DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL BENZIMIDAZOLE CHALCONE DERIVATIVES AS ANTITUBERCULAR AGENTS TARGETING *GLUTAMINE SYNTHETASE 1*

A Dissertation submitted to
THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY
CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the Degree of
MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
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Under the guidance of
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COLLEGE OF PHARMACY
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TAMIL NADU

CERTIFICATE

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EXAMINERS

1.

2.

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LIST OF ABBREVIATIONS

TB	Tuberculosis
M.tb	Mycobacterium tuberculosis
HIV	Human immune deficiency virus
AIDS	Acquired immumo deficiency syndrome
DOTS	Directly observed therapy short-course
MDR-TB	Multi drug resistance tuberculosis
XDR-TB	Extensively drug resistance tuberculosis
GS I	Glutamine synthetase I
IR	Infrared spectroscopy
NMR	Nuclear magnectic resonance imaging
LC-MS	Liquid chromatography-mass spectrometry
MABA	Microplate Alamar Blue assay
WHO	World Health Organization
CADD	Computer aided drug designing
TDR-TB	Total drug resistance tuberculosis
PDB	Protein data bank

LIST OF TABLES

LIST OF FIGURES

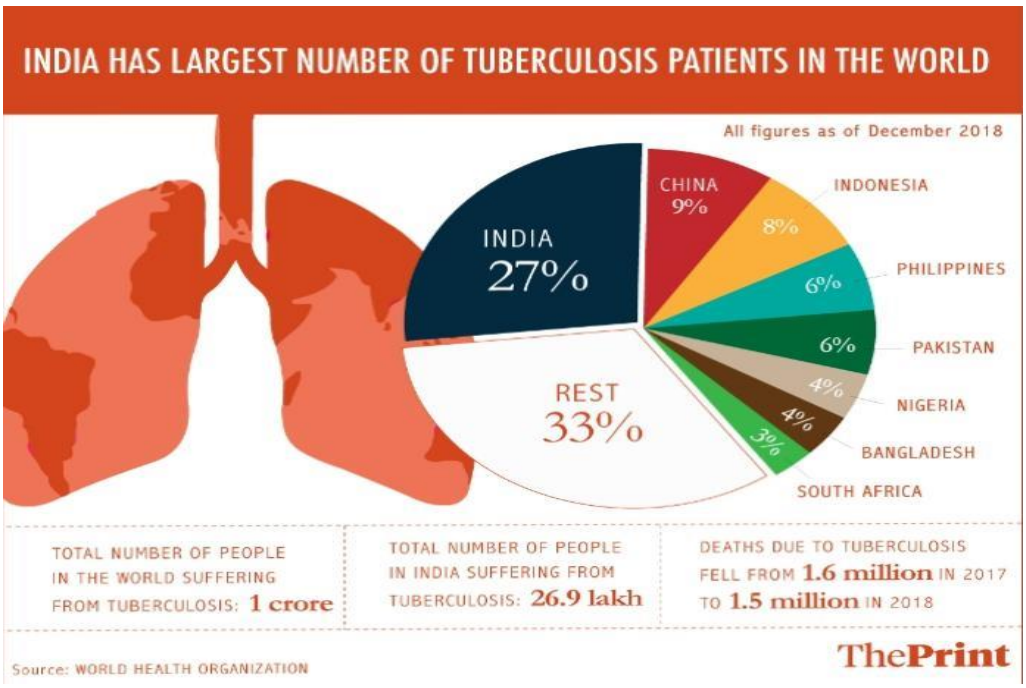
CONTENTS

INTRODUCTION

Tuberculosis[1] (TB) is an infectious disease caused by bacteria ***Mycobacterium tuberculosis*** that are spread from person to person through air. TB usually affects the lungs, and it can also affect other parts of the body, such as the brain, the kidneys, or the spine. Most infections do not have symptoms, and the TB bacteria remain inactive for a lifetime without causing disease, in which case it is known as latent tuberculosis. About 10% of latent infections progress to active disease which, if left untreated, kills about half of those infected.

GLOBAL IMPACT OF TB

Tuberculosis remains a worldwide public health problem despite the fact that the causative organism was discovered more than 100 years ago and highly effective drugs and vaccine are available making tuberculosis curable and preventable disease. TB occurs in every part of the world. In 2020, the largest number of new TB cases occurred according to WHO in the South-East Asian Region. In 2020, 86% of new TB cases occurred in the 30 high TB burden countries. Eight countries accounted for two thirds of the new TB cases: India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa[2].



WHO report on Tuberculosis cases across worldwide [2].

SYMPTOMS

Signs and symptoms of active TB include[3]:

- Coughing for three or more weeks
- Coughing up blood or mucus
- Chest pain, or pain with breathing or coughing
- Unintentional weight loss
- Fatigue
- Fever
- Night sweats
- Chills
- Loss of appetite

DOTS THERAPY

DIRECTLY OBSERVED TREATMENT SHORT COURSE

Tuberculosis (TB) requires at least six months of treatment. If treatment is incomplete, patients may not be cured and drug resistance may develop. Directly Observed Therapy (DOT) is a specific strategy, endorsed by the World Health Organization, to improve adherence by requiring health workers, community volunteers or family members to observe and record patients taking each dose[4].

EFFECT OF COVID PANDEMIC IN TUBERCULOSIS MANAGEMENT

The Covid-19 pandemic has had devastating effects on every aspect of global health, but tuberculosis services have been disproportionately affected. Tuberculosis deaths have increased because of reduced access to care. In 2020, there were roughly 1.5 million tuberculosis deaths worldwide, representing the first year-over-year increase in tuberculosis deaths since 2005[5].

BCG VACCINE

BCG, or bacille Calmette-Guerin, is a vaccine for tuberculosis (TB). BCG is used in many countries with a high prevalence of TB to prevent childhood tuberculo meningitis and miliary

disease. The BCG vaccine should be considered only for very select persons who meet specific criteria and in consultation with a TB expert. Treatment of Latent TB infection substantially reduces the risk that TB infection will progress to disease[6].

CO – INFECTION:

Risk of developing Tuberculosis is more in the Immuno compromised individuals. HIV/AIDS infection makes an individual more immune compromised. People with HIV Infection are 16.27 times more vulnerable to develop the TB infection than the non-HIV individuals. The risk of death is also twice in these cases. The progress of one disease speeds up by the other[7].

CURRENT TREATMENT AGAINST TUBERCULOSIS:

About one third of the world's population has latent tuberculosis, caused by Mycobacterium tuberculosis infection. DOTS is highly effective at promoting successful treatment.

A Regimen of Isoniazid (INH), Rifampin (RIF), Pyrazinamide (PZA), and either Ethambutol (EMB) or Streptomycin (SM) is usually the drugs of choice for the treatment of latent TB. M. tuberculosis strains can be multidrug resistant TB (MDR TB), extensively drug resistant TB (XDR TB), or totally drug- resistant TB (TDR TB)[8].

MULTIDRUG-RESISTANT TB (MDR TB)

Multidrug-resistant TB (MDR TB) is caused by TB bacteria that is resistant to at least isoniazid and rifampin, the two most potent anti TB drugs. These drugs are used to treat all persons with TB disease[8].

EXTENSIVELY DRUG-RESISTANT TB (XDR TB)

Extensively drug-resistant TB (XDR TB) is a rare type of MDR TB that is resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin).Because XDR TB is resistant to the most potent TB drugs, patients are left with treatment options that are much less effective[8].

THE ETIOLOGICAL AGENT:

Mycobacterium *tuberculosis* is the causative agent of tuberculosis in mammals including Humans. The Mycobacterium tuberculosis complex consists of strains of five species-**M. *tuberculosis*, M. *canettii*, M. *africanum*, M.*microti*, and M. *bovis*** and two subspecies-**M. *caprae*** and **M. *pinnipedii***[9-10]**.**

MYCOBACTERIUM TUBERCULOSIS

Mycobacterium tuberculosis has an unusual, waxy coating on its cell surface primarily due to the presence of mycolic acid.It is a non motile ,rod shaped bacteria. *M. tuberculosis* is highly aerobic and requires high levels of oxygen. The rods are 2-4 µm in length and 0.2-0.5 µm in width.It is a small bacillus that can withstand weak disinfectants and can survive in a dry state for weeks. Its unusual cell wall is rich in lipids such as mycolic acid, is likely responsible for its resistance to desiccation and is a key virulence factor[11].

TAXONOMY CLASSIFICATION

Kingdom **:** Bacteria

Phylum **:** Actinobacteria

Class **:** Actinobacteria

Order **:** Actinomycetales

Suborder **:** Corynebacterineae

Family **:** Mycobacteriaceae

Genus **:** Mycobacterium

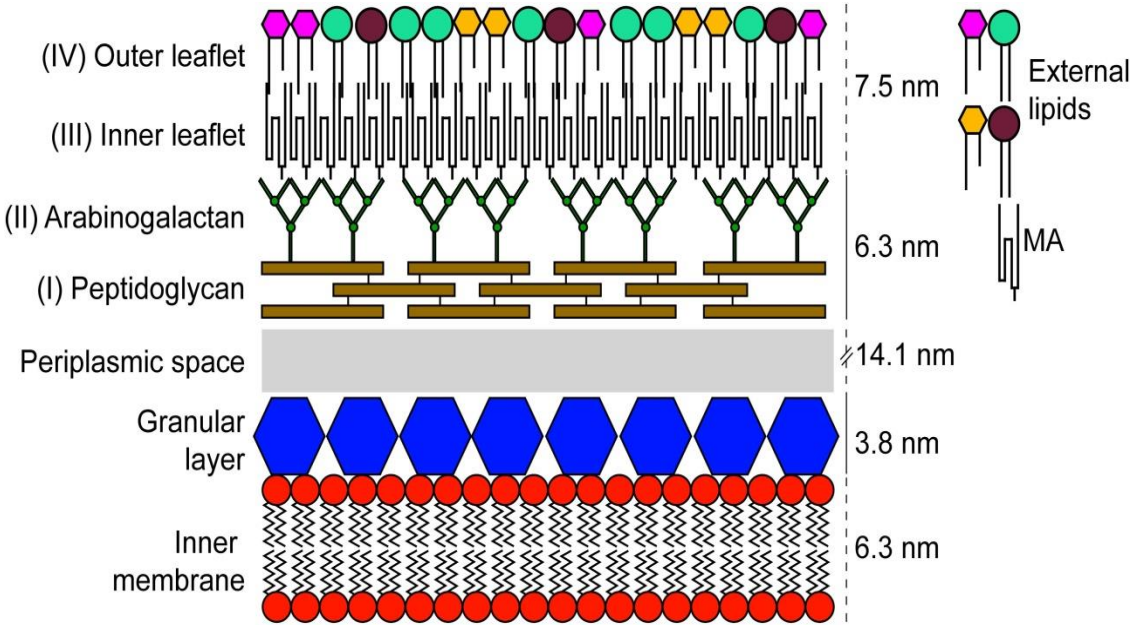
Species **:** M.tuberculosis

Synonym **:** Tubercle bacillus Koch

Mycobaterium tuberculosis[13]

MYCOBACTERIAL CELL WALL:

The cell wall is a major virulence factor of Mycobacterium tuberculosis and contributes to its intrinsic drug resistance. Cryo-electron microscopy showed that the mycobacterial cell wall lipids form an unusual outer membrane. Identification of the components of the uptake and secretion machinery across this membrane is critical for understanding the physiology and pathogenicity of Tuberculosis and for the development of better anti-tuberculosis drugs[12].

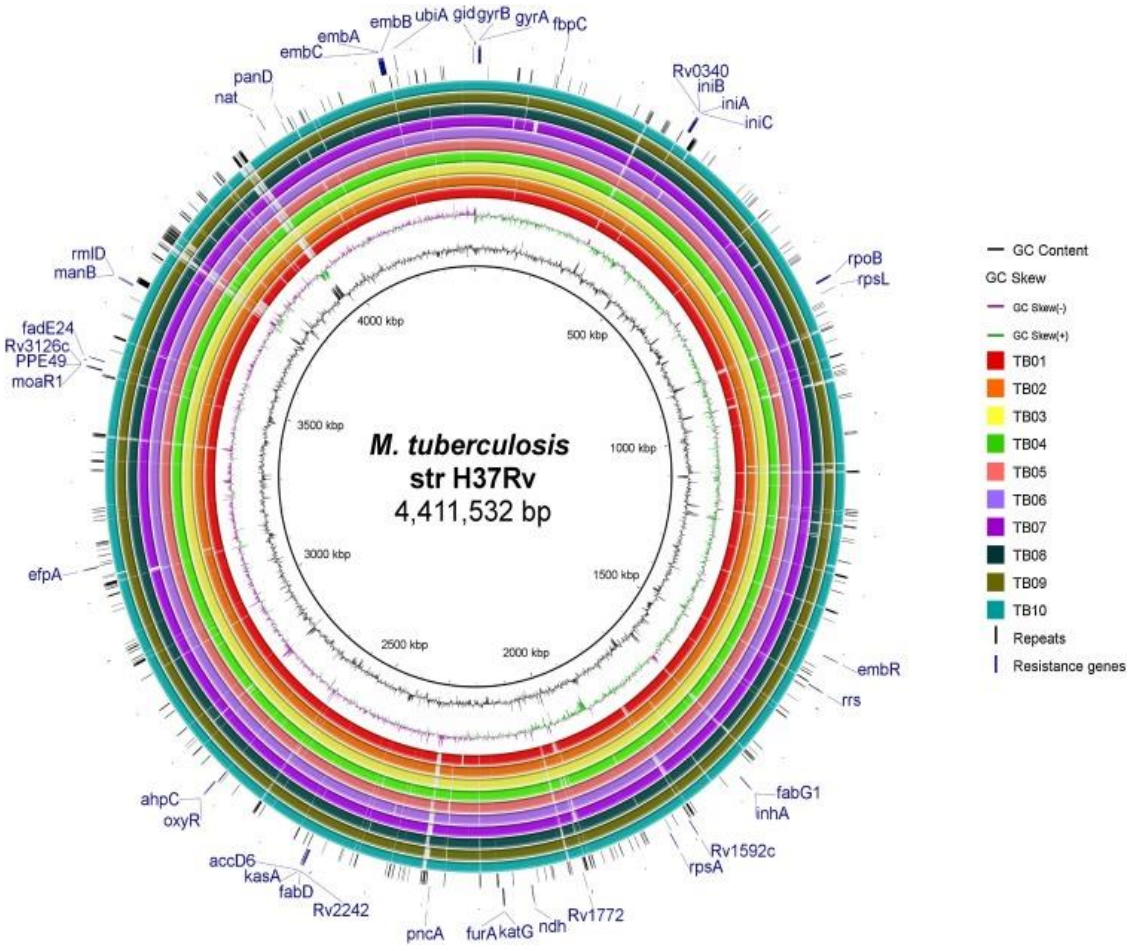


Acid fast mycobacterial cell wall[14]

GENOME:

Mycobacterium tuberculosis has circular chromosomes containing 4,200,000 nucleotides long. The G+C content of 65% .The genome of M. tuberculosis was studied using the strain M. tuberculosis H37Rv. The genome contains about 4000 genes. Genes that code for lipid metabolism are a very important part of the bacterial genome. Eight percent of the genome is involved in this activity. The different species of the M. tuberculosis complex show a 95-100% DNA relatedness based on studies of DNA homology. The sequence of the 16S rRNA gene are exactly the same for all species.

Plasmids in M. tuberculosis are important in transferring virulence because genes on the plasmids are more easily transferred than genes located on the chromosome. One such 18kb plasmid in the M.tuberculosis H37Rv strain was proven to conduct gene transfers[15].



Genome of Mycobacteriumm *tuberculosis*[16].

New agents should ideally have the following attributes:

- A novel mechanism of action to attenuate cross resistance.
- Rapid bactericidal activity to reduce duration of therapy.
- Optimised pharmacokinetic, pharmacodynamic properties for once daily oral administration.
- Low potential for drug drug interaction to allow combination therapy especially with other TB drugs and current anti HIV therapeutics
- Excellent safety profile to allow for use in children and pregnant women.

BIOLOGICAL TARGET

Glutamine synthetase I is an enzyme that plays an essential role in the metabolism of nitrogen by catalysing the condensation of glutamate and ammonia to form glutamine. Glutamine synthetase (GS; EC 6.3.1.2) has three metal ions in the active site between the two pockets, which are necessary for stability and catalytic activity. Glutamine synthetase I also known as γ-glutamyl: ammonia Ligase[18].

Extracellular mycobacterial glutamine synthetase may also affect pH modulation in phagosomes and consequently prevent phagosome lysosome fusion. Numerous studies indicate that inhibition of mycobacterial glutamine synthetase is feasible therapeutic strategy.

ENZYME PROFILE:

PROTEIN NAME : Glutamine synthetase 1

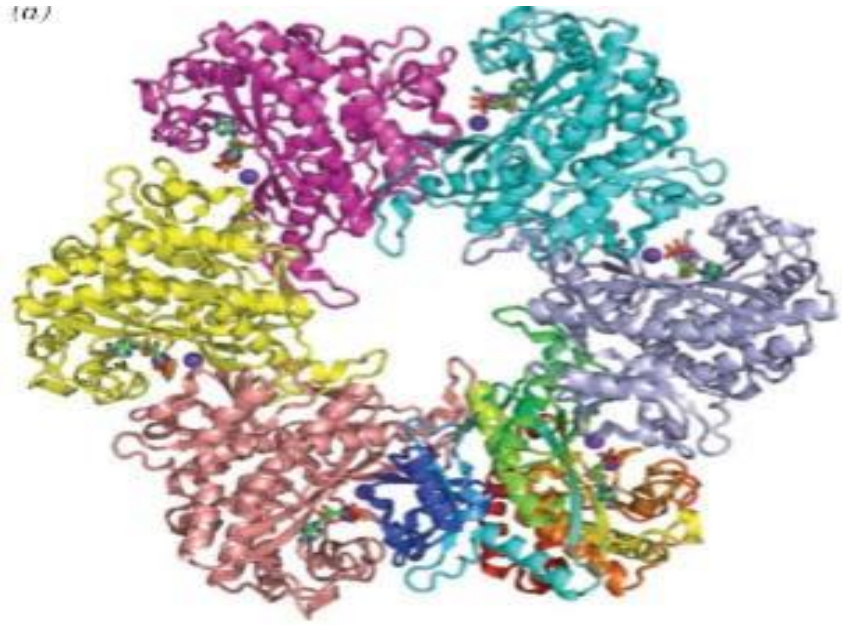
CLASSIFICATION : Ligase

CHAINS : A, B, C, D, E, F

TOTAL STRUCTURE WEIGHT : 332264.16

GENE NAME : glnA1 glnA Rv2220 MTCY190.31MTCY427.01

FUNCTION : catalyzes the ATP dependent condensation between glutamate and ammonia, to give glutamine.



