"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL ALDIMINES OF BENZIMIDAZOLE AS ANTITUBERCULAR AGENTS"

A dissertation submitted to

THE TAMIL NADU Dr.M.G.R MEDICAL UNIVERSITY
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In partial fulfillment of the requirements
for the award of the degree of

MASTER OF PHARMACY

in

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Submitted by

261215707

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

Chennai- 600 003

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CERTIFICATE

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Dr.A.Jerad Suresh, M.Pharm., Ph.D., M.B.A
Principal,
College of Pharmacy,
Madras Medical College,
Chennai-600003

Place: Chennai
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Dedicated to the
Almighty
and my family

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CONTENTS

LIST OF ABBREVIATIONS USED

TB	Tubercle Bacillus
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immuno Deficiency Syndrome
BCG	Bacille Calmette Guerin
DOT	Directly Observed Therapy
MDR-TB	Multidrug-resistant TB
XDR-TB	Extensively drug-resistant TB
LTBI	Latent Tuberculosis Infection
GS	Glutamine Synthase
CADD	Computer-Aided Drug Design
SAR	Structure-Activity Relationship
QSAR	Quantitative Structure-Activity Relationship
QSPR	Quantitative Structure Property Relationship
SBDD	Structure based drug design
LBDD	Ligand based drug design
ADME	Absorption, Distribution, Metabolism and Excretion
DNA	Deoxy Ribonucleic Acid
PSA	Polar Surface Area
ATP	Adenosine Triphosphate
ADP	Adenosine Diphosphate
OSIRIS	Optical, Spectroscopic and Infrared Remote Imaging System
GLIDE	Grid Based Ligand Docking With Energetics
G Score	Glide Score
OPLS	Optimized Potential for Liquid Simulations
TPSA	Total Polar Surface Area
Log P	Partition Co-Efficient
WHO	World Health Organization
MIC	Minimum Inhibitory Concentration
PDB	Protein Data Bank
TLC	Thin Layer Chromatography
NMR	Nuclear Magnetic Resonance

IR	Infrared Spectroscopy
REMA	Resazurin Micro Plate Assay
MABA	Micro Plate Alamar Blue Assay
DMSO	Dimethyl Sulphoxide

Introduction

BACKGROUND

Tuberculosis (TB) is the most common cause of infectious disease–related mortality worldwide. Tuberculosis (TB) is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent.[1]

The World Health Organization estimates that 2 billion people have latent TB, while another 3 million people worldwide die of TB each year. In 2012, 8.6 million people fell ill with TB and 1.3 million died from TB.[2]

In addition, the prevalence of drug-resistant TB is also increasing worldwide. Co-infection with HIV has been an important factor in the emergence and spread of resistance.[22] New TB treatments are being developed and new TB vaccines are currently under investigation.[3] TB is a major global health threat, and we must improve the existing treatment regimen to control the spread of TB.

INTRODUCTION

Tuberculosis (TB) is caused by bacteria (*Mycobacterium tuberculosis*) that most often affect the lungs. Tuberculosis is curable and preventable. It has been recorded in history since the Greco-Roman and Egyptian civilizations.[4] In 1882, German Physician Robert Koch isolated the bacterium.

Tuberculosis is contagious and airborne. It is spread from person to person through the air. When people infected with lung TB cough, sneeze or spit, they force the TB germs into the air. A healthy person needs to inhale only a few of these germs to become infected.[4]

MYCOBACTERIUM TUBERCULOSIS

Mycobacterium tuberculosis is caused by the rod-shaped, non–spore-forming, aerobic bacterium.[22] Being an aerobic bacterium meaning it needs oxygen to survive. During active TB disease, *Mtb* complexes are always found in the upper air sacs of the lungs due to this reason.

Mycobacterium typically measure 0.5 μm by 3 μm, are classified as acid-fast bacilli and have a unique cell wall structure crucial to their survival.[4]

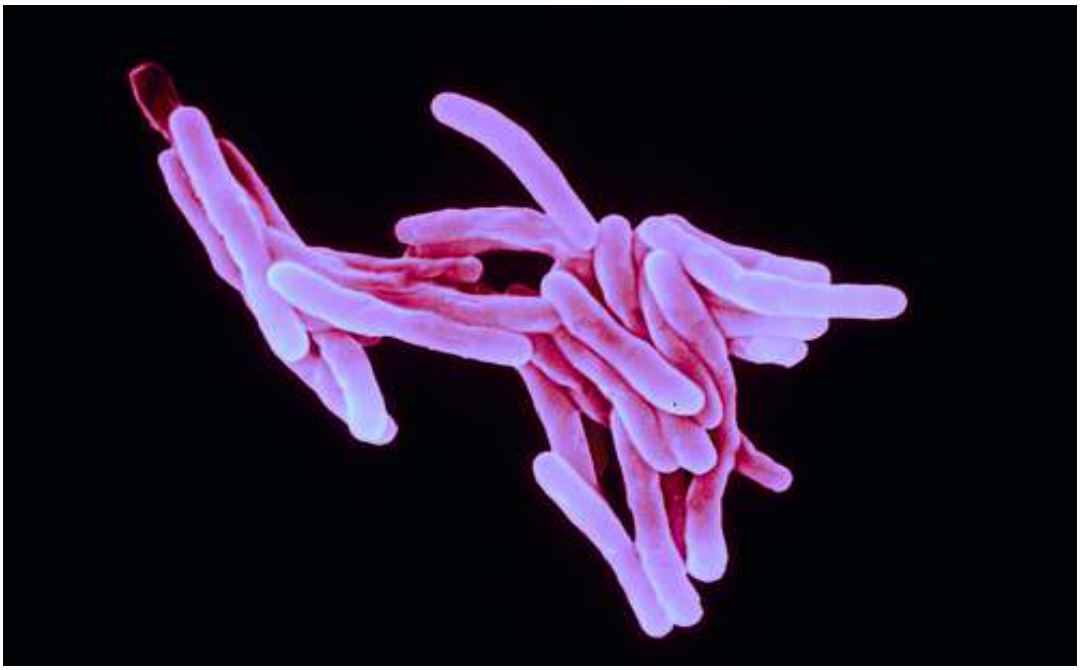


Fig 1: Electron microscope image of *Mycobacterium tuberculosis*

Cell wall

The well developed cell wall contains a considerable amount of a fatty acid, mycolic acid, covalently attached to the underlying peptidoglycan-bound polysaccharide arabinogalactan, providing a peculiar lipid barrier. This barrier is responsible for many of the medically challenging physiological characteristics of tuberculosis, including resistance to antibiotics and host defense mechanisms. The composition and quantity of the cell wall components affect the bacteria's virulence and growth rate. The peptidoglycan polymer confers cell wall rigidity.[4]

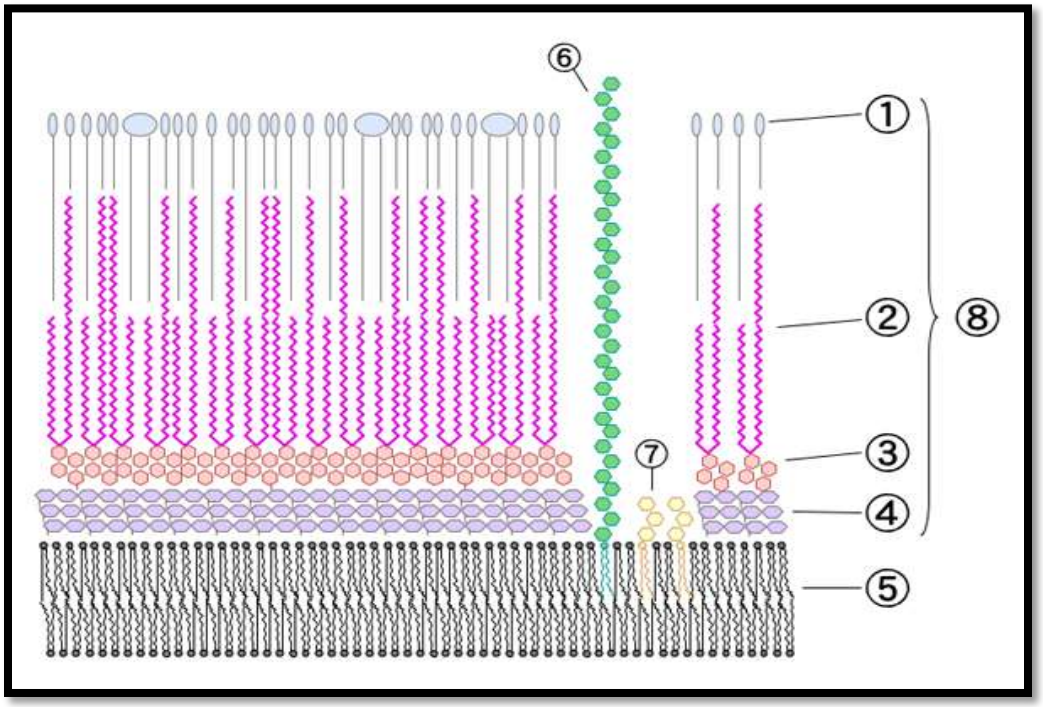


Fig 2: Mycobacterial cell wall (1-outer lipids, 2-mycolic acid, 3-polysaccharides, 4-peptidoglycan, 5-plasma membrane, 6- lipoarabinomannan, 7-phosphatidyllinositol mannoside, 8- cell wall skeleton)

PATHOPHYSIOLOGY

After inhalation, the infectious droplets settle throughout the airway. The majority of the bacilli are trapped in the upper parts of the airways as there are mucus-secreting goblet cells here.

Bacteria in droplets that bypass the mucociliary system which reach the alveoli are surrounded and engulfed by alveolar macrophages. These macrophages are a part of the innate immune system and provide an opportunity for the body to destroy the invading mycobacteria and prevent infection. Macrophages are the readily available phagocytic cells that combat many pathogens without requiring a previous exposure to the pathogen.

The mycobacterial lipoarabinomannan is a vital ligand for a macrophage receptor. The complement system also plays a major role in the phagocytosis of the bacteria. The complement protein C3 binds to the cell wall and enhances recognition of the mycobacteria by macrophages. Opsonization by C3 is rapid. The subsequent phagocytosis by macrophages initiates a cascade of events that results in either successful control of the infection, followed by latent tuberculosis, or progression to active disease, called primary progressive tuberculosis.

After being ingested by macrophages, the mycobacteria continue to multiply slowly, with bacterial cell division occurring every 25 to 32 hours. Regardless of whether the infection becomes controlled or progresses, initial development involves production of proteolytic enzymes and cytokines by macrophages in an attempt to degrade the bacteria.[4]

Released cytokines attract T lymphocytes, the cells that constitute cell-mediated immunity. Macrophages then transfers mycobacterial antigens on their surface to the T cells. This initial immune process continues for 2 to 12 weeks; the microorganisms continue to grow until they reach sufficient numbers to fully elicit the cell-mediated immune response.

For persons with intact cell mediated immunity, the next step is formation of granulomas around the organism. These lesions are formed from an accumulation of activated T lymphocytes and macrophages.This environment destroys macro phages and produces early solid necrosis at the center of the lesion. By 2 or 3 weeks, the necrotic environment resembles soft cheese, and termed caseous necrosis, and is characterized by low oxygen levels, low pH, and limited nutrients. This condition restricts further growth of the organism and establishes latency.

In patients infected with *Mtb,* droplets can be coughed up from the bronchus and infect other people. If discharge into a vessel occurs, extra pulmonary tuberculosis is likely to occur. Bacilli may also drain into the lymphatic system and collect in the tracheobronchial lymph nodes of the affected lung, where the organisms can form new caseous granulomas.[4]

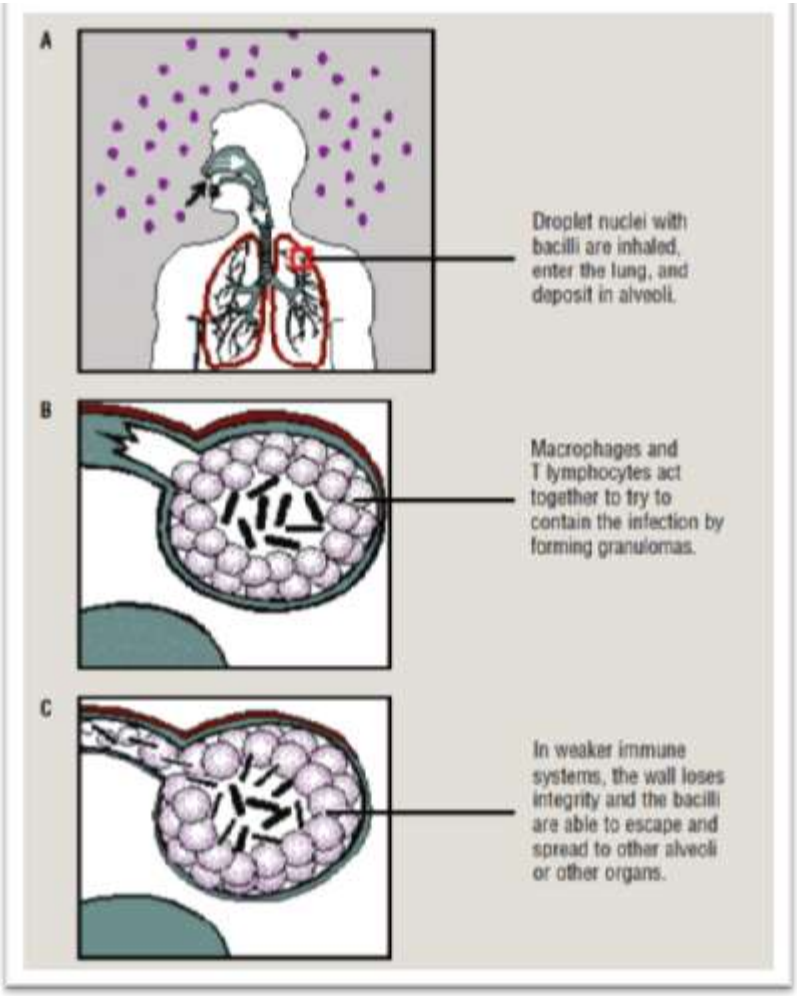


Fig 3: Pathophysiology of tuberculosis: inhalation of bacilli (A), containment in agranuloma (B), and breakdown of the granuloma in less immunocompetent individuals (C).

THE NEED FOR NOVEL TUBERCULOSIS DRUGS

- to improve current treatment by shortening the total duration of treatment .
- to improve the treatment of MDR TB[5]
- to provide for more effective treatment of latent tuberculosis infection.[4]
- New drugs to improve current drugs that facilitate compliance by providing for less intensive supervision are also of great interest.
- Discovery of a compound that would reduce both the total length of treatment and the frequency of drug administration.[6]

- MDR TB must be treated with a combination of "second line" drugs, which are not only more expensive but also much more toxic and less effective than the drugs used in standard therapy.

MEDICINAL CHEMISTRY

Medicinal Chemistry concerns with the discovery, development, identification and interpretation of the mode of action of biologically active compounds at the molecular level. Medicinal Chemistry is also concerned with the study, identification and synthesis of the metabolic products of these drugs and related compounds. It is a multi disciplinary approach drawing out theoretical chemistry, organic chemistry, analytical chemistry, molecular biology, pharmacology and bio-chemistry.[7]

DRUG DISCOVERY

The process of drug discovery is very complex and requires an interdisciplinary effort to design effective and commercially feasible drugs. Earlier drug discovery has been a trial-and-error process.The process of drug development has evolved with time. New understanding of the quantitative relationship between structure and biological activity ushered the beginning of computer-aided drug design.[8] With the help of computers, a new era has begun in drug discovery. The development cost will be cut by almost a third. The development times are reduced.

LEAD AND LEAD OPTIMIZATION

A Lead is defined as a compound, usually a small organic molecule that demonstrates desired biological activity on a validated molecular target. Lead optimization is a technique of refining 3D structures of drug molecules and promoting the binding of drug to protein active sites. In this technique the modification of the structure of the drug molecules is done by docking every specific structure of a drug compound in active site of protein, and calculating the extent of their interactions.[13] Optimization aids in the structural modification of newer molecules in order to improve the physico-chemical properties and biological activity for a given set of compounds in the library.[8] Further structural modification improves the affinity, reactivity towards target and enhances stability during metabolism.

COMPUTER AIDED DRUG DESIGN

Computer-aided drug design uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules. The fundamental goal is to predict whether a given molecule will bind to a target. Molecular mechanics or molecular dynamics are most often used to predict the conformation of the small molecule and to model conformational changes in the biological target. This provides semi-quantitative prediction of the binding affinity. .[9]

Ideally the computational method should be able to predict the affinity before a compound is synthesized. The reality however is that present computational methods are imperfect and provide at best only qualitatively accurate estimates of affinity. Therefore in practice it still takes several iterations of design, synthesis, and testing before an optimal molecule is discovered. On the other hand, computational methods have accelerated discovery by reducing the number of iterations required and in addition have often provided more novel small molecule structures.

Drug design with the help of computers may be used at any of the following stages of drug discovery:

1. hit identification using virtual screening (structure- or ligand-based design)
2. Hit-to-lead optimization of affinity and selectivity (structure-based design, QSAR, etc.)
3. lead optimization, optimization of other pharmaceutical properties while maintaining affinity[9]

In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and compound 3D structure information are used to analysis.

RATIONAL DRUG DESIGN

Rational drug design can be broadly divided into two categories:

(A) Development of small molecules with desired properties for targets, biomolecules (proteins or nucleic acids), whose functional roles in cellular processes and 3D structural information are

known. This approach in drug design is well established and is being applied extensively by the pharmaceutical industries.

(B) Development of small molecules with predefined properties for targets, whose cellular functions and their structural information may be known or unknown. Knowledge of unknown targets (genes and proteins) can be obtained by analyzing global gene expression data of samples untreated and treated with a drug using advanced computational tools.

Once a target is identified, then both approaches (A) and (B) for development of small molecules require examination of several aspects. These aspects include the evaluation of binding scores (affinity/specificity), balance between hydrophilicity/lipophilicity, absorption, distribution, metabolism and excretion (ADME), electrophilic, nucleophilic, and radical attack (biodegradation), toxicity of the parent small molecules, and products due to biotransformation in the different phases of metabolism, quantitative structure–activity relationship (QSAR), and quantitative structure–property relationship (QSPR) respectively.[10]

TYPES OF DRUG DESIGN

Advances in computational techniques and hardware have facilitated the application of *in silico* methods in the discovery process. Drug Design can be categorized as two types:

- Structure based drug design (SBDD)
- Ligand based drug design (LBDD).

Ligand Based Drug Design

It is also called indirect drug design. Ligand based drug design is an approach used in the absence of the receptor 3D information and it relies on knowledge of molecules that bind to the biological target of interest. 3D quantitative structure activity relationships (3D QSAR) and pharmacophore modeling are the most important and widely used tools in ligand based drug design. They can provide predictive models suitable for lead identification and optimization.[11]

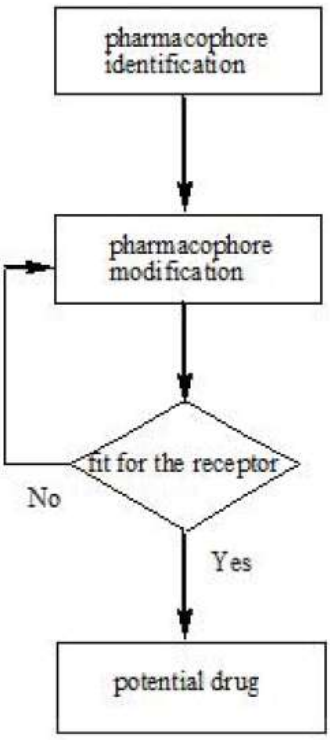


Fig 4: Ligand based drug design

Structure Based Drug Design

SBDD is the approach where the structural information of the drug target is exploited for the development of its inhibitor. Receptor structure(s) is a prerequisite for this method. Most commonly the structure of the receptor is determined by experimental techniques such as X-ray crystallography or NMR. If the structure of the protein drug target is not available, protein structure can be predicted by computational methods like threading and homology modeling.

Threading (also called as fold) is a modeling approach used to model proteins that do not have homologous proteins with known structure. In threading, a given amino acid sequence is searched for compatibility with the structures in a database of known folds. The structure of the query protein is built from these folds.

Homology modeling (also called as comparative) is an approach that relies on a clear relationship or homology between the sequence of the target protein and at least one known structure. The process of homology modeling of proteins consists of the following steps:

Identification of homologous protein with known 3D structure(s) that can serve as template; sequence alignment of target and template proteins; generation of model for the target based on the 3D structure of the template and the alignment; model refinement and validation. Over the years, homology modeling has become the main alternative to get a 3D representation of the target in the absence of crystal structures.[11]

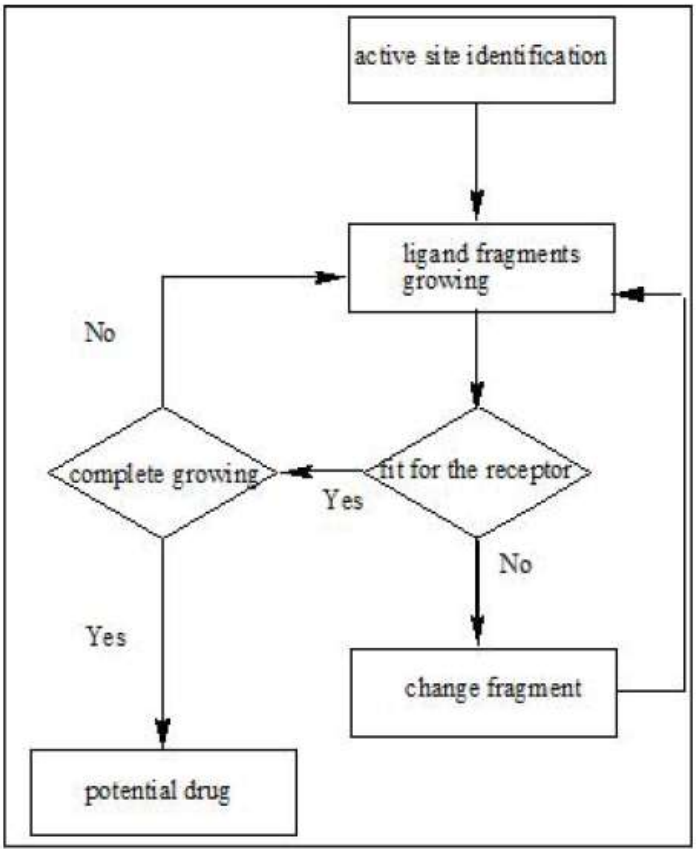


Fig 5: Structure based drug design

DOCKING

Docking involves the fitting of a molecule into the target structure in a variety of positions, conformations and orientations. Molecular docking is used to predict the structure of the intermolecular complex formed between two molecules.[12] The small molecule called Ligand usually interacts with protein's binding sites. Binding sites are areas of protein known to be active in forming of compounds. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. It also predicts the strength of

the binding, the energy of the complex; the types of signal produced and calculate the binding affinity between two molecules using scoring functions. The most interesting case is the type protein-ligand interaction, which has its applications in medicine.[13]

TYPES OF DOCKING

- **Lock and Key\Rigid Docking [13]** – In rigid docking, both the internal geometry of the receptor and ligand is kept fixed and docking is performed.
- **Induced fit\Flexible Docking [13]** - An enumeration on the rotations of one of the molecules (usually smaller one) is performed. Every rotation the surface cell occupancy and energy is calculated; later the most optimum pose is selected.

