"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL ANTI TUBERCULAR AGENTS TARGETING L, D-Transpeptidase-2"

A dissertation submitted to

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In partial fulfillment of the requirements

For the award of the degree of

MASTER OF PHARMACY

In

PHARMACEUTICAL CHEMISTRY

Submitted by 261415713

Under the Guidance of

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CERTIFICATE

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Place : Chennai

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

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CONTENTS

LIST OF ABBREVIATIONS

TLC	Thin Layer Chromatography
IR	Infrared
H^1-NMR	Proton Nuclear Magnetic Resonance
LC-MS	Liquid Chromatography and Mass Spectroscopy
GC-MS	Gas Chromatography and Mass Spectroscopy
Gm	Gram
δ	Delta
Sec	Seconds
Rf	Retention Factor
m.p	Melting Point
Mol.For	Molecular Formula
Mol.Wt	Molecular Weight
DMF	Dimethyl Formamide
DMSO	Dimethyl Sulphoxide
◦C	Degree Celsius
SEM	Standard Error Mean
m\e	Mass per charge Ratio
STD	Standard
CFU ML-1	Colony Forming Unit per Milliliter
UV	Ultra Violet
MIC	Minimum Inhibitory Concentration
mg\kg	Milligram per kilogram
µg	Microgram
b.w	Body Weight
min	Minutes
TB	Tuberculosis
MDR-TB	Multi Drug Resistance TuBerculosis
MABA	Microplate Alamar Blue Assay

INTRODUCTION

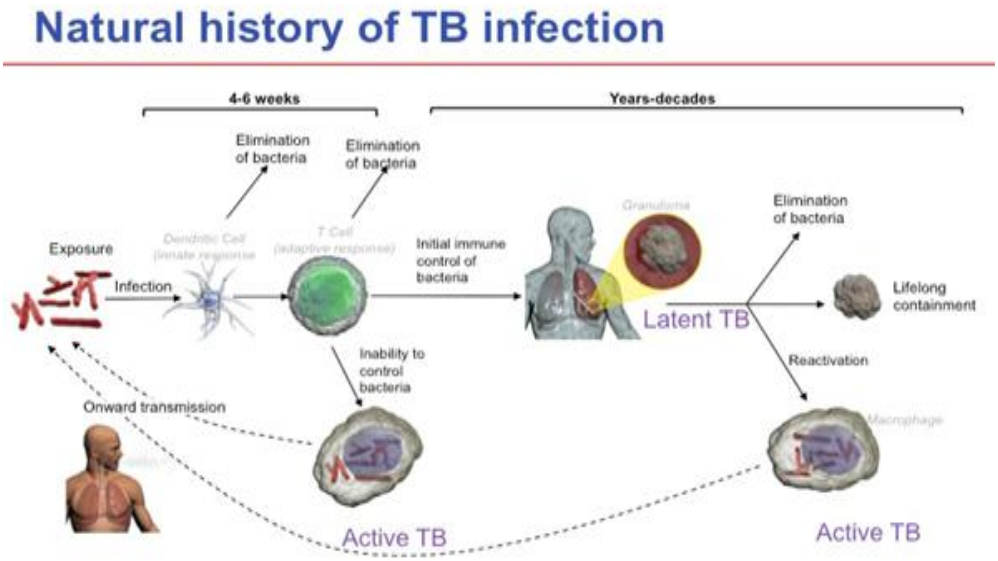
Tuberculosis or TB is the most common infectious disease. In the past Tuberculosis also called as phthisis or phthisis pulmonalis. TB is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent.[1] In addition, the prevalence of drug-resistant TB is also increasing worldwide. Co-infection with HIV has been an important factor in the emergence and spread of resistance. [2] New TB treatments are being developed and new vaccines are currently under investigation. [3] TB is a major global health threat, and there is need to improve the existing treatment regimen to control the spread of TB.

Tuberculosis is an airborne disease. It is spread from person to person through the air (vehicle).Tuberculosis is most often affect the lungs.[4]

HISTORY

Tuberculosis is the second deadliest disease (first HIV/AIDS). The World Health Organization estimates, that 2 billion people have latent TB, while another 3 million people worldwide die of TB in every year. In 2013, 8.2 million peoples fell ill with TB and 1.2 million peoples died. [5]

Figure 1[6]



TUBERCULOSIS

M. tuberculosis and seven very closely related mycobacterial species (*M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canetti* and *M. mungi*) together comprise what is known as the *M. tuberculosis* complex. Most, but not all, of these species have been found to cause disease in humans. In the United States, the majority of TB cases are caused by *M. tuberculosis*. *M. tuberculosis* organisms are also called tubercle bacilli. (7)

M. tuberculosis is carried in airborne particles, called droplet nuclei, of 1– 5 microns in diameter. Infectious droplet nuclei are generated when persons who have pulmonary or laryngeal TB disease cough, sneeze, shout, or sing. Depending on the environment, these tiny particles can remain suspended in the air for several hours. *M. tuberculosis* is transmitted through the air, **not** by surface contact. Transmission occurs when a person inhales droplet nuclei containing *M. tuberculosis*, and the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs. (8)

TYPES OF TUBERCULOSIS

- Active tuberculosis
- Inactive tuberculosis

Active tuberculosis

Active tuberculosis means the bacteria are active in the body i.e., the immune system is unable to stop these bacteria from causing illness

Inactive tuberculosis

It is also known as latent TB. If a person has a latent TB, their body has been able to successfully fight against the bacteria and stop them from causing illness. People have latent TB do not feel sick, do not have symptoms and cannot spread tuberculosis. In the people who have HIV, the inactive TB may become active TB if their immune system becomes weakened. (9)

PATHOPHYSIOLOGY

Humans are the only known reservoir for Mycobacterium tuberculosis. TB is transmitted by airborne droplet nuclei, which may contained fewer than 10 bacilli. TB exposure occurs by sharing common airspace with an individual who is the infection stage of TB.

Inhalation

The bacteria are inhaled. The majority of the bacteria will become lodged in the upper respiratory tract , namely the nose and throat, where their survival is difficult. Some of the smaller particles, Hhough, will make it into the lungs and alveoli where infection sets in. Alternatively, the bacteria might be ingested.[10]

Bacteria multiplication

In the alveoli, the bacteria are engulfed by inactivated macrophages, white blood cells present within tissues, where they multiply until the macrophage bursts. The Mycobacterium tuberculosis replicates very slowly, only once a every twenty four hours, and takes up to one month to form a colony.

T-cell activation

Dendritic cells are a key part of the mammalian immune system. When dendritic cells detect foreign substance entering the body, they engulf and bring them to the lymph nods where they present the antigens to certain white blood cells called T-cells. If the T-cell has a specific receptor for the presented antigen it will become activated to release potent molecules, such as interferon-gamma and tumour necrosis factor, which in turn stimulate macrophages and other T cells to produce a cell-mediated response against the bacteria carrying those antigens.

Tubercle formation

The T-cells return to the site of infection through the blood stream, where they contribute to the formation of a tubercle or granuloma. The TB tubercle is made up of a core of infected macrophages, a surrounding ring of

foamy macrophages and an outside ring of T-cells, all enveloped in fibrous shell. In some cases, an individual's immune system is unable to defend against the bacteria by creating a tubercle to isolate it. Primary progressive tuberculosis occurs as a result. This is mostly seen in young children or individuals with much suppressed immune systems.

When contained inside the granuloma the bacteria are inactive and the case of tuberculosis is considered to be latent. The bacteria are contained in the granuloma until the immune system is weakened, breaking down the outer ring of the tubercle, releasing the bacteria inside. In this situation, the case of tuberculosis has been reactivated and is known as secondary progressive tuberculosis. Only approximately 3-5% of immune-competent individuals will develop secondary progressive tuberculosis within two years of the primary infection, and a further 3-5% will develop it after two years.[11]

Cavitation and tubercle break-down

In some cases, the damaged cells at the center of the granulation liquefy. The bacteria grow well in this liquid, multiplying outside of macrophages, their typical hosts. As they multiply, the tubercle enlarges. This can cause nearby tissue in the lungs to die and rupture, forming a cavity, or the tubercle to burst spreading bacteria further around the lungs or the body. This would also be considered a case of secondary progressive tuberculosis. The immune system will responds as per steps 3& 4 when the bacteria are recognized in their new locations.[12]

A small number of tubercle bacilli enter the bloodstream and spread throughout the body. The tubercle bacilli may reach any part of the body ,including areas where TB disease is more likely to develop (such as the brains, larynx, lymph node, spine, bone, or kidney).[13]

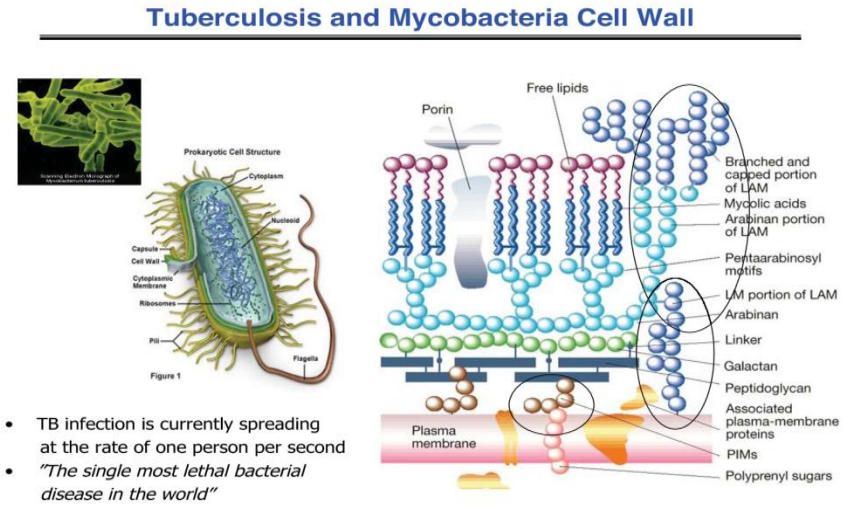
EPIDEMIOLOGY

The highest incidences are seen in those countries of Africa, Asia, and latin America. The world health organization estimates that nine million

people get TB every year, of whom 95% live in developing countries. An estimated 2-3 million people die from TB every year.

8.6 million people fell ill with in 2012, including 1.1 million cases among people living with HIV. In 2012, 1.3 million people died from TB, including 320 000 among people who were HIV positive. The mortality rate has decreased to 45% since 1990, and the 2015 global target of a 50% reduction in mortality is now within reach.[14]

Figure 2[15]

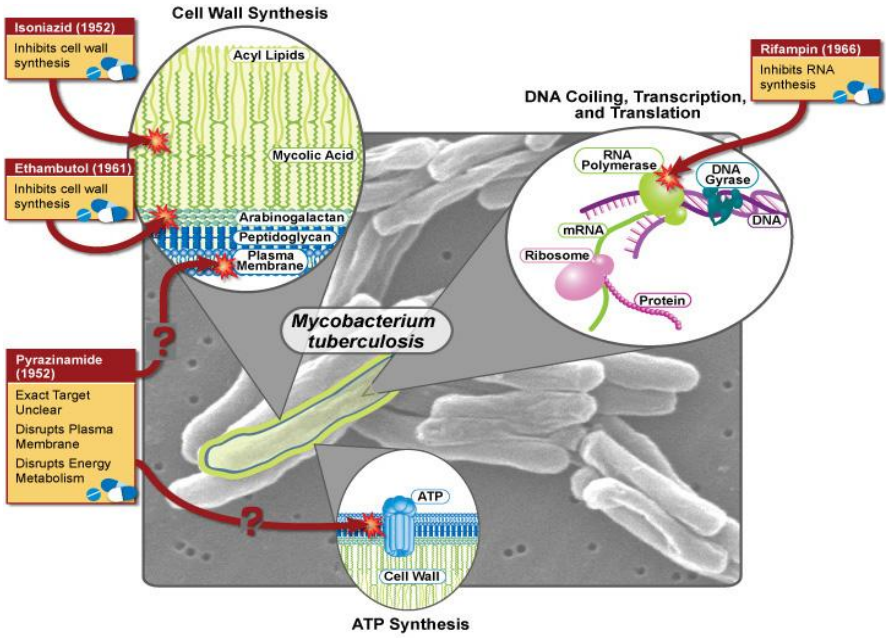


GENOME

Countless millions of people have died from Tuberculosis, a chronic infectious disease caused by the Tubercle Bacillus. The complete genome sequence of the best-characterized strain of *Mycobacterium tuberculosis*, H37Rv, has been determined and analyzed in order to improve our understanding of the biology of this slow-growing pathogen and to help the conception of new prophylactic and therapeutic interventions.[16] The genome comprises 4,411,529 base pairs, Contains around 4,000 genes, and have a very high guanine + cytosine content that is reflected in the biased amino-acid content of the proteins. *M. tuberculosis* differs radically from other bacteria in that a very large portion of its coding capacity is devoted to the production of enzymes involved in lipo genesis and lipolysis, and to two new families of

glycine-rich proteins with a repetitive structure that may represent a source of antigenic variation.[17]

Figure 3 [18]



LIFECYCLE OF MYCOBACTERIUM TUBERCULOSIS [19]

There's 5 Stages of Tuberculosis

1) Onset (1-7 Days): The Bacteria is inhaled.

2) Symbiosis (7-21 Days): If the Bacteria do not get killed then it reproduces.

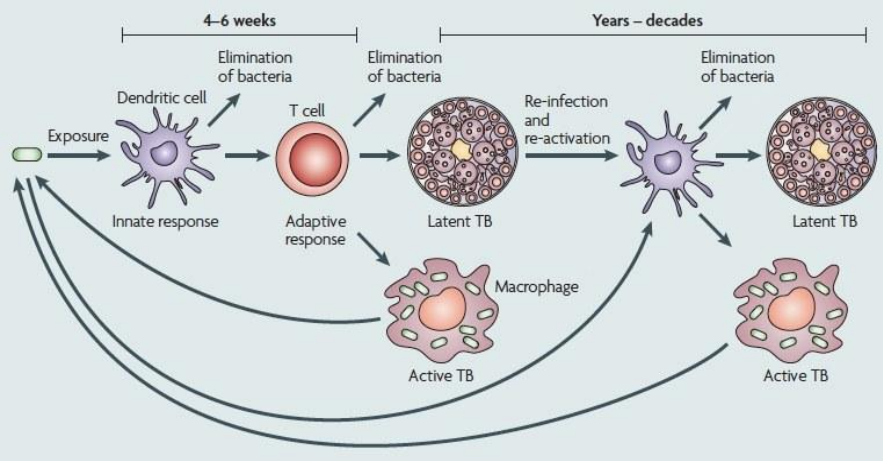
3) Initial Caseous Necrosis (14-21 Days): Tuberculosis starts to develop when the Bacteria slow down at reproducing, they kill the surrounding non activated Macrophages and run out of cells to divide in. The Bacteria then produces anoxic conditions and reduces the PH. The Bacteria can't reproduce anymore but can live for a long time.

4) 4. Interplay of Tissue Damaging and Macrophage Activating Immune Response (After 21 days): Macrophages surround the tubercle but some may be inactive. Tuberculosis then uses it to reproduce which causes it to grow. The tubercle can break off and spread around. If it spreads in

the blood one can develop tuberculosis outside the lungs, this is called Miliary Tuberculosis[5].

5) **Liquification and Cavity Formation:** The tubercles at one point will liquefy, which will make the disease spread faster, not everyone will get to this stage. Only a small percent of people will get to this stage.

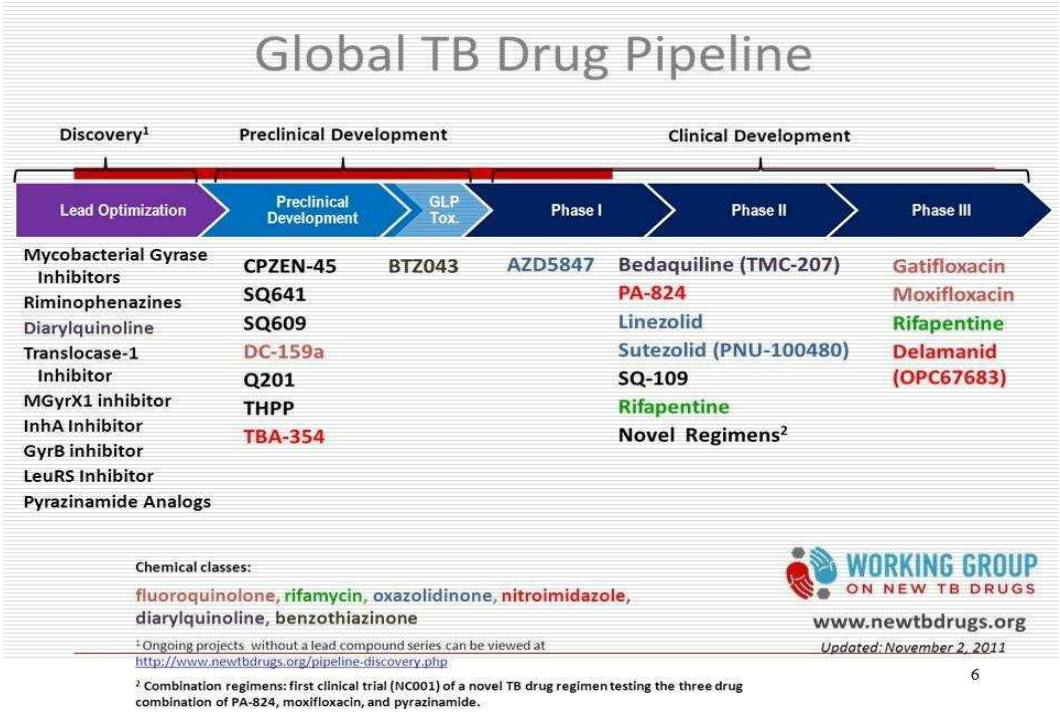
Figure 4[(20)]



CURRENT STATUS OF TUBERCULOSIS:

Tuberculosis (TB) continues to remain one of the most pressing health problems in India. India is the highest TB burden country in the world, accounting for one fifth of the global incidence - an estimated 1.96million cases annually. Approximately 2.9million people die from tuberculosis each year worldwide; about one fifth of them in India alone .Nearly 500,000 die from the disease – more than1000 per day–one every minute.[(21)] The disease is a major barrier to social and economic development. An estimated 100 million workdays are lost due to illness. The society and the country also incur huge cost due to TB–nearly US$ 3 billion in indirect cost and US$ 300 million in direct costs. The situation is more complicated considering that disproportionately affects the young population in India. TB mortality in the country has reduced from an estimated 42 per lakh population in 1990 to 28 per lakh population in 2006, and the prevalence of TB in the country has reduced from 568 per lakh population in 1990 to 253 per lakh population by the year 2014.[(22)]

Figure 5[23]



MEDICAL CARE

The tuberculosis vaccine, known as Bacilli Calmette-Gurine (BCG) may prevent the spread of tuberculosis and tuberculosis meningitis in children, but the vaccine does not necessarily protect against pulmonary tuberculosis.

The chemotherapy of infectious disease, using sulfonamide and penicillins, had been underway for several years, but these molecules were ineffective against Mycobacterium tuberculosis. From 1943 streptomycin was used in treatment. Following streptomycin, p-aminosalicylic acid (1949), isoniazid (1952), pyrazinamide (1954), cycloserine (1955),ethambutol (1962), and rifampin, pyrazinamide, and ethambutal provide effective TB treatment.[24]

DIRECTLY OBSERVED THEARAPY

For initial empiric treatment of tuberculosis (TB), start patients on a 4-drug regimen: Isoniazid, rifampin, pyrazinamide, and either ethambutol or streptomycin. Once the TB isolate is known to be fully susceptible, ethambutol (or streptomycin if used as a fourth drugs) can be discontinued.

After 2 months of therapy (for a fully susceptible isolate), pyrazinamide can be stopped. isoniazid plus rifampin are continued as daily or intermittent therapy for 4 more months. If isolated isoniazid resistance is documented, discontinue isoniazid and continue treatment with rifampin,pyrazinamide, and ethambutol for the entire 6 months. Therapy must be extentded if the patient has cavitary disease or remains culture-positive after 2 months of treatment.[25]

Directly observed therapy (DOT) is recommended for all patients. Patients on the above regimens as DOT can be switched to 2- to 3-times per week dosing after an intial 2 weeks of daily dosing. Patients on twice –weekly dosing must not miss any dose. Prescribe daily therpy for patients on self – administered medication.[26]

DRUG RESISTANT OF TB

TB organisms resistant to the antibiotics used in its treatment are widespread and occur in all countries. Drug resistance emerges as a result of inadequate treatment and once TB organisms acquire resistance they can spread from person to person in the same way as drug sensitive TB.

MDR-TB

Multidrug-resistance TB (MDR-TB) is caused by organisms that are resistant to the most effective anti-TB drugs (isoniazid and rifampicin). MDR-TB results from either infection with organisms which are already drug-resistance or may develop in the course of a patient's treatment.[27]

XDR-TB

Extensively drug-resistance TB (XDR-TB) is a form is TB caused by organisams that are resistant to isoniazid and rifampicin (i.e. MDR-TB) as well as any fluoroquinolone and any of the second-line anti-TB injectable drugs (amikacin,kanamycin or capreomycin).

These forms of TB do not respond to the standard six month treatment with first-line anti-TB drugs and can take two years or more to treat with drugs that are less potent, more toxic and much more expensive.[28]

THE NEED FOR NOVEL TUBERCULOSIS DRUGS

- To improve the treatment of MDR-TB.
- To provide more effective treatment of latent tuberculosis infection.
- XDR TB disease is resistant to first-line and second-line drugs, patients are left with treatment options that are more toxic, more expensive, and much less effective.
- Discovery of a compound that would reduce both the total duration of treatment and the frequency of drug administration.
- With multidrug resistant cases of tuberculosis increasing globally, better antibiotic drugs and novel drug targets are becoming an urgent need.[28]

ENZYME

TARGET : L, D-TRANSPEPTIDASE-2

- Traditional β-lactam antibiotics that inhibit D,D-transpeptidases are not effective against mycobacteria, because mycobacteria rely mostly on L,D-trans peptidases for biosynthesis and maintenance of their peptidoglycan layer.
- This reliance plays a major role in drug resistance and persistence of Mycobacterium tuberculosis (Mtb) infections.
- The crystal structure at **1.7 Å** resolution of the Mtb L,D-transpeptidase containing a bound peptidoglycan fragment, provides information about catalytic site organization as well as substrate recognition by the enzyme.
- Based on structural, kinetics, and calorimetric data, a catalytic mechanism for LdtMt2 in which both acyl- acceptor and acyl-doner substrates reach the catalytic site from the same, rather than different,entrance has been proposed by Sabri B. Erdemli et al.

- This information provides vital insights that facilitate development of drugs targeting this validated yet unexploited enzyme.[29]

Figure 6[29]

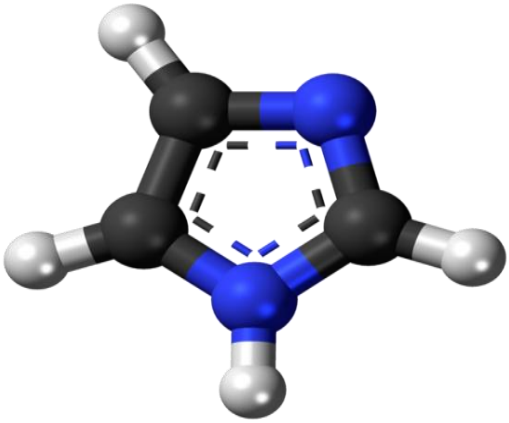
BASIC NUCLEUS INTRODUCTION

HETEROCYCLIC CHEMISTRY:

Heterocyclic structures always are a part in the field of research and development in organic chemistry. Millions of heterocyclic structures are found to exist having special properties and biological importance. Among various compounds, we have chosen imidazole, a fused diazole heterocyclic structure. This ring system is present in important biological building- blocks, such as histidine, and the related hormone histamine. Many drugs contain an imidazole ring.[30]

IMIDAZOLE NUCLEUS:

Imidazole is a planar five-member heterocyclic ring with 3C and 2N atom and in ring N is present in 1st and 3rd positions. The imidazole ring is a constituent of several important natural products, including purine, histamine, histidine and nucleic acid. Imidazole derivatives have occupied a unique place in the field of medicinal chemistry. The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery.[31] The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents.[32]

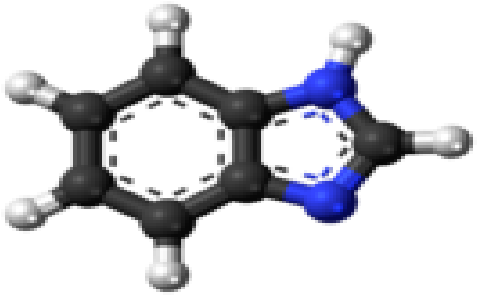


BENZIMIDAZOLE

NUCLEUS

Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. The most prominent benzimidazole compound nature is *N*-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B12.[33]

Benzimidazole also has fungicidal properties. It acts by binding to the fungal microtubules and stopping hyphal growth. It also binds to the spindle microtubules and blocks nuclear division.[34]



On the basis of various literature surveys Imidazole and Benzimidazole derivatives shows various pharmacological activities.[35],[36]

- ❖ Anti-tubercular activity.[37]
- ❖ Anti-fungal and Anti-bacterial activity.
- ❖ Anti-inflammatory activity and analgesic activity.

- Anti-depressant activity.
- Anti-cancer activity.[38]
- Anti-viral activity.
- Antileishmanial activity.

On view of the importance of the imidazole and benzimidazole nucleus. It was decided to design molecule based on the imidazole and benzimidazole nucleus.

DRUG DISCOVARY,DESIGN AND DEVELOPMENT

DRUG DISCOVERY

The process of drug discovery is very complex and requires interdisciplinary effort to design effective and commercially feasible drugs. Earlier drug discovery was 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.[39] With the help of computers, a new era has begun in drug discovery. The development cost will be cut by almost a third. The development times are reduced.

LEAD AND LEAD OPTIMIZATION

A Lead is defined as a compound, usually a small organic molecule that Demonstrated desired biological activity on a validated molecular target. Lead optimization is a technique of refining 3D structures of drug molecules and promoting the binding of drug to protein active sites. In this technique the modification of the structure of the drug molecules is done by docking every specific structure of a drug compound in active site of protein and calculating the extent of their interactions.[40] Optimization aids in the structural modification of newer molecules in order to improve the physico-chemical properties and biological activity for a given set of compounds in the library.[39]Further structural modification improves the affinity, reactivity towards target and enhances stability during metabolism.

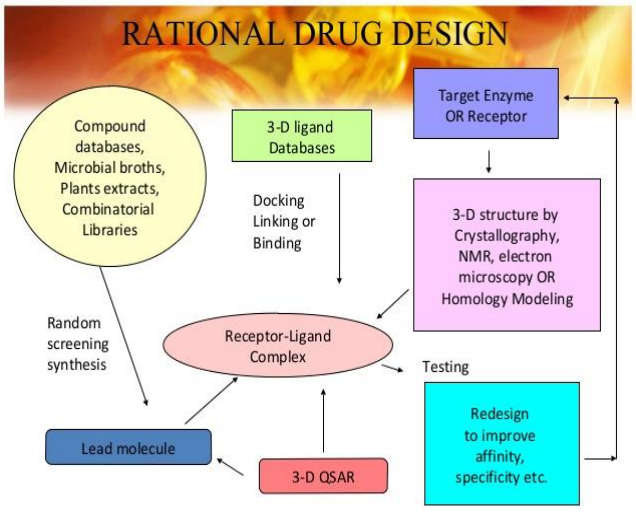
Computer aided drug design[41]

Computer aided drug design uses computational chemistry to discover, enhance or study drugs and related biologically active molecules. The most fundamental goal is to predict whether a given molecule will bind to a target and if so how strongly. 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. This provides semi-quantitative prediction of the binding affinity. Also, knowledge-based scoring function may be used to provide binding affinity estimates. These methods use linear regression, machine learning, neural nets or other statistical techniques to derive predictive binding affinity equations by fitting experimental affinities to computationally derived interaction energies between the small molecule and the target.[42]

Rational drug design[43]

Drug design is a process which involves the identification of a compound that displays a biological profile and chemical synthesis of the new chemical entity are optimized. Drug designing is otherwise known as rational drug design and it is a method of finding new medications, based on the biological receptors and target molecules. The objective of drug design is to find a chemical compound that can fit to a specific cavity on a protein target both geometrically and chemically.

Figure 7[44]



Types of drug design

- Ligand based drug design
- Structure based drug design

Ligand based drug design

Ligand based drug design is an indirect approach which 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.

Structure based drug design

Structure based drug design is a direct approach which relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography and 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, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist or various automated computational procedures to suggest new drug candidates.[45]

Docking

Docking is simply refer to the ability to position a ligand in the active or a designated site of a protein and calculates the specific binding affinities. Ligand-protein docking as evolved so remarkably throughout the past decade that docking single or multiple small molecules to a receptor site is now routinely used to identify ligands. Optimal docking procedures need to be fast, generate reliable ligand geometries, rank the ligand conformation

correctly (scoring) and thereby, estimate the binding energy. A number of studies have shown that docking algorithms are capable of finding ligands and binding conformations at a receptor site close to experimentally determined structures. These algorithms are equally applicable to the identification of multiple proteins to which a small molecule can bind. The application of this approach may facilitate the prediction of either unknown and secondary therapeutic target proteins their side effects and toxicity of particular drugs. In computational structure-based drug design, the evaluations of scoring functions are the cornerstones to the success of design and discovery. Many approaches have been explored to improve their reliability and accuracy, leading to three families of scoring functions. These are force-field-based, knowledge-based, emprical-based.[46]

Scoring function

Scoring functions are normally parameterized (or trained) against a data set consisting of experimentally determined binding affinities between molecular species similar to the species that one wishes to predict.

Types

1) **Force field based -** Force-field affinities are estimated by summing the strength of intermolecular vanderwaals and electrostatic interactions between all atoms of the two molecules in the complex.

2) **Emprical -** Based on coming the number of various types of interactions between the binding partners. Counting may be based on the number of ligand and receptor atoms in contact with each other or by calculating the change in solvent accessible surface area complex compared to the uncomplexed ligand and protein. These interaction terms of the function may include for example: hydrophobic-hydrophobic contacts, hydrophobic-hydrophilic contacts, number of hydrogen bonds, number of rotatable bonds immobilized in complex formation.

3) **Knowledge-based** (also known as statistical-potentials) - Based on statistical observations of intermolecular close contacts in large 3D databases which are used to derive "potentials of mean force". This method is founded on the assumption that close intermolecular interactions between certain types of atoms or functional groups that occur more frequently than one would expect by a random distribution are likely to be energetically favourable and therefore contribute favourably to binding affinity.(47)

Absorption, Distribution, Metabolism and Excretion (ADME) analysis For a drug to be pharmacologically active and exert the action it should possess appropriate pharmacokinetic properties like Absorption, Distribution, Metabolism and Excretion. In the field of drug research and development many drug failures do occur, such that the drug may fail to undergo those properties satisfactorily.

This has to be ruled out earlier in the process of drug discovery. Many *In vitro* studies are more frequently used to evaluate ADME properties. Some computational methods (*In silico* tools*)* have been evolved to investigate the most suitable drug molecules.

In silico modeling serves for two main functions in predicting those (ADME) properties i.e,

- Earlier investigation of designing compounds and compound libraries in order to reduce the risks at later.
- Optimizes the screening and testing, most probably by focusing only on the more active compounds.
- A deep rooted knowledge in understanding the relationship of ADME parameters and the underlying (drug likeness property) molecular structural features to which it depends on.
- It enhances in elaborating this session of interest to the area of posology where it gives information about the drug dosage and

frequency. This in turn reflects on issues on bioavailability, crossing various biological membranes like brain, ocular and dermal penetration.

These are the essential factors and criteria to look in, for a drug to be pharmacologically active and execute as the most successful clinical candidate in the pharmaceutical research.

Prediction of ADME related parameters

Absorption

To investigate this property in *in silico* model used simple parameters like log D (diffusion coefficient) and polar surface area are the descriptors for hydrogen bonding capacity and log P (partition coefficient) values should fall under the prescribed values asper the rule of thumb which determines the absorption.

Bioavailability

Factors like size and shape of molecule, lipophilicity and flexibility determines the bioavailability.

Blood Brain Barrier Penetration

In order for a drug to cross the blood brain barrier (molecule targeted to brain). Rule of thumb says log P values should be closer to 2 with a molecular mass of <450 Da and or with a polar surface area (PSA) <100 A are likely to possess.

Dermal and Ocular Penetration

For dermal and ocular route it should satisfy the existing parameters like log P (partition coefficient) for aqueous solubility, molecular weight and molecular flexibility.

Metabolism

Various *in silico* approaches exist in evaluating the metabolism namely QSAR and 3D QSAR etc. apart from those computational chemists have

updated the structural details in the data bases and tools for predicting metabolism. Simultaneously it reveals the metabolic information as well as the toxicity related to the molecular fragments by which the drug molecule undergoes the metabolism.[48]

Evaluation of *in silico* toxicity

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

In silico approaches like OSIRIS® property explorer predicts carcinogenicity, mutagenicity, teratogenicity, immune toxicology, irritation, sensitization etc. In addition, hepato, neuro and cardiotoxicity are evaluated in newly updated *in silico* tools.

AIM AND PLAN OF WORKS

Objective of the Present Study

AIM

The aim of this project is to develop potential antimycobacterial agents.

OBJECTIVES

The objective of the project is to design and synthesize some compounds which will act on L,D Transpeptidase 2 and inhibit the cell wall synthesis of *M.tuberculosis*.

THE PLAN OF WORK

- Design of L,D Transpeptidase 2 inhibitors by docking studies
- Insilico Prediction of Drug Likeness and Toxicity
- Laboratory synthesis of the top G score compounds
- Characterization of the synthesized compounds by
    - Infrared Spectrometry
    - Nuclear Magnetic Resonance Spectroscopy
    - Mass Spectrometry
- Determination of In-vitro anti tubercular activity of synthesized compounds.

