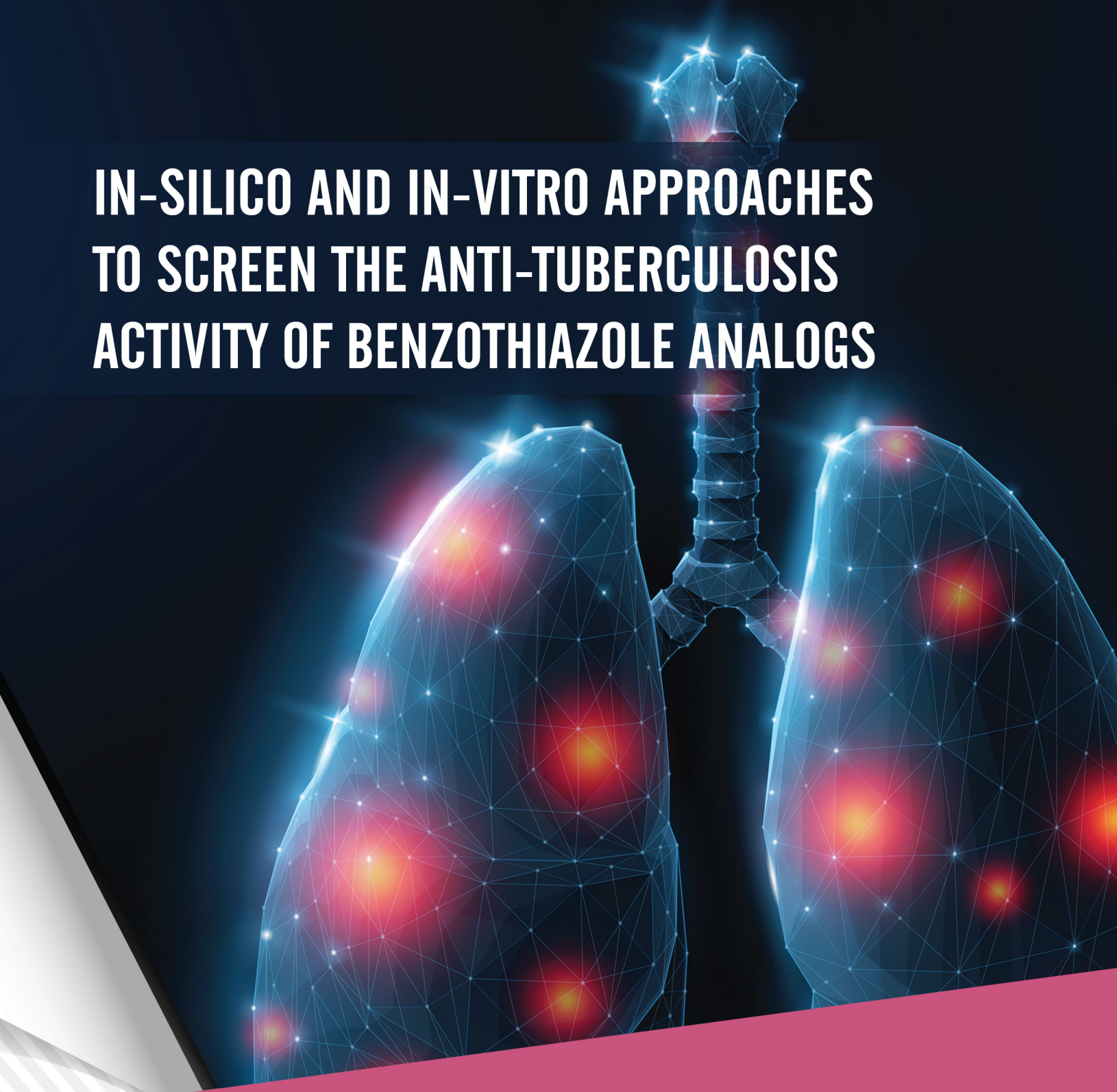
IN-SILICO AND IN-VITRO APPROACHES TO SCREEN THE ANTI-TUBERCULOSIS ACTIVITY OF BENZOTHIAZOLE ANALOGS



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FOREWORD

Tuberculosis is a serious infectious disease, which is caused by the *Mycobacterium tuberculosis*, generally it affects the lungs. Tuberculosis is spread from one person to another through microscopic droplets released into the air, it can happen through coughs, speaks, sneezes, spits, laughs from the active tuberculosis patient. According to the recent survey on tuberculosis by world Health Organization, in 2018, 10 million people fell ill with TB, and 1.5 million died from the disease. These numbers are alarming the need for more efforts to fight against the tuberculosis.

The book entitled "*In-Silico* and *In-Vitro* Approaches to Screen the Anti-tuberculosis Activity of Benzothiazole Analogs" comprises of six chapters, with extensive literature documentation on the benzothiazole analogs and corresponding structure activity relations, further authors enumerated the *in-vitro* and *in-silico* methodology for the screening the benzothiazole molecules to the anti-TB regime. Further various chapters explained the synthesis of the different types of the benzothiazole scaffold moiety and their tuberculosis activities with neat descriptions of the methodology. Additionally, each chapter includes the synthetic schemes, with mechanism for the benzothiazole derivatives.

I am happy to recommend this book to students and researchers of the Universities and research centers as a reference book. This book is written in the way that it is student friendly with the help of the diagrams, tables, reaction schemes and mechanisms. This book helps to get the glimpse on tuberculosis diseases and the wide information to carry out further research on the benzothiazole. I sincerely believe that, this book has been prepared with scientific skills and experimental details and will serve as a useful document for the researchers.

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PREFACE

Tuberculosis (TB) continues to have a remarkable impact on human healthcare worldwide, even though availability of anti-tubercular treatment (ATT) from the last five decades. The worldwide statistical data on tuberculosis was surprised to know that, nearly one-third of the world population is infected with tuberculosis and newly 1% of the population of each year adds to this number and also causes about 1.7 million deaths every year. Tuberculosis is an infectious disease spread by the *Mycobacterium tuberculosis,* a small, aerobic, nonmotile *bacillus*, which divides every 16 to 20 hours, it is a extremely slow rate compared with the other bacteria. Whatever drugs used for the treatment of tuberculosis diseases were developed in the mid of last century. There is need of more advanced treatment of TB because changes in the environmental conditions, genetic modification in human beings and also in tuberculosis species which influences the medication procedure and also treat the multi drug resistance bacteria.