Mycobacterial Glutamine synthetase I (GS 1)[17]

MECHANISM:

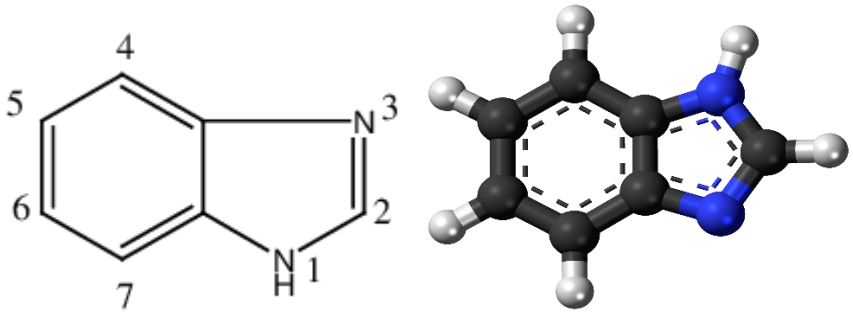
Glutamine synthase (GS) catalyzes the ATP dependent condensation of glutamate with ammonia to yield glutamine. This mechanism takes place in two steps:

The first step is the formation of the activated intermediate γ-glutamyl phosphate. The Mg2+ ion coordinates the γ-phosphate oxygen of ATP to allow phosphoryl transfer to the γcarboxylate group of glutamate, yielding the intermediate (acyl phosphate). ADP and Pi do not dissociate until ammonia binds and glutamine is released. The presence of ADP causes a conformational shift in GS that stabilizes the γ-glutamyl phosphate moiety[25].

This is followed by a second step deprotonation of ammonium, which allows ammonia to attack the intermediate from its nearby site to form glutamine. The inhibition of GS secreted by M. tuberculosis is sufficient to halt the growth of the bacterium, suggesting that TB-GS might be a valid target for anti-tuberculosis drug-design. The structure of TB-GS is currently being solved to aid in the design of novel inhibitors for this enzyme[19]. The development of new classes of anti-tuberculosis drugs and new drug targets is of global importance, since attacking the bacterium using multiple strategies provides the best means to prevent resistance.

BASIC NUCLEUS

Benzimidazole is a fused heterocycle with 2 nitrogens in 1,3 positions and molecular formula $C_7H_6N_2$. This bicyclic compound consists of the fusion of benzene and imidazole. In benzimidazole imino nitrogen is assigned position 1, tertiary nitrogen is assigned position 3.



Fusion of benzene ring to imidazole increases the possibilities of electron delocalization.The basicity is lowered and the activity is very slightly increased. Ring system is stable towards heat and towards oxidizing agent. Benzimidazole resists hydrogenation. Benzimidazole is more susceptible to nucleophilic attack due to electron accepting properties of fused carbocyclic ring system. Alkylation affords 1-alkyl benzimidazole and 1,3 dialkyl benzimidazolium salts.no diene like properties are shown by fused ring systems[31-33].

Derivatives of Benzimidazole have various biological activity like

- Anti bacterial
- Anti viral including Anti HIV activity
- Anti helminthics
- Anti fungal
- CNS depressant
- Anti emetic
- Anti tumour

LITERATURE REVIEW

LITERATURES RELATED TO TUBERCULOSIS

1. **Chaw, Liling et al., (2020)**[21] assessed the Global TB burden and the focus of majority of Countries on the Latent tuberculosis infection by performing a literature survey.
2. **Canetti et al., (2020)**[22] briefly outlined the complication of Anti-TB treatment in the HIV co-infected patients. The new drug regimen for the co-treatment was clearly explained.
3. **Migliori, G. Battista et al., (2020)**[23] evaluated the risks associated with the treatment of Drug-resistant tuberculosis and their clinical management.

LITERATURES REGARDING TARGET GLUTAMINE SYNTHETASE I

4. David Eisenberg et al.,(2000)[24] reported the structural and functional relationships of Glutamine synthetases1.
5. Wojciech W.krajewski et al.,(2005)[25] Summarised the glutamine synthetase catalysts the ligation of glutamate and ammonia to form glutamine with the hydrolysis of ATP. The enzymes are central component of bacterial nitrogen metabolism and a separate potential drug target.
6. Marcus A.Hortwitz et al., (2003)[26] investigated a novel antibiotic strategy targeting the glutamine synthetse enzyme of mycobacterium tuberculosis. The feasibility of inhibiting mycobacterial tuberculosis GS I enzyme plays a vital role in both cell wall synthesis and nitrogen metabolism.

LITERATURES REGARDING BENZIMIDAZOLE:

7. Rohit verma et al., (2021)[27] reported the synthesis , characterization of 2- substituted benzimidazole derivatives and evaluated their anti-bacterial potency.
8. Araujo D.M.L et al.,(2018)[28] reported synthesis of a series of (Z)-3-((1H- benzo imidazol-2-yl) methyl) substituted thiazolidine 2,4 Dione and evaluated their anti tubercular activity.
9. Jerad Suresh et al.,(2016)[29] reported the microwave assisted synthesis of novel imidazole and benzimidazole derivatives. The synthesized molecules were effective in inhibiting enzyme mycolic acid synthase 2.

10. Fatmah A.S alasmary et Al.,(2015)[30] reported synthesis and evaluation of selected Benzimidazole derivatives with substitutents at position 1,2 and 5 as potent anti microbial agents.
11. Chavan BB et Al.,(2012)[31] reported synthesis and biological evaluation of novel Benzimidazole derivatives (2,3-dihydro-2-[2 acetoxy] phenyl) 1H benzo (d) imidazole with aspirin as potent anti microbial and anti fungal agents.
12. Sundari and co workers (2004)[32] synthesized 3,5 diaryl 4-(2- ethoxy benzimidazol-2-yl) tetra hydro 1,4 thiazin 1,1 dioxide derivatives studied their antimicrobial and anti fungal and anti histamine activity.
13. Khan et al., (2003)[33] synthezied 2-(4-Aryl-2-thiazolyl amino) benzimidazole and studied their anti bacterial and antifungal activity.

LITERATURES REGARDING CHALCONES AS ANTI-TUBERCULAR AGENTS

14. Ashok babu kasetti et al.,(2021)[34] reported the synthesis , characterization and evaluated thiazole chalcones for antitubercular and antiproliferative activities. Chalcones containing 2,4-difluorophenyl and 2,4-dichlorophenyl groups, showed potential antitubercular activity higher than the standard pyrazinamide.
15. Rambabu anandham et al., (2018)[35] reported the synthesis , characterization and evaluated C-dimethylated chalcones for anti-tubercular agents. Synthesized compounds had found more potent than standard streptomycin and ciprofloxacin.
16. Vibhute YB et al.,(2003)[36] synthesized the 2-Hydroxy chalcones and reported their anti-bacterial activity.

LITERATURES REGARDING THE COMPUTER AIDED DRUG DESIGN:

17. Fernando, D.Prielo Martinez (2019)[37] outlined the importance of the computational drug design methods in the Drug Discovery process.
18. Hachem, El.Nehme et al., (2017)[38] visualized Docking as an essential computation tool for the Structure based drug design.
19. Forli Stefano (2016)[39] elaborated on the Docking and virtual screening methods provided by the AutoDock Suite. The procedure involved in docking the compounds against the protein target was elucidated
20. Tien, Sheng et al., (2015)[40] established the importance of drug likeness of a molecule and the scope of predicting ADMET properties.

21. Lipinski et Al (2001)[41] estimate solubility and permeability in drug discovery and development settings.
22. Leonardo G ferreira et al (2015)[42] discussed the strategies by which molecular docking and structure based drug design. The approach would lead to the identification of novel bioactive compounds.

LITERATURES EXPLAINING THE MICROPLATE ALAMAR BLUE ASSAY (MABA) & ACUTE TOXICITY STUDIES:

23. Cho, Sanghyun (2015)[43] briefly explained the procedure involved in determining the Drug susceptibility by MABA.
24. Jose de Jesus alba Romero et Al[44] ., applied the Alamar Blue assay to determine the susceptibility to anti tuberculosis pharmaceuticals.
25. JonssonJonsson, martina et al., (2013)[45] determined the acute toxicity of the mycotoxin by following the OECD Guidelines 423 [Acute Oral Toxic Method].

AIM AND OBJECTIVE

AIM:

The aim of this project is to design molecules with potent anti-tubercular activity.

OBJECTIVE:

The aim will be achieved by synthesis of some novel molecules containing benzimidazole as a basic nucleus which will possess antimycobacterial activity, capable of inhibiting cell wall synthesis by inhibiting Glutamine synthetase 1.

THE PRESENT STUDY INCLUDES THE FOLLOWING

- Sketching of Benzimidazole derivatives using chemsketch.
- In-silico Drug likeness prediction.
- In-silico Toxicity Assessment.
- Design of Glutamine synthetase 1 inhibitors by docking studies using Autodock (1.5.6 version) software.
- Laboratory synthesis of those compounds with top Docking Scores.
- Characterization of the synthesized compounds by
    - ✓ Infrared Spectroscopy.
    - ✓ H1 NMR Spectroscopy.
    - ✓ Melting point.
    - ✓ LC-MS
- Determination of In-vitro anti -tubercular activity of the synthesized compounds by
- Microplate Alamar Blue Assay (MABA).
- Acute Toxicity on Albino wister rats.

PLAN OF THE WORK

Based on the Literature review, Glutamine synthetase-I was selected as target

Designing of novel Benzimidazole derivatives

Toxicity Prediction by OSIRIS property Explorer

In silico assessment of Drug likeness by Molinspiration

Docking against the target using AutoDock tools

Laboratory Synthesis of molecules

Characterization of the synthesized compounds by TLC, Melting point, IR,

NMR & LC-MS.

In vitro Anti-tubercular activity determination by MABA.

Determination of Acute oral toxicity

Patenting the Highly effective novel Anti-tubercular molecules.

MATERIALS AND METHODS

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

- Sketching of Benzimidazole derivatives using chemsketch.
- In-silico Drug likeness prediction.
- In-silico Toxicity Assessment.
- Docking studies by using AutoDock tool.
- Synthesis of the designed molecules.
- Characterization of the synthesized molecules.
- Biological evaluation of the synthesized molecules

IN-SILICO SCREENING OF DRUG LIKENESS

MOLINSPIRATION[48]

The designed and docked molecules are screened in silico using MOLINSPIRATION Chemo informatics software to evaluate drug likeness. It is the online software available for calculation of important molecular properties log P,total polar surface area, number of hydrogen bond donors and acceptors and prediction of bioavailability score for the most important drug targets (GPCR ligands, Kinase inhibitors, ion channel modulators, nuclear receptors).

LIPINSKI'S RULE[46]

Lipinski's rule of five is a rule of thumb to evaluate drug likeness, ie., to determine if a chemical compound with a certain pharmacological or biological activity has the properties that would make it a likely orally active drug in humans. Christopher A. Lipinski formulated the rule in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules.

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. active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule. The modification of the molecular structure often leads to drugs with higher molecular weight, more rings, more rotatable bonds, and a higher lipophilicity.

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

- ✓ Partition coefficient of log P less than 5
- ✓ Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- ✓ Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- ✓ Molecular weight under 500 daltons
- ✓ An additional rule was proposed by Veber: Not more 10 rotatable bonds.

IN-SILICO TOXICITY PREDICTION

OSIRIS

In silico toxicity prediction for the molecules is done using OSIRIS Property Explorer a JAVA based online tool[49].

- The tool predicts toxicity related parameters like mutagenicity, tumerogenicity, skin irritation and reproductive effects.
- The prediction is based on the fragment contribution group present in the structure of the molecule.
- Properties with high risks of undesired effects like mutagenicity indicates red, whereas a green color indicates drug-conform behavior.

DRUG DESIGN:

Rational drug design is the process of finding new medications based on theknowledge of a biological target. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it[56].

LEAD IDENTIFICATION/OPTIMIZATION :

Lead identification/optimization is the one of the most important steps in drug development. The iterative process of inventing new chemical structures to identify an improved drug lead with the goal of progressing the compound as a preclinical candidate.

The chemical structure of the lead compound is used as a starting point for chemical modifications to improve potency, selectivity and pharmacokinetic parameters[50-51].

COMPUTER AIDED DRUG DESIGN[52] :

The most important goal in drug design is to predict how strongly the given molecule will interact with a target. Molecular mechanics or molecular dynamics is most often used to estimate the strength of the intermolecular interaction between the small molecule and its biological target. Drug design with the help of computers may be used at any of the following stages of drug discovery:

1. Hit identification using virtual screening (structure- or ligand-based design).

2. Hit-to-Lead optimization of affinity and selectivity (structure-based design, QSAR, etc.).

3. Lead optimization of other pharmaceutical properties while maintaining affinity. In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and compound 3D structure information are used for analysis.

TYPES OF DRUG DESIGN:

There are two major types of drug design.

- ligand-based drug design
- structure-based drug design

LIGAND-BASED

Ligand-based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.

STRUCTURE-BASED

Structure-based drug design (or direct drug design) relies on knowledge of the three-dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not

available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.

TARGET ENZYME: GLUTAMINE SYNTHETASE 1

The crystal structure of the enzyme is downloaded from the Protein Data Bank (An Information Portal to Biological Macromolecular Structure) (**PDB id- 4acf**). The target enzyme Glutamine synthetase 1 from Mycobacterium tuberculosis, is one of the key enzymes involved in glutamine synthesis, which is critical for the survival and growth of Mycobacterium tuberculosis. This target enzyme was selected from the In-silico target identification pipeline for Mycobacterium tuberculosis[53].

CRITERIA TO SELECT THE BEST PROTEIN STRUCTURE FROM PDB [55]

1. Resolution must be minimum possible (This will ensure the better quality of protein structure).

2. Domain completeness. The PDB structure is examined and confirmed that the under-study domain's full structure is available. Partial domain will lead to false interpretations.

3. Variant /Mutations. We need to look for whether the structure is a wild type or a mutant/variant. In case of a mutant structure requirement we may have to introduce required mutations manually and model them.

4. Side Chain Completeness (is of secondary importance). Structures determined through old techniques might have (Not always) missing side chains due to flaw in tech or manual error. Right 3D confirmation of side chains is critical in small ligand binding thus ensuring their completeness is important. As a possible solution we may look for latest structure availability of the same.

5. To get out the right docking result removal (As per case study) of ligand / crystalline water/ co factor elements from PDB file is important. According to the above criteria the Glutamine synthetase I enzyme 4acf in pdb format was downloaded which has a X-ray resolution of 2.00A^{o} and the structure was detected by X-ray Diffraction method.

DOCKING[57-58]

Docking program involves the prediction of variety of positions, conformations and orientations of ligand ie. small molecule within a target binding sites. Docking mode is known as pose. Docking methods fit a ligand into a binding site of biomolecular target by combining and optimizing variables like steric, hydrophobic and electrostatic complementarity and also estimating the free energy of binding (scoring)

MOLECULAR DOCKING BY AUTODOCK

Molecular Docking is an effective and competent tool for in silico screening. AutoDock 4.2.5.1 is a computational procedure for predicting the interaction of ligands with target. The precise interaction of such agents or candidate molecules with their targets is important for the drug discovery process.

- ✓ Protein preparation
- ✓ Ligand preparation
- ✓ Receptor grid generation
- ✓ Ligand docking (screening).

PROTEIN PREPARATION

Read molecule from the file (allows reading of PDB coordinate files.)

Edit -Charges – Compute Gasteiger (for arbitrary molecules)

Edit – Hydrogen –Merge non polar

Save as .pdb in AutoDock folder

LIGAND PREPARATION

Ligand –Input from file

Ligand – Torsion –choose torsion: Rotatable bonds are shown in green, and non-rotatable bonds are shown in red.

Ligand – Torsion –set number of torsion: sets the number of rotatable bonds in the ligand by leaving the specified number of bonds as rotatable.

Ligand – Output – save as .pdbqt in AutoDock folder.

GRID PREPARATION

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

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

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

File – Close saving current

Grid – Output – save as .gpf (grid parameter file.

DOCKING

Docking –Open the macromolecules – set rigid file name.

Docking – ligand – open the ligand.

Docking –search parameters – genetic algorithm parameters : this command opens a panel for setting the parameters used by each of the search algorithms, such as temperature schedules in simulated annealing and mutation/crossover rates in genetic algorithms.

Docking – docking parameters: opens a panel for setting the parameters used during the docking calculation, including options for the random number generator, options for the force field, step sizes taken when generating new conformations, and output options.

