DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUTION OF SOME NOVEL HETEROCYCLIC DERIVATIVES AS ANTI - TUBERCULAR AGENTS

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY
Chennai

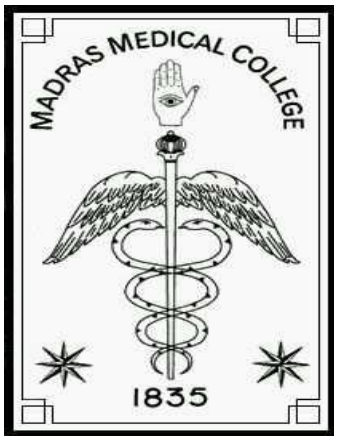
In partial fulfilment of the requirements
For the award of the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
261415712

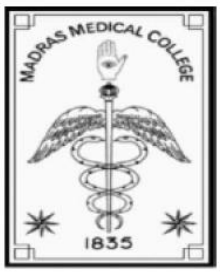
Under the Guidance of

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 2016



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

CERTIFICATE

This is to certify that the dissertation entitled "DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL HETEROCYCLIC DERIVATIVES AS ANTI- TUBERCULAR AGENTS" submitted by the candidate bearing the register No:261415712 in partial fulfillment of the requirements for the award of 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 2015-2016 at the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai- 600 003.

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

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

CERTIFICATE

This is to certify that the dissertation entitled "DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL HETEROCYCLIC DERIVATIVES AS ANTI- TUBERCULAR AGENTS" submitted by the candidate bearing the register No:261415712 in partial fulfillment of the requirements for the award of 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 2015-2016 under my guidence at the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai- 600 003.

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

`ACKNOWLEDGEMENT

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

I consider this as an opportunity to express my gratitude to all the dignitaries who have been involved directly or indirectly with the successful completion of this dissertation. The satisfaction that accompanies the successful completion of any task would be incomplete without mention of the people who made it possible with constant guidance, support and encouragement that crows all effort with success.

I express my immense gratitude to Govt of Tamilnadu for providing me the Monthly scholarship. I express my thanks to the Dean **Dr.R.Vimala M.D.,** Madras Medical College, for permitting me to undertake the project during the period of my academic study.

It is with great pleasure that I place on record a deep sense of gratitude and heartfelt thanks to my guide **Prof. Dr. A. Jerad Suresh M.Pharm., Ph.D., MBA**, Principal, Head, Professor, Department of Pharmaceutical chemistry, College of Pharmacy, Madras Medical College, Chennai-03 for his help, support and constant encouragement throughout the progress of this work. It was really a great experience working under him. His guidance was of immense help in my project work without which it would have been an unachievable task.

It’s a great pleasure for me to thank all the teaching staff members **Dr. V. Niraimathi, M.Pharm., Ph.D.**, **Dr.R.Priyadharshini, M.Pharm., Ph.D., Mrs.T.Saraswathy, M.Pharm., Dr.P.G.Sunitha, M.Pharm., Ph.D., Dr.M.Sathish, M.Pharm., Ph.D.,** Tutors in Pharmacy, Department of Pharmaceutical Chemistry for their gracious support and encouragement in making this work successful.

I extend my thanks to all non-teaching staff members **Mr.S.Baskar, Mr.Umapadhi, Mrs.Maheshwari and Mrs.K.Murugeshwari** Department of

Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03, for their assistance during my come work.

My sincere thanks to all the research scholars **Mr. K.M.Noorulla**, **Ms.P.R.Surya and Mrs. R.Devi** Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03.

I am grateful thanks to **Dr. Kishore G Bhat,** for his support in carrying out the in-vitro evaluation of anti-tubercular activity.

I would like to thank my friends, **Mrs.G.Sathyavani, M.Neelakandan, N.Ramya, P.Mugilarasi, R.Kalaiselvi, P.Karunya, R.Pandiyan, R.Ravikumar** and also the other department friends for them co- operation for completing my project.

I have no words to express my pleasure in thanking my best friend's **P.PalPandi, C.Saravanan, M.S.Hemraj, R.Dinesh Kumar, K.Vimalraj, M.Ganesh Kumar,** who stood beside me in each and every step during my project and given me constant support.

I would like to thank **my seniors** and to **my juniors** and **UG friends** for their kind support and co-operation.

Most of all I would like to thank **my beloved parents, brother, family members** and my dearest friends for their priceless support, love and encouragement throughout the entire tenure of this course.

CONTENTS

S. NO	TITLE	PAGE NO
1.	INTRODUCTION	
	TUBERCULOSIS	1
	ENZYME PROFILE	10
	HETEROCYCLIC CHEMISTRY	13
2.	AIM AND OBJECTIVE	16
3.	LITERATURE REVIEW	17
4.	METERIALS AND METHODS	
	DOCKING STUDIES	24
	SYNTHETIC INVESTIGATION	31
	CHARACTERIZATION	33
	BIOLOGICAL EVALUATION	36
5.	RESULTS AND DISCUSSION	38
6.	SUMMARY AND CONCLUSION	65
7.	BIBLIOGRAPHY	67

LIST OF ABBREVIATIONS

IR	Infrared
H1-NMR	Proton Nuclear Magnetic Resonance
LC-MS	Liquid Chromatography and Mass Spectroscopy
GC-MS	Gas Chromatography and Mass Spectroscopy
Gm	Gram
δ	Delta
Sec	Seconds
Rf	Retention Factor
m.p	Melting Point
Mol.For	Molecular Formula
Mol.Wt	Molecular Weight
◦C	Degree Celsius
SEM	Standard Error Mean
m\e	Mass per charge Ratio
STD	Standard
CFU ML-1	Colony Forming Unit per Milliliter
UV	Ultra Violet
MIC	Minimum Inhibitory Concentration
mg\kg	Milligram per kilogram
µg	Microgram
b.w	Body Weight
min	Minutes
TB	Tuberculosis
MDR-TB	Multi Drug Resistance TuBerculosis
MABA	Microplate Alamar Blue Assay
TLC	Thin Layer Chromatography

INTRODUCTION

TUBERCULOSIS

Tuberculosis (TB) is caused by a bacterium called *Mycobacterium tuberculosis*. The bacteria usually attack the lungs, but TB bacteria can attack any part of the body such as the kidney, spine, and brain. If not treated properly, TB disease can be fatal. [(1)]

Consumption, phthisis, scrofula, Pott's disease and the white plague are all terms used to refer to tuberculosis throughout history. [(2)]

Spreadability of TB [(1)]

TB is spread through the air from one person to another. The TB bacteria are put into the air when a person with TB disease of the lungs or throat coughs, sneezes, speaks, or sings. People nearby may breathe in these bacteria and become infected. TB is NOT spread by

- Shaking someone's hand
- Sharing food or drink
- Touching bed linens or toilet seats
- Sharing toothbrushes
- Kissing

CURRENT STATUS OF TUBERCULOSIS

TB BURDEN GLOBALLY [(20)]

According to WHO global tuberculosis report

Tuberculosis (TB) is contagious and airborne**.** It ranks alongside HIV/AIDS as a leading cause of death worldwide. **9.6 million** People fell ill with TB in 2014, including

1.2 million People living with HIV. In 2014, 1.5 million people died from TB, including 0.4 million among people who were HIV-positive.

TB is one of the top five killers of women among adult women aged 20–59 years.

480 000 women died from TB in 2014, including 140 000 deaths among women who were HIV-positive. 890 000 men died from TB and 5.4 million fell ill with the disease.

An estimated 1 million children became ill with TB and 140 000 children died of TB in 2014.

TB Burden in India [20]

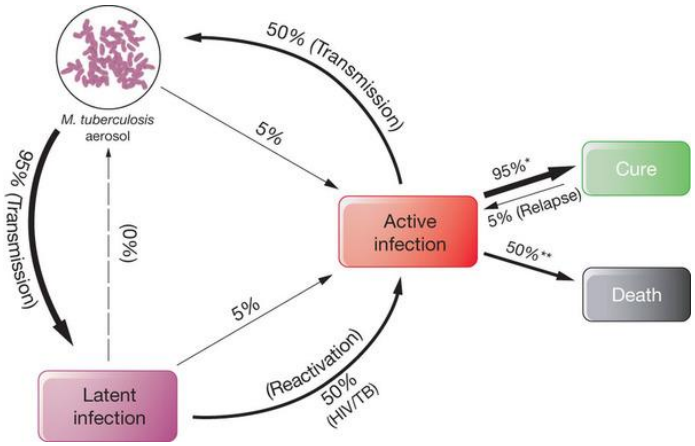
Each year 12 lakh (1,200,000) Indians are notified (that is reported to the RNTCP) as having newly diagnosed TB. In addition at least 2.7 lakh (270,000) Indians die. Some estimates calculate the deaths as being twice as high. TB can affect any age, caste or class but cases are mainly poor people and mostly men. Slum dwellers, tribal populations, prisoners and people already sick with compromised immune systems are over-represented.

Globally, a TB associated death happens every 20 seconds. India has 20 per cent of the global burden of TB. TB is one of the leading causes of mortality in India, with nearly 1000 deaths a day.[21]

Latent TB Infection and TB Disease [1]

Not everyone infected with TB bacteria becomes sick. As a result, two TB-related conditions exist: latent TB infection and TB disease.

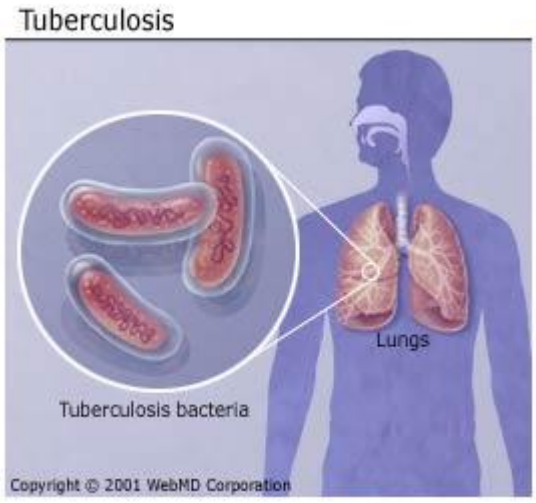
Figure: 1[3]



Latent TB Infection

TB bacteria can live in the body without making you sick. This is called latent TB infection. In most people who breathe in TB bacteria and become infected, the body is able to fight the bacteria to stop them from growing. People with latent TB infection do not feel sick and do not have any symptoms. People with latent TB infection are not infectious and cannot spread TB bacteria to others. However, if TB bacteria become active in the body and multiply, the person will go from having latent TB infection to being sick with TB disease.[1]

Figure: 1[3]



TB Disease

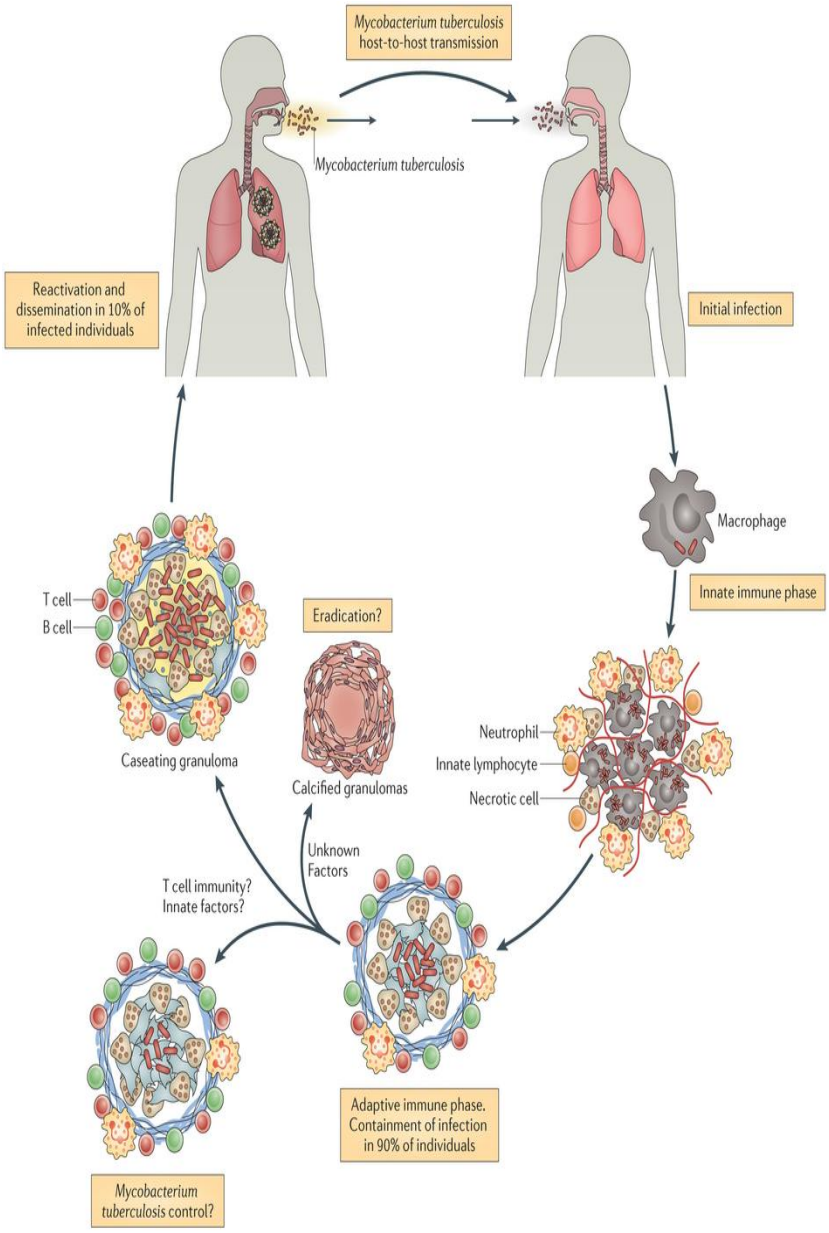
TB bacteria become active if the immune system can't stop them from growing. When TB bacteria are active (multiplying in your body), this is called TB disease. People with TB disease are sick. They may also be able to spread the bacteria to people they spend time with every day. [1]

Many people who have latent TB infection never develop TB disease. Some people develop TB disease soon after becoming infected (within weeks) before their immune system can fight the TB bacteria. Other people may get sick years later when their immune system becomes weak.

For people whose immune systems are weak, especially those with HIV infection, the risk of developing TB disease is much higher than for people with normal immune system.

In a country like ours, where the TB bacteria are so prevalent, it is imperative to maintain hygiene to prevent the spread of the disease. Spitting in public or coughing or sneezing without covering the mouth should be completely discouraged. [4]

Figure: 2 [5]



Some people are known to have a higher risk of progressing from latent TB to TB disease. These include:

- Infants and children aged less than 4 years
- People infected within the previous two years
- People infected with HIV
- People who have certain clinical conditions or conditions which compromise their immune system, such as people with diabetes, and people with chronic renal failure.

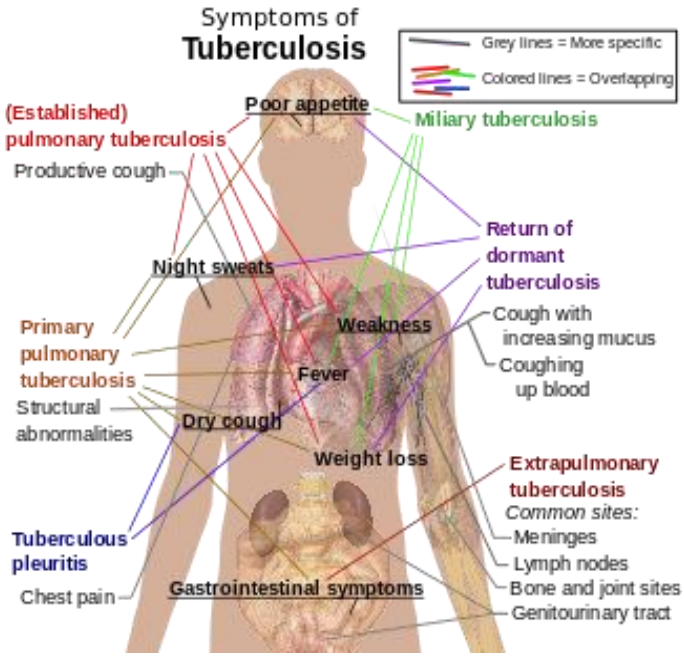
SYMPTOMS OF TB [6]

Will generally have no symptoms if are infected with tuberculosis (TB), unless have active TB disease. In fact, may not even be aware that you have a latent TB infection until it's revealed through a skin test, perhaps during a routine check-up.

If do have active TB disease, may have these symptoms:

- Overall sensation of feeling unwell.
- Cough, initially with yellow or green mucus, and possibly with bloody sputum later in the disease.
- Fatigue.
- Shortness of breath.
- Weight loss.
- Fever.
- Night sweats.
- Pain in the chest, back or kidneys, or in all three.

Figure: 3[7]



MYCOBACTERIA [8]

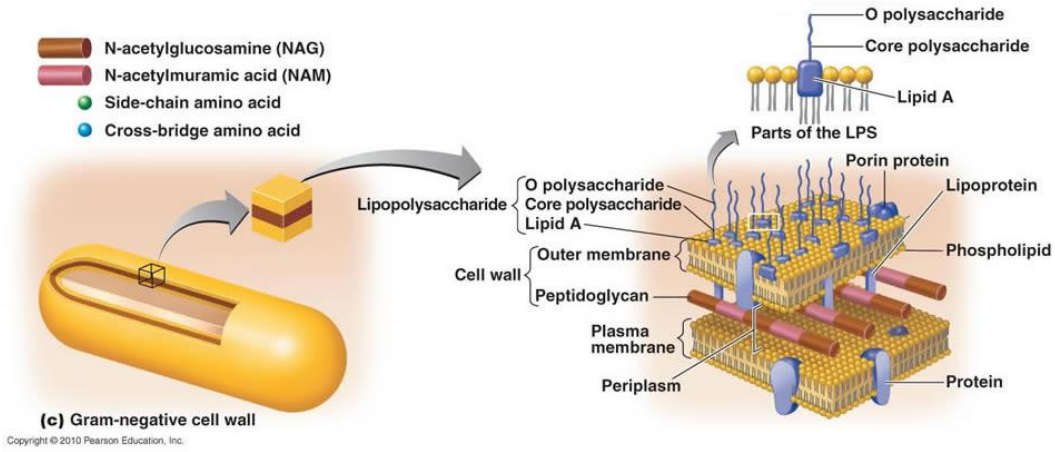
Kingdom	:	Bacteria.
Phylum	:	Actinobacteria.
Class	:	Actinobacteria.
Order	:	Actinomycetales.
Suborder	:	Corynebacterineae.
Family	:	Mycobacteriaceae.
Genus	:	Mycobacterium.
Species	:	Mycobacterium tuberculosis.
Synonym	:	Tubercle bacillus Koch 1882**.**

Mycobacterial cell wall:

The cell wall is a major virulence factor of *Mycobacterium tuberculosis* and contributes to its intrinsic drug resistance. [9] Cryo-electron microscopy showed that the mycobacterial cell wall lipids form an unusual

outer membrane. [10] 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.[11] Although the genome of *Tuberculosis* appears to encode over 100 putative outer membrane Proteins, only a few have been identified and characterized. The membranes contain Mycolic acids, Peptidoglycan, and Arabinogalactan. [12]

Figure 4[13]



GENOME: [14]

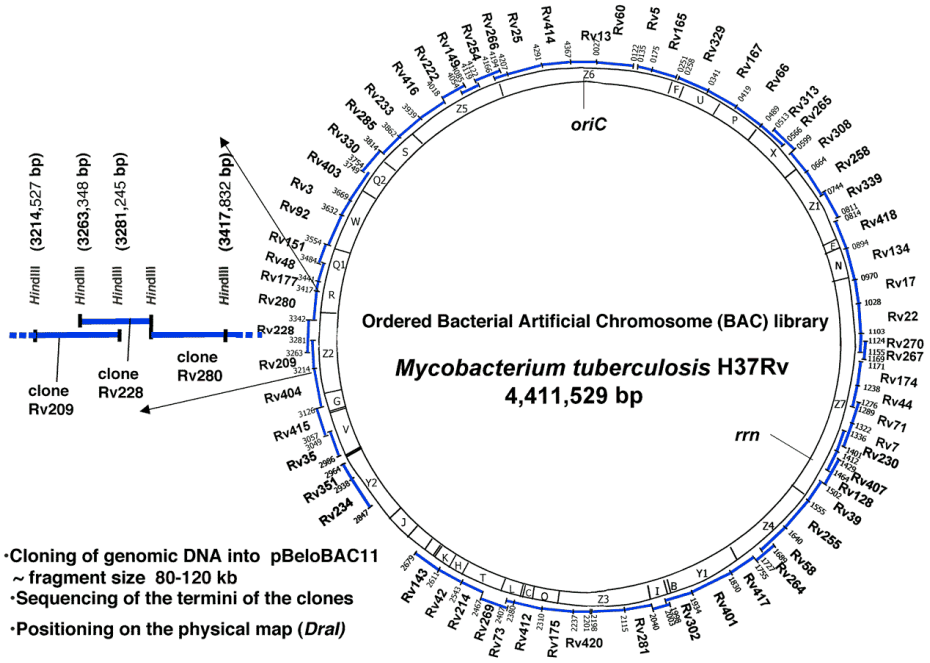
Mycobacterium tuberculosis has circular chromosomes of about 4,200,000 nucleotides long. The G+C content is about 65%. [15]

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, and 8% of the genome is involved in this activity. [16]

The different species of the *Mycobacterium tuberculosis* complex show a 95-100% DNA relatedness based on studies of DNA homology, and the sequence of the 16S rRNA gene are exactly the same for all the species. So some scientists suggest that they should be grouped as a single species while others argue that they should be grouped as varieties or subspecies of *M. tuberculosis*. [17]

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.

