"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL BENZIMIDAZOLE (SCHIFF' BASE DERIVATIVES AS ANTI-TUBERCULAR AGENTS AGAINST INHA"

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
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In partial fulfilment of the requirements for the award of degree of

MASTER OF PHARMACY

Submitted by

J.ROBERT DILTON

Reg No: 261615706

Under the guidance of

Dr. A. JERAD SURESH M. Pharm., Ph.D., M.B.A

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COLLEGE OF PHARMACY
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CHENNAI-03

MAY 2018

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CERTIFICATE

This is to certify that the dissertation entitled ***"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL BENZIMIDAZOLE (SCHIFF'S BASE) DERIVATIVES AS ANTITUBERCULAR AGENTS AGAINST INHA"*** ***submitted*** *by* ***J.ROBERT DILTON*** *bearing the* ***Reg. No. 261615706*** *in partial fulfilment of the requirements For the award of the degree of* ***MASTER OF PHARMACY in PHARMACEUTICAL CHEMISTRY*** *by* ***The Tamilnadu Dr. M.G.R Medical University*** *is a bonafide work done by him during the academic year 2017-2018 at the* ***Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-3****.*

Dr. A. JERAD SURESH, M.Pharm., Ph.D., M.B.A.,
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Acknowledgement

"Gratitude makes sense of our past, brings peace for today and creates a vision for tomorrow"

Foremost, I want to offer this endeavor to our GOD ALMIGHTY for the wisdom he bestowed upon me, the strength, peace of my mind and good health in order to finish this research.

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CONTENTS

LIST OF ABBREVIATIONS

TB	Tubercule Bacilli
WHO	World Health Organization
DOTS	Directly Observed Treatment Schedule
LC-MS	Liquid Chromatography and mass spectroscopy
GC-MS	Gas Chromatography and Mass Spectroscopy
MDR-TB	Multi Drug Resistant –TB
XDR- TB	Extensively Drug Resistant –TB
CADD	Computer Aided Drug Design
SAR	Structure Activity Relationship
QSAR	Quantitative Structure Activity Relationship
ADME	Adsorption, Distribution, Metabolism and Excretion
PSA	Polar Surface Area
OSIRIS	Optical, Spectroscopic and Infrared Remote Imaging System
SCORE	Docking Score
TPSA	Total Polar Surface Area
Log P	Partition Coefficient
MIC	Minimum Inhibitory Concentration
PDB	Protein Data Bank
TLC	Thin Layer Chromatography
IR	Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
MABA	Micro plate Alamar Blue Assay
NRA	Nitrate Reductase Assay

Introduction

INTRODUCTION

TUBERCULOSIS

Tuberculosis is a common and an infectious disease which is caused by the organisms *"Mycobacterium tuberculosis" (M. tuberculosis)* .[1] Tuberculosis (TB) is the second major cause of death due to an infectious disease in adults worldwide with nine million new cases and close to 1.8 million deaths annually . TB is caused by Mycobacterium tuberculosis, an airborne pathogen transmitted among humans which infects macrophages in the lungs.[2]

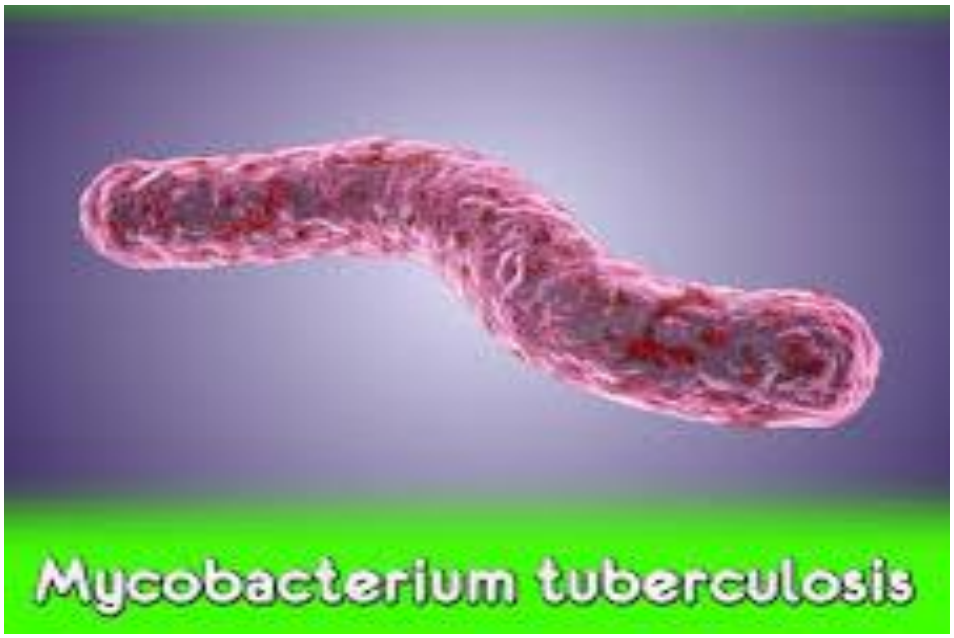

Figure 1[3] :*Mycobacterium tuberculosis*

BACKGROUND

TB was first discovered by Robert Koch in 1882. Robert Koch received the Nobel Prize in physiology or medicine for this discovery in 1905; The Bacterium is also known as "Koch's Bacillus".[6] Tuberculosis is the second deadliest disease (first HIV/AIDS). In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. In 2006, WHO launched the new stop TB strategy. The core of this strategy is DOTS (directly observed treatment schedule).The strategy is to be implemented over 10 years, as described in the global plan to stop TB 2006-2015.[4]

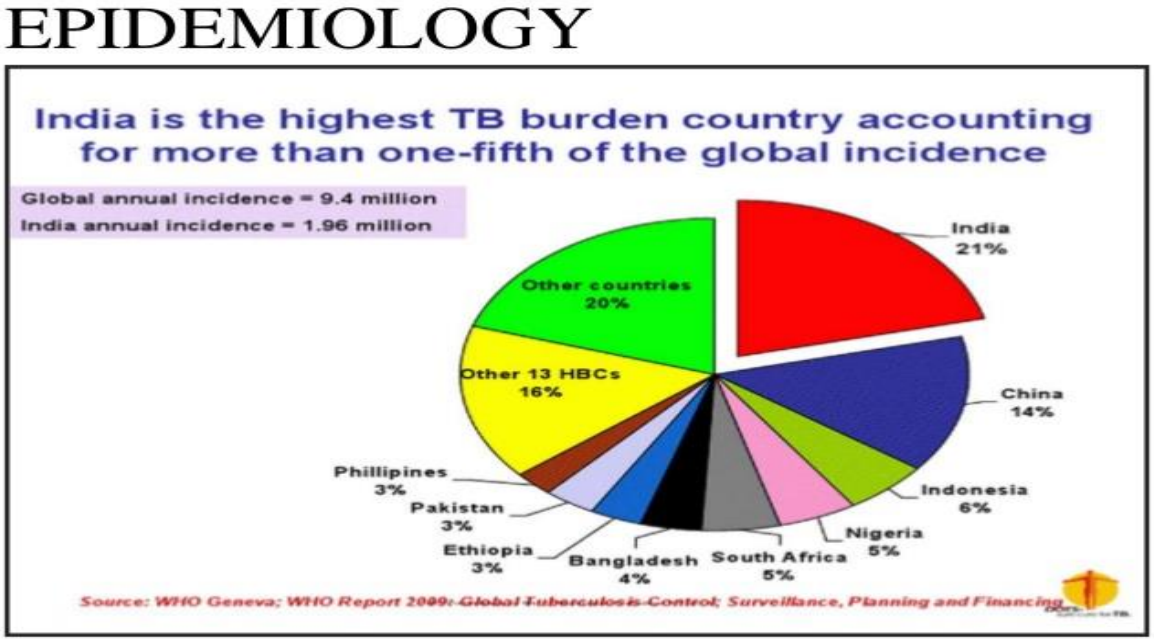

Figure 2[5] :*Epidemiology of Mycobacterium tuberculosis*

HISTORY

The timeline in the history of tuberculosis is depicted below.

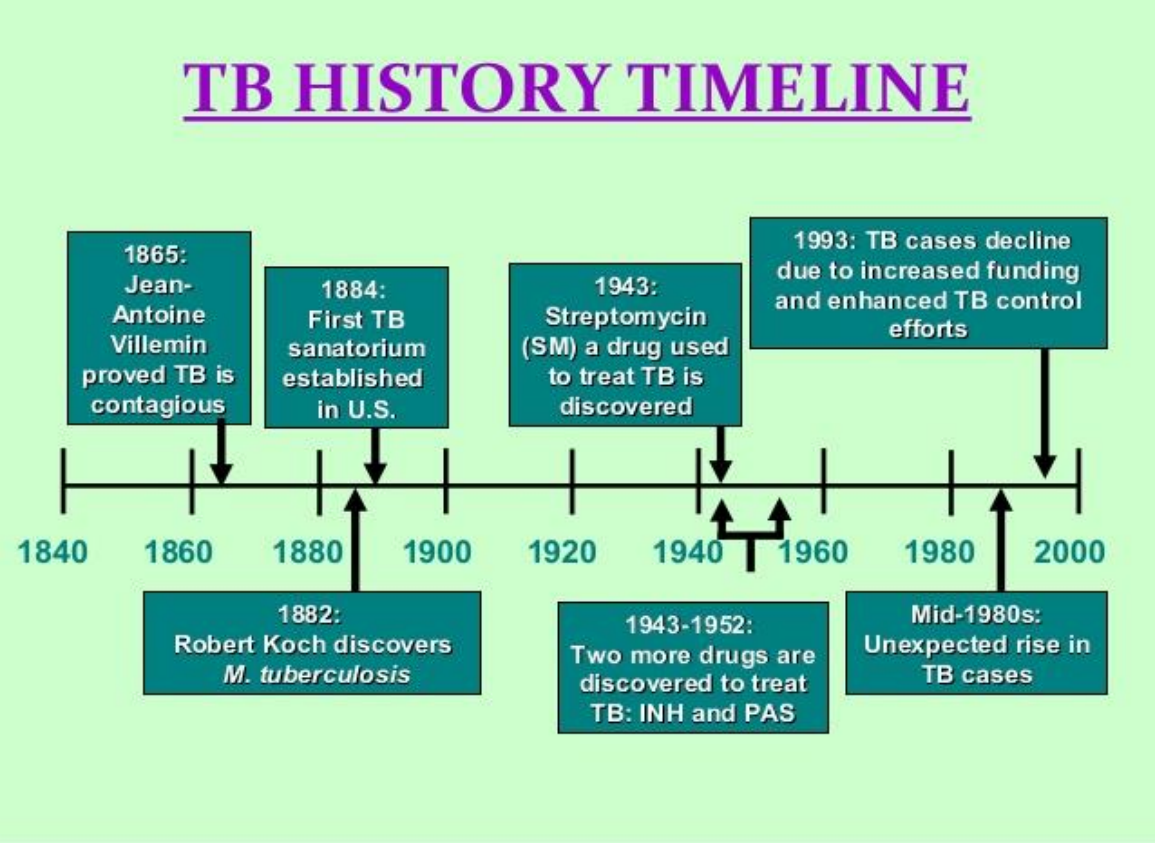

Figure3[7] : History of TB

MYCOBACTERIA

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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.[9]

SCIENTIFIC CLASSIFICATION [9]

Kingdom : Bacteria.

Phylum : Actinobacteria.

Class : Actinobacteria.

Order : Actinomycetales.

Suborder : Corynebacterineae.

Family : Mycobacteriaceae.

Genus : Mycobacterium.

Species : Mycobacterium tuberculosis.

Synonym : Tubercle bacillus Koch 1882**.**

Mycobacterium Tuberculosis is the rod-shaped, spore forming aerobic bacterium.[8]Mycobacterium tuberculosis has an unusual, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which makes the cell impervious to gram staining.

CELL WALL STRUCTURE

The cell wall structure of Mycobacterium tuberculosis deserves special attention because it is unique among prokaryotes, and it is major determinant of virulence for the bacterium.[10] Mycobacterium Tuberculosis has a tough cell wall that prevents passage of nutrients into and excreted from the cell, therefore giving it the characteristic of slow growth rate[11] .The cell wall complex contains **peptidoglycan**, but otherwise it is composed of complex lipids. The lipid fraction of MTB's cell wall consist of three major components, mycolic acids, cord factor, and waxD.[10]

The cell wall contains three classes of Mycolic acids: Alpha, keto, and methoxymycolates.[11]Mycolic acids are unique alpha branched lipids found in cell walls of mycobacterium and corynebacterium. The cell wall also contains lipid complexes including acyl glycolipids and sulfolipids. Beneath the cell wall there are layer of arabinogalacton and peptidoglycan that lie just above the plasma membrane.

The **Cordfactor**is toxic to mammalian cells and is also an inhibitor of polymorphonuclear leukocytes(PMN) migration.**Freund's adjuvant** is a solution of antigen emulsified in mineral oil and used as an immunopotentiator . The **complete form**, Freund's Complete Adjuvant is composed of inactivated and dried mycobacteria , whereas the **incomplete form** lacks the mycobacterial components **Wax D** in the cell envelopes the major component of Freund's completeadjuant(CFA).[10]

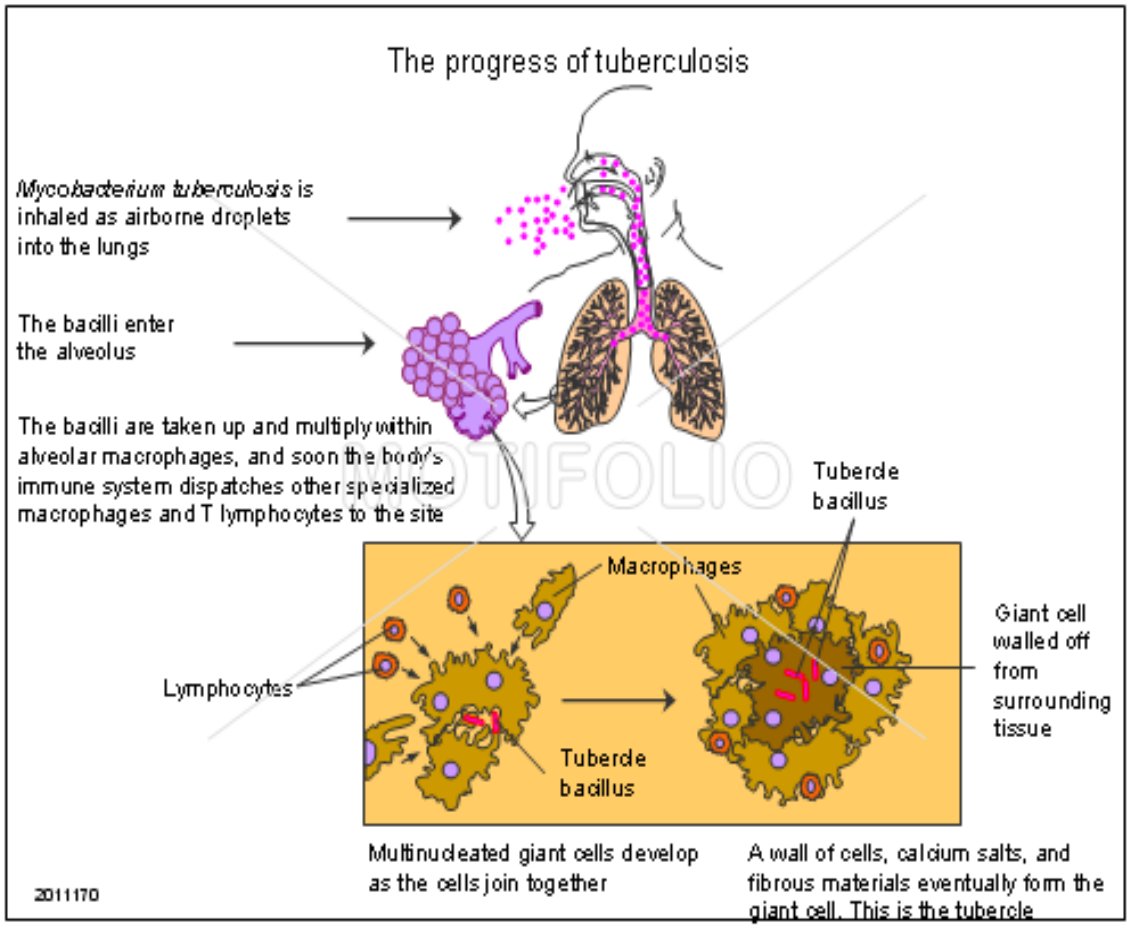


Figure 4[12] :*Mycobacterium tuberculosis cell wall structure*

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.[13]

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.[14]

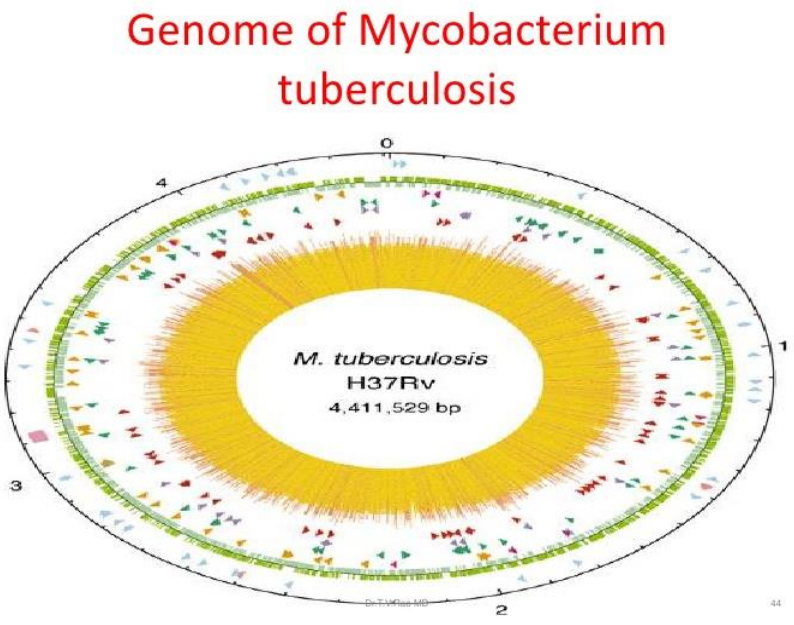


Figure 5[15]: ***Genome of Mycobacterium tuberculosis***

MODE OF TRANSMISSION

TB is usually spread through cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0µm in diameter. A single sneeze can release up to 40,000 droplets. They transmit the disease, since the infectious dose of tuberculosis is very small. [6]

PATHO PHYSIOLOGY

Mycobacterium tuberculosis is classified ***as acid- fast gram- positive*** bacteria due to their lack of an outer cell membrane. [10] It divides every 15-20 hours. Which is extremely slow compared to other bacteria, it is a small bacilli that can withstand weak disinfectants and can survive in a dry state for weeks.[13]

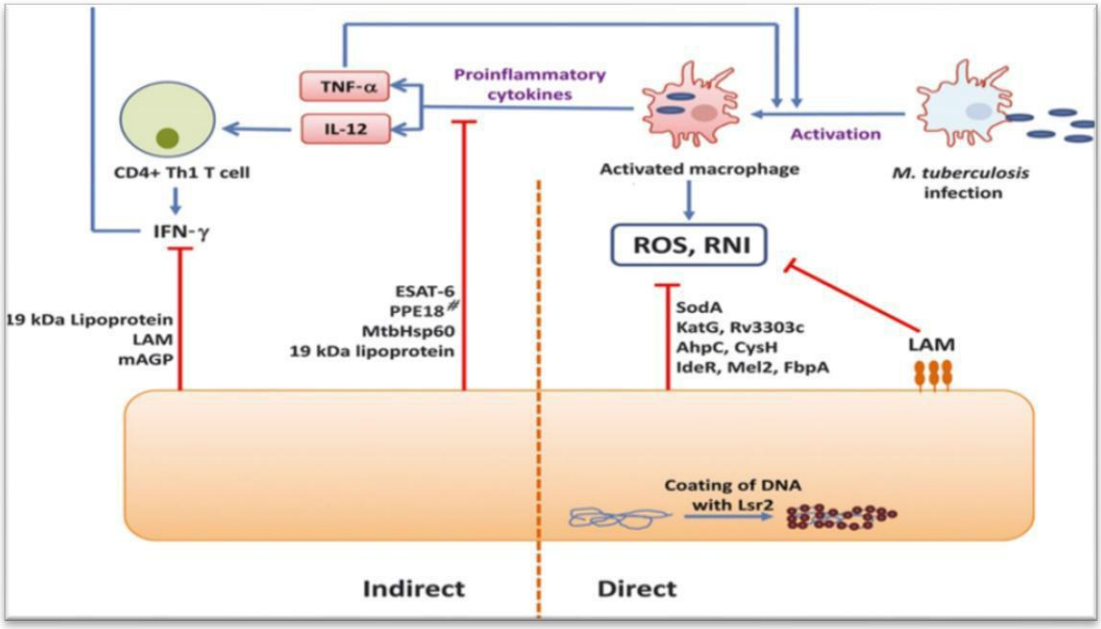


Figure 6[16] : ***Pathophysiology of Mycobacterium tuberculosis***

DRUG -RESISTANT TB

Drug resistant TB disease can develop in two different ways called primary and secondary resistance. Primary resistance occur in person who are initially exposed to and infected with resistant organism. Secondary resistance or acquired resistance develops during TB therapy either because the patient was treated with an inadequate regimen or did not take the prescribed regimen appropriately or because of other conditions such as drug mal absorption or drug-drug interactions that led to low serum levels.