STEPS INVOLVED IN DOCKING

1. Building the Receptor: The 3D structure of the receptor can be downloaded from PDB and then the structure should be processed.Processing should include removal of the water molecules from the cavity, stabilizing the charges, filling the missing residues, generation the side chains etc according to the parameters available. The receptor should be biological active and stable state.

2. Identification of the Active Site: After the receptor is built, the active site within the receptor should be identified. The receptor may have many active sites but the one of the interest should be selected. Most of the water molecules and heteroatom if present should be removed.

3. Ligand Preparation: Ligands can be obtained from various databases like ZINC, PubChem or can be sketched using tools such as Chemsketch. While selecting the ligand, the Lipinski's Rule of 5 should be applied. The rule is important for drug development where a pharmacologically active lead structure is optimized stepwise for increased activity and selectivity, as well as drug-like properties as described.

4. Docking: This is the last step, where the ligand is docked onto the receptor and the interactions are checked. The scoring function generates score depending on which the best fit ligand is selected.

SCORING FUNCTION

These are mathematical methods used to predict the strength of the non-covalent interaction called as binding affinity, between two molecules after they have been docked. Scoring functions have also been developed to predict the strength of other types of intermolecular interactions, for example between two proteins or between protein and DNA or protein and drug. These configurations are evaluated using scoring functions to distinguish the experimental binding modes from all other modes explored through the searching algorithm.[13]

ADME ANALYSIS

For a drug to be pharmacologically active and exert the action it should posses pharmacokinetic properties like absorption, distribution, metabolism and excretion. In the field of drug research and development many drug failures do occur, as they do not undergo these properties satisfactorily. This has to be ruled out earlier in the process of drug discovery. Many in-vitro studies are more frequently used to evaluate ADME properties. Some computational methods (in silico tools) have been evolved to investigate the most suitable drug molecules.

Prediction of ADME related properties

Absorption:

To investigate this in silico models uses simple parameters like log D (diffusion coefficient) and polar surface area are the descriptors foe hydrogen bonding capacity and log P (partition co efficient) values should fall under the prescribed values as per the Rule of Five, which determines the absorption.[14]

Bioavailability:

Size and shape of the molecule, lipophilicity and flexibility determines the bioavailability of the molecule.

Metabolism:

Various in silico approaches are existing in evaluating the metabolism namely QSAR and 3D QSAR. Apart from those computational chemists have updated the structural details in the data bases and tools for predicting metabolism. [14]

EVALUATION OF INSILICO TOXICITY

Toxicity is one of the major criteria to be considered for a molecule to shine as a successful clinical candidate in pharmaceutical research. About 20-40 % of drug failure comes under this category. Commercial in silico tools estimates toxicity and provides information by the use of QSAR (parameters and descriptors), scientific literatures and to some extent in abstracting issues from humans.[15]

In silico approaches like OSIRIS property explorer predicts the carcinogenicity, mutagenicity, teratogenicity, immune toxicology, irritation, sensitization, etc. Newly updated tools help in evaluating hepato, neuro and cardio toxicity.

CHARACTERIZATION

IR SPECTROSCOPY

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic chemists. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample.[16] Different functional groups absorb characteristic frequencies of IR radiation. IR spectroscopy is an important and popular tool for structural elucidation and compound identification.

The possible characteristic bands of the nucleus are

1. 3300-3540 cm-1 N-H Stretching Vibration
2. 3670-3230 cm-1 O-H Stretching Vibration

3. 1690-1630 cm-1 C=N Stretching Vibration
4. 2975-2840 cm-1 C-H Aliphatic Stretching Vibration
5. 3100-3000 cm-1 C-H Aromatic Stretching Vibration

NMR SPECTROSCOPY

NMR is the most powerful analytical tool currently available to an organic chemist. NMR allows characterization of a very small amount of sample (10mg), and does not destroy the sample (non-destructive technique). NMR spectra can provide vast information about a molecule's structure and can very often be the only way to prove what the compound really is. Typically though, NMR is used in conjunction with other types of spectroscopy and chemical analysis to fully confirm a complicated molecule's structure. It involves the interaction of the electromagnetic radiation and the hydrogen of the nucleus when placed in an external static magnetic field.[17]

Some basic characteristic peaks of the nucleus

1. Aromatic and hetero aromatic compounds 6-8.5 δ
2. Alcoholic hydroxyl protons 1-5.5 δ
3. Aldehyde protons 9-10 δ

MASS SPECTROSCOPY

Mass Spectrometry is an analytic technique that utilizes the degree of deflection of charged particles by a magnetic field to find the relative masses of molecular ions and fragments. It is a powerful method because it provides a great deal of information and can be conducted on tiny samples. Mass spectrometry has a number of applications in organic chemistry. They are:

- ✓ Determining molecular mass
- ✓ Finding out the structure of an unknown substance
- ✓ Verifying the identity and purity of a known substance
- ✓ Providing data on isotopic abundance[16]

BIOLOGICAL EVALUATION

Anti tubercular Activity [18]

There are various high throughput assays available for screening of new chemical entities against tuberculosis. They are:

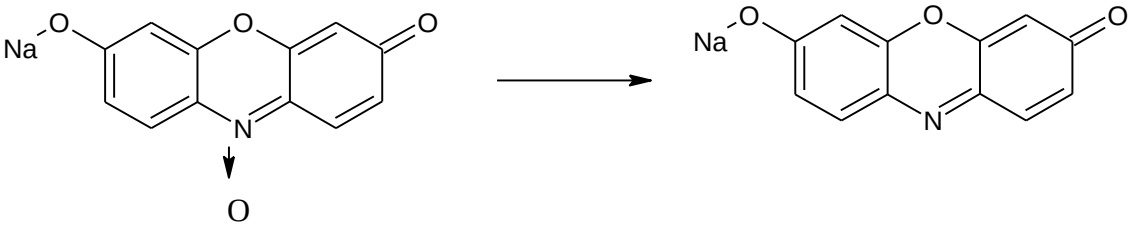
- Microplate Alamar Blue Assay
- BACTEC Assay
- Luciferous reporter phage assay
- REMA Assay
- Broth Dilution Assay
- Middle brook(7H 9,7H 10,7H 11) Agar Dilution Assay

THE ALAMAR BLUE ASSAY

Alamar Blue monitors the reducing environment of the living cell. The active ingredient is resazurin (IUPAC name: 7-hydroxy-10-oxidophenoxazin-10-ium-3-one), also known as diazo-resorcinol, azoresorcin, resazoin, resazurine, which is water-soluble, stable in culture medium, is non-toxic and permeable through cell membranes. Continuous monitoring of cells in culture is therefore permitted.

Growth is measured quantitatively by a visual colour change and the amount of fluorescence produced is proportional to the number of the living cells which is determined by colorimetric and fluorimetric methods.

Chemistry



Resazurin (oxidized) Resorufin (reduced)

Redox principle [19]

This assay is an indirect colorimetric DST method for determining the MIC of TB drug strains of Mycobacterium tuberculosis. The redox indicator Alamar blue monitors the reducing environment of the living cell. It turns from blue to pink in the presence of mycobacterial growth. As the indicator dye accepts electrons it changes from the blue, oxidized, non fluorescent state to the pink, reduced, fluorescent state. The oxidation-reduction potential of Alamar Blue is +380 mV at pH 7.0, 25 °C. Alamar Blue, therefore, can be reduced by NADPH (Eo = 320 mV), FADH (Eo = 220 mV), FMNH (Eo = 210 mV), NADH (Eo = 320 mV), as well as the cytochromes (Eo = 290 mV to +80 mV). In addition to mitochondrial reductases, other enzymes (such as the diaphorases (EC 1.8.1.4, dihydrolipoamine dehydrogenase), NAD (P) H: quinine oxidoreductase and flavin reductase located in the cytoplasm and the mitochondria may be able to reduce Alamar Blue.

ADVANTAGES

- It has accurate time course measurement.
- It has high sensitivity and linearity.
- It involves no cell lysis.
- It is ideal for use with post measurement functional assay.
- It is flexible as it can be used with different cell models.
- It is scalable and can be used with fluorescence and/or absorbance based instrumentation platforms.
- It is non toxic, non-radioactive and is safe for the user.

APPLICATIONS:

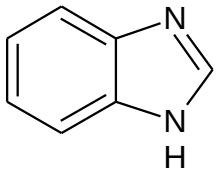
- Especially meant for studies on Mycobacterium tuberculosis.
- Used extensively in cell viability and cytotoxicity studies.

HETEROCYCLIC CHEMISTRY

Heterocyclic structures always are a part in the field of research and development in organic chemistry. Millions of heterocyclic structures are found to exist having special properties and biological importance. Among various compounds, I have chosen benzimidazole, a fused heterocyclic structure.

BENZIMIDAZOLE NUCLEUS

It is a fused heterocyclic moiety. It is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic in nature which consists of the fusion of benzene and imidazole.[20] Nowadays this is a moiety of choice which possesses many pharmacological properties.



1*H*-benzimidazole

The benzimidazole moiety is a versatile lead molecule. Literature survey conveys that benzimidazole derivatives are found to have wide range of biological activities like

- Antimicrobial & antibacterial action
- HIV Inhibitory activity
- Anti Ulcer Activity
- Antiproliferative activity
- Antitumor activity
- Anti-inflammatory activity
- Antioxidant activity
- Antiprotozoal activity
- Androgen Receptor antagonist
- Anti convulsant Agents

GLUTAMINE SYNTHASE – THE TARGET

Glutamine synthetase catalyzes the ligation of glutamate and ammonia to form glutamine, with the hydrolysis of ATP. The enzyme is a central component of bacterial nitrogen metabolism and is a potential drug target.[25]

Glutamate + ATP + NH_3 → Glutamine + ADP + phosphate

Structure

Glutamine Synthetase can be composed of 8, 10, or 12 identical subunits separated into two face-to-face rings. Bacterial GS are dodecamers with 12 active sites between each monomer. Each active site creates a 'bifunnel' which is the site of three distinct substrate binding sites: nucleotide, ammonium ion, and amino acid.ATP binds to the top of the bifunnel that opens to the external surface. Glutamate binds at the bottom of the active site. The middle of the bifunnel contains two sites in which divalent cations bind (Mn^{+2} or Mg^{+2}). One cation binding site is involved in phosphoryl transfer of ATP to glutamate, while the second stabilizes active GS and helps with the binding of glutamate. Hydrogen bonding and hydrophobic interactions hold the two rings of GS together. Each subunit possesses a C-terminus and an N-terminus in its sequence. The C-terminus (helical thong) stabilizes the GS structure by inserting into the hydrophobic region of the subunit across in the other ring. The N-terminus is exposed to the solvent. [21]

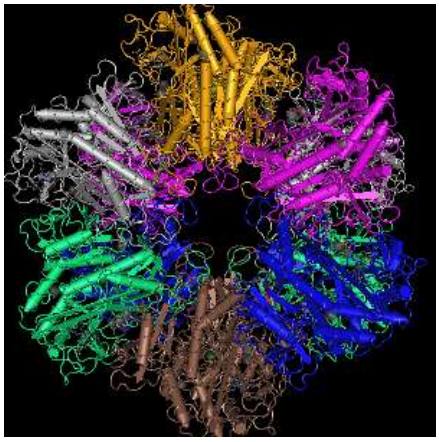


Fig 6: 4 ACF

Mechanism

GS catalyzes the ATP-dependent condensation of glutamate with ammonia to yield glutamine. The hydrolysis of ATP drives the first step of a two-part, concerted mechanism.ATP phosphorylates glutamate to form ADP and an acyl-phosphate intermediate, γ-glutamyl phosphate, which reacts with ammonia, forming glutamine and inorganic phosphate. ADP and P_i do not dissociate until ammonia binds and glutamine is released.[21]

ATP binds first to the top of the active site near a cation binding site, while glutamate binds near the second cation binding site at the bottom of the active site. The presence of ADP causes a conformational shift in GS that stabilizes γ-glutamyl phosphate atom. Ammonium binds strongly to GS only if the acyl-phosphate intermediate is present. Ammonium, rather than ammonia, binds to GS because the binding site is polar and exposed to solvent. In the second step, deprotonation of ammonium allows ammonia to attack the intermediate from its nearby site to form glutamine. Phosphate leaves through the top of the active site, while glutamine leaves through the bottom (between two rings). [21]

Aim and Objective

AIM

The aim of this project is to discover molecules with potential anti-tubercular activity.

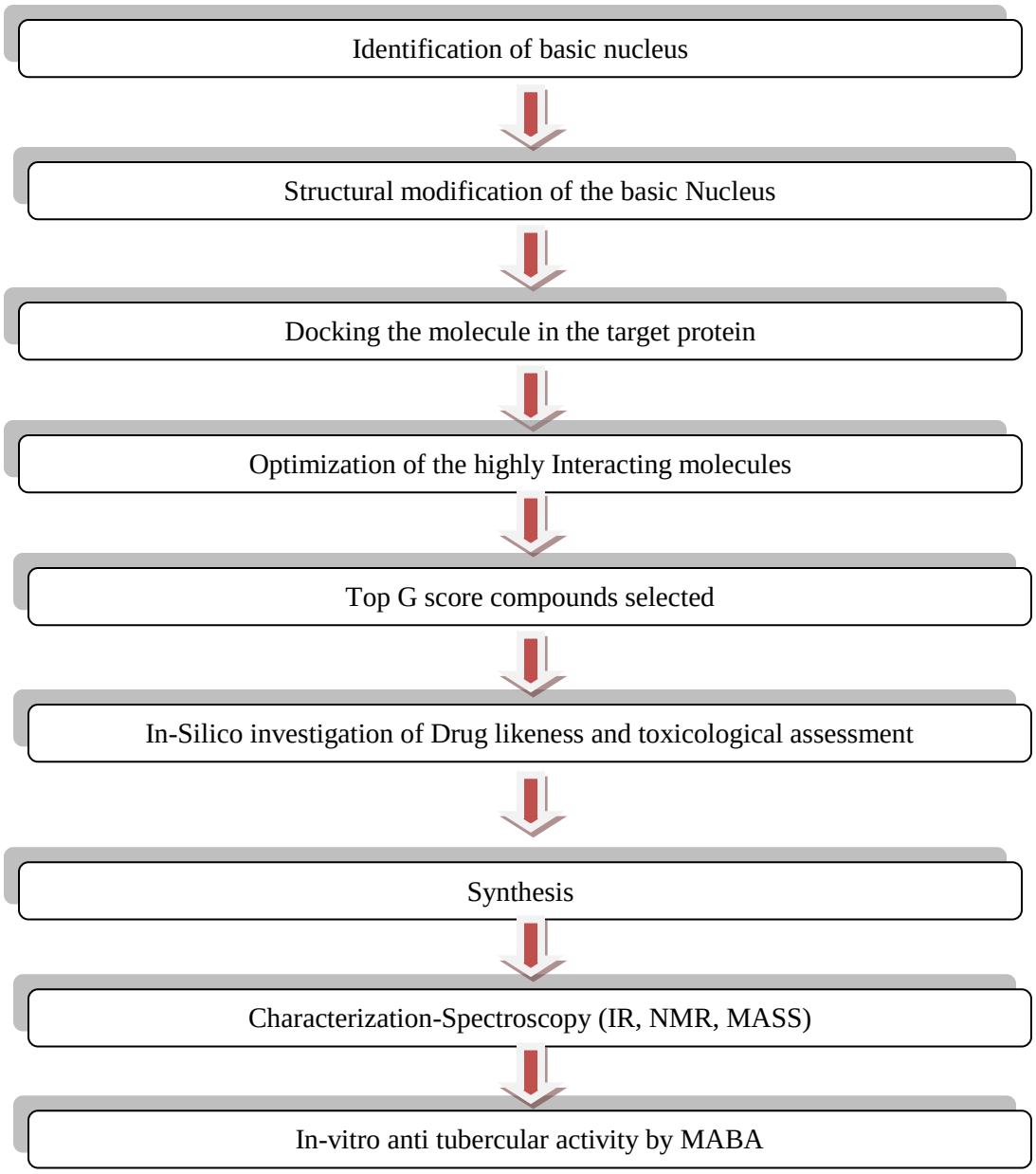
OBJECTIVE

The compounds are designed and docked against a specific crucial target, Glutamine Synthase 1, which is involved in the cell wall biosynthesis and nitrogen metabolism. The synthesized compounds are expected to act on the same.

The plan of work includes:

- Design of glutamine Synthase 1 inhibitors by docking studies.
- Insilico Drug Likeness Prediction
- Insilico Toxicity Assessment
- Laboratory synthesis of the compounds with top G.Score
- Characterization of the synthesized compounds by
    - Infrared Spectroscopy
    - ^{1}H Nuclear Magnetic Resonance Spectroscopy
    - Mass Spectroscopy
- In-vitro anti tubercular activity of synthesized compounds (MABA)

The whole study was carried out according to this flow chart.



Literature Review

LITERATURE REVIEW

1) Reviews related to the target- Glutamine Synthase

Berlicki L.; Kafarski [22] studied about the Glutamine synthetase enzyme which catalyses the formation of glutamine from glutamate and ammonium ion. It is one of the most important enzymes in nitrogen metabolism. The first part of the review presents the long-dating research on inhibitors of glutamine synthetase. Analysis of their structure-activity relationships is presented in some detail. The second part of the paper is dedicated to potential medical applications of glutamine synthetase inhibitors, which is proved as effective anti-tuberculosis agent with high selectivity towards the pathogen.

Marcus A.Horwitz et al [23] assessed the role of glutamine synthetase (GS), in the pathogenicity of *Mycobacterium tuberculosis*, glnA1 was constructed via allelic exchange. The mutant had no detectable GS protein or GS activity and was auxotrophic for L-glutamine. In addition, the mutant was attenuated for intracellular growth in human THP-1 macrophages. Based on growth rates of the mutant in the presence of various concentrations of L-glutamine the importance of the enzyme was known. These studies demonstrate that *glnA1* is essential for *M. tuberculosis* virulence.

Olof Lagerlund [24] synthesized some potential anti tubercular agents which targeted Glutamine Synthase. Glutamine Synthetase (GS), is one of the latest targets of *M.tb* which catalyses the formation of glutamine from glutamic acid. In this work, novel GS inhibitors and new Pd (0) - catalyzed methods have been developed.

Wojciech W. Krajewski et al [25] summarized that Glutamine synthetase catalyzes the ligation of glutamate and ammonia to form glutamine, with the hydrolysis of ATP. The enzyme is a central component of bacterial nitrogen metabolism

and is a potential drug target. This study provides the first reported structure for a taut form of the M. tuberculosis enzyme. The phospho compound, generated in situ by an active enzyme, mimics the phosphorylated tetrahedral adduct at the transition state. Some differences in ligand interactions of the protein with both phosphorylated compound and nucleotide are observed compared with earlier structures; a third metal ion also is found.

Marcus A.Horwitz et al [26] investigated on a novel antibiotic strategy targeting the Glutamine Synthase enzyme of Mycobacterium tuberculosis using the highly demanding guinea pig model of pulmonary tuberculosis. The feasibility of inhibiting *M. tuberculosis* glutamine synthetase (GS), an enzyme that plays a key role in both nitrogen metabolism and cell wall biosynthesis was studied.

2) Reviews related to Benzimidazole derivatives and its potential biological activities

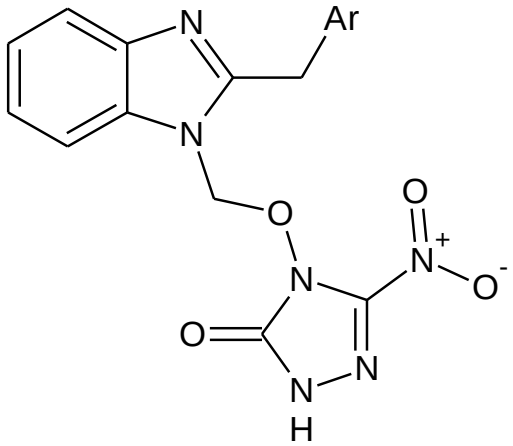
S. Sharma et al [27] studied various heterocyclic moieties and their antitubercular activity. Tuberculosis, a contagious infection caused by Mycobacterium tuberculosis, still remains the leading cause of the worldwide death among the infectious disease. Different heterocyclic moieties have been studied, synthesized and evaluated worldwide against M. tuberculosis to show their antitubercular activity.

Ramanpreet Walia et al [20] reviewed on the benzimidazole derivatives .Benzimidazole derivatives play important role in medical field with so many pharmacological activities such as antimicrobial, antiviral, antidiabetic and anticancer activity. The potency of these clinically useful drugs in treatment of microbial infections and other activities encouraged the development of some more potent and significant compounds. This review is summarized to know about the chemistry of different derivatives of substituted benzimidazoles along with their pharmacological activities.

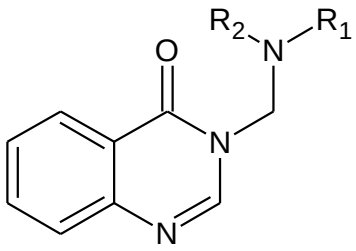
Kalyankar TM et al [28] have reviewed the various studies conducted on the benzimidazole derivatives. Benzimidazole derivatives play very efficient role in the medical field with plenty of therapeutic activities. Benzimidazole is an effective compound and there are a number of reviews available for biochemical and pharmacological studies which confirmed that these molecules are useful against a wide variety of microorganisms. Benzimidazole and its derivatives have been showing hopeful activity in the treatment of several diseases and for these reasons they achieved much attention as important pharmacophore and privileged structure in medicinal chemistry.

