Chapter 1:

Synthesis of novel Thiosemicarbazones and their biological activities
Section 1:

Introduction
Heterocyclic compounds, a main class of pharmacologically active agents, are the basis of life and their synthesis has always been full of excitement and challenges. Nitrogen heterocycles in particular exhibit diverse biological and pharmacological activities due in part to the similarities with many natural and synthetic molecules with known biological activities. Among them heterocyclic compounds containing nitrogen atoms are considered to be one of the most effective. Development of novel, economically viable and efficient synthesis protocols for attractive heterocyclic scaffolds is perhaps the ultimate goal of synthetic organic chemists in search of new pharmaceutical lead structures. Perhaps the most widely studied application of heterocycles is the preparation of biologically active and medicinally important molecules. The successful treatment of various ailments ranging from malaria to cancer to heart disease is often triggered by the presence of various heterocyclic compounds in extracts derived from plants, animals and insects. Heterocyclic derivatives such as morphine alkaloids, β-lactam antibiotics and benzodiazepines are just a few familiar examples from various pharmaceuticals featuring a heterocyclic component. Compounds with imidazole ring systems have many pharmaceutical activities and play important role in biochemical processes.

Benzimidazole nucleus is a constituent of many bioactive heterocyclic compounds that are of wide interest because of their diverse biological and clinical applications. Moreover, benzimidazole derivatives are structural isosters of naturally occurring nucleotides, which allows them to interact easily with the biopolymers of the living system. This created interest in researchers who have synthesized variety of benzimidazole derivatives and screened them for their various biological activities. Benzimidazole derivatives have found applications in diverse therapeutic areas including anti-ulcers, anti-hypertensives, anti-virals, anti-
fungals, anti-cancers, and anti-histaminics. Moreover, these fused heterocycles have been studied as new non-nucleoside topoisomerase-I poisons, human immuno deficiency virus-1 reverse transcriptase inhibitors and potent DNA gyrase inhibitors. They can also act as ligands to transition metals for modelling biological systems. In addition to this, benzimidazoles are very important intermediates in organic reactions. Therefore; preparation of new molecules containing benzimidazole has attracted considerable attention in recent years. Benzimidazole structures are classified under several classes of drugs, based on the possible substitution at different positions of the benzimidazole nucleus.

Benzimidazole derivatives recently attracted medicinal chemists in exploring their potential as anticancer agents. Kumar et al. prepared a series of carbomethoxy-substituted benzimidazole derivatives of UK-1 [a bis (benzoxazole) natural product] (i) isolated from a strain of Streptomyces and evaluated its cytotoxicity by Alamar Blue cytotoxicity assays against MCF-7, HL-60, HT-29 and PC-3 cell lines. Pt(II) complexes bearing 2-phenyl benzimidazole, (ii), and 2- mercapto methyl benzimidazoles, (iii), were found to be cytotoxic against human RD Rhabdomyosarcoma cell line and less mutagenic against Salmonella thyphimurium strains TA 98 and TA 100, which made them to be considered as potential antitumour agents. (Figure 1)
In a study of some novel fused heterocyclic compounds as eukaryotic topoisomerase II inhibitors, it was observed that 5-methylcarboxylate-2-phenylthiomethylbenzimidazole was more active than the reference drug etoposide.\(^{21}\) (Figure 2)

1, 2- Disubstituted benzimidazoles are gifted with an extensive range of biological applications.\(^ {22}\) They have emerged as potent non-nucleoside inhibitors of HIV-1 reverse transcriptase\(^ {23}\) and specific inhibitors of the NS5B polymerase of the hepatitis - C virus (HCV).\(^ {24}\) Furthermore, they can be used as agonists against \(\gamma\)-butyric acid A receptor (GABA).\(^ {25}\) Moreover, they show potent thrombin inhibitory activity\(^ {26}\) and anti-bacterial activity against gram-positive bacteria.\(^ {27}\)