MDR TB is caused by organism resistant to both isoniazid and rifampicin which are the most effective anti Tb drugs.

XDR TB is a relatively rare type of drug resistant TB, which is resistant to both isoniazid and rifampicin, plus any other fluoroquinolone and at least one of three injectable second line (i.e. amikacin, kanamycin, or capreomycin). [14]

Need for new anti-TB drugs

- To improve the treatment of MDR-TB.[15]
- To improve current treatment by shortening the total duration of the treatment.
- The recent rise in TB cases and especially the increase of drug resistant mycobacteria indicate an urgent need to develop new anti-TB drugs.
- There is a need to design new drugs that are more active against slowly growing and non growing persistent bacilli.
- Discovery of compound that would reduce both the length of treatment and the frequency of drug administration.[16]
- To provide more effective treatment for latent tuberculosis infection.

- New drug to improve current that would reduce both the total length of treatment and the frequency of drug administration.

ENZYME PROFILE:

ENZYME NAME	**: ENOYL-ACP REDUCTASE[17]**
CLASSIFICATION	**: OXIDO REDUCTASE**
POLYMER	**: 1**
TYPE	**: Protein**
CHAINS	**: A, B**
ORGANISM	**: Mycobacterium tuberculosis**
PROTEOME	**: Chromosome**
FUNCTIONAL CATEGORY	**: Type II fatty acid biosynthesis pathway**

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 pm. [18]

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.[19]

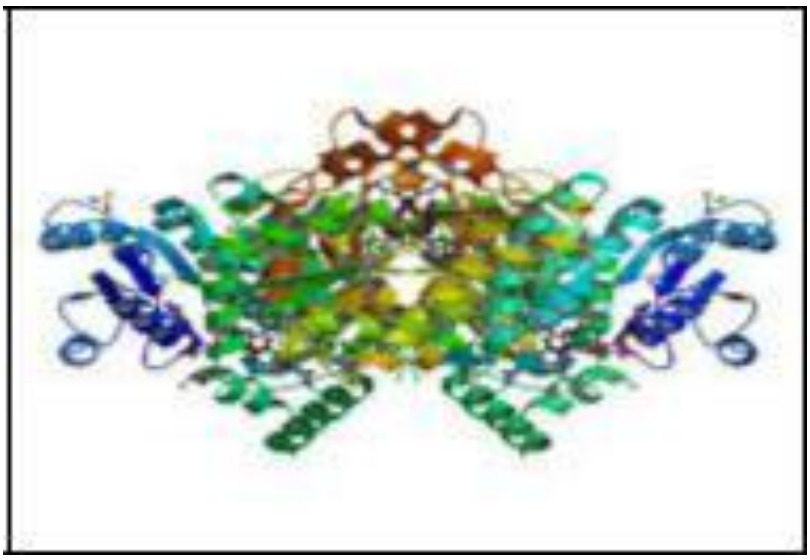


Figure 7[20]*: Crystal structure of Inh A (enoyl ACP) R*

BASIC NUCLEUS INTRODUCTION

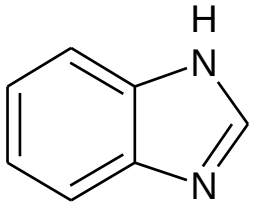
Heterocyclic structures always are a part in the field of research and development in organic chemistry. Millions of heterocyclic structures are found to exist having special properties and biological important.

Benzimidazole could be a heterocyclic aromatic chemical compound. It's a crucial pharmacophore and privileged structure in medicative chemistry. It plays a awfully vital role with lots of helpful therapeutic activities such as: antiulcers, antihypertensives, analgesic,medication, antivirals, antifungals and anticancers

The review of the literature shows that the benzimidazole derivatives square measure effective compound and range of reviews on the market for organic chemistry and medical specialty studies conformed that their molecules square measure helpful against a good style of micro-organisms. Due to their importance. [21]

Benzimidazole derivatives exhibit pharmacological activities such as antimicrobial, antiviral, anticancer, anti-inflammatory, analgesic, etc. The substituted benzimidazoles are summarized in this review to know about the chemistry as well as pharmacological activities.

The benzo derivative of imidazole is refered to as benzimidazole . Although benzimidazole is the commonest name of the parent compound of the series, other names such as benzimidazole and 1,3-benzodiazole (1) are often used.



Benzimidazole derivative are associated with various types of pharmacokinetic and pharmaco dynamic properties. Benzimidazole nucleus is one of the bioactive heterocyclic compounds that exhibit a range of biological activities .Specifically, this nucleus is a constituent of vitamin B_{12} .The pharmacological activities of the benzimidazole containing moiety have been well documented .Albendazole, Mebendazole and Thiabendazole are widely used as anthelmintic drugs.[22]

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. The development cost will be cut by almost third. The development times are reduced.[23]

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 technique of refining 3D structures of drug molecules and promoting the binding of drug to protein active sites. In this technique modification of a structureof the 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.[24] 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.[23] 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

- Structure based drug design
- 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 modelling.

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 to the biological target of interest. 3D quantitative structure activityrelationship (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.[25]

COMPUTER AIDED DRUG DESIGN

Computer aided drug design use as computational chemistry to discover, enhance or study drugs and related biologically active molecules. Molecular mechanics or molecular dynamics are most often used to predict the confirmation of the small molecule and to model conformational changes in the biological target but may occur when the small molecules binds to it.

Molecular mechanics methods may also be used to provide semi quantitative prediction of the binding affinity also knowledge based scoring function may be used to provide binding affinity estimates.[26]

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

- **Hit identification** using virtual screening (structure or ligand based-baseddesign)
- **Hit-to-lead optimization** of affinity and selectivity (structure based design,QSAR, etc)
- **Lead optimization**, optimization of other pharmaceutical properties whilemaintaining affinity.

In order to overcome the insufficient prediction of binding affinity calculated by resent scoring functions, the protein-ligand interaction and compound 3D structure are used to analysis [27]

AIM AND PLAN OF WORK

AIM AND OBJECTIVE

AIM:

To develop the novel and potent anti-tubercular agents

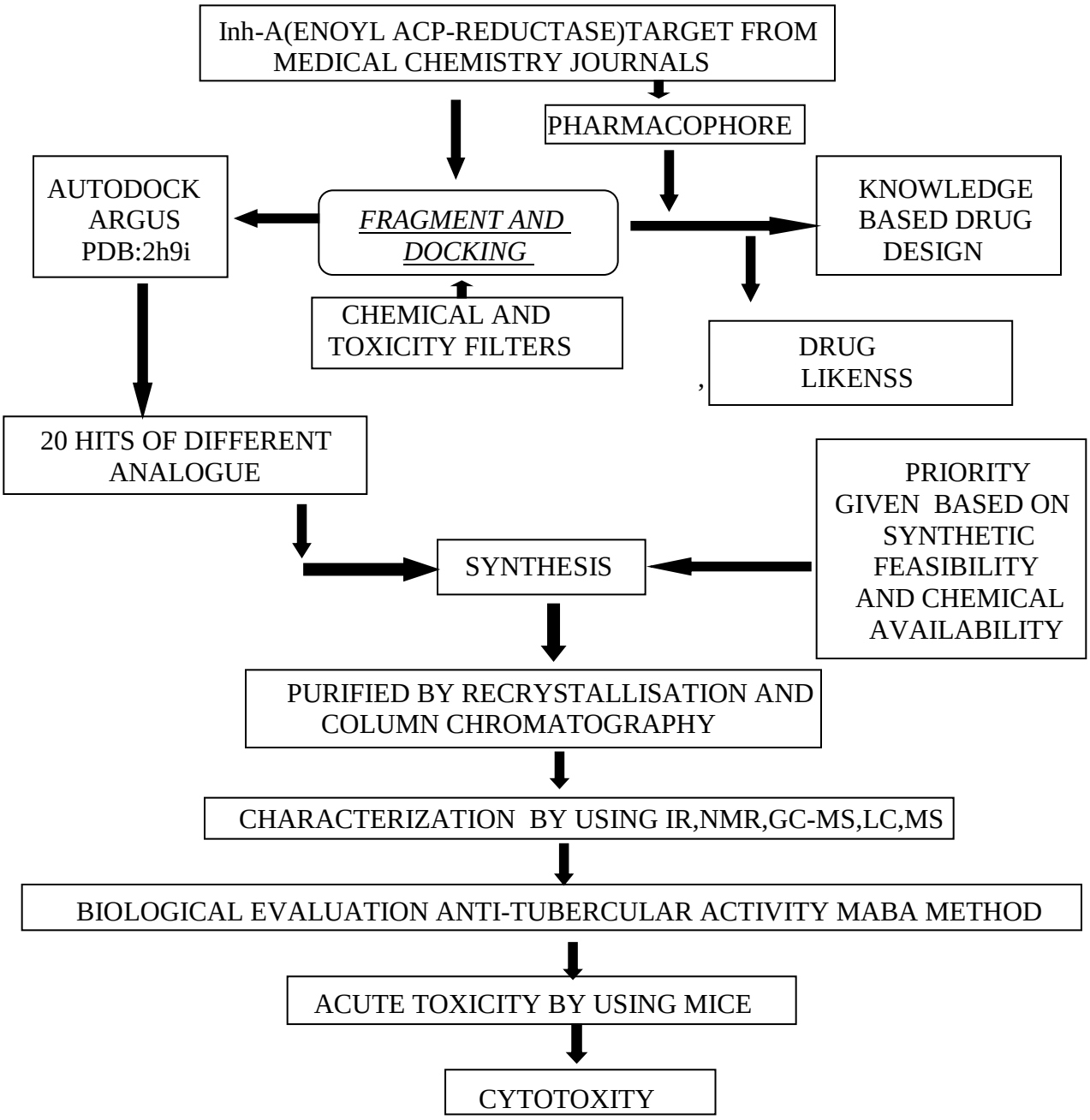
OBJECTIVE:

The plan of work includes:

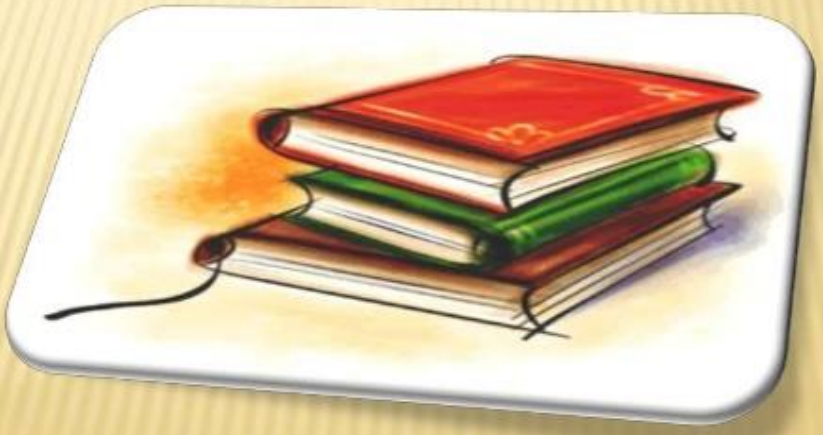
- Design of InhA (enoyl-ACP reductase) inhibitors by docking studies using Argus lab 4.0® software.
- Insilico Drug likeness prediction.
- Insilico Toxicity Assessment.
- Laboratory synthesis of those compounds with top Docking Scores.
- Characterization of the synthesized compounds by
  - Infrared Spectroscopy.
  - H1 Nuclear Magnetic Resonance Spectroscopy.
  - Melting point.
  - GC-Mass Spectroscopy.
  - LC-Mass Spectroscopy.
- In-vitro anti -tubercular activity of synthesized compounds (MABA).

PLAN OF WORK

The present study will be carried out based on the flow chart given below:



LITERATURE REVIEW



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[28]
2. **Frieden TR, GR *et al.,***(2003) has shown that "Impact of national consultants on successful expansion of effective tuberculosis control in India.[29]
3. **Williams, B.G *et al.,* (**2010) studied the "The Population Dynamics and Control of Tuberculosis**.**[30]
4. **Leimane V**,***et al***., (2005) described the "Clinical outcome of individualized treatment of multidrug-resistant tuberculosis [31]
5. **Balabanova Y**,***et al.,* (**2006) made a report about "The Directly Observed Therapy Short-Course (DOTS) strategy"[32]
6. **Keane, J, *et al.,***(1997) explained that "Mycobacterium Tuberculosis promotes Human alveolar macrophage apoptosis, Infection and immunity."[33]
7. **Lonnroth K, *et al.,***(2006) has studied the Productive engagement of private providers in tuberculosis control. [34]
8. **Duncan k, *et al.,*** (2004) developed, "Prospects for New Anti-Tubercular drugs.[35]

9. **Pierpalo de colombai., *et al.,***(2007) has described The Global Plan to Stop TB.[36]

10. **Ruohonen RP, *et al.,*** (2002) performed the "Implementation of the DOTS strategy for tuberculosis.[37]

11. **Bumburidi E, *et al.,*** (2006) explained the Disease Control and Prevention (CDC)in this article, "Progress toward tuberculosis control and determinants of treatment outcomes.[38]

12. **M, Coker RJ *et al.,*** (2007) studied the Reform of tuberculosis control and DOTS within public health systems.[39]

Literature review for drug design :

13. **Alfred burger, *et al.,***(1984) Textbook of guide to chemical basis of drug design(John Wiley& Sons).[44]

14. **Edward.C.Olson**,***et al.,***(1979)Textbook of Computer assisted Drug Design(American Chemical Society).[45]

15. **Wilson & Giswold's *et al.,***(2011) Text book of Organic, Medicinal and Pharmaceutical chemistry[46]

16. **Romono T. Kroemeret *et al..,***(2003)**"**An introduction into ligand–receptor docking".[47]

17. **Lipinski CA *et al.,*** (2001) developed an experimental and computational approach to estimate solubility and permeability in drug discovery and development settings.[48]

18. **Madsen *et al.,*** (2002) Textbook of Drug Design and Discovery.[49]

The Review of Literature related to the desired chemical entities*:*

19. **Anu Kajal et al.** reported Schiff base a versatile pharmacophore. Schiff bases are condensation product of primary amine with carbonyl compound gaining important 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**. [50]**

20. **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**.[51]**

21. **Angelo De Fathima et al**. Schiff base: A short reviews of the antimicrobialactivities 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**.[52]**

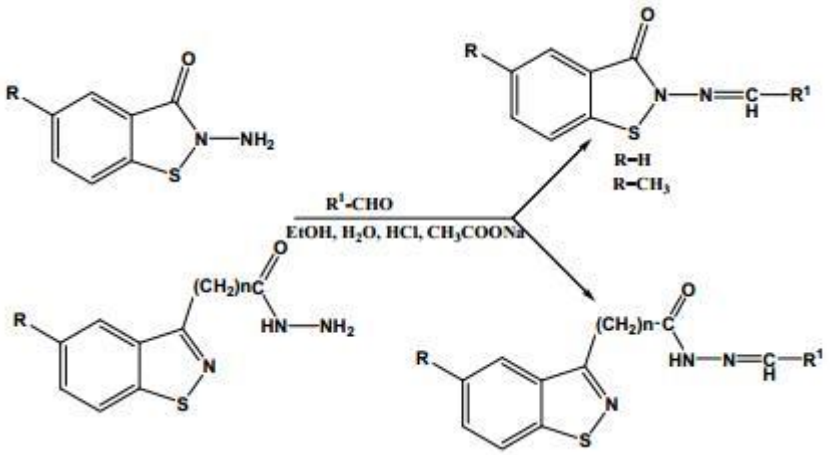
22. **S.K. Ghosh et al. Synthesis of Schiff base in aqueous medium** a green alternative approaches with effective mass yield and high reaction rates. Schiff base constitute a class of pharmaceutical and medicinally important molecules. Various **S**chiff bases by stirring 1,2 diamino benzene with various aromatic aldehydes in water as solvent**.[53]**

23. **Ina Boltz et al**. Novel Schiff bases derived from 5 amino barbituric acid: synthesis and a solid state structure for this purpose 1,3 and dimethyl and 1butyl 5amino butyric acid are condensed with p-nitro and p-N,N dimethyl amino cinnamaldehyde respectively**.[54]**

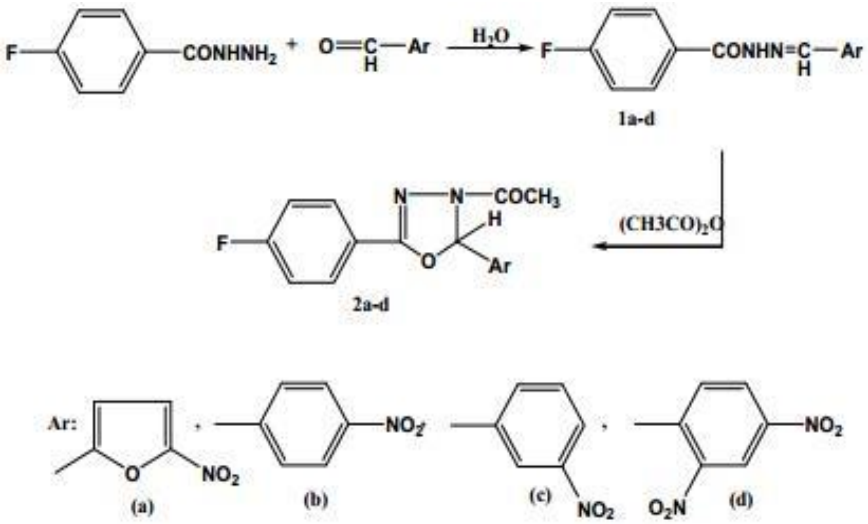
24. **Shelar Mahendra Devidas et al**. Novel One–pot synthesis of schiff basecompounds derived from different diamine & aromatic aldehydes catalyzaed by P2O5/Sio2 Under free solvent condition at room temperature. A potential method for one-pot synthesis of schiff base compounds derived from different aldehyde and diamine by using catalyst P2O5/Sio2. **[55]**

25. **Hamid Latif Siddiqui et al.** synthesis of spectroscopic studies of new Schiffbases. Five novel Schiff bases have been prepared from o-formyl phenoxyacetic acid and a ries of amino thiazoles to form a number of potentially biologically active compounds.**[56]**

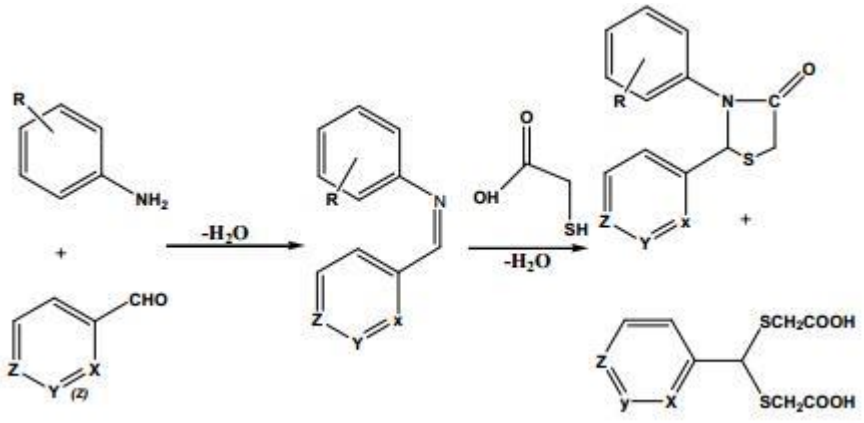
26. 1.2-benzisothi- azole hydrazides as well as their cyclic and acyclic 1.2-benzisothiazole parent hydrazides.**[57]**



27. Schiff bases by reacting 5-chloro-licylaldehyde and primary amines. The compounds were assayed for antibacterial (Bacillus subtilis, Esh- erichia coli, Pseudomonas fluorescence and Staphylo- coccus aureus). **[58]**