Maste M. M. et al [29] aimed to synthes

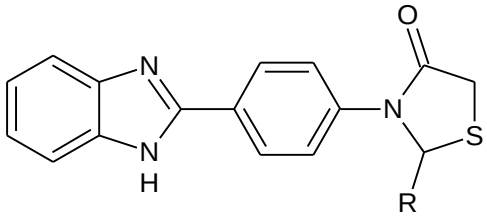
ize some benzimidazole acetic acid derivatives and also the derivatives associated with 1, 2,4 triazolone and was investigated for their biological activities. All the compounds were screened for their anti tubercular and antimicrobial activities by standard methods. Results reveal that compounds show promising anti tubercular activity at both the concentration compared to standard drug streptomycin.



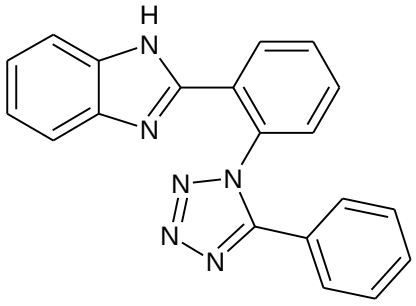
M.Vijey Aanandhi et al [30] synthesized novel molecules with benzimidazole nucleus and further formed a Schiff base and evaluated the antimicrobial activity.



Panneer Selvam T et al [31] synthesized a novel series of 2-substituted benzimidazole derivatives were synthesized and characterized. The compounds were screened for antibacterial and antifungal activity. The Minimum Inhibitory Concentrations was determined by vagar streak dilution method.

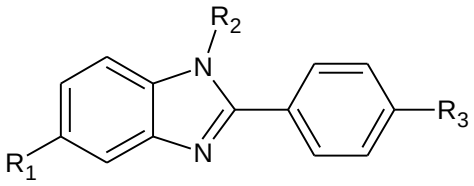


Ahamed A. Jafar et al [32] synthesized benzimidazole derivatives and screened them for antimicrobial activity. They synthesized 1H-tetrazol-1-yl) phenyl)-1H-benzo[d] imidazole by a series of conventional methods. Antimicrobial activity against bacteria and fungi was studied. The results of preliminary biological tests showed that of these compounds possess good biological activities.

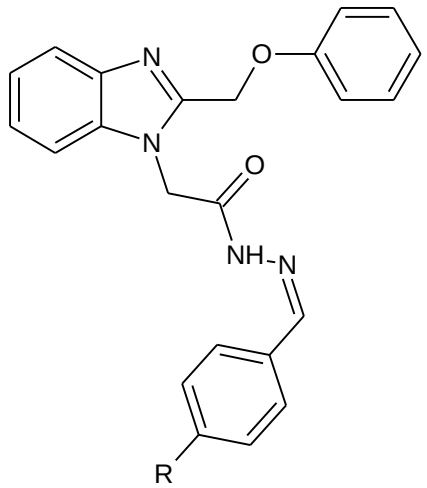


P.C Santhosh et al [33] have studied the versatility of the benzimidazole nucleus. Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. Benzimidazole is an extension of the well elaborated imidazole system has been used as carbon skeleton for N-heterocyclic carbines. They are found to have varied biological activities.

Hamdan S. Al-Ebaisat [34] synthesized a set of six novel benzimidazoles compounds. The biological activity of these compounds as fungicides was tested against three commercially known fungicides (C. albicans, patient isolate C. glabrata and C. krusei).The biological activity of two compounds was found to be comparable to that of the commercially available fungicides

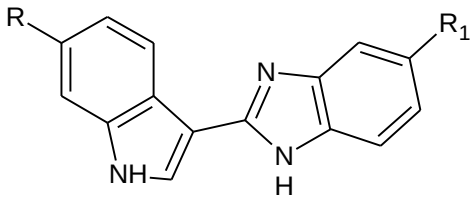


Mohammad Shaharyar et al [35] synthesized 2-[2- (phenoxymethyl)-1H-benzimidazol-1-yl] acetohydrazide and few derivatives and screened the synthesized compounds for anticonvulsant activity.



R. Walia et al [36] have summarized the chemistry of different derivatives of substituted benzimidazoles along with their pharmacological activities. In the present research the new N-(2-(1H-benzo[d]imidazol-2-yl) phenyl)-N-phenylbenzamide derivatives were synthesised. The reaction was carried out between o-phenylenediamine with substituted anthranilic acids. The resultant compounds were then refluxed with benzoyl chloride in the presence of pyridine to yield the product.The antimicrobial activity was evaluated against bacteria and fungi were studied.

Y Radha et al [37] syntheised, characterized and evaluated some benzimidazole derivatives for antimycobacterial, cytotoxic and diuretica activity.Novel benzomidazole derivatives possessing two different structural moieties, pyridinyloxyphenyl benzimidazoles and indolyl benzimidazole were synthesized.

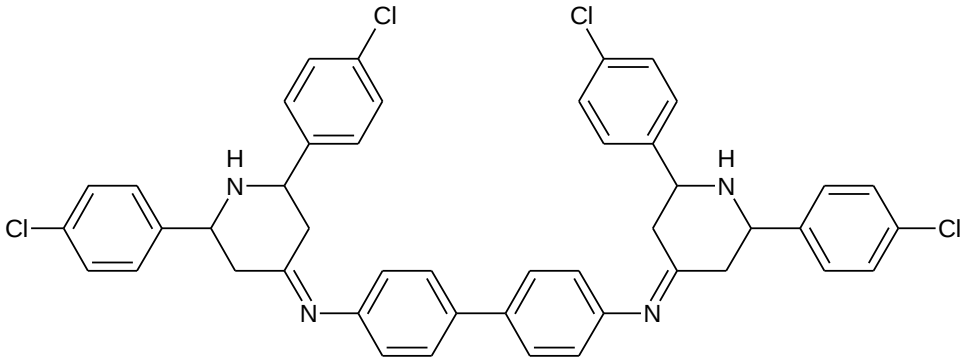


3) Reviews related to Schiff bases and their biological activities

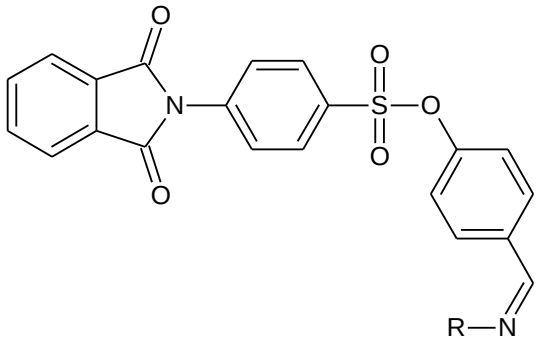
Vaibhav Sharma et al [38] reviewed on the chemistry and biological activities of Schiff bases. Schiff bases are the compounds which are mainly formed by the condensation of the aldehydes and amines. These compounds can be synthesized by various synthetic routes. Pharmacological actions of Schiff compounds which have been reported in previous studies are antimicrobial, antimalarial, antitubercular, anticancer, anthelmintic, analgesic, anti-inflammatory etc.

Khlood Fahed Hamak [39] synthesized Schiff base and evaluated it for antimicrobial activity.Schiff base were synthesis by the reaction of 2,6-bis (4-chlorophenyl) pipridone-4 with benzidine in ratio (2:1) and reaction of 3,5- dimethyl-2,6- diphenyl piperidone-4 with 1,2-

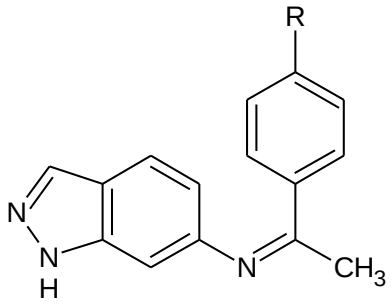
Phenylendiamine and 2-aminobenzenethiol in ratio (1:1). All synthesized compound were characterized and evaluated for their *in vitro* antibacterial activities, against Gram positive (*Staphylococcys aureus*) and Gram negative (*Escherichia.coli, Enterobacter, Salmonella, Klebsiella*) bacteria.



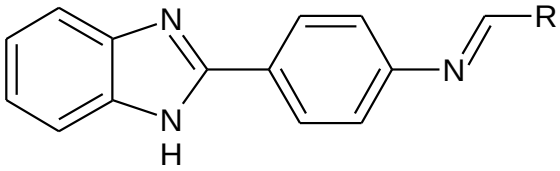
Ahlam Marouf Al-Azzawi et al [40] synthesized a series of new Schiff bases linked to phthalimide through phenyl sulfonate moiety via multistep synthesis. Also a series of new Schiff bases linked to phthalimide through methylene group have been synthesized via reaction of 4-phenyl phenacyl bromide with phthalimide potassium salt producing compound which in turn introduced in reaction with different primary aromatic amines affording the new Schiff bases.



Kalpesh S. Parikh et al [41] designed and synthesized Schiff bases from acetopheneone.6-Amino Indazole condensed with various aromatic acetophenone. Finally the product was characterized by conventional and instrumental methods.



Amira S. Abd El-A et al [42] used efficient green chemistry for synthesizing a series of Schiff bases incorporating 4-1*H*-benzo[*d*] imidazole moiety, by microwave technique and heating conventional procedures which are used for their preparation. This work aims to make a comparison between conventional and microwave irradiation methods. The design of selected newly Schiff bases is defined by molecular modeling. The compounds were characterized and the evaluations of anticancer activities of compounds were done against human cancer cell lines.



Michael J. Hearn et al [43] synthesized and characterized Schiff bases of isoniazid and studied their antimycobacterial activity. Few structural modification of the isonicotinic acid hydrazide (INH) was performed which provides lipophilic adaptations of the drug in which the hydrazine moiety of the parent compound has been chemically blocked from the deactivating process of N2-acetylation by N-arylaminoacetyl transferases.

4) Reviews related to biological evaluation of anti tubercular activity by MABA.

Scott G.Franzblau et al [45] studied MIC determination by MABA. A colorimetric, microplate-based Alamar blur Assay (MABA) method was used to determine the MICs of Isoniazid, rifampin, streptomycin and ethambutol for 34 Peruvian Mycobacterium tuberculosis isolates and the H37Rv strain by using bacterial suspensions prepared directly from media. The MABA is a

simple, rapid, low-cost, appropriate technology which does not require expensive instrumentation and which makes use of a nontoxic, temperature-stable reagent.

Sephra N.Rampresad [46] studied the various applications of Alamar blue as an indicator. Alamar Blue is an important redox indicator that is used to evaluate metabolic function and cellular health. The Alamar blue bioassay is being utilized to access cell viability and cytotoxicity in a range of biological and environmental system and in a number of cell types including bacteria, yeast, fungi,protozoa.

Jose de Jesus Alba-Romero et al [47] applied the Alamar Blue assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. The results showed that the MABA test is fast and easy to apply. It is a very reliable method of determining the drug susceptibility to pharmaceuticals.

Materials and Methods

MATERIALS AND METHODS

A. DRUG DESIGN

A process of design and discovery of new chemical entities is done using an automated docking program GLIDE- Grid Based Ligand Docking with Energetics Maestro 9.4.047 Schrodinger Suite.

GLIDE is one of the docking programs which predicts the binding modes of ligand to a protein (target).It searches the ligands having maximum favourable interactions with a receptor usually a protein, Ligand is a single molecule where as the receptor may include proteins, metals and cofactors.

It runs on rigid and flexible docking modes. The latter generates conformations automatically for the input of each ligand and gives out the best fit pose of the molecule which has been docked to the receptor.

The docking procedure involves

- Protein Preparation
- Ligand Preparation
- Receptor Grid Generation
- Ligand Docking

Protein Preparation

Protein preparation and refinement studies were performed on Glutamine Synthase 1.
The protein is downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) protein Data Bank with the following PDB id: 4ACF, resolution 2 Å using the protein preparation wizard of Maestro 9.4.047. A typical PDB structure consists of heavy atoms, metal ions, co factors, waters and can be multimeric.

The next step is to pre-process the protein structure by assignment of bond orders, tautomeric states and by the addition of polar hydrogen atoms.

Then only the ligand- receptor subunit is retained for the binding interaction of ligand and all the water molecules are deleted.

The final step is minimization of the protein structure by using force field OPLS _2005 to reorient side chain hydroxyl groups and alleviate potential steric clashes.

Ligand Preparation

The ligands were exported in a MDL file format into LigPrep tool of Maestro 9.4.047 version for ligand preparation.

Ligand preparation involves optimization of geometric parameters prior to docking. The molecules designed for docking should have a 3D representation with realistic bond lengths and bond angles with filled valencies. Preparation process involves a series of steps which includes conversions, applying corrections and generating variations to the structures, there by rendering optimization.

Receptor Grid Generation

To perform the docking calculations more efficiently, the position, site and constraints of the receptor are mentioned to generate a receptor grid. In receptor grid generation the ligand is used to define the grid position and size. The receptor grid determines the size and position of the active site. This process is done using the Receptor Grid generation tool of Maestro 9.4.047 after the protein preparation process.

Ligand Docking

Docking was carried out by choosing the ligand grid base name file and the receptor grid base name file as output files which contains the 3D structures of the synthesized compounds and the receptor grid generated from prepared protein has the 3D structure of the enzyme respectively.

The docking process calculates the binding energies of a particular conformation of the ligand to the receptor. Accessing the affinity of various ligands with particular receptor was carried out by Glide in the extra precision mode (XP).

Docking Score

The compounds are ranked based on G score which is obtained by the consideration of both rewards and penalties when the molecule interacts with the target.

Rewards are represented by negative values. The basic parameters which are considered as rewards include lipophilic pair term, hydrophobic enclosure reward, hydrophobically packed hydrogen bond, hydrophobically packed correlated hydrogen bond, hydrogen bond pair term, chlorine and bromine, electrostatic rewards and sitemap ligand/receptor. Penalties value should be less for the best compounds. The basic parameters which are considered as penalties include hydrophobic ligand groups, rotatable bond and similarity.

B. IN-SILICO SCREENING OF DRUG LIKENESS

For a drug to be pharmacologically active and exert the action it should posses pharmacokinetic properties like absorption, distribution, metabolism and excretion. In the field of drug research and development many drug failures occur due to unfavourable ADME properties. This has to be ruled out earlier in the process of drug discovery. Some computational methods (in silico tools) have been evolved to investigate the most suitable drug molecules before synthesis.

Lipinski's rule of five also known as the **Pfizer's rule of five** is a rule to evaluate drug likeness. It is used to predict whether a molecule is likely to be orally bio-available or to evaluate drug likeness.[48]

Lipinski's rule

It is used to predict whether a molecule is likely to be orally bio-available or to evaluate drug likeness. The rule was formulated by Christopher A. Lipinski in 1997.The rule states that for drug likeness the molecule should have the following properties:

- Molecular weight less than 500 Daltons
- Calculated log P value less than 5
- Less than 10 hydrogen bond acceptor groups(eg.-O-, -N-,etc)
- Less than 5 hydrogen bond donor groups (eg. NH, OH, etc)
- Less than 10 rotatable bonds

The designed and docked molecules are screened in silico using **Molinspiration Cheminformatics Software** to evaluate drug likeness. This tool is quick and easy to use. It is a software available online for calculation of important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors and others), as well as prediction of bioactivity score for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors)[49]

C. IN SILICO TOXCITY PREDICTION

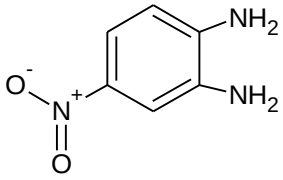
In silico toxicity prediction is done using OSIRIS Property Explorer. It is free software available for access in the Organic Chemistry Portal [15]. Using this prediction tool, mutagenicity, tumorogenicity, skin irritancy, and reproductive effects can be calculated. The prediction properties relies on a precompiled set of structure fragment that gives rises to toxicity alerts in case they are encountered in the structure currently drawn. These fragment lists is created by rigorously shredding all compounds in the data base known to be active in a certain toxicity class. During the shredding any molecule is first cut at every rotatable bonds leading to a set of core fragments [53]. Osiris software is used to calculate various drug relevant properties of chemical structures. The results are colour coded. The green colour represents that the compound is non-toxic. Yellow and red colour indicates moderate and severe toxicity of the chemicals respectively.

D. SYNTHETIC METHODOLOGY

REACTANT PROFILE

1. 4-NITRO O-PHENYLENEDIAMINE

Structure:



Synonym: 4-nitrobenzene-1,2-diamine

Molecular Formula: $C_6H_7N_3O_2$

Molecular Weight: 153.13

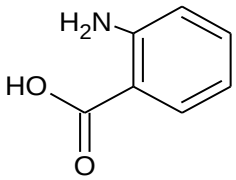
Solubility: Soluble in DMSO, methanol

Description: Orange red powder

Melting Point: 201°C

2. ANTHRANILIC ACID

Structure:



Synonym: 2-aminobenzoic acid

Molecular Formula: $C_7H_7NO_2$

Molecular Weight: 137.13

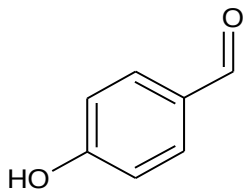
Solubility: Soluble in chloroform, ether, ethanol, hot water.

Description: Pale yellow or tan crystalline powder

Melting Point: 148°C

3. Para- HYDROXY BENZALDEHYDE

Structure:



Synonym: 4- hydroxyl benzaldehyde

Molecular Formula: $C_7H_6O_2$

Molecular Weight: 122.12

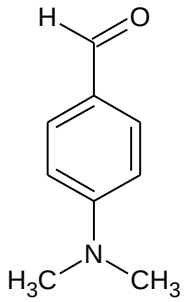
Solubility: Soluble in chloroform, acetonitrile, methanol and hot water.

Description: Yellow to tan powder

Melting Point: 114°C

4. Para- DIMETHYL AMINO BENZALDEHYDE

Structure:



Synonym: 4- (dimethylamino) benzaldehyde

Molecular Formula: $C_9H_{11}NO$

Molecular Weight: 149.18

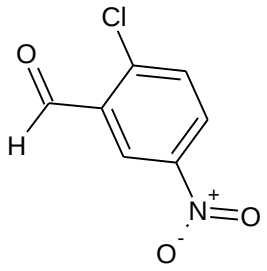
Solubility: Soluble in chloroform,acetonitrile,hot ethanol.Slightly soluble in water.

Description: White crystalline powder

Melting Point: 73°C

5. 2-CHLORO 5-NITRO BENZALDEHYDE

Structure:



Synonym: 2- chloro-5-nitro benzaldehyde

Molecular Formula: $C_7H_4ClNO_3$

Molecular Weight: 185.56

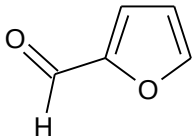
Solubility: Soluble in DMSO,chloroform, acetonitrile.

Description: Light yellow to beige yellow crystalline powder

Melting Point: 76°C

6. FURFURALDEHYDE

Structure:



Synonym: Furan-2-Carbaldehyde

Molecular Formula: $C_5H_4O_2$

Molecular Weight: 96.08

Solubility: Soluble in methanol.Slightly soluble in water.

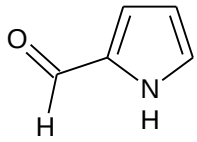
Description: Colourless Oil

Melting Point: -37°C

Boiling Point: 162°C

7. PYRROLE 2 CARBOXALDEHYDE

Structure:



Synonym: **2-Formylpyrrole**

Molecular Formula: C_5H_5NO

Molecular Weight: 95.10

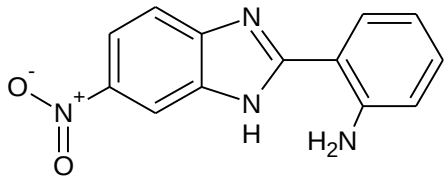
Solubility: Soluble in chloroform, DMSO, methanol. Insoluble in water.

Description: Light yellow crystalline powder

Melting Point: 44°C

INTERMEDIATE PROFILE

Structure:



Synonym: 2-(6-Nitro-1H-benzimidazol-2-yl)aniline

Molecular Formula: $C_{13}H_{10}N_4O_2$

Molecular Weight: 254.24

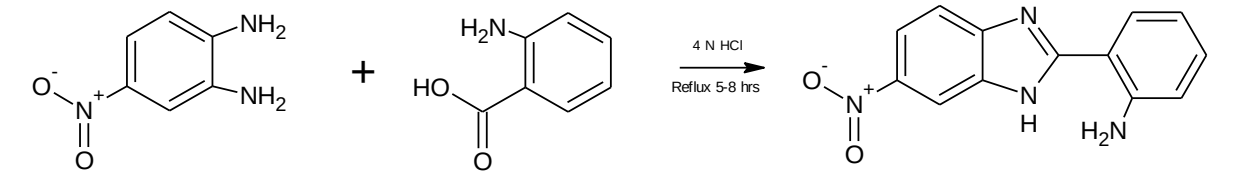
Solubility: Soluble in DMSO, ethanol. Insoluble in water.