Figure 5[18]



LIFECYCLE OF MYCOBACTERIUM TUBERCULOSIS [19]

The 5 Stages of Tuberculosis are

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

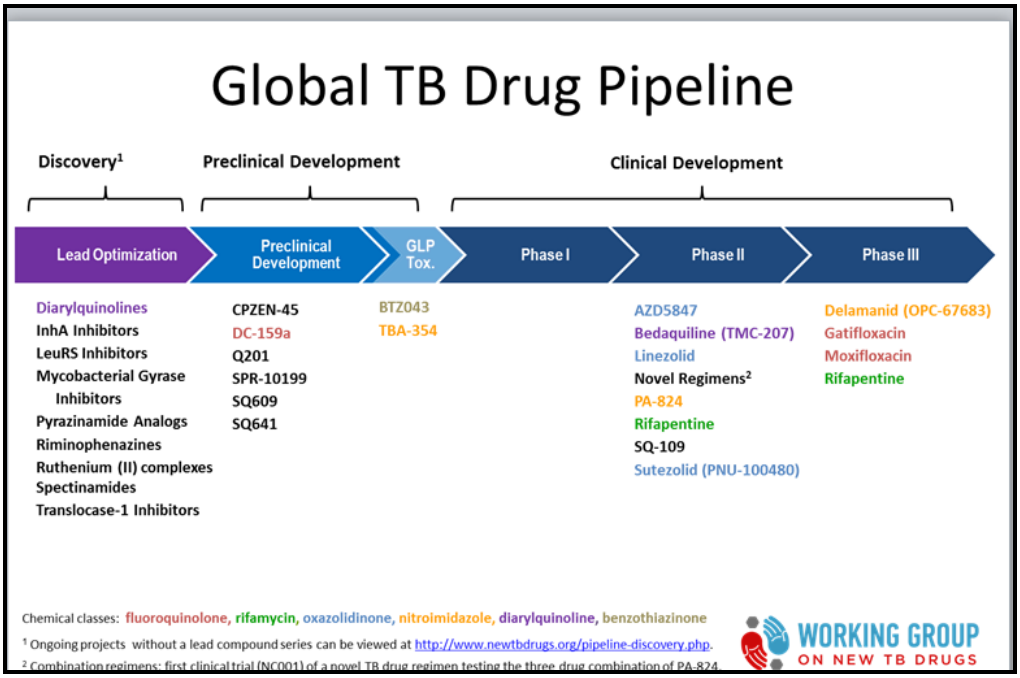
2) **Symbiosis (7-21 Days):** If the Bacteria do not get killed then it reproduces.

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

4) **Interplay of Tissue Damaging and Macrophage Activating Immune Response (After 21 days):** Macrophages surround the tubercle but some may be inactive. Tuberculosis then uses it to reproduce which causes it to grow. The tubercle can break off and spread around. If it spreads in the blood one can develop tuberculosis outside the lungs, this is called Miliary Tuberculosis.

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

Figure: 6 [22]



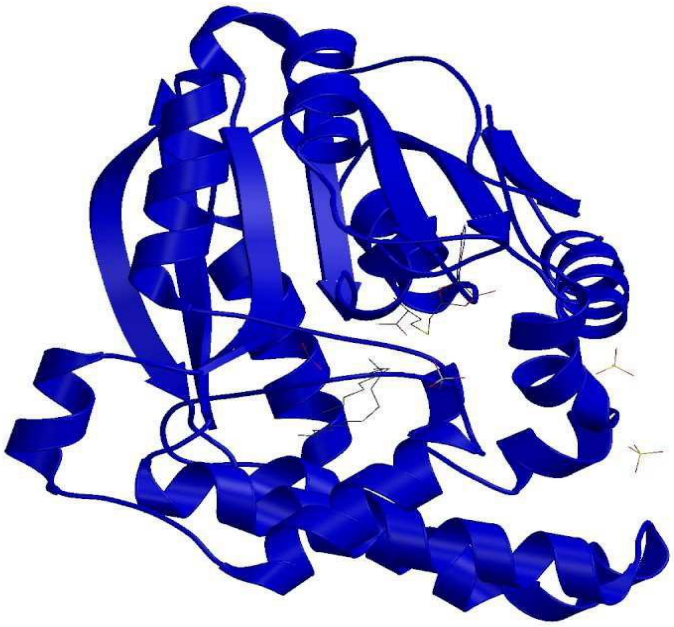
ENZYME PROFILE

CYCLOPROPANE SYNTHASE CmaA2 [23]

PDB ID	:	1KPI
Classification	:	Transferase
Structure weight	:	35797.58
Molecule	:	Cyclopropane-fatty-acyl-phospholipid synthase-2
Polymer	:	1
Chains	:	A
Type	:	Protein
Lenth	:	302
Organism	:	Mycobacterium tuberculosis
Gene Name	:	cmaA2 cma2 CMAS-2 Rv0503c MTCY20G9.30c

The crystal structures of cyclopropane synthase CmaA2 is closely related with root mean square deviation (RMSD) between the Ca atoms of the core region of less than 0.7Å. The core region which contains a seven stranded b-sheet which are all parallel apart from b7 which runs antiparallel. The α-helices flank each side of the sheet and run in the same N- to C- orientation. The two long α-helices lie adjacent to the C- terminal ends of the b-sheet, which encloses SAM/SAH cofactor binding site. In cyclopropane synthase the overall polypeptide fold are similar to other SAM-Mtases in the protein database, such as catechol-O methyltransferases and DNA methyltransferases. [24]

Figure 7 [24]



The structure of cyclopropane enzymes is revealed by a tunnel approximately 15Å by 10 Å wide which extends from the surface of the protein to the cofactor binding site. The tunnel is exclusively lined with hydrophobic residues and believed to be the binding site for the acyl substrate and is virtually identical in the three enzymes CmaA1, CmaA2 and PcaA. The important interactions between the acyl chain and the protein include Leu192, Ile169, Phe200, Ile195, Leu205, Leu236, Tyr232, Leu278 and Phe273. It reveals that the reactive group may sit in the active binding site and the length of the acyl chain which these enzymes may accept apart from the interactions of the co-crystal structure with the lipid. [25]

The cyclopropane synthase CmaA2 and other enzyme are the part of FASII pathway for the biosynthesis of mycolic acids in mycobacteria and these enzymes acts on a long acyl chain. which linked to acyl carrier protein (AcpM). The mechanism for distal versus proximal substrate specificity is based on the differing modes of binding of acyl-AcpM. Recent studies shows the cyclopropane synthases of M.tuberculosis is considered as a novel class of persistence genes and the need of new inhibitors for the persistent phase of

tuberculosis infection and the absence of the cyclopropanated lipids in human results.

Cyclopropane synthases as an attractive target for the new drug development. Despite the apparent non-redundancy of the CmaA2 and other cyclopropane synthases, the similarity of this family of enzymes in the mode of binding substrates and in their catalytic mechanism is very clear. This makes the prospect of a single drug effectiveness against multiple targets is highly possible, so that the chance for the development of drug resistance is less. [26]

NEED FOR NEW DRUGS [27]

The firstline treatment for TB comprises of Rimpampicin, Isoniazid, Pyrazinamide and Ethamabutol adminsitered in combination for 6- 9 months, delivered under the DOTS programme. The duration of the treatment and the side effects of the drugs are major reasons for non-compliance which in return catalyses the emergence of Multi Drug Resistant (MDR) and Extremely Drug Resitant (XDR) strains of TB. In such drug resistant cases we have to rely on a regimen of drugs which are not only less effective but are also significantly more toxic and to top it all have to be administered for extended period up to 18 months.

The recent reports of extreme varieties of XDR TB from Mumbai raise an alarm bell. Co-morbity situations like TB with HIV or TB in diabetic patients, both of which are prevalent in India, make treatment even more complex. Thus there is an urgent need to introduce not only new TB drugs but also new regiments that are effective and can also reduce the duration of therapy.[27]

LACK OF INNOVATION

There is a serious lack of innovation of new drugs in TB as no new drugs have been introduced in the last 50 years. This is because of the lack of a sizable market attractive for the large pharmaceutical companies to invest. The global TB drug market is estimated to be only US$ 300-400 million while

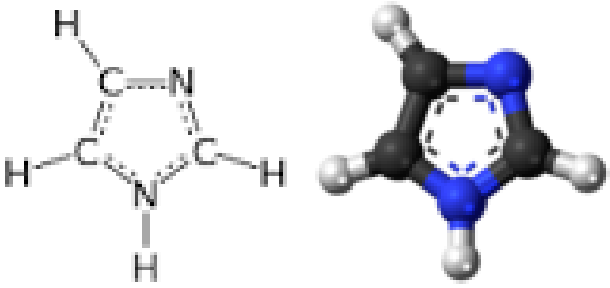
the cost of discovery and development of a drug is estimated to be manifolds of this figure.[27]

BASIC NUCLEUS INTRODUCTION HETEROCYCLIC CHEMISTRY

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

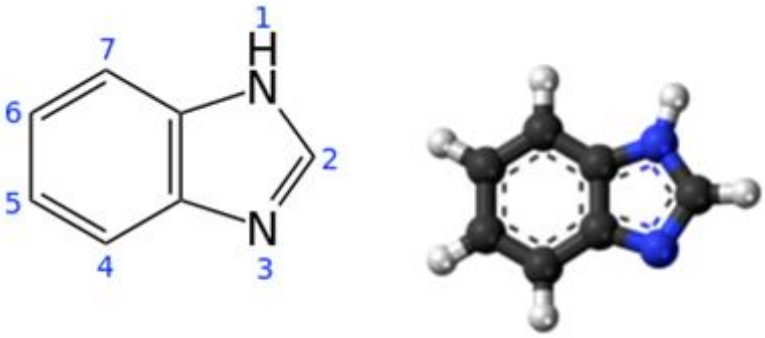
IMIDAZOLE NUCLEUS

Imidazole is a planer five-member heterocyclic ring with 3C and 2N atom and in ring N is present in 1st and 3rd positions. Imidazole derivatives have occupied a unique place in the field of medicinal chemistry. The incorporation of the imidazole nucleus is an important synthetic strategy in drug discovery. [28] The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents.



BENZIMIDAZOLE NUCLEUS

Benzimidazole is a heterocyclic aromatic organic compound. This bicyclic compound consists of the fusion of benzene and imidazole. The most prominent benzimidazole compound nature is *N*-ribosyl-dimethylbenzimidazole, which serves as an axial ligand for cobalt in vitamin B12.[29] Benzimidazole also has fungicidal properties. It acts by binding to the fungal microtubules and stopping hyphal growth. It also binds to the spindle microtubules and blocks nuclear division. [30]



1*H*-benzimidazole

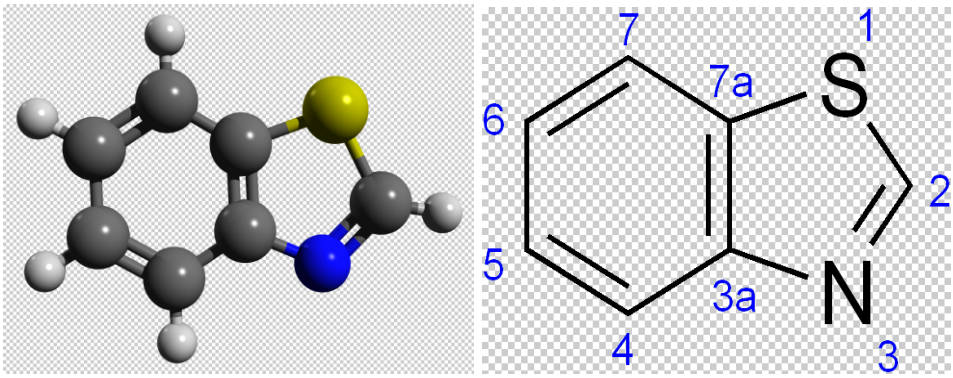
BENZOTHIAZOLES

Benzothiazoles consist of a 5-membered 1, 3-thiazole ring fused to a benzene ring. The nine atoms of the bicycle and the attached substituents are coplanar.[31]

Benzothiazole is one of the most important heterocyclic compound, weak base, having varied biological activities and still of great scientific interest now a days. They are widely found in bioorganic and medicinal chemistry with application in drug discovery.

Benzothiazole is a privileged bicyclic ring system. Due to its potent and significant biological activities it has great pharmaceutical importance; hence, synthesis of this compound is of considerable interest. The small and simple benzothiazole nucleus if present in compounds involved in research aimed at evaluating new products that possess interesting biological activities.

Benzothiazole moites are part of compounds showing numerous biological activities such as antimicrobial, anticancer, anthelmintic, anti-diabetic activities etc, they have also found application in industry as anti-oxidants, vulcanisations accelerators. Various benzothiazoles such as 2-substituted benzothiazole received much attention due to unique structure and its uses as radioactive amyloidal imagining agents, and anticancer agents.[32]



On the basis of various literature surveys Imidazole and Benzimidazole, Benzthiazole derivatives shows various pharmacological activities. [33), (34)]

- Anti-tubercular activity. [(35)]
- Anti-fungal and Anti-bacterial activity.
- Anti-inflammatory activity and analgesic activity.
- Anti-depressant activity.
- Anti-cancer activity. [(36)]
- Anti-viral activity.
- Antileishmanial activity.

On view of the importance of the imidazole and benzimidazole nucleus. It was decided to design nucleus based on the imidazole and benzimidazole nucleus. Morethan 200 different molecules with the imidazole and benzimidazole scaffolds are drawn and docked.

AIM AND PLAN OF WORK

OBJECTIVE OF THE PRESENT STUDY

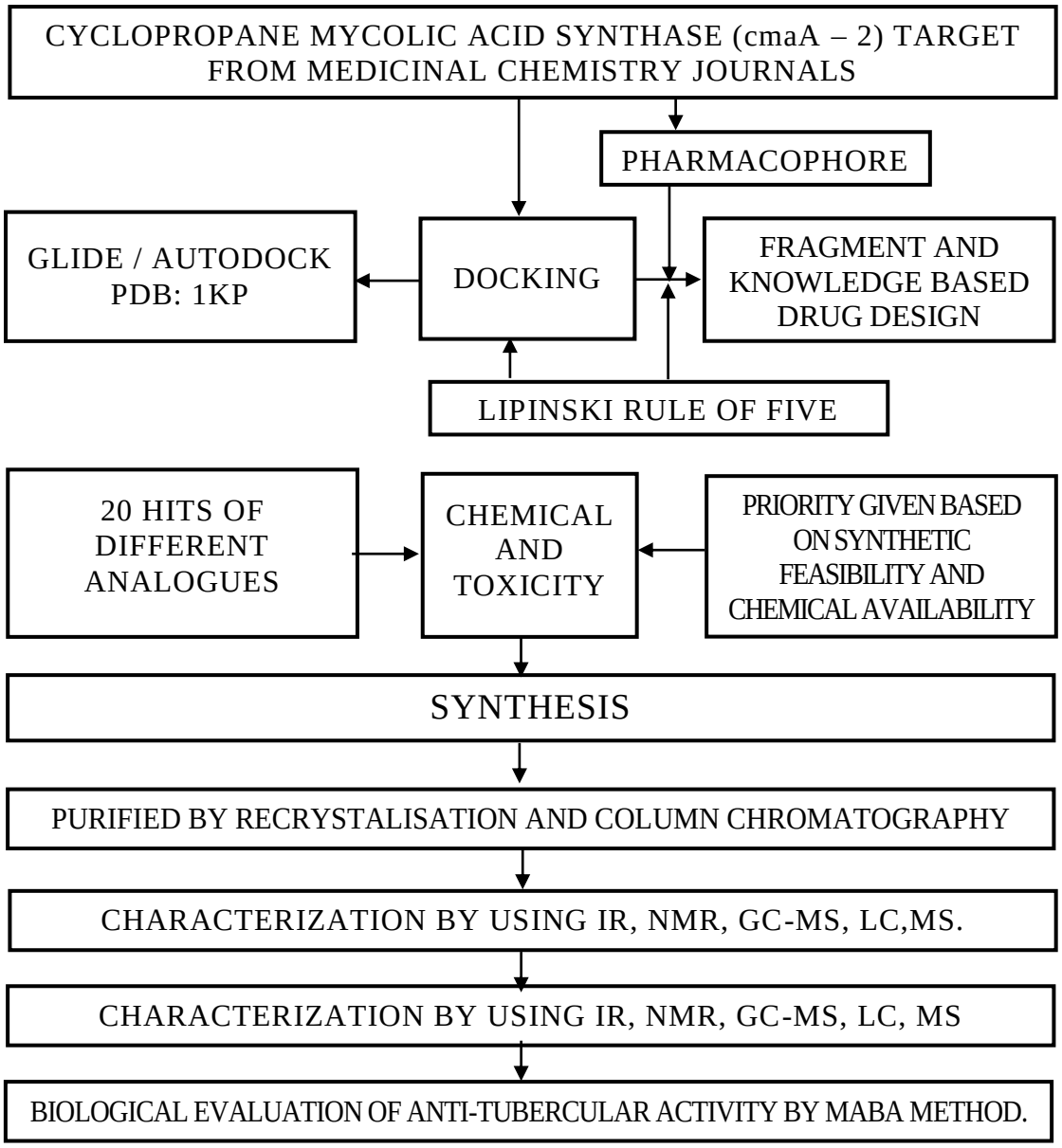
AIM

To develop novel Anti-tubercular molecules, which inhibit Cyclopropane mycolic acid synthase-2 (cmaA-2).

OBJECTIVE

The Objective of the project is to Design, Synthesis, and Characterize and biologically evaluates some novel Anti-tubercular molecules.

WORK FLOE OF THE STUDY



REVIEW OF LITERATURE

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

1) **De Souza MVN, et al.,(**2006)[68] Current status and future prospects for new therapies for Pulmonary Tuberculosis.

2) **Duncan k,et al.,** (2004)[69] Prospects for New Anti-Tubercular drugs. **Van der Geize,R.et al**.,(2007)"AGene Cluster Encoding Cholesterol Catabolismin a Soil Actinomycete Provides Insight into Mycobacterium Tuberculosis Survival in Macrophages."

