Chapter-2
Characterization Techniques and Synthesis, Characterization of ethyl 4-((1H-benzotriazol-1-yl) methyl amino) benzoate (3) and 4-((1H-benzotriazol-1-yl)methylamino)benzohydrazide (4)

The present chapter comprises three sections. **Section-A** comprises characterization techniques used to characterize the produced compounds (shown in following chapters). **Section-B** comprises the synthesis and characterization of 1H-benzotriazole(1) and ethyl-p-amino benzoate(2). **Section-C** comprises the synthesis and characterization of ethyl 4-((1H-benzotriazol-1-yl) methylamino) benzoate (3) and 4-((1H-benzotriazol-1-yl)methylamino)benzohydrazide (4).

![Scheme 2.1 Image]

Scheme 2.1
Section-A

Techniques Used for Characterization of Compounds

2.1 Elemental Analysis

The majority of organic compounds are composed of a relatively small number of elements. The most important ones are: carbon, hydrogen, oxygen, nitrogen, sulphur, chlorine, etc.

Elementary quantitative organic analysis is used to determine the content of carbon, hydrogen, nitrogen, and other elements in the molecule of an organic compound.

2.2 Introduction to Spectrometry:

Fundamental to modern techniques of structure determination is the field of spectroscopy: the study of the interaction of matter and light (or other electromagnetic radiations). Spectroscopy has been immensely important to many areas of chemistry and physics. For example, much of what is known about orbitals and bonding comes from spectroscopy. But spectroscopy is also important to the laboratory organic chemist because it can be used to determine unknown molecular structures. Although this presentation of spectroscopy will focus largely on its applications, some fundamentals of spectroscopy theory must be considered first.

2.3 Infrared Spectroscopy:

Infrared spectroscopic technique [1-4] is of an immense importance to organic chemists for the identification of the presence of functional groups in the organic compounds although it does not provide the complete information regarding the molecular structure of
the organic compounds. However it is used for the identification of the compounds.

Infrared spectroscopic technique gives the information about the molecular vibrations or more precisely on the transitions between rotational and vibrational energy levels in the molecule and due to this characteristic; it is of immense help to organic chemists.

When infrared light is passed through a sample, some of the frequencies are absorbed while other frequencies are transmitted through the sample. The absorption of infrared radiation depends on increasing the energy of vibration or rotation associated with co-valent bond in a molecule.

Absorption of radiation in the infrared region results in the excitation of bond deformations, either stretching or bending. Various stretching and bending vibrations occur at certain quantized frequencies. When infrared light of that frequency is incident or impart on the molecule, energy is absorbed and the amplitude of that vibration is increased.

“An infrared spectrum is obtained when the frequency of molecular vibrations corresponds to the frequency of the infrared radiations absorbed.”

The material under study is usually in the form of a solid, a neat liquid or a solution. Sometimes, however, a compound in the gas or vapor phase is studied. Under these conditions, in addition to changes in vibrational energy, simultaneous changes in rotational energy can occur and consequently some fine structures may be observed on the vibrational band. Infrared spectrum of a compound represents its energy absorption pattern in the infrared region and is obtained by plotting percentage absorbance or transmittance of
infrared radiation as a function of wavelength or wave number over a particular range.

Infrared spectroscopy is usually divided into three regions.

- Near infrared (overtone region) – between 12500cm\(^{-1}\)-4000cm\(^{-1}\)
- Middle infrared (fundamental vibrational region) – between 4000cm\(^{-1}\)-667cm\(^{-1}\)
- Far infrared (pure rotational region) – between 667cm\(^{-1}\)-50cm\(^{-1}\)

The normal or middle infrared region is particularly meant for organic chemists since the vibrations induced in organic molecules are absorbed in this region. This fundamental vibrational region is divided into the functional group region (4000cm\(^{-1}\)-1400cm\(^{-1}\)) and finger print region (1400cm\(^{-1}\)-667cm\(^{-1}\)). The normal and far infrared regions contain absorptions due to fundamental harmonic and combination bands.

The use of linear-in-frequency instruments results in a considerable expansion of the high frequency end of the infrared region, resulting in an increased ability to resolve bands and define their positions. The position of absorption in the spectrum is usually expressed in terms of wave number (cm\(^{-1}\)) of the absorbed light.

The infrared spectrum is the simplest, most rapid and often most reliable means for assigning a compound to its class. It can also provide a variety of information on structure, symmetry, purity, structural and geometrical isomers and hydrogen bonding.
2.3.1 Anticipated Infrared Frequencies for Heterocyclic products of Aryl azo pyrazole-benzotriazole combined molecules.

