DESIGN, SYNTHESIS,CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL BENZIMIDAZOLE DERIVATIVES AS ANTI-TUBERCULAR AGENTS TARGETING *InhA* (Enoyl Acyl Carrier Protein Reductase) ENZYME

A Dissertation Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI-600032

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by

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CHENNAI-600 003

MAY– 2019

COLLEGE OF PHARMACY

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CHENNAI-600 003

TAMIL NADU

CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL BENZIMIDAZOLE DERIVATIVES AS ANTI TUBERCULAR AGENTS TARGETING InhA (Enoyl Acyl Carrier Reductase Protein)ENZYME"** submitted by **SANGEETHA K, Reg. No: 261715707** to The Tamil Nadu Dr. M.G.R. Medical University, examination is evaluated.

EXAMINERS

1.

2.

Place: Chennai-03

Date

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LIST OF ABBREVIATIONS

TB	Tubercle Bacillus
WHO	World Health Organization
HIV	Human Immuno Deficiency Syndrome
MDR-TB	Multi Drug Resistant TB
PMN	Polymorphonuclear leukocytes
CFA	Freund's Complete adjuvant
SBDD	Stucture Based Drug Design
RO5	Rule of Five
BCG	Bacillus Calmette Guerin
μm	Micro meter
μg/ml	Micro gram/ milli litre
MTB	*Mycobacterium tuberculosis*
DNA	Deoxyribo Nucleic Acid
InhA	Enoyl acyl carrier protein reductase
ADME	Absorption, Distribution, Metabolism, Excretion
CADD	Computer Aided Drug Design
QSAR	Quantitative Structural Activity Relationship
MABA	Microplate Alamar Blue Assay
OSIRIS	Optical, Spectroscopic and Infrared Remote Imaging System
PT 70	2-(o-Tolyloxy)-5-hexyl phenol
ppm	Parts Per million
NAD+	Nucleoside Adenine Dinucleotide
PDB	Protein Data Bank
IR	Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance Spectroscopy

GC-MS	Gas Chromatography Mass Spectrometry
LC-MS	Liquid Chromatography Mass Spectrometry
FBS	Fetal Bovine Serum
DMEM	Dulbecco's Modified Eagle Medium
SRB	Sulphorhodamine-B
TCA	Trichloroacetic acid
TLC	Thin Layer Chromatography
Rf	Retention Factor
w/v	Weight/volume
mM	MilliMoles
nm	Nanometer

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INTRODUCTION

TUBERCULOSIS

Tuberculosis is a common and an infectious disease which is caused by the organism *"Mycobacterium tuberculosis" (M. tuberculosis)*[1]. Tuberculosis (TB) is the second major cause of death in adults worldwide. The causative microorganism Mycobacterium tuberculosis is an airborne pathogen transmitted among humans which infects macrophages in the lungs. [2]

History:

The German microbiologist Robert Koch announced that Mycobacterium tuberculosis caused TB in 1882[3]. This revolutionary finding, along with the later discoveries of tuberculin in 1890 and the Bacillus-Calmette Guerin (BCG) vaccine in 1908 and anti tuberculosis drugs starting in 1943, offered hope for the eradication of a disease deadlier than the plague. Mortality rates significantly declined from the early to mid-20th century.

The World Health Organization (WHO) estimates that there are 10.4 million new cases and 1.7 million deaths annually, including 0.4 million deaths in people with HIV infection, of which 95% occur in low and middle-income countries. The WHO End TB Strategy has set the ambitious target of reducing the incidence of tuberculosis disease by 90% by 2035[4].

EPIDEMIOLOGY:

All countries and age groups are affected by tuberculosis but most cases (90%) in 2016 were in adults and almost two-thirds were accounted by seven countries: INDIA, Indonesia, China, Philippines, Pakistan, South Africa and Nigeria. The sex ratio (male to female) was 1:9 and 10% of patients with newly diagnosed tuberculosis were also HIV- positive.[5]

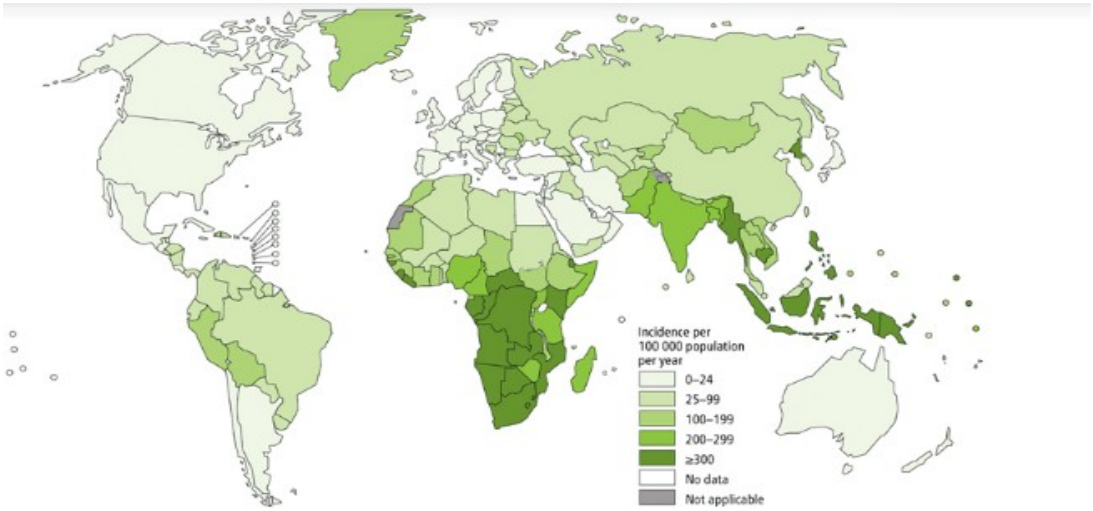


Figure 1: Global epidemiology of tuberculosis[6]

TB BURDEN IN 2016 AND RECENT TRENDS

Globally 23% of the world's population were estimated to be infected with M.tuberculosis complex in 2014, equivalent to a best estimate of of 1.7 billion people. Global estimates of TB incidence and deaths in the period 2000-2016 are shown in (fig.2). TB affects all countries; the number of incident cases relative to total population size varied from under 10 per 100,000 population in most high income countries to above 500 in a few countries including the Democratic people's Republic of Korea, Lesotho, Mosambique, Phillipines and South Africa[7].

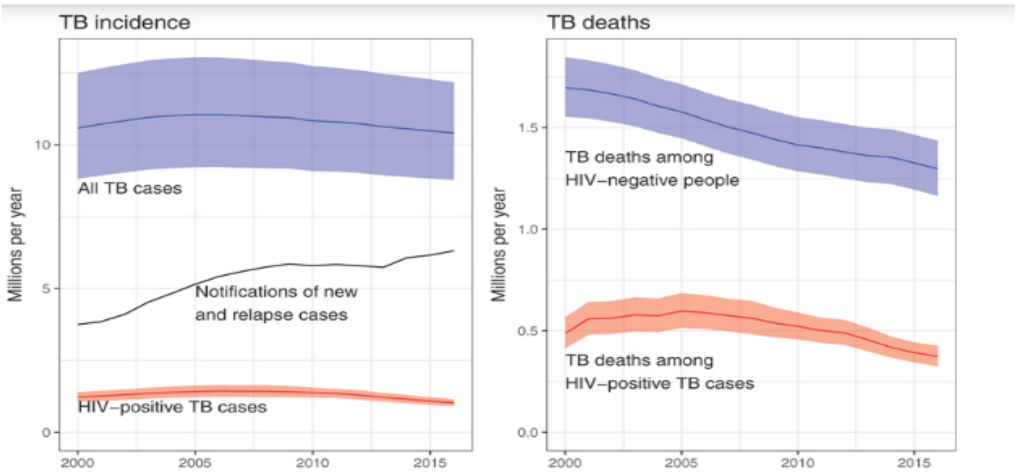


FIGURE 2: TB incident cases during 2000-2016[8]

The number of incident cases per 100,000 population has been falling slowly at an average rate of 1.4% per year during the period 2000-2016 and 1.9% between 2015 and 2017. Regionally, the TB incidence rate is falling fast in the WHO European Region (4.6%).

MULTI DRUG RESISTANT TB

MDR TB means that the TB bacteria that a person is infected with are resistant to two of the most important TB drugs, **Isoniazid (INH) and Rifampicin.** Globally in 2016 about 490,000 people are estimated to have become ill with MDR-TB. Almost half of these cases (47%) were in India, China or the Russian Federation[9].

Need for new anti-TB drugs

- To improve the treatment of MDR-TB.[10]
- To prevent the complexity and toxicity of the current TB drug regimes.
- The recent rise in TB cases and especially the increase of drug resistant mycobacteria indicate an urgent need to develop new anti-TB drugs.
- To afford shorter and simple multi drug regimes for drug sensitive TB
- Discovery of compound that would reduce both the length of treatment and the frequency of drug administration.[11]
- To provide short, simple and easily tolerable safe regimes for latent tuberculosis infection[12].

MYCOBACTERIA

Mycobacterium tuberculosis and seven very closely related mycobacterium species (M.bovis, M.africanum, M.microti, M.caprae, M.pinnipedii, M. Canetti and M. mungi) are together known as M.tuberculosis complex[13]

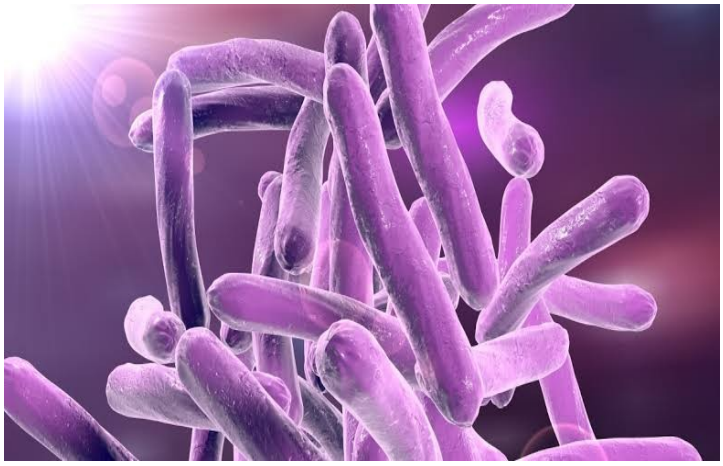


Figure 3: Mycobacterium tuberculosis[14]

SCIENTIFIC CLASSIFICATION [15]

Kingdom	:	Bacteria
Phylum	:	Actinobacteria.
Class	:	Actinobacteria.
Order	:	Actinomycetales.
Suborder	:	Corynebacterineae.
Family	:	Mycobacteriaceae.
Genus	:	Mycobacterium.
Species	:	Mycobacterium tuberculosis.
Synonym	:	Tubercle bacillus Koch 1882

CELL WALL STRUCTURE

The cell wall structure of Mycobacterium tuberculosis is specifically unique among the prokaryotes and it is a major determinant of virulence for the bacterium. The cell wall complex contains,

- **Peptidoglycan**
- **Complex lipids**

Over 60% of the mycobacterial cell wall is made up of lipids. The lipid fraction of MTB's cell wall consists of three major components, mycolic acid, cord factor and wax-D.

Mycolic acids are unique alpha- branched lipids found in cell walls of Mycobacterium and corynebacterium. They occupy 50% of the dry weight of mycobacterial cell envelope. Mycolic acids are strong hydrophobic molecules that form a lipid shell around the organism and affect permeability properties at the cell surface. Mycolic acids are the significant determinant of virulence in MTB. Probably, they prevent attack of the mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytic granule.

Cord factor is toxic to mammalian cells and is also inhibitor of Polymorphonuclear leukocytes (PMN)migration. It is most abundantly produced in virulent strains of MTB.

Wax-D in the cell envelope is the major component of Freund"s complete adjuvant (CFA)[16]Freund's adjuvant is a solution of antigen emulsified in mineral oil and used as an immune potentiator. The complete form, Freund's Complete Adjuvant is composed of inactivated and dried mycobacteria (usually M.*tuberculosis*)

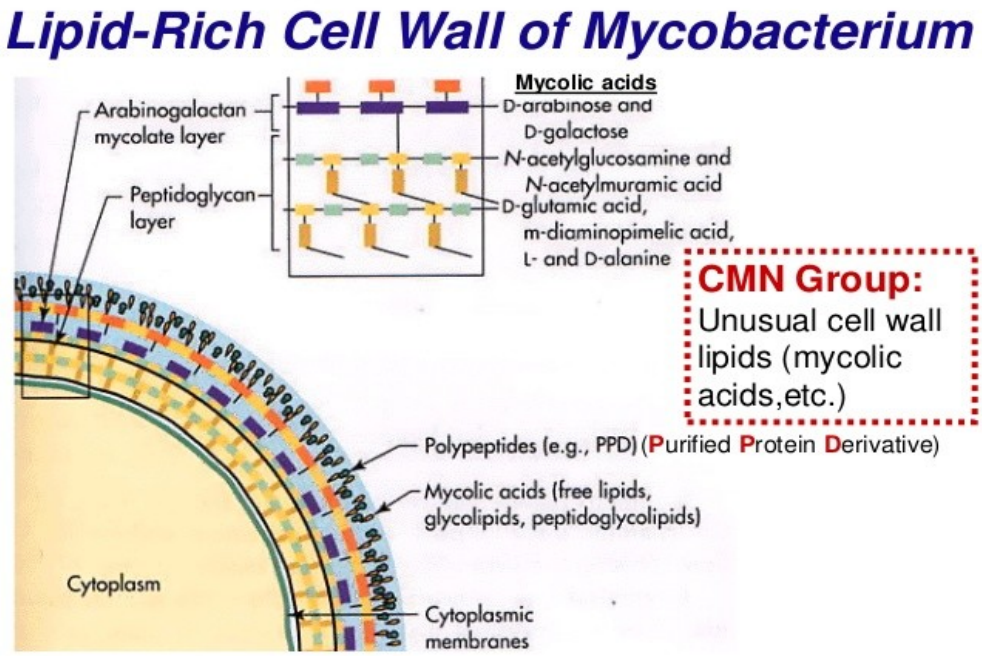


FIGURE 4: Cell wall of Mycobacterium tuberculosis[17]

GENOME

Mycobacterium Tuberculosis genome encodes about 190 transcriptional regulators, including sigma factors, two–component system and more than 140 transcription regulators. Several regulators have been found to respond to environmental distress, such as extreme cold or heat, iron starvation, and oxidative stress[18].

Mycobacterium Tuberculosis has circular chromosomes of about 4,200,000 long nucleotide. The genome was studied generally using the strain Mycobacterium Tuberculosis H37RV.The genome has about 4000 genes. Genes that code for lipid metabolism are very important part of the bacterial genome and 8% of the genome is involved in its activity[19].

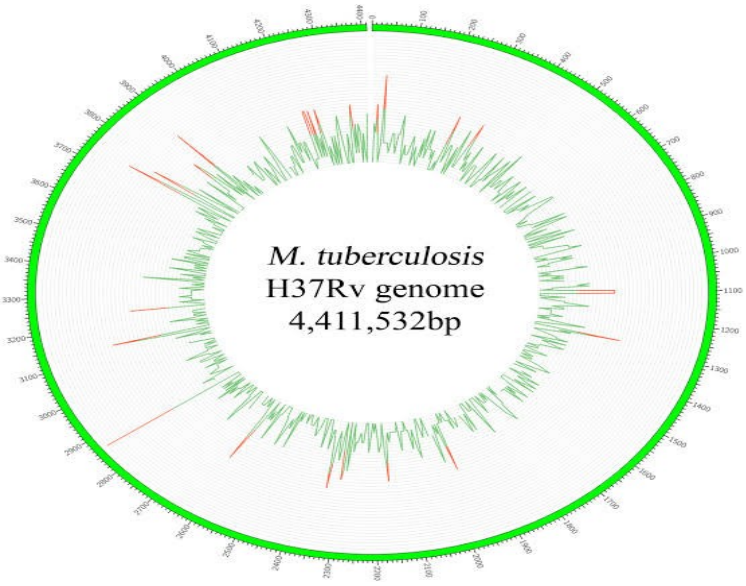


FIGURE 5: Genome of M.Tuberculosis[20]

ENZYME PROFILE:

ENZYME NAME	**:**	**ENOYL-ACP REDUCTASE[21]**
CLASSIFICATION	**:**	**OXIDO REDUCTASE**
POLYMER	**:**	**1**
TYPE	**:**	**Protein**
CHAINS	**:**	**A, B**
ORGANISM	**:**	**Mycobacterium tuberculosis**
PROTEOME	**:**	**Chromosome**
FUNCTIONAL CATEGORY	**:**	**Type II fatty acid biosynthesis pathway**

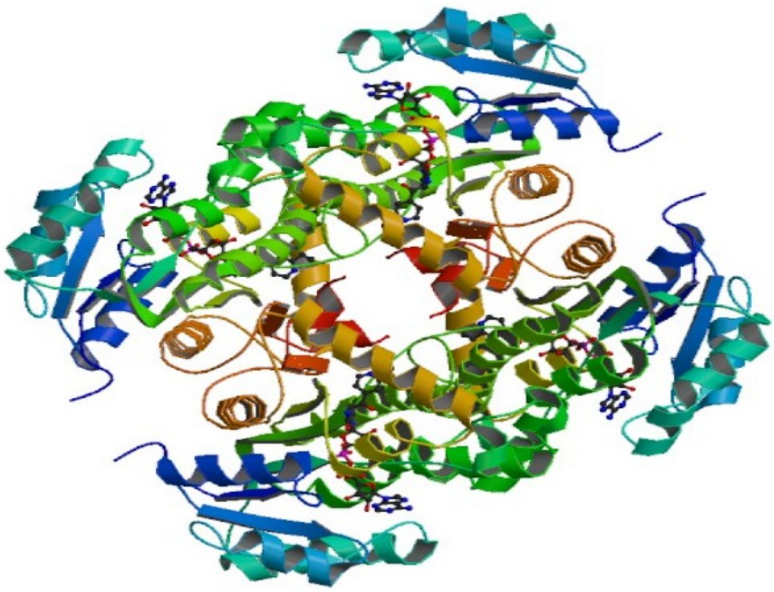


FIGURE 6: Enoyl ACP protein reductase enzyme[21]

InhA, the enoyl-ACP reductase in Mycobacterium tuberculosis is an attractive target for the development of novel drugs against tuberculosis, a disease that kills more than two million people each year.

InhA is the target of the current first line drug isoniazid for the treatment of tuberculosis infections. Compounds that directly target InhA and do not require activation by the mycobacterial catalase-peroxidase Kat G are promising candidates for treating infections caused by isoniazid-resistant strains.

However, these compounds are rapid reversible inhibitors of the enzyme, and based on the knowledge that long drug target residence times are an important factor for *in vivo* drug activity, which set out to generate a slow onset inhibitor of InhA using structure-based drug design. 2-(o-Tolyloxy)-5-hexylphenol (PT70) is a slow, tight binding inhibitor of InhA with a K (1) value of 22 ppm. [22] Crystal structure of the ternary complex between InhA, NAD(+), and PT70 reveals the molecular details of enzyme-inhibitor recognition and supports the hypothesis that slow onset inhibition is coupled to ordering of an active site loop, which leads to the closure of the substrate-binding pocket.