The review on following works provided basic information about the target enzyme, 1KPI and its function:

3) **Maria Loreto Incandela., et al,** (2013) reported that 1KPI, a new taxonomic marker in mycobacteria. [70]

4) **Liao RZ *et al, (1978)*** Mechanism of Mycolic acid cyclopropane synthase. They demonstrated that the reaction starts via the transfer of a methyl to the substrate double bond, followed by the transfer of a proton from the methyl cation to the bicarbonate present in the active site. [26]

5) **George KM, *et al., (2008)*** the biosynthesis of Cyclopropanated mycolic acids in Mycobacterium tuberculosis. Identification and functional analysis of CMAS-2 revealed the gene whose product cyclopropanates the proximal double bond was cloned by homology to a putative cyclopropane synthase identified from the Mycobacterium leprae genome sequencing project. This gene, named cma2, was sequenced and found to be 52% identical to cma1 (which cyclopropanates the distal double bond) and 73% identical to the gene from M. leprae. Both cma genes were found to be restricted in distribution to pathogenic species of mycobacteria. Expression of cma2

in Mycobacterium smegmatis resulted in the cyclopropanation of the proximal double bond in the alpha 1 series of Mycolic acids. [71]

6) **Dominique Guianvarc'h*, et al., (2009)*** Identification of inhibitors of the E. coli Cyclopropane fatty acid synthase from the screening of a chemical library. [72]

7) **Christine *et al. (2007),*** Synthesis and evaluation of analogues of S-adenosyl-L-methionine, as inhibitors of the E. coli Cyclopropane fatty acid synthase. [73]

8) **Cécile Asselineau *et al. (2003)*** reviewed the biosynthesis of Mycolic acids by mycobacteria. [12]

9) **Michael S. Glickman *et al., (2012)*** revealed that Mycobacterium tuberculosis lacking all Mycolic acid, cyclopropanation is viable but highly attenuated and hyper inflammatory in mice. [10]

10) **Chih-chin Huang *et al***. (2012), Mycolic acids (PcaA, CmaA1, and CmaA2) are major components of the cell wall of *Mycobacterium tuberculosis*. Several studies indicate that functional groups in the acyl chain of Mycolic acids are important for pathogenesis and persistence. [10]

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

11) **Deepak. D. Borkar., et al. (2012),** Design and Synthesis of *p*-hydroxy benzohydrazide Derivatives for their Antimycobacterial Activity. [74]

12) **Romono T. Kroemeret *et al.(2003),*** An introduction into ligand–receptor docking. It illustrates the basic underlying concepts. [75]

13) **Andrew Worth *et al. (1998),*** Distribution, Metabolism and Excretion (ADME) properties, which are often important in discriminating between the toxicological profiles of parent compounds and their metabolites/degradation products. [76]

14) **Lipinski CA et al., (2001)** A experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. (55)

15) **Lipinski CA (2004)** A Lead and drug-like compounds and the role of fine resolution. (56)

16) **Madsen et al., (2002)** Textbook of Drug Design and Discovery. (77)

The review on following works provided ideas for synthesis of the desired benzothiazole nucleous for anti tubercular agents:

17) **RuhiAli and NadeemSiddiqui** Journal of Chemistry Volume 2013 (2013), Article ID 345198, 12 pages http://dx.doi.org/ 10.1155/2013/345198 Indian journal of pharmaceutical sciences. Review Article: Biological Aspects of Emerging Benzothiazoles: A Short Review(78)

18) **S. T. Asundaria and K. C. Patel,** "Synthesis, characterization and antimicrobial activity of thiazole, benzothiazole and pyrimidine derivatives bearing sydnone moieties," Pharmaceutical Chemistry Journal, vol. 45, no. 12, pp. 725–731, 2012(79)

19) **K. Bolelli, I. Yalcin, T. Ertan-Bolelli et al.,** "Synthesis of novel 2-[4-(4-substitutedbenzamido/phenylacetamido) phenyl]benzothiazoles as antimicrobial agents," Medicinal Chemistry Research, vol. 21, no. 11, pp. 3818–3825, 2012(80)

20) **P. K. Sharma, M. Kumar, and V. Mohan,** "Synthesis and antimicrobial activity of 2H-pyrimido[2,1-b]benzothiazol-2-ones," Research on Chemical Intermediates, vol. 36, no. 8, pp. 985–993, 2010. (81)

21) **C. Sheng, J. Zhu, W. Zhang et al.,** "3D-QSAR and molecular docking studies on benzothiazole derivatives as Candida albicans N-myristoyltransferase inhibitors," European Journal of Medicinal Chemistry, vol. 42, no. 4, pp. 477–486, 2007..(82)

22) **B. S. Soni, M. Ranawat, R. Sharma, A. Bhandari, and S. Sharma,** "Synthesis and evaluation of some new benzothiazole derivatives as potential antimicrobial agents," European Journal of Medicinal Chemistry, vol. 45, no. 7, pp. 2938–2942, 2010(83)

23) **P. K. Sahu, P. K. Sahu, S. K. Gupta, D. Thavaselvam, and D. D. Agarwal,** "Synthesis and evaluation of antimicrobial activity of 4H-pyrimido[2,1-b] benzothiazole, pyrazole and benzylidene derivatives of curcumin," European Journal of Medicinal Chemistry, vol. 54, pp. 366–378, 2012 (84)

24) **I. H. R. Tomi, J. H. Tomma, A. Daraji, and A. Al-Dujaili,** "Synthesis, characterization and comparative study the microbial activity of some heterocyclic compounds containing oxazole and benzothiazole moieties," Journal of Saudi Chemical Society, 2012(85)

25) **V. S. Padalkar, B. N. Borse, V. D. Gupta et al.,** "Synthesis and antimicrobial activity of novel 2-substituted benzimidazole, benzoxazole and benzothiazole derivatives," Arabian Journal of Chemistry, 2012.(86)

26) **S. Gilani, K. Nagarajan, S. P. Dixit, M. Taleuzzaman, and S. A. Khan,** "Benzothiazole incorporated thiazolidin-4-ones and azetidin-2-ones derivatives: synthesis and in vitro antimicrobial evaluation,"Arabian Journal of Medicinal Chemistry, 2012.(87)

27) **Arpana Rana, N Siddiqui, SA Khan Year :** 2007 Volume : 69 | Issue : 1 Page : 10-17 Benzothiazoles: A new profile of biological activities(88)

28) **G. Navarrete-Vazquez, M. Ramírez-Martínez, S. Estrada-Soto et al**., "Synthesis, in vitro and in silicoscreening of ethyl 2-(6-substituted benzo[d]thiazol-2-ylamino) -2-oxoacetates as protein-tyrosine phosphatase 1B inhibitors," European Journal of Medicinal Chemistry, vol. 53, pp. 346–355, 2012.(89)

29) **G. A. Pereira, A. C. Massabni, E. E. Castellano** et al., "A broad study of two new promising antimycobacterial drugs: Ag(I) and Au(I) complexes with 2-(2-thienyl)benzothiazole," Polyhedron, vol. 38, no. 1, pp. 291–296, 2012 .(90)

30) **V. N. Telvekar, V. K. Bairwa, K. Satardekar, and A. Bellubi**, "Novel 2-(2-(4-aryloxybenzylidene) hydrazinyl)benzothiazole derivatives as anti-tubercular agents," Bioorganic and Medicinal Chemistry Letters, vol. 22, no. 1, pp. 649–652, 2012.(91)

31) **L. Katz,** "Antituberculous compounds. III. Benzothiazole and benzoxazole derivatives," Journal of the American Chemical Society, vol. 75, no. 3, pp. 712–714, 1953(92)

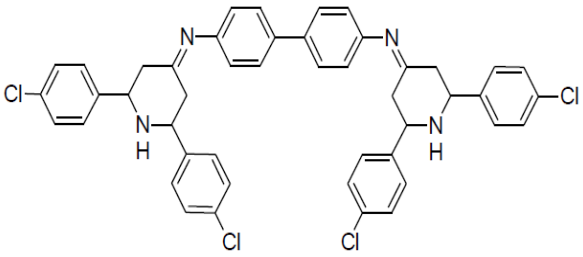
32) **Y. Cho, T. R. Ioerger, and J. C. Sacchettini,** "Discovery of novel nitrobenzothiazole inhibitors forMycobacterium tuberculosis ATP phosphoribosyl transferase (HisG) through virtual screening," Journal of Medicinal Chemistry, vol. 51, no. 19, pp. 5984–5992, 2008. (93)

33) **Kamuran Görgün, Handan Can Sakarya, and Müjgan Özkütük** The Synthesis, Characterization, Acid Dissociation, and Theoretical Calculation of Several Novel Benzothiazole Schiff Base Derivatives J. Chem. Eng. Data, 2015, 60 (3), pp 594–601(94)

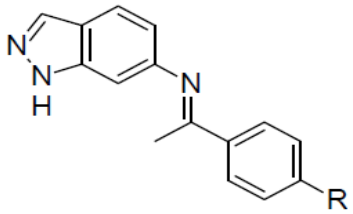
34) **Mahmood-ul-Hassan a, Zahid H. Chohanb* & Claudiu T. Supuran** Anti Bacterial Co(Ii) And Ni(Ii) Complexes Of Benzothiazole-Derived Schiff Bases DOI:10.1081/SIM-120014861 c pages 1445-1461 Article from Tayler And Francies Online (95)

35) **Vaibhav Sharma et al [38]** reviewed on the chemistry and biological activities of Schiff base. Schiff bases are the compounds which are mainly formed by the condensation of the aldehydes and amines. These compounds can be synthesized by various synthetic routes. Pharmacological actions of Schiff compounds which have been reported in previous studies are antimicrobial, antimalarial, antitubercular, anticancer, anthelmintic, analgesic, etc. (96)

36) **Khlood Fahed Hamak s**ynthesized Schiff base and evaluated it for antimicrobial activity. Schiff base were synthesis by the reaction of 2,6-bis(4-chlorophenyl)piperidone-4 with benzidine and reaction of 3,5-dimehyl-2,6-diphenyl piperidone-4 with 1,2-phenylenediamine. All synthesized compound were characterized and evaluated for their in vitro antibacterial activities, against gram positive (Staphylococcus aureus) and gram negative (Escheria coli).[97]



37) **Kalpesh S. Parikh et al.,** designed and synthesized Schiff bases from acetophenone. 6-amino imidazole Condensed with various aromatic acetophenone. Finally the product was characterized by conventional and instrumental methods. [98]



38) **Micheal J. Hearn et al** synthesized and characterized Schiff base of isoniazid and studied their biological activity. Few structural modification of the isonicotinic acid hydrazide (INH) was performed which provides lipophilic adaptations of the drug in which the hydrazine moiety of the parent compound has been chemically blocked from the deactivating process of N2-acetylation by N-aryl amino acetyl transferase.

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

39) **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. (64), (66), (67)

40) **Todd P. Primm., et., al(2007),** Recent Advances in Methodologies for the Discovery of Antimycobacterial Drugs. (99)

41) **Sephra N.Ramprasad** [71] studied the various applications of Alamar blue as an indicator. Alamar blue is an indicator that is used to evaluated metabolic function and cellular health. The Alamar blue bioassay is being utilized to access cell viability and cytotoxicity in a biological and environmental system and in a number of cell types including bacteria, yeast, fungi, and protozoa.(100)

42) **Jose d Jesus Alba-Romero et al** [72] applied the Alamar blue assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. The results showed that the MABA test is fast and easy to apply. It is very reliable method to determining the drug susceptibility to pharmaceuticals.(101)

MATERIALS AND METHODS

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

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

DOCKING STUDIES DRUG DESIGN:[37]

Drug design, sometimes referred to as **rational drug design** or simply **rational design**, is the inventive process of finding new medications based on the knowledge of a biological target.[38] 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.

Drug design frequently but not necessarily relies oncomputer modeling techniques.[39] This type of modeling is often referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure - based drug design.[39] In addition to small molecules, biopharmaceuticals and especially therapeutic antibodies are an increasingly important class of drugs and computational methods for improving the affinity, selectivity, and stability of these protein-based therapeutics have also been developed.[40]

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 optimization of other pharmaceutical properties while maintaining affinity

TYPES

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**.[39]

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.[41]

Structure-based

Structure-based drug design (or **direct drug design**) relies on knowledge of the three dimensional structureof the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy.[42] If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates.[43]

Binding site identification

Binding site identification is the first step in structure based design.[44],[45] If the structure of the target or a sufficiently similar homolog is determined in the presence of a bound ligand, then the ligand should be observable in the structure in which case location of the binding site is trivial. However, there may be unoccupied allosteric binding sites that may be of interest. Furthermore, it may be that only apoprotein (protein without ligand) structures are available and the reliable identification of unoccupied sites that have the potential to bind ligands with high affinity is non-trivial.

Scoring functions

Structure-based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying the principles of molecular recognition. Selective high affinity binding to the target is generally desirable since it leads to more efficacious drugs with fewer side effects. Thus, 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 (and known antitargets) and use the predicted affinity as a criterion for selection.[46]

One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Bohm.[47],[48] This empirical scoring function took the form:

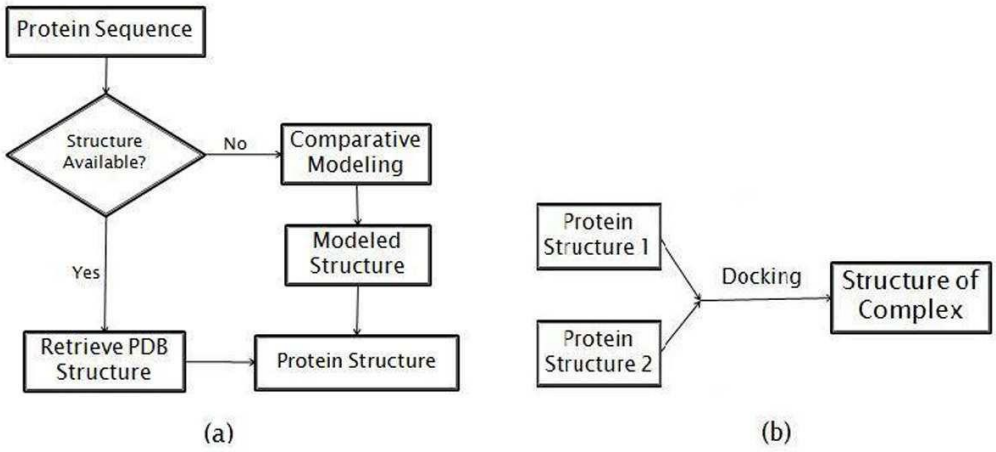
$$\Delta G_{\text{bind}} = \Delta G_0 + \Delta G_{\text{hb}} \Sigma_{h-bonds} + \Delta G_{\text{ionic}} \Sigma_{ionic-int} + \Delta G_{\text{lipophilic}} |A| + \Delta G_{\text{rot}} NROT$$

where:

- ΔG_0 – empirically derived offset that in part corresponds to the overall loss of translational and rotational entropy of the ligand upon binding.
- ΔG_{hb} – contribution from hydrogen bonding
- ΔG_{ionic} – contribution from ionic interactions
- ΔG_{lip} – contribution from lipophilic interactions where $|A_{lipo}|$ is surface area of lipophilic contact between the ligand and receptor

- ΔG_{rot} – entropy penalty due to freezing a rotatable in the ligand bond upon binding

Figure 1 [49]



STEPS INVOLVED IN DOCKING [50], [51], [52]

Docking is done by using ARGUS LAB Software

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

PROTEIN PREPARATION

Step: 1

- Protein (pdb) ID is entered in the protein data bank. (1KPI)
- I clicked the download files and select pdb as text file.
- Saved the downloaded pdb (text file) to the desktop.

Step: 2

- After I Opened Argus lab file→Open→Imported pdb file from the desktop.

- 3D Structure of the protein will appeared in the workspace of Argus lab.
- Left side of the screen shows molecular tree view.
- Open pdb → Open 'residues' → Open 'misc'
- From 'Misc' delete the inhibitor and hetero residues [Note: Do not delete Co-factor]
- Then I Opened water press shift, selected all water molecules and deleted.
- Added hydrogen atoms.
- Go to Calculation on the toolbar → energy by UFF method → start.
- Saved the prepared protein as *.agl file format in the desktop.

2. Q-SITE FINDER

Step: 1

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

Step: 2

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

3. LIGAND PREPARATION

- Draw the structure from Chem sketch and save as MDL Mol format.
- Imported the ligand into workspace of Argus lab.
- Cleaned Geometry, Cleaned Hybridisation.
- I Selected the ligand, Right click on the mouse Make a group from the residues give name ligand Ok.

4. DOCKING PROCEDURE

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

5. VISUALIZATION / INTERPRETATION OF DOCKING

Molegro Molecular viewer will help in analysing the energies and interaction of the binding.

- View → Secondary Structure view.
- View → Hydrogen bond interaction.
- Ligand map → Interaction overlay.

TOXICITY 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 color shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green color indicates drug-conform behavior. [54]

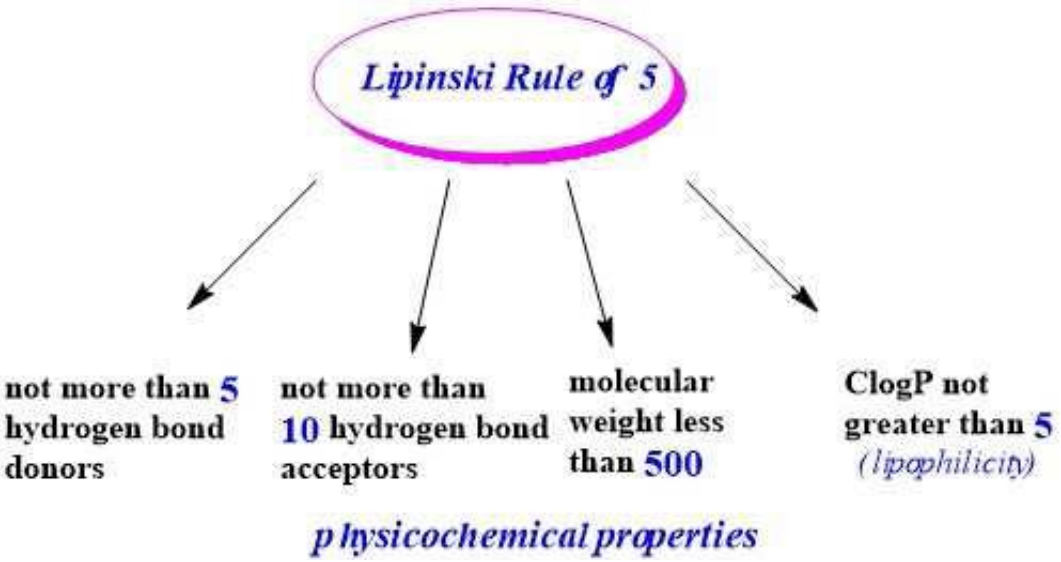
Molecular property prediction includes

- Toxicity risk assessment
- Clog P prediction
- Solubility prediction
- Molecular weight
- Drug likeness prediction
- Drug likeness score

LIPINSKI'S RULE OF FIVE (55), (56)

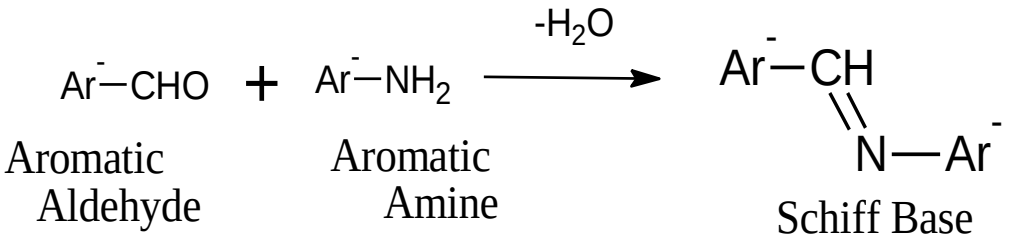
- **Lipinski's rule of five** also known as the **Pfizer's rule of five** or simply the **Rule of five** (RO5) is to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans.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

Figure 2 [57]



SYNTHETIC METHODOLOGY

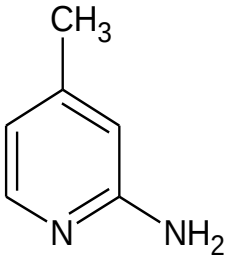
Scheme



Synthesis of Schiff base

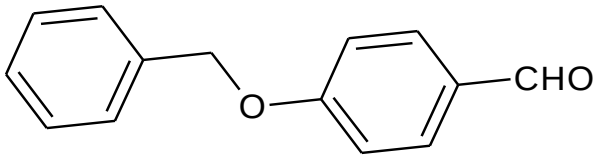
Equimolar quantity of 6-nitro-1H-benzimidazol-2-amine and substituted aromatic aldehydes is reflux for 10-15 hours in 20ml of ethanol. Completion of the reaction is monitor by TLC. After the completion of the reaction, the mixture was pour into a beaker containing ice cold water. The precipitate is form then filter the precipitate and dry it. The product is recrystallize by using ethanol. [58]

REACTANT PROFILE



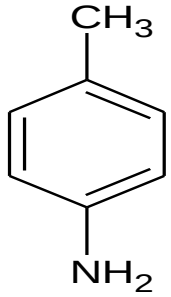
MOLECULAR FORMULA	:	$C_6H_8N_2$
MOLECULAR WEIGHT	:	108.14
BOILING POINT	:	230^0C
MELTING POINT	:	96-99^0C

BENZYLOXY BENZALDEHYDE



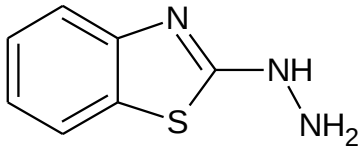
MOLECULAR FORMULA	:	$C_{14}H_{12}O$
MOLECULAR WEIGHT	:	212.24
MELTING POINT	:	70-72^0C

***p*-TOLUDINE**



MOLECULAR FORMULA	:	C_7H_9N
MOLECULAR WEIGHT	:	107
BOILING POINT	:	200^0C

2-HYDRAZINO BENZOYHIAZOLE

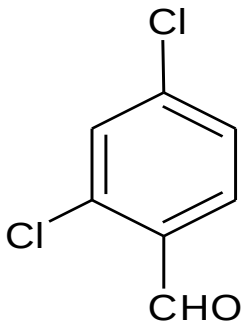


MOLECULAR FORMULA : $C_7H_7N_3S$

MOLECULAR WEIGHT : 165.