Starcevic et al. prepared a set of heterocyclic benzimidazole derivatives bearing amidino substituents at C-5 of benzimidazole ring, by introducing various heterocyclic nuclei (pyridine, N-methyl pyrrole or imidazole) at C-2 and evaluated their antiviral activity towards
coxacakieviruses and echoviruses. Fairly strong activity was observed with 2-(1-methyl-1\textsubscript{H}-pyrrol-2-yl)-1\textsubscript{H}-benzimidazole-5-carboxamidine hydrochloride, (i) and n-isopropyl-2-pyrindin-2-yl-1\textsubscript{H}-benzimidazole-5-carboxamidine, (ii) towards adenovirus, which make them to be considered as leads against adenoviral replication. \textsuperscript{28} (Figure 3)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{As Anti-viral agent}
\end{figure}

Most important use of benzimidazoles is as anti-helmentic agents. In 1960, a broad-spectrum group of benzimidazoles were discovered having specific activities against the gastrointestinal helminths. But out of several thousand compounds only three have gained enormous recognition and wide acceptance, namely: Albendazole, Mebendazole and Thiabendazole. They are widely used across the globe for the management and treatment of intestinal nematode infections. \textsuperscript{29-30}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{Anthelmentic drugs containing Benzimidazole}
\end{figure}
Recently, 2-aryl benzimidazoles have also proved their potency as Anxiolytics 31. Also, 5-substituted (amino)-2-phenyl-1-(2-carboxybiphenyl-4-yl) benzimidazoles as anti-hypertensive agents. 32 They are non-peptide angiotensin- αII receptor antagonists. Thus, Benzimidazole is an important pharmacophore in medicinal chemistry.

Thiosemicarbazones are compounds of considerable interest because of their important chemical properties and potentially beneficial biological activities. Thiosemicarbazones belong to a class of compounds that occupy a wide range of biological activities and have been studied for their activity against tuberculosis, 33 bacteria, 34 virus 35-36 and most important against various cancerous cells. According to the IUPAC nomenclature, 37 these compounds, usually obtained by condensation of an aldehyde, or a ketone with a thiosemicarbazide, may be named by adding the class name “thiosemicarbazone” after the name of the condensed aldehyde or ketone. In the same way bis(thiosemicarbazones) are derived from dicarbonyl compounds and two thiosemicarbazide moieties. The basic structure of thiosemicarbazone compounds and IUPAC numbering scheme is shown in Fig. (5). SAR studies showed that a large number of Thiosemicarbazones of a N-heterocyclic carboxaldehydes have low π-electron density at the side chain part and the ring N-atom should be reasonably a good electron pair donor to transition metals for the formation of co-ordination compounds. 38

![Figure 5: Basic structure of Thiosemicarbazone](image)

R₁, R₂, R₃, R₄ = H, Alkyl or Aryl group
Thiosemicarbazone ligands may exist as thione/thiol tautomeric forms owing to the intramolecular proton transfer, Fig. (6)

![Figure 6: Tautomeric forms of Thiosemicarbazones](image)

A review of thiosemicarbazone structures\(^\text{39}\) shows that in solid state these molecules are almost planar, with the sulphur atom trans to the azomethine nitrogen atom (configuration E). Although there are several electronic and steric factors that may contribute to the adoption of this arrangement, the most important is probably that the trans arrangement places the amine (4N) and azomethine (1N) nitrogen atoms in relative positions suitable for intramolecular hydrogen bonding. However in most of the complexes the thiosemicarbazone moiety coordinates to the metal ion in the cis configuration through the thione/thiol atom and the azomethine nitrogen atom. The coordination capacity of thiosemicarbazones can be further increased, if the parent aldehyde or ketone contains additional functional group in position suitable for chelation. Particularly, compounds in which the thiosemicarbazone side-chain is attached in position to a N-heterocyclic ring, namely N-heterocyclic thiosemicarbazones, have shown substantial in vitro activity against various human tumour lines. The (N)-TSCs possess a conjugate NNS donor set which favour the coordination to metal ions forming two five-membered chelate rings of a partially conjugate character and these particular structural characteristic seems to be essential for biological activity\(^\text{40}\). The coordination chemistry of thiosemicarbazones appear to be
very interesting from the point of view of both the number of metals forming complexes with them and the stabilization of various (less common) oxidation states of metals. Moreover many of their biological activities of the thiosemicarbazones often have been attributed to their ability of chelation with endogenous metals \(^{41-42}\). Moreover the aromatic ring can enter into the interaction with biomolecules modifying the biological activity \(^{43}\). Therefore the modification of the structure of (N)-TSC derivatives gives the possibility of synthesizing novel compounds and exploring their biological activities. The (N)-TSC skeleton can be modified around three positions, Fig. (7): the heterocyclic ring, the 4N-substituents on the thiosemicarbazone moiety and chelation with metal ions. A large number of different (N)-TSCs, introducing structural variation have been synthesized in order to verify if the change in the new structural motifs have positively modulated the biological activity.

Figure 7: Thiosemicarbazone skeleton

Some synthetic derivatives of Thiosemicarbazone already exist in market like, Triapine, Marboran, etc. \(^{44}\) Triapine is a potent ribonucleotide reductase inhibitor and used in cancer treatment. Triapine
(3-aminopyridine-2-carboxaldehyde thiosemicarbazone) (figure 8) is a tridentate chelator that ligates Fe via a sulphur and two nitrogen atoms\textsuperscript{45-46}. Triapine is one of the most comprehensively assessed iron chelators with anti-tumor activity. A recent study reported that the Triapine-Fe (II) complex was significantly more active at inhibiting ribonucleotide reductase than free Triapine\textsuperscript{47}. Marboran is a good Anti-viral agent. It has activity against pox viruses, Maloney leukaemia viruses and recently against HIV\textsuperscript{48}. Thiosemicarbazones are also used as the inhibitors of Topoisomerase-II which is an important enzyme in DNA replication process\textsuperscript{49}. Thiosemicarbazones have all the activities due to their chelating property, and with the consideration that recently some thiosemicarbazones having anti-malarial activity have been synthesized\textsuperscript{50}.

![Triapine and Methisazone](image)

Figure 8: Some Thiosemicarbazone drugs

The recent investigations have proved that thiosemicarbazone derivatives containing chitosan, a polysachcaride have the good anti-oxidant activity. The 2-[hydrazine thiosemicarbazone] - chitosan is a potential anti – oxidant which prevents the oxidation of the cellular oxidisable substrates\textsuperscript{51}.
Figure 9: Anti-oxident derivative of thiosemicarbazone

The discovery of the anti-tuberculosis activity of a member of benzaldehyde thiosemicarbazones was first announced by Dogmak and his co-workers in 1946. Their results have been confirmed experimentally as well as clinically by a number of researchers. Unfortunately, the more recent articles have shown that the thiosemicarbazones so far developed clinically may produce serious toxic effects which suppressed their usefulness. Therefore, satisfactory chemotherapy of tuberculosis may result from the combination of thiosemicarbazones with other drugs 52.

As concerns malaria, Biot et al. report the design, synthesis, and antimalarial activity of chimeras of thiosemicarbazones and ferroquine (Figure 10). The authors started from the known potent antimalarial activity of thiosemicarbazones, which were in the past abandoned for the heavy side effects. Then, they synthesize molecules in which a ferroquine, another molecule recently discovered with antimalarial properties, was inserted. The compounds were tested against four strains of the malaria parasite Plasmodium falciparum and against the parasitic cysteine protease falcipain-2. The major contributor to the antimalarial activity seems to be the aminoquinoline thiosemicarbazone part. The most active derivatives against all strains of P. falciparum were the chimeras of thiosemicarbazones and ferroquine analogues but in some cases also the corresponding organic derivatives were similarly active 53.
Figure 10: Chimeras of Thiosemicarbazones and Ferrocene as antimalarial agents
**General methods of preparation of Benzimidazole:**

Benzimidazoles and Thiosemicarbazones can be synthesized by conventional heating methods as well as by green chemistry methods.

- **Conventional methods:**
  
  There are many synthetic processes available in the literature.

1. One of the most common way to synthesize Benzimidazole is condensation of an arylene diamine with a carbonyl compounds like acid, ester, aldehydes, etc.\(^{54}\)

   ![Condensation reaction](image)

2. A convenient method for the synthesis of 2-substituted benzimidazoles and benzothiazoles offers short reaction times, large-scale synthesis, easy and quick isolation of the products, excellent chemoselectivity, and excellent yields as main advantages\(^ {55}\).

   ![Synthesis of 2-substituted benzimidazoles](image)

3. A NaH-mediated reaction of carbonitriles and 1, 2-phenylenediamine allows the formation of Benzimidazoles\(^ {56}\).

   ![NaH-mediated reaction](image)
4. 1,2- disubstituted benzimidazoles can also be synthesized by direct one-step condensation of o-phenylenediamines with aldehydes under the influence of different acid catalysts such as Alumina Methanesulfonic acid (AMA) \(^{57}\), Poly Phosphoric Acid (PPA), \(^{58}\) etc.

\[ \begin{align*}
\text{NH}_2 & \quad \text{NH} \\
\text{R}_1 & \quad \text{O} \\
\end{align*} \]

5. Ion-exchange resins like Amberlite IR-120 are also used as catalyst in benzimidazole synthesis \(^{59}\). Also, Alcohols can be converted into benzimidazoles by transition metal catalysed hydrogen transfer reactions. Ruthenium (Ru) and Iridium (Ir) are used as catalysts \(^{60}\).

\[ \begin{align*}
\text{R} \quad \text{OH} & \quad \text{NH}_2 \\
\text{NH}_2 & \quad \text{O} \\
\end{align*} \]

6. Thiosemicarbazones can also be synthesized by the condensation of the aldehyde and the thiosemicarbazide in the presence of methanol \(^{61}\).

\[ \begin{align*}
\text{NH} & \quad \text{S} \\
\text{R} \quad \text{OH} & \quad \text{NH} \\
\end{align*} \]

7. Thiosemicarbazides can be prepared by isothiocyanates and hydrazine. This is a general method for the preparation which is modified for the synthesis of various substituted thiosemicarbazones \(^{61-62}\). Thiosemicarbazides then reacts with carbonyl compounds and forms the final product.
**Green Chemistry Methods:**

Green Chemistry is the utilization of a set of principles that reduces or eliminates the use or generation of the hazardous substances in the manufacture of chemical products. So, any method which does not involve the use of harmful chemical reagents and solvents is classified under the green methods. Nowadays, Industrial chemistry is widely adopting the “Green Concept” to protect the human health and environment\(^6^3\).

One of the thrust areas for achieving this target is to develop alternative reaction pathway and medium to accomplish the desired transformation with minimal by-products as well as reduction in the use of conventional organic reagents. Among those developing methods the use of microwaves as an energy source for the reaction is becoming an attractive alternative. Microwave heating in the laboratory began to gain wide acceptance following papers in 1986, although the use of microwave heating in chemical modification can be traced back to the 1950s\(^6^4\). After that many research articles have appeared proving the utility of microwave induced organic reaction enhancement (MORE) in regular organic synthesis.

**Principle:**\(^6^5\)

Microwave irradiation is electromagnetic irradiation in the frequency range 0.3 to 300 GHz corresponding to wavelengths of 1 mm to 1 m. The microwave region lies between the infrared and radio frequencies. Wavelengths between 1 and 25 cm are mostly used for RADAR transmissions and the remaining is used for telecommunications. All the domestic microwave ovens and microwave reactors for chemical synthesis...
that are available today operate at a frequency of 2.45 GHz (12.2 cm) in order to avoid the interference with telecommunications, wireless networks and cellular phone networks.

Figure 11: Electric and magnetic components in the microwaves.

Microwaves are electromagnetic waves which consist of an electric and magnetic field component perpendicular to each other, (Figure 11), though only the electric field transfers energy to heat a substance. Any interaction from the magnetic field is insignificant. Microwave chemistry involves ability of a specific material (solvent or reagent) to absorb microwave energy and convert it into heat. This is known as Dielectric heating. There are two fundamental processes by which the energy is transferred into heat. They are as follows:

1. Dipolar polarization and 2. Ionic conduction.

Dipolar polarization is due to interaction in which polar molecules or species try to align with the rapidly changing electric field of the microwave. The motion of the molecule as it tries to align the field results in energy transfer. The ability of this mechanism depends on the polarity of the molecules and their ability to align with the field follows
relaxation to their initial state. In short, any polar substances which are present will undergo this mechanism of energy transfer.