InhA (enoyl-[acyl-carrier-protein] reductase), involved in mycolic acid synthesis is a target of front-line anti-tubercular drugs, such as isoniazid and ethionamide.[23]

BASIC NUCLEUS INTRODUCTION

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.

Benzimidazole is a heterocyclic aromatic compound. This bicyclic compound is a fusion of benzene and imidazole. Although benzimidazole is the commonest name of the parent compound of the series, other names such as benzimidazole and 1,3-benzodiazole are often used.

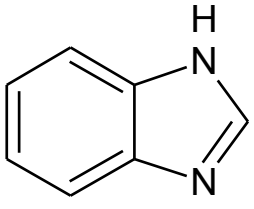
PROPERTIES:

CHEMICAL FORMULA: $C_7H_6N_2$

MOLAR MASS :118.14 g/mol

MELTING POINT :170-172˚C

ACIDITY :12.8



Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities like anti-microbial, anti-viral, anti-diabetic and anti-cancer activity[24]. It's a crucial pharmacophore and privileged structure in medicinal chemistry.

Benzimidazole derivatives are associated with various types of pharmacokinetic and pharmacodynamic properties. Literature survey reveals that the various derivatives of benzimidazole have been synthesized for their pharmacological activities.

Specifically, this nucleus is a constituent of vitamin $B_{12.}$ Albendazole, Mebendazole and Thiabendazole are widely used as anthelmintic drugs.[25]

DRUG DISCOVERY

The process of drug discovery is very complex and requires 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 with the help of computers, a new era has begun in drug discovery.[26]

COMPUTER AIDED DRUG DESIGN

Computer aided drug design in computational chemistry is to discover, enhance or study drugs and related biologically active molecules. 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 when the small molecules binds to it.

Molecular mechanics methods may be used to provide semi quantitative prediction of the binding affinity and also knowledge based scoring function may be used to provide binding affinity estimates.[29]

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 modification of the structure of drug molecules is done by docking every specific structure of a drug compound in active site of protein and calculating the extent of the interaction.[27] Optimization aids in the several modification of newer molecules in order to improve the physico-chemical properties and biological activity for a given set of compounds in the library. Further structural modification improves the affinity, reactivity towards target and enhances stability during metabolism.

TYPES OF DRUG DESIGN

Advances in computation techniques and hardware have facilitated the application of in-silico methods in the discovery process drug design can be categorized as two types

1. Structure based drug design
2. 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 inhibitors 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 threading and homology modeling.

Ligand based drug design:

Ligand based drug design is also called indirect drug design. It is an approach used in the absence of the receptor 3D information and it relies on knowledge of molecules to the biological target of interest. 3D quantitative structure activity

relationship (3D QSAR) and pharmacophore modelling are the most important and widely used tools in the ligand based drug design. They can provide protective models suitable for lead identification and optimization.[28]

REVIEW OF LITERATURE

In order to know the current status regarding the advances in TB the literature pertaining to the disease, design, synthesis, characterisation and biological evaluation were reviewed.

Literature review on Tuberculosis research :

1. Robert Koch., (2008) has outlined the History of Tuberculosis [30]

2. Frieden TR, GR *et al.,* (2003) has shown that "Impact of national consultants on successful expansion of effective tuberculosis control in India.[31]

3. Williams, B.G *et al*., (2010) studied the "The Population Dynamics and Control of Tuberculosis**.**[32]

4. Leimane V, ***et al***., (2005) described the "Clinical outcome of individualized treatment of multidrug-resistant tuberculosis [33]

5. Balabanova Y, ***et al*., (**2006) made a report about "The Directly Observed Therapy Short-Course (DOTS) strategy"[34]

6. Gulrez Shah azhar (2012) made a systematic review about " DOTS for TB relapse in India"[35]

7. Ibrahim Abubakar.,et al.,(2018) studied about the " Prognostic value of interferon –γ release assays and tuberculin skin test in predicting the development of active tuberculosis"[36]

8. Yigzaw Alemu Limenih, et al.,(2018)studied about the "Survival analysis of time to cure on multi drug resistance tuberculosis patients in Amhara region, Ethiopia"[37]

9. Maryam B. Haddad, et al.,(2018) estimated the " Local prevalence of latent Tuberculosis Infection United States, 2011-2015"[38]

Literature review for drug design :

10. Alfred burger, *et al.,* (1984) Textbook of guide to chemical basis of drug design (John Wiley& Sons).[39]

11. Edward.C.Olson, ***et al.,*** (1979)Textbook of Computer assisted Drug Design (American Chemical Soceity).[40]

12. Wilson & Giswold's *et al.,* (2011) Text book of Organic,Medicinal and Pharmaceutical chemistry[41]

13. Romono T. Kroemeret *et al..,* (2003) **"**An introduction into ligand–receptor docking".[42]

14. Lipinski CA *et al*., (2001) developed an experimental and computational approach to estimate solubility and permeability in drug discovery and development settings.[43]

15. Madsen *et al*., (2002) Textbook of Drug Design and Discovery[44]

Literature review related to the desired chemical entities*:*

16.Anu Kajal et al. reported Schiff base a versatile pharmacophore. Schiff bases are condensation product of primary amine with carbonyl compound gaining importants day by day in present scenario. Schiff bases are compound carrying imine or azo methane. (-C=N-) functional group and are found to be versatile pharmacophore for design and development of various bio active lead compounds**. [45]**

17.Ruchi Agarwal et al. Schiff base complexes derived from thiosemicarbazones, synthesis characterisation and the biological activity. The Schiff base anisasldehyde thiosemicarbazone,3,4 dimethoxy benzaldehyde thiosemicarbazone, thiophene 2 aldehyde thiosemicarbazone.**[46]**

18.Angelo De Fathima et al. Schiff base: A short reviews of the antimicrobial activities Schiff base are aldehyde or ketone like compounds in which the

carbonyl group is replaced and imine or azomethane group. The widely used for industrial purposes and biological activities.[47]

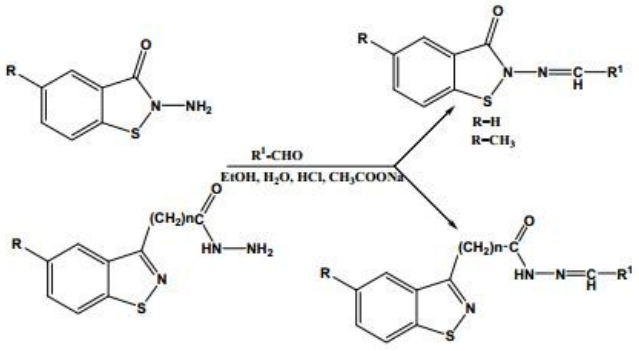
19.S.K. Ghosh et al. Synthesis of Schiff base in aqueous medium a green alternative approaches with effective mass yield and high reaction rates. Schiff base constitute a class of pharmaceutical and medicinaly important molecules. Various **S**chiff bases by stirring 1,2 diamino benzene with various aromatic aldehydes in water as solvent**.**[48]

20.Ina Boltz et al. Novel Schiff bases derived from 5 amino barbituric acid: synthesis and a solid state structure for this purpose 1,3-dimethyl and 1-butyl-5-amino butyric acid are condensed with p-nitro and p-N,N dimethyl amino cinnamaldehyde respectively**.**[49]

21.Shelar Mahendra Devidas et al. Novel One –pot synthesis of schiff base compounds derived from different diamine & aromatic aldehydes catalyzaed by P2O5/Sio2 Under free solvent condition at room temprature. A potential method for one-pot synthesis of schiff base compounds derived from different aldehyde and diamine by using catalyst P2O5/Sio2. [50]

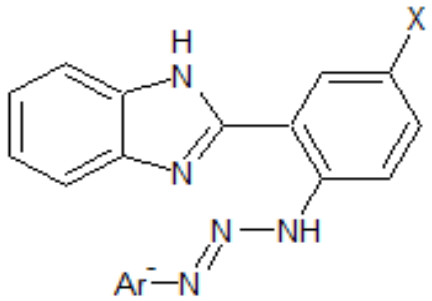
22.Hamid Latif Siddiqui et al. synthesis of spectroscopic studies of new Schiff bases. Five novel Schiff bases have been prepared from o-formyl phenoxyacetic acid and a series of amino thiazoles to form a number of potentially biologically active compounds**.**[51]

23.1.2-benzisothiazole hydrazides as well as their cyclic and acyclic 1.2-benzisothiazole parent hydrazides.[52]

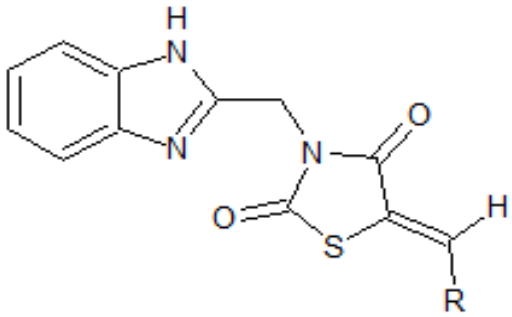


Literature review on benzimidazole

24. Sujit Kumar Mohanty *et al.*,(2018) reported synthesis of novel azo derivaties of benzimidazole as potent antibacterial and anti tubercular agents[53].

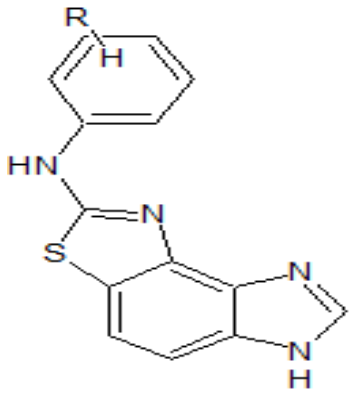


25. Araujo D.M.L *et al.*, (2018) reported synthesis of a series of (Z)-3-((1H-benzo[d] imidazol-2-yl)methyl)-substituted thiazolidine 2,4-dione and evaluated their mycobacterium inhibitory activity against *Mycobacterium Tuberculosis* H37Rv strain[54].

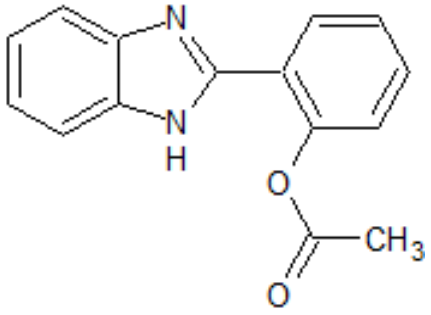


26. Fatmah A.S. Alasmary *et al.*, (2015) reported synthesis and evaluation of selected Benzimidazole derivatives with substituents at position 1,2 and 5 as potential antimicrobial agents[55].

27. Vasu N *et al.*, (2013) reported synthesis of a series of various novel phenyl –(6H-thiazolo[4,5-e] benzoimidazole-2-yl)-amines and screened for their antibacterial and anti fungal activity[56].



28. Chavan B B *et al.*, (2012) reported synthesis and biological evaluation of novel benzimidazole derivative (2,3- dihydro-2-[(2-acetyloxy) phenyl]-1H-benzo [d] Imidazole)with aspirin as potent antimicrobial and antifungal agents[57].



Review of Literature related to the evaluation of anti tubercular activity by MABA

29.Scott G Franzblau. et al. studied MIC determination by MABA. A colorimetric, Microplate Based Alamar Blue Assay (MABA) method was used to determine the MICs of Isoniazid, Rifampin, Streptomycin and Etambutol for 34 peruvian Mycobacterium tuberculosis isolates and the H37Rv strain by using bacterial suspensions prepared directly from media. The MABA is a simpe, rapid, low cost, appropriate technology which does not require extensive instrumentation and which makes use of a nontoxic, temperature stable reagent. **[58]**

30.Sephra N Rampresad. et al. studied the various applications of Alamar Blue as an indicator. Alamar Blue is a 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, and protozoa. [59]

31.Jose de Jesus Alba-Romero et al. applied the Alamar Blue Assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. [60]

Literature review on target enzyme Inh A (enoyl ACP reductase) Inhibitors:

32.Luckner, S.R *et al*., (2010) reported that Inh A, the enoyl-ACP reductase in Mycobacterium tuberculosis is an attractive target for the development of novel drugs against tuberculosis.[61]

33.Argyrou, A. *et al*., (2007) developed new insight into the mechanism of action of and resistance to isoniazid: interaction of Mycobacterium tuberculosis enoyl-ACP reductase with INH-NADP.[62]

34.Dias, M.V. *et al*., (2007) studied the crystallographic studies on the binding of isonicotinyl-NAD adduct to wild-type and isoniazid resistant 2-trans-enoyl-ACP (CoA) reductase from Mycobacterium tuberculosis.[63]

35.Vilcheze, C. ***et al*.,** (2006) developed the transfer of a point mutation in Mycobacterium tuberculosis.[64]

36.Argyrou, A. *et al*., (2006) has shown the dihydrofolate reductase from mycobacterium tuberculosis is inhibited by the acyclic 4R isomer of INH-NADP a derivative of the prodrug isoniazid.[65]

From the literature review the following points are concluded

- TB is the deadliest disease across the world wide which has to be treated.
- Inh A (enoyl ACP- Reductase) is the most attractive target enzyme to treat Mycobacterium tuberculosis.
- It is clear that Benzimidazole scaffold has a tremendous activity against the Mycobacterium tuberculosis from the literature review.

AIM AND OBJECTIVE

AIM:

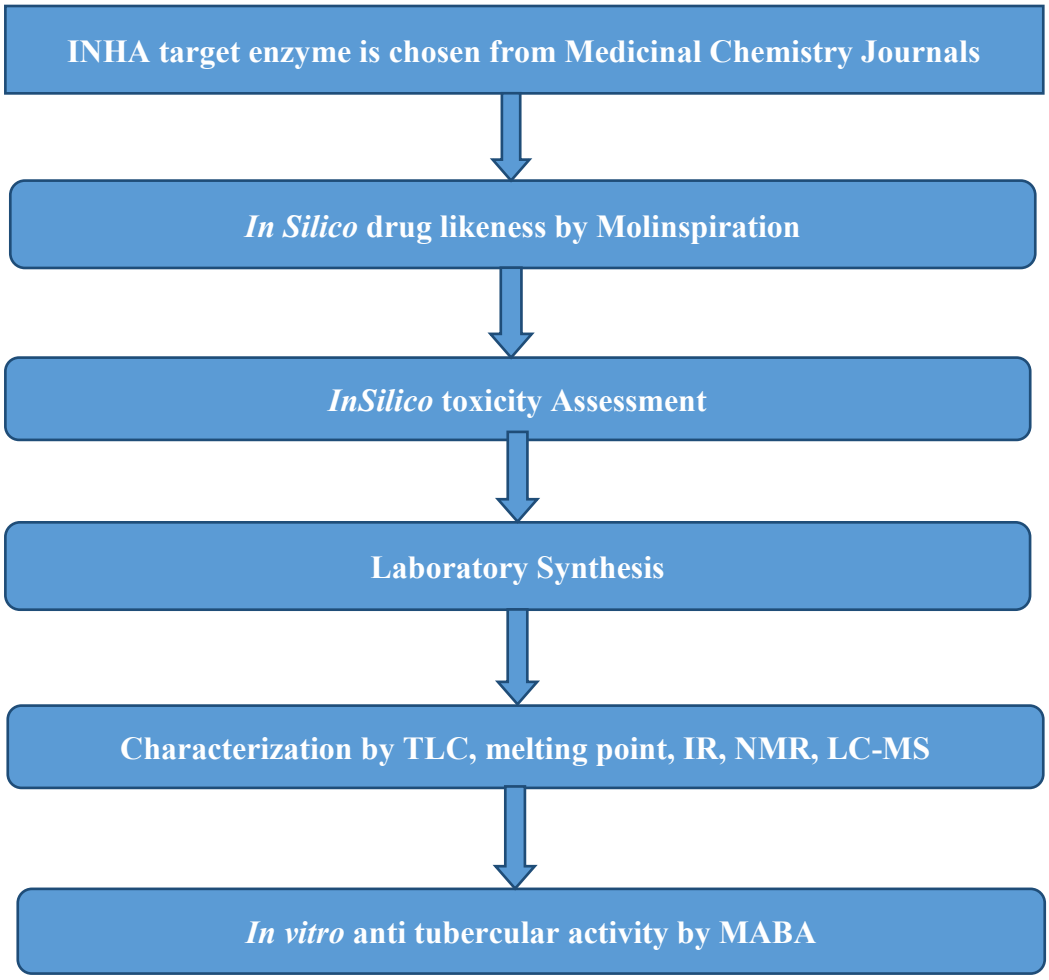
To develop the novel and potent anti-tubercular agents

OBJECTIVE:

The plan of work includes:

- Design of InhA inhibitors by docking studies using autodock tools
- *Insilico* drug likeness prediction
- *Insilico* toxicity assessment
- Laboratory synthesis of those compounds with top docking scores
- Characterization of the synthesized compounds by
  - Infrared spectroscopy
  - H1 nuclear magnetic spectroscopy
  - Melting point
  - GC mass spectrometry
  - LC mass spectrometry
- *Invitro* anti tubercular activity of synthesized compounds(MABA)

PLAN OF WORK


INHA target enzyme is chosen from Medicinal Chemistry Journals
In Silico drug likeness by Molinspiration
InSilico toxicity Assessment
Laboratory Synthesis
Characterization by TLC, melting point, IR, NMR, LC-MS
In vitro anti tubercular activity by MABA

MATERIALS AND METHODS

The Project is to be carried out in the following phases.

- Drug design by using Autodock tools®.
- Synthesis of the designed molecules.
- Characterization of the synthesized molecules.
- Biological evaluation of the synthesized molecule.

DRUG DESIGN PROCESS

A) Molecular Docking by AUTODOCK®

AutoDock Tools® 1.5.6 is an automated docking software designed to predict how small molecules such as substrates or drug candidates bind to a receptor of known 3D structure.

The current version of AutoDock Tools, using the Lamarckian Genetic Algorithm and empirical free energy scoring function, typically will provide reproducible docking results for ligands with approximately 10 flexiblebonds.

The quality of any docking results depends on the starting structure of both the ligand and protein. The protein and ligand structure should be prepared prior to docking[66]. The following steps were followed;

- Protein preparation
- Ligand preparation
- Receptor grid generation
- Ligand docking (Screening)

Preparation of Protein:

- Read molecule from the file (allows reading of PDB coordinate files.)
- Edit -Charges – Compute Gasteiger (for arbitrary molecules)
- Edit – Hydrogen –Merge non polar.
- Save as **.pdb** in AutoDock folder.

Preparation of Ligand:

- Ligand-Input from file
- Ligand–Torsion–choose torsion: Rotatable bonds are shown in green, and non-rotatable bonds are shown in red. Bonds that are potentially rotatable but treated as rigid, such as amide bonds and bonds that are made rigid by the user, are shown in magenta.
- Ligand–Torsion–set number of torsion: sets the number of rotatable bonds in the ligand by leaving the specified number of bonds as rotatable.
- Ligand–Output–save as **.pdbqt** in AutoDock folder

Grid preparation:

Grid–Macromolecule-open (open the pdb file that has been saved and then save it in **pdbqt** extension in AutoDock folder)

1) Grid–Set map types –open ligand : tools to define the atom types for the grids that will be calculated

2) Grid – Grid box – launches interactive commands for setting the grid dimensions and center (Set dimension of 60: 60:60 – Center :center on macromolecule)

3) File – Close saving current

4) Grid – Output – save as **.gpf** (grid parameter file)

5) Open command prompt **[cd AutoDock**

cd 4.5.3

autogrid4.exe –p a.gpf –l a.glg]

Preparation of Docking Parameters:

- Docking –macromolecules – set rigid file name
- Docking – ligand – open
- Docking –search parameters – genetic algorithm parameters : this command open a panel for setting the parameters used by each of the search algorithms, such as temperature schedules in simulated annealing and mutation/crossover rates in genetic algorithms.
- Docking – docking parameters: opens a panel for setting the parameters used during the docking calculation, including options for the random number

generator, options for the force field, step sizes taken when generating new conformations, and output options.