MELTING POINT : 198-202^{0}C

2, 4 DICHLORO BENZALDEHYDE



MOLECULAR FORMULA : $C_7H_4Cl_2O$

MOLECULAR WEIGHT : 175.01

BOILING POINT : 233^{0}C

MELTING POINT : 64-69^{0}C

CHARACTERIZATION PHYSICAL EVALUATION:

1) Physical properties of the synthesized compounds are evaluated, such as

- ❖ Nature
- ❖ Solubility
- ❖ Molecular formula
- ❖ Melting point

- ❖ Boiling point
- ❖ Colour
- ❖ Molecular weight

2) Further the synthesized compounds are Characterized by following Spectroscopic methods. Such as

IR SPECTROSCOPY[59]

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic chemists. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation. IR spectroscopy is an important and popular tool for structural elucidation and compound identification. The possible characteristic bands of the nucleus are

- ❖ 3540-3300 cm-1 N-H Stretching Vibration
- ❖ 3670-3230 cm-1 O-H Stretching Vibration
- ❖ 1690-1630 cm-1 C=N Stretching Vibration
- ❖ 2975-2840 cm-1 C-H Aliphatic Stretching Vibration
- ❖ 3100-3000 cm-1 C-H Aromatic Stretching Vibration

NMR SPECTROSCOPY[60]

NMR is the most powerful analytical tool currently available to an organic chemist. NMR allows characterization of a very small amount of sample (10mg), and does not destroy the sample (non-destructive technique). NMR spectra can provide vast information about a molecule's structure and can very often be the only way to prove what the compound really is. Typically though, NMR is used in conjunction with other types of spectroscopy and chemical analysis to fully confirm a complicated molecule's structure. It involves the interaction of the electromagnetic radiation and the

hydrogen of the nucleus when placed in an external static magnetic field. Some basic characteristic peaks of the nucleus

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

MASS SPECTROSCOPY[61]

Mass Spectrometry is an analytic technique that utilizes the degree of deflection of charged particles by a magnetic field to find the relative masses of molecular ions and fragments. It is a powerful method because it provides a great deal of information and can be conducted on tiny samples. Mass spectrometry has a number of applications in organic chemistry. They are:

- Determining molecular mass
- Finding out the structure of an unknown substance
- Verifying the identity and purity of a known substance
- Providing data on isotopic abundance

HYPHENATED TECHNIQUE[62],[63]

1.GC-MS

It is a combined technique, used for molecular weight determination. Gas chromatography and mass spectroscopy combined to form GC-MS.

2.LC-MS:

LC-MS is an analytical chemistry technique that combines with physical seperation capabilities of liquid chromatography with mass analusis capabilities of mass chromatography. It has very high sensitivity.

BIOLOGICAL EVALUATION

Anti-tubercular Activity

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

- Micro plate Alamar Blue Assay
- BACTEC Assay
- Luciferous Reporter Phage assay
- REMA Assay
- Broth Dilution Assay
- Middle brook(7H 9,7H 10,7H 11) Agar Dilution Assay.

THE ALAMAR BLUE ASSAY

Alamar Blue monitors the reducing environment of the living cell. The active ingredient is resazurin (IUPAC name: 7-hydroxy-10-oxidophenoxazin-10-ium-3-one), also known as diazoresorcinol, azoresorcin, resazoin, resazurine, which is water-soluble, stable in culture medium, is non-toxic and permeable through cell membranes. Continuous monitoring of cells in culture is therefore permitted. Growth is measured quantitatively by a visual colour change and the amount of fluorescence produced is proportional to the number of the living cells which is determined by colorimetric and fluorimetric methods.[64]

APPLICATIONS

- Especially meant for studies on Mycobacterium tuberculosis.
- Used extensively in cell viability and cytotoxicity studies.[65]

PROCEDURE for Anti-TB activity using Alamar Blue Dye[66],[67]

- The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).
- This methodology is non-toxic, uses a thermally stable reagent and shows Good correlation with propotional and BACTEC radiometric method
- 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.
- The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
- The final drug concentrations tested were 100 to 0.2 µg/ml.
- Plates were covered and sealed with parafilm and incubated at 37ºC for five days.
- 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.
- A blue color in the well was interpreted as no bacterial growth, and pink Color was scored as growth.
- The MIC was defined as lowest drug concentration which prevented the Color change from blue to pink.

RESULT AND DISCUSSION

Two hundred molecules which were sketched using chemsketch.® Then it were docked against the MTB enzyme cyclopropane mycolic acid synthase 2 by using Argus lab 4.0.1® softwere. The molecules with best docking score and good interaction were selected and synthesised.

The molecules were also docked against the following targets,

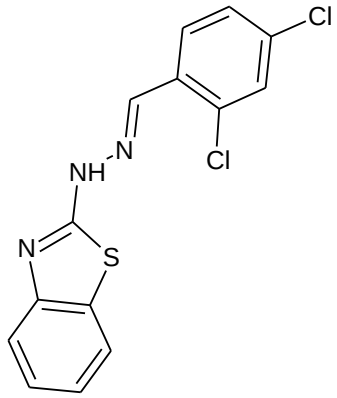
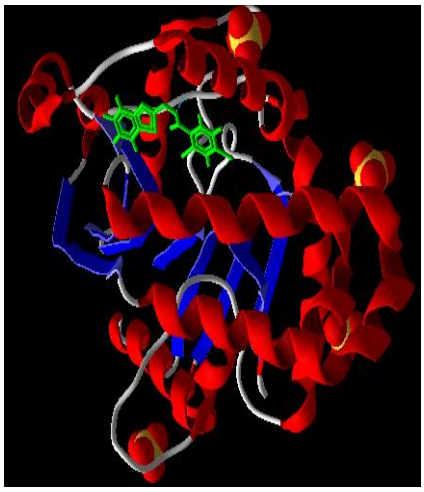
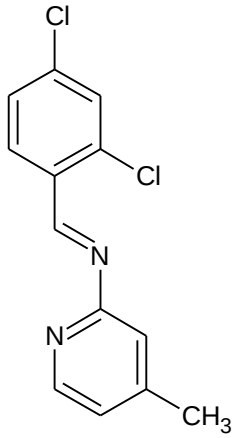
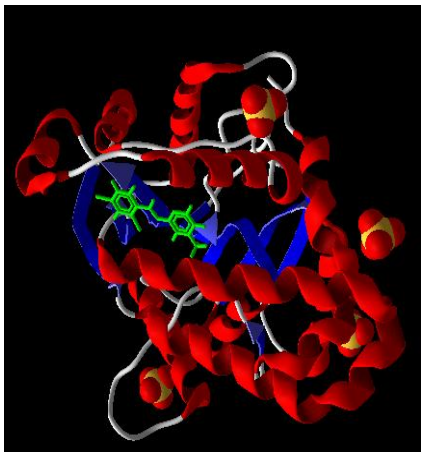
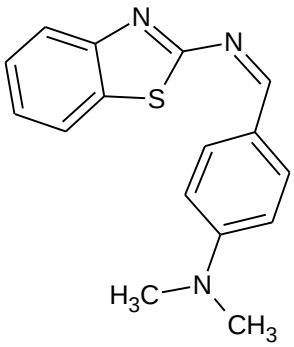
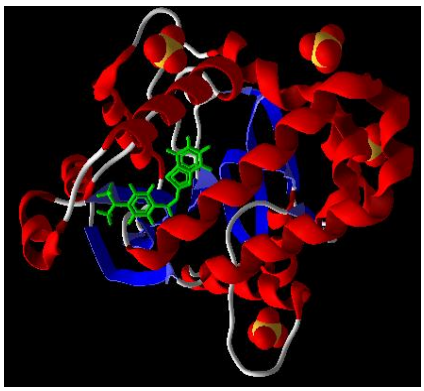
- Oxidoreductase (4TRM)
- Cyclopropane fatty acid synthase (3HEM)
- Thymidilate synthase (3GWC)

The resultant scores are tabelled below,

Table No: 1

Name of the targets	Docking scores(kcal/mol)				
	M1	**M2**	**M3**	**M7**	**M11**
cyclopropane mycolic acid synthase 2(1KPI)	-10.5407	-9.79903	-8.445	-11.140	-9.2508
Oxidoreductase (4TRM),	-9.6345	-6.1127	-5.875	-10.664	7.6654
Cyclopropane fatty acid synthase (3HEM)	-8.6543	-5.6759	-11.564	-8.4538	-9.876
Thymidilate synthase (3GWC	-9.9983	-11.7685	-7.560	-6.0029	-4.9876

Interaction of the docked molecules with the enzyme cyclopropane mycolic acid synthase 2 (1KPI)

M1		-10.5407 kcal/mol	
M2		-9.79903 kcal/mol	
M3		-8.445 kcal/mol	

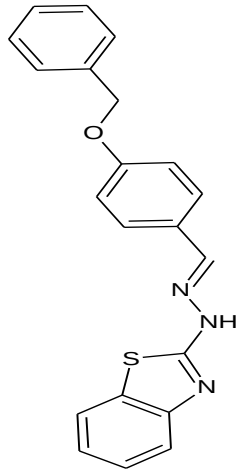
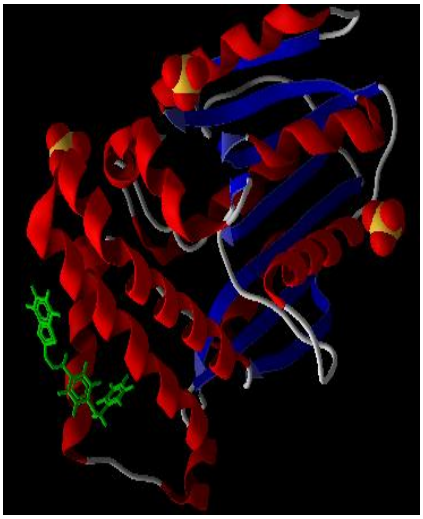
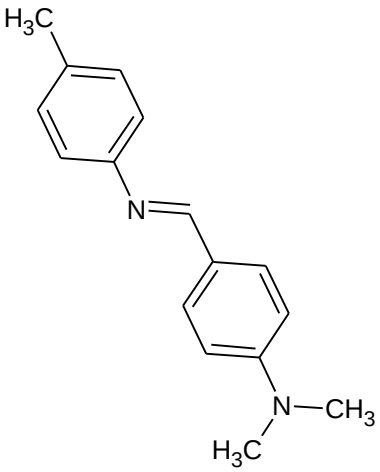
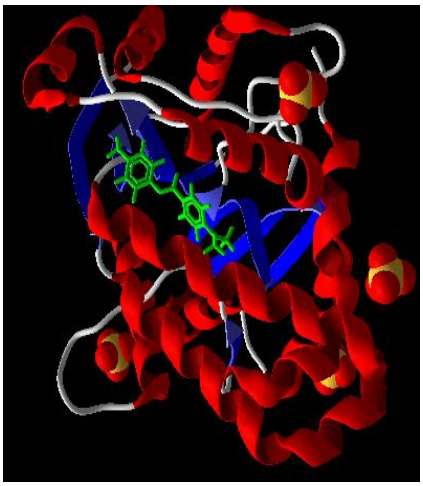
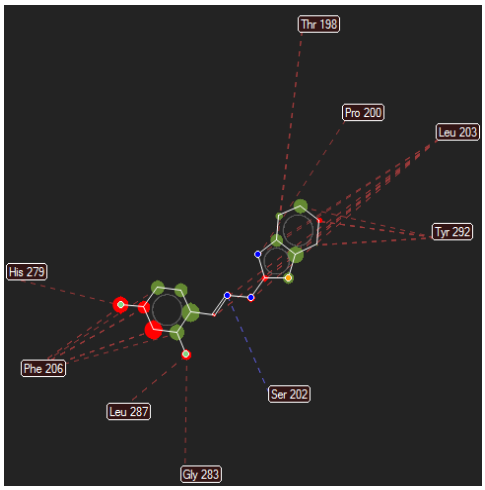
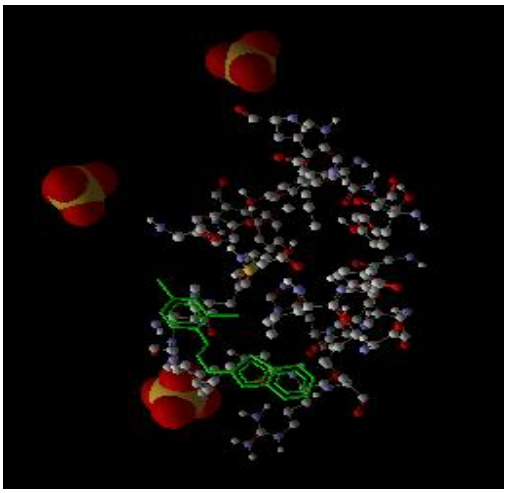
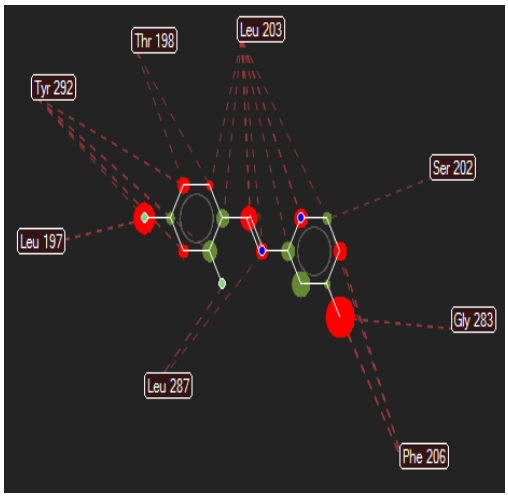
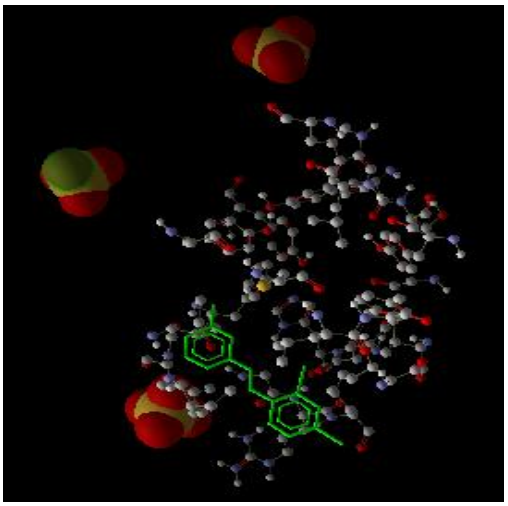
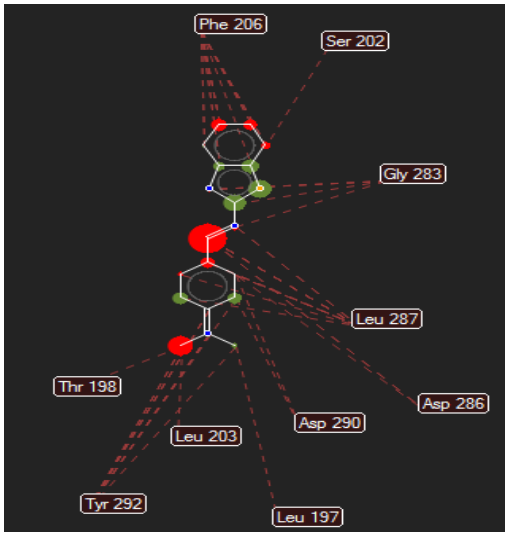
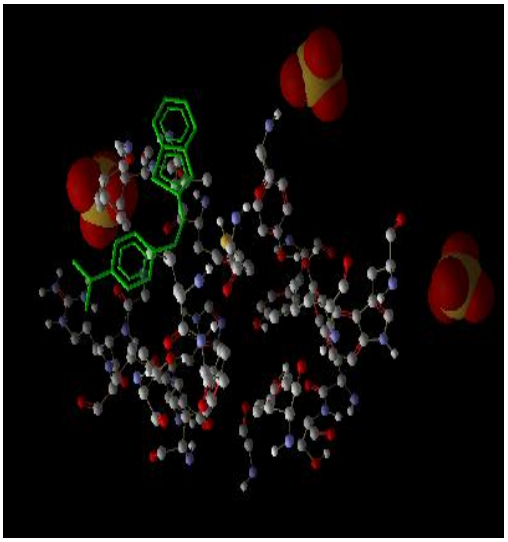
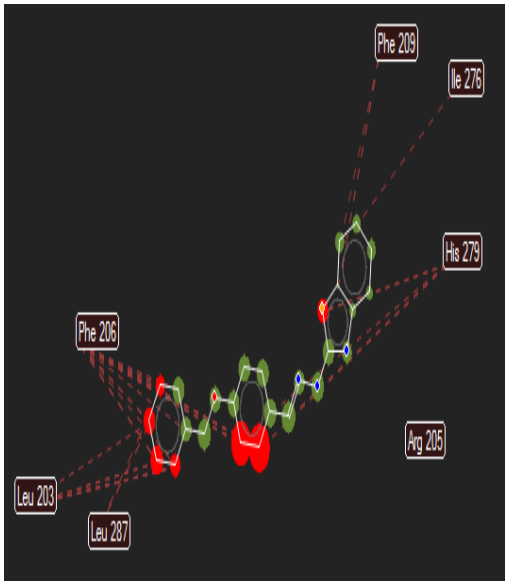
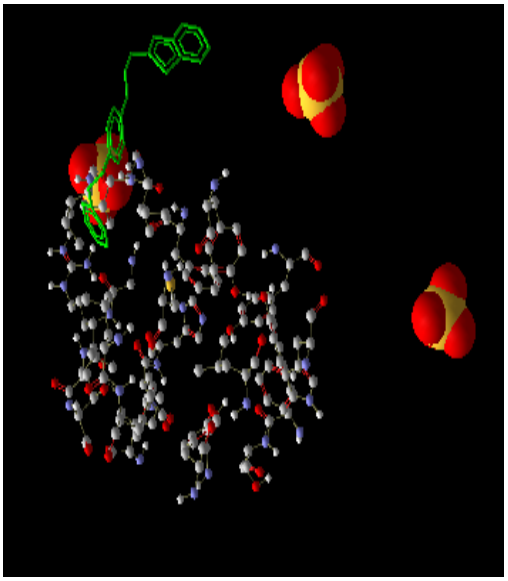
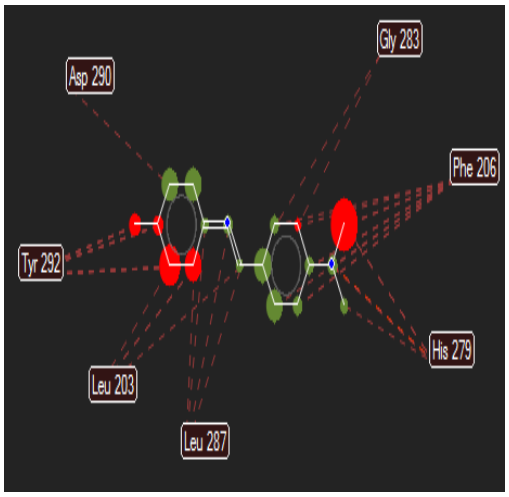
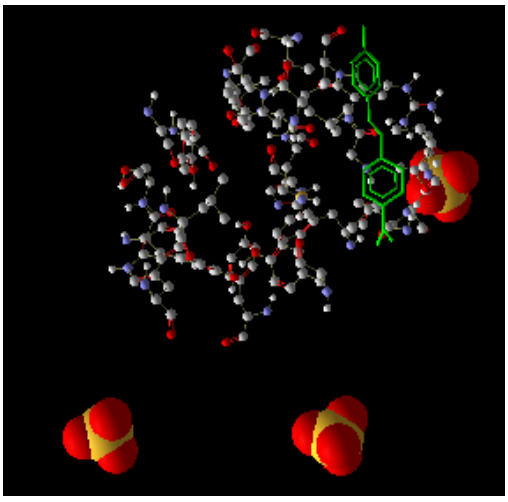
M7		-11.1405 kcal/mol	
M11		-9.2508 kcal/mol	

TABLE 1: 1KPI INTERACTION WITH LIGAND

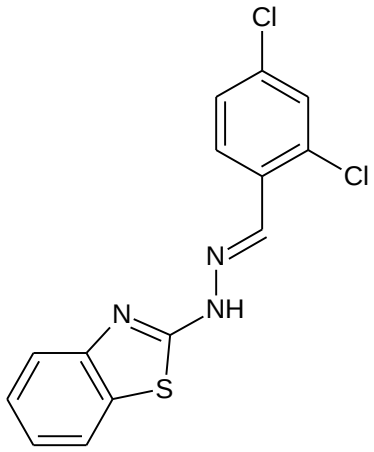
S.NO	INTERACTION WITH AMINO ACIDS	HYDROGEN BOND INTERACTION
M1		
M2		
M3		

M7	 Phe 209 Ile 276 His 279 Phe 206 Arg 205 Leu 203 Leu 287	
M11	 Gly 283 Asp 290 Phe 206 Tyr 292 His 279 Leu 203 Leu 287	

