DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SACCHARIN DERIVATIVES AS ANTI- TUBERCULAR AGENTS TARGETING GLUTAMINE SYNTHETASE

SYNOPSIS OF THE THESIS

Submitted to

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

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

MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by

M . ANGURAJ

Reg no:261915701

Under the guidance of

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

Principal, Professor & Head

Department of Pharmaceutical Chemistry

College of Pharmacy, Madras Medical College.

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

COLLEGE OF PHARMACY, MADRAS MEDICAL COLLEGE

CHENNAI-600 003

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

CERTIFICATE

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Dr.A.JERAD SURESH, M.Pharm.Ph.D.,M.B.A.,
Principal & Head,
Department of Pharmaceutical Chemistry,
College of pharmacy,
Madras Medical college,
Chennai 600003.

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EXAMINERS

1.

2.

ACKNOWLEDGMENT

“Gratitude makes sense of our past, brings peace for today and

creates a vision for tomorrow”

I am grateful to **almighty** for giving me the strength to successfully complete this dissertation.

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INDEX

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

TB	Tuberculosis
WHO	World Health Organization
HIV	Human Immuno Virus
AIDS	Acquired Immuno Deficiency Syndrome
DOTS	Directly Observed Treatment Short-Course
MDR-TB	Multi Drug Resistant- Tuberculosis
XDR-TB	Extensively Drug Resistant- Tuberculosis
ATP	Adenosine Triphosphate
IR	Infrared spectroscopy
NMR	Nuclear Magnetic Resonance Imaging
LC-MS	Liquid Chromatography-Mass Spectrometry
MABA	Micro plate Alamar Blue assay
DMSO	Dimethyl Sulfoxide
CADD	Computer Aided Drug Designing
MIC	Minimum Inhibitory concentration
LBDD	Ligand Based Drug Design

INTRODUCTION

INTRODUCTION

The discovery of drugs and drug molecules has always been the aim of Pharmaceutical sciences. Despite the advances in medical and pharmaceutical sciences, there are still many diseases which are incurable or can only be treated symptomatically, and at a great economic and social cost owing to only moderately effective therapeutic agents. Furthermore, there are many contagious diseases like Malaria, Typhoid, and Tuberculosis which are becoming increasingly the resistant against antibiotics[1]. Therefore, there is still a great need for drugs with fewer undesired or toxic side-effects, agents useful in prophylaxis and drugs which will cause as little as possible harmful contamination in the already polluted environment.

Heterocyclic rings, which have been the unique basis for the activity of most of the drugs of natural origin gave the lead to the discovery of many synthetic drugs which possessed the heterocyclic rings. Their fused analogs represent an important class of heterocyclic compounds and exist in numerous natural products displaying a wide range of biological and pharmaceutical activities. On intensive research heterocyclic derivatives continue to yield newer medicinal agents.

TUBERCULOSIS

Tuberculosis (TB) is one of the most common infectious diseases known to mankind. About 32% of the world's population is infected by ***Mycobacterium tuberculosis*** – the main causal agent of TB. Every year, approximately 8 million of the infected people develop active Tuberculosis. The incidence of Tuberculosis infection has steadily risen in the last decade (WHO progress report, 2011); World Health Organization estimates that about 30 million people will be infected by ***M. tuberculosis*** within the next 20 years. The re-emergence of Tuberculosis infection has been further complicated by an increase in the prevalence of drug-resistant Tuberculosis.

Robert Koch a German physician first isolated ***Mycobacterium tuberculosis*** in 1882 who received the Nobel Prize for this discovery. Tuberculosis germs are most commonly found in the lungs, but sometimes they can move to other parts of the body.

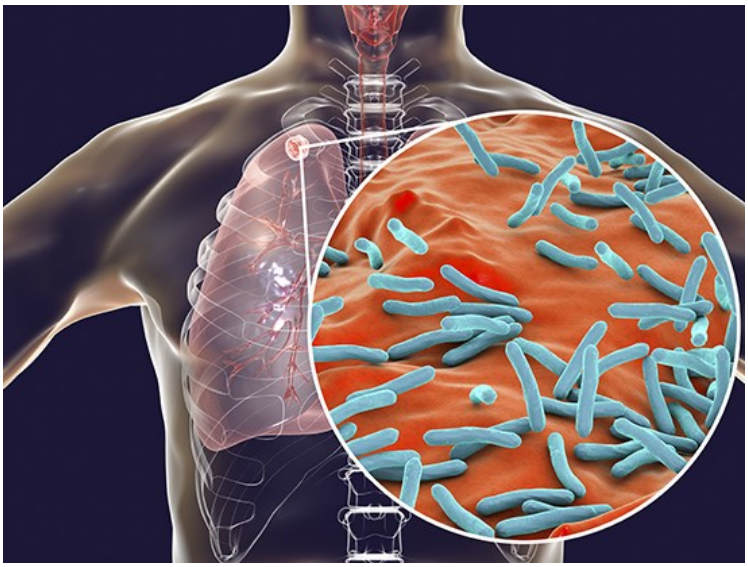


Fig. 1 Mycobacterium tuberculosis

Many years ago, this disease was referred to as ‘consumption’ because without effective treatment, those patients often would waste away. Anti-tubercular drugs play a crucial role in the successful treatment of tuberculosis.

There has been no new drugs registered to treat Tuberculosis in the last 40 years. Bedaquiline and delamanid appear to be promising new anti-TB drugs. Due to a mechanism of action that is different from that of other available drugs, their efficacy has appeared optimal in cases of adults with resistant pulmonary TB. This reflects that the inherent difficulties for discovery and clinical testing of new agents and the lack of pharmaceutical industry research in this area. Tuberculosis is often divided into pulmonary (respiratory) and extra pulmonary (non respiratory) infection. Pulmonary Tuberculosis exclusively involves lungs whereas extra pulmonary Tuberculosis includes brain, bone, liver kidney etc. But the treatment is same for both the types.

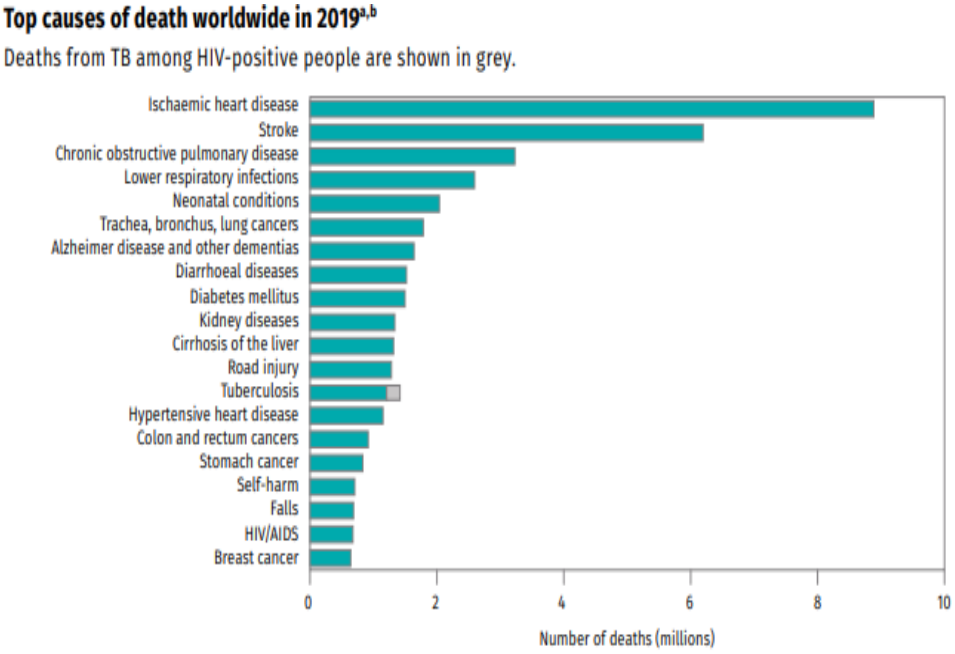


Fig.2 Top cause of death world wide in 2019[1]

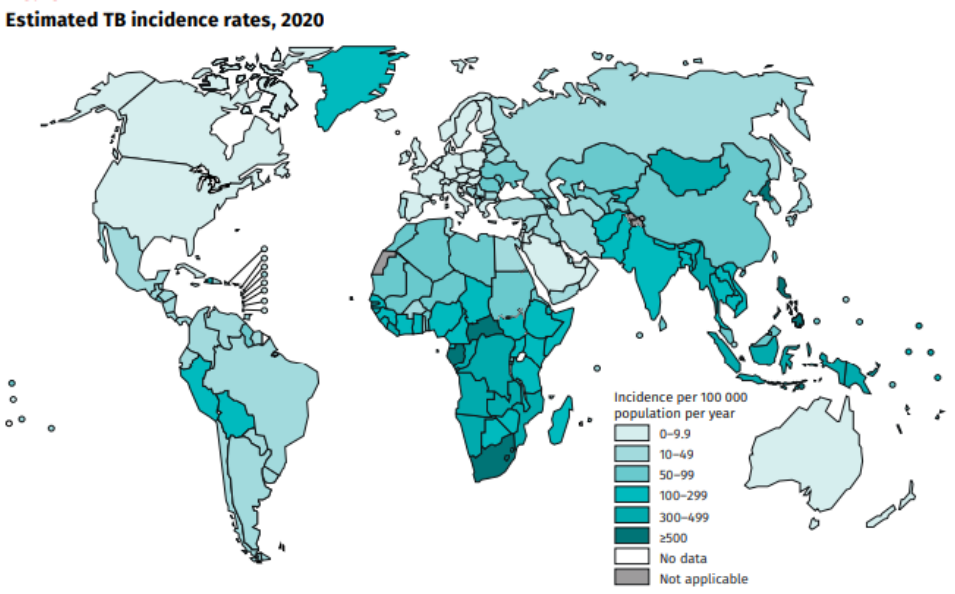


Fig. 3 Estimated TB incidence rates, 2020[1]

HISTORY OF TUBERCULOSIS:

- Tuberculosis is known to exist since antiquity. But until 1820, it was not identified as a single disease. The name tuberculosis was coined by 'J.L.Schonlein'. [3]
- In the earlier times, Tuberculosis was commonly known by the terms such as Phthisis, Pulmonalis and White Plague. [4]
- Pulmonary tuberculosis was called as Phthisis which means Consumption in Greek. The common term consumption was used to denote the severe weight loss produced as a result of this deadly disease. [4]
- In 1882, Robert Koch identified the organism causing the disease and described it as Mycobacterium tuberculosis. [5]
- In the 19th and early 20th centuries, TB was more common among the urban poor and the major concern was on the prevention and treatment of the disease. [5]
- Due to this, Medical Research Council initially focused more on the tuberculosis research in 1913. [5]
- The hope of eradication of TB arose with the development of Streptomycin in the year 1946. But, the rise of drug resistant strains in 80's has increased the need for newer therapeutic agents. [6]
- Development of resistance to the drugs used in the TB treatment regimen resulted in the emergence of MDR (Multi-Drug Resistant) TB and XDR (Extensively Drug Resistant) TB. [6]
- Co-infections like HIV, COVID-19 and the metabolic disorders like Diabetes worsens the body's natural immunity and renders the management of tuberculosis very challenging.

CLASSIFICATION OF TUBERCULOSIS: [7]

Based on the site of infection:

i. Pulmonary Tuberculosis infects the lung parenchyma and tracheobronchial tree.

ii. Extra pulmonary or Miliary Tuberculosis infects the organs other than lungs such as pleura, lymph nodes, intestine, genitourinary tract, meninges of the brain, joint & bones.

Based on the resistance to drugs:

i. Mono drug resistance TB – A TB patient whose biological specimen is resistant to only one of the first line drug.

ii. Poly drug resistance TB – Resistance to more than one of the first line drugs.

iii. Multi drug resistant (MDR) TB – Resistance to Isoniazid & Rifampicin and with or without the resistance to other first line drugs.

iv. Extensively drug resistant (XDR) TB – In addition to the Isoniazid & Rifampicin resistance, when the specimen was resistant to any of the fluoroquinolones (levofloxacin/ofloxacin/moxifloxacin) and any of the second line injectable drugs such as Kanamycin, Amikacin and Capreomycin, then it is termed as XDR TB.

ANTI-TB TREATMENT: [8]

In India, Revised National TB Control Program (RNTCP) was initiated in 1997 to provide proper treatment guidelines across the entire nation. Now, it is updated as RNTCP- II to provide services for TB/HIV & MDR-TB. It utilizes the Directly Observed Treatment Shortcourse (DOTS) strategy recommended by the WHO in the mission against Tuberculosis infection. It carries out the Five-year TB National Strategic Plan (NSP) by the Government of India. The national goal announced in 2017 was 'Elimination of TB in India by 2025'. In 2020, RNTCP was rechristened as National Tuberculosis Elimination Programme (NTEP).

Vision & Goals of NSP:

- TB free India with zero deaths, disease and poverty due to TB.
- To achieve a rapid decline in the burden of TB, Mortality & Morbidity while working towards the elimination of TB in India by 2025.

Anti-TB Drugs: [9]

I. First line drugs:

- Isoniazid
- Rifampicin
- Ethambutol
- Pyrazinamide

II. Second line drugs:

- Ethionamide, Cycloserine, Clofazimine
- Fluoroquinolones – Levofloxacin, Moxifloxacin, Gatifloxacin
- Injectable antibiotics – Amikacin, Kanamycin, Streptomycin
- Add on agents – Para amino salicylic acid, Bedaquiline, Delamanid,etc.,

CO – INFECTION: [10]

Risk of developing Tuberculosis is more in the Immunocompromised individuals. HIV/AIDS infection makes an individual more immunocompromised. People with HIV Infection is 16.27 times more vulnerable to develop the TB infection than the non-HIV individuals. The risk of death is also twice in these cases.

Providing treatment for both the infection i.e., HIV- Anti RetroViral therapy (ART) & Anti-TB therapy at a time results in several problems such as,

- Drug-drug interactions
- Cumulative drug toxicities
- High pill burden
- The Immune Reconstitutive Inflammatory Syndrome (IRIS).