- Docking- output –Lamarkian GA –save as **.dpf (**docking parameter file**)**
- Open command prompt **[autodock4.exe –p a.dpf –l a.dlg]**

Visualization / Interpretation of Docking

- Analysis –Docking – open .dlg (docking log file) file
- Analysis – macromolecule open
- Analysis – Confirmation –Play and Play ranked by energy : Play- will use the order of conformations as they were found in the docking calculations, and Play Ranked By Energy will order the conformations from lowest energy to highest energy.
- Analysis – Load : Information on the predicted interaction energy is shown at the top, and individual conformations
- Analysis – Docking – show interaction: specialized visualization to highlight interactions between the docked conformation of the ligand and the receptor.

B) MOLECULAR PROPERTY PREDICTION:

The designed and docked molecules were screened *insilico* using MOLINSPIRATION® Software to evaluate the druglikeness. Molinspirsation® is used to calculate the important properties such as log P, polar surface area, number of hydrogen bond donor and acceptors.

Molecular toxicity property prediction includes.

- Drug likeness prediction
- clogP prediction.
- Solubility prediction.
- Molecular weight.
- Drug likeness score

DRUG LIKENESS:

Druglikeness may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and of course presence of various pharmacophoric features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others. Simple count criteria (like limits for molecular weight, logP , or number of hydrogen bond donors or acceptors) have also relatively limited applicability and are useful only to discard obvious non-drugs [67]

A model compound for the lipophilic cellular membrane is octanol (a lipophilic hydrocarbon), so the logarithm of the **octanol/water partition coefficient**,

known as **LogP**, is used to predict the solubility of a potential oral drug. This coefficient can be experimentally measured or predicted computationally, in which case it is sometimes called **cLogP**

clogP PREDICTION:

The logP value of a compound, which is the logarithm of its partition **coefficient** between n-octanol and water log (octanol/water), is a well established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high logP values cause poor absorption or permeation. clogP value must not be greater than 5.0 for permeability.

SOLUBILITY PREDICTION:

Aqueous solubility is one of the most important physico-chemical properties in modern drug discovery. It has impact on ADME-related properties like drug uptake, distribution and even oral bioavailability. Solubility can also be a relevant descriptor for property-based computational screening methods in the drug discovery process.[68]

MOLECULAR WEIGHT:

Optimizing compounds for high activity on a biological target almost often goes along with increased molecular weights. However, compounds with higher weights are less likely to be absorbed and therefore to ever reach the place of action. Thus, trying to keep molecular weights as low as possible should be desirable for every drug molecule.

LIPINSKI'S RULE OF FIVE [69]

The rule was formulated by Christopher A. Lipinski in 1997. **Lipinski's rule of five** also known as the **Pfizer's rule of five** or simply the **Rule of five** (RO5) is to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely

orally active drug in humans. The rule is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized step-wise to increase the activity and selectivity of the compound as well as to ensure drug-like physicochemical properties are maintained as described by Lipinski's rule.

Candidate drugs that conform to the RO5 tend to have lower attrition rates during clinical trials and hence have an increased chance of reaching the market.

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria**:**

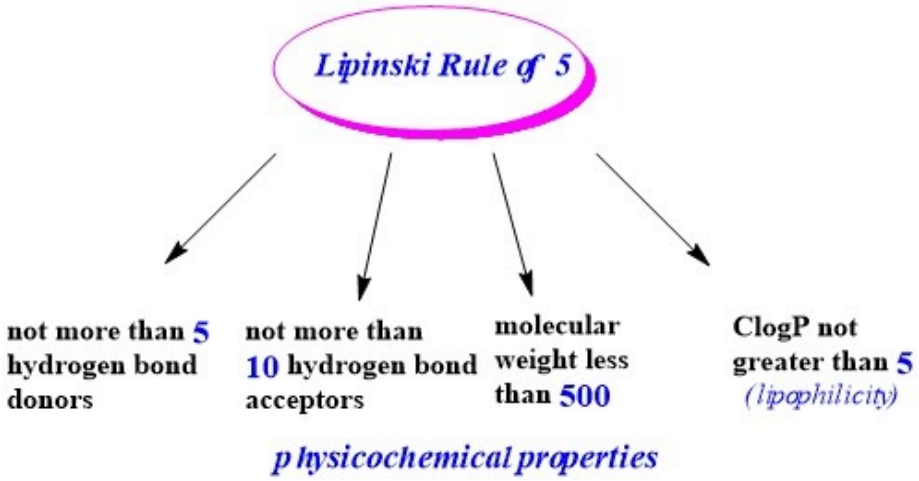


Figure 7 : Lipinski rule of five[70]

VARIANTS

In an attempt to improve the predictions of druglikeness, the rules have spawned many extensions,

1. Partition coefficient log P in −0.4 to +5.6 range
2. Molar refractivity from 40 to 130
3. Molecular weight from 180 to 500
4. Number of atoms from 20 to 70 (includes H-bond donors [e.g. OH and NH] and H-bond acceptors

Also the 500 molecular weight cut off has been questioned. Polar surface area and the number of rotatable bonds has been found to better discriminate between

compounds that are orally active and those that are not for a large data set of compounds in the rat. In particular, compounds which meet only the two criteria of:

1. 10 or fewer rotatable bonds and
2. Polar surface area not greater than 140 A^2 are predicted to have good oral bioavailability.

C) TOXICITY RISK ASSESSMENT:

All the docked molecules can be subjected to the toxicity risk assessment by using OSIRIS® program, which is available online. The OSIRIS® property Explorer is an integral part of Actelion's in house substance registration system. Prediction results are color coded in which the red colour shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green colour indicates drug-conform behaviour.

On drawing a structure the toxicity risk predictor will start looking for potential toxicity risks as long as the currently drawn structure is a valid chemical entity. Toxicity risk alerts are an indication that the drawn structure may be harmful concerning the risk category specified [71]

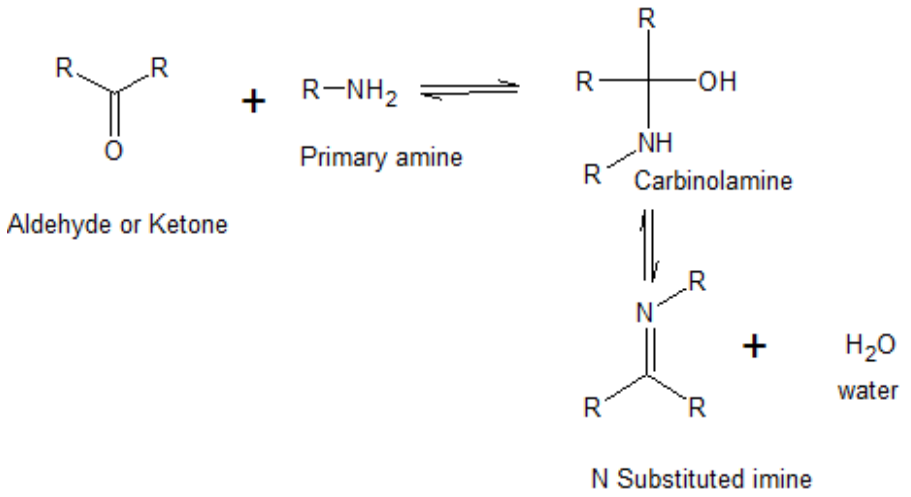
SYNTHETIC METHODOLOGY

The designed and docked compounds are synthesized by the following scheme which is presented below.

SYNTHESIS

The compounds with top docking score were selected for synthesis as per the scheme below. The necessary chemicals of laboratory grade for the synthesis were procured from Sigma Aldrich and synthesis was carried out.

SCHEME:

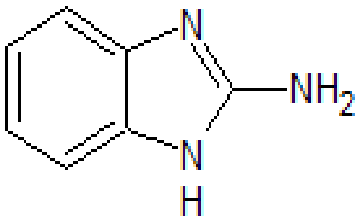


PROCEDURE

A mixture of 0.01 mole 2-amino benzimidazole and 0.01 mole of aromatic aldehyde is dissolved in dimethyl formamide and refluxed for 6-7 hr. The reaction mixture is slowly poured over crushed ice. The solid mass thus separated is filtered, dried, and purified by recrystallization from methanol. The progress of the reaction was characterized by TLC[72].

REACTANT PROFILE:

1. 2-amino benzimidazole



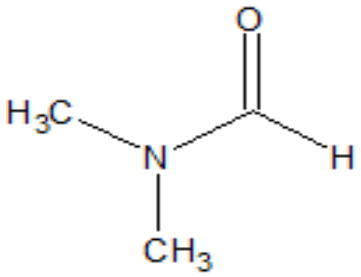
Molecular formula : $C_7H_7N_3$

Molecular weight : 133.154g/mol

Description : white powder

Melting point : 435°F

2. Dimethyl formamide



Molecular formula : C_3H_7NO

Molecular weight : 73.10g/mol

Description : Colourless liquid.

Melting point : 61°C

Boiling point : 154°C

3. Sulphuric acid

H_2SO_4

Molecular formula : H_2SO_4

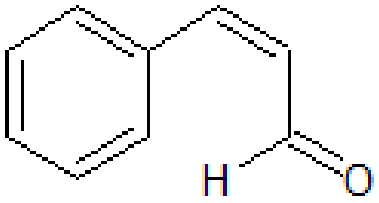
Molecular weight : 98.07g/mol

Description : Colourless liquid.

Melting point : 10^oC

Boiling point : 337°C

4. Cinnamaldehyde



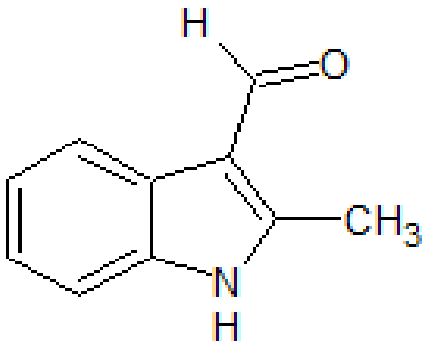
Molecular formula : C_9H_8O

Molecular weight : 132.16 g/mol

Description : yellow oil

Melting point : 265.6 K

5. 2-methyl Indole-3-Carboxaldehyde



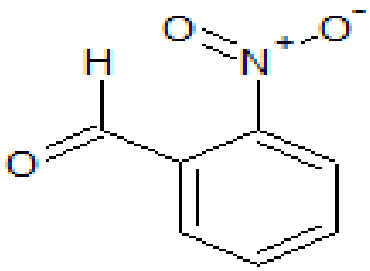
Molecular formula : $C_{10}H_9NO$

Molecular weight : 159.18 g/mol

Description : Brown powder

Melting point :200-201˚C

6. 2-nitro benzaldehyde



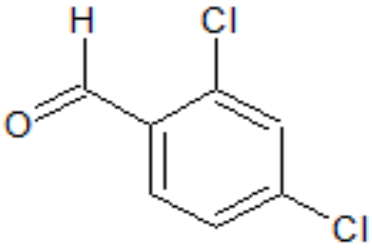
Molecular formula : $C_7H_5NO_3$

Molecular weight : 151.12 g/mol

Description : Pale yellow crystalline powder

Melting point :43˚C

7. 2,4-dichloro benzaldehyde



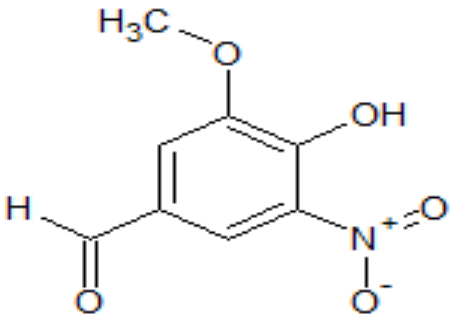
Molecular formula : $C_7H_4Cl_2O$

Molecular weight : 175.008 g/mol

Description : white powder

Melting point : 451˚F

8. 5-Nitro vanillin



Molecular formula : $C_8H_7NO_5$

Molecular weight : 197.146 g/mol

Description : white powder

Melting point : 173-175˚C

CHARACTERIZATION

The compound is checked for purity by TLC method and sharp melting point.

PHYSICAL EVALUATION:

1. Physical properties of the synthesized compounds are evaluated, such as

   - Colour
   - Nature
   - Solubility
   - Molecular weight
   - Molecular formula
   - Melting point
   - Boiling point

2. Further the synthesized compounds are to be characterized by the following Spectroscopic and Spectometric methods.
   - IR Spectroscopy by ABB MB 3000-PH FT-IR Spectrometer
   - ^{1}H NMR Spectroscopy by 500MHZ Bruker Topspin using DMSO-d6
   - LC-MS Spectrometry by Agilent technologies, 6230B Time of Flight (TOF)

IR SPECTROSCOPY

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic chemists for detection of functional compounds and mixtures for compound comparison. The spectrum obtained in minutes using a few mg of the compound which can also be recovered. IR spectroscopy is an important and popular tool for structural elucidation and compound identification. Infrared

spectrum shows percentage transmittance versus frequency expressed as wave numbers [73]

1. 3540-3300 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

NMR SPECTROSCOPY

Nuclear magnetic resonance (NMR) spectroscopy is the important analytical technique available for organic chemist. It involves the interaction of the electromagnetic radiation and the hydrogen of the nucleus when placed in an external static magnetic field. NMR spectra will provide detailed information about a molecule's structure and will prove what the compound really is. NMR is a non-destructive technique. The NMR spectra were recorded on 300 MHz BRUKER Advance III NMR spectrometer. DMSO and $CDCl_3$ was used as a solvent [74]

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

HYPHENATED TECHNIQUE:

LC-MS

LC-MS is a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectroscopy. While liquid chromatography seperates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity [76]

BIOLOGICAL EVALUATION

Anti-tubercular Activity

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
- ✓ Resazurin Micro plate Assay(REMA)
- ✓ Broth Micro Dilution Assay
- ✓ Middle brook (7H 9,7H 10,7H 11) Agar Dilution Assay.
- ✓ Nitrate Reductase Assay

MICROPLATE ALAMAR BLUE ASSAY (MABA) [77]

ORGANISM USED : *M.tuberculosis* H37Rv.
INDICATOR : Alamar blue dye.

Principle:

MABA is an indirect colorimetric method for determining the MICs of TB drugs for strains of mycobacterium tuberculosis. In this assay, the redox indicator Alamar blue monitors the reducing environment of the living cells. It turns from blue to pink in the presence of mycobacterium growth.

Procedure:

1) The anti-mycobacterial activity of the compounds are to be 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 proportional and BACTEC radiometric method.
3) Briefly, 200 ml of sterile deionized water is 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 100µl of the Middle brook 7H9 broth and serial dilution of compounds are placed directly on plate.
5) The final drug concentrations tested is made up to 100 to 0.2µg/ml.
6) Plates are covered and sealed with Para film and incubated at 37°C 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 24hrs.
8) A blue colour in the well is interpreted as no bacterial growth, and pink colour was scored as growth.

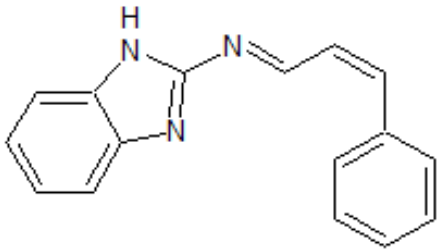
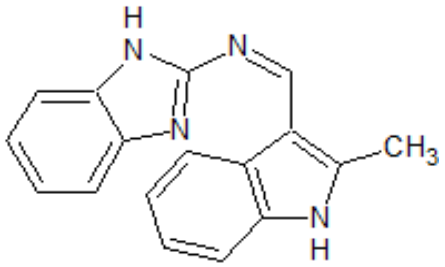
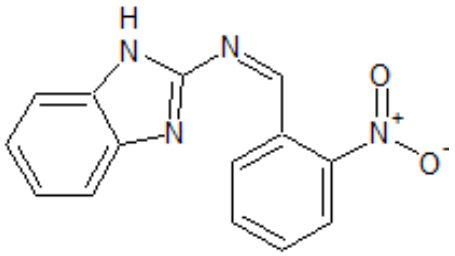
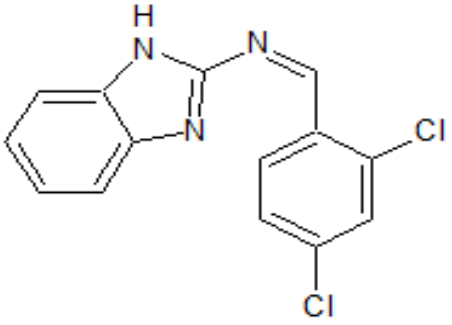
The MIC was defined as the lowest drug concentration which prevents the colour change from blue to pink

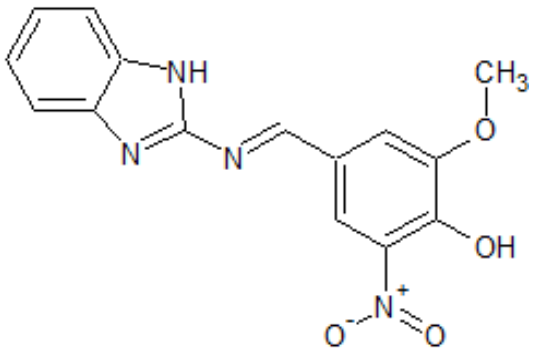
RESULTS AND DISCUSSION

ACTIVITY PREDICTION:

More than 100 compounds drawn using Chemsketch® were docked against the enzyme InhA using AUTODOCK Tools® 1.5.6 software. The molecules with good docking score and favourable interactions were selected, screened and further synthesized.