In the earlier days, natural substances were used for their nutritional value and also for the treatment of diseases. We can use the traditional medicines to cure the various diseases, but it has some limitations *viz*, purification difficulties from the raw source, difficult to get in large quantity *etc.* From the 19th century, new methods were used for the treatment of diseases with synthetic drugs, modification of the natural products through the synthetic process adopted for the synthesis of useful semi synthetic drugs. The improvement in the life style has been greatly influenced by the advancement in the field of medicinal chemistry, the useful applications of life surviving medicines makes much advanced inventions in the field of synthetic chemistry.

Now-a-days computer aided applications *i.e. in-silico* tool is the modern trend of application in the drug designing investigations, which gives the idea regarding how synthetic compound interact with particular protein and mechanism of action. *In-silico* tool is also helpful to relate the structure and activity of particular series of the organic molecules. To know the molecular interaction with protein, here discussed the molecular docking study enoyl acyl carrier reductase (InhA) of *M. tuberculosis* (PDB ID: 1ZID) and decaprenyl phosphoryl-D-ribose oxidase (DprE1), (PDB ID: 4FDO).

In this book we have made an attempt to search the alternate drug molecule for the substituent. Here we have taken the benzothiazole moiety and elucidate the Quantitative structural activity relationship, *in-silico* computer aided drug design for the tuberculosis and followed by the *in-vitro* methods for analysis. In the first chapter of the book we have briefly explained the importance of the benzothiazole moiety in the medicinal chemistry and corresponding QSAR study. Second chapter covers the structural activity relationship models, *in-silico* and *in-vitro* procedure for the tuberculosis drugs. In this section, briefly explained various concepts regarding tuberculosis, causes, symptoms and present treatment. In the later chapters covers the synthesis, anti-TB activity of the benzothiazole derivatives such as, benzothiazole diamide, benzothiazole guanidinyl derivatives, benzothiazole conjugated pyrazole derivatives and benzothiazole azo-ester derivatives.

This hand book gives the information regarding TB diseases, methods of *in-silico* and *in-vitro* for anti-tuberculosis and importance of the benzothiazole in TB regime. Totally this book is suitable for, those who are interested in discovering the tuberculosis drug, researcher and post graduate students.

I am thankful to the efficient team of Bentham Science Publishers especially, Humaira

Hashmi, In-charge Book Department, Editorial Manager Publications and his team valuable suggestions during the construction of this book.

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CHAPTER 1

Introduction to Benzothiazole as a Drug Moiety

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Abstract: Benzothiazole (1, 3-benzothiazole) is one of the important fused heterocyclic compounds, which is a weak base having varied biological activities. The unique methyne center present in the thiazole ring makes benzothiazole as the most bioactive heterocyclic compound. The benzothiazole subunit is the commonly found in many natural bioactive compounds and semisynthetic drugs. Benzothiazole and its analogous found to exhibit variety of bio-activities, also with less toxic effect and their derivatives showed the enhanced activities, which has proven Benzothiazole scaffold is one of the prominant moiety in the medicinal chemistry, presence of hetero atoms such as sulphur and nitrogen makes the receptor cite in the parent moiety for the observed activity. Benzothiazole derivatives possess various pharmacological activities such as anti-viral, anti-microbial, anti-allergic, anti-diabetic, anti-tumor, anti-inflammatory, anthelmitic and anti-cancer activities. Which makes chemistry of benzothiazole is makes the prominent, rapidly developing and interesting field in the medicinal chemistry. In this chapter, briefly explained the importance, common methods of synthesis of the benzothiazole scaffold and also explained the popular benzothiazole molecules which have applications in various fields of the chemistry. Main objective of this chapter to explore various pharmacological activities containing benzothiazole moieties and rationalized the activities based on the structural variations with respect to the tuberculosis activity. The studies on benzothiazole derivatives reveal that, substitution on the C-2 and C-6 positions are the reasons for variety of biological activities.

Keywords: Activity, Benzothiazole, Cyclization, Docking, Docking score, DprE1, Guanidinyl, H37Rv Strain, Heterocycle, IC_{50}, InhA, Luciferin, MIC, MTT, Pyrazole, SAR, TB activity, Thiazole, Triazole, Tuberculosis.

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1. INTRODUCTION

The compound derived from the heterocyclic compounds have important role in the field of medicinal chemistry. The various activities and less toxicity to the normal cells makes these compounds are privileged structures in the medicinal chemistry. The presence of the hetero atom in the ring helps to make the interactions with DNA of various microorganisms. In pharmaceutical industries more than 75% of the top two hundred drugs contain heterocyclic units in their structure. In many of the heterocyclic compounds, benzene ring is fused with five membered hetero atom containing ring. These structures are the more consideration by the researcher because of the enhanced activity towards the various microorganisms [1]. In indole six membered rings fused with pyrole moiety, where as in benzothiazole six membered benzene ring fused with thiazole moiety, some of such structures are shown in Fig. (**1**).

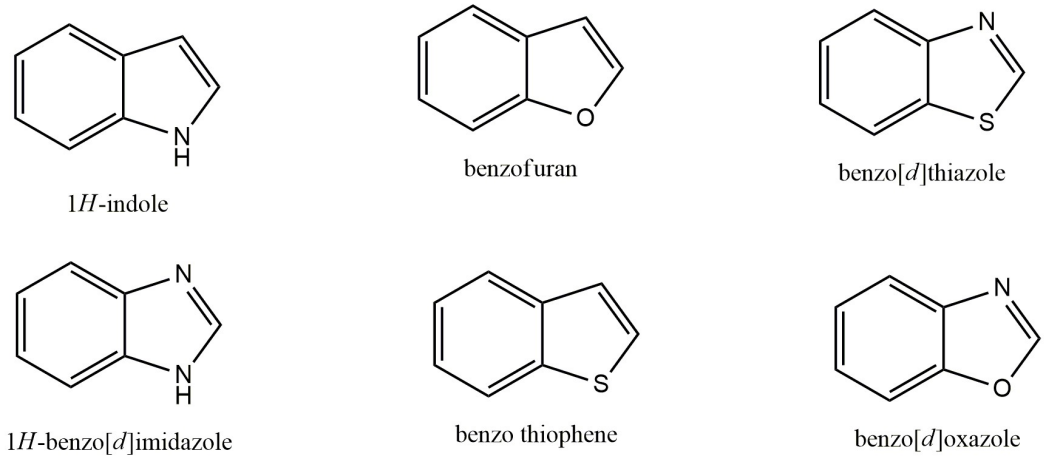

Fig. (1). Fused heterocyclic compounds.

