LC-MS):

It is unlikely that the laboratory organic chemist will be required to record mass spectra of compounds produced in the laboratory as they will normally be obtained through a centralized service.

Probably the most common use of mass spectrometry by the organic chemist is for the accurate determination of molecular weight. A second important use is to provide information about the structure of compounds by an examination of the fragmentation pattern. So for sake of convenience the selected samples have been scan for LC-MS. LC-MS of selected one sample of each series has been carried out on LC-MSD Trap-SL 01046 instrument using CH$_3$CN solvent.

On the basis of structure of known reactants and their reactive sites [8], the structures of all ligands are as shown in chapter-2. The structures are confirmed by LC-MS data and typical spectra shown in Figures: 3.8 to 3.10.
Figure: 3.8
MASS spectrum of ligand L-1
Figure: 3.9
MASS spectrum of ligand L-4
Figure: 3.10
MASS spectrum of ligand L-7
3.5 RESULTS AND DISCUSSION

Newly prepared ligands L-1 to L-12 were synthesized as shown in chapter-2 of the thesis. As we already know that the hydrazide and its derivatives are the crucial material and having broad range of biological activity. Hence, here newly prepared ligands L-1 to L-12 were characterized by elemental analysis, Infrared spectroscopy, Nuclear magnetic resonance spectroscopy and Mass spectroscopy.

Their structures were confirmed by analytical and spectral data. The C, H and N contents of the prepared compounds were consistent with their predicted structures as shown in Scheme-2.1. The infrared spectra of prepared ligands show the band in the region 1610 -1620 cm\(^{-1}\) and 1580 – 1590 cm\(^{-1}\) for \(-\text{C}=\text{O}\)- and \(-\text{C}=\text{N}\)- linkage. Also it shows broad band near 3400 cm\(^{-1}\) and \(-\text{N}-\text{N}\)- band near 1060 cm\(^{-1}\).

The nuclear magnetic resonance spectrum of the prepared ligands shows singlet at 3.36 ppm for \(-\text{CH}_2\)- bridge proton, 2.4 ppm for \(-\text{CH}_3\text{-C}=\text{N}\)- proton, 11.2 ppm for \(-\text{NH}\)- proton and 7.10 to 8.00 ppm for multiplet aromatic rings. All other signals are at their respective positions in the NMR spectrum.

The Mass spectrum of samples (i.e. Ligand L-1, L-4 and L-7) show the peak at 220.8, 207.2 and 283.0 respectively, which is consistent of molecular weight of these sample. All these facts confirm the structures of newly prepared ligands.
REFERENCES:


