CHAPTER I

General Introduction and Review of Literature
1.1 Overview of Heterocyclic Compounds:

History of heterocyclic compounds starts with early nineteenth century along with the progress of organic chemistry. The major developments and expansion of the research in the field of heterocyclic chemistry has taken place during the time of II World War, because of development of the new drug molecules to imperative cure of the diseases. Among 50% of the chemical compounds recorded in the chemical abstracts contains the heterocyclic fragments in their structure. In the pharmaceutical industry, over 75% of the top two hundred drugs are from heterocyclic family.

A heterocyclic compound is one which contains cyclic ring made up of more than one kind of the atoms. In some of the cyclic compounds like benzene, naphthalene, cyclohexanol etc, the rings are made up of only carbon atoms, such compounds are called homocyclic compounds. If the rings contain other kinds of atoms, in addition to carbon atom like Nitrogen, Sulfur, Oxygen, etc. these compounds generally consist of small (3 and 4-membered) and common (5 and 6-membered) ring systems, which are called as heterocyclic compounds. The presences of the heterocyclic compounds are widespread in nature and available from both natural and synthetic sources. They have attained a lot of importance, because, they are associated with the wide spectrum of the physiological activities in the biological world. Because of their applications in the field of Medicine, Pharmacy, Agriculture and other industrial applications, they became hottest and exponentially growing branch of the science.

There is a vast distribution of the heterocyclic compounds in natural compounds like alkaloids, terpenoids, flavonoids etc, and also a major component in the living systems like nucleic acids, vitamins, enzymes, coenzymes, ATP, sarotin, heme, chlorophylls etc. Many of the heterocyclic compounds form the stable complexes with the metals and these complexes have significant role in the biochemical processes like, Heme, Chlorophyll, etc. The compounds derived from the heterocyclic moiety play an important role in the modern drug discovery, which are extensively applied in the field of medicinal chemistry, because of their varied biological activities like antiviral, anticancer, antimicrobial, anti-tuberculor, anti-malarial, anti-oxidant etc. Hence, heterocyclic compounds became most important, over the organic compounds, because they exhibit lower toxicity to the human cell lines.
1.2 Fused Heterocyclic Compounds:

In several compounds, benzene is fused with the five-membered heterocyclic systems such as Indole, Benzothiazole, Benzimidazole, Benzoazazole (fig 1.1), which have been synthesized and studied extensively, because of their pharmacological activities.\(^1\)

![Image of fused heterocyclic compounds](image)

Figure 1.1. Fused heterocyclic compounds

1.3. Introduction to Benzothiazoles:

Benzothiazole (1, 3-benzothiazole) is one of the heterocyclic compounds, weak base and having varied biological activities. They are rarely available in nature, from the source of marine or terrestrial organisms as a natural compound. Benzothiazole analogues are present in the aroma of the tea leaves and also found in flavor component produced in fungi \textit{viz. Aspergillus clavatus, Polyporous frondosus etc.}\(^2\-3\)

Benzene ring is fused with thiazole at 4, 5 positions to form the basic structure of benzothiazole.

![Image of numbering of atoms in benzothiazoles](image)

Figure 1.2. Numbering of atoms in benzothiazoles

The two rings together constitute the basic nucleus of 1, 3 benzothiazole.\(^4\) The resultant compound is a planar and the various positions of the atoms are numbered in
such a way that, sulfur atom is numbered 1, followed by methylene carbon atom and then nitrogen of the ring as represented in fig 1.2.

During the last decade, the structure activity concept has been emerged as central concept for the new drug discovery and is a fruitful approach to understand the relationship between the structure of synthesized chemical compound and their affinity towards the biological systems. Benzothiazole is such a model structure and has wide spectrum of activity for the diverse biological receptor and hence, it became the most valuable scaffold and tailing fragment for the design and synthesizing the target based drugs.\textsuperscript{5-6} The diversified molecular structure of the benzothiazole and remarkable biological activity makes one of the important compounds in heterocyclic chemistry and has received overwhelming response.\textsuperscript{7}

The unique methyne center present in the thiazole ring makes benzothiazole as the most important heterocyclic compound and some of the compounds containing benzothiazoles are also found in nature. This electron-withdrawing moiety is thermally stable with diversified applications in various fields of chemistry, such as thioflavin used as a coloring agent and some benzothiazole moieties are used as drugs in pharmaceuticals, for example Riluzole. During the vulcanization of rubber, 2-mercaptobenzothiazole has been used as the accelerator and the ring is a potential component in nonlinear optics (NLO). Benzothiazole molecules are capable of binding multiple receptors with high affinity, because presence of the sulfur and nitrogen hetero-atoms in thiazole unit.\textsuperscript{8} These types of the Nitrogen and Sulfur containing heterocycles play important roles in not only life science but also in other industrial sector related to speciality and fine chemicals.

1.4 Benzothiazoles of Biological interest:

In 1950’s a number of 2-amino benzothiazoles were intensively studied as central muscle relaxants. When the pharmacological profile of Riluzole [6-(trifluoro methoxy) benzothiazol-2-amine], as a Glutamate neurotransmission inhibitor was discovered then, biologist’s attention was drawn to this series. After that, benzothiazole scaffolds were extensively synthesized, studied for the wide variety of biological activities and found the diverse chemical reactivity and broad spectrum activity of benzothiazole moiety.
Riluzole (Rilutek) is a drug used to treat amyotrophic lateral sclerosis marketed by Sanofi-Aventis. It delays the onset of ventilator-dependence or tracheostomy in selected patients and may increase the survival period for three to four months.

1. Riluzole

2-Mercaptobenzothiazoles are used in polymer chemistry, dyes, drugs and accelerators of rubber vulcanization and as corrosion inhibitor.

2. 2-Mercaptobenzothiazole

Luciferin is a bioluminescent compound having benzothiazole derivative in the structure and found in fireflies. On oxidation of luciferin in presence of luciferase enzyme, produces oxyluciferase and liberates energy in the form of light.

3. Luciferin

Thioflavin T is a benzothiazole salt, which is commonly used for visualizing and quantification of the misfolded proteins in the tissues.

4. Thioflavin T

Lubeluzole (Prosynap) is a drug which acts as an indirect NMDA (N-Methyl-D-aspartate receptor) antagonist. It prevents the release of glutamate, inhibits nitric oxide synthesis and blocks Calcium and Sodium ion channels. It has neuroprotective
effects particularly in hypoxic conditions that would be effective for preventing damage from acute stroke.

5. Lubeluzole

2-Thiocyano methyl thiobenzothiazole (TCMTB) has applications in the treatment of soil and seeds against various diseases of field crops, treatment of certain vegetables and ornamentals and also used as a substitute for chlorophenols in wood preservation and leather production.

6. 2-Thiocyano methyl thiobenzothiazole

Methabenzthiazuron (MBTU) is one of the benzothiazole analogues and used as herbicide for the winter corn crops and also is used as an active ingredient for commercially available Tribunil and Ormet. The above compound is specialty chemical in slimicides, which is used in the paper and pulp industry.

7. Methabenzthiazuron (MBTU)

2-Amino benzothiazole is the starting material for the manufacture of the dyes, its azo derivatives show the intense color, so used as coloring agent. 2-substituted derivatives constitute a large number of xenobiotics.

Benzothiazole derivatives are used in all the branches of Chemistry, in polymer chemistry it catalyses the formation of sulfide linkage between the unsaturated
elastomeric polymers, the obtained polymer was more flexible and larger cross-linked material, as compared to the synthesized by other conventional methods.\textsuperscript{13}

![Popular benzothiazole molecules](image)

**Fig 1.3. Popular benzothiazole molecules**

### 1.5 Objectives:

- Synthesis of benzothiazole and their derivatives with structure modifications by appropriate methods.
- Synthesis of benzothiazole derivatives with aromatic and heterocyclic substituents.
- Characterization of synthesized compounds through spectroscopic techniques, \textit{viz.}, IR, NMR, Mass Spectroscopy.
- To evaluate the synthesized compounds for their biological activities.

### 1.6 Chemistry of Benzothiazoles:

In 1887, mercapto benzothiazole was synthesized by A.W. Hofmann by the reactions of carbon disulfide on $o$-amino thiophenol. Because of the simple cyclization, a
number of methods have been reported and updated with different catalysts and different reaction conditions. 2-Substituted benzothiazoles can be synthesized by condensation reaction of o-amino thiophenol with substituted carboxylic acid, aldehydes, nitriles or acid chlorides with different cyclization reagents such as I₂, ZrOCl₂•8H₂O, TMSCl, PCC, Cerium Ammonium Nitrate (CAN), Br₂, nano ceria, boron trifluoride etherate etc.¹⁴

\[
\text{NH}_2\text{SH} + \text{R-FG} \rightarrow \begin{array}{c}
\text{N} \\
\text{S} \\
\text{R}
\end{array}
\]

where R- acids, aldehydes, nitriles, imidates, orthoesters, anhydrides or lactones groups
where (a) Strong acids/ milder reagents / oxidative reagents / different catalyst.


2-Aryl substituted benzothiazoles were effectively synthesized by the Jacobsen cyclization method based on potassium ferricyanide radical cyclization of benzanilides.¹⁵ Even microwave assisted methods also developed for synthesis of benzothiazole from the o-aminophenol with p-chloro cinnamaldehyde and reactions of o-aminothiophenol with β-amino thiophenol, reactions of dimethylsulfide with o-aminophenol were carried out for the synthesis of benzothiazole, radical cyclization of benzyne intermediates and Grignard reactions of aryl thiocyanates.

\[
\begin{array}{c}
\text{R}^1 \text{N} \text{O} \\
\text{R}^2 \text{N} \text{O}
\end{array} \rightarrow \begin{array}{c}
\text{R}^1 \text{N} \text{S} \\
\text{R}^2 \text{N} \text{S}
\end{array} \rightarrow \begin{array}{c}
\text{N} \\
\text{S}
\end{array}
\]

where (b) Lawesson's reagent, C₆H₅Cl, (c) K₃Fe(CN)₆, aq. NaOH, 90 °C

Scheme 1.2. General methods for synthesis of benzothiazole aryl derivatives.

**Cuputo et. al.,** (2011) synthesized two sets of benzothiazole derivatives having aryl amide substitution on C-2 carbon atom or an aryl urea substitution. In the synthesized compounds, five of them showed the in-vitro anticancer activity. 6-Trifluoro methoxy
and para substituted compound & p-cyano bearing moiety marked for the enhanced activity against Leukemia & Melanoma cell lines at $10^{-5}$ M concentration.\(^\text{16}\)

![Diagram of compound synthesis](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OCH$_3$</td>
<td>2-F</td>
<td>6-F</td>
</tr>
<tr>
<td>2</td>
<td>OCF$_3$</td>
<td>4-F</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>OCF$_3$</td>
<td>4-OCH$_3$</td>
<td>H</td>
</tr>
<tr>
<td>4</td>
<td>OCF$_3$</td>
<td>4-CN</td>
<td>H</td>
</tr>
<tr>
<td>5</td>
<td>OCF$_3$</td>
<td>2-F</td>
<td>6-F</td>
</tr>
</tbody>
</table>

where (i) KSCN, CH$_3$COOH, Br$_2$ (ii) aryl chloride NaH, DMF (iii) aryl isocyanate, CH$_2$Cl$_2$

**Scheme 1.3. Synthesis of Benzothiazole aryl amide and aryl urea derivatives**

Benzothiazole guanidine propanoic acid derivatives and their Schiff’s bases were synthesized by [Venkatesh & Tiwari, (2011)](#) and evaluated their cytotoxicity against the HeLa cell lines and antimicrobial activity.
In the synthesized compounds, 3-[3-(6-hydroxy benzothiazole guauidino) propanoic acid showed remarkable (IC$_{50}$ = 1.8 µM) HeLa cytotoxicity activity. Sulfonamide & Bromo substituted compound showed MIC up to 6.25 µg/mL for antimicrobial activity. The compounds exhibited superior activity for *vibrio cholera, monascus purpureus* pathogen.$^{17}$

**Chauhan et. al.,** (2015) synthesized a series 4-formyl pyrazole substituted 6-chloro benzothiazole by Vilsmeier Haack cyclization reaction. The synthesized compounds exhibited antimicrobial activity, MIC at 100 µg/mL and *p*-hydroxy substituted compounds had inhibition ability up to 94% compared to the standard.$^{18}$
where (i) glacial acetic acid, KSCN, Br₂ stirring 10 hrs, b) NH₂NH₂, H₂O, ethylene glycol, reflux for 3 hrs, (iii) ethanol, reflux 5 hrs, (iv) DMF, POCl₃, Microwave Irradiation.

Yea⁶ et. al., (2012) synthesized Azo-bridged benzothiazole ester derivatives and studied their anisotropic properties. Here, aliphatic carboxylic acids were employed for the synthesis of ester derivatives and the resultant compound exhibited the liquid crystal nematic isotropic behavior.

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**Scheme 1.6. Synthesis of Benzothiazole guanidine derivatives**

1.6 Pharmacological activities of the Benzothiazole analogs:

In olden days, natural substances were used for their nutritional value and also for the treatment of diseases. But drawback of the natural substances is that of toxic or lethal effects. From the 19th century, new methods were brought by the treatment of diseases with synthetic drugs, even though the modification of the natural products through the synthetic process gives the useful semi synthetic drugs. The improvement of the life
style has been greatly influenced by the advancement in the field of medicinal chemistry, which makes much advanced invention in synthetic chemistry.

The investigation and development of the new molecules have the main aim to search the better activities in lower concentrations. Now-a-day’s problems with multi-drug resistant micro-organisms have reached an alarming level in many countries. So, in recent years, research is taking place to develop the drug molecule with the activities for several microorganisms. So, we have discussed the comprehensive overview in the development of benzothiazole derivatives based on their role in medicinal chemistry and also explained the Structure Activity Relationship to understand the molecular diversity.

1.6.1. Benzothiazole as Antimicrobial agent:

Certain species of microorganisms capable of causing several diseases and infections to animals like amoebiasis, typhoid, malaria, common cold, cough, tuberculosis, influenza, syphilis, AIDS etc, which threaten the mankind, is a matter of scientific concern. In the history of medicinal chemistry, many of the attempts have been made to develop new structural models for the invention of more effective antimicrobials, but still, benzothiazole derivatives remain as one of the important compounds against microbes.\(^{20}\)

Sutoris and co-workers (1977) synthesized the 2- and 2, 6- di substituted thiobenzothiazoles which were found to exhibit good antimicrobial activity against the non-specific bacterial flora, mycobacteria and pathogenic fungi. The remarkable activity was found in 2- allyl benzothiazole, 2-allyl-6-nitro benzothiazole and 2-allyl, 6-nitro benzothiazole derivatives.\(^{21}\)

Some 6-fluoro-7- (substituted) sulfonamido benzothiazole derivatives were synthesized by Sreenivas et. al., (1998) and screened for a series of bacterial and fungal strains and majority of the compounds exhibited moderate activity against \(S.\) \(aureus, S.\) \(albus\) and \(C.ablicans.\)^{22}
Gopkumar et al., (2001) synthesized the benzothiazolyl carboxamido pyrazoline derivatives. The derived compounds were tested for anti-microbial activity. SAR studies revealed that, compound with chlorine and p-methoxy phenyl group, showed remarkable activity for *S. aureus* and compound with methyl and p-methoxy phenyl group, ranked for the activity against *S. aureus, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis*.  

2-(3, 4-Difluoro-benzyl sulfanyl)-4-fluoro benzothiazole was synthesized by Huang et al., (2006) and exhibited very good antifungal activities against *R. solani, B. cinereapers* and *D. gregaria* for a series of polyfluorinated 2-benzyl thiobenzothiazoles.  

Bhusari et al., (2008) synthesized the Benzene sulfonamide conjugated benzothiazole derivatives as antimicrobial and anti-TB agents. The compounds containing chloro and carboxyl substitutions marked for the antimicrobial activity
against *B. subtilis* and *E. coli*. The chloro and methoxy substituted derivatives acted as antifungal agents against *C. albicans*. The compounds with Chloro and Bromo acted as more potent than the compound with nitro group for the anti-TB activities.25

Argyropoulou *et al.*, (2009) synthesized benzothiazole containing phenyl sulfonamide at C-2 position and assayed for their *in-vitro* antimicrobial assay for some of the selected microorganisms, which have remarkable antimicrobial activity against the gram positive bacteria and showed the MIC values over the range of 0.3-100 µg/mL. Here, synthesized compounds were most sensitive to *Bacillus subtilis*. Here, benzothiazole sulfonamide with nitro- and amino- groups at the fourth position of the phenyl ring were the more potent compounds of the series.26

Bondock *et al.*, (2009) synthesized the pyrazole, isoxazole and pyridine derivatives incorporating benzothiazole moiety and resultant compounds were tested for the *in-vitro* antimicrobial assay of gram positive, gram negative and fungi species. Here, majority of the compounds exhibited moderate to excellent activities, with MIC over the range of 3.125-100 µg/mL. Incorporating benzothiazole to the pyrimidine derivatives through the acid amine coupling produces enhancement of the activity against gram-positive bacteria. Some of the pyrazole conjugated benzothiazole derivatives also showed remarkable activities compared to the standard.27
**Soni et. al.,** (2010) synthesized the Schiff’s bases of triazole conjugated benzothiazoles and screened for a series of microorganisms. The results reveal that, compound with 4-hydroxy, 4-dimethyl amino, 3,4- dimethoxy groups on the aromatic ring showed better antimicrobial activities among the synthesized compounds. The activities go on decreasing with the 2-nitro, 3-nitro and 2-cloro groups on the benzene ring. In other words, electron donating groups on para position show enhanced activity and electron withdrawing groups decrease the activity. 

**Sharma et. al.,** (2010) synthesized the benzothiazole pyramidino derivatives and investigated against a series of microorganisms.

The fusion of the benzothiazole and pyrimidine resulted in the formation of a new heterocyclic systems and showed remarkable activity towards the *B. coagulans, P.*
aeruginosa, and S. aureus. From the synthesized compounds, 6-methoxy substituted derivatives showed enhanced activity with MIC value 25 µg/mL.29

Saeed et al., (2010) synthesized the benzothiazole thiourea derivatives and screened for antimicrobial and anticancer activities. The synthesized compounds exhibited a wide range of activities for the tested microorganisms, where some of the compounds had the superior activity on the fungi than bacteria. Benzothiazole has nitro group on 5th position and has comparable MIC value of 10 µg/mL for bacteria and 5 µg/mL for fungi. SAR study reveals that, the derivatives with conjugation of thiophene and morpholine enhance the activity compared to the derivatives without substitution and also observed that, presence of electron withdrawing groups on benzothiazole enhance the activity in the present case. These molecules were also evaluated for their MTT based cytotoxicity assays and showed most potent activity for MCF-7 and HeLa cells, IC50 values were over the range of 18-46 µM.30

\[
\begin{align*}
\text{MIC (E. coli)} & = 15 \text{ µg/mL} \\
\text{MIC (C. glabrata)} & = 10 \text{ µg/mL} \\
\text{MIC (C. glabrata)} & = 5 \text{ µg/mL}
\end{align*}
\]

Rao et al., (2010) synthesized the 6-methoxy, 2-amino benzothiazole phosphonate derivatives by microwave assisted one pot reaction, as an antimicrobial and antioxidant agent. Substitution of the nitro and bromo on phosphonates of the phenyl group had more activity compared to the standard Penicillin.

\[
\begin{align*}
\text{IC}_{50} (\text{HeLa}) & = 46.46 \text{ µM} \\
\text{IC}_{50} (\text{HeLa}) & = 18.10 \text{ µM}
\end{align*}
\]
MIC (B. subtilis) = 50 µg/mL  
MIC (S. aureus) = 3.125 µg/mL  
Anti-oxidant IC₅₀ = 65.26 µg/mL

Thiophene and n-butyl phosphonate, compounds recorded the lower activity. Antioxidant property of the compounds was determined by ferric thiocyanate method and majority of the derivatives exhibited excellent activity, IC₅₀ values were over the range of 86 µg/mL to 96.8 µg/mL concentrations.³¹

Some of the hydrazine 2-substituted benzothiazole derivatives (Alang et al., 2010) showed better antifungal activity and the compounds with halogen substitution in ortho position showed the enhanced activity, the activity decreased with methoxy substitution in para position.³²

Singh et al., (2013) synthesized a series of triazole conjugated benzothiazole derivatives and evaluated their antimicrobial activities. Compound 29 had evidence for maximum activity against all bacterial strains with MIC values over the range of 1.56-12.56 µg/mL, which had two fold efficiency compared to standard drug Ciprofloxacin (MIC 6.25 µg/mL). Compound 30 was most active against fungal species with MIC values over the range of 1.56-12.5 µg/mL. SAR study of the series of compounds revealed that, the presence of electron withdrawing group flouro, chloro and presence of both showed the superior activity compared to the presence of electron donating groups such as methyl, methoxy and phenyl groups.³³
MIC (S. aureus) = 3.12 µg/mL  
MIC (C. neoformans) = 1.56 µg/mL  
MIC (E. faecalis) = 3.12 µg/mL

1.6.2 Benzothiazole as anticancer agent:

One of the major goals of the medicinal chemistry is to treat the life-threatening cancer disease. Cancer is most dangerous to the human health, it causes the second largest death after the cardiovascular diseases. In 2007, 13% of the deaths occurred by cancer over worldwide. Cancer has threatened to the developing countries because of changes in the lifestyle. There are about 200 types of cancers that affect the human population. Excessive neovascularization leads to the uncontrolled growth of the cells which is the major reason for cancer and depends upon the angiogenesis.

Ionizing radiations and chemotherapeutics are both therapies to cure the disease and they interfere with cell division and cause the DNA lesions. But, they cause damage to the normal cells. Even though much advancement has taken place in cancer research and development of various cancerostatic drugs, but there are two major limitations. 1) Lack of selectivity to cancer cells and causes side effects. 2) Development of multidrug resistant anticancer compounds.

The above fact made the scientists to synthesize more and more new target molecules to cure the cancer disease. Now-a-day’s extensive research has been devoted to develop the new effective anticancer therapeutics, involving surgical techniques of cancer treatment like radiation and chemotherapy. In the present day’s a lot of efforts have been taking place to develop and identify the new novel anti-tumor-specific therapies, which are able to selectively decrease the migration of cancer cells.
cancer biology, many class of the compounds were tested to get more potent drugs, among them benzothiazole derivatives get more attention towards the cancer research. In several attempts, scientists modify the existing molecules which improve their antitumor activities.

Easmon and co-workers (1997) synthesized a series of benzothiazolyl hydrazone derived from acetyl pyridines and screened for a series of cell lines to study their antiproliferation activity. These compounds were found to exhibit the moderate activity for tested cell lines and showed excellent activity for Burkitt’s lymphoma cells.\cite{37}

\[\text{IC}_{50} \text{ (MCF-7)} = 0.49 \mu\text{M}, \text{IC}_{50} \text{ (MDA 468)} = 0.72 \mu\text{M}\]

Shi et. al., (2001) synthesized the 2-(4-amino phenyl) benzothiazole sulfamate salt prodrugs and they found that, degradation of the amine occurs in strongly acidic condition and salts are less active compared to their parent amines against the human cell lines (MCF-7 and MDA-468) by \textit{in-vitro} studies.\cite{38} For the free amines of the above compounds have the IC$_{50}$ values were in the range between 0.0001–0.001 µM, but for the salts 0.42– 9.0 µM.\cite{39} Activity was higher for methyl substitution and decreased in the order of Iodo, chloro and no substitution on phenyl group.

\[\text{IC}_{50} \text{ (MCF-7)} = 0.49 \mu\text{M}, \text{IC}_{50} \text{ (MDA 468)} = 0.72 \mu\text{M}\]

Hutchinson et. al., (2002), synthesized a series of water soluble L-Lysyl and L-Alanyl amide prodrugs of the 2-(4-amino phenyl) benzothiazoles which were synthesized and tested for \textit{in-vivo} antitumor activity on mice and dogs. From the series, lysyl-amide of the 2-(4-amino-3-methyl phenyl)-5-fluoro benzothiazole (NSC 710305, popularly called as Phortress), has been a more potent candidate for
antitumor disease and selected for the phase 1 clinical evaluation.\textsuperscript{40} This compound showed the activity against breast tumors, regardless of estrogen receptor status and ovarian, renal, lung, and colon cancer cells. In the same year, for the above compound, Drug Development Group had approved a clinical candidate and provided the permission for synthesis, formulation and pharmacokinetics study. In 2004, Pharminox obtained the right to develop the molecule and phase I clinical evaluation studies are still going on in UK.\textsuperscript{41}

\textbf{Song et. al.} (2005) synthesized a series of 2, 3, 4-trimethoxy acetophenoxime conjugated Benzothiazoles which were synthesized by the reaction of oxime and acyl chloride in the alkaline medium. The bioassay of the synthesized compounds were showed moderate activity when they were tested by \textit{in-vitro} MTT assay. Antiproliferation activity of the $p$-methyl substituted compounds showed the activity against PC3 and A431 cells, at 71.3 % and 69.9 % respectively for 10 $\mu$g/mL concentration.\textsuperscript{42}

\textbf{Kok et. al.} (2008) synthesized Phthalimide derivatives of the benzothiazole by one-pot condensation reaction and screened for \textit{in-vitro} cytotoxicity on human carcinoma cell lines. These compounds were reducing the 50 % cellular adenosine triphosphate (ATP) content which was found to be around 25 $\mu$g/mL concentration on 48 hours of investigations.\textsuperscript{43}
Havrylyuka et al. (2010) synthesized the 4-thiazolidinone conjugated benzothiazole by Knoevenagel condensation reaction. Synthesized compounds were subjected to *in-vitro* anticancer activity, where two of them showed the activity for leukemia, lung, colon, melanoma, CNS, renal, ovarian, prostate and breast cancers cell lines. Here 2-[2-[3-(benzothiazol-2-yl amino)-4-oxo-2-thioxothiazolidin-5-ylidenemethyl]-4-chloro phenoxy] -N-(4-methoxy phenyl) - acetamide (36) was the promising candidate for anticancer activity with logGI₅₀ and logTGI values -5.38 and -4.45 respectively.⁴⁴

Kumbhare et al. (2012) synthesized novel series of triazoles and isoxazole linked 2-phenyl benzothiazole and screened for anticancer activity for A549, colo-205, MCF-7 cell lines by MTT assay. SAR study revealed that, the introduction of fluorine atom especially CF₃ in the third position of the target triazole compound showed enhanced cytotoxic activity and also isoxazole moiety with trifluoro methoxy substituted compound showed good activity.⁴⁵
Lindgren et al., (2014) synthesized the (E)-2-benzothiazole hydrazones and screened for their in-vitro antiproliferative activity against the HL-60 (leukemia), MDAMB-435 (breast) and HCT-8 (colon) human cancer cell lines. They studied the anticancer ability of the synthesized compounds by theoretical and experimental methods. Theoretically (Gaussian 09W program) the compound with electron donating groups showed the enhanced activity compared to the electron withdrawing groups. In the experimental MTT assay, compounds with dihydroxy groups exhibited remarkable activity for all the three cell lines, with IC$_{50}$ values ranging from 0.59 to 11.18 mM.\textsuperscript{46}

\begin{table}
\begin{tabular}{|c|c|c|c|c|}
\hline
Compound & IC$_{50}$ in MCF-10A & IC$_{50}$ in A549 & IC$_{50}$ in Colo-205 & IC$_{50}$ in MCF-7 \\
\hline
37 & 31.07 ± 2.73 & 11.07 ± 0.62 & 10.78 ± 0.69 & 18.94 ± 0.97 \\
38 & 43.87 ± 2.57 & 23.87 ± 0.57 & 19.87 ± 0.62 & 26.62 ± 1.28 \\
\hline
\end{tabular}
\end{table}

IC$_{50}$ values are in µM

Moustafa et al., (2016), synthesized a series of 6-fluoro-2-substituted Schiff’s bases, studied for computational molecular modeling with the ATP binding sites and screened for in-vitro cervical antitumor activity by MTT assay against cervical cancer (Hela) and kidney fibroblast cancer (COS-7) cell lines.

\begin{table}
\begin{tabular}{|c|c|}
\hline
Compound & IC$_{50}$ \\
\hline
39 & IC$_{50}$ (HL-60) 0.59 mM \\
40 & IC$_{50}$ (MDAMB-435) 6.27 mM \\
 & IC$_{50}$ (HCT-8) 11.18 mM \\
\hline
\end{tabular}
\end{table}

\begin{table}
\begin{tabular}{|c|c|}
\hline
Compound & IC$_{50}$ \\
\hline
41 & IC$_{50}$ (HeLa) = 2.41 µmol/L \\
 & IC$_{50}$ (COS-7) = 4.31 µmol/L \\
42 & IC$_{50}$ (HeLa) = 22 µmol/L \\
 & IC$_{50}$ (COS-7) = 45.6 µmol/L \\
\hline
\end{tabular}
\end{table}
The structure activity correlation of the synthesized compounds to the tested cell lines indicates, the presence of 2-(4-hydroxy-2-methoxy benzylidene) hydrazine moiety at second position of the benzothiazole which increased the activity, for both the cell lines. Replacement of 4-hydroxy with 4-methoxy group reduced the activity, by changing the positions of hydroxy and methoxy groups also decreased the activity. In addition, the presence of 2-(3-methyl benzylidene) hydrazino group showed superior activity, because of the hydrophobic interaction with receptor.\textsuperscript{47}

\textbf{Lad et. al.,} (2017) synthesized a novel series of 4- and 5- substituted methyl sulfonyl benzothiazole derivatives, which were tested for antimicrobial and anti-cancer activities. Some of the compounds showed the MIC values in the range of 4 to 50 \( \mu g/mL \). These antimicrobial compounds were screened for cancer activity for HeLa cell lines. The calculated IG\textsubscript{50} values were in the range of 0.2-0.6 \( \mu M \).\textsuperscript{48}

\begin{align*}
43 & \quad \text{IG}_{50} = 0.22 \ \mu M \\
44 & \quad \text{IG}_{50} = 0.6 \ \mu M
\end{align*}

\textbf{1.6.3 Benzothiazole as anti-tubercular agent:}

\textit{Mycobacterium tuberculosis} causes the Tuberculosis diseases and are spread through the air. The main reason to cause the tuberculosis infectious disease by TB pathogen mainly affects the lungs and causes the respiratory-related problems. It is the deadly disease, which threaten the under developing and developing countries. Statistical data of World health organization on tuberculosis is surprising to know that, nearly one-third of the world population is infected by tuberculosis and about 2 million deaths occur in every year. The tuberculosis is often common for the people who are suffering from HIV/AIDS. The lack of the efficient anti- \textit{Mycobacterium} agents against \textit{Mycobacterium tuberculosis} is made to develop new multi-resistant drug molecules.\textsuperscript{49} Katz in 1952 synthesized 2-hydrazino benzothiazole derivatives from
cyclization of 2-Amino thiophenol and tested for anti tubercular activity and exhibited the moderate activity.\textsuperscript{50}

A series of 2-benzyl sulfanyl conjugated benzothiazoles were synthesized by Koci et al.,\textsuperscript{(2002)} and screened for the in-vitro anti-tubercular activity. In the series, dinitro benzyl substituted compound (46) had excellent Mtb MIC value (2 \(\mu\)mol/L) and \(p\)-cyano, 2-nitro, benzyl substitutions decreased the activity.\textsuperscript{51}

\[
\begin{align*}
\text{45} & \quad \text{46}
\end{align*}
\]

Vicini and co-worker (2003) synthesized the Benzoisothiazole and benzothiazole Schiff’s bases and screened for anti-mycobacterium tubercular activity by MTT method, but in fact, the compound did not show the notable activity, but exhibited antimicrobial, cytotoxicity and HIV-1 activities.\textsuperscript{52}

Rahman et al.,\textsuperscript{(2007)} synthesized the benzothiazole urea and thiourea derivatives and tested for their cytotoxicity and antimicrobial activities including mycobacterium tuberculosis. From the synthesized compounds, benzothiazole morpholine thiourea derivative (47) exhibited the highest cytotoxicity by reducing the growth of 76 \% of the MCF-7 cell lines at 10 \(\mu\)g/mL and the same compound had 37 \% growth inhibition for mycobacterium tuberculosis H\textsubscript{37}Rv strain at 6.25 \(\mu\)g/mL.\textsuperscript{53}

\[
\begin{align*}
\text{47}
\end{align*}
\]

Huang and co-workers (2009) described the synthesis of benzothiazole isoxazole carboxamide derivatives and tested against \textit{Mycobacterium tuberculosis} H\textsubscript{37}Rv strain. Some of the compounds exhibited superior activity and inhibit Mtb growth at micromolar concentrations with MIC values in the range of 1.4 to 1.9 \(\mu\)M concentrations. The hydroxamate showed the remarkable activity against \textit{T.b. rhodesiense}. Variation in the substitution in the amide site was well suited for enhancing the activity, ranging from small dimethyl amino group to substituted amino acid esters for good anti-TB activity.\textsuperscript{54}
Benzothiazolo conjugated naphthyridone carboxylic acids exhibited both *in-vitro* and *in-vivo* anti-TB activities, against mycobacterium tuberculosis H₃₇Rv strain and multi-drug-resistant mycobacterium tuberculosis (MDR-TB). Compound 48 showed most active compound in a series of *in-vitro* studies with MIC value of 0.19 µM and 0.04 µM against mycobacterium tuberculosis and MDR-TB, which was superior activity compared to the standard drug Gatifloxacin and Isoniazid. In an *in-vivo* animal model, these compounds showed the MIC value 2.81 µM for lung and spleen tissues and they exhibited the activity at 50 mg/kg dosage. Piperidine conjugation showed very well anti-TB and cytotoxic activities.⁵⁵