1.1. Introduction to Benzothiazole

Benzothiazole is a class of fused heterocyclic compound, it is formed by the fusion of benzene ring with five membered thiazole ring at 1 and 4th position. The resultant compound is 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. (**2**). The benzothiazole compound is neutral exist in liquid form and insoluble in the aqueous solutions. Benzothiazole exists in all eukaryotes, ranging from yeast to humans. Naturally benzothiazole found in various food and beverages such as safflower, guava, potato, and fruits *etc*. Benzothiazole has the major role in plant metabolite, a xenobiotic and an environmental contaminant.

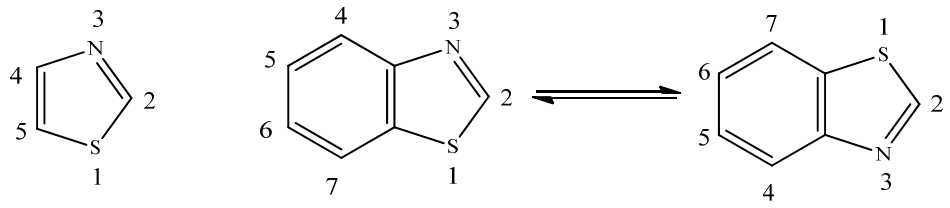

Fig. (2). Numbering of atoms in benzothiazole.

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). The presence of the sulfur and nitrogen hetero-atoms in thiazole unitof Benzothiazole makes molecules is capable of binding with multiple receptors with high affinity [2, 3]. 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.

1.2. Biology of the Benzothiazole

The benzothiazole chemistry begins with the synthesis of 2-Mercapto-benzothia zole by A. W. Hofmann an accidental invention. The chemistry of benzothiazole was accelerated in the mid of 20th century. The various important benzothiazole moieties are found in the different fields of medicines and biological sciences. Table **1** indicates the prominent moieties in the different fields.

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 [5].

CHAPTER 2

Structural Activity Relationship and *In-Silico* Methods in Drug Design

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Abstract: Bioactivity of the synthesized compounds depends upon the molecular morphology of the compounds. The presence of the number of heteroatoms and ring size and shape of the molecules influences the corresponding biological activity. To predict the biological activity, structural activity relationship is one of the traditional non-computational method and from this methods, activity relation with structure can correlate. In this chapter discussed the source of the SAR, reliability of the method and general information. To predict the activity of the molecule, various computational methods were also developed, this method is called *in-silico* method. In continuation of this chapter, molecular docking, source of molecular docking, different types of docking, various interactions, docking process and applications are discussed.

Keywords: Algorithm, Benzothiazole, Binding sites, Docking, DprE1, Drug design, Dynamics, HTS, InhA, *In-silico*, *In-vitro*, Ligand, MTT assay, Nitroimidazole, Pyrrole, PZase, QSAR model, Receptor, Rigidification, SAR, Tuberculosis.

1. STRUCTURAL ACTIVITY RELATIONSHIP

Structure Activity Relationships (SAR) is the traditional non-computational method and can be used to predict biological activity from two dimensional molecular structures. It is the collection of the molecules and their associated activities. Synthesis of new target oriented compounds for various diseases helpful to do required modification in the existing molecule.

The biological characteristics of the new designed compound can be predicted from comparing the molecular structure of the designed compound with the simi-

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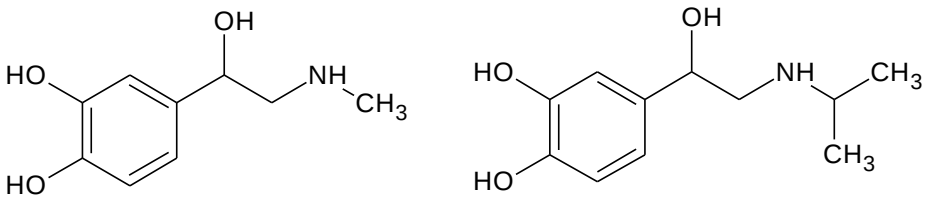
lar structure available in the literature. These are because the similar compounds have similar physical, chemical and biological properties. So there is a relationship between the molecular structure and their biological activity. The analysis of SAR gives the idea regarding determination of the group or atom, or functional group which is responsible for the evoking of targeting biological effect in the organism. This allows the modification of the effect or potency of bioactive compounds by changing its chemical structures. Qualitative predictions are based on the comparison of the valid measured data from the literature from one or more analogue with the chemical structure of interest. The level of the toxicity like, less toxic, moderate toxic and more toxic used to qualitatively can be assumed from this method. Qualitative and quantitative predictions are usually in the form of a regression equation and it gives the predicted dose response data. CDD vault (Collaborative Drug Discovery Inc.) is the SAR tool, detects the correlation and builds the model used for the evaluation of the chemical structure to predict the biological activity.

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 relationships 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.

1.1. Substituent Modification

Consider the two identical structures of the adrenaline and isoprenaline, isoprenaline is the *N* -isopropyl derivatives of the adrenaline. Isoprenaline has the greater selectivity β adrenoreceptor agonist compare to the adrenaline. Isoprenaline is also used for the treatment of brandycardia, heart block and rarely for asthma. By changing the isopropyl group instead of the methyl enhances the activity, this indicates the *N*-substitution enhances the activity.



Structural Simplification: SAR technique allows the structural simplification by knowing which part of the moiety is responsible for the activity and unwanted part of the structure. For an example in many cases the auxochrome is not responsible for the bioactivity, which can be omitted from the structure without affecting the activity.

Rigidification: For better biological activity, particular stereo specific conformation is also important. Molecules have different biological activity in different conformations. Rigidification restrict the conformational, so the rigidness of the molecule is also important.

1.1.1. Source of SAR

The source of the SAR data can be sub divided into two categories, one is those based on the statistical data (*e.g*. regression models) and another one is based on the physical approaches (*e.g*., pharmacophore models). Depending upon what extent and how much details are explored on the SAR model which influences the selecting model for carry out the SAR Study. SAR statistical data of two dimensional descriptor ignores stereochemistry. Chiral carbon exhibits the stereochemistry, which exhibit the different conformations and influences the activity. So three dimensional approaches are generally more informative, that gives the information regarding the ligand-receptor interactions with particularly stereo-chemical conformational information. In most of the biological system it is unreasonable to expect linear relationships because the *in-vivo* activity is the complex and multiple process. In all, the method depends on the analysis of the set of the previous reported molecules. From the SAR one can build the model as a predictive method. Such virtual screening can apply the designed molecule and can obtain the numerical prediction of the activity. Key to such an exploration depends upon understanding the ability of how exactly the structure of molecules is correlated with observed activity to specific structural species [1, 2].

