“DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL ANTI TUBERCULAR AGENTS TARGETING DECAPRENYLPHOSPHORYL-BETA-D-RIBOSE 2’EPIMERASE-1”

A dissertation submitted to

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

In the partial fulfilment of the requirements
For the award of the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by

Reg. No. 261415716

Under the guidance of

Dr. A. Jerad Suresh, M.Pharm., Ph.D., M.B.A.,

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

APRIL-2015-2016

COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003
TAMIL NADU

CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL ANTI- TUBERCULAR AGENTS TARGETING DECAPRENYL PHOSPHORYL-BETA-D-RIBOSE 2' EPIMERASE-1"** submitted by the candidate bearing the register No:**261415716** in partial fulfillment of the requirements for the award of degree of **MASTER OF PHARMACY IN PHARMACEUTICAL CHEMISTRY** by the Tamil Nadu **Dr. M.G.R Medical University**is a bonafide work doneby her during the academic year 2015-2016 at the **Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai- 600 003.**

Dr. A .JERAD SURESH
Principal,
Professor and Head,
Department of Pharmaceutical Chemistry,
College of Pharmacy,
Madras Medical College,
Chennai- 600 003.

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Dr. A .JERAD SURESH
Principal,
Professor and Head,
Department of Pharmaceutical Chemistry,
College of Pharmacy,
Madras Medical College,
Chennai- 600 003.

CONTENTS

ACKNOWLEDGEMENT

First and foremos00t I thank the Almighty for all the love, grace and blessings he has bestowed upon me and for strengthening me throughout, to successfully complete this dissertation.

I take this opportunity to express my gratitude to the people who were instrumental in the successful completion of this project.

I express my immense gratitude to Government of Tamilnadu for providing me the monthly scholarship.

At this moment of accomplishment, first of all I express my deep sense of gratitude to **Mrs. Vimala M.D.,** Dean, Madras Medical College, Chennai-3 for providing all facilities during the period of study.

It is my privilege and great pleasure to express my gratitude to my esteemed Principal and Guide **Dr. A. Jerad Suresh, M.Pharm., Ph.D., M.B.A.,** College of Pharmacy, Madras Medical College, Chennai-3. This work would not have been possible without his able guidance, support and encouragement. Under his guidance I successfully overcame many difficulties and learned a lot. His passion for perfection combined with eagerness and enthusiasm to impart knowledge to students is what I am in awe of. His knowledge and commitment to the highest standards inspired and motivated me. I thank him immensely for his guidance.

I submit my thanks to **Mrs. T. Saraswathy M.Pharm., Mrs. R. Priyadarshini M.Pharm., Ph.D., Mrs. P.G Sunitha M.Pharm., Ph.D, and Mr. Sathish M.Pharm., Ph.D,** Tutors, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College for their timely help and cooperation towards completing this project.

I feel obliged in thanking **Mr.T.Siva kumar, Mr.S.Baskar and Mrs.V.Mala,** Lab Supervisor **Mrs.S.Maheshwari, K.Murugeswari, Mrs.V.Geetha and Mr.K.N.Umapathi,** Lab Technicians Department of Pharmaceutical Chemistry, Madras Medical College for helping me to do this project.

I sincerely thank **Mrs.Devi, Mr.K.M.Noorulla M.pharm , Ms.P.R.Suriya,** Scholars for their support, valuable suggestions and comments during my project.

I convey my thanks to **Dr. Kishore Bhatt,** Professor and Head, Department of Microbiology, Maratha Mandal's Institute of Dental Science and Research Institute, Belgaum, Karnadaka and SASTRA University & IIT for helping me to carry out the spectral studies in due time.

My thanks are also due to my dear friends **N. Shantha sheela, S. Navaneetha, S. C. Suruthi, S. Nandhini, P. Vijayalakshmi, W. Nivetha, K. Madhesh, R. Kalaiselvi, M .Neelakandan, R. Sathyavani,** for their constant help and motivation. Without their support this project would not have materialized.

I heartily thanks to my dear friends **P.Karunya, P.Elamathi** for their care, support and encouragement throughout.

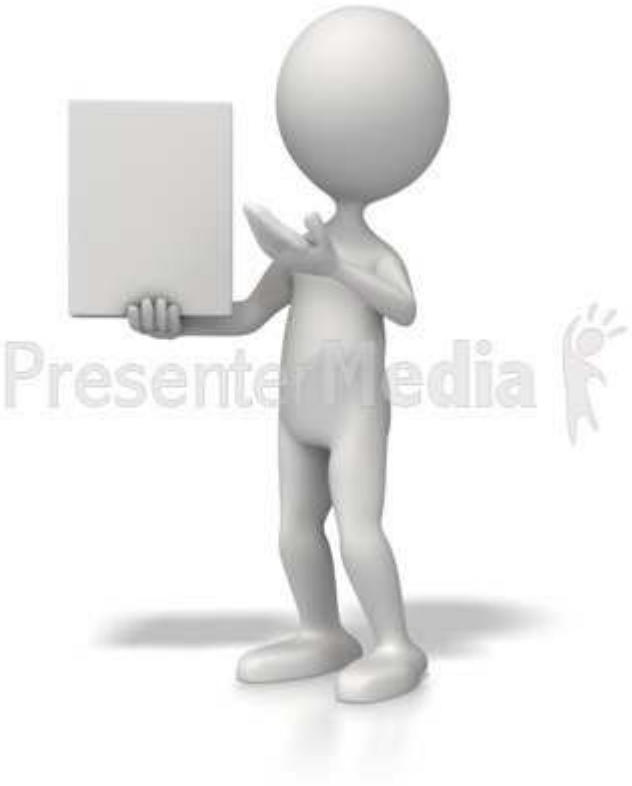
I take immense pleasure in thanking all my seniors, juniors and friends from all other departments.

Finally yet importantly, I Wish to express my heartfelt thanks to my beloved **Father, Mother, sister** and my **Husband** for their blessings, encouragement and wishes for the successful completion of this dissertation.

LIST OF ABBREVIATIONS

TB	Tubercule Bacillus
HIV	Human Immuno Deficiency Syndrome
AIDS	Acquired Immuno Deficiency Syndrome
BCG	Bacilli Chalmette Guerin
DOTS	Directly Observed Treatment Short-Course
MDR-TB	Multi Drug Resistant
XRD-TB	Extensively Drug Resistant-TB
LTBI	Latent Tuberculosis Infection
DprE1	Decaprenylphosphoryl-beta-d-ribose 2' Epimerase 1
CADD	Computer Aided Drug Design
QSAR	Quantitative Structural Activity Relationship
PSA	Polar Surface Area
OSIRIS	Optical, Spectroscopic and Infrared Remote Imaging System
OPLS	Optimized Potential For Liquid Simulation
TPSA	Total Polar Surface Area
SBDD	Structure Based Drug Design
LBDD	Ligand Based Drug Design
Log P	Partition Co-Efficient
WHO	World Health Organization
MIC	Minimum Inhibitory Concentration
PDB	Protein Data Bank
TLC	World Health Organization
IR	Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
GC-MS	Gas Chromatography-Mass spectroscopy
REMA	Resazurin Micro Plate Assay
MABA	Micro Plate Alamar Blue Assay
NRA	Nitrate Reductase Assay

DEDICATED TO MY FAMILY AND FRIENDS



INTRODUCTION

1. INTRODUCTION

BACKGROUND

Tuberculosis is a major disease causing death every year 1.8 million worldwide and represents the leading cause of mortality resulting from a bacterial infection. Introduction in the 60's of first-line drug regimen resulted in the control of the disease and TB was perceived as defeated.

In 2011, tuberculosis (TB) remained the second cause of death from infectious disease worldwide. It mainly affects the poorest countries of Africa and Southeast Asia. In 2010, according to the world health organization (WHO), TB incidence and prevalence were estimated at 8.8 and 12 million cases respectively about 1.1 million among HIV-negative people and 0.35 million among HIV-positive people died from TB. Most importantly, one third of the world population is infected with latent infection and 10% of those infected people will develop active TB in their life.

The directly observed treatment short- course (DOTS), a multiple therapy program developed by WHO is one of the most efficient weapons against the global TB epidemic. Nevertheless, the treatment success rate struggles to reach the target of 85% .Unfortunately; first-line treatment can fail due to poor compliance which leads to the emergence of multidrug resistance (MDR) strains of M.Tuberculosis. The number of TB drugs in preclinical and clinical development is today higher than that during the past 40 years.[1]

TB IN INDIA

India is the country with the highest burden of TB, with World Health Organization (WHO) statistics for 2014 giving an estimated incidence 2.2 million cases of TB for India out of a global incidence of 9 million. The estimated TB prevalence figure for 2014 is given as 2.5 million. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB.[2]

CURRENT THERAPIES

Tuberculosis (TB) is caused by bacteria mycobacterium tuberculosis that most often affect the lungs. Tuberculosis is curable and preventable. In 1882, The German physician Robert Koch isolated the bacterium. Tuberculosis is contagious and airborne disease.

In 1944, streptomycin was to treat tuberculosis (TB).This amino glycoside interferes with protein biosynthesis through an interaction with the small 30S subunit of the ribosome. The discovery of Para amino salicylic acid in 1946 was quickly followed by the important discovery of Isoniazide (INH), as one of the most active anti-TB drugs used today. Inhibition of mycolic acids biosynthesis, one of the essential components of the mycobacterium cell wall was determined as the mechanism of action. Pyrazinamide (PZA) appeared as a potential Anti-TB drug in 1952.

The TB treatment in the 1980s was a great success as it allowed to shorten the duration of the therapy from 9 to 6 months. Ethambutol (EMB) and Rifampin (RIF), the two last derivatives used in the TB first-line treatment, were discovered during the

60's .Ethambutol is an ethylenediamine discovered in 1961, which affects the cell wall by specifically targeting the polymerization of arabinogalactan and lipoarabinomannan. Finally, Rifampin appeared as a drug of choice for TB treatment around 1970, by acting on replicating and non-replicating mycobacteria.This derivative belongs to the rifampicin family and inhibits bacterial RNA synthesis by binding to the b-subunit of the DNA-dependent polymerase.

The current standard regimen (DOTS) for TB recommended by WHO is a combination of isoniazide, rifampicin, ethambutol, and pyrazinamide for 6 months therapy. To treat MDR-TB, WHO recommended the use of second-line drugs which include amino glycosides (kanamycin, amikacin), Capreomycin, Cycloserin, Para-aminosalicylic acid, Thionamides (Ethionamide, Proethionamide) and fluoroquinolones (Ciprofloxacin, Ofloxacin, Levofloxacin).[1]

BACTERIOLOGICAL PROFILE OF MYCOBACTERIUM TUBERCULOSIS

SPECIES

The mycobacterium tuberculosis complex consists of Mycobacterium bovis, Mycobacterium africanoum and Mycobacterium canettii, Mycobacterium capre, Mycobacterium microti and Mycobacterium pinnipedi.[3]

Tuberculosis (TB) is caused by the obligate human pathogen, Mycobacterium tuberculosis.Mycobacteria are a distinctive rod shaped, non spore forming aerobic bacteria that share a common property of a lipid-rich cell-wall that avidly retains carbol fushion dye in the presence of acidic alcohol (acid fast staining). Mycobacteria typically measures 0.5µm to 3µm.[4]

CELL STRUCTURE

Mycobacterium tuberculosis is a slow-growing aerobic rod-shaped bacterium. The cell wall is composed of two segments, upper and lower. Beyond the membrane is Peptidoglycon (PG) in covalent attachment to arabinogalactan (AG), which in turn is attached to mycolic acids with their long meromycolate and short α chain. This cell wall core complies of the mycolyl arabinogalactan-petidoglycon (mAGP) complex. The upper segements is composed of free lipids, some with longer fatty acids complementing the longer chains. Interspersed with the cell-wall proteins are the phosphatidylinositol mannosides (PIMs), the phthiocerol containing lipids, lipomannan (LM), and lipoarabinomannan (LAM).When cell walls are disrupted, for instance when extracted with various solvents, free lipids,proteins,LAM and PIMs are solubilised and the mycolic acid-arabinogalactan-peptidoglycon complex remains as the insoluble residue.[5]

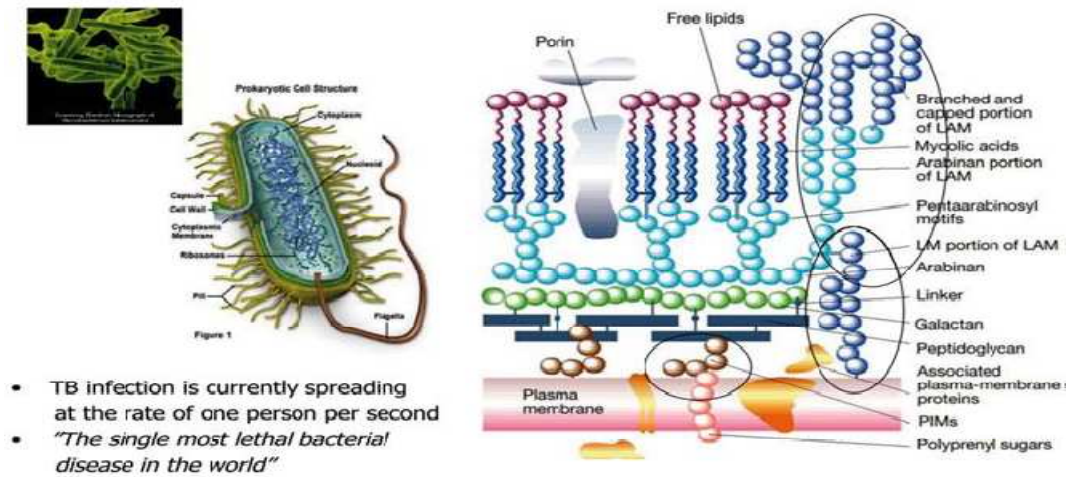


Fig 1. Structure of cell wall

GENOME STRUCTURE

Mycobacterium tuberculosis H37RV was first isolated in 1905. It has remained pathogenic and is the most widely used strain in tuberculosis research. The complete genome sequence and annotation of the strain was published in 1998.Approximately 9%of the M.tuberculosis genome consists of two related families of genes that have been named the PE and PPE families. These names derive from the presence of conserved proline glutamate(PE) or proline-proline-glutamate(PPE) residues near the N terminus of the predicted proteins of the family.These predicted protein share a conserved N terminal domain of 110 amino acids (PE) or 180 aminoacids (PPE)with divergent C-terminal sequence. A Subfamily of the PE family has been designated the PGRS proteins based on the C-terminal extension rich in a repetitive glycine/alanine (GA) motif. A large portion of the coding capacity of the M.tuberculosis genome is putatively involved with lipid biosynthesis or lipid degradation.[4]

CHOLESTROL CATABOLISM

Cholesterol metabolism has been studied extensively because of its possible therapeutic application in Tuberculosis (TB) infection. It has been shown numerous times that TB infections require cholesterol for virulence in vivo, because Mycobacterium tuberculosis (MTB), the causative agent, utilizes cholesterol as an electron and carbon source during infection.[6]