In the event of causing impaired host defences, Diabetes has been the cause of 15% of active TB cases. TB patients suffering from diabetes have severe cavitating disease, adverse treatment outcomes and a high risk of mortality

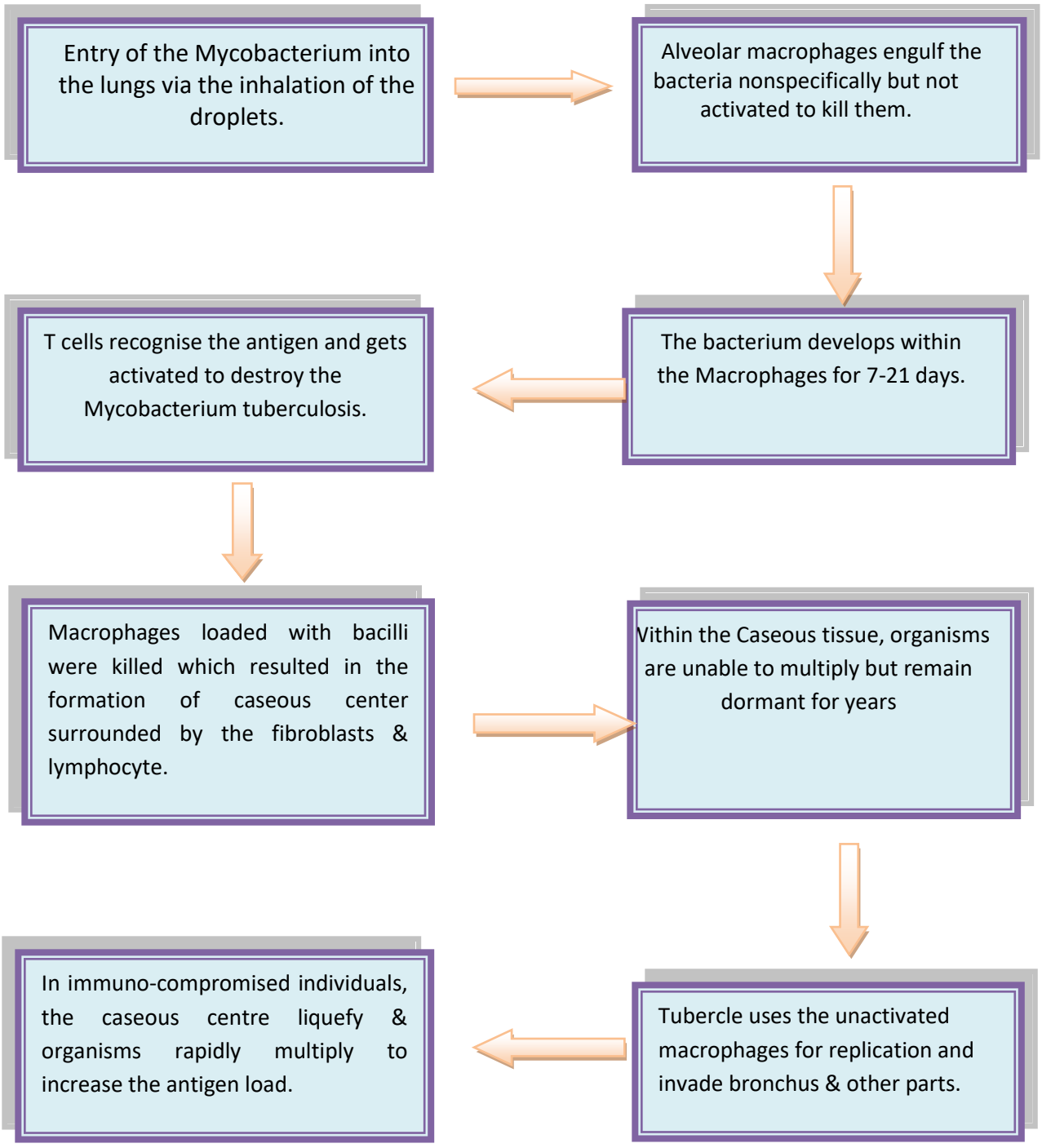
CAUSATIVE ORGANISM:

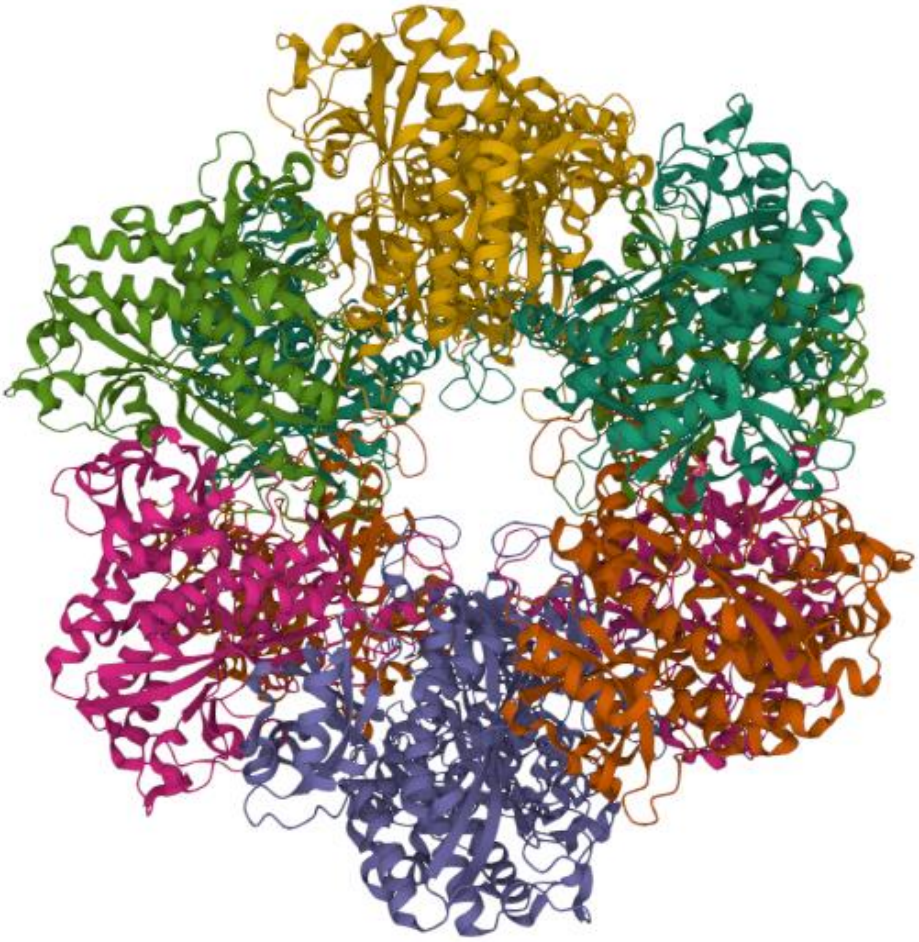
A small aerobic bacterium which causes the deadliest disease is Mycobacterium tuberculosis.

Table 01: Hierarchial classification of Mycobacterium tuberculosis

Domain	Bacteria
Phylum	Actinobacteria
Class	Actinobacteria
Order	Actinomycetales
Family	Mycobacteriaceae
Genus	Mycobacterium
Species	***Mycobacterium tuberculosis***

EVENTS IN THE TUBERCULOSIS INFECTION: [11]





ENZYME PROFILE

BIOLOGICAL TARGET

GLUTAMINE SYNTHETASE 1 (GS) [12], [13]

Glutamine synthetase 1 (GS) is an enzyme that plays an essential role in the metabolism by catalyzing the condenation of glutamate and ammonia to form glutamine.

Glutamate + ATP + NIH_3 ⟶ Gilutamine + ADP + phosphate

Glutamine Synthetase is composed of 8, 10, or 12 identical subumits separated into face-to-face rings. Bacterial GS are dodecamers with 12 active sites between each monomer. Each active site creates a 'tunnel' which is the site of three distinct sabstrate binding sites nucleotide, ammonium ion, and amino acid, ATP binds to the top of the bifunnel that opens to the external surface of GS. Glutamate binds at the bottom of the active site. The middle of the bifunnel contains two sites in which divalent cations bind (Mn 2 or Mg+2), One cation binding site is involved in phosphoryl transfer of ATP to glutamate, while the second stabilizes active GS and helps with the binding of glutamate"

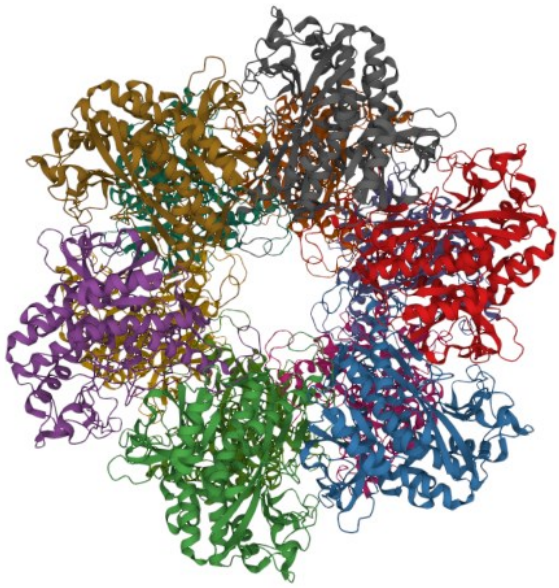


Fig.4 3D structure of Glutamine Synthetase (PDB ID – 4ACF)

Glutamine synthetase [L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] is one potentially important determinant of M. tuberculosis pathogenesis. Glutamine synthetase may influence host-pathogen interaction in two key respects.

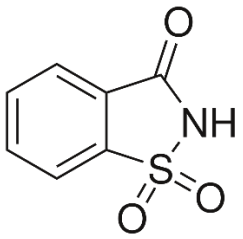
- Because of its central role in nitrogen metabolism, the enzyme may influence the ammonia level within infected host cells and hence contribute to the pathogen's capacity to inhibit phagosome-lysosome fusion and phagosome acidification.
- Glutamine synthetase mediates the extracellular catalysis of glutamine which suggests a direct invalvement of the enzyme in the synthesis of the cell wall structure poly(L-glutamic acid glutamine) which is found in pathogenic but not nonpathogenic mycobacteria.

Extracellular MBGS may also affect pH modulation in phagosomes and consequently prevent Phagosome-lysosome fusion. Numerous studies indicate that inhibition of MBGS is a feasible therapeutic strategy).

BASIC SCAFFOLD

BASIC NUCLEUS: SACCHARIN [14]

Saccharin, also called ortho-sulfo benzoic Acid Imide, is an organic compound employed as a non-nutritive sweetening agent. It occurs as insoluble saccharin or in the form of various salts, primarily sodium and calcium. Saccharin has about 200–700 times the sweetening power of granulated sugar and has a slightly bitter and metallic aftertaste.



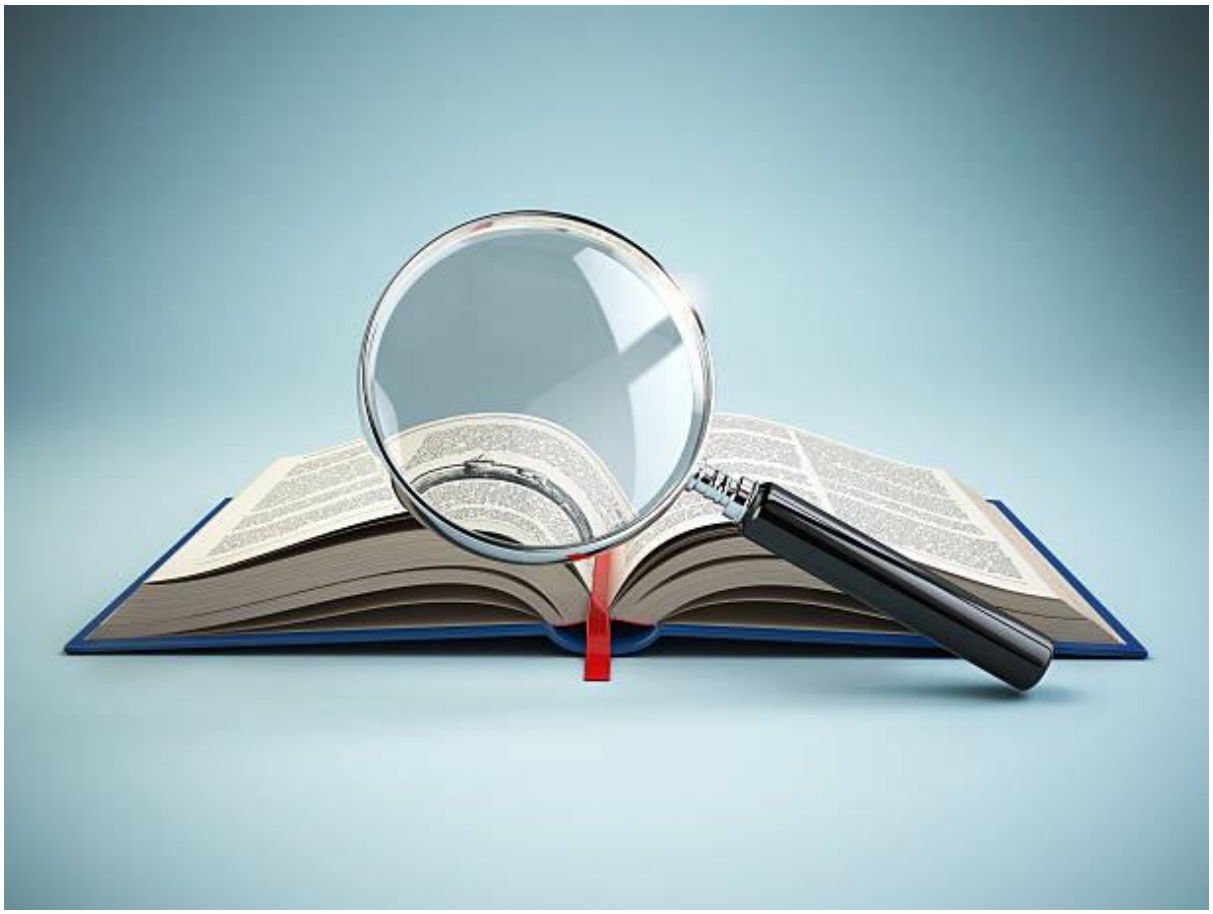
Saccharin was discovered by the chemists Ira Remsen and Constantin Fahlberg in 1879, while they were investigating the oxidation of o-toluenesulfonamide. Fahlberg noticed an unaccountable sweet taste to his food and found that this sweetness was present on his hands and arms, despite his having washed thoroughly after leaving the laboratory. Checking over his laboratory apparatus by taste tests, Fahlberg was led to the discovery of the source of this sweetness—saccharin. Saccharin became the first commercially available artificial sweetener. It is still made by the oxidation of o-toluene sulfonamide, as well as from phthalic anhydride.

Pharmacological activity

On the basis of various literature surveys saccharine derivatives shows various pharmacological activity.

- Hyperglycemia
- Hypertension
- carbonic anhydrase inhibitors

Inspite of the availability of fourth generation antibiotics & several Anti-TB drugs, we are still in a need of a novel drug to act against the actively dividing and dormant bacilli because of the, Complexity and toxicity of the current TB drug regimen. Development of resistant strains against the available drugs is another reason.



LITERATURE SURVEY

LITERATURE REVIEW

The purpose of Literature Review is to:

- Establish a theoretical frame work for a topic/ subject area.
- Define Key terms and terminology.
- Identify studies, Models, Case studies etc supporting a topic.
- Define/establish an area of Study.

Literatures related to the target disease Tuberculosis and its management:

1. **Cardenas, J.A. Garido et al.,** [15] studied the progression of research in the area of treatment for Tuberculosis. The authors performed Bibliometric analysis to determine the number of research papers published in the field of Tuberculosis treatment Globally.