1.1.2. Evolution of QSAR

Modern QSAR practices are based on the publication of the Hansch *et al*., in

CHAPTER 3

Mycobacterium Tuberculosis and *In-Vitro* Methods for Screening Anti-TB Drugs

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Abstract: Tuberculosis is a most infectious dangerous health problems caused by the *Mycobacterium tuberculosis*, the species mainly affects the lungs, it can also spreads to various parts of the body like the brain and the spine and second biggest killer in the world. In this chapter, we have discussed about the *Mycobacterium tuberculosis* and its various types and symptoms of the tuberculosis diseases. Detailed history of the evolution of the tuberculosis treatment has been discussed, followed by the prominent drug molecules and their classification. To screen the molecules to tuberculosis, different methodologies are required; here various *in-vitro* and *in-vivo* methods have been discussed in-order to evaluate the drug capacity against the tuberculosis.

Keywords: Alamar Blue assay, Benzothiazole, Dubos broth Pyrazinamide, *in-vitro*, *in-vivo,* H37Rv strain, Ligand, LJ media, McFarland, MDRX, MTT assay, Nitroimidazole, Pyrazinamide, Pyrrole, PZase, Resazurin, Rifampicin, Streptomycin, Tuberculosis.

1. *MYCOBACTERIUM TUBERCULOSIS*

The improved pervasiveness of irresistible sicknesses undermines total population. Tuberculosis (TB) keeps on remarkably affecting human medicinal services around the world, despite the fact that accessibility of Anti-tubercular treatment (ATT) from the last 50 years. The worldwide statistical data on tuberculosis was surprised to know that almost 33% of the total population is tainted with tuberculosis and in recent year increase of 1% of the number of inha-

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bitants in every year [1] and furthermore causes around 1.7 million deaths consistently [2].

TB is an most dangerous health issue, spread by the *Mycobacterium tuberculosis*, (*Mycobacterium bovis* and *Mycobacterium africanum* are belongs to the same family and can causes tuberculosis) a small, aerobic, nonmotile *bacillus*, which undergo doubling every 16 to 20 hours, compare to other bacteria, this is the extremely slow rate of multiplication. In nature *Mycobacterium tuberculosis* grows only inside of the cells of the host organism. *Mycobacterium tuberculosis* mainly affects the lungs, it can also spreads various other part of the body like the brain and the spine and second biggest killer in the world. The statistical graphical representations are shown in Fig. (**1**).

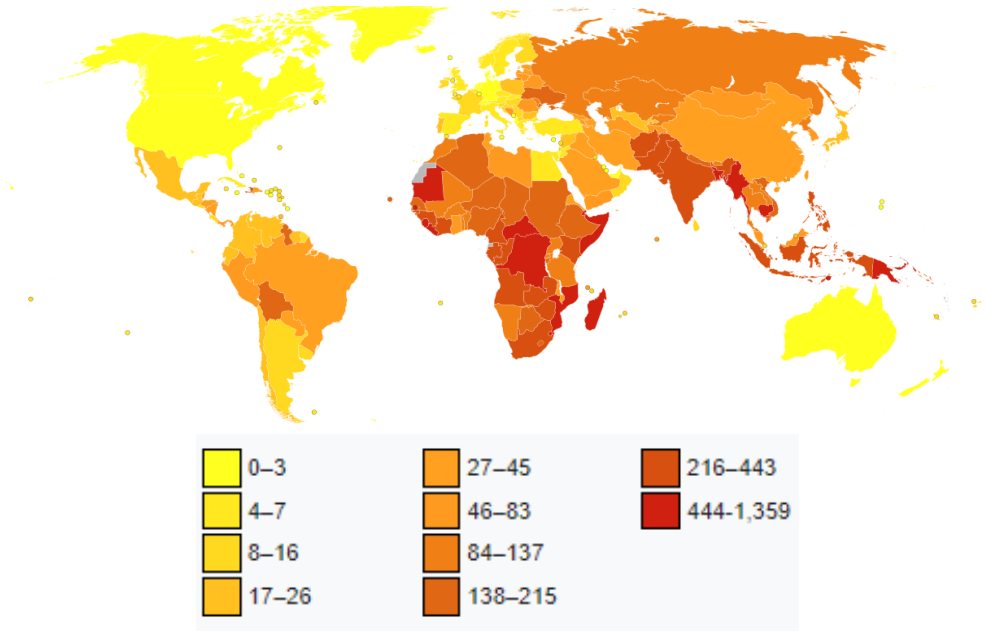

Fig. (1). World wide tuberculosis deaths per million person in 2012. Courtesy: WHO 2009 report.

The microorganisms that cause tuberculosis are mycobacterium that has a common group that belongs is called *M. tuberculosis* complex. This complex involves the accompanying species:

i. *M. tuberculosis*
ii. *M. bovis* (subsp. *bovis* and *caprae*)

iii. vaccine strain *M. bovis* BCG *(Bacille Calmette-Guérin)*
iv. *M. africanum*
v. *M. canettii*
vi. *M. microti*
vii. *M. pinnipedii*

Feeling debilitated or powerless, loss of craving and weight reduction, chills, fever, and night sweats, chest pain *etc*. are some of the symptoms of the tuberculosis diseases. TB spreads through air when a person with TB, sneezes, spits, laughs, or talks. Despite the fact that tuberculosis is a repairable illness, it is critical to grow new multi-target arranged medication compounds with lower poisonous in nature and lesser measurement. The absence of productive enemy of Mycobacterium operators against *Mycobacterium tuberculosis* is made to grow new multi-safe medication drug molecules.

Regimes for the treatment of tuberculosis diseases must contain multiple drugs to which bacteria are sensitive. Treatment with a single drug which can lead to the development of bacterial resistant to that drug. Tuberculosis is the major threat to the HIV infected people and it leads to death, it was estimated that, quarter million deaths occur in this group [3]. In 2013, over nine million new tuberculosis infected patients, 4,80,000 people being affected by the multi-drug resistant (MDR) *Mycobacterium tuberculosis* strains [4]. If the *Mycobacterium tuberculosis* strains show the *in-vitro* resistance to one of the standard drug like, isoniazid and rifampicin then it is called MDR strain, while extensively drug resistance (XDR-TB) is resistant to at least one of the fluoroquinolone and one injectable second line anti-TB drug along with isoniazid and rifampicin [5 - 7].