PATHOPHYSILOGY OF M.TUBERCULOSIS

Mycobacterium tuberculosis is spread by small airborne droplets, called droplet nuclei. Once inhaled, infectious droplets settle throughout the airways. The majority of the bacilli are trapped in the upper parts of the airways where the mucus-secreting globlet cells exist. Bacteria in droplets that bypass the mucociliary system and reach the alveoli are quickly surrounded and engulfed by alveolar spaces. The Mycobacterium lipoarabinomannan is a key ligand for a macrophage receptor.The complement of C3 bind to the cell wall and enhance recognition of the mycobacteria by macrophages. Opsonization by C3is rapid, even in the air spaces of a host with no previous exposure to M.tuberculosis. After being ingested by the macrophages, the mycobacteria continue to multiply slowly, with bacterial cell division occurring every 25 to 32 hours. Regardless of whether the infection becomes controlled or progress, initial development involes protection of proteolytic enzymes and cytokines by macrophages in an attempt to degrade the bacteria. Released cytokinins attract T lymphocytes to the site, the cells that constitute cell-mediated immunity.Macrophages then presents mycobacterial antigen on the surface to the T cells.This initial immune process continues for 2 to 12 weeks;the microorganisms continue to grow until they reach the sufficient numbers to fully elicit the cell-mediated immune response, which can be detected by skin test. For persons with intact cell mediated immunity, the next defensive step is formulation of granulomas around the M.tuberculosis organism. These nodular-type lesions form an accumulation of activated T lymphocytes and macrophages, which creates a microenvironment that limites replication and the spread of the mycobacteria. M.tuberculosis organism can change their phenotypic expression, such as protein regulation, to enhance survival. By 2 or 3 weeks, the necrotic environment resembles soft cheese, often referred to caseous necrosis, and is characterized by low oxygen levels, low P^H and limited nutrients. M.tuberculosis requires oxygen to grow. Ziehl-neelsen or acid-fasting staining is used.[7]

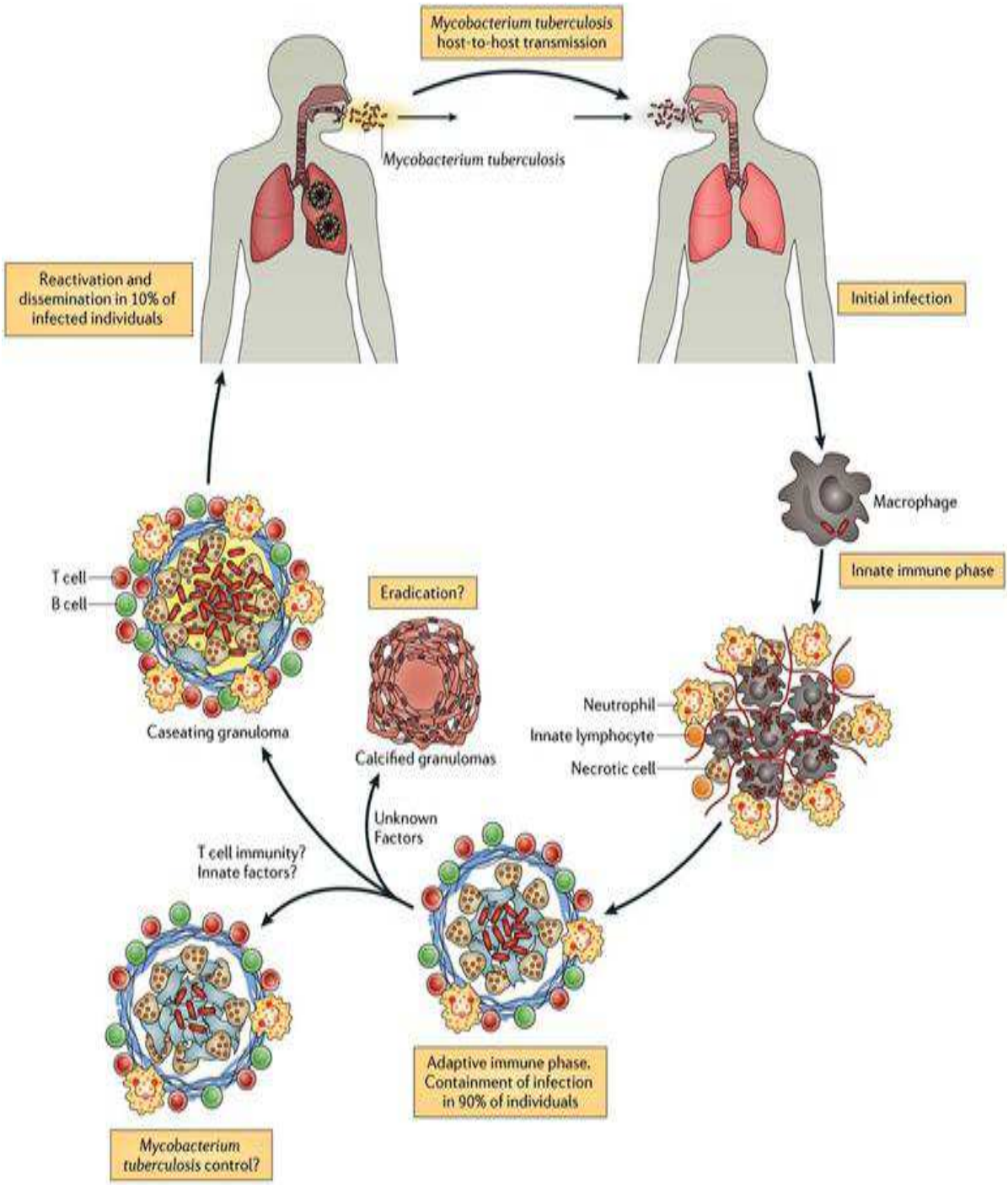


Fig. 2. Pathophysiology of M.Tuberculosis

MEDICINAL CHEMISTRY

In the so called pre-scientific period, natural products having a history as folk remedies were in use, but little of the drug therapy of today is based on these remedies. Some of natural products currently used either themselves or as derivatives, were often used originally for other purpose, such as arrow poisons, part of religious or other rituals, or even cosmetics. Examples of such products include opium, belladonna, cinchona bark, ergot, curare, nutmeg, Calabar bean, foxglove and squill.

Development of drug therapy could not progress until knowledge of anatomy and physiology had reached the status of science. The empiric observation of Harvey and Sydenham were of great importance to this development in the seventeenth century. The work of magendie (1783-1855), an instructor of anatomy in Paris, probably represents the earliest application of the experimental medicine.

Following the French revolution, the study and classification of disease made considerable progress. Ineffective remedies were recognized and discarded. In Germany, much of the drug discovery in the nineteenth century resulted from the investigation in the chemical industries mainly concerned with dyes. It was not until the twentieth century, that the search for new drugs entities or classes took place in university laboratories.

The concept of the drug-receptor interaction has undergone much modification from 1960s to 1990s.The use of computer graphics to portray drug-receptor interaction has also been a notable interaction has been a notable development of the decade.

The approaches to practice of Medicinal Chemistry has developed from an empiric one involving organic synthesis of new compounds, based largely on modification of structure of known activity, to one that is more logical and less intuitive is mostly because of advancement in Molecular Biology, Pharmacology, and Enzymology.[8]

The primary objective of medicinal chemistry is to design and discover new compounds that are suitable for use as drugs.[9]

ENZYME PROFILE

Resistance against currently used Antitubercular therapeutics increasingly undermines efforts to contain the worldwide tuberculosis (TB) epidemic. Recently, benzothiazinone (BTZ) inhibitors have shown nanomolar potency against both drug-susceptible and multidrug-resistant strains of the tubercle bacillus. However, their proposed mode of action is lacking structural evidence. The crystal structure of the BTZ target, FAD-containing oxido-reductase Mycobacterium tuberculosis DprE1, which is essential for viability.

Different crystal forms of ligand-free DprE1 reveal considerable levels of structural flexibility of two surface loops that seem to govern accessibility of the active site. Structures of complexes with the BTZ-derived nitroso derivative CT325 reveal the mode of inhibitor binding.

More recently nitro-benzothiazinone (BTZs) have emerged as a promising class of inhibitors, effective against both drug-susceptible and MDR/XDR strains of Mycobacterium tuberculosis at significantly lower minimum inhibitory concentrations (MICs) than either isoniazid or Rifampicin, in combination with reduced toxicity.

Biochemical studies showed that rv3790 and the neighboring gene rv3791 code for proteins that act in concert to catalyze the epimerization of decaprenylphosphoryl ribose (DPR) to decaprenylphosphoryl arabinose (DPA) a precursor for arabinan synthesis without which a complete mycobacterial cell wall cannot be produced.

DPA is the sole known donor substrate for a series of membrane-embedded Arabinosyl transferases, including the Ethambutol targets EmbC, EmbA, and EmbB (9). Essentiality of DPA supply and lack of alternative synthetic pathways position DprE1, which is highly conserved in mycobacteria, and DprE2 at a critical

intersection of cell wall biosynthesis. A motion confirmed by transposon mutagenesis. This situation has led DprE1as a magic drug target.[10]

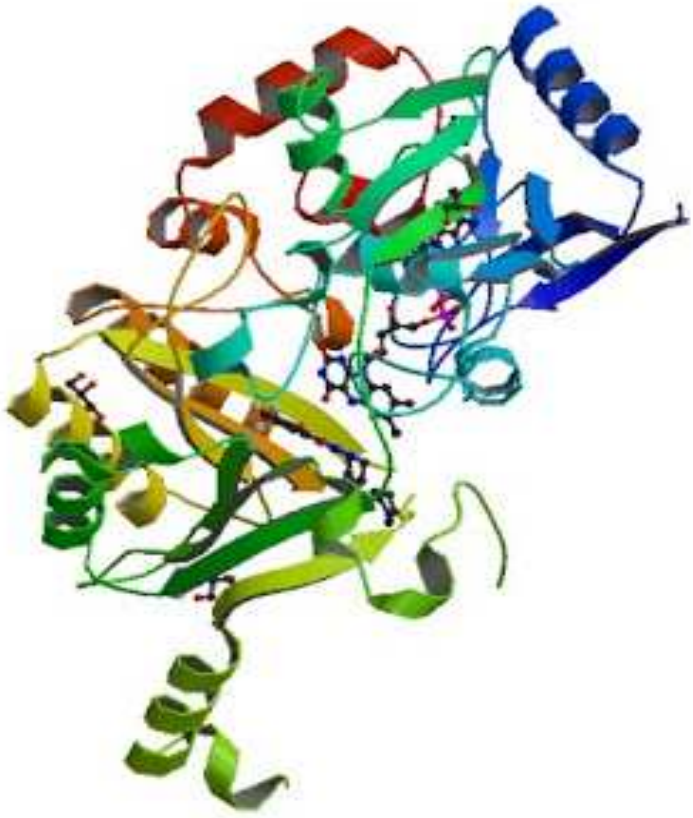


Fig 3. DprE1 enzyme

General annotation[11]

Table. 1

Gene name	dprE1
Rv number	Rv3790
Type	CDS
Function	Together dprE2(Rv3791,catalyzes epimerization of decarprenyl phosphoryl ribose (DPA) to decaprenyl phosphoryl arabinose (DPA) in arabinan synthesis.
Product	Decaprenylphosphoryl-beta-D-ribose 2'-epimerase-1
Molecular mass	50163.2
Isoelecric point	7.769
Gene length(bp)	1386
Protein length	461
Location(kb)	4235.78
Functional category	Lipid metabolism
Proteomics	Identified in the membrane fraction of M.tuberculosis H37Rv using ID-SDS-PAGE and uLC-MS/MS(see Gu et al.,2003). Identified in the membrane fraction of M.tuberculosis H37Rv using 2DLC/MS (see Mawuenyega et al., 2005). Identified by mass spectroscopy in triton X-114 extracts of M.tuberculosis H37Rv(see Malen et al.,2010).Identified by mass spectroscopy in the membrane protein fraction and whole cell lysates of M.tuberculosis H37Rv but not the culture filtrate(See de souza et al.,2011).Translation start site supported by proteomics data(See kelkar et al.,2011)
Mutation	Essential gene by Himar-based transposon mutagenesis inH37Rv strain(See Sassetti et al.,2003).Essential gene for in vitro growth of H37Rv, by sequencing of Himar-based transposon mutagenesis (See Griffin et al.,2011)

DRUG DESIGN PROFILE

The phrase "drug design" is to some extent a misnomer.[12] what is really meant by drug design is ligand design (i.e., design of a small molecule that will bind tightly to its target). Drug design involves the design of small molecules that are complementary in shape and charge to the bimolecular target with which they interact and therefore will bind to it. Drug design is sometimes referred to as rational drug design or more simply rational design is the inventive process of finding new medications based on the knowledge of a biological target. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit.

There are two major types of drug design. The first is referred to as ligand-based drug design and the second, structure-based drug design.[12]

TYPES OF DRUG DESIGN

LIGAND BASED DRUG DESIGN

Ligand-based drug design or indirect drug design relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. In other words, a model of the biological target may be built based on the knowledge of what binds to it, and this model in turn may be used to design new molecular entities that interact with the target. Alternatively, a quantitive structure-activity relationship (QSAR), in which a correlation between calculated properties of molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs. [12]

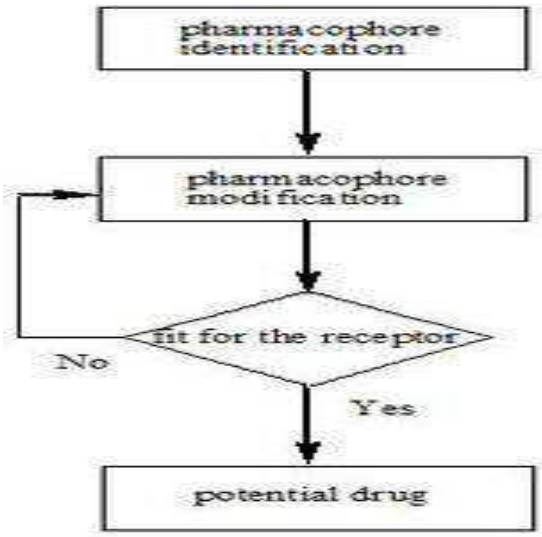


Fig. 4

STRUCTURE BASED DRUG DESIGN

Structure based drug design or direct design relies on knowledge of the three dimensional structure of the biological target obtained through methods such as X-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of medicinal chemist. Current methods for structure based drug design can divided roughly into two categories. The first category is about finding ligand for a given receptor, which is usually referred as database searching. In this case, a large number of potential ligand molecules are screened to find those fitting the binding pocket of the receptor. The key advantage of database searching is that it saves synthetic effort to obtain new lead compounds. Another category of structure based drug design methods is about building ligand, which is usually referred as receptor-based drug design. In this case, ligand molecules are built up within the constraints of the binding pocket by assembling small pieces in a stepwise manner. These pieces can be either individual atoms or molecular fragments.[12]

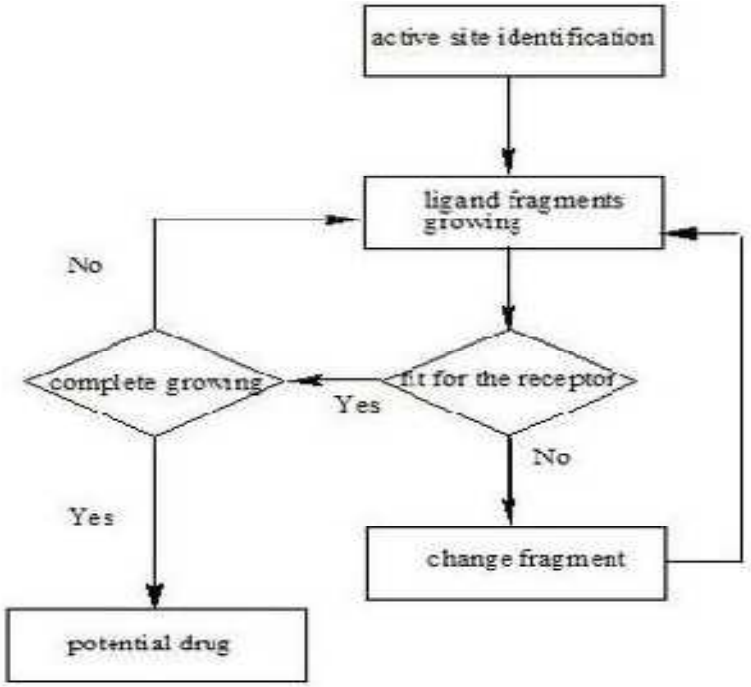


Fig. 5

ACTIVE SITE IDENTIFICATION

Active site identification is the first step. It analyzes the protein to find the binding pocket, derives key interaction sites within the binding pocket, and then prepares the necessary data for ligand fragment link. Both ligand and protein atoms need to be classified and their atomic properties should be defined, basically, into four atomic types;

- Hydrophobic atom: All carbons in hydrocarbon chains or in aromatic groups.
- H-bond donor: Oxygen and nitrogen atoms bonded to hydrogen atom [s].
- H-bond acceptor: Oxygen and sp2 or sp hybridized nitrogen atoms with lone electron pair [s].
- Polar atom: Oxygen and nitrogen atoms that are neither H- bond donor nor H-bond acceptor, sulfur, phosphorus, halogen, metal, and carbon atoms bonded to hetero-atom[13]

SCORING METHOD

Structure-based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying accepted principles of molecular recognition. The basic assumption underlying structure-based drug design is that a good ligand molecule should bind tightly to its target.[12]

SCREENING AND DESIGN

The process of finding a new small molecule (ligand) against a chosen target for a particular disease usually involves high-throughput screening (HTS).