PHYSIO CHEMICAL PROPERTIES OF SYNTHESISED SAMPLES

SAMPLE CODE: M1

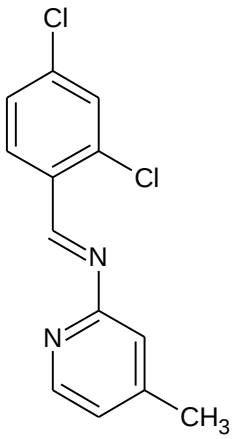
IUPAC: 2-[(2*E*)-2-(2,4-dichlorobenzylidene)hydrazinyl]-1,3-benzothiazole



Molecular Formula	:	$C_{14}H_9Cl_2N_3S$
Formula Weight	:	322.21236
Appearance	:	light yellow
Melting point	:	230^0C
Solubility	:	Methanol, Chloroform, Ethyl acetate
Composition	:	C (52.19%) H (2.82%) Cl (22.01%) N (13.04%) S (9.95%)
Molar Refractivity	:	85.59 ± 0.5 cm^3
Molar Volume	:	219.7 ± 7.0 cm^3
Parachor	:	596.9 ± 8.0 cm^3
Index of Refraction	:	1.707 ± 0.05
Surface Tension	:	54.4 ± 7.0 dyne/cm
Density	:	1.46 ± 0.1 g/cm^3
Surface Tension	:	40.9 ± 7.0 dyne/cm

SAMPLE CODE: M2

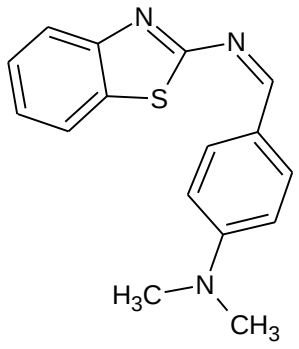
IUPAC: (*E*)-1-(2,4-dichlorophenyl)-*N*-(4-methylpyridin-2-yl)methanimine



Molecular Formula	:	$C_{13}H_{10}Cl_2N_2$
Formula Weight	:	265.1379
Composition	:	C(58.89%) H(3.80%) Cl(26.74%) N(10.57%)
Appearance	:	light yellow
Melting point	:	900C
Solubility	:	Methanol, Chloroform, Ethyl acetate
Molar Refractivity	:	72.48 ± 0.5 cm3
Molar Volume	:	212.0 ± 7.0 cm3
Parachor	:	536.3 ± 8.0 cm3
Index of Refraction	:	1.599 ± 0.05
Polarizability	:	28.73 ± 0.5 10-24cm3
Density	:	1.25 ± 0.1 g/cm3
Polarizability	:	33.93 ± 0.5 10-24cm3

SAMPLE CODE: M3

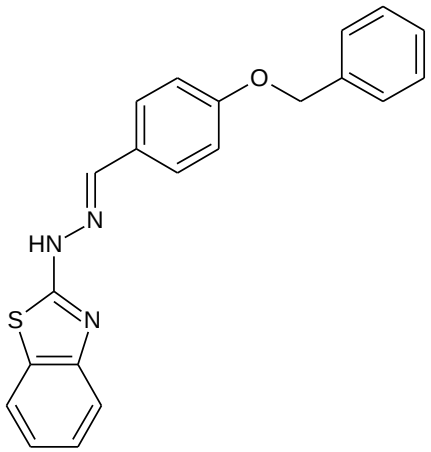
IUPAC: 4-[(*Z*)-(1,3-benzothiazol-2-ylimino)methyl]-*N*,*N*-dimethylaniline



Molecular Formula	:	$C_{16}H1_5N_3S$
Formula Weight	:	281.3754
Composition	:	C(68.30%) H(5.37%) N(14.93%) S(11.40%)
Appearance	:	light yellowish colour
Melting point	:	2300C
Solubility	:	Methanol, Chloroform, Ethyl acetate
Molar Refractivity	:	86.14 ± 0.5 cm3
Molar Volume	:	237.6 ± 7.0 cm3
Parachor	:	615.8 ± 8.0 cm3
Index of Refraction	:	1.644 ± 0.05
Surface Tension	:	45.0 ± 7.0 dyne/cm
Density	:	1.18 ± 0.1 g/cm3
Polarizability	:	34.14 ± 0.5 10-24cm3

SAMPLE CODE: M7

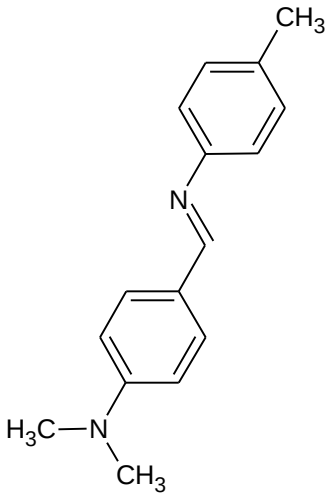
IUPAC: 2-{(2*E*)-2-[4-(benzyloxy) benzylidene] hydrazinyl}-1, 3-benzothiazole



Molecular Formula	:	$C_{21}H_{17}N_3OS$
Formula Weight	:	359.44418
Composition	:	C(70.17%) H(4.77%) N(11.69%) O(4.45%) S(8.92%)
Appearance	:	Light brown colour
Melting point	:	2700C
Solubility	:	Methanol, Chloroform, Ethyl acetate
Molar Refractivity	:	107.49 ± 0.5 cm3
Molar Volume	:	291.6 ± 7.0 cm3
Parachor	:	773.9 ± 8.0 cm3
Index of Refraction	:	1.658 ± 0.05
Surface Tension	:	49.5 ± 7.0 dyne/cm
Density	:	1.23 ± 0.1 g/cm3
Polarizability	:	42.61 ± 0.5 10-24cm3

SAMPLE CODE: M11

IUPAC: *N*, *N*-dimethyl-4-{(*E*)-[(4-methylphenyl)imino]methyl}aniline



Molecular Formula	:	$C_{16}H_{18}N_2$
Formula Weight	:	238.32752
Composition	:	C (80.63%) H (7.61%) N (11.75%)
Appearance	:	Dark yellow
Melting point	:	1100C
Solubility	:	Methanol, Chloroform, Ethyl acetate
Molar Refractivity	:	77.63 ± 0.5 cm3
Molar Volume	:	246.0 ± 7.0 cm3
Parachor	:	593.8 ± 8.0 cm3
Index of Refraction	:	1.543 ± 0.05
Surface Tension	:	33.9 ± 7.0 dyne/cm
Density	:	0.96 ± 0.1 g/cm3
Polarizability	:	30.77 ± 0.5 10-24cm3

IR SPECTRUM

The samples were prepared by the KBr pellet techniques & spectrum obtained by using FT-IR SHIMADZU.

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

The reaction involves between the aldehydes and amines to give the yield of Schiff base. Therefore it is expected that there should be no absorption band corresponding to either the aldehyde or the amine.

An absorption band corresponding to C=N stretching -1550-1680cm^{-1}.

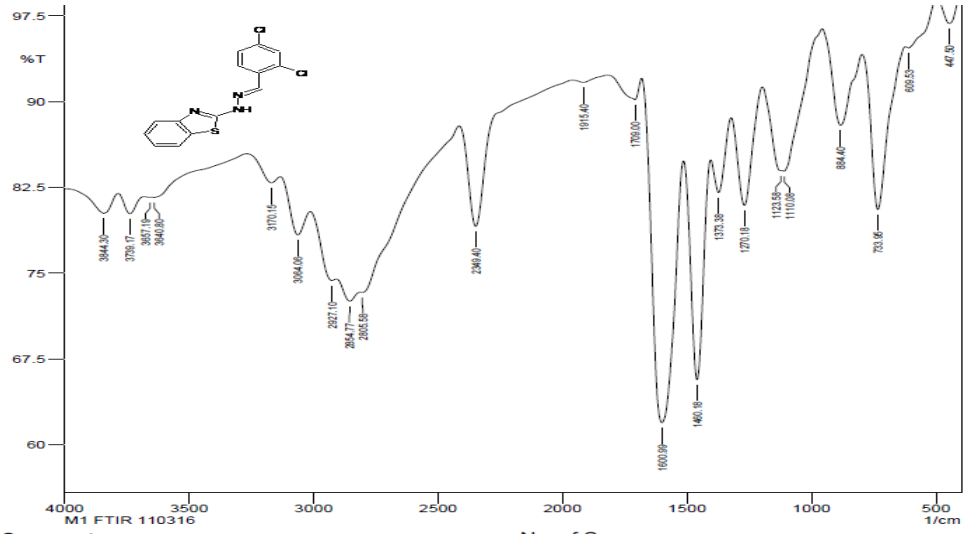
Table No: 2

Absorption bands	M1	M2	M3	M4	M5
Aldehydes	X	X	X	X	X
Amines	X	X	X	X	X
C=N	✓	✓	✓	✓	✓

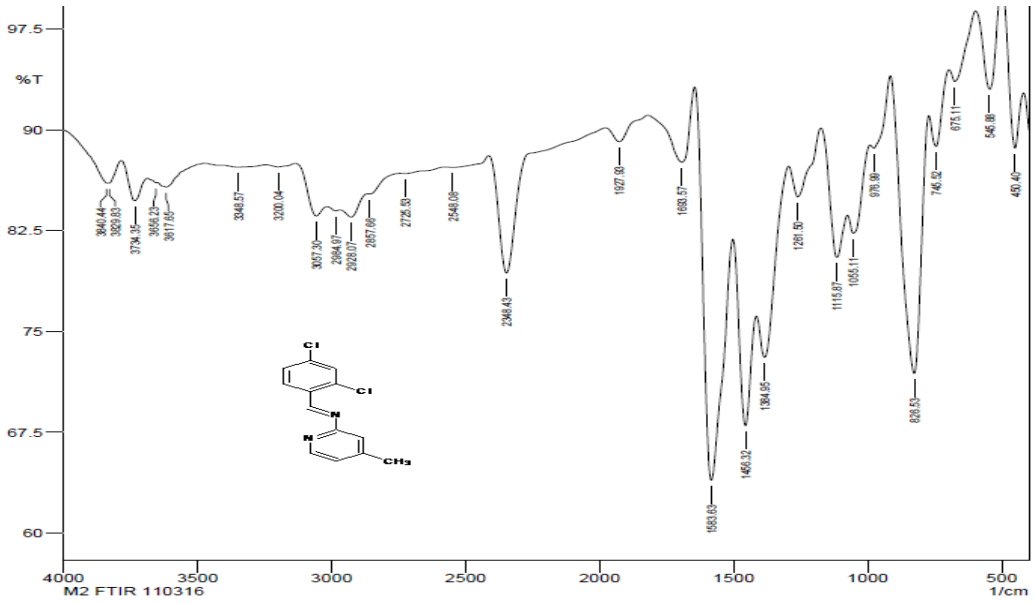
(✓) - Indicates presence of functional groups

(X) - Indicates absence of functional groups

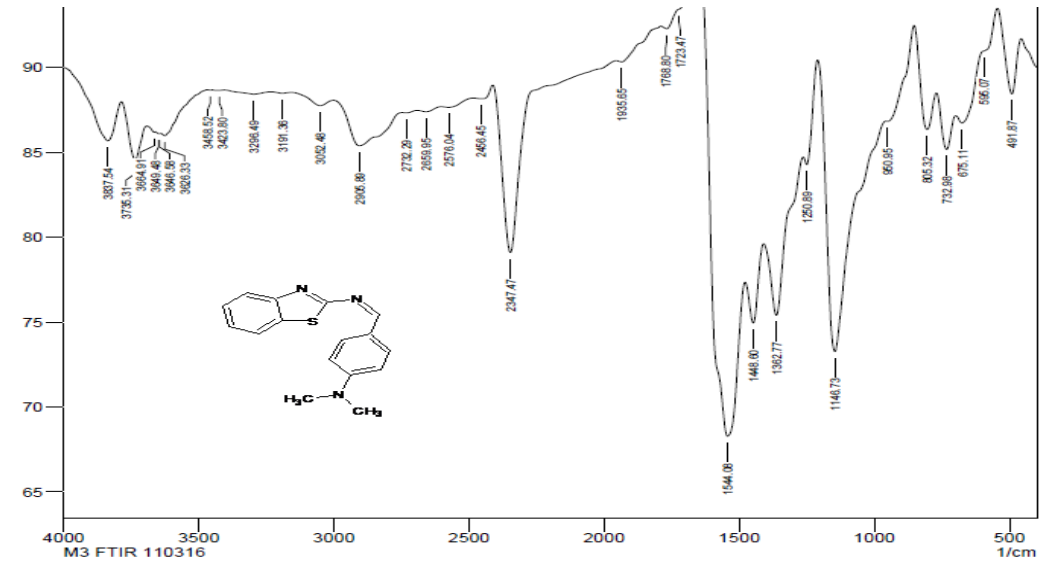
SAMPLE CODE: M1



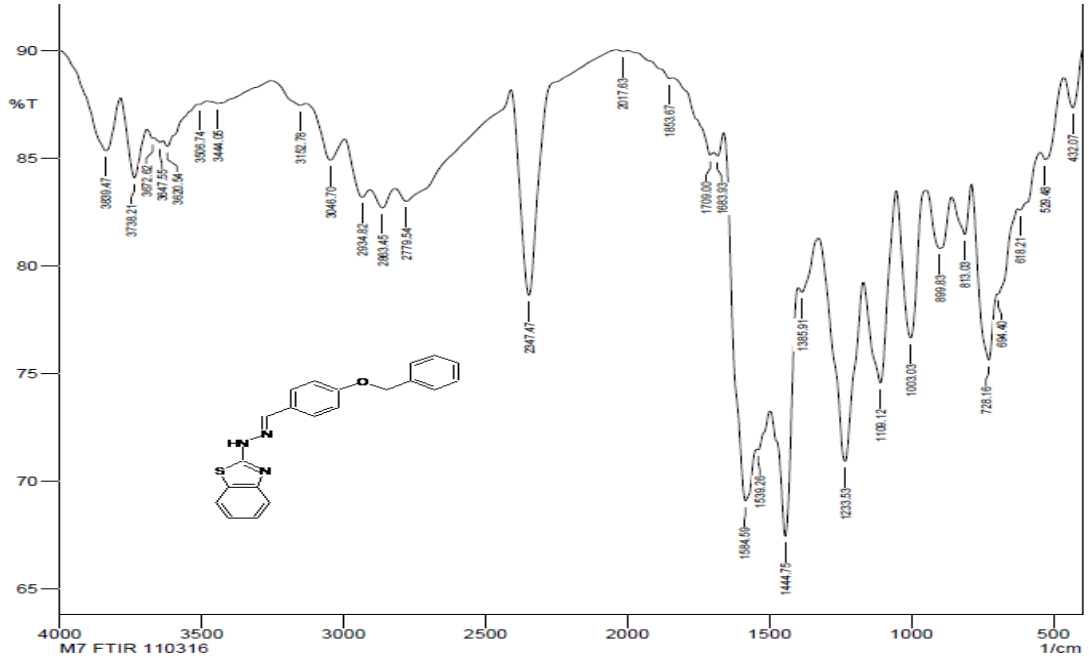
SAMPLE CODE: M2



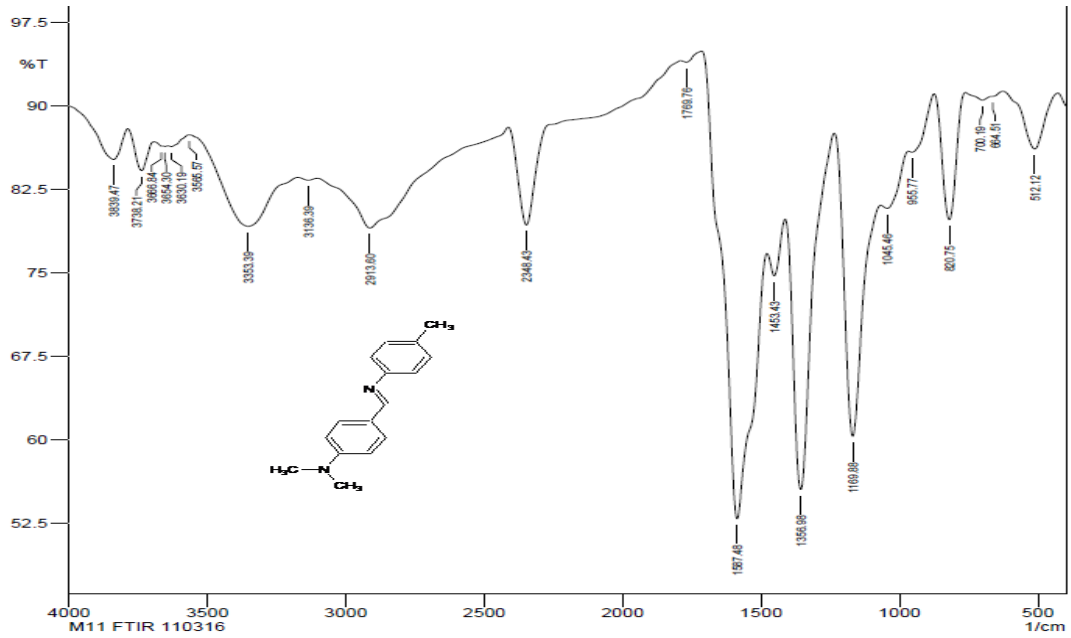
SAMPLE CODE: M3



SAMPLE CODE: M7



SAMPLE CODE: 11



LC-MS SPECTRUM

The molecular weight of synthesised compounds are compared by using LC-MS Spectrum.

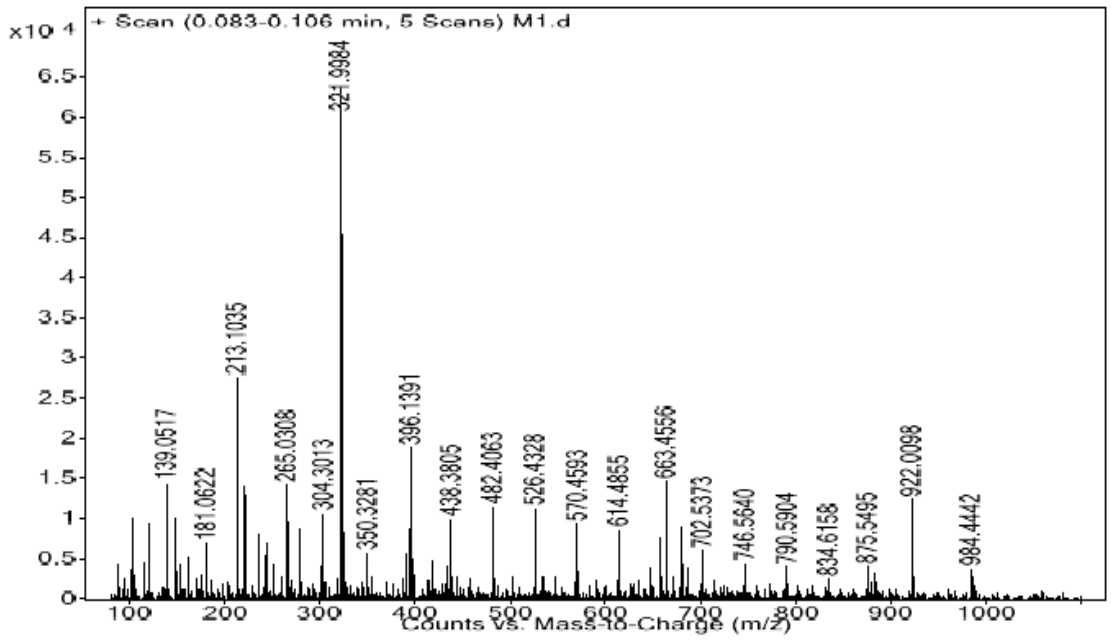
LC-MS results.

