"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL 3, 4-DIHYDROPYRIMIDIN-2(1H)-ONE AS ANTI TUBERCULAR AGENTS"

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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

Reg. No. 261215704

Under the Guidance of

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

Principal, Professor and Head

Department of Pharmaceutical Chemistry

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

APRIL 2014

CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTRIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL 3, 4-DIHYDROPYRIMIDINE-2(1H)-ONE AS ANTITUBERCULAR AGENTS"** submitted by the candidate bearing the **Reg. No. 261215704** in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY in PHARMACEUTICAL CHEMISTRY** by **The Tamilnadu Dr. M.G.R Medical University** is a bonafide work done by him during the academic year 2013-2014 at the **Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-3**.

Dr. A. JERAD SURESH, M.Pharm., Ph.D., M.B.A.,
Principal,
Professor and Head,
Department of Pharmaceutical chemistry,
College of Pharmacy,
Madras Medical College,
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Principal,
Professor and Head,
Department of Pharmaceutical chemistry,
College of Pharmacy,
Madras Medical College,
Chennai- 3.

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Dedicated to my

Family & Samarjit

CONTENTS

A. BACKGROUND

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

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

The directly observed treatment short-course (DOTS), a multidrug therapy program developed by WHO is one of the most efficient weapons against the global TB epidemic. Nevertheless, the treatment success rate struggles to reach the target of 85%. Unfortunately, first-line treatment can fail due to poor compliance which leads to the emergence of multidrug resistant (MDR) strains of M. tuberculosis. Anti-tuberculosis agents in current development trigger a large panel of biological pathways such as cell wall synthesis, protein synthesis or membrane energy production. The number of TB drugs in preclinical and clinical development is today higher than during the past 40 years.

Current Therapies:

In 1944, streptomycin was the first compound used to treat TB. This amino glycoside interferes with protein biosynthesis through an interaction with the small 30S subunit of the ribosome. The discovery of para-aminosalicylic acid in 1946 was quickly followed by the important identification of isoniazid (INH), one of the most active anti-TB drug used today. Inhibition of mycolic acids biosynthesis, one of the essential components of the mycobacterial cell wall was

determined as isoniazid mechanism of action. Pyrazinamide (PZA) appeared as a potential TB drug in 1952. Its introduction in the TB treatment in the 1980s was a great success as it allowed to shorten the duration of the TB therapy from 9 to 6 months. Ethambutol (EMB) and rifampin (RIF), the two last derivatives used in the TB first-line treatment, were discovered during the 60's. Ethambutol is an ethylenediamine discovered in 1961, which affects the cell wall by specifically targeting the polymerization of arabinogalactane and lipoarabinomannane. Finally, rifampin appeared as a drug of choice for TB treatment around 1970, by acting on replicating and non-replicating mycobacteria. This derivative belongs to the rifamycine family and inhibits bacterial RNA synthesis by binding to the b-subunit of the DNA-dependent polymerase.

The current standard regimen (DOTS) for TB recommended by WHO is a combination of isoniazid, rifampin, ethambutol and pyrazinamide for a 6 months therapy. To treat MDR-TB, WHO recommends the use of second-line drugs which include aminoglycosides (kanamycin, amikacin), capreomycin, cycloserin, para-aminosalicylic acid, thioamides (ethionamide (ETH), prothionamide), and fluoroquinolones[1] (ciprofloxacin, ofloxacin, levofloxacin). Ethionamide and prothionamide are isoniazid analogues discovered in 1956. [1]

Fluoroquinolones gatifloxacin and moxifloxacin (Phase 3) oxazolidinone linezolid and nitroimidazole metronidazole (Phase 2) have been repurposed for tuberculosis. New chemical entities have also progressed in clinical development based on optimization of known chemical scaffolds: this is the case for nitroimidazole derivatives OPC-67683 and PA-824 both in phase 2, substituted ethylenediamine SQ109 (phase 2), oxazolidinone analogues PNU- 100480 (phase 2) and AZD5847 (phase 1). Finally, promising diarylquinoline TMC207, first compound of a new class of antituberculosis drugs is currently being evaluated in Phase 2.[1]

B. BACTERIOLOGICAL PROFILE OF MYCOBACTERIUM TUBERCULOSIS:

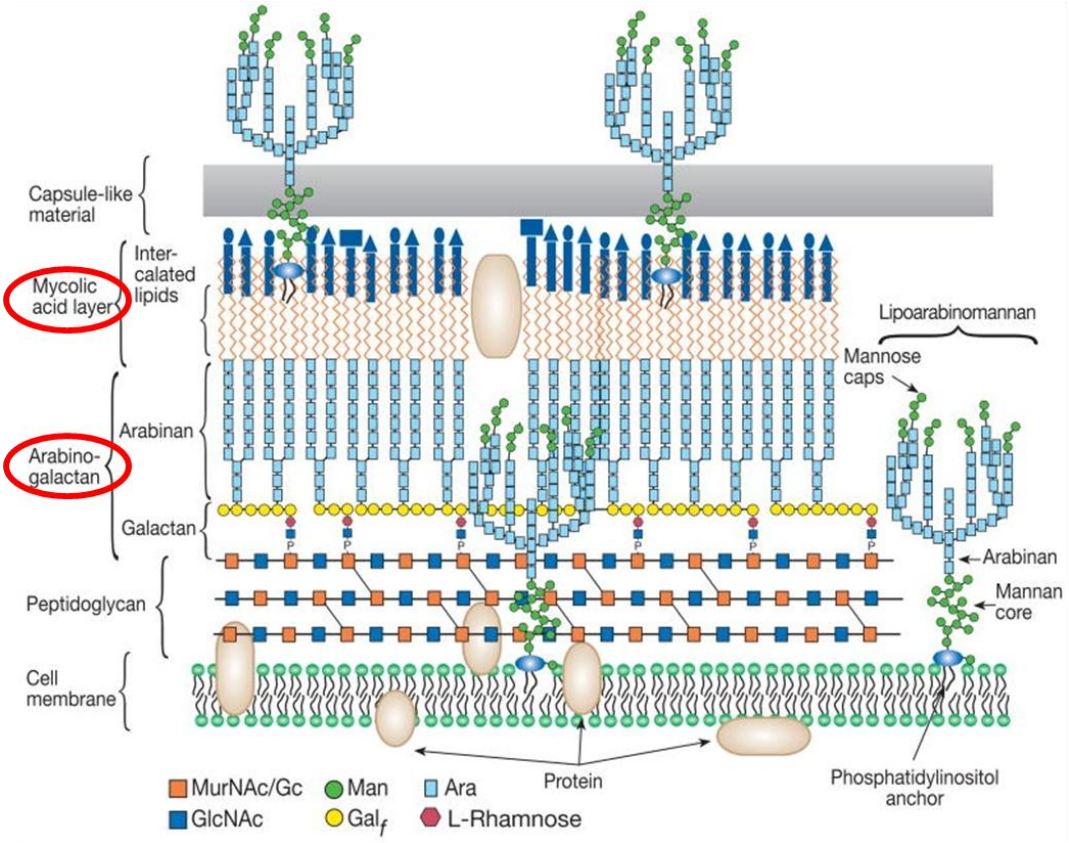
SPECIES:

The Mycobacterium tuberculosis complex (MTC) consists of Mycobacterium africanum, Mycobacterium bovis, Mycobacterium caprae, Mycobacterium microti, Mycobacterium pinnipedii and Mycobacterium canettii. [2]

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

CELL STRUCTURE:

In 1882, Robert Koch identified Mycobacterium tuberculosis as the causative agent of TB. M. tuberculosis belongs to the order Actinomycetales. Mycobacterium tuberculosis is a slow-growing aerobic rod-shaped bacterium. The cell wall is composed of two segments, upper and lower. Beyond the membrane is peptidoglycan (PG) in covalent attachment to arabinogalactan (AG), which in turn is attached to the mycolic acids with their long meromycolate and short α-chains. This is the cell wall core the mycolyl arabinogalactan–peptidoglycan (mAGP) complex. The upper segment is composed of free lipids, some with longer fatty acids complementing the shorter α-chains, and some with shorter fatty acids complementing the longer chains. Interspersed with the cell-wall proteins are the phosphatidylinositol mannosides (PIMs), the phthiocerol containing lipids, lipomannan (LM), and lipoarabinomannan (LAM). When cell walls are disrupted, for instance extracted with various solvents, the free lipids, proteins, LAM, and PIMs are solubilized, and the mycolic acid–arabinogalactan– peptidoglycan complex remains as the insoluble residue. [4]



GENOME STRUCTURE:

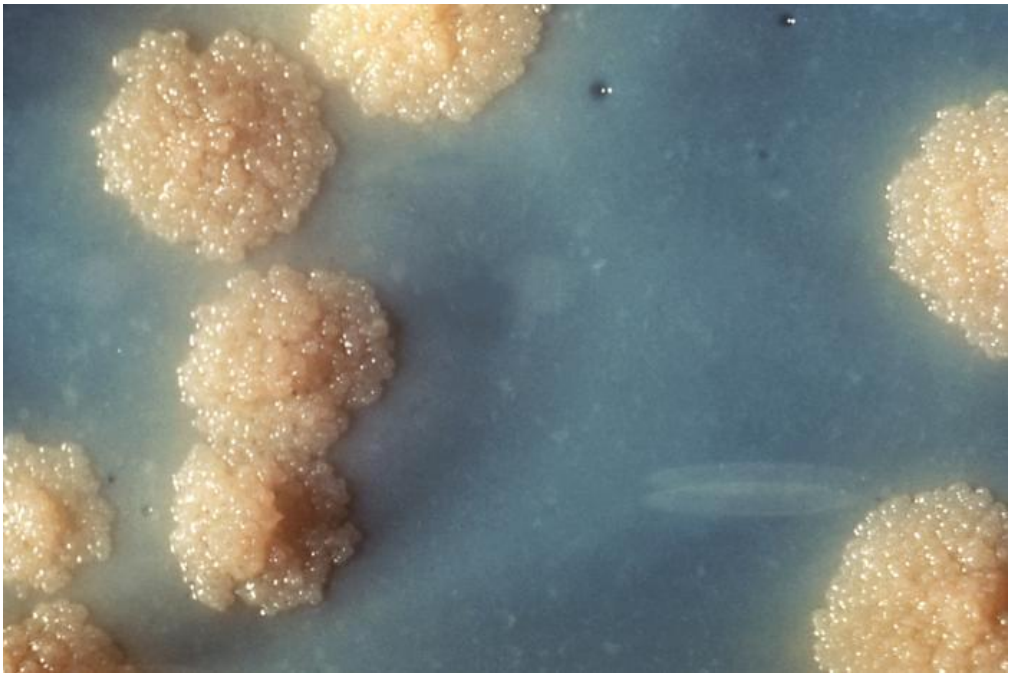
Mycobacterium tuberculosis H37Rv was first isolated in 1905, has remained pathogenic and is the most widely used strain in tuberculosis research. The complete genome sequence and annotation of this strain was published in 1998 (Cole et al., 1998⇓). Approximately 9% of the M. tuberculosis genome consists of two related families of genes that have been named the PE and PPE families (cole et al., 1998). These names derive from the presence of conserved proline glutamate (PE) or proline-proline-glutamate (PPE) residues near the N terminus of the predicted proteins of the family. These predicted protein share a conserved N terminal domain of ~ 110 amino acids (PE) or 180 aminoacdis(PPE) with divergent C-terminal sequences. A subfamily of the PE family has been designated the PGRS proteins based on C-terminal extensions rich in a repetitive glycine/alanine (GA) motif. A large portion of the coding capacity of the M. tuberculosis genome is putatively involved with lipid biosynthesis or lipid degradation. [3]

As per March 2013 in TubercuList there are [5]

- 4,411,532 bp of DNA sequence representing the whole Mycobacterium tuberculosis chromosome (strain H37Rv)
- 4,018 protein genes
- 13 pseudogenes
- 45 tRNA genes
- 3 rRNA genes
- 30 ncRNA genes
- 2 miscRNA genes
- Gene density: 0.91 genes per kb
- Average length: 1,002 bases per gene
- Protein coding bases: 4,027,296
- Protein coding percentage: 91.2%
- GC percentage: 65.9 %

CHOLESTEROL CATABOLISM:

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



M. tuberculosis bacterial colonies

Scanning Electron Microscopic Image of M.tuberculosis

PATHOPHYSIOLOGY OF M.tuberculosis:

Mycobacterium tuberculosis is spread by small airborne droplets,called droplet nuclei.

Once inhaled, the infectious droplets settle throughout the airways. The majority of the bacilli are trapped in the upper parts of the airways where the mucus-secreting goblet cells exist.

Bacteria in droplets that bypass the mucociliary system and reach the alveoli are quickly surrounded and engulfed by alveolar macrophages, the most abundant immune effector cells present in alveolar spaces. The mycobacterial lipoarabinomannan is a key ligand for a macrophage receptor. The complement protein C3 binds to the cell wall and enhances recognition of the mycobacteria by macrophages. Opsonization by C3 is rapid, even in the air spaces of a host with no previous exposure to *M tuberculosis.* After being ingested by macrophages, the mycobacteria continue to multiply slowly, with bacterial cell division occurring every 25 to 32 hours. Regardless of whether the infection becomes controlled or progresses, initial development involves production of proteolytic enzymes and cytokines by macrophages in an attempt to degrade the bacteria. Released cytokines attract T lymphocytes to the site, the cells that constitute cell-mediated immunity. Macrophages then present myco - bacterial antigens on their surface to the T cells. This initial immune process continues for 2 to 12 weeks; the microorganisms continue to grow until they reach sufficient numbers to fully elicit the cell-mediated immune response, which can be detected by a skin test.

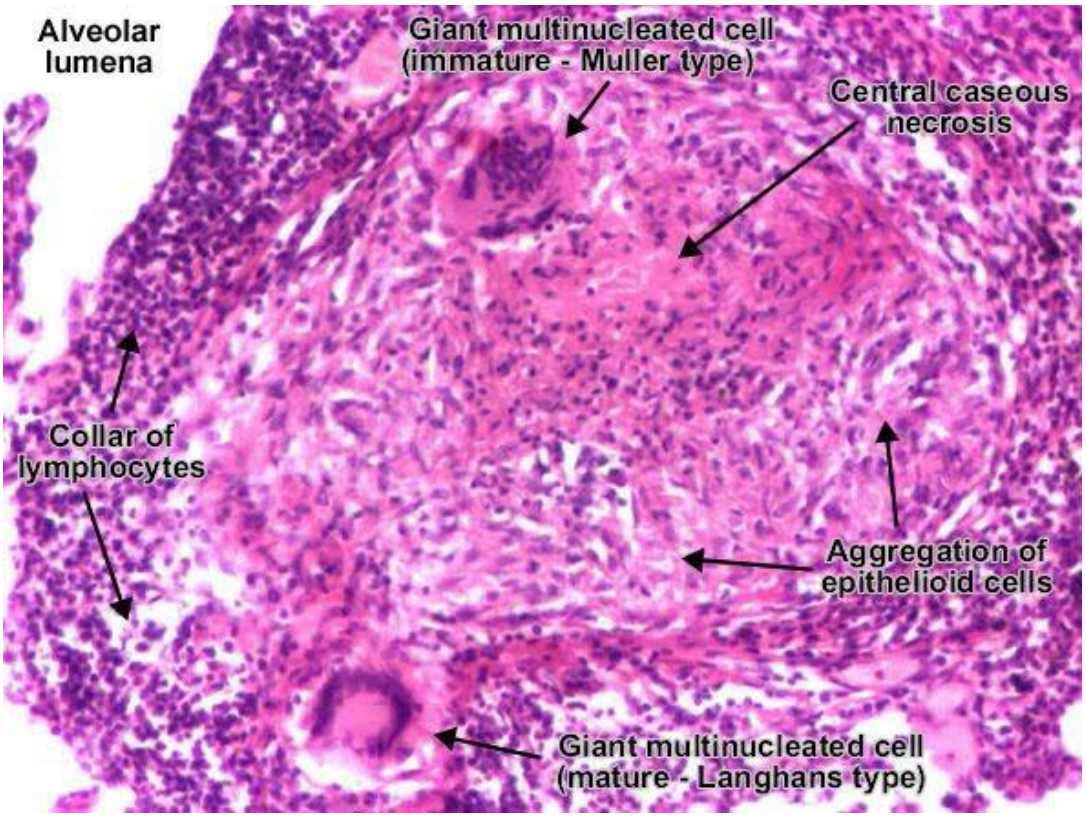
For persons with intact cell mediated immunity, the next defensive step is formation of granulomas around the *M tuberculosis* organisms. These nodular-type lesions form from an accumulation of activated T lymphocytes and macrophages, which creates a microenvironment that limits replication and the spread of the mycobacteria.

M tuberculosis organisms can change their phenotypic expression, such as protein regulation, to enhance survival. By 2 or 3 weeks, the necrotic environment resembles soft cheese, often referred to caseous necrosis, and is characterized by low oxygen levels, low pH, and limited nutrients. [6]

M. tuberculosis requires oxygen to grow. Ziehl-Neelsen staining or acid-fast staining, is used. [6]

MICROSCOPY:

Owing to their high lipid content in cell walls, M.tuberculosis cannot be identified by Gram's staining. Acid-Fast staining or Ziehl-Neelsen staining is employed to identify the organisms. M. tuberculosis is characterized by caseating granulomas containing Langhans giant cells, which have a "horseshoe" pattern of nuclei. Organisms are identified by their red color on acid-fast staining.



Acid-Fast staining showing caseating granulomas containing Langhans giant cells, which have a "horseshoe" pattern of nuclei.

DIAGNOSIS:

Tuberculin skin test

Tuberculin skin test, also called as Mantoux skin test, has been used for the diagnosis of tuberculosis for more than a century. Despite the numbers of logistic and performance problems and poor specificity, TST is still performed as a routine diagnostic method. The purified protein derivative (PPD) antigens, that are used for TST are highly homologous to

antigens of Mycobacterium bovis bacillus Calmette-Guerin (BCG) vaccine and nontuberculosis mycobacteria (NTM) antigens. The test is based on a delayed-type hypersensitivity (DTH) response to a complex cocktail of M. tuberculosis antigens, known as purified protein derivative (PPD). The induration of more than 5mm, recorded 48 to 72 hours after injection of PPD, is considered as positive. [7]

Interferon Gamma Releasing Assay (IGRA)

IFN is a cytokine that plays a critical role in resistance to Mycobacterium tuberculosis infection and MTB infected individuals respond to MTB antigen stimulation by releasing increased amounts of this cytokine from effector memory cells, methods based on measuring the IFN production by antigen stimulated human T lymphocytes have been developed. More sensitive and specific tests such as cell-mediated immunity-based interferon-gamma (IFN-γ) release assays (IGRAs) have also been developed that detect T cell responses after stimulation by two M. tuberculosis-specific antigens, early secreted antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10). Two commercial IGRAs, whole blood, ELISA-based QuantiFERON (QFN)-TB Gold assay (Cellestis Ltd., Carnegie, Australia) and peripheral blood mononuclear cell (PBMC) and enzyme-linked immunospot (ELISPOT) technology-based T SPOT-TB (Oxford Immunotec, Oxford, UK) test have also been developed and approved by Food and Drug Administration (FDA) for detecting LTBI. [7]

C. MEDICINAL CHEMISTRY- AN INTRODUCTION AND A HISTORY

Medicinal chemistry, "tries to be based on the ever increasing hope that biochemical rationales for drug discovery may be found" [8]. Pharmaceutical Chemistry is a subject concerned primarily with modification of structures having known physiologic and pharmacologic effects and with analysis of drugs [8]

In the so-called pre-scientific period, natural products having a history as folk remedies were in use, but little of the drug therapy of today is based on these remedies. Some of the natural products currently used either themselves or as derivatives, were often used originally for other purposes, such as arrow poisons, part of religious or other rituals, or even cosmetics. Examples of such products include opium, belladonna, cinchona bark, ergot, curare, nutmeg, calabar bean, foxglove and squill. [8]

Development of drug therapy could not progress until knowledge of anatomy and physiology had reached the status of science. The empiric observations of Harvey and Sydenham were of great importance to this development in the seventeenth century. The work of Magendie (1783-1855), an instructor of anatomy in Paris, probably represents the earliest application of the experimental medicine. [8]

Following the French Revolution, the study and classification of diseases made considerable progress. Ineffective remedies were recognized and discarded. In Germany, much of the drug discovery in the nineteenth century resulted from the investigations in the chemical industries mainly concerned with dyes. It was not until twentieth century, that the search for new drug entities or classes took place in university laboratories. [8]

The concept of the drug-receptor interaction has undergone much modification from1960s to the 1990s. The use of computer graphics to portray drug-receptor interaction has also been a notable development during the past decade. [8]

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

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

The discovery or design of a new drug not only requires a discovery or design process, but also synthesis of the drug, a method of administration, the development of tests and procedures to establish how it operates in the body and safety assessment. Drug discovery also requires fundamental research into biological and chemical nature of the diseased state. [9]

D. DRUG DESIGN PROFILE:

The phrase "drug design" is to some extent a misnomer [10]. What is really meant by drug design is ligand design (i.e., design of a small molecule that will bind tightly to its target). Drug design involves the design of small molecules that are complementary in shape and charge to the bimolecular target with which they interact and therefore will bind to it. Drug design, sometimes referred to as rational drug design or more simply rational design is the inventive process of finding new medications based on the knowledge of a biological target. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit.

There are two major types of drug design. The first is referred to as ligand-based drug design and the second, structure-based drug design. [10]

TYPES OF DRUG DESIGN:

LIGAND BASED DRUG DESIGN:

Ligand-based drug design or indirect drug design relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. Quantitative structure-activity relationship (QSAR), in which a correlation between calculated properties of molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analogs. [10]

STRUCTURE BASED DRUG DESIGN:

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. Using the structure of the biological target, drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. As experimental methods such as X-ray crystallography and NMR develop, the amount of information concerning 3D structures of biomolecular targets has increased. Current methods for structure-based drug design can be divided roughly into two categories. The first category is about finding ligands for a given receptor, which is usually referred as database searching. In this case, a large number of potential ligand molecules are screened to find those fitting the binding pocket of the receptor. The key advantage of database searching is that it saves synthetic effort to obtain new lead compounds. Another category of structure-based drug design methods is about building ligands, which is usually referred as receptor-based drug design . In this case, ligand molecules are built up within the constraints of the binding pocket by assembling small pieces in a stepwise manner. These pieces can be either individual atoms or molecular fragments. [10]

ACTIVE SITE IDENTIFICATION:

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

- **Hydrophobic atom**: All carbons in hydrocarbon chains or in aromatic groups.
- **H-bond donor**: Oxygen and nitrogen atoms bonded to hydrogen atom(s).
- **H-bond acceptor**: Oxygen and sp^2 or sp hybridized nitrogen atoms with lone electron pair(s).
- **Polar atom**: Oxygen and nitrogen atoms that are neither H-bond donor nor H-bond acceptor, sulfur, phosphorus, halogen, metal, and carbon atoms bonded to hetero-atom(s). [10]

SCORING METHOD:

Structure-based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying accepted principles of molecular recognition. The structure-based drug design is that a good ligand molecule should bind tightly to its target. One of the most important principles for designing or obtaining potential new ligands is to predict the binding affinity of a certain ligand to its target. [10]

SCREENING AND DESIGN:

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

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

- increase activity against the chosen target
- reduce activity against unrelated targets

- improve the drug likeness or ADME properties of the molecule.

This process will require several iterative screening runs, during which, it is hoped, the properties of the new molecular entities will improve, and allow the favoured compounds to go forward to in vitro and in vivo testing for activity in the disease model of choice. A range of parameters can be used to assess the quality of a compound, or a series of compounds, as proposed in the Lipinski's Rule of Five. Such parameters include calculated properties such as cLogP to estimate lipophilicity, molecular weight, polar surface area and measured properties, such as potency, in-vitro measurement of enzymatic clearance etc. Some descriptors such as ligand efficiency (LE) and lipophilic efficiency (LiPE) combine such parameters to assess druglikeness.

Other methods, such as virtual high throughput screening, where screening is done using computer-generated models and attempting to "dock" virtual libraries to a target, are also often used.

Another important method for drug discovery is drug design, whereby the biological and physical properties of the target are studied, and a prediction is made on the sorts of small molecules that might (e.g.) fit into an active site. One example is fragment-based lead discovery (FBLD). Novel pharmacophores can emerge very rapidly from these exercises. In general, computer-aided drug design is often used to try to improve the potency and properties of new drug leads. [8]

COMPUTER-AIDED DRUG DESIGN

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

Molecular mechanics methods may also be used to provide semi-quantitative prediction of the binding affinity. Also, knowledge-based scoring function may be used to provide binding affinity estimates.

Drug design with the help of computers may be used at any of the following stages of drug discovery: [10]

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**: optimization of other pharmacokinetic properties while maintaining affinity.

In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and a compound's 3D structure information are used for analysis. [10]

MOLECULAR PROPERTY PREDICTION:

All the data set molecules were subjected to the toxicity risk assessment by using Osiris program, which is available online. The OSIRIS property Explorer shown in this page is an integral part of Actelion's in house substance registration system. It allows drawing chemical structures and also calculates various drug relevant properties whenever a structure is valid. Prediction results are color coded in which the red colour shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green colour indicates drug-conform 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 currently drawn structure is a valid chemical entity. Toxicity risk alerts are an indication that the drawn structure may be harmful concerning the risk category specified. The prediction process relies on a precomputed set of structural fragment that give rise to toxicity alerts in case they are encountered in the structure currently drawn. These fragment lists were created by rigorously shreddering all compounds of the RTECS database known to be active in a certain toxicity class like mutagenicity, Tumorigenicity, Irritating effects and Reproductive effects. [11]

clogP PREDICTION:

The logP value of a compound, which is the logarithm of its partition coefficient between n-octanol and water $\log(c_{octanol}/c_{water})$, is a well established measure of the compound's hydrophilicity. Low hydrophilicities and therefore high logP values cause poor absorption or permeation. clogP value must not be greater than 5.0 for permeability. [11]

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. [11]

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. [11]

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**". [11]

LIPINSKI'S RULE OF FIVE: **[12,13]**

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

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

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

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

E. ENZYME PROFILE

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

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

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

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

DPA is the sole known donor substrate for a series of membrane-embedded arabinosyltransferases, including the ethambutol targets EmbC, EmbA, and EmbB (9). Essentiality of DPA supply and lack of alternative synthetic pathways position DprE1, which is highly conserved in mycobacteria, and DprE2 at a critical intersection of cell wall biosynthesis, a notion confirmed by transposon mutagenesis. This situation has led DprE1 as a magic drug target. [14]

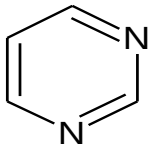
General annotation [15]

Gene name	dprE1
Rv number	Rv3790
Type	CDS
Function	Together with DPRE2|Rv3791, catalyzes epimerization of decaprenylphosphoryl ribose (DPR) to decaprenylphosphoryl arabinose (DPA) in arabinan synthesis
Product	Decaprenylphosphoryl-beta-D-ribose 2'-oxidase
Molecular mass (Da)	50163.2
Isoelectric point	7.769
Gene length (bp)	1386
Protein length	461
Location (kb)	4235.78

Functional category	lipid metabolism

Proteomics	Identified in the membrane fraction of M. tuberculosis H37Rv using 1D-SDS-PAGE and uLC-MS/MS (See Gu et al., 2003). Identified in the cell membrane fraction of M. tuberculosis H37Rv using 2DLC/MS (See Mawuenyega et al., 2005). Identified by mass spectrometry in Triton X-114 extracts of M. tuberculosis H37Rv (See Malen et al., 2010). Identified by mass spectrometry in the membrane protein fraction and whole cell lysates of M. tuberculosis H37Rv but not the culture filtrate (See de Souza et al., 2011). Translational start site supported by proteomics data (See Kelkar et al., 2011).
Mutation	essential gene by Himar1-based transposon mutagenesis in H37Rv strain (see Sassetti et al., 2003). Essential gene for in vitro growth of H37Rv, by sequencing of Himar1-based transposon mutagenesis (See Griffin et al., 2011).

F. BIOLOGICAL ACTIVITIES OF DIHYDYDROPYRIMIDINONES

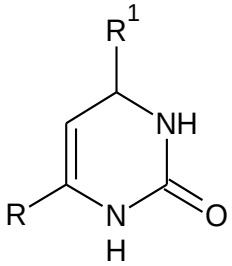


Pyrimidine is a six membered heterocyclic ring having two nitrogen (N) atoms in their ring. Pyrimidine having molecular formula of C4H4N2 Pyrimidine is a colourless compound, having melting point 22.50C and boiling point 1240C. Pyrimidines can be considered best as derivatives of pyridine and, to a lesser extent, as cyclic amidines. Pyrimidine, which accepts two protons under extremely acidic conditions (pKa1 1.3 pKa2 6.9), is much weaker base than pyridine (pKa 5.23), imidazole (pKa 7.2), or amidines in general.

In medicinal chemistry pyrimidine derivatives have been very well known for their therapeutic applications. The presence of a pyrimidine base in thymine, cytosine and uracil, which are the essential building blocks of nucleic acids, DNA and RNA is one of the possible reasons for their activities. [16]

DIHYDROPYRIMIDINONES

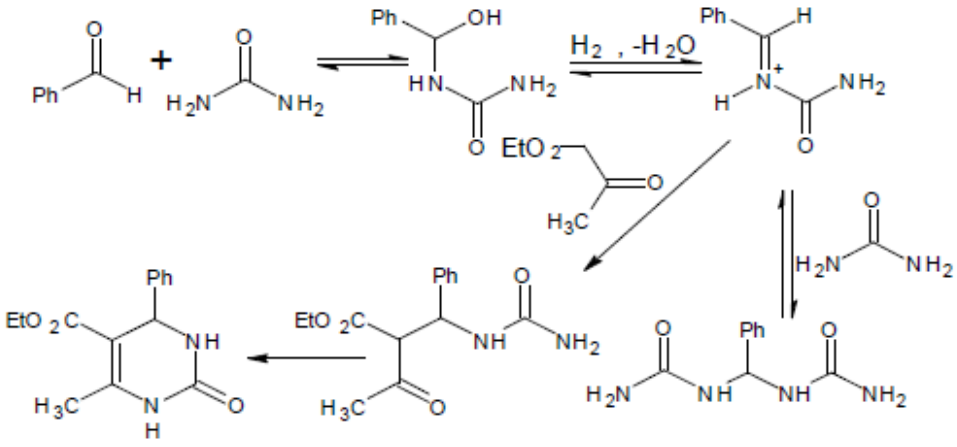
The first synthesis of dihydropyrimidinones was reported by Biginelli in 1893.



In 1893, P Bigenelli reported on the acid catalyzed cyclo-condensation reaction of ethyl acetoacetate, benzaldehyde, and urea . The reaction was carried out by simply heating a mixture of the three components dissolved in ethanol with a catalytic amount of hydrochloric acid at reflux temperature. The product of this novel one-pot, three components synthesis that precipitated on cooling of the reaction mixture was identified as 3,4-dihydropyrimidin-2*(1H)*-one and this reaction is known as “Biginelli reaction”, or “Biginelli Condensation”, or as “Biginelli dihydropyrimidine synthesis”. [17]

The first mechanism for the synthesis of dihydropyrimidine (Biginelli reaction) were conducted by Folkers and Johnson in 1933. In 1973, a second mechanistic proposal was suggested by Sweet and Fissekis. In 1997, the mechanism was provided by Kappe whose proposal is currently the accepted mechanism for the Biginelli reaction.

Kappe35(1997)

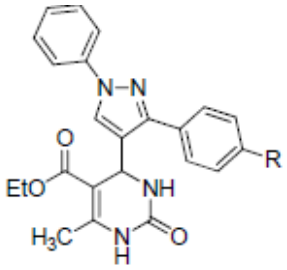


BIOLOGICAL ACTIVITY

Biginelli type dihydropyrimidines have received a considerable amount of attention, due to interesting pharmacological property associated with calcium channel blocker activity, antihypertensive activity, antibacterial and antimicrobial activity.

ANTI-TUBERCULAR ACTIVITY :

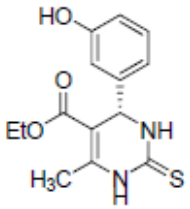
Dihydro pyrimidines also were evaluated for their antitubercular activity against Mycobacterium tuberculosis H37Rv. ethyl 4- [3-(4-fluorophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5 carboxylate and ethyl 4-[3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl]-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **63** were shown to be the most active compounds and found to be more potent than isoniazid. [18]



R=F, NO_2

ANTI TUMOR ACTIVITY;

Monastrol is the first Biginelli compound which has excellent anticancer activity, further a series of compounds for their ability to inhibit Eg5 activity has been investigated using two in vitro steady-state ATPase assays (basal and microtubule-stimulated) as well as a cell-based assay. [18]

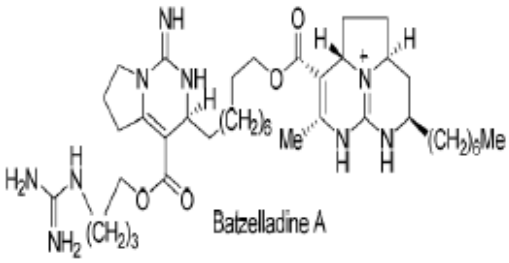


(S)-monastrol

33

ANTI HIV AGENTS:

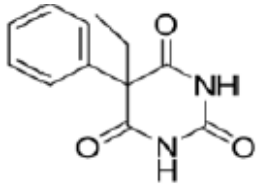
Batzelladine derivatives of DHPMs obtained from marine natural source have promising anti HIV activity. These low molecular weight derivatives inhibit the binding of HIVgp-120 to CD4 cells. [18]



Batzelladine A

ANTI EPILEPTICS:

Phenobarbital is well known drug for epilepsy. Biginelli compounds have similar structural framework and have shown promising anti-epilepsy activity. [18]



phenobarbital

AIM AND OBJECTIVE OF THE STUDY

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

DOCKING:

Several chemical libraries containing various scaffolds will be sketched and docked against the 3D structure of DprE1. The compounds for the synthesis will be chosen based on the high G-Score and their feasibility in synthetic chemistry.

SYNTHESIS:

Based upon the Docking parameters, the following compounds will be synthesized

A. Synthesis of 4-(4-hydroxyphenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one(MBS2)
B. Synthesis of 4-(4-chlorophenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one (MBS3)
C. Synthesis of 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one (MBS 6):

CHARACTERIZATION:

The above compounds will be characterized by using Infrared Spectroscopy, Nuclear Magnetic Spectroscopy and Mass spectroscopy.