Description: Dark red needle shaped crystals

Melting Point: 90°C

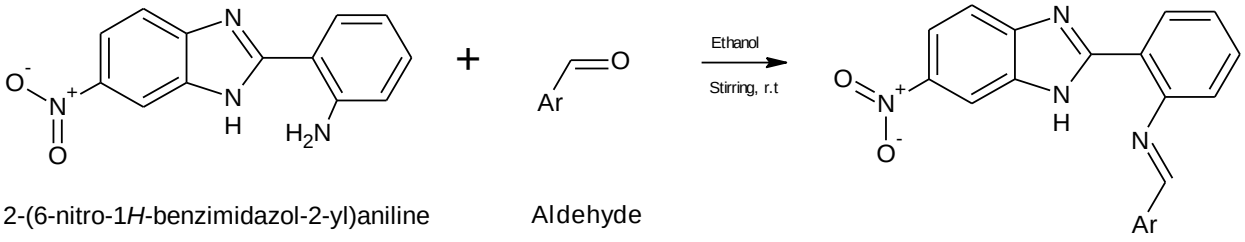
SYNTHESIS [51]

STEP – 1



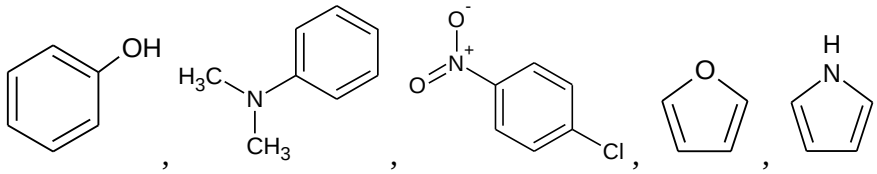
4-nitrobenzene-1,2-diamine + 2-aminobenzoic acid → 2-(6-nitro-1*H*-benzimidazol-2-yl)aniline

STEP – 2



2-(6-nitro-1*H*-benzimidazol-2-yl)aniline + Aldehyde → Product

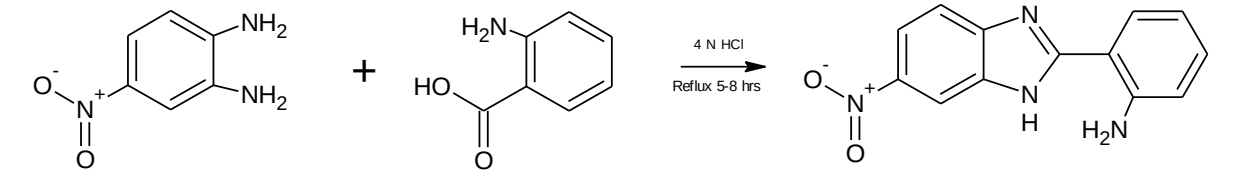
Ar-



PROCEDURE

STEP 1: PREPARATION OF 2-(6-NITRO-1*H*-BENZIMIDAZOL-2-YL) ANILINE[36]

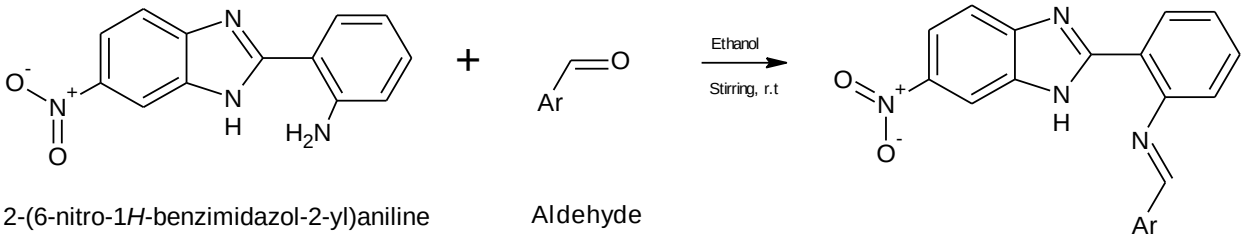
3.06 g (0.02 mole) of 4-nitro o- phenylene diamine and 2.64g (0.02 mole) anthranilic acid was taken in a round bottom flask. 50 ml of 4 N Hydrochloric acid was added to it. It was refluxed for 5-8 hours. To check the completeness of the reaction monitoring was done by TLC.After completion of the reaction, the mixture was poured into crushed ice. Sodium hydroxide was added drop wise till the solution was neutral to litmus. The precipitate obtained was filtered, washed with water, dried and recrystallised using ethanol.



4-nitrobenzene-1,2-diamine 2-aminobenzoic acid 2-(6-nitro-1*H*-benzimidazol-2-yl)aniline

STEP 2: PREPARATION OF SCHIFF BASE [50]

0.01 mole of the product obtained in step 1 and 0.01 mole of the aldehyde were taken in a round bottom flask. 50 ml of ethanol was added to it and was stirred at room temperature for 10 to 15 hours. The progress of the reaction was monitored by TLC. The product formed was filtered and dried. It was recrystallised using ethanol



2-(6-nitro-1*H*-benzimidazol-2-yl)aniline Aldehyde Product

RECRYSTALLISATION

To the synthesized compound, warm ethanol was added and it was heated. The hot solution was filtered and the filtrate was collected in a china dish and allowed to cool. On cooling, crystals appeared.

THIN LAYER CHROMATOGRAPHY

The reactants and products were dissolved in ethanol. It was spotted on the TLC plate.

Stationary phase: Pre-coated Silica gel GF plates
Mobile phase: Toluene: Ethyl Acetate: Formic Acid (5:4:1)
Detection: UV chamber

A single principle spot for the product and the absence of secondary spots and spots for parent compounds confirmed the purity of the compound.

CHARACTERISATION

The synthesized compounds were identified by the following methods:

1. **MELTING POINT**

The melting point of the synthesized compound was determined by one end open capillary tube method. The temperature at which the compound starts losing its crystallinity and changes from solid to liquid form was found and recorded.

2. **IR SPECTROSCOPY**

IR Spectroscopy helps to ascertain the presence and absence of the functional group. The synthesized compound was made into a pellet with potassium bromide by pressed pellet

technique using pellet press (Model No: M15) .The pellet was mounted on the pellet disc and percentage transmittance was recorded in ABB IR Spectrophotometer (Model No: MB 3000).

3. NMR SPECTROSCOPY

Proton NMR Spectroscopy helps us to study the number of equivalent protons and their environment thereby we can ascertain the structure of the molecule. The NMR spectra was recorded on 300 MHz BRUKER Advance III NMR Spectrometer. DMSO was used as solvent.

4. MASS SPECTROSCOPY

Mass Spectroscopy enables us to establish the molecular mass of the compound. The mass spectra of the synthesized compounds were recorded in Q-Tof-Mass Spectrometer (Q-Tof micro hybrid quadrupole time of flight mass spectrometer) with electrospray ionization (ESI) and in JEOL GCMATE II GC-MS.

E. IN VITRO ANTITUBERCULAR ACTIVITY

Anti-TB activity using Alamar Blue Dye [52]

PROCEDURE

1) The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).
2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.
3) Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation.
4) The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
5) The final drug concentrations tested were 100 to 0.2 µg/ml.
6) Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
7) After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
8) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

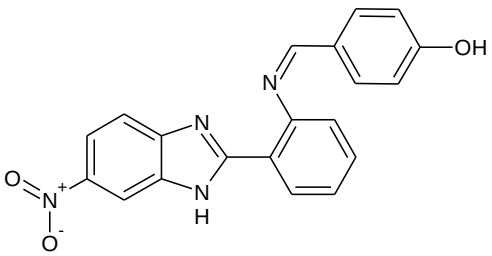
Results and Discussion

RESULTS AND DISCUSSION

A. DRUG DESIGN

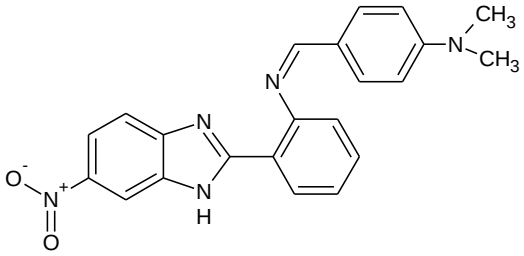
The designed molecules were docked against the selected target- Glutamine Synthase 1.Extra precision mode molecular docking was executed for perfect docking of the ligand into the cavity of the protein having active site. During the docking procedure different poses of the ligand were generated and the ligands were docked in different poses. The best docked pose was selected based on the G.Score generated and the interactions between the protein and the ligand. There were hydrogen bond interactions between all the five molecules and the active site. Hydrophobic interactions are also seen.

DOCKING SCORE OF THE COMPOUNDS



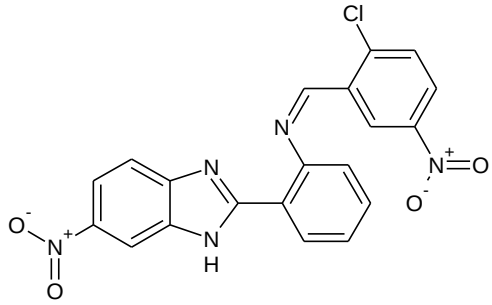
Compound Id: SSS-HBZ

G.Score: -6.72 kcal/mol



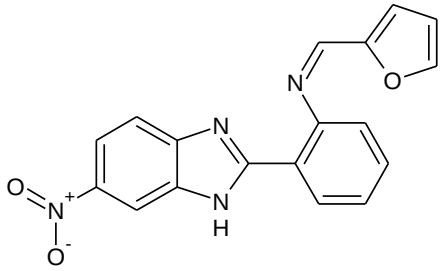
Compound Id: SSS-DMAB

G.Score: -4.97kcal/mol



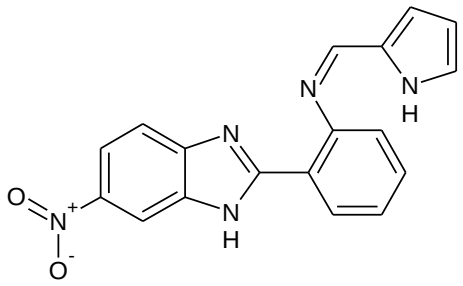
Compound Id: SSS-CNBZ

G.Score: -6.65 kcal/mol



Compound Id: SSS-FUR

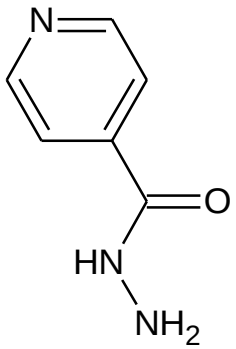
G.Score: -6.64kcal/mol



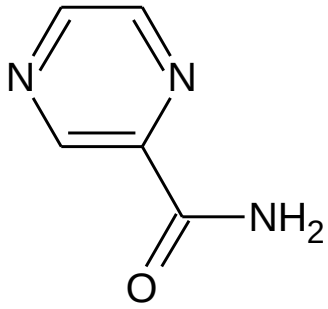
Compound Id: SSS-PC

G.Score: -5.49kcal/mol

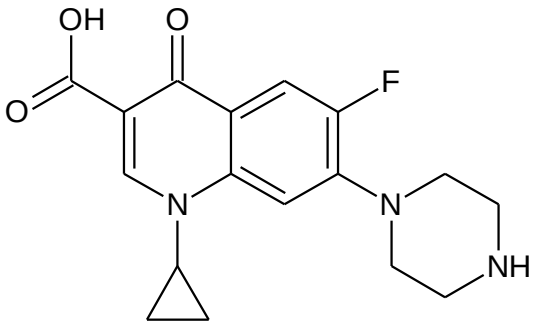
DOCKING SCORE OF THE STANDARD DRUGS



Isoniazide
G.Score: -4.4 kcal/mol



Pyrazinimide
G.Score: -4.1 kcal/mol



Ciprofloxacin
G.Score: -6.5 kcal/mol

COMPOUNDS DOCKED AGAINST THE PROTEIN 4ACF

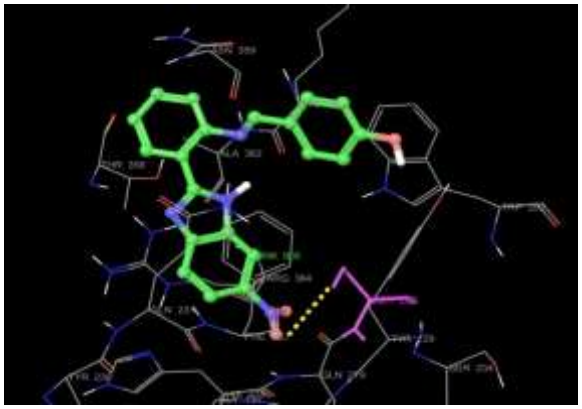


Fig 7: SSS- HBZ with 4 ACF

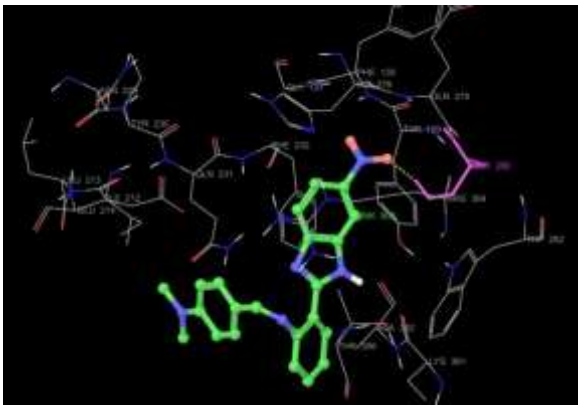


Fig 8: SSS-DMAB with 4 ACF

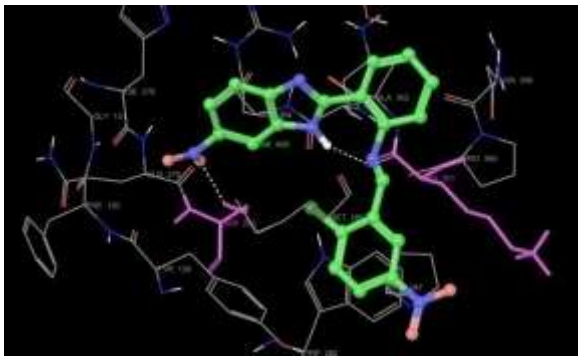


Fig 9: SSS- CNBZ with 4 ACF

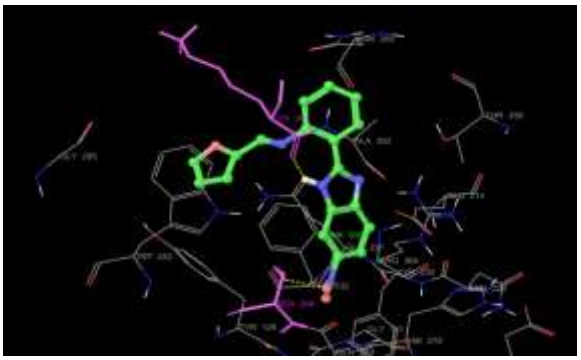


Fig 10: SSS-FUR with 4 ACF

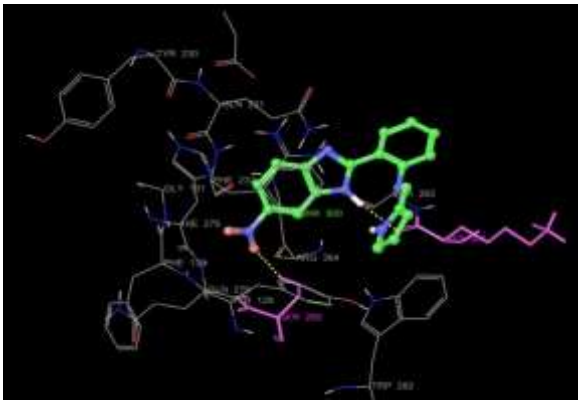
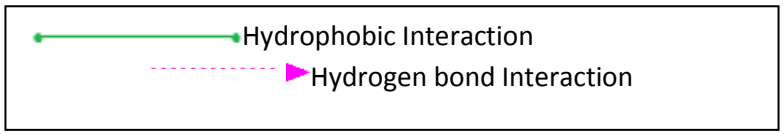


Fig 11: SSS-PC with 4 ACF

INTERACTIONS BETWEEN THE COMPOUNDS AND THE PROTEIN

Key:



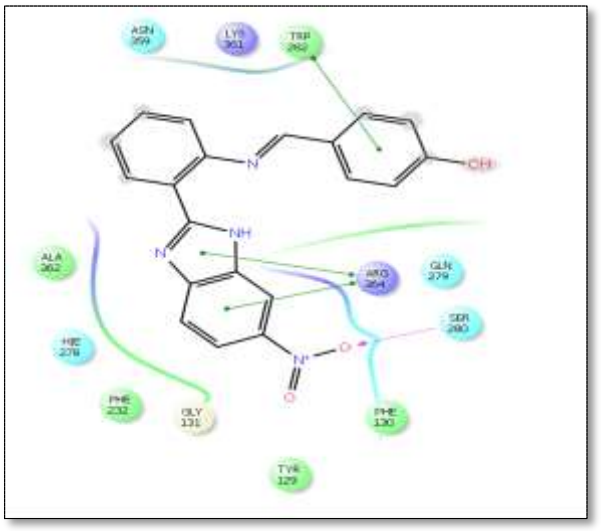


Fig 12: Interactions of SSS- HBZ with 4ACF

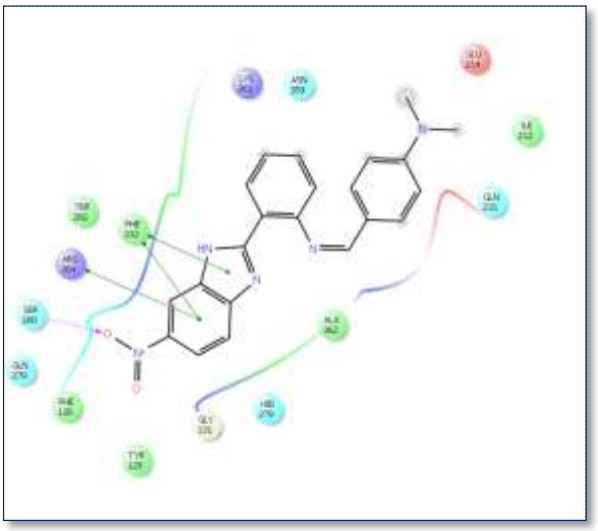


Fig 13: Interactions of SSS-DMAB with 4ACF

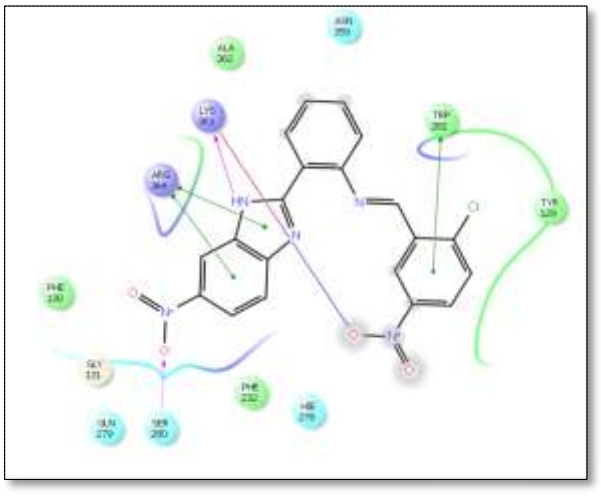


Fig 14: Interactions of SSS- CNBZ with 4ACF

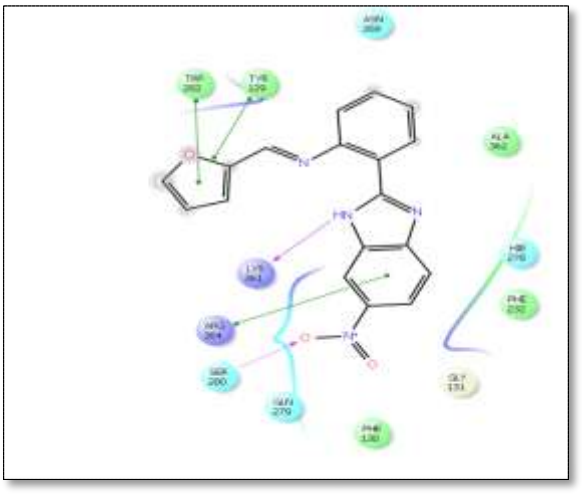


Fig 15: Interactions of SSS-FUR with 4ACF

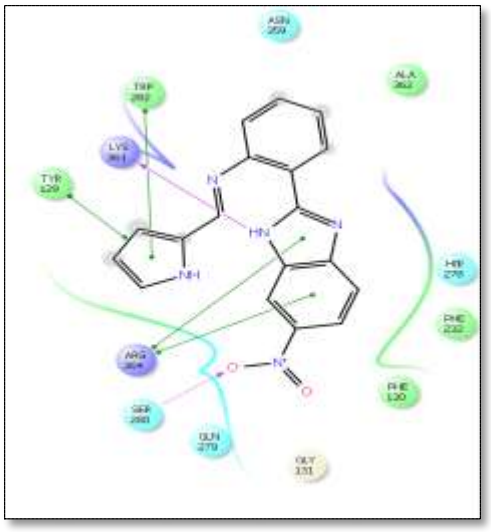


Fig 16: Interactions of SSS- CNBZ with 4ACF

Table 1: Rewards

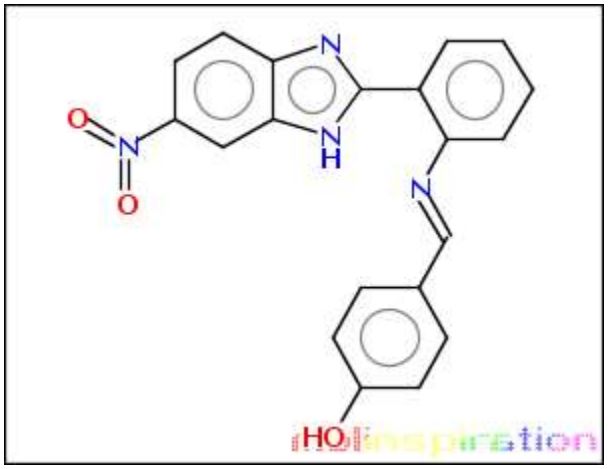
Ligand	G.Score	Dock Score	Lipophilic EvdW	PhobEn	PhobEn HB	PhobEn PairHB	HBond	Electro	Sitemap	iCat	ClBr	LowMW
SSS-HBZ	-6.72	-6.49	-4.1	-0.47	-1.5	0	-1.26	-0.21	-0.15	0	0	-0.31
SSS-DMAB	-4.97	-4.74	-3.34	0	-1.5	0	-0.66	-0.29	-0.12	0	0	-0.22
SSS-CNBZ	-6.65	-6.42	-4.7	-0.68	-1.5	0	-0.66	-0.11	-0.21	0	0	-0.09
SSS-FUR	-6.94	-6.71	-4.17	-0.53	-1.5	0	-0.66	-0.15	-0.24	0	0	-0.39
SSS-PC	-5.49	-5.26	-3.91	0	-1.5	0	-0.66	-0.19	-0.19	0	0	-0.4

Table 2: Penalties

Ligand	Penalties	HBPenal	ExposPenal	RotPenal	EpikStatePenalty	Similarity	Activity
SSS-HBZ	0	0	1.09	0.19	0.23	1	-6.72
SSS-DMAB	0	0	0.98	0.17	0.23	1	-4.97
SSS-CNBZ	0	0	1.16	0.14	0.23	1	-6.65
SSS-FUR	0	0	0.49	0.22	0.23	1	-6.94
SSS-PC	0	0	1.13	0.22	0.23	1	-5.49

B. IN-SILICO SCREENING OF DRUG LIKENESS

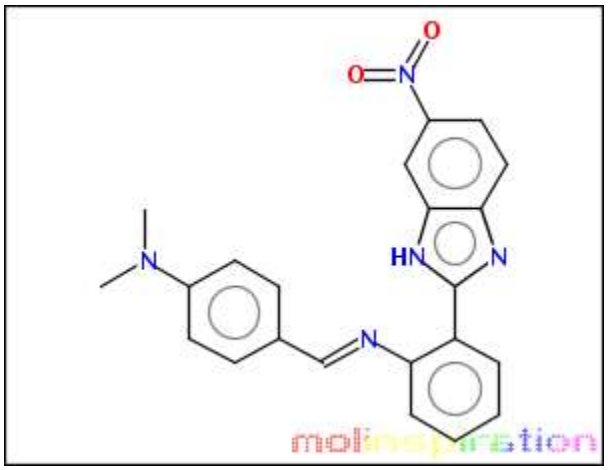
All the 5 compounds were screened for drug likeness by using Molinspiration Cheminformatics Software. On screening it was found that the ADME parameters were within the range.