Docking- output –Lamarkian GA –save as .dpf (docking parameter file)

Open command prompt

[autogrid4.exe –p grid.gpf –l grid.glg]

[autodock4.exe –p dock.dpf –l dock.dlg]

VISUALIZATION

Analysis –Docking – open .dlg (docking log file) file

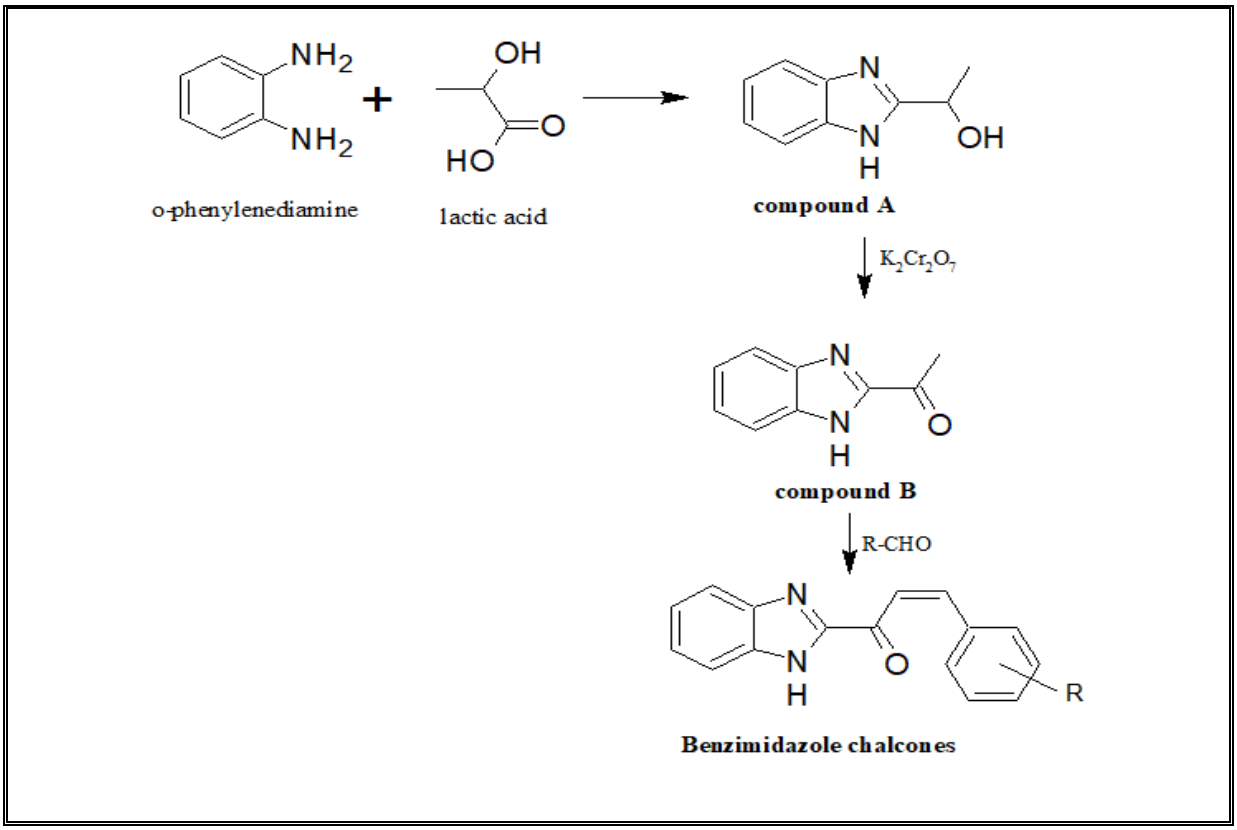
Analysis – open the macromolecule

Analysis – Confirmation –Play and Play ranked by energy: Play- will use the order of conformations as they were found in the docking calculations, and Play Ranked By Energy will order the conformations from lowest energy to highestenergy.

Analysis – Load : Information on the predicted interaction energy is shown at the top and the individual conformations

Analysis – Docking – show interaction: specialized visualization to highlight interactions between the docked conformation of the ligand and the receptor.

SYNTHETIC SCHEME



SYNTHETIC METHODOLOGY

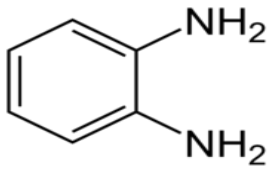
Step 1: Ortho phenylenediamine (0.25 mol) with lactic acid (0.36 mol) is refluxed in RBF for 2.30 hrs. The reaction mixture is cooled to room temperature and made just alkaline with 10% sodium hydroxide. The crude pink product was filtered and added to the 400 ml boiling water. 2g decolourising charcoal was added to it and heated for 5-7 mins. The mixture was filtered rapidly and washed with water to obtain the white crystals of compound A.

Step 2: To compound A (8.1 gm, 50mM) in dil H2SO4 (5%, 40 ml) was added at RT a solution of K2Cr2O7 (9.8 gms, 50mM) in water (60 ml) and conc. H2SO4 (20 ml) in a dropwise fashion, over a period of 20 mins. The reaction mixture was stirred for 2 hrs. The solid was filtered and washed with water. The precipitate was resuspended in water (50 ml) and treated very carefully with aq. NH3 to a pH of 6.0 – 6.5 and filtered. The residue was washed with water and recrystallized with ethyl acetate.

Step 3: Compound B (0.01mol) and aromatic aldehyde (0.01mol) was dissolved in ethanol (20ml) and 10% sodium hydroxide was added. The reaction mixture was stired at room temperature for 6-7 hrs.The completion of reaction was monitored by TLC. The mixture was poured into crushed ice and acidified with concentrated hydrochloric acid. The solid mass obtained was filtered an recrystallized with ethanol.

REACTANT PROFILE

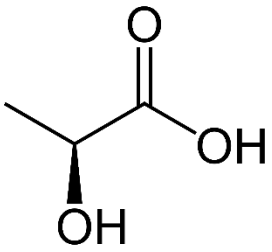
O-PHENYLENE DIAMINE



O-Phenylene diamine

MOLECULAR FORMULA	$C_6H_8N_2$
MOLECULAR WEIGHT	108.1 $g.mol^{-1}$
MELTING POINT	102 ^{o}-104^{o} C
DESCRIPTION	Pale brownish yellow crystals

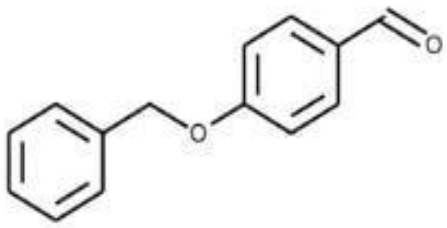
LACTIC ACID



Lactic acid

MOLECULAR FORMULA	$C_3H_6O_3$
MOLECULAR WEIGHT	90.078 $g.mol^{-1}$
BOILING POINT	122 ^{o} C
DESCRIPTION	clear colourless liquid

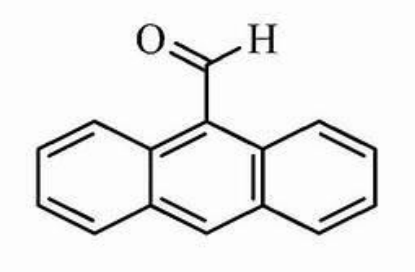
4-BENZYLOXY BENZALDEHYDE



4-Benzyloxy benzaldehyde

MOLECULAR FORMULA	$C_{14}H_{12}O_2$
MOLECULAR WEIGHT	212.24 $g.mol^{-1}$
MELTING POINT	71 ^{o} C
DESCRIPTION	Pale white powder

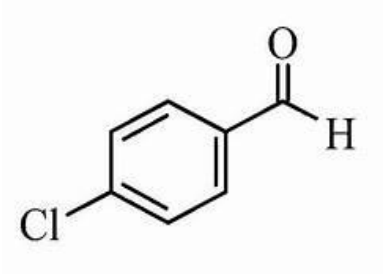
9-ANTHRALDEHYDE



9-Anthraldehyde

MOLECULAR FORMULA	$C_{15}H_{10}O$
MOLECULAR WEIGHT	206.24 $g.mol^{-1}$
MELTING POINT	$104^{o} – 105^{o}$ C
DESSCRIPTION	Yellow powder

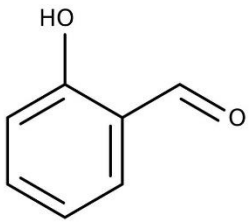
PARA-CHLORO BENZALDEHYDE



4-Chloro benzaldehyde

MOLECULAR FORMULA	C_7H_5OCl
MOLECULAR WEIGHT	140.567$g.mol^{-1}$
MELTING POINT	47^{o}C
DESCRIPTION	Colourless crystals

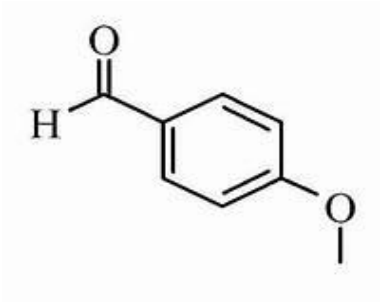
SALICYLALDEHYDE



Salicylaldehyde

SYNONYM	2-Hydroxy benzaldeyde
MOLECULAR FORMULA	$C_7H_6O_2$
MOLECULAR WEIGHT	122.12 $g.mol^{-1}$
BOILING POINT	196^o C
DENSITY	$1.17g/cm^3$
DESCRIPTION	Colourless oily liquid

p-ANISALDEHYDE



4-Anisaldehyde

SYNONYM	4-Methoxy benzaldehyde
MOLECULAR FORMULA	$C_8H_8O_2$
MOLECULAR WEIGHT	136.15 $g.mol^{-1}$
BOILING POINT	248^o C
DENSITY	$1.12\ g/cm^3$
DESCRIPTION	Pale yellow oily liquid

CHARACTERIZATION

PHYSICAL EVALUATION:

The physical properties synthesized compounds are evaluated as follows

- ✓ Colour
- ✓ Nature
- ✓ Solubility
- ✓ Melting point

IR SPECTROSCOPY

IR spectroscopy deals with the infrared region of the electromagnetic spectrum, i.e. light having a longer wavelength and a lower frequency than visible light. Infrared Spectroscopy generally refers to the analysis of the interaction of a molecule with infrared light. The major use of infrared spectroscopy is to determine the functional groups of molecules, relevant to both organic and inorganic chemistry.

REGIONS OF THE INFRARED SPECTRUM

Most of the bands that indicate functional group are found in the region from 4000 cm^{-1} to 1300 cm^{-1} . Their bands can be identified and used to determine the functional group of an unknown compound. Bands that are unique to each molecule, similar to a fingerprint, are found in the fingerprint region, from 1300 cm^{-1} to 400 cm^{-1} .These bands are only used to compare the spectra of one compound with another.

NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

Nuclear Magnetic Resonance (NMR) spectroscopy is a technique used to determine the content and purity of a sample as well as its molecular structure. NMR is the most powerful technique used to obtain structural information about a compound. 1H NMR and 13C NMR spectroscopies are used for analyses of all organic compounds.

MASS SPECTROMETRY

Mass spectrometry is an analytical technique used to establish the molecular structure and the molecular weight of the analyte under investigation. In this technique, the compound under investigation is bombarded with a beam of electrons producing ionic fragments of the original molecule. The relative abundance of the fragment ion formed depends on the stability of the ion and of the lost radical. The resulting charged particles are then separated according to their masses. Mass spectrum is a record of information regarding various masses produced and their relative abundances.

HYPHENATED TECHNIQUES

A technique where a separation technique is coupled with an online spectroscopic detection technology is known as hyphenated technique. Chromatography produces pure or nearly pure fractions of chemical components in a mixture. Spectroscopy produces selective information for identification using standards or library spectra. These hyphenated techniques offer shorter analysis time, higher degree of automation, higher sample throughput, better reproducibility, reduction of contamination because it is a closed system.

Various hyphenated techniques are

- GC-MS
- LC-MS
- LC-FTIR
- LC-NMR
- CE-MS

LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (LC-MS)

LC-MS is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography - MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity. This tandem technique can be used to analyze biochemical, organic, and

inorganic compounds commonly found in complex samples of environmental and biological origin.

BIOLOGICAL EVALUATION

MICROPLATE ALAMAR BLUE ASSAY (MABA)

The anti-microbial activities of the synthesized compounds are determined by MABA method. The organism used in the studies is Mycobacteria tuberculosis (Vaccine strain, H37 RV strain): ATCC No- 27294[59-61].

PROCEDURE:

1. The antimycobacterial activity of compounds were assessed against M. Tuberculosis using microplate Alamar Blue assay (MABA).

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

3. 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

4. The 96 wells plate received 100µl of the Middle brook 7H9 broth and serial dilution of compounds were placed directly on plate.

5. The final drug concentrations tested was made up to 100 to 0.8µg/ml and thereafter extended beyond 0.8 µg/ml.

6. Plates were covered and sealed with Para film and incubated at 37°C for five days.

7. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to the plate and incubated for 24hrs.

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

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

TOXICOLOGICAL EVALUATION

ACUTE TOXICITY[63-64]

Animal : Either sex of Albino wistar rats

Acute toxicity studies are designed to determine the dose that will produce either mortality or serious toxicological effects when given once or as multiple administrations.The method is used to evaluate the acute oral toxicity (OECD Guidelines423).

PROCEDURE:

1. The oral toxicity of the synthesized compounds was performed by acute toxic class method.
2. Based on the activity of the synthesized compounds, animals were grouped (3 animals for each compound).
3. As per OECD guidelines, the dosage is selected (2000mg/kg).
4. The mice were fasted overnight prior to dosing.
5. Following the period of fasting the animals were weighed and the synthesized compounds were administered orally at the dose of 2000 mg/kg body weight.
6. Animals were observed individually after dosing atleast during the 1st 4 hrs and daily thereafter, for a total of 14 days.
7. If no mortality or toxic signs are noted then synthesized compounds is considered to be safe (non-toxic).

RESULT AND DISCUSSION

TOXICITY STUDIES

Toxicity prediction was carried out using the online software OSIRIS® property Explorer which predicts carcinogenicity, mutagenicity, teratogenicity, irritation, etc. The In-silico toxicity assessment of the selected five molecules are summarized below.

Based on the colour code,

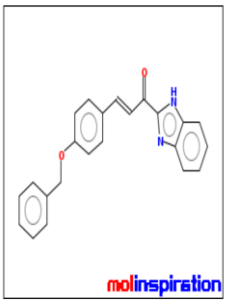
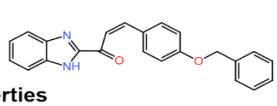
GREEN - NON TOXIC

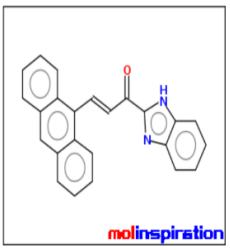
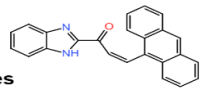
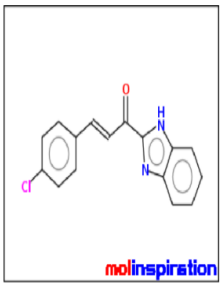
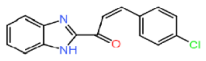
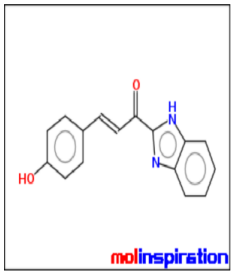
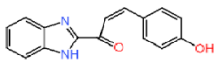
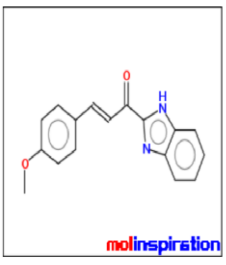
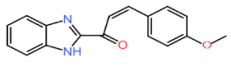
RED - UNDESIRED EFFECT

PREDICTION OF DRUG LIKENESS:

The designed molecules were screened In-silico using Molinspiration® cheminformatics to evaluate drug likeness. All the five compounds were found to comply with LIPINSKI's rule of five.

Table 1 : DRUG LIKENESS AND TOXICITY PREDICTION

COMPOUND CODE	IN-SILICO DRUG LIKENESS PREDICTION	IN-SILICO TOXICITY PREDICTION
VS 1	molinspiration miSMILES: O=C(C=Cc2ccc(OCc1ccccc1)cc2)c4nc3ccccc3[nH]4  Molinspiration property engine miLogP 4.89 TPSA 54.99 natoms 27 MW 354.41 nON 4 nOHNH 1 nviolations 0 nrotb 6 volume 323.87	OSIRIS Property Explorer Predicted toxicity risks mutagenic tumorigenic irritant reproductive effective  Predicted properties cLogP 4.14 Solubility -4.68 Molweight 354.41 TPSA 54.98 Druglikeness -5.76 H bond acceptor 4 H bond donor 1 Nb stereocenters 0 Nb rotatable bonds 6 Drug-Score 0.40

VS2	molinspiration miSMILES: O=C(C=Cc2c1ccccc1cc3ccccc23)c5nc4ccccc4[nH]5  Molinspiration property engine miLogP 4.93 TPSA 45.75 natoms 27 MW 348.40 nON 3 nOHNH 1 nviolations 0 nrotb 3 volume 314.66	OSIRIS Property Explorer Predicted toxicity risks mutagenic tumorigenic irritant reproductive effective  Predicted properties cLogP 5.18 Solubility -6.55 Molweight 348.40 TPSA 45.75 Druglikeness 0.53 H bond acceptor 3 H bond donor 1 Nb stereocenters 0 Nb rotatable bonds 3 Drug-Score 0.12
VS3	molinspiration miSMILES: O=C(C=Cc1ccc(Cl)cc1)c3nc2ccccc2[nH]3 1-(1H-benzimidazol-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one  Molinspiration property engine miLogP 4.09 TPSA 45.75 natoms 20 MW 282.73 nON 3 nOHNH 1 nviolations 0 nrotb 3 volume 240.21	OSIRIS Property Explorer Predicted toxicity risks mutagenic tumorigenic irritant reproductive effective  Predicted properties cLogP 3.40 Solubility -4.07 Molweight 282.73 TPSA 45.75 Druglikeness 3.38 H bond acceptor 3 H bond donor 1 Nb stereocenters 0 Nb rotatable bonds 3 Drug-Score 0.87
pVS4	molinspiration miSMILES: O=C(C=Cc1ccc(O)cc1)c3nc2ccccc2[nH]3 1-(1H-Benzimidazol-2-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one  Molinspiration property engine miLogP 2.93 TPSA 65.98 natoms 20 MW 264.28 nON 4 nOHNH 2 nviolations 0 nrotb 3 volume 234.69	OSIRIS Property Explorer Predicted toxicity risks mutagenic tumorigenic irritant reproductive effective  Predicted properties cLogP 2.45 Solubility -3.04 Molweight 264.28 TPSA 65.98 Druglikeness 2.74 H bond acceptor 4 H bond donor 2 Nb stereocenters 0 Nb rotatable bonds 3 Drug-Score 0.91
VS5	molinspiration miSMILES: COc3ccc(C=CC(=O)c2nc1ccccc1[nH]2)cc3 1-(1H-Benzoimidazol-2-yl)-3-(4-methoxy-phenyl)-propenone  Molinspiration property engine miLogP 3.46 TPSA 54.99 natoms 21 MW 278.31 nON 4 nOHNH 1 nviolations 0 nrotb 4 volume 252.22	OSIRIS Property Explorer Predicted toxicity risks mutagenic tumorigenic irritant reproductive effective  Predicted properties cLogP 3.55 Solubility -3.84 Molweight 276.34 TPSA 45.75 Druglikeness 2.22 H bond acceptor 3 H bond donor 1 Nb stereocenters 0 Nb rotatable bonds 4 Drug-Score 0.83

DOCKING STUDY

Five hundred molecules were docked against the target enzyme Glutamine synthetase 1 using autodock® tool 4.2.5.1 software. Molecules were screened on the basis of gooddocking scores and good binding interaction. They are tabulated below.