Table 1: The molecules with good docking score were mentioned below:

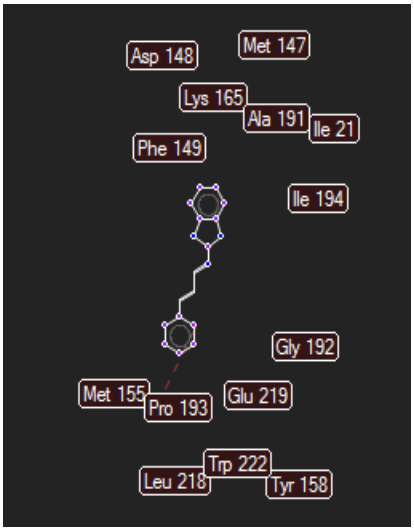
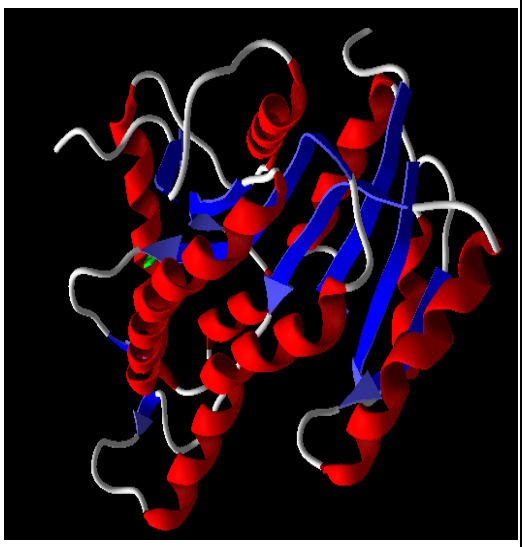
CODE NAMES	STRUCTURE	DOCKING SCORE
SC		-6.77 Kcal/mol
SM		-8.38 Kcal/mol
SJ		-6.73 K cal/mol
SN		-8.04 K cal/mol

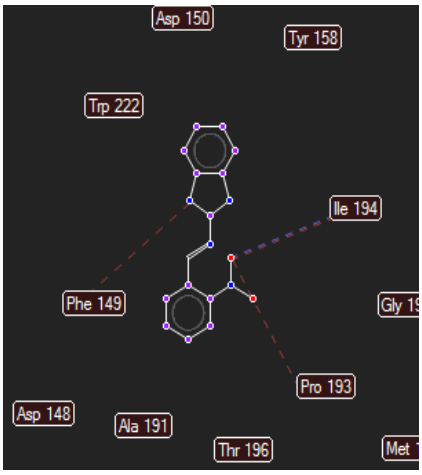
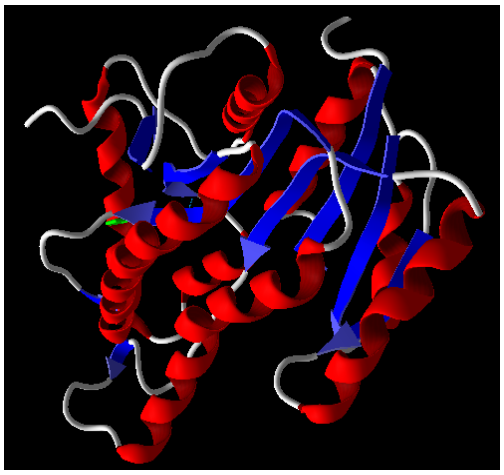
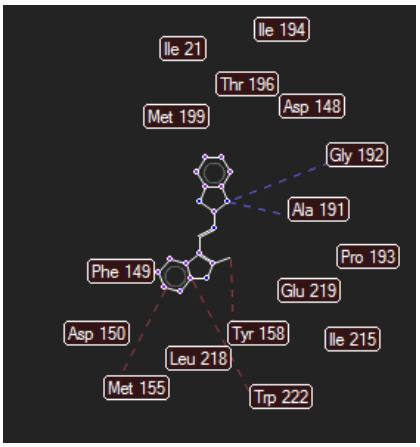
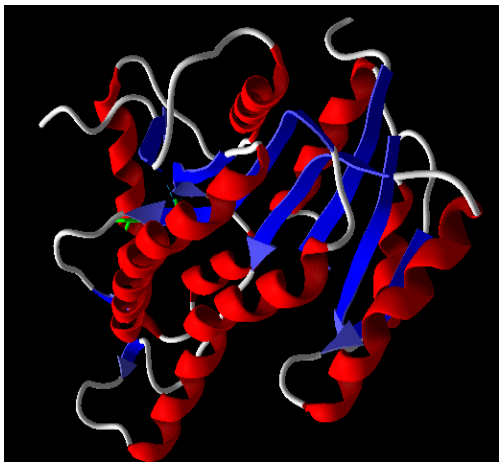
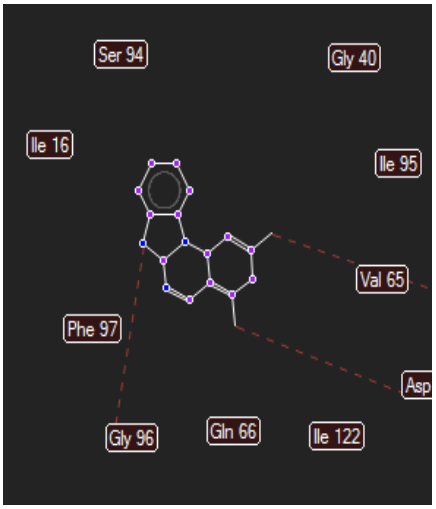
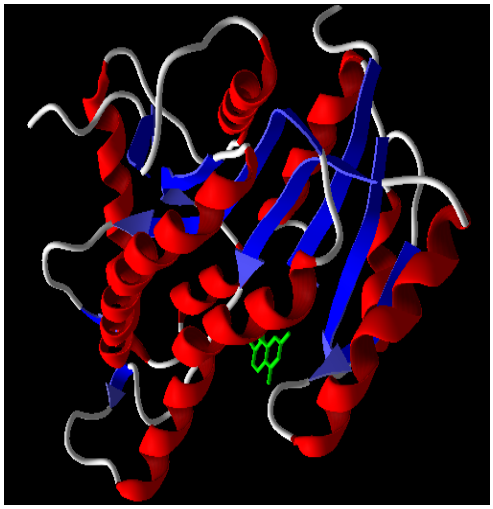
SE		-6.1 K cal/mol

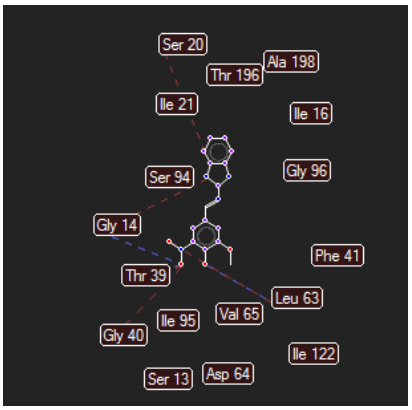
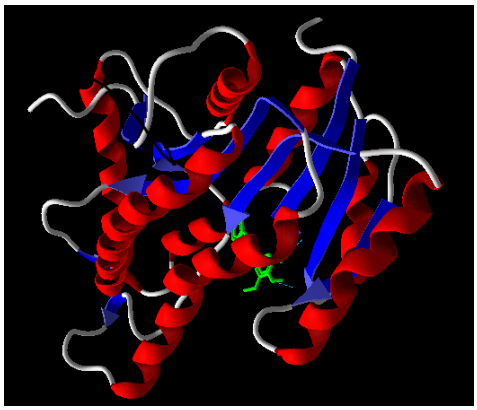
DOCKING VIEW AND INTERACTION OF THE DOCKED MOLECULES WITH THE ENZYME:

During the docking using AUTODOCK Tools® 1.5.6 a complete systemic search of the conformations, orientations and position of a compound in the defined binding site was concluded. Unwanted poses using scoring and energy optimization were eliminated. The best poses were selected on the basis of the scoring function and the quality of pose orientation within the active site aminoacids.

TABLE 2: INTERACTION WITH AMINOACID

SAMPLE CODE	INTERACTION WITH AMINO ACID	DOCKING VIEW
SC		

SJ	 Asp 150 Tyr 158 Trp 222 Ile 194 Phe 149 Pro 193 Asp 148 Ala 191 Thr 196	
SM	 Ile 194 Ile 21 Thr 196 Asp 148 Met 199 Gly 192 Ala 191 Pro 193 Phe 149 Glu 219 Asp 150 Tyr 158 Ile 215 Leu 218 Met 155 Trp 222	
SN	 Ser 94 Gly 40 Ile 16 Ile 95 Val 65 Phe 97 Gly 96 Gln 66 Ile 122	

SE	 Ser 20 Ala 198 Thr 196 Ile 21 Ile 16 Ser 94 Gly 96 Gly 14 Phe 41 Thr 39 Leu 63 Val 65 Ile 95 Gly 40 Ile 122 Ser 13 Asp 64	

VARIANTS:

PREDICTION OF TOXICITY (INSILICO):

Toxicity prediction was done by OSIRIS® property explorer,the online software of Thomas Sander Actelion Pharmaceuticals Ltd,Switzerland.

The chemical structure was drawn in OSIRIS property explorer to show the biological property of the compound. Properties with high risks of undesired effects like mutagenicity,tumerogenicity and reproductive effect were indicated in red.Green colour was used to indicate drug conform behavior.

The following are the results of toxicity prediction for five selected molecules based on docking score:

TABLE 3: INSILICO TOXICITY PREDICTION

Compound code	Mutagenic	Tumorigenic	Irritant	Reproductive effect
SC	--	--	--	--
SJ	--	--	--	--
SM	--	--	--	--
SN	--	--	--	--
SE	--	--	--	--