![Chemical structure of compound 48](image)

Mtb H₃₇Rv MIC = 0.19 µM, MIC (MDR-TB) = 0.04 µM

Benzothiazole triazole conjugated pyridine compounds were synthesized by Patel et al., (2010) and evaluated for their *in-vitro* antitubercular activity against mycobacterium tuberculosis, H₃₇Rv strain by using Lowenstein-Jensen medium. Synthesized compounds containing 6-methoxy benzothiazole (50) showed superior activity (50 µg/mL) against Mtb. The presence of the halogen on benzothiazole showed moderate activity and electron withdrawing groups decreased the activity, but from the mesomeric effect, compound with chloro group (49) had MIC value 25 µg/mL.⁵⁶

![Chemical structures of compounds 49 and 50](image)

Mtb H₃₇Rv MIC = 25 µg/mL  
MIC = 50 µg/mL
Wang et al., (2013) synthesized Thiophene conjugated benzothiazole at 2\textsuperscript{nd} position via amide bond (51), which was found to inhibit biofilm formation by Mtb H\textsubscript{37}Rv strain and also showed inhibitory activity for planktonic culture condition and showed the MIC value as 0.01 µg/mL.\(^{57}\)

A series of 1, 2,3 triazole conjugates of mercapto benzothiazole were synthesized by Fauzia et al., (2014) and their antitubercular activity was determined against mycobacterium tuberculosis H\textsubscript{37}Rv strain by in-vitro broth dilution method. From the synthesized compounds, triazole conjugated series were more active compared to amide linkage moiety. Compounds with Nitro (52) and Chloro (53) substitutions on aromatic rings exhibited potential activity and had MIC value 8 µg/mL. The docking studies indicated the above compounds acting as DprE1 inhibitors. Bactericidal activity of the compounds showed the utility of sulphur rich benzothiazole moiety as potent ligand against TB.\(^{58}\)

Landge et al., (2015) synthesized 2-substituted benzothiazole, studied for anti-tubercular activity and identified the synthesized compounds to exhibit the potent anti-mycobacterium activity through the specific inhibition of decaprenylphosphoryl-\(\beta\)-D-ribose 2\textsuperscript{-}oxidase (DprE1). They also established mode of binding and specific target linkage using co-crystallization and protein mass spectroscopy.\(^{59}\)
**1.6.4 Anti-inflammatory activity of Benzothiazoles:**

Nonsteroidal anti-inflammatory drugs (NSAIDs) were available to small animal practitioners from many years, but the proper application of them remained unknown. Anti-inflammatory drugs are mainly involved in the reduction of inflammation. Endogenous chemical factors derived from plasma or cells and triggered by the inflammatory stimulus, mediate the vascular and cellular responses for both acute and chronic inflammations. Nonsteroidal anti-inflammatory drugs (NSAIDs), were used to provide antianalgesic, anti-inflammatory and antihypertic capabilities, but the action of mechanism was yet to be known clearly and are under investigations.

The main working principles of the anti-inflammatory drugs are to inhibit the effect of enzymes called cyclooxygenase (COX) enzyme and lisyl oxidase (LOX), which help to produce other chemicals called prostaglandins. Because of these prostaglandins, pain and inflammation occur. So, reducing in production of prostaglandins, results in lowering the pain and inflammation. A wide variety of the heterocyclic moiety has been explored to reduce the anti-inflammatory process at any of the stages. In literature, many of benzothiazole derivatives are reported for the anti-inflammatory activity. Some of the important findings are discussed here.

**Singh et al.,** (1986), synthesized the (2-dimethyl pyrazole)-6-substituted benzothiazoles (56) and found to display the moderate anti-inflammatory activity. 2-Aryl substituted 6-hydroxy benzothiazoles (57) were prepared by **Sawhney et al.**, (1987) and this series of compounds exhibited low to moderate activity.
3-Pyridyl methyl substituted -2-amino-6-hydroxy benzothiazoles were synthesized by Hibi et. al., (1994) and anti-inflammatory activities were reported. Synthesized compounds exhibited dual inhibitory action against leukotriene B₄ and thromboxane A₂. These compounds inhibit the results of, direct action on 5-lipoxygenase and TXA2 synthetase. Here, the position of the 3-pyridyl methyl group played a vital role in the activity. Compound 6-hydroxy-5,7-dimethyl-2-(methyl amino)-4-(3-pyridyl methyl) benzothiazole (58) showed the superior activity.64

Papadopoulu et. al., (2005) synthesized the benzothiazolyl amide derivatives of 4-phenyl-piperazine and tested for anti-inflammatory activity. The ability of the synthesized compounds on inflammation was evaluated by carrageenan-induced mouse paw oedema model and compounds inhibited 44-74.1 % of inflammation.65

Benzothiazole conjugated spiro indole compounds were synthesized by Kaur et. al, (2010) and screened for their in-vitro anti-inflammatory and analgesic activities. Series of compounds were synthesized, among them 5-chloro indolyl benzothiazole was marked for most potent compound for anti-inflammatory activity (72 % Oedema inhibition at 100 mg/kg), 7-chloroindolyl benzothiazole demonstrated for potent analgesic activity (69.2 % at 100 mg/kg). SAR studies for the above series of the compounds reveals that, bromo and chloro-substituted derivatives showed good activity and presence of the oxadiazole ring (60 a & b) increased the activity.66
Shafi et al., (2012) synthesized 2-mercapto benzothiazole based bis-heterocycles and their anti-inflammatory and anti-nociceptive activities were calculated by cyclooxygenase (COX) assay and carrageenan-induced hind paw oedema. Electron withdrawing groups on substituted aromatic rings para and ortho positions showed enhanced activity and electron donating groups exhibited the decreased activity.  

A series of benzothiazole thio substituted analogs were synthesized from Bylis-Hillman bromides method under the solvent-free conditions at room temperature. Synthesized compounds were evaluated for their inhibition ability by calorimetric COX inhibition screening assay. Benzothiazole trifluoro compound (61) showed 77% inhibition at 100 mg and IC$_{50}$ value 2.93 µmolar concentrations, the activity got enhanced with electron withdrawing groups and had more inhibition capacity when a compound containing benzothiazole unit compared to benoxazole and pyramidine moieties. 

A series of benzothiazole conjugated with heterocycles were synthesized by Abbas et al., (2015). Anti-inflammatory activity of the compounds were assessed by carrageenan rat paw edema model and observed that, higher activity was compared to standard and duration of action was significantly longer. 2-Phenyl hydrazine benzothiazole derivatives (62) and 2-substituted hydrazino Schiff’s base (63) were much active compounds with percentage inhibition 95.16 % and 84.54 %
respectively. These compounds also exhibited anti-neoceptive activity, 2-substituted hydrazino Schiff’s base (63) is the most potent in the series.\(^6^9\)

![Chemical structures](image)

**1.6.5 Benzothiazole as antioxidant agent:**

Antioxidant-rich foods play a significant role as health protecting factors. Scientific investigations reported that, antioxidants can reduce the risk of chronic diseases including cancer and heart diseases. Highly reactive free radicals and oxygen species are rich in the biological systems with the broad spectrum of sources. Some of the oxygen derived free radicals such as nitric oxide (NO\(^•\)), superoxide (O\(_2\)^•\(–\)), Hydroxy (OH\(^•\)) and peroxy (RO\(_2\)^•\(–\)) are carcinogenic and affect the disorderness in the human body by oxidizing nucleic acids, proteins, lipids or DNA. The ability of the compounds to trap this kind of the free radicals is called antioxidants. Antioxidant compounds like phenolic acids, polyphenols inhibit the oxidative mechanism and stop the degenerative mechanism.\(^7^0\)

Series of the 6-substituted benzothiazoles were synthesized by Cressier et. al., (2009), their *in-vitro* antioxidant capacity was evaluated by DPPH and ABTS assay. Here thiol derivatives of benzothiazole (64) had IC\(_{50}\) value 0.046 mM, aminothiole derivatives of benzothiazole (65) showed 1.39 mM IC\(_{50}\) value.\(^7^1\)

![Chemical structures](image)

Some of the spiro indolinone's incorporated with benzothiazoles were synthesized by Karali et. al., (2010). All the compounds had high degree of potency by inhibiting lipid peroxidation and showed very good radical scavenging activity against DPPH and ABTS assay methods. Some of the compounds had IC\(_{50}\) value 1.30 mM.
concentration and also exhibit ferric ion reducing capacity. The enhanced activity can be observed in the derivatives with methyl substitution (66 and 67).