Majorly, this tuberculosis is affected in low income countries and pharmaceutical companies showed relatively less interest in developing new drugs for anti-TB diseases [8]. The clinical outcome of the MDR/X-DR-TB trials is largely sub-optimal and their treatment takes long time, toxic and expensive. These are serious difficulties to treat many TB disease cases, controlling and eliminating the disease [9]. A recent survey demonstrate that, in Germany MDR-TB related average costs exceeds the € 50,000 per patients, in Europe its more than € 1,60,000 [10], these study also reveals that, the success rate of TB treatment is only 54%. Every day this clinical trial faces the challenges, adverse effect, lack of clinical experiences, limited availability of adequate diagnostics, changing in lifestyle, changing in conditions and new problems in patient.

The World Health Organization recently started the innovative ‘End TB Strategy’ for supporting the TB elimination, with a vision that the world is free from TB, with zero deaths, suffering and diseases due to TB. The new policies of WHO

CHAPTER 4

Synthesis and Anti-TB Activity Screening of Benzothiazole Amide Derivatives

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Abstract: Synthesis of diamide derivatives are carried out by the phenylalanine initially phenylalanine reacts with the aromatic amine by the acid amine coupling followed by de-protection of the boc and further reacts with the benzothiazole carboxylic acid by the formation of the amide coupling in the presence HATU as coupling reagent. Further series of the diamide benzothiazole derivative compounds were screened for the *in-silico* and *in-vitro* anti TB activity. In *in-silico* method compounds show a good docking score with respect to the standard drug. *In-vitro* Alamar blue assay demonstrate some of the compounds marked for the superior activity with minimum inhibitory concentration 1.6 µg/mL.

Keywords: Alamar Blue Assay, Amide, Anti-TB, Benzothiazole, Boc, Diethyl oxalate, DIPEA, Docking, DprE1, HATU, InhA, Isoniazid, MIC, Phenyl alanine, Pyrazinamide, T3P, Trifluoro acetic acid, Triethylamine, Tuberculosis.

1. INTRODUCTION

The amide functionality is wide spread in small or complex molecules of natural and synthetic compounds [1]. Amide functional group was found not only in enzymes, proteins, hemoglobin and immune protection antibodies but also is the basis for the versatile and widely used synthetic drugs and polymers [2]. In medicinal chemistry, more than 25% of compounds contain the carboxamide group and have high polarity, are neutral and stable, have conformational diversity and possess hydrogen bonding with accepting and donating properties,which makes them popular and reliable functional groups in synthetic organic chemistry [3].

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The functionality of amides are observed in different fields of chemistry such as, polymers (Nylon 6 6, urea formaldehyde polymer *etc*.), Pharmaceutical drugs (acetaminophen, phenacetin, empirin *etc*.), insecticides (carbaryl, isoprocarb *etc*.) and also as solvents, for example dimethyl formamide.

Paracetamol, lidocaine, cinchocaine, lipitor *etc*. are the amide containing drugs used for different medications. Paracetamol is used for pain and fever relieving; lidocaine ointment is used for different parts of the body to cause numbness and also used for pain relieving. Cinchocaine is used as spinal and topical anesthesia and also used in veterinary drugs, for example Somulose for horse and cattle. Lipitor reduces cholesterol levels and triglycerides in the blood. The marketed drugs of amide compounds are shown in Fig. **(1)**.

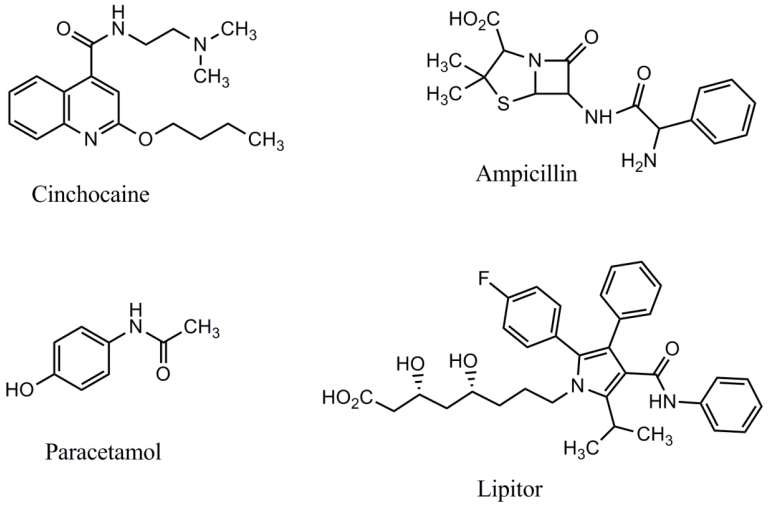


Fig. (1). Marketed drugs containing amide functional groups.

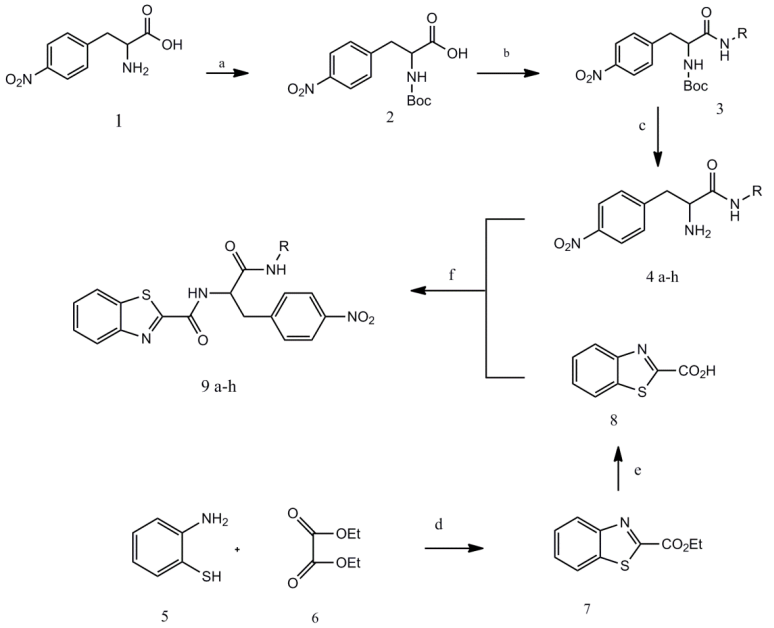
Amides are one of the important building blocks of synthetic chemistry and exhibit numerous biological activities [4]. They have been reported for antibiotic [5], anti-malaria [6], anticancer [7] and antimicrobial activities [8]. So, amide bond transformation becomes the most executed transformation in organic chemistry. Many of the reported scientific investigations confirm that nitrogen and sulfur containing heterocyclic compounds are potential towards cancer, viral and fungal diseases. The bioactivity of the benzothiazole can be enhanced by constructing amide bonds with the 4-nitro phenylalanine amide derivatives. Benzothiazole is the fused heterocyclic compound containing nitrogen and sulfur hetero atoms in the ring and having the varied biological activities. The amino acids conjugated with heterocycles have proved enhanced activities [9, 10].