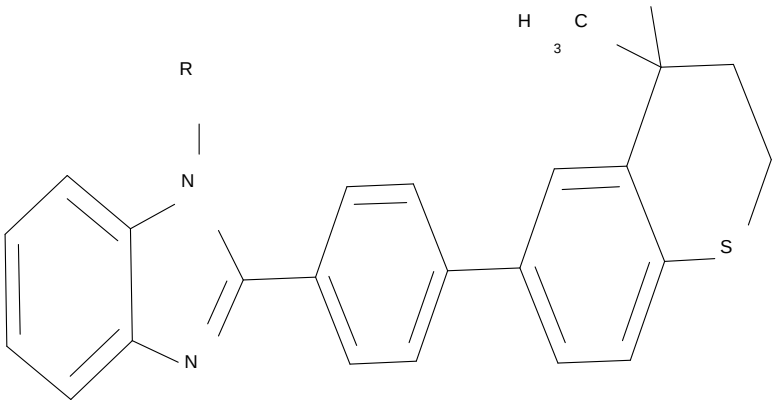


28. Aromatic Schiff bases and 2,3-di- ia and co-workers have reported the synthesis two neryl-1,3-thiazolidin-4-one (Scheme 11) derivatives have been prepared and tested for anti-inflammatory and antinociceptive activities. **[59]**

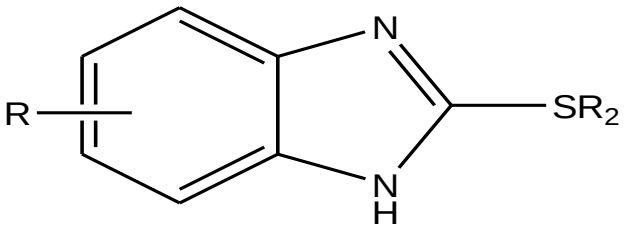


Literature review for benzimidazole :

29. **Kumar *et al*** reported a series of novel and functionalized benzimidazole derivatives shown anti-diabetic activity against DPP-IV and PTP-IB.[60]

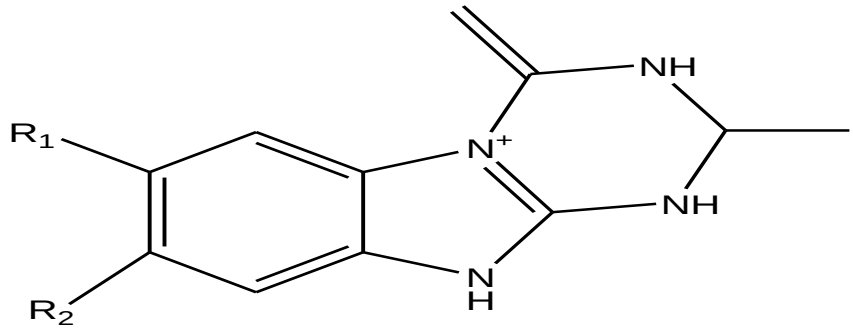


30. **Kazimierczuk *et al*** reported Synthesis of substituted 2-polyfluroalkyl and 2-nitrobenzyl sufanyl benzimiazole Compounds were evaluated for their activity against mycobacterium strains.[61]

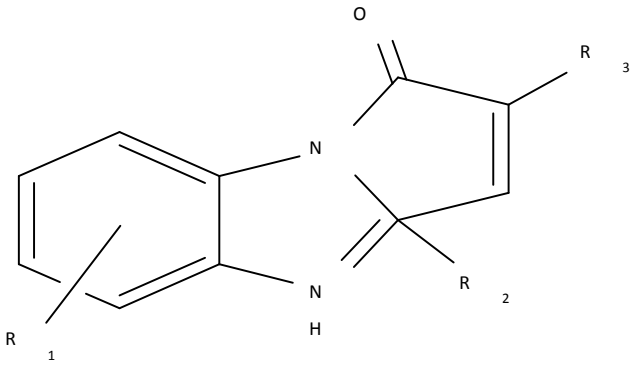


R1=Cl,Br
R2=methy nitro benzene

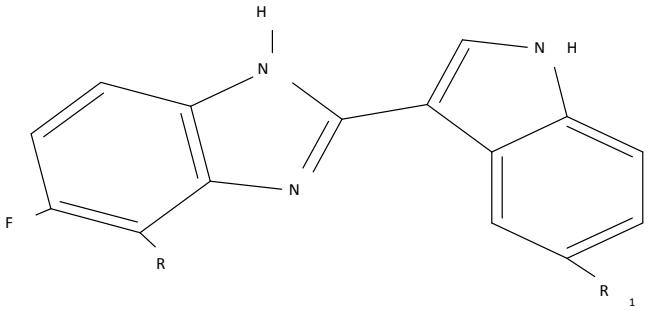
31. **Sondhi *etal*·** reported Synthesis of pyrimido [1,6-*a*] benzimidazole derivatives **[62]**



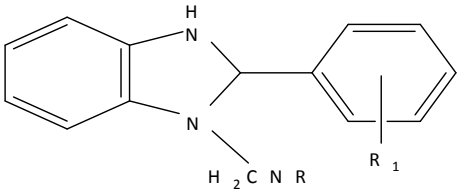
32. **Chimrri *et al*** reported this synthesis of novel 1*H*-pyrrolo (1,2-a)benzimidazole-1-one derivative**.** **[63]**



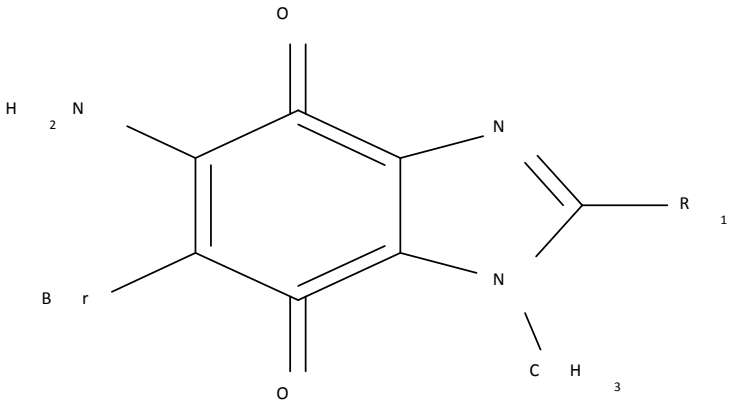
33. **Alagoz *et al*** reported Synthesis of some 6-flouro-5-substituted benzimidazole.[64]



34. **Leonardo *et al***reportedSynthesis and anti-inflammatory activity of phenyl benzimidazole.·[65]



35. **Gellis *et al*** reported some new benzimidazole-4,7-diones substituted at 2-position were synthesized·[66]



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

36. **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 simple, rapid, low cost, appropriate technology which does not require extensive instrumentation and which makes use of a nontoxic, temperature stable reagent. **[67]**

37. **Sephra N Rampresad. et al.** studied the various applications of Alamar Blue asan 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. **[68]**

38. **Jose de Jesus Alba-Romero et al.** applied the Alamar Blue Assay to determinethe susceptibility to anti-tuberculosis pharmaceuticals. **[69]**

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

39. **Luckner, S.R *et al***., (2010) reported that Inh A, the enoyl-ACP reductase inMycobacterium tuberculosis is an attractive target for the development of novel drugs against tuberculosis.[70]

40. **Argyrou, A. *et al*.,** (2007) developed new insight into the mechanism of action ofand resistance to isoniazid: interaction of Mycobacterium tuberculosis enoyl-ACP reductase with INH-NADP.[71]

41. **Dias, M.V. *et al*.,** (2007) studied the crystallographic studies on the binding ofisonicotinyl-NAD adduct to wild-type and isoniazid resistant 2-trans-enoyl-ACP (CoA) reductase from Mycobacterium tuberculosis.[72]

42. **Vilcheze, C.*et al*., (**2006) developed the transfer of a point mutation in Mycobacterium tuberculosis.[73]

43. **Argyrou, A. *et al*.,** (2006) has shown the dihydrofolate reductase frommycobacterium tuberculosis is inhibited by the acyclic 4R isomer of INH-NADP a derivative of the prodrug isoniazid.[74]

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.


MATERIALS &
METHODS

MATERIALS AND METHODS

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

- Drug design by using Argus lab 4.0.
- Synthesis of the designed molecules.
- Characterization of the synthesized molecules.
- Biological evaluation of the synthesized molecule

DRUG DESIGN PROCESS

A) DOCKING STUDY

The synthesized compounds are docked against the target protein by using Argus lab® software. The flow chart of the docking study is presented below

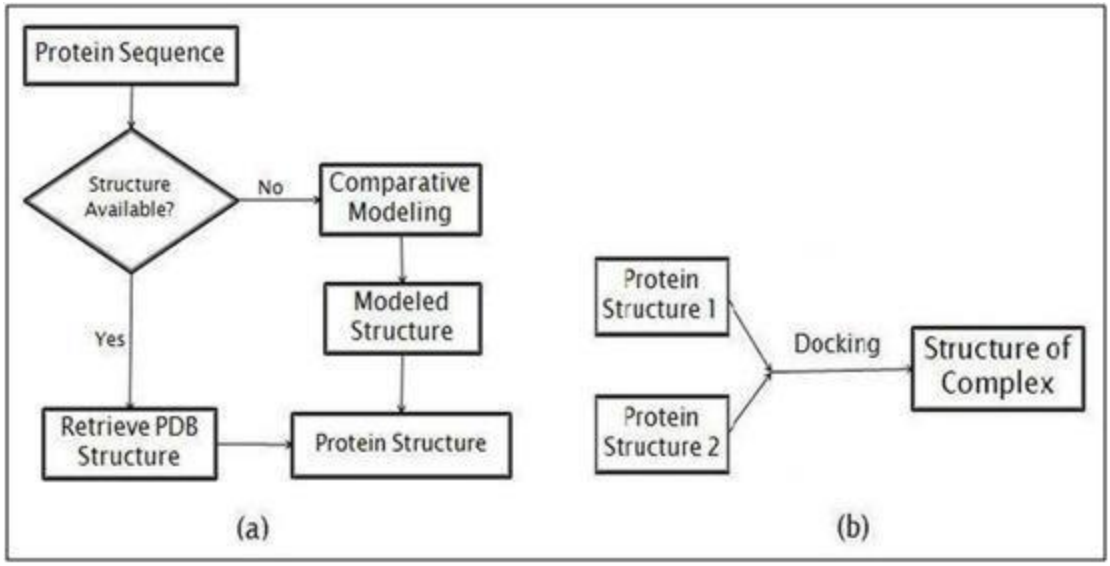


Figure 8[76] : Molecular docking and Drug design

B) **MOLECULAR PROPERTY PREDICTION:**

The designed and docked molecules were screened insilico using MOLINSPIRATION® Software to evaluate the drug likeness. 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 predicition.
- 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 [93]

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 beexperimentally 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 (coctanol/cwater), 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.[77]

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 the desire of every drug forger.

LIPINSKI'S RULE OF FIVE [78]

The rule was formulated by Christopher A.Lipinski in 1997. **Lipinski's ruleof five** also known as the **Pfizer's rule of five** or simply the **Rule of five** (RO5) is to evaluate druglinkness 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**:**

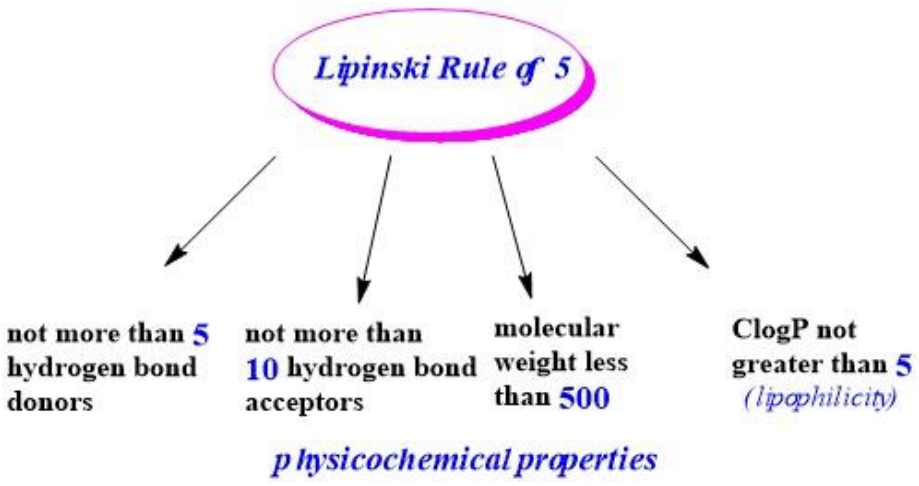


Figure 9[43] : Lipinski rule of five

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. OHs and NHs] and H-bond acceptors [e.g. Ns and Os])

Also the 500 molecular weight cutoff 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 no 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 [79]

ACUTE ORAL TOXICITY STUDY:

Acute oral toxicity study (Limit Test) was designed as per the OECD guidelines(423).

Principles and purpose

The main purpose of acute toxicity is to evaluate the degree of toxicity in a quantitative and qualitative manner.

Experimental Animals

Six healthy adult Albino mice were weighing between 20-25g were selected for the study. For all the six animals food, but not water was withheld overnight prior to dosing.

Selection of dose levels and administration of dose:

Being synthetic molecules, the mortality was unlikely at the highest starting dose level (2000mg/kg/b.w). Hence a limit test one dose levels of 2000mg/kg/b.w was conducted in all animals as per the OECD guidelines (423).

Procedure:

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be

determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

COMPUTER-AIDED DRUG DESIGN [80]

Computer-aided drug design uses computational chemistry 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 that may occur when the small molecule binds to it. Molecular mechanics methods may also be used to provide semi quantitative prediction of the binding affinity. Also, knowledge-based scoring function may be used to provide binding affinity estimates. Drug design with the help of computers may be used at any of the following stages of drug discovery**:**

- **hit identification** using virtual screening (structure or ligand-based design)
- **hit-to-lead** optimization of affinity and selectivity (structure-based design, QSAR, etc.)
- **lead optimization:** optimization of other pharmacokinetic properties while maintaining affinity. In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and a compound‟s 3D structure information are used for analysis.

TYPES OF DRUG DESIGN:[81]

Drug design is carried out by either of the two methods. SBDD is opted when the structure of the target protein is known i.e. established by a PDB ID.

- Ligand based drug design.
- Structure based drug design.

STEPS INVOLVED IN DOCKING [40],[41],[42]

Docking can be carried out using a number of software available for the purpose. GLIDE® (Schrodinger) AUTODOCK® and ARGUS LAB® are a few of them. The steps involved in general are

1. Protein preparation.
2. Selection of active site (Q-Site finder).
3. Ligand Preparation.
4. Docking Procedure.
5. Visualization / Interpretation of Docking.

1. PROTEIN PREPARATION

The various operations of the protein preparation are tabulated below.

Step: 1

- ✓ Enter protein (pdb) ID in the protein data bank. (2X22)
- ✓ Go to download files and select pdb as text file.
- ✓ Save the download pdb (text file) to the desktop.

Step: 2

- ✓ Open Argus lab file – Open Import pdb file from the desktop.
- ✓ 3D Structure of the protein will appear in the workspace of Argus lab.
- ✓ Left side of the screen shows molecular tree view.
- ✓ Open pdb – Open „residues‟ – „Open misc‟
- ✓ From „Misc‟ delete the inhibitor and hetero residues [Note**:** Do not delete Co-factor] Open water press shift, select all water molecules and delete.
- ✓ Add hydrogen atoms.
- ✓ Calculation on the toolbar – energy by UFF method start. Save the prepared protein as *.agi file format in the desktop

2. Q-SITE FINDER

Step: 1

- ✓ Open Q-Site finder through online.
- ✓ Upload / Import the PDB format of the Protein.
- ✓ Find all the active site and make a list out of the common amino acid residues.

Step: 2

- ✓ Open residues – open Amino acids.
- ✓ Press control and select the amino acid which listed from the Q-Site finder.
- ✓ Make sure that all amino acid residues listed are selected.
- ✓ Right click on the mouse – make a group from the selected residues
- ✓ Give name Binding site – Ok

3. LIGAND PREPARATION

The various operations of the ligand preparation are tabulated below

- ✓ Draw the structure from Chemsketch and save as MDL Mol format.
- ✓ Import the ligand into workspace of Argus lab.
- ✓ Clean Geometry – Clean Hybridisation.
- ✓ Select the ligand, Right click on the mouse
- ✓ Make a group from the residues give name ligand – Ok.

4. DOCKING PROCEDURE

The procedure for docking are tabulated below

- ✓ Select the set up a Dock Ligand calculation from the toolbar.
- ✓ Argus Dock as the Docking Engine.
- ✓ Dock was selected as calculation type.
- ✓ Flexible for the scoring function.
- ✓ Calculation size.
- ✓ Start docking.
- ✓ Save the Docked protein Ligand complex as Brookhaven pdb files (*.pdb)

5. VISUALIZATION / INTERPRETATION OF DOCKING

The visualization of docking is tabulated below

- ✓ **Molegro Molecular viewer** helps in analyzing the energies and interaction of the binding.
- ✓ "View Secondary" helps to view the Structure.
- ✓ "View" – Hydrogen bond interaction.
- ✓ "Ligand map" – Interaction overlay.

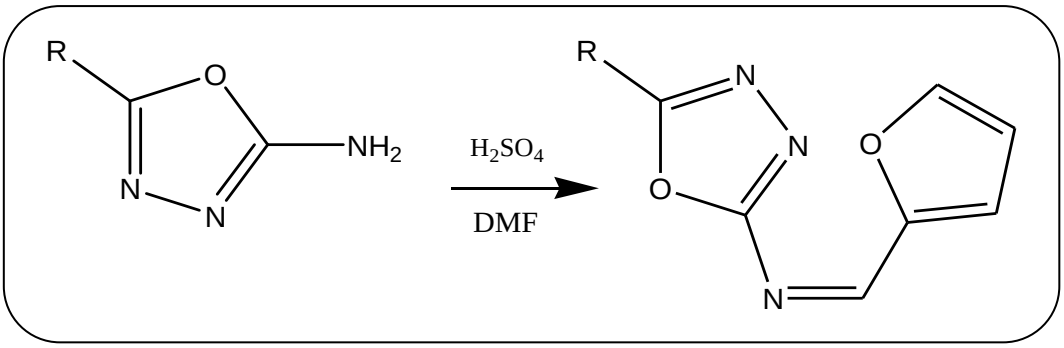
SYNTHETIC METHODOLOGY

The designed and docked compounds are synthesized by the following scheme which 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 [75]

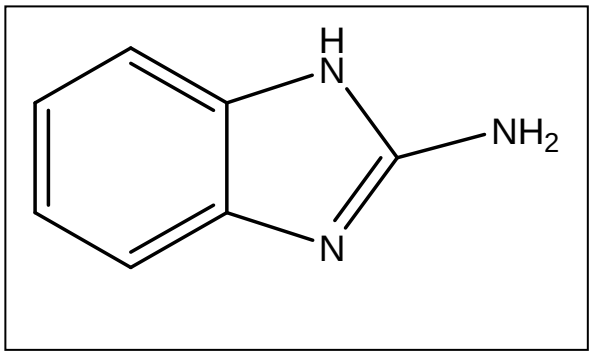


PROCEDURE

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.

REACTANT PROFILE

2-Aminobenzimidazole



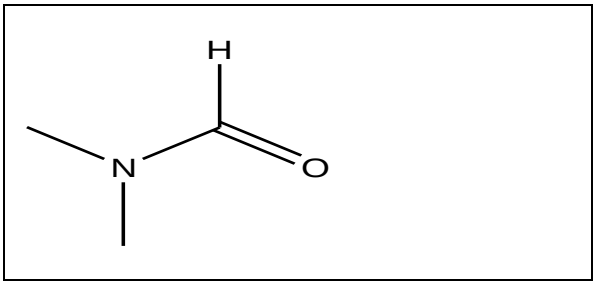
Molecular formula : $C_7H_7N_3$

Molecular weight : 133.154g/mol

Description : white powder.

Melting point : $435^{o}F$

Dimethylformamide



Molecular formula : C_3H_7NO

Molecular weight : 73.10g/mol

Description : Colourless liquid.

Melting point : $61^{o}C$

Boiling point : 154°C

Sulphuric acid

H_2SO_4

Molecular formula : H_2SO_4

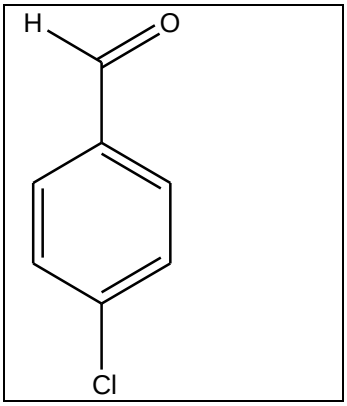
Molecular weight : 98.07g/mol

Description : Colourless liquid.