2. **Chaw, Liling et al.,** [16] assessed the Global TB burden and the focus of majority of Countries on the Latent tuberculosis infection by performing a literature survey.

3. **Canetti et al.,** [17] briefly outlined the complication of Anti-TB treatment in the HIV co-infected patients. The new drug regimen for the co-treatment was clearly explained.

4. **Migliori, G. Battista et al.,**[18] evaluated the risks associated with the treatment of Drug-resistant tuberculosis and their clinical management.

5. **Daley, L.Charley** [19] outlined the current approaches to control Tuberculosis and the several gaps needed to be closed for the eradication of the disease.

6. **Pezella, A.Thomas** [20] highlighted the epidemiology of TB since ancient times and the evolution of the Tuberculosis treatment.

Reviews related to the target- Glutamine synthetase I:

7. **Gunter Harth and Marcus A. et al., [21]** Horwitz demonstrated that inhibitors of extracellular Glutamine synthetase block the growth of M. tuberculosis and other pathogenic mycobacteria. Remarkably, the inhibitors were selective for pathogenic mycobacteria, which export Glutamine synthetase. The inhibitor reduced Glutamine synthetase activity in the

extracellular milieu of M. tuberculosis cultures by 80% but had little effect on cell-associated Glutamine synthetase38.

8. **Marcus A. Horwitz et al., [22]** assessed the role of Glutamine synthetase (GS), in the pathogenicity of Mycobacterium tuberculosis; glnA1 was constructed via allelic exchange. The mutant had no detectable Glutamine synthetase protein or Glutamine synthetase activity and was auxotrophic for L-glutamine. In addition, the mutant was attenuated for intracellular growth in human THP-1 macrophages. Based on growth rates of the mutant in the presence of various concentrations of L-glutamine the importance of the enzyme was known. These studies demonstrate that glnA1 is essential for M.tuberculosis virulence39.

9. **Lukasz Berlicki et al., [23]** studied about the Glutamine synthetase enzyme which catalyses the formation of glutamine from glutamine and ammonium ion. It is one of the most important enzymes in nitrogen metabolism. The first part of the review presents the longdating research on inhibitors of Glutamine synthetase. The second part of the paper is dedicated to potential medical applications of Glutamine synthetase inhibitors, which is proved as effective anti-tuberculosis agent with high selectivity towards the pathogen40.

10. **O Lagerlund et al., [24]** synthesized some potential anti-tubercular agents which targeted Glutamine synthetase (GS), which is one of the latest targets of M.tuberculosis which catalyses the formation of glutamine from glutamic acid. In this work, novel Glutamine synthetase inhibitors and new Pd (O) - catalyzed methods have been developed41.

11. **Wojciech W. Krajewski et al.,[25]** summarized that Glutamine synthetase catalyzes the ligation of glutamate and ammonia to form glutamine, with the hydrolysis of ATP. The enzyme is a central component of bacterial nitrogen metabolism and is a potential drug target. This study provides the first reported structure for a tauto form of the tuberculosis enzyme. The phospho compound, generated in situ by an active enzyme, mimics the phosphorylated tetrahedral adduct at the transition state. Some differences in ligand interactions of the protein with phosphorylated compound and nucleotide were observed compared with the earlier structures; a third metal ion also was found42.

Literatures regarding the Computer Aided Drug Design:

12. **Fernando, D.Prielo Martinez** [26] outlined the importance of the Computational drug design methods in the Drug Discovery process.

13. **Hachem, El.Nehme et al.,** [27] visualized Docking as an essential computation tool for the Structure based drug design.

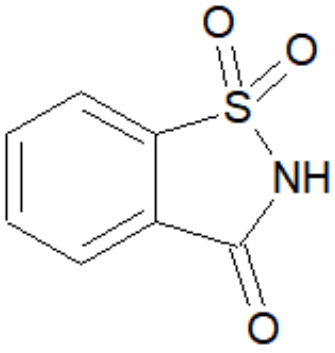
14. **Forli Stefano** [28] covered the Docking and virtual screening methods provided by the AutoDock Suite. The procedure involved in docking the compounds against the protein target was elucidated.

15. **Tien, Sheng et al.,** [29] established the importance of drug likeness of a molecule and their prediction by various in silico filters.

16. **Abdul, Wadood et al.,** [30] focused on the importance of the in silico methods in drug design. To prevent the failure of a novel compound in the later stages of drug discovery, in silico methods are used.

Literatures reviewed for synthesizing the designed molecules from the Basic scaffold:

17. **Daniel G. Stark et al,. [31]** presented a broad review on the various methods of synthesis of Isatin, their physical and chemical properties, applications in organic synthesis and their Pharmacological activities.

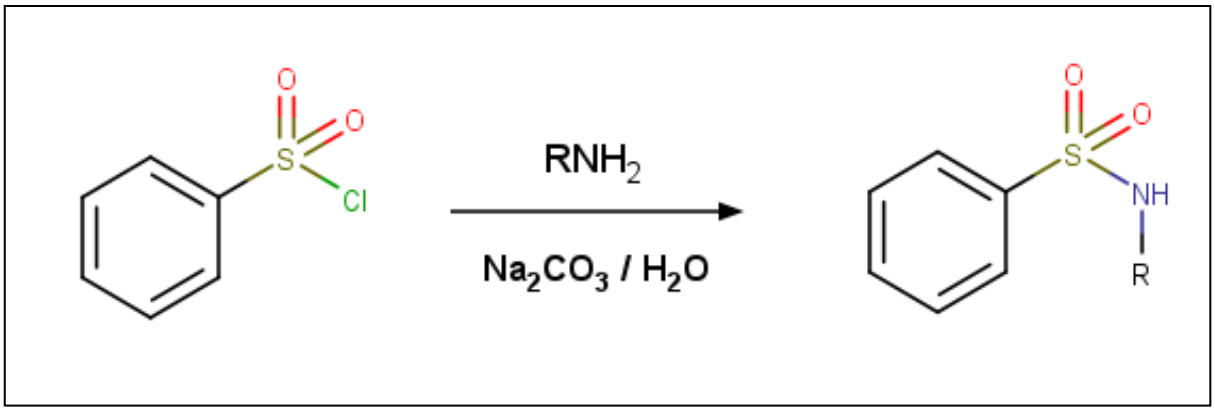


18. Bassin.J.P et al.,[32] described the chlorosulphonation of aromatic compounds. Chlorosulphonation of the compounds is carried out using the chlorosulphonic acid in an ice cold condition 0-5o C. The replacement of the aromatic hydrogen by the chlorosulfonyl group is achieved by the following method:

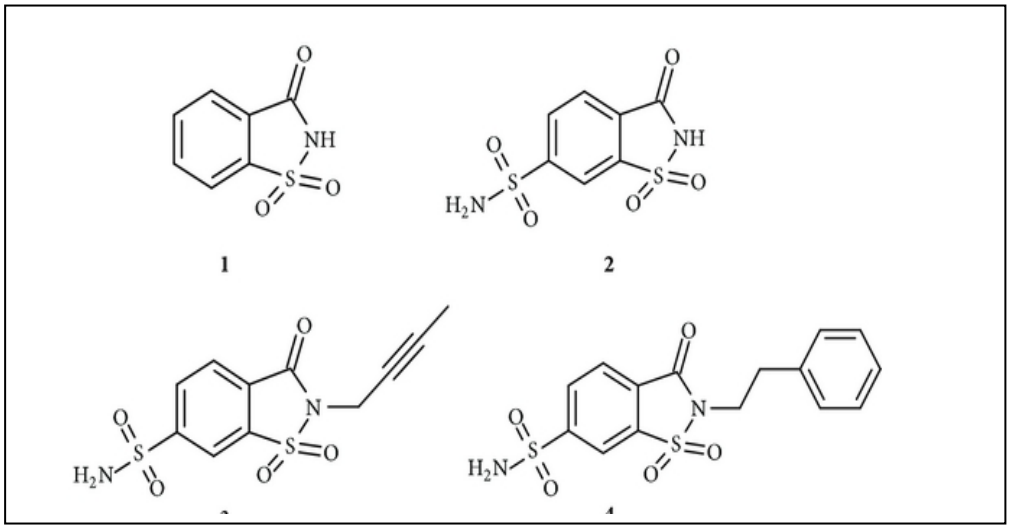
ArH + ClSO3H ArSO3H + HCl ↑

ArSO3H + ClSO3H ArSO3Cl + H2SO4

19. Zainab, Almarhoon et al.,[33] synthesized Sulphonamide derivatives from Sulphonyl chloride by following a simpler laboratory process. Sulphonyl chlorides are allowed to react with the amines in the presence of eco-friendly solvents such as water or ethanol in a basic condition to yield suphonamide derivatives.



20. **Vaida Morkūnaitė et al.,[34]** describe the series of modified saccharin sulfonamides have been designed as carbonic anhydrase (CA) inhibitors and synthesized. Their binding to CA isoforms I, II, VII, XII, and XIII was measured by the fluorescent thermal shift assay (FTSA) and isothermal titration calorimetry (ITC).



21. **JM Wilson et al., [35]** The present work demonstrates that the high-activity zinc metalloenzyme, carbonic anhydrase (CA II) from bovine erythrocytes is inhibited by the cyclic sulfimide, saccharin, and 2-and 4-carbobenzoxybenzene sulfonamide.

AIM AND OBJECTIVES

AIM AND OBJECTIVE

AIM:

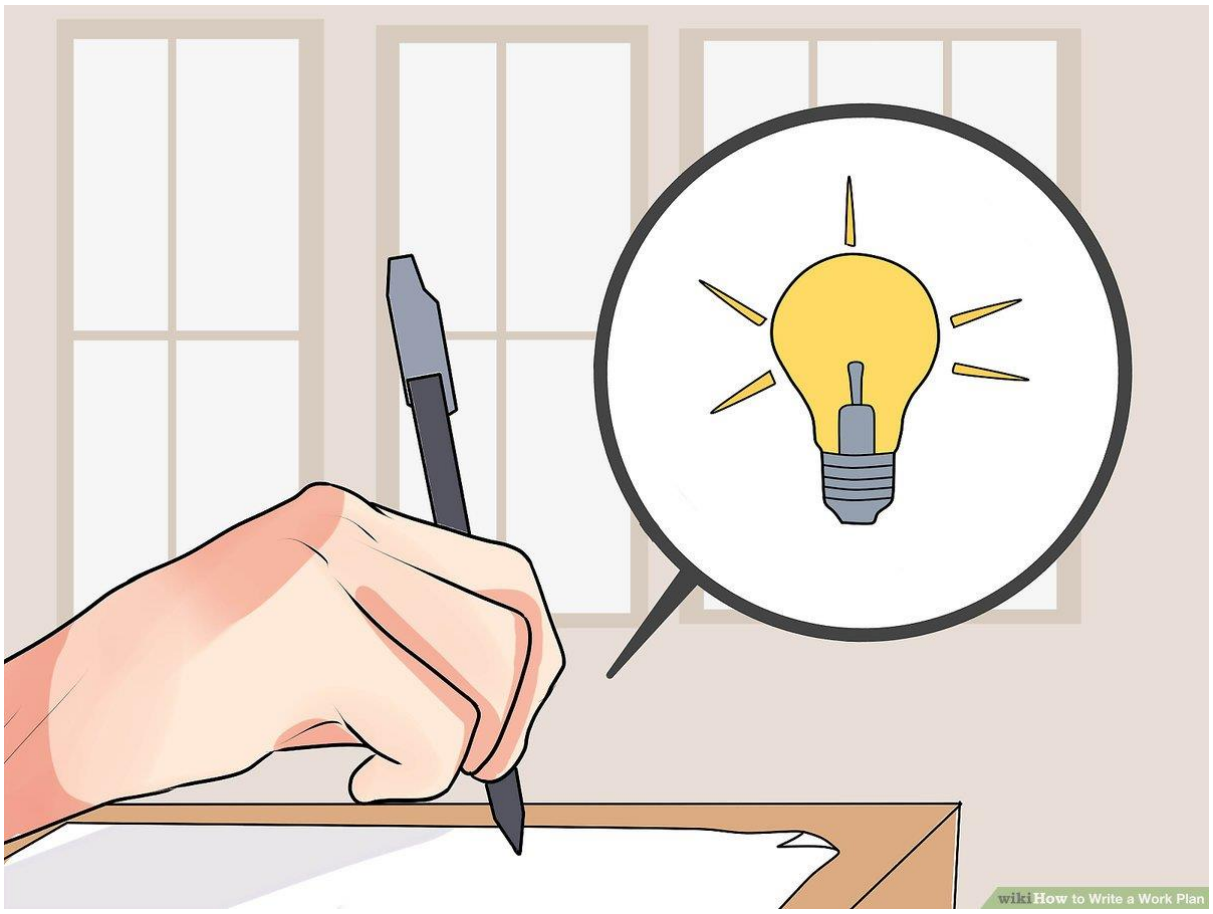
To develop the novel and potent anti-tubercular agents against Glutamine synthetase 1 enzyme.

OBJECTIVE:

To design molecules, synthesize, characterize and determine antitubercular activity, acute toxicity and cytotoxicity.