![Chemical structures](image)

IC$_{50}$ DPPH for 66: 1.30 mM, ABTS: 1.02 mM, Reducing Power: 1.36 mM
IC$_{50}$ DPPH for 67: 0.98 mM, ABTS: 0.98 mM, Reducing Power: 0.70 mM

Fluoro benzo pyrazoline derivatives synthesized by the Hazra et al., (2011), showed antioxidant activity at 0.01 mM concentration with DPPH methods for the $p$-OCH$_3$ (68) and $p$-OH (69) substituted compounds. The same compounds showed ferric ion reduction capacity at 2-4 mM concentrations. Here, the substitution of the pyrazoline bridged benzothiazole derivatives increased the activity. Substitution with electron donating groups at 4$^{th}$ positions increased the activity.

![Chemical structures](image)

DPPH IC$_{50}$ = 0.010 mM
Ferric ion reduction IC$_{50}$ = 1.866 mM
DPPH IC$_{50}$ = 0.010 mM
Ferric ion reduction IC$_{50}$ = 1.866 mM

Some of the benzothiazole isothiourea derivatives also were examined for the antioxidant activity (Laura et al., 2016) by DPPH scavenging and Fenton reaction method. (E)-5-[(benzothiazol-2-yl imino) (methyl thio) methyl amino]-2-hydroxy benzoic acid (70) showed 37% scavenging at 0.013 mM concentration compared to the standard which had superior activity, hence was evaluated for ex-vivo acetaminophen-induced hepatotoxicity model. These compounds demonstrated for the reduced glutathione content, decreased malondialdehyde levels and also capable of
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inhibiting the cytochrome P450, producing the protective reactive intermediate by scavenging the radicals.\textsuperscript{74}

Benzothiazole thiazolidinedione -2-acetamides were synthesized and screened for DPPH radical scavenging, superoxide anion scavenging activity (SASA), lipid peroxidation (LPI) and hemolysis Inhibition (EHI) assay. DPPH radical scavenging EC\textsubscript{50} values ranged from 20-60 µM, which were superior to the standard (Ascorbic acid 40.28 µM). Benzothiazole-2-amine compounds exhibited good antioxidant DPPH, LPI, EHI activities. 6-Methyl-benzothiazole-2-amine derivative (72) showed very good IL-1β inhibition activity, with IC\textsubscript{50} value 6.38 µM. Benzothiazole derivatives with methoxy, nitro substituents had good antioxidant activity.\textsuperscript{75}

\begin{align*}
\text{DPPH EC}_{50} &= 52.18 \mu M \\
\text{EC}_{50} (\text{SASA}) &= 52.18 \mu M \\
\text{EC}_{50} (\text{EHI}) &= 107.28 \mu M
\end{align*}

\begin{align*}
\text{IC}_{50} (\text{IL-1β}) &= 7.03 \mu M \\
\text{Ferric ion reduction IC}_{50} &= 1.866 \text{mM}
\end{align*}

1.6.6 Benzothiazole as antimaterial agent.

Malaria is one of the foremost serious diseases mainly affecting the tropical and subtropical countries and has become the serious public health concern issue in the developing countries. According to the statistical data of World Health Organization, in 2015, there were 214 million cases of malaria detected. At least 300 million people are affected all over the world. Malaria is one of the most dangerous to humans and
developing the new drug molecules becomes the major issue globally. Among the plasmodium species, *Plasmodium falciparum* is the most dangerous and deadliest.\textsuperscript{76}

Rhodacyanine dye linked with a variety of heterocyclic moieties exhibited potent antimalarial activity and moderately selective cytotoxicity. Rhodacyanine moiety balances the molecular hydrophobicity required for the activity, compound 73 showed the EC\textsubscript{50} value at 12 nM concentration.\textsuperscript{77}

![Image of molecule 73](image)

EC\textsubscript{50} (*P. falciparum*) = 1.2 \times 10^{-8} M

Rhodacyanine derivatives of benzothiazoles were synthesized by Pudhom\textit{ et al.}, (2006), which showed \textit{in-vitro} *Plasmodium falciparum* K1 anti-malarial activity and \textit{in-vivo} activity against *P. berghei* in mice. Compound 74 and 75 had high activity and promising candidate for clinical studies. Compound 74 showed 88.7 \% plasmodium suppression at 25 mg/ kg/d and 75 showed the 89.0 \% suppression at 10 mg/kg/d dosage.\textsuperscript{78}

![Image of molecules 74 and 75](image)

Benzothiazole conjugated amodiaquine derivatives showed excellent anti-plasmodium activity W\textsubscript{2} and K\textsubscript{1} chloroquine resistance strains of the *Plasmodium falciparum* and IC\textsubscript{50} values were over the range of 7 to 22 nM concentrations. The results indicated that, protonatable amine was responsible for the potential activity. Benzothiazole analogues were superior compared to the indole, pyridine analogs.\textsuperscript{79}
IC$_{50}$ ($P. falciparum$ W$_2$) = 0.012 µM, 0.013 µM, 0.04 µM

IC$_{50}$ ($P. falciparum$ K$_1$) = 0.014 µM, 0.007 µM, 0.025 µM

Benzothiazole hydrazone derivatives were synthesized by Sarkar et. al., (2015), and they found that compounds were potent against malaria parasite $P. falciparum$ 3D7 strain and K1 strain. These were dose-dependent anti-malarial and haem polymerization inhibitory active compounds which also showed in-vivo antimalarial activity in a murine model. Here, lethal multiple-drug-resistant strain of $Plasmodium$ yoelii was used to infect Swiss albino mice. Hydroxy and di-hydroxy substituted compounds showed in-vitro anti-malarial IC$_{50}$ values at 27.1 and 16.95 µM respectively. Same compounds also exhibited the iron chelating activity and their IC$_{50}$ values were 78.11 µM and 16.95 µM respectively.
6-Amino-2-substituted benzothiazole compounds were synthesized as anti-malarial agents against *P. falciparum*. The QSAR models were developed on account of electronic structure. Benzothiazole with dimethylamine (81) and diethylamine (82) substituted compounds were marked for potent compounds in the series, they showed IC$_{50}$ value at 0.05 µg/mL concentrations.\textsuperscript{81}

![Image](image1.png)

81 82

Spiro oxindoles conjugated -2-amino benzothiazoles were found to exhibit the activity against *Plasmodium falciparum* and their MIC values were in the range of 0.5 - 0.8 µg/mL, comparable with the Choloroquine (MIC 0.02 µg/mL). These compounds had very good MIC values because of the combined effect of benzothiazole and spiro oxindole.\textsuperscript{82}

![Image](image2.png)

83

**1.6.7 Benzothiazole as antidiabetic agent:**

Diabetes is the serious chronic disease when the pancreases are not able to produce enough insulin or not able to utilize produced insulin. Diabetes leads to complications in various parts of the body and increases the risk of dying in early age. It can cause the heart attack, stroke and kidney failure, vision loss *etc*. In 2014, it was estimated that, globally 422 million adults were living with diabetes and estimated that diabetic patients number is increasing year by year. WHO reported that, diabetes will be the 7\textsuperscript{th} leading cause for the death in 2030. Even though, the much advanced invention has taken place in the field of pharmacy and medicinal chemistry, diabetes has remained as life threatening disease.\textsuperscript{83} Benzothiazole derivatives exhibit the antidiabetic activities and importance of them is discussed here.
Indole conjugated benzothiazole derivatives were synthesized for aldose reductase inhibitor in which 84 showed the IC$_{50}$ value at 5 nM, which superior to the standard. It lowers the sorbital level in nerve and lens and showed ED$_{50}$ value of 4.5 mg/kg/d in 5 days STZ-induced diabetic model.$^{84}$

Benzothiazole thiazolidine diones conjugated with the alkyl groups on exocyclic nitrogen were synthesized by Jeon et al., (2006), and found the activity against peroxisome proliferator activated receptor-γ (PPARγ). Compounds containing methyl group on the exocyclic nitrogen demonstrated for the most potent PPARγ agonist. It was observed that, on increasing the length of $N$ alkyl carbon chain substitution decreased the PPARγ activity, but increased the inhibition action of NO$^+$ production.$^{85}$