BIOLOGICAL EVALUATION:

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

REVIEW OF LITERATURE

The purpose of a literature review is to:

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

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

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

Sarah M. Batt, et al., (2012) Structural basis of inhibition of Mycobacterium tuberculosis DprE1 by benzothiazinone inhibitors.[14]

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

Donald R Ronning., et, al., (2013) Targeting the mycobacterial envelope for tuberculosis drug development[20]

Véronique Dartois., et, al.,(2012) reviewed the Role of Transport Mechanisms in Mycobacterium tuberculosis Drug Resistance and Tolerance[21]

Sarala Menon, et al, (2012), studied the Drug resistance profiles of *Mycobacterium tuberculosis* isolates to first line anti-tuberculous drugs.[22]

P.Mudassar., et al.,(2011) Had a Brief review on Multi Drug resistant Mycobacterium tuberculosis.[23]

Christian Lienhardt., et al.,(2010) Had a review on New drugs and new regimens for the treatment of tuberculosis: review of the drug development pipeline and implications for national programmes.[24]

Sarah L. Kinnings., et al,(2010), reviewed the Mycobacterium tuberculosis Drugome and Its Polypharmacological Implications.[25]

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

Yaghoub Sarrafi., et al., (2013), Mesoporous SBA-15 nanoparticles: An efficient and eco-friendly Catalyst for onepot synthesis of 3, 4-dihydropyrimidin-2(1H)-ones under solvent-free conditions. [26]

Shrivastav Pranav S., et al.,(2013)Application of La(III)-, Pr(III)-, Nd(III)- AND Sm(III)-polychelates as catalyst for biginelli reactions. [27]

Manjusha V. Yadav., et al.,(2013), Microwave assisted Biginelli's synthesis of 3,4-dihydropyrimidin-2(1*H*)-ones using 1, 3, 5-triazine-2, 4, 6-triyltrisulfamic acid as heterogeneous and recyclable catalyst[28]

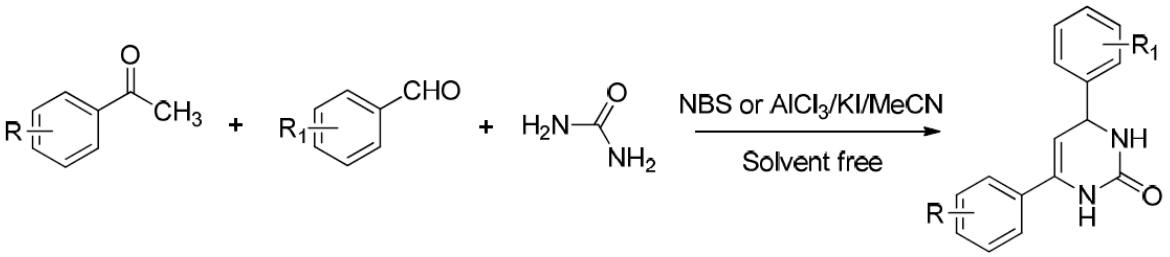
IBRAHIM A. I. ALI., et al., (2013) synthesized, One-pot Synthesis of 3,4-dihydropyrimidin-2-(1*H*)-one / thiones, bearing Sugar Side Chain Using Samarium Chloride as a Catalyst. [29]

Rajendra P. Pawar., et al., (2013), an efficient synthesis of 4-aryl-substituted 3,4-dihydropyrimidin-2(1*h*)-ones using nanocomposite ferrite catalyst. [30]

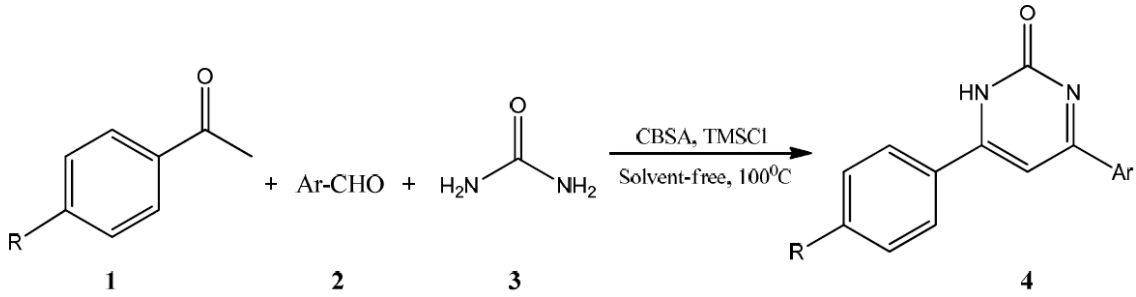
Boudjemaa Boumoud., et al., (2013) synthesized a One-Step Multicomponent Synthesis of2-Oxo-Quinolin-3-yl-Dihydropyri-midinoneand 2-oxo-1,2-Dihydroquinolin-3-yl-tetrahydroquinazolinedioneDerivatives. [31]

K.Padmaja., et al., (2013) reviewed biological activities of dihydro pyrimidinones / thiones. [32]

Jagir S. Sandhu., et al.,(2012), had review on Past, present and future of the Biginelli reaction: a critical perspective. [18]



Srinivasa Rao Jetti., et al., (2012),synthesized a Carbon-Based Solid Acid as an Efficient and Reusable Catalyst for the Synthesis of 4,6-Diarylpyrimidin-2(1*H*)-ones under solvent-free conditions. [33]



M. Jalali., et al,(2012) studied and reported Antimicrobial evaluation of some novel derivatives of 3,4- dihydropyrimidine-2(1H)-one.[34]

Ridha BEN SALEM.,et al.,(2012) synthesized some 3,4-dihydropyrimidinones catalyzed by ammonium chloride or montmorillonite ksf without solvent under ultrasonic irradiation. [35]

Mahesh P. Davadra., et al.,(2012) An efficient three component one-pot synthesis of some new tetrahydroindeno-[1,2-*d*]pyrimidinone and dihydro-*1H*-indeno[1,2-*d*]pyrimidine derivatives using Antimony (III) chloride as a catalyst and investigation of their antimicrobial activity. [36]

Shivaji Pandit., et al.,(2009) synthesized some 5 unsubstituted -3,4-dihydropyridine-2-(1h)- ones using nbs as a catalyst under solvent free conditions. [37]

Majid M. Heravi., et al., (2008), Three-component one-pot synthesis of 4,6-diarylpyrimidin-2(1H)-ones under solvent-free conditions in the presence of sulfamic acid as a green and reusable catalyst. [38]

D. Subhas Bose., et al.,(2005) Had a New protocol for Biginelli reaction-a practical synthesis of Monastrol. [39]

Jung Yun Do., et al., (2008), Synthesis of 3,4-Dihydropyrimidin-2(*1H*)-ones Using HClO4-SiO2 as a Heterogeneous and Recyclable Catalyst. [40]

M. P. Kaushik., et al.,(2007) Synthesized 4-Aryl Substituted 3,4-Dihydropyrimidinones Using Silica-chloride Under Solvent Free Conditions. [41]

K. VIJAY, S. GANAPATY., et al.,(2010), Synthesis, Characterization and Biological Evaluation of Some Dihydropyrimidinones [42]

S. Kakaei., et al.,(2010), Green procedure for synthesis of 3, 4 dihydropyrimidinones using 12-molybdophosphoric acid, as a catalyst and solvent free condition under microwave irradiation [43]

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

T.S. Chitre, et al., (2011), Design, Synthesis, Docking and Anti-mycobacterial activity of some novel thiouracil derivatives as thymidine monophoshate kinase (TMPKmt) inhibitors.[44]

Himaja.M., et al., (2011), Synthesis, Docking studies and Antioxidant activity of Linear Tetrapeptide Fayv.[45]

Deepak. D. Borkar., et al.(2012), Design and Synthesis of *p*-hydroxybenzohydrazide Derivatives for their Antimycobacterial Activity. [46]

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

David A. J. Moore., et al., (2008), Inter- and Intra-Assay Reproducibility of Microplate Alamar Blue Assay Results for Isoniazid, Rifampicin,Ethambutol, Streptomycin, Ciprofloxacin, and Capreomycin Drug Susceptibility Testing of Mycobacterium tuberculosis. [47]

Todd P. Primm., et., al(2007), Recent Advances in Methodologies for the Discovery of Antimycobacterial Drugs.[48]

MATERIALS AND METHODS

1. DOCKING:

A. PROTEIN PREPARATION:

A typical PDB file consists only of heavy metals, can contain waters, cofactors and metal ions and can be multimeric. The structure generally has no information on bond orders, topologies, or formal atomic charges. Terminal groups can also be misaligned, because the X-ray structure analysis cannot easily distinguish between O and NH2 ion. Ionization and tautomeric states are usually unassigned. GLIDE® calculations use an all atom force-field for accurate energy evaluation. Thus, GLIDE® requires bond orders and ionization states to be properly assigned and performs better when side chains are reoriented when necessary and steric clashes are relieved. [49,50]

The steps by which protein preparation was carried out:

- The ligand/protein co crystallized structure, from PDB was imported into maestro. The preparation component of a protein preparation facility requires an identified ligand.

- The protein-ligand complex was identified for its form as dimer or other multimer containing duplicate binding sites and duplicate chains that were redundant, redundant binding sites and the associated chains were removed by picking and deleting molecules or chains.
- Located waters that need to be kept, and others were then deleted. The waters that bridge between the ligand and protein were sometime retained and all the other waters (except those coordinated to metals) were deleted. If waters are added then hydrogen would be automatically added and the orientations of water molecules were checked once again.
- Adjust the protein, metal ions, and cofactors were adjusted. Problems in the PDB protein structure that need to be repaired are done. Covalent bonds from metal ions to the protein are changed to zero-order bonds, and the formal charges on the metal and the ligating groups are adjusted to appropriate values.

- A restrained minimization of the protein structure run reorients side chain hydroxyl groups and alleviates potential steric clashes. The minimization is restrained to the input protein coordinates by a user-selected RMSD tolerance.
- The ligand bond orders and formal charges were adjusted.

B. LIGAND PREPARATION: [49,51]

Ligand preparation is designed to prepare high quality, 3D structures for large numbers of drug like molecules. The structures that are docked must have actual ligand structures and should meet the following conditions:

- They must be three dimensional.
- They must have realistic bond lengths and bond angles.
- They must each consist of a single molecule that has no covalent bonds to the receptor, with no accompanying fragments, such as counter ions and solvent molecules.
- They must have all their hydrogens.
- They must have an appropriate protonation state for physiological pH values (around 7).

The LigPrep process consists of a series of steps that perform conversions, apply corrections to the structures, eliminate unwanted structures, and optimize the structures. The simplest use of LigPrep produces a single low-energy 3D structure with correct chiralities for each successfully processed input structure. LigPrep can also produce a number of structures from each input structure with various ionization states, tautomers, stereochemistries, and ring conformations, and eliminate molecules using various criteria including molecular weight or specified numbers and types of functional groups present.

C. RECEPTOR GRID GENERATION: [49,51]

The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. For receptors that adopt more than one conformation on binding, grids must be prepared for each conformation to ensure that possible actives are not missed. The receptor grid defines the receptor structure by excluding any co-crystallized ligand that might be present, determine the position and the size of the active site as it will be represented by receptor grids, set up glide constraints, and set up

flexible hydroxyl groups. Receptor grid generation requires a prepared structure: an all-atom structure with appropriate bond orders and formal charges.

D. LIGAND DOCKING:

Glide ligand docking jobs require a set of previously calculated receptor grids and one or more ligand structures. The ligand structures must satisfy the conditions listed above. Information on setting up grid generation jobs is given under. Preparation of the ligands before docking is strongly recommended. LigPrep or macro Model can be used to prepare ligands. If a correct Lewis structure cannot be generated for a ligand, it is skipped by the docking job.

E. DOCKING AND SCORING FUNCTION: [49,50]

The ligands were docked with the active site using the 'Extra precision' Glide algorithm. Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. Final scoring of docked ligand is carried out on the energy-minimized poses Glide Score scoring function. Glide Score is based on ChemScore, but includes a stericclash term and adds buried polar terms devised by Schrödinger to penalize electrostatic mismatches.

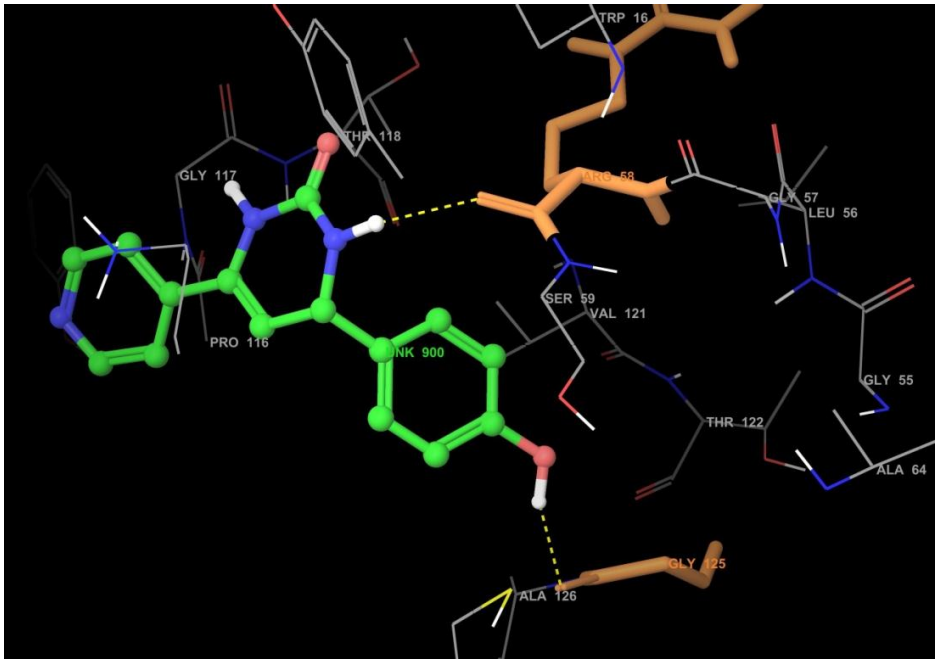
GScore = 0.065*vdW + 0.130*Coul + Lipo + Hbond + Metal + BuryP + RotB + Site Where, vdW: - Van der Waal energy; Coul: - Coulomb energy; Lipo: - Lipophilic contact term; HBond: - Hydrogen-bonding term; Metal: - Metal-binding term; BuryP: - Penalty for buried polar groups; RotB: - Penalty for freezing rotatable bonds; Site: - Polar interactions at the active site; and the coefficients of vdW and Coul are: - a = 0.065, b = 0.1

DOCKING OF dprE1:

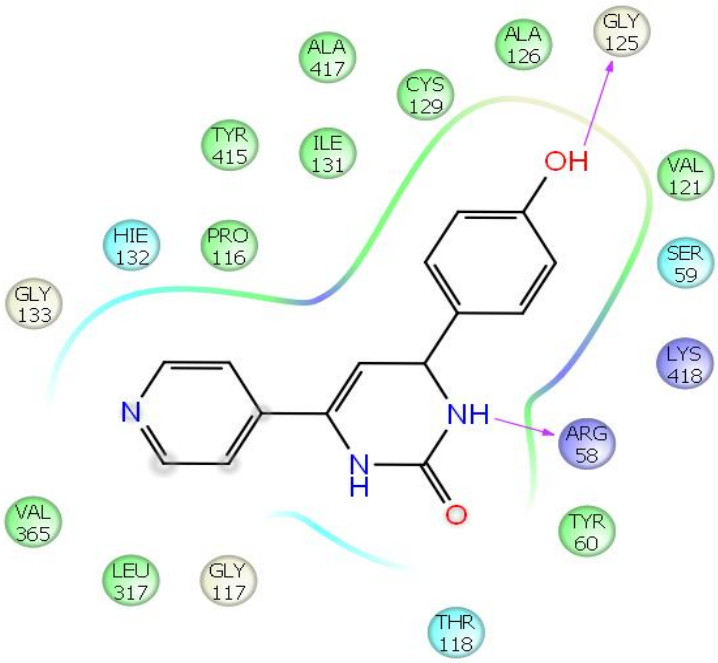
Crystal structure of (PDB code-4FDO) was used for the study. The 3D structurewas downloaded from the Protein Data Bank (PDB) and loaded to the Maestro®Workbench. The hydrogen atoms were added to the proteins and further minimization was performed using protein preparation wizard. Structure based docking studies were carried out using GLIDE® on dprE1inhibitors and generated best docking poses. During the docking, GLIDE® initially performs a complete systematic search of the conformation, orientation, and position of a compound in the defined binding site and eliminates unwanted poses using scoring and energy optimization. The best poses were selected based on the scoring functions and quality of pose orientation within the active site amino acids. The docking scores were examined and the compounds showing high docking score were chosen for synthesis.