The plan of work includes:

- *Insilico* Drug likeness prediction.
- Design of Glutamine synthetase 1 inhibitors by docking studies using Autodock Tool (1.5.6 version) software.
- *Insilico* Toxicity Assessment.
- Laboratory synthesis of those compounds with top Docking Scores.
- Characterization of the synthesized compounds by
    - Melting point.
    - Infrared Spectroscopy.
    - GC-Mass Spectroscopy.
    - LC-Mass Spectroscopy.
    - H1 Nuclear Magnetic Resonance Spectroscopy.
- *In-vitro* anti -tubercular activity of synthesized compounds (MABA).
- Acute Toxicity on mice

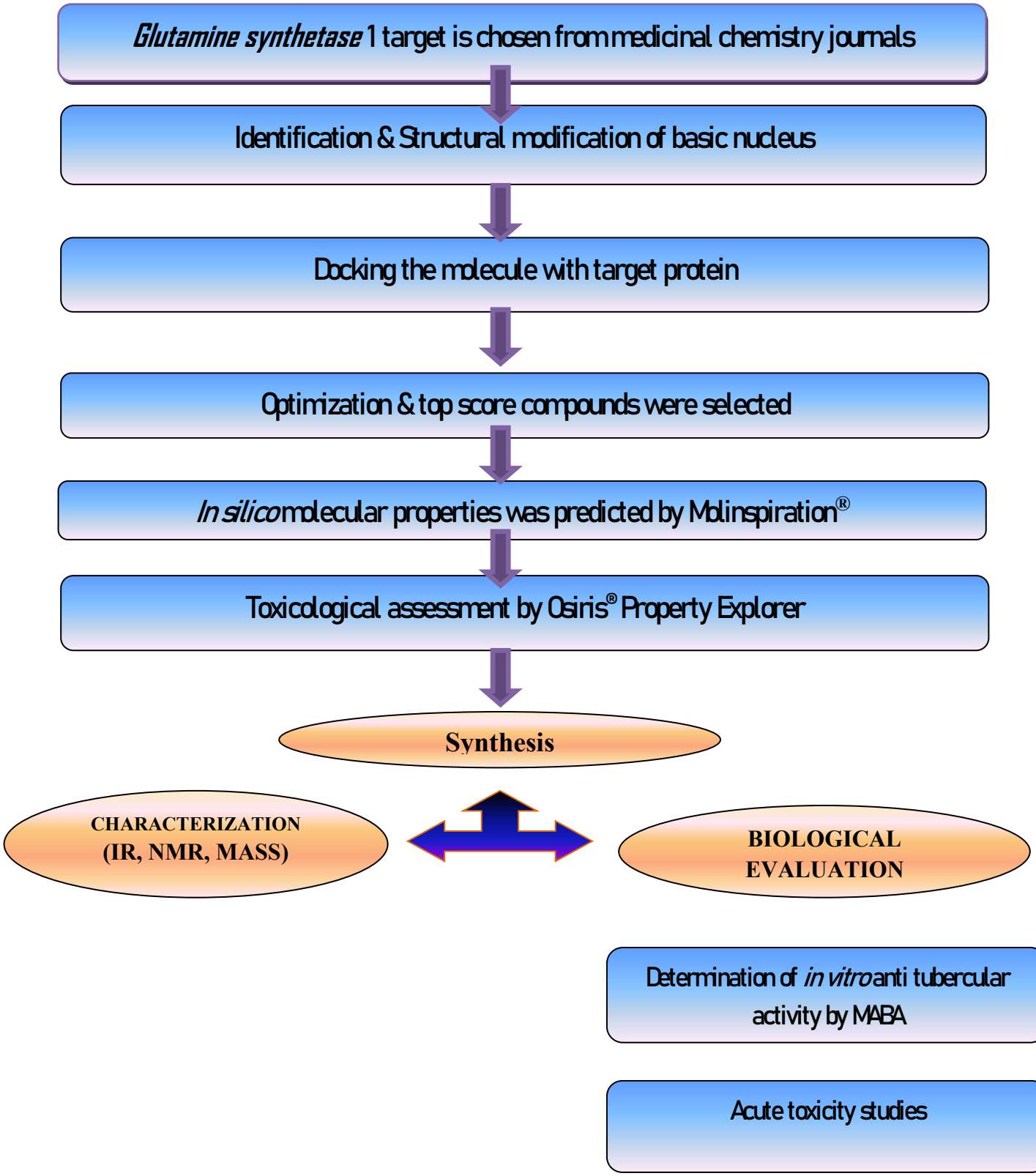


PLAN OF WORK

PLAN OF WORK

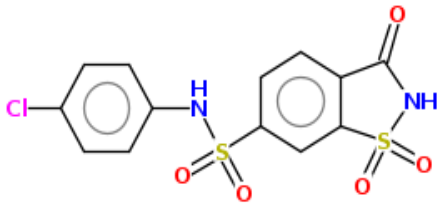
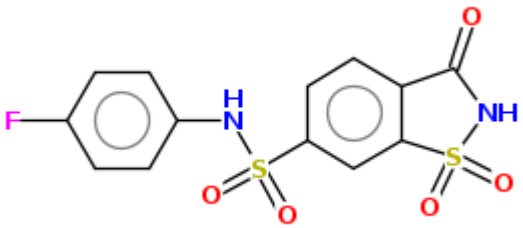
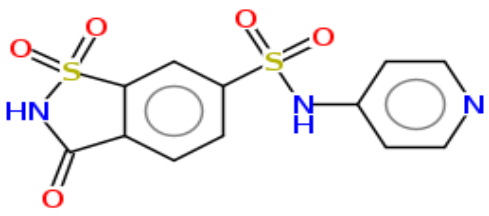
- Design of Glutamine synthetase I inhibitors by docking studies using AutoDock Tools 4® software.
- *In silico* Drug likeness prediction using Molinspiration®.
- *In silico* Toxicity Assessment using Osiris Property Explorer®.
- Laboratory synthesis of those compounds with top Docking Scores.
- Characterization of the synthesized compounds by,
    - TLC method
    - Melting point.
    - Infrared Spectroscopy.
    - LC-Mass Spectroscopy.
    - H1 Nuclear Magnetic Resonance Spectroscopy.
- *In-vitro* anti -tubercular activity of synthesized compounds (Microplate Alamar Blue Assay).
- Acute Oral Toxicity studies
- *In-vitro* cytotoxicity studies.

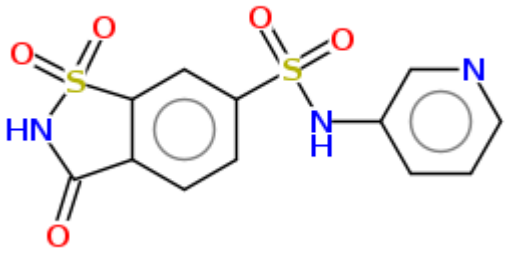
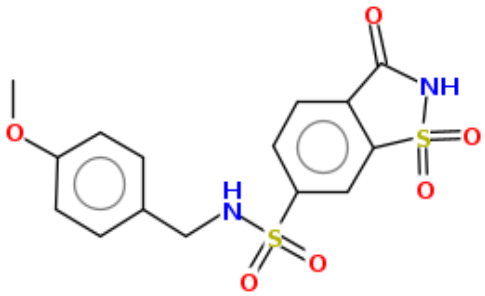
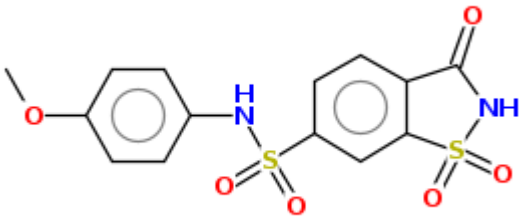
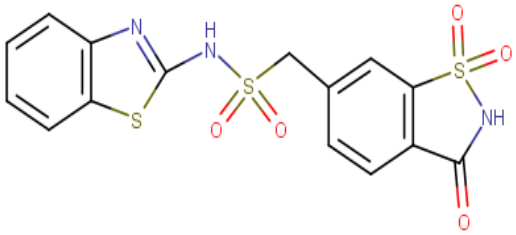
WORK PLAN


Glutamine synthetase 1 target is chosen from medicinal chemistry journals
Identification & Structural modification of basic nucleus
Docking the molecule with target protein
Optimization & top score compounds were selected
In silico molecular properties was predicted by Molinspiration®
Toxicological assessment by Osiris® Property Explorer
Synthesis
CHARACTERIZATION (IR, NMR, MASS)
BIOLOGICAL EVALUATION
Determination of *in vitro* anti tubercular activity by MABA
Acute toxicity studies

LIST OF COMPOUNDS TO BE SYNTHESIZED:

Table 1. List of compounds to be synthesized

SAMPLE CODE	STRUCTURE	IUPAC NAME
AMA-1		N-(4-chlorophenyl) -1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide
AMA-2		N-(4-fluorophenyl)-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide.
AMA-3		1,1,3-trioxo-N-(pyridin-4-yl)-2,3-dihydro-1 lambda 6,2-benzothiazole-6-sulfonamide

AMA-4		**1,1,3-trioxo-N-(pyridin-3-yl)-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide**
AMA-5		**N-[(4-methoxyphenyl)methyl]-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide**
AMA-6		**N-(4-methoxyphenyl)-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide**
AMA-7		**N-(1,3-benzothiazol-2-yl)-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide**

MATERIALS AND METHODS

MATERIALS AND METHOD

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

- Drug design by using Molinspiration, Autodock® tools, Osiris®, Pass prediction.
- Synthesis of the designed molecules.
- Characterization of the synthesized molecules.
- Biological evaluation of the synthesized molecules - MABA activity, Acute oral toxicity.

COMPUTER AIDED DRUG DESIGN:

Drug discovery is the process by which drugs are discovered and/or designed. Rational Drug Design (RDD) is the inventive process of finding new molecules based on the knowledge of the biological target. Computer Aided Drug Design (CADD) computational tools and software`s are used to simulate drug receptor interactions.

Computer-aided drug design (CADD) techniques are used for the rapid assessment of chemical libraries in order to guide and speed up the early-stage development of new active compounds. CADD entails a vast number of computational methodologies like virtual screening, virtual library design, lead optimization and de novo design. The use of these methods depends on the nature of the target and the available information on the system[38]

Computer aided drug design (CADD) has offered valuable tools in the identification of compounds, minimizing the risk of later rejection of lead compounds. Even though high throughput screening (HTS) usually offers several hit compounds, success rates are often very low and many of the identified compounds are later rejected due to their physicochemical properties[39].

A binding interaction between a small molecule ligand and an enzyme protein results in activation or inhibition of the enzyme, which results in agonism or antagonism. Identification of new ligands for a given receptor by searching large databases of 3D structures of small molecules to find those fitting the binding pocket of the receptor using docking programs. This method is known as virtual screening[40].

DOCKING:

Docking program is used to fit the ligand molecule into the target structure in a variety of positions, conformations and orientations. Docking mode is known as pose. Each pose is scored based on its complementarities to the target in terms of shape and properties such as electrostatics in order to identify the most favorable energetic pose.

SCORING FUNCTIONS[41]:

One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Böhm. This empirical scoring function took the form:

ΔG0 – empirically derived that in part corresponds to the overall loss of translational and rotational entropy of the ligand upon binding.

ΔGhb – contribution from hydrogen bonding

ΔGionic – contribution from ionic interactions

ΔGlip – contribution from lipophilic interactions where is surface area of lipophilic contact between the ligand and receptor

ΔGrot – entropy penalty due to freezing a rotatable bond in the ligand bond upon binding

ΔGbind = -RT ln Kd

$$Kd = \frac{[Ligand]\,[Receptor]}{[Complex]}$$

ΔGbind = ΔG desolvation + ΔG motion + ΔG configuration + ΔG interaction

Where:

ΔG desolvation is the enthalpic penalty for removing the ligand from solvent

ΔG motion is the entropic penalty for reducing the degrees of freedom when a ligand binds to its receptor

ΔG configuration is the conformational strain energy required to put the ligand in its "active"

conformation

ΔG interaction is the enthalpic gain for "resolvating" the ligand with its receptor. According to Gibbs free energy equation, the relation between dissociation equilibrium constant, Kd, and the components of free energy was built.

TYPES

Direct and indirect designs are the two strategies usually employed in drug design process.

There are two major types of drug design.

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

LIGAND-BASED [42]

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

STRUCTURE-BASED

Structure-based drug design (or **direct drug design**) relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.

TARGET ENZYME

Glutamine synthetase I was selected as the target enzyme for the study. The crystal structure of the enzyme was downloaded from the Protein Data Bank (An information Portal to Biological Macromolecular Structure) PBD ID – 4ACF.

MOLECULAR DOCKING BY AUTODOCK®:

Autodock® tool 4.2.5.1 is an automated procedure for predicting the interaction of ligands with bio macromolecular targets. Progress in biomolecular x-ray crystallography

continues to provide important protein and nucleic acid structures. These structures could be targets for bioactive agents in the control of animal and plant diseases, or simply key to the understanding of fundamental aspects of biology. The precise interaction of such agents or candidate molecules with their targets is important in the drug discovery process.

In any docking scheme, two conflicting requirements must be balanced: the desire for a robust and accurate procedure, and the desire to keep the computational demands at a reasonable level. The ideal procedure would find the global minimum in the interaction energy between the substrate and the target protein and exploring all available degrees of freedom (DOF) for the system.

AutoDock® tool combines two methods to achieve these goals: rapid grid-based energy evaluation and efficient search of torsional freedom. The current version of AutoDock® using the Lamarckian Genetic Algorithm and empirical free energy scoring function, typically provides reproducible docking results for ligands with approximately 10 flexible bonds. The quality of any docking result depends on the starting structure of both the protein and the potential ligand. The protein and ligand structure need to be prepared to achieve the best docking results. [43]

STEPS INVOLVED IN DOCKING

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

DOCKING PROCEDURE

Preparation of protein:

- Read molecule from the file (allows reading of PDB coordinate files.)
- Edit -Charges – Compute Gasteiger (for arbitrary molecules)
- Edit – Hydrogen –Merge non polar
- Save as .pdb in AutoDock® folder

Preparation of Ligand:

- Docking – docking parameters: opens a panel for setting the parameters used during the docking calculation, including options for the random number generator, options for the force field, step sizes taken when generating new conformations, and output options.
- Ligand –Input from file
- Ligand – Torsion –choose torsion: Rotatable bonds are shown in green, and non-rotatable bonds are shown in red. Bonds that are potentially rotatable but treated as rigid, such as amide bonds and bonds that are made rigid by the user, are shown in magenta.
- Ligand – Torsion –set number of torsion: sets the number of rotatable bonds in the ligand by leaving the specified number of bonds as rotatable.
- Ligand – Output – save as .pdbqt in AutoDock folder.

Preparation of Docking Parameters:

- Docking –Open the macromolecules – set rigid file name.
- Docking – ligand – open the ligand.
- Docking –search parameters – genetic algorithm parameters : this command opens a panel for setting the parameters used by each of the search algorithms, such as temperature schedules in simulated annealing and mutation/crossover rates in genetic algorithms.
- Docking- output –Lamarkian GA –save as dock.dpf (docking parameterfile) Open command prompt [autodock4.exe –p drug.dpf –l drug.dlg]

Visualization / Interpretation of Docking:

- Analysis –Docking – open .dlg (docking log file) file
- Analysis – open the macromolecule
- Analysis – Confirmation –Play and Play ranked by energy : Play- will use the order of conformations as they were found in the docking calculations, and Play Ranked By Energy will order the conformations from lowest energy to highest energy.

- Analysis – Load : Information on the predicted interaction energy is shown at the top and the individual conformations
- Analysis – Docking – show interaction: specialized visualization to highlight interactions between the docked conformation of the ligand and the receptor.

Lipinski's rule

Lipinski's rule is used to predict if a molecule is likely to be orally bioavailable or to evaluate drug likeness. The rule was formulated by Christopher A. Lipinski in1997.The rule states that for drug likeness the molecule should have the following properties.

The rule states that most "drug-like" molecules have

- Not more than 5 Hydrogen bond donors
- Not more than 10 Hydrogen bond acceptors
- Molecular weight less than 500 Daltons
- Calculated log P value less than 5
- Less than 10 rotatable bonds

Also the 500 molecular weight cutoff has been questioned. Polar surface area and the number of rotatable bonds has been found to better discriminate between compounds that are orally active and those that are not for a large data set of compounds in the rat. In particular, compounds which meet only the two criteria of: 1. 10 or fewer rotatable bonds and 2. Polar surface area no greater than 140 Å are predicted to have good oral bioavailability

IN SILICO TOXICITY PREDICTION OSIRIS: [36]

- *In silico* toxicity prediction is done using **OSIRIS®** property explorer.
- It is free (JAVA based online) software available for access in the Organic Chemistry Portal.
- The tool can be used to predict mutagenicity, tumorigenicity, skin irritation and reproductive effects.