Melting point : 10^oC

Boiling point : 337°C

P-Chlorobenzaldehyde



Molecular formula: $C_7H_5CLN_3$

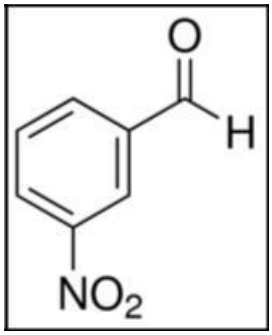
Molecular weight: 140.56g/mol.

Description: White crystalline solid

Melting point: 117°C

Boiling point: 415°F

3-Nitro Benzaldehyde



Molecular formula: C7H5NO3

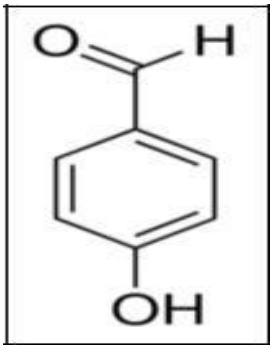
Molecular weight: 151.12g/mol.

Description: Pale yellow powder

Melting point: 43°C

Boiling point: 152°C

P-Hydroxy Benzaldehyde



Molecular formula : C7H6O2

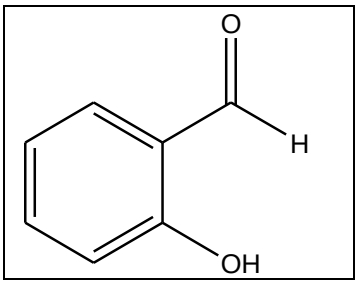
Molecular weight : 122.12g/mol.

Description : Light brown.

Melting point : 112-116°C

Boiling point : 196°C

Salicylaldehyde



Molecular formula : $C_7H_6O_2$

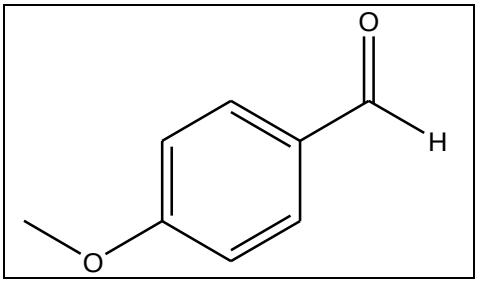
Molecular weight : 122.12g/mol.

Description : Colourless liquid.

Melting point : 7°C

Boiling point : 197°C

Anisaldehyde



Molecular formula: $C_8H_8O_2$

Molecular weight: 136.15g/mol.

Description: SlightlyYellow liquid.

Melting point: 1°C

Boiling point: 248°C

CHARACTERIZATION

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

PHYSICAL EVALUATION:

Physical properties of the synthesized compounds are evaluated, such as

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

Further the synthesized compounds are to be characterized by the following Spectroscopic and Spectometric methods.

IR SPECTROMETRY

Infrared (IR) spectrometry is one of the most common spectroscopic techniques used by organic chemists for detection of functional compounds and mixtures and 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 per cent transmittance versus frequency expressed as wave numbers **[82]**

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 was used as a solvent [83]

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

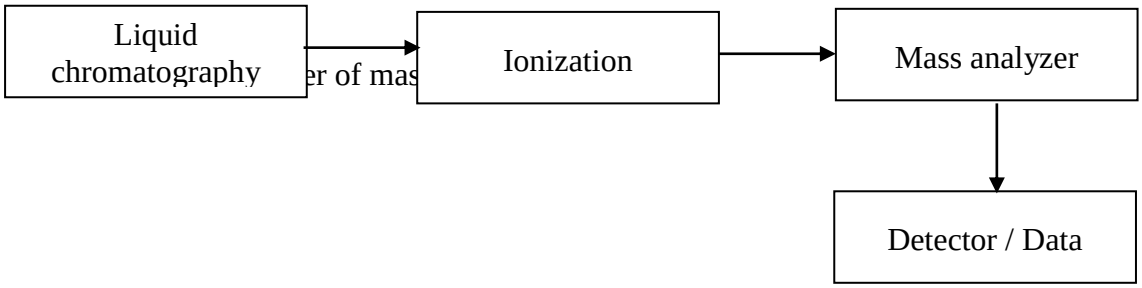
HYPENATED TECHNIQUE:

GC-MS:

Gas chromatography-mass spectrometry is a hyphenated technique, consisting of two analytical procedures in sequence, namely a gas chromatography (GC) separation followed by Mass spectroscopy (MS) detection. The purpose of GC step is to separate multiple compounds in a sample so that they reach the MS detector one at a time.[85]

LC-MS

LC-MS is a hyphenated technique, combining the separation power of HPLC,



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) [86]

- ✓ The anti- mycobacterial activities of the synthesized compounds were determined by MABA method. The organism used in the studies M.tuberculosis H37Rv.
- ✓ Alamar blue dye is used as an indicator for the determination of viable cells.

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^{o}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 is defined as lowest drug concentration which prevents the colour change from blue to pink **[87]**


results
discussion!!
researchpedia.info

RESULTS AND DISCUSSION

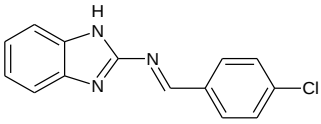
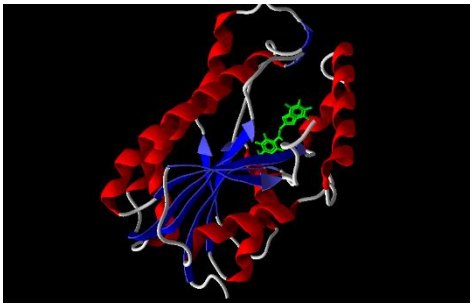
RESULTS OF DRUG DESIGN

PREDICTION FOR ACTIVITY

(INSILICO DRUG DESIGN)

More than 200 compounds were docked against the MTB enzyme Inh A (Enolyl Acyl Carrier Protein) (2h9i) by using Argus lab 4.0.1® software.The compounds with the best docking score and good interaction molecules were selected and screened. The docking score and the view for different compound is presented below.

Table 1: Docking Score And View Using Argus Lab 4.0®

Compound Code	Structure	Docking Score	Docking View
RJ		**-7.83 Kcal/mol**	

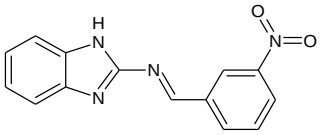
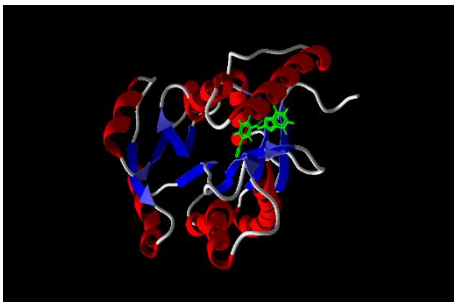
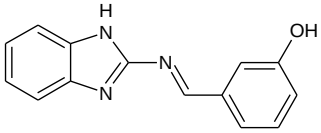
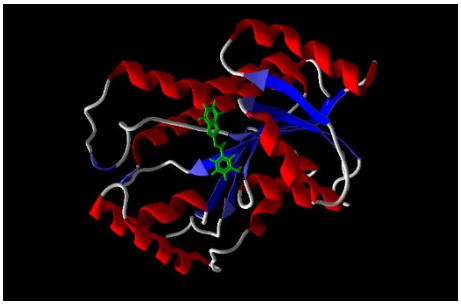
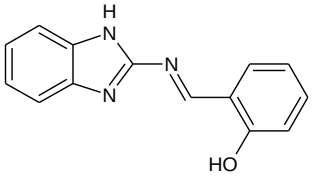
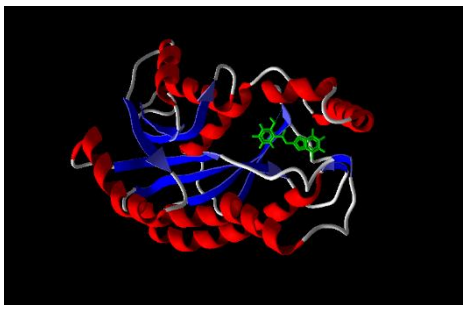
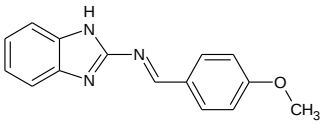
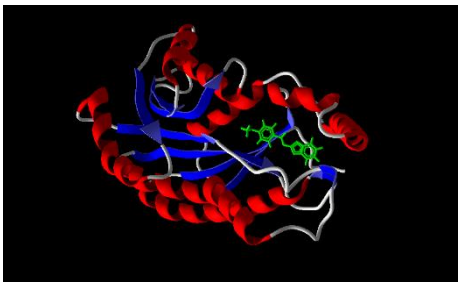
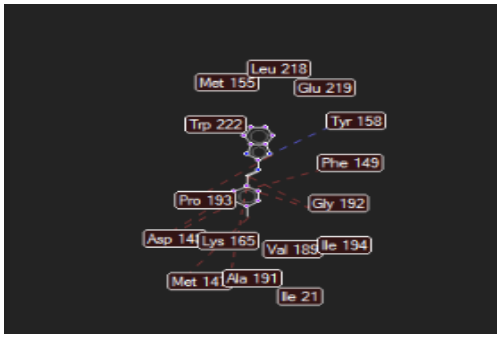
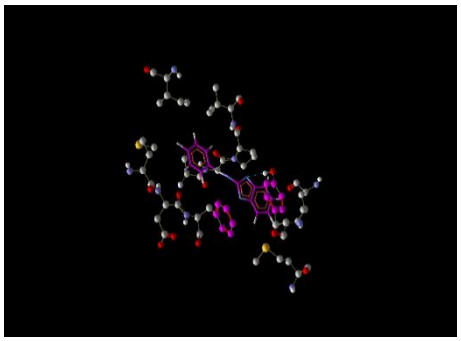
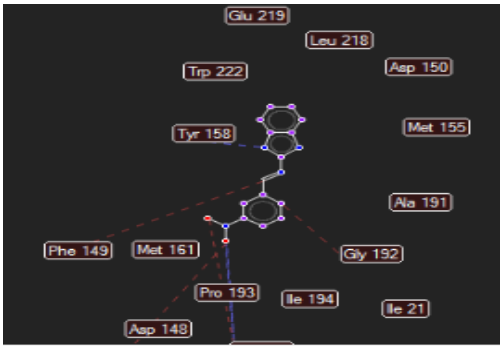
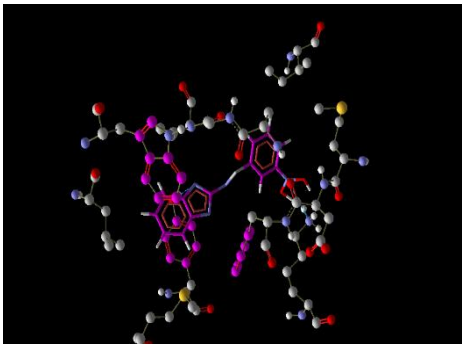
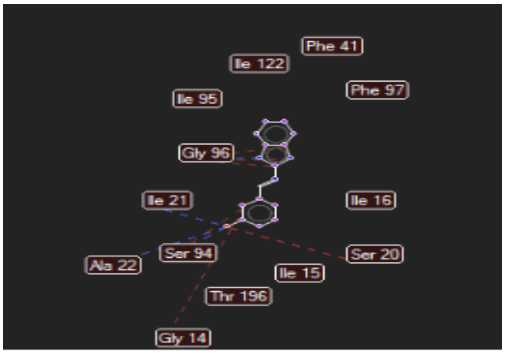
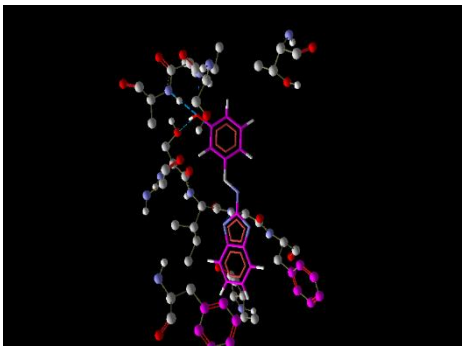
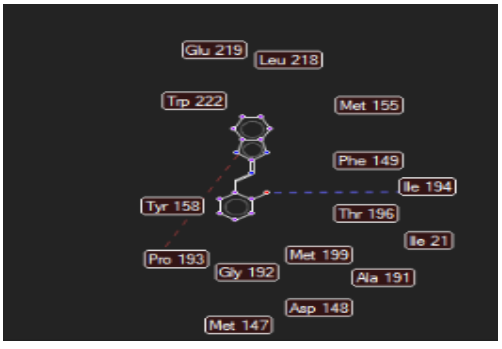
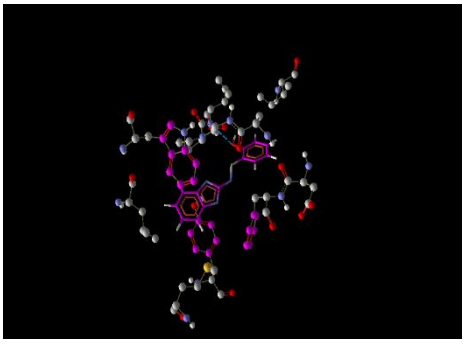
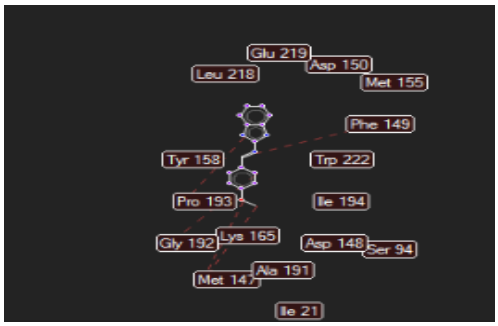
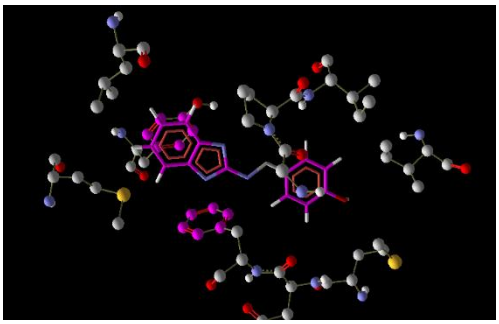
RK		**-8.52 Kcal/mol**	
RM		**-7.35 Kcal/mol**	
RN		**-7.54 Kcal/mol**	
RO		**-7.64 Kcal/mol**	

Table 2 : 2h9i Interaction With Ligand

Compound Code	Interaction with Aminoacid	Hydrogen bond Interaction
RJ		
RK		
RM		
RN		

Compound Code	Interaction with Aminoacid	Hydrogen bond Interaction
RO		

PREDICTION FOR TOXICITY

(INSILICO)

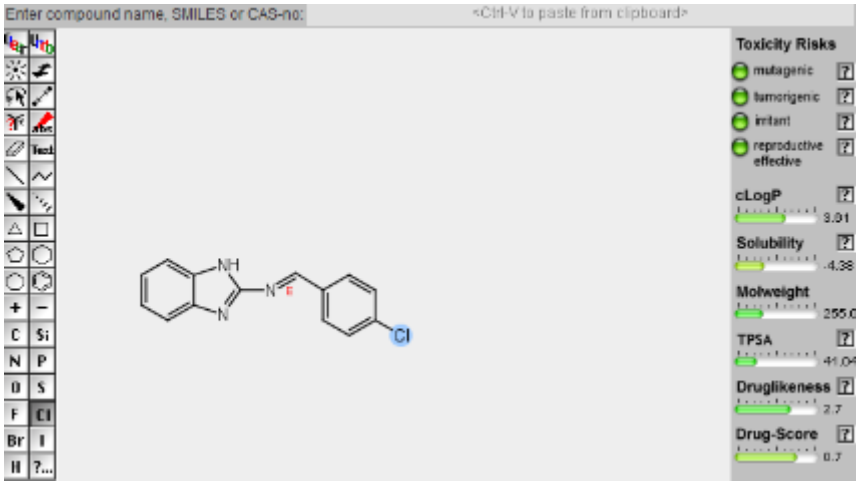
OSIRIS® Property Explorer is the online software of Thomas Sander, Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, and 4123 Allschwil, Switzerland. This applet predicts physico-chemical properties and detects potential toxicity risks for any drawn chemical structure in real time. The prediction result is indicated by color codes. For predicting properties of a chemical compound the structure was drawn and *Property Explorer* starts calculating properties as soon as a chemical structure is valid. Properties with high risks of **undesired effects** like mutagenicity or a poor intestinal absorption are shown in **red.** Whereas **green** colour indicates the **drug conform** behaviour.

TABLE 3 : PREDICTION OF TOXICITY USING OSIRIS® PROPERTY EXPLORER

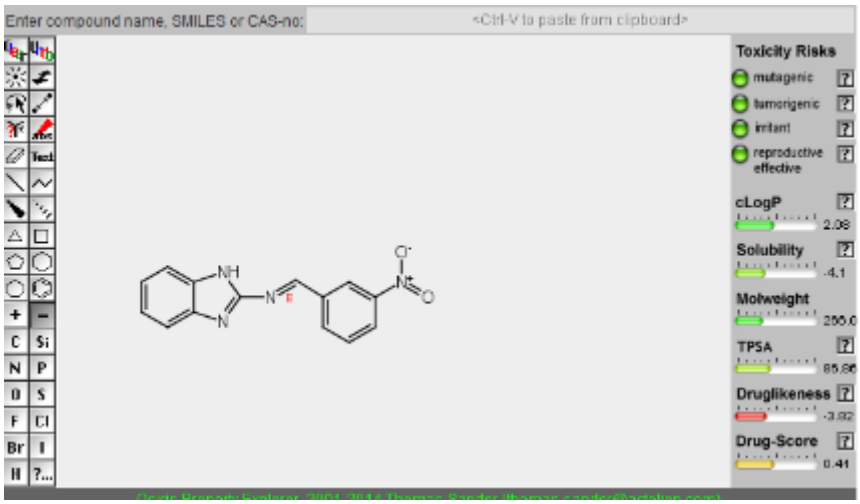
TOXICITY PARAMETERS	COMPOUND CODE				
	RJ	RK	RM	RN	RO
MUTAGENIC	Nil	Nil	Nil	Nil	Nil
TUMOROGENIC	Nil	Nil	Nil	Nil	Nil
IRRITANT	Nil	Nil	Nil	Nil	Nil
REPRODUCTIVE EFFECT	Nil	Nil	Nil	Nil	Nil

Snap shot of the results of insilico toxicity predictions is given below:

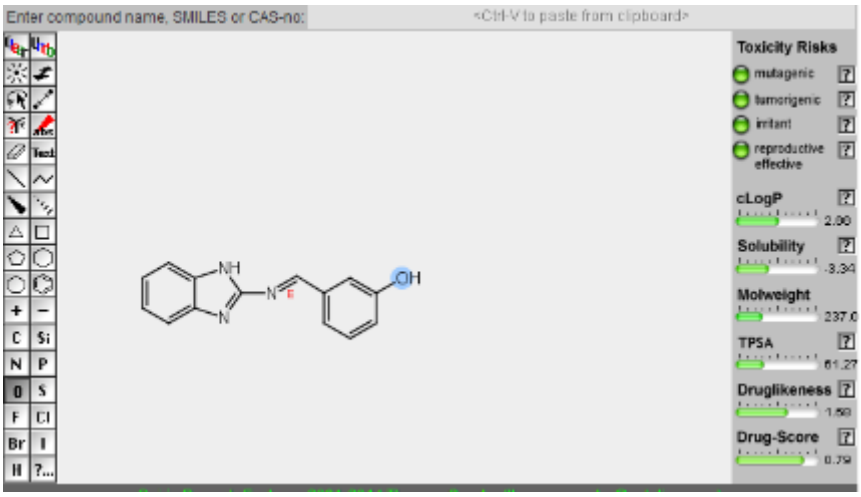
Sample Code: RJ



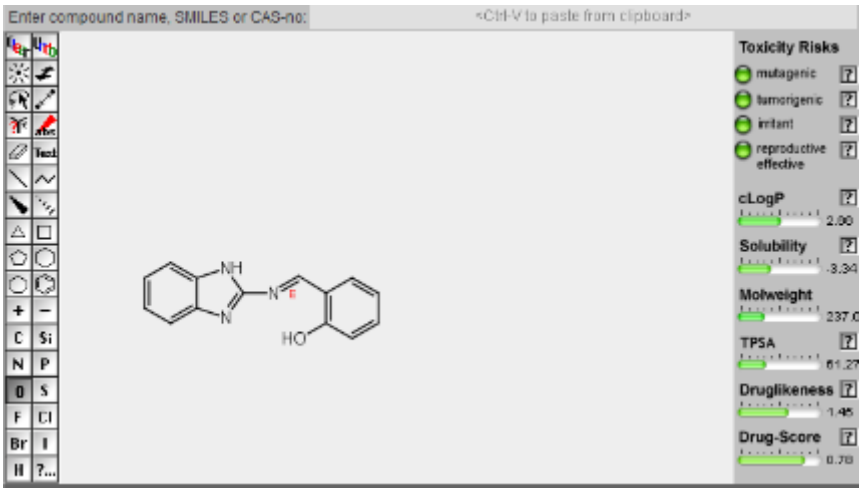
Sample Code: RK



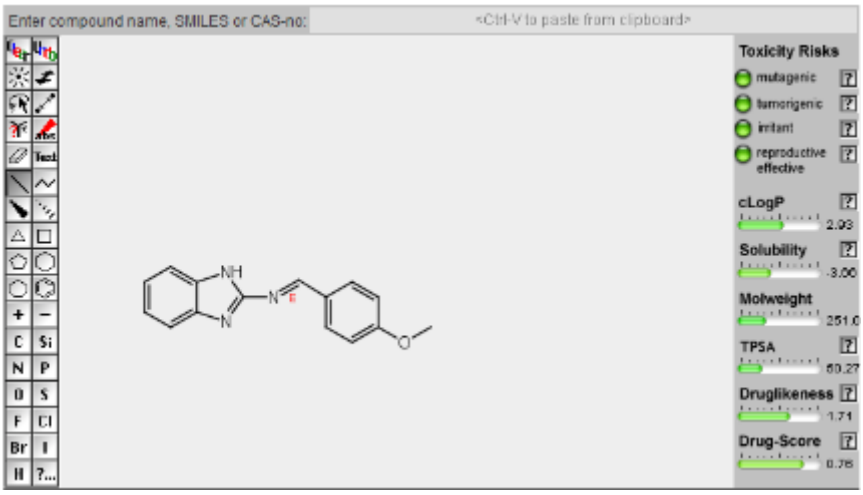
Sample Code: RM



Sample Code: RN



Sample Code: RO



None of the componds RJ,RK,RM,RN and RO exhibited any form of toxicity.