INTERACTIONS

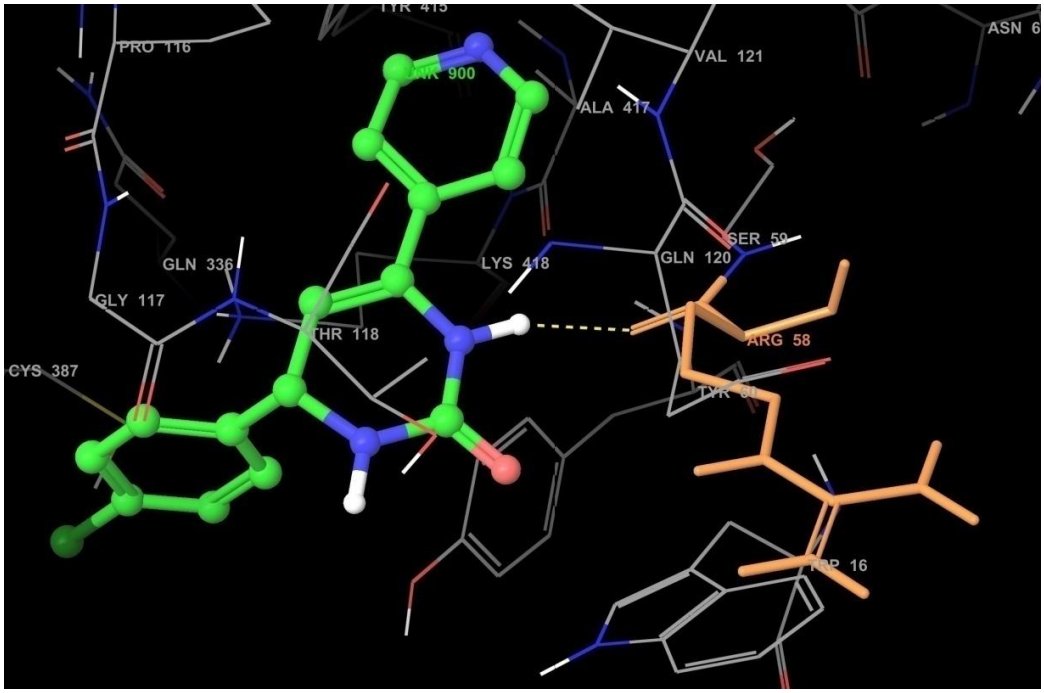
The following are the pictographic representation of the docked compounds (MBS2,MBS3, MBS6) with dprE1 respectively



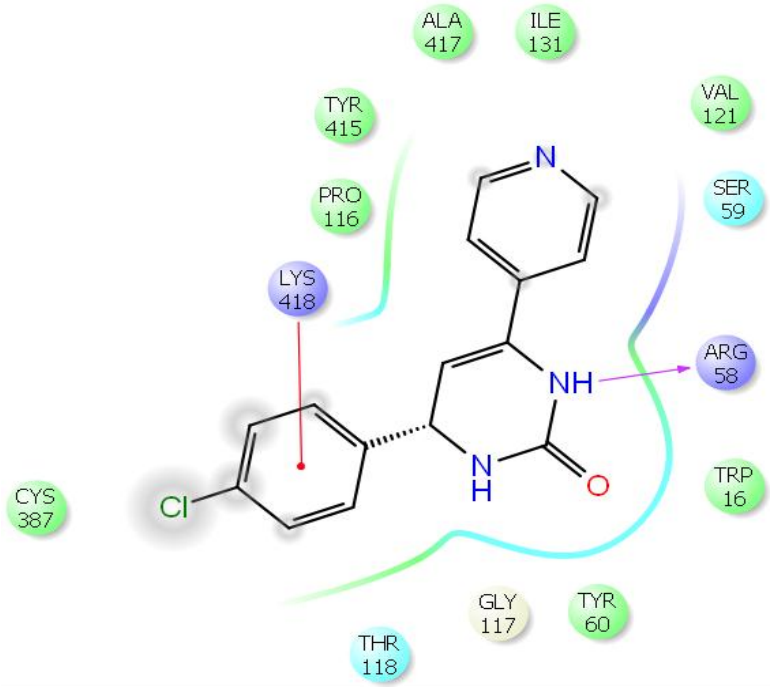
MBS2 Docked with dprE1



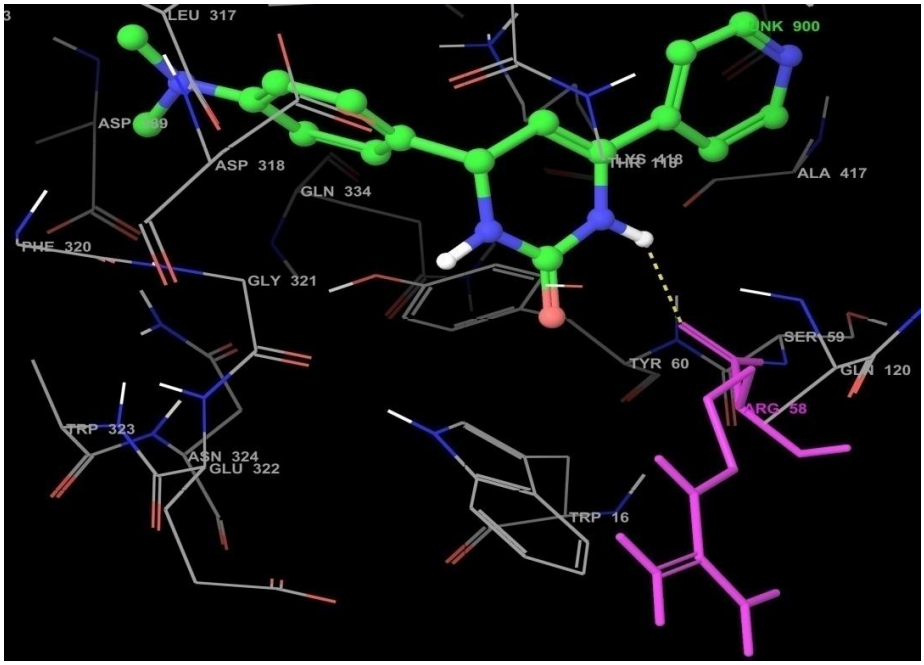
Ligand interaction diagram of MBS2



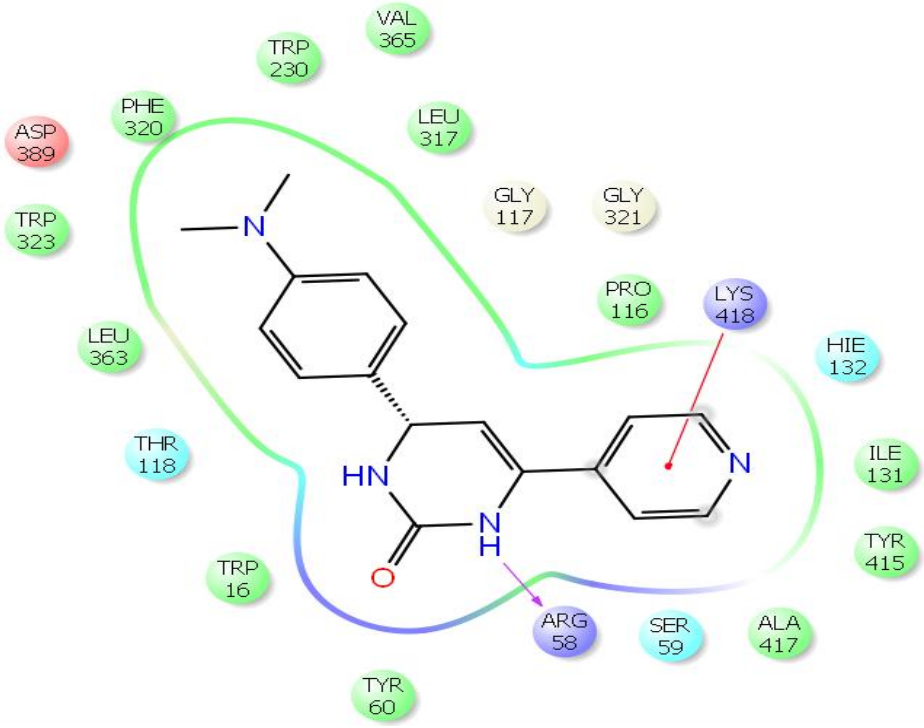
MBS3 docked with dprE1



Ligand Interaction diagram of MBS3



MBS6 docked with dprE1

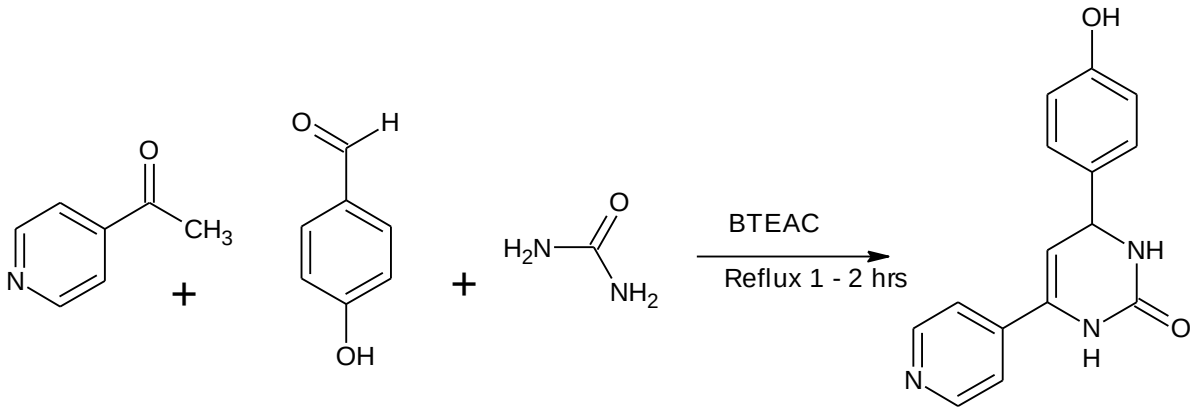


Ligand Interaction Diagram of MBS6

2. SYNTHETIC METHDOLOGY

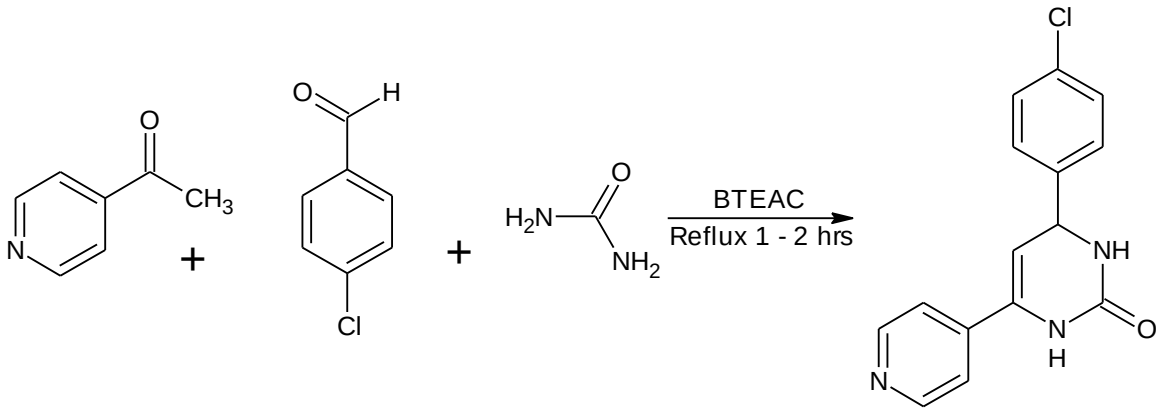
Synthesis of 4-(4-hydroxyphenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1*H*)-one(MBS2)

A mixture containing 1mmol of 4-Acetyl pyridine, 1 mmol of 4 hydroxy benzaldehyde, urea 1.5 mmol and benzytriethylammonium chloride 10 mol% was refluxed for 2hrs, completion of the reaction was confirmed by TLC, then the reaction mixture was poured onto crushed ice and stirred for 5-10min. The solid separated was filtered under suction, washed with ice-cold water, and then recrystallized from hot ethanol.



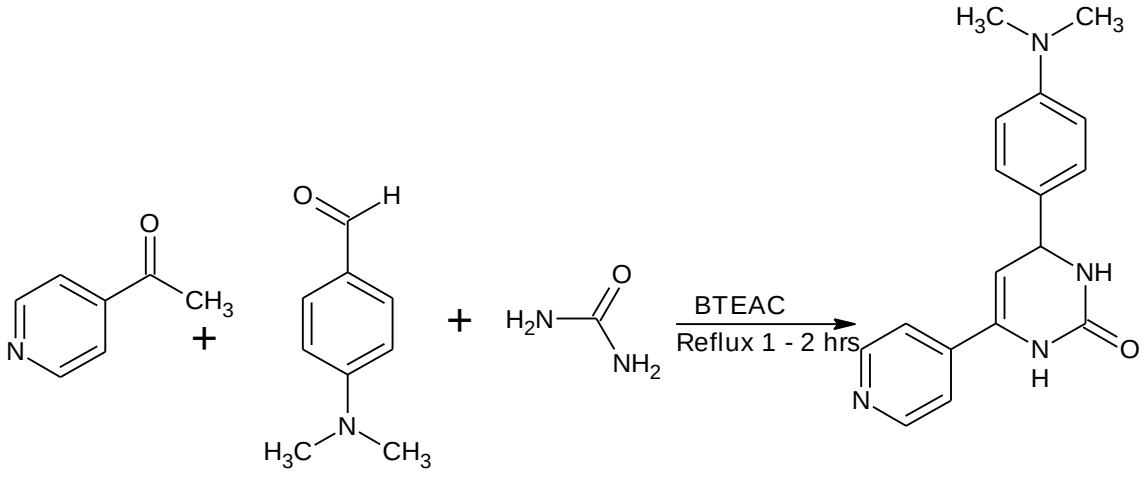
Synthesis of 4-(4-chlorophenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1*H*)-one (MBS3)

A mixture containing 1mmol of 4-Acetyl pyridine, 1 mmol of 4 chloro benzaldehyde, urea 1.5 mmol and benzytriethylammonium chloride 10 mol% was refluxed for 2hrs, completion of the reaction was confirmed by TLC, then the reaction mixture was poured onto crushed ice and stirred for 5-10min. The solid separated was filtered under suction, washed with ice-cold water, and then recrystallized from hot ethanol.



Synthesis of 4-(4-dimethylamino)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1*H*)-one (MBS6)

A mixture containing 1mmol of 4-Acetyl pyridine, 1 mmol of dimethyl aminobenzaldehyde, urea 1.5 mmol and benzytriethylammonium chloride 10 mol% was refluxed for 2hrs, completion of the reaction was confirmed by TLC, then the reaction mixture was poured onto crushed ice and stirred for 5-10min. The solid separated was filtered under suction, washed with ice-cold water, and then recrystallized from hot ethanol.



JUSTIFICATION OF PURITY

A. THIN LAYER CHROMATOGRAPHY:

Precoated aluminium TLC plates were used. Solutions of the reactants and products were prepared by dissolving them in methanol.

Stationary Phase : Precoated Silica Gel Plates

Mobile Phase : Chloroform : Ethanol (3:7)

Visualization : Iodine Vapors and UV chamber

A single spot not corresponding to the parent compound was noticed and hence the purity of the synthesized compounds was justified.

B. MELTING POINT:

The melting points of synthesized compounds were determined by open tube capillary method with an aid of a melting point apparatus. The melting points were sharp and were presented uncorrected.

CHARACTERISATION STUDIES

A. INFRA RED (IR) SPECTROSCOPY:

The recrystallised compounds were subjected to IR spectral analysis for functional group identification using KBr pellet method. Infra Red Spectra was recorded using ABB (MB 3000) spectrophotometer.

B. NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY:

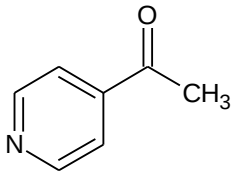
$_1$H NMR spectra was recorded on Bruker Advance III 500*MHz* NMR spectrometer. Samples were prepared by dissolving a minute quantity of pure compounds in DMSO. Chemical shifts were reported in parts per million (ppm)

C. MASS SPECTROSCOPY:

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

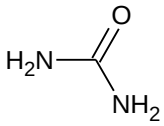
3. REACTANT PROFILE

A. 4-Acetyl Pyridine



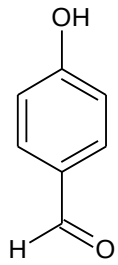
Molecular Formula:	C_7H_7NO
Formula Weight:	121.14
Boiling Point:	212^oC
Description:	Light yellow liquid

B. Urea



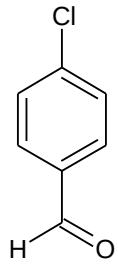
Molecular Formula	CH_4N_2O
Molecular Weight	60.06
Description	White solid
Melting point	133-135 ^oC

C. 4 Hydroxy benzaldehyde



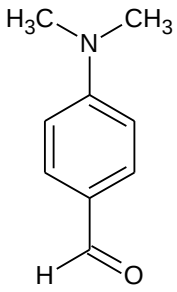
Molecular Formula	$C_7H_6O_2$
Molecular Weight	121.12
Description	Light yellow to light brown
Boiling point	191 ^oC

D. P Chloro benzaldehyde



Molecular Formula	C_7H_5ClO
Molecular Weight	140.57
Description	White crystalline solid
Melting point	46 ^oC

E. Dimethylamino Benzaldehyde



Molecular Formula	$C_9H_{11}NO$
Molecular Weight	149.19
Description	White crystalline powder
Melting point	72-75 ^oC

F**: Benzyltriethylammonium chloride**

Molecular Formula	C13H22ClN
Molecular Weight	227.77
Description	white to light yellow crystal powder
Melting point	239 ^oC

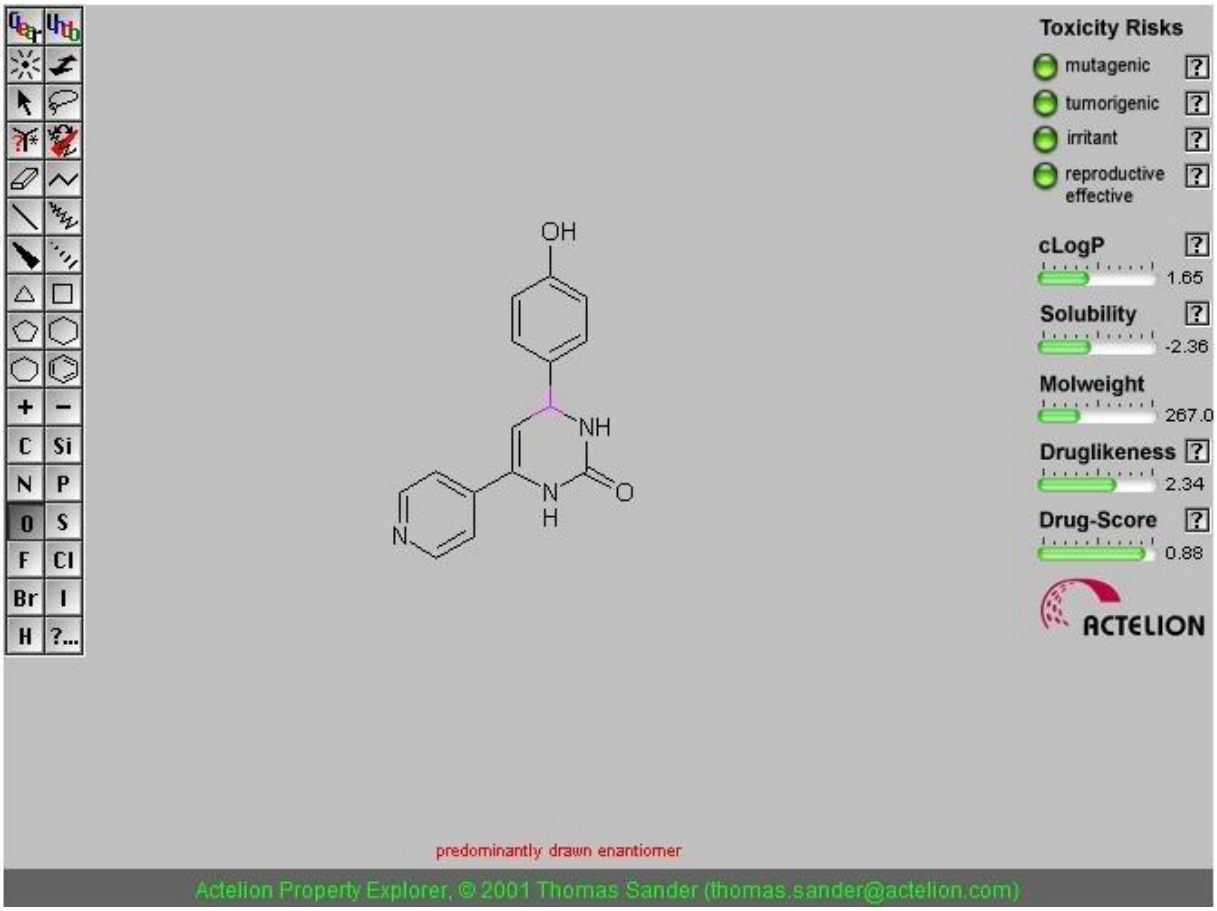
4. TOXICITY RISK ASSESSMENT

All the data set molecules were subjected to the toxicity risk assessment by using Osiris program, which is available online. The OSIRIS property Explorer shown in this page is an integral part of Actelion's in house substance registration system. It allows drawing chemical structures and also calculates various drug relevant properties whenever a structure is valid. Prediction results are color coded in which the red colour shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green colour indicates drug-conform behavior.