The present thesis comprises the study of Aryl azo pyrazole and benzotriazole clubbed nuclei:

Hence, prior to characterize these compounds by IR spectroscopy it is necessary to predict the anticipated frequencies of each moiety.

**Aryl azo pyrazole-benzotriazole clubbed molecules:**

pyrazole is a heterocyclic compound. The bands due to –CH₂NH bridge is at nearer to 3100 cm⁻¹. The corresponding N-H in plane and out of plane bending vibrations occurs at 1630 and 699 cm⁻¹ respectively. The other band due to aromatic segments is appeared at their respective position. The bands due to –CH₂ are at 2850, 2920, 1470 cm⁻¹. The bands due to ethyl ester is appeared at near 1165 cm⁻¹ and band at near to 1685 attributed to secondary amide (-CONH) group. The other unknown bands are due to substitution in aromatic segments.

2.4 Proton Nuclear Magnetic Resonance Spectroscopy:

Nuclear magnetic resonance (NMR) spectroscopy 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 PMR 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 (µ).

The spinning nucleus of a hydrogen atom (¹H or proton) is the simplest and is commonly encountered in organic compounds. The hydrogen nucleus has a magnetic moment, µ= 2.79268 and its spin
number \((l)\) 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 \(h\nu \Delta E = h\nu\).

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 radiations 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.

2.5 Carbon-13 Spectroscopy:

Besides the PMR spectroscopy, the CMR spectroscopy is now more précised method to determine the structure or organic molecules. Considerably greater sensitivity is required for ¹³C than for ¹H due to low natural abundance of ¹³C and the lower magnetic moment compared to that of the proton. However, greater resolution is possible with ¹³C.
In contrast to $^1$H spectra, it is not possible to determine the relative ratio of carbon atoms in a compound by integration of the peak areas in the $^{13}$C FT-NMR spectrum. There are two reasons for this. The first result results from the different relaxation times of carbon atoms in different environments. This means that some atoms with long relaxation times may still be partly saturated when the next pulse of radiation is received, and the resulting absorption peak areas will not be proportional to the number of different carbon atoms. Carbon atoms without hydrogen attached have longer relaxation times and are therefore likely to give rise to peaks of lower intensity in the spectrum. The second reason is due to the Nuclear Overhauser Effect (NOE). This is the enhancement of some signals in the $^{13}$C spectrum as a result of the spin-decoupling process which is used to produce the normal, noise-decoupled spectrum by removing the interaction between carbon and hydrogen nuclei. The NOE is not the same for all nuclei. The maximum effect is for carbon atoms with hydrogen attached. The consequence is that carbon atoms without hydrogen attached appear without any NOE enhancement. As a result of these two effects, it is often possible to identify by inspection, as a result of their lower intensity, those peaks in the $^{13}$C spectrum which result from carbon atoms not attached to hydrogen, including those in aromatic rings which carry a substituent.

A considerable amount of the data is available which correlates the position of absorptions in the $^{13}$C NMR spectrum with the structure of an organic molecule, and it is these imperial correlations which provide the main basis for the use of the technique in structure determination. The values for the chemical shifts are normally related to the tetramethylsilane carbon absorption, with positive values increasing to lower field (corresponding to the $\delta$ scale in PMR spectroscopy). The vast majority of absorptions fall in a range of 200 ppm between the carbonyl absorptions at low field and the methyl absorptions at high field.
Hybridization of the carbon atom has a significant effect on the chemical shifts. sp$^3$-hybridized carbon absorbs at high field (0-60 ppm downfield from TMS), sp$^2$-carbon at low field (80-200 ppm) and sp$^1$-carbon at intermediate values. The precise position of absorption of a particular atom is largely determined by the electronic effects of any substituents, and the fact that these are approximately additive enables fairly accurate predictions of chemical shifts to be made, provided that similar compounds of known structure are available for reference purposes.