Molinspiration property engine
v2013.09

miLogP	4.486
TPSA	107.099
natoms	27.0
MW	358.357
nON	7
nOHNH	2
nviolations	0
nrotb	4
volume	306.294

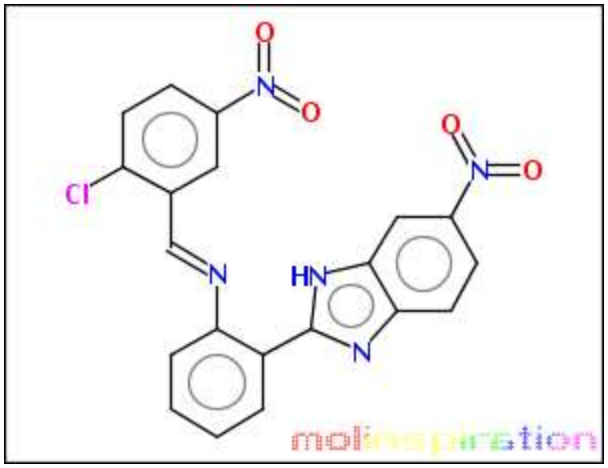
Fig 17: Screenshot of SSS-HBZ screening of drug likeness in Molinspiration



Molinspiration property engine
v2013.09

miLogP	5.067
TPSA	90.109
natoms	29.0
MW	385.427
nON	7
nOHNH	1
nviolations	1
nrotb	5
volume	344.182

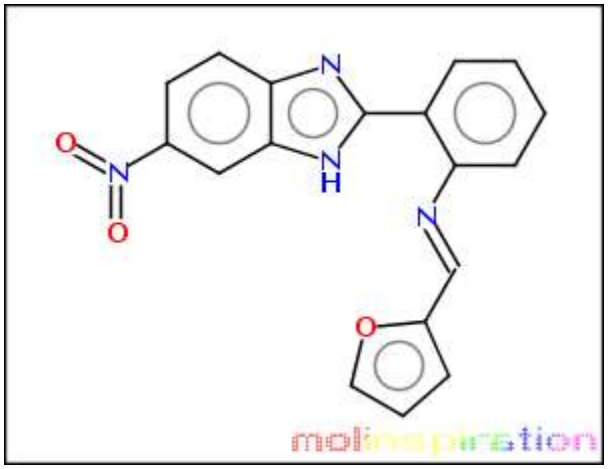
Fig 18: Screenshot of SSS-DMAB screening of drug likeness in Molinspiration



Molinspiration property engine
v2013.09

miLogP	5.53
TPSA	132.695
natoms	30.0
MW	421.8
nON	9
nOHNH	1
nviolations	1
nrotb	5
volume	335.146

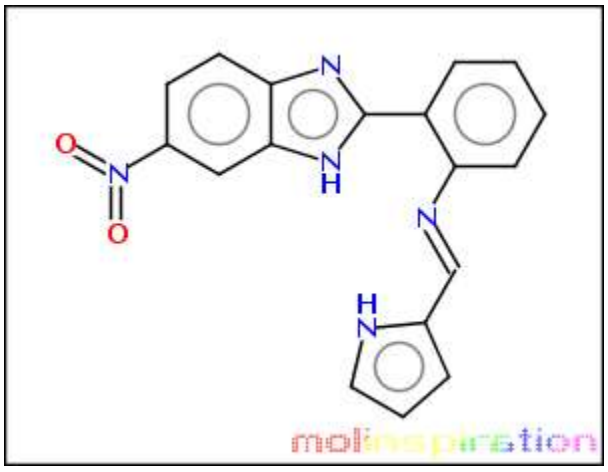
Fig 19: Screenshot of SSS-CNBZ screening of drug likeness in Molinspiration



Molinspiration property engine
v2013.09

miLogP	4.222
TPSA	100.011
natoms	25.0
MW	332.319
nON	7
nOHNH	1
nviolations	0
nrotb	4
volume	279.844

Fig 20: Screenshot of SSS-FUR screening of drug likeness in Molinspiration



Molinspiration property engine v2013.09

miLogP	4.119
TPSA	102.662
natoms	25.0
MW	331.335
nON	7
nOHNH	2
nviolations	0
nrotb	4
volume	283.262

Fig 21: Screenshot of SSS-PC screening of drug likeness in Molinspiration

Compound	miLogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb	Volume
SSS-HBZ	4.486	107.099	27.0	358.357	7	2	0	4	306.294
SSS-DMAB	5.067	90.109	29.0	385.427	7	1	1	5	344.182
SSS-CNBZ	5.53	132.695	30.0	421.8	9	1	1	5	335.146
SSS-FUR	4.222	100.011	25.0	332.319	7	1	0	4	279.844
SSS-PC	4.119	102.662	25.0	331.335	7	2	0	4	283.262

Table 3: Values for In-silico drug likeness assessment

C. IN SILICO TOXCITY PREDICTION

In silico toxicity prediction was done for the filtered 5 compounds using OSIRIS Property Explorer. This software is available for access in the Organic Chemistry Portal [15]. Using this prediction tool, mutagenicity, tumorogenicity, skin irritancy, and reproductive effects were calculated. The results were colour coded. The green colour represents that the compound is non-toxic. Yellow and red colour indicates moderate and severe toxicity of the chemicals respectively.

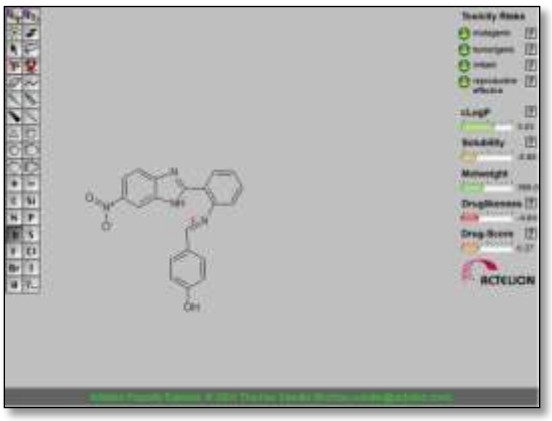


Fig 35: Toxicity prediction for SSS-HBZ

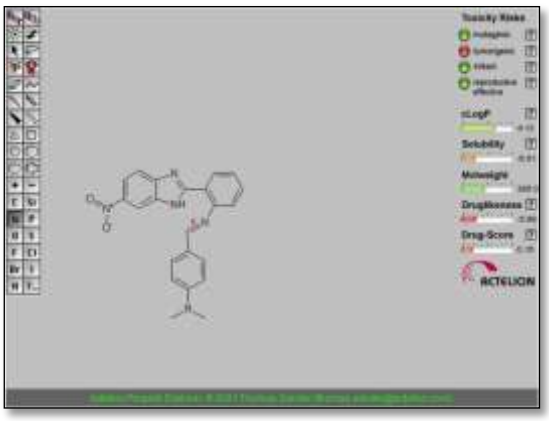


Fig 36: Toxicity prediction for SSS-DMAB

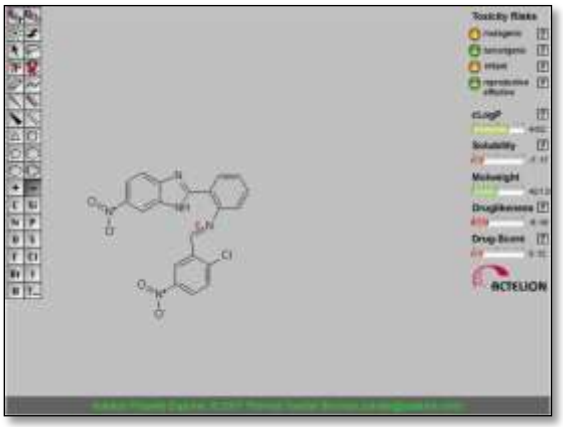


Fig 37: Toxicity prediction for SSS-CNBZ

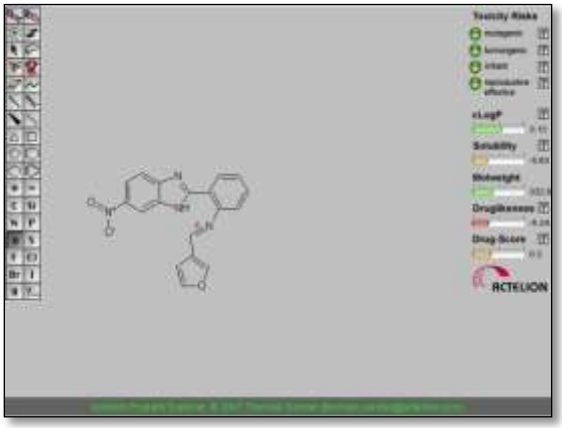


Fig 38: Toxicity prediction for SSS-FUR

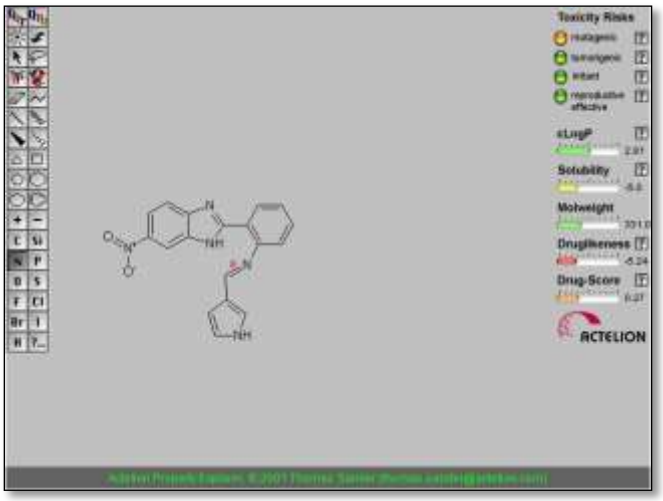


Fig 37: Toxicity prediction for SSS-PC

Compound Code	Mutagenicity	Tumorogenicity	Skin Irritancy	Reproductive Effect
SSS-HBZ	Green	Green	Green	Green
SSS-DMAB	Green	Red	Green	Green
SSS-CNBZ	Yellow	Green	Yellow	Green
SSS-FUR	Green	Green	Green	Green
SSS-PC	Yellow	Green	Green	Green

Table 6: Toxicity Predicition using OSIRIS

Compounds SSS-HBZ and SSS-FUR were predicted to be non-toxic. SSS-CNBZ and SSS-PC were predicted to be moderately mutagenic and SSS-DMAB was predicted to be severely tumorogenic.

D. SYNTHESIS AND CHARACTERIZATION

The synthetic scheme was drawn for the filtered 5 compounds from docking and the procedure for synthesis was collected from literature. The necessary chemicals of laboratory grade were procured and synthesis was carried out after the optimization of the reaction conditions. Products were obtained with a yield of about 77% and then recrystallised. The physical properties such as appearance, solubility, melting point were recorded uncorrected and tabularized below.

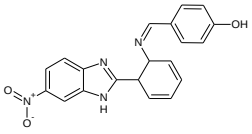
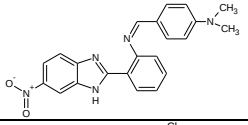
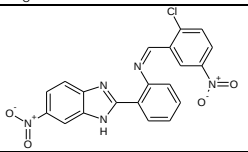
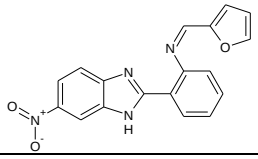
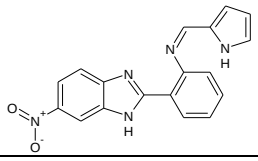
Compound	Molecular Weight	Molecular Structure	Solubility	Melting Point	Appearance
SSS-HBZ	360.36		Dimethyl Sulphoxide, Acetonitrile	217ºC	Brown coloured powder
SSS-DMAB	385.41		Dimethyl Sulphoxide, Acetonitrile	165ºC	Dark red coloured powder
SSS-CNBZ	421.79		Dimethyl Sulphoxide, Acetonitrile	150 ºC	Dark brown coloured powder
SSS-FUR	332.31		Dimethyl Sulphoxide, Acetonitrile	128 ºC	Brown coloured crystalline powder
SSS-PC	331.32		Dimethyl Sulphoxide, Acetonitrile	200 ºC	Dark Reddish crystals

Table 4: Physical Properties of the synthesized compounds

Further the products were characterized and the purity was justified by taking the melting point, running TLC, IR Spectroscopy, NMR Spectroscopy and Mass Spectroscopy. The results are interpreted along with the spectra below.

COMPOUND SSS-HBZ

a. IR SPECTROSCOPY

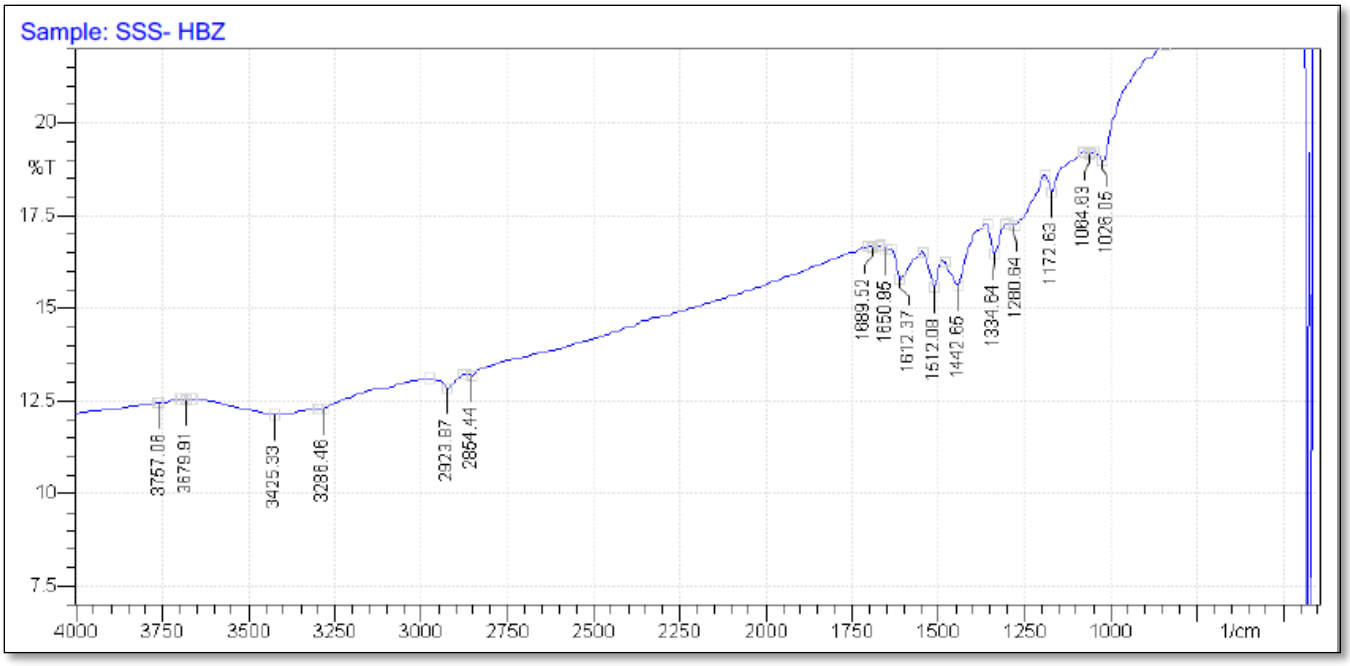


Fig 22: IR Spectra of SSS-HBZ

Compound	IR Absorption Region	Interpretation
SSS-HBZ	1442.65 cm^{-1}	C=C Aromatic Stretching
	2923.87 cm^{-1}	C-H Aliphatic Stretching
	3286.46 cm^{-1}	N-H 2º Amine Stretching
	3425.33 cm^{-1}	O-H Hydroxyl Stretching
	1334.61 cm^{-1}	N=O (Ar-NO_2) Stretching
	1512.08 cm^{-1}	C=N Stetching in Heterocyclic Rings

b. NMR SPECTROSCOPY

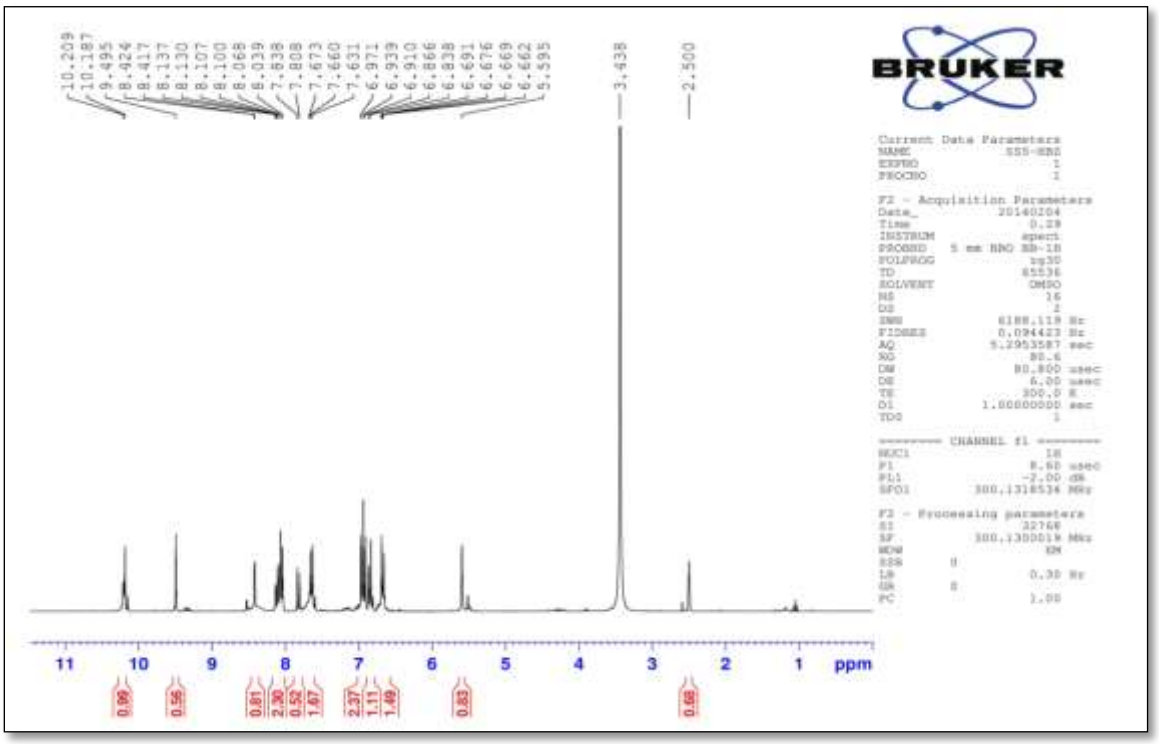


Fig 23: NMR Spectra for SSS- HBZ

Compound	^{1}H NMR Data
SSS-HBZ	δ 2.45- 2.53 (s, 1H, Aliphatic H) δ 5.52- 5.61 (s, 1H, Hydroxyl H) δ 6.53- 6.78 (d, 1H, Aromatic H) δ 6.78- 6.89 (d, 1H, Aromatic H) δ 6.89- 7.02 (t, 2H, Aromatic H) δ 7.58- 7.78 (d, 2H, Aromatic H) δ 7.78- 7.89 (d, 1H, Aromatic H) δ 8.01- 8.17 (m, 2H, Aromatic H) δ 8.38- 8.48 (s, 1H, Aromatic H) δ 9.45- 9.55 (s, 1H, Aromatic H) δ 10.12- 10.28 (m, 1H, Aromatic H)

c. MASS SPECTROSCOPY

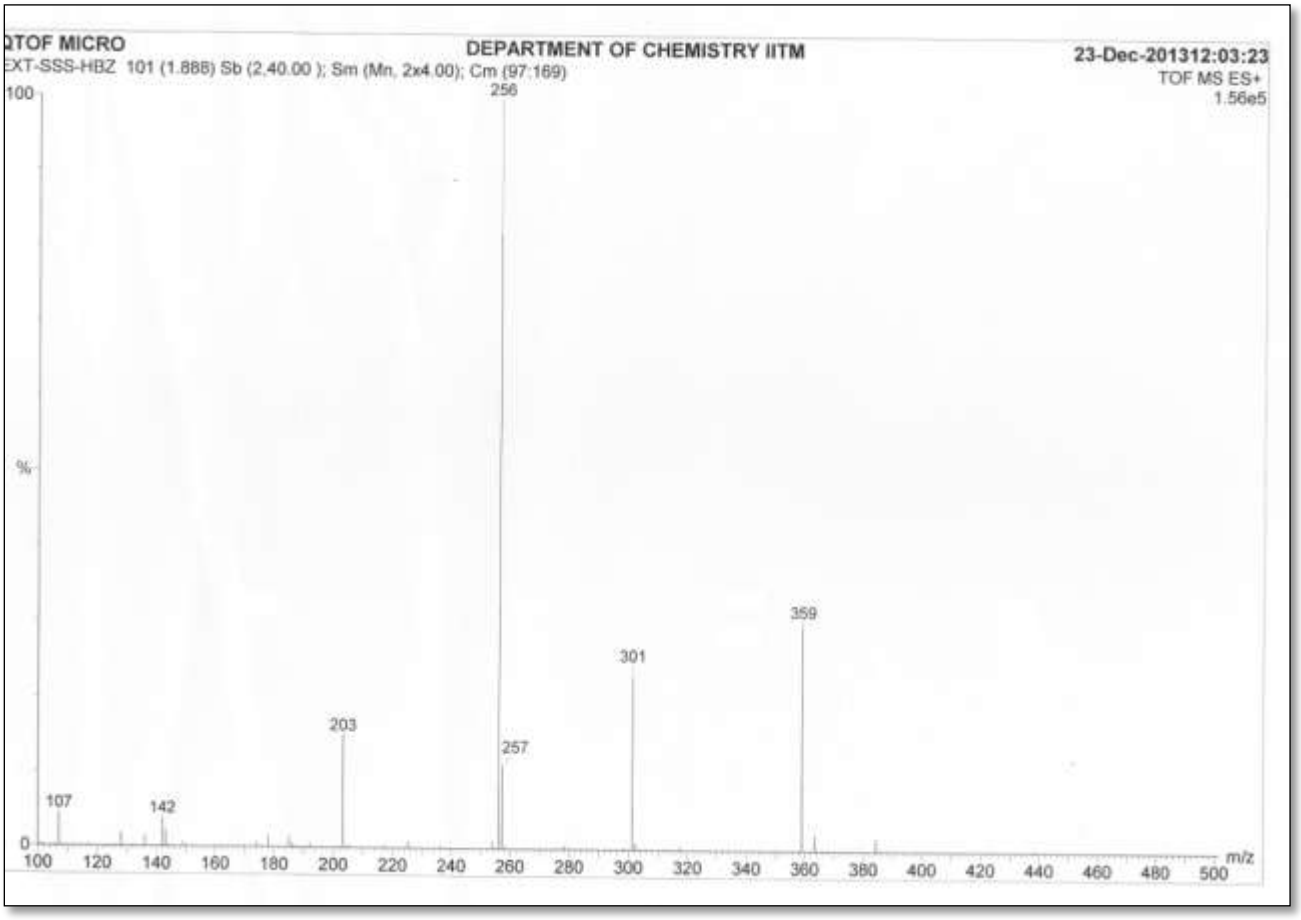


Fig 24: Mass Spectra for SSS- HBZ

Compound	Mol. formula / Mol. Wt calculated	m/e value Relative abundance
SSS-HBZ	$C_{20}H_{14}N_4O_3$ / 358.35	359 (M^+) 256 (B)