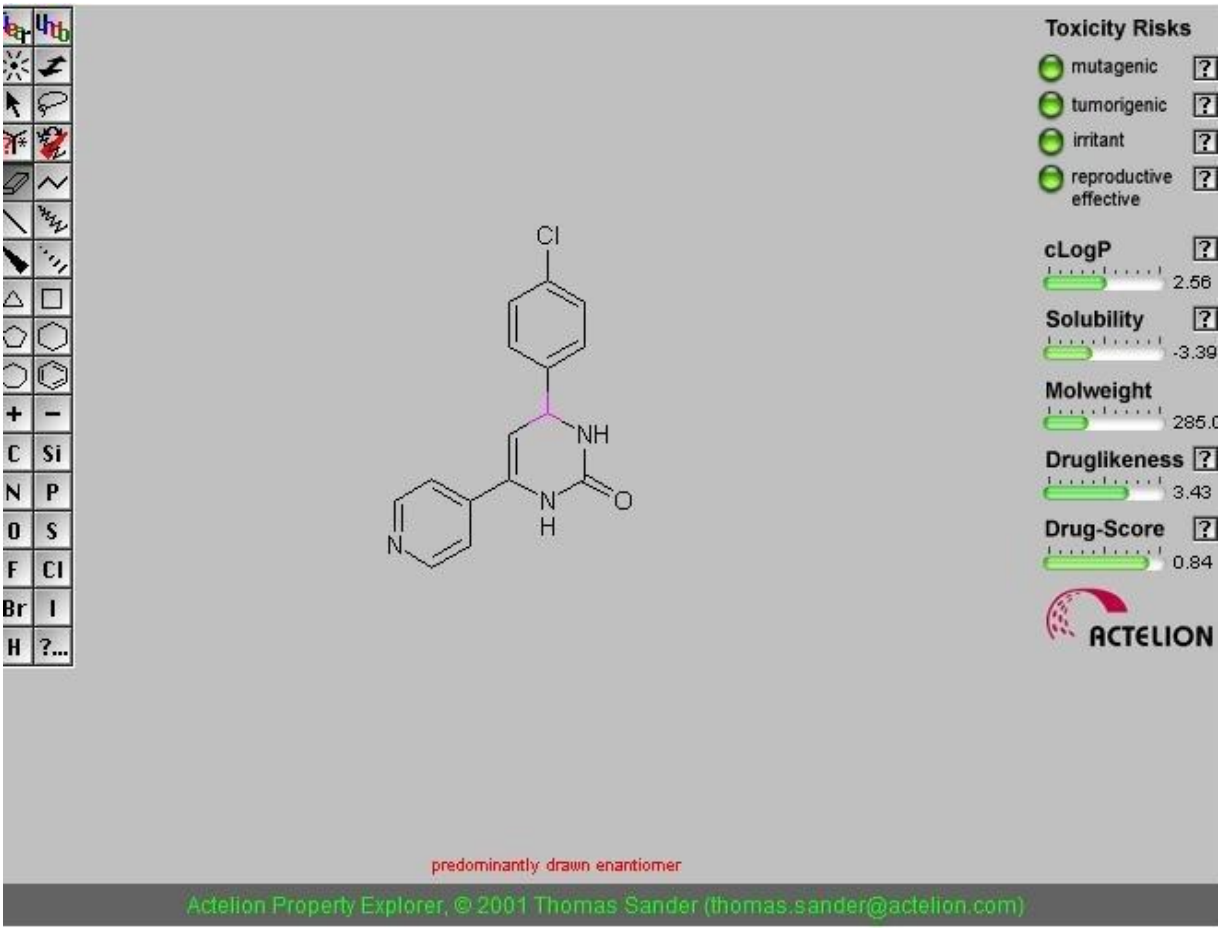
Molecular property prediction includes

- Toxicity risk assessment
- clogP predicition
- Solubility prediction
- Molecular weight
- Drug likeness prediction
- Drug likeness score

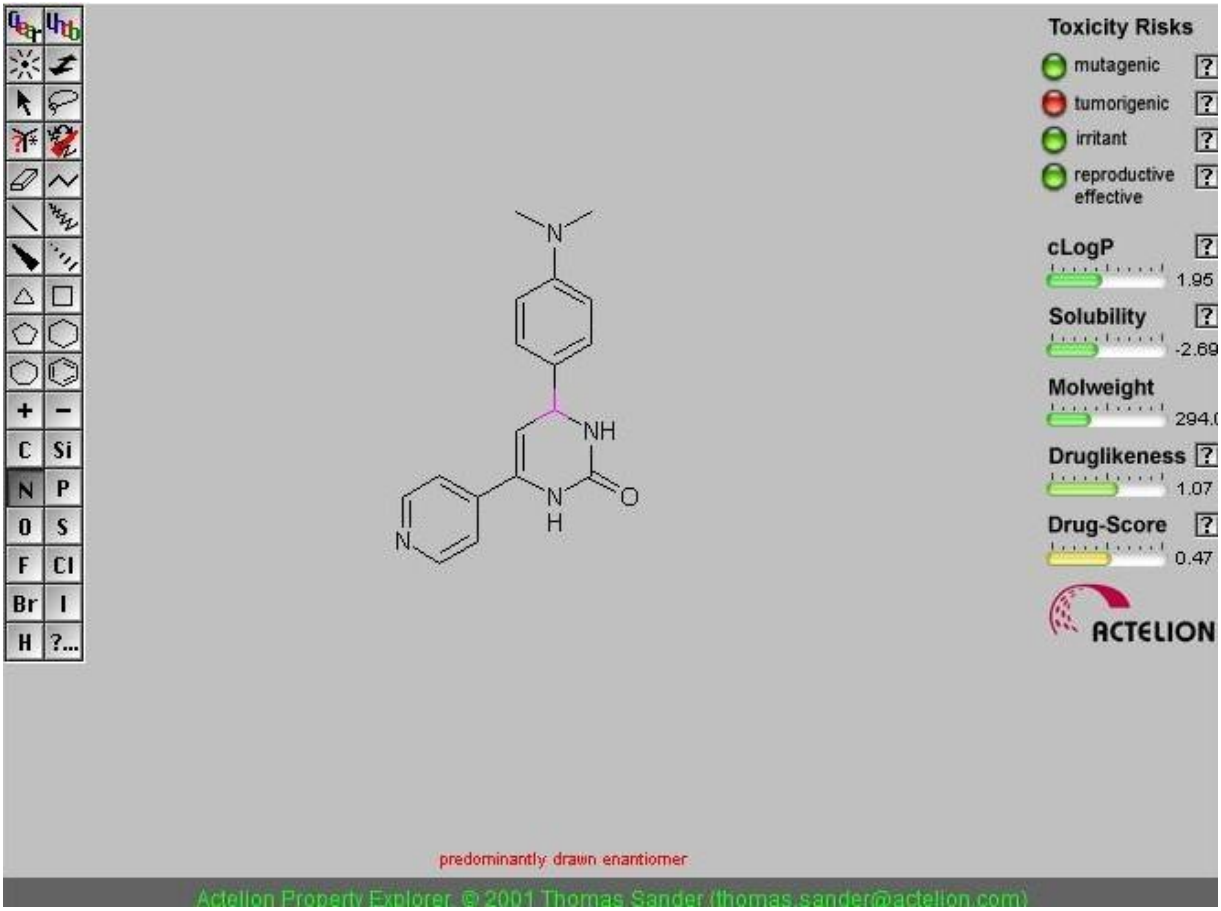
Toxicity assessment for 4-(4-hydroxyphenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one(MBS2)



Toxicity Assessment for 4-(4-chlorophenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one (MBS3)



Toxicity Assessment for Synthesis of 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one (MBS 6):



MICROBIOLOGICAL ASSAY

Microbial assays or microbiological assays is a type of bioassay and are designed to analyse the compounds or substances which have effect on micro-organisms.

Microbiological assay is defined as the determination or estimation of concentration or potency of an antibiotic by means of measuring and comparing the area of zone of inhibition or turbidity produced by test substance with that of standard over a suitable microbe under standard conditions.

So as definition says the hypothesis is that when an antibiotic is administered, there is inhibition in the growth of microbe as indicated by decrease in area of zone of microbial colony on nutrition media or decrease in turbidity due to decrease in microbial concentration.

TYPES OF MICROBIOLOGICAL ASSAY:

REDOX BASED METHODS:
Microplate Alamar blue assay.
Resazurin Microtiter Assay, REMA, or Microdilution Resazurin Assay, MRA.
Tetrazolium Dyes, Tetrazolium Microplate Assay, TEMA.

REPORTER GENE-BASED METHODS:
Green Fluorescent Protein Microplate Assay, GFPMA;
Luciferase Assays;
Beta-Galactosidase Assays.

OTHER METHODS:
BACTEC 460 TB;
Nitrate Reductase Assay, NRA;
Disk Diffusion; Visual Microbroth, or Broth Microdilution;
Malachite Green;
STC Agar; Flow Cytometry.

MICROPLATE ALAMAR BLUE ASSAY:

MABA is clearly the standard in the field for HTS of compounds against mycobacteria, and is the most widely cited. The primary reference for the method is Collins and Franzblau in 1997. In that study, MABA was effective with MTB H37Rv, H37Ra and M. avium strain ATCC 25291, a relatively virulent isolate. MABA is reliable with clinical isolates of MTB and MAC. MABA has also been applied to M. kansasii and M. malmoense, as well as M. leprae. In addition to screening and sensitivity applications, MABA has also been used in patient treatment follow-up. MABA can operate in simple colorimetric mode with visual reading (blue to pink change indicates viability, with the MIC recorded as the lowest compound concentration in wells which remain blue). Newly synthesized product was assayed in vitro for anti tubercular activity.

Evaluation of the products for their in vitro antitubercular activity against Mycobacterium Tuberculosis H37Rv using MicroplateAlamar Blue Assay (MABA) biological test was done. This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods. [48, 52]

Anti-TB activity using Alamar Blue Dye

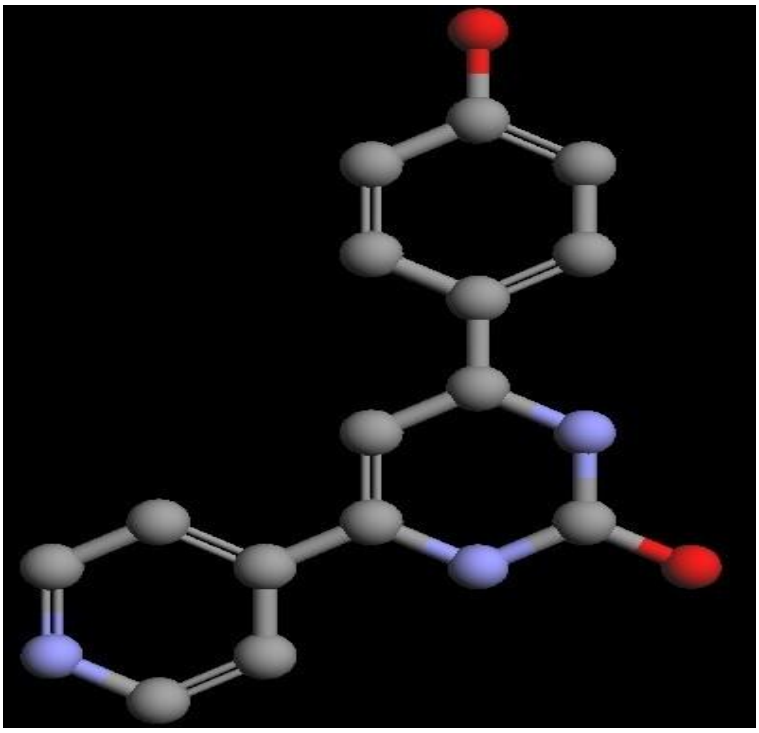
The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).

1. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.
2. Briefly, 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.
3. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
4. The final drug concentrations tested were 100 to 0.2 µg/ml.
5. Plates were covered and sealed with parafilm and incubated at 37ºC for five days.

6. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
7. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
8. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. [52,53]

PRODUCT PROFILE:

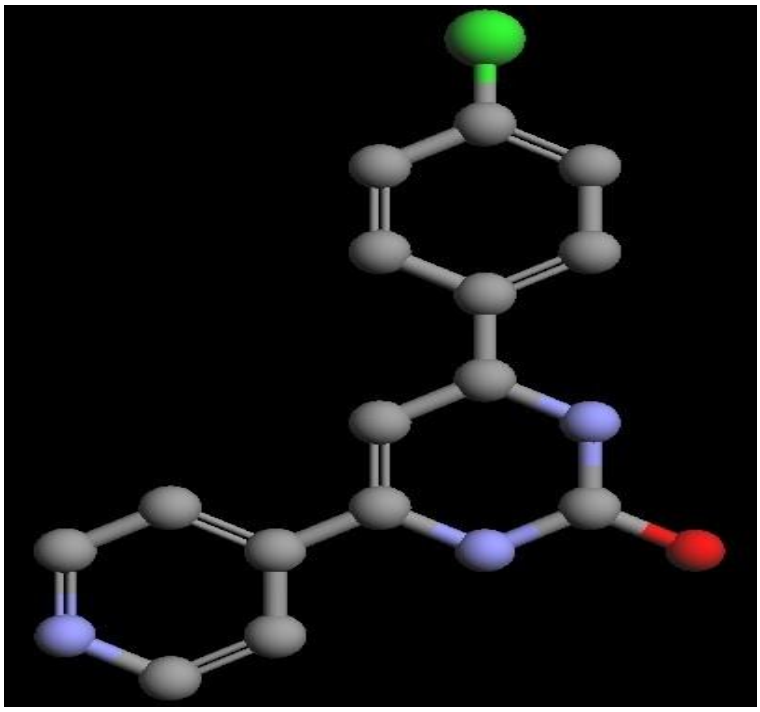
4-(4-hydroxyphenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1*H*)-one(MBS2)



PHYSICO- CHEMICAL PROPERTIES:

Description:	Brick Red colour solid
Solubility:	Soluble in Ethanol, Methanol.
Molecular Formula:	$C_{15}H_{13}N_3O_2$
Formula Weight :	267.28262
Composition:	C(67.40%) H(4.90%) N(15.72%) O(11.97%)
Molar Refractivity:	73.73 ± 0.3 cm^3
Molar Volume:	206.3 ± 3.0 cm^3
Parachor:	562.5 ± 6.0 cm^3
Index of Refraction:	1.633 ± 0.02
Surface Tension:	55.2 ± 3.0 dyne/cm
Density:	1.295 ± 0.06 g/cm^3
Polarizability:	29.23 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass:	267.100777 Da
Nominal Mass:	267 Da
Average Mass:	267.2826 Da

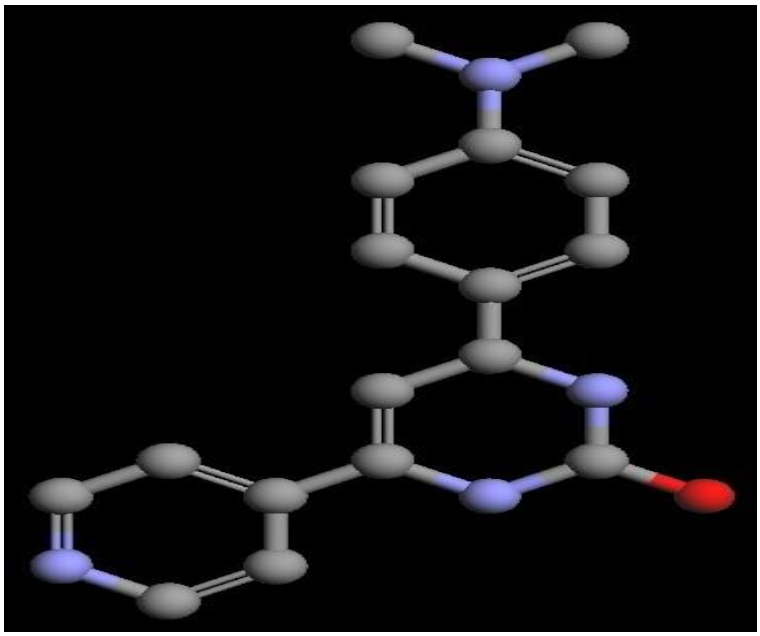
Synthesis of 4-(4-chlorophenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1*H*)-one (MBS3)



PHYSICO-CHEMICAL PROPERTIES:

Description:	Pale Brown colour solid
Solubility:	Soluble in Ethanol, Methanol, DMSO.
Molecular Formula:	$C_{15}H_{12}ClN_3O$
Formula Weight:	285.72828
Composition:	C(63.05%) H(4.23%) Cl(12.41%) N(14.71%) O(5.60%)
Molar Refractivity:	76.74 ± 0.3 cm^3
Molar Volume:	219.8 ± 3.0 cm^3
Parachor:	584.4 ± 6.0 cm^3
Index of Refraction:	1.615 ± 0.02
Surface Tension:	49.9 ± 3.0 dyne/cm
Density:	1.299 ± 0.06 g/cm^3
Polarizability:	30.42 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass:	285.06689 Da
Nominal Mass:	285 Da
Average Mass:	285.7283 Da

Synthesis of 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1*H*)-one (MBS 6):



PHYSICO- CHEMICAL PROPERTIES:

Description:	Orange color Solid.
Solubility:	Soluble in Ethanol, Methanol.
Molecular Formula	$C_{17}H_{18}N_4O$
Formula Weight	294.35102
Composition	C(69.37%) H(6.16%) N(19.03%) O(5.44%)
Molar Refractivity	86.16 ± 0.3 cm^3
Molar Volume	245.8 ± 3.0 cm^3
Parachor	651.9 ± 6.0 cm^3
Index of Refraction	1.618 ± 0.02
Surface Tension	49.4 ± 3.0 dyne/cm
Density	1.197 ± 0.06 g/cm^3
Polarizability	34.15 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	294.148061 Da
Nominal Mass	294 Da
Average Mass	294.351 Da

RESULTS AND DISCUSSION

DOCKING STUDIES:

To predict the possible binding modes and enzyme inhibition mechanism, compounds were docked onto the active sites of dprE1using GLIDE, the grid-based ligand docking with energetics software from Schrödinger. The best pose was selected based on Glide score and the favorable interactions formed between the compound and amino acid residues of the dprE1 active site. All the ligands in the complex structures showed the hydrogen bond interactions with **ARG 58, ARG 58, LYS 418.** This clearly indicates that these hydrogen bonded amino acids play a crucial role in inhibition activity. The docking scores for these compounds are reported on the table.