The structure-activity relationship (SAR) is to improve certain features of the lead compound:

- Increase activity against the chosen target
- Reduce activity against unrelated targets
- Improve the drug likeness or ADME properties of the molecule.

This process will require several iterative screening runs, during which, it is hoped, that properties of the new molecular entities will improve, and allow the favored compounds to go forward to in vitro and in vivo testing for activity in the disease model of choice. A range of parameters can be used to assess the quality of a compound, or a series of compounds, as proposed in the Lipinski's Rule of Five. Such parameters include calculated properties such as clog P to estimate lipophilicity, molecular weight, polar surface area and measured properties, such as potency, in-vitro measurement of enzymatic clearance etc. Some descriptors such as ligand efficiency (LE) and lipophilic efficiency (LiPE) combine such parameters to assess drug likeness.Other methods, such as virtual high through put screening, where screening is done using computer-generated models and attempting to "dock" virtual libraries to a target, are also often used.

Another important method for drug discovery is FBLD drug design, whereby the biological and physical properties of the target are studied, and a prediction is made on the sorts of small molecules that might (e.g.) fit into an active site. The fragment-based lead discovery (FBLD), novel pharmacophore can emerge very rapidly from these exercises. In general, computer-aided drug design is often used to try to improve the potency and properties of new drug leads.[12]

COMPUTER-AIDED DRUG DESIGN

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: [12]

- 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.[12]

MOLECULAR PROPERTY PREDICTION

All the data set molecules were subjected to the toxicity risk assessment by using Osiris program, which is available online. The OSIRIS property Explorer shown in this page is an integral part of Actelion's in house substance registration system. It allows drawing chemical structures and also calculates various drug relevant properties whenever a structure is valid. 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 behave

Molecular property prediction includes

- Toxicity risk assessment
- Clog prediction
- Solubility prediction
- Molecular weight
- Drug linkers prediction
- Drug linkers

TOXICITY RISK ASSESSMENT

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. The prediction process relies on a precomputed set of structural fragment that give rise to toxicity alerts in case they are encountered in the structure currently drawn. These fragment lists were created by rigorously shreddering all compounds of the RTECS database known to be active in a certain toxicity class like mutagenicity, Tumorigenicity, Irritating effects and Reproductive effects. [13]

Clog P 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. (13)

SOLUBILITY PREDICTION

The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption and therefore the general aim is to avoid poorly soluble compounds.(13)

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. (13)

DRUG LIKENESS

Drug likeness is a qualitative concept used in drug design for how "drug like" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. A drug like molecule has properties such as: Solubility in both water and fat, as an orally administered drug needs to pass through the intestinal lining after it is consumed, carried in aqueous blood and penetrate the lipid cellular membrane to reach the inside of a cell. 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". (13)

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. (13)

SOLUBILITY PREDICTION

The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. Typically, a low solubility goes along with a bad absorption and therefore the general aim is to avoid poorly soluble compounds. (13)

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. (13)

DRUG LIKENESS

Drug likeness is a qualitative concept used in drug design for how "drug like" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. A drug like molecule has properties such as: Solubility in both water and fat, as an orally administered drug needs to pass through the intestinal lining after it is consumed, carried in aqueous blood and penetrate the lipid cellular membrane to reach the inside

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LIPINSKI'S RULE OF FIVE[14-15]

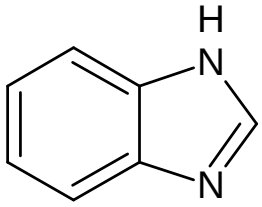
Lipinski's rule of five also known as the Pfizer's rule of five or simply the Rule of five (RO5) is to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. The rule was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

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active.

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

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms).
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular mass less than 500 Daltons.
- An octanol-water partition coefficient log P not greater than 5.

BIOLOGICAL ACTIVITY OF BENZIMIDAZOLE



Benzimidazole is the heterocyclic compound formed from benzene and imidazole ring wide interest because of their diverse biological activity and clinical applications, they are remarkably effective compounds both with respect to their inhibitory activity and their favorable selectivity ratio. Reported nucleus is a constituent of vitamin-B12. It occurs as white crystals. It is used as muscle relaxant.[16]

SYNONYMS[17]

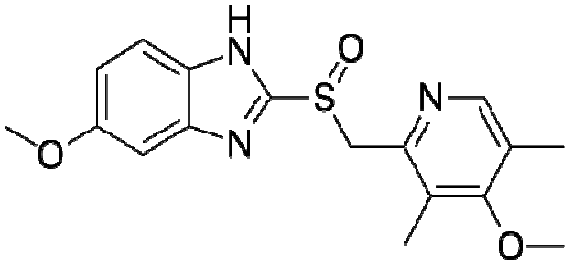
- Benziminazole,
- 3-benzodiazole,
- Azindole,
- Benzoglyoxaline,
- 3-azaindole,
- 1,3-diazaindene
- Meting point 170-1720^{0}C

BIOLOGICAL ACTIVITY[18]

- Antimicrobial,
- Antiviral,
- Antidiabetic,
- Antiulcer,
- Anticancer.

Anti-ulcer drugs

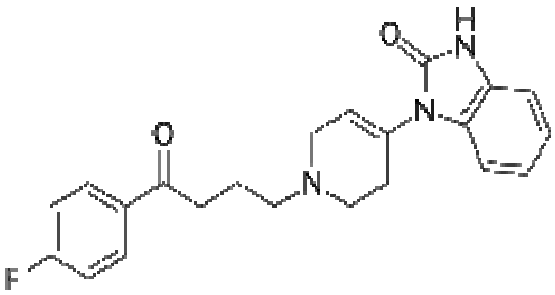
These are the drugs which inhibits both basal and stimulated gastric acid secretion. Some drugs containing benzimidazole nucleus are Pantoprazole, Rabeprazole, Lansoprazole, Omeprazole etc Ex. Omeprazole



Anti-psychotic agents

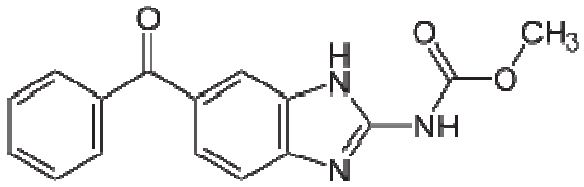
In psychosis thinking of patient becomes illogical, bizarre and loosely organized. Patient has difficulty in understanding reality and their own conditions. Some drugs containing benzimidazole nucleus are droperidol, pimozide, and benperidol.

Ex. Droperidol



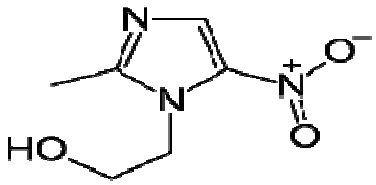
Anthelmintic

These are the drugs that either kill or expel infesting helminthes. Some drugs containing benzimidazole nucleus are Thibendazole, Mebendazole, and Albendazole etc. Ex. Mebendazole



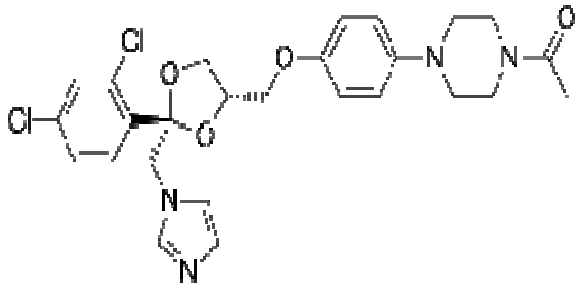
Anti-protozoal agents

These are the drugs which are used to treat the amoebiasis caused by E.histolytica. They exert cytotoxicity by damaging DNA and result in DNA helix destabilization strand breakage. The antiprotozoal drugs containing imidazole nucleus are metronidazole, benznidazole. Ex. Metronidazole



Antifungal

These are the drugs used for superficial and deep fungal infections. Fungal infections are termed mycoses and in divided in to superficial infections (skin, nails, and scalp) and systemic infections (deeper tissues and organs) some conditions are blastomycosis, histoplasmosis, candidiasis, coccibiomycosis etc. superficial fungal infections can be classified in to the dermatomycoses and candidiasis. Most common antifungal agents containing imidazole nucleus are Clotriamazole, Miconazole, Ketoconazole.[18]. Ex. Ketaconazole



REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The Purpose of Literature Review is to:

- Establish a theoretical frame work for a topic /subject area
- Define key terms and terminology
- Identify studies, models, case studies etc supporting a topic
- Define /establish an area of study

The review on following works provided basic information about the target enzyme, DprE1 and its function

Sarah M. Batt., *et al.,* (2012) Structural basis of inhibition of Mycobacterium tuberculosis DprE1 by benzothiazinone inhibitors .[10]

Maria Loreto Incandela., *et.al.,(*2013) reported that DprE1, a new taxonomic marker in mycobacteria.[19]

The following works throws a light upon the various genomic aspects of M.tuberculosis and also various targets intended for drug action:

Donald R Ronning., *et al.,* (2012) Targeting the mycobacterial envelope for tuberculosis drug development.[20]

Sarala Menon., *et al.,* (2012), studied the Drug resistance profiles of Mycobacterium tuberculosis isolates to first line anti-tuberculous drugs.[21]

P.Mudassar., *et al.,* (2011) Had a Brief review on Multi Drug resistant Mycobacterium tuberculosis.[22]

Christian Lienhardt., *et al.,* (2010) Had a review on New drugs and new regimens for the treatment of tuberculosis: review of the drug development pipeline and implications for national programmes.[23]

Sarah L. Kinnings., *et al.*, (2010), reviewed the Mycobacterium tuberculosis Drugome and Its Polypharmacological Implications.[24]

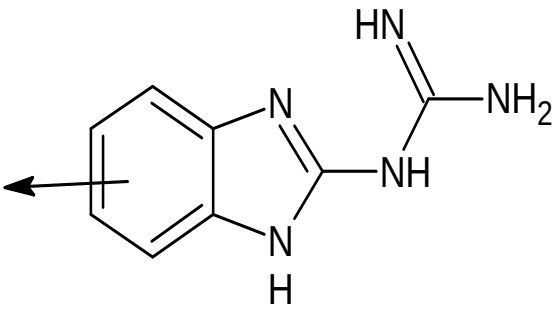
The review on following works provided ideas for synthesis of the desired chemical entities:

Elina R. Marinho.,et al The reaction of *o*-phenylenediamine with ethoxymethylene compounds and aromatic aldehyde,(2009).[25]

A. M. Soliman, Synthesis and biological activity of dihydroimidazole and 3,4-dihydrobenzo[4,5]imidazo[1,2-a][1,3,5]triazins, (2012) .[26]

Shaaban K Mohamed, Eco-Friendly Synthesis of Pyrimidine and Dihydropyrimidinone Derivatives under Solvent Free Condition and their Anti-microbial Activity, (2013) [27]

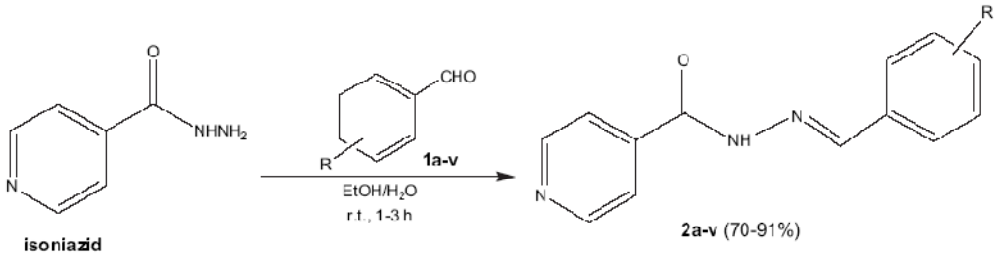
Babu K,Synthesis of novel benzimidazole derivatives.[28]



SHIFF BASE:

Zhaoqi Yang, Pinhua Sun, Compare of three ways of synthesis of simple Schiff base, 2006.[29]

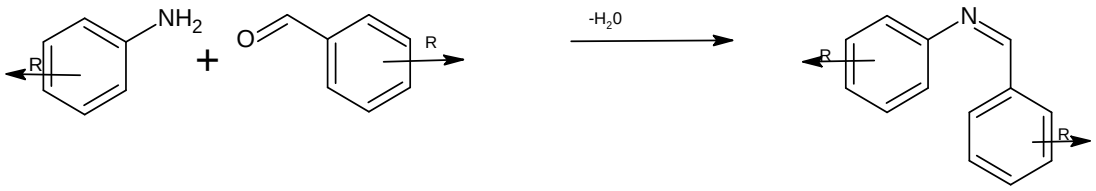
Marcus Vinı´cius Nora de Souza ,carried out Synthesis and antimycobacterial activity of (E)-N0-(monosubstituted-benzylidene) isonicotino hydrazide derivatives,2008.[30]



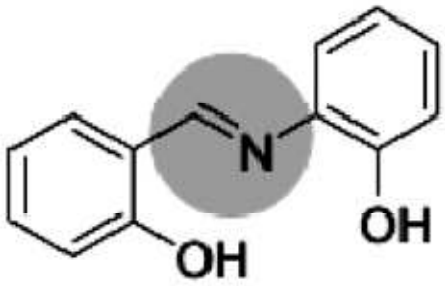
A.Mobinikhaled, et al.,An efficient synthesis of Schiff bases containing Benzimidazole moiety catalyzed by transition metal nitrates,(2010).[31]

Mostafa M. H. Khalil,et al., Synthesis and characterization of a novel schiff base metal complexes and their application in determination of iron in different types of natural water, 2012.[32]

Sandeep Miglani, et al., The rapid synthesis of schiff-bases without solvent under microwave irradiation and their antimicrobial activity, (2012).[33]



K. Brodowska,et al., Schiff bases – interesting range of applications in various fields of science,(2014).[34]



Panneer Selvam .T et al., synthesized a novel series of 2-substituted benzimidazole derivatives were synthesized and characterized.[35]

The following literatures were surveyed in depth to provide supporting data for the drug design study

LaurieAT.,et.al.,(2005) Q site finder"on energy based method for the prediction of protein –ligand binding site" Bioinformatics,[36]

http ; // www.modelling leads.ac.uk /q site finder / [37]

Sanju joy ,parvathy S Nair.,et.al.,(2006) "*Detailed* comparison of Pro-ligdocking efficiency of GOLD" , a commercial package and Argus lab, a licensable freeware [insilico biology 6,0053 [2006] [38]

The review on following works revealed the basics of Alamar blue assay for evaluating the anti-mycobacterial action.