DOCKING SCORES OF THE STANDARD DRUG

Pyrazinamide - 5.69 kj/mol Rifampicin - 5.45 kj/mol

Table 2 :DATA ON DOCKING STUDIES:

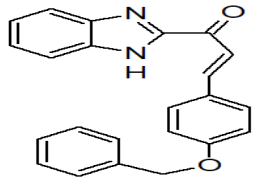
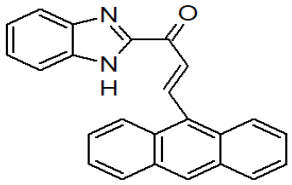
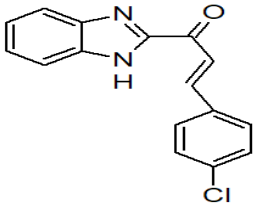
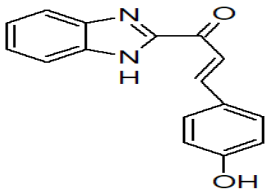
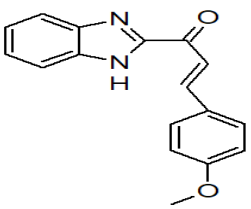
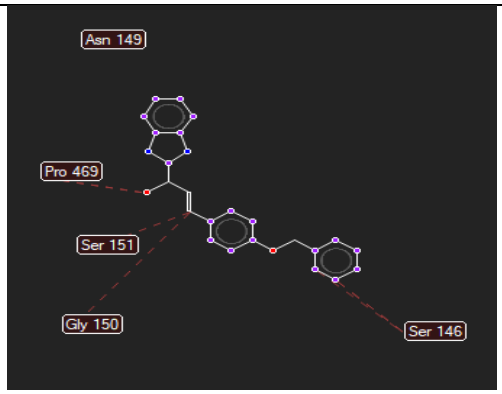
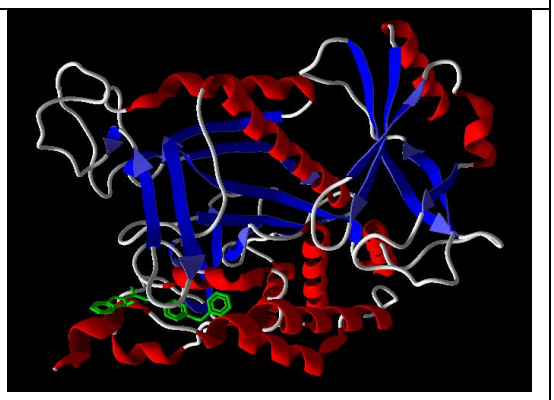
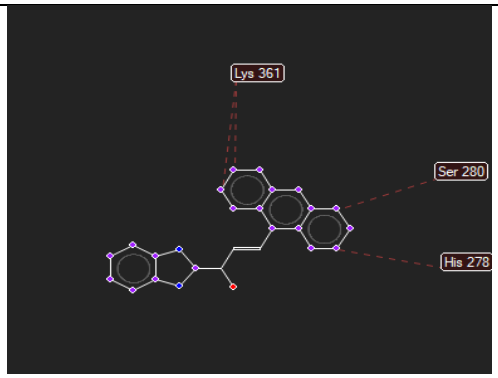
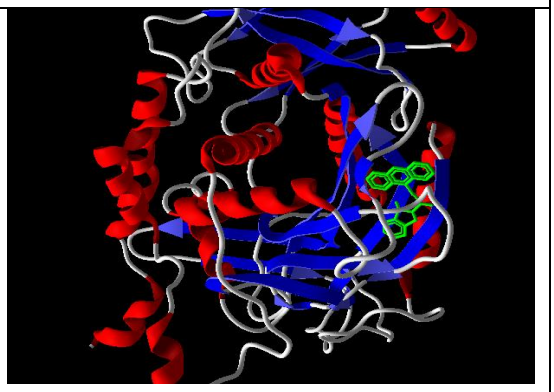
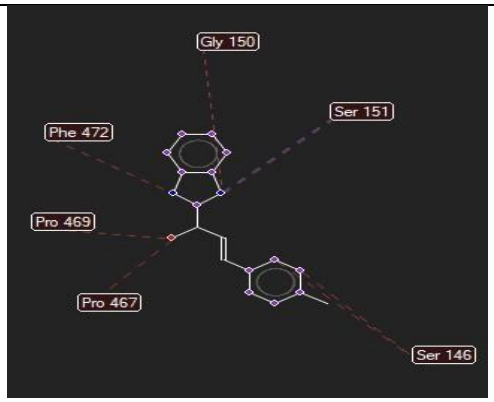
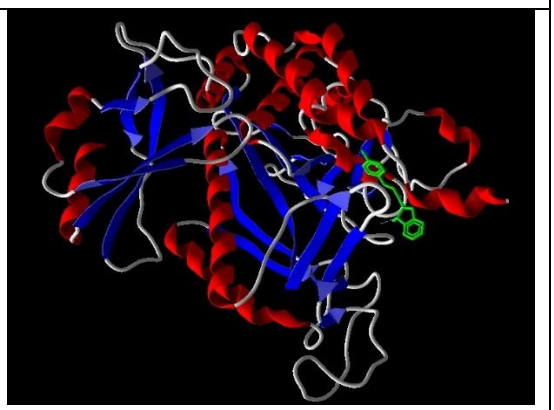
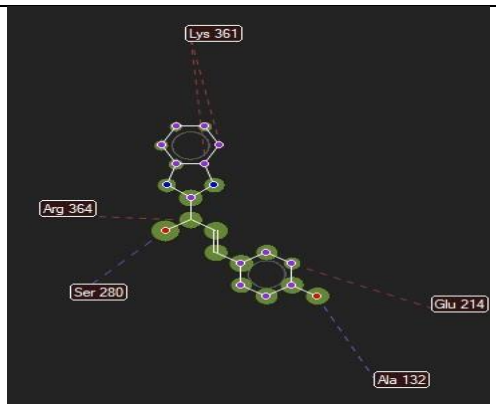
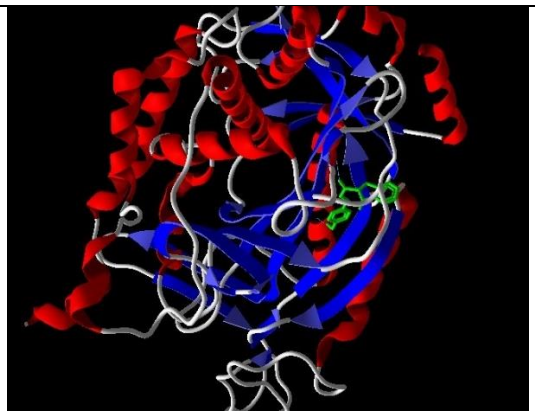
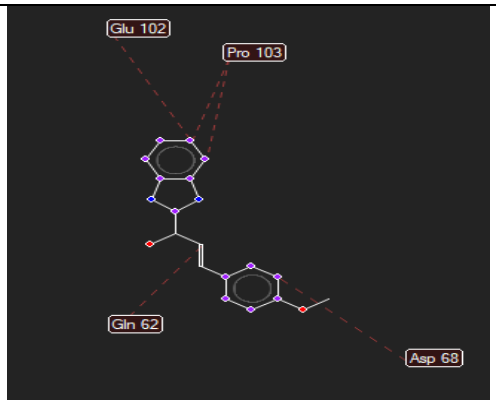
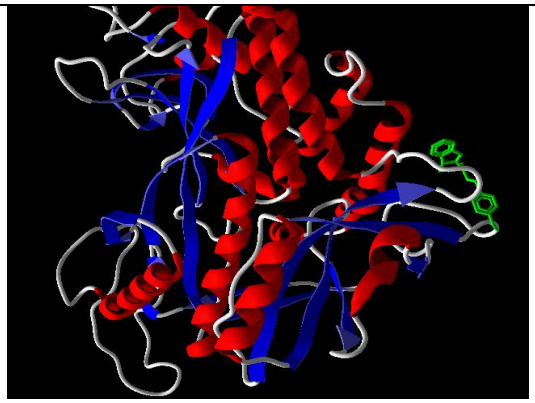
S.NO	COMPOUND CODE	STRUCTURE	DOCKING SCORE Kj/mol
1	VS 1		-7.82
2	VS 2		-8.44
3	VS 3		-8.53
4	VS 4		-7.26
5	VS 5		-5.66

Table 3:Interaction of molecules with amino acids and docking view

COMPOUND CODE	INTERACTION WITH AMINOACIDS	DOCKING VIEW
VS 1		
VS 2		
VS 3		
VS 4		

VS 5		

RESULTS OF SYNTHESIZED COMPOUNDS:

The selected molecules were synthesized with satisfactory yield. Purity of the synthesized compounds was ensured by repeated recrystallization.

Melting point was determined using digital melting point apparatus. TLC was carried out on precoated plates, with a suitable solvent system to determine purity. The Solvent system used was **Methanol : Chloroform (9:1).** Rf value of the products were found to be different from the Rf value of the reactants.

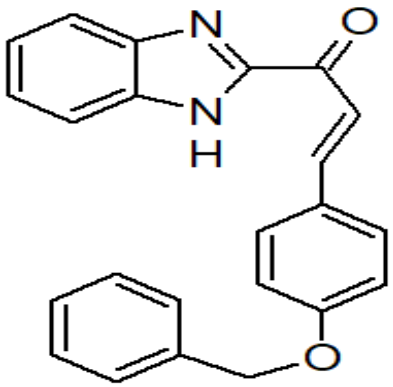
The characterization was carried out using sophisticated instruments like IR, NMR, and Mass spectroscopy.

Table 4 :DATA OF THE SYNTHESIZED COMPOUNDS

S.NO	COMPOUND CODE	COLOUR	PERCENTAGE YIELD	MELTING POINT	Rf VALUE
1	VS 1	Pale yellow	85 %	185^{o} C	0.87
2	VS 2	Orange	88%	199^{o} C	0.76
3	VS 3	Pale yellow	82%	188 ^{o}C	0.72
4	VS 4	Brown	75%	161 ^{o}C	0.52
5	VS 5	Pale yellow	85%	152 ^{o}C	0.64

PRODUCT PROFILE

COMPOUND CODE : VS 1



Molecular Formula	$C_{23}\ H_{18}\ N_2\ O_2$
Molecular Weight	354.42
Appearance	Pale yellow colour
Composition :	C(77.95%) H(5.12%) N(7.90%) O(9.03%)
Molar Refractivity	108.64 ± 0.3 cm^3
Molar Volume	280.1 ± 3.0 cm^3
Parachor	777.3 ± 4.0 cm^3
Index of Refraction	1.702 ± 0.02
Surface Tension	59.2 ± 3.0 dyne/cm
Density	1.264 ± 0.06 g/cm^3
Dielectric Constant	Not available
Polarizability	43.06 ± 0.5 $10^{-24} cm^3$

IR SPECTRUM OF SAMPLE :VS 1

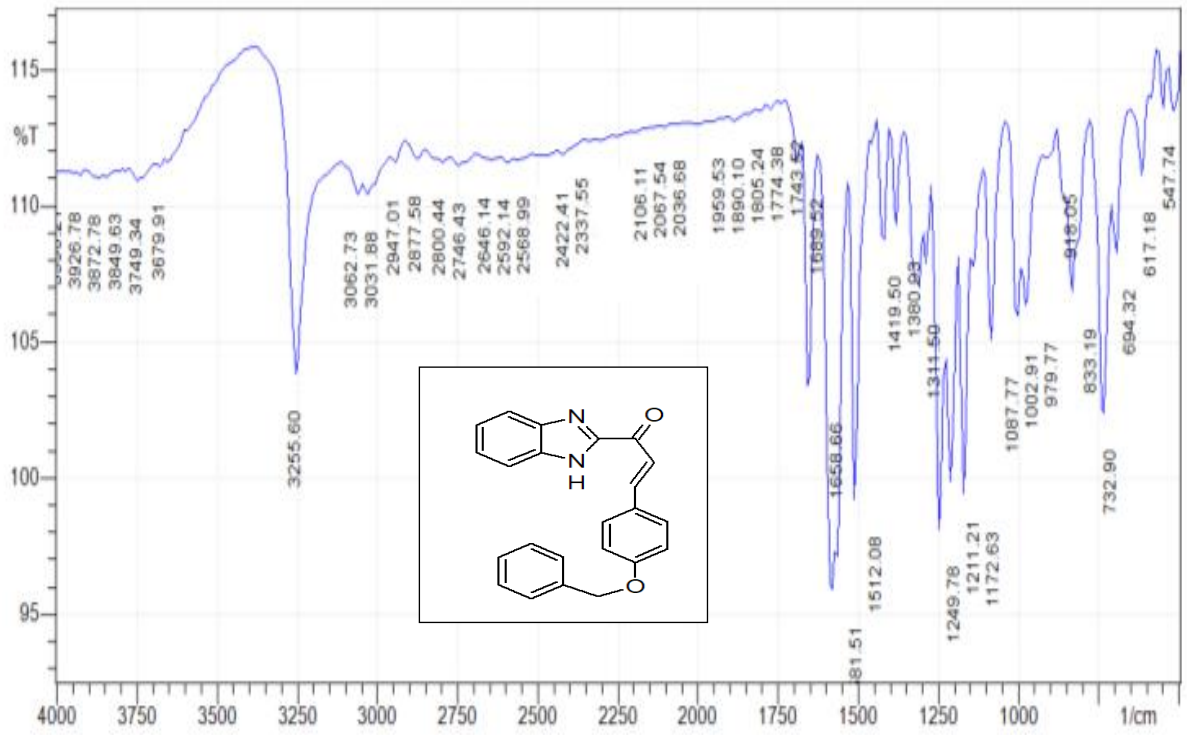


Table 5:IR INTERPRETATION OF SAMPLE VS 1

S.NO	WAVENUMBER (cm^{-1})	FUNCTIONAL GROUP
1	1658.66	C=O stretching
2	1581.51	C=C stretching
3	3062.88	Ar-C-H stretching
4	3255.60	N-H stretching

NMR OF SAMPLE VS 1

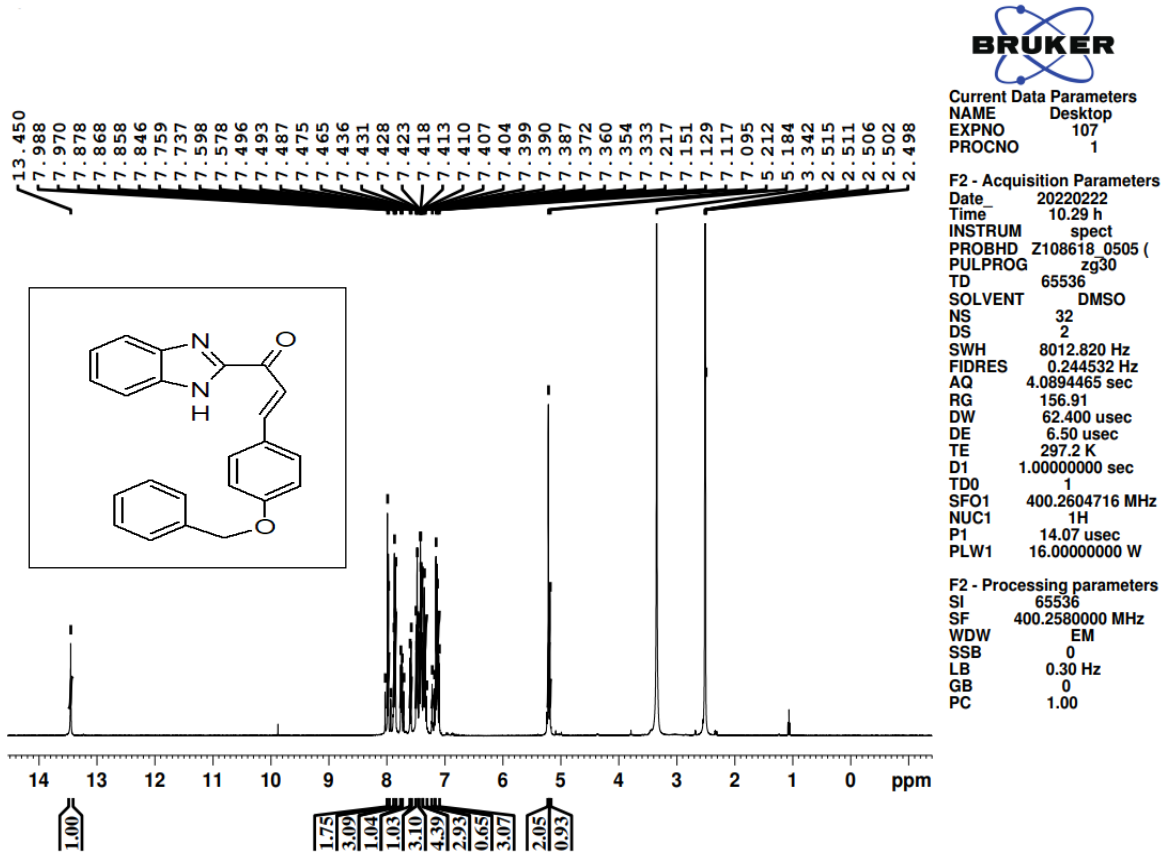
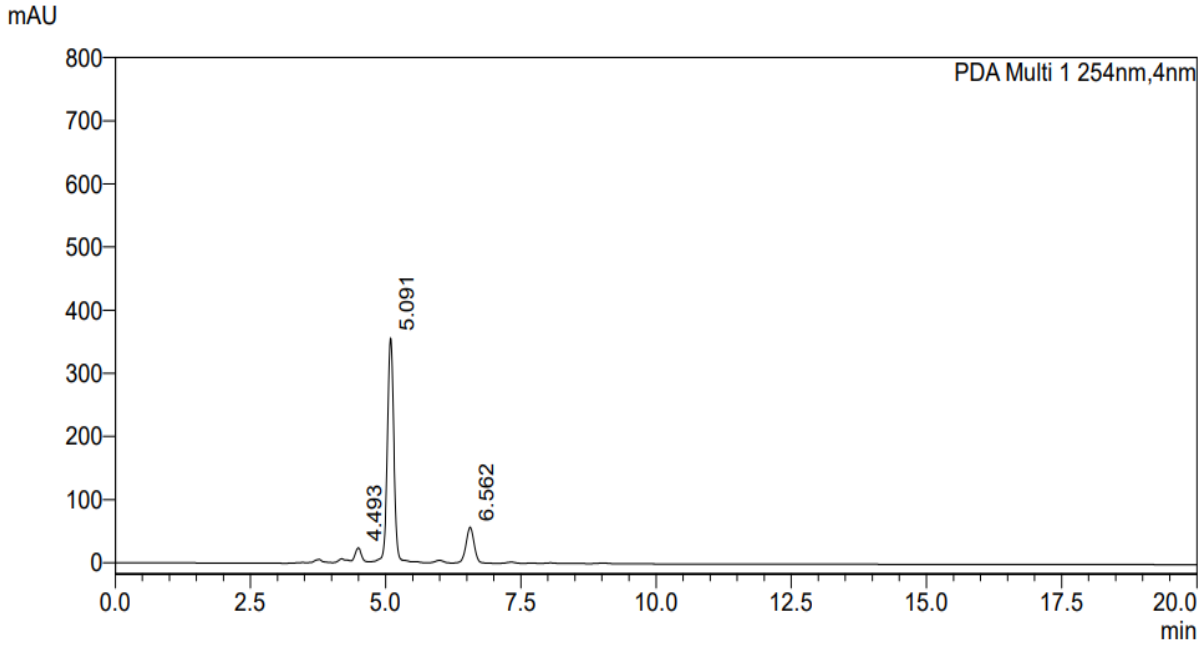