- The prediction properties relies on a precompiled set of structure fragment that gives rises to toxicity alerts in case they are encountered in the structure currently drawn.
- Compounds with undesired effects are shown in red colour, whereas green colour indicates the drug conform behavior.

MOLINSPIRATION®: [37]

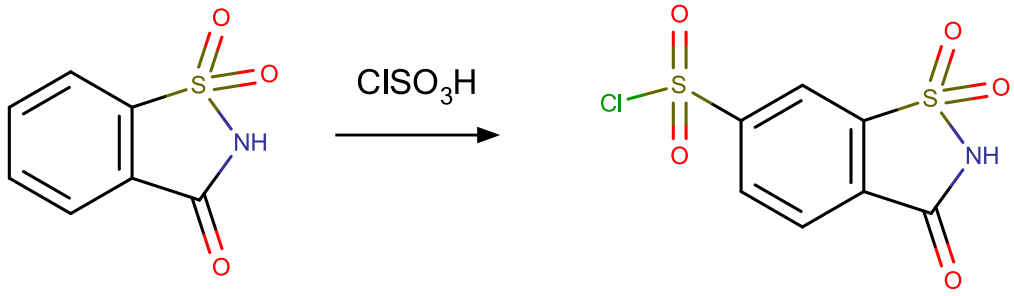
- ✓ The designed and docked molecules are screened *in silico* using **MOLINSPIRATION®** cheminformatics software to evaluate drug likeness.
- ✓ It is a software available online for calculation of important molecular properties log P, polar surface area, number of hydrogen bond donors and acceptors and others, as well as rediction of bioavailability score for the most important drug targets (GPCR ligands, Kinase inhibitors, ion channel modulators, nuclear receptors).

SYNTHETIC SCHEME:

GENERAL SYNTHETIC SCHEME:

STEP 1: Chlorosulphonation

An excess (4:1) of Chlorosulphonic acid was added to the Saccharine. The mixture was mixed well and kept in the refrigerator over-night. Then, the mixture was poured over crushed ice and the precipitated Saccharine-5Chloro Sulphonic acid was filtered and dried.

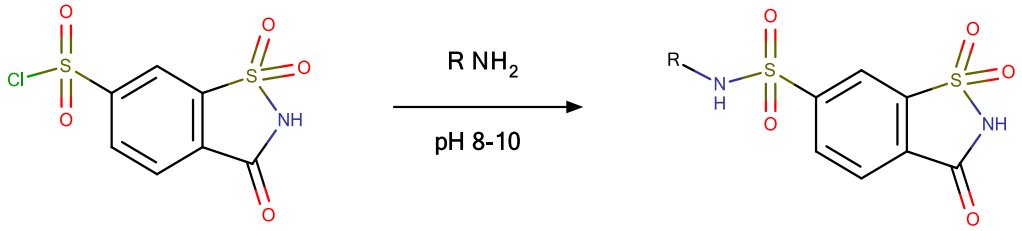


Saccharine

Saccharine 5- Chloro sulphonic acid

STEP 2: Sulphonamide Synthesis

Various substituted amines were dissolved in ethanol and added slowly to the Saccharine-5-sulphonic acid with constant stirring for 2 hours. The pH was maintained at 8 using Sodium carbonate solution. After 2 hours, the mixture was neutralized to pH 2 with Concentrated hydrochloric acid and poured over crushed ice. The precipitated Sulphonamides were filtered and dried.

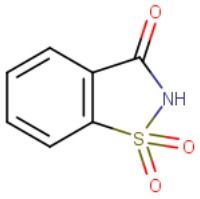


Saccharine 5- Chloro sulphonic acid

Saccharine 5-Sulphonamide

REACTANT PROFILE

SACCHARINE



IUPAC Name : 1,1-dioxo-1,2-benzothiazol-3-one

Molecular Formula : C7H5NO3S

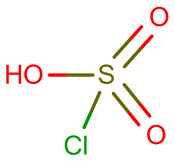
Molecular Weight : 183.19

Appearance : white crystals or crystalline powder.

Melting point : 228.8 °C

Density : 0.828 g/cm^3

CHLOROSULPHONIC ACID



IUPAC Name : Sulfurochloridic acid

Molecular Formula : SO2(OH)Cl

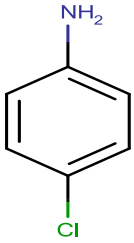
Molecular Weight : 116.53

Appearance : Colorless colored fuming liquid with a pungent

Melting point : 152 °C

Density : 1.75 g/cm^3

p-CHLORO ANILINE



IUPAC Name : 1-amino-4-chloro benzene

Molecular Formula : C6H6ClN

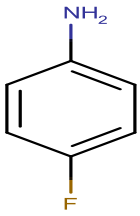
Molecular Weight : 127.57 g/mol

Appearance : White coloured solid

Melting point : 72.5°C

Density : 1.43 g/cm^3

p-FLUORO ANILINE



IUPAC Name : 1-amino-4-fluoro benzene

Molecular Formula : C6H6FN

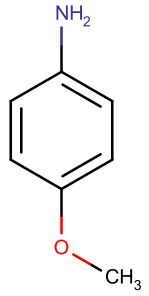
Molecular Weight : 111.12 g/mol

Appearance : Light coloured oily liquid

Boiling point : 182^o C

Density : 1.17 g/cm^3

p-ANISIDINE



IUPAC Name : 1-amino-4-methoxy benzene

Molecular Formula : C7H9NO

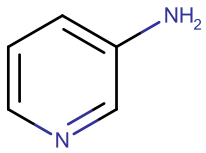
Molecular Weight : 123.15 g/mol

Appearance : Reddish brown solid

Melting point : 57.2° C

Density : 1.07 g/cm^3

3-AMINO PYRIDINE



IUPAC Name : Pyridin-3-amine

Molecular Formula : C5H6N2

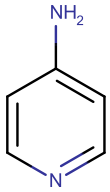
Molecular Weight : 94.11g/mol

Appearance : Yellow brown crystals

Melting point : 64.5° C

Density : 1.107g/cm^3

4-AMINO PYRIDINE



IUPAC Name : Pyridin-4-amine

Molecular Formula : C5H6N2

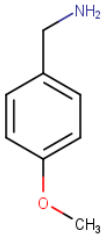
Molecular Weight : 94.11 g/mol

Appearance : White crystalline solid

Melting point : 155° C

Density : 1.26 g/cm^3

4-METHOXY BENZYLAMINE



IUPAC Name : (4-methoxyphenyl)methanamine

Molecular Formula : C8H11NO

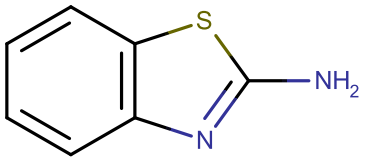
Molecular Weight : 137.18

Appearance : Clear Colourless To Pale Yellowish Liquid

Boiling point : 236.5 °C

Density : 1.05 g/mL

2 – AMINO BENZTHIAZOLE



IUPAC Name : 1,3-benzothiazol-2-amine

Molecular Formula : C7H6N2S

Molecular Weight : 150.20

Appearance : an odorless gray to white powder

Melting point : 126-129 °C

Density : 1.2162 (rough estimate)

5. CHARACTERIZATION STUDIES:

Determination of Melting point: Purity of the synthesized compounds was evaluated by determining the melting point using the Digital Melting point apparatus (GUNA-SNo.1125).

Thin Layer Chromatography (TLC): Pre-coated silica plates were used for TLC. Various proportion of different solvents were tried as Mobile phase and by trial and error method Methanol : Chloroform in the ratio of 9:1 was chosen as the mobile phase. TLC was performed to confirm the completion of the synthetic reaction by comparing the Rf value of the reactants and products.

PHYSICAL EVALUATION:

The physical properties of the synthesized compounds are evaluated as follows

- Colour
- Nature
- Solubility
- Melting point

Spectroscopic Evaluation:

The synthesized compounds were also characterized by the following spectral studies.

- IR Spectroscopy
- Nuclear Magnetic Spectroscopy(^{1}H- NMR)
- Liquid Chromatography – Mass Spectrometry(LC-MS)

INFRARED SPECTROSCOPY: [44]

Infrared (IR) or Vibrational spectroscopy involves measuring the interaction of Infrared radiation with the samples (solid, liquid, gas) by absorption, emission or reflection. It is often used to study the chemical structures or functional groups which give rise to characteristic bands in particular frequency and intensity. A common instrument used to measure the IR spectrum is the **Fourier Transform Infrared Spectrometer (FTIR).**

It relies on the fact that chemical molecules absorb certain frequencies that are characteristic to their structure. For a molecule to absorb the infrared energy, there must be a change in the dipole moment and that influences the intensity of the infrared absorptions.

The infrared portion of the electromagnetic spectrum is divided into,

i. **near IR** - 14000-4000 cm-1 (1.4-0.8µm)

ii. **mid IR** - 4000-400 cm-1 (30-1.4 µm)

iii. **far IR** - 400-10 cm-1 (1000-30 µm)

The number of observed absorptions may be increased by combination tones and overtones of the functional vibrations and decreased by molecular symmetry & spectrometer limitations. Unique patterns are found in the region of **1450-600cm^{-1}** and it is referred as Fingerprint region. Absorption bands in the **4000-1400cm^{-1}** region are mainly due to the stretching vibrations of the diatomic units and it is referred as **Group frequency or Functional group region.**

Table 02: Group Frequencies in Infrared Region[45]

ABSORPTION (cm-1)	APPEARANCE OF PEAK	FUNCTIONAL GROUP	COMPOUND CLASS
3500	Medium	N-H Stretching	Primary amine
3350-3310	Medium	N-H Stretching	Secondary amine
2000-1650	Weak	C-H Stretching	Aromatic compounds
1685-1666	Strong	C=O Stretching	Conjugated ketones
1648-1638	Weak	C=C Stretching	Alkene
1650-1580	Medium	N-H Bending	Amine
1465	Medium	C-H Bending	Alkane
1370-1335	Strong	S=O Stretching	Sulphonamides
1400-1000	Strong	C-F Stretching	Fluoro compounds
900-700	Strong	C-H Stretching	Benzene derivatives
850-550	Strong	C-Cl Stretching	Chloro compounds

The IR Spectrum of the synthesized compounds were obtained from the ABB MB3000 FT-IR Spectrometer.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR): [46]

One of the important techniques used to obtain **the physical, chemical, electronic and structural information about the molecule** is NMR Spectroscopy. This research technique exploits the magnetic properties of certain atomic nuclei including **1H(Proton), 13C(Carbon), 15N(Nitrogen) and 19F(Fluorine).** The qualitative information about a molecule can be

obtained based on the chemical shift produced in the resonance frequencies of nuclear spins in the sample. NMR Spectroscopy is based on the principles such as,

- ❖ All atomic nuclei have their own spin and are electrically charged
- ❖ In the presence of an external magnetic field, transfer of energy from the base level to the higher energy level occurs at a wavelength that coincides with radio frequencies.
- ❖ When the spin returns to its base ground state emission of energy at the same frequency is possible.
- ❖ NMR Spectrum for the sample molecule is processed by measuring the signal which matches the transfer frequency.

The amount of energy and the frequency of Electromagnetic radiation required for resonance to occur depends on the strength of the applied external magnetic field and the type of nucleus involved in the study.

CHEMICAL SHIFT: The NMR Spectrum is displayed as a plot of the applied radio frequency versus the absorption. The position in the plot at which the nuclei absorbs the electromagnetic radiation is called the Chemical shift.

- ➢ Left side of the plot – Downfield or De shielded side
- ➢ Right side of the plot – Upfield or Shielded side

TMS (Tetramethylsilane) is commonly used as a standard reference and assigned a chemical shift of zero. The δ-scale is expressed as parts per million (ppm).

$$\delta = \frac{frequency\ of\ the\ signal - frequency\ of\ the\ standard}{spectrometer\ frequency} \times 10^6$$

In the Proton NMR spectrum, the peaks are splitt into groups due to the coupling between the adjacent protons in the sample molecule. The interaction between the pair of protons is measured by Coupling constant (J).

Thus, in a NMR spectrum

- ➢ The location of the peak (chemical shift) provides information about the local chemical environment adjacent to the proton.

- The integration ratio or intensity of the peak depends on the number of equivalent type of protons in the molecule.
- The coupling pattern or multiplicity is based on the number of protons on the adjacent carbon atom.

This information gives us an idea about the molecule.

Table 03 [47]: Chemical Shift values in NMR

Types of protons	δ (ppm)
R-CH3	0.9
R-CH2-R	1.3
Ar-CH3	2-3
R2N-CH3	2.3
RO-CH3	3.8
R-CH2-F	4.5
Ar-H	7.3

The NMR Spectrum of the compounds were obtained from 400MHz Bruker Topspin – Advanced NMR Instrument using deuterated DMSO as solvent.

HYPHENATED TECHNIQUES: [48]

Coupling of different analytical techniques i.e., commonly a chromatographic technique is combined with a spectroscopic technique and are known as hyphenated techniques. Components in the sample mixture are separated by the chromatographic technique and then each component enters into the spectroscopic analysis via a suitable interphase.

1) Separation – separation

2) Separation – identification

3) Identification – identification.

DOUBLE HYPHENATED TECHNIQUES	TRIPLE HYPHENATED TECHNIQUES
LC-MS	LC-API-MS
LC-NMR	ESI-MS-MS
LC-IR	LC-NMR-MS
GC-MS	APCI-MS-MS
GC-FTIR	LC-PDA-MS

Table 04 LC-MS [Liquid Chromatography – Mass Spectrometry]: [49], [50]

LC-MS is an analytical technique which combines the physical separation of liquid chromatography (HPLC) with the Mass Spectrometry by an interface. To obtain only few fragment ions with the molecular ion, soft ionization techniques are utilized in the LC-MS. For confirming the identity of the compound single LC-MS run is not sufficient. Scanning speed of the MS is influenced by the Chromatographic resolution, therefore to achieve accurate integration, it is suggested to have 10 scans across the chromatographic peak.

The hyphenated technique LC-MS includes,

- LC – Liquid chromatography separates the sample mixture into individual components
- Interface – transfers the separated component from LC Column to MS ion source
- MS – Mass spectrometry identifies the structure of the individual components

Liquid chromatography involves the separation of components in the liquid mixture by distributing them between the two immiscible phases (Stationary & Mobile phase). Typically in HPLC, 20µl of sample is injected into the mobile phase by a high pressure pump. The sample mixed with the mobile phase permeates through the stationary bed and gets separated based on their differential affinities towards the stationary & mobile phases and gets eluted from the column at different times (Rt). **Reverse Phase Partition Chromatography** (Non-polar stationary phase and polar mobile phase) is widely used.