David A. J. Moore., *et al.,* (2008), Inter- and Intra-Assay Reproducibility of Micro plate Alamar Blue Assay Results for Isoniazid, Rifampicin, Ethambutol, Streptomycin, Ciprofloxacin, and Capreomycin Drug Susceptibility Testing ocobacterium tuberculosis. [39]

Todd P. Primm., *et al.,* (2007), Recent Advances in Methodologies for the Discovery of Antimycobacterial Drugs.[40]

AIM AND OBJECTIVES

3. AIM AND OBJECTIVES

With the ongoing progress in protein crystallography and NMR, structure-based drug design is gaining increasing importance in the search for new drugs. Modeling starts from the 3D structure of a target protein in order to construct molecules which are complementary to a binding site, in their geometry as well as in the pattern of their physicochemical properties around the molecules The present study relates to the synthesis of various aryl carboxylic acid derivatives and subsequent screening for their anti-tubercular activity. Due to several toxic effects of isoniazid, attempts were made to eliminate the toxicophore and substituting with a group contributing to the anti-tubercular action. This work also aims the same motive and the compounds were synthesized according to the developed and valid synthetic route.

DOCKING

A chemical data base consisting 500 compounds of various scaffolds had been sketched and docked against the protein target of DPrE1. Based upon the docking score the 50 compounds were selected. For further modifications /derivatisation in order to achieve improved binding affinity and then once again docked against the same target.

Based upon the docking score the first 20 compounds with diverse scaffolds were selected. From these 20 compounds 5 top ranked compounds were selected based upon the Insilco toxicity prediction and then synthetic feasibility**.**

The compounds are as follows:

COMPOUND1:

N-1*H*-benzimidazol-2-yl-*N*'-{(*Z*)-[4-(dimethylamino)phenyl]methylidene} guanidine

COMPOUND2:

N-1*H*-benzimidazol-2-yl-*N*'-[(*Z*)-(4-hydroxyphenyl)methylidene]guanidine

COMPOUND3:

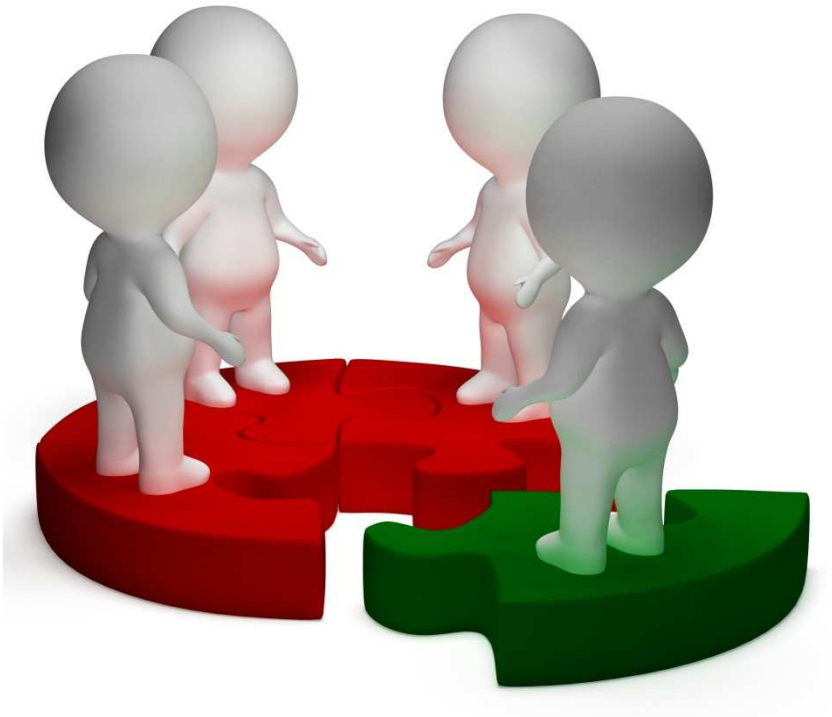
N-1*H*-benzimidazol-2-yl-*N*'-[(*Z*)-(2,4-dichlorophenyl)methylidene]guanidine

CHARACTERIZATION

The above compounds will be characterized by using infrared spectroscopy, nuclear magnetic spectroscopy and mass spectroscopy.

BIOLOGICAL EVALUATION

The synthesized compounds will be screened for their anti-tubercular activity by various in-vitro methods.



MATERIALS AND METHODS

4.METERIALS AND METHODS

I) DOCKING

1) MOLECULAR DOCKING BY ARGUS LAB SOFTWARE

Docking analysis of synthesized compounds i.e. ligands will be carried out by **Argus lab**^{R} docking software. Docking allows the medicinal chemist to virtually screen a set of compounds and predict the strongest binding capacity based on various scoring function. It explores ways in which two molecules such as ligand and receptor (protein) fit together and docks to each other well. The molecule binding to a receptor inhibits its function and thus acts as drug.

Argus lab 4.0 distributed freely for windows platforms by planaria software. It is an introductory molecular modeling package with academics. Argus docking engine implementry in Argus lab approximates an exhaustive search method which is similar to DOCK and GLIDE. Flexible ligand docking is possible with Argus lab, where the ligand is described as torsion tree and grids are constructed that overlay the binding site. The accuracy of the Argus lab docking algorithm takes into account, the key features such as the nature of the binding site and the number of rotatable bonds to the ligand.

Molegro molecular viewer

Molegro molecular viewer is an application which helps in analyzing the energies and interaction of the binding site.

Q-site finder

Q-site finder is an energy-based method for protein-ligand binding site prediction. During prediction we use the crystal structures of macromolecules (receptor) with small substrates (pdb ID). Identifying the location of binding sites on a protein is of fundamental importance for a range of applications including molecular docking. It uses the interaction energy between the protein and a simple vanderwaals probe to locate energetically favorable binding sites.

A. **PREPARATION OF PROTEIN:**

STEP 1

- Enter protein pdb ID (3VAE) in the protein data bank in the protein data bank.
- Go to download files and select pdb as text file.
- Save the downloaded pdb text file to 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, do not delete cofactor
- Open water press shift, select all water molecules and delete.
- Add hydrogen atoms.
- Go to calculation on toolbar → energy by UFF method → start.
- Save the prepared protein as *.agl file format in the desktop.

B. IDENTIFICATION/ SELECTION OF ACTIVE SITE

STEP 1

- Q-site finder was opened through online.
- The pdb format of the protein was imported.
- Found all the active site and make a list out of the common amino acid residues.

STEP 2

- residues → open amino acids was opened.
- Control was selected and select the amino acids which were listed from the Q-site finder.
- Make sure that all the amino acid residues listed are selected.

- Right click on the mouse → make a group from the selected residues → give name → Binding site → OK.

C. PREPARATION OF LIGANDS

- Drawn the structure from chem. Sketch and save as MDL mol format.
- The ligand wae imported into workspace of Argus lab.
- Clean geometry → clean hybridization. Select the ligand; right click on the mouse → make a group from the residues → give name→ ligand→ OK.
- The ligand was selected; right click on the mouse → make a group from the residues → give name→ ligand→ OK.

D. DOCKING PARAMETER :

- Calculation was selected from the toolbar → Dock a ligand.
- 'Argus Dock' as the Docking engine.
- 'Dock' was selected as calculation type.
- 'Flexible' for the ligand.
- Ascore as the scoring function.
- Calculation size.
- Docking was started.

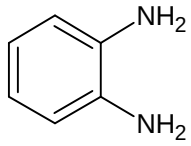
Save the Docked protein Ligand complex as Brookhaven pdb files (*.pdb)

E. VISUALIZATION/INTERPRETATION OF DOCKING :

- Molegro molecular viewer will help in analyzing the energies and interaction of the binding.

II) REACTANT PROFILE[41]

O-PHENYLENE DIAMINE:



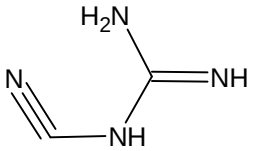
Molecular Formula :$C_6H_8N_2$

Molecular Weight :108.14

Description : brown in colour

Melting point :102-104^{0}C

CYANOQUANIDINE:



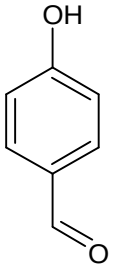
Molecular Formula :$C_2H_4N_2$

Molecular Weight :84.08

Description : White crystals

Melting point :209^{0}C

4-HYDROXYL BENZALDEHYDE:



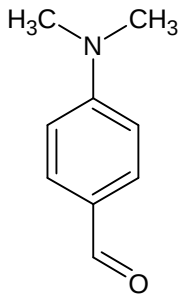
Molecular Formula :$C_7H_6O_2$

Molecular Weight :121.12

Description : Light yellowish to light brown

Melting point :191^{0}C

N,N' DIMETHYL BENZALDEHYDE:



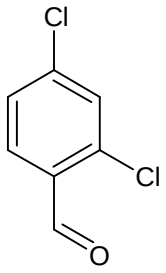
Molecular Formula :$C_9H_{11}NO$

Molecular Weight :149.19

Description : White crystalline powder

Melting point :72-75^{0}C

2,4 DICHLORO BENZALDEHYDE:



Molecular Formula :$C7H_4Cl_2O$

Molecular Weight :175.01

Description : White crystalline powder

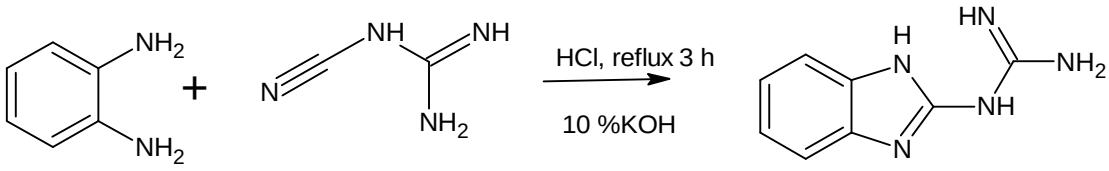
Melting point :233^0 C

3.SYNTHETIC METHODOLOGY

STEP1

Synthesis of 2-benzimidazolylguanidine[26]

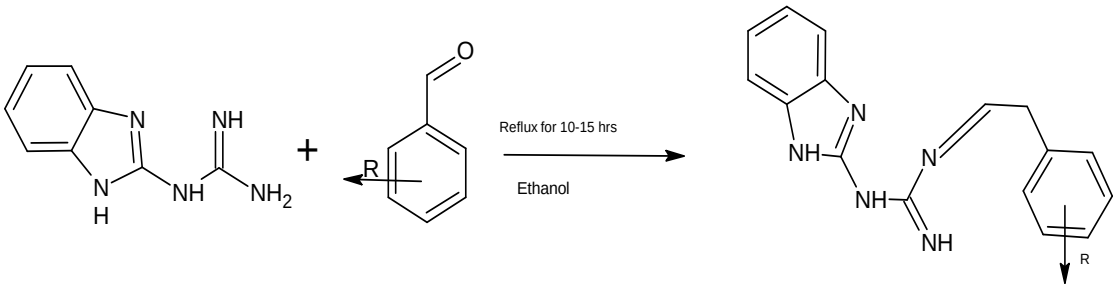
A mixture of *o*-phenylenediamine (100 mmol), cyanoguanidine (100 mmol) and concentrated Hydrochloric acid (20ml) in water (200 ml) is heated under reflux for 3 hrs. The reaction mixture is cooled at 0°C and KOH (10%; 50 ml) was added slowly. The precipitates of 2-guanidinobenzimidazole is collected by filtration, washed with water, dried and recrystallized by ethanol.



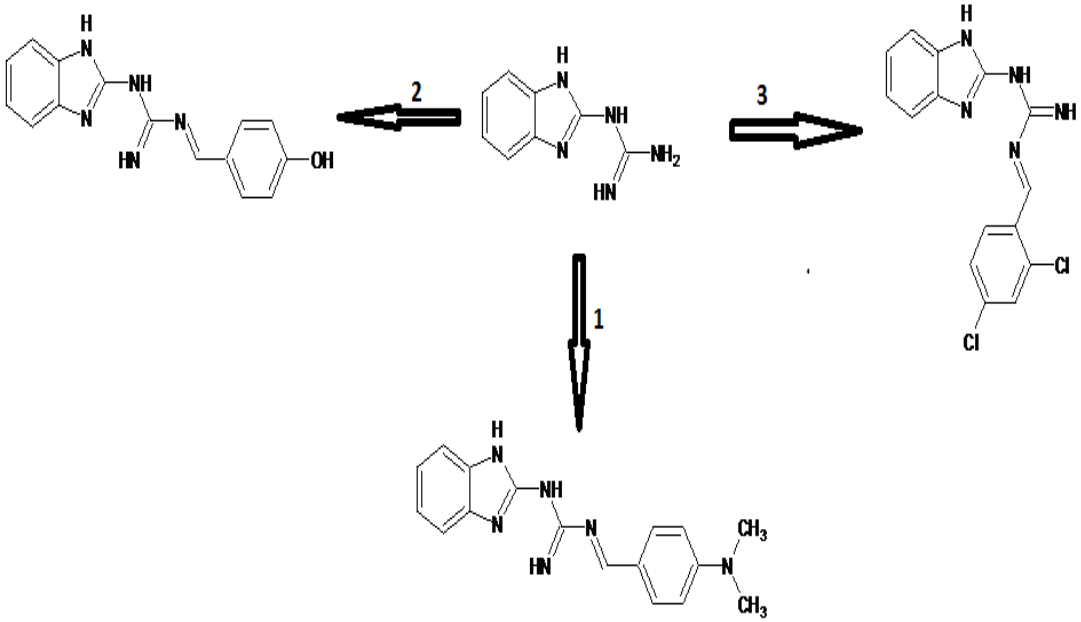
STEP 2

Synthesis of Schiff base[30]

An equimolar quantity of 2-benzimidazoylguanidine and substituted aromatic aldehyde is refluxed for 10-15 hours in 20ml ethanol. Completion of reaction is monitored by TLC. After completion of the reaction the content is poured into ice cold water. The precipitate collected by filtration, washed with water and dried. Recrystallization is carried out by ethanol.



SYNTHESIS OF COMPOUNDS:



2-benzimidazolylguanidine reacts with

1. N,N' dimethylaminobenzaldehyde
2. p-hydroxybenzaldehyde
3. Dichlorobenzaldehyde

JUSTIFICATION OF PURITY:

A.THIN LAYER CHROMATOGRAPHY

Precoated aluminium TLC plates are used. Solutions of the reactants and products are prepared by dissolving them in methanol. Stationary Phase: Precoated Silica Gel Plates. Mobile Phase: Chloroform: Methanol (3:2) Visualization: Iodine Vapors and UV chamber A single spot not corresponding to the parent compound is indicative. Absence of other spots justifies the purity of the product.

B. MELTING POINT

The melting points of synthesized compounds are determined by open tube capillary method with an aid of a melting point apparatus. The melting points were Sharp melting point are indicate the purity of the product.

CHARACTERISATION STUDIES

A. INFRA RED (IR) SPECTROSCOPY

The recrystallised compounds are subjected to IR spectral analysis for functional group identification using KBr pellet method. Stretching and bending vibrations for the new functional are indicated. Absence of the vibrational bands for the parent functional group is ensured.

B. NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

1H NMR spectra are recorded on samples are prepared by dissolving a minute quantity of pure compounds in DMSO. Chemical shifts are reported in parts per million (ppm)

C. **MASS SPECTROSCOPY**:

Mass Spectra is recorded on Shimadzu HPLC-MS using Electron Spray Ionization Technique and was quantified using La Solutions Software 7.0, Samples are prepared by dissolving a minute quantity of pure compounds in methanol. The fragmentation patterns are reported in m/z values.

MICROBIOLOGICAL ASSAY:

Microbial assays or microbiological assays is a type of bioassay and are designed to analyze the compounds or substances which have effect on micro-organisms. Microbiological assay is defined as the determination or estimation of concentration or potency of an antibiotic by means of measuring and comparing the area of zone of inhibition or turbidity produced by test substance with that of standard over a suitable microbe under standard conditions. So as definition says the hypothesis is that when an antibiotic is administered, there is inhibition in the growth of microbe as indicated by decrease in area of zone of microbial colony on nutrition media or decrease in turbidity due to decrease in microbial concentration.[43,44]

TYPES OF MICROBIOLOGICAL ASSAY:

REDOX BASED METHODS:

Micro plate Alamar blue assay. Resazurin Microtiter Assay, REMA, or Micro dilution Resazurin Assay, MRA. Tetrazolium Dyes, Tetrazolium Microplate Assay, TEMA.

REPORTER GENE-BASED METHODS:

Green Fluorescent Protein Micro plate Assay, GFPMA; Luciferase Assays; Beta-Galactosidase Assays.

OTHER METHODS: BACTEC 460 TB

Nitrate Reductase Assay, NRA; Disk Diffusion; Visual Micro broth, or Broth Micro dilution; Malachite Green; STC Agar; Flow Cytometr

MICROPLATE ALAMAR BLUE ASSAY:

MABA is clearly the standard in the field for HTS of compounds against mycobacteria, and is the most widely cited. The primary reference for the method is Collins and Franzblau in 1997. In that study, MABA was effective with MTB H37Rv, H37Ra and M. avium strain ATCC 25291, a relatively virulent isolate. MABA is reliable with clinical isolates of MTB and MIC. MABA has also been applied to M. kansasii and M. malmoense, as well as M. leprae. In addition to screening and sensitivity applications, MABA has also been used in patient treatment follow-up. MABA can operate in simple colorimetric mode with visual reading (blue to pink

change indicates viability,with the MIC recorded as the lowest compound concentration in wells which remain blue). Newly synthesized product are assayed in vitro for anti-tubercular activity. Evaluation of the products for their in vitro antitubercular activity against Mycobacterium Tuberculosis H37Rv using MicroplateAlamar Blue Assay (MABA) biological test is done. This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods.[40,42]

Anti-TB activity using Alamar Blue Dye:

1. The anti-mycobacterial activity of compound are assessed against M. tuberculosis using micro plate 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, 200µl of sterile deionized water is added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
4. The 96 wells plate receive 100 µl of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on plate.
5. The final drug concentrations tested are 100 to 0.8 µg/ml.
6. Plates were covered and sealed with par film and incubated at 37ºC for five days.
7. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
8. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
9. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.[42,43]

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Results of drug design

Two hundred molecules, which are sketched using chem sketch were docked against the MTB enzyme Decaprenylphosphoryl-beta-D-ribose 2'-epimerase-1by using Argus lab 4.0.1 software. The molecules with best docking score and good interactions were selected and synthesized.

The molecules were also docked against the following targets

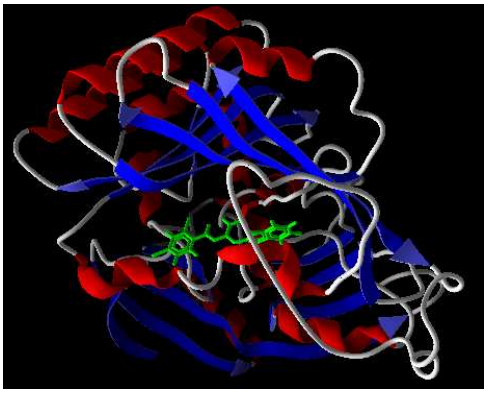
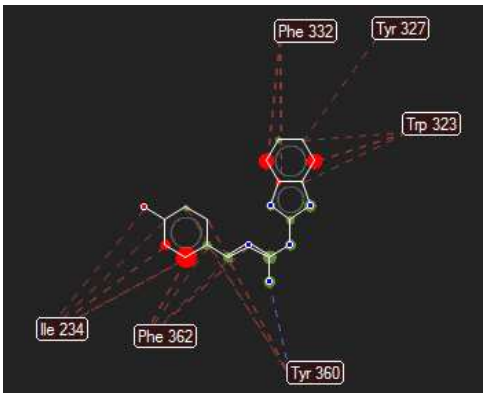
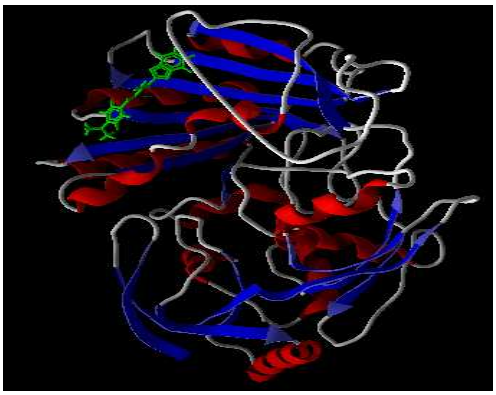
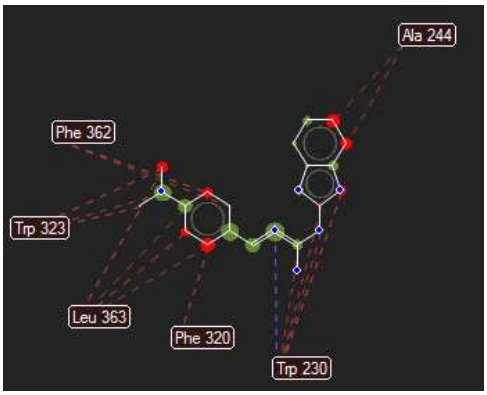
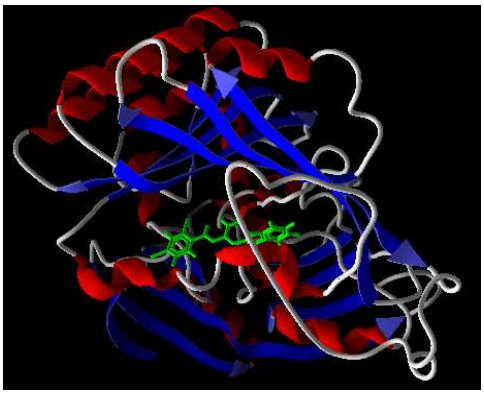
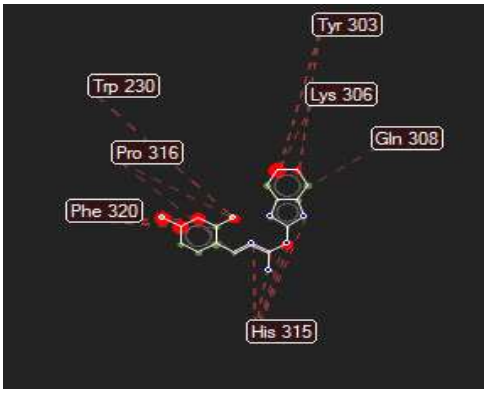
1. Methoxy mycolic acid
2. Glutamine synthetase 1
3. Cyclopropane mycolic acid synthase 2

Table no: 2

Name of the target	Docking score		
	R1	R2	R3
Decaprenylphosphoryl-beta-D-ribose 2'-epimerase-1	-7.62223	-8.02866	-8.52044
Methoxy mycolic acid	-6.62823	-8.029316	-7.92264
Glutamine synthetase 1	-6.85964	-8.38081	-7.37541
Cyclopropane mycolic acid synthase 2	-8.06321	-7.36964	-8.30648

Interactions of the docked molecules with the enzyme Decaprenylphosphoryl-beta-D-ribose 2'-epimerase-1.

Table no: 3

Sample code	Docking view	Interactions with amino acids
R1		
R2		
R3		

Product profile

Compound R1

Physicochemical Properties of *N*-1H-Benzimidazol-2-Yl-*N*'-[(*E*)-(4 hydroxy phenyl) methylidene] Guanidine

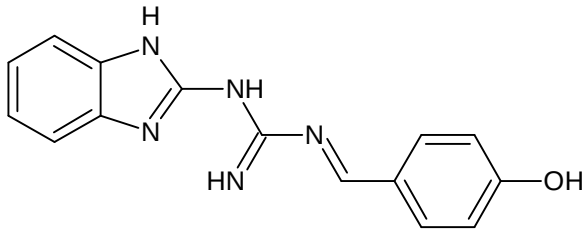


Table no: 4

Description	Reddish brown colour solid
Solubility	Soluble in methanol,Ethanol
Molecular Formula	$C_{15}H_{13}N_5O$
HFormula Weight	279
Composition	C(64.51%) H(4.69%) N(25.07%) O(5.73%)
Molar Refractivity	79.01 ± 0.5 cm
Molar Volume	201.4 ± 7.0 cm^3
Parachor	560.9 ± 8.0 cm^3
Index of Refraction	1.713 ± 0.05
Surface Tension	60.1 ± 7.0 dyne/cm
Density	1.38 ± 0.1 g/cm^3
Polarizability	31.32 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	279.11201 Da
Nominal Mass	279 Da
Average Mass	279.2966 Da
M+	279.111461 Da
M-	279.112559 Da
[M+H]+	280.119286 Da
[M+H]-	280.120384 Da
[M-H]+	278.103636 Da
[M-H]-	278.104734 Da

Compound R2

Physicochemical Properties of *N*-1H-Benzimidazol-2-Yl-*N*'-[(Z)-(4 dimethyl amino) phenyl] methylidene}Guanidine

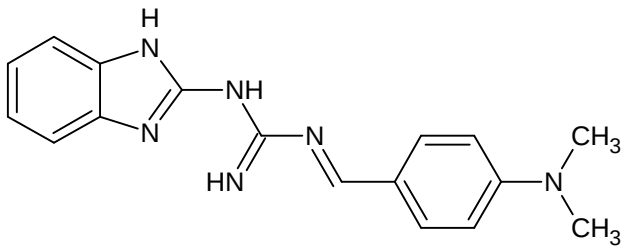


Table no: 5

Description	Orange in colour
Solubility	Soluble in methanol,Ethanol
Molecular Formula	$C_{17}H_{18}N_6$
Formula Weight	306
Composition	C(66.65%) H(5.92%) N(27.43%)
Molar Refractivity	90.96 ± 0.5 cm^3
Molar Volume	245.3 ± 7.0 cm^3
Parachor	651.6 ± 8.0 cm^3
Index of Refraction	1.663 ± 0.05
Surface Tension	49.7 ± 7.0 dyne/cm
Density	1.24 ± 0.1 g/cm^3
Polarizability	36.06 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	306.159295 Da
Nominal Mass	306 Da
Average Mass	306 Da
M+	306.365 Da
M-	306.158746 Da
[M+H]+	307.166571 Da
[M+H]-	307.167668 Da
[M-H]+	305.152018 Da
[M-H]-	305.150921 Da

Compound R3

Physicochemical properties of N-1H-benzimidazole-2-yl N'{(z)[2,4(dichloro)phenyl]methylidine} guanidine.

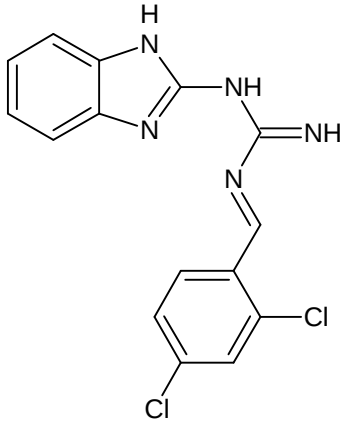


Table no: 6

Description	light white in colour.
Solubility	soluble in ethanol,methanol
Molecular Formula	C_{15} H $_{11}$Cl $_{2}$$N_5$
HFormula Weight	332
Composition	C(54.23%)H(3.34%)Cl(21.35%)N(21.08%)
Molar Refractivity	87.35 ± 0.5 cm^3
Molar Volume	222.7 ± 7.0 cm^3
Parachor	613.0 ± 8.0 cm^3
Index of Refraction	1.713 ± 0.05
Surface Tension	57.3 ± 7.0 dyne/cm
Density	1.49 ± 0.1 g/cm^3
Polarizability	34.63 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	331.039151 Da
Nominal Mass	331 Da
Average Mass	331.1234Da
M^+	332.1565
M^-	332.1678
$[M+H]^+$	332.1498
$[M+H]^-$	332.1685
$[M-H]^+$	332.5845
$[M+H]^-$	332.8796

CHARACTERIZATION:

Newly synthesized compounds were characterized by

- IR
- NMR
- GC-MS

IR SPECTROSCOPY

The samples were prepared by the KBr pellet technique and spectrum obtained from ABB(MB 3000) spectrophotometer

The spectra were examined for the absence of the functional group region of parent compound and examined for presence of the vibrational absorption band for the new functional group.

Our reaction involves reaction between Aldehyde and amine to yield of Schiff bases.