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

The present study carried out based on the below flow

Identification of the lead compound

↓

Lead optimization

↓

Docking of the molecule to the target protein

↓

Top G score compounds selected

↓

Insilico drug likeness and toxicity risk assessment

↓

Synthesis

↓

Justification of purity-TLC, GC

↓

Characterization – Spectroscopy (IR,NMR,GC- MS,LC-MS)

↓

Invitro anti-tubercular activity

REVIEW OF LITERATURE

The Review of Literature related to the genomic of the Mycobacterium Tuberculosis:

1) **Rahul Jain *et al.,(2005)*** Tuberculosis (TB) is one of the most devastating diseases primarily due to several decades of neglect, and presents a global health threat of escalating proportions. TB is the second leading infectious causes of mortality today behind only HIV/AIDS. (49)

2) **James C. Sacchettini *et al., (2004)*** worked on TB drug discovery. Addressing issues of persistence and resistance by reviewing the recent developments of some of the pathways involved in a persistent infection and pathogenesis of mycobacterium tuberculosis, which reveal new targets for drug development. (50)

The Review of Literature related to the target enzyme, 4GSU and its function:

3) **Kim et al.,(2013)** worked on Structure basis for the inhibition of Mycobacterium tuberculosis L, D-transpeptidase by meropenem, a drug effective against extensively drug-resistant strain.(51)

4) **Hyoun Sook Kim., et al.(2012**) reported on structure basis for the inhibition of Mycobacterium Tuberculosis L,D-transpeptidase by meropenem, a drug effective against(52)

The Review related to the drug design study:

5) **Deepak. D. Borkar., et al. (2012),** Design and Synthesis of *p*-hydroxy benzohydrazide Derivatives for their Antimycobacterial Activity. (53)

6) **Romono T. Kroemeret *et al.(2003),*** An introduction into ligand–receptor docking. It illustrates the basic underlying concepts. (54)

7) **Andrew Worth *et al.(1998),*** Distribution, Metabolism and Excretion (ADME) properties, which are often important in discriminating

between the toxicological profiles of parent compounds and their metabolites/degradation products. (55)

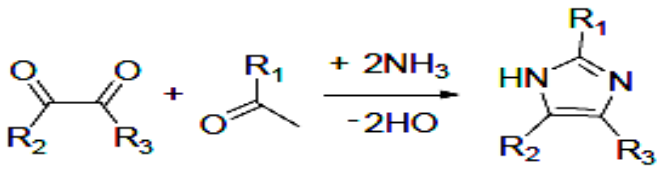
8) **Lipinski CA et al., (2001)** A experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. (56)

9) **Lipinski CA (2004)** A Lead and drug-like compounds and the role of fine **resolution**. (57)

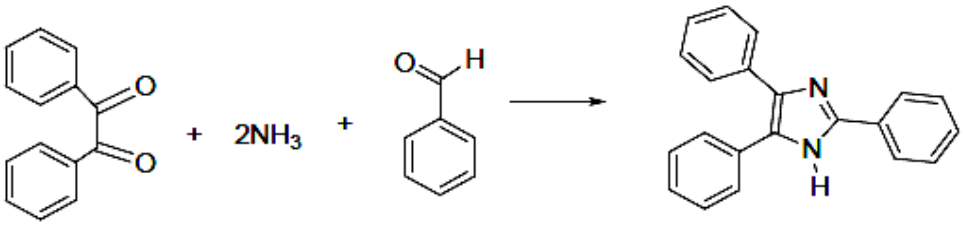
10) **Madsen et al., (2002)** Textbook of Drug Design and Discovery. (54) The review on following works provided ideas for synthesis of the desired chemical entities:

The Review of Literature related to the desired chemical entities:

11) **Debus et al.(1858),** Debus Synthesis of imidazole by using glyoxal and formaldehyde in ammonia. This synthesis, while producing relatively low yields, is still used for creating C substituted Imidazoles. (58)

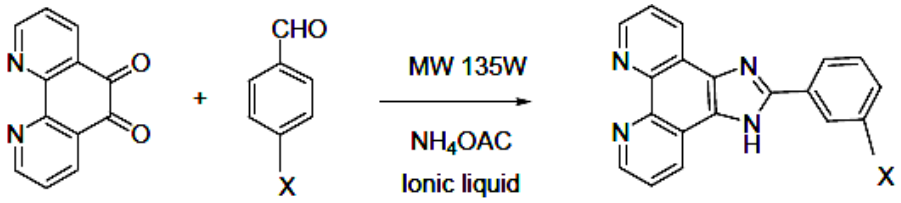


12) **Radiszewski et al.** Radiszewski reported the condensation of a dicarbonyl compound, benzil and α- keto aldehyde **or** benzaldehyde in the presence of ammonia, to yield 2, 4, 5 triphenylimidazole. (59)

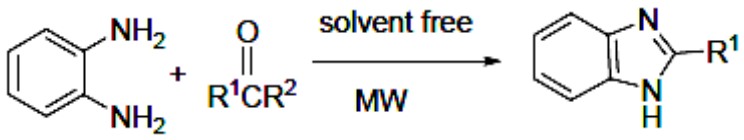


13) **Qasim *et al* (2011)** Synthesis of 2- phenylimidazo [4,5-f] [1,10] Phenanthroline derivatives, by reacting dicarbonyl compound and *p*-substituted benzaldehyde. This is a type of acid catalyzed reaction with excellent yields in a neutral ionic liquid, 1-methyl-3-

heptylimidazolium tetrafluoroborate [(HeMIM) BF4], under solvent free and microwave assisted conditions. This particular reaction accompanies all the merits of microwave reactions like easy workup, better yield, and environment friendly reaction. (60)

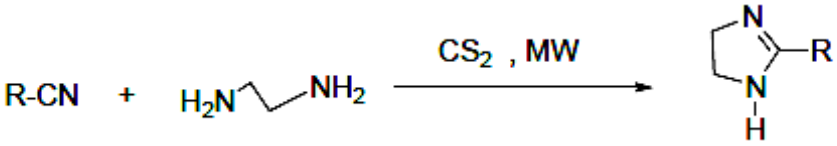


14) **Na Zhao *et al* (2005)** Reported an efficient and a quick microwave-assisted synthesis of benzimidazoles and trisubstituted imidazoles. (61)

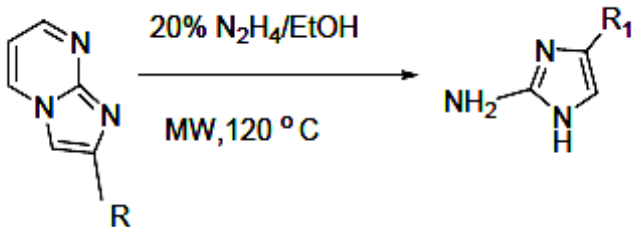


a) R^1= $C_6H_5OCH_2$- , R^2=OH
b) R^1=2,4-$(Cl)_2$ $C_6H_3OCH_2$ - , R^2 = OH
c) R^1=-CH_3 ,R^2 =-CH_2COOEt
d) R^1=C_6H_5- ,R^2 =OH
e) R^1= -CH_3 ,R^2=OEt

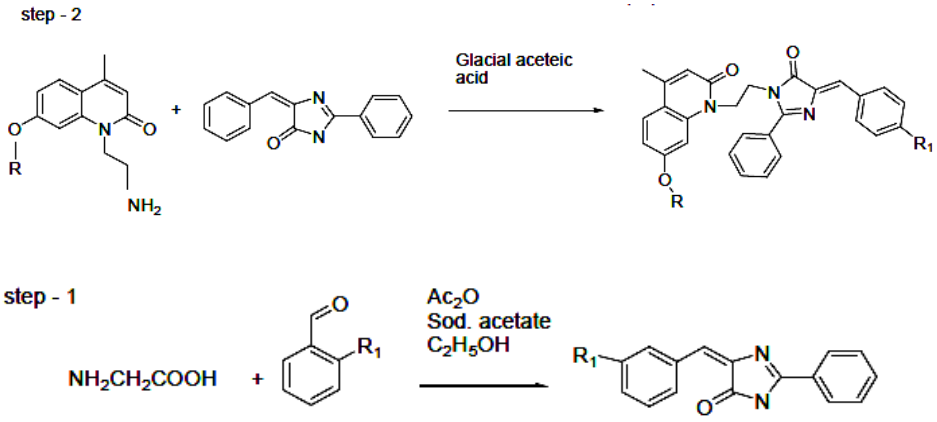
15) **Pathan *et al* (2006)** Reported the reaction of alkyl cyanide with ethylenediamine in the presence of carbon disulphide gives 2-substituted 2 imidazolines under microwave irradiation. (62)



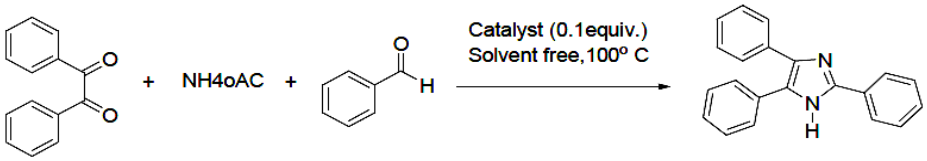
16) **Ermolat *et al* (2009**) Explained the synthesis of mono and disubstituted-2-amino-1H imidazoles Via microwave assisted hydrazinolysis of substituted imidazo[1,2a]pyrimidines.(63)



17) **Raghavendra *et al* (2011)** A Series of 1-(2-((18Z)-4-substituted benzylidene-4, 5-dihydro-5- oxo-2-phenylimidazol-1-yl) ethyl)-1, 2-dihydro-4-methyl-2-oxoquinolin-7-yl imidazole quinoline analogs is synthesized by condensation of substituted imidazole and substituted quinolone. [64]

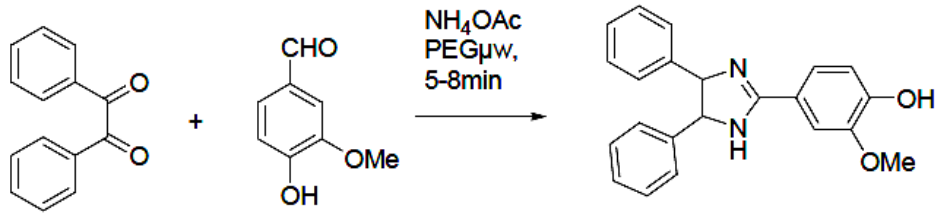


18) **Safari *et al* (2010)** (NH4)6.Mo.7O2. 4H2O was used as an efficient catalyst for an improved and rapid synthesis of 2,4,5-trisubstituted imidazoles by a three-component, one-pot condensation of benzil, aryl aldehydes and ammonium acetate in good yields under solvent-free conditions using microwave irradiation. The reactions in conventional heating conditions were compared with the microwave-assisted reactions. [60]

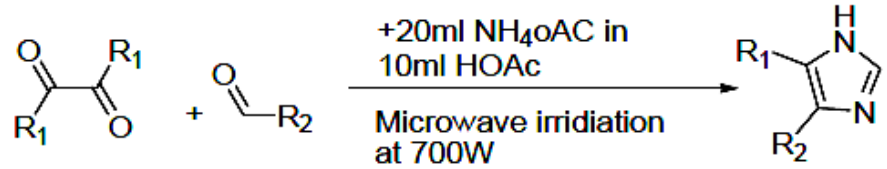


19) **Nalage *et al* (2010)** Described an efficient and green procedure for the synthesis of 2, 4, 6-triaryl- 1H-imidazole in polyethylene glycol by condensing benzil and 3-methoxy- 4- hydroxyl benzaldehyde under

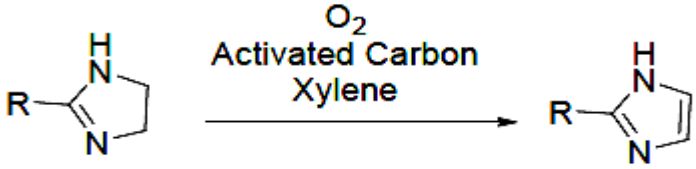
microwave irradiation in excellent yield. Polyethylene glycol is non-toxic, reusable, inexpensive and easily available. [65]



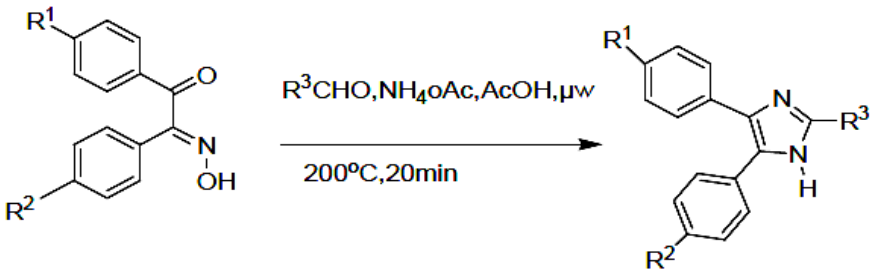
20) **Wahyuningrum *et al* (2007)** Explained 4,5-substituted imidazole **derivatives** have been synthesized utilizing microwave assisted organic synthesis (MAOS) method, by reacting with suitable diketone and some aldehyde or ketone, in order to investigate their corrosion inhibition mechanism on carbon steel surface. [66]



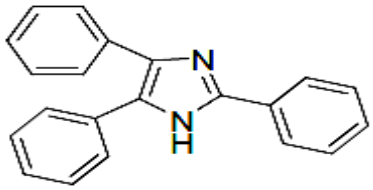
21) **Kawashita *et al* (2009),** A variety of hetero aromatic compounds such as 2-substituted imidazoles were synthesized by oxidative aromatization of 2-substituted imidazolines using the activated carbon and molecular oxygen system. [67]



22) **Sparks *et al* (2004)** Synthesis of 2,4,5-Triaryl-imidazoles directly from the ketooxime in moderate to good yields via cyclization to the N-hydroxyimidazole and an unprecedented in situ thermal reduction of the N-O bond upon microwave irradiation at 200°C for 20 min. [68]



23.Satyajit *et al* (2010) A series of 2-substituted-4,5-diphenyl imidazoles were synthesized by refluxing benzil with different sub-stituted aldehydes in the presence of ammonium acetate and glacial acetic acid. Compounds were screened for anthelmintic activity. [69]



Review of Literature related to the target: L,D-Transpeptidase

23) **Wen-Juan Li et al.,**[70]Crystal structure of L,D-transpeptidase LdtMt2 in complex with meropenem reveals the mechanism of carbapenem against *Mycobacterium tuberculosis.*

24) **Sabari b. erdemli et al,**[71] studied that the traditional β-lactam antibiotics that disrupt the D,D-transpeptidases are not effective against mycobacteria. As mycobacteria rely mostly on β-lactam insensitive L,D-transpeptidases for biosynthesis and maintenance of their peptidoglycan layer. Based on the structural, kinetic and calorimetric data, catalytic mechanism for LdtMtb2 has been proposed.

25) **Lauriane Lecoq et al,**[72] Dynamics Induced by β-Lactam Antibiotics in the Active Site of Bacillus subtilis L,D-Transpeptidase .

26) **Soumya De and Lawrence P. McIntosh** [73] investigated the structural and dynamic basis for the unexpected inhibition of peptidoglycan crosslinking L,D-transpeptidases by carbapenam antibiotics.

27) **Dominic Both et al,**[74] studied the crystal structure of the protein and summarized that he transpeptidase LdtMt2 catalyzes the formation of the cross links characteristics of the peptidoglycan layer in the Mycobacterium tuberculosis cell wall.

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

28) **Scott G. Franzblau et al**,[75] studied MIC determination by MABA. A colorimetric, Microplate Based Alamar Blue Assay (MABA) method was used to determine the MICs of Isoniazid, Rifampin, Streptomycin and Etambutol for 34 peruvian Mycobacterium tuberculosis isolates and the H37Rv strain by using bacterial suspensions prepared directly from media. The MABA is a simpe, rapid, low cost, appropriate technology which does not require extensive instrumentation and which makes use of a nontoxic, temperature stable reagent.

29) **Sephra N. Rampresad et al,**[76] studied the various applications of Alamar Blue as an indicator. Alamar Blue is a redox indicator that is used to evaluate metabolic function and cellular health. The Alamar Blue Bioassay is being utilized to access cell viability and cytotoxicity in a range of biological and environmental system and in a number of cell types including bacteria, yeast, fungi, and protozoa.

30) **Jose de Jesus Alba-Romero et al,**[77] applied the Alamar Blue Assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. The result showed that the MABA test is fast and easy to apply. It is a very reliable method of determining the drug susceptibility to pharmaceuticals.

MATERIALS AND METHODS

DRUG DESIGN

Docking program is used to fit the ligand molecules into the target structure in a variety of positions, confirmations, and orientations. Docking mode is known as pose. Each pose is scored, based on its complementarity to the target in terms of shape and electrostatic properties. This is done to identify the most favorable energetical pose.

The quality of any docking result depends on the starting structure of both the protein and the potential ligand. The protein and ligand structures need to be prepared to achieve the best docking results.[78]

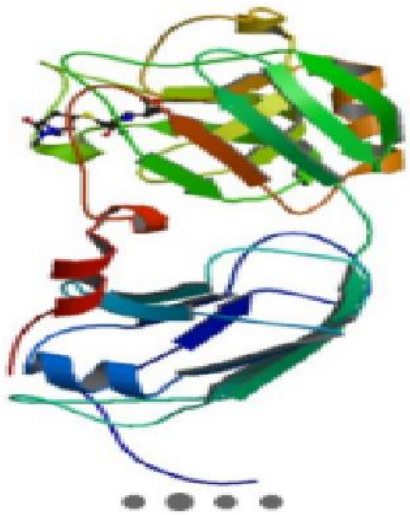
MOLECULAR DOCKING BY DRUG DESIGN

Argus lab 4.0 is distributed freely for windows platforms by planaria software. It is an introductory molecular modeling package for academics. Argus docking engine implement 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 in the ligand.[79]

Protein Preperation

The crystallized structure of the receptor/protein is imported from Protein DataBank (PDB). The PDB id: 4GSU it refers to the target L,D Transpeptidase 2. The PDB file was selected based on its species, X-ray crystallography or NMR spectroscopy, resolution value 2.8 A, external ligand and presence of co-factor.[80].The water molecules, co-factor and unwanted chains were deleted. And the energy minimization has been done so that it will be ready for grid generation.

Figure 8 [81]



TYPES OF DRUG DESIGN: [82]

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

Ligand Based Drug Design

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

De Novo Drug Design

De novo is a Latin expression meaning "from the beginning". Active site of drug targets when characterized from a structural point of view will shed light on its binding features. This information of active site composition and the orientation of various amino acids at the binding site can be used to design ligands specific to that particular target. Computational tools that can analyze protein active site and suggest potential compounds are extensively used for *de novo* design methods.

Structure Based Drug Design(SBDD)

If the approach where the structural information of the drug target is exploited for the development of its inhibitor. Receptor structure(s) is a prerequisite for this method. Most commonly the structure of the receptor is determined by experimental techniques such as X-ray crystallography or NMR. If the structure of the protein drug target is not available, protein structure can be predicted by computational methods like threading and homology modeling. Threading (also called as fold) is a modeling approach used to model proteins that do not have homologous proteins with known structure.[83]

DOCKING

Docking involves the fitting of a molecule into the target structure in a variety of positions, conformations and orientations. Molecular docking is used to predict the structure of the intermolecular complex formed between two molecules.[84] The small molecule called ligand usually interacts with the protein binding site.There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. It also predicts the strength of the binding, the energy of the complex; the types of the complex; the types of the signal produced and calculates the binding affinity between two molecules using scoring functions. The most interesting case is the type protein-ligand interaction, which has its applications in medicine.[85]

TYPES OF DOCKING

- **Lock And Key or Rigid Docking** [84] - In rigid docking, both the internal geometry of the receptor and ligand is kept fixed and docking is performed.

- **Induced Fit or Flexible Docking**[84] - an enumeration on the rotations of one of the molecules (usually smaller one) is performed. Every rotation the surface cell occupancy and energy is calculated; later the most optimum pose is selected.

STEPS INVOLVED IN DOCKING [86],[87],[88]

Docking is done by using ARGUS LAB Software.

1) Protein preparation.

2) Selection of active site (Q-Site finder).

3) Ligand Preparation.

4) Docking Procedure.

5) Visualization / Interpretation of Docking.

PROTEIN PREPARATION

Step:1

- The PDB id(4GSU) is entered in the protein data bank.
- The pdb files are downloaded and text file selected.
- The downloaded pdb (text file)is saved in the desktop.

Step: 2

- In the Argus lab software, the pdb file is imported from the desktop and the 3D structure of the protein appears in the workspace of Argus lab.
- The molecular tree view will appear on left side of screen and the pdb is opened and residues ,miscellaneous are viewed.
- Miscellaneous option is selected and the inhibitor and hetero residues and water molecules deleted.
- The hydrogen atoms are added
- The energy is selected on the calculation toolbar by UFF method and started.
- The prepared protein is saved as *.agi file format in the desktop.

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 favourable binding sites.

LIGAND PREPARATION

- The structure is drawn from Chem sketch and save as MDL Mol format. The ligand is imported into the workspace of Argus lab.

- The Geometry and Hybridisation is cleaned.

- Identify the location of ligand and make a group from the residues and name as ligand.

DOCKING PROCEDURE

- Then set up a Dock Ligand calculation which is selected from the toolbar.

- Argus Dock is selected from Docking Engine.

- Dock is selected as calculation type.

- Flexible is selected from the scoring function.

- The docking is started and docked protein ligand complex is saved as Brookhaven pdb files (*pdb)

VISUALIZATION / INTERPRETATION OF DOCKING

Molegro Molecular viewer

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

- Secondary structure and hydrogen bond interaction,ligand map interaction is viewed.

MOLECULAR PROPERTY PREDICTION: (89)

All the docked molecules are subjected to the toxicity risk assessment by using OSIRIS®program, which is available online. The OSIRIS® property Explorer 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.
- clogP predicition.
- Solubility prediction.
- Molecular weight.
- Drug likeness prediction.
- Drug likeness score.

TOXICITY RISK ASSESSMENT

On drawing a structure the toxicity risk predictor will start looking for potential toxicity risks as long as the 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 Registry of Toxic Effects of Chemical Substance (RTECS) database known to be active in a certain toxicity class like mutagenicity, Tumorigenicity, Irritating effects and Reproductive effects. (89)

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

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.

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.

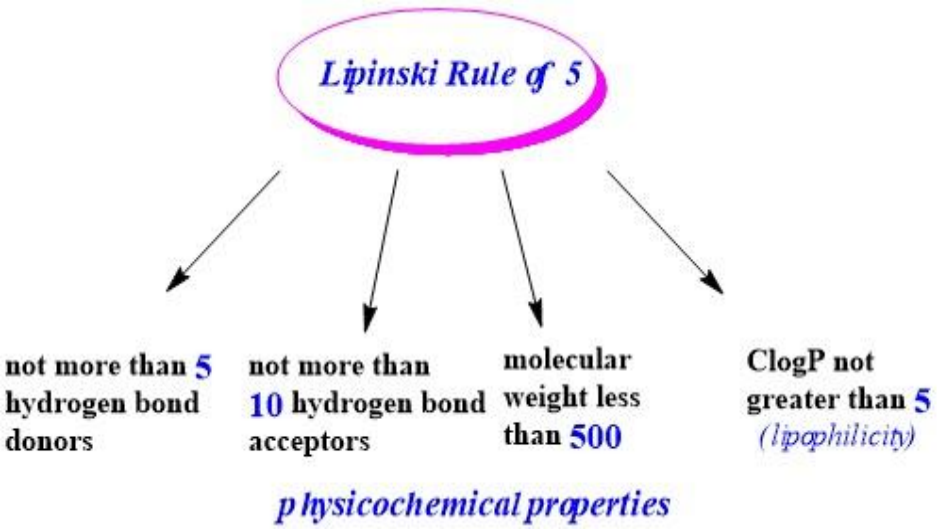
DRUG LIKENESS

Druglikeness is a qualitative concept used in drug design for how "druglike" 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 druglike 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**".

LIPINSKI'S RULE OF FIVE (90), (91)

Lipinski's rule of five also known as the **Pfizer's rule of five** or simply the **Rule of five** (RO5) is to evaluate druglikeness or d etermine 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:

Figure9(91)

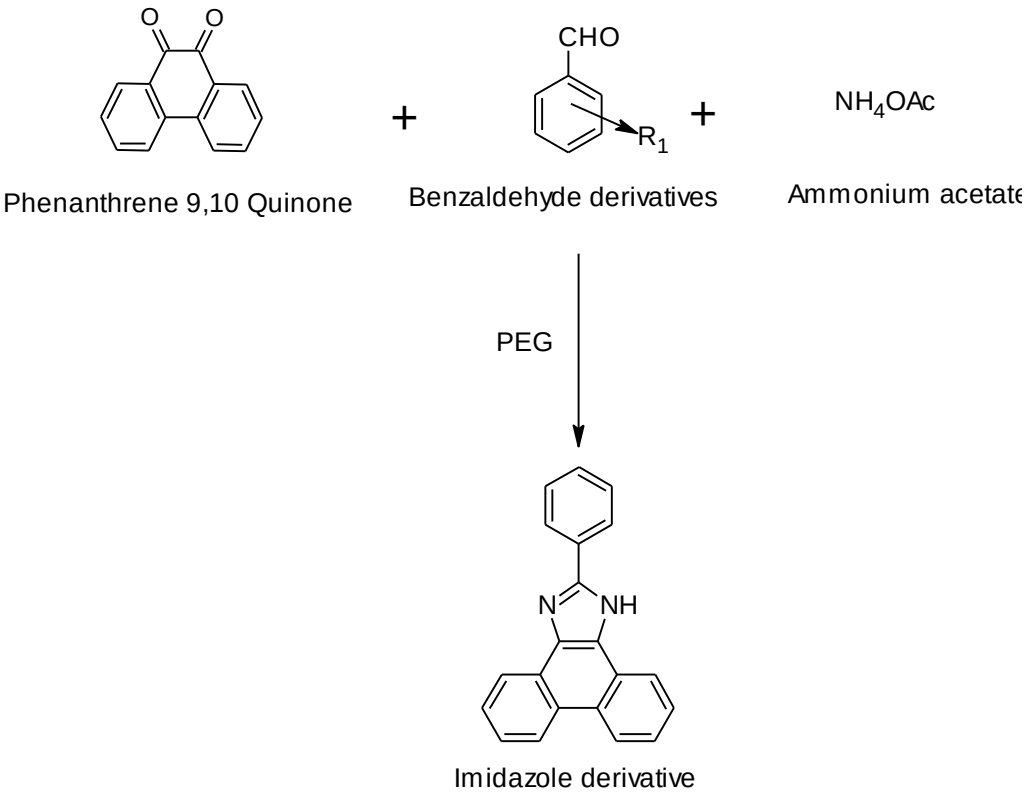


SYNTHETIC INVESTIGATION

Scheme-1

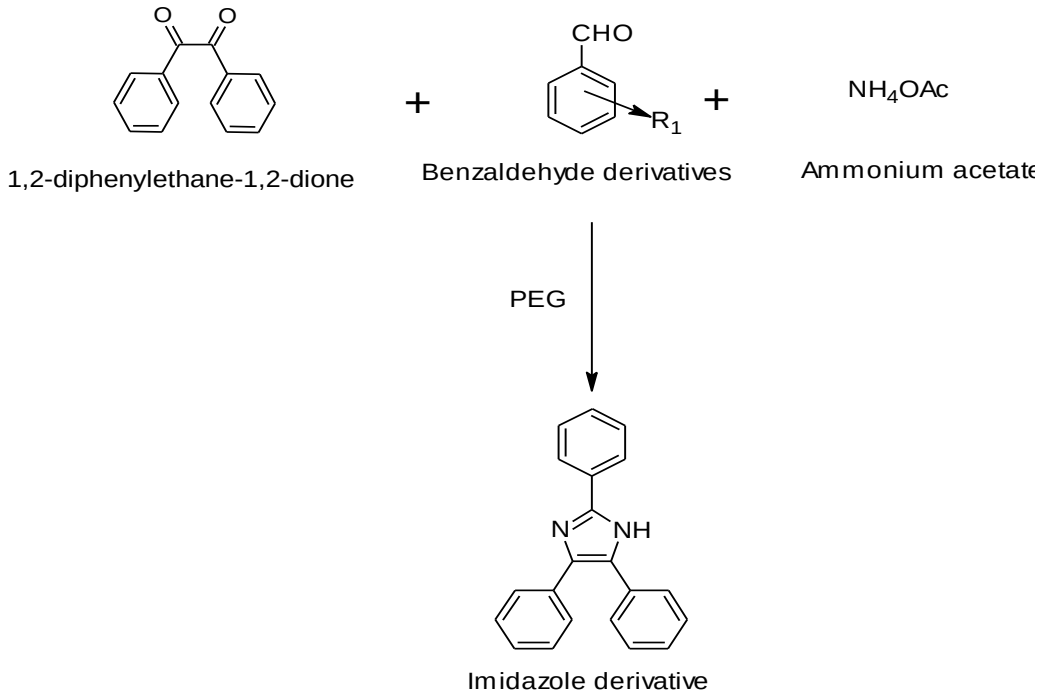
A Mixture of 1 mole of Phenanthrene 9,10-dione, 1 mole of Substituted aromatic aldehyde and 2 mole of Ammonium acetate is taken in a round bottom flask with Poly ethylene glycol which acts as a catalyst and the mixture is refluxed. On completion of reaction as monitored by TLC at an interval of 30 minutes. The sticky mass is transferred to acetone: water (6:4)

mixture. The precipitate that is obtained is filtered, dried and recrystallized. It is finally purified by using column choromatography. (92)



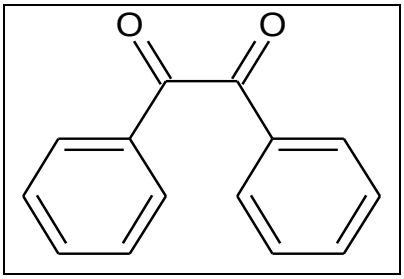
Scheme-2

A mixture of 1 mole of Benzil, 1 mole of Substituted aromatic aldehyde and 2 moles of Ammonium acetate is taken in a round bottom flask with Poly ethylene glycol which acts as a catalyst and the mixture is refluxed. On completion of reaction, as monitored by TLC at an interval of 30 minutes. The sticky mass is transferred to acetone: water (6:4) mixture. The precipitate which is obtained is filtered, dried and recrystallized. It is finally purified by using column choromatography. (93)



Reactant profile

Benzil



Molecular formula : $C_{14}H_{10}O$

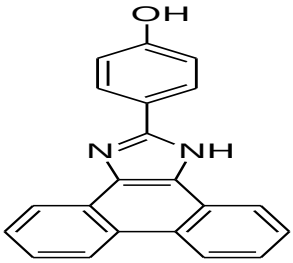
Moleculae weight : 208.21g/mol

Discription : Orange red crystals

Melting point : 209-212℃

Boiling point : 360℃

Phenanthrene 9,10-dione



Molecular formula : $C_{14}H_8O_2$

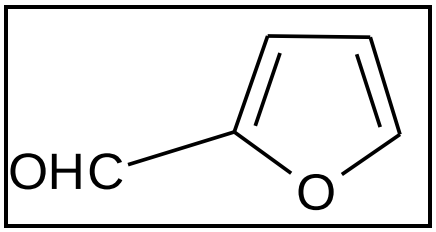
Moleculae weight : 210.22g/mol.

Discription : Yellow Crystals

Melting point : 94-96^{0}C

Boiling point : 348^{0}C

Furaldehyde



Molecular formula : $C_5H_4O_2$

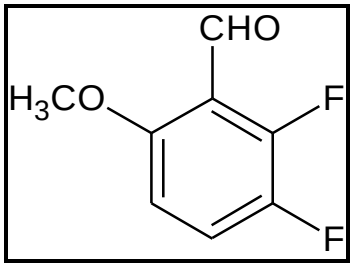
Moleculae weight : 96.08g/mol.

Discription : Reddish brown oily liquid

Melting point : -37^0C

Boiling point : 162^0C

2,3 difluro-6-methb oxy benzaldehyde



Molecular formula : $C_8H_6F_2O_2$

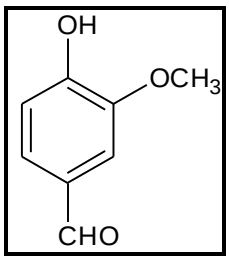
Moleculae weight : 172.12g/mol.

Discription : white colour powder

Melting point : 55^0C

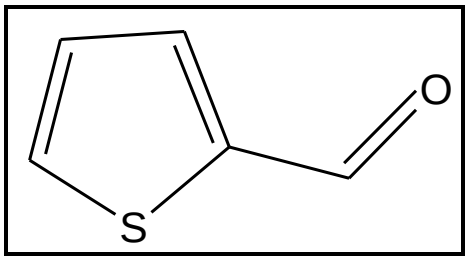
Boiling point : 233.5℃

Vanillin



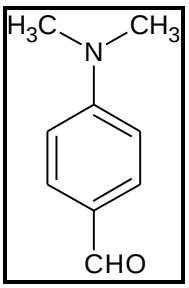
Molecular formula	:	C8H8O3
Moleculae weight	:	152.14g/mol.
Discription	:	white crystalls
Melting point	:	81oC
Boiling point	:	285.5℃

Thiophene 2 carboxyaldehyde



Molecular formula	:	C_5H_4OS
Moleculae weight	:	112.15g/mol.
Discription	:	brown colour liquid
Melting point	:	78^0 C
Boiling point	:	196.5^0C

4-Dimethylaminobenzaldehyde



Molecular formula : $C_9H_{11}NO$

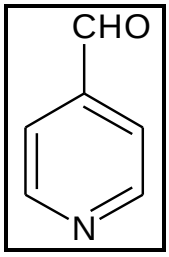
Moleculae weight : 149.18g/mol.

Discription : Crystalline powder

Melting point : 74 ^{0}c

Boiling point : 176-177 c

4-Pyridine carboxyaldehyde



Molecular formula : C_5H_5NO

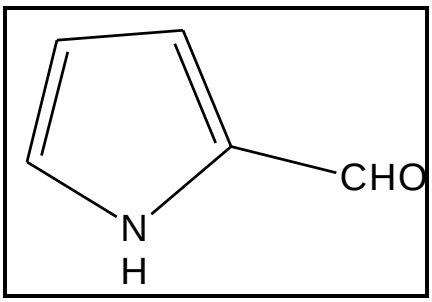
Moleculae weight : 107.11g/mol.

Discription : yellow to brown liquid.

Melting point : -4 to 2

Boiling point : 198

Pyrrole 2 Carboxyaldehyde



Molecular formula : C_5H_5NO

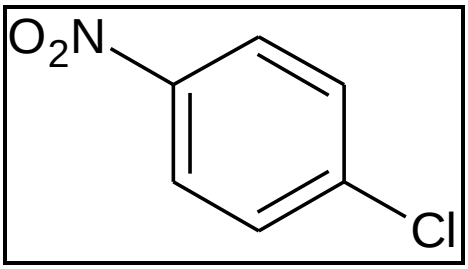
Moleculae weight : 95.0g/mol.

Discription : Pale yellow crystalline solid

Melting point : 48 c

Boiling point : 218 c

2-Chloro 5- Nitro Benzaldehyde



Molecular formula : $C_6H_4\,CLNO_2$

Moleculae weight : 157.55g/mol.

Discription : Clear slightly yellow powder

Melting point : 75-77 c

Boiling point : 290.3 c

CHARACTERIZATION PHYSICAL EVALUATION:

Physical properties of the synthesized compounds are evaluated,such as

1) Colour
2) Nature
3) Solubility
4) Molecular weight
5) Molecular formula
6) Melting point
7) Boiling point

THIN LAYER CHROMATOGRAPHY

The precoated aluminum TLC-GF binder was used. Solution of the reactants and products in ethanol are prepared. Various mobile phases are tried out of which methanol and chloroform was found to be suitable. Stationary phase: precoated silica gel GF plates

IR SPECTROSCOPY [94]

The IR spectroscopy analysis is used to determine the chemical functional groups present in the sample. Different functional groups absorb characteristic frequencies of IR radiation. The synthesized compounds are suitably prepared and IR spectrum was recorded using of FT-IR spectrophotometry(model no: MB 3000)in the range of 4000-500cm^{-1} by kBr pellet technique.

^{1}H NMR SPECTROSCOPY

Proton NMR spectra was recorded on BRUKER Advance 500 NMR spectrometry using the solvent Deuterated Dimethyl Sulphoxide. Chemical shift are reported in parts per million, relative to TMS as an internal standard.

MASS SPECTROSCOPY[94]

The mass spectroscopy, the synthesized compound under investigation is bombarded with a high beam of electrons producing ionic fragments of the original species. The relative abundance of the fragment ion formed depends on the stability of ion and of the last radical. The resulting charged particles are then separated according to their masses. The mass spectra was recorded on a Q-Tof-Mass Spectroscopy (Q-Tof micro hybrid quadrupole time of flight mass spectrometer) with electro spray ionization (ESI) and in JEOL GCMATE II GC-MS.

HYPHENATED TECHNIQUE [95]

GC-MS

The gas chromatograph coupled to a mass spectrometer, by which complex mixture of compounds can be separated, identified and quantified. GC is used to determine the purity of compounds by looking for additional peaks in a sample that are not present in the pure compound. The smaller structure have lower boiling point and will thus elute faster than those with higher boiling point.

Factors affecting the separation of compounds in GC-MS

Higher boiling point of compounds,

Polarity of compounds versus the polarity of stationary phases on column.

Column type (polar-non polar)

Amount of material injected,

High temperature and High flow rate decrease the retention time, but also decreased the quality of separation.

LC-MS

Liquid chromatography with mass spectroscopy is used to characterize the non-volatile compounds but GC-MS is used to characterize only the volatile compounds.

BIOLOGICAL EVALUATION Anti-tubercular Activity

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

- Resazurin Micro plate Assay (REMA)
- Nitrate Reductase Assay (NRA)
- Micro plate Alamar Blue Assay (MABA)
- 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)
- Middle Brook 7H11 Agar dilution Assay
- Broth Micro dilution Method
- BACTEC system
- Luciferase Reporter Phage Assay

THE ALAMAR BLUE ASSAY

Alamar Blue monitors the reducing environment of the living cell. The active ingredient is resazurin (IUPAC name: 7-hydroxy-10-oxidophenoxazin-10-ium-3-one), also known as diazoresorcinol, azoresorcin, resazoin, resazurine, which is water-soluble, stable in culture medium, is non-toxic and permeable through cell membranes. Continuous monitoring of cells in culture is therefore permitted. Growth is measured quantitatively by a visual colour change and the amount of fluorescence produced is proportional to the number of the living cells which is determined by colorimetric and fluorimetric methods.[96]

Figure10[87]

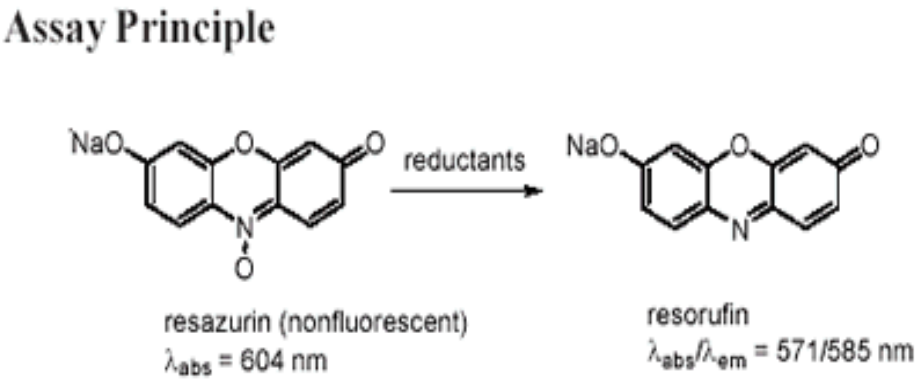


Figure B9 Reduction of resazurin converts the essentially nonfluorescent dye to the highly red fluorescent resorufin.

Redox principle

This assay is an indirect colorimetric DST method for determining the MIC of TB drug strains of Mycobacterium tuberculosis. The redox indicator Alamar blue monitors the reducing environment of the living cell. It turns from blue to pink in the presence of mycobacterial growth. As the indicator dye accepts electrons it changes from the blue, oxidized, non fluorescent state to the pink, reduced, fluorescent state. The oxidation-reduction potential of Alamar Blue is +380 mV fore, can be reduced by NADPH (Eo = 320 mV), FADH (Eo = 220 mV), FMNH (Eo = 210 mV), NADH (Eo = 320 mV), as well as the cytochromes (Eo = 290 mV to +80 mV). In addition to mitochondrial reductases, other enzymes (such as the diaphorases (EC 1.8.1.4, dihydrolipoamine dehydrogenase), NAD (P) H: quinine oxidoreductase (EC 1.6.99.2) and flavin reductase (EC 1.6.99.1) located in the cytoplasm and the mitochondria may be able to reduce Alamar Blue. (98)

ADVANTAGES

- It has accurate time course measurement.
- It has high sensitivity and linearity.
- It involves no cell lysis.

- It is ideal for use with post measurement functional assay.
- It is flexible as it can be used with different cell models.
- It is scalable and can be used with fluorescence and/or absorbance based instrumentation platforms.
- It is nontoxic, non-radioactive and is safe for the user.

APPLICATIONS

Especially meant for studies on Mycobacterium tuberculosis.

Used extensively in cell viability and cytotoxicity studies. (99)

ACUTE TOXICITY STUDIES

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short space of time (usually less than 24 hours). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance.

Acute toxicity studies in animals are usually necessary for any compound intended for human use. The information obtained from these studies is useful in choosing doses for repeat-dose studies, providing preliminary identification of target organs of toxicity, and, occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for Phase I human studies, and provide information relevant to acute overdosing in humans.[100]

This acute toxicity study was designed as per the OECD Guidelines for Testing of Chemicals, Acute Oral Toxicity (Acute Toxic Class Method), Guideline 423.

PRINCIPLE OF THE TEST

The test is based on a stepwise procedure with the use of a minimum number of animals per step. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using 3 animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

- ❖ No further testing is needed.
- ❖ Dosing of three additional animals, with the same dose.
- ❖ Dosing of three additional animals at the next higher or the next lower dose level

DESCRIPTION OF THE METHOD:

Experimental animals

Healthy adult female Albino mice (14 nos.) weighing between 20-25g are used for the research. For all the animals, food, but not water is withheld overnight prior to dosing.

Housing and lighting conditions

The temperature in the experimental animal room is maintained at 22ºC (±3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal[(101)]

PROCEDURE

Administration of doses

The synthesised compounds are administered initially at a starting dose of 2000mg/kg body weight in 1% CMC and observed 14 days for any mortality due to acute toxicity. The test substance is administered in a single dose by using a 19 gauge oral tube.

Testing procedures

The test compounds are administered to the animal to identify doses causing no adverse effects and doses causing major (life-threatening) toxicity. The use of vehicle for control groups should be considered. For compounds with low toxicity, maximum feasible dose should be administered.

OBSERVATION

The animals are observed individually after dosing once, during the first 30 minutes, periodically for the first 24 hours, with special attention

given during the first 4 hours & daily there-after, for a total of 14 days. The following clinical observation are made and recorded.

Toxic Signs

Whatever a mice showed observed any toxic signs

Body Weight

Individual body weights are recorded for all the animals once in a week.

Cage Side Observation

The faeces colour, faeces consistency, changes in skin & fur, eyes & mucous membrane (nasal) of the animal are observed once in a week.

Physical examination

Salivation, lacrymation, perspiration, piloerection, micturition and defecation. ***Central nervous system:*** Ptosis, drowsiness, gait, eye prominence, eyelid closure, convulsions, biting, straub's test, motor incoordination, writhing, stereotypy, aggression, righting reflex, pinna reflex, corneal reflex, tremors and convulsions.

FIGURE 11[(101)]

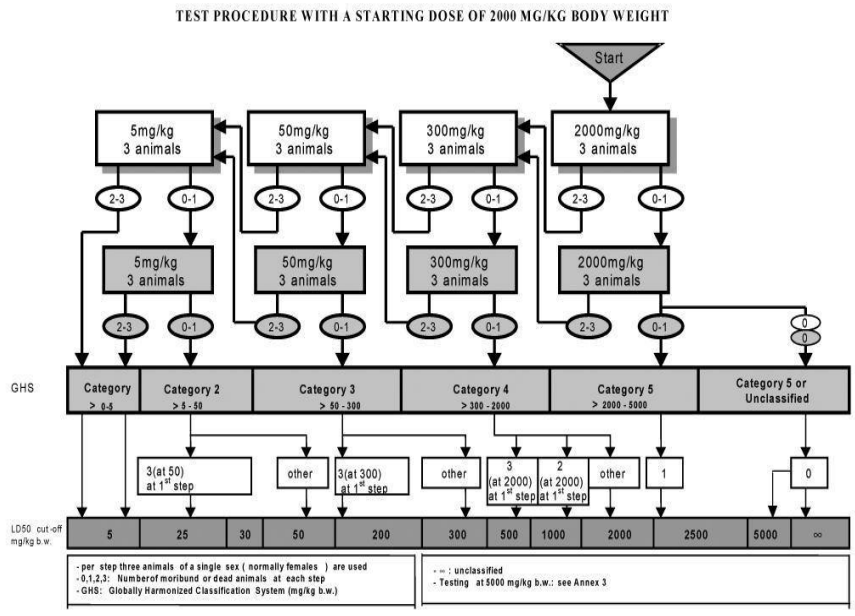


Fig 22- OECD Guidelines for Performing Animal Toxicitystudies

IN VITRO ANTITUBERCULAR ACTIVITY

MICROPLATE ALAMAR BLUE ASSAY (MABA) [108,109,110]

- The anti-mycobacterial activities of the synthesized compounds are determined by MABA method. The organism used is M.tuberculosis H37Rv

- Alamar blue dye is used as an indicator for the determination of viable cells.

Principle

MABA is an indirect colorimetric Drug Susceptibility Test method for determining the Maximum Inhibitory Concentrations 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 compounds are assessed against M. tuberculosis using Micro Plate Alamar Blue assay (MABA).

2) This methodology is non-toxic, and uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

3) 200µl 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 receive 100 µl of the Middle brook 7H9 broth and serial dilution of compounds is placed directly on plate.

5) The final drug concentrations tested is 100to0.8 µg/ml.

6) Plates are covered and sealed with 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 is added to the plate and incubated for 24 hrs.

8) A blue color in the well is interpreted as no bacterial growth, and pink color was scored as growth.

The MIC is defined as lowest drug concentration which prevents the color change from blue to pink.

RESULTS AND DISCUSSION

RESULTS OF DRUG DESIGN

DRUG LIKENESS

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

The rule is important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity,as well as drug-like properties as described by Lipinski rule. The modification of the molecular structure often leads to drug with higher molecular weight, more ring, more rotatable bonds, and higher polarity.

Lipinski rule says that ,an orally active drug has no more than one violation of the following criteria:

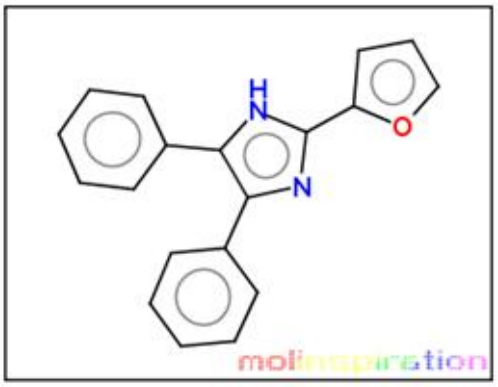
- Not more than 5 hydrogen bonds doners (nitrogen or oxygen atoms with one or more hydrogen bond)
- Not more than 5 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular weight under 500 daltons
- A partition coefficient log p less than 5

LIPINSKI RULE AND SYNTHESIZED COMPOUNDS

Compound	Molecular Formula	Molecular weight	Hydrogen bond acceptor	Hydrogen bond donor	Partition coefficient
9-F	$C_{19}H_{14}N_2O$	286.32	3	1	3.47
9-DF	$C_{22}H_{16}F_2N_2O$	362.37	5	1	5.05
9-V	$C_{22}H_{18}N_2O_2$	342.39	4	2	4.34
9-S	$C_{19}H14N_2S$	302.39	3	1	4.84
9-PD	$C_{20}H_{13}N_3$	295.33	3	1	3.52

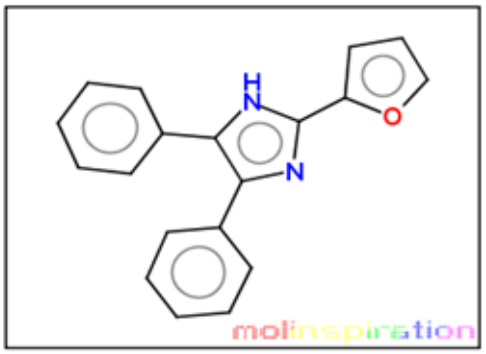
INSILICO DRUG LIKENESS PREDICTION

SAMPLE CODE-9F



Molinspiration property engine v2014.11

miLogP	4.53
TPSA	41.82
natoms	22
MW	286.33
nON	3
nOHNH	1
nviolations	0
nrotb	3
volume	260.67



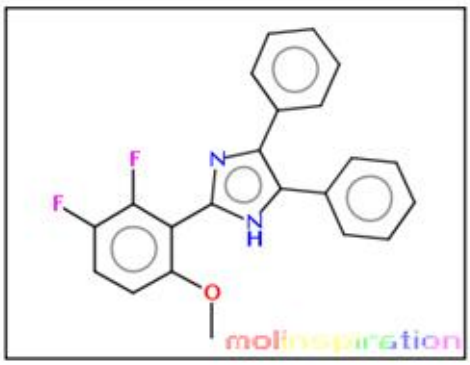
Molinspiration bioactivity score v2014.03

GPCR ligand	0.19
Ion channel modulator	0.00
Kinase inhibitor	0.24
Nuclear receptor ligand	-0.32
Protease inhibitor	-0.62
Enzyme inhibitor	0.10

Get data as text (for copy / paste).

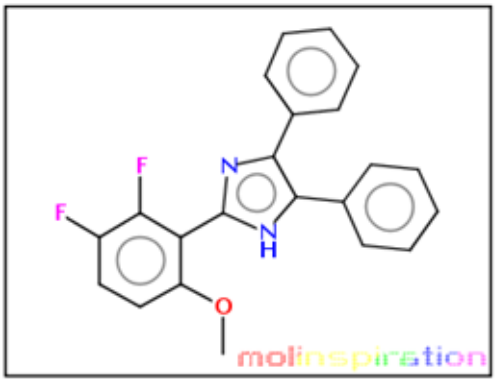
Get 3D geometry BETA

SAMPLE CODE-9DF



Molinspiration property engine v2014.11

miLogP	5.63
TPSA	37.92
natoms	27
MW	362.38
nON	3
nOHNH	1
nviolations	1
nrotb	4
volume	314.51



Molinspiration bioactivity score v2014.03

GPCR ligand	0.12
Ion channel modulator	0.00
Kinase inhibitor	0.37
Nuclear receptor ligand	-0.02
Protease inhibitor	-0.33
Enzyme inhibitor	0.19

Get data as text (for copy / paste).

Get 3D geometry BETA

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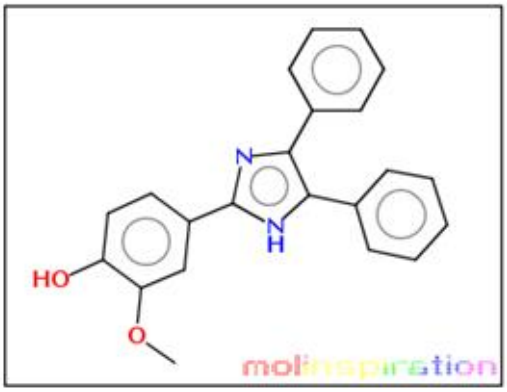
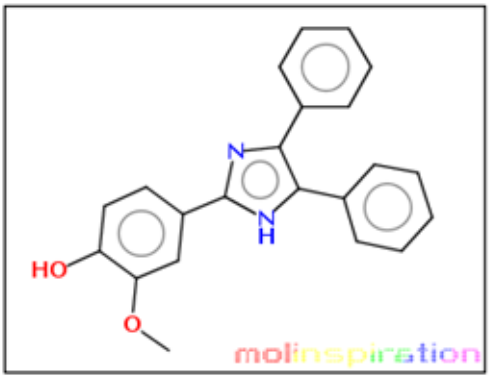


Molinspiration property engine v2014.11

miLogP	4.73
TPSA	58.15
natoms	26
MW	342.40
nON	4
nOHNH	2
nviolations	0
nrotb	4
volume	312.66



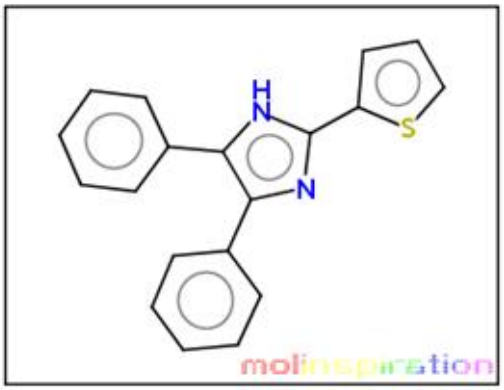
Molinspiration bioactivity score v2014.03

GPCR ligand	0.11
Ion channel modulator	0.02
Kinase inhibitor	0.39
Nuclear receptor ligand	-0.05
Protease inhibitor	-0.38
Enzyme inhibitor	0.18

Get data as text (for copy / paste).

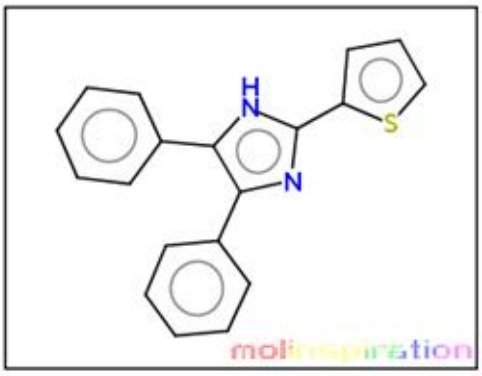
Get 3D geometry BETA

SAMPLE CODE-9S



Molinspiration property engine v2014.11

miLogP	5.17
TPSA	28.68
natoms	22
MW	302.40
nON	2
nOHNH	1
nviolations	1
nrotb	3
volume	269.81



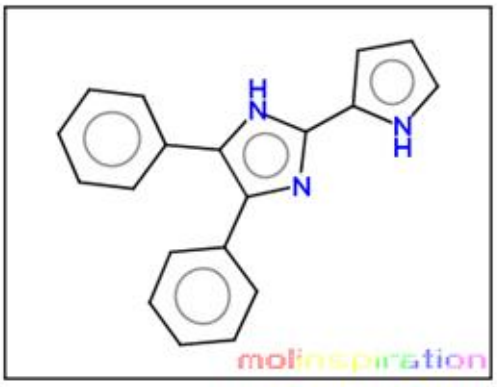
Molinspiration bioactivity score v2014.03

GPCR ligand	0.05
Ion channel modulator	-0.00
Kinase inhibitor	0.42
Nuclear receptor ligand	-0.20
Protease inhibitor	-0.40
Enzyme inhibitor	0.17

Get data as text (for copy / paste).

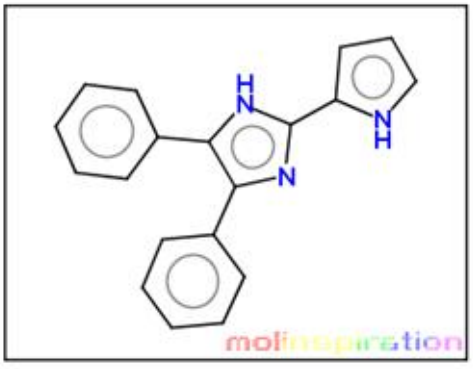
Get 3D geometry BETA

SAMPLE CODE-9P



Molinspiration property engine v2014.11

miLogP	4.18
TPSA	44.47
natoms	22
MW	285.35
nON	3
nOHNH	2
nviolations	0
nrotb	3
volume	264.08



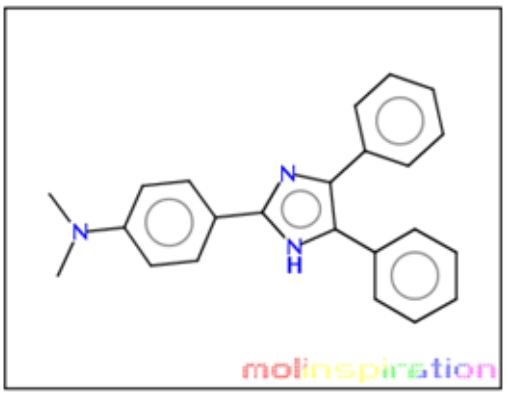
Molinspiration bioactivity score v2014.03

GPCR ligand	0.36
Ion channel modulator	0.38
Kinase inhibitor	0.70
Nuclear receptor ligand	0.02
Protease inhibitor	-0.25
Enzyme inhibitor	0.43

Get data as text (for copy / paste).

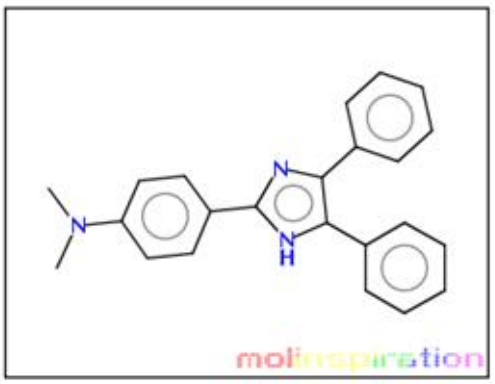
Get 3D geometry BETA

SAMPLE CODE-9DM



Molinspiration property engine v2014.11

miLogP	5.49
TPSA	31.92
natoms	26
MW	339.44
nON	3
nOHNH	1
nviolations	1
nrotb	4
volume	325.00



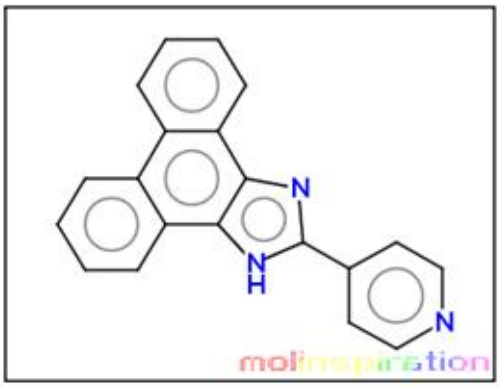
Molinspiration bioactivity score v2014.03

GPCR ligand	0.14
Ion channel modulator	0.03
Kinase inhibitor	0.39
Nuclear receptor ligand	-0.07
Protease inhibitor	-0.32
Enzyme inhibitor	0.15

Get data as text (for copy / paste).

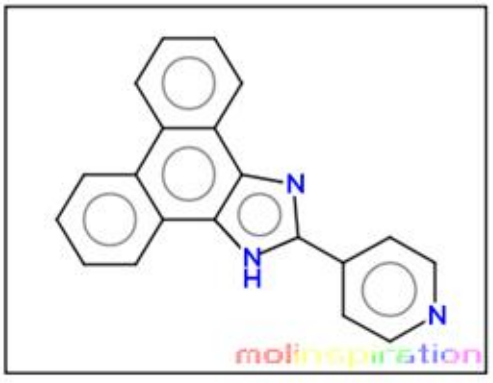
Get 3D geometry BETA

SAMPLE CODE-9PD



Molinspiration property engine v2014.11

miLogP	4.53
TPSA	41.58
natoms	23
MW	295.35
nON	3
nOHNH	1
nviolations	0
nrotb	1
volume	264.10



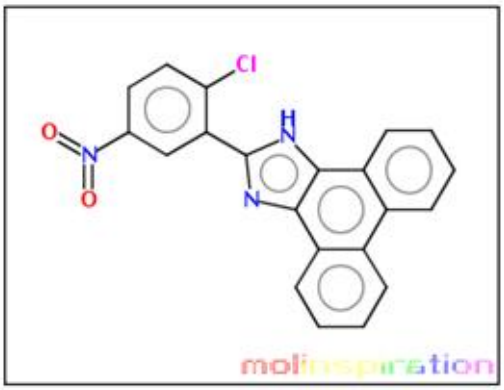
Molinspiration bioactivity score v2014.03

GPCR ligand	0.30
Ion channel modulator	0.34
Kinase inhibitor	0.61
Nuclear receptor ligand	0.03
Protease inhibitor	-0.02
Enzyme inhibitor	0.38

Get data as text (for copy / paste).

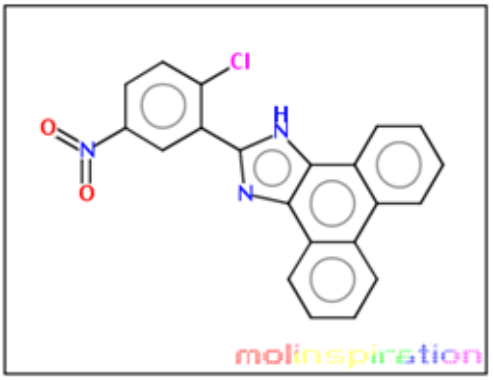
Get 3D geometry BETA

SAMPLE CODE-CN



Molinspiration property engine v2014.11

miLogP	6.38
TPSA	74.51
natoms	27
MW	373.80
nON	5
nOHNH	1
nviolations	1
nrotb	2
volume	305.13



Molinspiration bioactivity score v2014.03

GPCR ligand	0.10
Ion channel modulator	0.12
Kinase inhibitor	0.29
Nuclear receptor ligand	-0.04
Protease inhibitor	-0.19
Enzyme inhibitor	0.13

Get data as text (for copy / paste).

Get 3D geometry BETA

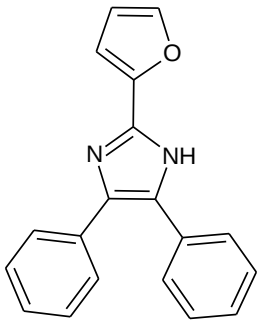
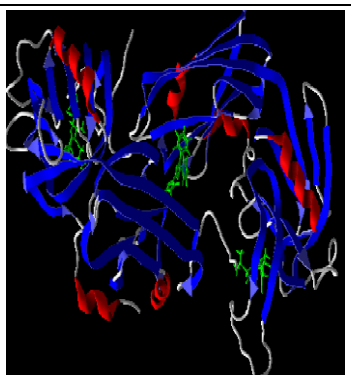
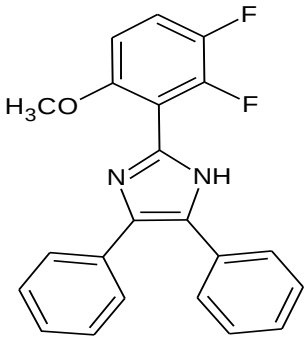
DOCKING STUDIES

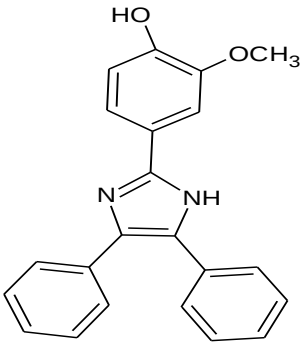
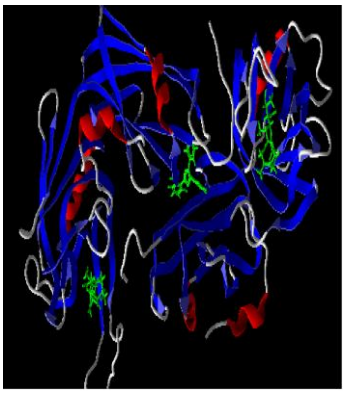
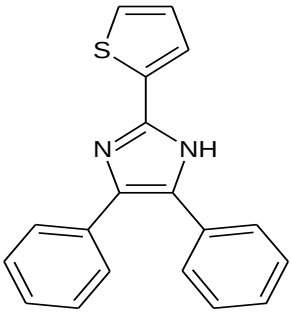
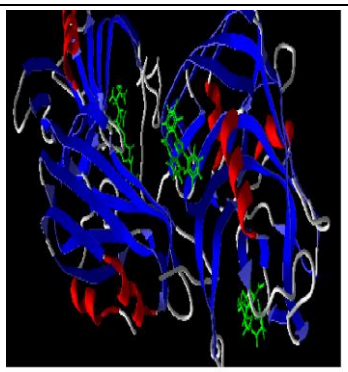
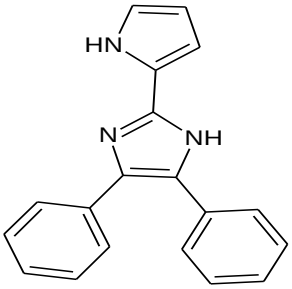
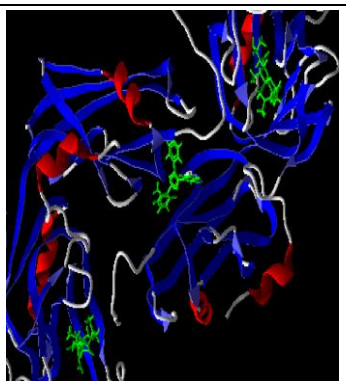
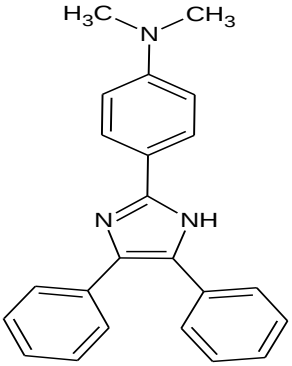
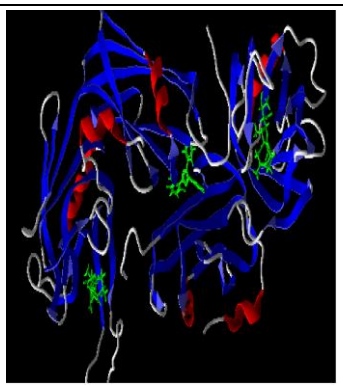
Morethan 200 scaffolds were docked against the MTB enzyme L,D-Transpeptidase (4GSU) by using Argus lab 4.0.1 software. The best docking Score -10 TO -15 and good interaction molecules are selected and screened.I have selected 8 compounds for synthesis which is having docking energy is -10 to -15. The compounds were also docked against the critical enzyme of the mycobacterium tuberculosis Diaminopimelate epimarase (dapF) and UDP-N-acetyl muramate-L-alanine ligase.

The docking score of the 3 target enzymes are listed bellow

Compounds	L,D-Transpeptidase	Diaminopimelate epimarase	UDP-N-acetyl muramate –L-alanine ligase
9-F	-9.247kcal/mol	-7.62kcal/mol	-8.90kcal/mol
9-DF	-10.62kcal/mol	-8.53kcal/mol	-9.94kcal/mol
9-V	-10.95kcal/mol	-7.30kcal/mol	-7.34kacl/mol
9-S	-9.985kcal/mol	-7.81kcl/mol	-8.21kcal/mol
9-P	-8.63kcal/mol	-8.96kcal/mol	-9.96kcal/mol
9-DM	-10.12kcal/mol	-8.46kcal/mol	-8.90kcal/mol
9-PD	-9.873kcal/mol	-7.71kcal/mol	-7.62kcal/mol
9-CN	-11.42kcal/mol	-8.62kocal/mol	-8.62kcal/mol

Table 1 : Docking Score and View

Compound code	Structure	Docking Score	Docking view
9-F		-9.247Kca/mol	
9-DF		-10.62kcal/mol	

Compound code	Structure	Docking Score	Docking view
9-V		-10.95kcal/mol	
9-S		-9.985kcal/mol	
9-P		-8.637kcal/mol	
9-DM		-10.12kcal/mol	

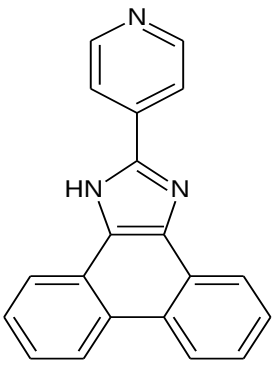
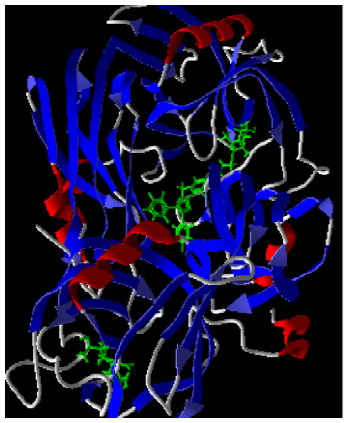
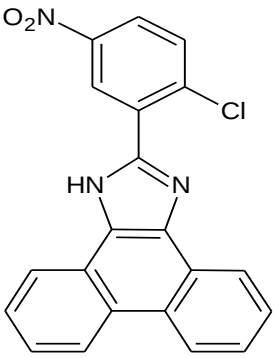
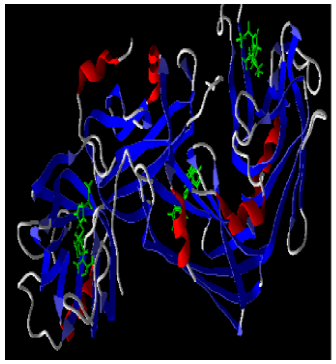
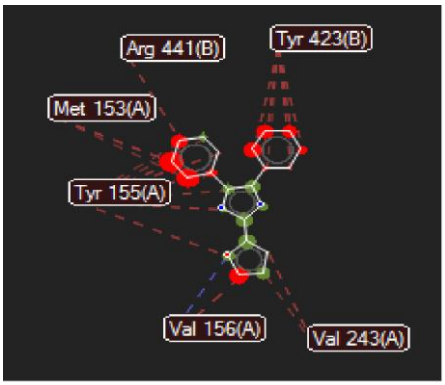
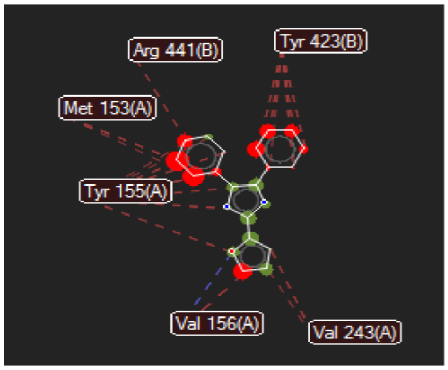
Compound code	Structure	Docking Score	Docking view
9-PD		-9.873kcal/mol	
9-CN		-11.42kcal/mol	

Table 2 : 4GSU Interaction with Ligand

Sample code-9F	Sample code-9DF
	

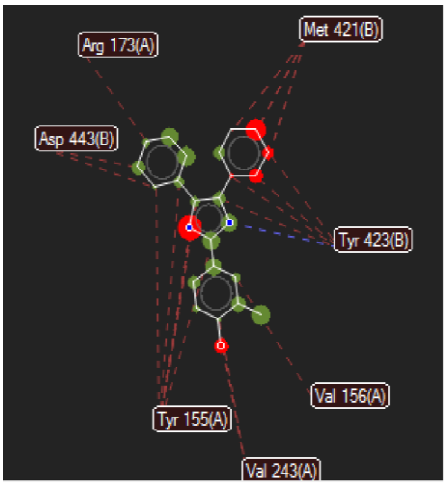
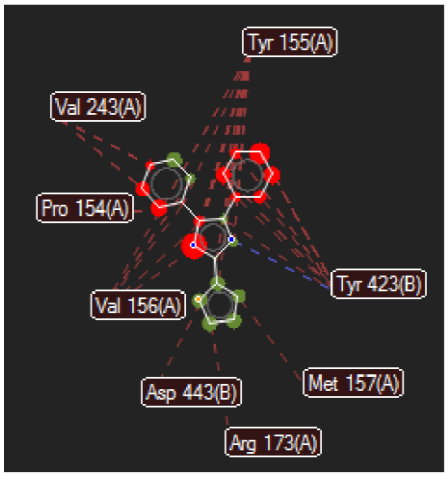
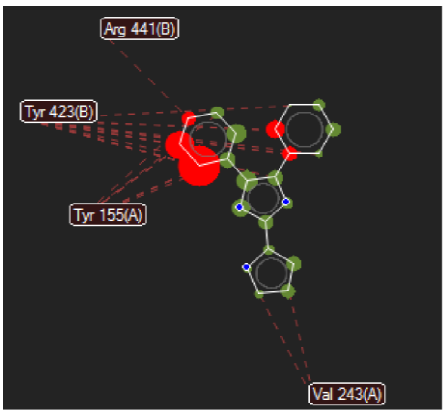
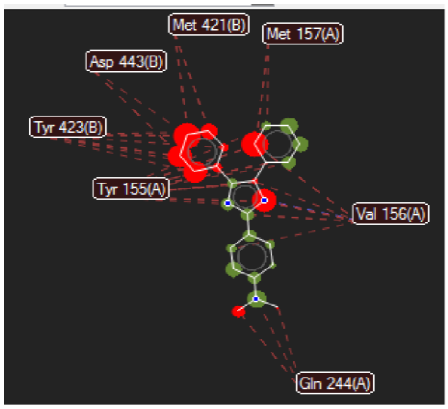
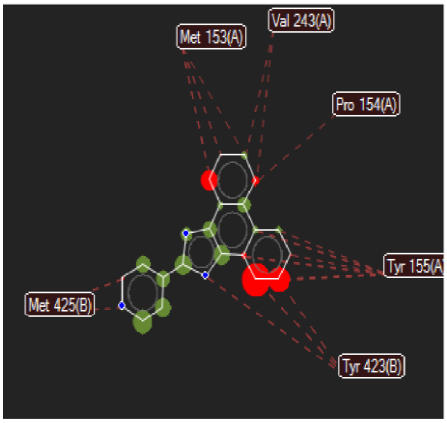
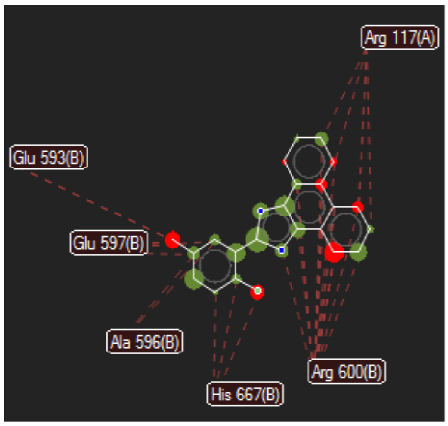
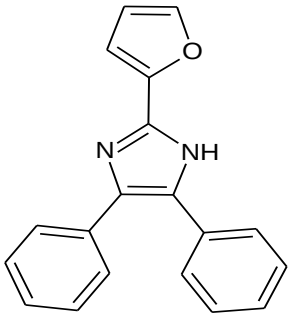
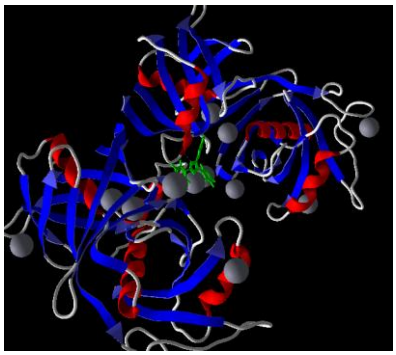
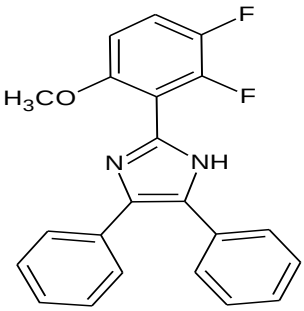
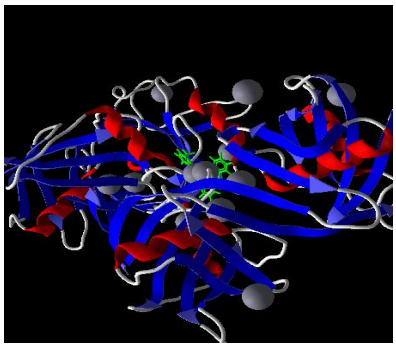
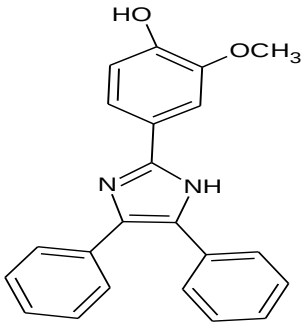
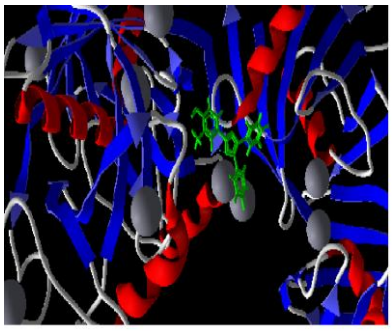
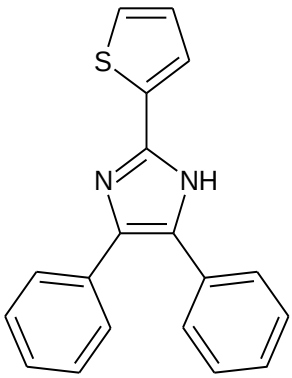
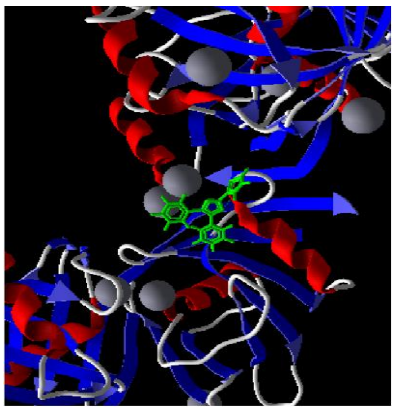
Sample code-9-v	Sample code-9s
	
Sample code-9P	**Sample code-9DM**
	
Sample code-9PD	**Sample code-9CN**
	

TABLE 3 : COMPOUNDS DOCKED AGAINST THE PROTEIN 4IKO

Compound code	Structure	Docking Score	Docking view
9-F		-7.62kcal/mol	
9-DF		-8.53kcal/mol	
9-V		-7.30kcal/mol	
9-S		-7.81kcal/mol	

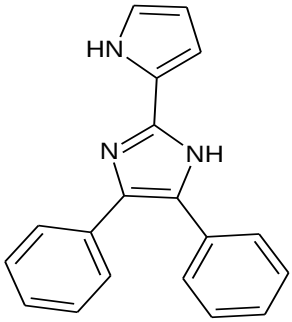
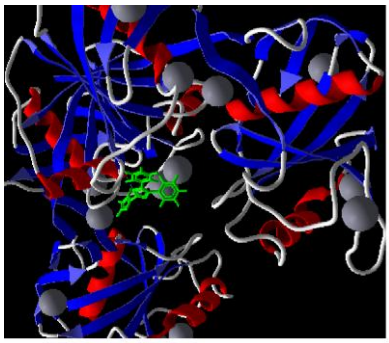
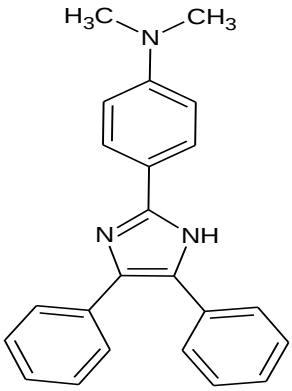
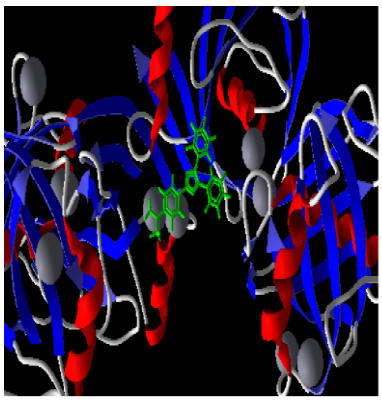
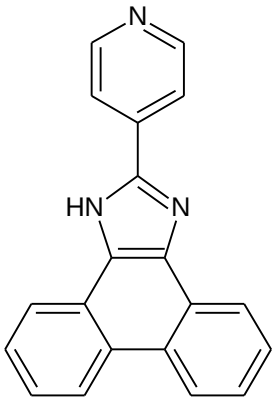
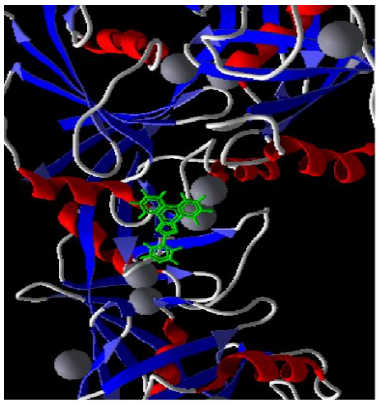
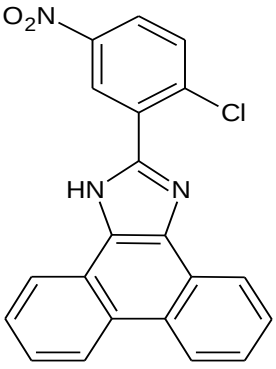
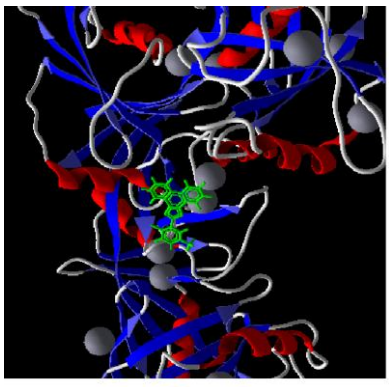
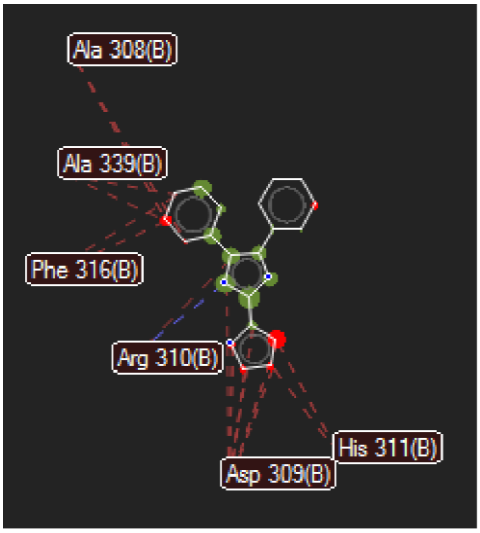
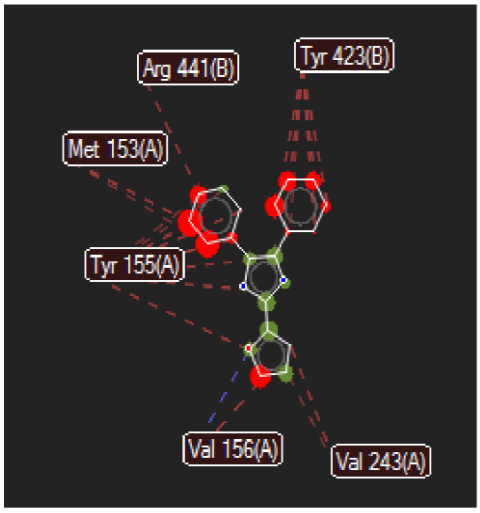
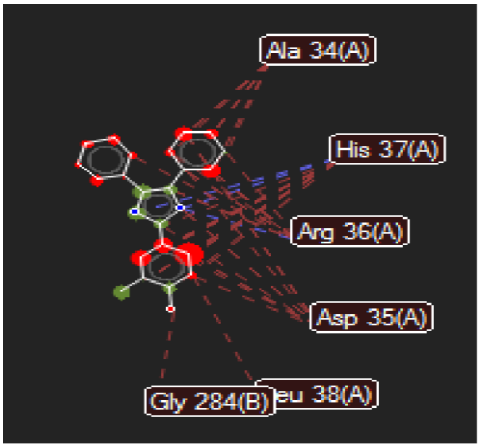
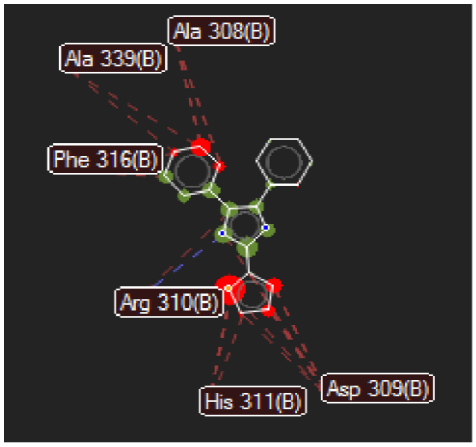
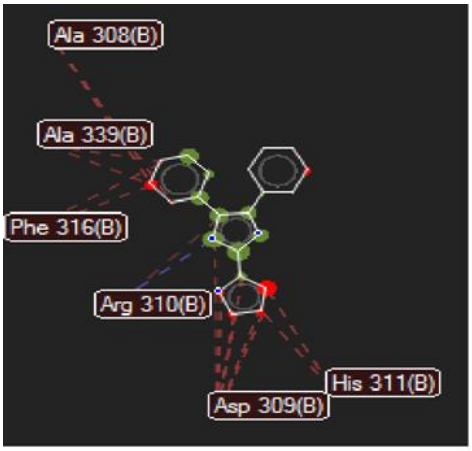
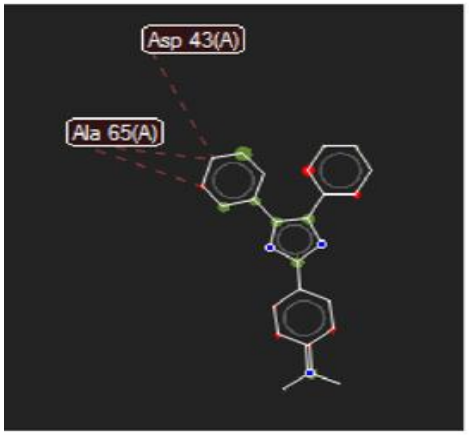
9-P		-8.96kcal/mol	
9-DM		-8.46kcal/mol	
9-PD		-7.71kcal/mol	
9-CN		-8.62kcal/mol	

Table 4: 4IKO Interaction with Ligand

Sample code-9F	Sample code-9DF
	
Sample code-9-v	**Sample code-9s**
	
Sample code-9P	**Sample code-9DM**
	

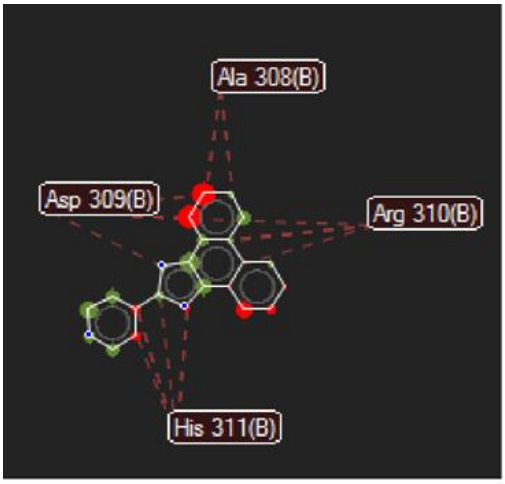
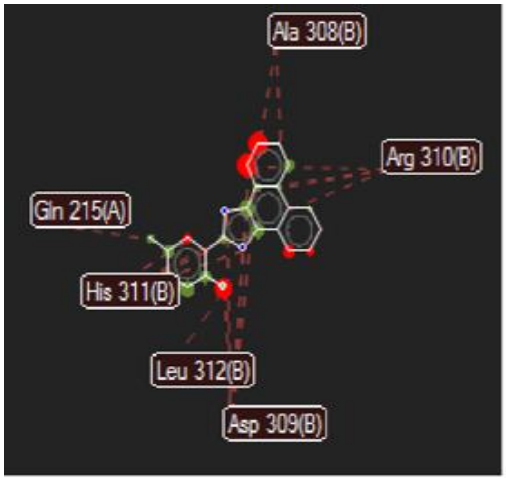
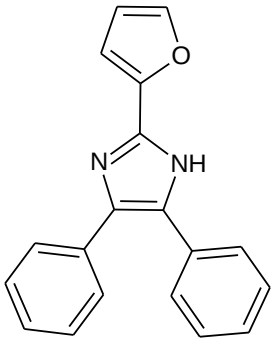
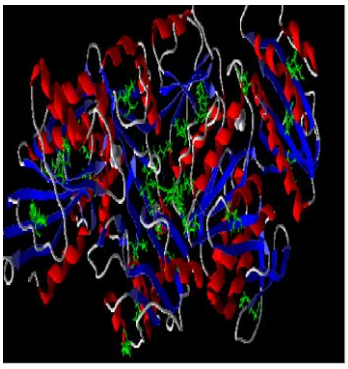
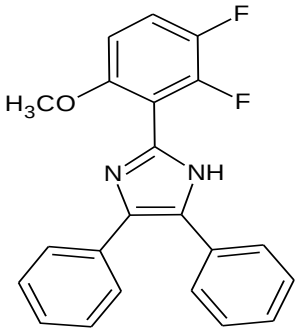
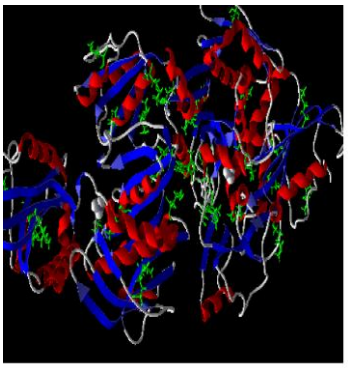
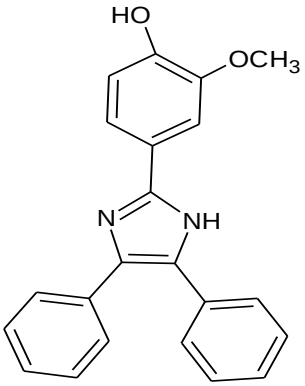
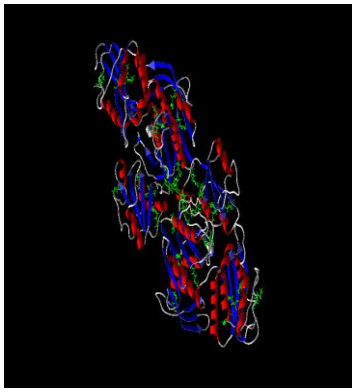
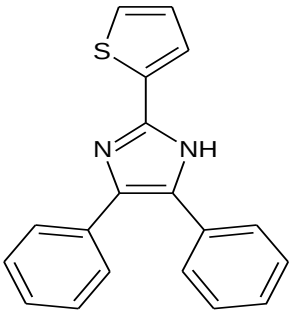
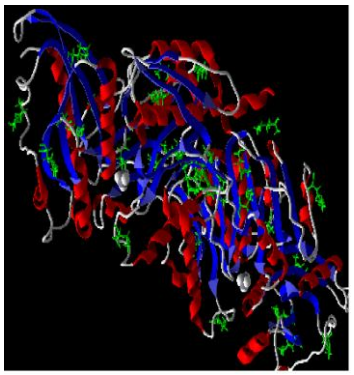
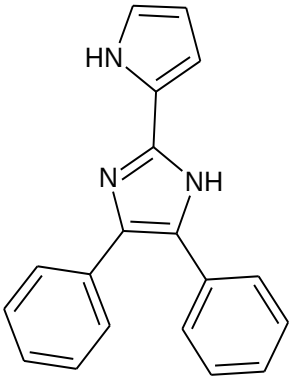
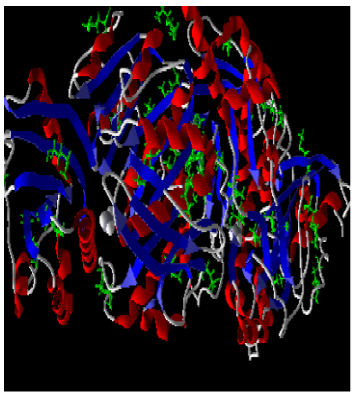
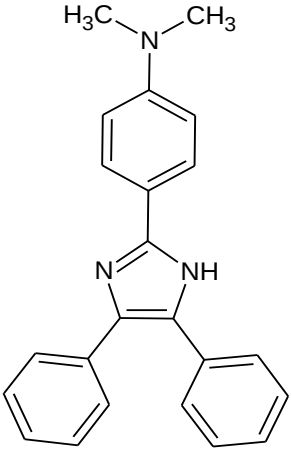
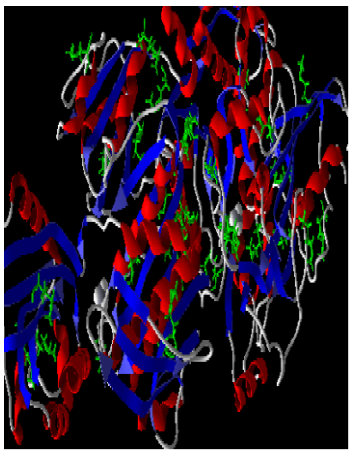
Sample code-9PD	Sample code-9CN
	

TABLE 5 :COMPOUNDS DOCKED AGAINST THE PROTEIN 2FOO

Compound code	Structure	Docking Score	Docking view
9-F		-8.90kcal/mol	
9-DF		-9.94kcal/mol	

9-V		-7.34kcal/mol	
9-S		-8.21kcl/mol	
9-P		-9.96kcl/mol	
9DM		-8.90kcal/mol	

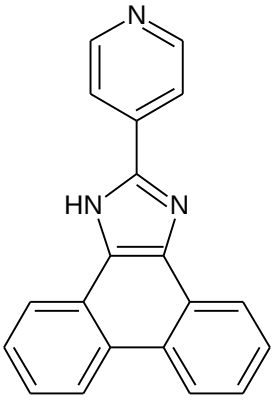
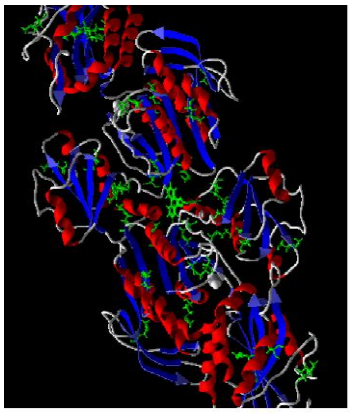
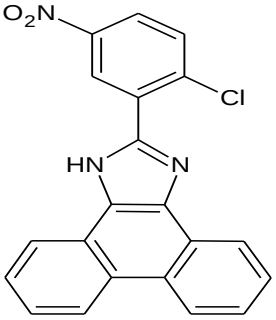
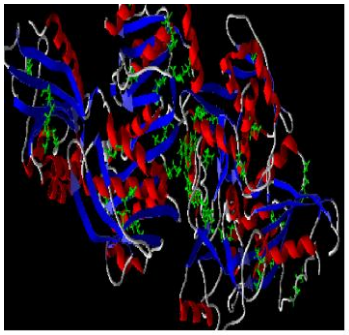
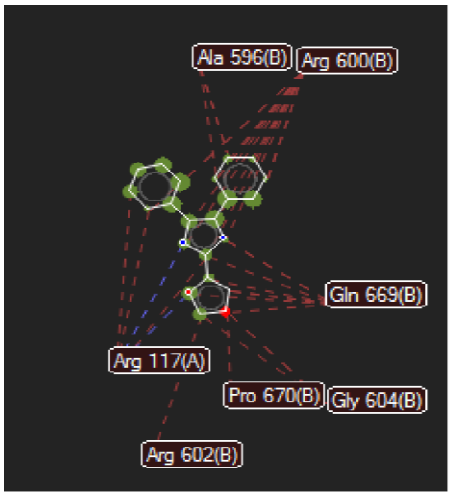
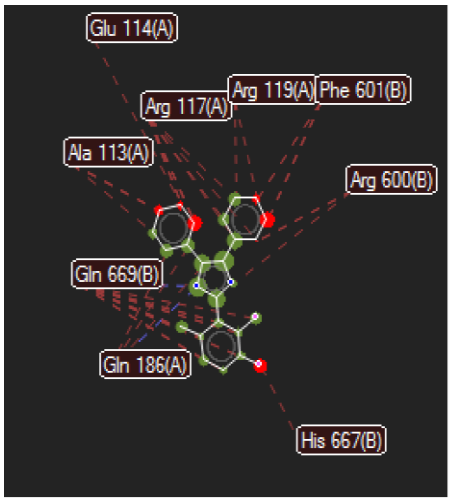
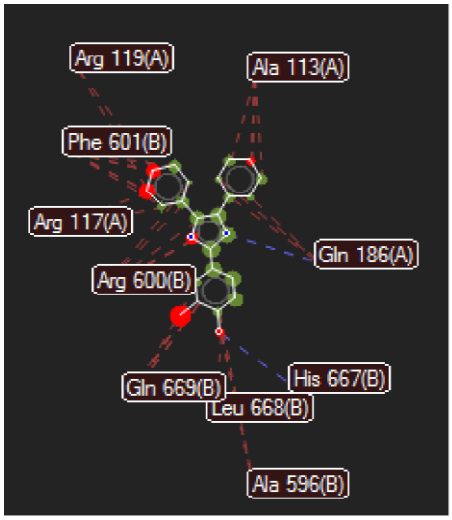
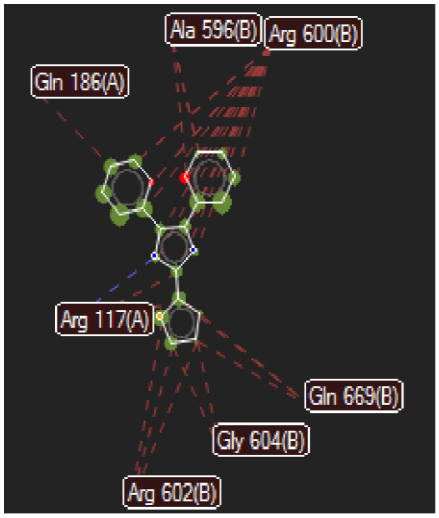
9-PD		-7.62kcal/mol	
9-CN		-7.62kcal/mol	

TABLE 6 :2FOO INTRACTION WITH LIGAND

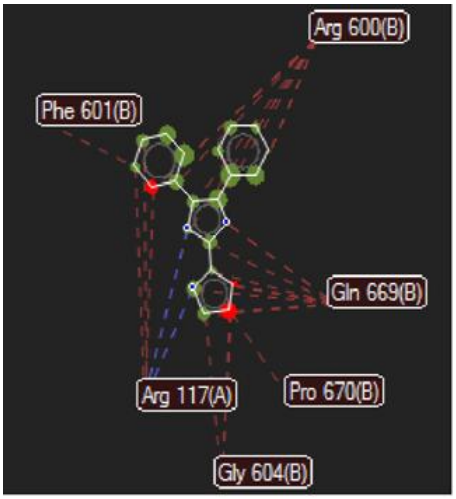
Sample code-9F	Sample code-9DF
	
Sample code-9-v	Sample code-9s



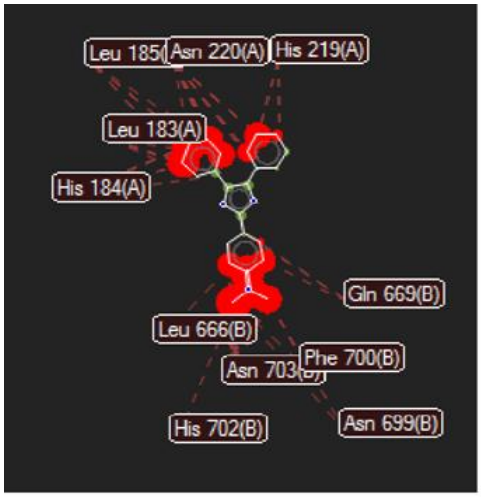
Sample code-9P



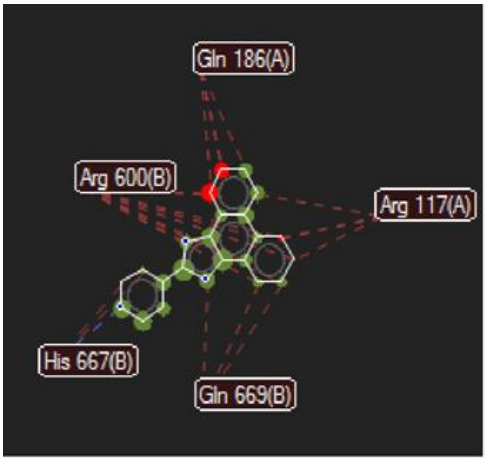
Sample code-9DM

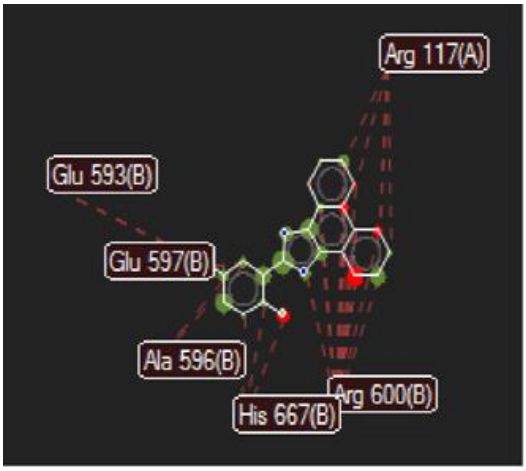


Sample code-9PD



Sample code-9CN





RESULTS OF SYNTHESIZED COMPOUNDS

The synthetic route was preferred by conventional method. The reaction was carried out by using 1 mole of phenanthrene 9,10 Dione,1mole of substituted aromatic aldehyde and 2 moles of ammonium acetate, poly ethylene glycol which is acts as catalyst the mixture is refluxed. This synthesis is involves modified palnour reaction leading to formation of Benzodiazole nucleus. One mole of benzil used as a another reactant this is leads to formation of imidazole nucleus.

CHARACTERISATION

The synthesized compounds were subjected to purification by recrystallization and TLC. The melting point of the synthesized compounds was founded. The characterization was carried out using sophisticated instruments like IR, NMR, and Mass spectroscopy and characteristic properties through the aid of computer software.

INFRARED ABSORPTION SPECTROSCOPY

IR spectrum of synthesized compounds the following functional groups like Ar-CH, NH, NO_2,C=NH,C=C,C-O-C-CH_3 can be ascertained.

The absorption band of the parent reactants are as follows

- Aldehyde : 2830 - 2695cm^{-1}
- Ketone : 1710 – 1720cm^{-1}
- Amines : 3000-3300cm^{-1}

The IR spectra is showed the absence of parent groups and the presence of a new functional groups are C=NH and NH_2 stretching are formed. For all the synthesized compounds 9-F,9-DF,9-V,9-S,9-P,9-DM,9-CN the absorption band for C=NH stretching at 2347.47cm^{-1} are obtained.

- IR absorption of the synthesized compounds 9-DM showed the presence of aliphatic C-H stretching vibration between 2960-2916cm^{-1},For all the synthesized compounds showed the presence of NH

stretching vibration between 3440-3390cm^{-1},aromatic CH stretching vibration between 3080-3060 cm^{-1}, C-N stretching vibration between 1220-1280 cm^{-1}are obtained.

- The absorption band for 9-CN compound showed the strong band at 1342cm^{-1} was indicate the presence of nitro group.

^{1}H NMR SPECTRA

The number of signals in an NMR spectrum denotes the number of the set of equivalent protons in a molecule . The position of the signals help us to know the nature of protons viz, aromatic, heteroaromatic, aliphatic, vinyl C-H groups.