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DRUG LIKENESS PREDICTION

(INSILICO)

The designed and docked molecules were screened insilico using **Molinspirationcheminformatics®** to evaluate drug likeness. Molinspiration®is used to calculate important molecular properties such as log P, polar surface area, number of hydrogen bond donors and acceptors.

Lipinski's rule of five explains the ability of the chemical compound with certain pharmacological or biological activity to make it orally active drug. This rule describes the important pharmacokinetics properties like adsorption, distribution, metabolism and excretion (ADME).

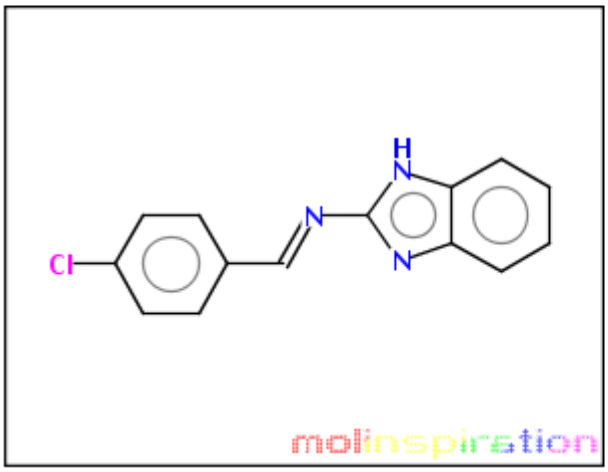
These drug likeness properties which were determined using molinspiration® are presented here.

INSILICO SCREENING OF DRUG LIKENESS

COMPOUND NAME: RJ

molinspiration

miSMILES: Clc3ccc(C=Nc2nc1ccccc1[nH]2)cc3
N-(4-Chlorobenzylidene)-1H-benzoimidazole-2-amine



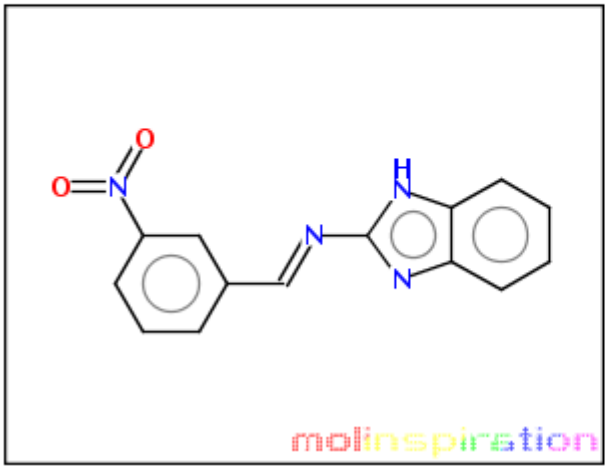
Molinspiration property engine

miLogP	3.96
TPSA	41.05
natoms	18
MW	255.71
nON	3
nOHNH	1
nviolations	0
nrotb	2
volume	217.07

COMPOUND NAME: RK

molinspiration

miSMILES: O=N(=O)c3cccc(C=Nc2nc1ccccc1[nH]2)c3
VYYONVIMXUTDEL-UHFFFAOYSA-N



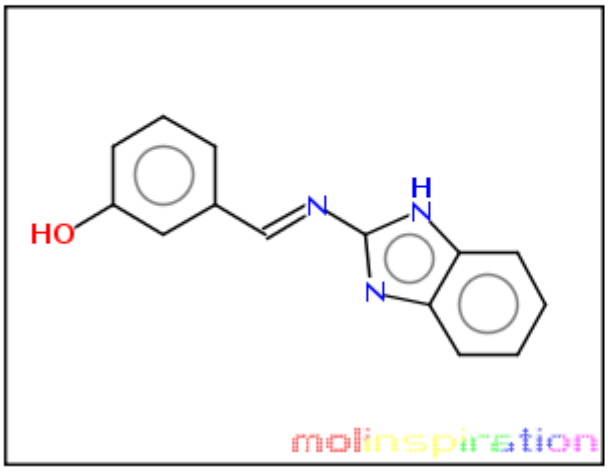
Molinspiration property engine

miLogP	3.22
TPSA	86.87
natoms	20
MW	266.26
nON	6
nOHNH	1
nviolations	0
nrotb	3
volume	226.87

COMPOUND NAME: RM

molinspiration

miSMILES: Oc3cccc(C=Nc2nc1ccccc1[nH]2)c3



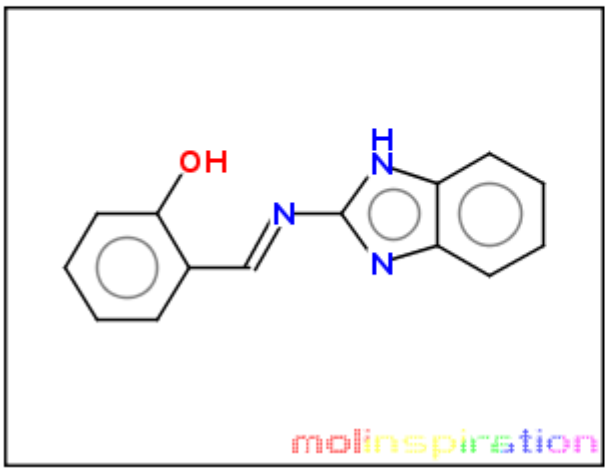
Molinspiration property engine

miLogP	2.78
TPSA	61.27
natoms	18
MW	237.26
nON	4
nOHNH	2
nviolations	0
nrotb	2
volume	211.55

COMPOUND NAME: RN

molinspiration

miSMILES: Oc1ccccc1C=Nc3nc2ccccc2[nH]3
2-(Salicylideneamino)-1H-benzoimidazole



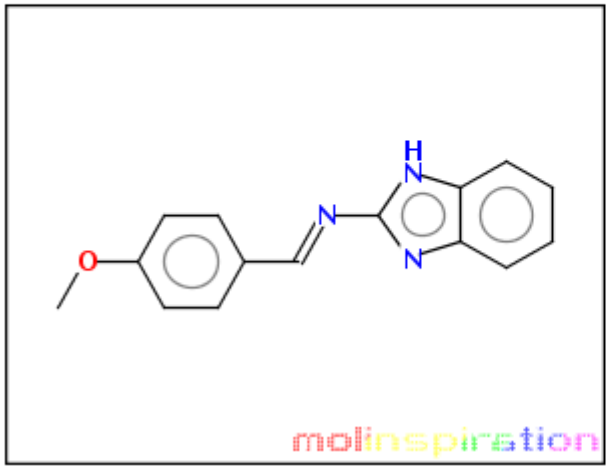
Molinspiration property engine

miLogP	3.22
TPSA	61.27
natoms	18
MW	237.26
nON	4
nOHNH	2
nviolations	0
nrotb	2
volume	211.55

COMPOUND NAME: RO

molinspiration

miSMILES: COc3ccc(C=Nc2nc1ccccc1[nH]2)cc3
DDNITXQZCRTCOD-UHFFFAOYSA-N



Molinspiration property engine

miLogP	3.34
TPSA	50.28
natoms	19
MW	251.29
nON	4
nOHNH	1
nviolations	0
nrotb	3
volume	229.08

SYNTHESIS

The selected 5 compounds were synthesized and recrystallised. Then the synthesized compounds were evaluated for their purity through melting point determination and checking for the absence of parent functional groups and the presence of the newly formed functional group. TLC was performed on different solvent system of different polarities to ensure presense of single spot.

TABLE 4: RESULTS OF SYNTHETIC EFFORTS

S.NO	Compound code	Molecular weight	Percentage Yield	Melting point
1	**RJ**	255.70g/mole	80%	180°C
2	**RK**	266.25 g/mole	75%	138°C
3	**RM**	237.26 g/mole	85%	143°C
4	**RN**	237.26 g/mole	70%	116°C
5	**RO**	251.28 g/mole	70%	148°C

The Rf value of the synthesized compounds varied from the Rf value of the reactants. Hence it is concluded that the reaction was completed.

CHARACTERISATION

The synthesized compounds were subjected to purification by recrystallization and TLC. The melting point of the synthesized compounds founded. 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 spectrums of the synthesized compounds were inspected for presence of the new functional group and absence of the functional group which induced the changes in the chemical reaction.

- The absorption band for RK compound shows that the strong band at 1373 cm^{-1} and 1365 cm^{-1} respectively indicating the presence of NO group.
- For all the synthesized compounds showing the presence of NH stretching vibration between 3450- 3100cm-1, aromatic CH stretching vibration between 2880-2600 cm^{-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 help us to know the nature of protons viz, aromatic, hetero aromatic, 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 11.02-11.35 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) hetero aromatic protons between (7.2-7.8) δ ppm.

Gas chromatography-Mass Spectrometry:

GC is used to determine the purity of compounds based on additional peaks in the sample which are not present in the pure compound. The purpose of the GC step is to separate multiple compounds in a sample so that they reach the MS detector one at a time.The molecular ion peak of the spectrum showing the synthesized compounds were formed.

The synthesized compounds are undergoing two types of cleavage namely

- HOMOLYTIC CLEAVAGE
- HETEROLYTIC CLEAVAGE

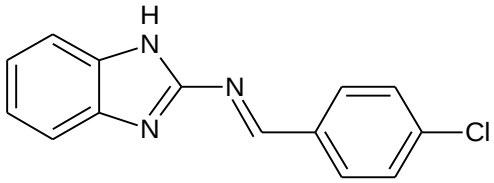
TABLE5: MOLECULAR WEIGHT OF THE SYNTHESIZED COMPOUNDS

NAME OF COMPOUNDS	CALCULATED MASS	ACTUAL MASS
RJ	255.99 g/mole	255.70 g/mole
RK	267.03g/mole	266.25 g/mole
RM	237.36 g/mole	237.26 g/mole
RN	237.99 g/mole	237.26 g/mole
RO	252.04 g/mole	251.28 g/mole

PRODUCT PROFILE

CODE: RJ

IUPAC Name:N-(-1H-BENZIMIDAZOLE-2-YL)-1-(CHLOROPHNYL)METHANIMINE



Molecular Formula**:** $C_{14}H_{10}CLN_3$

Formula Weight**:** 255.702g/mol

Appearance**:** Yellow colour.

Melting Point**:** 180^{o}C

Solubility**:** Chloroform, Methanol, Ethanol.

Percentage Yield**:** 80%

Composition**:** C [65.76%], H[3.94%], CL[13.86%], N[16.43%]

Molar Refractivity**:** 73.18±0.5 cm^3

Molar Volume**:** 195.9±7.0 cm^3

Parachor**:** 518.6±8.0 cm^3

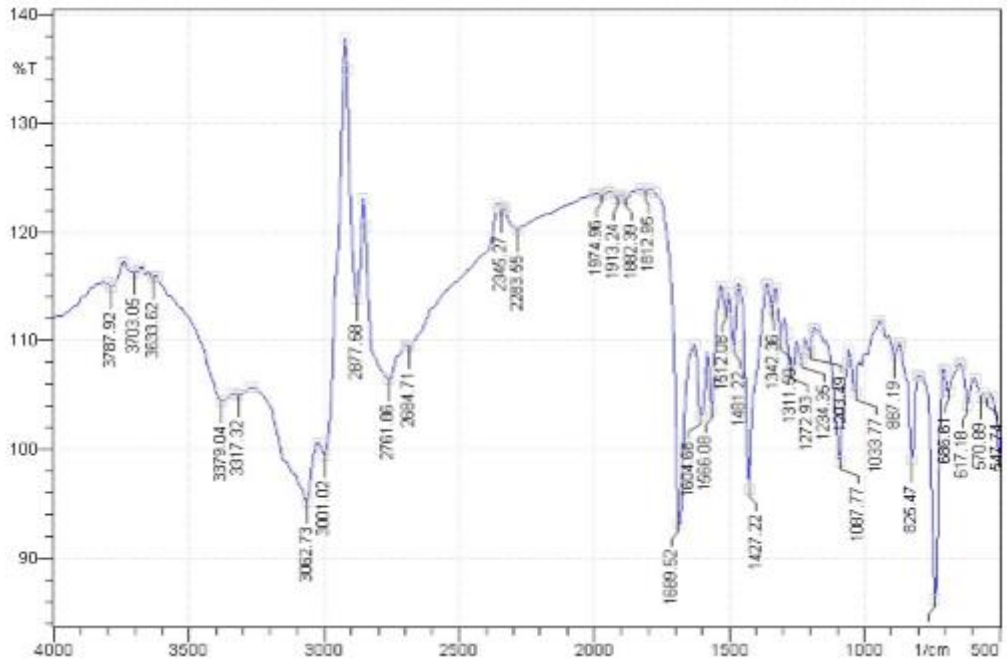
Index of Refraction**:** 1.669±0.05

Surface Tension**:** 49.1±7.0 dyne/cm

Density**:** 1.30±0.1g/ cm^3

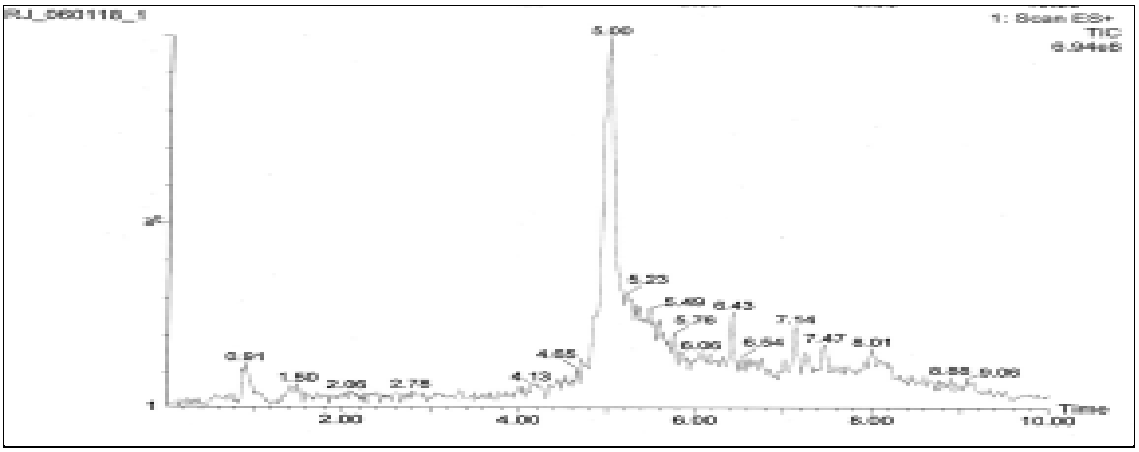
Polarizability**:** 29.01±0.5 10^{-24} cm^3

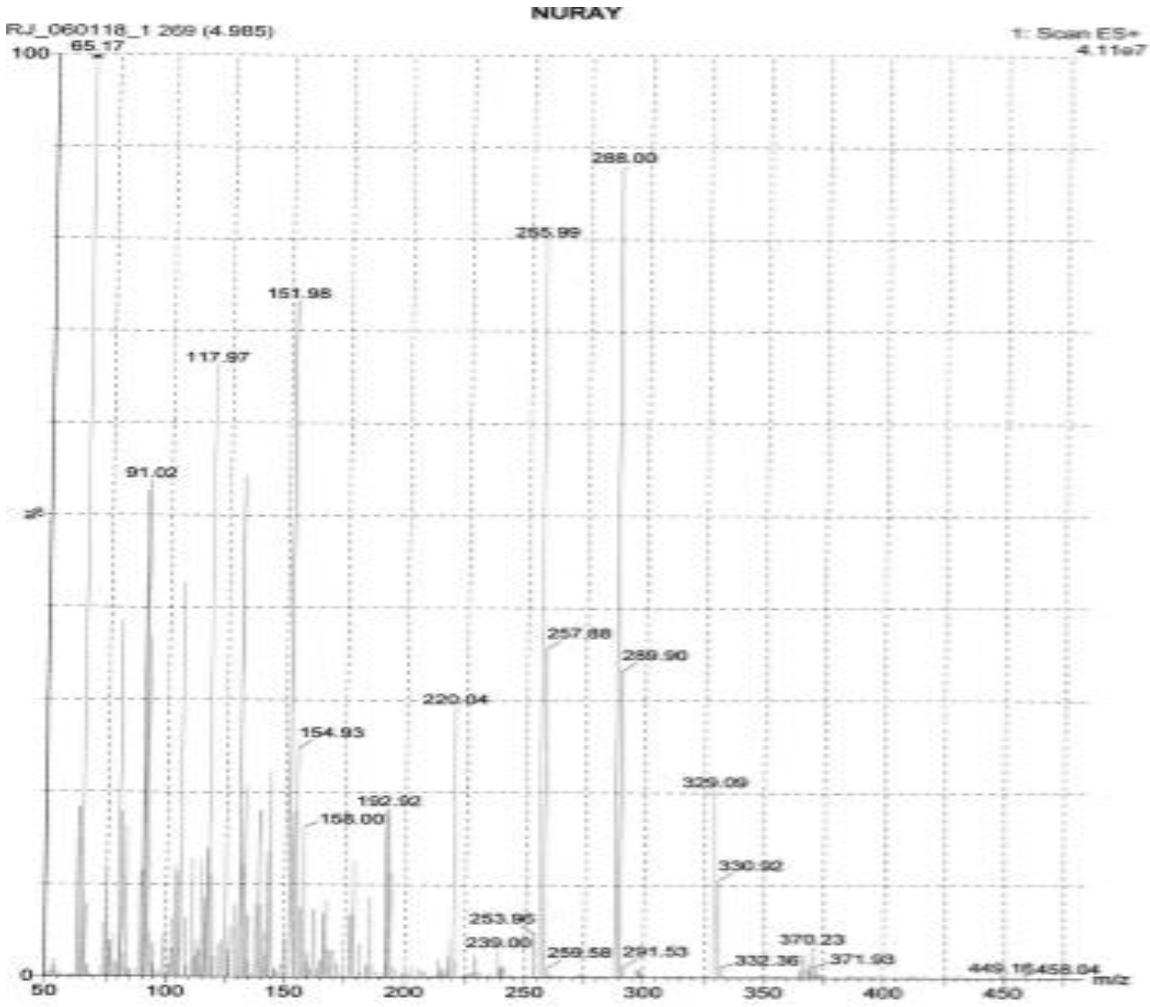
IR SPECTRUM: RJ



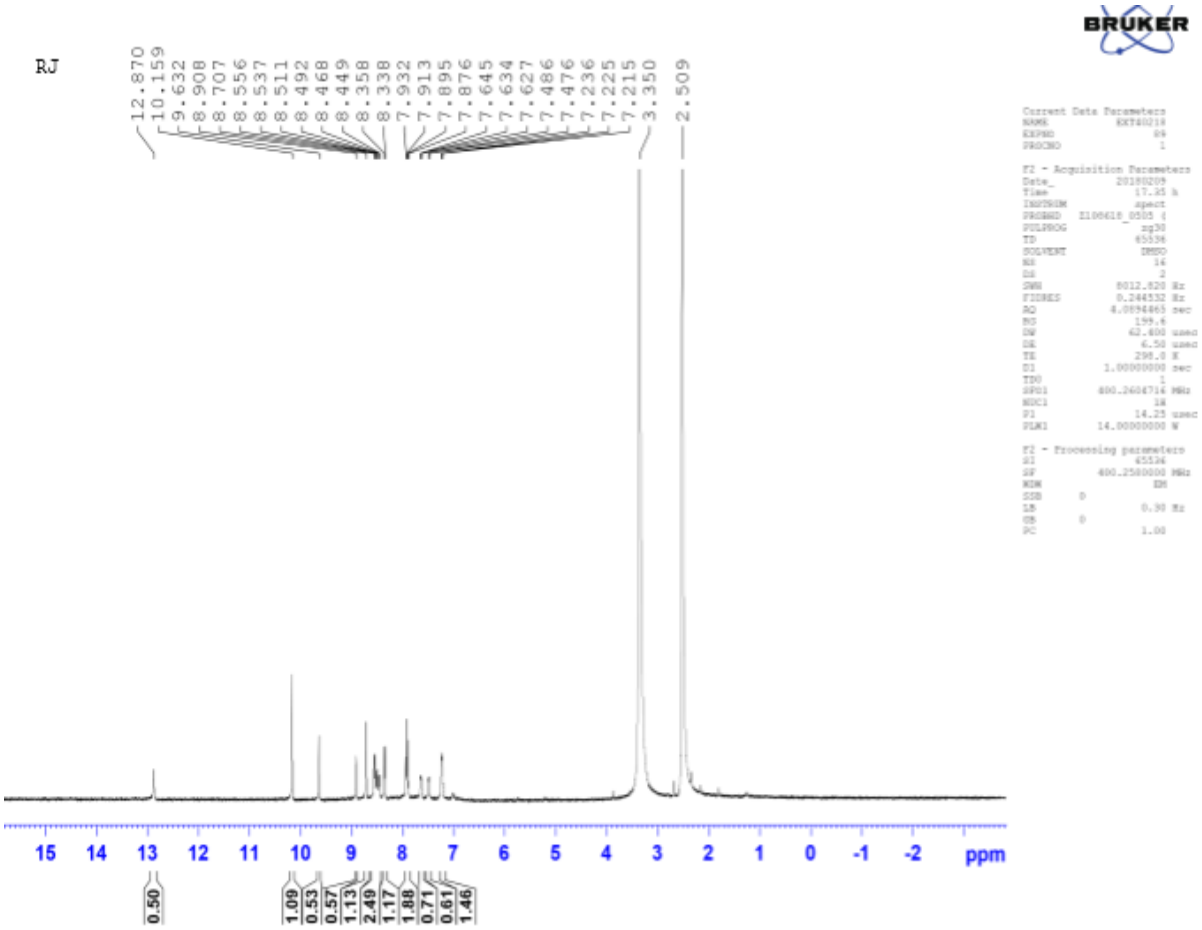
S.No	Wave number (cm^{-1})	Functional group
1	743	-C-CL Stretching
2	2684	-C=N Stretching
3	2877	-C-N Stretching
4	3317	-N-HStretching