Rewards:

Ligand	G Score	Dock Score	Lipophilic EvdW	Phob En	Phob EnHB	Phob EnPairHB	HBond	Electro	Site map	PiCat	ClBr	Low MW
MBS2	-7.38	-	-4.17	0	-1.5	0	-1.01	-0.25	-0.17	0	0	-0.5
MBS3	-6.27	-	-3.29	-0.26	-1.5	0	-0.67	0.08	-0.37	0	0	-0.5
MBS6	-6.97	-	-4.41	-0.3	-1.5	0	-0.22	0.15	-0.38	0	0	-0.5

Penalities:

Ligand	Penalties	HB Penal	Expos Penal	Rot Penal	EpikState Penalty	Similarity	Activity
MBS2	0.01	0	0	0.21	--	-	-7.38
MBS3	0.05	0	0	0.19	--	1	-6.27
MBS6	0.02	0	0	0.18	--	1	-6.97

Comparison of Glide score of compounds with Standard Drugs:

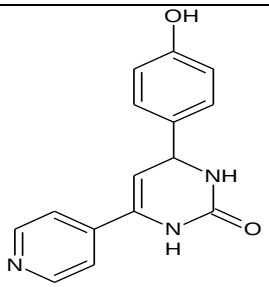
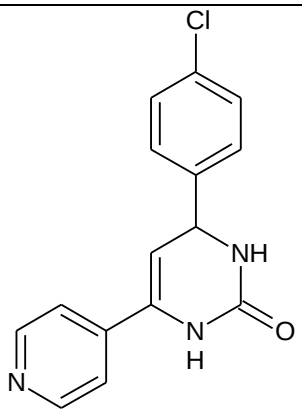
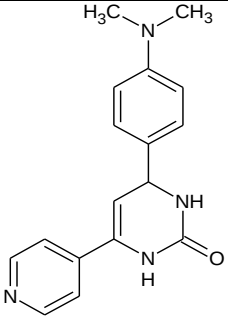
Docking score of the 3 compounds were compared with that of standard drugs, that is Pyrazinamide, Ciprofloxacin and Streptomycin, and the results were shown below.

COMPOUNDS	DOCKING SCORE(Glide)kcal/mol
Pyrazinamide	-5.8
Ciprofloxacin	-3.8
Streptomycin	-5.7
INH	-5.0
MBS 2	-7.8
MBS 3	-6.2
MBS 6	-6.7

SYNTHESIS AND CHARACTERIZATION

The 1H-NMR spectra were recorded on Bruker AC Spectrometer using DMSO as Solvent. The data are given in parts per million(ppm) and are referenced to an internal standard of tetramethylsilane (TMS,δ 0.00 ppm). Peak Multiplicity is reported as s (singlet), d (doublet), dd (double doublet), t (triplet), and m(multiplet). Melting points were measured on SH apparatus.

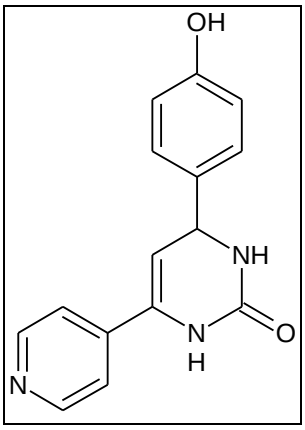
Physical Properties of Synthesized compound:

S.No	Compound ID	Structure	Mass	Solubility	Appearance
1.	**MBS-2**		266.0	Ethanol, Methanol, DMSO.	Pale Orange solid
2.	**MBS-3**		284.01	Ethanol, Methanol, DMSO.	Pale Brown solid
3.	**MBS-6**		293	Ethanol, Methanol, DMSO.	Orange solid

CHARACTERIZATION STUDIES:

Compound 1: MBS 2

Analytical data of 4-(4-hydroxyphenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one



Molecular Formula: $C_{15}H_{13}N_3O_2$

Melting Point: 105-110^{o}c

Yield: 85%

IR V $^{cm-1}$ (KBr): 3379 cm^{-1} (OH str)

3217 cm^{-1}(N-H str)

3062 cm^{-1}(C-H str)

1596 cm^{-1} (C=O str)

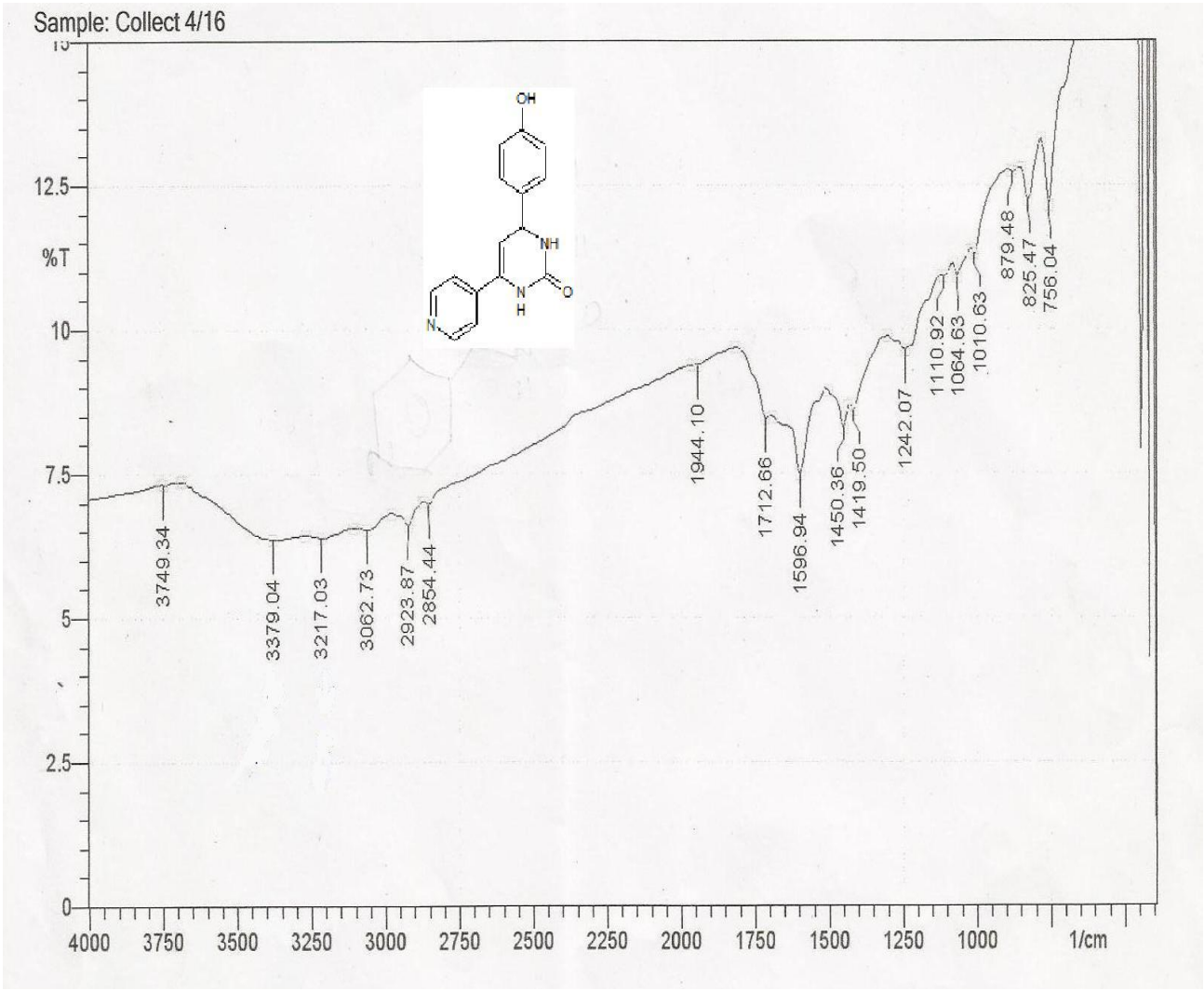
1450 cm^{-1}(C=C str)

1242 cm^{-1}(C-N str)

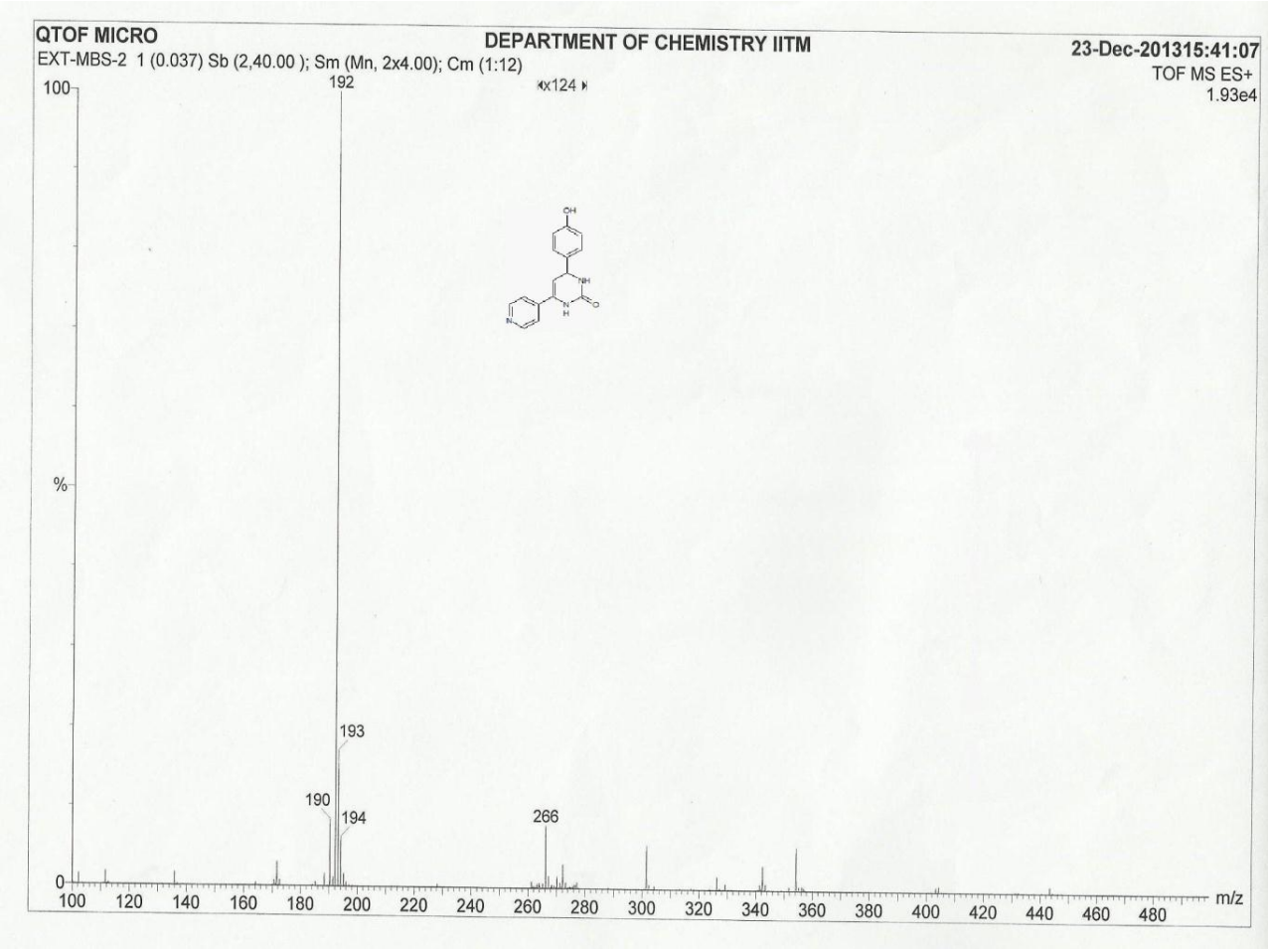
MASS (m/z): 266(M+ peak)

NMR: δ 1.20 – 1.40 [m, 1H,Aliphatic CH]
δ 2.4 – 2.5 [s, 1H,Aliphatic CH]
δ 6.90 – 6.97 [m, 2H,Ar - CH]
δ 7.50 – 7.51[s,1H,Ar-H]
δ 7.98 – 8.78 [m, 5H, Ar – H]
δ 9.0 – 9.4 [1H,OH]

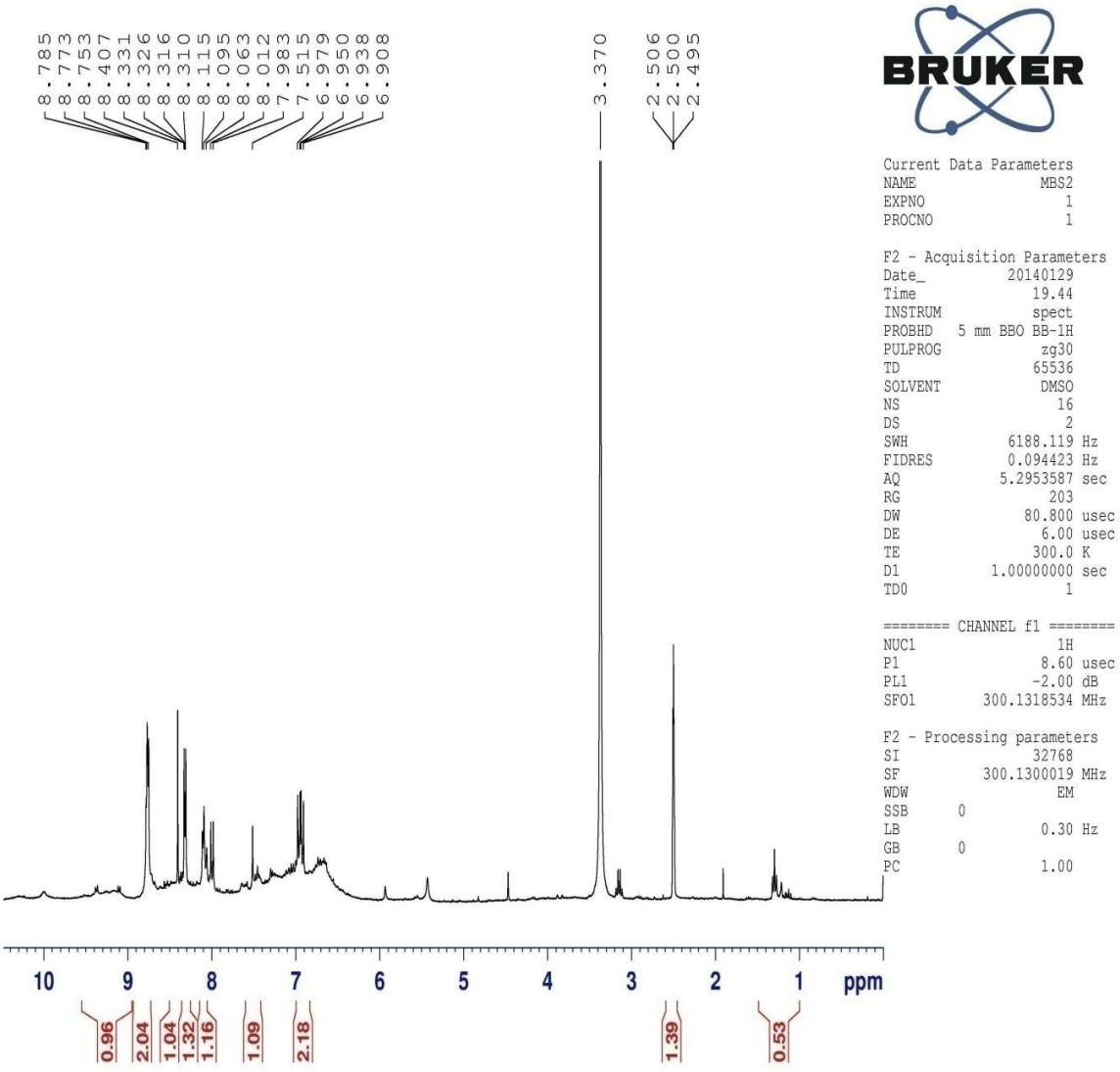
IR SPECTRA OF 4-(4-hydroxyphenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one