- The absorption band for aldehydes are

  2800-2700cm^{-1}

  1700-1750cm^{-1}

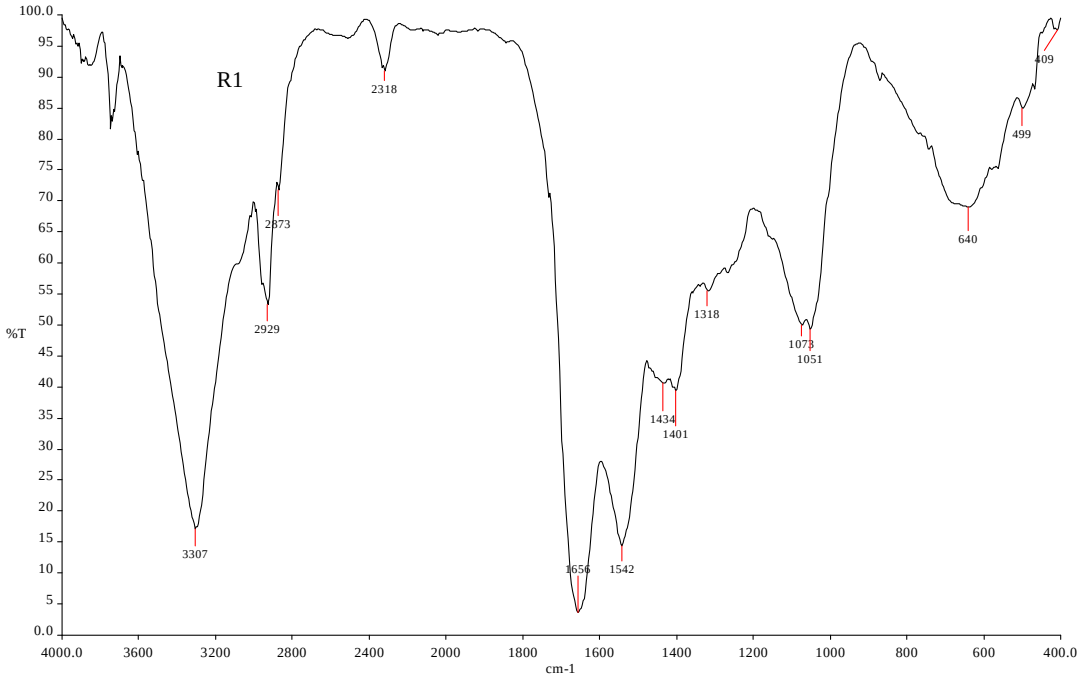
- The absorption band for amine 3400-3600 cm-1
- The absorption of newly synthesized –C=N- is 1650-1600cm^{-1}

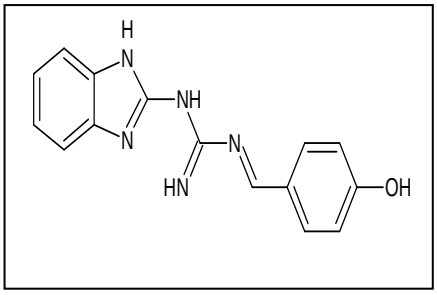
Table no: 7

IR absorption band	R1	R2	R3
Aldehyde	-	-	-
Amine	-	-	-
-C=N-	+	+	+

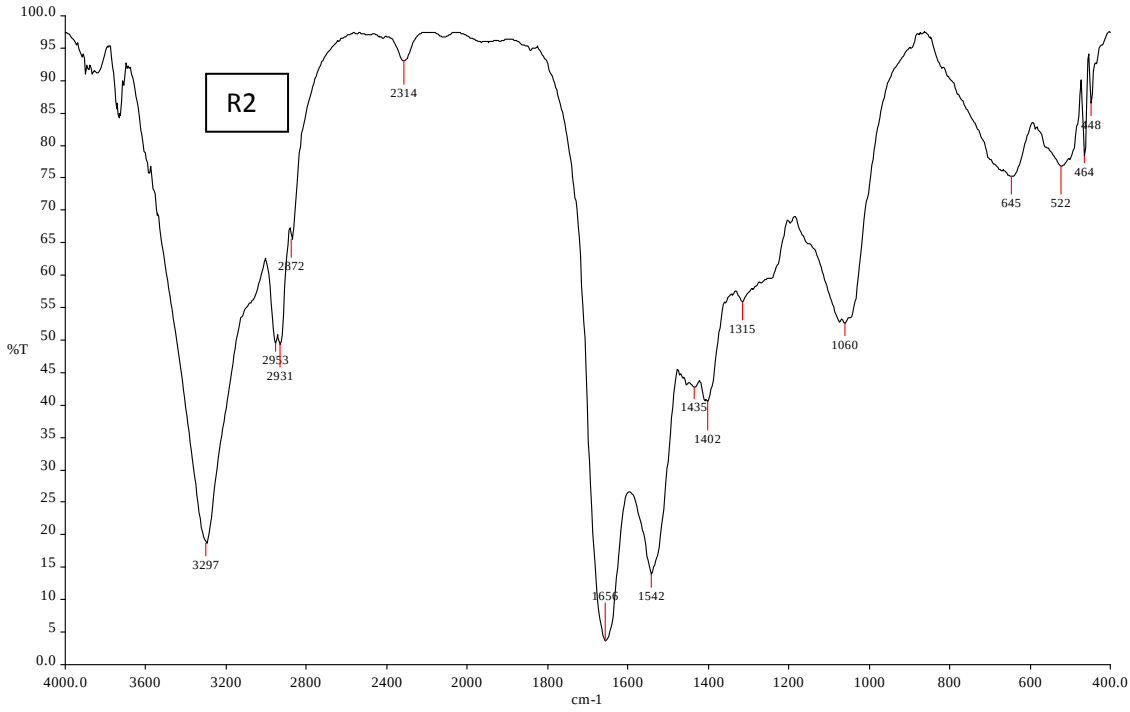
(+) indicates presence
(-) indicates absence

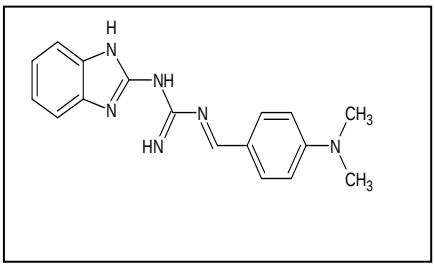
IR SPECTRUM OF R1:



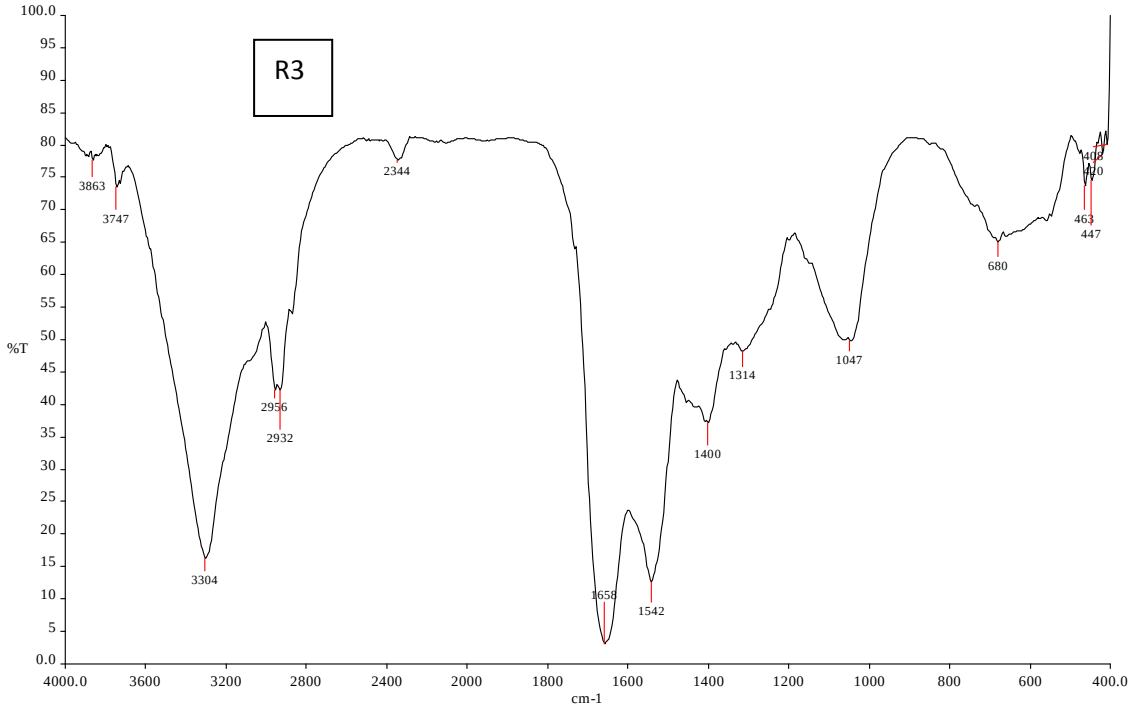


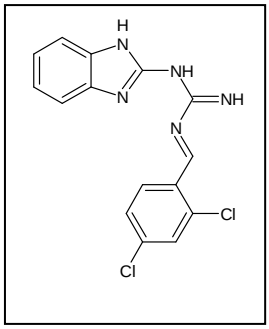
IR SPECTRUM OF R2:





IR SPECTRUM OF R3:





NMR

1H NMR spectra are recorded on Bruker Advance III 500 MHz NMR spectrophotometer

COMPOUND R1

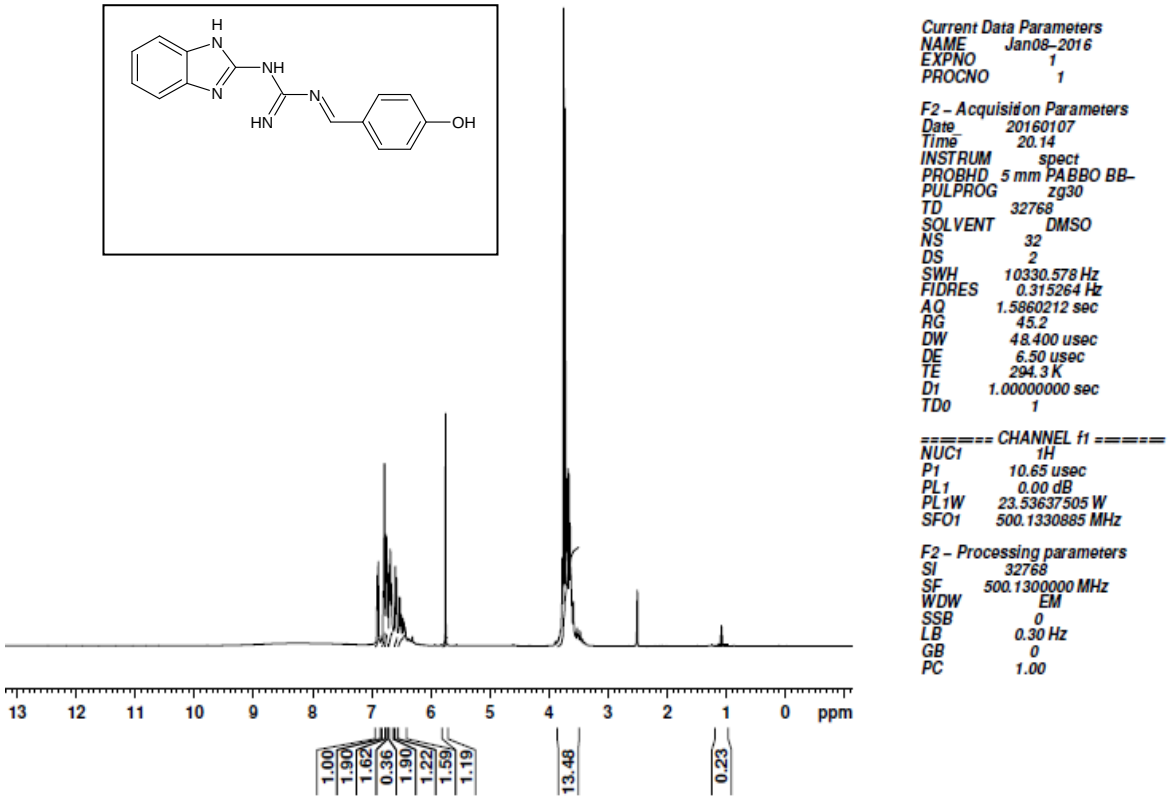


Table no: 8

TYPE OF PROTON	NO OF PROTON	DELTA(δ)	NATURE OF PEAK
-NH	3	3.4-4.1	Multiplate
-OH	1	5.4	Singlet
Ar-H	8	6.3-7.1	Multiplate

COMPOUND R2

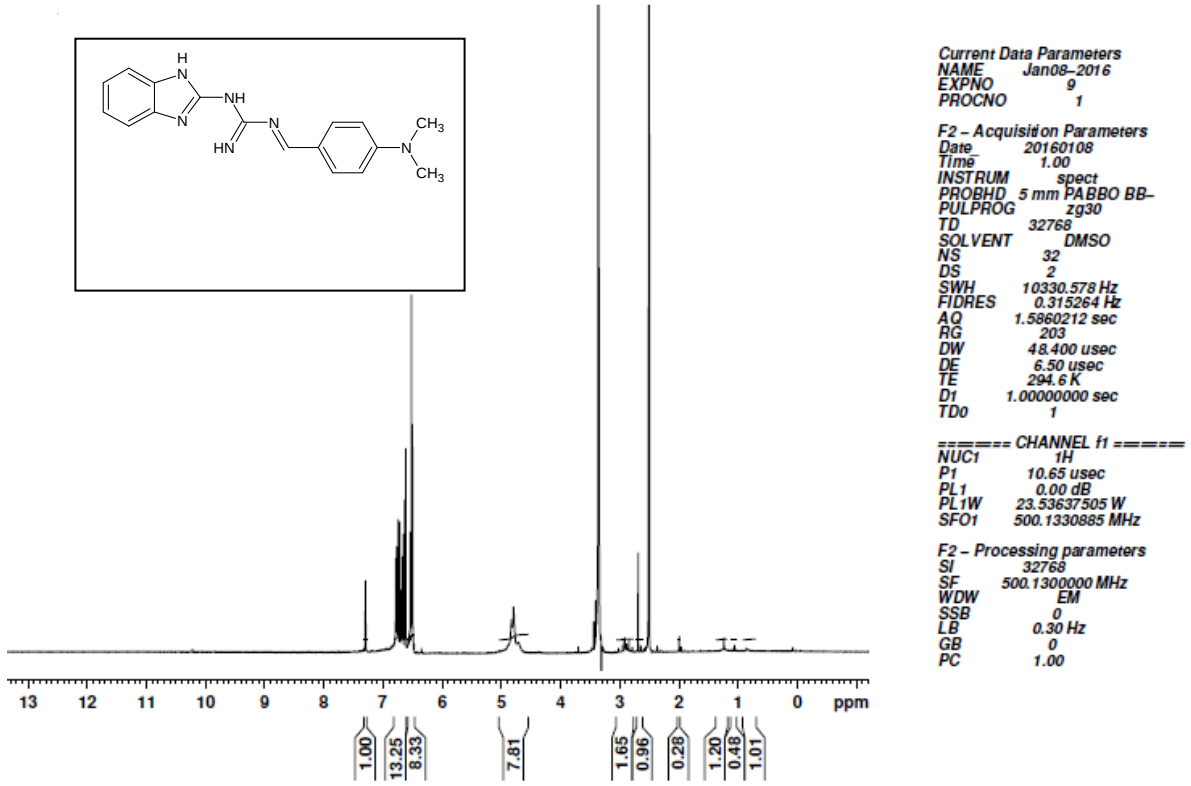


Table no:9

TYPE OF PROTON	NO OF PROTON	DELTA(δ)	NATURE OF PEAK
-NH	3	3.2-3.7	Multiplate
$-N(CH_3)_2$	4	2.8-3.1	Singlet
Ar-H	8	6.4	Singlet

COMPOUND R3

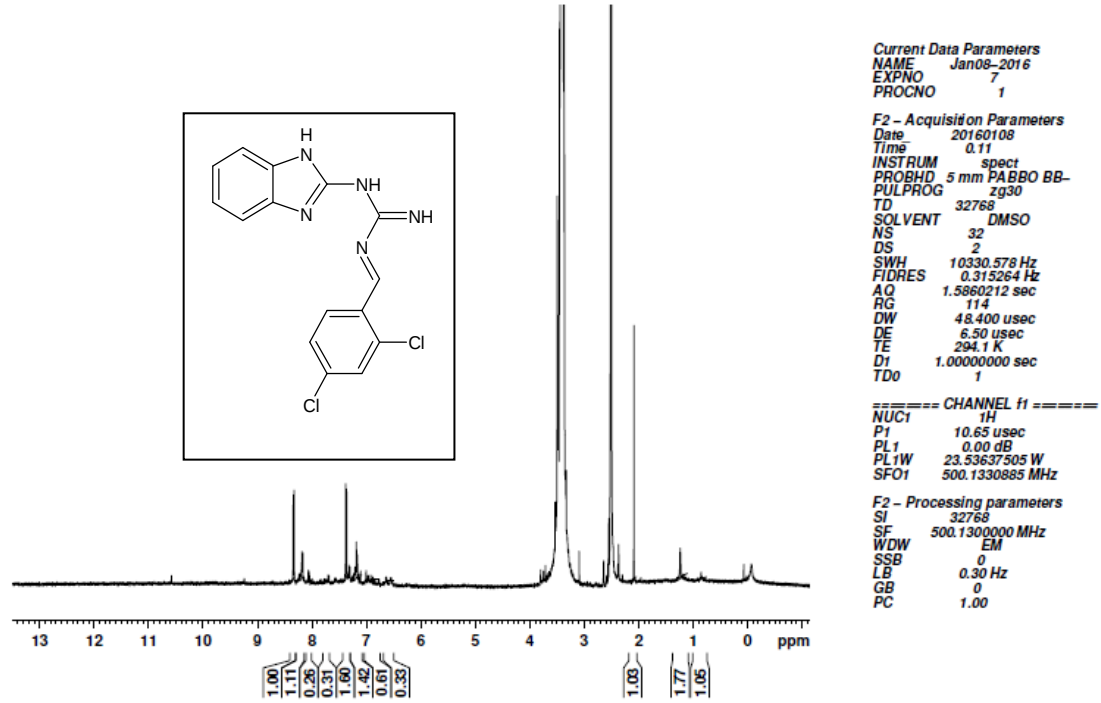


Table no:10

TYPE OF PROTON	NO OF PROTON	DELTA(δ)	NATURE OF PEAK
-NH	1	2.2-2.4	Singlet
Ar-H	7	7.8-8	Multiplate
=CH-	1	8.2	Singlet