LC-MS SPECTRUM :RJ





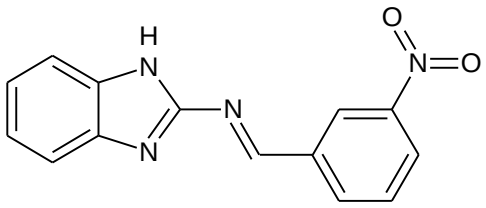
H[1] NMR SPECTRUM :RJ



S.No	δ VALUE (RJ)	NATURE OF PEAK	NUMBER OF PROTONS
1	δ7.3	Doublet	2 Protons
2	δ7.9-8.0	Doublet	2 Protons
3	δ8.3-8.6	Multiplet	4 Protons
4	δ8.9	Singlet	1 Protons
5	δ10.2	Singlet	1 Protons

CODE: RK

IUPAC Name: N-(1H-BNZIMIDAZOLE-2-YL)-1-(3-NITROPHENYL)METHANIMINE



Molecular Formula**:** $C_{14}H_{10}N_4O_2$

Formula Weight**:** 266.2548g/mol

Appearance**:**yellowish orange.

Melting Point**:** 138^{o}C

Solubility**:** Chloroform, Methanol, Ethanol.

Percentage Yield**:** 75%

Composition**:** C [63.15%], H [3.79%], N [21.04%], O [12.02%]

Molar Refractivity**:** 74.724±0.5 cm^3

Molar Volume**:** 191.9±7.0 cm^3

Parachor**:** 535.2±8.0 cm^3

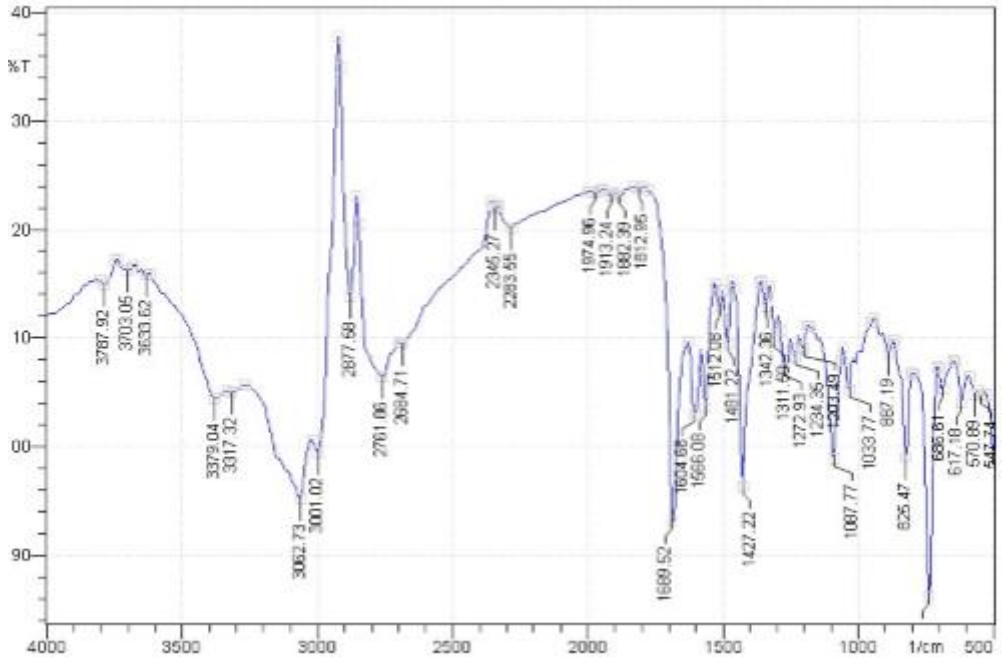
Index of Refraction**:** 1.700±0.05

Surface Tension**:** 60.4±7.0 dyne/cm

Density**:** 1.38±0.1g/ cm^3

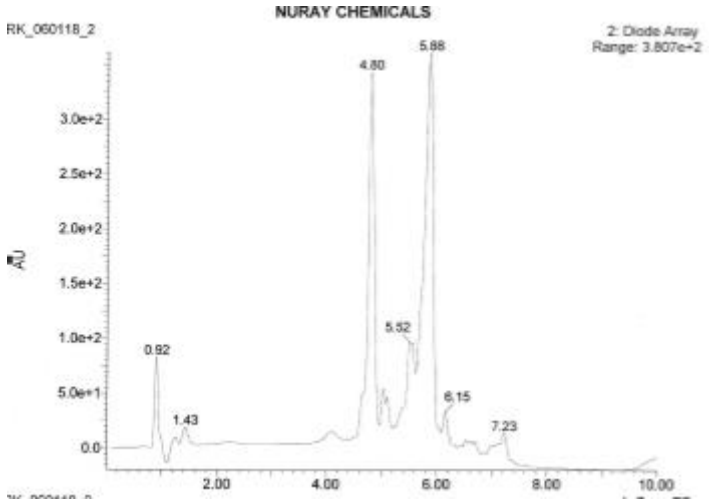
Polarizability**:** 29.43±0.5 $10^{-24}cm^3$

IR SPECTRUM: RK



S.No	Wave number (cm^{-1})	Functional groups
1	1510	-NO_2 Stretching(Aromatic)
2	3317	-N-H Stretching
3	3078	-C-H Stretching
4	2746	-C=N Stretching

LC-MS SPECTRUM: RK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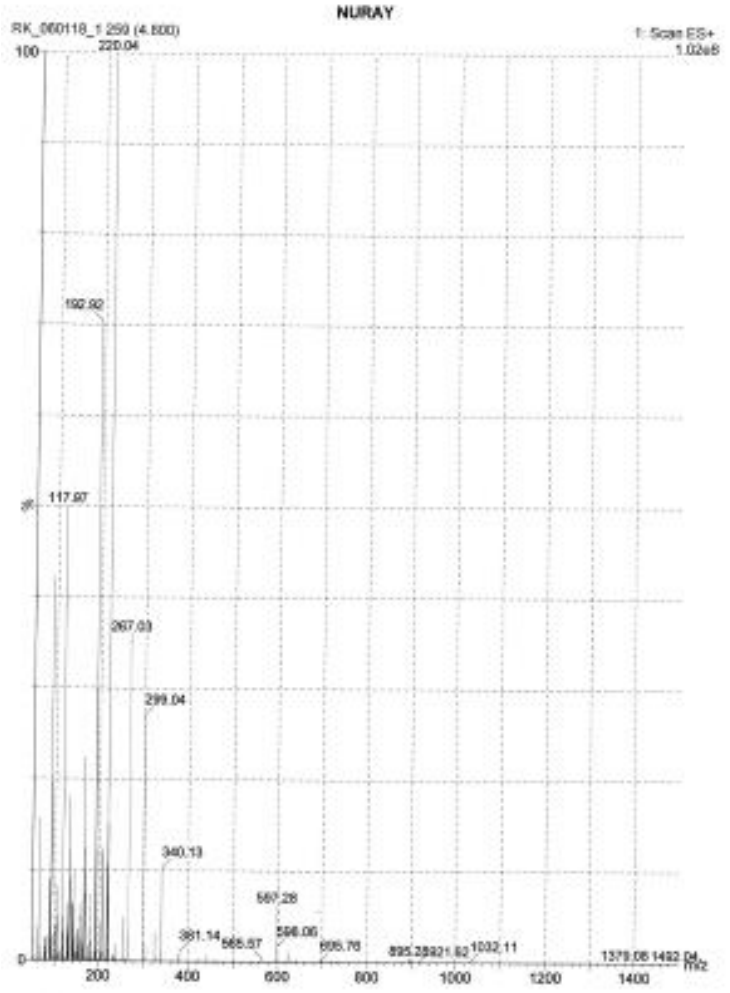
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565.57
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695.76
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400
600
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1000
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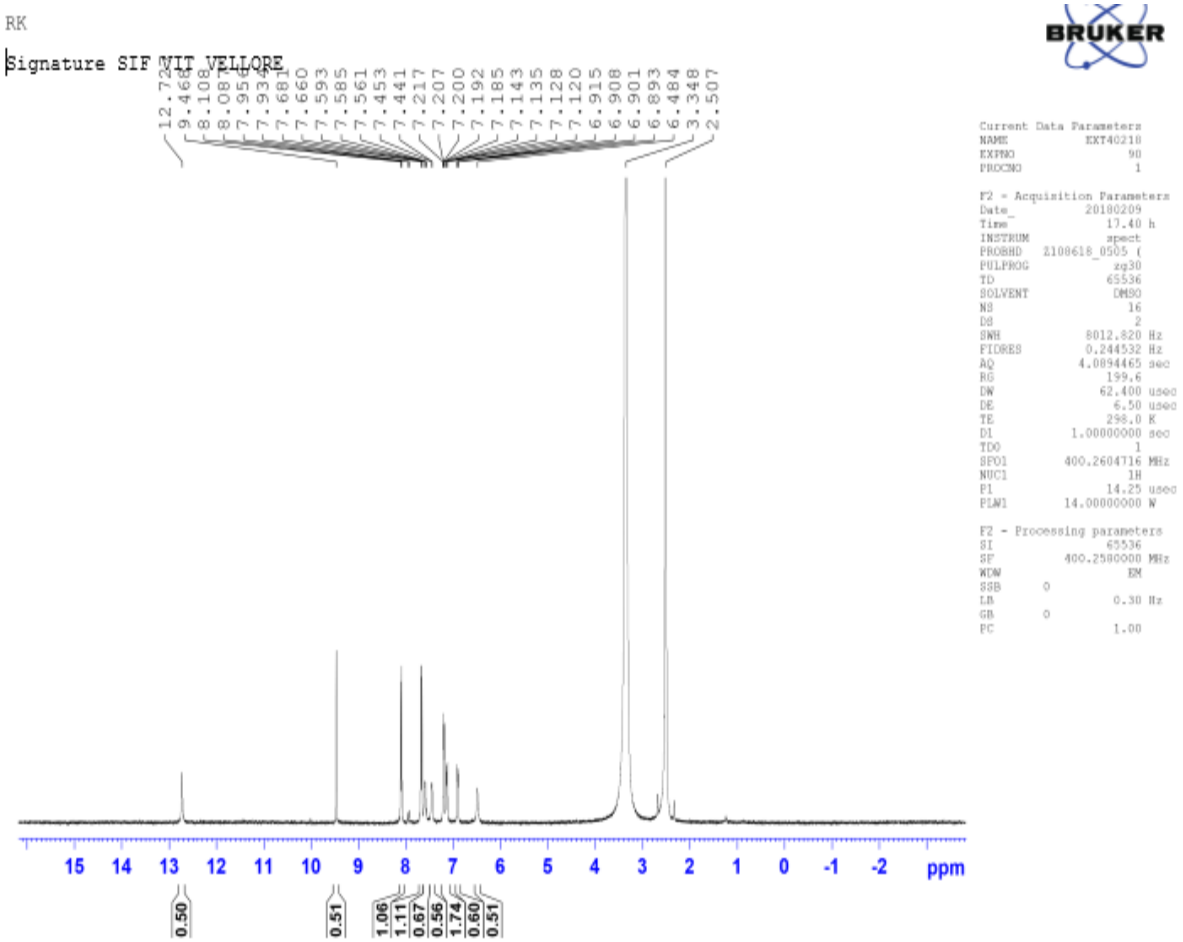
m/z

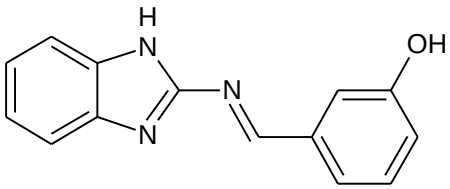
H^1 NMR SPECTRUM :RK



S.No	δ VALUE (RK)	NATURE OF PEAK	NUMBER OF PROTONS
1	δ6.5	Singlet	1 Protons
2	δ6.9	Singlet	1 Protons
3	δ7.1-7.2	Doublet	2Protons
4	δ7.4-7.7	Doublet	2 Protons
5	δ8.2	Doublet	2 Protons
6	δ9.5	Singlet	1 Protons
7	δ12.8	Singlet	1 Protons

CODE: RM

IUPAC Name: 3-[(E)-(1H-BENZIMIDAZOLE-2-YLIM INO)METHYL]PHENOL



Molecular Formula**:** $C_{14}H_{11}N_3O$

Formula Weight**:** 237.25g/mol

Appearance**:** Red colour.

Melting Point**:** 143^{o}C

Solubility**:** Ethanol, Chloroform

Percentage Yield**:** 85%

Composition**:** C [70.87%], H [4.67%], N [17.71%], O [6..74%]

Molar Refractivity**:** 69.43±0.5cm^3

Molar Volume**:** 183.88.0cm^3

Parachor**:** 495.4±8.0cm^3

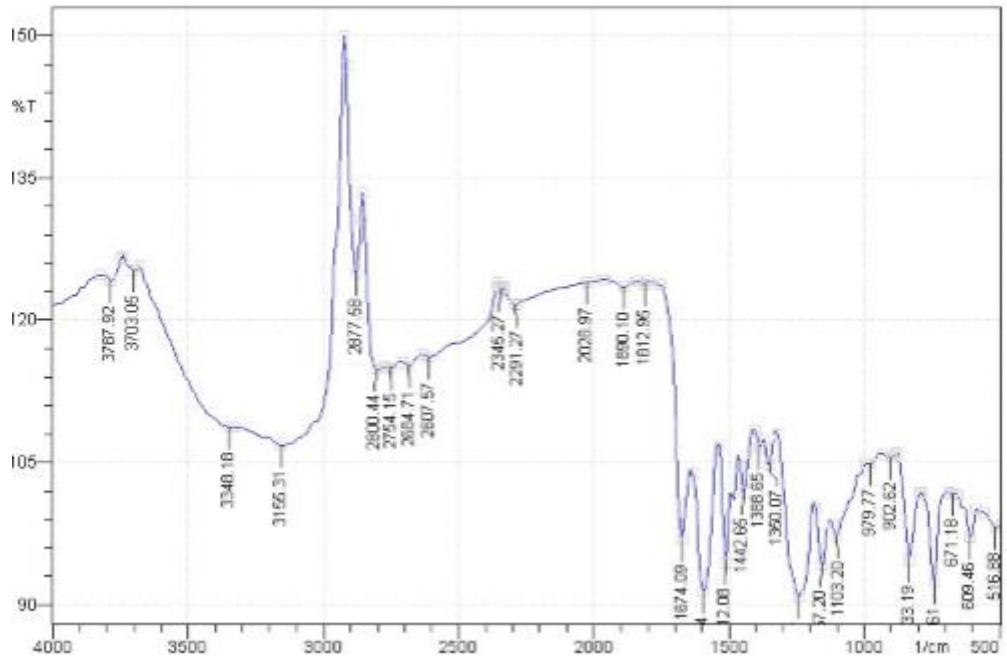
Index of Refraction**:** 1.679±0.05

Surface Tension**:** 52.07±7.0 dyne/cm

Density**:** 1.29±0.1g/cm^3

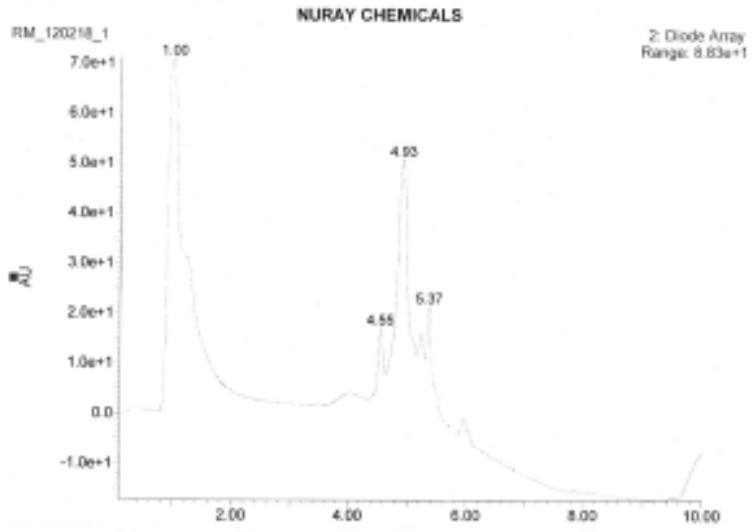
Polarizability**:** 27.52±0.5 10^{-24}cm^3