MASS SPECTRA OF 4-(4-hydroxyphenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one

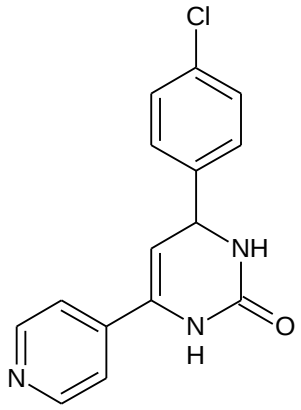


NMR SPECTRUM OF 4-(4-hydroxyphenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one



Compound 2: MBS 3

Analytical data of 4-(4-chlorophenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one



Molecular Formula: $C_{15}H_{12}N_3OCl$

Melting Point: 95-97^{o}c

Yield: 90%

IR V $^{cm-1}$ (KBr) : 3209 cm^{-1}(N-H str)

3031 cm^{-1} (C-H str)

1596 cm^{-1} (C=O str)

1450 cm^{-1}(C=C str)

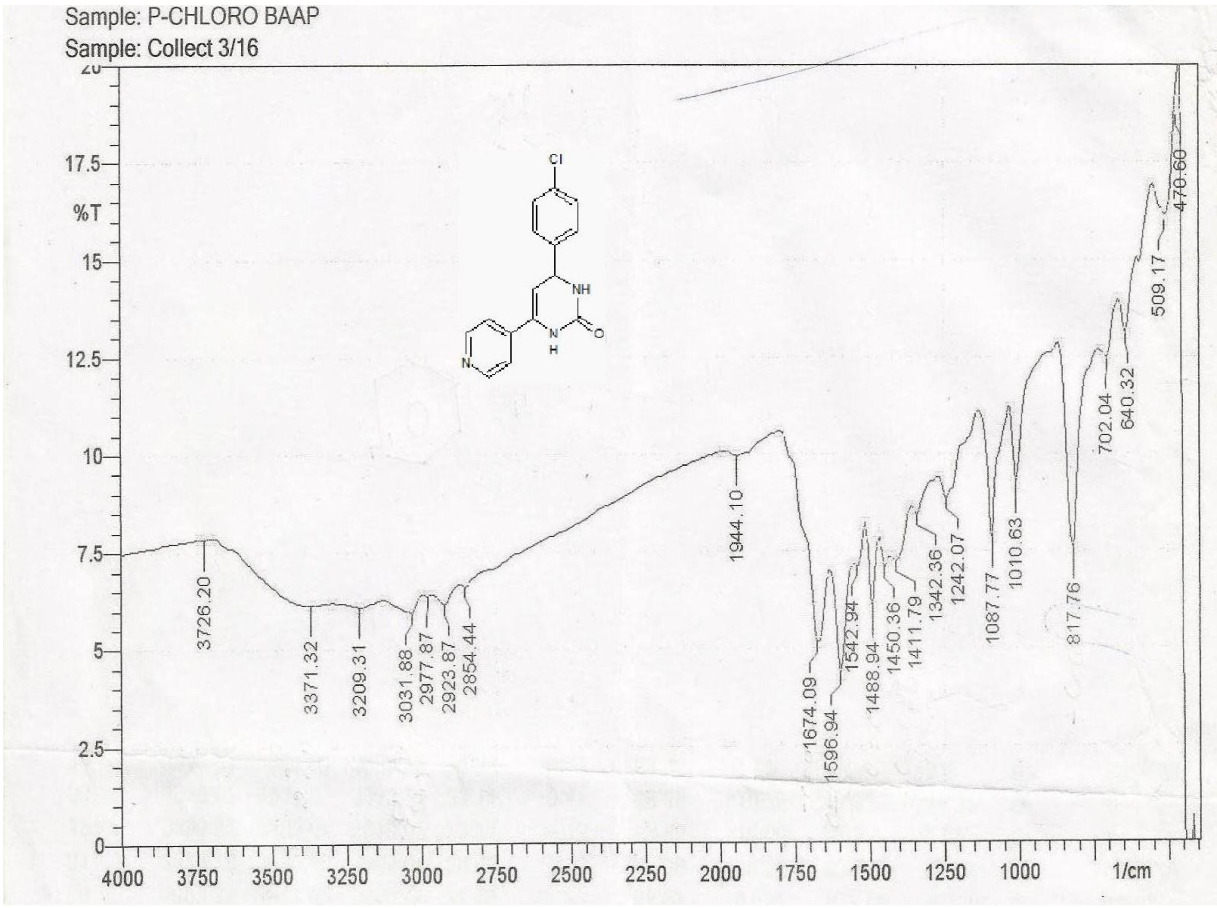
1242 cm^{-1}(C-N str)

817 cm^{-1}(C-Cl str)

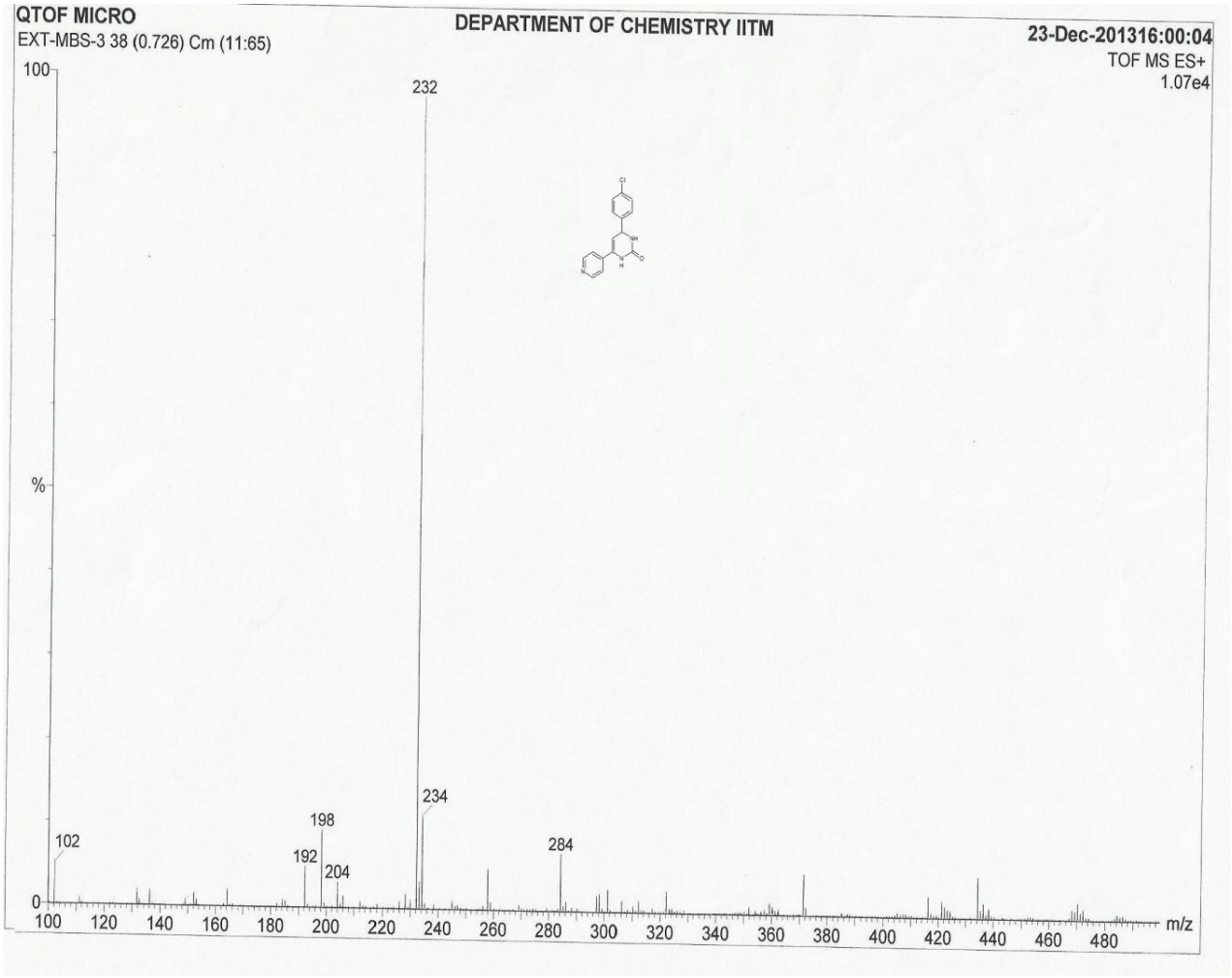
Mass (m/z): 284 (m+ peak)

NMR: δ 1.1 – 2.5 [d,2H,Aliphatic CH]
δ 3.4 – 3.5 [s, 2H,NH]
δ 7.2 – 8.7 [m, 8H,Aromatic]

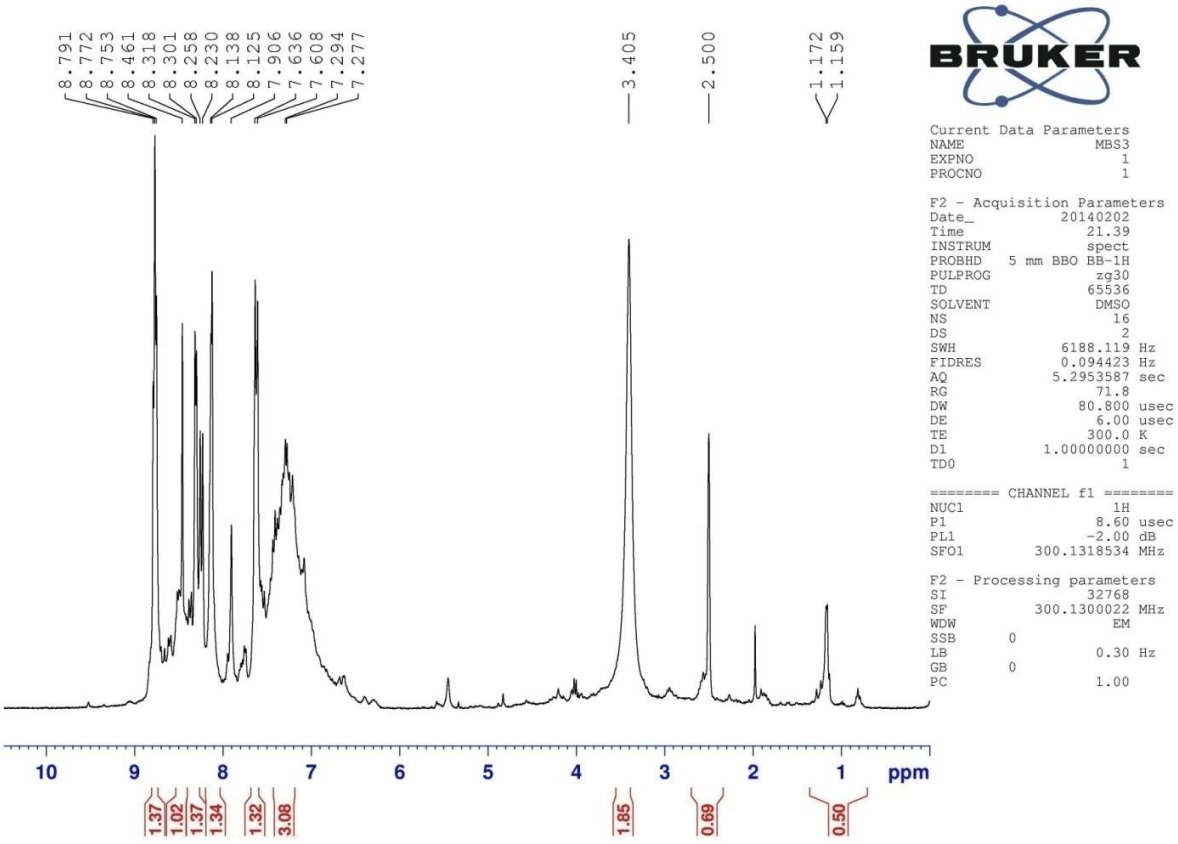
IR SPECTRUM OF 4-(4-chlorophenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one



MASS SPECTRUM OF 4-(4-chlorophenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one

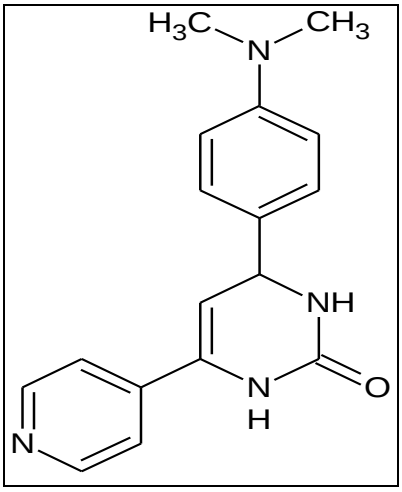


NMR SPECTRUM OF 4-(4-chlorophenyl)-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one



Compound 3: MBS 6

Analytical data of 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one.



Molecular Formula: $C_{17}H_{18}N_4O$

Melting Point: 102 ^{o}c

Yield: 75%

IR V $^{cm-1}$ (KBr) : 3379 cm^{-1}(N-H str)

3031 cm^{-1}(C-H str)

2923 cm-1(methylene CH Str)

1596 cm^{-1} (C=O str)

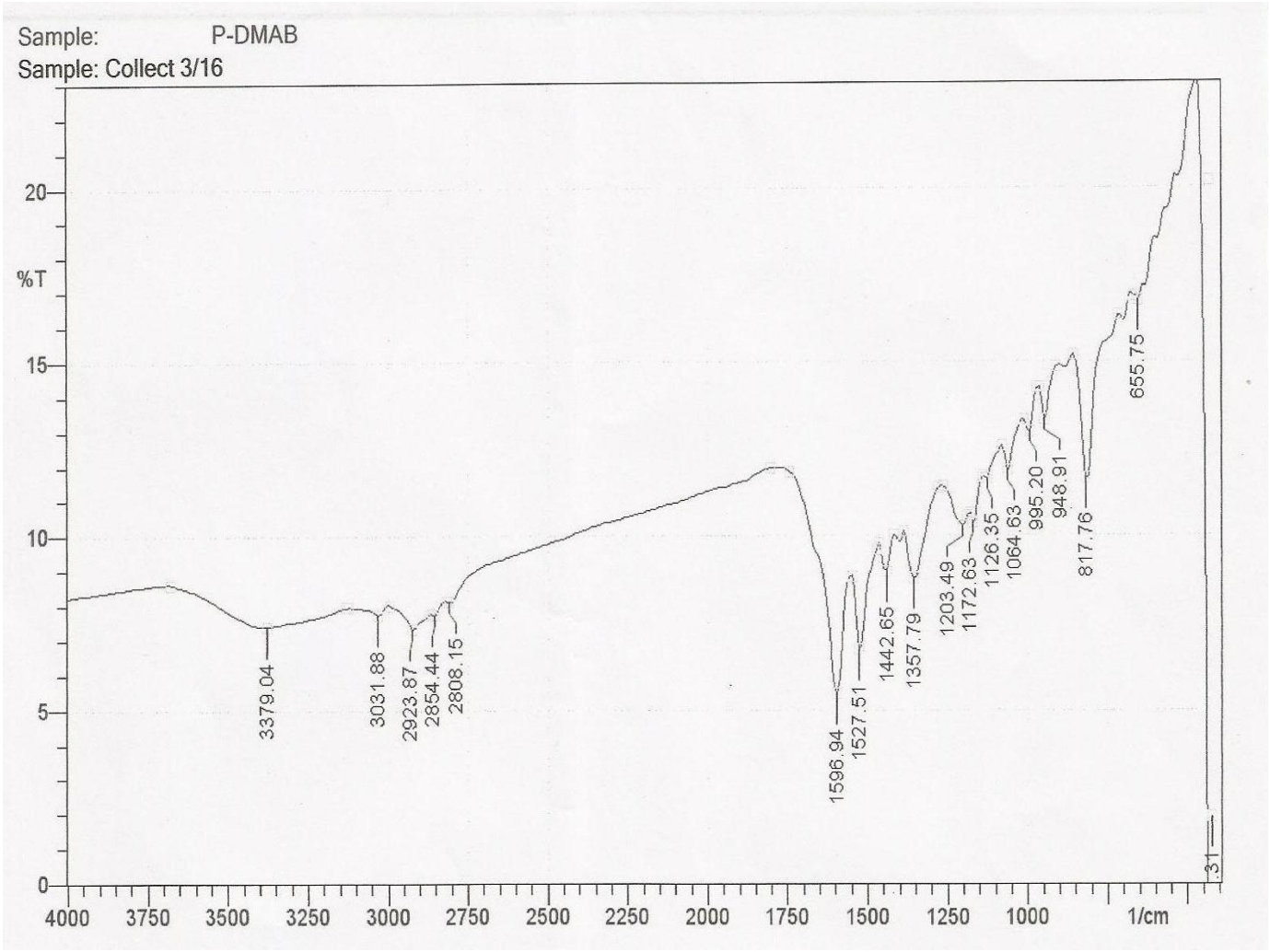
1442 cm^{-1}(C=C str)

1357 cm^{-1}(C-N str)