COMPOUND SSS-DMAB

a. IR SPECTROSCOPY

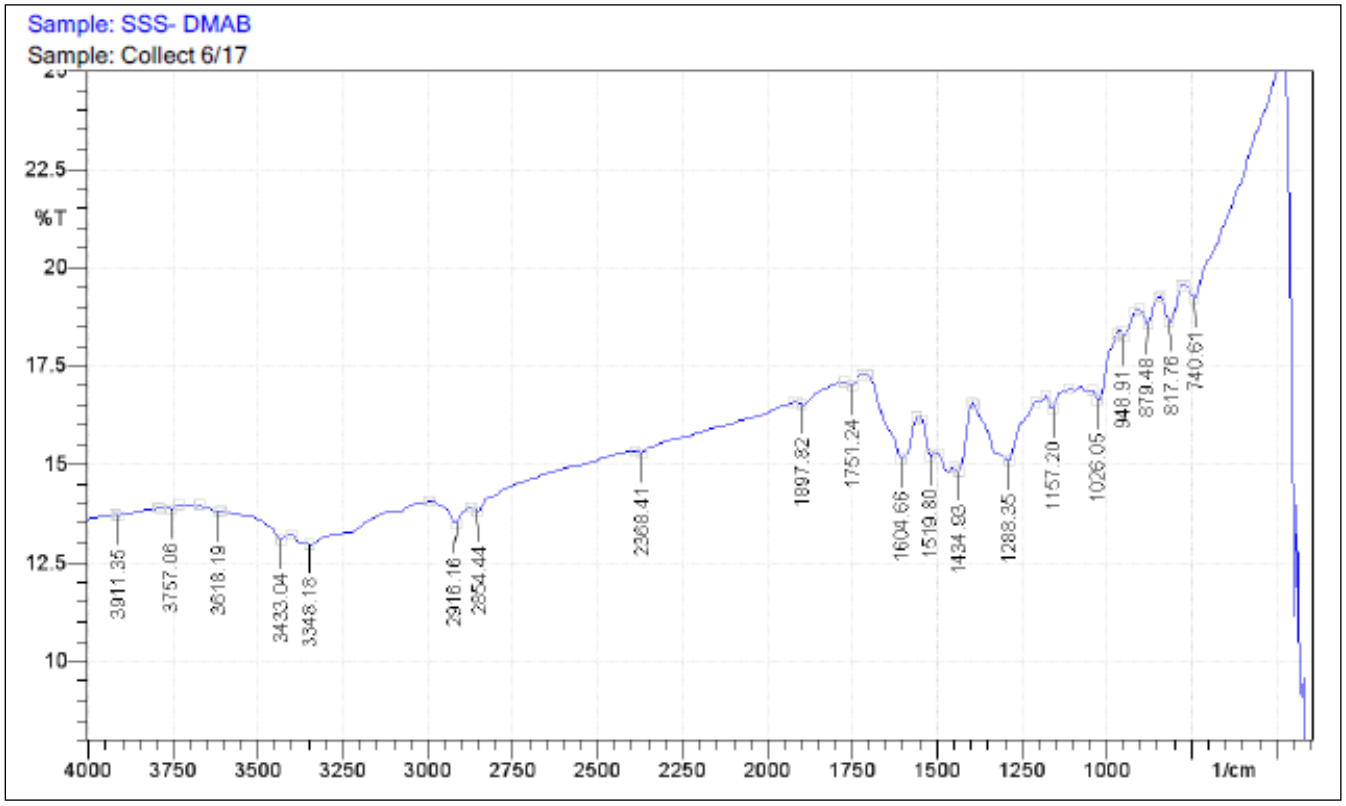


Fig 25: IR Spectra of SSS-DMAB

Compound	IR Absorption region	Interpretation
SSS-DMAB	1434.93 cm^{-1}	C=C Aromatic Stretching
	2916.16 cm^{-1}	C-H Aliphatic Stretching
	3348.18 cm^{-1}	N-H 2º Amine Stretching
	1288.35 cm^{-1}	N=O (Ar-NO_2) Stretching
	1519.80 cm^{-1}	C=N Stetching in Heterocyclic Rings
	1026.05 cm^{-1}	C-N Stretching

b. NMR SPECTROSCOPY

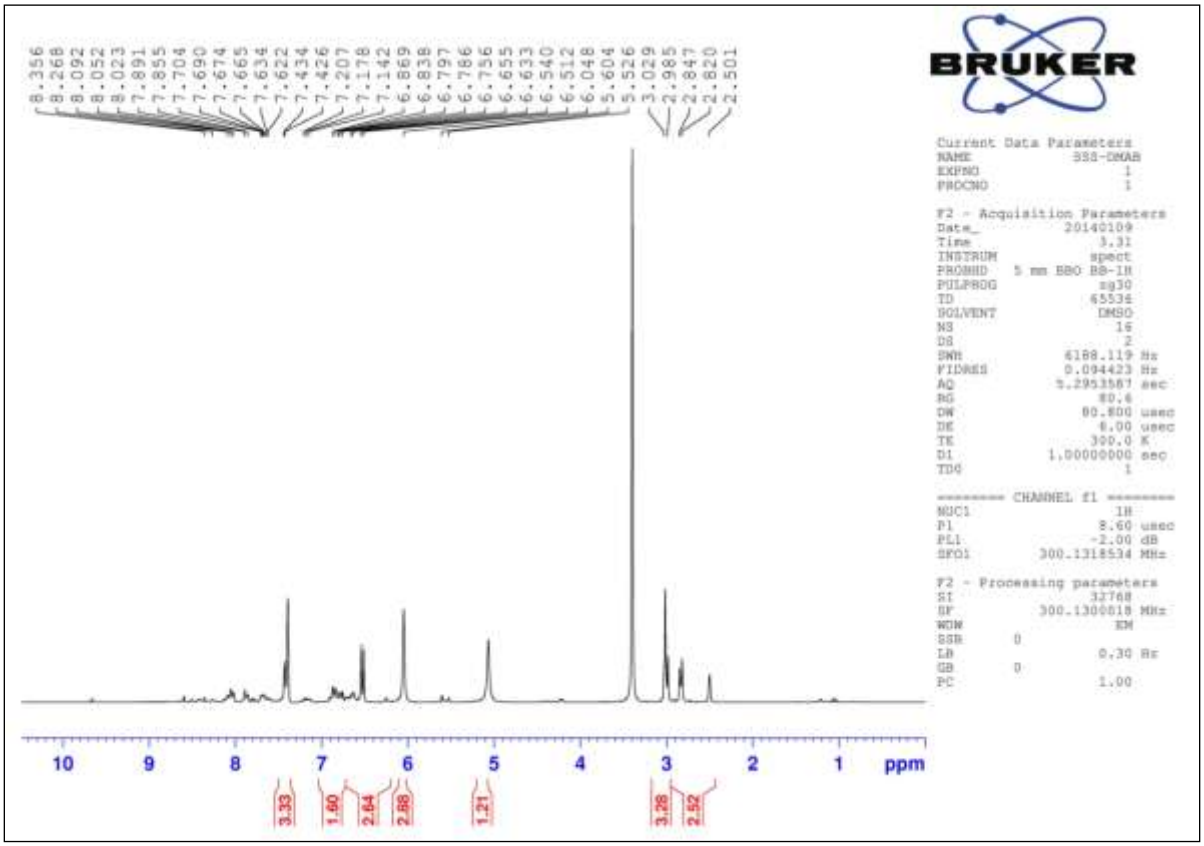


Fig 26: NMR Spectra for SSS- DMAB

Compound	^{1}H NMR Data
SSS-DMAB	δ 2.45- 2.97 (d, 3H, Aliphatic H) δ 2.97- 3.18 (d, 3H, Aliphatic H) δ 5.08- 5.19 (s, 1H, Aliphatic H) δ 6.02- 6.11 (s, 1H, Aromatic H) δ 6.18- 6.75 (d, 2H, Aromatic H) δ 6.75-7.01 (m, 2H, Aromatic H) δ 7.34- 7.49 (d, 1H, Aromatic H)

c. MASS SPECTROSCOPY

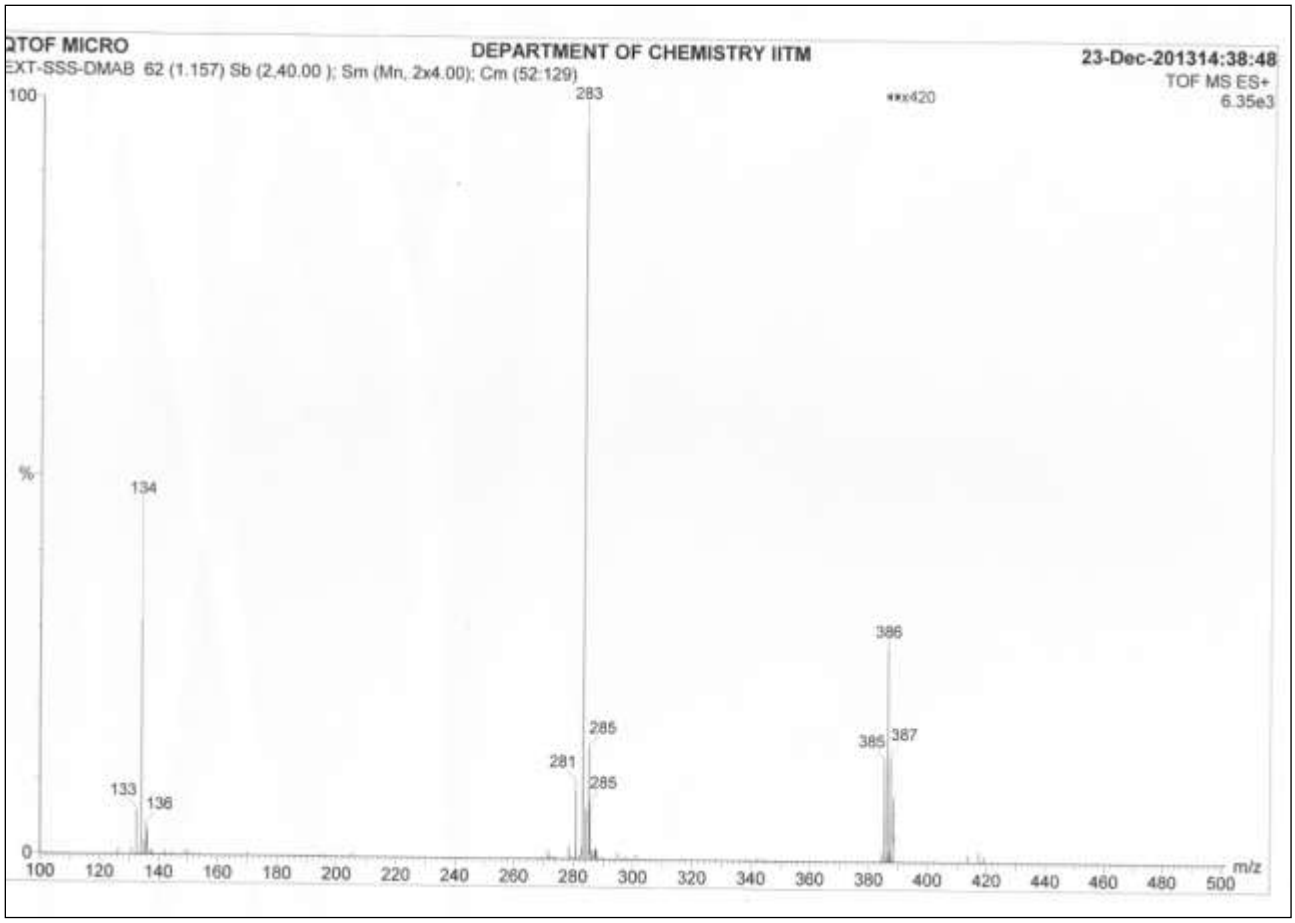


Fig 27: Mass Spectra for SSS- DMAB

Compound	Mol. formula / Mol. Wt calculated	m/e value Relative abundance
SSS-DMAB	$C_{22}H_{19}N_5O_2$ / 385.41856	386 (M^+) 283 (B)

COMPOUND SSS-CNBZ

a. IR SPECTROSCOPY

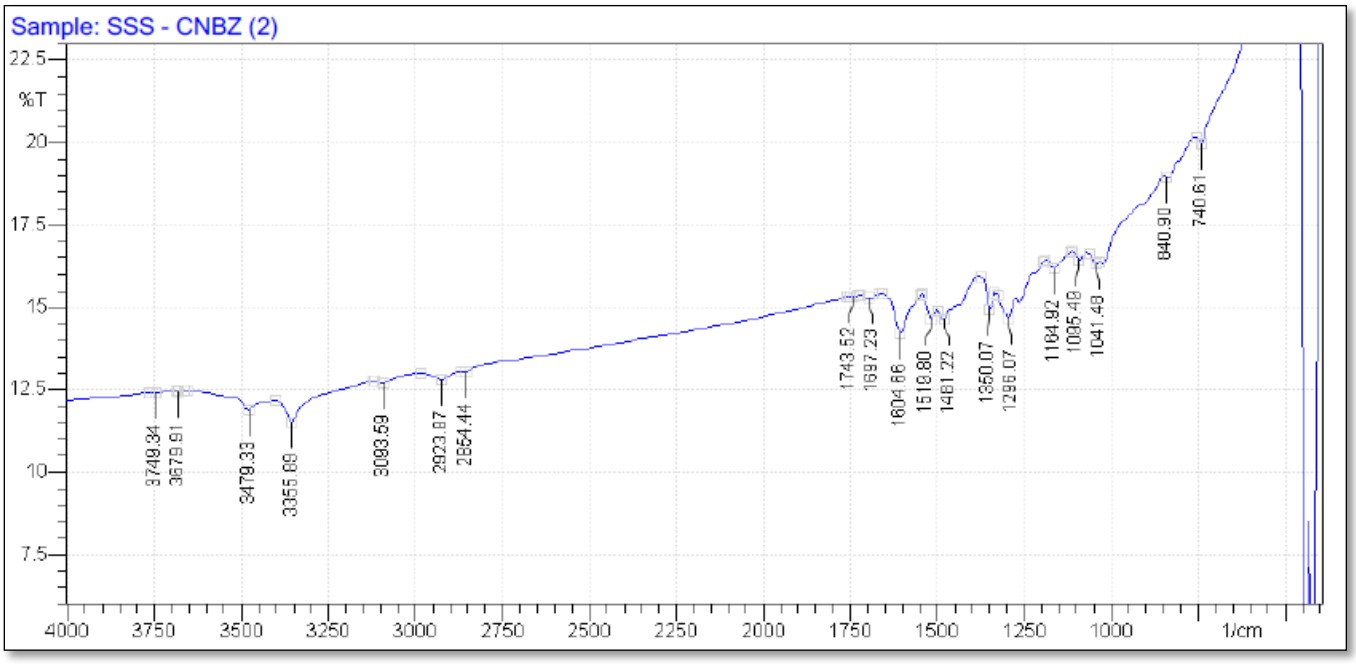


Fig 28: IR Spectra of SSS-CNBZ

Compound	IR Absorption region	Interpretation
SSS-CNBZ	1481.22 cm^{-1}	C=C Aromatic Stretching
	2923.87 cm^{-1}	C-H Aliphatic Stretching
	3093.59 cm^{-1}	C-H Aromatic Stretching
	3355.89 cm^{-1}	N-H 2º Amine Stretching
	1350.07 cm^{-1}	N=O (Ar-NO_2) Stretching
	1519.80 cm^{-1}	C=N Stetching in Heterocyclic Rings
	740.61 cm^{-1}	C-Cl Stretching

b. NMR SPECTROSCOPY

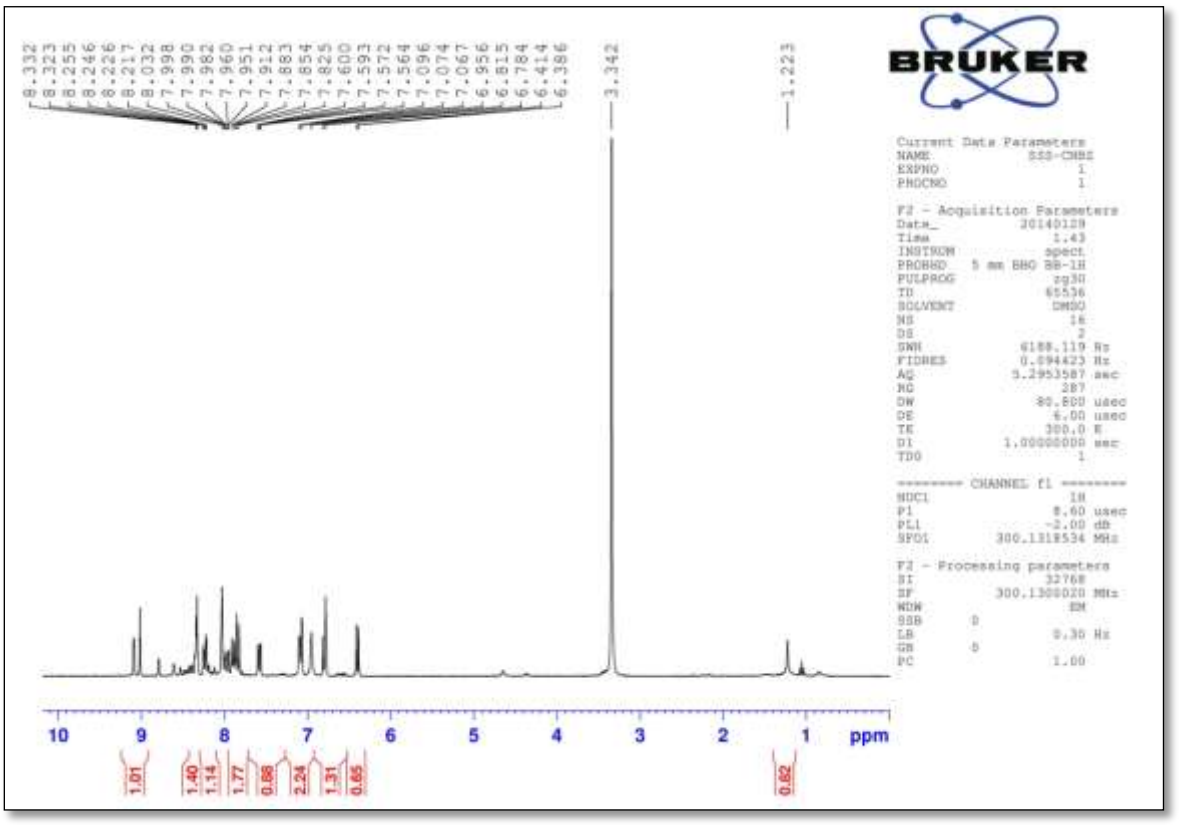


Fig 29: NMR Spectra for SSS- CNBZ

Compound	^{1}H NMR Data
SSS-CNBZ	δ 1.11-1.40 (s, 1H, Aliphatic H) δ 6.31-6.51 (d, 1H, Aromatic H) δ 6.52-6.95 (d, 1H, Aromatic H) δ 6.95-7.28 (d, 2H, Aromatic H) δ 7.28-7.69 (d, 1H, Aromatic H) δ 7.69-7.97 (m, 2H, Aromatic H) δ 8.10-8.31 (m, 1H, Aromatic H) δ 8.31-8.42 (s, 1H, Aromatic H) δ 8.91-9.22 (s, 1H, Aromatic H)

c. MASS SPECTROSCOPY

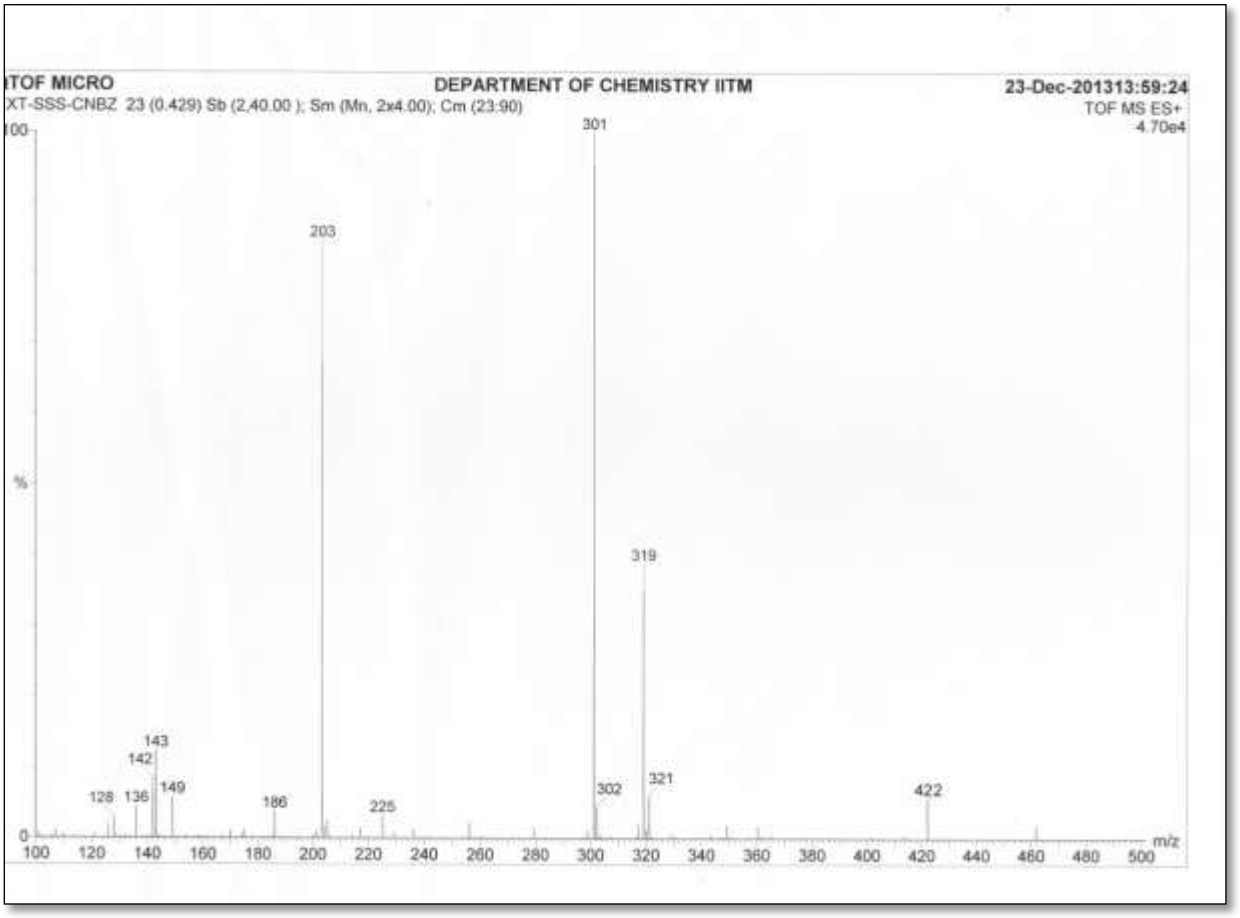


Fig 30: Mass Spectra for SSS- CNBZ

Compound	Mol. formula / Mol. Wt calculated	m/e value Relative abundance
SSS-CNBZ	$C_{20}H_{12}ClN_5O_4$/ 421.79338	422 (M^+) 301 (B)