NMR Data In δ scale pertaining to the synthesized compounds

The ^{1}H NMR spectral data of all the synthesized compounds are in conformity with the structure assigned. A singlet at 9.74-10.02 was observed for all compounds conforming the presence of N-H proton. High deshielding was due to the presence of electro negative nitrogen atom and additional conjugation. So lesser value of applied field is needed to resonate the proton. In general protons adjacent to N-are strongly deshielded. All the compounds showed multiplet and doublet signals for the presence of aromatic protons between (6.20-8.59) δppm and heteroaromatic protons between (6.20-8.3) δppm.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

GC is used to determine the purity of compounds by looking for additional peaks in a sample that are not present in the pure compound. The smaller structure have lower boiling point and will thus elute faster than those with higher boiling point.

The synthesized compound of 9-F is 100% Purity ,which is having signal at m/e 285.35 is molecular ion peak. There is no additional peak present in this compound. The synthesized compound of 9-DF is 96% purity, which is having signal at m/e 361.37 is the molecular ion peak and also the

base peak. molecular ion peak can be a base peak but base peak should not be a molecular ion peak.

GENERAL MASS SPECTRAL DETAILS OF THE SYNTHESIZED COMPOUND

The synthesized compounds are undergoing two types of cleavage namely

1) HOMOLYTIC

2) HETEROLYTIC

Under hemolytic cleavage the compound basically undergoes mode II cleavage. In all the synthesized compounds hetero atom is singly bonded carbon atom, So parent/molecular ion is formed by removal of one electron from the molecule (mode I)

Heterolytic cleavage is the most important fragmentation pattern observed in the synthetic compounds. The cleavage of C-N bond is more difficult than C-C bond. So the positive charge is carried by carbon and not heteroatom. In the set of all synthetic compounds having a different molecular ion of mass 9-F=286.32,9DF=362.37 was found which might be due to C-N cleavage.

TABLE 7 : MOLECULAR WEIGHT OF SYNTHESIZED COMPOUNDS

NAME OF COMPOUNDS	CALCULATED MASS	ACTUAL MASS
9-F	286.32g/mole	286.22g/mole
9-DF	362.37g/mole	362.18g/mole
9-V	342.39g/mole	342.22g/mole
9-S	302.39g/mole	302.06g/mole
9-P	285.34g/mole	285.28g/mole
9-DM	339.43g/mole	339.48g/mole
9-PD	295.37g/mole	296.12 g/mole
9-CN	373.79g/mole	374.02g/mole

RESULTS OF LC-MASS

Liquid chromatography with mass spectroscopy was used to characterize the non-volatile compounds. The synthesized compounds is characterized in lc-ms. These are compounds (9-v,9-s,9-p,9-cn) showed 99% purity with molecular ion peek.

DISCUSSION

- Fifty compounds were selected using chemsketch and docked against the target enzyme L,D-Transpeptidase-2 .The top 10 compounds with good G-score and ligand interaction and least toxicity were selected and synthesized.

- The synthesized compounds are characterized using IR, GC-MASS, LC-MASS, NMR spectroscopy.

- The synthesized compound of 9-DF was found to be 100% purity and 9-DF is 95% purity was determined by GC-MS spectroscopy.

- All the other compounds 9-v,9-s,9-p,9-dm,9-pd,9-cn was found to be 99% purity.

- All the compounds were tested for the invitro anti-tb activity. The 9-DF compound was found to be the most sensitive at the least concentration 1.6µg/ml and compounds 9-s,9-v,9-p,9-dm,9-pd,9-cn showed minimum inhibitory concentration at 12.5µg/ml.

- Acute Toxicity Studies

There was no death no death of any animal in the acute toxicity study performed by the ICH guidelines over 14 days period. The animals showed no significant toxicity.

Mechanism Of Action

The L,D-Transpeptidase-2 enzyme catalyse the formation of peptidoglycan cross-links of the mycobacterium tuberculosis cell wall and

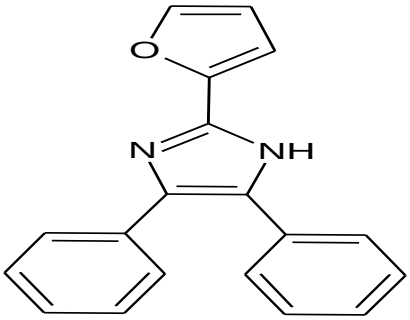
facilitates the resistance against classical β-lactams. The synthesized compounds inactivate the L,D-Transpeptidse-2 enzyme.

The compounds were also docked against the following critical enzyme of the mycobacterium tuberculosis.

- Diaminopimelate epimarase (dapF).
- UDP-N-acetyl muramate-L-alanine ligase
- If it is clear by the docking studies that the synthesized compounds act by the inhibiting L,D-Transpeptidase. The compounds 9-F,9-DF,9-V,9-DM,9-CN rather than the enzymes Diaminopimelate epimarase (dapF). UDP-N-acetyl muramate-L-alanine ligase.

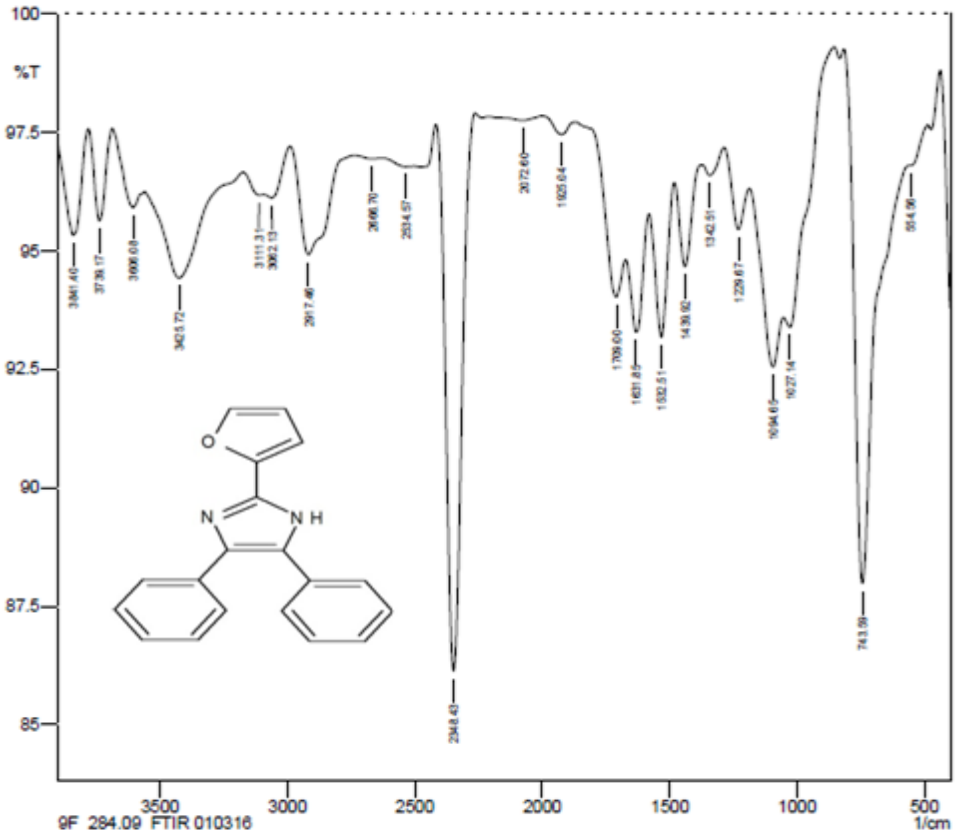
PRODUCT PROFILE

SAMPLE CODE:9-F



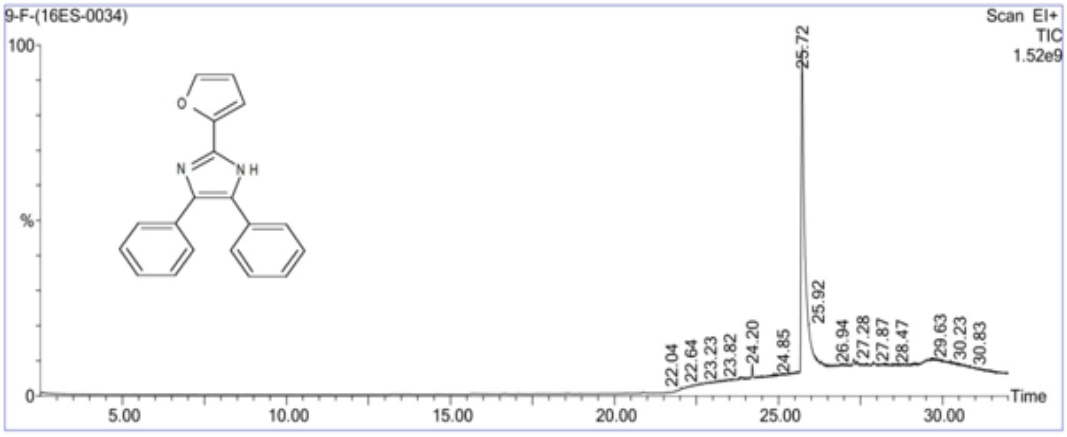
IUPAC Name	:	2-(furan-2-yl)-4,5-diphenyl-1*H*-imidazole
Molecular Formula	:	$C_{19}H_{14}N_2O$
Formula Weight	:	286.32g/mol
Appearance	:	black colour crystals
Melting Point	:	308ºC
Solubility	:	Chloroform, Methanol, Dimethyl Sulfoxide
Percentage Yield	:	95%
Composition	:	C[79.70%], H[4.93%], N[9.78%], O[5.59%]
Molar Refractivity	:	84.85±0.3cm3
Molar Volume	:	239.6±3.0cm3
Parachor	:	635.0±4.0cm3
Index of Refraction	:	1.626±0.02
Surface Tension	:	49.3±3.0 dyne/cm
Density	:	1.194±0.06g/cm3
Polarizability	:	33.63±0.510-24cm^3
Monoisotopic Mass	:	286.110613 Da
Nominal Mass	:	286 Da
Average Mass	:	286.3273 D

IR Spectrum: 9-F

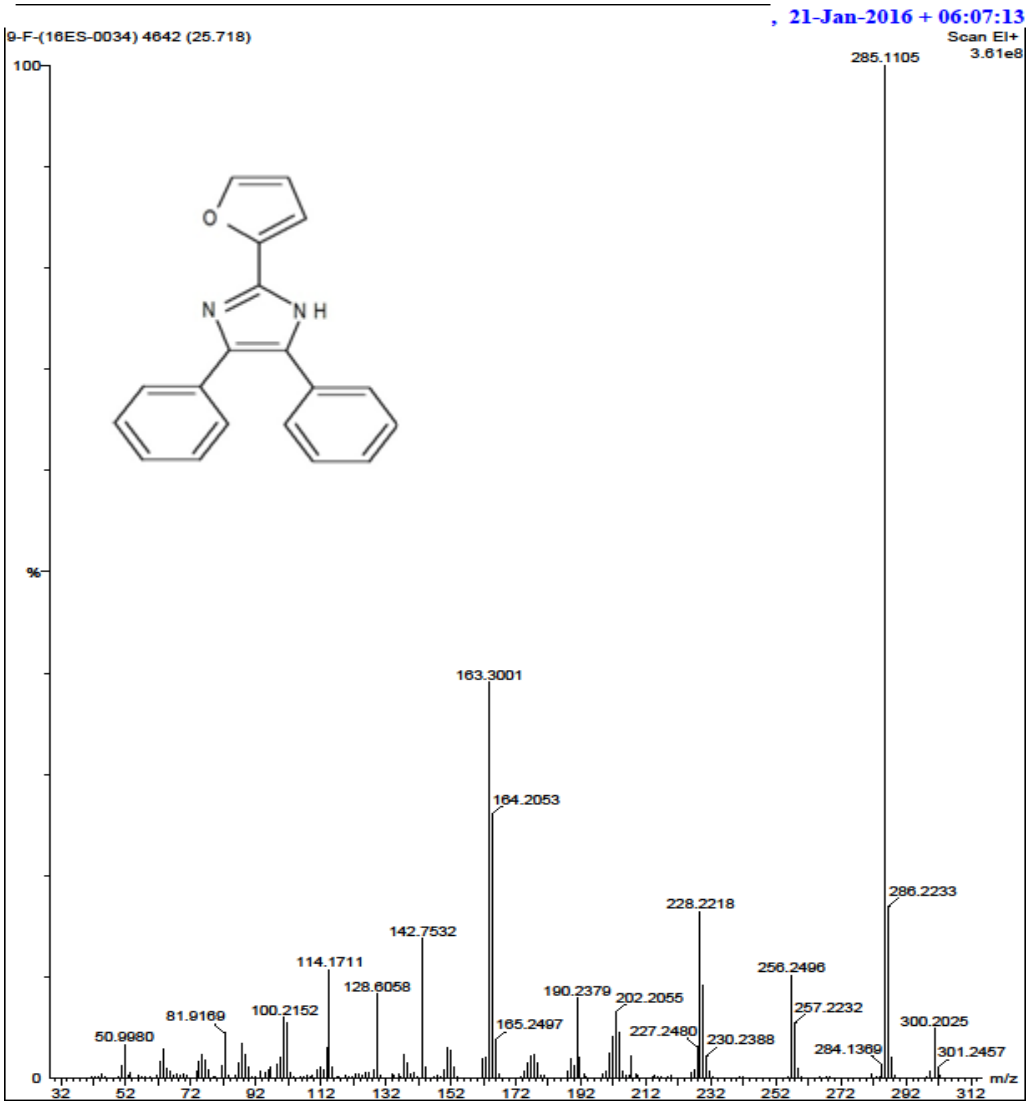


S.NO	WAVE NUMBER (cm-1)	FUNCTIONL GROUPS
1	3062.13cm-1	-CH Stretching
2	1532.51cm-1	-C=C Stretching
3	1229.67cm-1	-C-C Stretching
4	2348.83cm-1	-C=N stretching
5	1094.65cm-1	-C-N Stretching
6	1027.14cm-1	-C-O Stretching
7	3425.72cm-1	-N-H Stretching

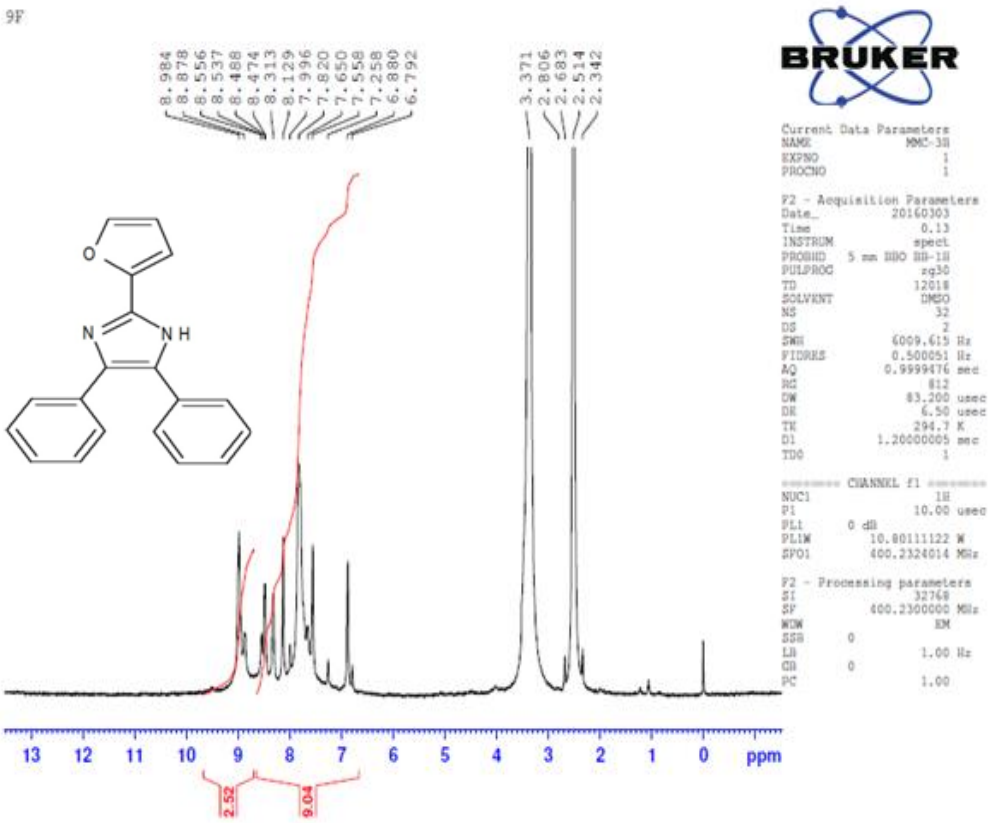
GC-MS Spectrum: 9-F



#	RT	Scan	Height	Area	Area %	Norm %
1	25.718	4642	1,402,922,112	163,691,232.0	100.000	100.00

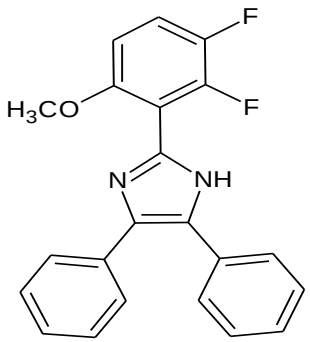


H_1 NMR Spectrum:-9F



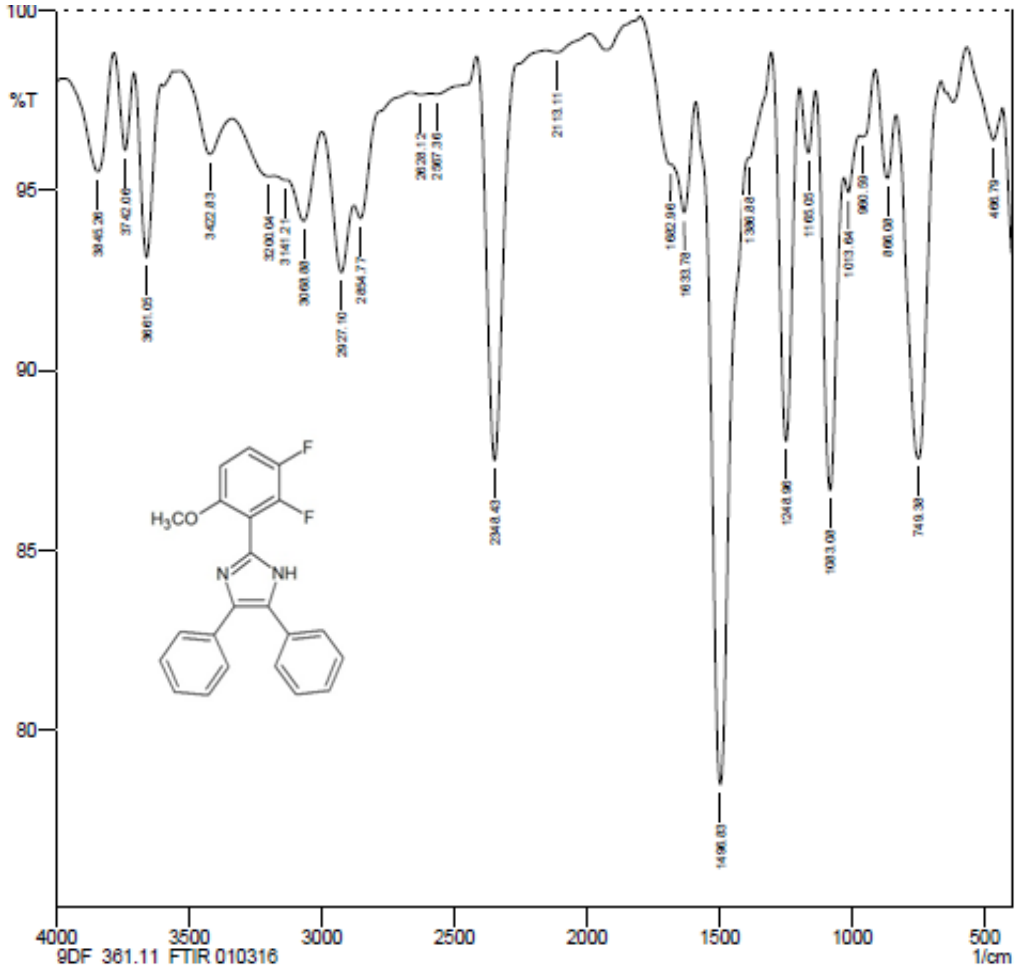
S.No	δ value	Nature of Peak	Number of Protons
1	δ2.4	Doublet	2 proton
2	δ 3.3	Singlet	1 proton
3	δ 8.9-9.1	Doublet	2 proton
4	δ 6.7-8.6	Multiplet	9protons

SAMPLE CODE: 9-DF



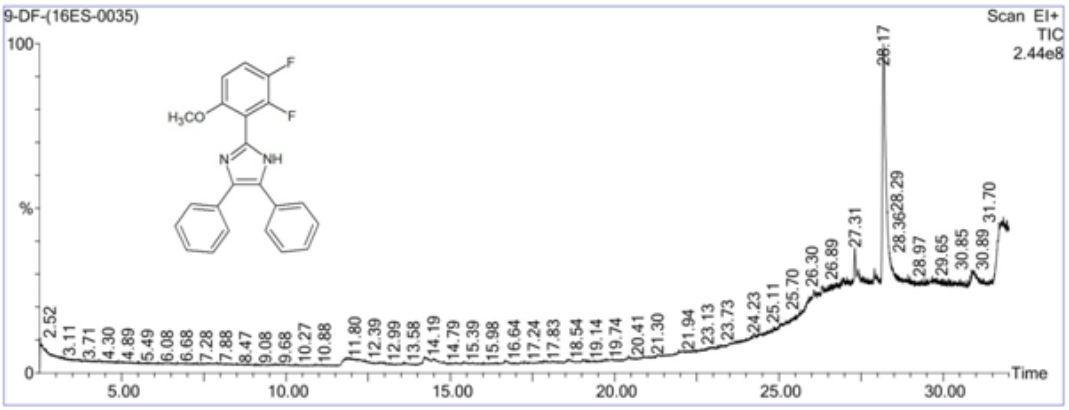
IUPAC Name	:	2-(2,3-difluoro-6-methoxyphenyl)-4,5-diphenyl-1*H*-imidazole
Molecular Formula	:	$C_{22}H_{16}F_2N_2O$
Formula Weight	:	362.37g/mol
Appearance	:	Yellow colour solid
Melting Point	:	310ºC
Solubility	:	Chloroform, Methanol, Dimethyl Sulfoxide
Percentage Yield	:	95%
Composition	:	C[79.92%], H[4.45%], F[10.49],N[7.73%], O[4.42%]
Molar Refractivity	:	99.21±0.3cm3
Molar Volume	:	289±3.0cm3
Parachor	:	752.0±4.0cm3
Index of Refraction	:	1.601±0.02
Surface Tension	:	45.6±3.0 dyne/cm
Density	:	1.252.±0.06g/cm3
Polarizability	:	39.33±0.510-24cm^3
Monoisotopic Mass	:	362.12307 Da
Nominal Mass	:	362 Da
Average Mass	:	362.372 D

IR Spectrum: 9-DF

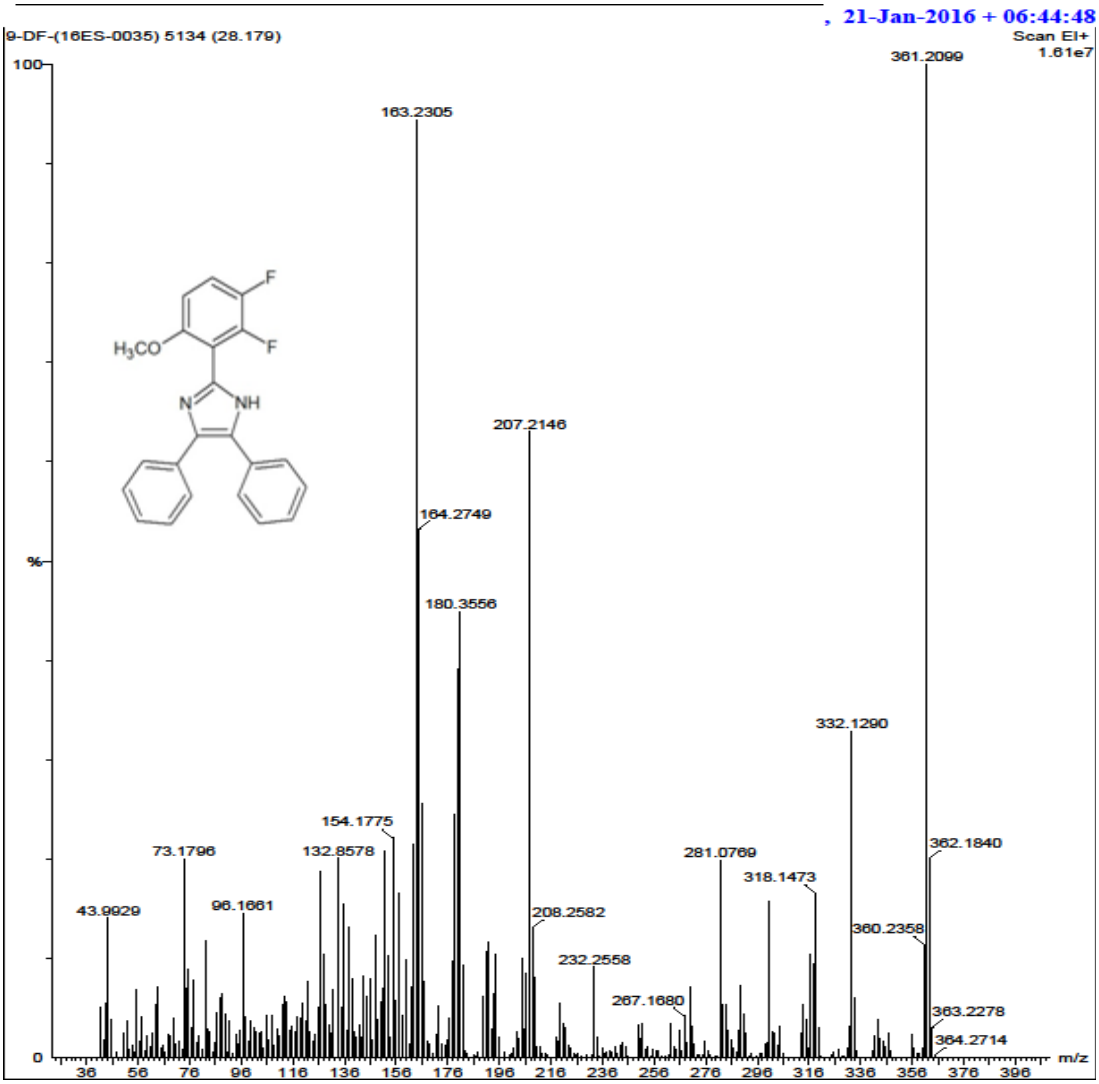


S.NO	WAVE NUMBER (cm^{-1})	FUNCTIONL GROUPS
1	3068.88cm^{-1}	-CH Stretching
2	2348.43cm^{-1}	-C=N Stretching
3	1496.83cm^{-1}	-C=C Stretching
4	1248.96cm^{-1}	-C-N Stretching
5	3422.83cm^{-1}	-N-H Stretching
6	2927.10cm^{-1}	-C-OCH_3 Stretching
7	1017.64cm^{-1}	-C-F Stretching

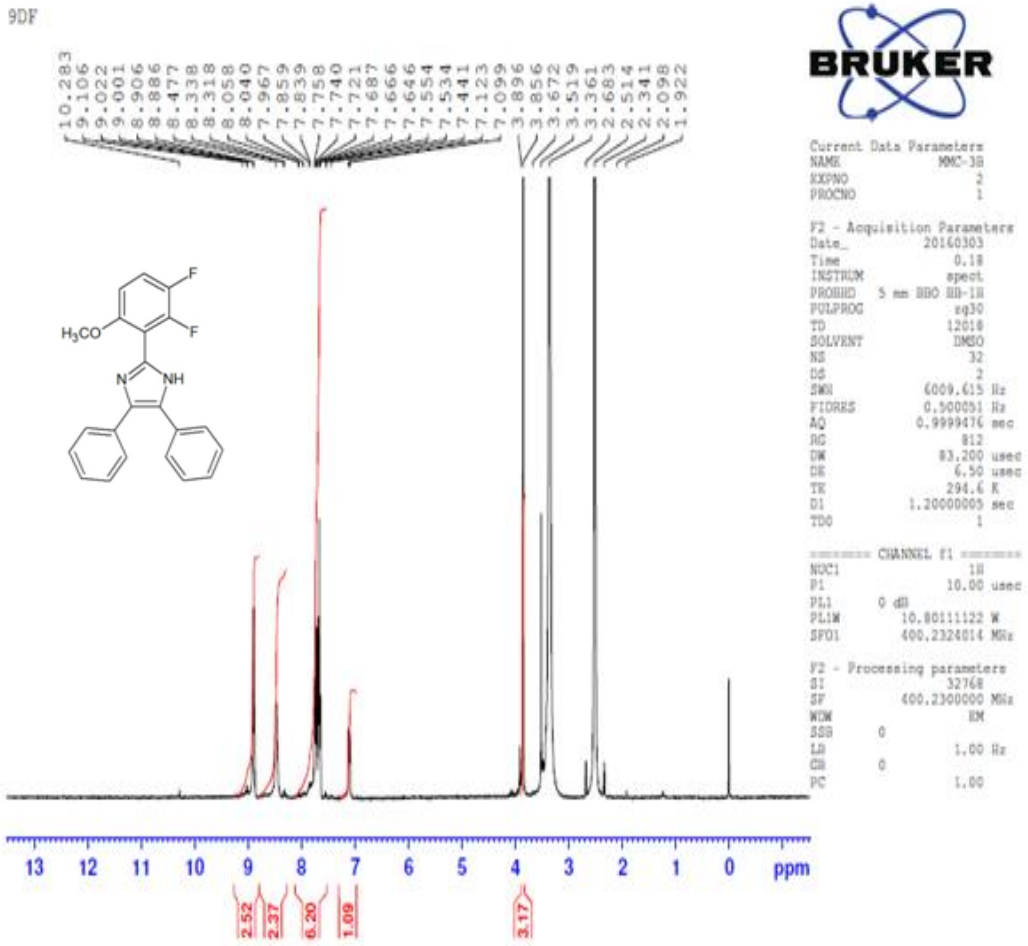
GC-MS Spectrum: 9-DF



#	RT	Scan	Height	Area	Area %	Norm %
1	27.308	4960	24,063,394	1,022,448.8	4.875	5.12
2	28.179	5134	173,894,304	19,951,186.0	95.125	100.00

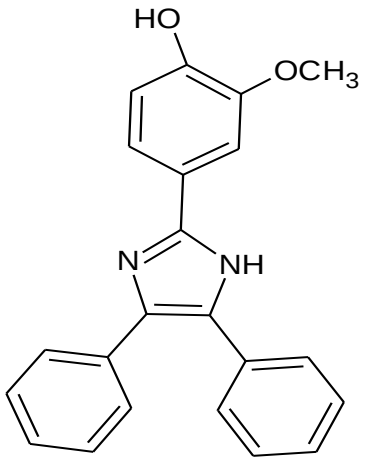


H_1 NMR Spectrum:-9DF



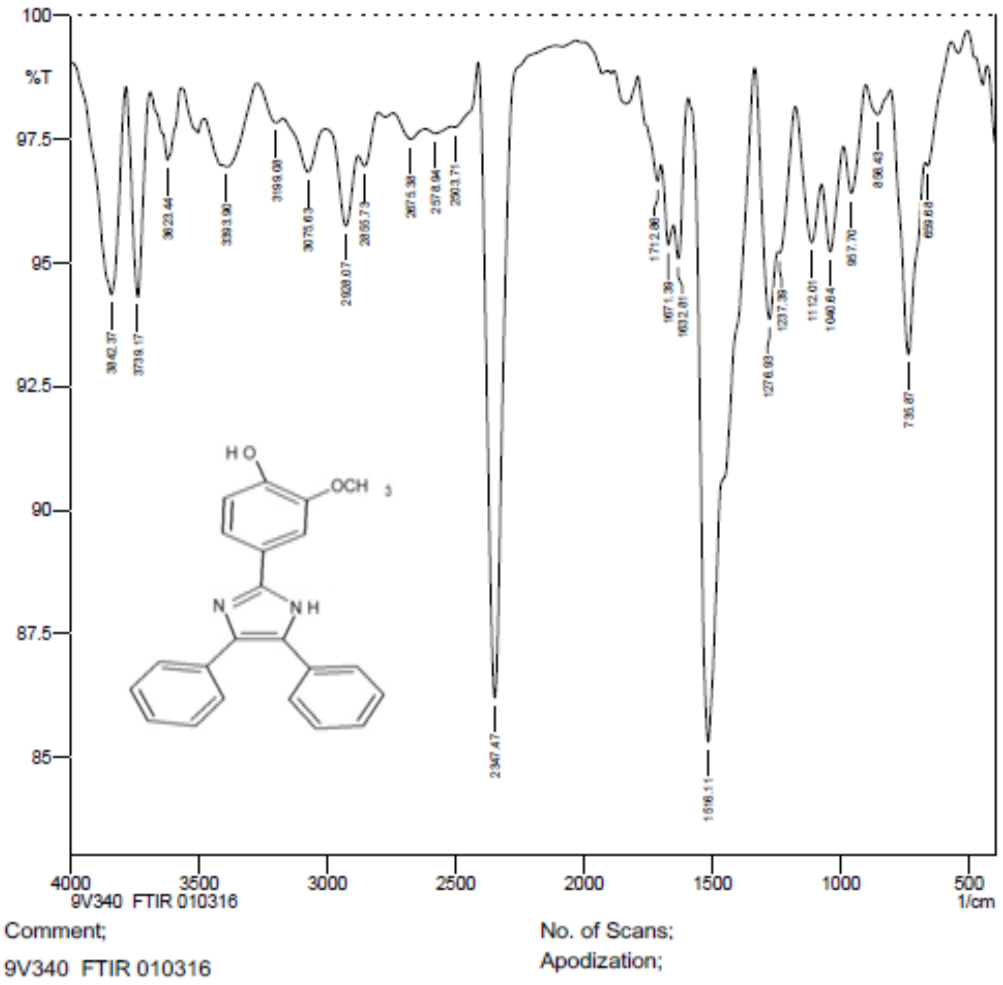
S.No	δ value	Nature of Peak	Number of Protons
1	δ2.4	singlet	1 proton
2	δ 3.5	singlet	1 proton
3	δ 4	singlet	3 proton
4	δ 7-9.1	Multiplet	11protons