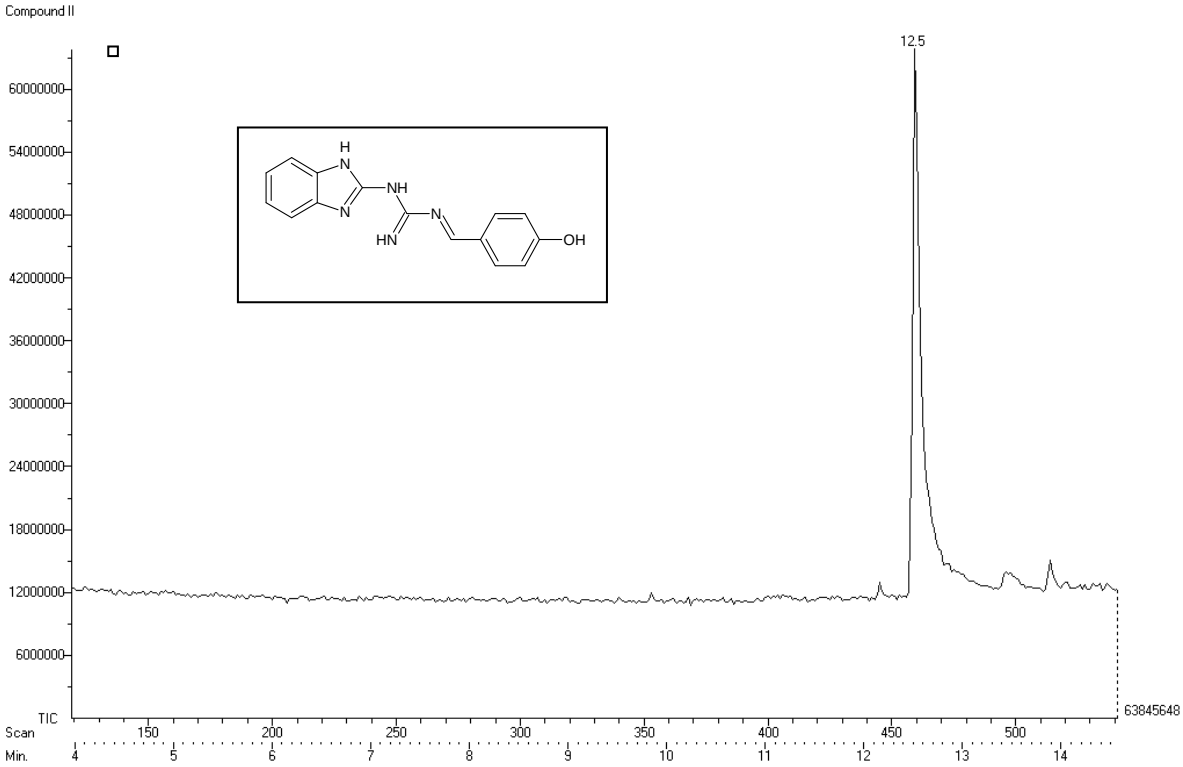
GC-MS:

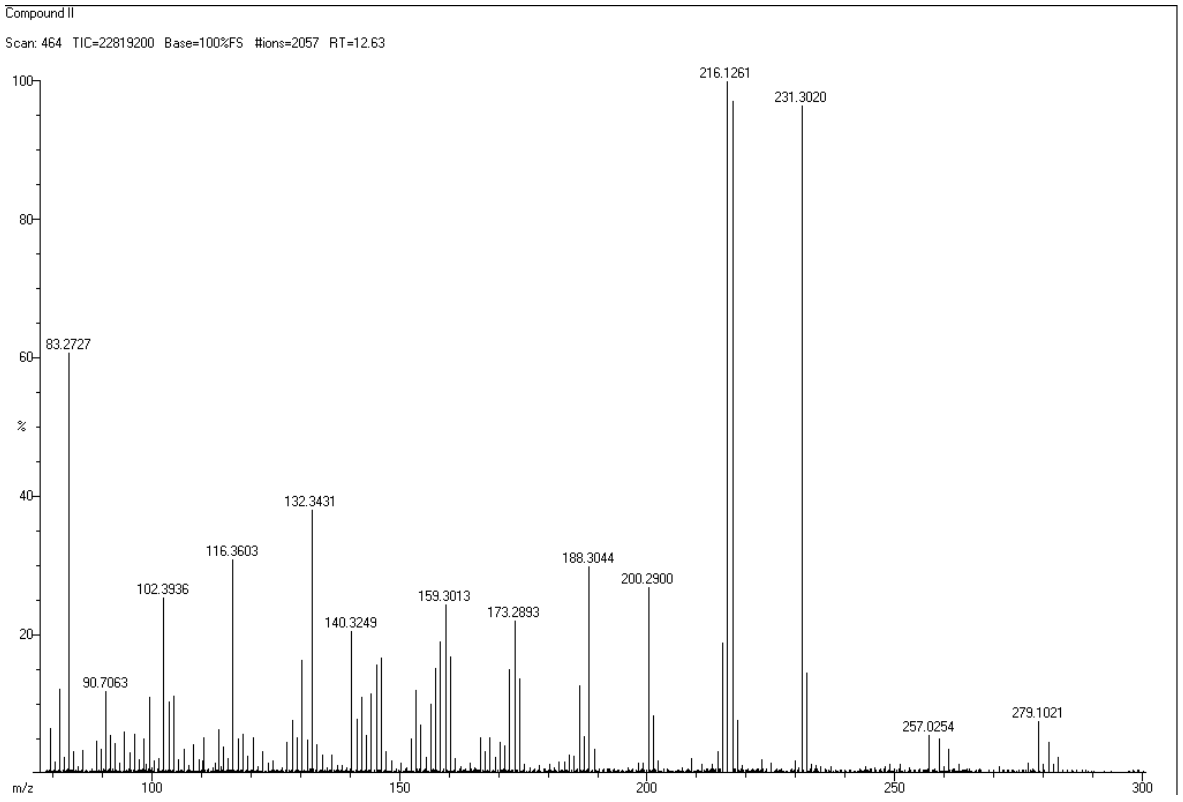
The molecular weight of the synthesized compounds were compared by GC-MS analysis.

Table no: 11

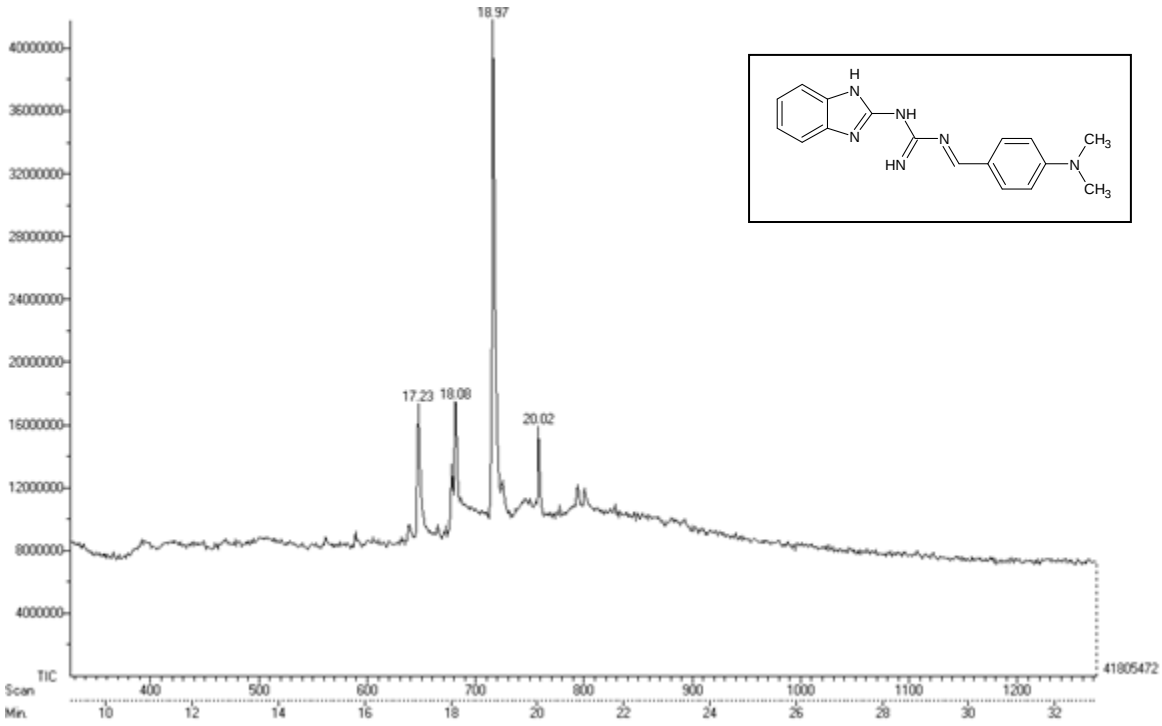
Compound	Calculated mass	Actual Mass
R1	279	279
R2	306	306
R3	332	331

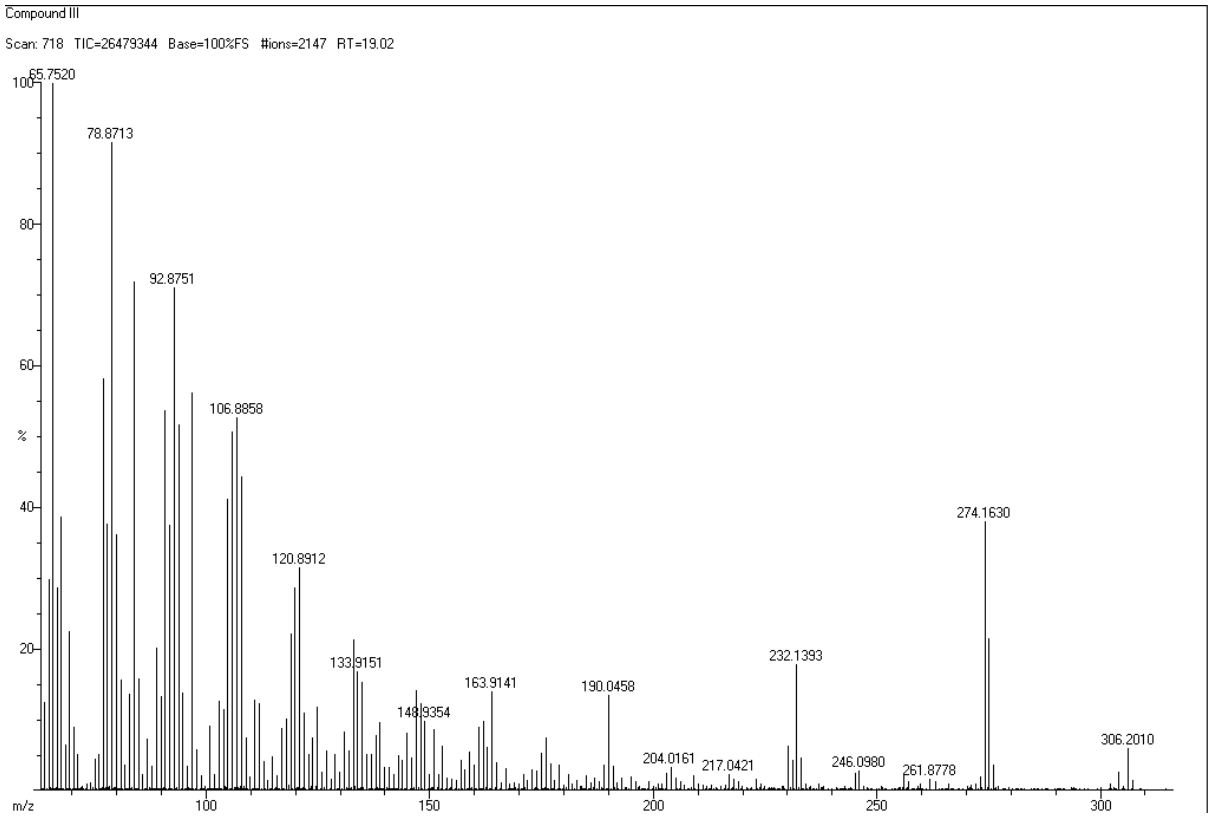
Compound R1:


Compound II
12.5
60000000
54000000
48000000
42000000
36000000
30000000
24000000
18000000
12000000
6000000
H
N
NH
N
HN
N
OH
TIC
63845648
Scan
150
200
250
300
350
400
450
500
Min.
4
5
6
7
8
9
10
11
12
13
14

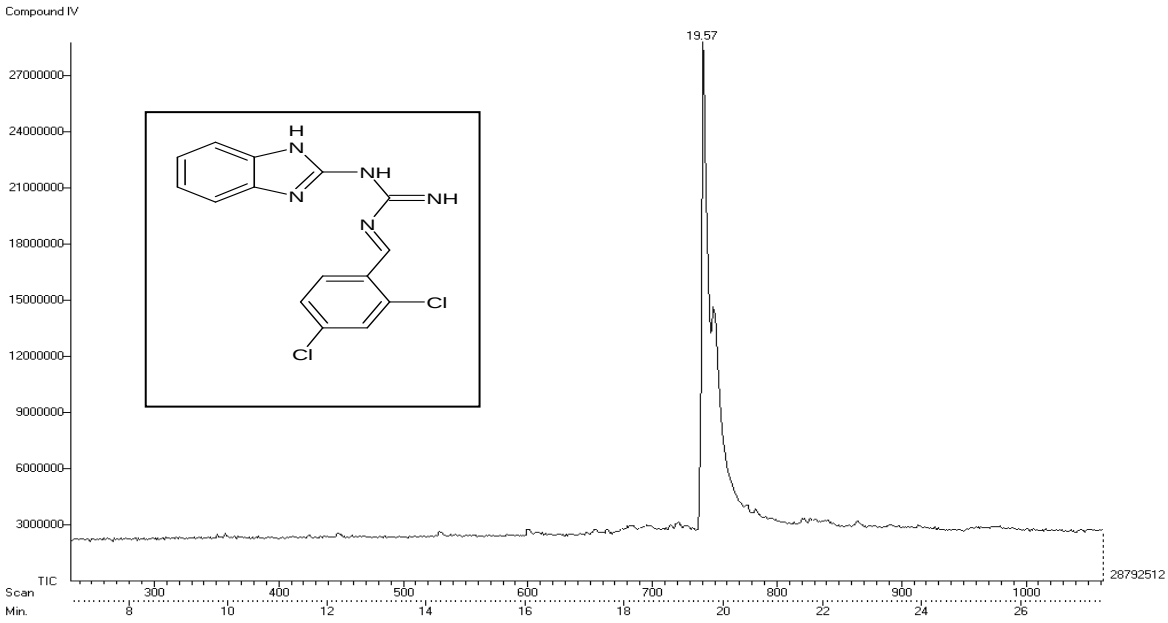

Compound II
Scan: 464 TIC=22819200 Base=100%FS #ions=2057 RT=12.63
100
80
60
40
20
%
216.1261
231.3020
83.2727
132.3431
116.3603
188.3044
200.2900
102.3936
159.3013
173.2893
140.3249
90.7063
257.0254
279.1021
m/z
100
150
200
250
300