The interface is designed to offer adequate nebulization and vapourization of the solvent, ionization of the sample, removal of the excess solvent vapour and the extraction of the ions. The transition of components from the High pressure environment of HPLC to High vacuum condition in the Mass analyzer is facilitated by the interface. The commonly used interfaces are,

- Electrospray Ionisation Interface (ESI),
- Atmospheric Pressure Chemical ionisation Interface (APCI).

MS is an analytical technique that measures the Mass to charge ratio (m/z) of the charged ions. The ions produced from the analyte are manipulated by the electric and magnetic fields to determine their m/z ratio. The basic components include,

- Ion source: Components in the sample mixture is ionized by electron/photon beams which fragments the sample molecules into gas phase ions and sent to analyzers.
- Mass analyzer: Ions are sorted according to their masses.
- Detector: Abundance of each ion is measured by the detection through amplification.

The synthesized compounds were characterized by LC-MS instrument – Agilent technologies 6230B TOF (Time of Flight).

BIOLOGICAL EVALUATION OF ANTITUBERCULAR ACTIVITY [51]

Susceptibility of the compounds to Mycobacterium tuberculosis can be detected either by observing the inhibition of growth or metabolism in a medium containing the test compound (Macroscopic observation of growth of the organism in a suitable medium or measurement of the metabolic activity or its product) or detecting the mutations in the genes as the result of the potency of the compound.

Various methods are available to determine the anti-tubercular activity of the synthesized compounds. Some of them are as follows,

- Microplate Alamar Blue Assay
- Microbroth dilution
- Radiometric BACTEC 460 ®
- Luciferase Reporter phage assay
- HPLC Mycolic acid assay

MICROPLATE ALAMAR BLUE ASSAY (MABA) [52]

The anti-microbial activity of the synthesized compounds is determined by MABA method. The organism used in the study is ***Mycobacteria tuberculosis*** (Vaccine strain, H37 RV strain): ATCC No- 27294.

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

Principle:

MABA is an indirect colorimetric method for determining the minimum inhibitory concentration of antitubercular drugs. In this assay, the redox indicator Alamar blue monitors the reducing environment of the living cells. It turns from blue to pink in the presence of mycobacterium growth.

Procedure:

1. This method is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.
2. Briefly, 200µl of sterile deionised water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during

incubation.

3. The 96 wells plate received 100µl of the Middle brook 7H9 broth and serial dilution of compounds were placed directly on plate.
4. The final drug concentrations tested was made up to 100 to 0.2µg/ml.
5. Plates were covered and sealed with Para film and incubated at 37^{o}C for five days.
6. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to the plate and incubated for 24hrs.
7. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.
8. The MIC is the lowest drug concentration which prevents the colour change from blue to pink.

PHARMACOLOGICAL EVALUATION

ACUTE TOXICITY [53] [54]

The main purpose of acute toxicity is to evaluate the adverse effects that occur after oral administration of the synthesized molecule. The acute oral toxicity studies were performed as per OECD guidelines 423(ACUTE TOXIC CLASS METHOD)

Animal requirements

SPECIES	:	ALBINO MICE
WEIGHT	:	20-25 g
SEX	:	FEMALE
DURATION OF STUDY	:	14 DAYS

SELECTION OF DOSE LEVEL:

Being synthetic molecules, the mortality was unlikely at the highest starting dose level (2000mg/kg) body weight. Hence a limit test one dose level of 2000mg/kg/b.w was conducted in all animals as per the OECD guidelines (423).

PROCEDURE: [55]

- Animals were fasted prior to dosing (food but not water withheld for 3-4 hours).
- Following the period of fasting each animal was weighed and the compounds were administered using a suitable intubation cannula.
- After dosing animals were observed for the first 30mins.
- Special attention were given during first 4hours and periodically observed for 24hrs.
- Animals were observed for signs of toxicity for 14 days.
- The signs of toxicity includes observational changes in colour of skin or fur, sleep, salivation, coma, motor activity, respiration etc.
- Statistical calculation were carried out using Graph Pad Prism 7 software.

EXPERIMENTAL DESIGN:

The animals were kept in their cages two days before dosing for the acclimatisation to the laboratory conditions

↓

Animals were fasted prior to dosing (food was withheld for 3-4 hours).

↓

The maximum dose of 2000 mg/kg is chosen for the study

↓

The test substance is accurately weighed & suspended in 0.1% Carboxy methyl cellulose (CMC) and administered using an intubation canula.

↓

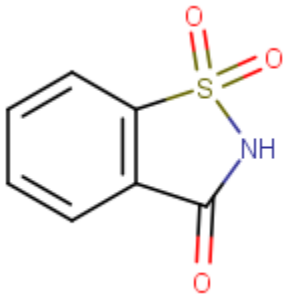
Changes in skin & fur, eyes & mucous membranes & respiratory, circulatory, autonomic & central nervous systems, and behaviour pattern were observed.

RESULTS AND DISCUSSIOM

RESULTS AND DISCUSSION

1. RESULTS OF DRUG DESIGN:

- Five hundred molecules were designed using ACD/Chem Sketch® (Freeware) software by performing structural modifications in the basic hetero nuclear scaffold Saccharin.
- Various functional groups were substituted in the 5th position of the hetero nucleus Saccharin.
- Energy minimization of the designed molecules was performed in Chem3D Ultra® software.
- The designed molecules were coded for identification.

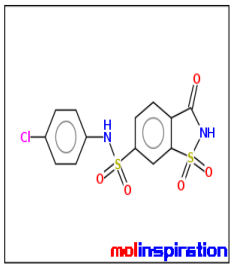
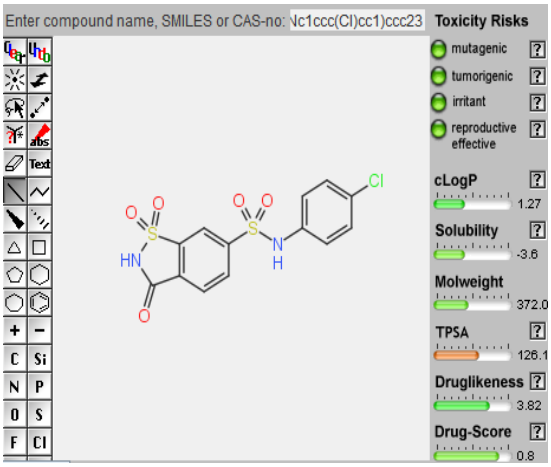
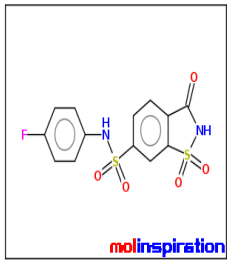
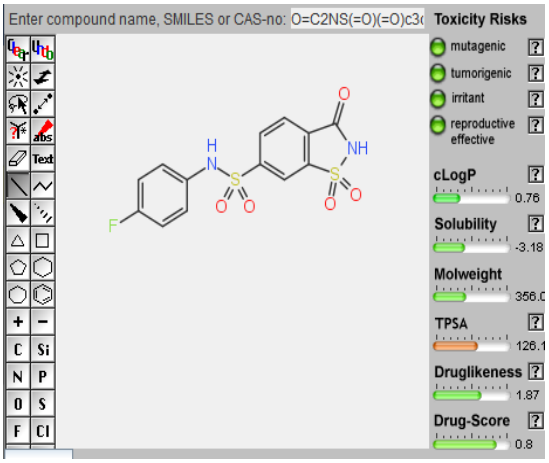


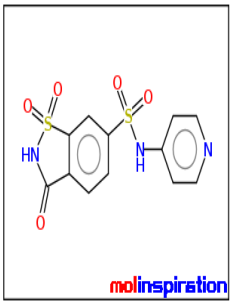
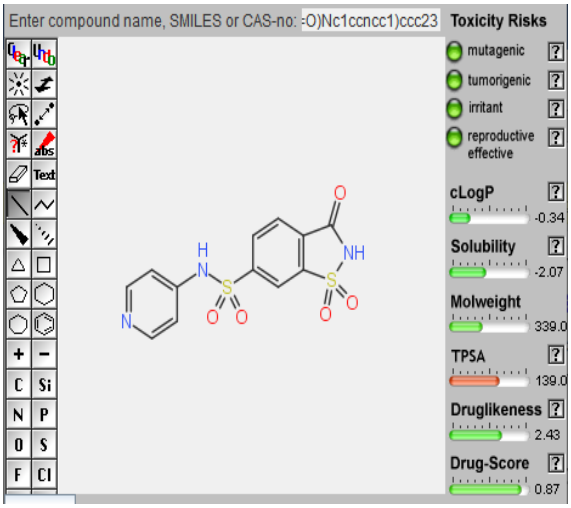
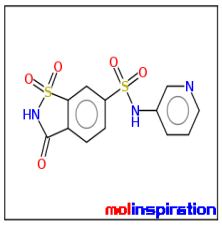
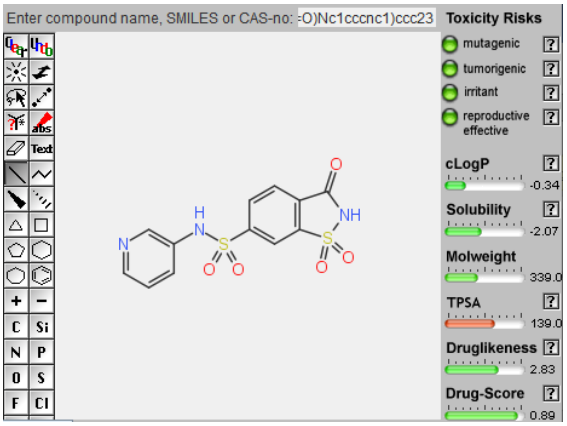
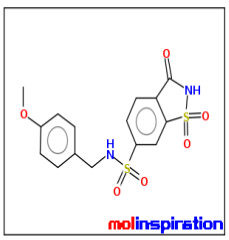
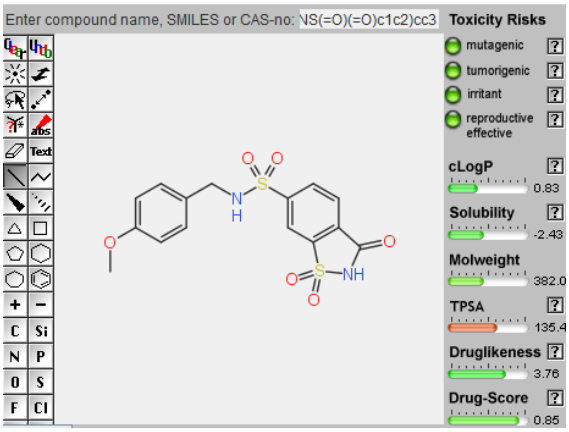
2. RESULTS OF *IN SILICO* DRUG LIKENESS AND TOXICITY PREDICTION:

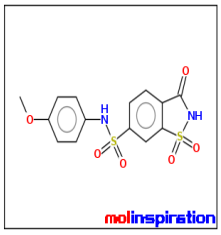
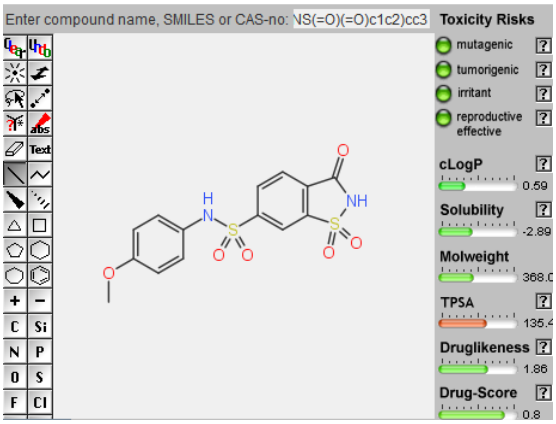
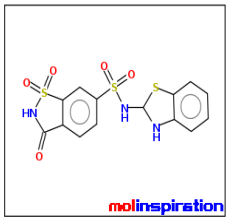
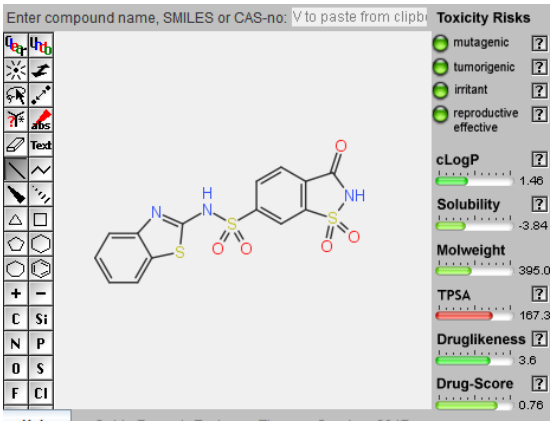
For a molecule to be orally active, it must have drug likeness properties conforming to the Lipinski's Rule of five and it must be non-toxic.

- The molecules were evaluated for their drug likeness behaviour using the online tool – **Molinspiration® Cheminformatics.**
- The toxicity of the molecules was assessed using **OSIRIS® Property Explorer**.
- From the results, 350 molecules were found to have Drug likeness property and no toxicity.
- In silico drug likeness and toxicity prediction results of some of the molecules which showed good binding energy in the docking studies performed later are shown here.