SAMPLE CODE: 9-V



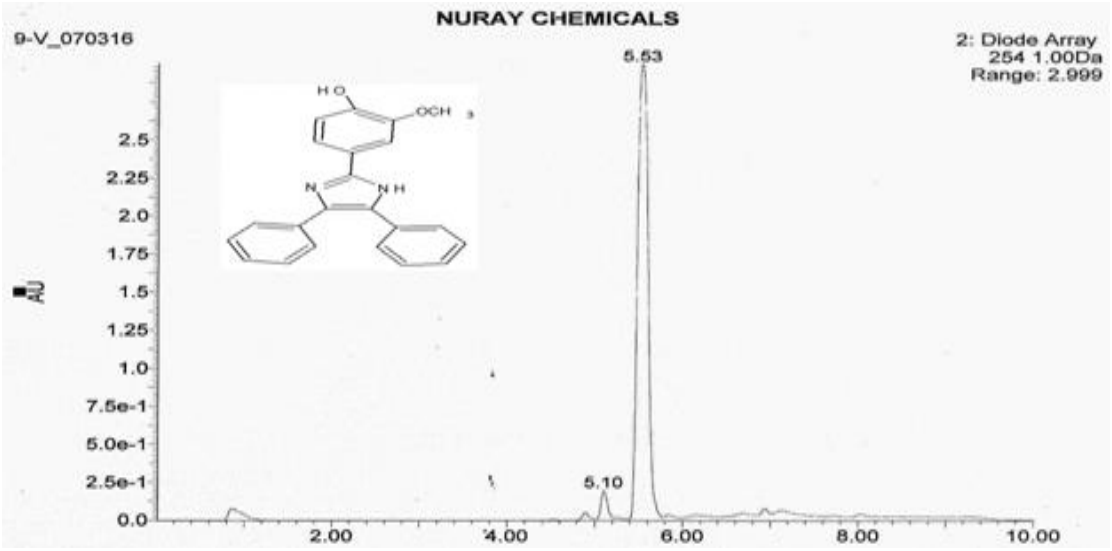
IUPAC Name	:	2-(4-hydroxy-3-methoxyphenyl)-4,5-diphenyl-1H-imidazole
Molecular Formula	:	C22H18N2O2
Formula Weight	:	342.39g/mol
Appearance	:	light grean colour solid
Melting Point	:	310ºC
Solubility	:	Chloroform, Methanol, Dimethyl Sulfoxide
Percentage Yield	:	98%
Composition	:	C[77.17%], H[5.30%], N[8.18], O[9.35%]
Molar Refractivity	:	101.11±0.3cm3
Molar Volume	:	279.2±3.0cm3
Parachor	:	752.77±4.0cm3
Index of Refraction	:	1.643±0.02
Surface Tension	:	52.7±3.0 dyne/cm
Density	:	1.226.±0.06g/cm3
Polarizability	:	40.08±0.510-24cm3
Monoisotopic Mass	:	342.136828 Da
Nominal Mass	:	342 Da
Average Mass	:	342.3905 Da

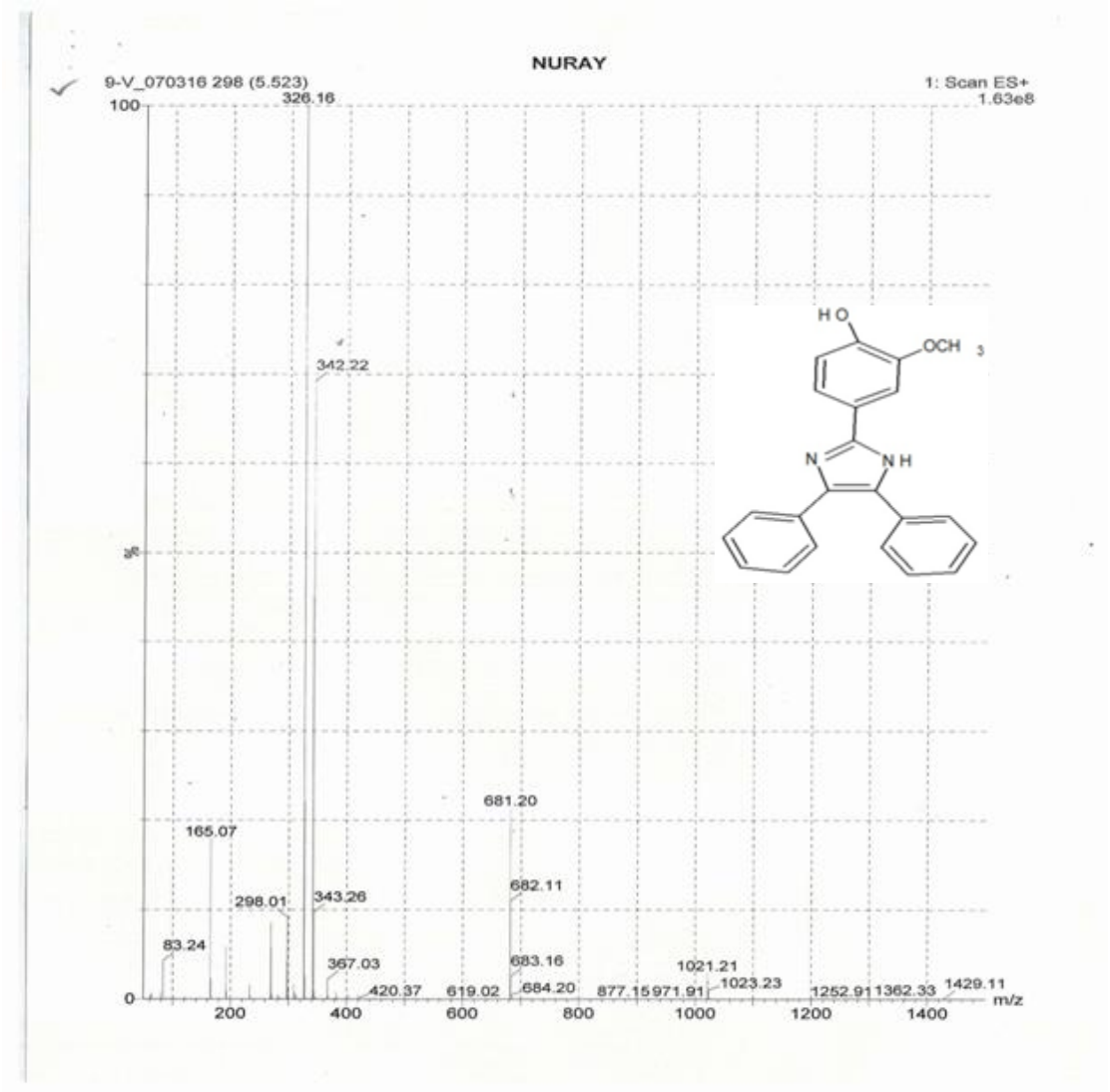
IR Spectrum: 9-V



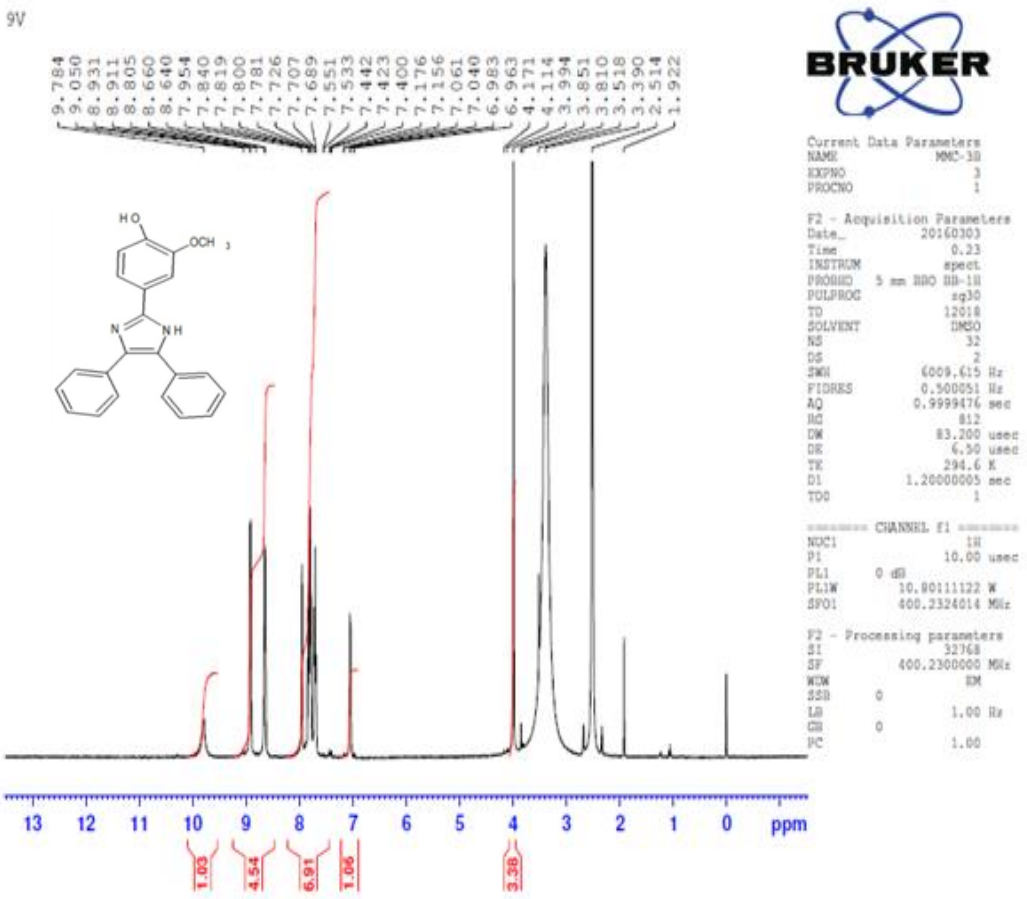
S. NO	WAVE NUMBER (cm-1)	FUNCTIONL GROUPS
1	3075.63cm-1	-CH Stretching
2	1516.11cm-1	-C=C Stretching
3	1276.93cm-1	-C-N Stretching
4	2347.47cm-1	-C=N Stretching
5	3623.44cm-1	-OH Stretching
6	2928.07cm-1	-C-OCH3 Stretching
7	3393.09cm-1	-N H Stretching

LC-MS Spectrum: 9-V



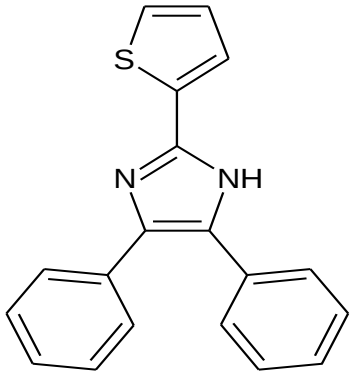


H_1 NMR Spectrum:-9V



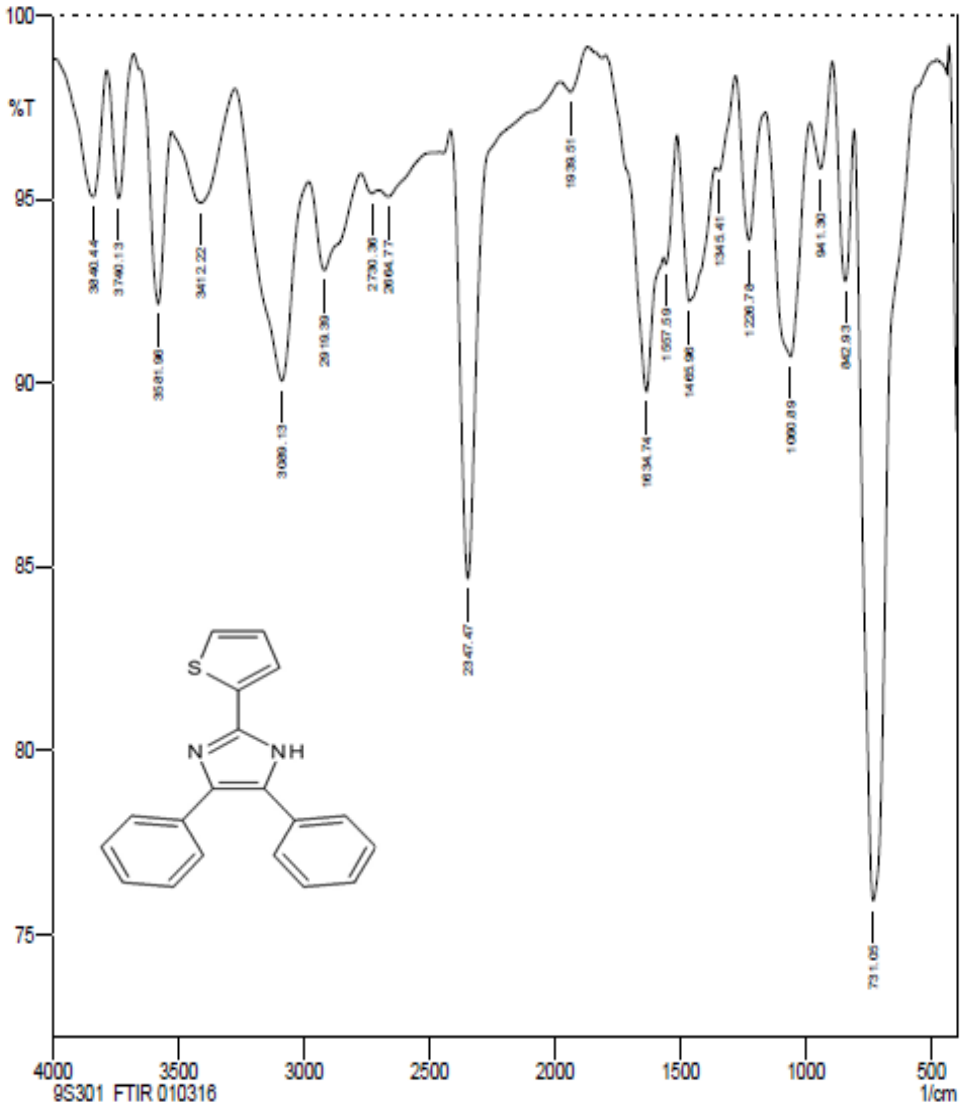
S.No	δ value	Nature of Peak	Number of Protons
1	δ 1.9	singlet	1 proton
2	δ 2.5	singlet	1 proton
3	δ3.5	singlet	1 proton
4	δ 4	singlet	3protons
5	δ 7-8.2	multiplet	7 protons
6	δ 8.8-10	multiplet	5protons

SAMPLE CODE: 9-S



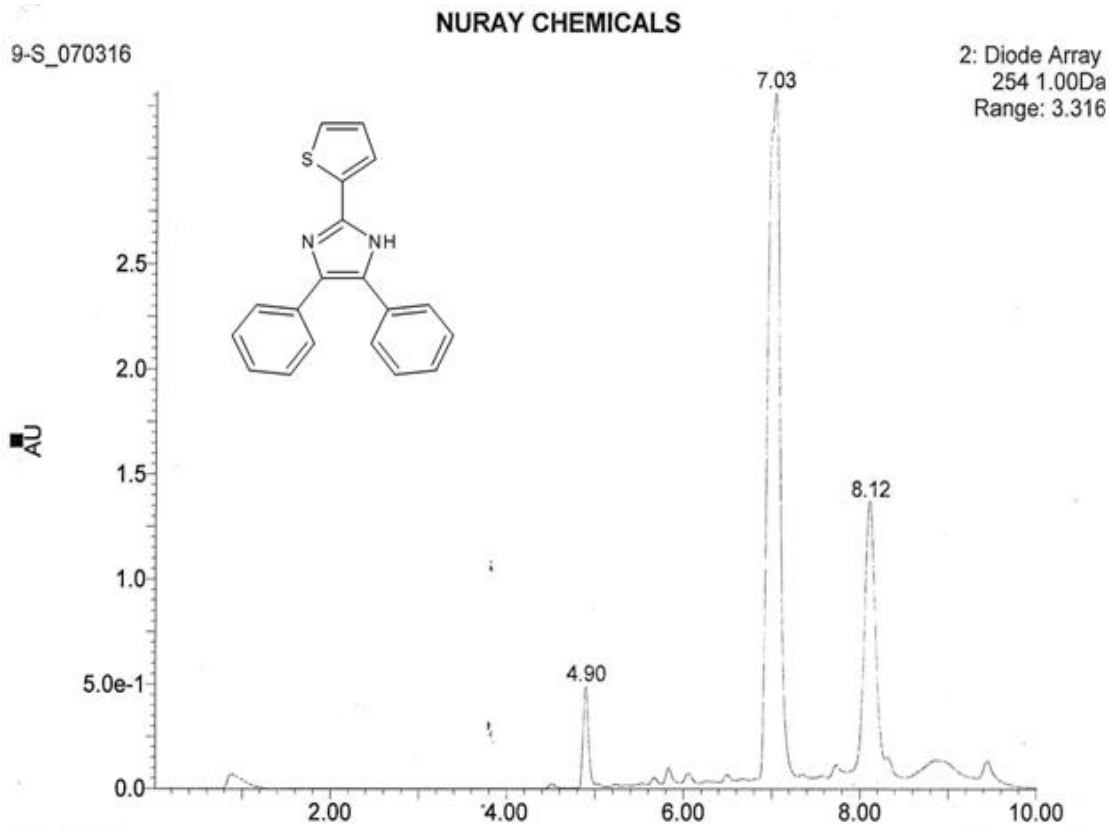
IUPAC Name	:	4,5-diphenyl-2-(thiophen-2-yl)-1H-imidazole
Molecular Formula	:	C19H14N2S
Formula Weight	:	302.39g/mol
Appearance	:	light yellow colour solid
Melting Point	:	320ºC
Solubility	:	Chloroform, Methanol, Dimethyl Sulfoxide
Percentage Yield	:	98%
Composition	:	C[75.47%], H[4.67%], N[9.26], S[10.60%]
Molar Refractivity	:	90.93±0.3cm3
Molar Volume	:	246.373±3.0cm3
Parachor	:	604.2±4.0cm3
Index of Refraction	:	1.660±0.02
Surface Tension	:	52.873±3.0 dyne/cm
Density	:	1.227.±0.06g/cm3
Polarizability	:	36.0570.510±0.510-24cm3
Monoisotopic Mass	:	302.087768 Da
Nominal Mass	:	302 Da

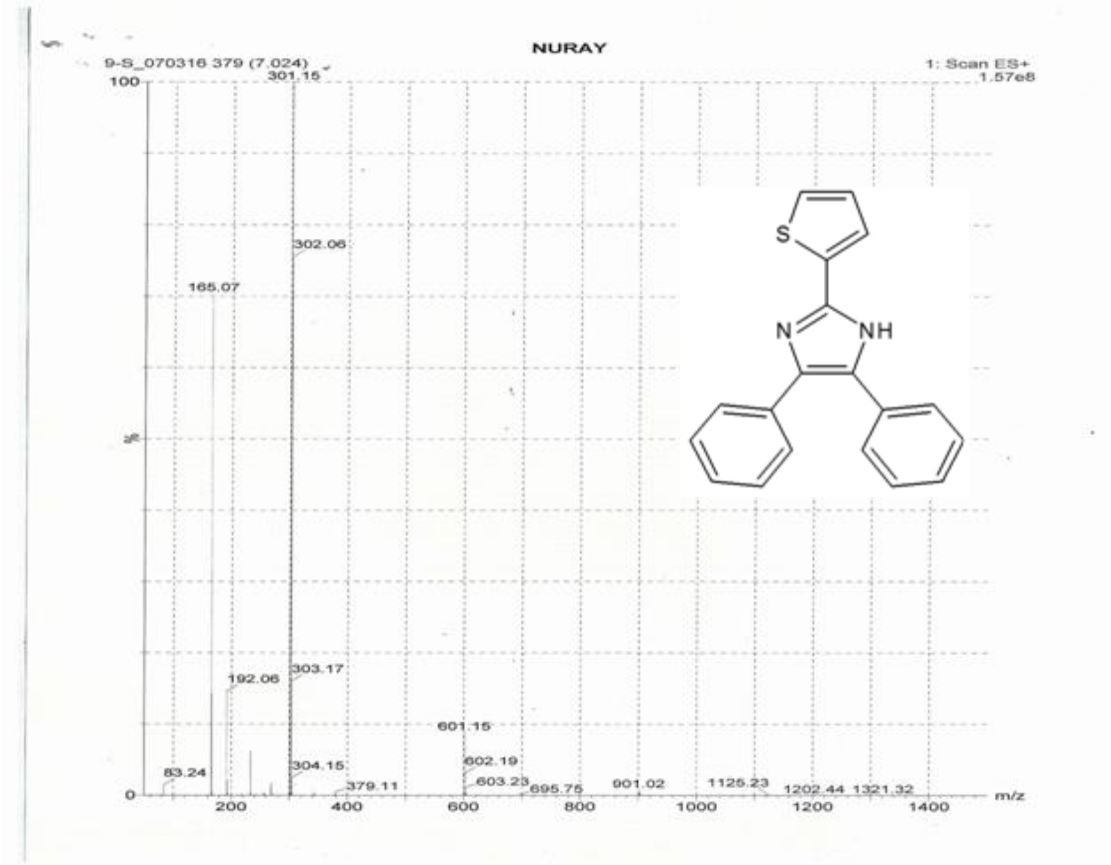
IR Spectrum: 9-S



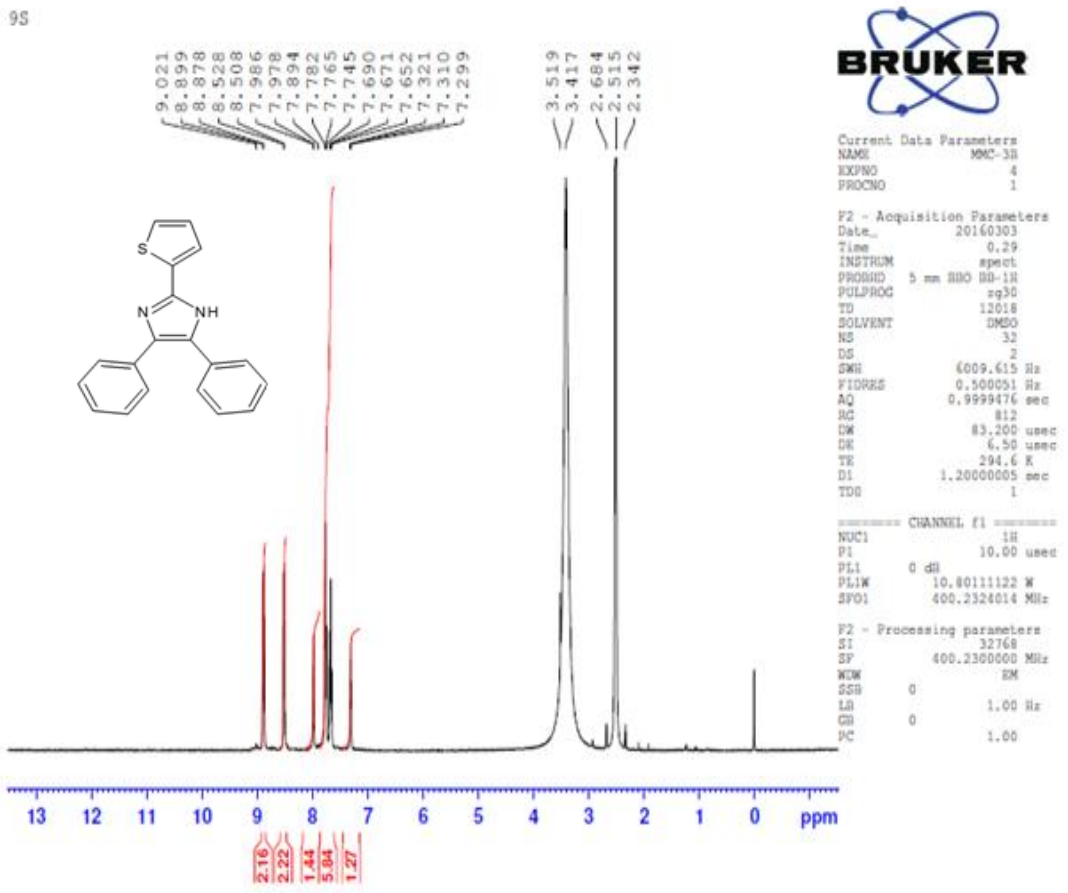
S.NO	WAVE NUMBER (cm-1)	FUNCTIONL GROUPS
1	3089.13cm-1	-CH Stretching
2	1634.74cm-1	-C=C Stretching
3	2347.47cm-1	-C=N Stretching
4	1226.79cm-1	-C-N stretching
5	3412.22cm-1	-N-H Stretching
6	731cm-1	-C-S Stretching

LC-MS Spectrum: 9-S



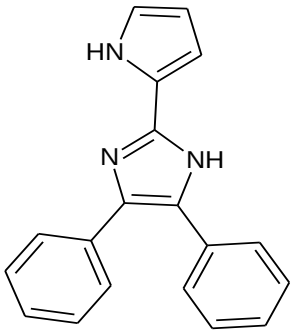


H_1 NMR Spectrum:-9S



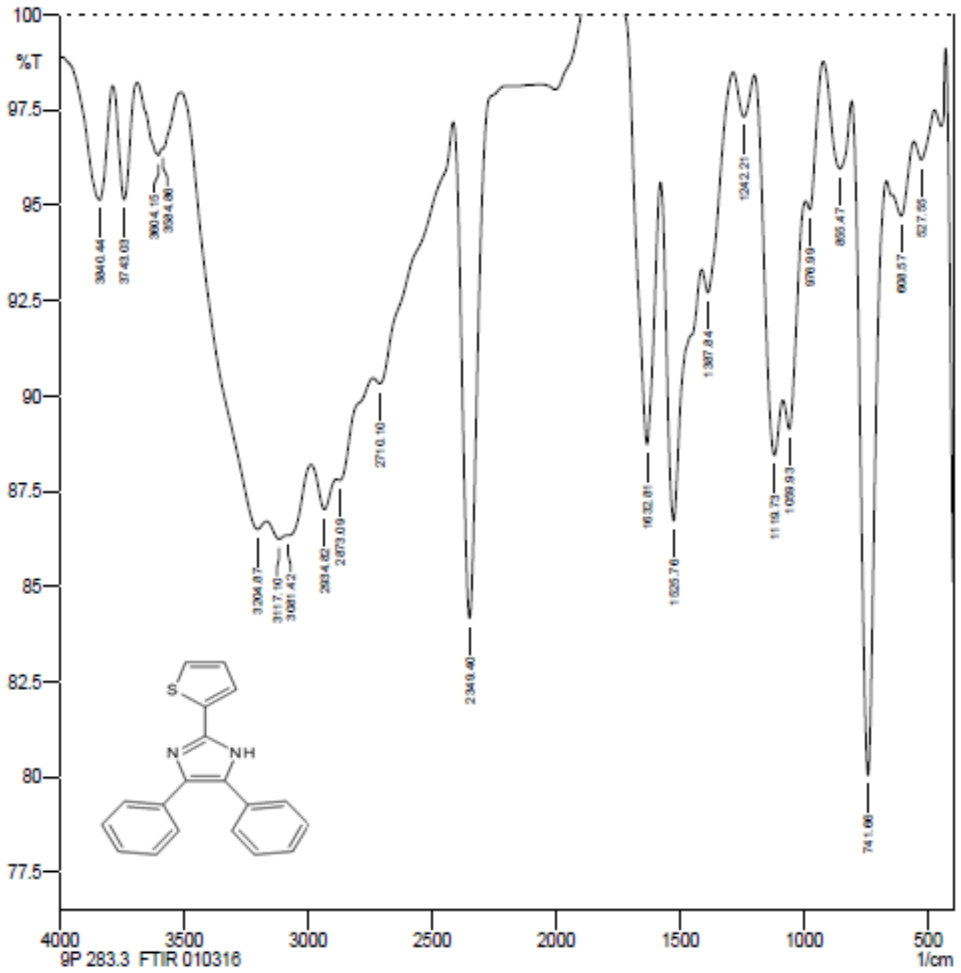
S.No	δ value	Nature of Peak	Number of Protons
1	δ2.4	singlet	1 proton
2	δ 3.5	singlet	1 proton
3	δ 7.4	singlet	1 proton
4	δ 7.5-9	Multiplet	11protons

SAMPLE CODE: 9-P



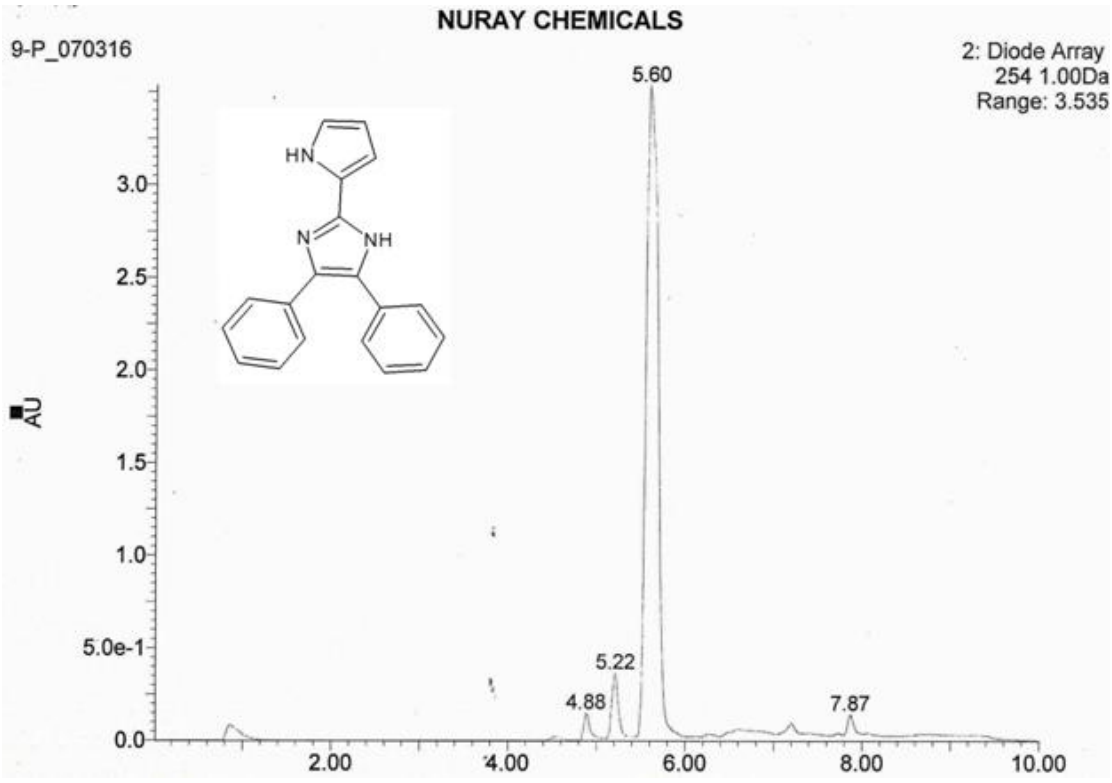
IUPAC Name	:	4,5-diphenyl-2-(1H-pyrrol-2-yl)-1H-imidazole
Molecular Formula	:	C19H15N3
Formula Weight	:	285.34g/mol
Appearance	:	black colour solid
Melting Point	:	305ºC
Solubility	:	Chloroform, Methanol, Dimethyl Sulfoxide
Percentage Yield	:	96%
Composition	:	C[79.98%], H[5.30%], N[14.73%],
Molar Refractivity	:	86.98±0.3cm3
Molar Volume	:	235.1±3.0cm3
Parachor	:	640.7±4.0cm3
Index of Refraction	:	1.661±0.02
Surface Tension	:	55.17±3.0 dyne/cm
Density	:	1.213.±0.06g/cm3
Polarizability	:	34.48±0.510-24cm3
Monoisotopic Mass	:	285.126597 Da
Nominal Mass	:	285 Da
Average Mass	:	285.3425 Da