The uncontrolled proliferation of cells rather than normal cell behavior causes the starting of cancer. The substance which has ability to arrest the cell division is known as anti-mitotic drug. Many of the 2-substituted benzothiazoles are reported in literature for the moderate antimitotic activity [11]. So, in the present section, explained synthesis of phenylalanine conjugated benzothiazoles through the amide bond formation and evaluated for their anti-TB activities.

2. SYNTHESIS OF BENZOTHIAZOLE AMIDE DERIVATIVES

2.1. Chemistry

The amino group of 4-nitro phenyl alanine was protected by the BOC-anhydride. T3P was used as the coupling reagent for the preparation of the amide derivatives of BOC protected 4-nitro phenylalanine and different aromatic amines. In the later step, the BOC protection was removed by Trifluoro acetic acid (TFA) to get a free amine group. The title compound was synthesized by the acid amine coupling of benzothiazole-2-carboxylic acid and 4-nitro phenylalanine amide derivatives with HATU as acid-amine coupling reagent as shown in Scheme **1**.



Scheme 1. Synthesis of Benzothiazole amide derivatives. Reagents: **(a)** BOC anhydride, NaOH, *tert*- Butyl alcohol, **(b)** R_1NH_2, T3P®, TEA, EtOAc **(c)** TFA, MDC **(d)** Reflux **(e)** LiOH, THF:H_2O (2:1) **(f)** HATU, DIPEA, DMF.

CHAPTER 5

Synthesis and Anti-TB Activity Studies of Benzothiazole Guanidinyl Derivatives

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Abstract: Compounds containing CN_3 group in the open chain or in the ring system are called guanidine and are found in a variety of compounds occurring in natural and synthetic sources. The presence of the guanidine sub unit in the synthesized compound helps to enhance the activity, which helps to interact with the various organisms through the hydrogen bonding. A series of Guanidinyl benzothiazole derivatives were synthesized and evaluated for their anti-mycobacterial activity and cytotoxicity. Anti-mycobacterial study indicates that all the synthesized compounds were appreciably active and many of the compounds have MIC values lower than the standard drugs. The guanidinyl group and electron donating group present in the molecule interacts with the microorganism and arrest the further growth, so synthesized compounds exhibit the excellent activity and some of the compound has MIC at 1.6 µg/mL. In order to rationalize the biological results of the synthesized compounds, molecular docking studies with enoyl acyl carrier reductase (InhA) of *M. tuberculosis* were performed and the synthesized compounds shows the remarkable docking score -5.85 to -9.27, which can be compared with the standard drug Isoniazid (INH) with -6.61 as docking score.

Keywords: Acetone, Activity, Alamar Blue Assay, Ammonium thiocyanate, Anti-TB, Benzothiazole, Benzoyl Chloride, Docking, DprE1, Guanidine, *in-vitro*, *in-silico*, InhA, IC_{50} value, Mercuric chloride, MIC, Pyrazinamide, Reflux, Triethyl amine, Tuberculosis , Tyrosine.

1. INTRODUCTION

One of the main aims of the synthetic chemistry is the development of new anti-in the market, microorganisms are developing the resistance towards the available

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drugs and also they have limitations to the new diseases, so, developing new drug molecules plays a major role in defeating the new diseases.

The compounds containing CN_3 group in their open chain or cyclic form are called guanidines and are found in a variety of compounds occurring in natural and synthetic sources [1]. Guanidinyl moieties are basic in nature and have the capacity to form the intermolecular contacts by the hydrogen bonding interactions [2]. The molecules containing gunidinyl groups are much interesting in synthetic chemistry, because nitrogen atoms have lone pairs of electrons and labile -NH hydrogen atom which facilitates to form coordination compounds. Guanidinyl moieties are interacting with the functional groups of enzymes or receptors from the electrostatic interactions by the hydrogen bond [3]. The large number of microbial agents, which originated from the natural and synthetic sources, has guanidinyl functionalities in their structures.

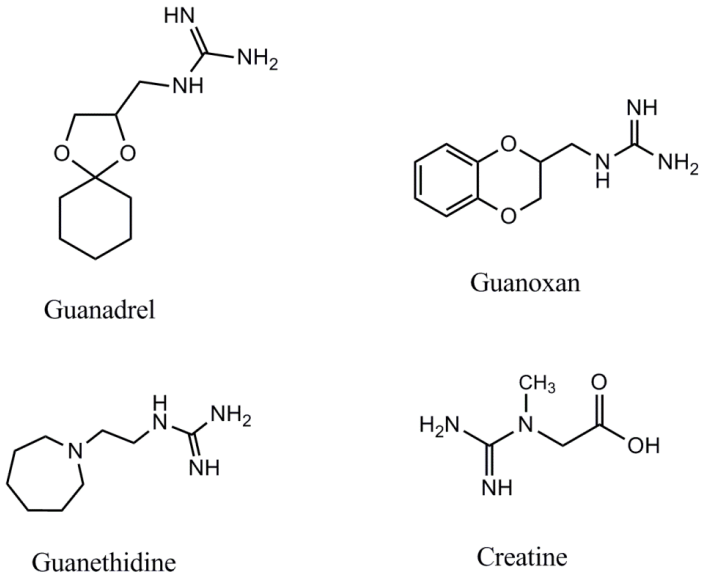


Fig. (1). Highly active guanidine drug molecules.

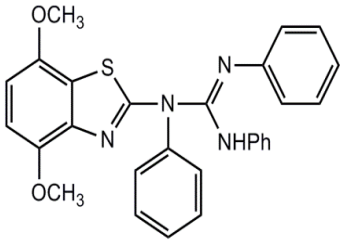
Guanidine's are physiologically active compounds which exhibit a variety of activities like, antidiabetic, antimicrobial, antiviral, anticancer, antibiotic and anti-inflammatory *etc*. [4 - 6]. The wide range of pharmacological and biological activities made the guanidine as one of the attractive pharmacophores. The presence of the CN_3 group makes the molecules to have efficient affinity towards various substituents, which makes a wide range of biochemical activities of the guanidine compounds. The drugs having less penetration in different membranes

can be enhanced by the introduction of guanidine groups. The guanidinyl moiety was also found in highly active drug molecules, which are shown in Fig. **(1)**.