Benzothiazole benzene sulphonamide derivatives were synthesized by Hermenegilda et al., (2008) and evaluated for anti-diabetic activity in a non-insulin dependent diabetes mellitus rat model. These compounds exhibited effective lowering of plasma glucose level. Compounds also exhibited in-vitro 11β-hydroxy-steroid dehydrogenase (11β-HSD1) inhibitory activity. Docking studies also indicated the presence of potential hydrogen bonding interactions with amino acids.$^{86}$
2-Aryl sulfonyl amino benzothiazoles were synthesized by Gabriel et al., (2009) by in-vitro protein tyrosine phosphate 1B (PTP-1B), which had inhibitory activity and showed IC$_{50}$ values in the micromolar range. Docking results also indicated the potential hydrogen bonding interaction. An in-vivo study also indicates the antihyperglycemic activity in type 2 diabetes mellitus and compounds also lowered the plasma glucose concentration.$^{87}$

Some of the benzothiazole -2- amino phenyl propane derivatives also showed the antidiabetic activity against α-amylase and acted as a glycosidase inhibitors. Bromo substitution on benzoyl group and electron donating groups on the sixth position of the benzothiazole marked for the superior activity.$^{88}$
1.6.8 Benzothiazole as antidepressant agent:

Depression is a severe, potentially life-threatening illness and it is characterized by loss of interest in pleasurable activities, feeling of worthlessness, loss of energy, gain or loss of weight, hypersomnia and thinking for suicide. Serotonin (5-HT), norepinephrine are the target for the treatment of depression. The second generation anti-depression drugs are involved in the discovery of inhibitors of selective serotonin uptake, noradrenaline re-up-take etc.

Several 2-substituted benzothiazole compounds exhibit dual acting 5-HT$_{1A}$ receptor and serotonin transport inhibitor anti-depressants. These compounds have low binding affinity to the dopamine transporter (DAT) and norepinephrine transporter (NET) which indicates the absence of cardiovascular side effects.$^{89}$

![Chemical Structures](image)

\[ \text{Ki (5-HT}_{1A}\text{)} = 28.3 \text{ nm} \quad \text{Ki (5-HT}_{1A}\text{)} = 7.3 \text{ nm} \]

\[ \text{SERT} = 81 \text{ nM} \quad \text{SERT} = 64 \text{ nM} \]

Wang et. al., (2014), synthesized the benzothiazole containing 1,3-dihydro dioxane derivatives and evaluated for their binding affinities to the 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor (subtypes of serotonin) and anti-depressant activity was also determined by Forced Swimming Test (FST) and Tail Suspension Test (TST). Compound 93 and 94 showed the binding affinity at 31 nM and 24 nM respectively.$^{90}$

![Chemical Structures](image)

1.6.9 Benzothiazole as anti-HIV agent:

In the present scenario, Human Immunodeficiency Virus (HIV), is most fatal disorder and till now not able to cure completely. According to the WHO survey (2015), 36.7
millions of people are living with HIV infections and the disease is growing exponentially. Chemotherapy and other medical aids are not well developed so far. The current treatment involves the inhibition of the human immunodeficiency virus type 1 (HIV-1), which is the source of acquired immunodeficiency syndrome (AIDS).91

Benzothiazole sulfonamide derivatives were synthesized by Nagarajan et al., (2003) and reported as inhibitors of the HIV-1. In benzothiazole derivatives, hydroxy or amino groups at the third positions showed the enhanced activity and replacement of the 4-butyl urea by benzothiazole sulfonamide compounds exhibited the improved antiviral activity than the parent compound.92

\[ EC_{50} (MT-4 \text{ cells}) = 9 \text{ nM} \]

\[ EC_{50} (MT-4 \text{ cells}) = 14 \text{ nM} \]

Delmas et al., (2004), synthesized (1, 3- Benzothiazol-2-yl) amino -9- (10 H) acridinone derivatives and showed the anti-HIV activities for MT-4 cells (infected with human immune deficiency virus strain HIV-1 and HIV-2 ROD). Here, compound 97 and 98 showed the EC50 values at 27.9 µM and 88.1 µM respectively.93

\[ EC_{50} (MT-4 \text{ cells}) = 27.9 \text{ µM} \]

\[ EC_{50} (MT-4 \text{ cells}) = 88.1 \text{ µM} \]

4-Nitro imidazole conjugated with benzothiazole was synthesized by the Soud et al., (2006) and the compound showed the in-vitro anti HIV-1 (Strain IIIb) and HIV-2 (strain ROD) activities in human T-lymphocyte (MT-4) cells. Compound 99 and 100 were most active in the series and inhibited the growth of the HIV-1 replications and their EC50 values were > 15 µg/mL.94
Benzothiazole conjugated 2-chromone acetamide derivatives were screened for anti-HIV activity by MTT method and showed the potential activity for HIV-1 with the EC$_{50}$ values 7 to 100 µg/mL. Compounds with hydroxy substitution were demonstrated as most active, with EC$_{50}$ value 7.0 µg/mL. The -OH group play a vital role in the formation of hydrogen bonding, so it shows the enhanced activity.$^{95}$

Several 4 and 6 substituted benzothiazole thiazolidine derivatives acted as the reverse transcriptase inhibitors, which play a major role in the HIV type-1 infections, halogen substitution on benzothiazole derivatives exhibit the superior activity than the alkoxy substituted compounds. Docking studies also support the stable binding of the most potential compounds, to the allosteric center of the reverse transcriptase. Compounds 103 and 104 showed the superior activity (IC$_{50}$ values 0.04 µM and 0.25 µM respectively) than the reference compound.$^{96}$
1.6.10 Benzothiazole as anticonvulsant agent:

Anticonvulsants are the diverse group of pharmacological agents. They are used in the treatment of epileptic seizures which act as mood stabilizers and also are used for the curing neuropathic pain. Epilepsy is more common in neurological disorders affecting the large number of the people. It was estimated that, 50 million people are affected by this disease.\(^{97-98}\) Derivatives with nitrogen heterocyclic system with other aromatic or heteroaromatic substituents are clinically active against epilepsy. Recently 2-Amino benzothiazoles emerged as a new class of anticonvulsants. Riluzole was the benzothiazole derivative, which was available for the clinical treatment for epilepsy and 2-amino benzothiazole acted moderately as anticonvulsant.\(^99\)

Derivatives of the Riluzole, 6-fluoro benzothiazole piperazine analogues were synthesized by Jimonet et. al., (1994) as a potential anticonvulsant and in-vitro neuroprotective agent. Compounds with fluoro and methoxy substitutions at 4\(^{th}\) position inhibit the L-glutamic acid induced seizures. Fluorine atoms at 2\(^{nd}\) and 3\(^{rd}\) positions decreased the activity.\(^{100}\)

\[
\begin{align*}
\text{R} &= F, \text{ED}_{50}(\text{L-glutamic acid induced}) = 2.2 \text{ mg/kg}, \text{ ED}_{50} (\text{MES}) = 2.3 \text{ mg/kg} \\
\text{R} &= \text{OMe}, \text{ED}_{50}(\text{L-glutamic acid induced}) = 2.5 \text{ mg/kg}
\end{align*}
\]

Benzothiazolone derivatives showed the anticonvulsant activity against seizures induced MES and pentylele tetrazole (scMet). Compounds 106 and 107 were most potent against MES-induced seizures with ED\(_{50}\) of 8.7 and 7.6 mg/kg and protective index PI < 26.9 and these compounds bind to \(\sigma_1\) receptors with nanomolar affinities.\(^{101}\)
Benzothiazole-2-semicarbazones were synthesized by the Siddiqui et al., (2007), and screened for the anticonvulsant activity by Maximal Electroshock Test (MES) and neurotoxicity test on the Swiss albino mice. Compounds 108, 109 & 110 had shown 100% protection in 0.5 hour and 4 hours intervals at 30 mg/kg dose levels. The substitutions like -CH$_3$, -OCH$_3$ at the aromatic rings with -NO$_2$ compounds showed activity, on the other hand, lesser lipophilic substituents like -Cl in aryl ring decreased the activity and none of the compounds showed the malfunctioning or toxicity to the liver.$^{102}$