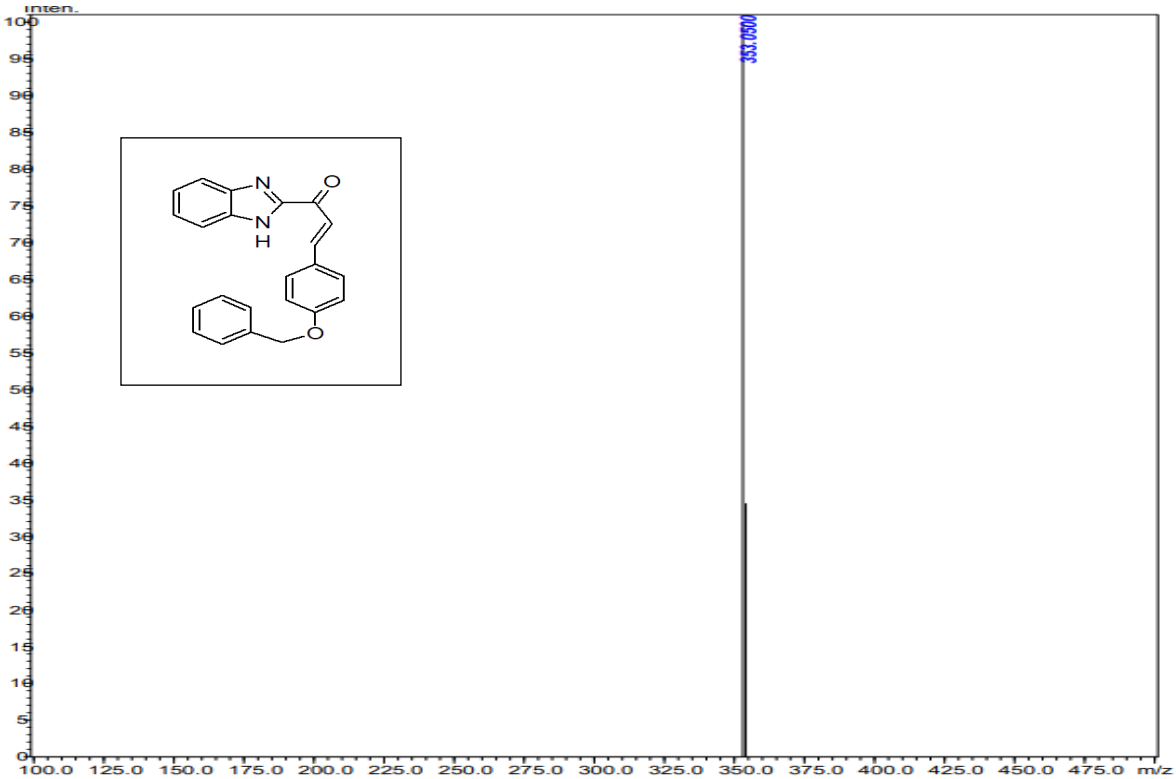
Table 6:H^1NMR INTERPRETATION OF SAMPLE VS 1

S.NO	δ VALUE	NATURE OF PROTON	NATURE OF PEAK	NO OF PROTON
1	13.5	N-H	Singlet	1 proton
2	7-7.4	Ar--H	Multiplet	3 protons
3	5-5.2	CH=CH	Doublet	2 protons
4	2.4	O-CH_2-	Singlet	2 protons

LC CHROMATOGRAM OF SAMPLE VS 1

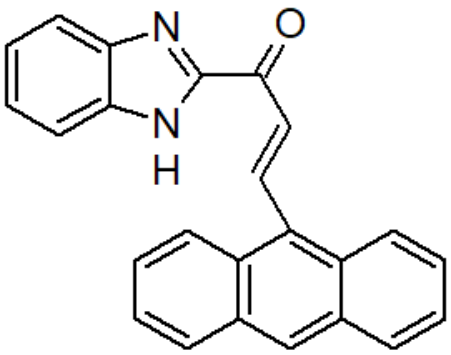


MASS SPECTRUM OF SAMPLE VS 1



MOLECULAR WEIGHT OF SAMPLE VS 1: 353.00

COMPOUND CODE : VS 2



Molecular Formula	C_{24} H_{16} N_2 O
Molecular Weight	348.40
Appearance	Orange red colour
Composition	C(82.74%) H(4.63%) N(8.04%) O(4.59%)
Molar Refractivity	113.16 ± 0.3 cm^3
Molar Volume	263.7 ± 3.0 cm^3
Parachor	756.2 ± 4.0 cm^3
Index of Refraction	1.804 ± 0.02
Surface Tension	67.6 ± 3.0 dyne/cm
Density	1.321 ± 0.06 g/cm^3
Dielectric Constant	Not available
Polarizability	144.86 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF SAMPLE : VS 2

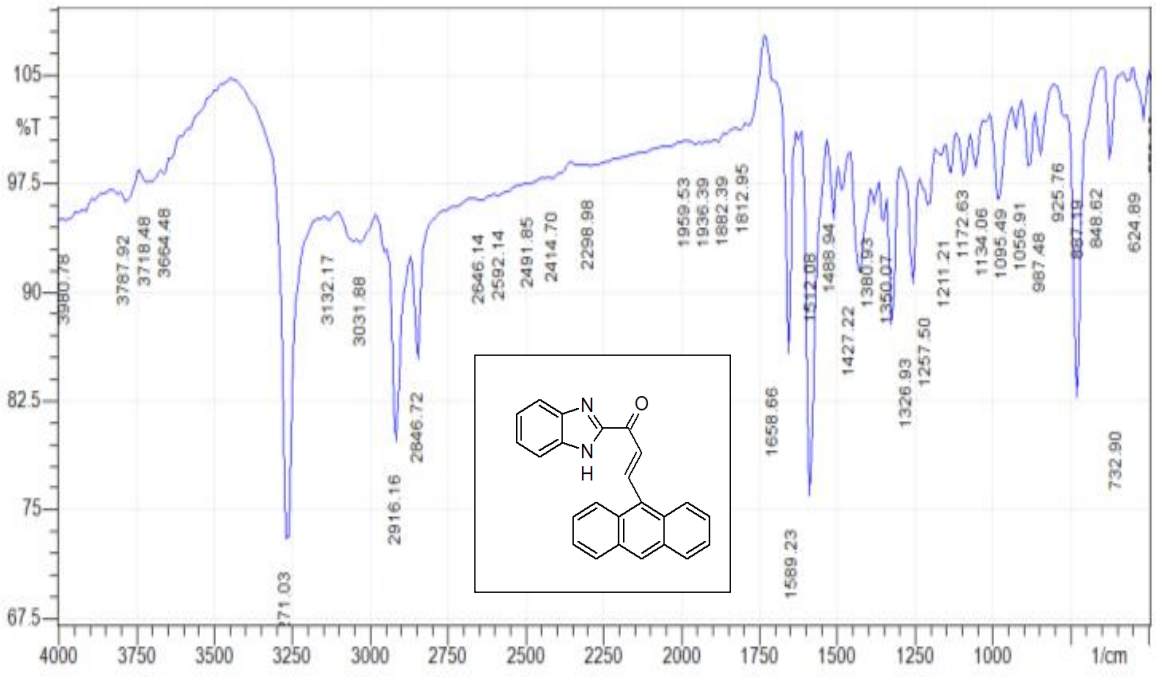


Table 7:IR INTERPRETATION OF COMPOUND VS 2

S.NO	WAVENUMBER (cm^{-1})	FUNCTIONAL GROUP
1	1658.66	C=O stretching
2	1589.23	C=C stretching
3	3031.88	Ar-C-H stretching
4	3271.03	N-H stretching

NMR OF SAMPLE VS 2

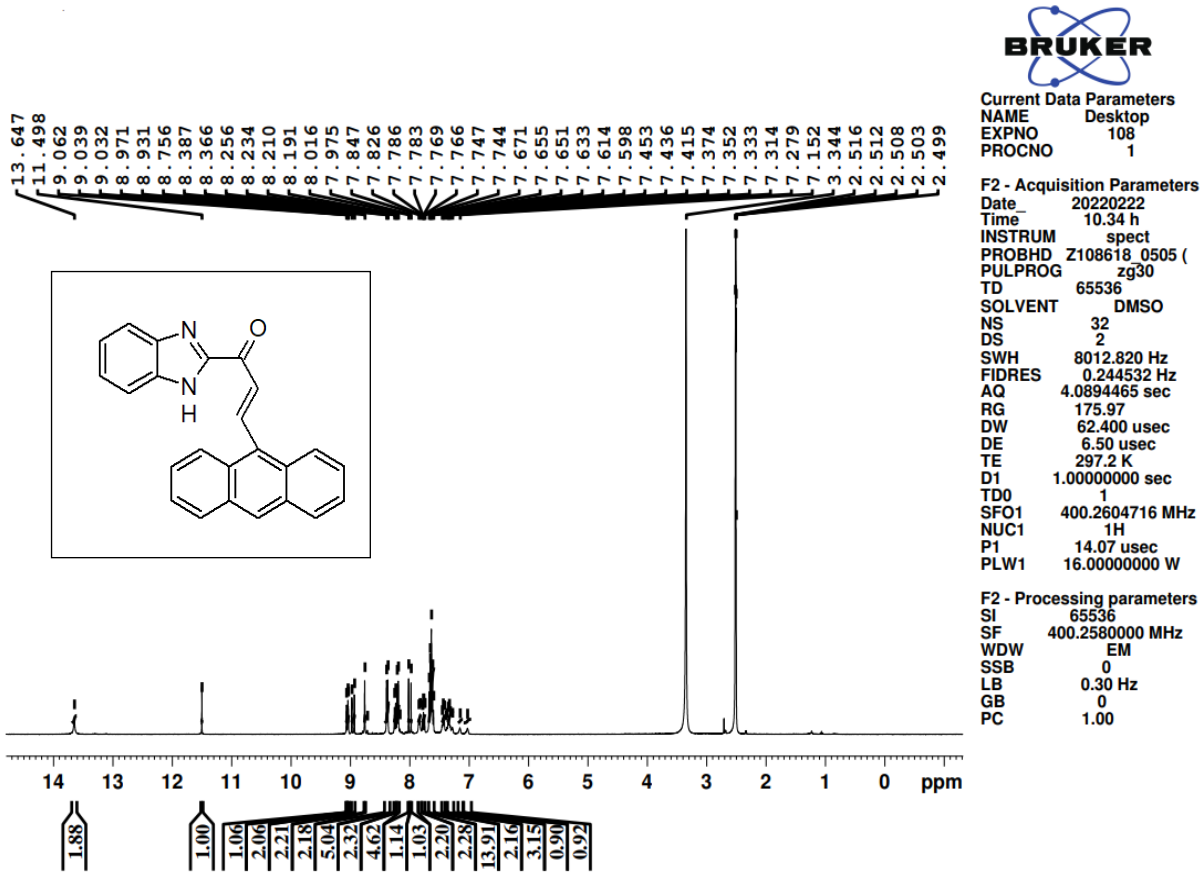
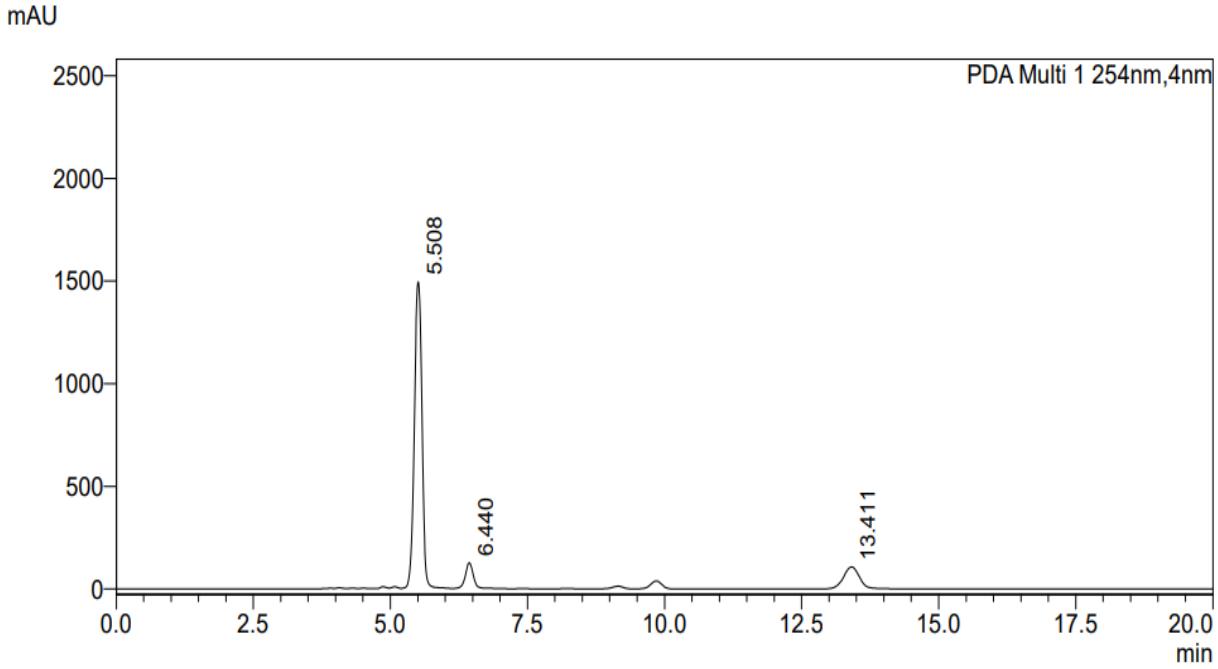


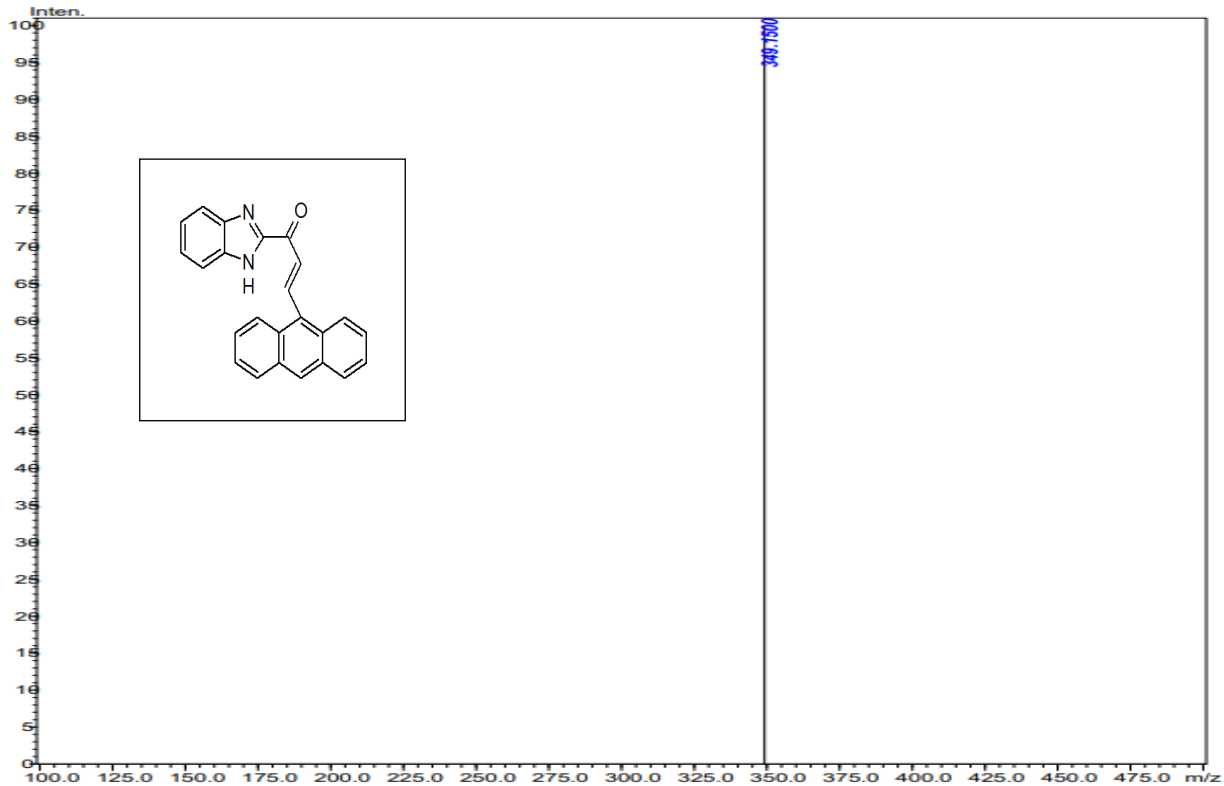
Table 8:H^1 NMR INTERPRETATION OF SAMPLE VS 2

S.NO	δ VALUE	NATURE OF PROTON	NATURE OF PEAK	NO OF PROTON
1	11.5	N-H	Singlet	1 proton
2	7.6-7.8	Ar--H	Multiplet	3 protons
3	3.5	CH=CH	Doublet	2 protons

LC CHROMATOGRAM OF SAMPLE VS 2

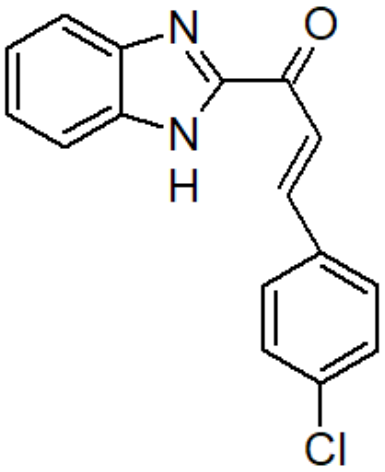


MASS SPECTRUM OF SAMPLE VS 2



MOLECULAR WEIGHT OF SAMPLE VS 2 : 349.15

COMPOUND CODE : VS 3



Molecular Formula	C_{16} H_{11} N_2 OCl
Molecular Weight	282.73
Appearance	Pale yellow colour
Composition	C(67.97%) H(3.92%) Cl(12.54%) N(9.91%) O(5.66%)
Molar Refractivity	82.37 ± 0.3 cm^3
Molar Volume	207.4 ± 3.0 cm^3
Parachor	584.4 ± 4.0 cm^3
Index of Refraction	1.725 ± 0.02
Surface Tension	63.0 ± 3.0 dyne/cm
Density	1.362 ± 0.06 g/cm^3
Dielectric Constant	Not available
Polarizability	32.65 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF SAMPLE :VS 3