The second pathway of energy transfer is by ionic conduction. This mechanism will occur if there are free ions present in the species being heated. The electric field will generate ionic motion of the charged particles which try to align the field. This phenomenon will cause the rapid heat transfer in the reaction.

Generally, heat transfer by ionic conduction is more than dipolar polarization.

**Energy Transfer Medium:**

In MORE technique, organic solvents serve as an energy transfer medium. Any solvent having high dielectric constant and high B.P. is excellent transfer medium for a variety of microwave induced organic reactions. High boiling solvents like N, N-dimethyl formamide (DMF), O-dichlorobenzene, 1, 2-dichloromethane are used commonly. Polar solvents with high dielectric constants absorb microwave energy better than non-polar solvents due to dipole rotation and are therefore, heated rapidly with higher energy transfer rates. Thus DMF and dichloromethane are heated much faster than hexane or carbon tetrachloride in a microwave oven. Superheating of liquids is common under microwave irradiation. Water for example reaches 105°C (5° C above actual boiling point) and acetonitrile reaches 120°C amazing 38°C higher than its boiling point. This superheating, which is not commonly seen in conventional heating, may help in increasing the rate of reaction. Rate of temperature increase is not only a function of dielectric properties but also the ionic strength, specific capacity, emissivity, geometry, sample volume and strength of the applied
field. In practice, and as general route, almost all types of organic reactions that require heat can be performed using microwaves.

Fluid salts or ionic liquids, consisting entirely of ions, absorb microwave radiation in a highly efficient manner and are particularly attractive additives because they are relatively inert and stable at temperature up to 200 °C, have a negligible vapour pressure 220-221 and dissolve to an appreciable extent in a wide range of organic solvents. Energy transfer between the polar molecules that couple with the microwave radiation and the non-polar solvent bulk is rapid and often provides an efficient mean of using non-polar solvents for synthesis using microwave irradiation.

Boiling points and dielectric constants of commonly used solvents are listed in table 1.

**Table 1: Dielectric constants and boiling point of different solvents**

<table>
<thead>
<tr>
<th>ETM</th>
<th>BP(°C)</th>
<th>DEC</th>
<th>ETM</th>
<th>BP(°C)</th>
<th>DEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100.0</td>
<td>78.50</td>
<td>Pyridine</td>
<td>115.5</td>
<td>12.30</td>
</tr>
<tr>
<td>Dimethyl sulphoxide</td>
<td>189.0</td>
<td>46.60</td>
<td>1,2-Dichloromethane</td>
<td>83.5</td>
<td>10.65</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>124.0</td>
<td>38.66</td>
<td>Ethyl acetate</td>
<td>77.1</td>
<td>6.02</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>82.0</td>
<td>37.50</td>
<td>Chlorobenzene</td>
<td>132.1</td>
<td>5.62</td>
</tr>
<tr>
<td>Diethyl formamide</td>
<td>153.0</td>
<td>36.71</td>
<td>Chloroform</td>
<td>61.7</td>
<td>4.81</td>
</tr>
<tr>
<td>Methanol</td>
<td>64.7</td>
<td>32.70</td>
<td>Diethyl ether</td>
<td>34.6</td>
<td>4.34</td>
</tr>
<tr>
<td>Ethanol</td>
<td>78.4</td>
<td>32.40</td>
<td>Toluene</td>
<td>110.6</td>
<td>2.38</td>
</tr>
<tr>
<td>Diethyl glycol</td>
<td>244.8</td>
<td>31.70</td>
<td>Benzene</td>
<td>80.1</td>
<td>2.27</td>
</tr>
<tr>
<td>Acetone</td>
<td>56.5</td>
<td>20.70</td>
<td>1,4-Dioxane</td>
<td>101.1</td>
<td>2.21</td>
</tr>
<tr>
<td>1-Butanol</td>
<td>117.7</td>
<td>17.80</td>
<td>n-Hexane</td>
<td>68.7</td>
<td>1.89</td>
</tr>
</tbody>
</table>