Table 05: *In silico* Drug-likeness and Toxicity prediction

SAMPLE CODE	*IN SILICO* DRUG LIKENESS PREDICTION	*IN SILICO* TOXICITY ASSESSMENT
AMA-1	molinspiration miSMILES: O=C2NS(=O)(=O)c3cc(S(=O)(=O)Nc1ccc(Cl)cc1)ccc23  Molinspiration property engine v2021.10 miLogP 1.65 TPSA 109.41 natoms 23 MW 372.81 nON 7 nOHNH 2 nviolations 0 nrotb 3 volume 264.80 Get data as text (for copy / paste). Get 3D geometry BETA	
AMA-2	molinspiration miSMILES: O=C2NS(=O)(=O)c3cc(S(=O)(=O)Nc1ccc(F)cc1)ccc23  Molinspiration property engine v2021.10 miLogP 1.13 TPSA 109.41 natoms 23 MW 356.36 nON 7 nOHNH 2 nviolations 0 nrotb 3 volume 256.19 Get data as text (for copy / paste). Get 3D geometry BETA	

AMA-3	molinspiration miSMILES: O=C2NS(=O)(=O)c3cc(S(=O)(=O)Nc1ccncc1)ccc23  Molinspiration property engine v2021.10 miLogP -0.32 TPSA 122.30 natoms 22 MW 339.35 nON 8 nOHNH 2 nviolations 0 nrotb 3 volume 247.10 Get data as text (for copy / paste). Get 3D geometry BETA	
AMA-4	molinspiration miSMILES: O=C2NS(=O)(=O)c3cc(S(=O)(=O)Nc1cccnc1)ccc23  Molinspiration property engine v2021.10 miLogP -0.10 TPSA 122.30 natoms 22 MW 339.35 nON 8 nOHNH 2 nviolations 0 nrotb 3 volume 247.10 Get data as text (for copy / paste). Get 3D geometry BETA	
AMA-5	molinspiration miSMILES: COc3ccc(CNS(=O)(=O)c2ccc1C(=O)NS(=O)(=O)c1c2)cc3  Molinspiration property engine v2021.10 miLogP 0.73 TPSA 118.64 natoms 25 MW 382.42 nON 8 nOHNH 2 nviolations 0 nrotb 5 volume 293.61 Get data as text (for copy / paste). Get 3D geometry BETA	

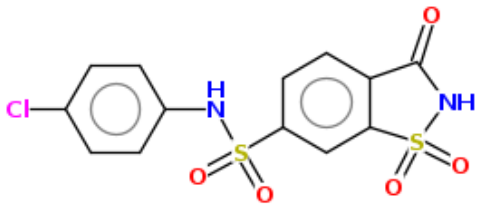
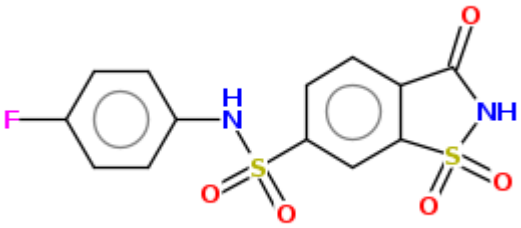
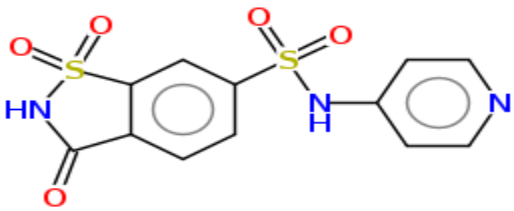
AMA-6	molinspiration miSMILES: COc3ccc(NS(=O)(=O)c2ccc1C(=O)NS(=O)(=O)c1c2)cc3  Molinspiration property engine v2021.10 miLogP 1.03 TPSA 118.64 natoms 24 MW 368.39 nON 8 nOHNH 2 nviolations 0 nrotb 4 volume 276.81 Get data as text (for copy / paste). Get 3D geometry BETA	
AMA-7	molinspiration miSMILES: O=C3NS(=O)(=O)c4cc(S(=O)(=O)NC2Nc1ccccc1S2)ccc34  Molinspiration property engine v2021.10 miLogP 0.96 TPSA 121.44 natoms 25 MW 397.46 nON 8 nOHNH 3 nviolations 0 nrotb 3 volume 287.78 Get data as text (for copy / paste). Get 3D geometry BETA	

- The above molecules showed no violations of Lipinski's rule and no toxicity risks (no red colour alert) were shown in OSIRIS® Property Explorer.
- The non-toxic, drug like molecules were subjected to Molecular Docking studies.

3. RESULTS OF MOLECULAR DOCKING:

- About 350 non-toxic molecules were docked against the target enzyme Glutamine Synthetase (PDB ID – 4ACF) of Mycobacterium tuberculosis to determine their effectiveness as an anti-tubercular agent.
- Five top scored molecules were further synthesized, characterized and evaluated.

Table 06: Code, Molecular Structure, Binding energy of the compounds.

SAMPLE CODE	STRUCTURE	DOCKING SCORE Kcal/mol
AMA-1		-7.05
AMA-2		-7.13
AMA-3		-8.59

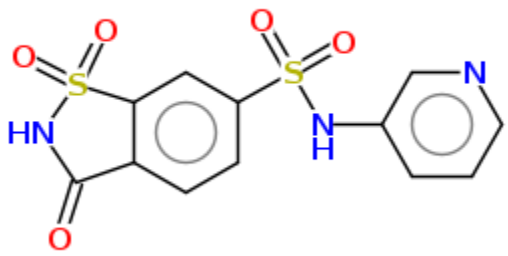
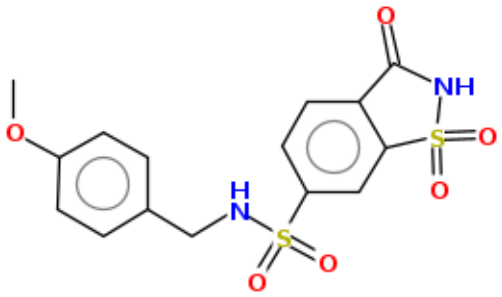
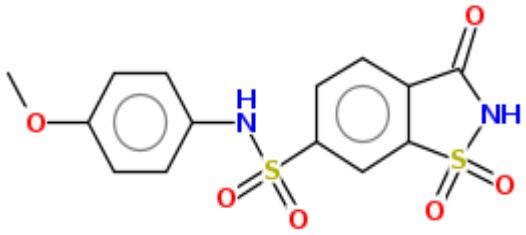
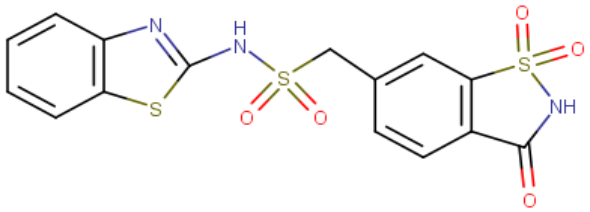
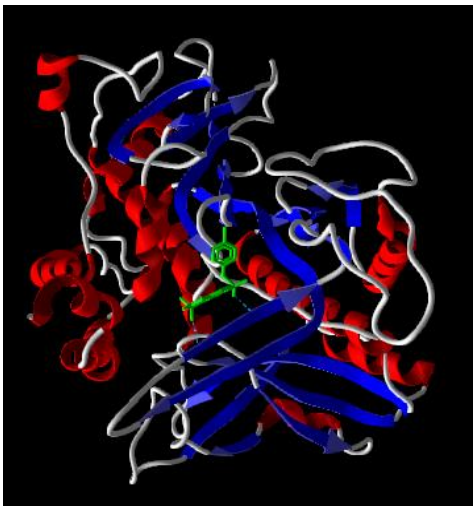
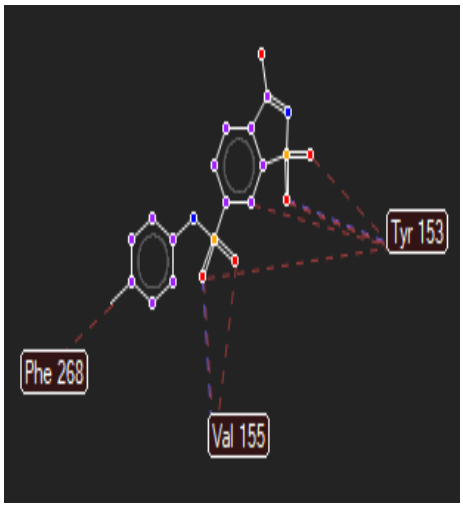
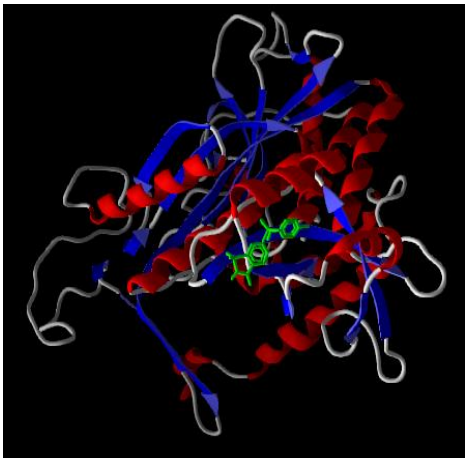
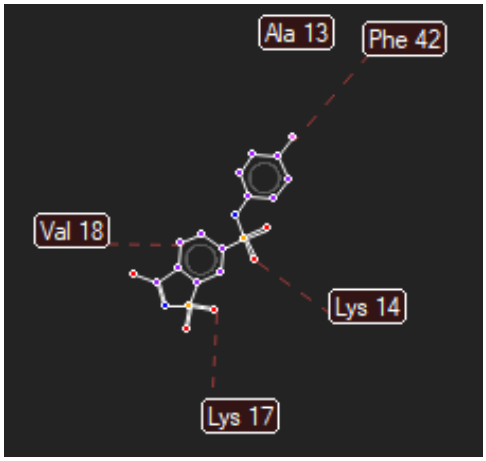
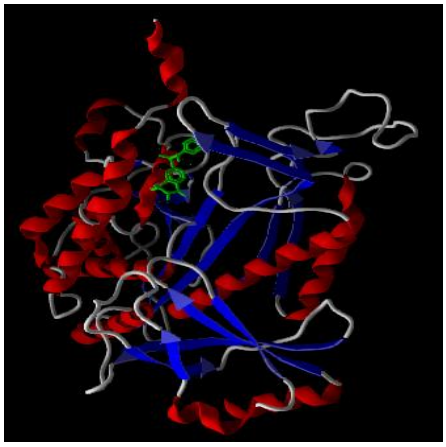
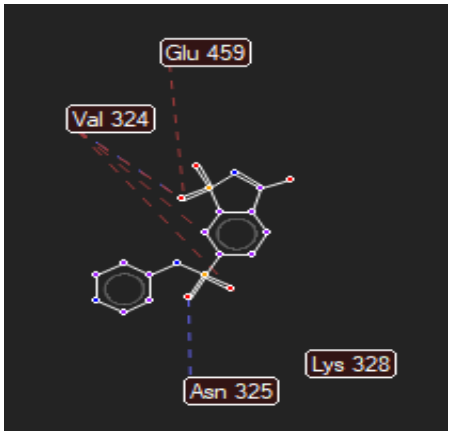
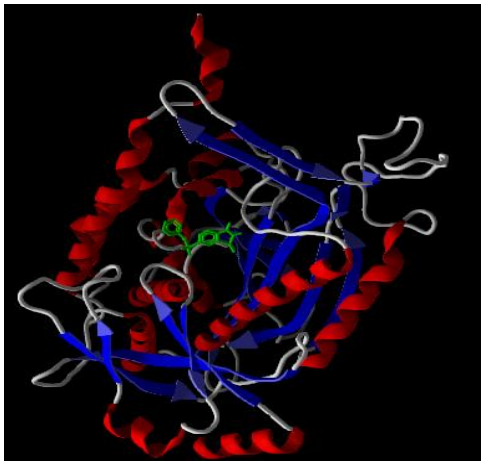
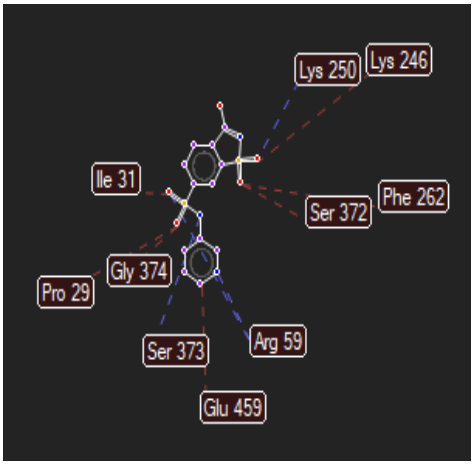
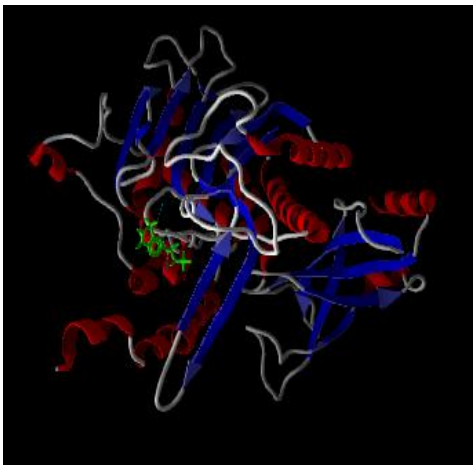
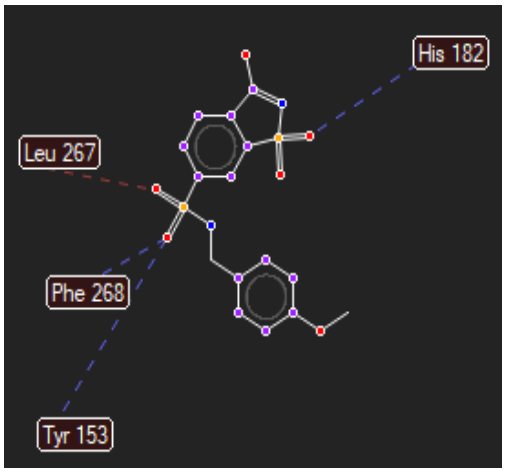
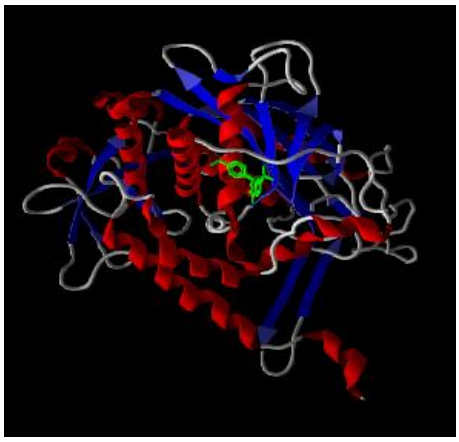
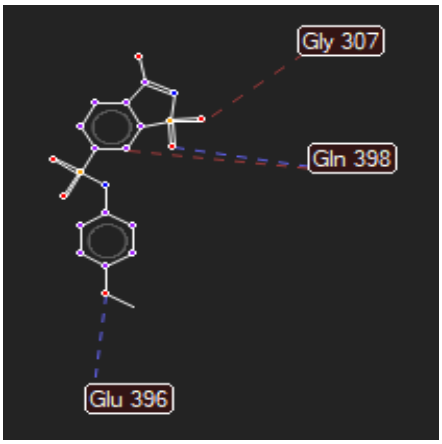
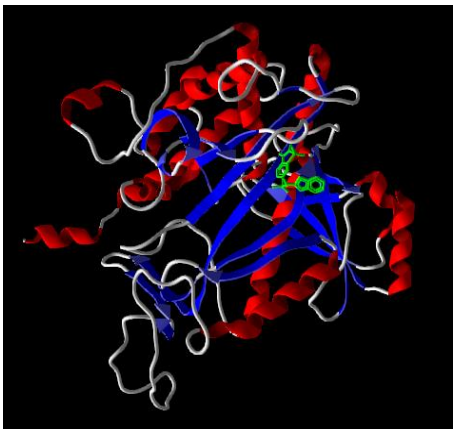
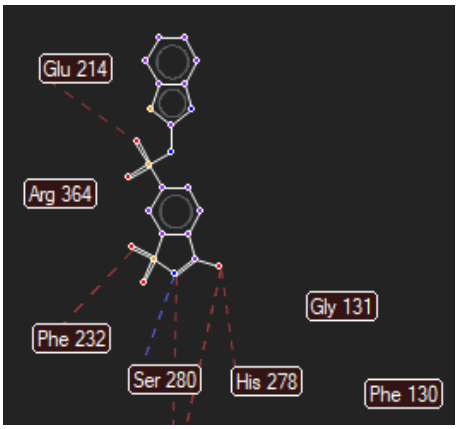
AMA-4		**-8.68**
AMA-5		**-8.05**
AMA-6		**-7.75**
AMA-7		**-8.35**

TABLE 07: INTERACTION WITH AMINOACID

SAMPLE CODE	INTERACTION WITH AMINO ACID	DOCKING VIEW
AMA-1		
AMA-2		

AMA-3		
AMA-4		
AMA-5		

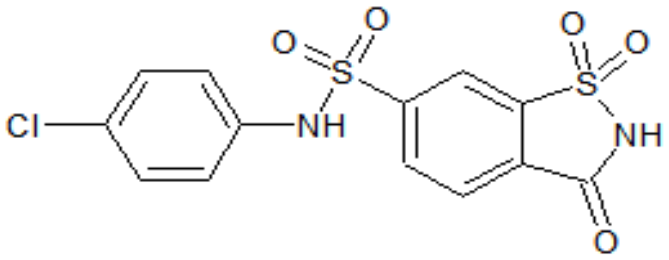
AMA-6		
AMA-7		

- Molecules which were found to be non-toxic in the *in silico* toxicity assessment tool were synthesized by suitable laboratory process and recrystallized using ethanol.
- Completion of the synthetic reaction was determined by comparing the Rf values of the reactants and products. Rf value of the products was found to be different from the Rf value of the reactants.
- Melting point of the products was determined by Digital Melting Point Apparatus(Guna S.No 1125). Sharp melting point denotes the purity of the compound.