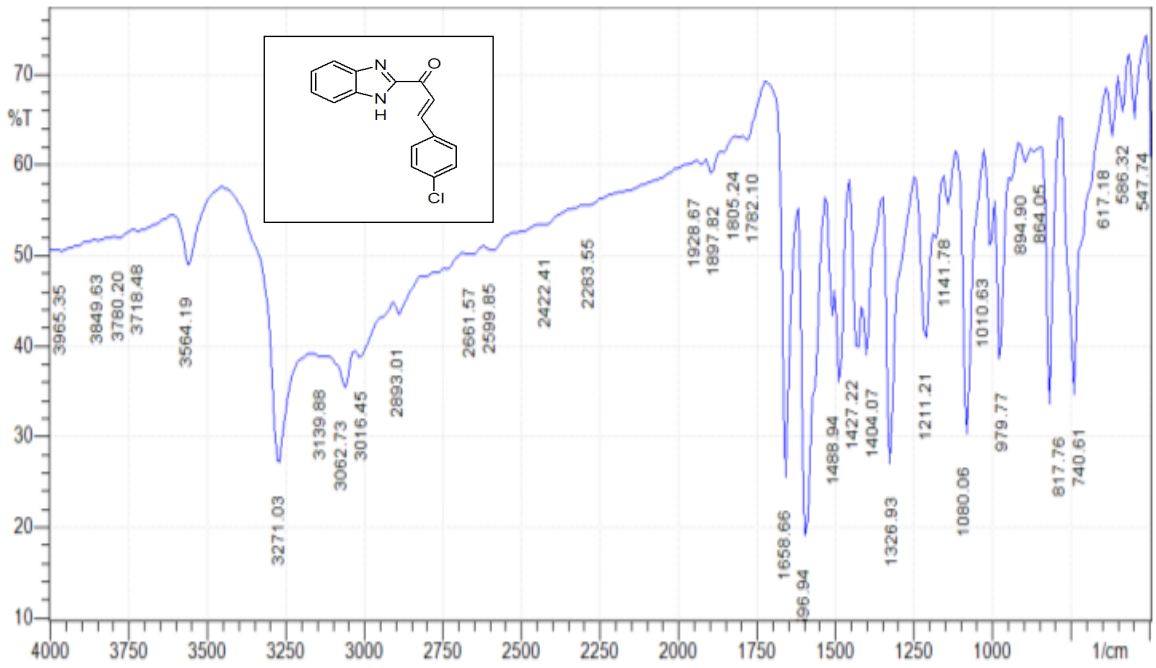


Table 9:IR INTERPRETATION OF SAMPLE VS 3

S.NO	WAVENUMBER (cm^{-1})	FUNCTIONAL GROUP
1	1658.66	C=O stretching
2	1596.94	C=C stretching
3	3016.45	Ar C-H stretching
4	3271.03	N-H stretching
5	740.61	C-Cl stretching

NMR OF SAMPLE VS 3

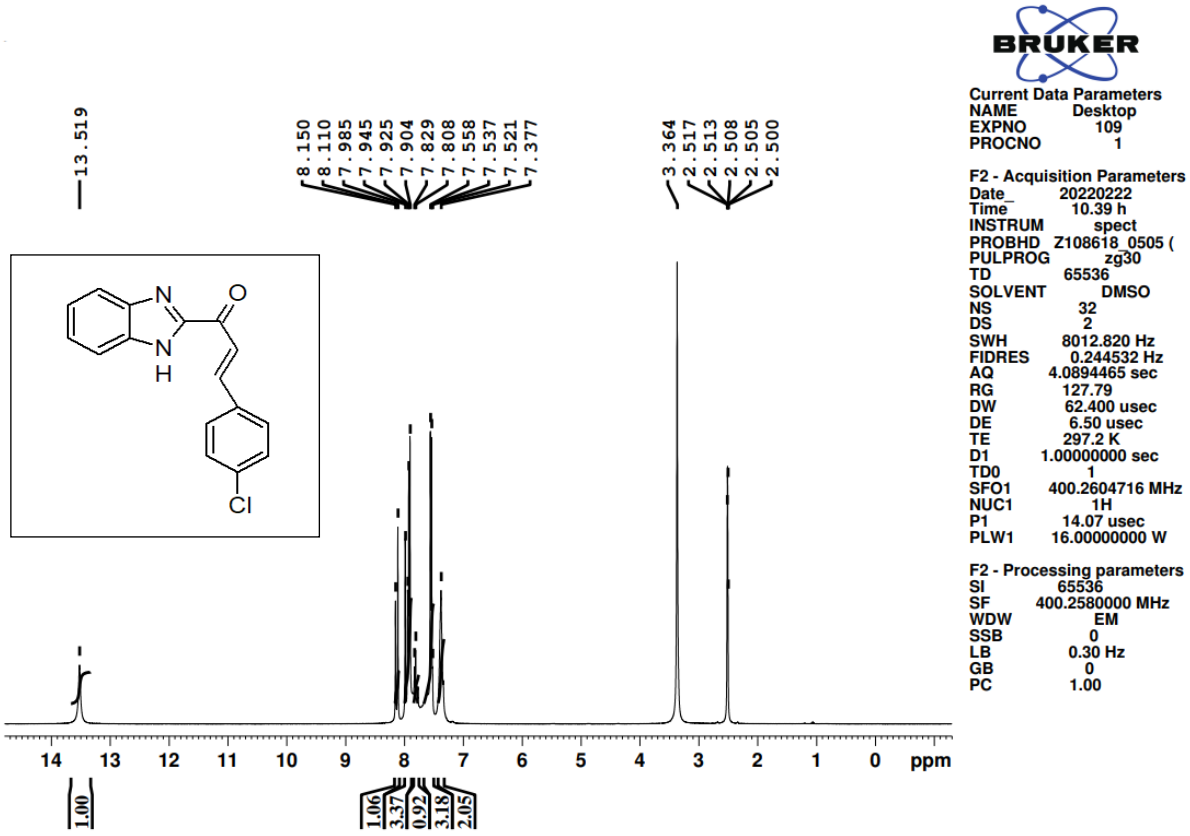
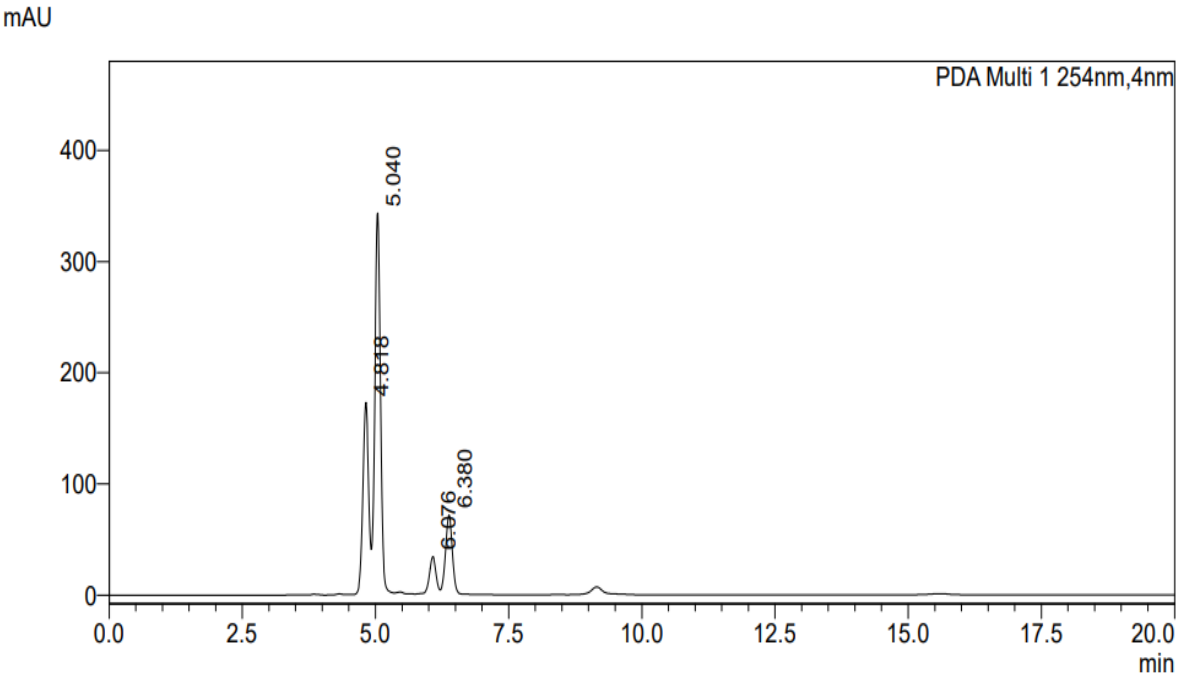


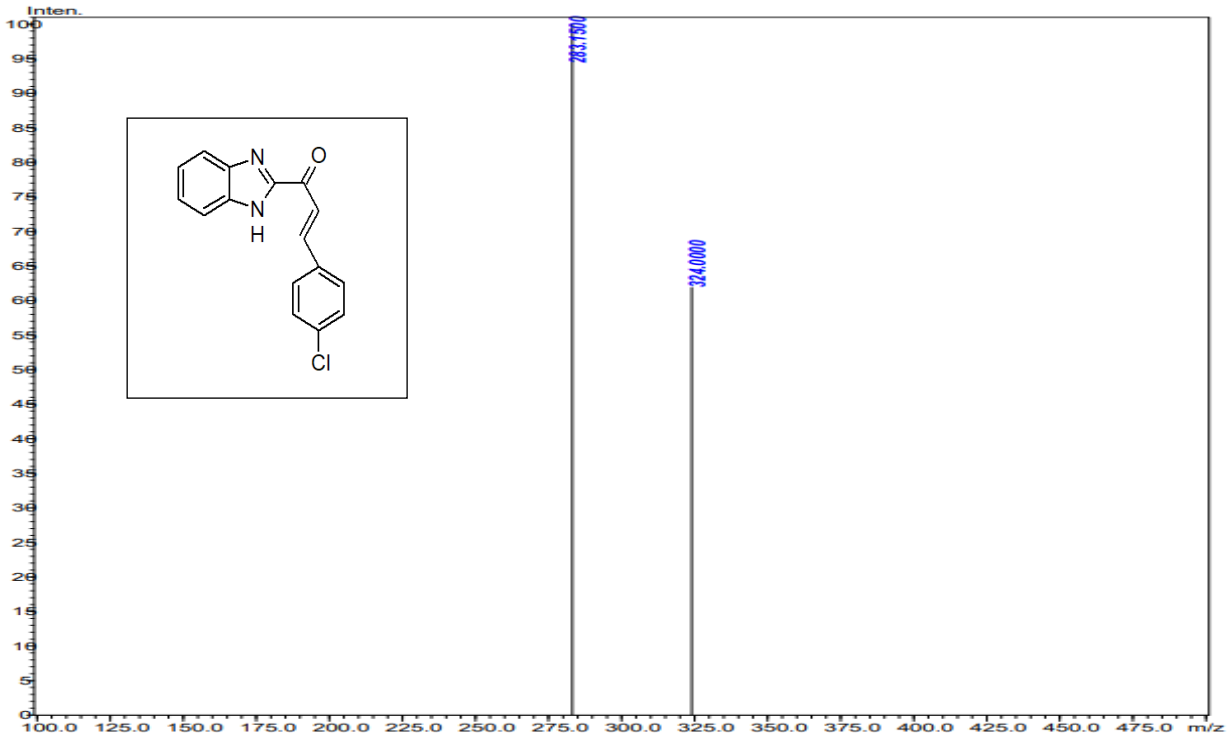
Table 10:H 1 NMR INTERPRETATION OF SAMPLE VS 3:

S.NO	δ VALUE	NATURE OF PROTON	NATURE OF PEAK	NO OF PROTON
1	13.5	N-H	Singlet	1 proton
2	7-7.4	Ar--H	Multiplet	3 protons
3	3.5	CH=CH	Doublet	2 protons

LC CHROMATOGRAM OF SAMPLE OF VS 3

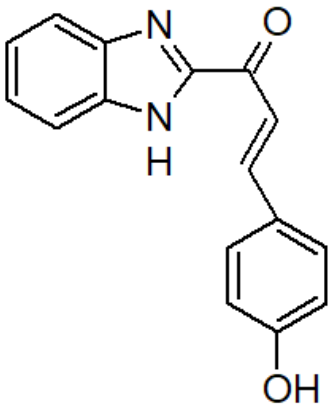


MASS SPECTRUM OF SAMPLE OF VS 3



MOLECULAR WEIGHT OF SAMPLE VS 3 : 283.15

COMPOUND CODE : VS 4



Molecular Formula	$C_{16}\ H_{12}\ N_2\ O_2$
Molecular Weight	264.28
Appearance	Brown colour
Composition	C(72.72%) H(4.58%) N(10.60%) O(12.11%)
Molar Refractivity	79.35 ± 0.3 cm^3
Molar Volume	193.9 ± 3.0 cm^3
Parachor	563.5 ± 4.0 cm^3
Index of Refraction	1.754 ± 0.02
Surface Tension	71.3 ± 3.0 dyne/cm
Density	1.362 ± 0.06 g/cm^3
Dielectric Constant	Not available
Polarizability	31.45 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF SAMPLE : VS 4

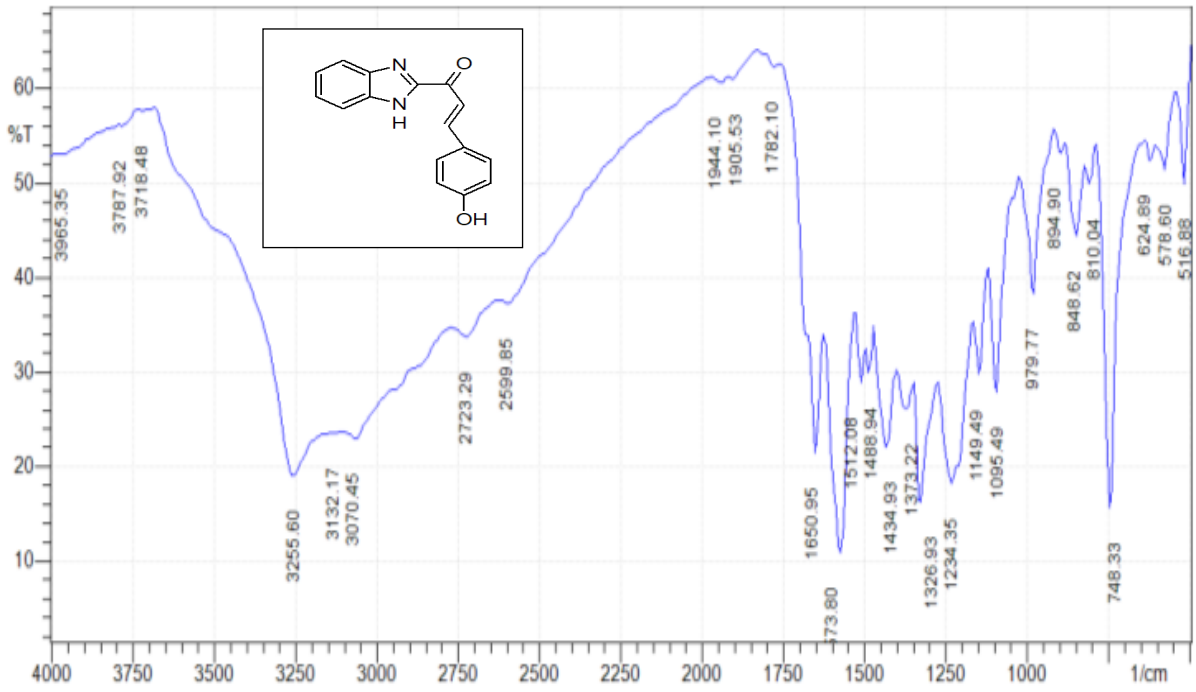


Table 11:IR INTERPRETATION OF SAMPLE VS 4

S.NO	WAVENUMBER (cm^{-1})	FUNCTIONAL GROUP
1	1650.95	C=O stretching
2	1573.80	C=C stretching
3	3070.45	Ar-C-H stretching
4	3255.60	N-H stretching
5	3126.93	Phenolic -OH stretching