Table No: 3

SAMPLES	CALCULATED MASS	ACTUAL MASS OF SYNTHESIZED COMPOUND
M1	322.2123 g/mol	321.9984 g/mol
M2	265.1329 g/mol	265.0306 g/mol
M3	281.3754 g/mol	282.1063 g/mol
M7	359.4441 g/mol	360.1167 g/mol
M11	238.3275 g/mol	239.1555 g/mol

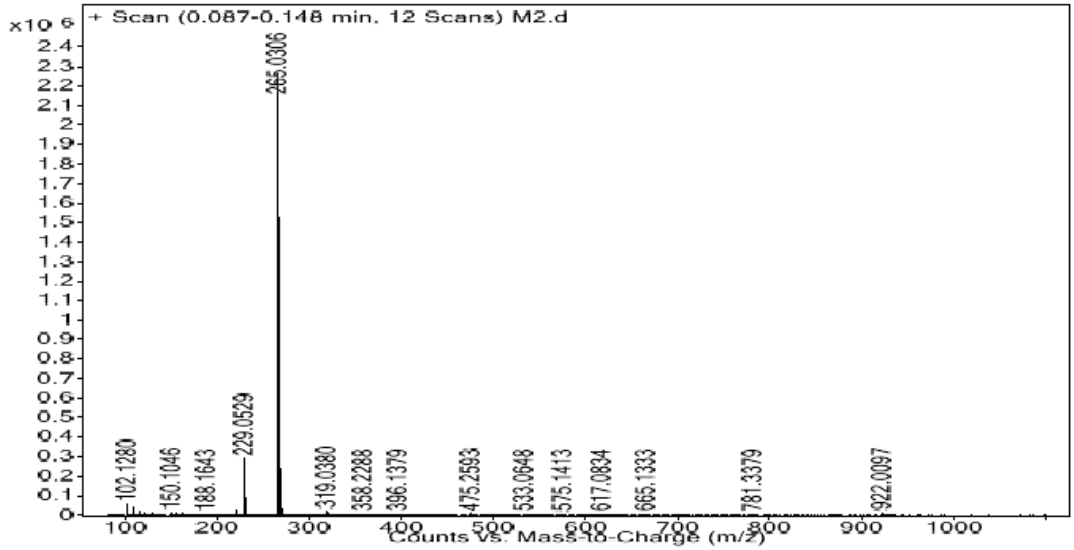
SAMPLE CODE: M1

AnalysisFileName	AcquiredTime	InstrumentName
M1.d	1-Mar-2016	LC-QTOF MS/MS



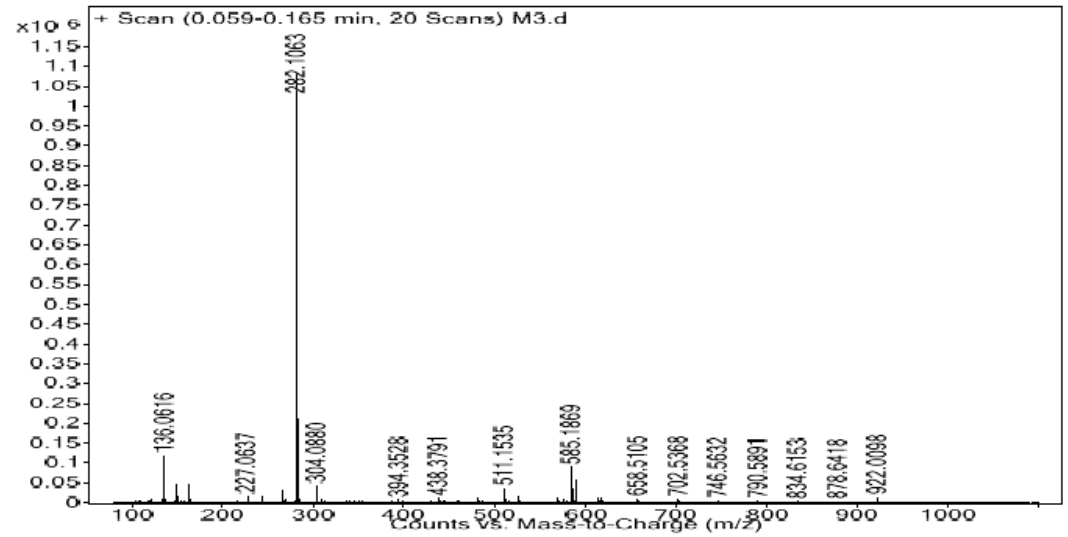
SAMPLE CODE:M2

AnalysisFileName	AcquiredTime	InstrumentName
M2.d	1-Mar-2016	LC-QTOF MS/MS



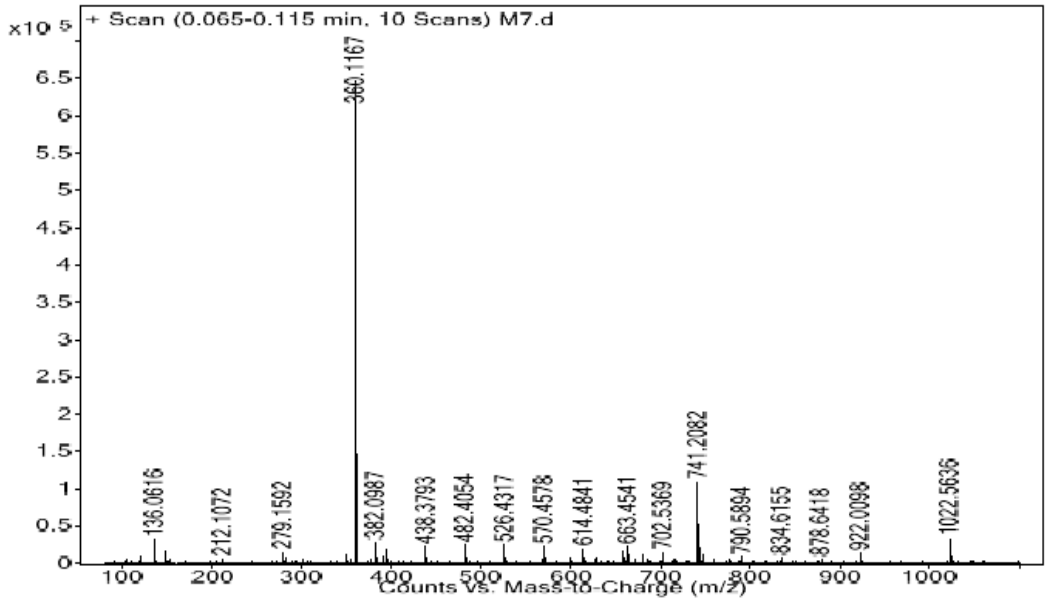
SAMPLE CODE:M3

AnalysisFileName	AcquiredTime	InstrumentName
M3.d	25-Feb-2016	LC-QTOF MS/MS



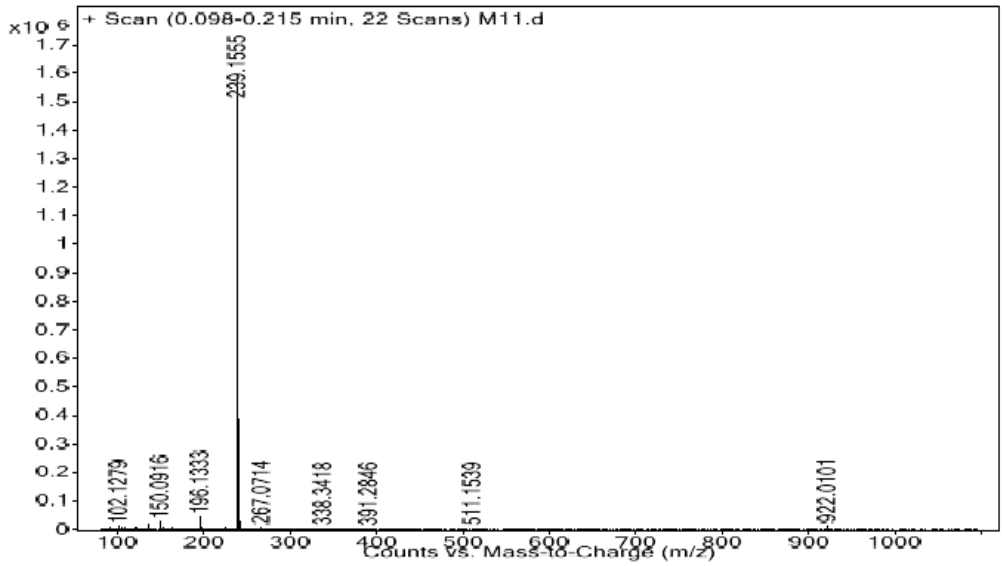
SAMPLE CODE:M7

AnalysisFileName	AcquiredTime	InstrumentName
M7.d	25-Feb-2016	LC-QTOF MS/MS



SAMPLE CODE:M11

AnalysisFileName	AcquiredTime	InstrumentName
M11.d	25-Feb-2016	LC-QTOF MS/MS



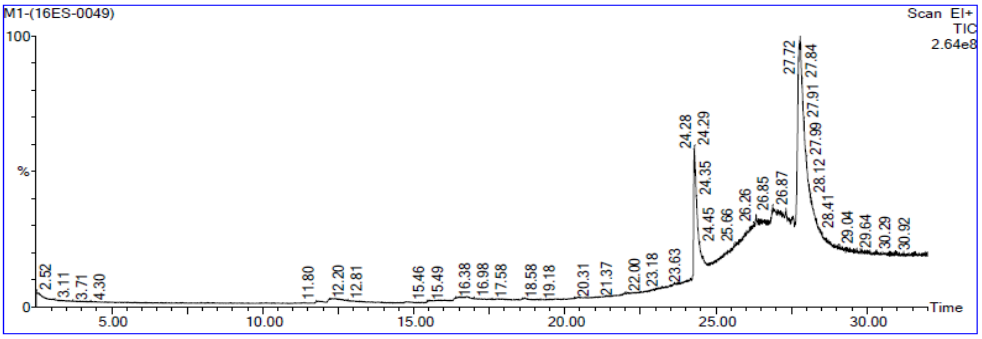
GC-MS SPECTRUM

The molecular weight of synthesised compounds are compared with the GC-MS results.

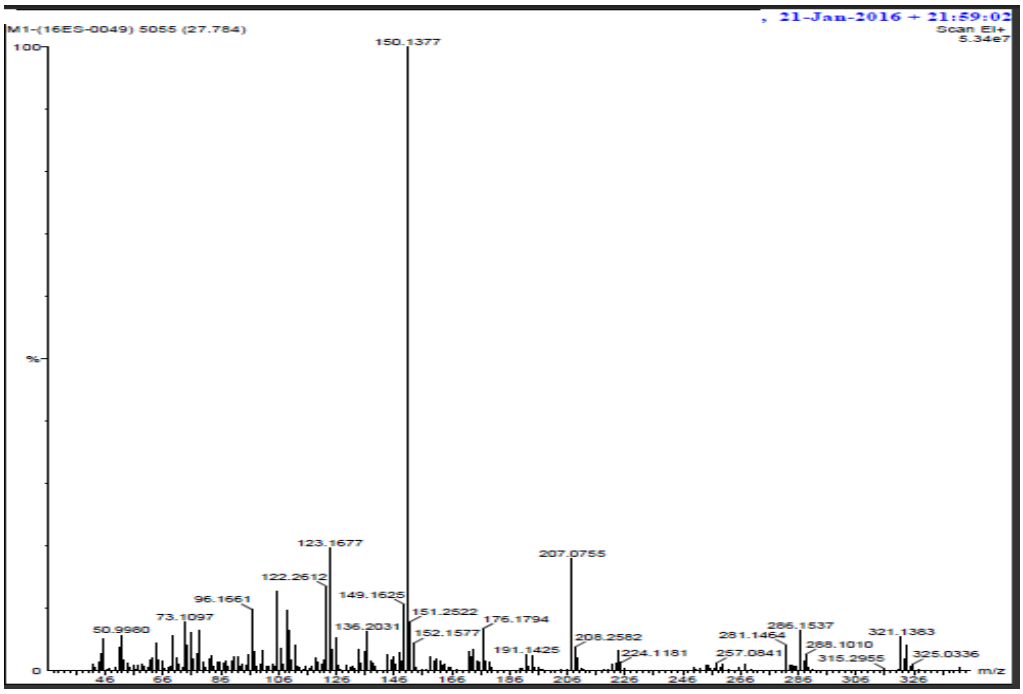
Table No: 4

SAMPLES	CALCULATED MASS	ACTUAL MASS OF SYNTHESISED COMPOUNDS
M1	322.21 g/mol	321.1360 g/mol
M2	265.13 g/mol	265.1512 g/mol

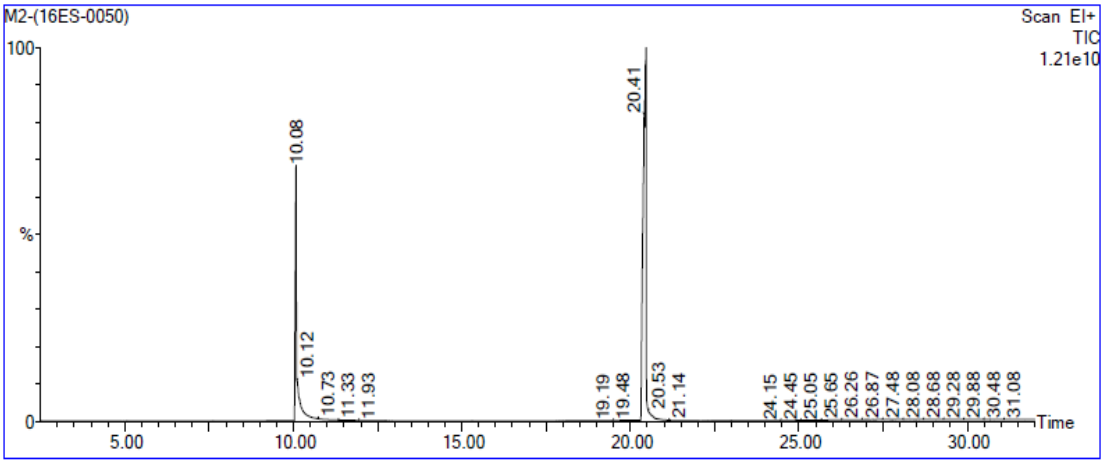
SAMPLE CODE:M1



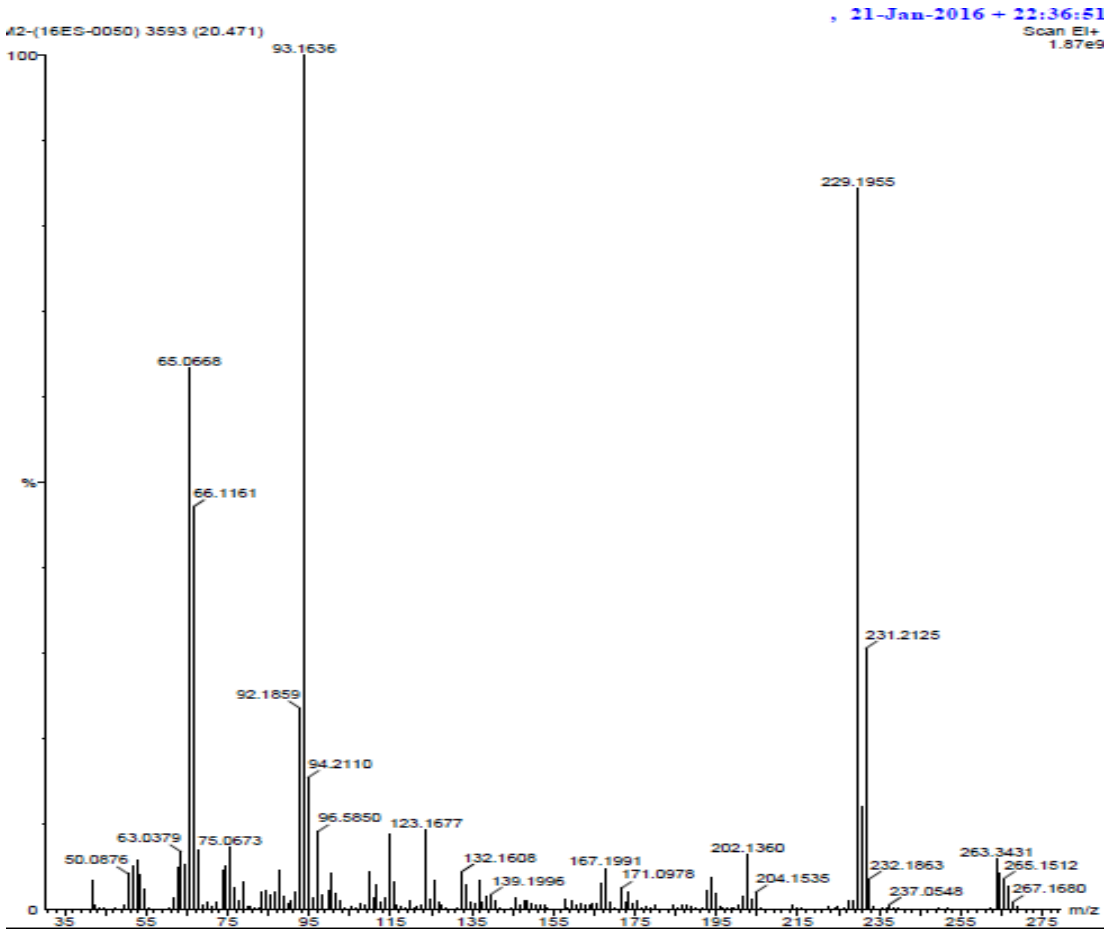
#	RT	Scan	Height	Area	Area %	Norm %
1	24.292	4357	127,968,832	17,303,806.0	22.664	36.39
2	26.328	4764	19,441,950	7,159,683.0	9.378	15.06
3	26.873	4873	18,400,436	4,337,891.5	5.682	9.12
4	27.784	5055	184,424,064	47,546,596.0	62.276	100.00



SAMPLE CODE: M2



#	RT	Scan	Height	Area	Area %	Norm %
1	10.081	1516	8,162,399,744	356,588,544.0	21.900	28.04
2	20.471	3593	12,037,055,488	1,271,704,960.0	78.100	100.00



NMR SPECTRUM

Our synthesised compounds were subjected to H^1 NMR instruments. Then the compounds are characterised by using NMR spectrum.