Where, ETM-Energy Transfer medium; BP-Boiling Point (°C); DEC-Dielectric constant (at 20/25°C).
On the basis of above principles, benzimidazole and thiosemicarbazones are synthesized with the help of microwave irradiation. Some of the methods are mentioned below:

1. Benzimidazoles are formed by condensation of 1, 2 – diamines with esters in the presence of ethylene glycol under microwave radiation

\[
\text{ benzimidazoles } + \text{ esters } \rightarrow \text{ benzimidazoles }
\]

2. Benzimidazoles are also generated by the reaction between carbonyl compounds and areylene diamine in the presence of acid catalysts like Poly Phosphoric Acid (PPA), Alumina Methanesulfonic Acid (AMA) etc. under the microwave radiation.

3. Recently, benzimidazoles were synthesized via cyclization of o-Pheneylenediamines by CO\textsubscript{2} in the presence of H\textsubscript{2}. Benzimidazoles were obtained in excellent yields using RuCl\textsubscript{2}(dppe)\textsubscript{2} as catalyst.

4. Thiosemicarbazones are also synthesized with the help of microwave by the condensation of the aldehydes and corresponding thiosemicarbazide.
5. Apart from the microwaves other methods are also used for the synthesis via green chemistry route by the use of ionic liquid as catalysts. Ionic liquids like imidazolium IL (butylimidazolium tetrafluoroborate, \([\text{Hbim}]\text{BF}_4\)) as medium\(^70\). Prolinium Nitrate \([\text{[Pro]}\text{NO}_2]\) can also be used as catalyst. This process is eco-friendly because ionic liquids are regenerated and can be reused\(^71\). They are rapidly used because of their inertness and high thermal stability up to 200 °C.

\[
\begin{array}{c}
\text{O} & + & \text{S} \\
\text{HN} & & \text{NH} \\
\text{HN} & & \text{NH} \\
\end{array}
\xrightarrow{\text{ethanol}}
\begin{array}{c}
\text{R} & \sim & \text{N} & \equiv & \text{NH} \\
\text{H} & & & & \text{NH}_2 \\
\end{array}
\]

\[
\begin{array}{c}
\text{NH}_2 & + & \text{H} & \xrightarrow{\text{[Pro]}\text{NO}_2} & \text{N} \\
\text{NH}_2 & & \text{H} & & \text{H} \\
\end{array}
\xrightarrow{\text{MW}}
\begin{array}{c}
\text{N} \\
\text{H} \\
\end{array}
\]

- **Comparision between conventional heating and microwave heating:**

  From the above discussion some of the points can be concluded, which clearly show the superiority of microwave method over the conventional methods which are cumbersome, more time consuming and hazardous to the environment. While the microwave method offers a simple, non-conventional route.

  - Their highly accelerated reaction rate is the main advantage which helps to carry out the reaction in much lesser time and in good yields.
  - Recent modifications in microwave technique have increased safety and practical usage of microwave in the laboratories.
In conventional methods, complex apparatus, longer heating times, large volume of solvents are required which have no control over energy output. Such conditions do not arise in the microwave method.

Solvents used in organic reactions are a major cause of pollution, many of which are proved as carcinogenic, mutagenic and allergen. But MORE is an Eco-friendly method, since it requires no solvents or very little solvent as medium. Very short duration of reaction time also results into less evaporation of the solvents and thereby preventing pollution.

Comparisons between energy transfers can be understood with the help of (Figure 11). It shows that in conventional heating heat transferred from the outside only, while microwave penetrates into the molecule deeply and creating rapid changing fields resulting into rapid heat transformation.

![Figure 12: Comparisons between energy transfers](image-url)
**General Reaction Scheme:**

Thiosemicarbazone derivatives have been widely studied for their importance in the field of pharmaceutical development. Generally their derivatives are water insoluble but if we introduce benzimidazole moiety in them their solubility increases in water. The reason between this phenomenon is that one extra hetero nitrogen is added into the molecule and due to that hydrophilicity is increased by formation of H-bonds. So, the plan of work is to explore the above compounds by synthesizing various derivatives. (Figure 8) (A)

![Chemical Structure](image)

Figure 13: Structure of the final compound which is modified by different substituents.