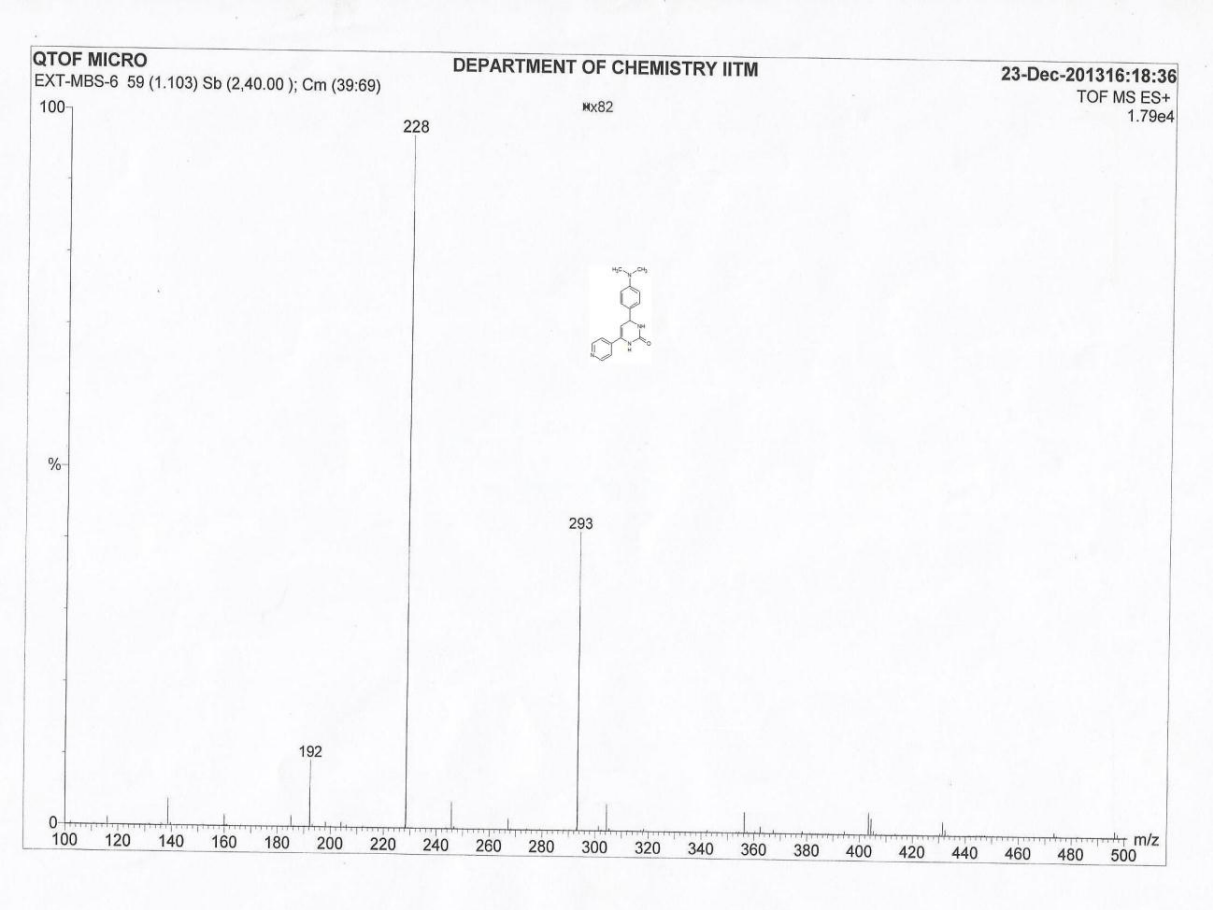
Mass (m/z): 294 (m+ peak)

NMR: δ 2.3 – 2.5 [s,1H,Aliphatic CH]
δ 2.8 - 3.0 [s,6H, $N(CH3)_2$]
δ 3.1 – 3.7 [s, 1H,CH]
δ 6.9 – 8.7 [M, 8H,Ar-CH]

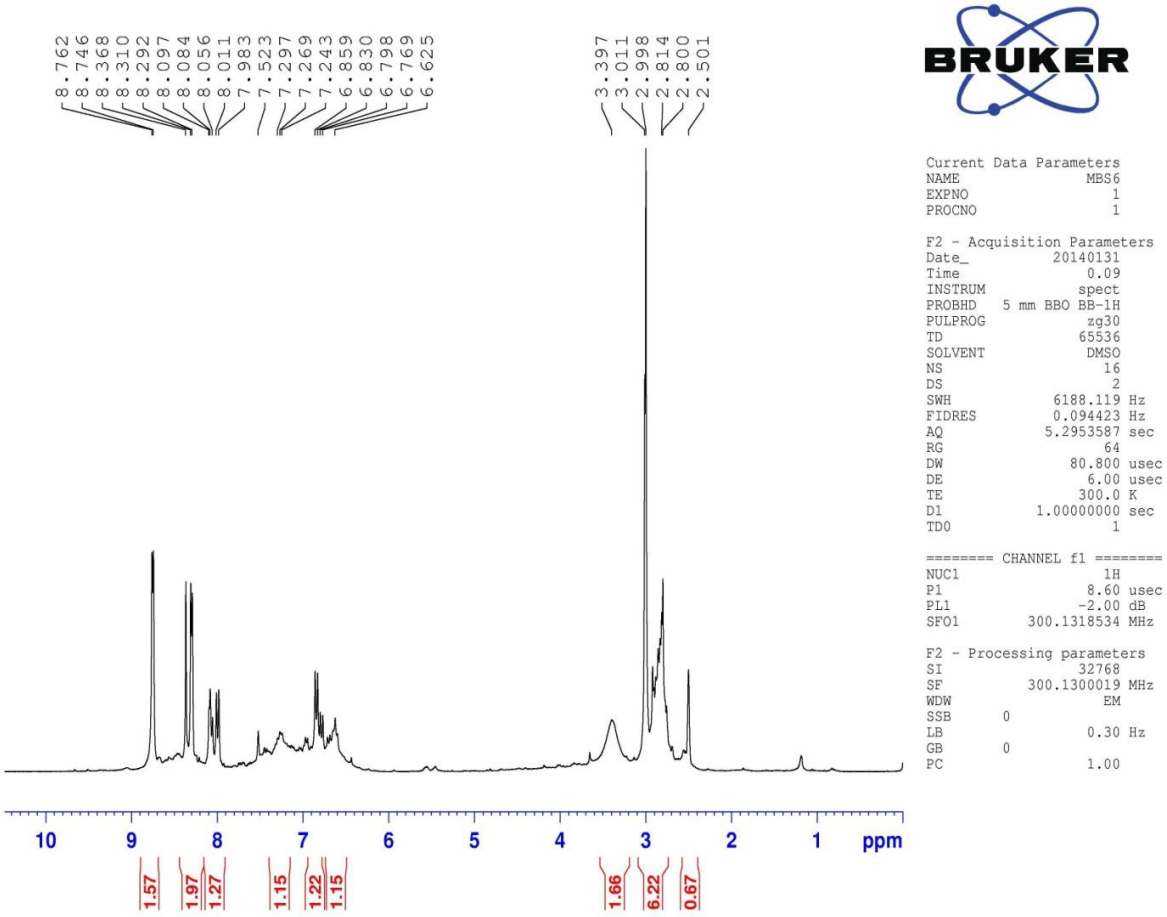
IR SPECTRUM OF 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one.



MASS SPECTRUM OF 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one.



NMR SPECTRUM OF 4-[4-(dimethylamino)phenyl]-6-(pyridin-4-yl)-3,4-dihydropyrimidin-2(1H)-one.

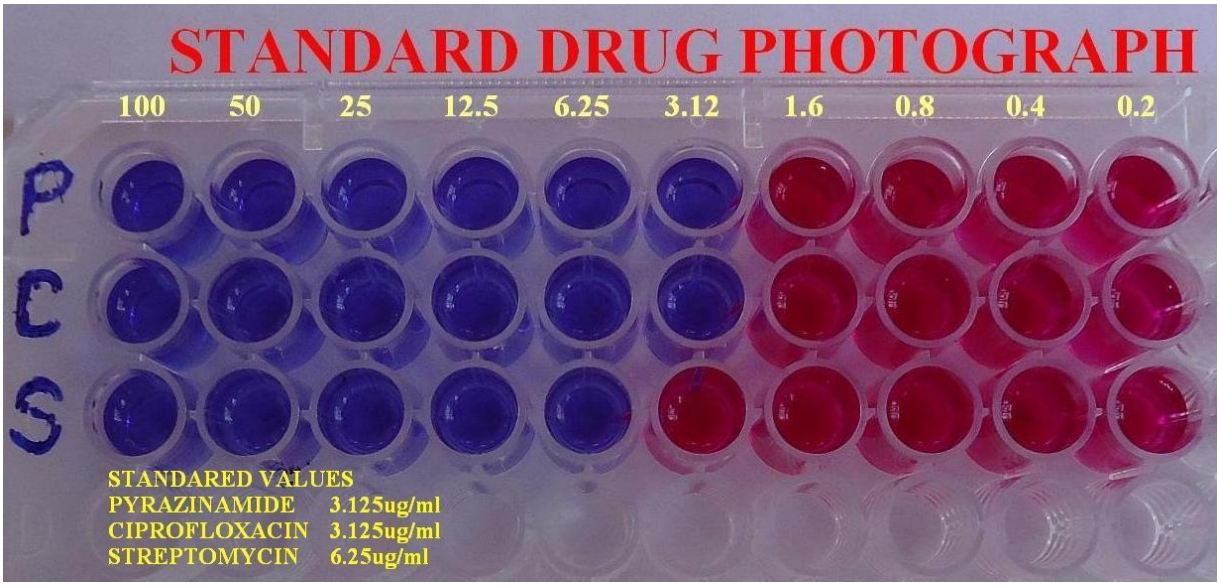


BIOLOGICAL SCREENING

The synthesized compounds were screened for their in-vitro anti mycobacterial activity by means of alamar blue assay. The compounds were tested in the concentration range of 100 to 0.8 µg/ml against *M.tuberculosis* H37Rv strain grown in Middlebrook 7H9 broth in 96 well titre plate. Pyrazinamide- 3.125µg/ml and Streptomycin- 6.25µg/ml were used as standards for comparison.

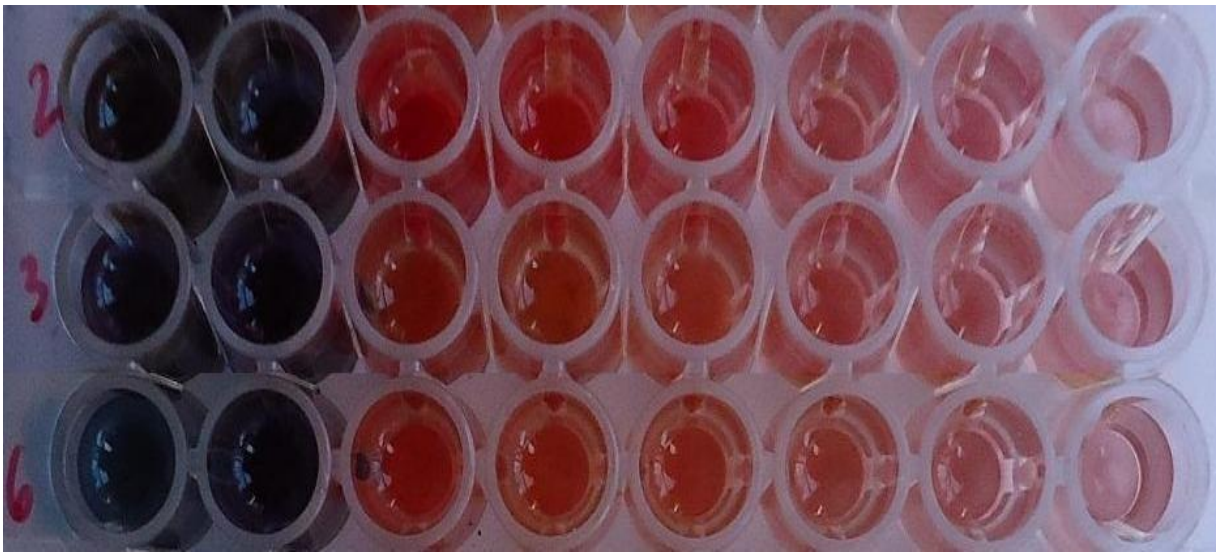
A blue color in the well was interpreted as no bacterial growth so it is termed as sensitive, and pink color was scored as growth and is referred as resistant. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

STANDARD DRUG PHOTOGRAPH



MABA REPORT OF THE SYNTHESIZED COMPOUNDS

Samples	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	6.25µg/ml	3.12µg/ml	1.6µg/ml	0.8µg/ml
MBS2	S	S	R	R	R	R	R	R
MBS3	S	S	R	R	R	R	R	R
MBS6	S	S	R	R	R	R	R	R



Anti Mycobacterial Activity of the Synthesized Compounds

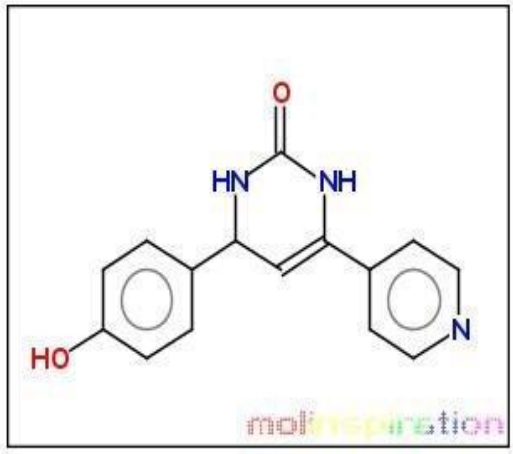
TOXICITY RISK ASSESSMENT:

The compounds were predicted to have low toxicity risks as per the OSIRIS Property Predictor Software. Prediction results are color coded and it indicates that all the three compounds has drug-conform behavior.

MOLINSPIRATION:

Molinspiration, a web based software was used to obtain parameter such as MiLogP, TPSA, drug likeness. MiLogP, is calculated by the methodology developed by Molinspiration as a sum of fragment based assistance and correction factors. MiLog P parameter is used to check good permeability across the cell membrane. TPSA is related to hydrogen bonding potential of compound. Number of rotatable bonds measures molecular flexibility. Molinspiration helps to conform about the Lipinski's rule of Five. It helps to know about that Drug likeness of compound, and also helps to know about the Pharmacokinetics profile of the drug entity, that includes absorption, distribution, metabolism and excretion ("ADME").

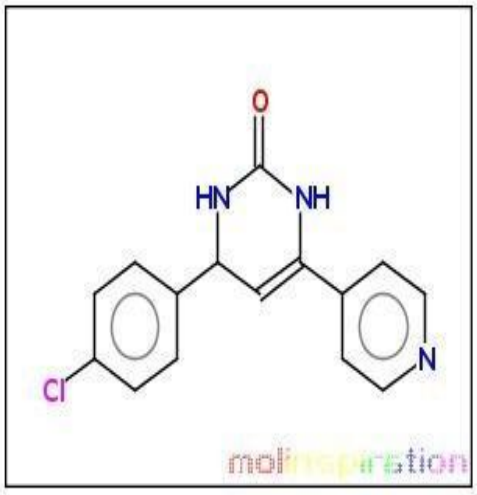
Molinspiration results of compound MBS 2, MBS 3 and MBS 6 are shown below.



Molinspiration property engine v2013.09

miLogP	1.266
TPSA	74.245
natoms	20.0
MW	267.288
nON	5
nOHNH	3
nviolations	0
nrotb	2
volume	236.505

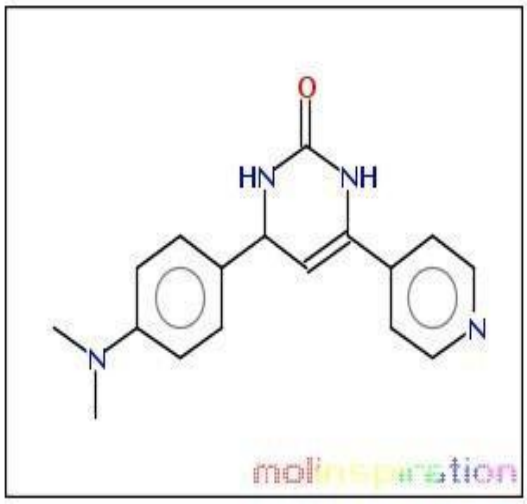
Molinspiration result of MBS 2



Molinspiration property engine v2013.09

miLogP	2.423
TPSA	54.017
natoms	20.0
MW	285.734
nON	4
nOHNH	2
nviolations	0
nrotb	2
volume	242.022

Molinspiration result of MBS 3



Molinspiration property engine v2013.09

miLogP	1.847
TPSA	57.255
natoms	22.0
MW	294.358
nON	5
nOHNH	2
nviolations	0
nrotb	3
volume	274.393

Molinspiration result of MBS 6

SUMMARY AND CONCLUSION

- Decaprenylphosphoryl-b-d-ribose 2'-Epimerase 1(DprE1) a enzyme of Oxido Reductase family is a critical enzyme for the growth of Mycobacterium tuberculosis H37Rv.
- From the review of literature DprE1 was chosen for our study for drug design.
- A database of 100 molecules with high potential of inhibiting the target possessing PDB ID of 4FDO were carefully chosen by making changes into the lead molecule 3,4-Dihydro pyrimidinones.
- The 3D structure of hopeful molecules were docked against the 3D structure of 4FDO using the docking platform Glide® (Grid Based Ligand Docking with Energetics).
- Three compounds with good Glide score(lower Binding energy) were selected for laboratory synthesis. Reaction conditions were optimized.
- The Compounds were labeled as MBS 2, MBS3, MBS6 were synthesized with satisfactory yield by Biginelli reaction.
- The purity of the synthesized compounds were evaluated by melting point and TLC. Recrystallization was done.
- The characterization of the synthesized compounds were done by Infrared, Nuclear magnetic resonance and Mass spectroscopic methods.
- The synthesized compounds were subjected to computer aided drug design (CADD) tool,for toxicity risk assessment by OSIRIS property calculator. The results are color coded as green color which confirms the drug likeness.
- The final pure compounds were screened for Antimycobacterial activity by in vitro method called Microplate Alamar Blue Assay(MABA).
- The Minimum Inhibitory Concentration (MIC) of the synthesized compounds were at 50mcg/ml against the MIC of known TB drugs Pyrazinamide: 3.125mcg/ml, Ciprofloxacin: 3.125mcg/ml and Streptomycin 6.25mcg/ml.

CONCLUSION

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

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

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