NMR OF SAMPLE VS 4

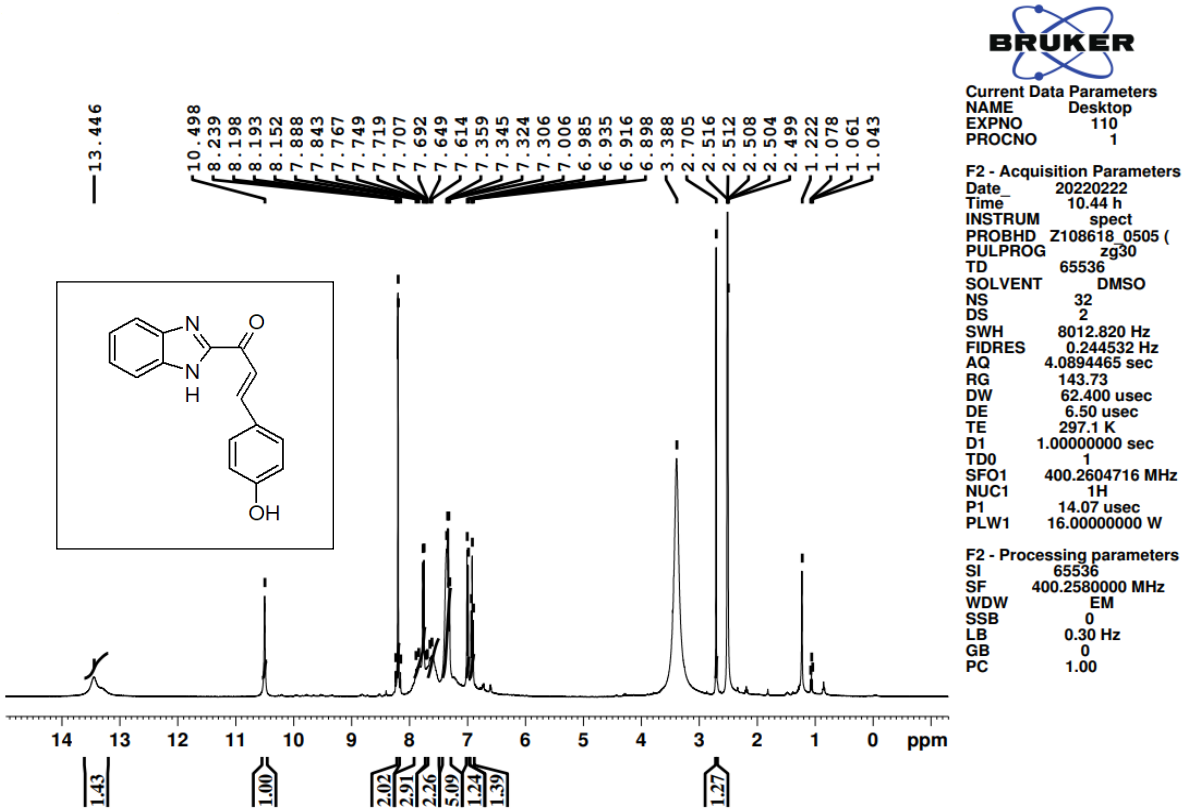
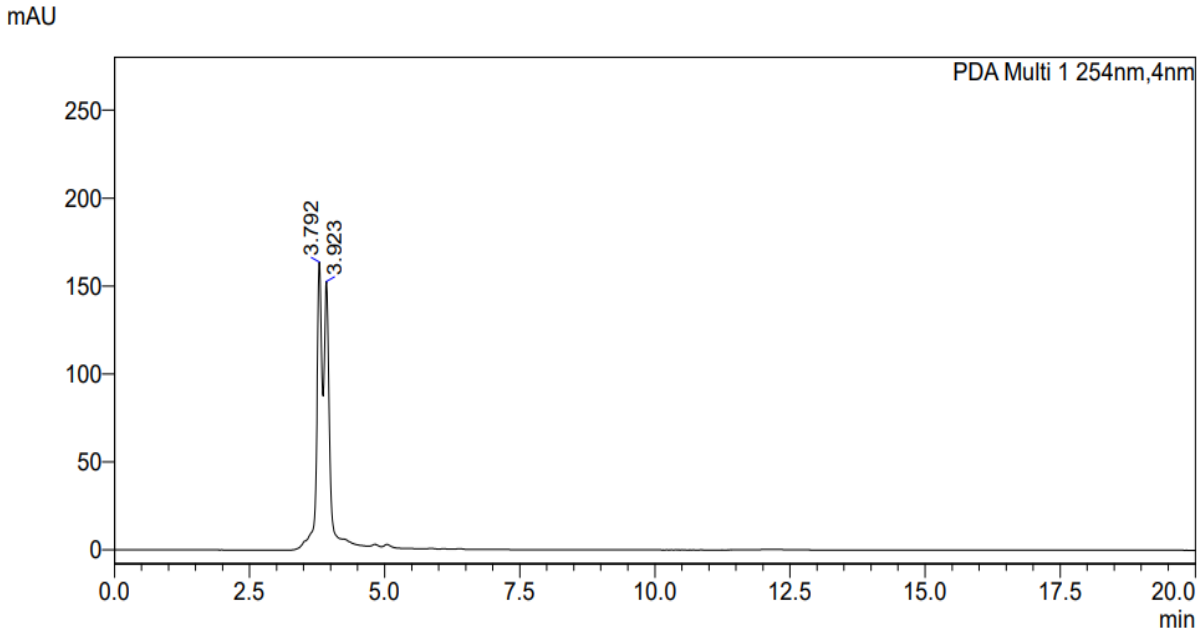


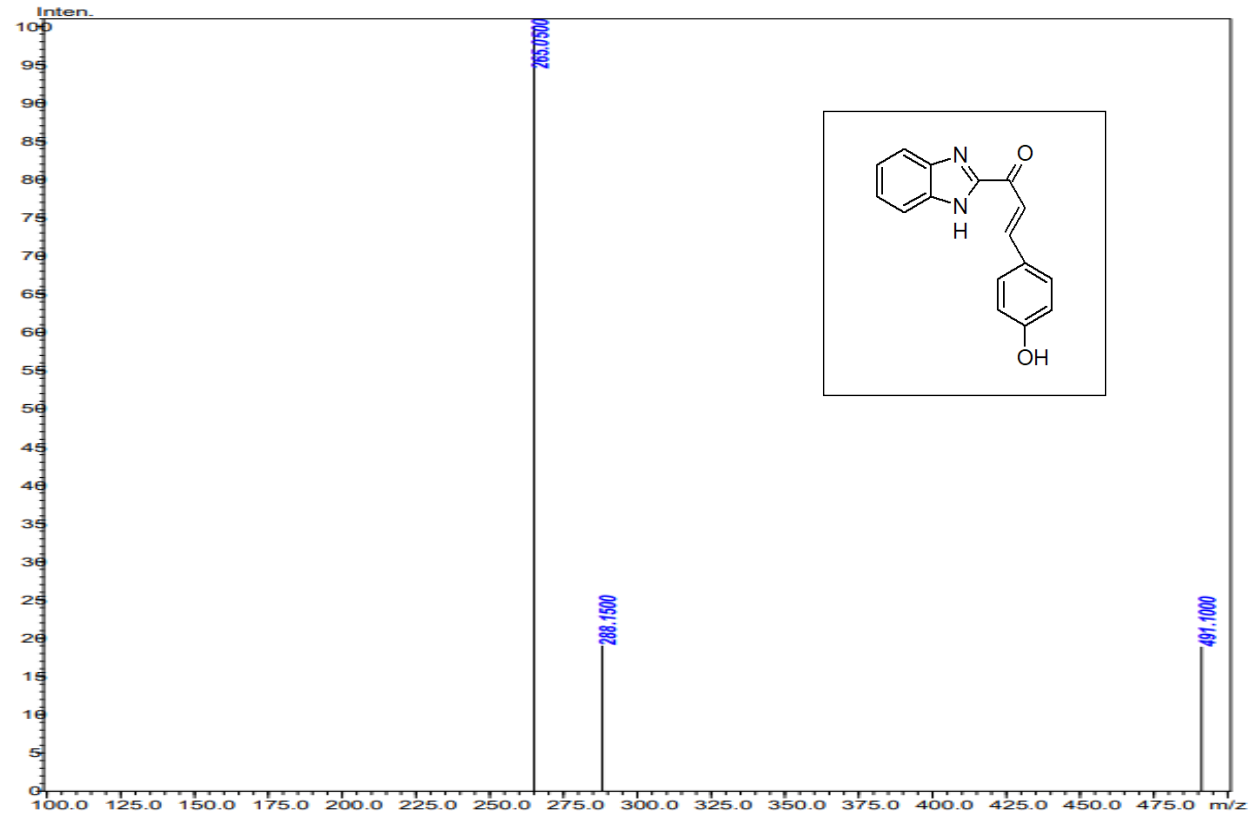
Table 12 :H[1]NMR INTERPRETATION OF SAMPLE VS 4

S.NO	δ VALUE	NATURE OF PROTON	NATURE OF PEAK	NO OF PROTON
1	13.4	N-H	Singlet	1 proton
2	7.3-7.6	Ar--H	Multiplet	3 protons
3	3.38	CH=CH	Singlet	2 protons
4	10.49	-OH	Singlet	1 proton

LC CHROMTOGRAM OF SAMPLE VS 4

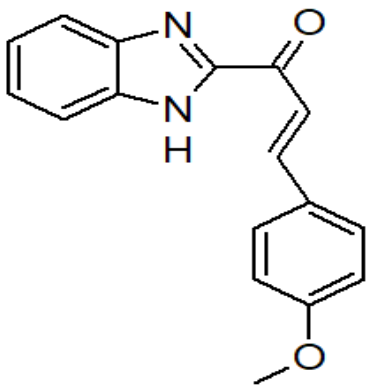


MASS SPECTRUM OF SAMPLE VS 4



MOLECULAR WEIGHT OF SAMPLE VS 4 : 242.25

COMPOUND CODE : VS 5



Molecular Formula	$C_{17}\ H_{14}\ N_2\ O_2$
Molecular Weight	278.34
Appearance `	Yellow colour
Composition	C(73.37%) H(5.07%) N(10.07%) O(11.50%)
Molar Refractivity	84.15 ± 0.3 cm^3
Molar Volume	219.4 ± 3.0 cm^3
Parachor	605.2 ± 4.0 cm^3
Index of Refraction	1.692 ± 0.02
Surface Tension	57.8 ± 3.0 dyne/cm
Density	1.267 ± 0.06 g/cm^3
Dielectric Constant	Not available
Polarizability	33.36 ± 0.5 $10^{-24} cm^3$

IR SPECTRUM OF SAMPLE : VS 5

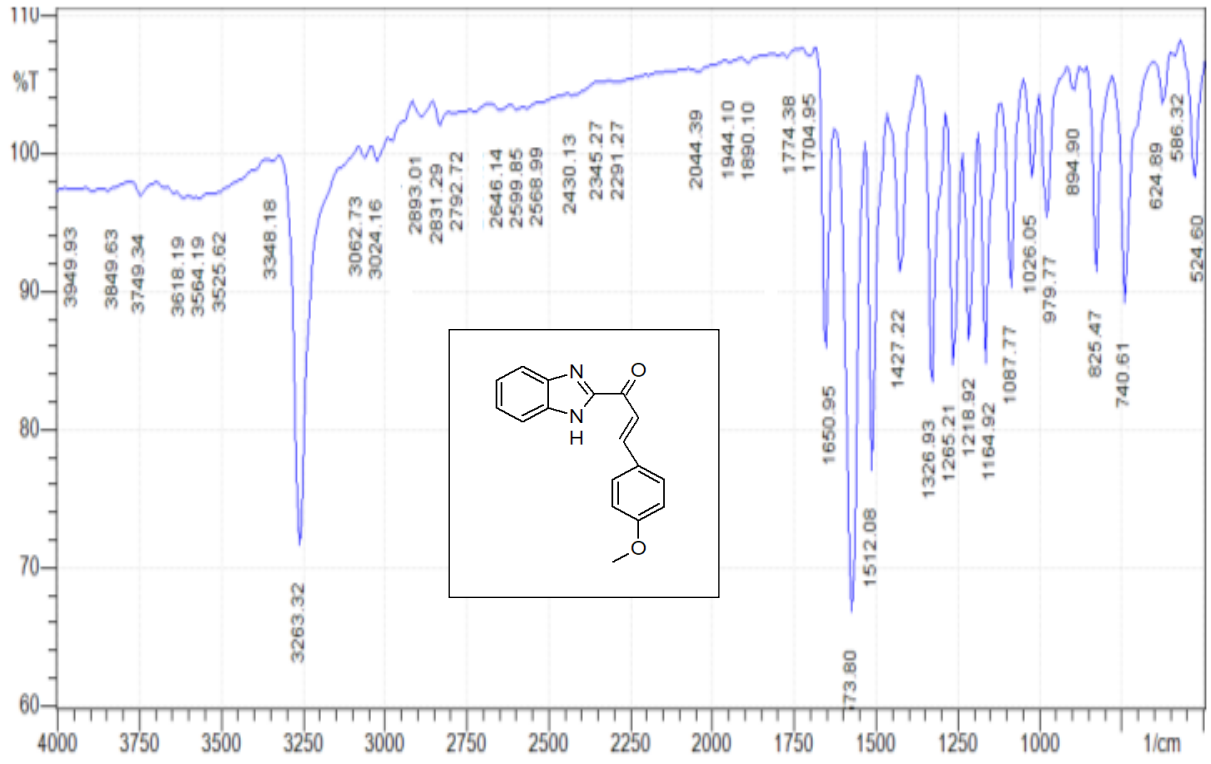


Table 13:IR INTERPRETATION OF SAMPLE VS 5

S.NO	WAVENUMBER (cm^{-1})	FUNCTIONAL GROUP
1	1650.95	C=O stretching
2	1512.08	C=C stretching
3	3024.16	Ar-C-H stretching
4	3263.32	N-H stretching
5	1087.77	C-O-C stretching

NMR OF SAMPLE VS 5

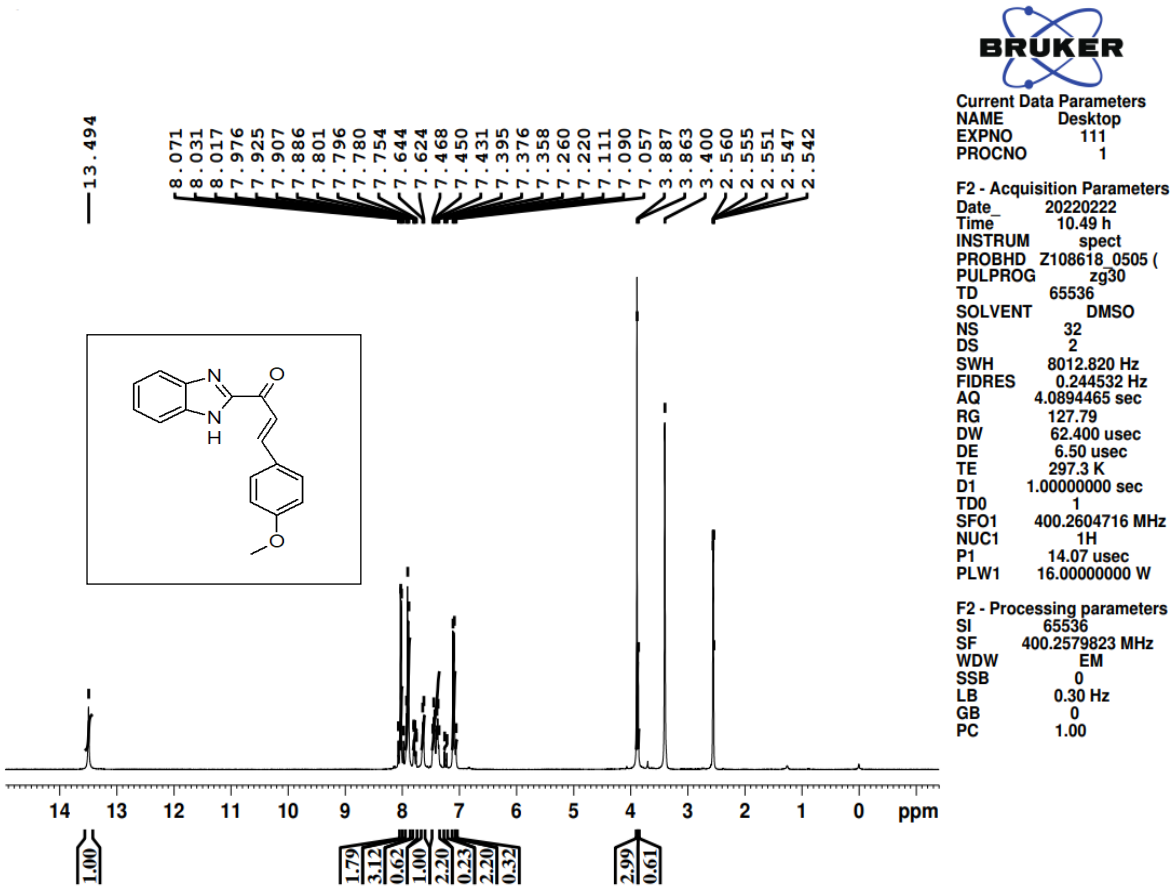
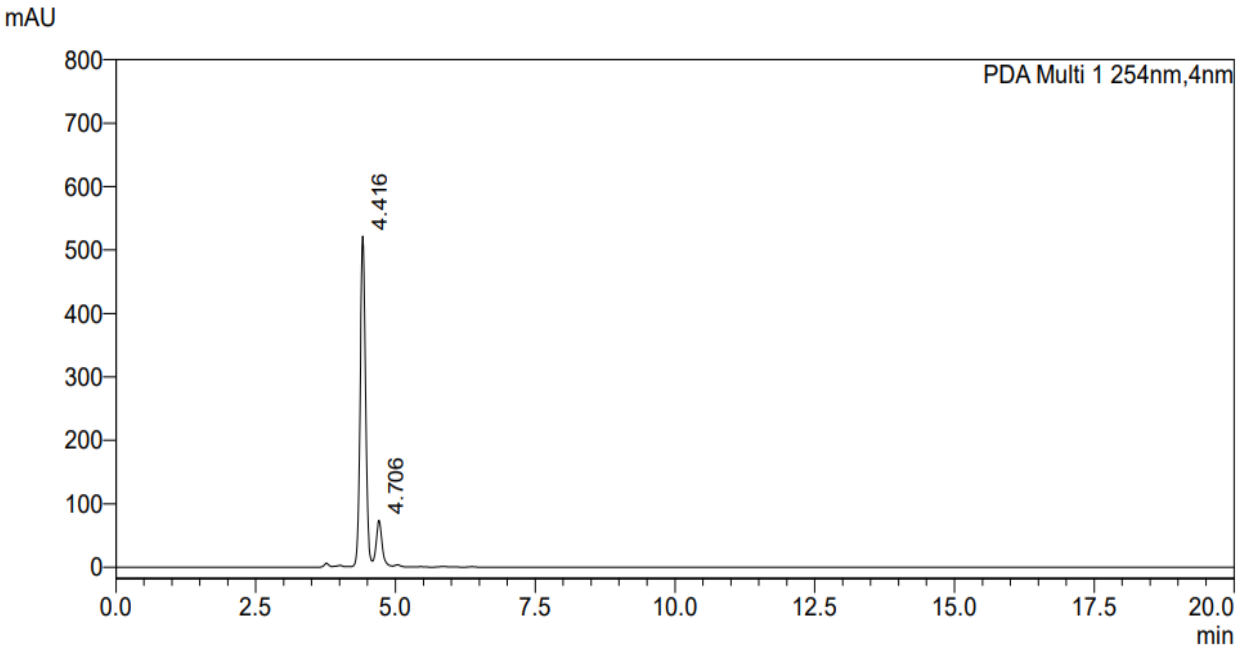