SAMPLE CODE: M3

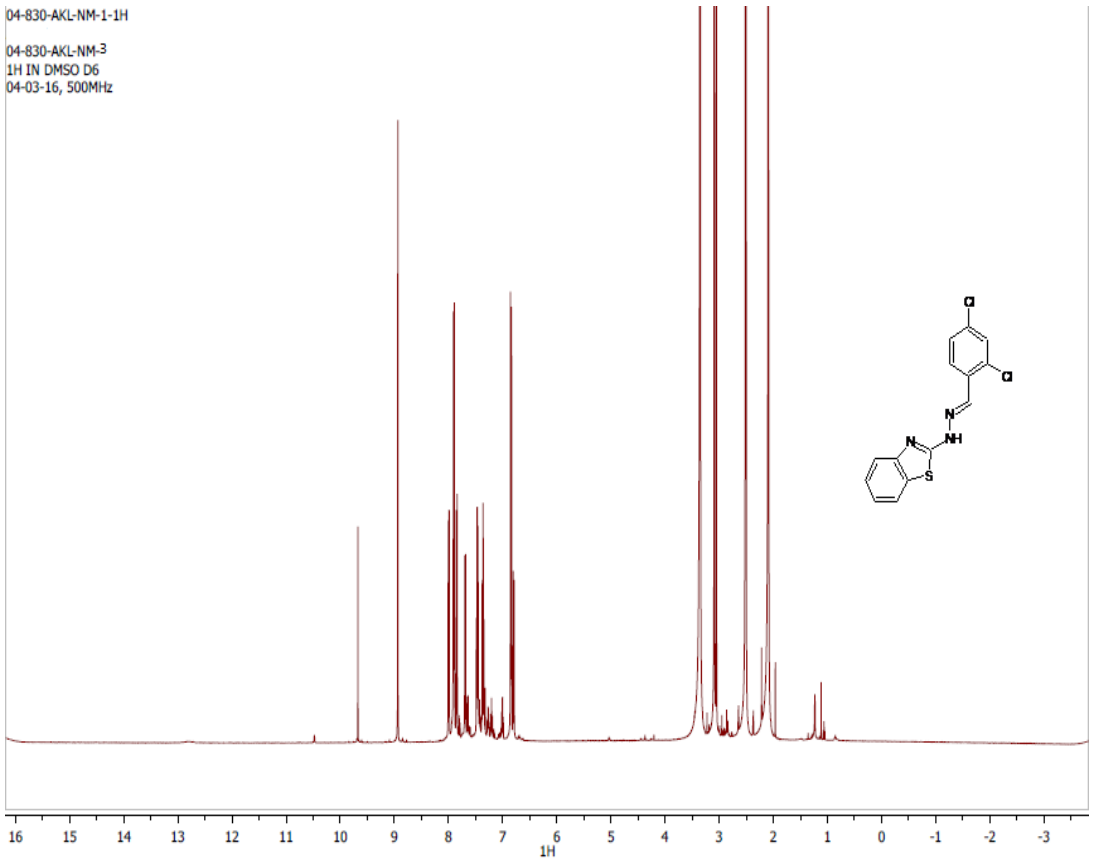
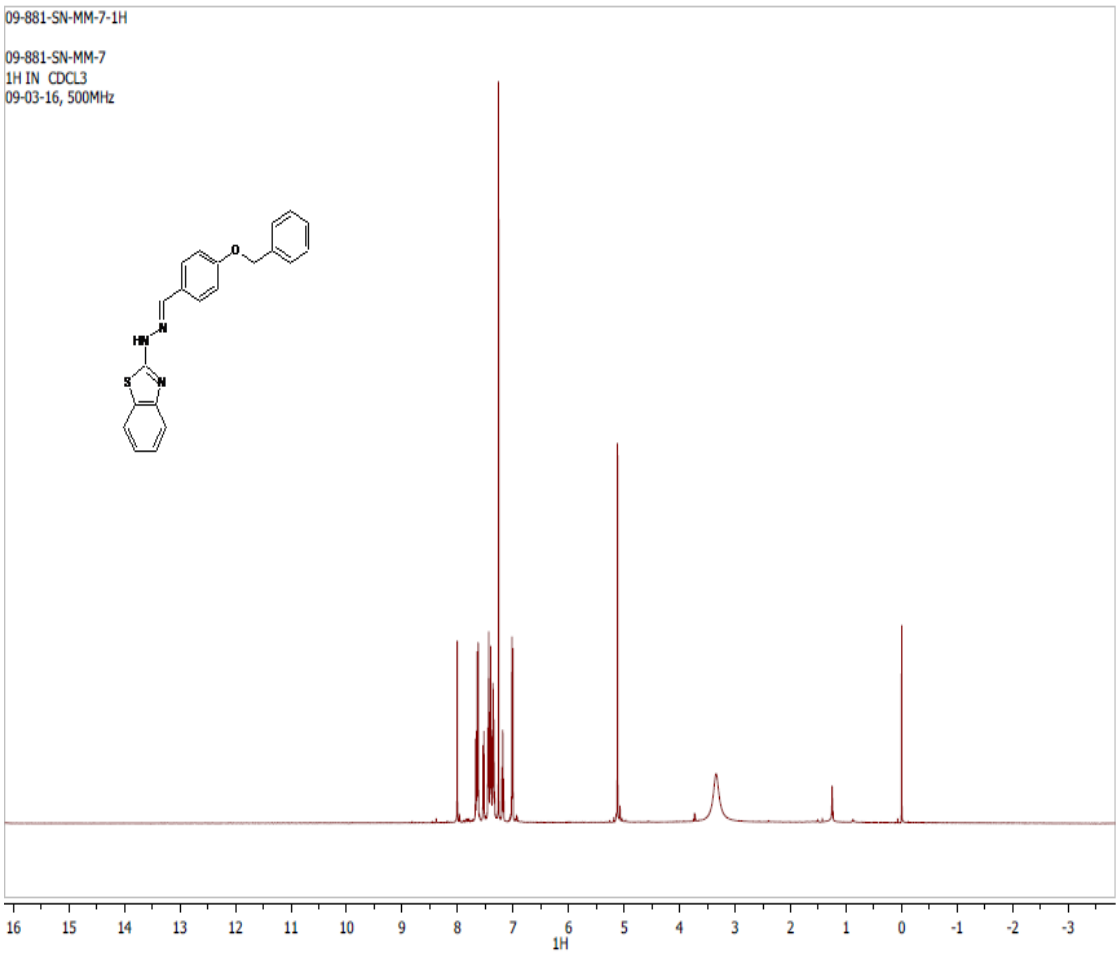


Table No: 5

TYPE OF PROTON	NO. OF PROTON	δ VALUES
Aromatic C-H	7	7.5
Benzylidene N=CH	1	8.0
NH	1	3.5

SAMPLE CODE:M7



TYPE OF PROTON	NO. OF PROTON	δ VALUES
Aromatic C-H	13	7.0-8.0
NH	1	3.5
CH2	2	5.0
Benzylidene N=CH	1	8.1

SAMPLE CODE:M11

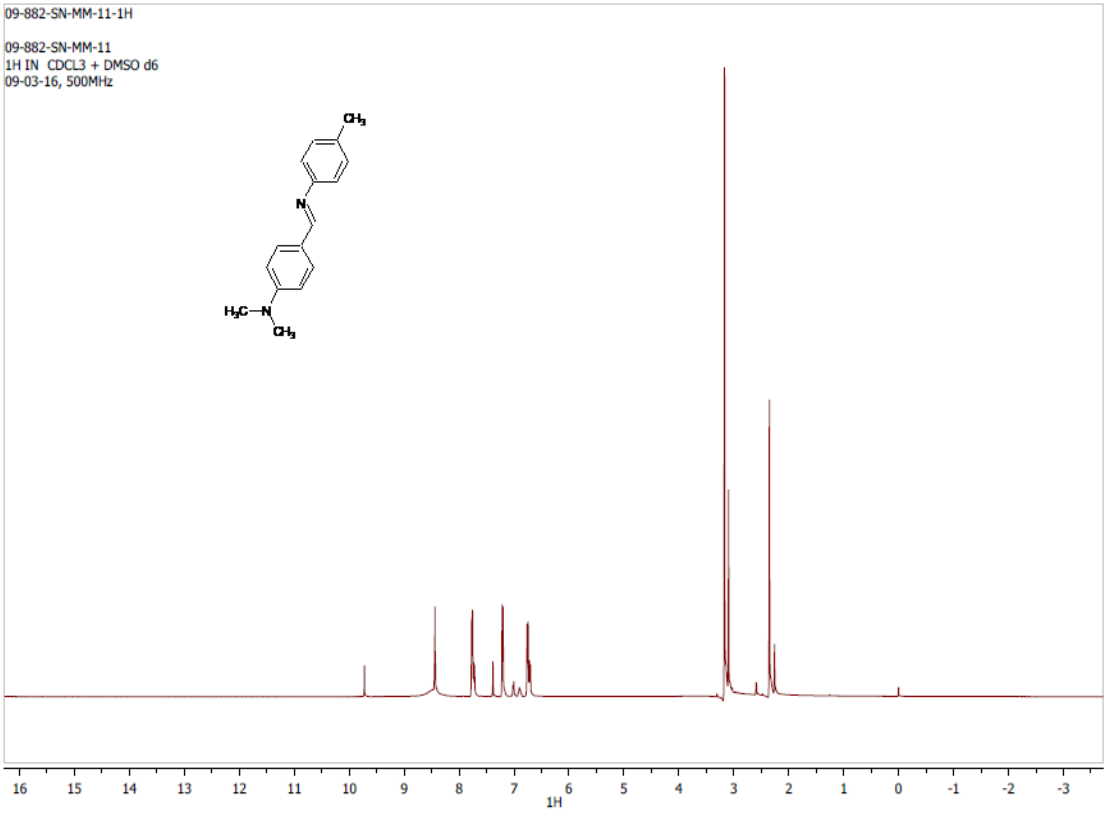


Table No: 6

TYPE OF PROTON	NO. OF PROTON	δ VALUES
N(CH3)	6	3.1
CH3	3	2.3
Aromatic (C-H)	8	6.5-8.0
C-H	1	3.1

TOXICITY STUDIES

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

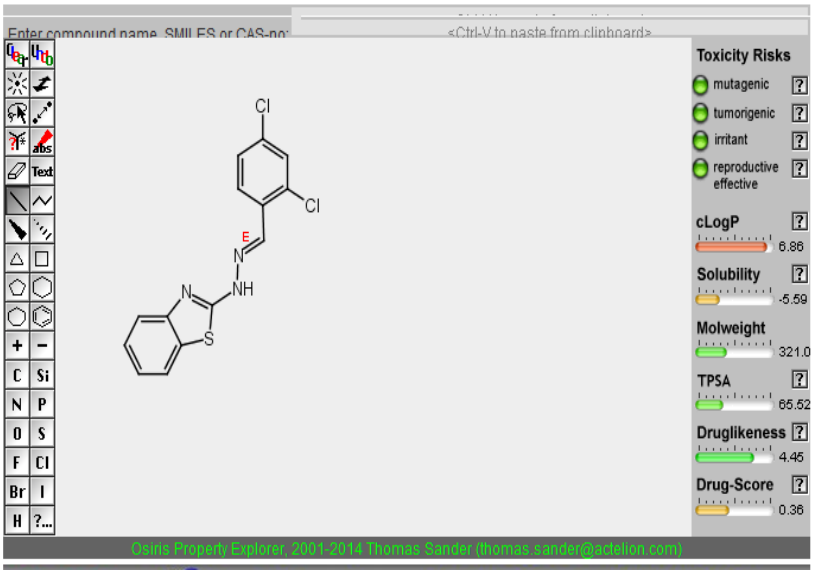
Table No: 7

SAMPLES	M1	M2	M3	M7	M11
MUTAGENIC	✓	✓	✓	✓	✓
TUMORIGENIC	✓	X	✓	X	✓
IRRITANT	✓	✓	✓	✓	✓
REPRODUCTIVE EFFECT	✓	✓	✓	✓	✓

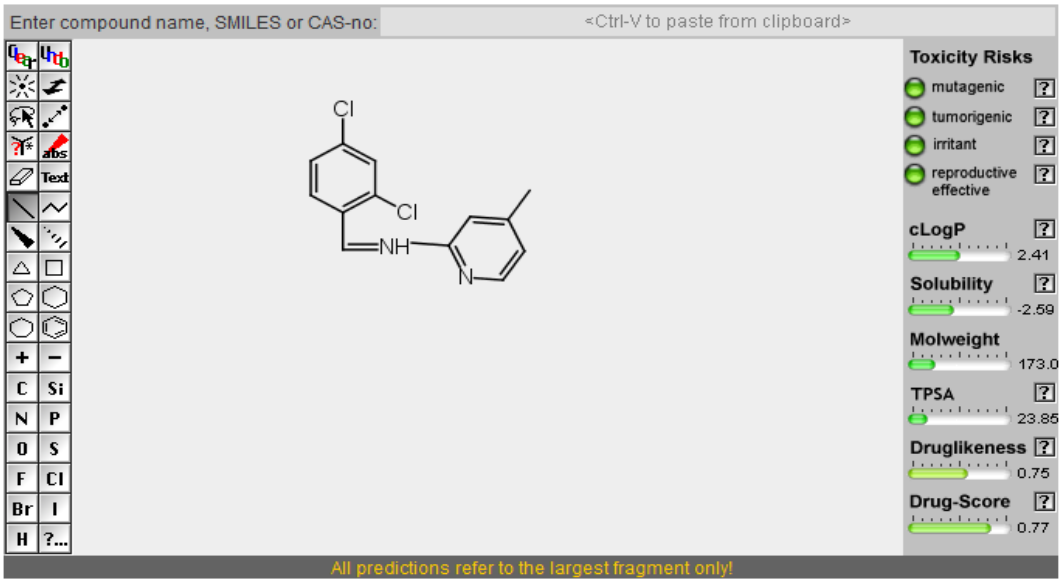
(✓) - Indicates absence of toxicity

(X) - Indicates presence of toxicity

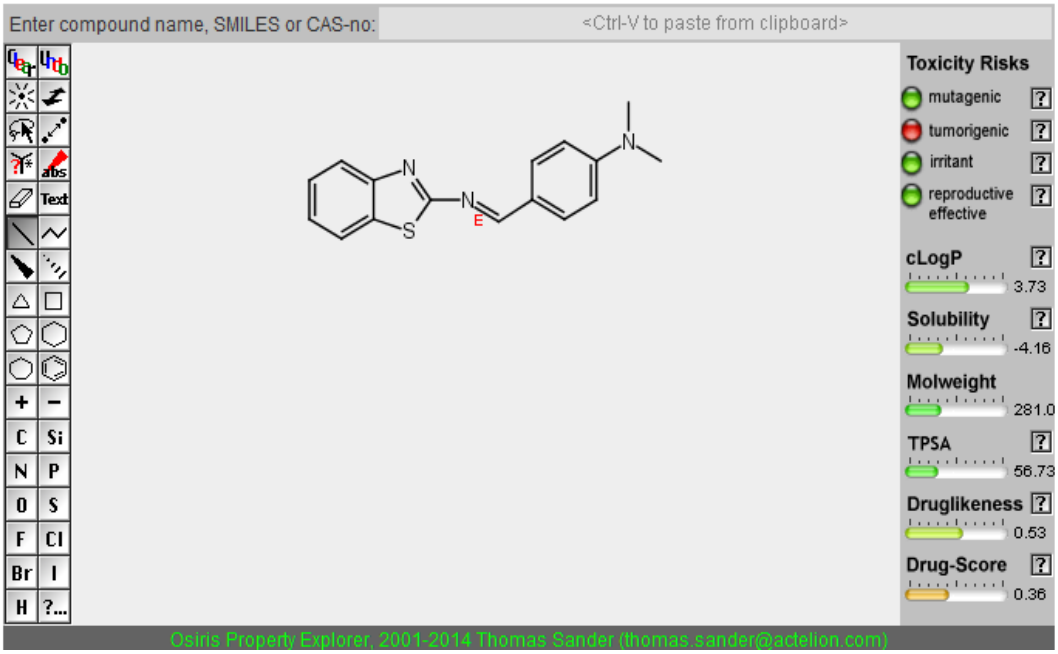
SAMPLE CODE: M1



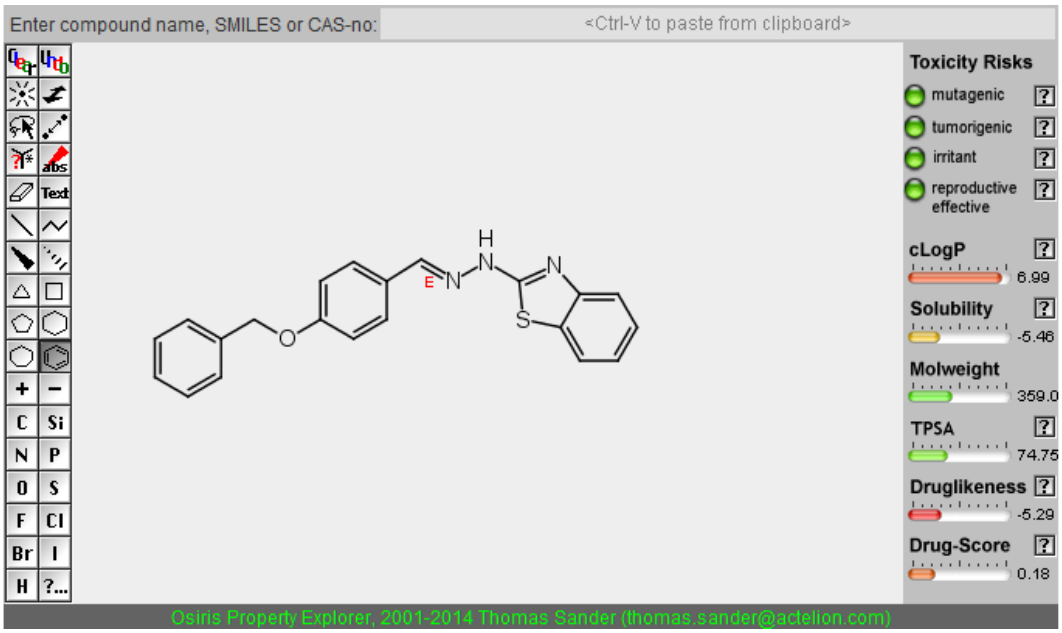
SAMPLE CODE: M2



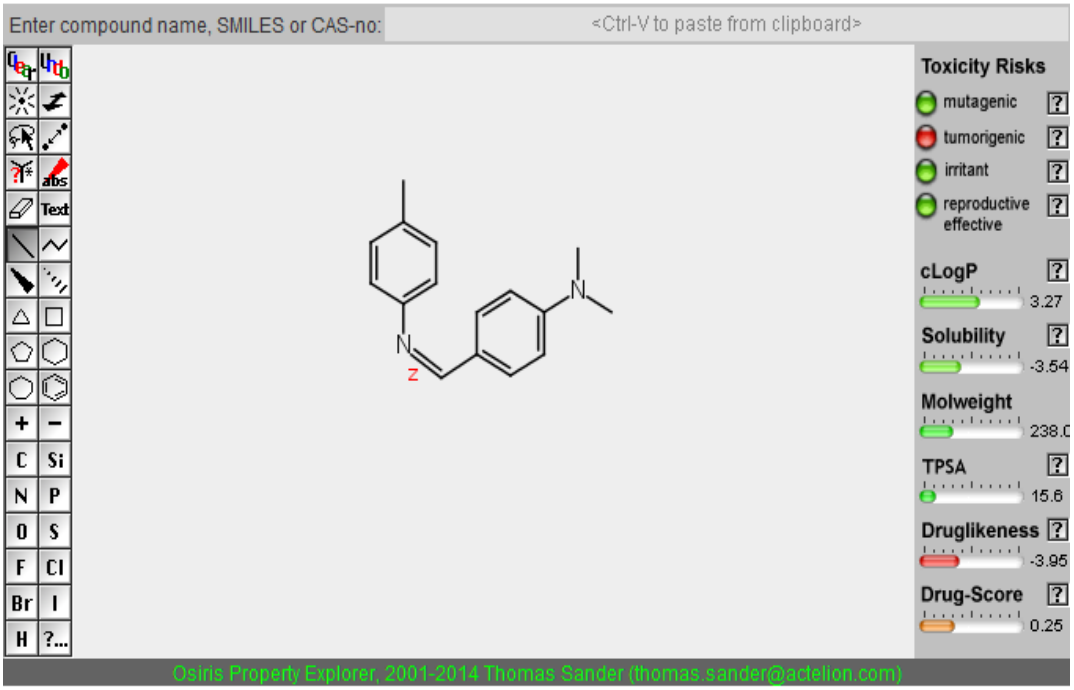
SAMPLE CODE: M3



SAMPLE CODE: M7



SAMPLE CODE: M11



BIOLOGICAL EVALUATION:

Anti TB results

Table No: 8

S. No	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	M1	S	R	R	R	R	R	R	R
2.	M2	S	S	R	R	R	R	R	R
3.	M3	S	S	R	R	R	R	R	R
4.	M7	R	R	R	R	R	R	R	R
5.	M11	S	R	R	R	R	R	R	R

Note:

S - Sensitive

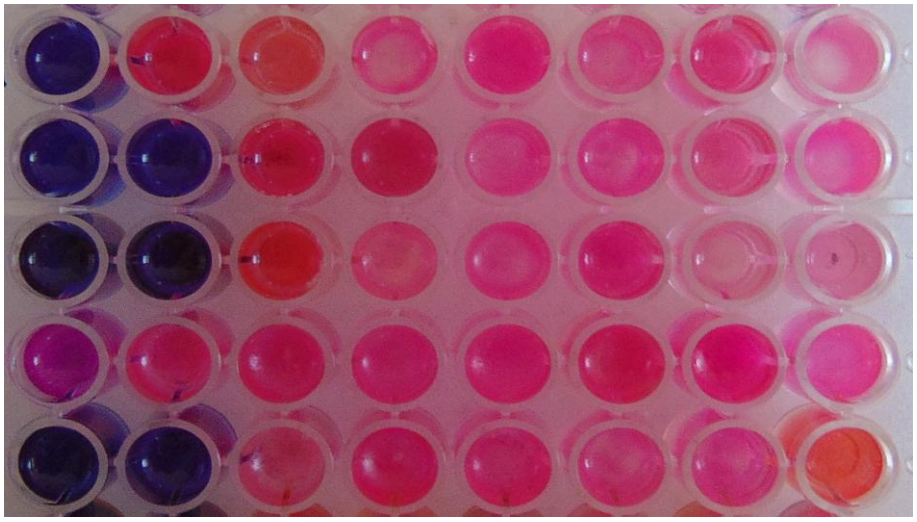
R - Resistant

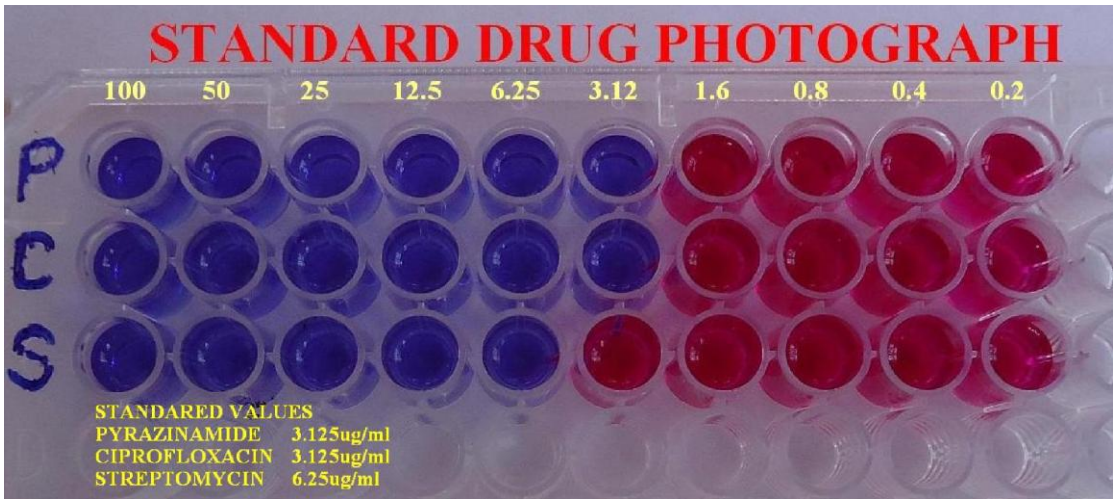
- Strain used: M.tuberculosis (H37 RV strain).
- Here are the standard values for the Anti-Tb test which was performed.
- Pyrazinamide- 3.125µg/ml.
- Streptomycin- 6.25µg/ml.
- Ciprofloxacin- 3.125µg/ml.