CODE NAME: SC

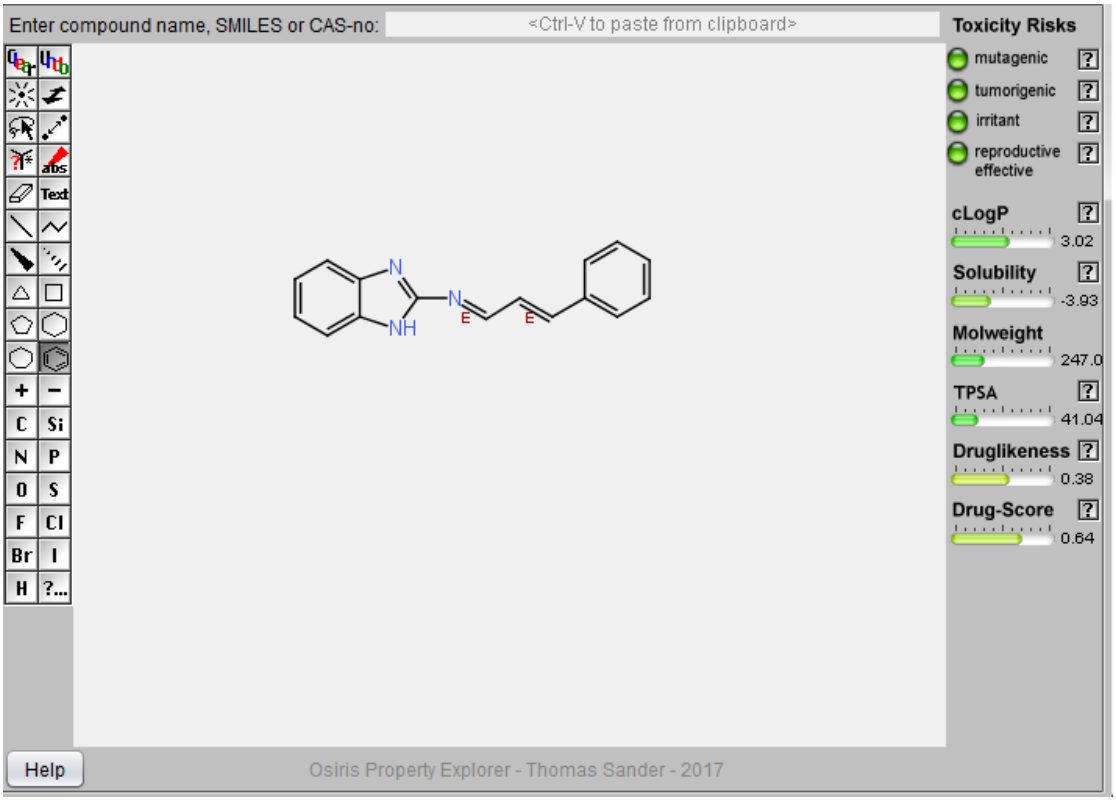


Figure 8 : Toxicity prediction of SC

CODE NAME: SJ

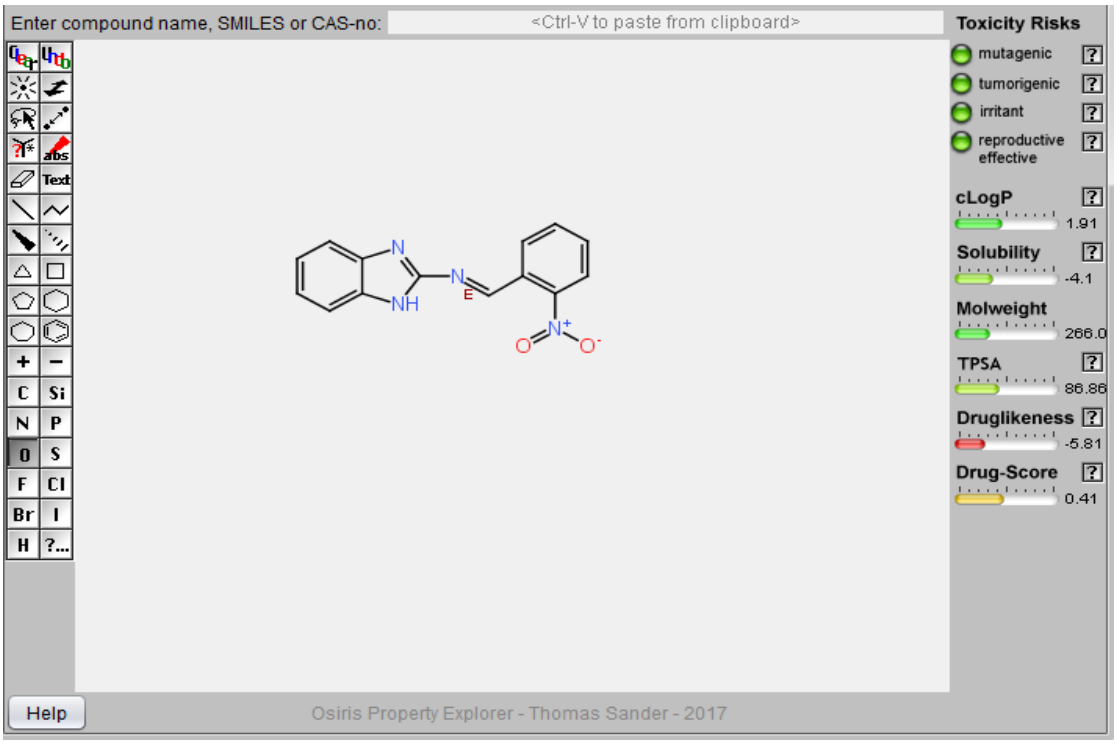


Figure 9 : Toxicity prediction of SJ

CODE NAME: SM

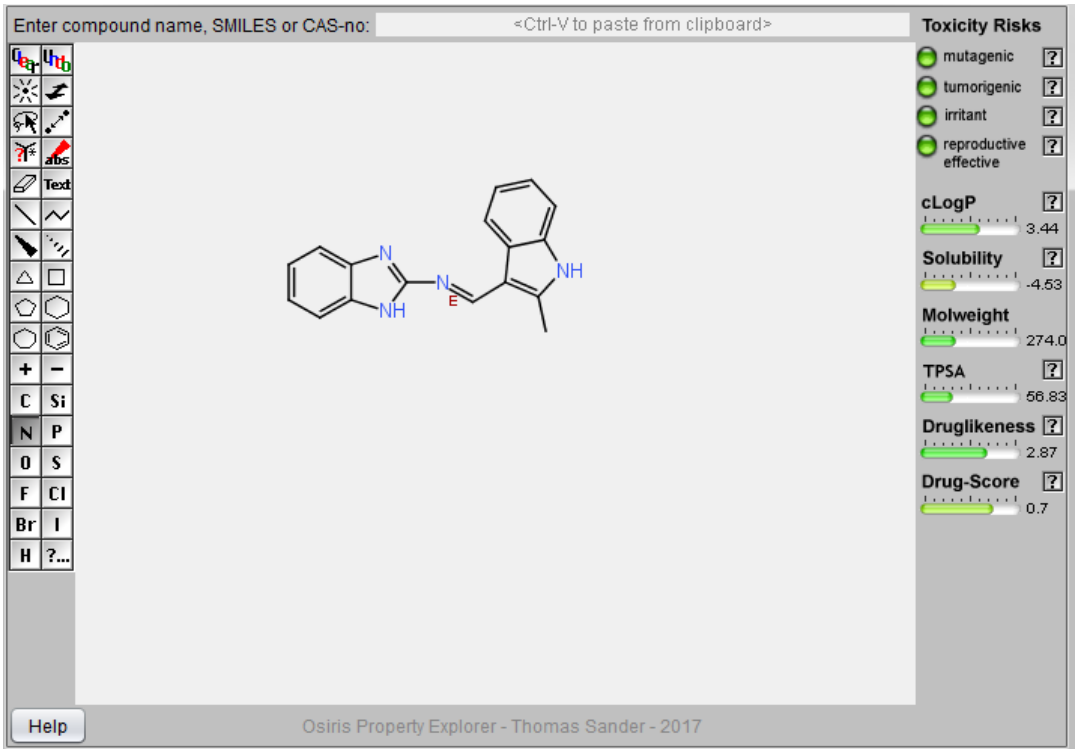


Figure 10: Toxicity prediction of SM

CODE NAME: SN

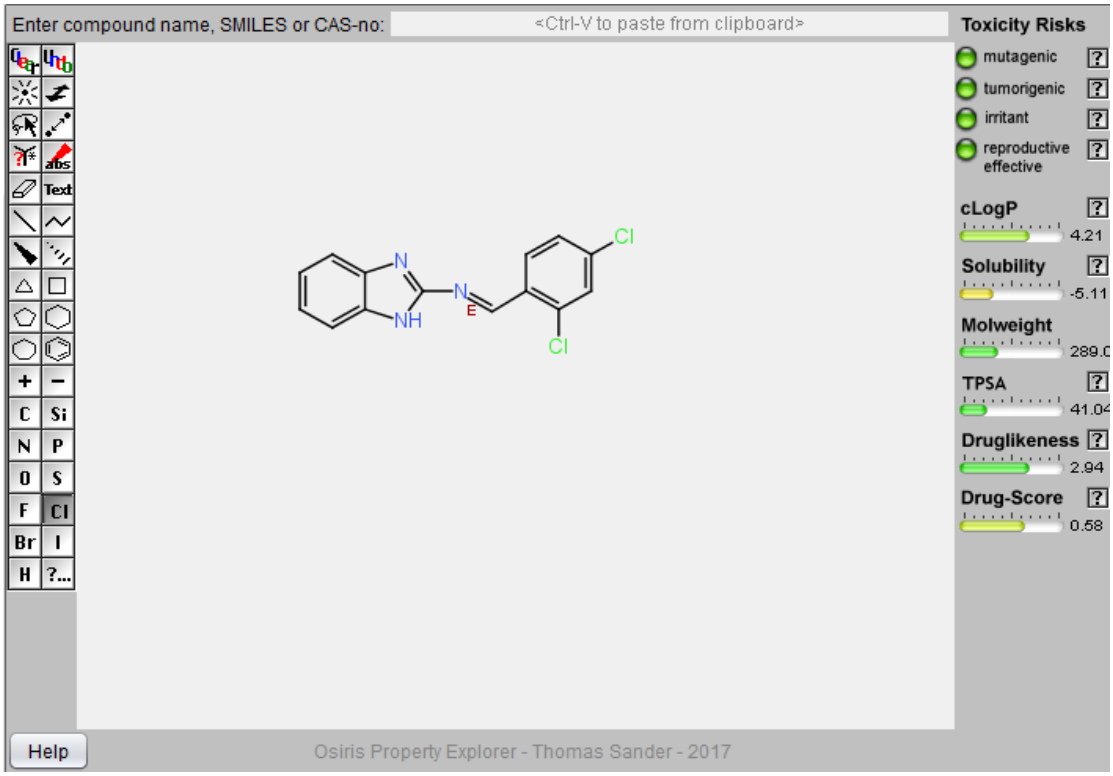


Figure 11 : Toxicity prediction of SN

CODE NAME: SE

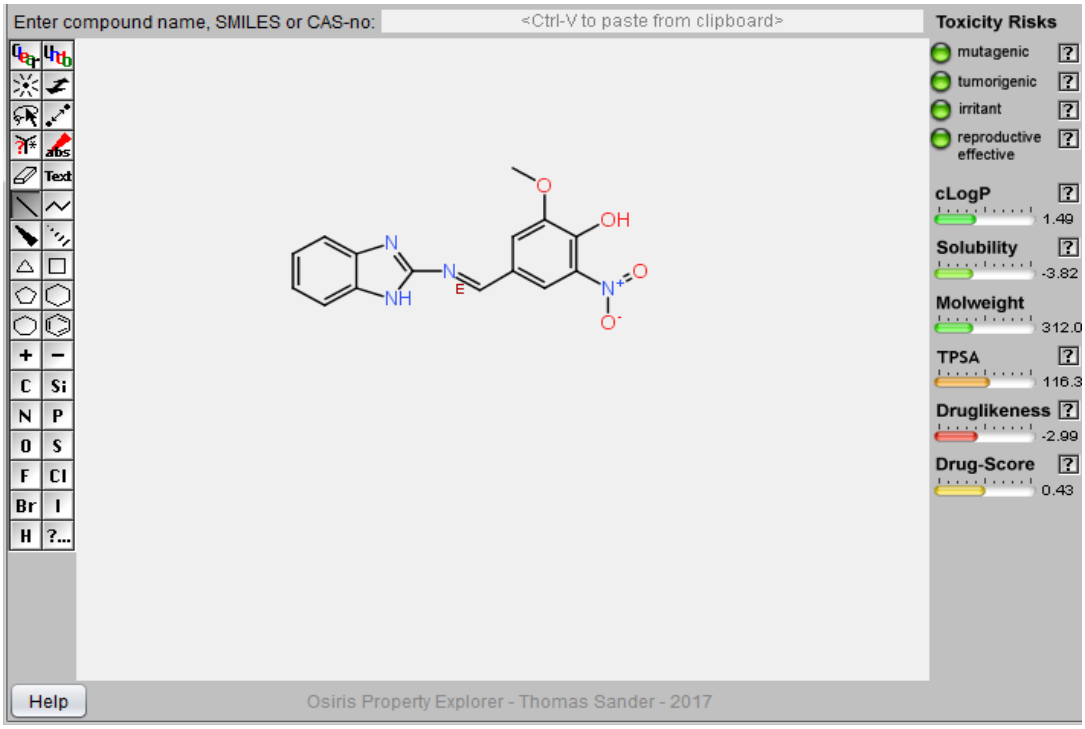


Figure 12 : Toxicity prediction of SE

None of the compounds showed violations and thus these compounds are further subjected for synthesis and characterization.

INSILICO SCREENING OF DRUG LIKENESS

Lipinski`s rule of five was used to evaluate drug likeness to determine if a chemical compound with certain pharmacological or biological activity has properties that would make it an orally active drug. The rule describes molecular properties important for a drugs pharmacokinetics in the human's body, including their absorption, distribution, metabolism, and excretion (ADME).However, the rule does not predict if a compound is pharmacologically active.

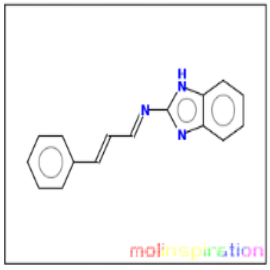
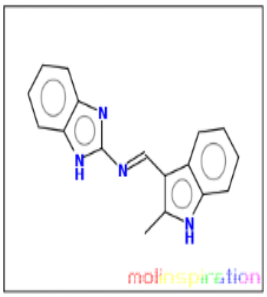
The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug- like properties as described by Lipinski rule.

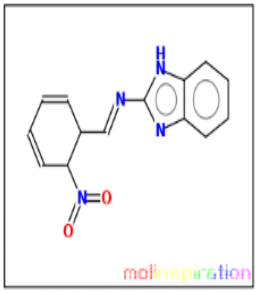
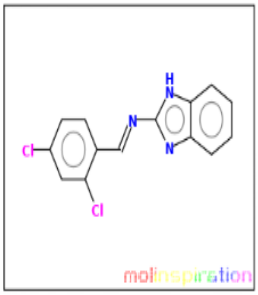
Lipinski rule says that, an orally active drug has not more than one violation of the following criteria:

- Partition coefficient log *P* in −0.4 to +5.6 range
- Molar refractivity from 40 to 130

- Molecular weight from 180 to 500
- Number of atoms from 20 to 70 (includes H-bond donors)
- 10 or fewer rotatable bonds and
- Polar surface area no greater than 140 Å2

TABLE 4: INSILICO DRUG LIKENESS PREDICION

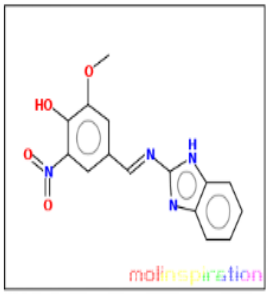
SAMPLE CODE	INSILICO DRUG LIKENESS PREDICTION
SC	molinspiration Calculation of Molecular Properties miSMILES: C(C=Nc2nc1ccccc1[nH]2)=Cc3ccccc3  Molinspiration property engine v2018.10 miLogP 4.04 TPSA 41.05 natoms 19 MW 247.30 nON 3 nOHNH 1 nviolations 0 nrotb 3 volume 230.95 Get data as text (for copy / paste). Get 3D geometry BETA
SM	molinspiration Calculation of Molecular Properties miSMILES: Cc2[nH]c1ccccc1c2C=Nc4nc3ccccc3[nH]4  Molinspiration property engine v2018.10 miLogP 3.65 TPSA 56.84 natoms 21 MW 274.33 nON 4 nOHNH 2 nviolations 0 nrotb 2 volume 249.07 Get data as text (for copy / paste). Get 3D geometry BETA

SJ	molinspiration Calculation of Molecular Properties miSMILES: O=N(=O)C1C=CC=CC1C=Nc3nc2ccccc2[nH]3  Molinspiration property engine v2018.10 miLogP 2.25 TPSA 86.87 natoms 20 MW 268.28 nON 6 nOHNH 1 nviolations 0 nrotb 3 volume 233.11 Get data as text (for copy / paste). Get 3D geometry BETA
SN	molinspiration Calculation of Molecular Properties miSMILES: Clc3ccc(C=Nc2nc1ccccc1[nH]2)c(Cl)c3 YEDFOJUXLWBFCV-UHFFFAOYSA-N  Molinspiration property engine v2018.10 miLogP 4.57 TPSA 41.05 natoms 19 MW 290.15 nON 3 nOHNH 1 nviolations 0 nrotb 2 volume 230.60 Get data as text (for copy / paste). Get 3D geometry BETA

SE

molinspiration Calculation of Molecular Properties

miSMILES:
COc3cc(C=Nc2nc1ccccc1[nH]2)cc(N(=O)=O)c3O



Molinspiration property engine v2018.10

miLogP	2.74
TPSA	116.33
natoms	23
MW	312.29
nON	8
nOHNH	2
nviolations	0
nrotb	4
volume	260.43

Get data as text (for copy / paste).

Get 3D geometry BETA

CHARACTERIZATION :

TLC PROFILE:

TLC is carried out by precoated plates (604 GF 254 MERCK),with a suitable solvent system

The selected 5 compounds were synthesized, recrystallised and purified .It was determined by its sharp melting point and single spot obtained in TLC. The Rf values are recorded accordingly.

TABLE 5: PRELIMINARY CHARACTERIZATION OF THE COMPOUNDS

Code name	Appearance	Mobile phase	Rf value	Melting point(˚c)
SC	DARK YELLOW	METHANOL:CHLOROFORM(9:1)	0.64	193-194
SM	PALE YELLOW	METHANOL:CHLOROFORM(9:1)	0.71	201-203
SJ	LIGHT BROWN	METHANOL:CHLOROFORM(9:1)	0.67	198-199
SN	DARK YELLOW	METHANOL:CHLOROFORM(9:1)	0.83	223-225
SE	BRICK RED	METHANOL:CHLOROFORM(9:1)	0.75	178-179

The characterization was carried out using sophisticated instruments like IR, NMR, and Mass spectrometry and characteristic properties through the aid of computer software.

Infrared Absorption Spectroscopy:

The IR spectrum of the synthesized compounds were inspected for presence of stretching/bending of the new functional group and absence of the functional group which induced the changes in the chemical reaction. All the compounds showed presence of the Imine formation.

- ✓ The spectrum of the compound SC showing the strong stretching vibration at 1620cm^{-1} indicates the presence of

C=N group.

All the compounds showing the Ar-CH stretching vibration between 3070- 3060cm^{-1}, N-H stretching vibration between 3220-3255 cm^{-1}, NO stretching vibration between

1120-1290cm^{-1}.

H^{1} NMR Spectroscopy:

Proton NMR spectroscopy help us to study the number of equivalent protons and their environment thereby we can ascertain the structure of molecule. The positions of the signals helps us to know the nature of protons viz, aromatic, heteroaromatic, aliphatic, vinyl C-H groups.The H^{1} NMR spectral data of all the synthesized compounds are in conformity with the structure assigned. A singlet at 11.02-11.35δ was observed for all compounds confirming the presence of N-H proton. All the compounds shows the multiplet and doublet signals for the presence of aromatic protons between (6.2-7.4 δ), heteroaromatic protons between (7.2-7.8) δppm.

LIQUID CHROMATOGRAPHY:

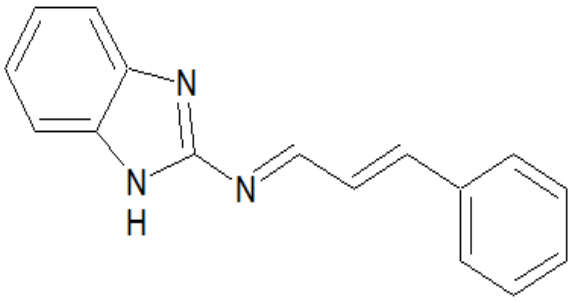
LC-MS was used to determine the purity of the compounds by looking for additional peaks in a sample that should not be present in the pure compound.

TABLE 6: MOLECULAR WEIGHT DETERMINATION BY MASS SPECTRA

S.NO	SAMPLE CODE	ACTUAL MASS (g/mol)	CALCULATED MASS (g/mol)
1	SC	245.95	247.30
2	SJ	266.95	266.26
3	SM	274.90	274.33
4	SN	291.80	290.15
5	SE	319.00	312.29

PRODUCT PROFILE

CODE NAME: SC



N-[(1*E*,2*E*)-3-phenylprop-2-en-1-ylidene]-1*H*-benzimidazol-2-amine

Molecular Formula	= $C_{16}H_{13}N_3$
Formula Weight	= 247.29452
Composition	= C(77.71%) H(5.30%) N(16.99%)
Molar Refractivity	= 77.79 ± 0.5 cm^3
Molar Volume	= 218.7 ± 7.0 cm^3
Parachor	= 567.0 ± 8.0 cm^3
Index of Refraction	= 1.629 ± 0.05
Surface Tension	= 45.1 ± 7.0 dyne/cm
Density	= 1.13 ± 0.1 g/cm^3
Polarizability	= 30.84 ± 0.5 $10^{-24} cm^3$

IR SPECTRUM OF SAMPLE CODE SC:

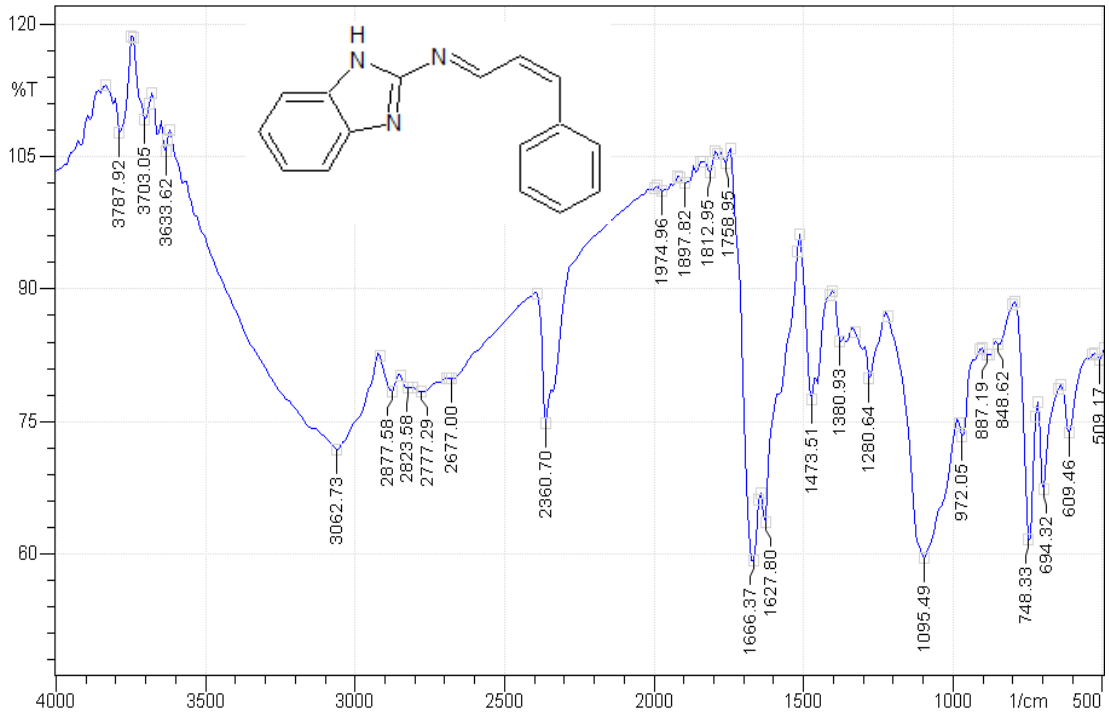
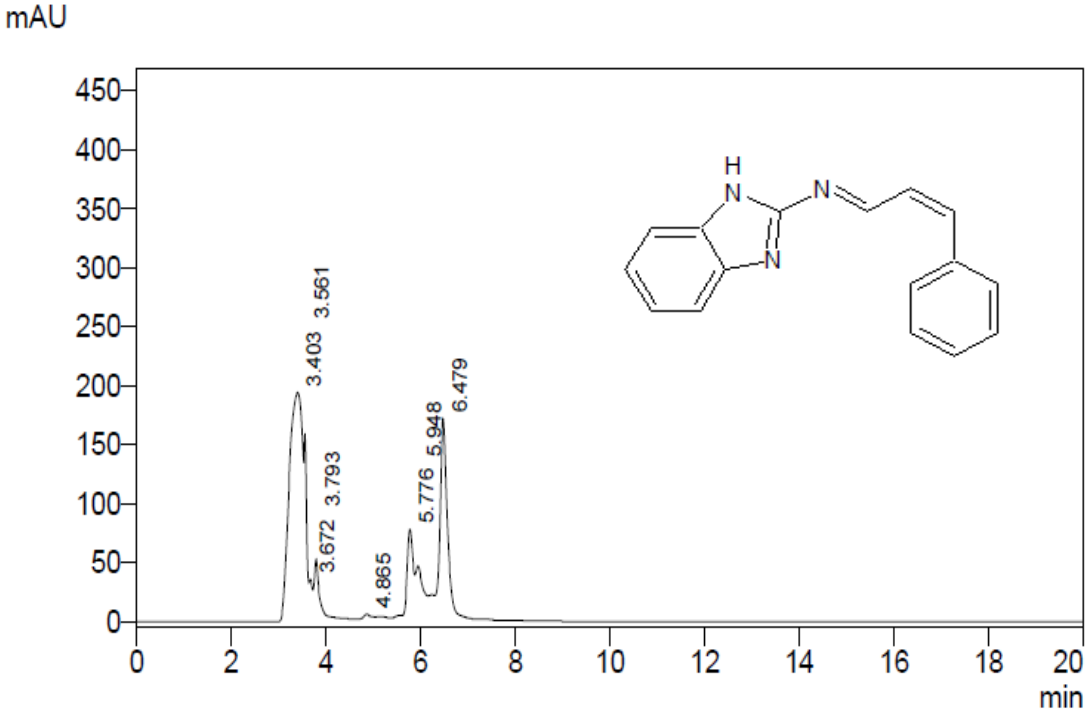
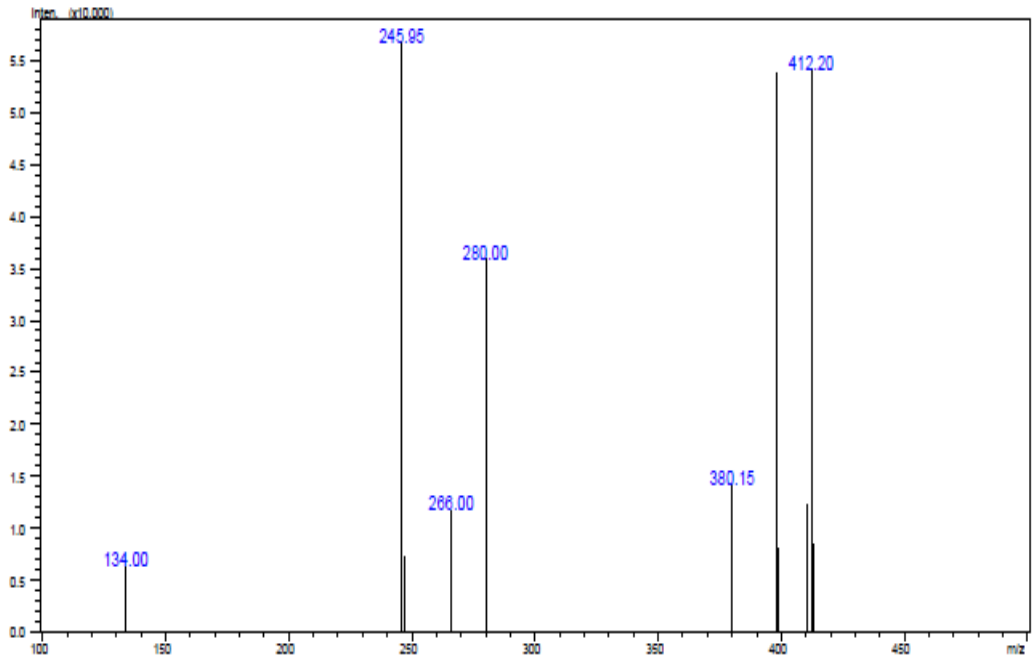


TABLE 7: IR INTERPRETATION OF SC

S.NO.	WAVENUMBER	TYPES OF VIBRATIONS	FUNCTIONAL GROUP
1	3062.73	C-H stretching	Alkyl group
2	1627.80	C=N stretching	Imine group
3	3633.62	N-H stretching	N-H group

LC-MS CHROMATOGRAM OF SC:





^{1}H NMR SPECTRUM OF SC

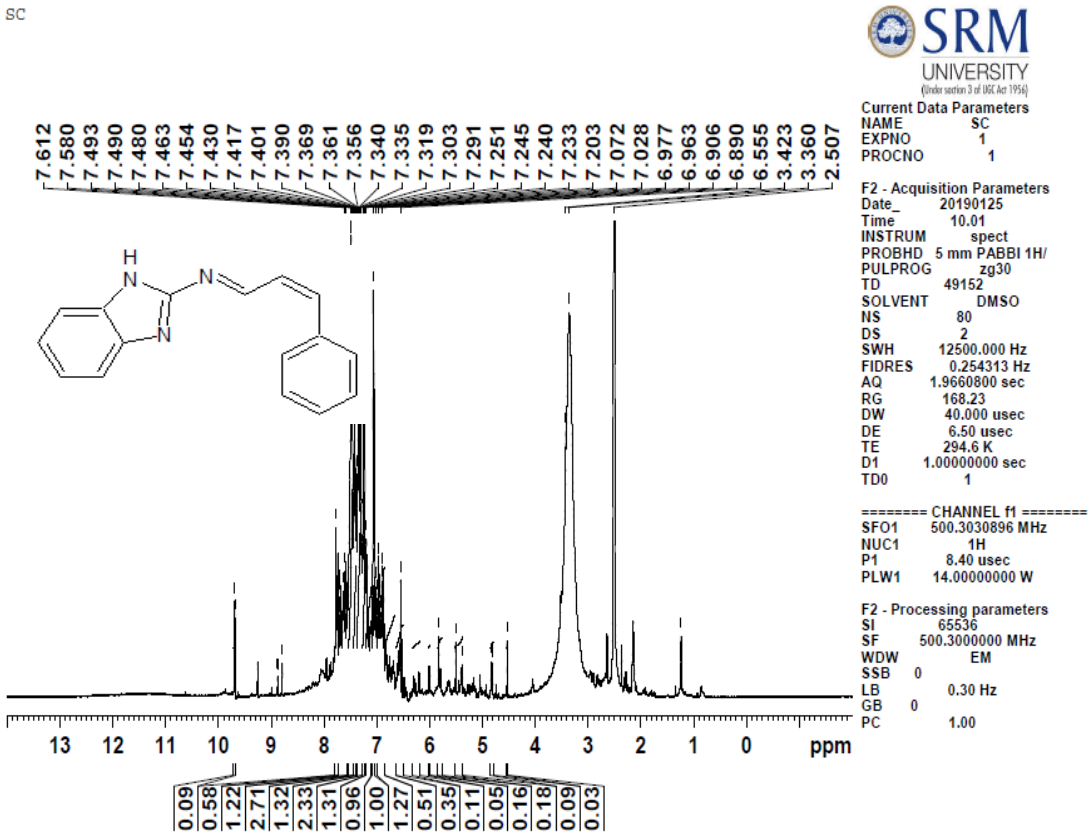
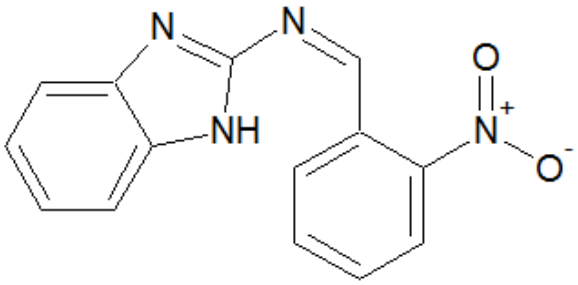


TABLE 8: ^{1}H NMR INTERPRETATION OF SC

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTONS
1	6-8.5	Multiplet	9
2	4.6-6.5	Multiplet	3
3	1-2.5	Singlet	1

CODE NAME: SJ



N-[(*Z*)-(2-nitrophenyl)methylidene]-1*H*-benzimidazol-2-amine

Molecular Formula	= $C_{14}H_{10}N_4O_2$
Formula Weight	= 266.2548
Composition	= C(63.15%) H(3.79%) N(21.04%) O(12.02%)
Molar Refractivity	= 74.24 ± 0.5 cm^3
Molar Volume	= 191.9 ± 7.0 cm^3
Parachor	= 535.2 ± 8.0 cm^3
Index of Refraction	= 1.700 ± 0.05
Surface Tension	= 60.4 ± 7.0 dyne/cm
Density	= 1.38 ± 0.1 g/cm^3
Polarizability	= 29.43 ± 0.5 $10^{-24} cm^3$

IR SPECTRUM OF SAMPLE CODE SJ

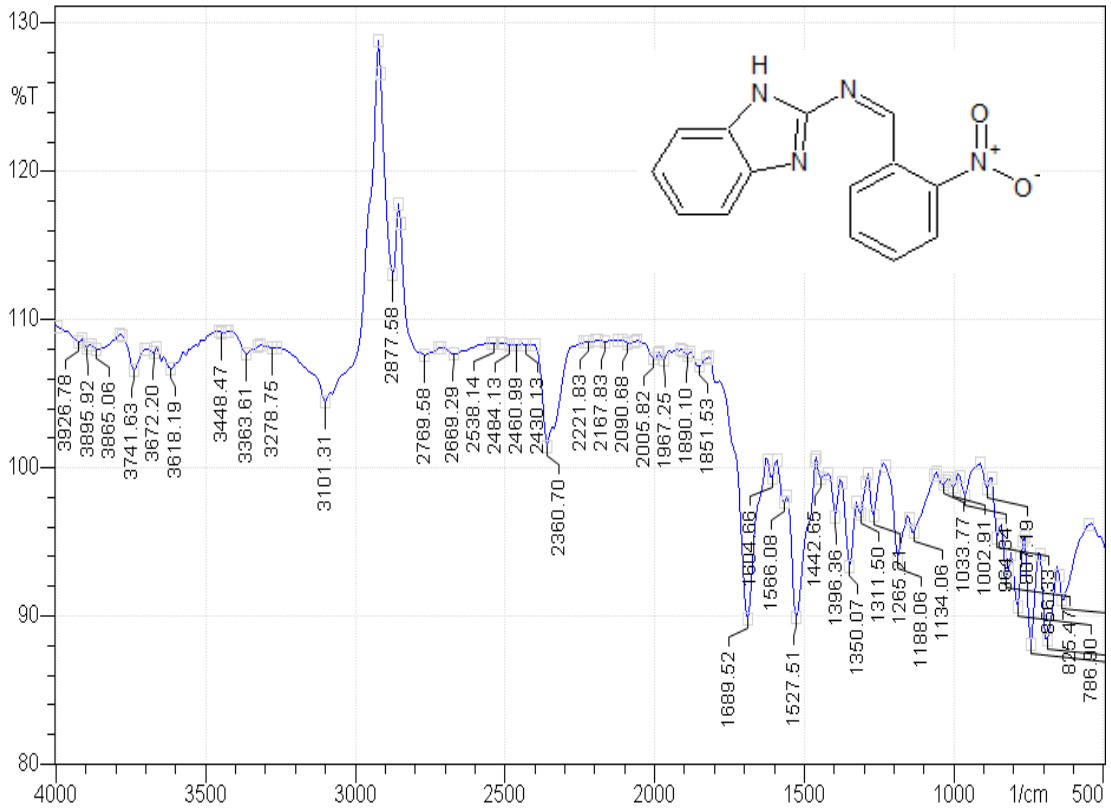
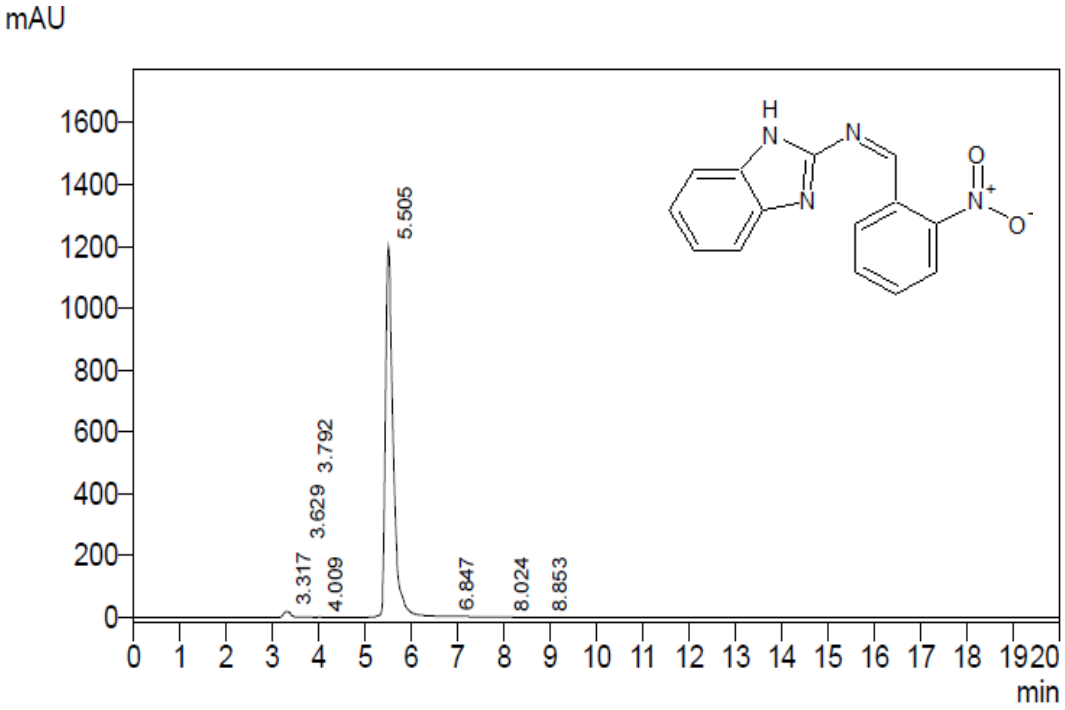
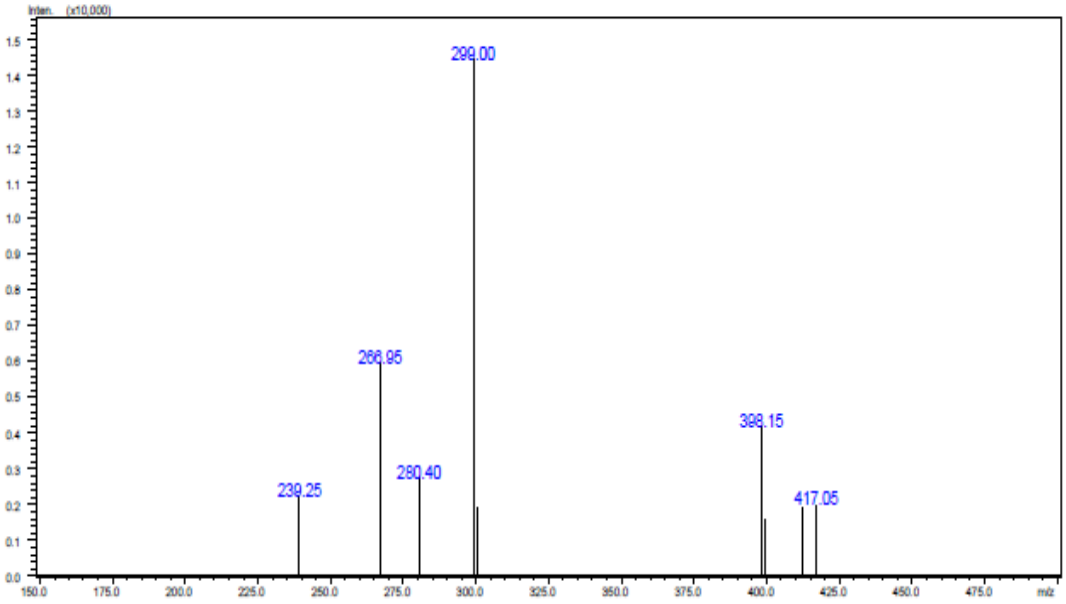


TABLE 9: IR INTERPRETATION OF SJ

S.NO	WAVENUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUP
1	1527.51 cm^{-1}	N=O stretching	Ar-NO_2 group
2	1689.52 cm^{-1}	C=N stretching	Imine group
3	3448.47 cm^{-1}	N-H stretching	N-H group

LC-MS CHROMATOGRAM OF SJ:





^{1}H NMR SPECTRUM OF SJ

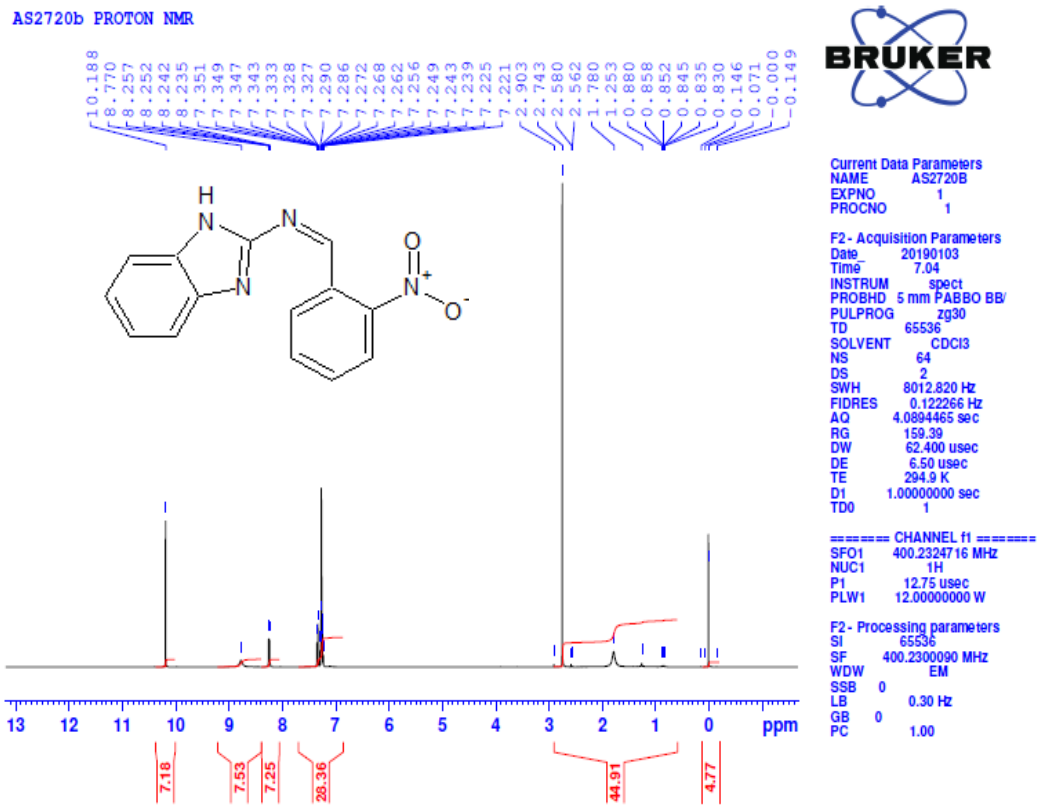
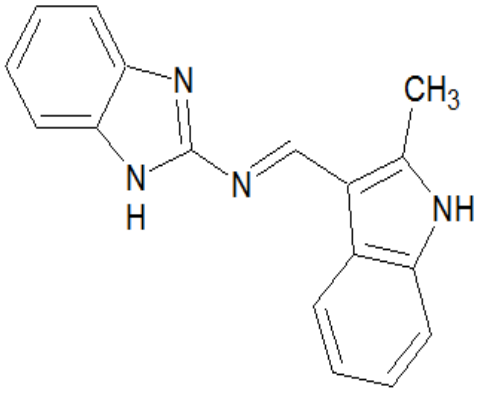


TABLE 10: ^{1}H NMR INTERPRETATION OF SJ

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTONS
1	6-9.0	Doublet	8
2	7.2-7.8	Multiplet	1
3	1-2.5	Singlet	1

CODE NAME: SM



N-[(*E*)-(2-methyl-1*H*-indol-3-yl)methylidene]-1*H*-benzimidazol-2-amine

Molecular Formula	= $C_{17}H_{14}N_4$
Formula Weight	= 274.31986
Composition	= C(74.43%) H(5.14%) N(20.42%)
Molar Refractivity	= 82.72 ± 0.5 cm^3
Molar Volume	= 210.1 ± 7.0 cm^3
Parachor	= 563.0 ± 8.0 cm^3
Index of Refraction	= 1.716 ± 0.05
Surface Tension	= 51.4 ± 7.0 dyne/cm
Density	= 1.30 ± 0.1 g/cm^3
Polarizability	= 32.79 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF SAMPLE CODE SM:

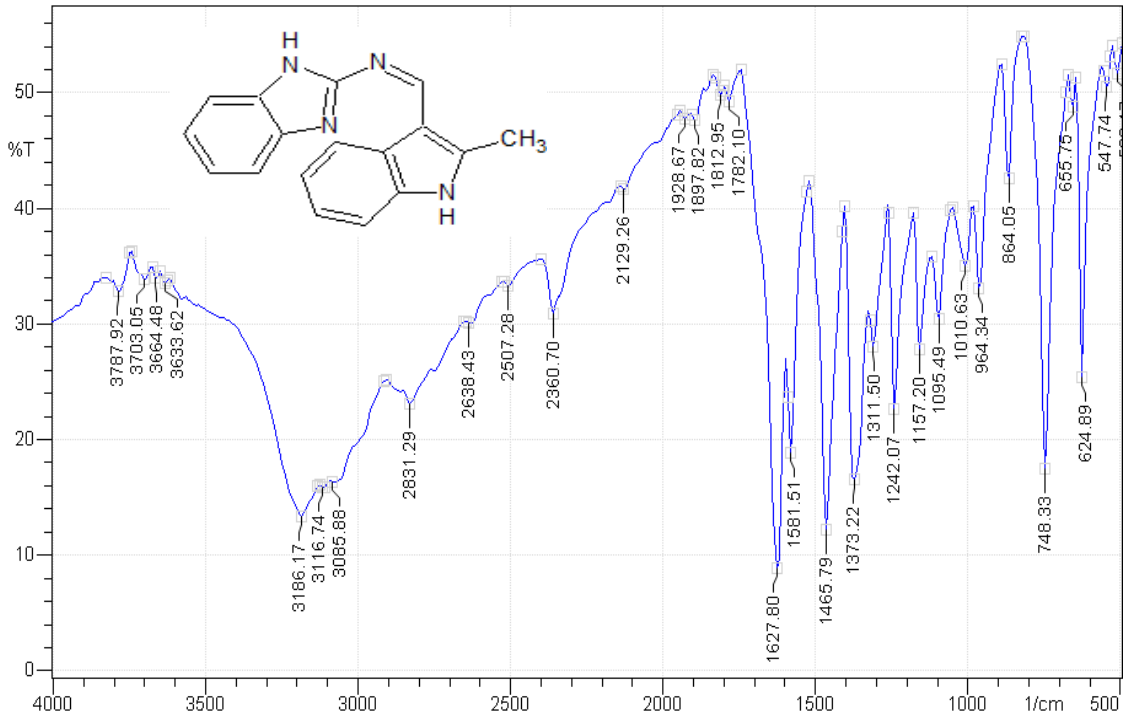
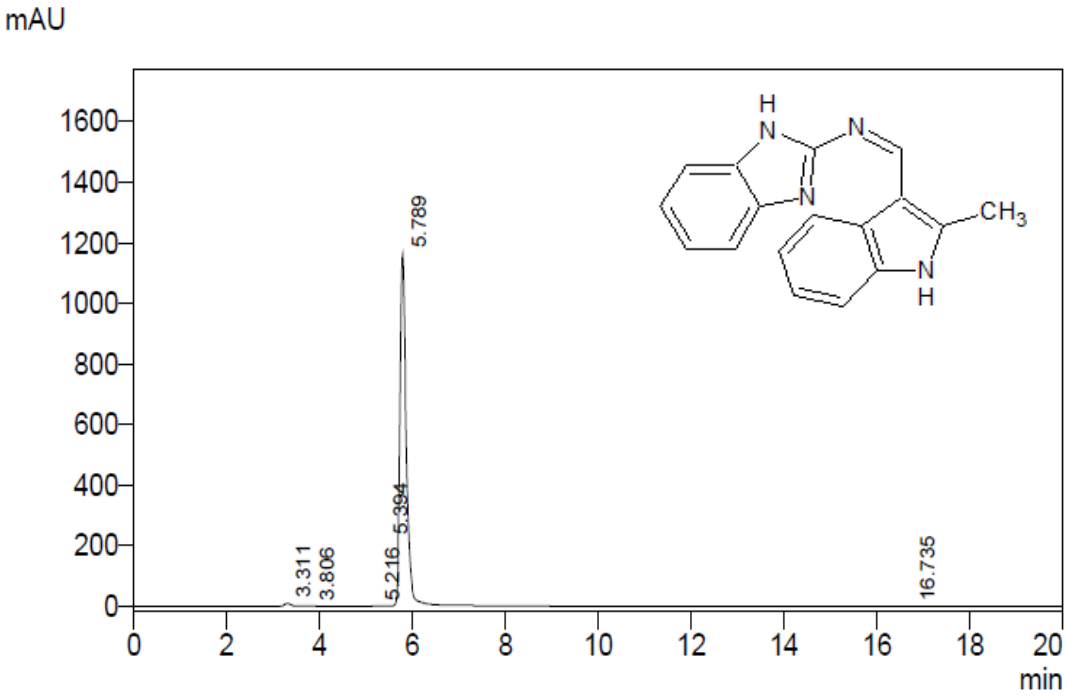
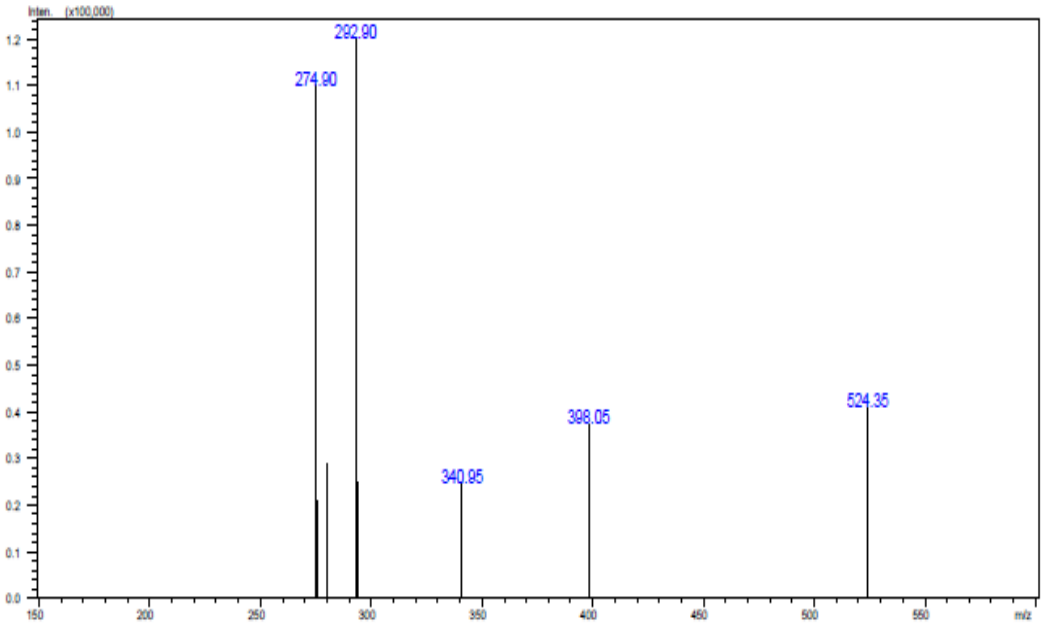


TABLE 11: IR INTERPRETATION OF SM

S.NO	WAVENUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUP
1	2831.29 cm^{-1}	C-H stretching	Alkyl group
2	1242.07 cm^{-1}	C-N stretching	C-N group
3	1627.80 cm^{-1}	C=N stretching	Imine group

LC-MS CHROMATOGRAM OF SM:





^{1}H NMR SPECTRUM OF SM

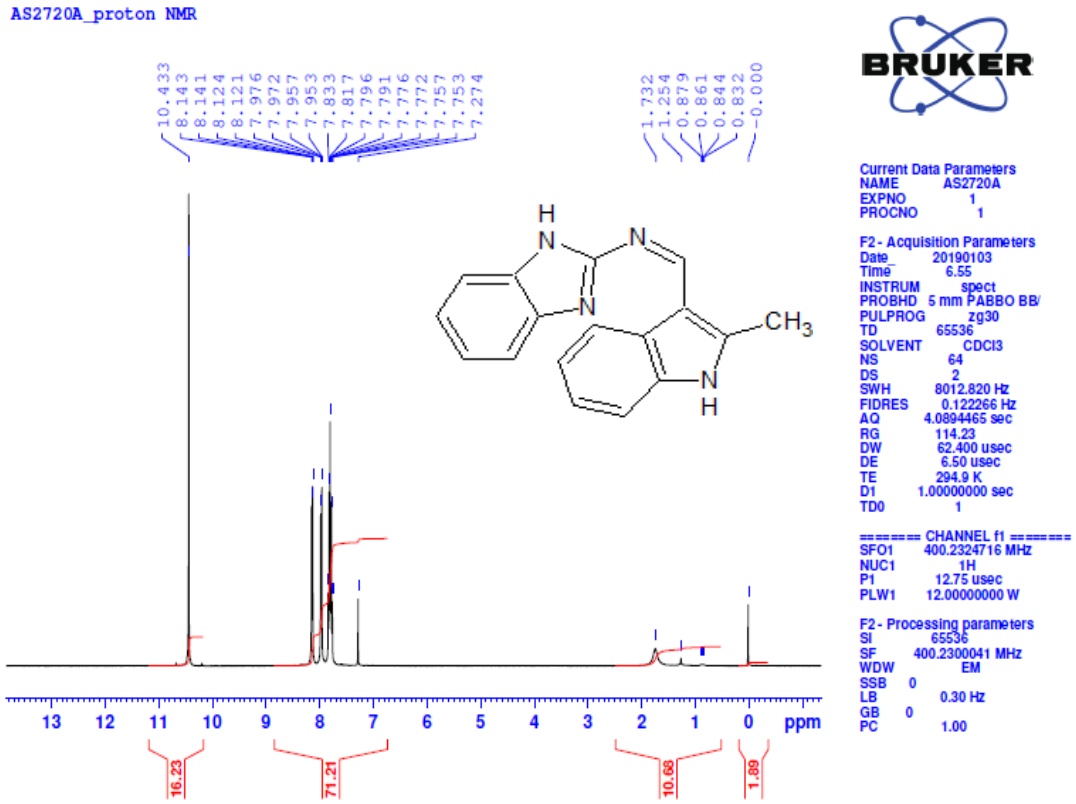
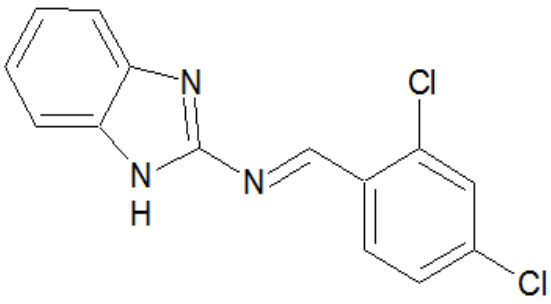


TABLE 12: ^{1}H NMR INTERPRETATION OF SM

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTONS
1	6-9.0	Multiplet	8
2	10-11.0	Singlet	2
3	7.2-7.8	Multiplet	4

CODE NAME: SN



N-[(*E*)-(2,4-dichlorophenyl)methylidene]-1*H*-benzimidazol-2-amine

Molecular Formula	= $C_{14}H_9Cl_2N_3$
Formula Weight	= 290.14736
Composition	= C(57.95%) H(3.13%) Cl(24.44%) N(14.48%)
Molar Refractivity	= 77.78 ± 0.5 cm^3
Molar Volume	= 205.2 ± 7.0 cm^3
Parachor	= 547.4 ± 8.0 cm^3
Index of Refraction	= 1.682 ± 0.05
Surface Tension	= 50.6 ± 7.0 dyne/cm
Density	= 1.41 ± 0.1 g/cm^3
Polarizability	= 30.83 ± 0.5 $10^{24} cm^3$

IR SPECTRUM OF SAMPLE CODE SN:

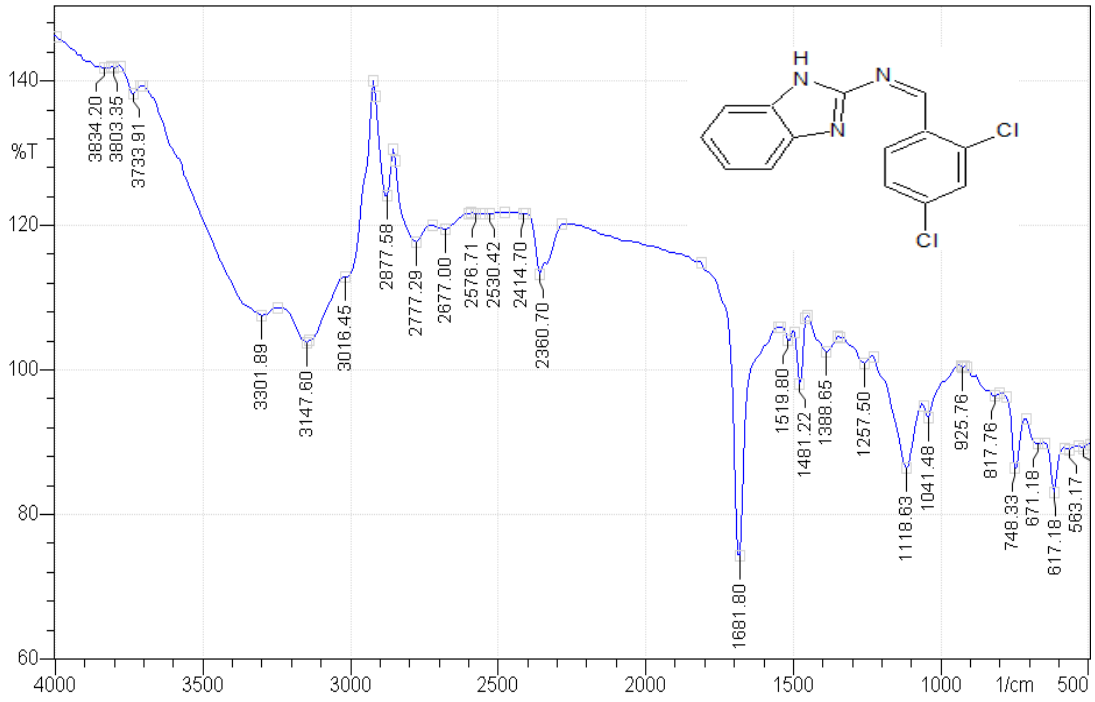
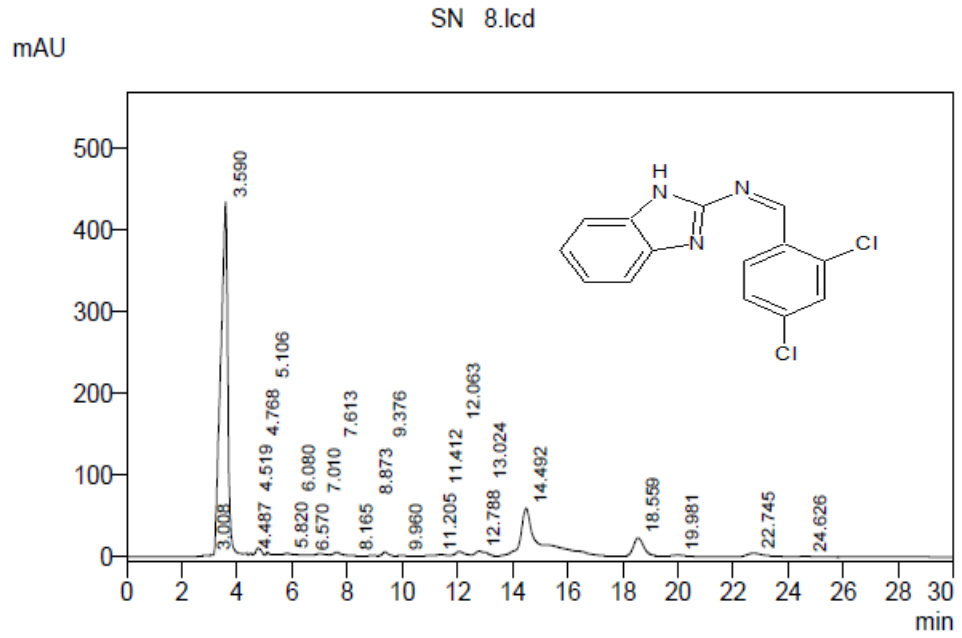
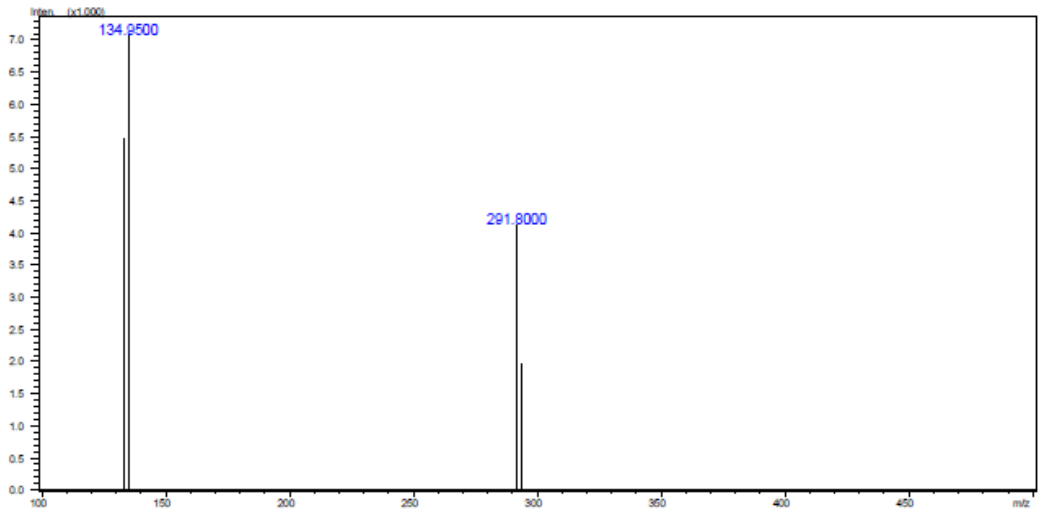


TABLE 13: IR INTERPRETATION OF SN

S.NO	WAVENUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUP
1	817.76 cm^{-1}	C-Cl stretching	C-Cl group
2	3016.45 cm^{-1}	Ar-H stretching	Aromatic hydrocarbon
3	1681.80 cm^{-1}	C=N stretching	Imine group

LC MS CHROMATOGRAM OF SN:





^{1}H NMR SPECTRUM OF SN

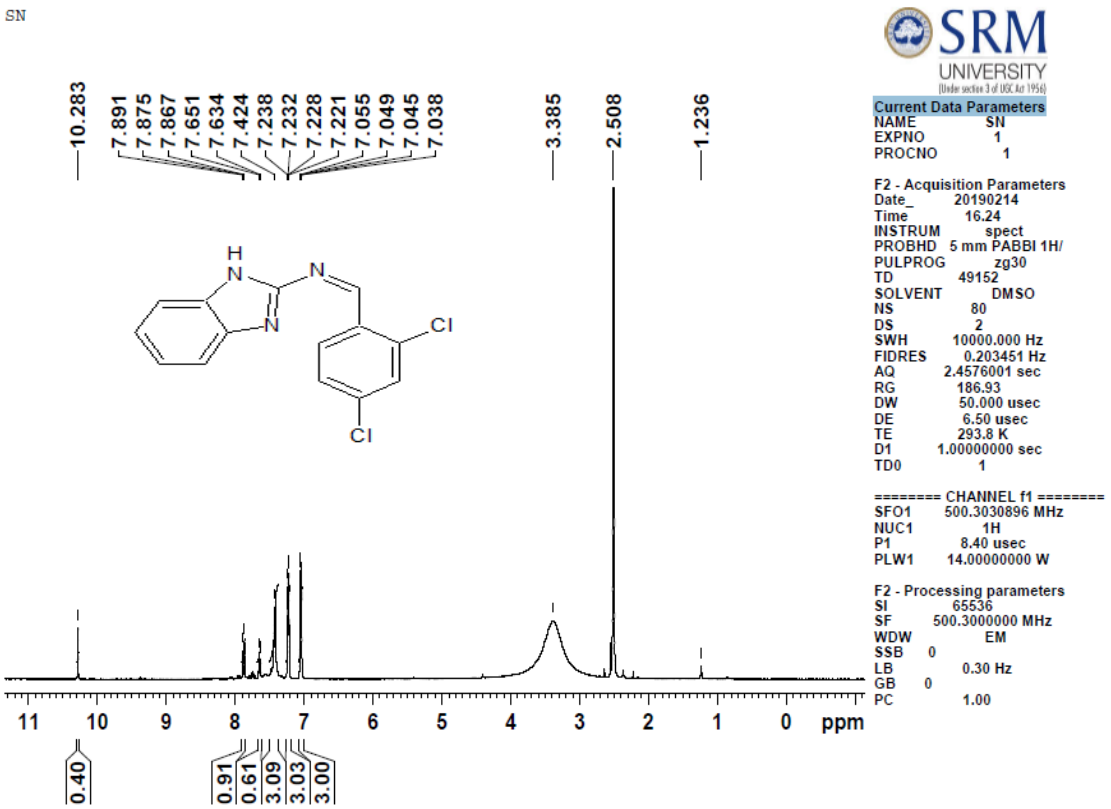
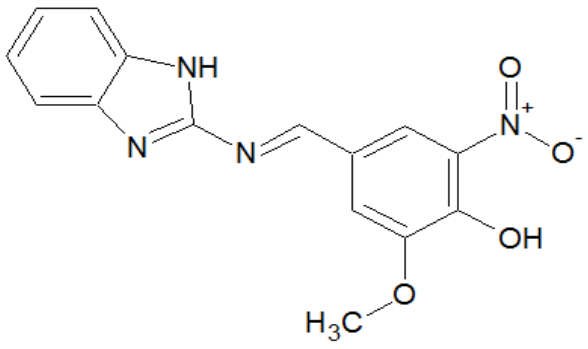


TABLE 14: ^{1}H NMR INTERPRETATION OF SN

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTONS
1	6.5-8	multiplet	7
2	10-11.5	Singlet	1