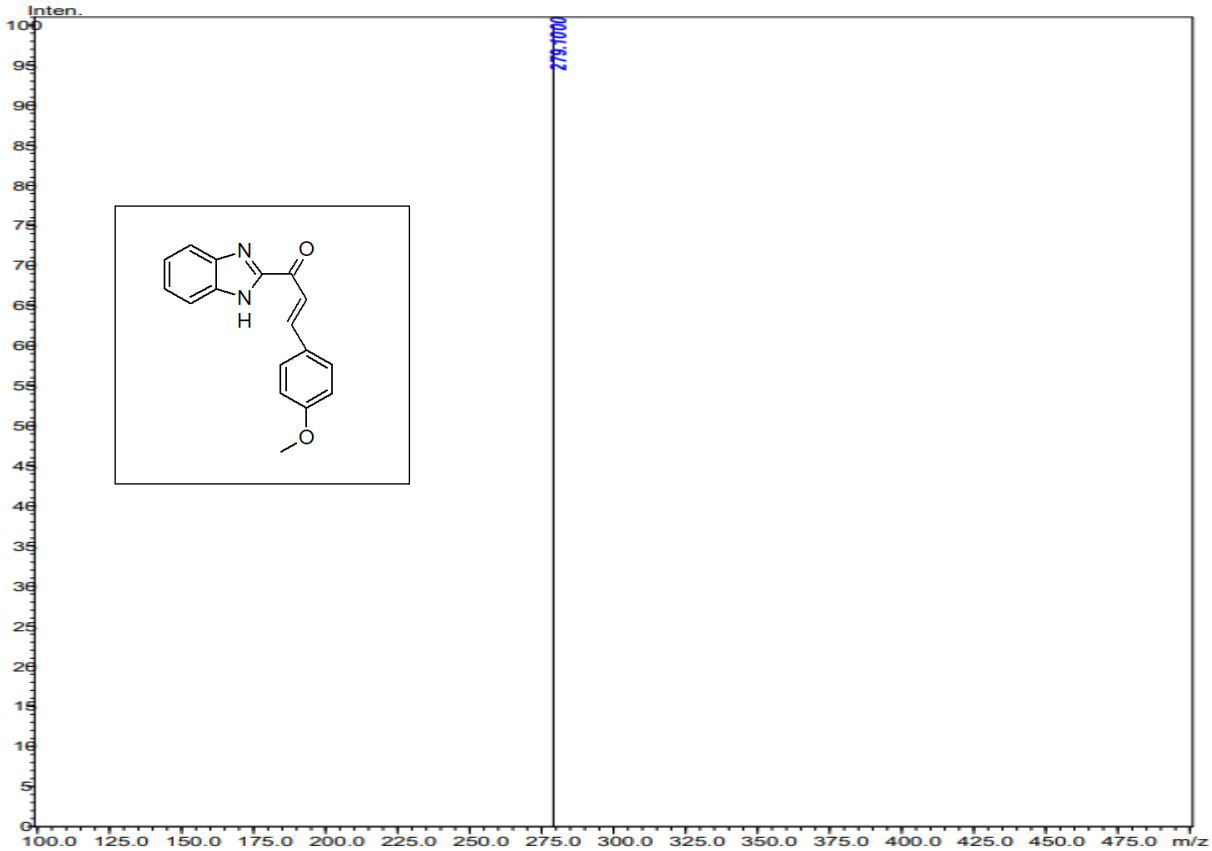
Table 14 :H[1]NMR INTERPRETATION OF SAMPLE VS 5

S.NO	δ VALUE	NATURE OF PROTON	NATURE OF PEAK	NO OF PROTON
1	13.49	N-H	Singlet	1 proton
2	7.4-7.6	Ar--H	Multiplet	3 protons
3	3.40	CH=CH	Doublet	2 protons
4	3.88	-O-CH_3	Singlet	3 protons

LC CHROMATOGRAM OF SAMPLE VS 5



MASS SPECTRUM OF SAMPLE VS 5



MOLECULAR WEIGHT OF SAMPLE VS 5 : 279.10

IR SPECTRUM:

- All the compounds were investigated for the absence of parent functional group and presence of newly formed functional groups.
- All the compounds showed strong stretching vibration between 1512.08 – 1596.94 cm^{-1} which indicates presence of chalcone functional group (C=C).

H 1 NMR SPECTRUM:

- The number of equivalent protons in a molecule was inferred from the presence of number of signals in the spectrum.
- The positions of the signals aid to indicate the nature of protons i.e aromatic, hetero aromatic, aliphatic or vinyl C-H protons etc.
- All the compounds contain multiple peaks from 6.6 to 8.9 which indicates the presence of aromatic and hetero aromatic protons.

LC-MS SPECTRUM:

- Liquid chromatography is used to determine the purity of the synthesized compounds.
- Based on the LC-MS report all the compounds were formed as M+1peak and the correct molecular weight was present.

Table 15: COMPARISON OF MOLECULAR WEIGHT

S.NO	COMPOUND CODE	CALCULATED MASS g/mol	ACTUAL MASS g/mol
1	VS 1	354.42	353.05
2	VS 2	348.40	349.15
3	VS 3	282.73	283.15
4	VS 4	264.28	265.05
5	VS 5	278.31	279.10

BIOLOGICAL EVALUATION

The Anti-tubercular activities of the synthesized compounds were determined by Microplate Alamar Blue Assay method (MABA). The organism used in the study was Mycobacterium tuberculosis H37Rv.

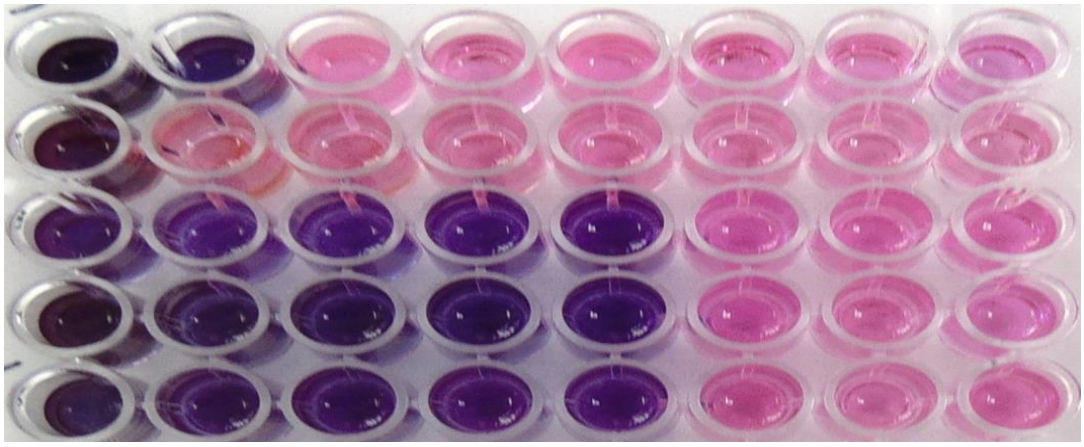
Table 16: DATA ON MABA ASSAY

S.NO	COMPOUND CODE	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml	0.8 µg/ml
1	VS 1	S	S	R	R	R	R	R	R
2	VS 2	S	R	R	R	R	R	R	R
3	VS 3	S	S	S	S	S	R	R	R
4	VS 4	S	S	S	S	S	R	R	R
5	VS 5	S	S	S	S	S	R	R	R

NOTE: S – Sensitive, R - Resistant

Strain used: Mycobacterium tuberculosis (Vaccine strain, H37Rv strain): ATTC No-27294.

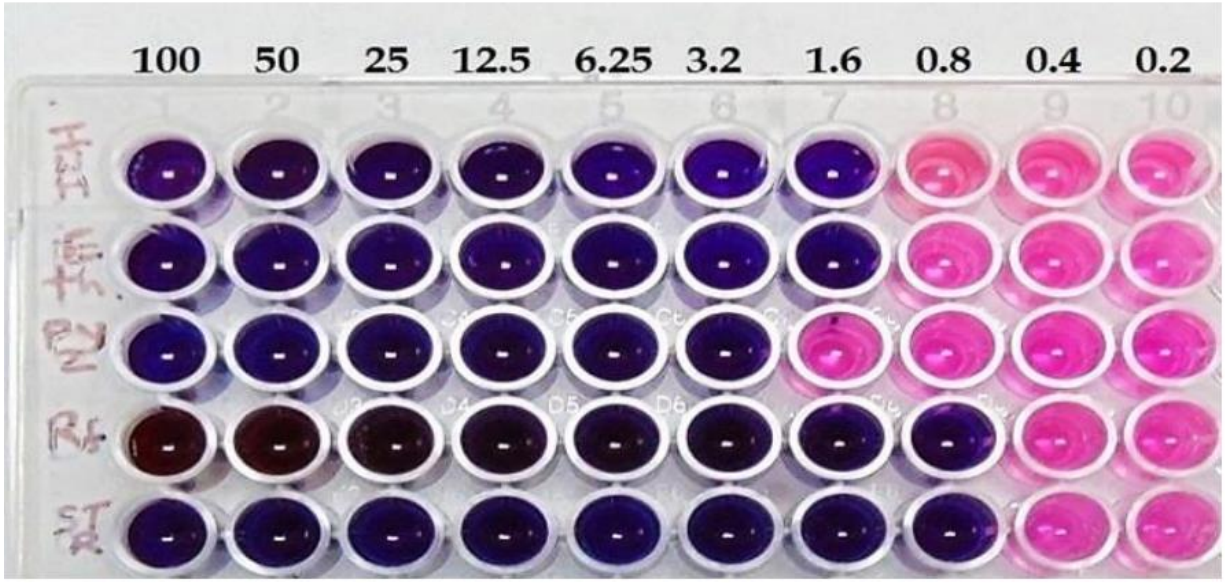
Table 17: Results of MABA Assay

CODE	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml	0.8 µg/ml
VS 1								
VS 2								
VS 3								
VS 4								
VS 5								

- ✓ The MIC of the sample **VS 1** was found to be **50 µg/ml.**
- ✓ The MIC of the sample **VS 2** was found to be **100 µg/ml.**
- ✓ The MIC of the **s**ample **VS 3, VS 4, VS 5** were found to be **6.25 µg/ml.**

MINIMUM INHIBITORY CONCENTRATION OF STANDARD ANTI-TUBERCULAR AGENTS :

1. Pyrazinamide 3.125 µg/ml
2. Isoniazid 1.6 µg/ml
3. Ethambutol 1.6 µg/ml
4. Rifampicin 0.8 µg/ml
5. Streptomycin 0.8 µg/ml



Anti-tubercular activity of standard drugs

ACUTE ORAL TOXICITY STUDY:

All the five compounds which were synthesized were found to be equally active or more active than the standard drugs. So all of them were taken up for acute oral toxicity study using albino mice following OECD guidelines (423). After administration of the compounds the animals were observed for behavioral signs of toxicity like motor activity, tremor etc. No significant toxic sign was observed during14 days following the administration. The results are tabulated below.

Table 18:ACUTE ORAL TOXICITY STUDY

S.NO	PARAMETER	RESULTS
1	Toxic signs	Absent
2	Pre-terminal deaths	Nil
3	Body weight	No specific change
4	Motor activity	Normal
5	Tremors	Absent
6	Convulsions	Absent
7	Straub reaction	Absent
8	Lacrimation and salivation	Normal
9	Sedation	Absent
10	Righting reflux	Present
11	Body temperature	Normal
12	Analgesia	Absent
13	Diarhoea	Absent
14	Skin colour	Appears normal
15	Respiration	Normal
16	Aggressiveness and restlessness	Absent

No significant toxic signs were observed and no mortality was reported up to 14 days of study. The five compounds were found to be non toxic and safe for oral administration upto dose of 2000 mg/kg of body weight.

SUMMARY

- Based on the various medicinal chemistry journals Glutamine synthetase1 was selected as the target for the study.
- A database of 500 molecules with high prospects of inhibiting the target Glutamine synthetase was carefully chosen by making changes to the known hit molecules, i.e Benzimidazole chalcones.
- The molecules were subjected to toxicity assessment by OSIRIS® property explorer.
- In-silico druglikeness properties of the designed molecules were determined by using the MOLINSPIRATION® software.
- 500 molecules were docked against the target protein using AutoDock 4 ® .
- Five molecules with good docking score [lower binding energy] and interactions were shortlisted and optimized for the synthesis.
- Compounds were synthesized with satisfactory yield and labelled as VS-1, VS-2, VS-3, VS-4 and VS-5.
- Purity of the synthesized compounds was ensured by repeated recrystallization and the compounds were evaluated by TLC and Melting point.
- The characterization of the synthesized compounds was done using Infra-red spectroscopy, Liquid Chromatography-Mass spectrometric methods [LC-MS] and Nuclear Magnetic Resonance [H1 NMR] spectroscopy methods.
- The pure compounds were screened for Anti-mycobacterial activity by in-vitro Microplate Alamar Blue Assay [MABA]. MIC of synthesized compound were found in the range of 100µg/ml – 6.25µg/ml.
- Acute oral toxicity study was conducted on albino wistar rats and all the compounds were found to be safe and non-toxic.

CONCLUSION

- ✓ Novel Benzimidazole chalcone derivatives were found to be capable of inhibiting the target enzyme Glutamine synthetase 1which is essential for the synthesis of mycobacterium cell wall.
- ✓ The docking score of designed compound ranges between -8.53 to -5.66 Kj/mol. There is no significant correlation between the docking score and activity of the compound.
- ✓ All the compounds inhibited the Mycobacterium tuberculosis at the range of 6.25µg/ml to 100µg/ml.
- ✓ The acute toxicity studies revealed that all the compounds found to be safe and non-toxic.
- ✓ The MABA test is carried out using H37Rv strain which is non- pathogenic. Hence further studied should be carried out using clinical isolates i.e, Pathogenic strain.
- ✓ Further structural refinement of the synthesized compounds is expected to yield promising drug candidate against Mycobacterium tuberculosis.

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This is to certify that Sangeetha v *has solemnly pledged and participated in online "**Corona Awarness programme**" organized by Pallavan Pharmacy College, Kolivakkam, Iyyengarkulam, for commitment of discharge the service to the nation as a responsible national*

28-4-2020

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IN CLINICAL RESEARCH & PHARMACOVIGILANCE

This is to certify that V. Sangeetha *attended the workshop entitled "Clinical Research & Pharmacovigilance" organized by Atria Biosciences held on* 28-04-2018. *This workshop provides a detailed introduction of Clinical Research, Pharmacovigilance and Clinical Data Management.*

Alamelu Murali

Mrs. ALAMELU MURALI, B.Pharm (PGDCR) MBA (HR)
Chancellor

No. 148, DR. Radhakrishnan Road,
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71st Indian Pharmaceutical Congress

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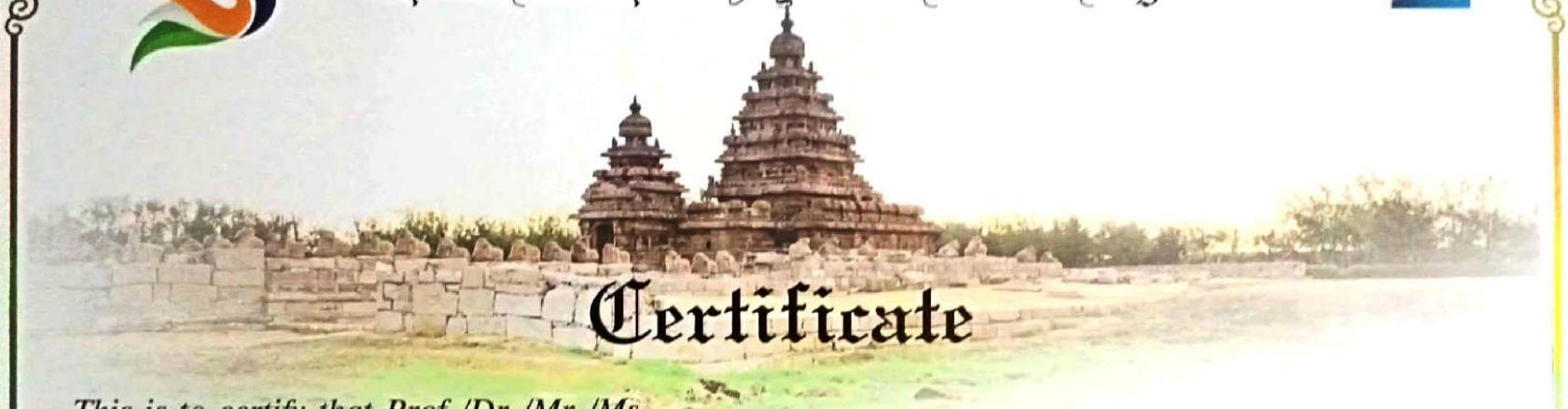

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And

Indian Medical Association, Tamil Nadu State Branch

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'INTERNATIONAL UPDATE ON COVID - 19'

Certificate of Participation

Dr./Mr./Ms. SANGEETHA.V

participated in the scientific deliberations of the ' ***International Update on COVID-19'***
held on the Tuesday ***17th March 2020*** *at 09:00 am at The Silver Jubilee Auditorium,*
The Tamilnadu Dr MGR Medical University, Guindy, Chennai 32

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MADRAS MEDICAL COLLEGE, CHENNAI – 600003

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

PROCEEDINGS

PRESENT: Dr. A. JERAD SURESH, M.Pharm., Ph.D., MBA

Roc. No: 5/AEL/IAEC/MMC/2022 Dated: 01-11-2021

Sub: IAEC, MMC, Ch-3 – Approval of Laboratory Animals – Regarding

Ref: IAEC Meeting held on 21-10-2021

This order is issued based on the approval by the Institutional Animal Ethics Committee Meeting held on 21-10-2021, Thursday.

Project Proposal ID Number	17/2021-2022
CPCSEA Registration Number	1917/GO/ReBi/2016/CPCSEA Valid till 19-9-2026
Name of the Researcher with ID Number	V. SANGEETHA 261915708
Name of the Guide	Dr. A. Jerad Suresh, M.Pharm., Ph.D., MBA
Project Title	Design, Synthesis, Characterization And Biological Evaluation Of Novel Benzimidazole – chalcones derivatives as Anti-tubercular agents targeting Glutamine Synthetase.
Date of submission of proposal to IAEC	07-10-2021
Date of IAEC meeting	21-10-2021
Date of submission of modified proposal to IAEC	22-10-2021
Date of Approval	21-10-2021
Validity of the Approved Proposal	One Year
Number & Species of Laboratory Animals Approved	**30 Wistar Rats Approved**

5/11/22

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai-600003

PRINCIPAL
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

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

Copy to:
Special Veterinary Officer, Animal Experimental Laboratory,
Madras Medical College, Ch-3.

INTRODUCTION

REVIEW OF LITERATURE

AIM AND OBJECTIVE

PLAN OF WORK

MATERIALS AND METHODS

RESULTS AND DISCUSSION

SUMMARY & CONCLUSION

BIBLIOGRAPHY

ANNEUXURES