COMPOUND SSS-FUR

a. IR SPECTROSCOPY

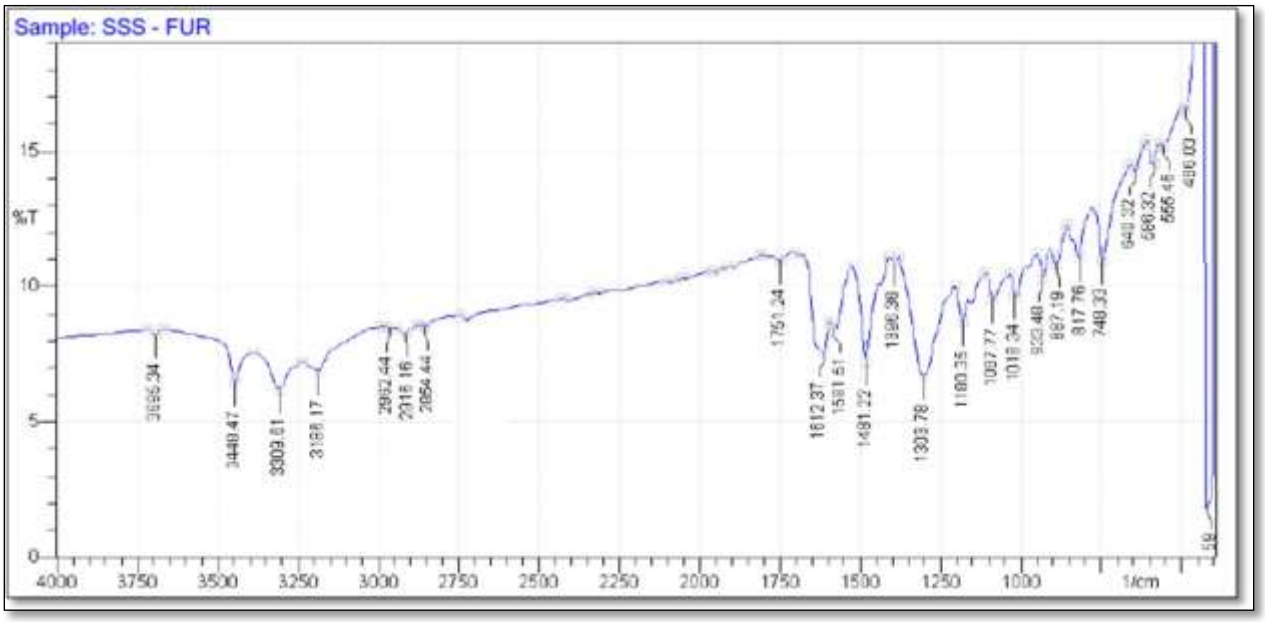


Fig 31: IR Spectra of SSS-FUR

Compound	IR Absorption region	Interpretation
SSS-FUR	1481.22 cm^{-1}	C=C Aromatic Stretching
	2916.16 cm^{-1}	C-H Aliphatic Stretching
	3186.17 cm^{-1}	C-H Aromatic Stretching
	3309.61 cm^{-1}	N-H 2º Amine Stretching
	1303.78 cm^{-1}	N=O (Ar-NO_2) Stretching
	1581.51 cm^{-1}	C=N Stetching in Heterocyclic Rings
	887.19 cm^{-1}	C-O Stretching in Heterocyclic Rings

b. NMR SPECTROSCOPY

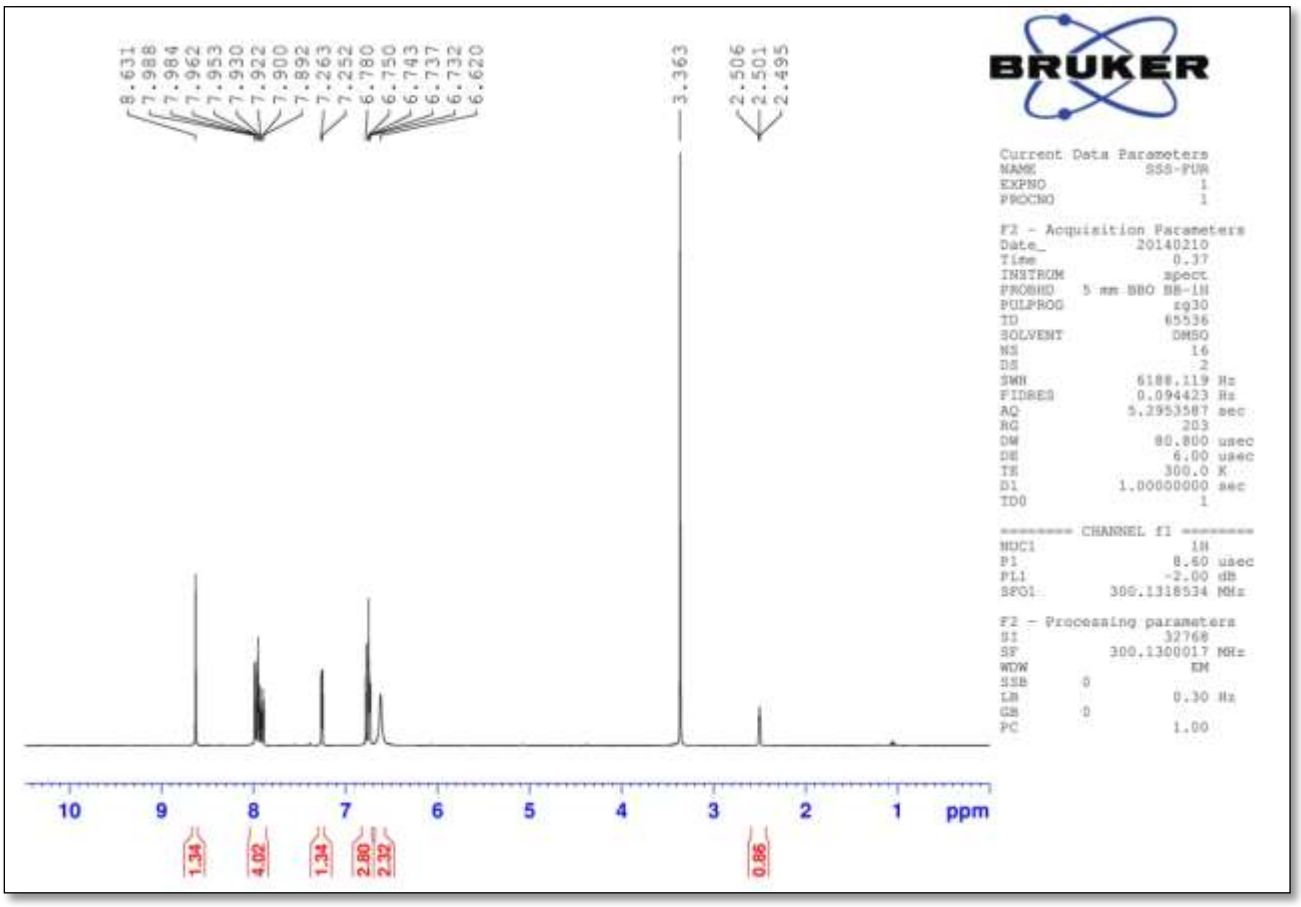


Fig 32: NMR Spectra for SSS- FUR

Compound	^{1}H NMR Data
SSS-FUR	δ 2.42- 2.59 (s, 1H, Aliphatic H) δ 6.56- 6.68(s, 2H, Aromatic H) δ 6.71- 6.82 (m, 3H, Aromatic H) δ 7.23- 7.29 (d, 1H, Aromatic H) δ 7.86- 8.03 (m, 4H, Aromatic H) δ 8.16- 8.66 (s, 1H, Aromatic H)

c. MASS SPECTROSCOPY

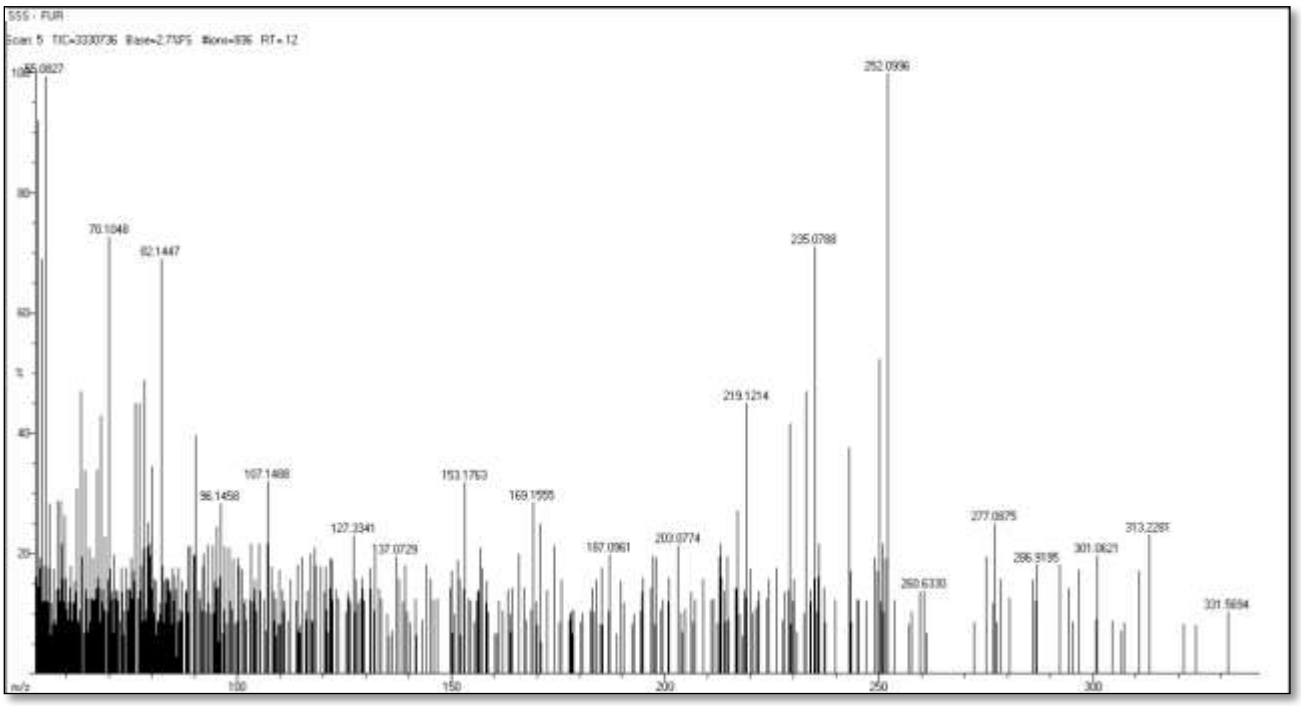


Fig 33: Mass Spectra for SSS- FUR

Compound	Mol. formula / Mol. Wt calculated	m/e value Relative abundance
SSS-FUR	$C_{18}H_{12}N_4O_3$ /332.31288	331.56 (M^+) 252.09 (B)

COMPOUND SSS-PC

a. IR SPECTROSCOPY

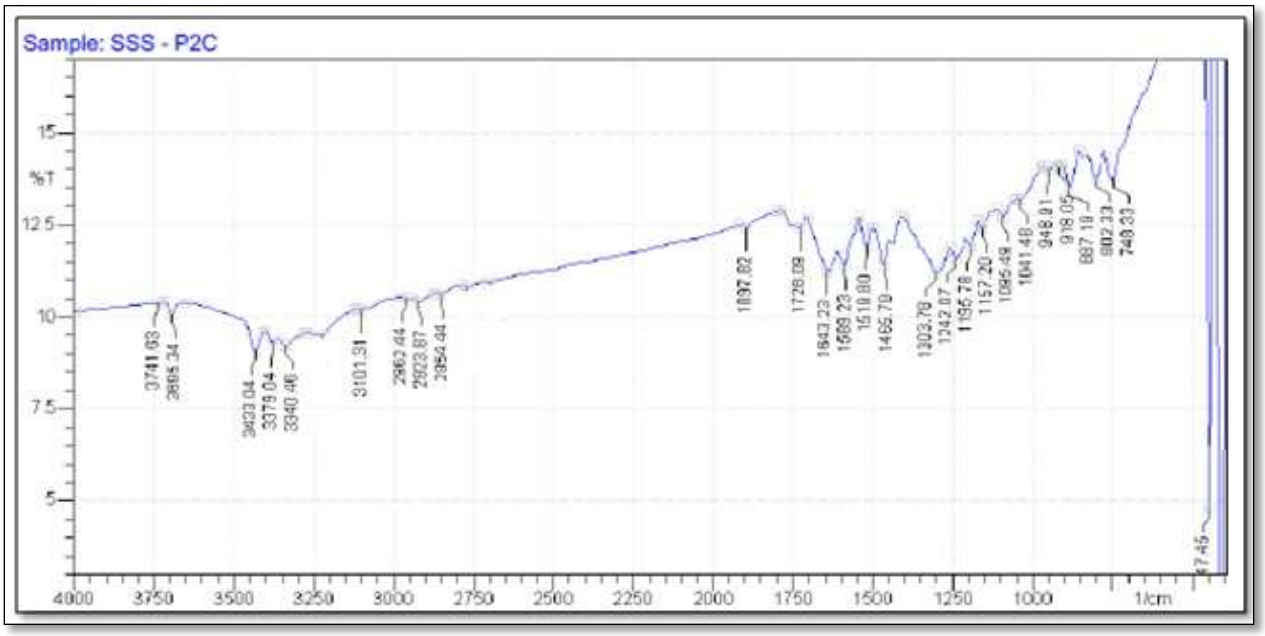


Fig 34: IR Spectra of SSS-PC

Compound	IR Absorption region	Interpretation
SSS-PC	1465.79 cm^{-1}	C=C Aromatic Stretching
	2923.87 cm^{-1}	C-H Aliphatic Stretching
	3101.31 cm^{-1}	C-H Aromatic Stretching
	3340.46 cm^{-1}	N-H 2º Amine Stretching
	1303.78 cm^{-1}	N=O (Ar-NO_2) Stretching
	1519.80 cm^{-1}	C=N Stetching in Heterocyclic Rings

b. NMR SPECTROSCOPY

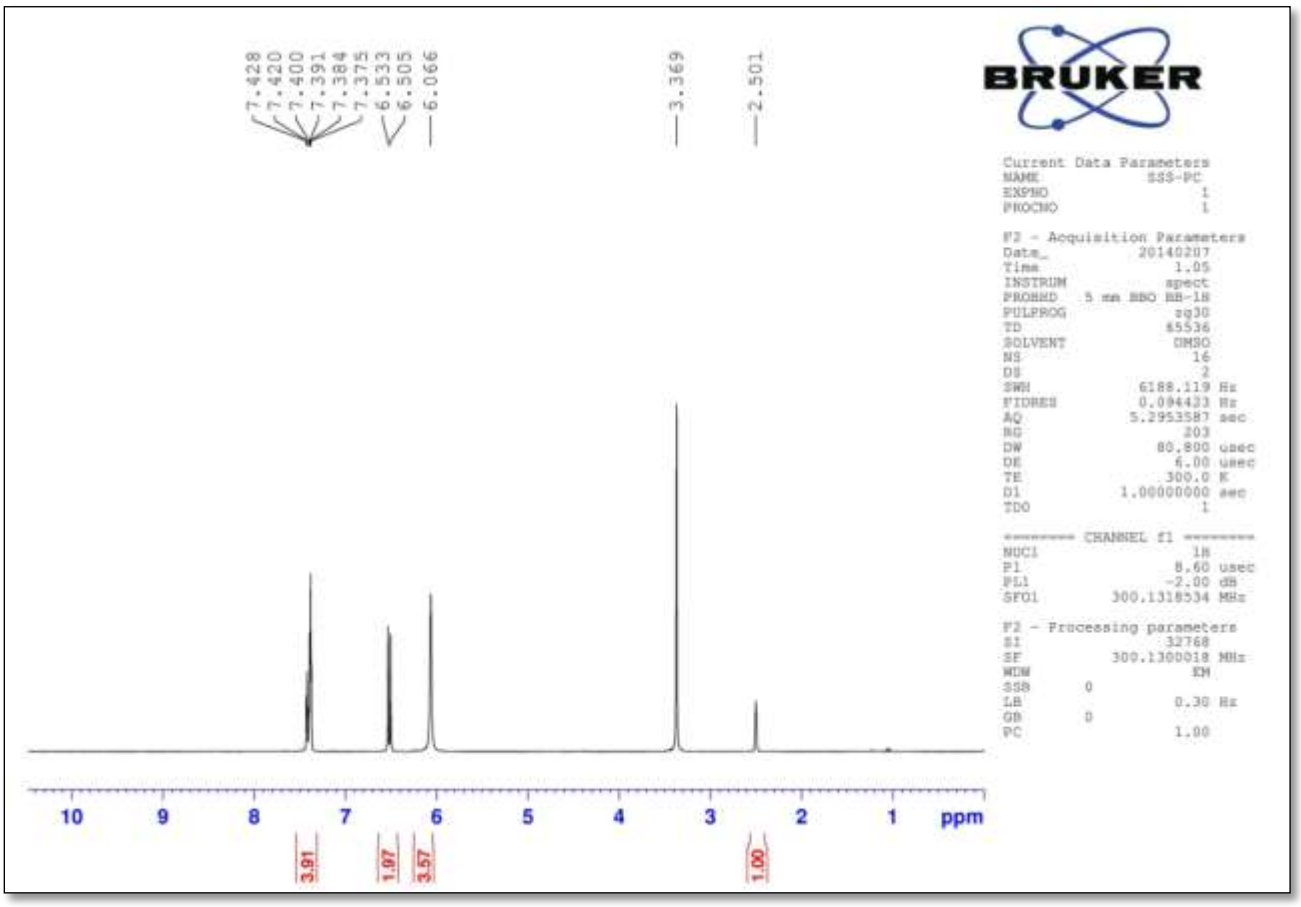


Fig 35: NMR Spectra for SSS- PC

Compound	^{1}H NMR Data
SSS-PC	δ 2.39- 2.55 (s, 1H, Aliphatic H) δ 6.05- 6.23 (s, 4H, Aromatic H) δ 6.42- 6.64 (d, 2H, Aromatic H) δ 7.30- 7.55 (m, 4H, Aromatic H)

c. MASS SPECTROSCOPY

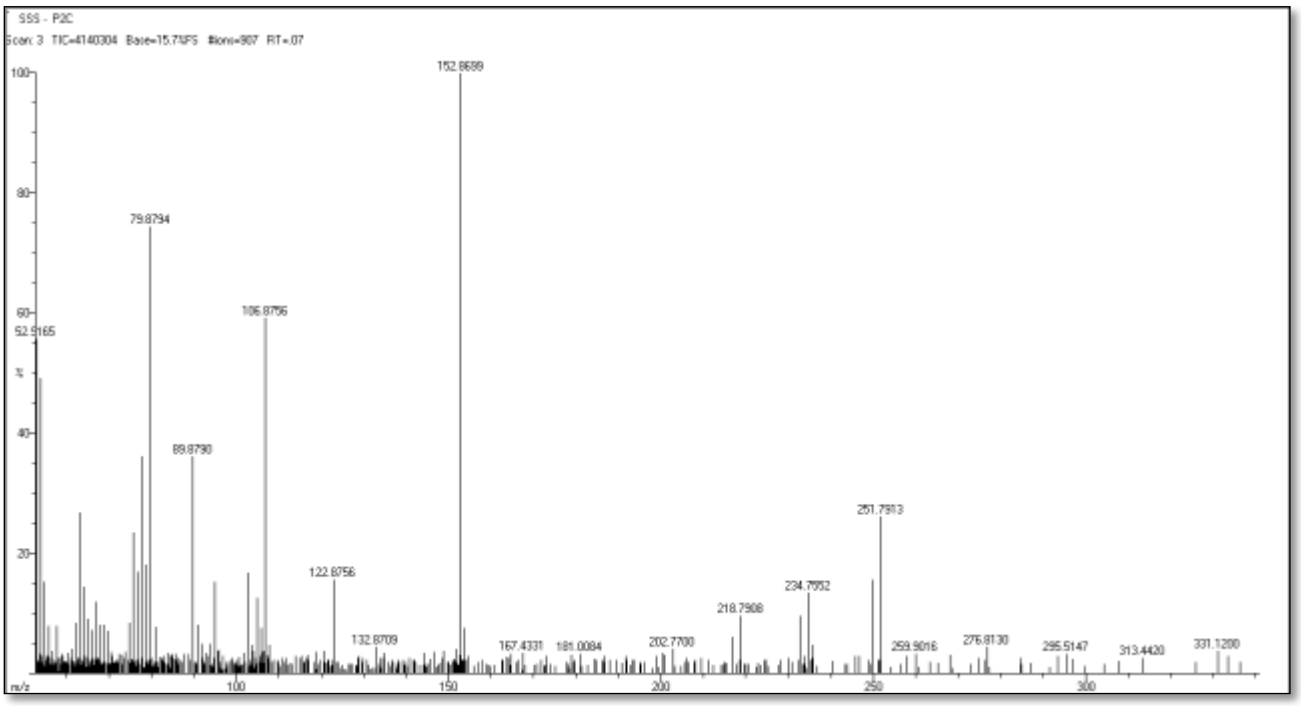


Fig 33: Mass Spectra for SSS- PC

Compound	Mol. formula / Mol. Wt calculated	m/e value Relative abundance
SSS-PC	$C_{18}H_{13}N_5O_2$ / 331.32812	331.12 (M^+) 152.86 (B)

E. IN VITRO ANTITUBERCULAR ACTIVITY

The anti-tubercular activity of the synthesized compounds was determined by Microplate Alamar Blue Assay (MABA) according to the procedure given in the materials and methods.