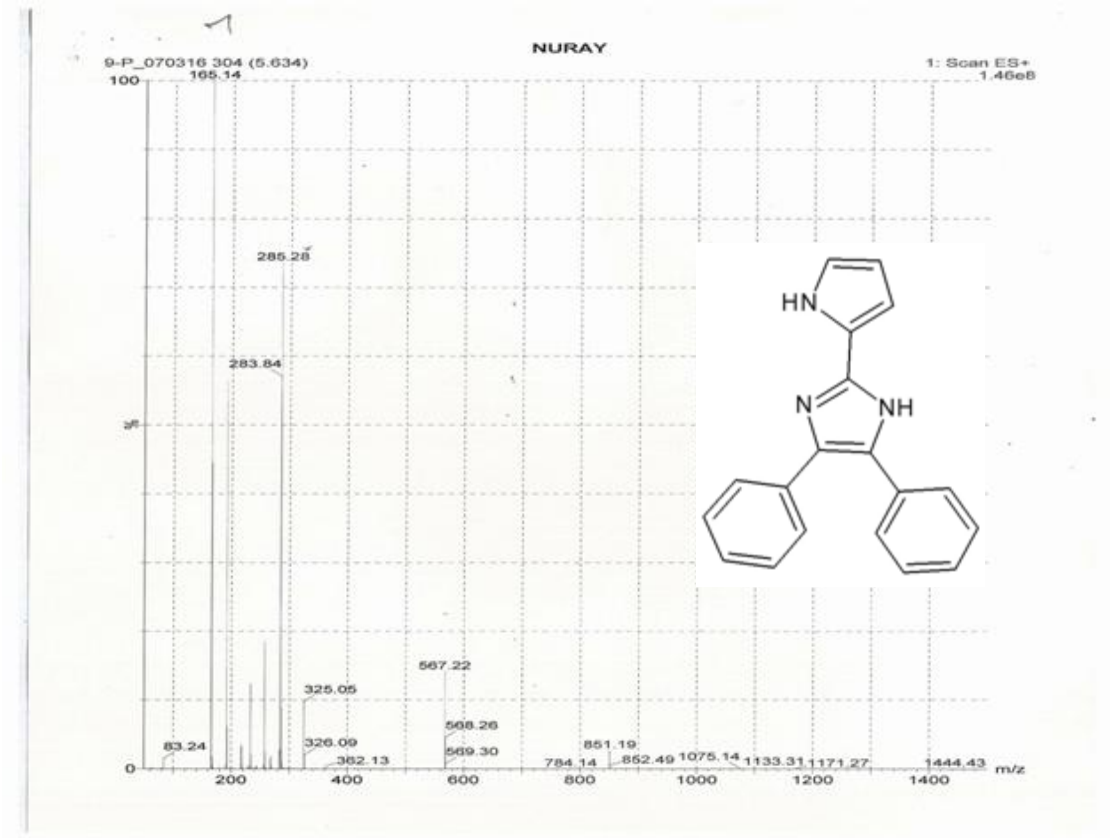
IR Spectrum: 9-P



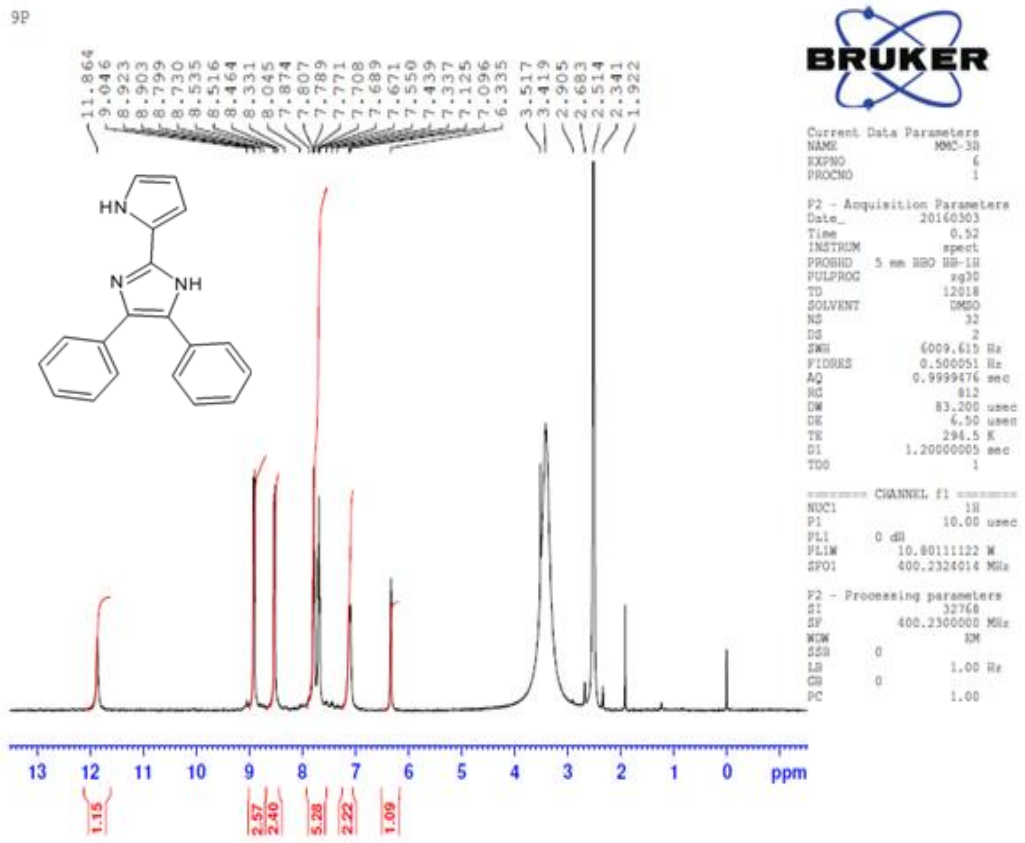
S.NO	WAVE NUMBER (cm-1)	FUNCTIONL GROUPS
1	3081.42cm-1	-CH Stretching
2	1525.76cm-1	-C=C Stretching
3	2349.4cm-1	-C=N Stretching
4	1242.21cm-1	-C-N stretching
5	3204.87cm-1	-NH stretching

LC-MS Spectrum: 9-P



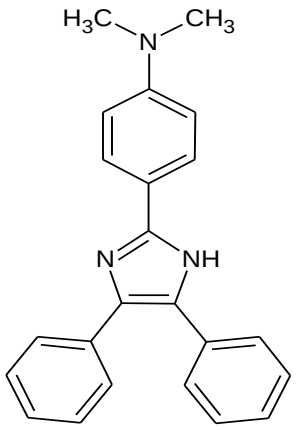


H_1 NMR Spectrum:-9P



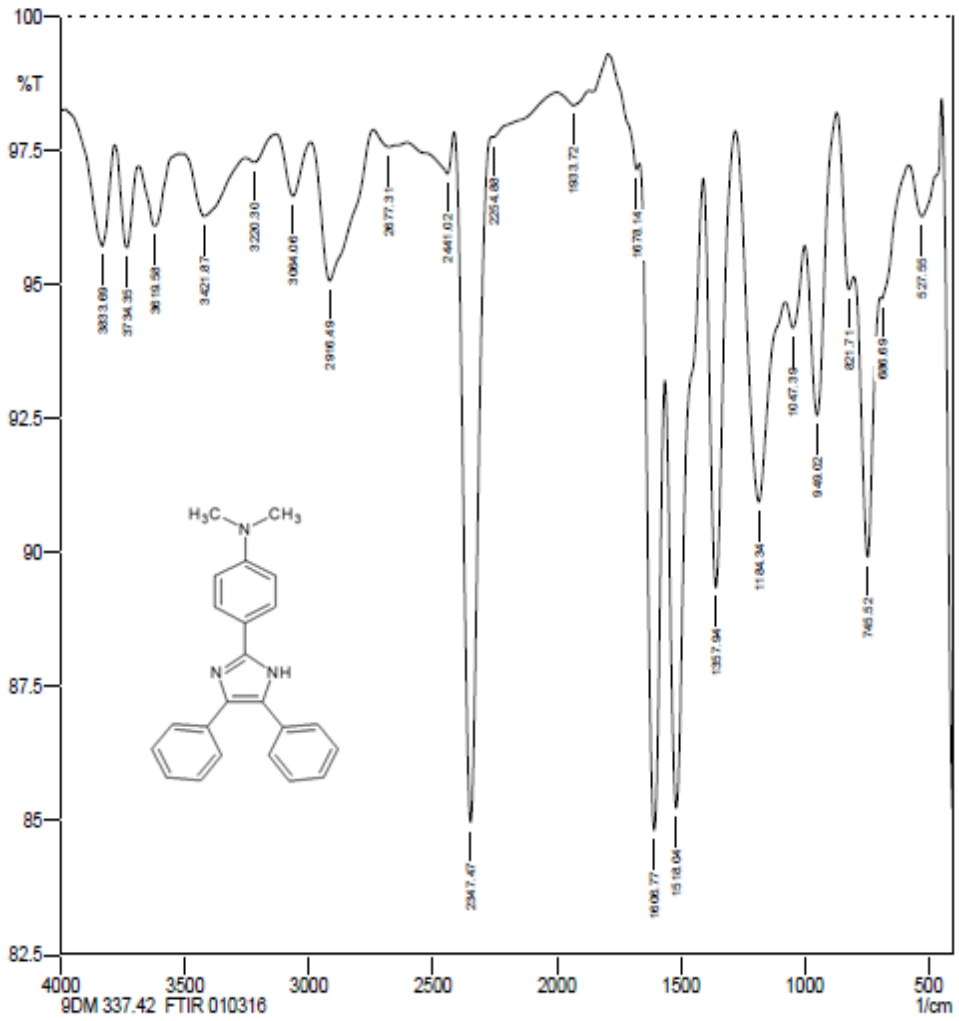
S.No	δ value	Nature of Peak	Number of Protons
1	δ1.9	singlet	1 proton
2	δ 2.6	singlet	1 proton
3	δ 3-4	singlet	1 proton
4	δ 6.3	singlet	1 protons
5	δ 7.2	singlet	2 protons
6	δ 7-9	multiple	11 protons
7	δ 11.8	Singlet	1 proton

SAMPLE CODE: 9-DM



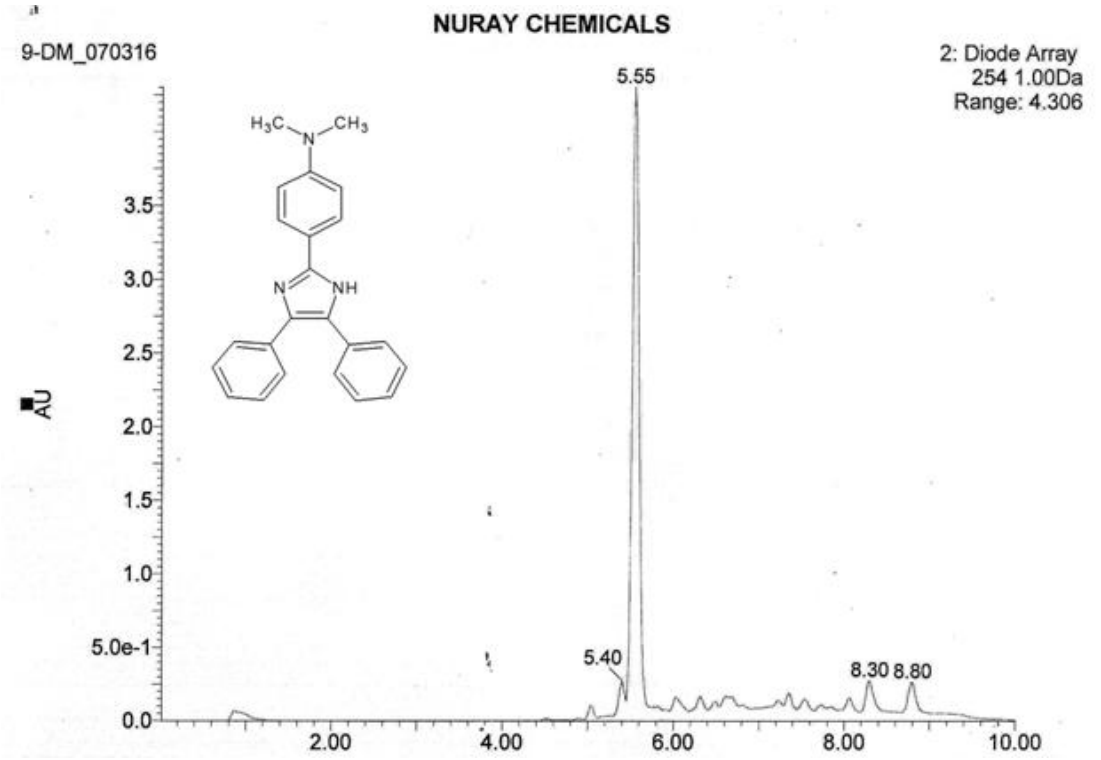
IUPAC Name	:	4,5-diphenyl-2-(4-dimethylamino-1-phenyl)-1H-imidazole
Molecular Formula	:	C23H21N3
Formula Weight	:	339.432g/mol
Appearance	:	Brown colour solid
Melting Point	:	335ºC
Solubility	:	Chloroform, Methanol, Dimethyl Sulfoxide
Percentage Yield	:	98%
Composition	:	C[81.38%], H[6.24%], N[12.38%],
Molar Refractivity	:	106.86±0.3cm3
Molar Volume	:	294.8±3.0cm3
Parachor	:	783.1±4.0cm3
Index of Refraction	:	1.644±0.02
Surface Tension	:	49.7±3.0 dyne/cm
Density	:	1.151.±0.06g/cm3
Polarizability	:	42.36±0.510-24cm3
Monoisotopic Mass	:	339.173548 Da
Nominal Mass	:	339 Da
Average Mass	:	339.4329 Da

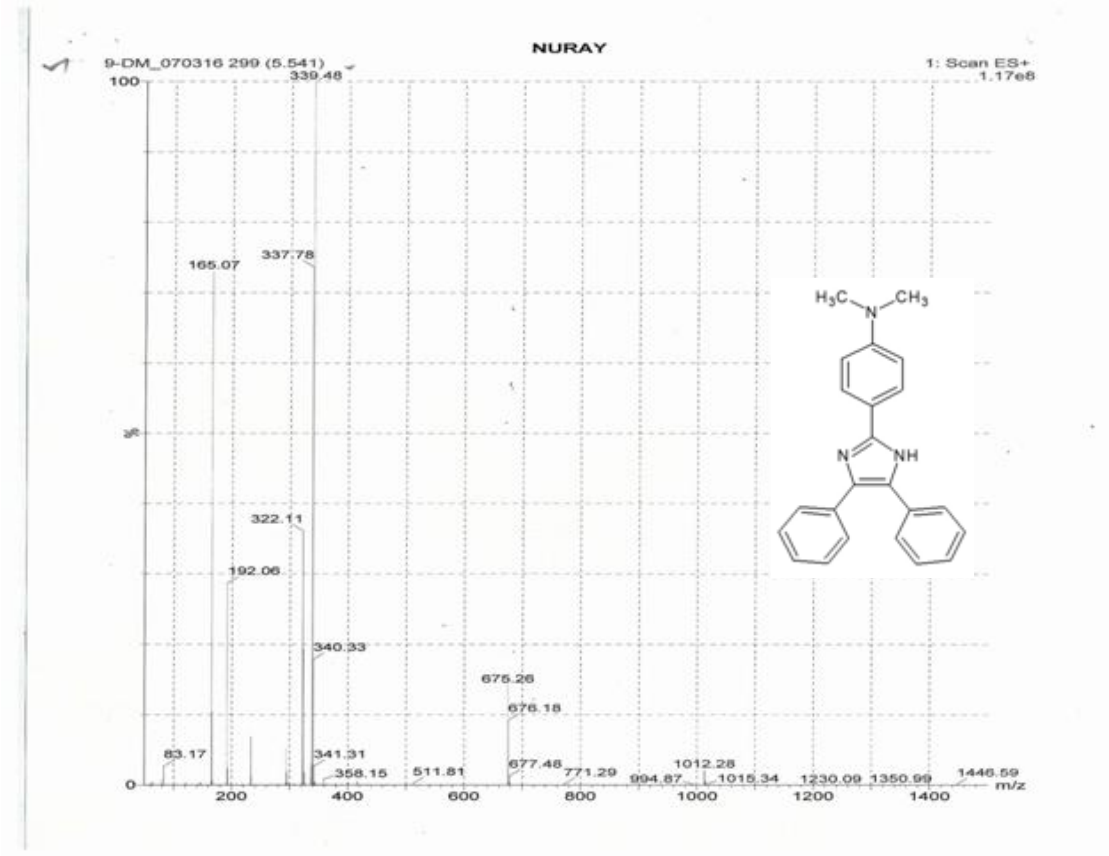
IR Spectrum: 9-DM



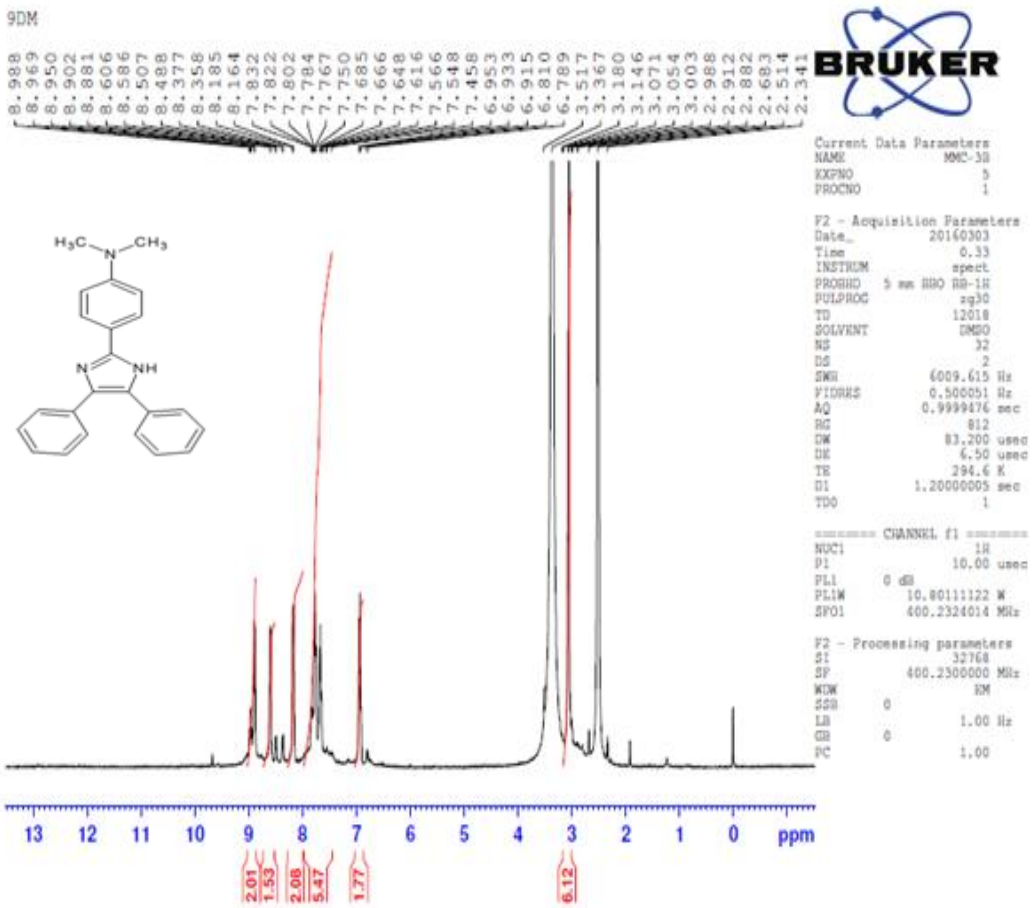
S.NO	WAVE NUMBER (cm-1)	FUNCTIONL GROUPS
1	3064.06cm-1	-CH Stretching
2	2916.49cm-1	-C-H Stretching
3	1184.34cm-1	-C-N Stretching
4	2347.47cm-1	-C=N stretching
5	1606.77cm-1	-C=C Stretching

LC-MS Spectrum: 9-DM



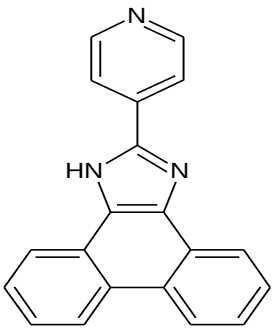


H_1 NMR Spectrum:-9DM



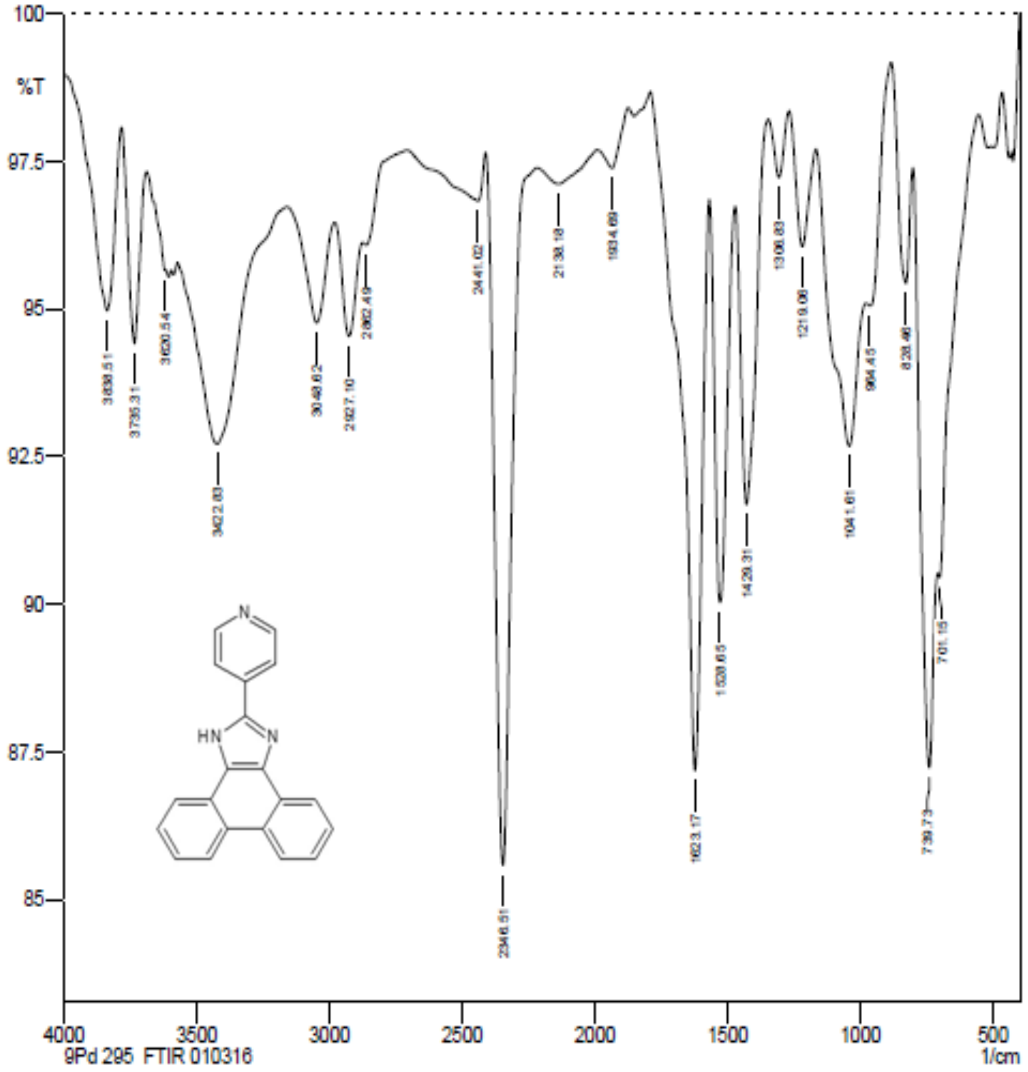
S.No	δ value	Nature of Peak	Number of Protons
1	δ1.8	singlet	1 proton
2	δ 2.5	singlet	2 proton
3	δ3	singlet	6 proton
4	δ3.2-4	singlet	1protons
5	δ7	singlet	1 protons
6	δ7.5-9	multiplet	10protons

SAMPLE CODE: 9-PD



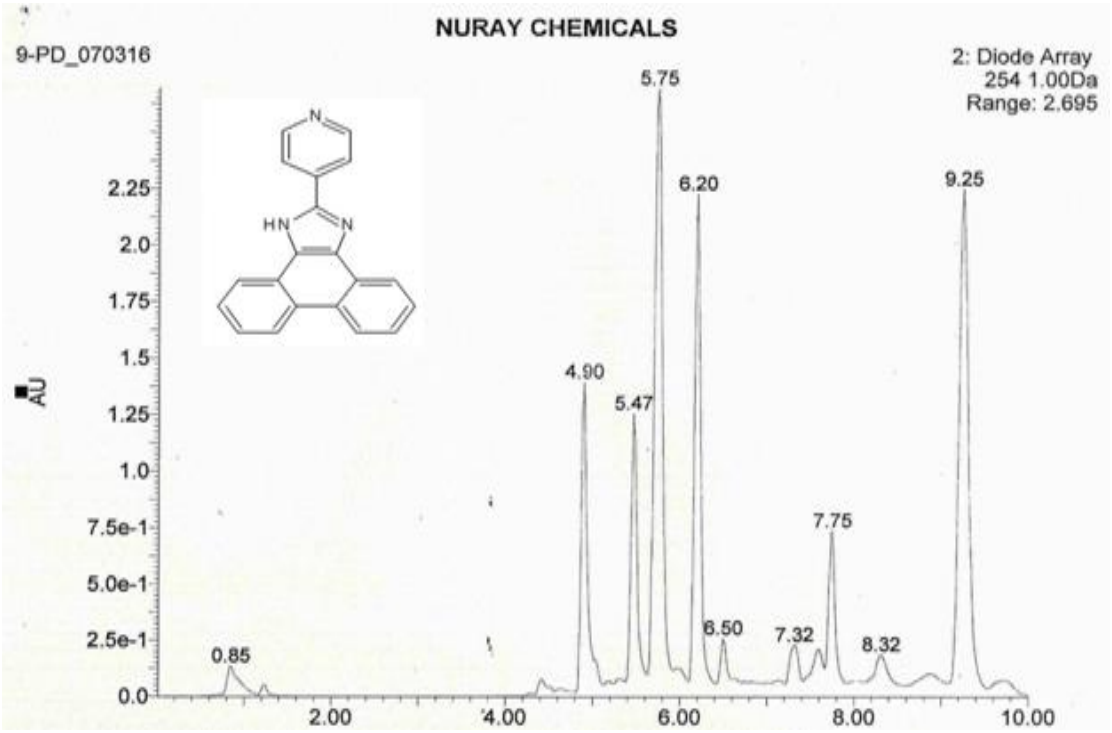
IUPAC Name	:	4-(4,5-diphenyl-1H-imidazol-2-yl)pyridine
Molecular Formula	:	C20H13N3
Formula Weight	:	295.37g/mol
Appearance	:	dark yellow colour solid
Melting Point	:	315ºC
Solubility	:	Chloroform, Methanol, Dimethyl Sulfoxide
Percentage Yield	:	97%
Composition	:	C[81.34%], H[4.44%], N[14.25],
Molar Refractivity	:	94.98±0.3cm3
Molar Volume	:	221.8±3.0cm3
Parachor	:	640.0±4.0cm3
Index of Refraction	:	1.801±0.02
Surface Tension	:	62.2±3.0 dyne/cm
Density	:	1.331±0.06g/cm3
Polarizability	:	37.65±0.510-24cm3
Monoisotopic Mass	:	295.110947 Da
Nominal Mass	:	295 Da
Average Mass	:	295.3373 Da

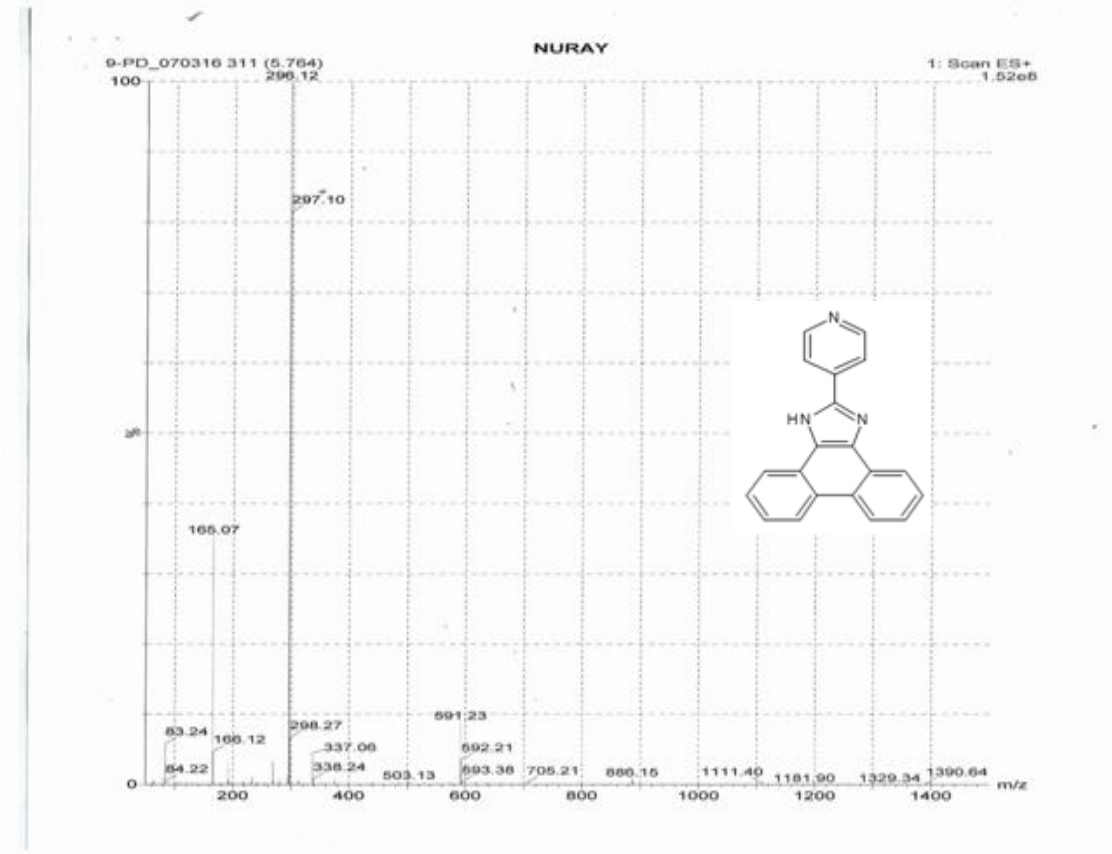
IR Spectrum: 9-PD



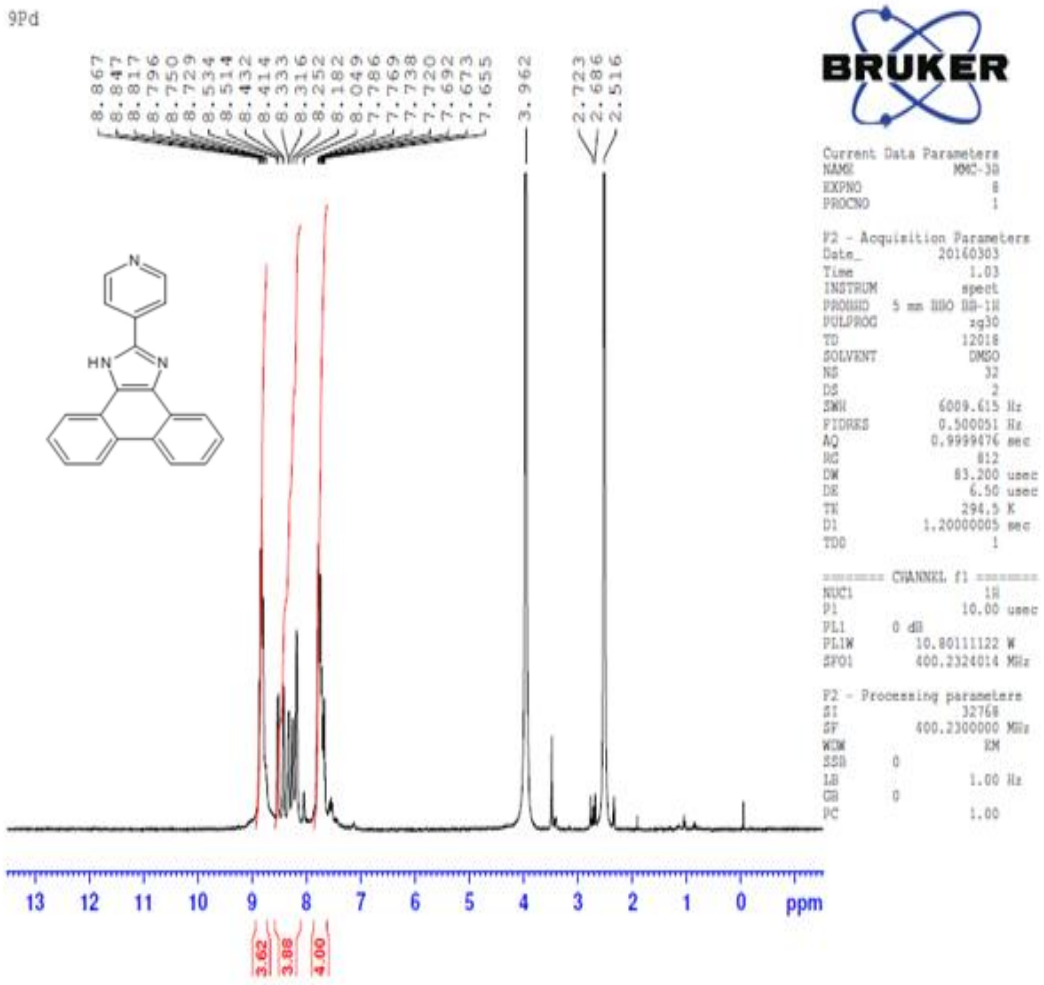
S.NO	WAVE NUMBER (cm-1)	FUNCTIONL GROUPS
1	3048.62cm-1	-CH Stretching
2	1528.65cm-1	-C=C Stretching
3	2346.51cm-1	-C=N Stretching
4	1219.06cm-1	-C-N stretching
5	3422.83cm-1	-N-H Stretching

LC-MS Spectrum: 9-PD



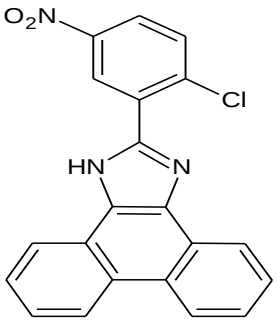


H_1 NMR Spectrum:-9PD



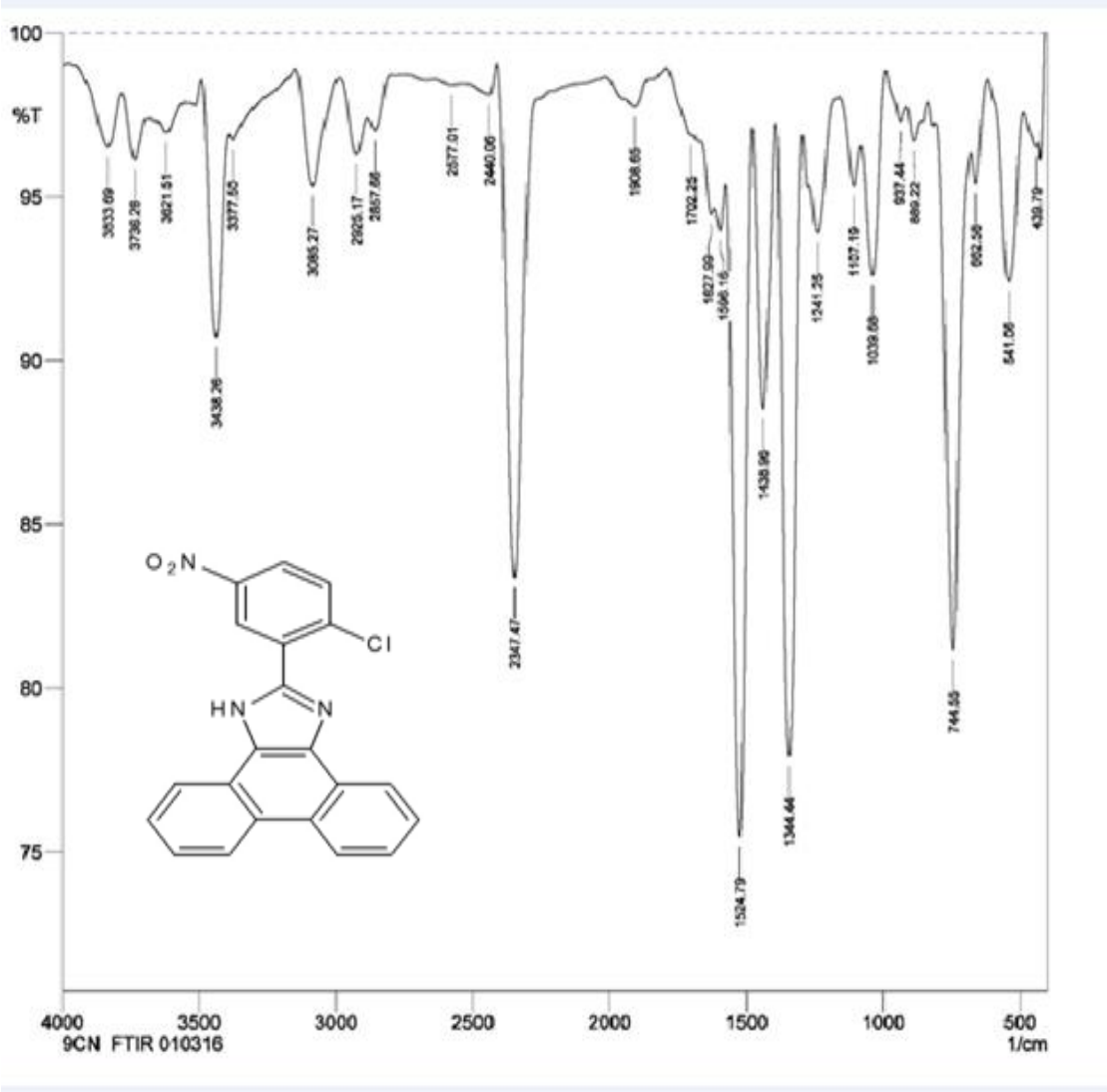
S.No	δ value	Nature of Peak	Number of Protons
1	δ2.5	singlet	1 proton
2	δ 3.5	singlet	1 proton
3	δ4	singlet	1 proton
4	δ7.6-9	Multiplet	10 protons