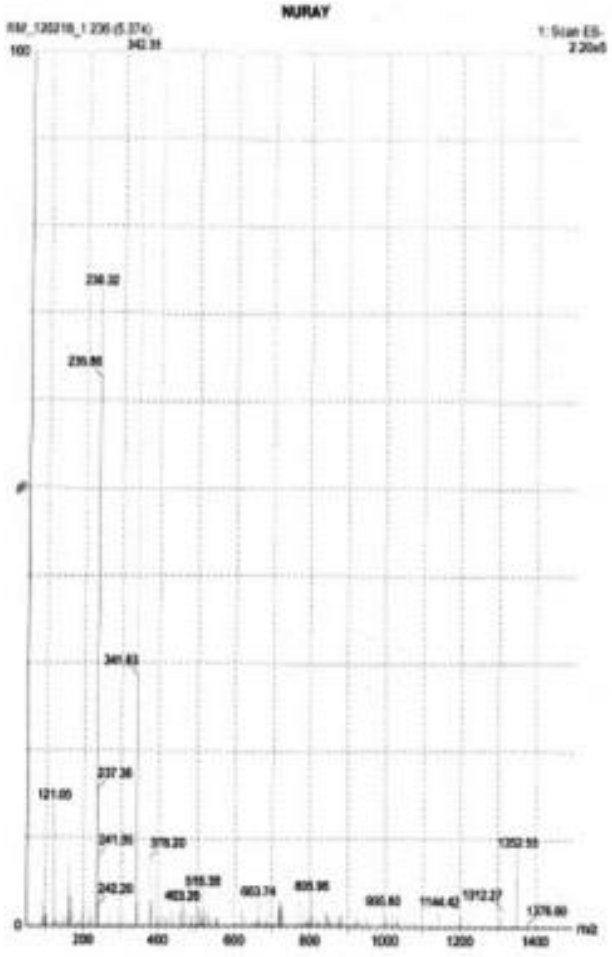
IR SPECTRUM :RM



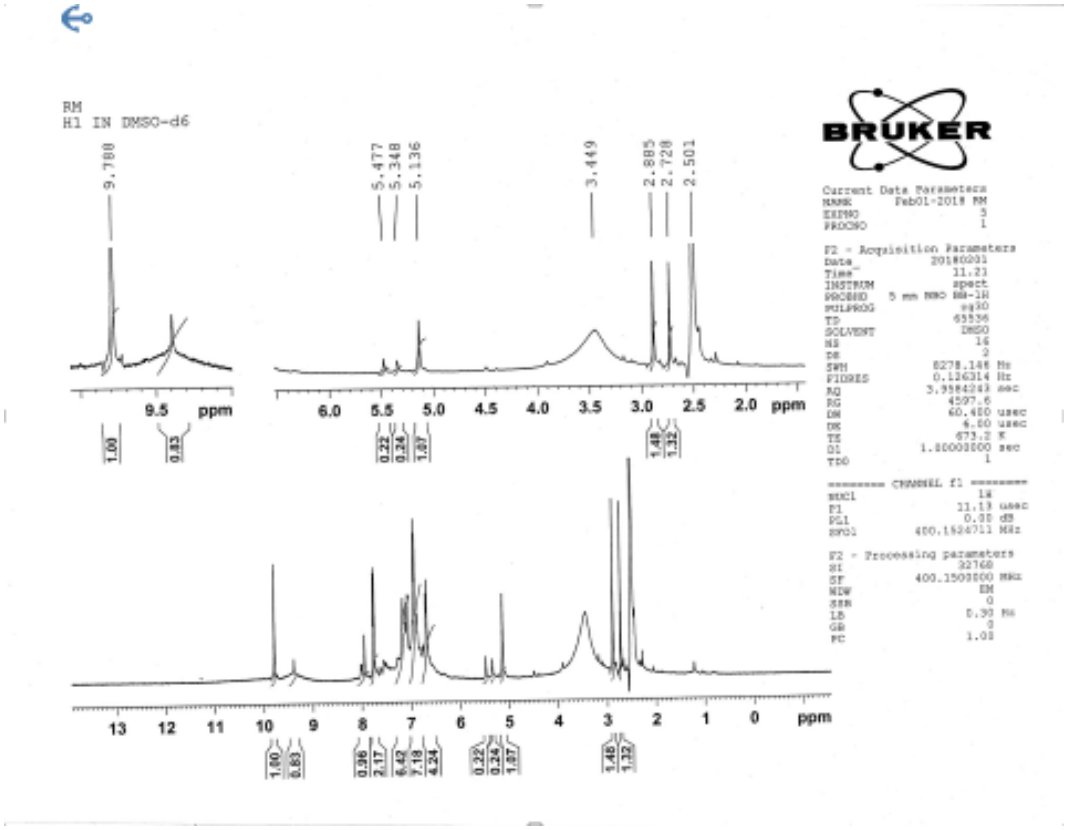
S.No	Wave number (cm^{-1})	Functional group
1	2607	-C=N Stretching
2	2877	-C-H Stretching
3	3348	-O-H Stretching
4	3155	-N-H Stretching

LC-MS SPECTRUM: RM





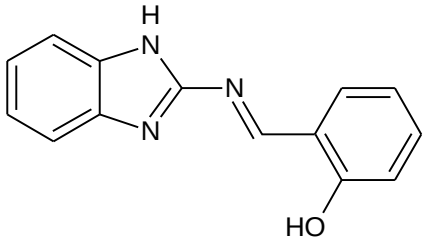
H^1 NMR SPECTRUM :RM



S.No	δ VALUE (PPM)	NATURE OF PEAK	NUMBER OF PROTONS
1	δ5.1	Singlet	1 Protons
2	δ6.-8.0	Multiplet	7 Protons
3	δ9.4	Singlet	1 Proton
4	δ10.9	Singlet	1 Proton

CODE: RN

IUPAC Name: 2-[(E)-(1H-BENZIMIDAZOL-2-YLIM INO)METHYL]PHENOL



Molecular Formula**:** $C_{14}H_{11}N_3O$

Formula Weight**:** 237.256g/mol

Appearance**:** Greenish yllow.

Melting Point**:** 116^{o}C

Solubility**:** Chloroform, Methanol, Ethanol.

Percentage Yield**:** 70%

Composition**:** C [70.8722%], H [4.67%], N [17.71%], O [6.74%]

Molar Refractivity**:** 69.43±0.5 cm^3

Molar Volume**:** 183.8±7.0 cm^3

Parachor**:** 495.4±8.0 cm^3

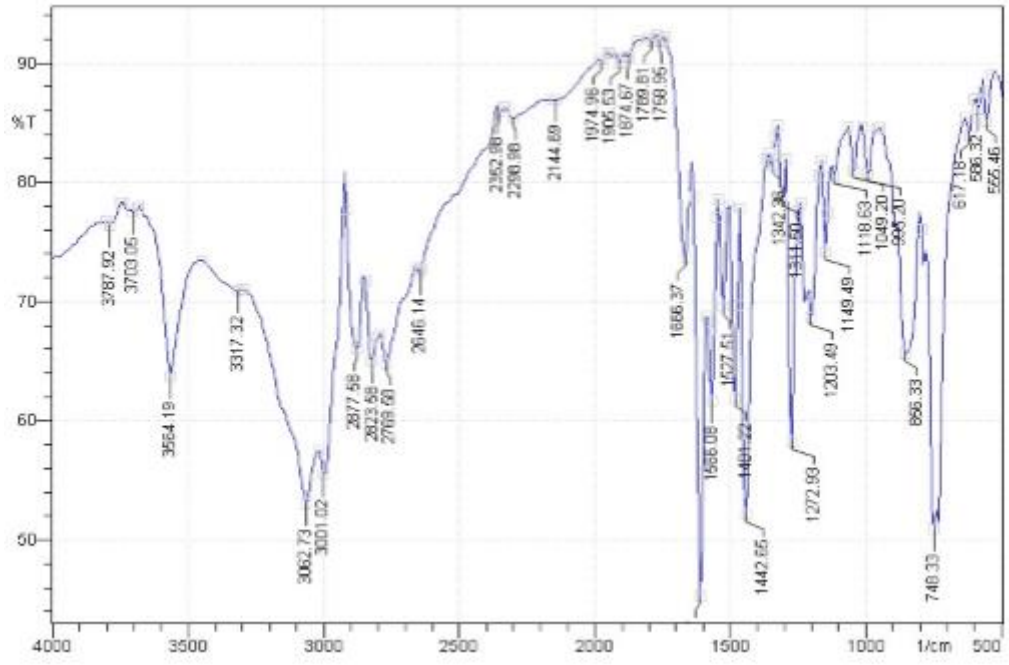
Index of Refraction**:** 1.679±0.05

Surface Tension**:** 52.7±7.0 dyne/cm

Density**:** 1.29±0.1g/ cm^3

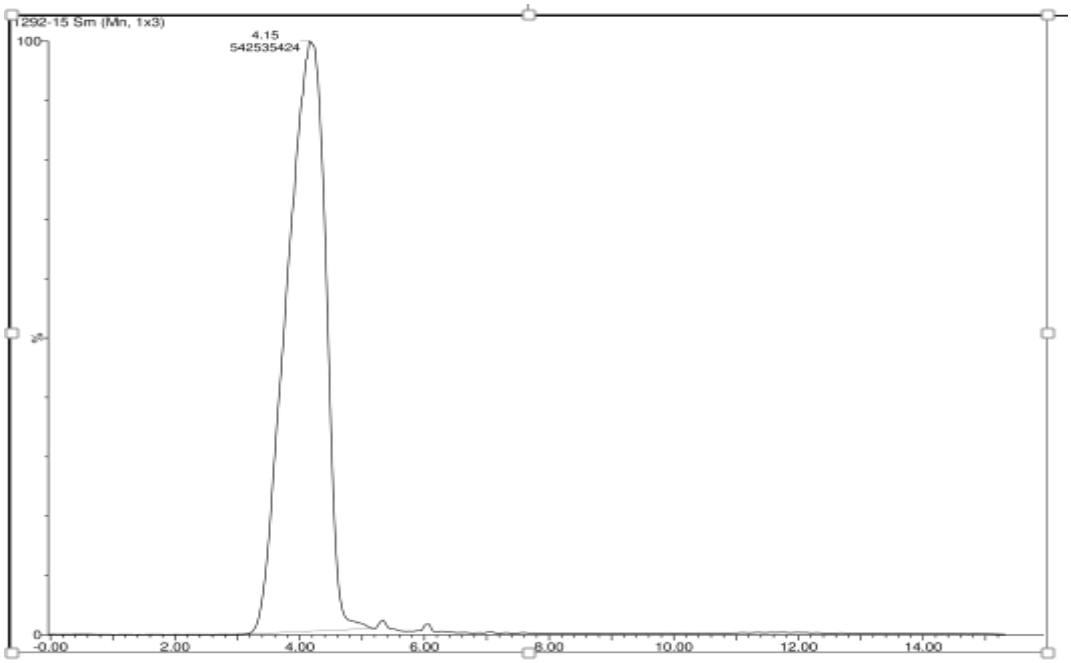
Polarizability**:** 27.52±0.5 $10^{-24}cm^3$

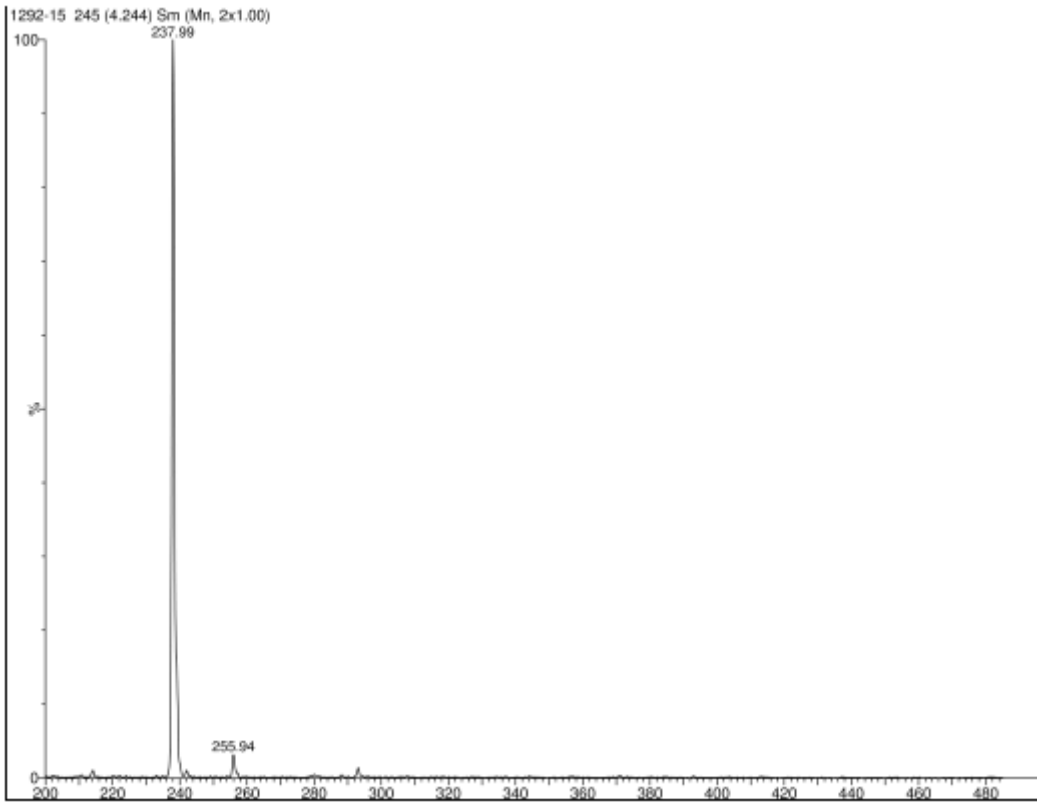
IR SPECTRUM :RN



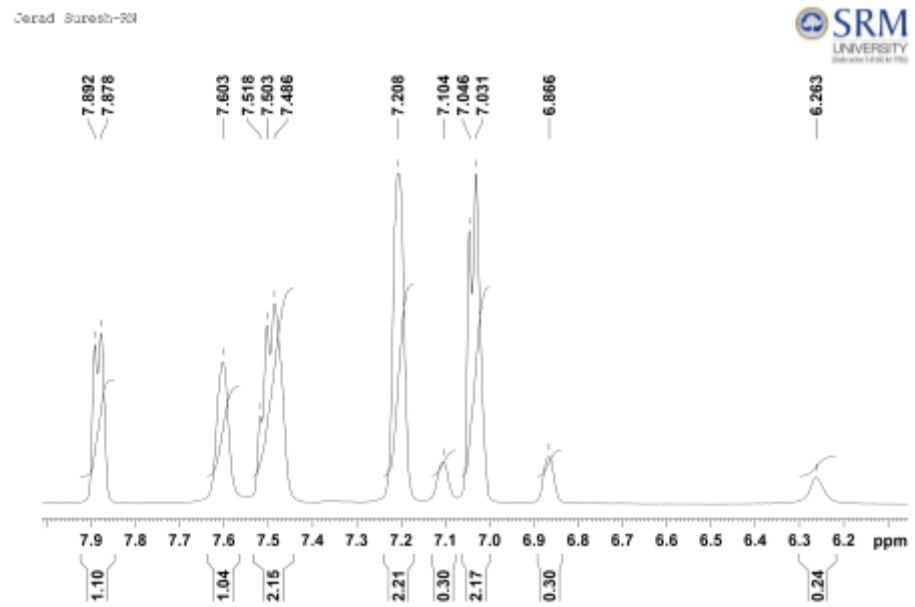
S.No	Wave Number (cm^{-1})	Functional group
1	3564	-O-H Stretching
2	3317	-N-H Stretching
3	2877	-C-H Stretching
4	2646	-C=N Stretching

LC-MS SPECTRUM :RN





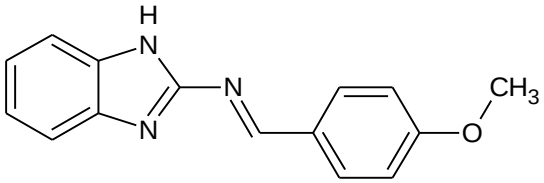
H^1 NMR SPECTRUM :RN



S.No	δ VALUE (PPM)	NATURE OF PEAK	NUMBER OF PROTONS
1	δ7.0	Doublet	2 Proton
2	δ7.2	Doublet	2 Protons
3	δ7.5	Triplet	3 Proton
4	δ7.6	Singlet	1 Proton
5	δ7.9	Doublet	2 Protons

CODE: RO

IUPAC Name: (E)-N-(1H-BENZIMIDAZOL-2-YL)-1-(4-METHOXYPHNYL)METHANIMINE



Molecular Formula**:** $C_{15}H_{13}N_3O$

Formula Weight**:** 251.28g/mol

Appearance**:** buff colour.

Melting Point**:** 148^{o}C

Solubility**:** Chloroform, Methanol, Ethanol.

Percentage Yield**:** 70%

Composition**:** C [71.70%], H [5.21%], N [16.72%], O [6.37%]

Molar Refractivity**:** 74.39±0.5 cm^3

Molar Volume**:** 208.3±7.0 cm^3

Parachor**:** 540.0±8.0 cm^3

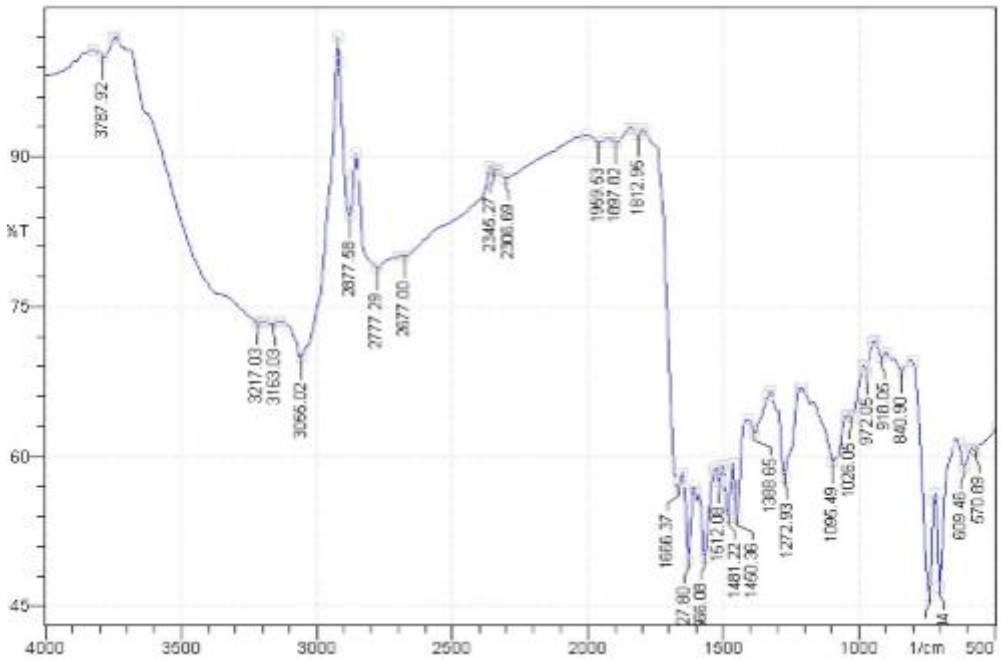
Index of Refraction**:** 1.632±0.05

Surface Tension**:** 45.1±7.0 dyne/cm

Density**:** 1.20±0.1g/ cm^3

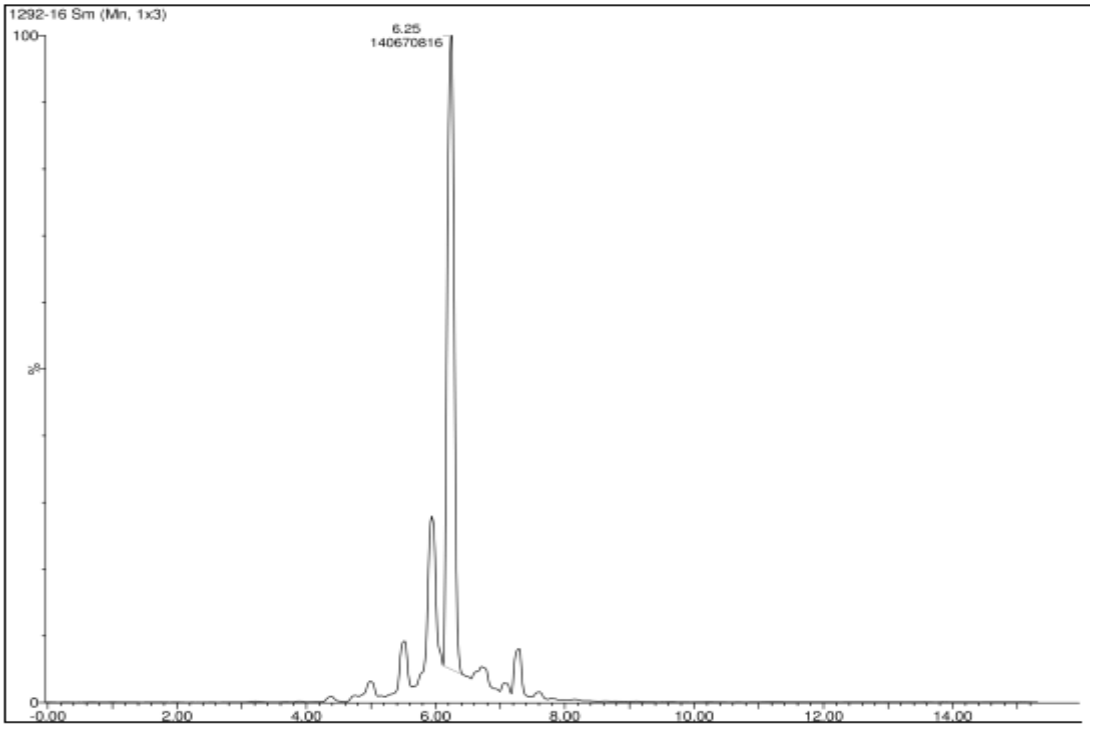
Polarizability**:** 29.49±0.5 10^{-24} cm^3