All the synthesized compounds showed antimycobacterial activity against the *M.tuberculosis* strain tested. The organism was susceptible to all the synthesized compounds and the minimum inhibitory concentration for the compounds varied between 100-50 µg/ml. Pyrazinamide (3.125 µg/ml), Streptomycin (6.25 µg/ml) and Ciprofloxacin (3.125 µg/ml) were used as standard. The data pertaining to this is presented as table.

Among the synthesized compounds all the compounds showed activity at 50 µg/ml except SSS-HBZ which showed activity at 100 µg/ ml. The picture below indicates the activity of the compound at the various dilutions.

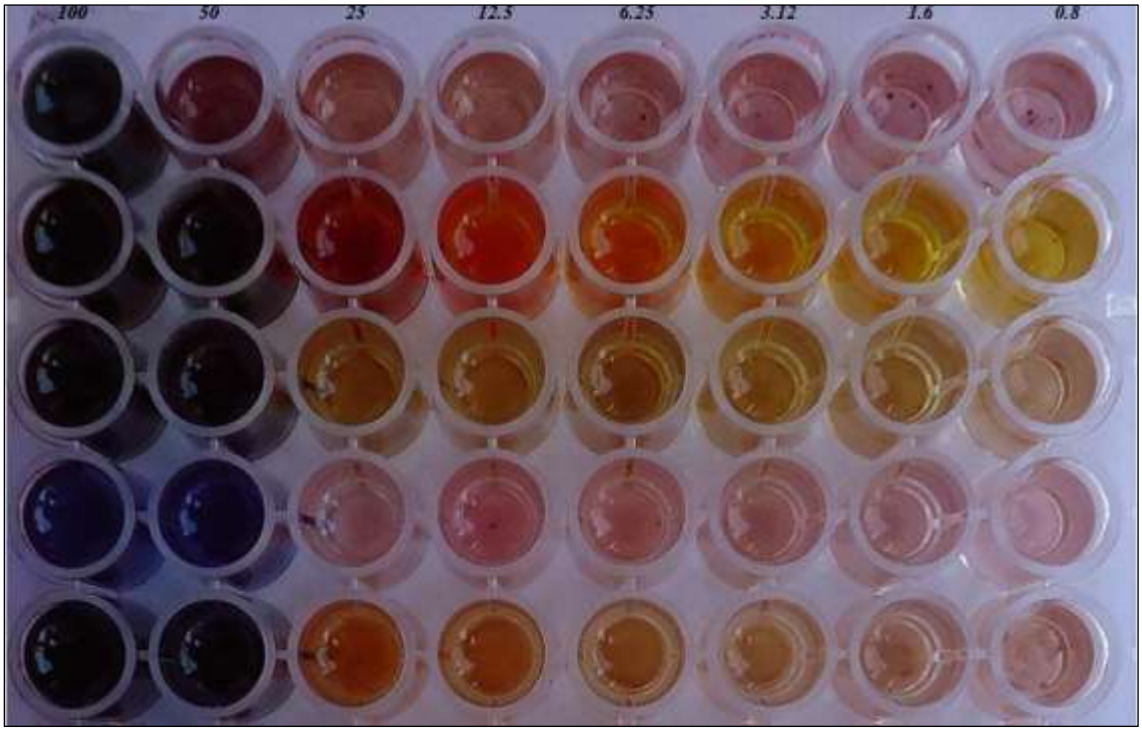


Fig 34: Anti tubercular activity by MABA for the synthesized compounds

Sl.No	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	SSS-HBZ	S	R	R	R	R	R	R	R
2.	SSS-DMAB	S	S	R	R	R	R	R	R
3.	SSS-CNBZ	S	S	R	R	R	R	R	R
4.	SSS-FUR	S	S	R	R	R	R	R	R
5.	SSS-PC	S	S	R	R	R	R	R	R

Table 5: Anti tubercular activity at various concentrations by MABA

NOTE:

S-Sensitive

R-Resistant

Strain Used: *M.tuberculosis* (H37 RV Strain)

Standard values:

Pyrazinimide- 3.125 µg/ml

Streptomycin-6.25 µg/ml

Ciprofloxacin- 3.125 µg/ml

Summary and Conclusion

SUMMARY

Glutamine Synthase I is a vital enzyme present in the cell wall of Mycobacterium tuberculosis H37Rv.It belongs to the Ligase family.

This enzyme was chosen as the target for the drug design study after thorough literature review. A database of 100 molecules with potential to inhibit the target (PDB id: 4ACF) was chosen by altering the lead molecule, 6-nitro benzimidazole.

The designed molecules were docked against the target chosen using Schrodinger's GLIDE® (Grid Based Ligand Docking with Energetics.

From among the docked molecules, 5 molecules with good Glide score were chosen for laboratory synthesis. The drug likeness and toxicity prediction was carried out for the filtered 5 compounds in silico.

Then further the compounds were synthesized. The reaction conditions were optimized.

The compounds were labeled as SSS-HBZ, SSS-DMAB, SSS-CNBZ, SSS- FUR and SSS-PC and synthesized and recrystallised.

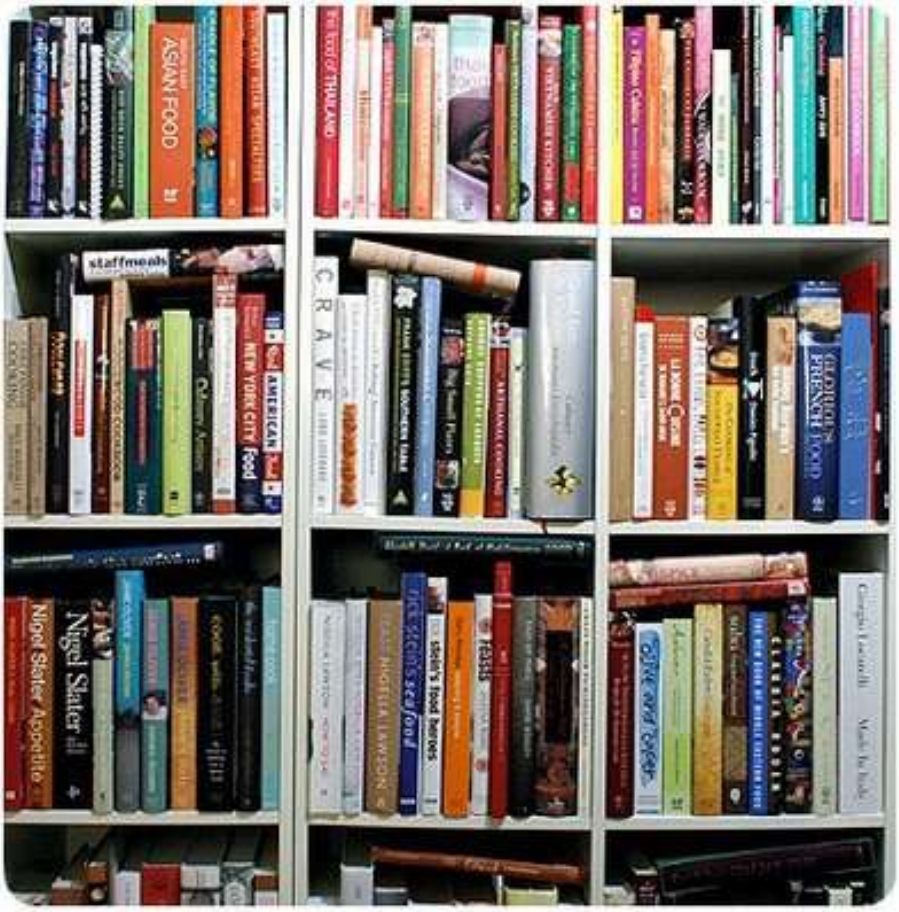
The purity of the synthesized compounds were evaluated by melting point and TLC and were characterized by Infrared Spectroscopy, Nuclear Magnetic Resonance Spectroscopy and Mass Spectroscopy.

The purified compounds were screened for antitubercular activity by *invitro* Micro Plate Alamar Blue Assay.

The Minimum Inhibitory Concentration (MIC) of all the synthesized compounds were at 50 µg /ml except SSS-HBZ which was at 100 µg/ml against the MIC of known TB drugs Pyrazinamide: 3.125 mcg/ml, Ciprofloxacin: 3.125 mcg/ml and Streptomycin 6.25 mcg/ml.

CONCLUSION

It is concluded that the synthesized compounds might effectively inhibit the chosen target, Glutamine Synthase 1 which is essential for the growth of the Mycobacterium tuberculosis. Further structural modifications of the synthesized compounds will aid in the development of potential molecules against the pathogen.



Bibliography

BIBLIOGRAPHY

1. Alimuddin Zumla, M.D., Ph.D., Mario Raviglione, M.D., Richard Hafner, M.D., C. Fordham von Reyn, M.D., Tuberculosis, *The New England Journa l of Medicine* 2013;368:745-55

2. World Health Organization, Global Tuberculosis Report 2012, ISBN 978 92 4 156450 2

3. Development of new vaccines and drugs for TB: Limitations and potential strategic errors, *Future Microbiology*, 2011 February ; 6(2): 161–177

4. Nancy A. Knechel, Tuberculosis: Pathophysiology, Clinical features and Diagnosis, *Crit Care Nurse* 2009; 29:34-43.

5. Ahmed Kamal, Shaik Azeeza, M. Shaheer Malik, Ahmad Ali Shaik and Maddamsetty V. Rao Efforts Towards the Development of New Antitubercular Agents: Potential for Thiolactomycin Based Compounds , *J Pharm Pharmaceutical Science* 11 (2): 56s-80s, 2008

6. Melvin K. Spigelman, New Tuberculosis Therapeutics: A Growing Pipeline , *Journal of Infectious Diseases*,(2007) 196 (Supplement 1): S28

7. Fulvio Guattieri, Organic and biomolecular chemistry- Vol II-Medicinal chemistry

8. Dipali Singh, Anushree Tripathi., An Overview Of Computational Approaches In Structure Based Drug Design, *Nepal Journal of Biotechnology*, Dec 2011, Vol 2, No: 1; 53-61.

9. Md. Mofizur Rahman, Md. Rezaul Karim, Md. Qamrul Ahsan, Abul Bashar Ripon Khalipha, Mohammed Raihan Chowdhury and Md Saifuzzaman.,Use of computer in

drug design and drug discovery: a review, *International Journal of Pharmaceutical and Life Sciences,* Volume 1, Issue 2, Serial 5: September 2012

10. Soma Mandal, Meenal Moudgil, Sanat K.Mandal, Rational Drug Design, *European Journal of Pharmacology* 625(2009) 90-100

11. Polamarasetty Aparoy, Kakularam Kumar Reddy, Pallu Reddanna, Structure and Ligand based drug design strategies in the development of Novel 5- LOX inhibitors, *Current Medicinal Chemistry*, 2012, 19,3763-3778

12. Burger's Medicinal Chemistry, sixth edition. Volume 1-8

13. Graham.L.Patrick. *An introduction to Medicinal Chemistry*, Fourth Edition, Oxford University Press. ISBN 0-19-855872-4

14. Lin J, Sahakian DC, De Morais SM, Xu JJ, Polzer RJ, Winter SM. The Role of Absorption, Distribution, Metabolism, Excretion And Toxicity In Drug Discovery. *Curr Top Med Chem*.2003; 3(10): 1125-1154

15. http://www.organic-chemistry.org/prog/peo/ retrieved on 22.12.2013

16. Y.R.Sharma., *Organic Spectroscopy*, Fourth Revised and Multicolour Edition 2012, S.Chand & Company ISBN : 81-219-2884-2

17. Gurudeep Chatwal and Sham K.Anand, *Instrumental Methods of Chemical Analysis*, Fifth And Enlarged Edition 2011, Himalaya Publishing House, ISBN: 978-93-5024-836-2

18. Sephra N.Rampersad, Multiple Applications of Alamar Blue as an Indicator of Metabolic Function and Cellular Health in Cell Viability Bioassays. *Sensors* 2012, 12, 12347-12360.

19. Shiqi Peng, Ming Zhao, Chunying Cui, Pharmaceutical Bioassays: Methods and Applications , WILEY, ISBN: 978-0-470-52219-6

20. Ramapreet Walia et al. Benzimidazole Derivatives- An Overview. *International Journal of Research in Pharmacy and Chemistry* 2011, 1 (3).

21. Goodsell, DS (June 2002). "Glutamine Synthetase". RCSB Protein Data Bank. Retrieved on 04/03/2014

22. Berlicki.L. ; Kafarski, P Inhibitors of glutamine synthetase and their potential application in medicine ,*Mini-reviews Med. Chem.* 2008, *8*, 869

23. Michael V. Tullius, Gunter Harth, and Marcus A. Horwitz ,Glutamine Synthetase GlnA1 Is Essential for Growth of *Mycobacterium tuberculosis* in Human THP-1Macrophages and Guinea Pigs, *Infection And Immunity*, July 2003, p. 3927–3936

24. Olof Lagerlund, Design and synthesis of novel Glutamine Synthase inhibitors and development of Palladium (0) - catalysed Aminicarbonylation, Uppsala Universitet, 2009. ISBN: 978-91-554-7492-8

25. Wojciech W. Krajewski et al Structure of Mycobacterium tuberculosis glutamine synthetase in complex with a transition-state mimic provides functional insights by. *Proceedings of the* National *Academy of Sciences of the United States of America.* July 26, 2005 vol. 102 no. 30 10499 –10504

26. Gunter Harth and Marcus A. Horwitz, Inhibition of *Mycobacterium tuberculosis* Glutamine Synthetase as a Novel Antibiotic Strategy against Tuberculosis: Demonstration of Efficacy In Vivo, *Infection And Immunity*, Jan. 2003, p. 456–464 Vol. 71, No. 1

27. S. Sharma , P.K. Sharma, N. Kumar, R. Dudhe, A review on various heterocyclic moieties and their antitubercular activity , *Biomedicine & Pharmacotherapy* 65 (2011) 244–251.

28. Kalyankar TM, Pekamwar SS, Wadher SJ, Tiprale PS and Shinde GH, Review on Benzimidazole Derivative. *International Journal of Chemical and Pharmaceutical Sciences* 2012, Dec., Vol. 3 (4)

29. Maste M. M., Jeyarani P., Kalekar M. C. and Bhat A. R. Synthesis and Evaluation of Benzimidazole Derivatives for Anti-tubercular and Antimicrobial Activities *Asian J. Research Chem.* 4(7): July, 2011; Page 1055-1058

30. Prem Shankar Misra, P.Shanmugasundaram, Rakhi Chaudhary and M.Vijey Aanandhi, Synthesis of 2-Phenyl Benzimidazole Derivatives and Their Schiff Bases as Possible Antimicrobial Agents. *Rasayan Journal Of Chemistry.*Vol.3, No.1 (2010),51-54

31. Panneer Selvam, T, P. P. Radhika, S. Janagaraj, A. Siva Kumar Synthesis of novel 2-substituted benzimidazole derivatives as potential anti microbial agents. *Research in Biotechnology, 2(3):50-57, 2011*

32. Ahamed A. Jafar, Kaliapillai N. Vijayakumar, Bathey R. Venkatramanb and Govinda Venkatesha, Synthesis, characterization and biological activity of some novel benzimidazole derivatives , *Orbital* 2009, 1 (4), 306-309

33. P.C Santhosh, S.N. Pandeya, Ashish K.Pathak, Benzimidazole: A versatile Chemical Entity, *IJRAP* 2001, 2 (6) 1726-1737.

34. Hamdan S. Al-Ebaisat Synthesis and Biological Activities of Some Benzimidazoles Derivatives *J. Appl. Sci. Environ. Manage*. Sept, 2011. Vol. 15 (3) 451 – 454

35. Mohammad Shaharya , Avijit Mazumder , Salahuddin, Ruchika Garg ,Rishabh D Pandey, Synthesis, characterization and pharmacological screening of novel benzimidazole derivatives , *Arabian Journal of Chemistry* (2011)

36. R. Walia, K. Dhamija, Vandana, Md J. Akhtar and Hs. Lamba, Synthesis of Novel Substituted Benzimidazole Derivatives as Potential Antimicrobial Agents , *International Journal of Pharmaceutical, Chemical and Biological Sciences* 2012, 2(3), 293-298

37. Y Radha, A Manjula B Madhava Reddy And B Vittal Rao, Synthesis and biological activity of novel benzimidazoles , *Indian Journal of Chemistry*, Vol 50 B, December 2011, pg: 1762-1773

38. Vaibhav Sharma, Dinesh Kumar Mehta, Suman Bala, Rina Das , A Review on Biologically Active Schiff Base Derivatives, *International Journal Of Universal Pharmacy And Bio Sciences* 2(4): July- August 2013.

39. Khlood Fahed Hamak, Synthesis, Characterization, Biological Evaluation And Anti-Corrosion Activity Of Some New Schiff Bases Derivatives Containing Piperidone-4. *Journal of Current Research in Science*, 2013, Vol. 1, No. 3, Pg: 137-143

40. Ahlam Marouf Al-Azzawi and Marwa Shawqi Abd Al-Razzak, Synthesis, Characterization and Antibacterial Screening of New Schiff bases linked to Phthalimide , *International Journal of Research in Pharmacy And Chemistry*, IJRPC 2013 3(3)

41. Kalpesh S. Parikh and Sandip P. Vyas., Design and Spectral Analysis of Novel Schiff Base derived with Acetophenone derivatives, Scholars Research Library. *Archives of Applied Science Research*, 2012, 4 (4): 1578-1580

42. Amira S. Abd El-A et al., Design, Synthesis and Anticancer Evaluation of Some Selected Schiff Bases Derived from Benzimidazole Derivative. *Global Journal of Pharmacology* 7 (2): 143-152, 2013

43. Michael J. Hearn et al., Preparation and antitubercular activities in vitro and in vivo of novel Schiff bases of isoniazid, *European Journal of Medicinal Chemistry* 44 (2009) 4169–4178.

44. Vera Klimesova´ et al., Synthesis and preliminary evaluation of benzimidazole derivatives as antimicrobial agents, *European Journal of Medicinal Chemistry* 37 (2002) 409–418.

45. Scott G.Franzblau, Richard S.Witzig, James C.McLaughlin, Patricia Torres, Guillermo Madico, Antonio Hernandez, Michelle Ferguson and Robert H.Gilman Rapid, Low-Technology MIC Determination with Clinical Mycobacterium tuberculosis Isolates by using the Microplate Alamar Blue Assay, Journal *of Clinical Microbiology* 1998, 36(2): 362.

46. Sephra N.Rampresad., Multiple Applications of Alamar Blue as an Indicator of Metabolic Function and Cellular Health in Cell Viability Bioassays by. *Sensors* 2012, 12, 12347-12360.

47. Jose de Jesus Alba-Romero et al., Application of the Alamar Blue assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. *African Journal of Microbiology Research* Vol.5 (26), pp.4659-4666, 16 November 2011.

48. http://en.wikipedia.org/wiki/Lipinski%27s_rule_of_five. Retrieved on 02.01.2014

49. http://www.molinspiration.com/cgi-bin/properties . Retrieved on 07.03.2014

50. Sunita Bhagat, Nutan Sharma, and Tejpal Singh Chundawat , Synthesis of Some Salicylaldehyde-Based Schiff Bases in Aqueous Media , *Hindawi Publishing Corporation, Journal of Chemistry*, Volume 2013, Article ID 909217, 4 pages.

51. Organic Chemistry by O.P Agarwal,47 th Edition, JOEL Publishing House, 2010

52. Maria C. S. Lourenco, Marcus V. N. DeSouza, Alessandra C Pinheiro, Marcelle de L Ferreira, Rasnisb B, Goncalves, Thais Cristina M Nogneira, Monica A Peralta, Evaluation of anti-Tubercular activity of nicotinic and isoniazid analogues. *ARKIVOC* 2007 (xv), 181-191.

53. C.N.Nalini, S. Raga Deepthi, N.Ramalakshmi and G.Uma, Toxicity Risk Assesment of Isatins, *RASAYAN J.Chem*. Vol.4, No.4 (2011), 829-833.