CODE NAME: SE



4-[(*E*)-(1*H*-benzimidazol-2-ylimino)methyl]-2-methoxy-6-nitrophenol

Molecular Formula	= $C_{15}H_{12}N_4O_4$
Formula Weight	= 312.28018
Composition	= C(57.69%) H(3.87%) N(17.94%) O(20.49%)
Molar Refractivity	= 80.90 ± 0.5 cm^3
Molar Volume	= 210.9 ± 7.0 cm^3
Parachor	= 591.1 ± 8.0 cm^3
Index of Refraction	= 1.693 ± 0.05
Surface Tension	= 61.6 ± 7.0 dyne/cm
Density	= 1.48 ± 0.1 g/cm^3
Polarizability	= 32.07 ± 0.5 $10^{-24} cm^3$

IR SPECTRUM OF SAMPLE CODE SE:

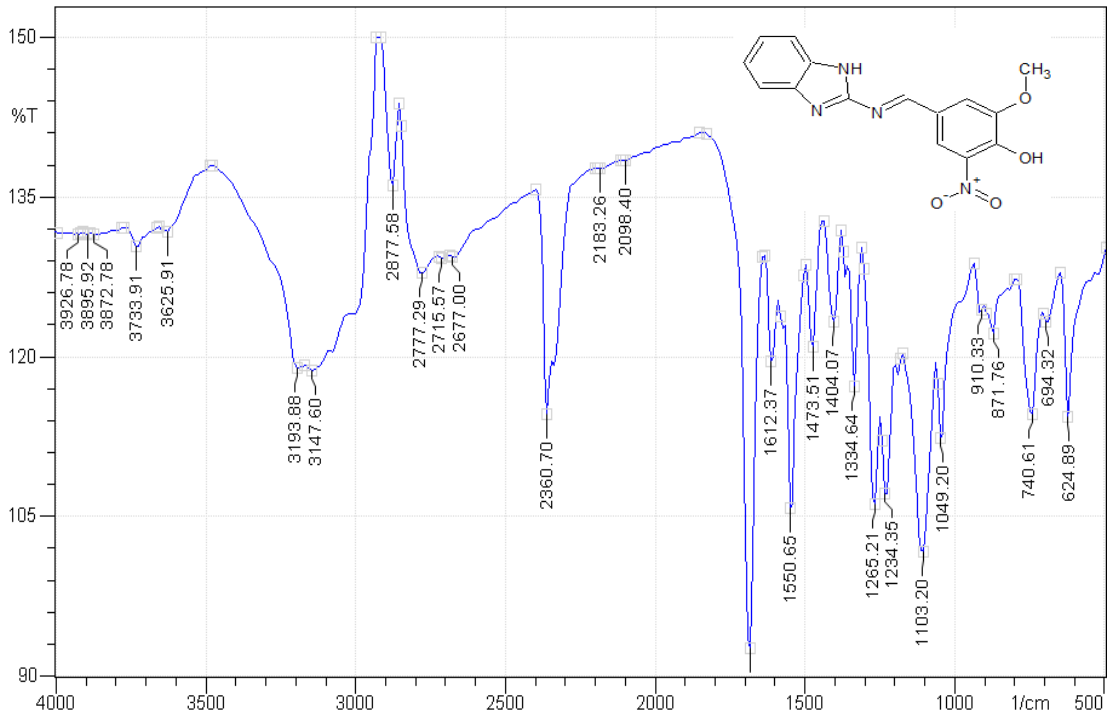
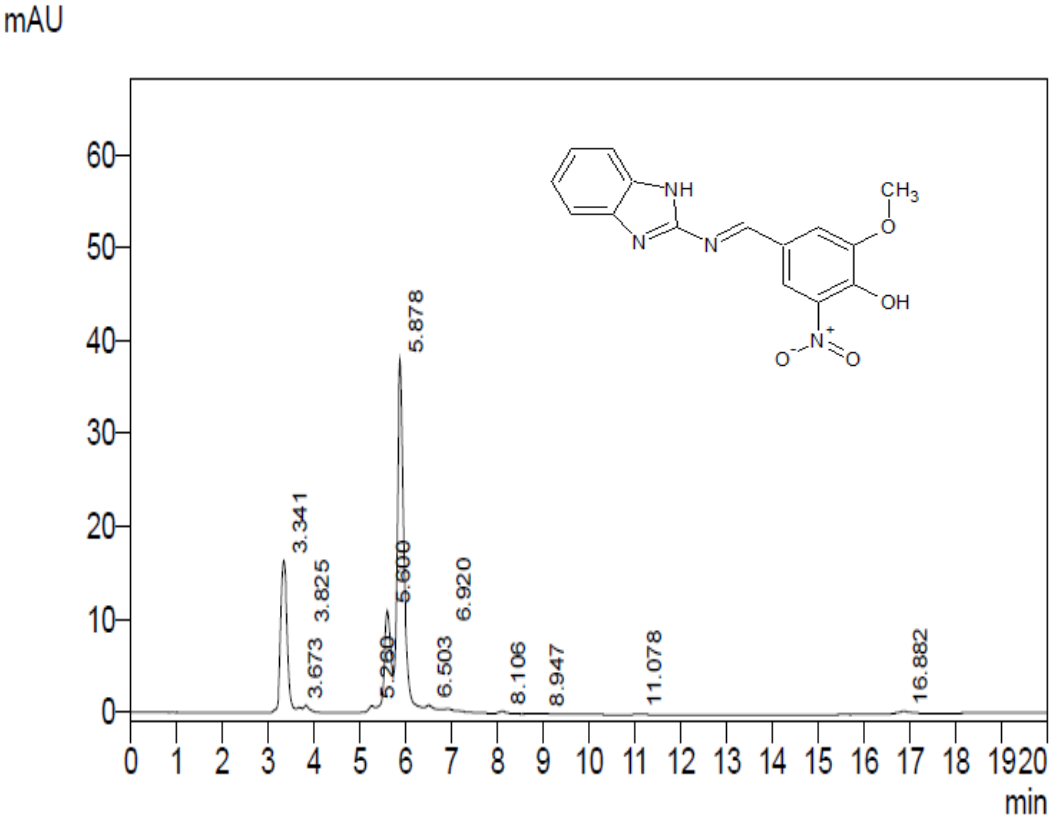
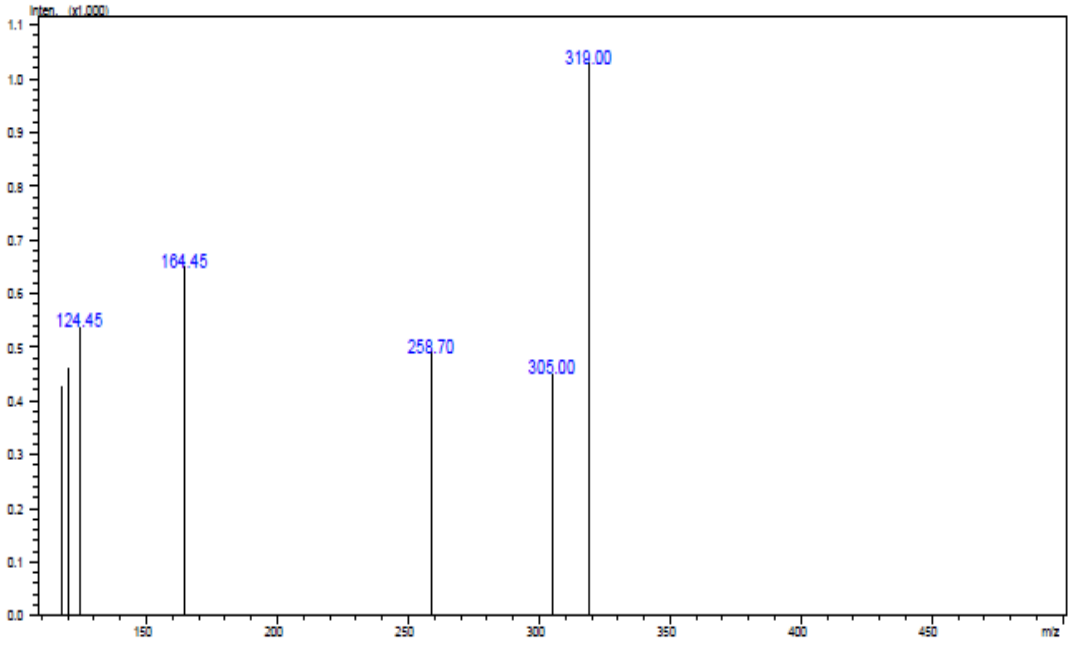


TABLE 15: IR INTERPRETATION OF SE

S.NO	WAVENUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUP
1	1550.65 cm^{-1}	N=O stretching	Ar-NO_2
2	3625.91 cm^{-1}	O-H stretching	Alcoholic group
3	1612.37 cm^{-1}	C=N stretching	Imine group

LC-MS CHROMATOGRAM OF SE:





^{1}H NMR SPECTRUM OF SE

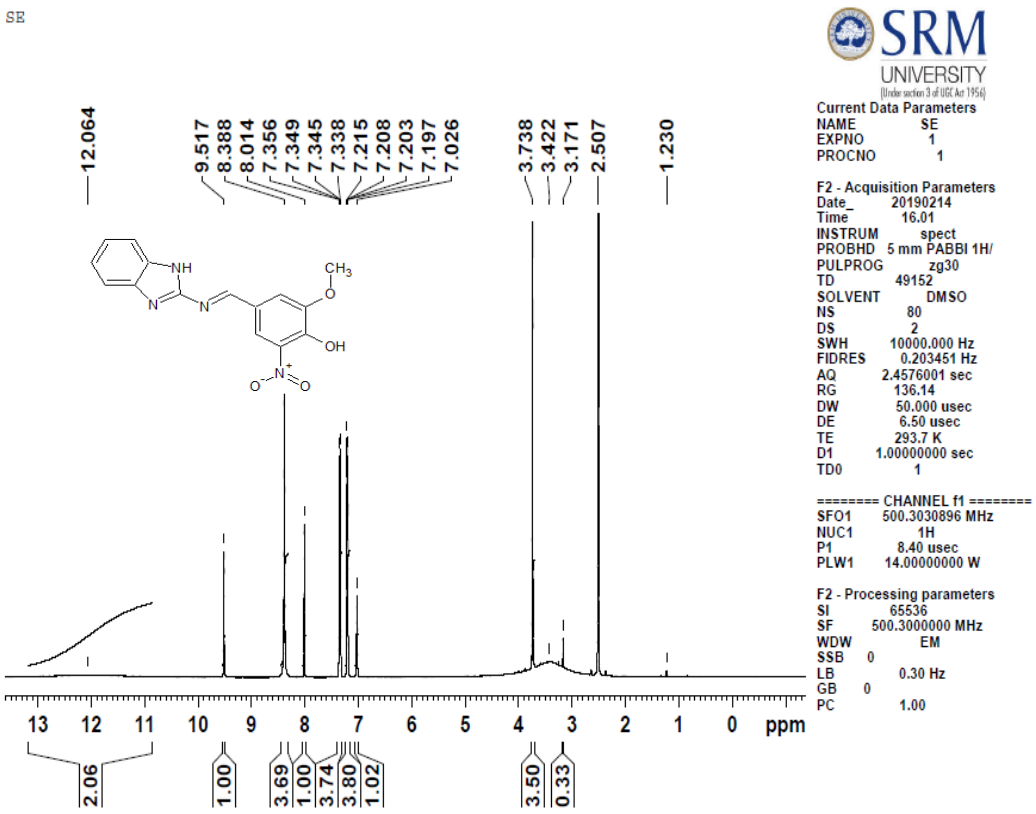


TABLE 16: ^{1}H NMR INTERPRETATION OF SE

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTONS
1	6.5-8	Doublet	6
2	1-2.5	Singlet	1
3	4-12.0	singlet	1
4	7.9-8.5	multiplet	3

BIOLOGICAL EVALUATION

MABA REPORT

Table 17: Biological Evaluation of the Synthesized Compounds

S.No	SAMPLE	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	SC	S	S	S	S	R	R	R	R
2	SJ	S	S	S	R	R	R	R	R
3	SM	S	S	S	R	R	R	R	R
4	SN	S	S	S	S	S	S	S	R
5	SE	S	S	S	S	S	S	S	R

NOTE: S - Sensitive R- Resistant

Standard Strain used: Mycobacteria tuberculosis (Vaccine strain, H37 RV strain): ATCC No-27294.

Standard values for the Anti-Tb test which was performed

Pyrazinamide- 3.125µg/ml

Ciprofloxacin-3.125µg/ml

Streptomycin- 6.25µg/ml

Compounds **SN and SE** were found to be less sensitive than pyrazinamide and ciprofloxacin(1.6 µg/ml)

Compound **SC** was found to have sensitivity at 12.5 µg/ml

The other 2 compounds **SJ** and **SM** were found to have sensitivity at 25 µg/ml

Table 18: MABA RESULTS

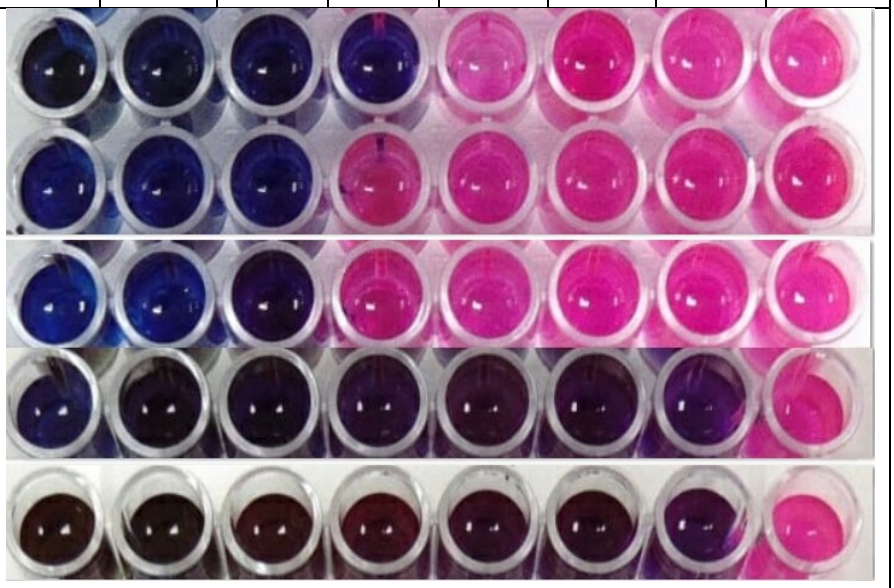
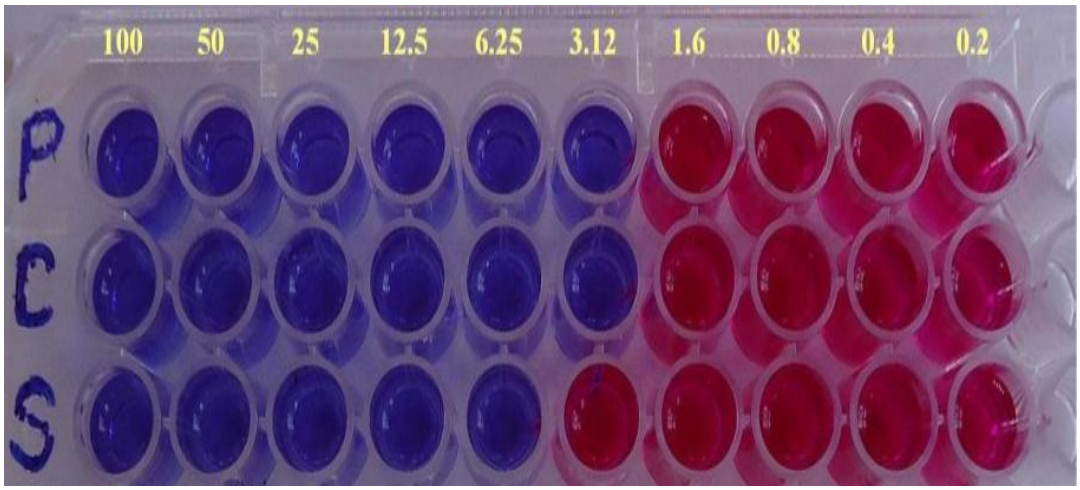
s. No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	SC								
2	SJ								
3	SM								
4	SN								
5	SE								

Fig 13: STANDARD DRUG PHOTOGRAPH



SUMMARY AND CONCLUSION

- ✓ InhA (Enoyl acyl carrier protein reductase), a critical enzyme for the cell wall synthesis of *Mycobacterium tuberculosis* was chosen for study after review of literature.
- ✓ Candidate molecules were designed and docked against *2h9i* protein using Autodock Tools® 1.5.6 software.
- ✓ Molecules with good Docking score (lower binding energy) and favourable interactions were checked for druglikeness and toxicity.
- ✓ The selected molecules were subjected to Toxicity prediction assessment by OSIRIS® software
- ✓ Compounds with good activity, druglikeness and absence of significant toxicity were shortlisted for synthesis.
- ✓ Compounds were synthesized by conventional method and labelled as SC, SJ, SM, SN, SE
- ✓ Purity of the synthesized compounds was ensured by repeated recrystallization with Methanol.
- ✓ Further the compounds were evaluated by TLC and Melting point were determined.
- ✓ The characterization of the synthesized compounds was done using Infra-Red (IR), Nuclear Magnetic Resonance Spectroscopy(^{1}H NMR) and Mass spectrometry (LC-MS).
- ✓ The pure compounds were screened for *In-vitro* Anti- tubercular activity by Micro plate Alamar Blue Assay (MABA). All compounds showed good anti-mycobacterial activity.
- ✓ The synthesized compounds were active at concentrations of 1.6-25.0µg/ml, which were comparable to the known anti-TB drugs: Pyrazinamide-3.125µg/ml, Ciprofloxacin - 3.125µg/ml and Streptomycin - 6.25µg/ml.

CONCLUSION

- ✓ The work concludes that the synthesized molecules are effective in inhibiting the target enzyme InhA , which is important for the growth of *Mycobacterium tuberculosis* cell wall.

- ✓ All the five compounds gave Docking score between -6.8 to -8.38 kcal/mol.

- ✓ There is a correlation between the docking score and actual activities of all the compounds which were tested and compared with the standard drugs. This goes to prove that *2h9i* is a critical enzyme for anti-mycobacterial activity.

- ✓ Further structural refinement to the structure of the synthesized compounds is expected to yield promising molecules against the pathogen *Mycobacterium tuberculosis.*

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SRI RAMACHANDRA UNIVERSITY
(Declared under section 3 of the UGC Act, 1956)
Accredited by NAAC with 'A' Grade
Porur, Chennai - 600 116.

in association with
Society for Ethnopharmacology, Chennai Chapter

CERTIFICATE

This is to certify that Dr./Mr./Ms. K. Sangeetha .....

has participated as a Resource person / Delegate in the Pre conference workshop on ***"Recent Advances in Analytical Techniques - Drugs & Pharmaceuticals"*** *held on 27th June 2017, organized by Sri Ramachandra University, Porur, Chennai.*

This carries 2 Credits.

D.Chand
Dr. D. CHAMUNDEESWARI
Principal &
Co-ordinator, SFE - Chennai Chapter

Dr. K.V. SOMASUNDARAM
Dean of Faculties

DEDICATED TO

THE ALMIGHTY AND

MY DAD & MOM

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