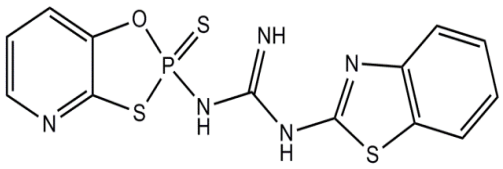
Similar to thiourea based compounds, there are several compounds incorporated with guanidine subunit, which have been reported to be potent antimicrobial agents. Kamoda *et al.,* (2006), reported the antibacterial activity of mono substituted aryl ester derivatives of guanidine (TG44), an anti-*H. pylori*agent against 54 clinical isolates of *H. pylori* and compared the activity with those of amoxicillin (AMX), clarithromycin (CLR) and metronidazole (MNZ) [7]. TG44 inhibited the growth of *H. pylori* in an MIC range of 0.0625 to 1 μg/mL.

Very less effort has been seen in the synthesis of benzothiazole guanidinyl moiety. Recently benzothiazole guanidines with methoxy substituent and 1- [2-(substituted Benzothiazole) -1, 3-diethyl-4-aryl guanidines (1) have been synthesized and screened for anti-inflammatory and analgesic activities [8, 9].

Katla *et al.,* (2013), synthesized the phosphorylated guanidine derivatives of different heterocyclic moieties and are screened for *in-vitro* and *in-vivo* anti-inflammatory activity. In the series, benzothiazole conjugated moieties (2) showed enhanced activity and had 77% inhibition in membrane stabilization test and 52% inhibition in *in-vivo* methods [10].



(1)



(2)

Siddiqui *et al*., (2004) synthesized the gunidinyl benzothiazoles and screened for anticonvulsant activity [11]. Anzini *et al.,* (2010) synthesized the gunidinyl derivatives of 6-trifluoro methoxy benzothiazole and they moderately acted as neuroprotective agents, which are applicable to brain diseases [12].

Benzothiazole moiety exhibits a variety of biological activities, so, they can solve the multi drug resistance infectious diseases. The enhancement of the activity of benzothiazole can be achieved by incorporating guanidinyl groups, which initiate the development of novel series of benzothiazole derivatives as shown in Schemes **1** and **2**. Here, we synthesized the benzothiazole guanidinyl compounds and screened for anti-TB activity.

CHAPTER 6

Synthesis and Anti-TB Activity Screening of Pyrazole Conjugated Benzothiazole Analogs

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Abstract: Hybrid derivatives have advantage over the other compounds, because they exhibit the enhanced bioactivity. In this chapter benzothiazole conjugated pyrazole hybrid compounds are taken the study. Pyrazole and benzothiazole scaffolds have their own advantage in the field of medicinal chemistry. In the present study, two series of the pyrazole conjugated benzothiazole derivatives were synthesized by Vilsmeier-Haack reaction, followed by the Schiff's base formation. The newly synthesized compounds were screened for the *in-vitro* and *in-silico* anti-TB activities. They show moderate antibacterial and antioxidant activities. Compounds containing OH, CH_3 and Cl groups exhibit the superior antibacterial activity in the series. Majority of the compounds exhibit the excellent *in-vitro* anti-TB activity, showing the MIC values up to 1.6 µg/mL. Some compounds were show the superior activity compared to the standard (INH and Cfx) and molecular docking studies were also carried out to know the molecular interaction with InhA protein.

Keywords: Acetophenone, Benzothiazole, Conjugation, Cyclization, Docking score, DprE1, Hybrid molecule, Imine, InhA, *In-silico*, *In-vitro*, Isoniazid, Molecular Docking, Phenyl hydrazine, Pyrazofurin, Pyrazole, Schiff's Base, Tuberculosis, Tyrosine, Vilsmeier-Haack.

1. INTRODUCTION

Heterocyclic compound with Nitrogen as a hetero atom represents the highly important class of the moiety in the drug design techniques and are widely used in the synthetic medicinal chemistry, drug discovery, medicines, agro chemistry and many more compounds are in the clinical trials. Ring size and suitable of the Nitrogen hetero atoms are also influences the observed activity. Among the var-

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ious ring sizes, five memberrings are suitable for drug design, their size, stability and tailoring of the nitrogen, carbon atoms highly influence the activity. Pyrazole is such a heterocyclic moiety and has wide applications in medicines, many of the parent derivatives are used as the drugs and available in the market for treating the various diseases. In pyrazole structure, two adjacent nitrogen atoms are present in the five member ring, and belong to the alkaloid family. Fig. **(1)** shows the pyrazole based structures, which is used for various treatments. In the structure of Pyrazofuran, pyrazole is linked with the furan moiety, which is a well-known antibiotic drug. The above structure gives the hint towards the hybrid structures and can give the enhanced activity towards the various pharmacological activities. From the various literature, we can observe that, by tailoring the pyrazole moiety with the suitable heterocyclic structure can increases the bioactivity and lowers the toxicity towards the normal cell lines [1, 2]. Benzothiazole is one such moiety, which has sulphur and nitrogen has hetero atom in the five member ring and followed by it is fused with six member aromatic ring and exhibit the various activities [3]. Pyrazole scaffold which is conjugated with other functionality are exhibit a wide spectrum of pharmacological activities *viz*, anti-cancer activity, anti-inflammatory [4], anti-tumor activity [5], anti-TB activity, antihypertensive [5] *etc*. The synthesis of pyrazole derivatives, which is conjugated with benzothiazole has a chance to enhance the biological activities.