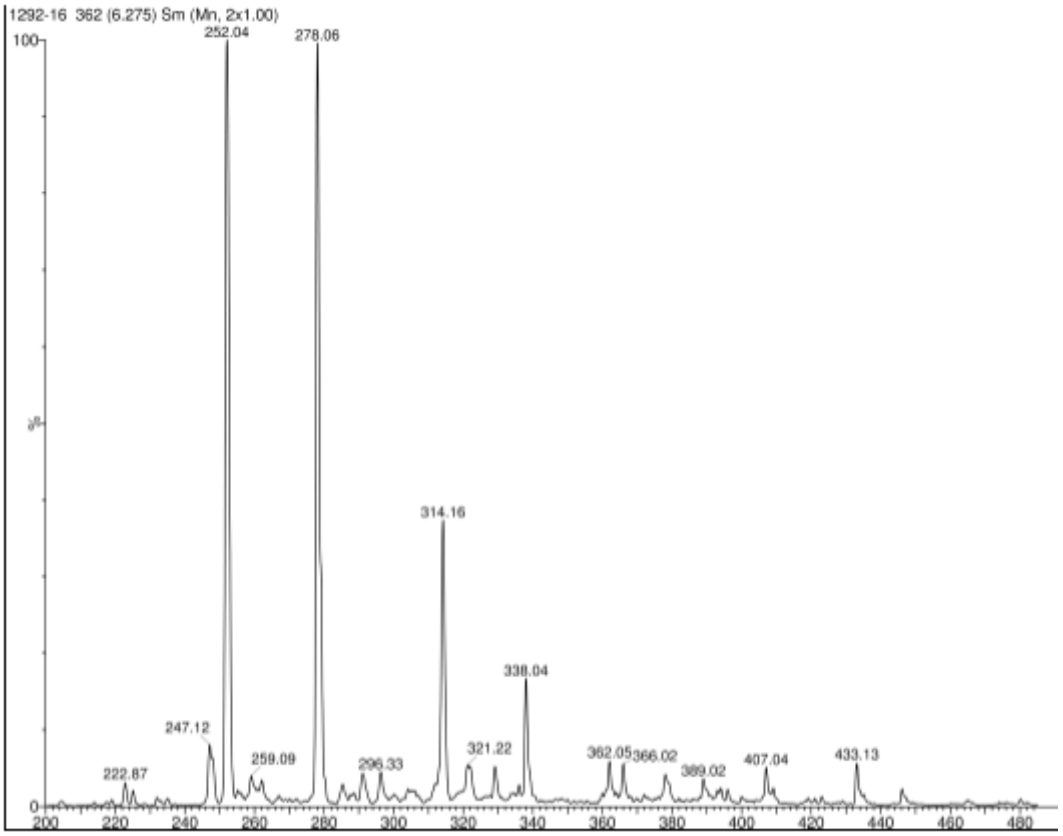
IR SPECTRUM : RO



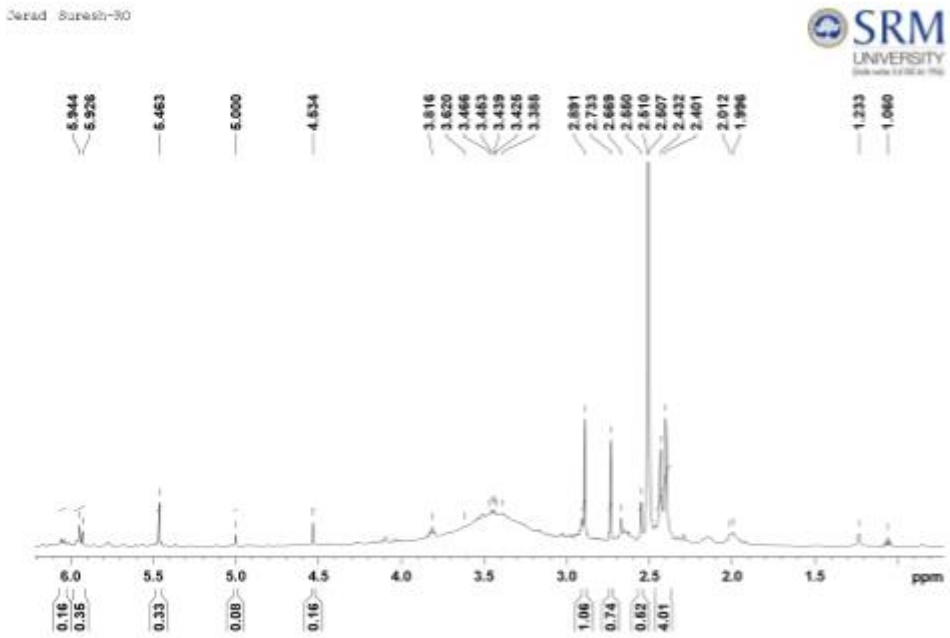
S.No	Wave Number (cm^{-1})	Functional group
1	2873	-O-CH_3 Stretching
2	3055	-C=H Stretching
3	2777	-C=N Stretching
4	3217	-N-H Stretching

LC-MS SPECTRUM : RO





H^1 NMR SPECTRUM : RO



S.No	δ VALUE (PPM)	NATURE OF PEAK	NUMBER OF PROTONS
1	δ4.5	Singlet	1 Protons
2	δ5.0	Singlet	1 Protons
3	δ5.5	Singlet	3 Protons
4	δ5.8	Triplet	3 Protons
5	δ6.0-6.2	Multiplet	5 Proton

BIOLOGICAL EVALUATION

The anti-tubercular activities of the synthesized compounds were determined by MicroplateAlamar Blue Assay method (MABA). The organism used in the study is Mycobacterium tuberculosis H37Rv. All the synthesized compounds showed anti-mycobacterial activity in varying degrees against the organism tested. The data pertaining to these observations are presented in the table[6] below.

TABLE 6: ANTI-TB RESULTS

SL. NO	SAMPLES	100 µg/ml	50µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	RJ	**S**	**S**	**S**	**S**	**S**	**S**	R	R
2.	RK	**S**	**S**	**S**	**S**	**S**	**S**	**S**	R
3.	RM	**S**	**S**	**S**	R	R	R	R	R
4.	RN	**S**	**S**	**S**	R	R	R	R	R
5.	RO	**S**	**S**	**S**	**S**	**S**	**S**	**S**	R

NOTE: S - Sensitive R - Resistant

Strain used: ***M.tuberculosis***(H37 RV strain): ATCC No- 27294.

Here are the ***standard values*** for the Anti-Tb test which was performed.

Pyrazinamide- 3.125µg/ml

Streptomycin- 6.25µg/ml

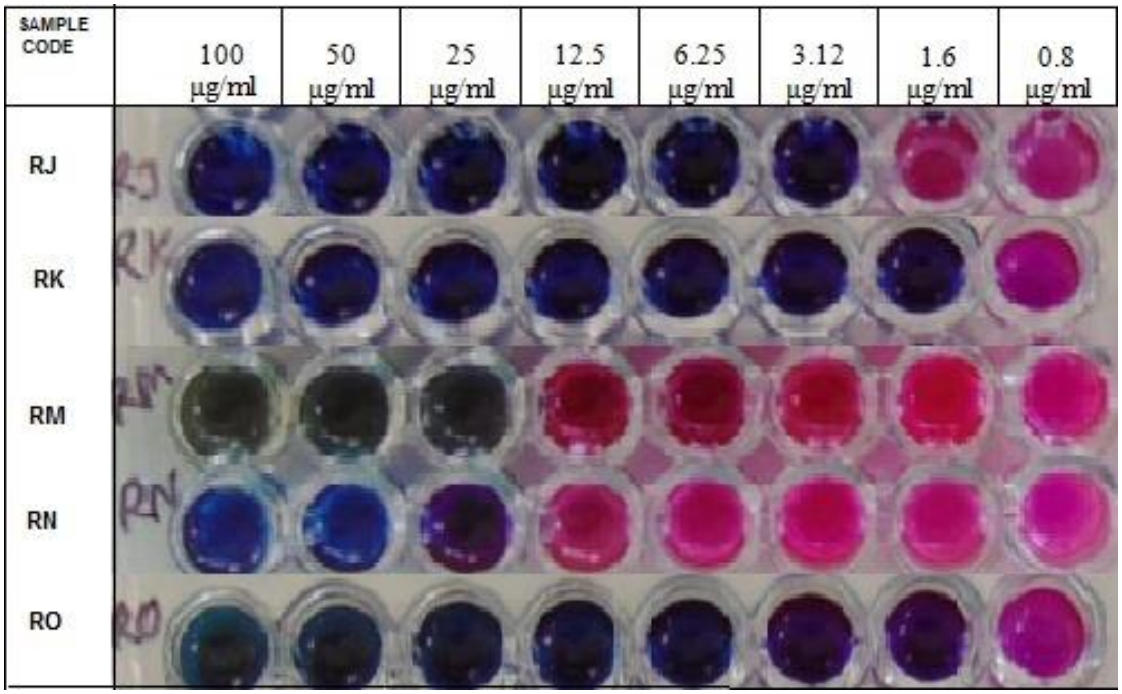
Ciprofloxacin-3.125µg/ml

Compounds RK and RO were found to be more activity than the standard drugs.They are active at 1.6µg/ml.

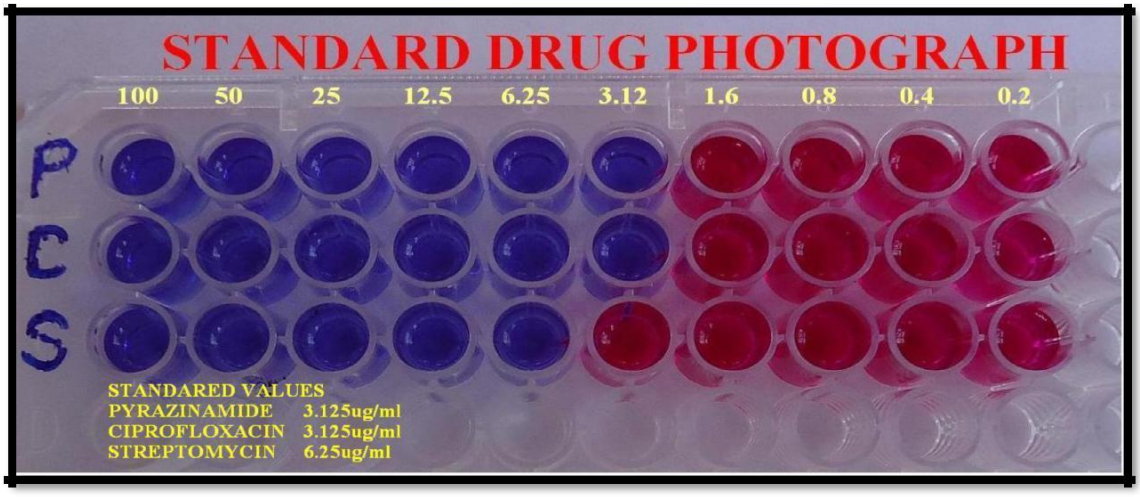
RK which shows activity at 3.125µg/ml was found to be as active as Pyrazinamide and Ciprofloxacin.

Compounds RM and RN were found to be less active than the standard drugs.

SAMPLE DRUG PHOTOGRAPH



STANDARD DRUG PHOTOGRAPH



ACUTE ORAL TOXICITY STUDY

Compounds RJ and RK which was found to be the active were taken for active toxicity study and the animals were observed for behavioral signs of toxicity like motor activity, tremor etc., and no significant toxic signs were observed during 14 days. The results of the acute toxicological studies revealed that the administration of 2 molecules by oral route upto 2000mg/kg/b.w did not produce any mortality and it was tolerated. The results of the observation are tabulated in table[7] below.

Table 7. Acute Oral Toxicity study

S.No.	PARAMETERS	RESULTS
1.	Toxic signs	Absent
2.	Pre-terminal deaths	Nil
3.	Body weight	No specific change
4.	Motor activity	Normal
5.	Tremors	Absent
6.	Convulsions	Absent
7.	Straub reaction	Absent
8.	Righting reflex	Present
9.	Lacrimation and Salivation	Normal
10.	Unusual vocalization	Absent
11.	Sedation	Absent
12.	Body temperature	Normal
13.	Analgesia	Absent
14.	Ptosis	Absent
15.	Diarrhoea	Absent
16.	Skin colour	Normal
17.	Respiration	Normal
18.	Scratching	Absent
19.	Aggressiveness and restlessness	Absent

CYTOTOXICITY EVALUATION:

Table 8. Cytotoxicity results

Concentration (µg/ml)	RJ	RK
500	98.14	96.96
250	97.99	91.42
125	92.51	49.09
64.5	72.92	26.22
31.25	40.99	9.99
IC50 from Prism	**37.63**	**111.2**

These two compounds, RJ and RK shows decreased IC50 Values of 37.63 and 111.2 µg/ml.

The IC_{50} for Rifampicin is 113 µg/ml on vero cell line. The reported values of the compounds were compared with standard drugs.

Therefore as compared to Rifampicin the synthesized compounds were found to be more cytotoxic

DISCUSSION

1. The synthesized compounds have best docking score against targeted enzymes and were synthesized in an appropriate manner, and the purity of the compounds were justified by single spot on TLC and sharp melting point.
2. Among 5 compounds, 2 of them were obtained at 98% and it was confirmed by LC-MS analysis (single peak) and molecular weight obtained at ± 1 variation (M+1 or M-1 peak). The presence of corresponding functional groups was obtained from FT-IR spectrum by obtaining specific absorption band in the spectra. The types of protons and no of protons were confirmed by H^1 NMR Spectra.
3. All the 5 compounds were obeyed Lipinski Rule of 5 and Rule of Seven for Druglikeness by Molinspiration®.
4. Osiris Property Explorer® showed that all the 5 compounds are non toxic.
5. The biological evaluation MABA of the compounds is denoted that the specific organism was sensitive at 25,1.6, 3.125 mcg/ml and showed better activity compared to standard drugs.
6. Acute toxicity study revealed that the selected compounds was relatively non toxic upto 2000mg/kg/b.w, indirectly pronouncing safety profile of the compounds.
7. The IC50 values of synthesized compounds was observed by Cytotoxicity study against Vero (African Green Monkey kidney cells) cell line.

   8.From the literature review the benzimidazole nucleus shows antitubercular activity. According to the results of my work NH amino on 3^{rd} position and N nitrogen in benzimidazole nucleus.

8. Cl on 4^{th} position of the nucleus having good antitubercular activity and NO_2 on 3^{rd} position of the second compound nucleus had given good activity.

   10.3^{rd} and 6^{th} position of OH group dcreased activity than other three compounds.

Summary & Conclusion

SUMMARY

- ✓ *Enoyl acyl carrier protein reductase* is a vital enzyme present in the cell wall of *Mycobacterium tuberculosis*.
- ✓ A database of 200 molecules with high prospects of inhibiting the target *Enoyl acyl carrier protein reductase* were carefully chosen by making changes to the known hit molecules, here the Benzimidazole nucleus waschosen.
- ✓ The designed molecules were docked against the target chosen using AutoDock 4®.
- ✓ Five molecules with good docking score [lower binding energy] and interactions were shortlisted for synthesis. Reaction conditions wereoptimized.
- ✓ The selected molecules were subjected to toxicity prediction assessment by OSIRIS® property explorer developed by Acetilon Pharmaceuticals limited which is available online. The results are color coded as green color which predicts the drug likeness and possibly betteractivity.
- ✓ The molecules were labelled as RJ, RK, RM, RN, RO and were synthesized with satisfactoryyield.
- ✓ The purity of the synthesized compounds was ensured by repeated recrystallization. Further the compounds were evaluated by TLC and Melting pointdetermination.
- ✓ The characterization of the synthesized compounds was done using Infra-red, Nuclear Magnetic Resonance [H1 NMR] and Mass spectrometric methods [LC-MS].
- ✓ All the Synthesized compounds exhibited molecular ion peak (M+) of varying intensities.
- ✓ The final pure compounds were screened for Anti-mycobacterial activity by in vitro method called Micro plate Alamar Blue Assay[MABA].

- ✓ The synthesized compounds showed sensitivity [Minimum inhibitory concentration] at 1.6mcg/ml. The standard drugs Pyrazinamide, Ciprofloxacin and Streptomycin exhibited anti-mycobacterial activity at 3.125 mcg/ml, 3.125 mcg/ml and 6.25mcg/ml concentrations respectively. This indicates that the synthesized compounds are as Potent as the standarddrugs.
- ✓ The selected compounds showed IC50 Values of 37.63,111.2μg/ml respectively for RJ,RK.

CONCLUSION

It is concluded that the synthesized compounds might effectively inhibit the chosen target *Enoyl acyl carrier protein reductase* which is essential for the *Mycobacterial tuberculosis*. Further structural modifications of the synthesized compounds will aid in the development of potential molecule against the pathogen.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Annexure

Proceedings of the Chairperson, Institutional Animal Ethics Committee, Madras Medical College, Chennai – 3.

Present: Dr.Sudha Seshayyan, M.B.B.S, M.S (Anatomy)

Roc. No. 20/ AEL/IAEC/MMC Date: 15.03.2018.

Sub: Animal Experimental laboratory – IAEC – research Project – approval – regarding.

Ref: IAEC meeting held on 06.09.2017.

The following order is based on the meeting held on 06.09.2017 and the addendum issued on 15.03.2018.

Project ID.	20/17.
CPCSEA registration number	1917 / ReBi/S/16/CPCSEA /25.10.2016
Name of the Researcher	J.ROBERT DILTON M. Pharm II year, Department of Pharmaceutical Chemistry
Name of the Guide	Dr.A.Jerad Suresh,M.Pharm, Ph.D.,
Title of the project	Design, Synthesis, Characterization and Biological Evaluation of some Novel Benzimidazole Derivatives as Anti-Tubercular Agents against InhA
Date of submission of proposal to IAEC	01.08.2017
Date on which IAEC conducted	06.09.2017
Date of submission of modified proposal (if applicable)	14.03.2018
Date on which approved	15.03.2018
Validity of the approved proposal	1 year
Remarks	Albino mice – Female 12 numbers approved.

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai -3

To,
Dr.A.Jerad Suresh,M.Pharm,Ph.D.,
Prof. & Head of Dept of Pharmaceutical Chemistry,
College of Pharmacy,
MMC, Chennai -3.

Copy to
Special Veterinary Officer, Animal Experimental Laboratory
Madras Medical College, Chennai – 3.

INDIAN PHARMACEUTICAL ASSOCIATION

NATIONAL CONVENTION 2017 - 18

Theme: Pharma Vision 2030: Planning the Future

Certificate of Participation

This is to certify that

Dr./Prof./Mr./Ms. J. ROBERT DILTON

participated in the National Convention 2017 - 18 held at B. S. Abdur Rahman Crescent Institute of Science & Technology, Chennai, 10th - 11th February 2018

Dr. S. Manivannan	J. Jayaseelan	T. Sathish
Chairman - LOC	IPA Convention Co-ordinator - LOC	Hon. Secretary - LOC

"Skill and Will to Make and Serve Quality Pill"

69th IPC 2017

CHANDIGARH

22nd - 24th December, 2017

PHARMA VISION 2020

PHARMACISTS FOR A HEALTHY INDIA

Certificate

This is to certify that

Prof./Dr./Mr./Ms. J. ROBERT DILTON

has participated as Delegate / Volunteer

in the 69th Indian Pharmaceutical Congress

held at Chitkara University, Rajpura from December 22nd to 24th, 2017.

Dr. Mahesh Burande	Dr. Shailendra Saraf	Dr. Dhirender Kaushik	Dr. Ashish Baldi
President - IPCA	Chairman - LOC	Organizing Secretary	Chairman, Registration Committee - LOC

Organised by : Indian Pharmaceutical Congress Association (IPCA)

Hosted by : Association of Pharmaceutical Teachers of India (APTI)

68th INDIAN PHARMACEUTICAL CONGRESS

ipc 2016 VISAKHAPATNAM

Theme: Quality Pharmaceuticals and Patient Welfare

PHARMA VISION 2020 — swasth bharat — PHARMACISTS FOR A HEALTHY INDIA

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. ROBERT DILTON

of MADRAS MEDICAL COLLEGE

has attended the 68th IPC as Registered Delegate held at AU College of Pharmaceutical Sciences,

Andhra University, Visakhapatnam, A.P. during 16th – 18th December 2016.

Mr. S.V. Veerramani	Dr. Rao Vadlamudi	Dr. T.V. Narayana	Dr. G. Nagarjuna Reddy
President, IPCA-2016	LOC, Chairman	LOC, Secretary	Chairman, Reg. Com.

Organised by: Indian Pharmaceutical Congress Association - Bangalore

Host: Indian Pharmaceutical Association - Mumbai

Venue: University Collage of Pharmaceutical Sciences