SAMPLE CODE: 9-CN



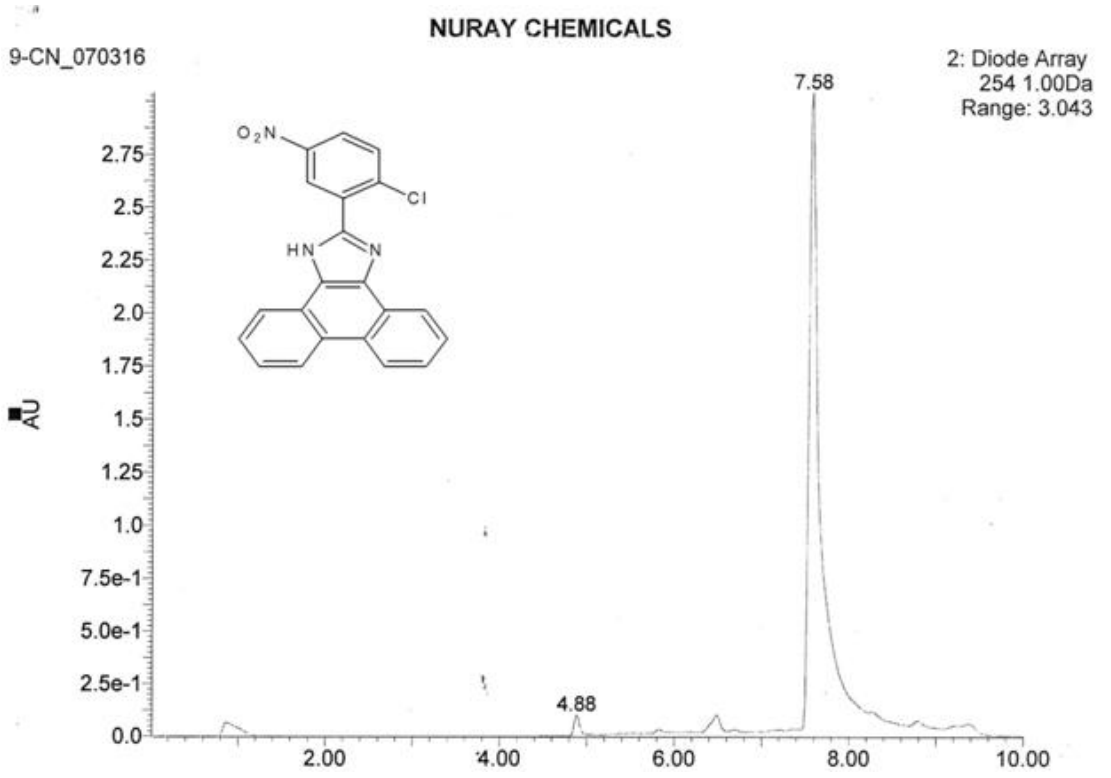
IUPAC Name	:	4,5-diphenyl-2-(2-chloro-5-nitro-1-phenyl)-1H-imidazole
Molecular Formula	:	C21H12CLN3O2
Formula Weight	:	373.7924g/mol
Appearance	:	light green colour
Melting Point	:	325ºC
Solubility	:	Chloroform, Methanol, Dimethyl Sulfoxide
Percentage Yield	:	97%
Composition	:	C[67.48%], H[3.24%], CL[9.48%],N[11.24%],
Molar Refractivity	:	108.337±0.3cm3
Molar Volume	:	252.4±3.0cm3
Parachor	:	737.27±4.0cm3
Index of Refraction	:	1.804±0.02
Surface Tension	:	72773.0±3.0 dyne/cm
Density	:	1.480.±0.06g/cm3
Polarizability	:	42.9470.510±0.510-24cm3
Monoisotopic Mass	:	373.061804 Da
Nominal Mass	:	373 Da
Average Mass	:	373.7919 Da

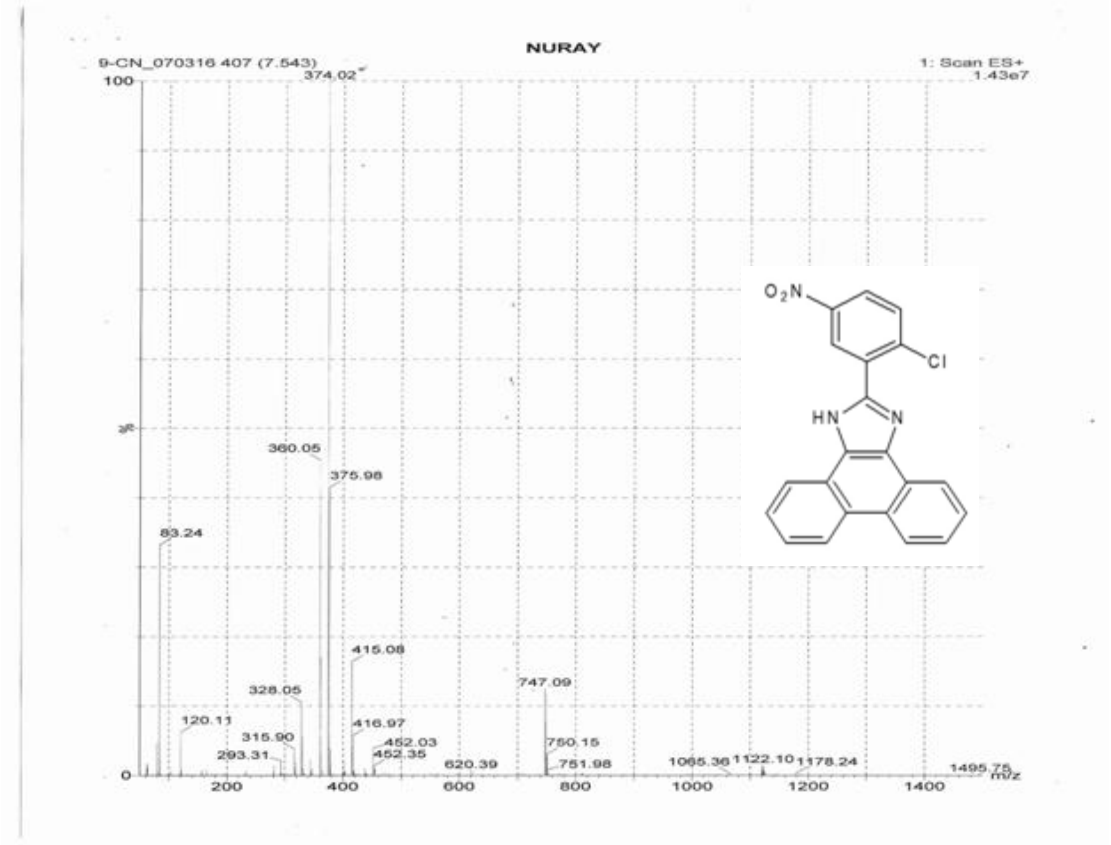
IR Spectrum 9-CN



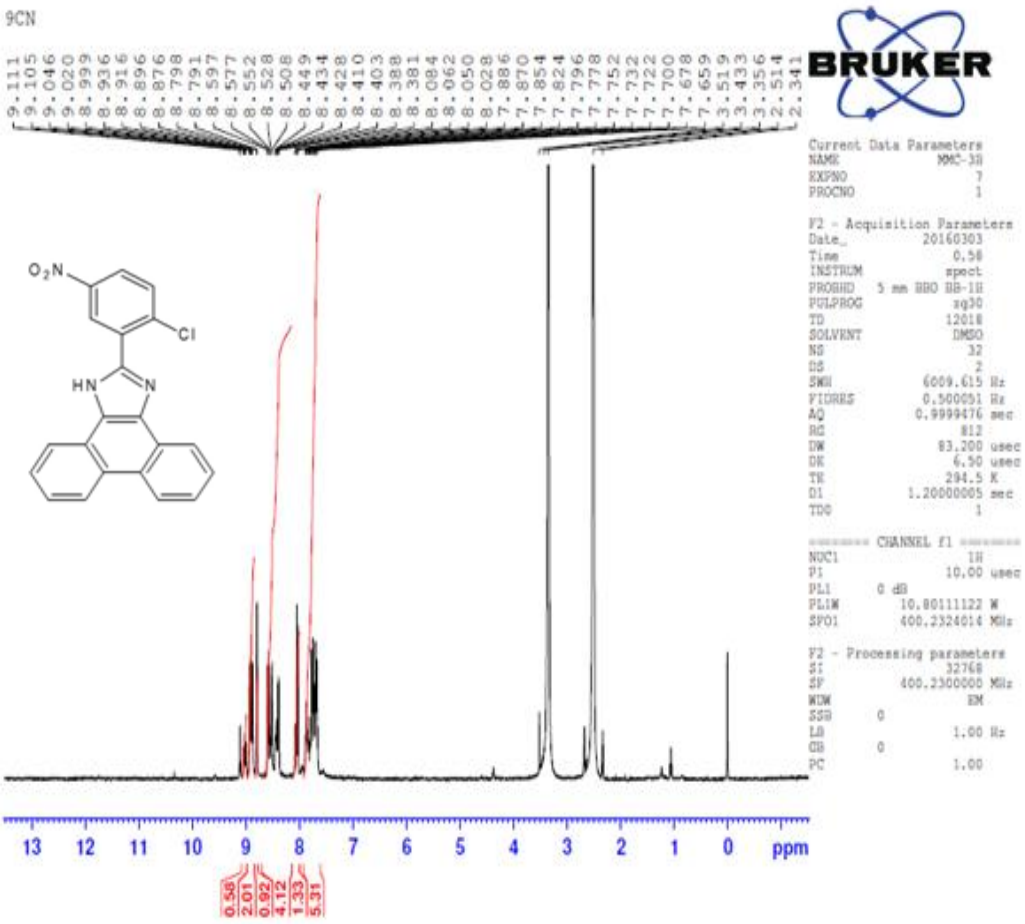
S.NO	WAVE NUMBER (cm-1)	FUNCTIONL GROUPS
1	3085.27cm-1	-CH Stretching
2	1524.79cm-1	-C-NO2 Stretching
3	1438.96cm-1	-C=C Stretching
4	2347.47cm-1	-C=N stretching
5	1241.25cm-1	-C-N Stretching
6	3438.26cm-1	-NH Stretching

LC-MS Spectrum: 9-CN





H_1 NMR Spectrum:-9CN



S.No	δ value	Nature of Peak	Number of Protons
2	δ 3.5	singlet	1 proton
3	δ7.6-8	multiplet	6 proton
4	δ8.5-9.2	Multiplet	6 protons

Biological Evaluation

Anti-TB Results

Sl. No	samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	9-F	S	S	S	S	R	R	R	R
2	9-DF	S	S	S	S	S	S	S	R
3	9-V	S	S	S	S	R	R	R	R
4	9-S	S	S	R	R	R	R	R	R
5	9-P	S	S	R	R	R	R	R	R
6	9-DM	S	S	R	R	R	R	R	R
7	9-PD	S	S	R	R	R	R	R	R
8	9-CN	S	S	R	R	R	R	R	R

Note: S-Sensitive

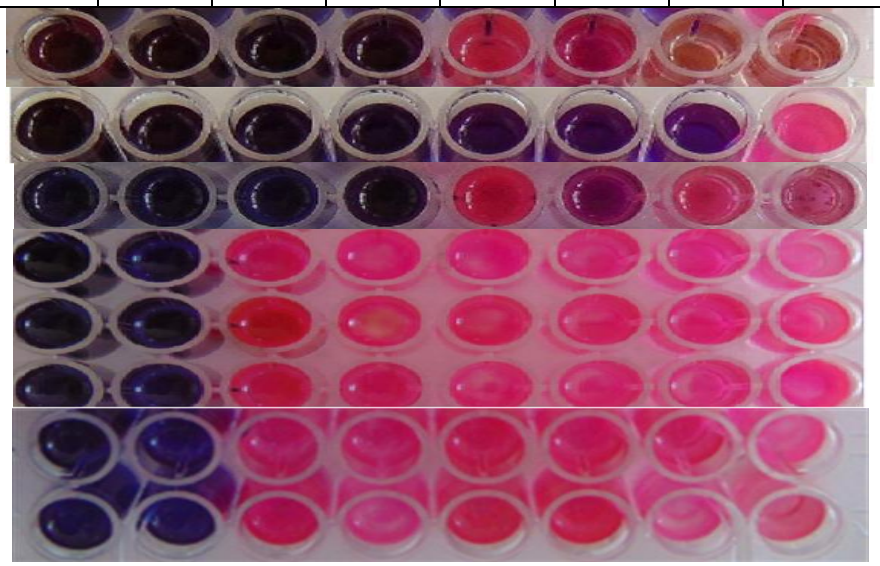
R-Resistant

Strain used: *M.tuberculosis* (H37 RV strain).

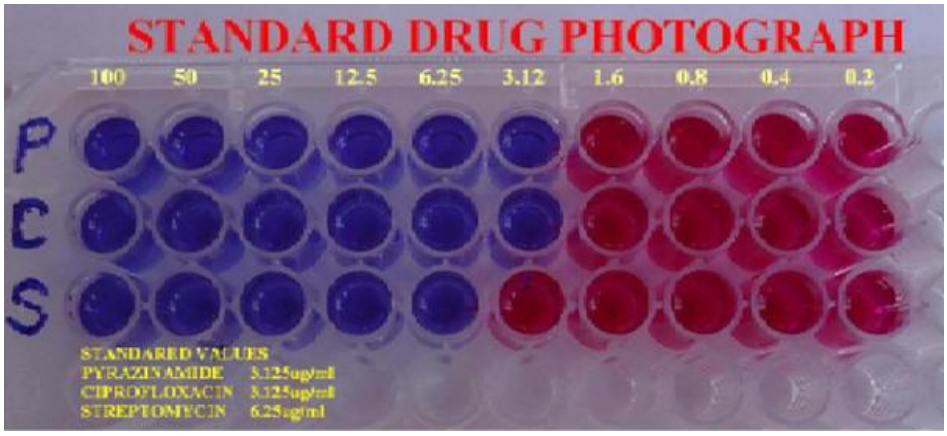
Here are the **standard values** for the Anti-Tb test which was performed.

- Pyrazinamide- 3.125µg/ml.
- Streptomycin- 6.25µg/ml.
- Ciprofloxacin- 3.125µg/ml.

SAMPLE DRUG PHOTOGRAPH

Sl. No	samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	9-F								
2	9-DF								
3	9-V								
4	9-S								
5	9-P								
6	9-DM								
7	9-PD								
8	9-CN								

STANDARD DRUG PHOTOGRAPH

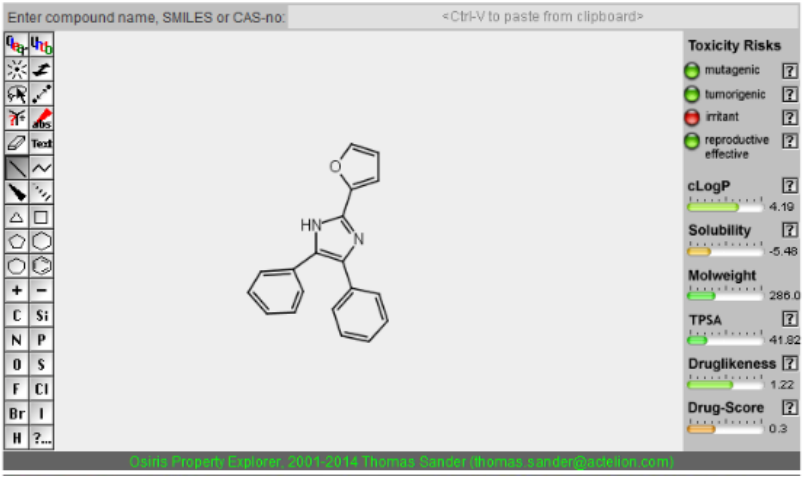


Toxicity Studies

Toxicity predicted by the OSIRIS Property Explorer the online software of Thomas Sander, Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, and 4123 Allschwil, Switzerland. The OSIRIS Property Explorer shown in this page is an integral part of Actelion's (1) inhouse substance registration system. It lets you draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Prediction results are valued and color coded. Properties with high risks of **undesired effects** like

mutagenicity or a poor intestinal absorption are shown in **red.** Whereas a **green** color indicates **drugconform** behavior.

Sample code : 9-F



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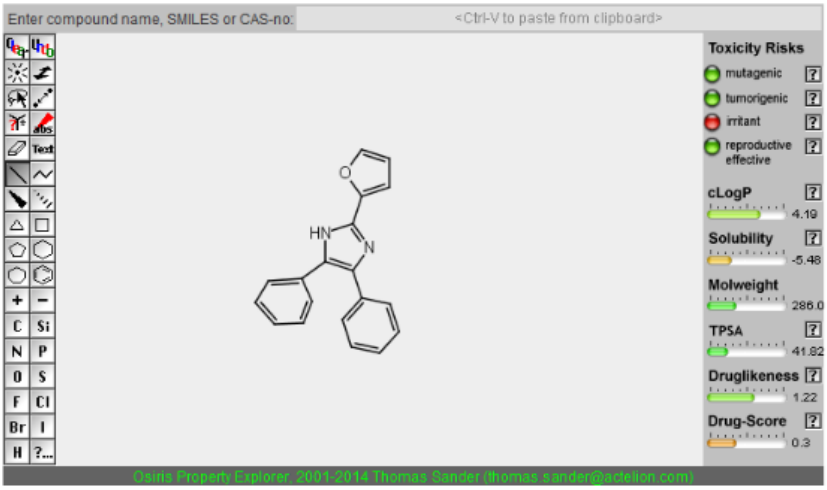
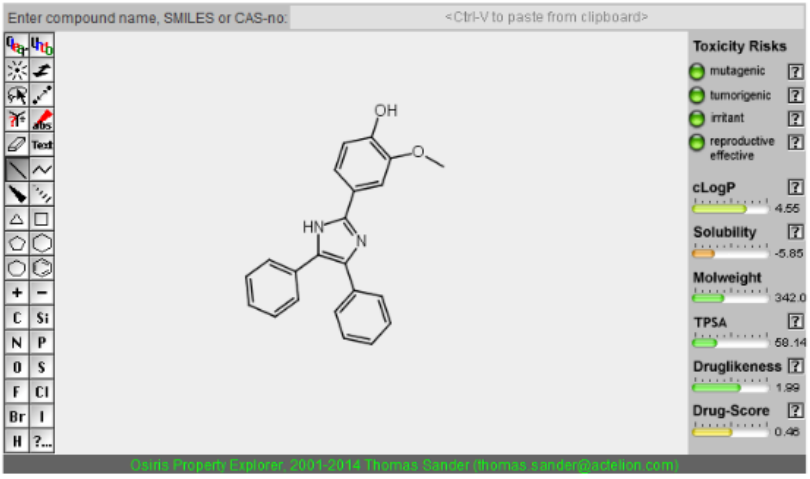
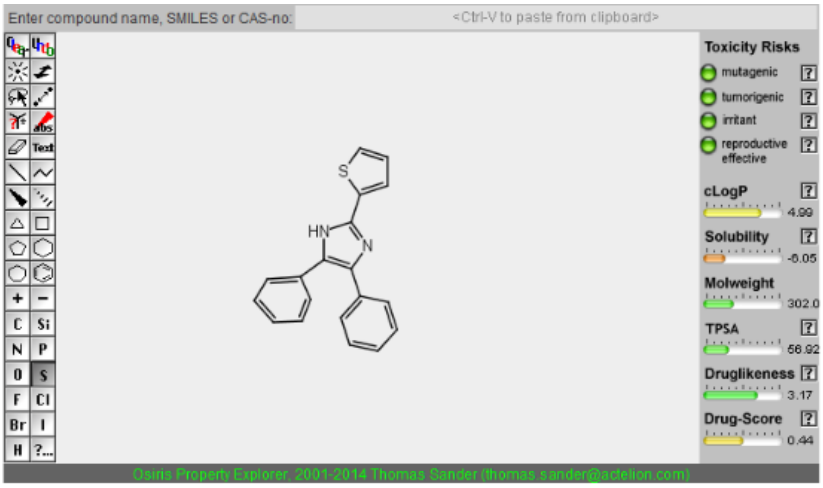


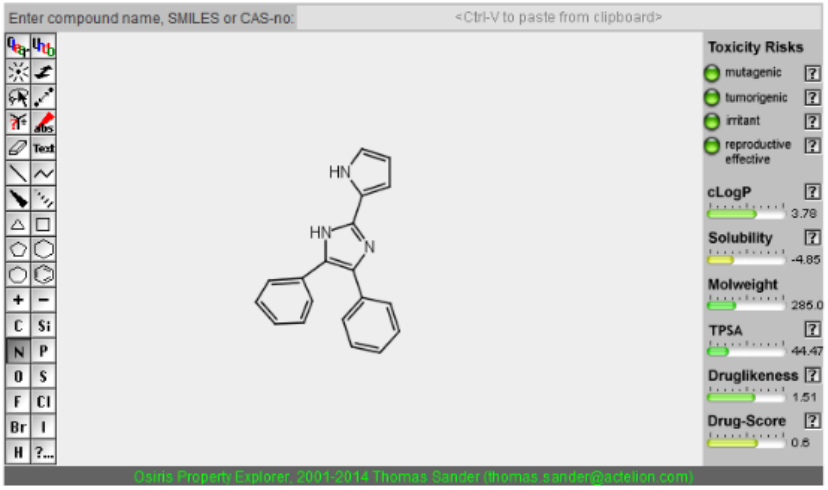
Sample code: 9-V



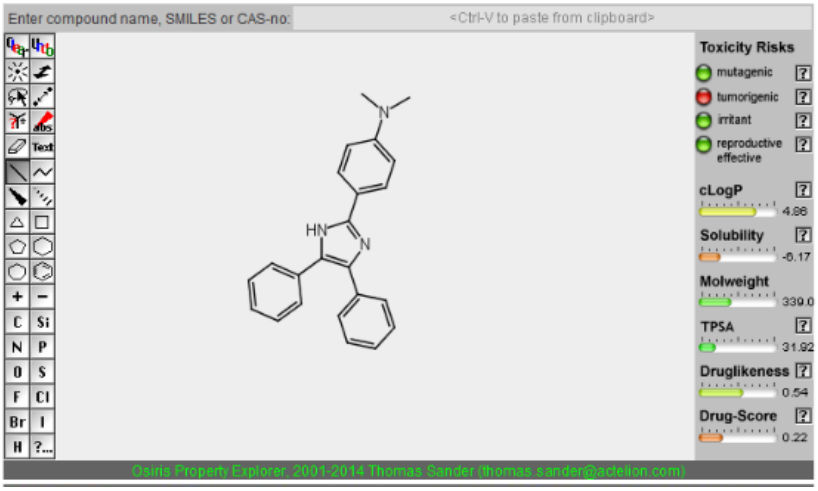
Sample code: 9-S



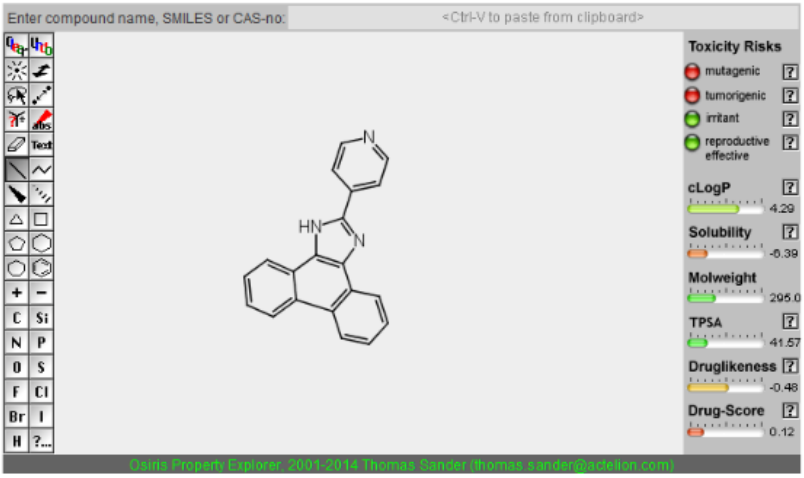
Sample code: 9P



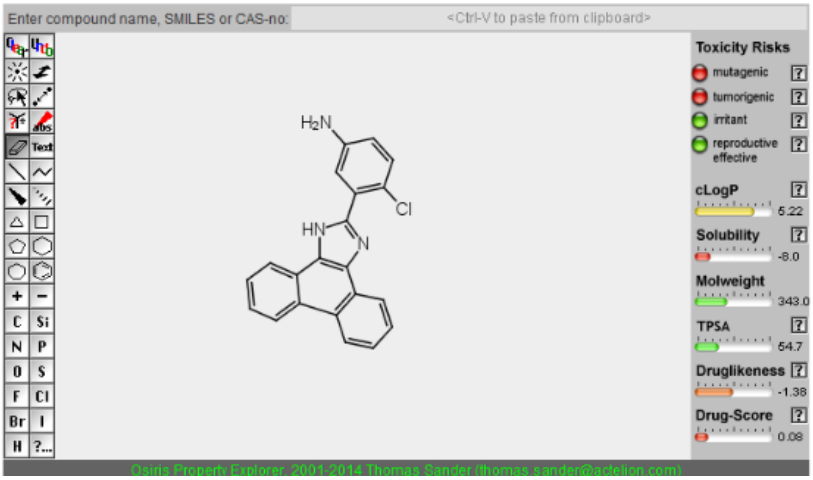
Sample code: 9-DM



Sample code: 9-PD



Sample code: -9-CN



PHARMACOLOGICAL STUDIES

ACUTE TOXICITY STUDY

Behavioural and physical observation of chemically synthesized compounds treated mice (2000mg/kg body weight)

TABLE : 8 Observation of acute toxicity study

OBSERVATION

S. No	Parameter	30min	4hr	24hrs	48hrs	7th Day	14th Day
1.	Sedation	Absent	Absent	Absent	Absent	Absent	Absent
2.	Excitation	Absent	Absent	Absent	Absent	Absent	Absent
3.	Jumping	Normal	Normal	Normal	Normal	Normal	Normal
4.	Writhing	Absent	Absent	Absent	Absent	Absent	Absent
5.	Piloerection	Absent	Absent	Absent	Absent	Absent	Absent
6.	Stereotypy	Absent	Absent	Absent	Absent	Absent	Absent
7.	Scratching	Normal	Normal	Normal	Normal	Normal	Normal
8.	Grooming	Normal	Normal	Normal	Normal	Normal	Normal
9.	Aggression	Absent	Absent	Absent	Absent	Absent	Absent
10.	Ptosis	Absent	Absent	Absent	Absent	Absent	Absent
11.	Exophthalmia	Absent	Absent	Absent	Absent	Absent	Absent
12.	Loss of righting reflux	Absent	Absent	Absent	Absent	Absent	Absent
13.	Loss o pinel reflx	Absent	Absent	Absent	Absent	Absent	Absent
14.	Loss of corneal	Absent	Absent	Absent	Absent	Absent	Absent
15.	Salivation	Absent	Absent	Absent	Absent	Absent	Absent
16.	Lacrimation	Absent	Absent	Absent	Absent	Absent	Absent
17.	Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal
18.	Color of the eye	Normal	Normal	Normal	Normal	Normal	Normal
19.	Tremors	Absent	Absent	Absent	Absent	Absent	Absent
20.	Diarrhoea	Absent	Absent	Absent	Absent	Absent	Absent
21.	Coma	Absent	Absent	Absent	Absent	Absent	Absent
22.	Straub's tail test	Absent	Absent	Absent	Absent	Absent	Absent
23.	Inflammation	Absent	Absent	Absent	Absent	Absent	Absent
24.	Gnawing	Normal	Normal	Normal	Normal	Normal	Normal
25.	Urination	Normal	Normal	Normal	Normal	Normal	Normal

The results of acute toxicity study are shown in table. There were no morbididity and mortality observed for chemically synthesized compounds treated animals upto 2000mg/kg.

FUTURE SCOPE OF STUDY

The synthesized compounds should significant anti-tubercular activity in MABA assay method. Hence the anti-tubercular study would deserve for further investigation of in-vivo anti-tubercular studies.

SUMMARY AND CONCLUSION

- L, D-Transpeptidase-2 (4GSU), a critical enzyme for the cell wall synthesis of Mycobacterium tuberculosis was chosen for study after review of literature.

- Candidate molecules were designed and docked against 4GSU protein using Argus lab 4.0software.

- Molecules with good Docking score (lower binding energy) and interactions were shortlisted for synthesis. The reaction conditions were optimized.

- The selected molecules were subjected to Toxicity prediction assessments by OSIRIS® software. The results are color coded as green color which confirms the drug likeness.

- Compounds were synthesized by conventional method and labeled as 9-F,9-DF,9-V,9-S,9-P,9-DM,9-PD,and 9-CN.

- Purity of the synthesized compounds was ensured by repeated recrystallization. Further the compounds were evaluated by TLC and Melting point determination.

- The characterization of the synthesized compounds was done using Infra-red, Nuclear Magnetic Resonance (H1 NMR) and Mass spectroscopic methods (LC-MS, GC-MS)

- The pure compounds were screened for *In-vitro* Anti- tubercular activity by Micro plate Alamar Blue Assay (MABA). All compounds showed a significant anti-mycobacterium activity.

- The synthesized compounds were active at 1.6 - 50µg/ml, which were comparable into the known anti-TB drugs: Pyrazinamide - 3.125µg/ml, Ciprofloxacin - 3.125µg/ml and Streptomycin - 6.25µg/ml

CONCLUSION

- Our work concludes that our synthesized molecules are effective in inhibiting the target enzyme L,D-Transpeptidase 2, which is important for the growth of Mycobacterium tuberculosis Cell wall.

- All the 8 compounds gave Docking score between -8.20 to -11.42kcal/mol. Pyrazinamide gave Docking score of -5.6 for 4GSU, Streptomycin gave Docking score of -7.4 for 4GSU and Ciprofloxacin gave Docking score of -5.9 for 4GSU. There is correlation between the score and activities of all the 8 compounds which were tested and compared with the standard drugs. This goes to prove that 4GSU is a critical enzyme for anti-mycobacterial activity.

- The minimum inhibitory concentration of the 8 synthesized compounds against H37RV ranged from 12.5 to 1.5.µg/ml. Which is better compared to that of the certain known Anti-TB agents Pyrazinamide-3.125µg/ml, Ciprofloxacin- 3.125µg/ml and Streptomycin- 6.25µg/ml. _ Further structural refinement to the structure of the synthesized compounds is expected to yield promising molecules against the pathogen Mycobacterium tuberculosis.

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CERTIFICATE

This is to certify that Ms. **S.MALA,** M.Pharm II year, Department of pharmaceutical chemistry, College of Pharmacy, Madras Medical College, Chennai – 600003 had submitted her protocol (Part B Application) 21/243/CPCSEA for the dissertation programme to the Animal Ethical Committee, Madras Medical College, Chennai – 600003.

TITLE: "DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL ANTI- TUBERCULAR AGENTS TARGETING L, D-Transpeptidase-2"

The Animal Ethical Clearance Committee experts screened her proposal No: 21/243/CPCSEA and have given clearance in the meeting held on 03-09-2014 at Dean's Chamber in Madras Medical College, Chennai – 600003. Her study involves only Swiss Albino mice.

Signature

14/3/2016

Dr. S.K. SEENIVELAN, B.V.Sc.,
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NATIONAL CONFERENCE ON
EMERGING AND RE-EMERGING VIRAL OUTBREAKS IN INDIA – CLINICAL CHALLENGES AND MANAGEMENT

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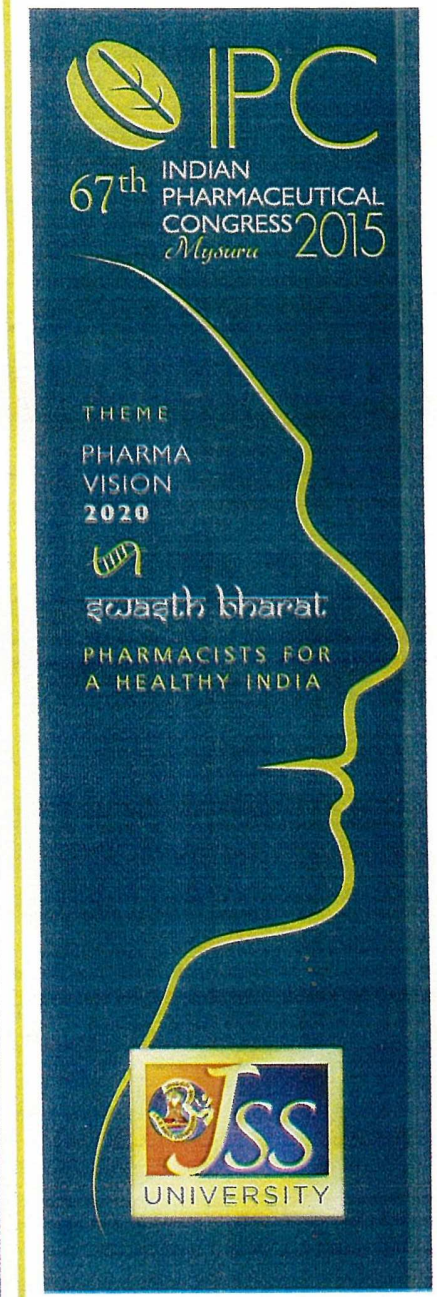

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