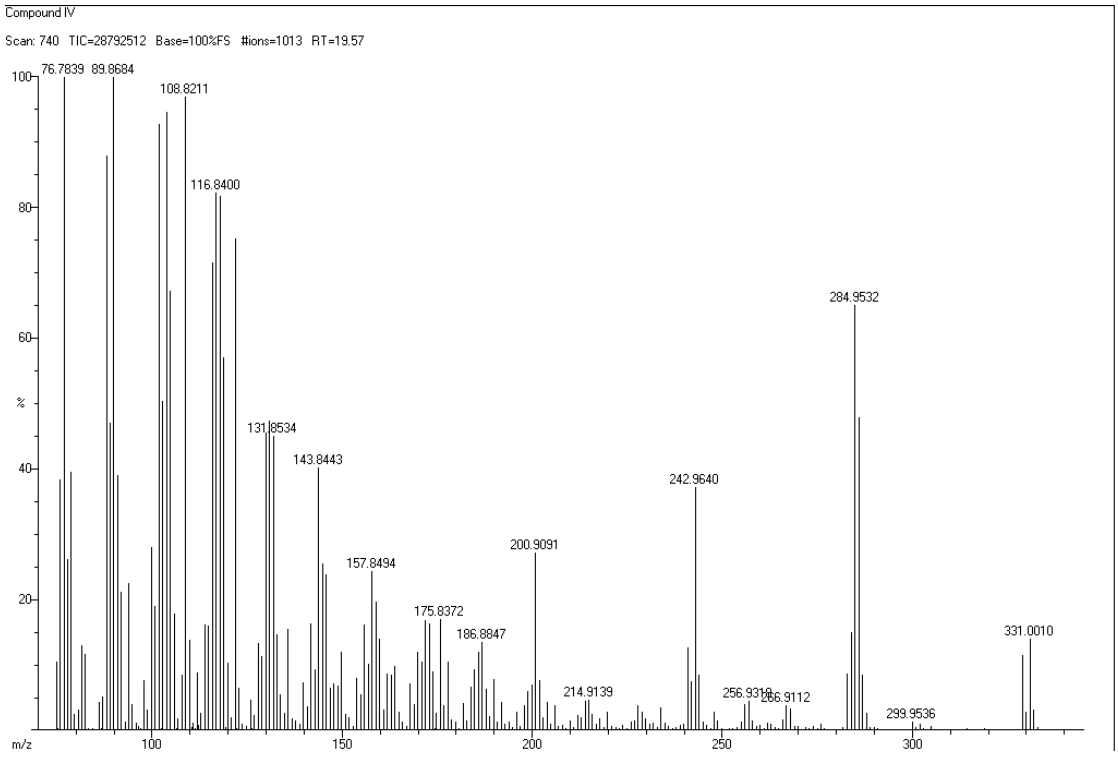
Compound R2:





Compound R3:


Compound IV
19.57
27000000
24000000
21000000
18000000
15000000
12000000
9000000
6000000
3000000
H
N
NH
N
NH
N
Cl
Cl
TIC
28792512
Scan
300
400
500
600
700
800
900
1000
Min.
8
10
12
14
16
18
20
22
24
26


Compound IV
Scan: 740 TIC=28792512 Base=100%FS #ions=1013 RT=19.57
100
80
60
40
20
%
76.7839
89.8684
108.8211
116.8400
131.8534
143.8443
157.8494
175.8372
186.8847
200.9091
214.9139
242.9640
256.9318
266.9112
284.9532
299.9536
331.0010
m/z
100
150
200
250
300

TOXICITY RISK ASSESSMENT

All the data set molecules were subjected to the toxicity risk assessment by using Osiris program, which is available online free of cost. The OSIRIS property Explorer shown in this page is an integral part of Actelion's in house substance registration system. It allows drawing chemical structures and also calculates various drug relevant properties whenever a structure is valid. 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 behavior.

Molecular property prediction includes

- Toxicity risk assessment
- Clog P predicition
- Solubility prediction
- Molecular weight

Table no: 12

Samples	R1	R2	R3
Mutagenic	+	+	+
Tumorigenic	+	-	+
Irritant	+	+	+
Reproductive effective	+	+	+

(+) indicates absence of toxicity

(-)indicates presence of toxicity

Fig. R1

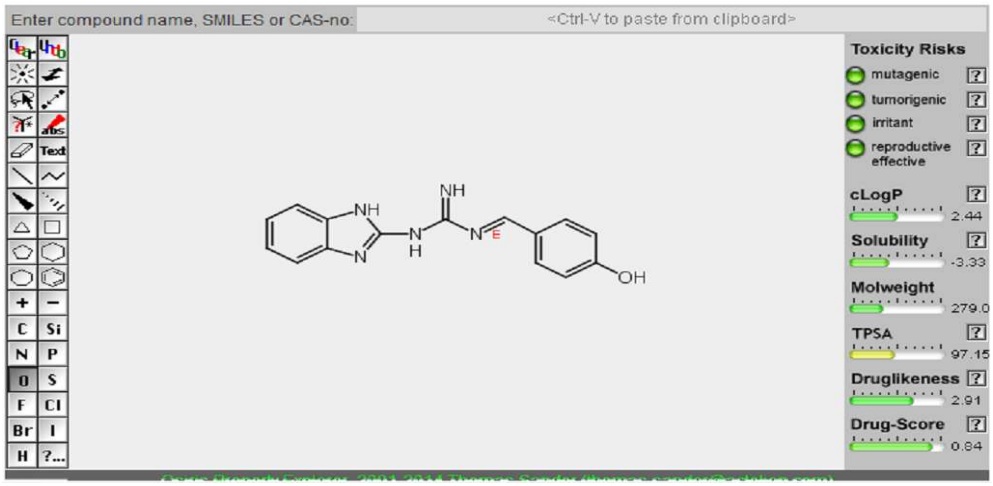


Fig. R2

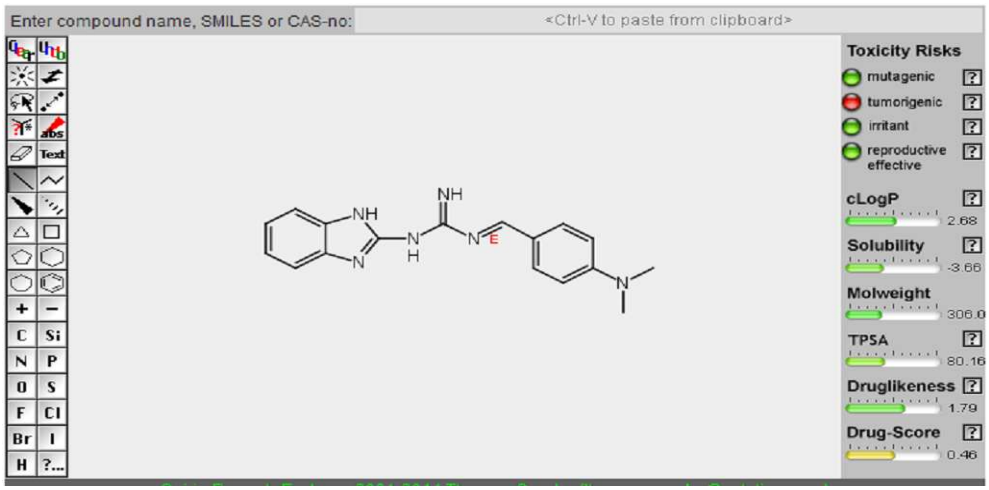
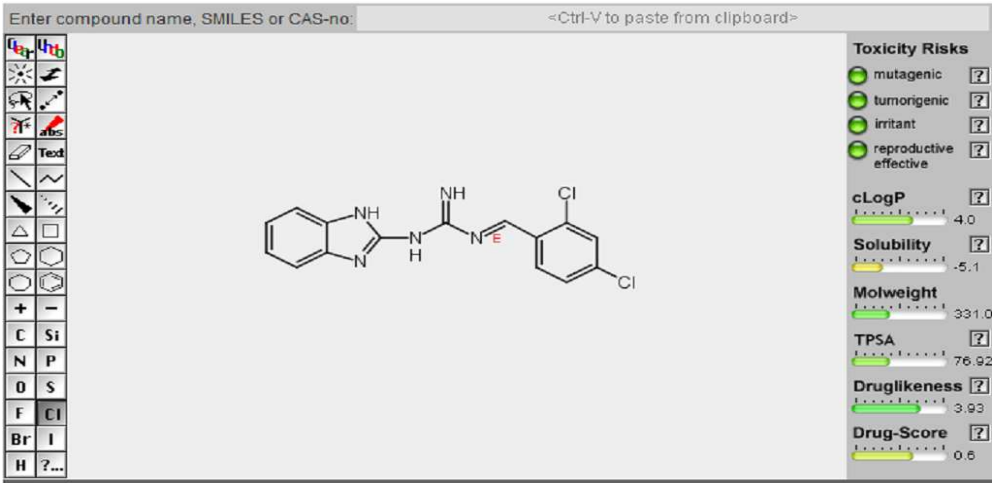


Fig. R3



BIOLOGICAL SCREENING

The synthesized compounds were screened for their in-vitro anti mycobacterial activity by means of alamar blue assay. The compounds were tested in the concentration range of 100 to 0.8 µg/ml against *M.tuberculosis* H37Rv strain grown in Middlebrook 7H9 broth in 96 well titre plate. Pyrazinamide- 3.125µg/ml and Streptomycin- 6.25µg/ml were used as standards for comparison. A blue color in the well was interpreted as no bacterial growth so it is termed as sensitive, and pink color was scored as growth and is referred as resistant. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Table no: 13

Compound	**Docking score**	**MIC value**
R1	-7.62223	50 µg/ml
R2	-8.02866	25 µg/ml
R3	-8.52044	50 µg/ml

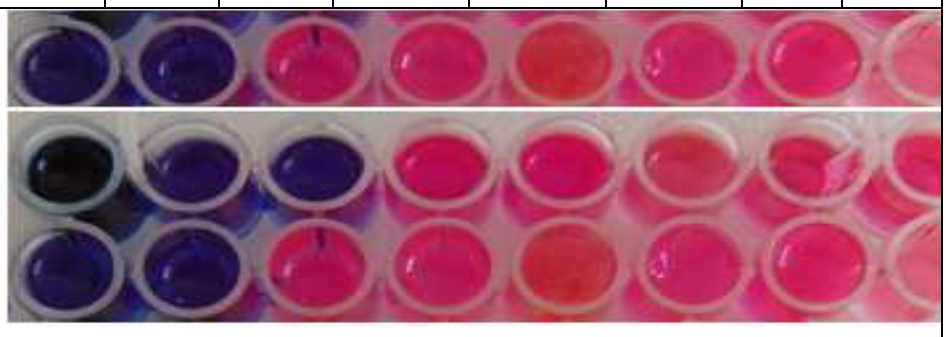
MABA REPORT OF THE SYNTHESISED COMPOUNDS

Table no: 14

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	R1	S	S	R	R	R	R	R	R
2	R2	S	S	S	R	R	R	R	R
3	R3	S	S	R	R	R	R	R	R

Fig. Standard drug photograph

Fig. synthesized compound photograph

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	R1								
2	R2								
3	R3								

SUMMARY AND CONCLUSION

SUMMARY

- Decaprenylphosphoryl-b-d-ribose 2'-Epimerase 1(DprE1) a enzyme of OxidoReductase family is a critical enzyme for the growth of Mycobacterium tuberculosis H37Rv.
- From the review of literature DprE1 was chosen for our study for drug design.
- A database of 100 molecules with high potential of inhibiting the target possessing PDB ID of 4FDO were carefully chosen by making changes to the lead molecule aryl carboxylic acid derivatives.
- The 3D structure of the molecules were docked against the 3D structure of DprE1 using the docking platform argus lab.
- Three compounds with good Docking score (lower Binding energy) were selected for laboratory synthesis. The reaction conditions were optimized.
- The Compounds were labeled as R1, R2, R3 were synthesized with satisfactory yield
- The purity of the synthesized compounds was ensured by repeated recrystallization and column chromatography.Further the compounds were evaluated by melting point and TLC.
- The characterizations of the synthesized compounds were done by Infrared, Nuclear magnetic resonance and Mass spectroscopic methods.
- The final pure compounds were screened for Antimycobacterial activity by in vitro method called Microplate Alamar Blue Assay (MABA).

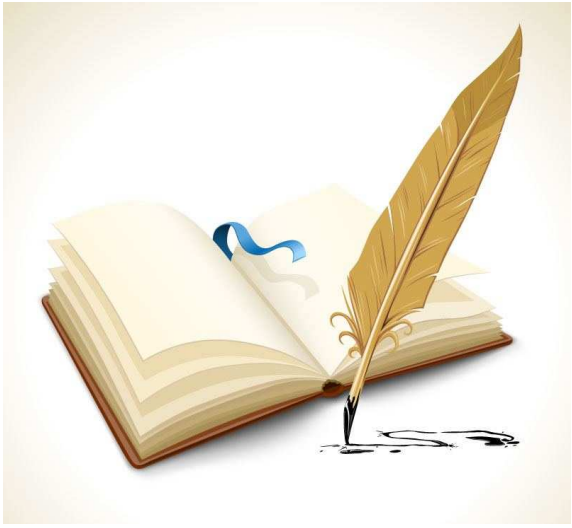
- The synthesized compounds were active at 25mcg/ml to <100mcg/ml, which are compared to that of the known anti-tubercular agents at 50mcg/ml against the MIC of known TB drugs. The synthesized compounds were lesser active than that of the standard TB drugs. Pyrazinamide: 3.125mcg/ml, Ciprefloxacin: 3.125mcg/ml and Streptomycin 6.25mcg/ml.

- The synthesized compounds were subjected to toxicity prediction assessments by OSIRS software. The results are coded as a green colour which confirms the drug likeness.

CONCLUSION

- Our work concludes that our synthesized molecules are effective in inhibiting Decaprenylphosphoryl-beta-D-ribose 2'-Epimerase 1(DprE1) which is important for the growth of Mycobacterium tuberculosis.

- Further structural refinement in the structure of the synthesized compounds will give new outlook to the development of promising molecules against the pathogen Mycobacterium tuberculosis.



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5. P.J.Brennan. Structure, function, and biogenesis of cell wall of Mycobacterium Tuberculosis. 2003; P. 83, 91-9.

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ANNEXURE

NATIONAL CONFERENCE ON
EMERGING AND RE-EMERGING VIRAL OUTBREAKS IN INDIA – CLINICAL CHALLENGES AND MANAGEMENT

CERTIFICATE

This is to certify that Mr./Ms./Dr. N.Ramya *has Participated in the National Conference on* **"EMERGING AND RE-EMERGING VIRAL OUTBREAKS IN INDIA – CLINICAL CHALLENGES AND MANAGEMENT"** *held from 20th-22nd JANUARY 2015 at CSIR-IICT-CCMB Auditorium, Hyderabad.*

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