Benzothiazole conjugated with pyrazole was synthesized by Amnerkar et al., (2010) and anticonvulsant activity was found out by maximal electroshock test. Some of the compounds also showed the activity for neurotoxicity, hepatotoxicity and behavioral study. 6-Methyl benzothiazole with N-methoxy pyrazole compounds exhibited the ED$_{50}$ value 25.46 µmol/kg (111), 50.21 µmol/kg (112) and were marked for the most potent compounds in the series. The 3D-QSAR study also supported the potentiality of these 2-Amino substituted benzothiazole derivatives as anticonvulsant agents.$^{103}$
Substituted benzothiazole amide derivatives were also evaluated for anticonvulsant and neuro-protective effects by Hassan et. al., (2012). Compounds were tested for anticonvulsant activity by Maximal Electroshock Seizure (MES) and also tested for phase II determination by Median Effective dose (ED$_{50}$), Median Toxic dose (TD$_{50}$) and protective Index (PI). Here, compounds containing electron releasing groups were more potential than with the electron withdrawing groups. Hydrophilic methoxy substituted derivatives were superior to the lipophilic methyl substituents and halogen substituent exhibited less neurotoxicity. Docking studies also indicated good binding capacity with Gamma Amino Butyric Acid aminotransferase (GABA-AT). $N$-(6-methoxy benzothiazol-2-yl)-4-oxo-4-phenyl butanamide (113) was the lead compound and exhibited the ED$_{50}$ value 40.96 mg/kg (for MES) with protective index 8.4 and chloro substituent did not show the neurotoxicity.$^{104}$

Benzothiazole 1,3,4-thiadiazole conjugates showed the *in-vitro* anticonvulsant activity with remarkable minimal neurotoxicity and subcutaneous phenylenetetrazole (scPTZ) test, capable of protecting against seizures at 3 mg/kg dose over the time of 40 hours. This was superior to the standard drug phenytoin, especially 115 and 116 showed 100% protection. The substituents like -OH and -OCH$_3$ on the aryl rings showed improved activity, because they were capable of forming intermolecular hydrogen bonding with the receptors.$^{105}$

ED$_{50}$ (MES) = 7.4 mg/kg  
ED$_{50}$ (scPTZ) = 10.8 mg/kg

ED$_{50}$ (MES) = 6.5 mg/kg  
ED$_{50}$ (scPTZ) = 8.5 mg/kg
1.6.11 Benzothiazole as anti-Alzheimer agent:

Alzheimer disease is a progressive neurodegenerative disorder. As of 2010, it has affected approximately 36 million people. There are many researchers who have developed potential therapies but to date, cholinesterase inhibitors (ChEIs) are the only class of the drugs in the market to treat the Alzheimer disease.\(^{106-107}\)

Alzheimer is a chronic neurodegenerative disease, which is characterized by the memory loss in the early stages, in later loose of ability to carry out conversation and respond to their environment. Amyloid aggregates play an important role in the development of Alzheimer’s disease, quantification of this amyloidal plaque can be detected by the Positron Emission Tomography (PET). Some of the benzothiazoles are used in the field of PET, as an imaging agents and a number of the compounds acted as inhibition agents.

**Ono et. al.,** 2009 synthesized the Benzothiazole-2-substituted derivatives exhibited the β-amyloid imaging probes (Aβ). In *in-vitro* binding experiments, they showed excellent affinity towards the Aβ (1-42) aggregates. These compounds exhibited the fluorescence properties and in the presence of β- amyloid plaques were clearly visualized in mouse and human brains. Compounds 117 and 118 had excellent inhibition with IC\(_{50}\) values 0.12 µM and 0.11µM respectively for Aβ aggregates.\(^{108}\)

![Chemical structures of 117 and 118](image)

Radioisotopes of fluoro ethoxy substituted benzothiazole analogues were synthesized by **Neumaier et. al.,** (2010), and showed the *in-vitro* competitive binding capacity to Aβ fibries. \([^{18}F]-2\)-[4’-(methylamino) phenyl]-6-fluoro ethoxy benzothiazole with Fluorine at 6\(^{th}\) position was most suitable as the amyloid imaging agent, which showed the remarkable binding affinity (Ki = 7.24 nM). This compound was capable of penetrating the blood- brain barrier and showed the high brain uptake.\(^{109-110}\)
Tacrine conjugated benzothiazole moieties were evaluated by *in-vitro*, as well as *ex-vivo* for the inhibition activities of acetylcholinesterase (AChE) and Aβ self-induced aggregations and they also acted against neuronal cell death protection at micromolar concentrations. Compound 120 exhibited the inhibition activity of AChE (IC$_{50}$ = 0.34 µM) and compound 121 was marked for the superior activity of the anti- Aβ$_{42}$- self-aggregations inhibition (61.3 % at 50 µM). These compounds also showed the interaction with catalytic action site and peripheral anionic site (PAS) of AChE in docking studies.$^{111}$

Tacrine linked with phenyl benzothiazole three carbon spacers (122) was the most potent compound for AChE inhibitor at IC$_{50}$ value at 0.017 µM concentration and also showed the Aβ aggregation inhibition activity 51.8 % at 20 µM concentration, the potentiality of the compound was proved by the Kinetic analysis and docking stimulations.$^{112}$

2-Substituted 6-Benzothiazoles were synthesized as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors. They exhibited good index of activity to
toxicity ratio. Isobutyl-(S)-1-[(R)-1-(6-fluoro benzo[d]thiazol-2-yl)ethyl carbamoyl] butyl carbamate (123a) and isopropyl-(S)-1-[(R)-1-(6-fluoro benzo[d]thiazol-2-yl)ethyl carbamoyl] butyl carbamate (123b) were demonstrated for the highest AChE inhibiting activity with inhibition at IC$_{50}$ concentration 20.22 and 21.31 µmol/L respectively. While ethyl-(S)-1-[(R)-1-(6-fluoro benzo[d]thiazol-2-yl) ethyl carbamoyl] butylcarbamate (123c) showed the highest BChE activity at 14.1 µmol/L IC$_{50}$ value.$^{113}$

From the above literature, the activity of the benzothiazole and their structural diversity can be correlated with each other. The enhancement of the biological activity in the series of the derivatives mainly depends on the substituent present in the molecule. Electron donating, electron withdrawing, hydrophilic and hydrophobic groups influence the activity of the synthesized compounds along with core moiety. Structure Activity Relationship (SAR) is the study of the relationship between a drug's molecular structure and the drug's biological activity. Structure activity relationship studies are significant for designing a pharmaceutical drug with the greatest potential activity and least side effects.

The relationship between the structure of the chemical species and pharmacological activity for a series of compounds depends on their structural characteristics in common, including shape, size, stereo-chemical arrangement and distribution of functional groups. Some other factors contributing to structure activity relationship include electronic effects, chemical reactivity, resonance and inductive effects.

The substituents at the second position of the benzothiazole ring, like mercapto, amide, hydrazine groups are mainly responsible for the bacterial activity and anti-inflammatory activities.$^{114}$
The presence of hydrophobic moieties in benzothiazole is conductive for cytotoxicity activity of benzothiazole compounds against the cancer cell lines. Benzothiazole containing amino, hydroxy, chloro groups show better anticancer activity.\(^\text{115}\)

\[
\text{R} = \text{NH}_2, \text{OH, Cl}
\]

The chloro substituent at 5\(^{th}\) position, methoxy substituent at 4\(^{th}\) and 6\(^{th}\) position in the benzothiazole ring system showed better anti-inflammatory activity (Venkatesh et al., 2009).\(^\text{116}\)

Anti-cancer activity of the compounds is also due to substitution at position 2\(^{nd}\) of amino benzothiazole.\(^\text{117}\) Benzothiazole compounds with prop-2-enamido derivatives and \(p\)-hydroxy phenyl substitution compounds show the most marked effect and possess significant cancer activity.

**1.7 Importance of Benzothiazole Derivatives:**

The importance of the Benzothiazole derivatives summarize as follows\(^\text{118}\)

- 2-Amino benzothiazole scaffold is one of privileged structure in medicinal chemistry.
- Many benzothiazole derivatives reported cytotoxicity on cancer cells.
- Benzothiazole derivatives show varied biological activities, so, new drug molecules which help to achieve new pharmacological profile action.
- They exhibit lower toxicity.
1.8 Proposed Research work:

From the above information, it is clear that Benzothiazole is one of the privileged scaffolds in the medicinal chemistry and plays a major role in drug discovery research. Many of the compounds with benzothiazole moiety are successfully synthesized and marked for prevention and treatment of the various diseases with high bioactivity and less toxicity. All these have strongly suggested that, there is a chance for infinite potential of benzothiazole derivatives which make us enlighten and carry out the extended research work on benzothiazole derivatives to get better pharmacological molecules.

Literature on benzothiazole derivatives reveals that, substitution on the C-2 carbon atom and C-6 are the reasons for variety of biological activities. Considering this, we planned the synthesis of molecules containing benzothiazole backbone (benzene ring + thiazole ring) with various substitution products on the C-2 and C-6 carbon atoms of the benzothiazole scaffold.

Figure 1.4 Proposed modification of benzothiazole structures

Looking at the previous research work and wide therapeutic applications of the benzothiazole scaffold, we were attracted for further investigations. Hence, the following objectives are proposed.
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