Only one of the three items of information normally available from PMR spectra (i.e. chemical shift, coupling constant and relative numbers of absorbing nuclei) is routinely available from the $^{13}$C spectrum, and that is the chemical shift. Quantitative coupling constants are not normally obtained, and relative numbers of nuclei cannot usually be derived from measurement of peak areas. The large $^{13}$C - $^1$H coupling constant (125-200 Hz for directly bonded protons) results in multiplets which overlap to a considerable extent, and in the absence of decoupling makes the spectrum difficult to analyze. Spectra are therefore normally spin-decoupled and each absorption appears as a sharp singlet; this technique is known as wide-band or noise decoupling. Although the sensitivity is thus increased, all the information normally available from spin-spin splitting pattern is lost. An alternative method of decoupling (off resonance decoupling) does however allow coupling of directly bonded carbon and hydrogen to be observed, although the separation of the peaks of the multiplets produced by this method is not equal to the true $^{13}$C - $^1$H coupling constant. It is thus possible to identify carbon atoms associated with methyl, methylene and methine groups since the absorptions appear as quartets, triplets and doublets respectively provided that the bonded hydrogen are equivalent.
2.6 Mass Spectroscopy:

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.

2.7 General Remarks for the Experimental Techniques:

- Melting points (°C) of all the compounds were measured by capillary method. All the melting points were 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).

- C, H, N and S contents of all the compounds were recorded on Thermofinigen 1101 Flash elemental analyzer.

- IR spectra were recorded in KBr pellets on Nicolet 760D spectrophotometer.

- PMR and CMR spectra were recorded on Bruker NMR spectrophotometer. PMR ad CMR chemical shifts are recorded in δ-value using TMS as an internal standard in CDCl₃/D₆-DMSO.

- LC-MS of selected one sample of each series has been carried out on LC-MSD Trap-SL 01046 instrument using CH₃CN solvent.
Section-B

2.8 Introduction:

The present section deals with the synthesis and characterization of 1H-benzotriazole (1) and ethyl-p-amino benzoate (2) by the process reported [5,6].

2.9 Materials:

All the chemicals used were of analytical grade and obtained from local market. The chemicals taken are listed in Table 2.1.

<table>
<thead>
<tr>
<th>Raw material</th>
<th>Structure</th>
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</thead>
<tbody>
<tr>
<td>1,2-phenylenediamine</td>
<td><img src="image1" alt="Structure" /></td>
</tr>
<tr>
<td>sodium nitrite</td>
<td>NaNO₂</td>
</tr>
<tr>
<td>acetic acid</td>
<td>CH₃COOH</td>
</tr>
<tr>
<td>p-amino benzoic acid</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>ethanol</td>
<td>CH₃CH₂OH</td>
</tr>
<tr>
<td>sodium carbonate</td>
<td>Na₂CO₃</td>
</tr>
</tbody>
</table>
2.10 General procedure for synthesis of 1H-benzotriazole (1)[5].

1,2-phenylene diamine (10.8g, 0.1 mol) was dissolved in mixture of glacial acetic acid (11.5ml,0.2 mol) and water(30ml) by slight warming. The mixture was col led to 15°C, stirred and a solution of sodium nitritre(7.5g,0.11mol) in water(15 ml)was added in one lot. The reaction mixture becomes warm and the tempure reaches to about 80°C. The colour changes from deep red to brown. Continue stirring for 15 minutes and the mixture cooled. The separated benzotriazole is filtered, washed with ice-cold water and crystallised from hot water.

2.11 General procedure for synthesis of ethyl-p-amino benzoate (2) [6].

Dry hydrogen chloride gas was passed into absolute ethyl alcohol (80 ml) till the alcohol was completely saturated. p-amino benzoic acid (12g,0.088 mol) was added and the mixture refluxed for 2 hrs under anhydrous conditions. The hot solution was pour into excess water (150ml) and sodium carbonate was added until the solution was neutral to litmus. The precipitated ethyl-p-amino benzoate was filtered and crystallized from R-spirit.

Table: 2.2: Characterization of 1H-benzotriazole (1) and ethyl-p-amino benzoate (2)

<table>
<thead>
<tr>
<th>No.</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>M.P. (°C)</th>
<th>Elemental analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%C</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Found</td>
</tr>
<tr>
<td>1</td>
<td><img src="image.png" alt="Structure" /></td>
<td>66</td>
<td>99</td>
<td>60.48</td>
</tr>
<tr>
<td>2</td>
<td><img src="image.png" alt="Structure" /></td>
<td>69</td>
<td>91</td>
<td>61.38</td>
</tr>
</tbody>
</table>
Section-C

2.12 Introduction:

The present section deals with the synthesis and characterization of ethyl-4-((1H-benzotriazol-1-yl) methyl amino) benzoate (3) and 4-((1H-benzotriazol-1-yl) methyl amino) benzo hydrazide (4)[7,8].