Table No: 9

Sample code	Docking score	Mic values
M1	-10.5407kcal/mol	100 µg/ml
M2	-9.79903kcal/mol	50 µg/ml
M3	-8.4453kcal/mol	>100 µg/ml
M7	-11.1405kcal/mol	50 µg/ml
M11	-9.2508kcal/mol	100 µg/ml

SAMPLE DRUG PHOTOGRAPH

S. No	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	M1								
2.	M2								
3.	M3								
4.	M7								
5.	M11								



DISCUSSION

- I have synthesised five different compounds of Schiff bases. The basic level of purity confirmation done by Melting point and TLC.
- Then I confirmed the synthesised compounds by various spectrums.
- After that I gone for the toxicity studies. In that only M3 and M11 compounds were have tumorigenic and rest of the synthesised compounds were non-toxic.
- Despite the compounds has best docking score, the resistant would be formed at the level of 25-50 µg/ml.
- In that M7 also has good docking score but it doesn't have sensitive even at the level of 100µg/ml.

SUMMARY AND CONCLUSION

- Cyclopropane Mycolic acid Synthase-2 (1KPI) a critical enzyme for the growth of Mycobacterium tuberculosis was chosen for our study after review of literature.

- A database of 200 molecules with high prospect of inhibiting the target 1KPI were carefully chosen by making changes to the known hit molecules, here the Imidazole nucleus and Benzimidazole nucleus.

- Candidate molecules were designed and docked against 1KPI protein using Argus lab 4.0.1 software.

- Five molecules with good Docking score (lower binding energy) and interactions were shortlisted for synthesis. Reaction conditions were optimized.

- The selected molecules were subjected to toxicity prediction assessment by OSIRIS software. The results are colour coded as green colour which confirms the drug likeness.

- The molecules were labelled as M1, M2, M3, M7 and M11 synthesized with satisfactory yield.

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

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

- The final pure compounds were screened for Anti- mycobacterial activity by in vitro method called Microplate Alamar Blue Assay (MABA).

CONCLUSION

- My work concludes that my synthesized molecules are slightly effective in inhibiting enzyme Cyclopropane Mycolic acid Synthase-2 (1KPI), which is important for the growth of Mycobacterium tuberculosis Cell wall.

- Among the four other enzymes the cyclopropane mycolic acid synthase 2 was the best enzyme for inhibiting Mycobacterium tuberculosis.

- Streptomycin gave Docking score of -7.4 for 1KPI and Ciprofloxacin gave Docking score of -5.9 for 1KPI. There is correlation between the score and activities of all the 5 compounds which were tested and compared with the standard drugs. This goes to prove that 1KPI is a critical enzyme for anti-mycobacterial activity.

- The Minimum inhibitory concentration of the 5 synthesized compounds against H37RV ranged from 50 µg/ml to >100 µg/ml which is compared to that of the certain known Anti-TB agents Pyrazinamide-3.125µg/ml, Ciprofloxacin- 3.125µg/ml and Streptomycin- 6.25µg/ml.

- Further structural refinement to the structure of the synthesized compounds is expected to yield promising molecules against the pathogen Mycobacterium tuberculosis.

BIBLIOGRAPHY

1) http://www.cdc.gov/tb/topic/basics/default.htm

2) Kumar v, abbas ak, fausto n, mitchell rn (2007), Robbins basic pathology (8th ed.)., Saunderselsevier. P.no. 516–522. ISBN 978-1-4160-2973-1.

3) www.webmdboots.com/a-to-z guides/tuberculosis introduction/

4) www.mdhil.com/tuberculosis-introduction/

5) Anil koul ***et al.,*** The Challenge of new Drug Discovery for Tuberculosis, Nature review article.Published 2011; Volume 469; Pg no: 483-490.

6) www.webmdboots.com/a-to-z guides/tuberculosis introduction/

7) http://en.m.wikipediea.org/wiki/tuberculosis

8) En.wikipedia.org/wiki/mycobacterium.tuberculosis.

9) Barry ce 3rd, ***et al.*** Mycolic acids: structure, biosynthesis and physiological functions. Prog lipid Res 1998;37:143–179.

10) Hong x, hopfinger aj. Construction, molecular modeling, and simulation of mycobacterium Tuberculosis cell walls. Biomacromolecules 2004;5:1052–1065.

11) Villeneuve m, ***et al.*** Conformational behavior of oxygenated mycobacterial mycolic acids frommycobacterium bovis bcg. Biochim biophys acta 2007;1768:1717–1726.

12) Glickman ms, *et al.* A novel mycolic acid cyclopropane synthetase is required for cording, Persistence, and virulence of mycobacterium tuberculosis. Mol cell 2000;5:717–727.

13) http://www2.nau.edu/~fpm/bio205/chapter4.htm

14) https://microbewiki.kenyon.edu/index.php/Mycobacterium_tuberculosis

15) Ouellet, H. et al. Mycobacterium tuberculosis CYP125A1, a steroid C27 monooxygenase that detoxifies intracellularly generated cholest-4-en-3-one. Mol. Microbiol. 77, 730 (2010).

16) William W. Mohn ***et al.*** The Actinobacterial mce4 Locus Encodes a Steroid Transporter. J. Biol. Chem. 283, 35368-35374 (2008).

17) Brzostek, A., Pawelczyk, J., Rumijowska-Galewicz, A., Dziadek, B. & Dziadek, J**.** Mycobacterium tuberculosis Is Able To Accumulate and Utilize Cholesterol. The Journal of Bacteriology 191, 6584-6591 (2009).

18) Fortune, s. M., a. Jaeger, d. A. Sarracino, m. R. Chase, c. M. Sassetti, d. R.sherman, b. R. Bloom, and e. J. Rubin. 2005. Mutually dependent secretionof proteins required for mycobacterial virulence. Proc. Natl. Acad. Sci.u. S. A. 102:1066–1068.

19) http://www.the life cycle of M.Tuberculosis.org.

20) Global tuberculosis report 2015.

21) http://www.thehindu.com/sci-tech/health/policy-and-issues/need-for-new-tb-drugs/article3220027.ece

22) Antigone barton., NIH-funded TB drug trial is latest effort to fill treatment pipeline by on December 12, 2012.

23) http://www.pdb.org

24) Hofmann, k., hsiao, c.-y. Y., henis, d. B., and panos, c., j. Biological Chemistry., Chapter 2, 217, 49 (1955).

25) Hofmann, k., henis, d. B., and panos, c., j. Biological Chemistry., 228, 349 (1957).

26) Dauchy, s., and asselineau, j., compt. Rend., 250, 2635(1960).

27) http://www.thehindu.com/sci-tech/health/policy-and-issues/need-for-new-tb-drugs/article3220027.ece

28) Sakai r, higa t, jefford cw, bernardinelli g (1986) j am chem soc 108:6404–6405.

29) http://benzimidazole.org

30) http://pharmacological action of benzimidazole.

31) http://en.m.wikipedia.org/wiki/benzimidazole

32) Swathi.Pathi, Swarna Latha.D, M.Sreenivasulu; A review on substituted benzothiazoles by Pharmatutor art - 1635

33) Y. Ozcan, s. Ide, i. Sakıyan, and e. Logoglu, j. Mol. Struc., 2003, 658, 207.

34) hadizadeh f and ghodsi r. Synthesis of novel n-substituted imidazolecarboxylic acid hydrazides as monoamine oxidase inhibitors. Farmaco (2005) 60: 237-240.

35) I. Sakiyan, n. Gunduz, and t. Gunduz, react. Inorg., met. Org. Chem., 2001, 31, 1175.

36) porsolt rd, bertin a and jalfre m. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. (1977) 229: 327-336.

37) www.wikipedia/drug design/

38) madsen U,Krogsgaard-larsen P,Liljfors T(2000). Textbook of drug design and discovery. Washington, DC: Taylor & Francis.

39) Reynolds CH, Merz KM, ***et al.,(***2010).Drug Design: Structure and Ligand based Approaches(1 ed.). Cambridge, UK.

40) Shirai H, Prades C, ***et al.,(*** Nov 2014). "Antibody informatics for drug discovery". Biochimice et Biophsica Acte 1844(11):2002-2015.

41) Gunner OF (2000). Pharmacophore Perception,Development,and use in drug design. La Jolla, Calif: International University Line.

42) Leach, Andrew R**.** ***et al.***,(2007). Structure based Drug Discovey; Page no: 126-153.

43) http://www.thehindu.com/sci-tech/health/policy-and-issues/need-for-new-tb-drugs/article3220027.ece

44) Yuan Y, ***et al.,*** (Dec (2014). " Binding site detection and drug ability prediction of protein targets for structure based Drug Design".

45) Leis S, ***et al.,(***2010). "In silico prediction of binding sites on proteins." Current Medicinal Chemistry17(15): 1550-62.

46) Warren GL, ***et al.,***(2011). Chapter 16: Scoring Drug Receptor Interactions.

47) Bohm HJ (Jun 1994). Journal of Computer Aided Molecular Design8(3):243-56.

48) Liu J, Wang R (23 March 2015). "Classification of current scoring funtions". Journal of Chemical Information and modeling 55(3): 475-482.

49) http://www.image.drug design.jpg.

50) Sajujoy, Parvathy S Nair, Ramkumar Hariharan, M.Radhakrishna pillai, "Detailed comparison of Protein-ligand docking efficiency of GOLD, a commercial package and Argus lab, a licensable freeware" (Insilico biology 6,0053 2006).

51) Laurie AT, Jackson RM, "Q-Site finder; an energy based method for the prediction of protein-ligand binding sites" Bioinformatics, (2005), 21(9) 1908-16.

52) http://www.rcsb.org/pdb.

53) Lewis RA (2011). "Chapter 4: The Development of Molecular Modeling Programs: The use and limitations of Physical Models". Royal society of chemistry. pp. 88-107.

54) http: // www.organic-chemistry.org/mog/peo. Retrieved on 22-12-2013.

55) Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (March 2001). "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings". Adv. Drug Deliv. Rev. 46 (2001): 3–26. doi:10.1016/S0169- 409X(00)00129-0. PMID 11259830.

56) Lipinski CA (December 2004). "Lead- and drug-like compounds: the rule-of-five revolution". Drug Discovery Today: Technologies 1 (4): 337– 341.doi:10.1016/ j.ddtec.2004.11.007.

57) http://www.image.lipinski.jpg

58) Vaibhav Sharma, Dinesh Kumar Mehta, SumanBala, Rina Das. A Review On Biological Active Schiff Base Derivatives, International Journal of Universal Pharmacy And Bioscience-2(4): July-August.

59) G. Dent, j.m. Chalmers, industrial analysis with vibrationalspectroscopy, royal society of chemistry, cambridge,1997.

60) James, t. L. 1975. Nuclear magnetic resonance in biochemistry. Academic press, new york.

61) Barber, m.; bordoli, r.s.; elliott, g.j.; sedgwick, r.d.; tyler, a.n. Anal. Chem. 1982, 54,645a-657a.

62) Gurdep chatwal, organic spectroscopy:hyphenated methods. Vol-1.

63) http://www.gc-ms.org

64) Tiballi, r. N., x. He, l. T. Zarins, s. G. Revankar, and c. A. Kauffman. 1995.use of a colorimetric system for yeast susceptibility testing. J. Clin. Microbiol.33:915–917. 60

65) Zabransky, r. J., a. R. Dinuzzo, and g. L. Woods. 1995. Detection ofvancomycin resistance in enterococci by the alamar mic system. J. Clin.microbiol. 33:791–793.

66) Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues. Arkivoc 2007(xv), 181-191.

67) Maria c. S. Lourenco, marcus v. N desouza, alessandra c pinheiro, marcelle de l.ferreira, rasnisb b, goncalves, thais cristina m nogneira, monica a peralta.

68) De Souza MVN, et al.,(2006) Current status and future prospects for new therapies for Pulmonary Tuberculosis.

69) Duncan k,et al., (2004) Prospects for New Anti-Tubercular drugs. Van der Geize,R.et al.,(2007)"AGene Cluster Encoding Cholesterol Catabolismin a Soil Actinomycete Provides Insight into Mycobacterium Tuberculosis Survival in Macrophages."

70) Maria Loreto Incandela., et al, (2013) reported that 1KPI, a new taxonomic marker in mycobacteria. 2011 Nov 1. doi: 10.1371

71) George KM, et al., (2008) the biosynthesis of Cyclopropanated mycolic acids in Mycobacterium tuberculosis., Jan 16, 2014 - 2008; 154: 1866–1875

72) Dominique Guianvarc'h, et al., (2009) Identification of inhibitors of the E. coli Cyclopropane fatty acid synthase from the screening of a chemical library. International Journal of Solids and Structures, Elsevier, 2009, 46, pp.2255-2264.

73) Christine et al. (2007), Synthesis and evaluation of analogues of S-adenosyl-L-methionine, as inhibitors of the E. coli Cyclopropane fatty acid synthase. 2007;282(15):11000–8.

74) Deepak. D. Borkar., et al. (2012), Design and Synthesis of p-hydroxy benzohydrazide Derivatives for their Antimycobacterial Activity. Vol. 56, No. 2.

75) Romono T. Kroemeret et al.(2003), An introduction into ligand–receptor docking. It illustrates the basic underlying concepts. Current Protein and Peptide Science, 2007, 8, 312-328

76) Andrew Worth *et al. (1998),* Distribution, Metabolism and Excretion (ADME) properties, which are often important in discriminating between the toxicological profiles of parent compounds and their metabolites/degradation products.

77) Madsen et al., (2002) Textbook of Drug Design and Discovery. Page no: 312-320.

78) RuhiAli and NadeemSiddiqui Journal of Chemistry Volume 2013 (2013), Article ID 345198, 12 pages http://dx.doi.org/10.1155/2013/345198 Indian journal of pharmaceutical sciences. Review Article: Biological Aspects of Emerging Benzothiazoles:

79) S. T. Asundaria and K. C. Patel, "Synthesis, characterization and antimicrobial activity of thiazole, benzothiazole and pyrimidine derivatives bearing sydnone moieties," Pharmaceutical Chemistry Journal, vol. 45, no. 12, pp. 725–731, 2012

80) K. Bolelli, I. Yalcin, T. Ertan-Bolelli et al., "Synthesis of novel 2-[4-(4-substituted benzamido /phenylacetamido) phenyl] benzothiazoles as antimicrobial agents," Medicinal Chemistry Research, vol. 21, no. 11, pp. 3818–3825, 2012

81) P. K. Sharma, M. Kumar, and V. Mohan, "Synthesis and antimicrobial activity of 2H-pyrimido[2,1-b]benzothiazol-2- ones," Research on Chemical Intermediates, vol. 36, no. 8, pp. 985–993, 2010.

82) C. Sheng, J. Zhu, W. Zhang et al., "3D-QSAR and molecular docking studies on benzothiazole derivatives as Candida albicans N-myristoyltransferase inhibitors," European Journal of Medicinal Chemistry, vol. 42, no. 4, pp. 477–486, 2007.

83) B. S. Soni, M. Ranawat, R. Sharma, A. Bhandari, and S. Sharma, "Synthesis and evaluation of some new benzothiazole derivatives as potential antimicrobial agents," European Journal of Medicinal Chemistry, vol. 45, no. 7, pp. 2938–2942, 2010

84) P. K. Sahu, P. K. Sahu, S. K. Gupta, D. Thavaselvam, and D. D. Agarwal, "Synthesis and evaluation of antimicrobial activity of 4H-pyrimido[2,1-b] benzothiazole, pyrazole and benzylidene derivatives of curcumin," European Journal of Medicinal Chemistry, vol. 54, pp. 366–378, 2012

85) I. H. R. Tomi, J. H. Tomma, A. Daraji, and A. Al-Dujaili, "Synthesis, characterization and comparative study the microbial activity of some heterocyclic compounds containing oxazole and benzothiazole moieties," Journal of Saudi Chemical Society, 2012

86) V. S. Padalkar, B. N. Borse, V. D. Gupta et al., "Synthesis and antimicrobial activity of novel 2-substituted benzimidazole, benzoxazole and benzothiazole derivatives," Arabian Journal of Chemistry, 2012.

87) S. Gilani, K. Nagarajan, S. P. Dixit, M. Taleuzzaman, and S. A. Khan, "Benzothiazole incorporated thiazolidin-4-ones and azetidin-2-ones derivatives: synthesis and in vitro antimicrobial evaluation,"Arabian Journal of Medicinal Chemistry, 2012.

88) Arpana Rana, N Siddiqui, SA Khan Year : 2007 | Volume : 69 | Issue : 1 | Page : 10-17 Benzothiazoles: A new profile of biological activitie

89) G. Navarrete-Vazquez, M. Ramírez-Martínez, S. Estrada-Soto *et al*., "Synthesis, in vitro and in silicoscreening of ethyl 2-(6-substituted benzo[d]thiazol-2-ylamino)-2-oxoacetates as protein-tyrosine phosphatase 1B inhibitors," European Journal of Medicinal Chemistry, vol. 53, pp. 346–355, 2012.

90) G. A. Pereira, A. C. Massabni, E. E. Castellano *et al.,* "A broad study of two new promising antimycobacterial drugs: Ag(I) and Au(I) complexes with 2-(2-thienyl)benzothiazole," Polyhedron, vol. 38, no. 1, pp. 291–296, 2012 ·

91) V. N. Telvekar, V. K. Bairwa, K. Satardekar, and A. Bellubi, "Novel 2-(2-(4-aryloxy benzylidene)hydrazinyl) benzothiazole derivatives as anti-tubercular agents," Bioorganic and Medicinal Chemistry Letters, vol. 22, no. 1, pp. 649–652, 2012.

92) L. Katz, "Antituberculous compounds. III. Benzothiazole and benzoxazole derivatives," Journal of the American Chemical Society, vol. 75, no. 3, pp. 712–714, 1953

93) Y. Cho, T. R. Ioerger, and J. C. Sacchettini, "Discovery of novel nitrobenzothiazole inhibitors forMycobacterium tuberculosis ATP phosphoribosyl transferase (HisG) through virtual screening," Journal of Medicinal Chemistry, vol. 51, no. 19, pp. 5984–5992, 2008.

94) Kamuran Görgün, Handan Can Sakarya, and Müjgan Özkütük The Synthesis, Characterization, Acid Dissociation, and Theoretical Calculation of Several Novel Benzothiazole Schiff Base Derivatives J. Chem. Eng. Data, 2015, 60 (3), pp 594–601

95) Mahmood-ul-Hassan a, Zahid H. Chohanb* & Claudiu T. Supuran Anti Bacterial Co(Ii) And Ni(Ii) Complexes Of Benzothiazole-Derived Schiff Bases DOI:10.1081/SIM-120014861 pages 1445-1461 Article from Tayler And Francies Online

96) Vaibhav Sharma, Dinesh Kumar Mehta, SumanBala, Rina Das. A Review On Biological Active Schiff Base Derivatives, International Journal of Universal Pharmacy And Bioscience-2(4): July-August.

97) Khlood Fahed Hamak et al., synthesized Schiff base and evaluated it for antimicrobial activity. International Jurnal of Chemical Technology., Res.2013,5(6). 2925.

98) Kalpesh s. parkh and sandip p. vyas., design and spectral analysis of novel Schiff base derived with acetophenone derivatives, scholars research library. Archives of applied science research, 2012,4(4): 1578-1580.

99) Todd P. Primm., et., al(2007), Recent Advances in Methodologies for the Discovery of Antimycobacterial Drugs. Current Bioactive Compounds 2007, 3, 000-000.

100) Sephra N. Ramprasad., Multible Application Of Alamar Blue As An Indicator Of Metabolic Function And Cellular Health In Cell Viability Bioassays By , Sensors 2012, 12, 12347-12360.

101) Jose D Jesus Alba-Romero Et Al., Application of the Alamar Blue Assay To Determine The Susceptibility To Antituberculosis Pharmaceuticals. African Journal of Microbiology Research Vol-5 (26), pp.4659-4666, 16 November 2011.