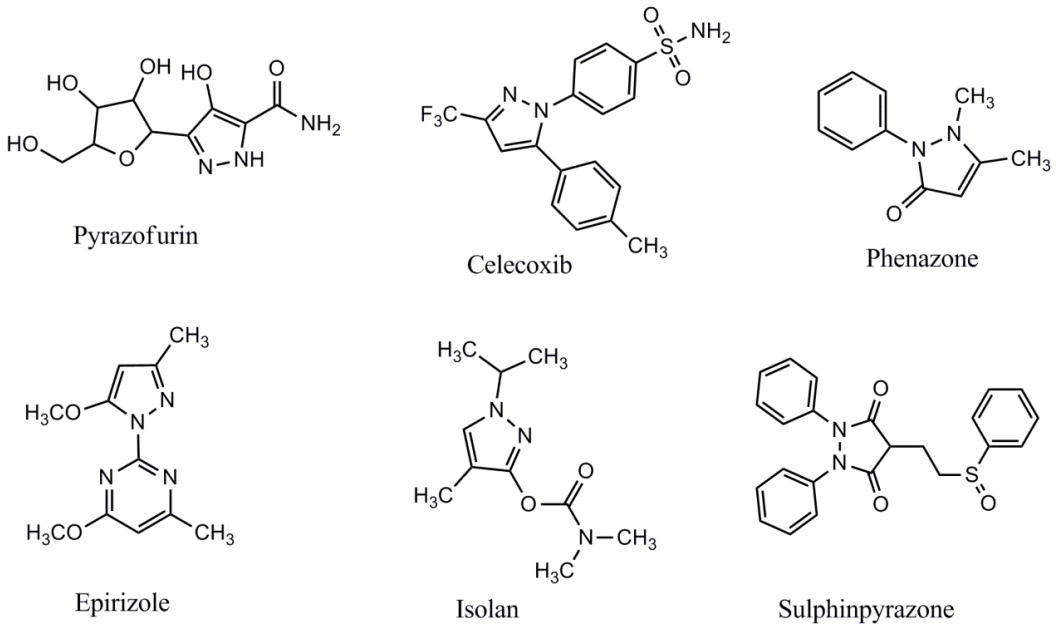
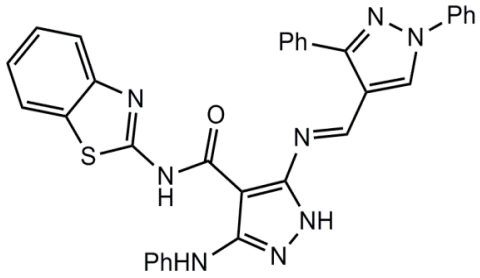


Fig. (1). Pyrazole containing molecules in medicine.

If the compound contains the azomethine (-CH=N-) group called imine, which were first synthesized by Hugo Schiff, a German chemist, called them as Schiff's bases [6]. Here, aldehyde or carboxylic acids condense with primary amines, with the elimination of water molecules. Schiff's bases are marked for the various biological activities [7]. The synthetic strategy of the Schiff's bases are applied in the condensation of the aryl/hetero aryl/or alkyl groups with primary amines as the azomethine linkages are wide spread in natural, natural derived or synthesized compounds.

Some of the benzothiazole conjugated heterocyclic compounds through the amide coupling were synthesized by the Bendock *et al.,*(2010), and screened for the antimicrobial activity. From the synthesized compounds, pyrazole amide derivatives of benzothiazole exhibited the excellent gram positive antibacterial activity with IC_{50} value 3.125 µg/mL concentration and moderate activities to other microbial strains [8]. They were also synthesized the Amidino benzothiazole derivatives showed antibacterial activity [9].

Pyrazole conjugated benzothiazole (1) exhibited the anticancer activity against liver and breast carcinoma cell lines [10].



IC_{50} value : 3.125 µg/mL (for *S. aureus* and *S. pyogenes*)

6-Fluoro benzothiazole pyrazole conjugated derivatives were synthesized by benzothiazole hydrazone, followed by the Vilsmeier-Haack cyclization reaction, the resultant compound acted as a good anti-microbial agent. In the series of synthesized compounds, hydroxy substituted derivatives showed the maximum inhibition rate (93%) and the activity decreased with $-NO_2$ substitution [11].

Pyrazole linked benzothiazoles were synthesized by Abdelgawad *et al*., (2014) and screened against the cancer cell lines and anti-prolifrations were arrested in cell lines of liver cancer (Hep G2) with the IC_{50} value 3.28 µM [12].

Benzothiazole conjugated sulfonamide pyrazole derivatives (2) were screened for the antihelmintic activity for *Perituma posthuma* earth worm and they showed the good activity [13].

SUBJECT INDEX

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Mahesh Bhat

Dr. Mahesh Bhat obtained a Ph.D. from the University of Mysore, Mysore, Karnataka, India. He has 10 years of research experience and 05 years of teaching experience.

He completed a B.Sc. degree from Poorna Prajna College, Udupi, with 86 % marks. During his bachelor's degree, he had received Sir C. V. Raman Scholarship from the Government of Karnataka. He successfully completed his Master's degree in Applied Chemistry from the Department of Chemistry, Mangalore University, Mangalore, Konaje, with distinction, and he was also a recipient of a gold medal for securing the first rank in Applied Chemistry during 2009-2011. He studied Organic, Inorganic, Physical, and Analytical Chemistry and carried out M.Sc. dissertation work at Kores India Limited, Roha, Maharashtra. After completing his Master's degree in 2011, he joined Syngene International Limited (A Biocon Company) and served as a research associate from June 2011 to April 2013. During that period, he had carried out the planning of the reaction schemes, total synthesis, purification, and characterization of the synthesized products.

In 2013, INSPIRE Fellowship (JRF) was awarded to him for the Ph.D. work, and he joined for research under guidance in May-2013. He successfully completed his course work for a Ph.D. degree in Chemistry during the first year. INSPIRE Fellowship (JRF) was upgraded to SRF during March-2016. He cleared the Karnataka State Level Eligibility Test for Lectureship in December 2016 in Chemical Sciences. In January-2018, he was awarded a Ph.D. from the University of Mysore.

Dr. Mahesh Bhat worked on the topic "Synthesis characterization and biological activity studies on Benzothiazole Derivatives." He synthesized many compounds that testify to his potential in the synthetic field. He has good research publications in reputed journals of international acclaim with good impact factors (Around 30 publications). Currently, he is working as an assistant professor in JSS College for Women, Saraswathipuram, Mysuru, since August-2017, where he teaches M.Sc. students of Chemistry.

S.L. Belagali

He obtained a Ph.D. degree from Southampton University, U.K, in 1992 with the research work on natural products synthesis & biological activity evaluation. In 1986, he joined Mangalore University and became the reader in 1995 at the Chemistry department. In October 1999, he joined as a professor & co-coordinator of the Environmental Science department at the Mysore University and also worked as a chairman of the Environmental Science department. He was also a registrar (Administration) at Rani Channamma University, Belagavi, for 15 months. He has guided 20 students for the doctoral degree and four students for the M.Phil degree. On his credential, there are more than 150 publications in national and international reputed journals.