2.13 Materials:

All the chemicals used were of analytical grade and obtained from local market. 1H-benzotirazole (1) and ethyl-p-amino benzoate (benzocain) (2) were taken which was synthesis and reported in section-B also formaldehyde was taken of pure grade for synthesis.

2.14 General procedure for synthesis of ethyl-4-((1H-benzotriazol-1-yl) methyl amino) benzoate (3)[7].

A mixture of 1H-benzotirazole (1) (0.02 mole), formaldehyde (0.02 mole) and ethyl-p-amino banzoate (benzocain) (2) (0.02 mole) in ethanol (50 ml) was heated under reflux for 4 hrs. Subsequently, ethanol was distilled off and the pasty mass obtained, which was isolated, and dried to give the desired product (3).

2.15 General procedure for synthesis of 4-((1H-benzotriazol-1-yl) methyl amino) benzo hydrazide (4)[8].

ethyl-4-((1H-benzotriazol-1-yl) methyl amino) benzoate (3) (0.05 mole) was refluxed with hydrazine hydrate (0.05 mole) in absolute ethanol for 10 to 12 hours. It was cooled and kept overnight. The solid so obtained was filtered and recrystallized from ethanol to give desired products (4).
**Compound-3**

![Chemical Structure](image)

Ethyl-4-((1H-benzotriazol-1-yl) methyl amino)benzoate

<table>
<thead>
<tr>
<th>Molecular Formula: C₁₆H₁₆N₄O₂</th>
<th><strong>Elemental Analysis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight: 296 gm/mole</td>
<td>%C %H %N</td>
</tr>
<tr>
<td>Melting Point: 146°C</td>
<td>Calculated 64.85 5.44 18.91</td>
</tr>
<tr>
<td>(Uncorrected)</td>
<td>Found 64.83 5.43 18.90</td>
</tr>
<tr>
<td>Yield: 69%</td>
<td></td>
</tr>
</tbody>
</table>

**Infrared Spectral Features (cm⁻¹)**

- ~3453 -N-H of methyl amine
- ~3362 Aromatic C-H stretching
- ~1725 C=O
- ~1270 C-O

**PMR spectral Features (δ,ppm)**

- 6.89-8.12 (8H,multiplet,aromatic C-H protons)
- 4.32 (2H,quartate,-OCH₂)
- 1.32 (3H,triplet,-OCH₂-CH₃)
- 5.69 (2H Singlet,CH₂ of CH₂NH)

**¹³CMR spectral Features (δ,ppm)**

- 61.1 OCH₂
- 114-149 Benzene
- 59.6 CH₂-N
- 170.4 C of –C=O
- 13.9 CH₃
Compound-4

4-((1H-benzotriazol-1-yl)methylamino)benzohydrazide

<table>
<thead>
<tr>
<th>Molecular Formula: C(<em>{14})H(</em>{14})N(_{6})O</th>
<th>Elemental Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight: 282.34 gm/mole</td>
<td>%C</td>
</tr>
<tr>
<td>Melting Point: 121(^\circ)C</td>
<td>Calculated</td>
</tr>
<tr>
<td>(Uncorrected)</td>
<td>Found</td>
</tr>
<tr>
<td>Yield: 71%</td>
<td></td>
</tr>
</tbody>
</table>

**Infrared Spectral Features (cm\(^{-1}\))**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
<th>PMR spectral Features ((\delta,\text{ppm}))</th>
<th>(\text{^13} \text{CMR spectral Features ((\delta,\text{ppm}))}</th>
</tr>
</thead>
</table>
| ~3150   | -N H of methyl amine | 6.89-8.14 (8H, multiplet, aromatic C-H protons) | Bangene
| ~3030   | Aromatic C-H stretching | 5.69 (2H, Singlet, CH\(_2\) of CH\(_2\)NH) | 114-149.2
| ~1725   | C=O | 59.6 | CH\(_2\)-N |
| ~1280   | C-O | 170.4 | C of ~C=O |
| ~1220   | C-N | | |

Department of Chemistry, S.P.University
Fig. 2.1 IR Spectrum of Compound 3

Fig. 2.2 IR Spectrum of Compound 4
Fig. 2.3 NMR Spectrum of Compound 3

Fig. 2.4 NMR Spectrum of Compound 4
Fig. 2.5 CMR Spectrum of Compound 3

Fig. 2.6 CMR Spectrum of Compound 4
Fig. 2.7 LC-MS of Compound 3

Fig. 2.8 LC-MS of Compound 4
References