Table 08: Data of the synthesized compounds AMA-1 to AMA -7

Sample code	Colour	Solubility	Mobile phase	Rf value	Melting point
AMA-1	White crystal	Methanol, Ethanol, DMSO, ACN	Methanol:Chloroform (9:1)	0.64	196-197
AMA-2	Pale green	Methanol, Ethanol, DMSO, ACN	Methanol:Chloroform (9:1)	0.71	198-199
AMA-3	White crystal	Methanol, Ethanol, DMSO, ACN	Methanol:Chloroform (9:1)	0.67	192-193
AMA-4	White crystal	Methanol, Ethanol, DMSO, ACN	Methanol:Chloroform (9:1)	0.75	200-201
AMA - 5	White crystal	Methanol, Ethanol, DMSO, ACN	Methanol:Chloroform (9:1)	0.83	195-196
AMA-6	Light Green	Methanol, Ethanol, DMSO, ACN	Methanol:Chloroform (9:1)	0.70	197-198
AMA-7	White powder	Methanol, Ethanol, DMSO, ACN	Methanol:Chloroform (9:1)	0.69	175-176

COMPOUND AMA-1



IUPAC Name: N-(4-chlorophenyl)-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide

Molecular Formula	$C_{13}H_9ClN_2O_5S_2$
Formula Weight	372.80396
Composition	C(41.88%) H(2.43%) Cl(9.51%) N(7.51%) O(21.46%) S(17.20%)
Molar Refractivity	83.92 ± 0.4 cm^3
Molar Volume	218.6 ± 3.0 cm^3
Parachor	642.6 ± 6.0 cm^3
Index of Refraction	1.693 ± 0.02
Surface Tension	74.5 ± 3.0 dyne/cm
Density	1.704 ± 0.06 g/cm^3
Dielectric Constant	Not available
Polarizability	33.27 ± 0.5 $10^{-24}cm^3$

Figure 05: IR Spectrum of Compound AMA-1

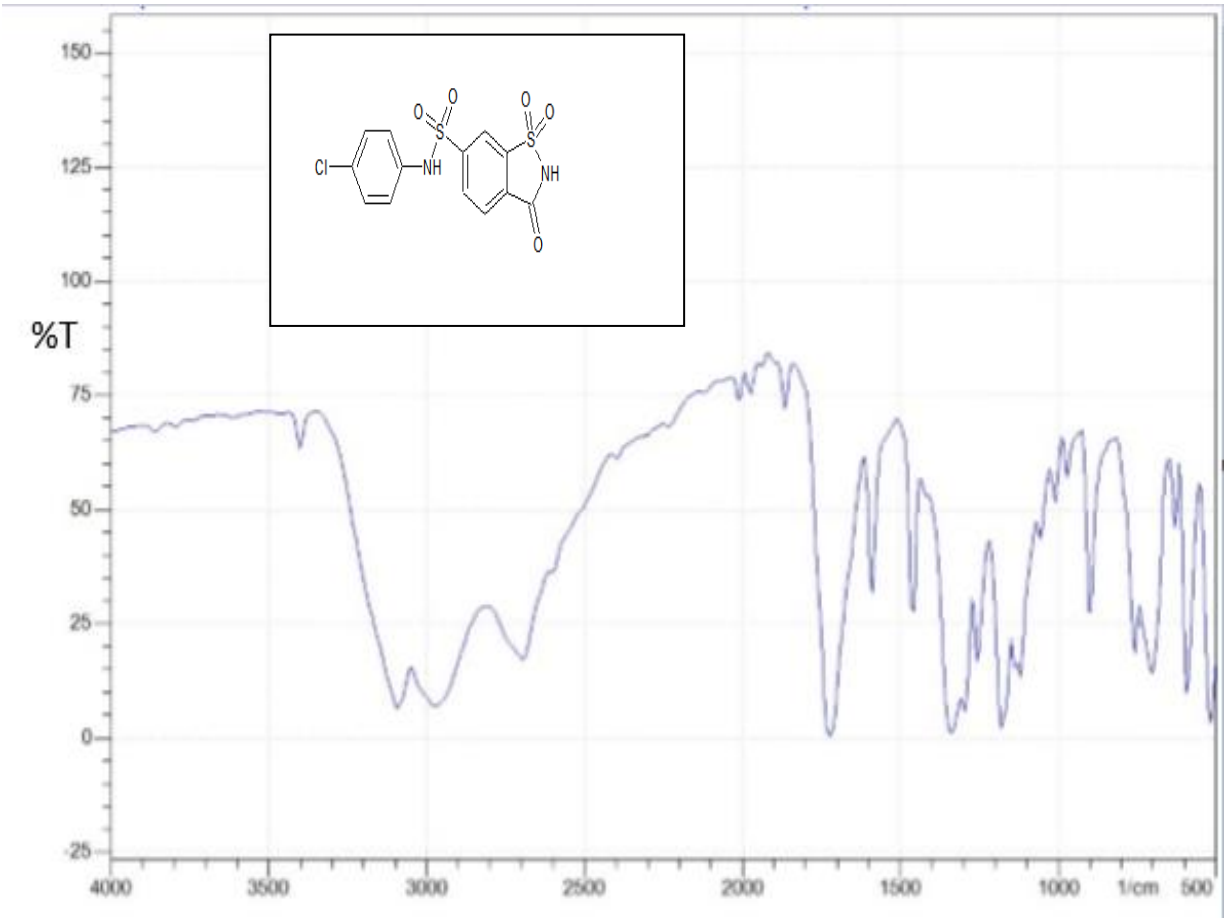


Table 09. Interpretation of IR Spectrum AMA-1

S.No	Wave Number(cm-1)	Functional Group
1	1475 and 1600	C=C(Aromatic)
2	1725	-C=O(Keto)
3	2970	-NH(Secondary amine)
4	1170 and 1370	S=O (Sulphonamide)
5	735	C-Cl (Chloro)
6	1725	-C=O(Keto)

Figure 06: ^{1}H NMR Spectrum of Compound AMA-1

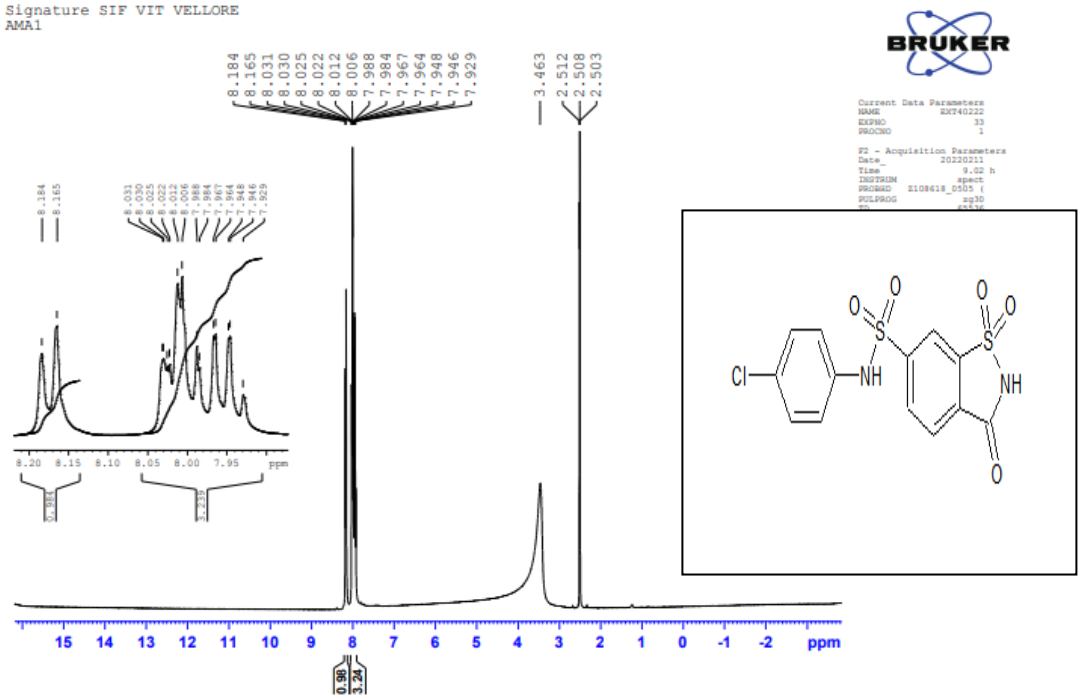


Table 10: Interpretation of 1H NMR Spectrum of Compound AMA-1

S.NO	δ VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
01.	3.463	-NH-	Singlet	2
02.	7.946 – 7.948	Ar-H	Doublet	1
03.	7.964 – 7.967	Ar-H	Doublet	1
04.	7.984 – 7.988	Ar-H	Doublet	1
05.	8.006 – 8.012	Ar-H	Doublet	1
06.	8.022 – 8.025	Ar-H	Doublet	1
07.	8.030 – 8.031	Ar-H	Doublet	1
08.	8.165 – 8.184	Ar-H	Doublet	1

LC-MS SPECTRUM OF AMA-1:

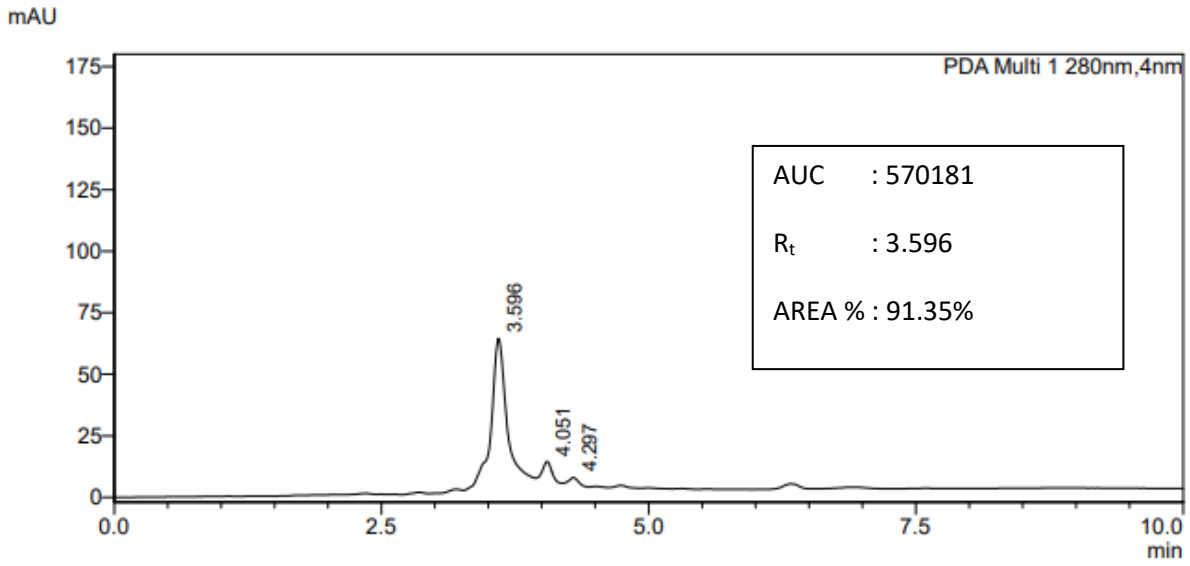


Figure 07: Chromatogram of Compound AMA-1

Calculated Molecular Weight: 372.80396g/mol

Actual Molecular Weight: 372.30000g/mol

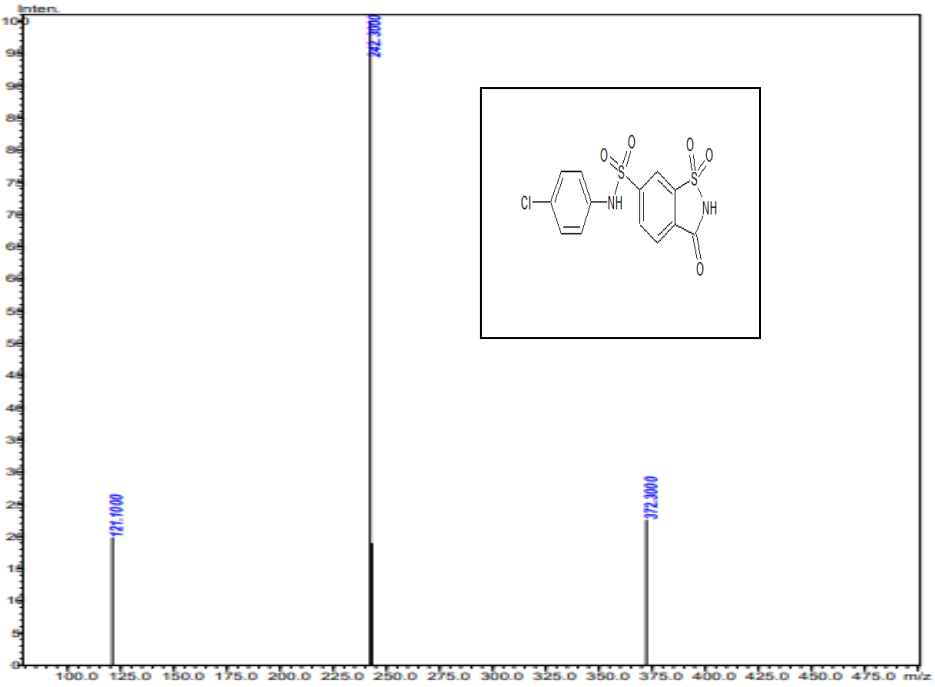
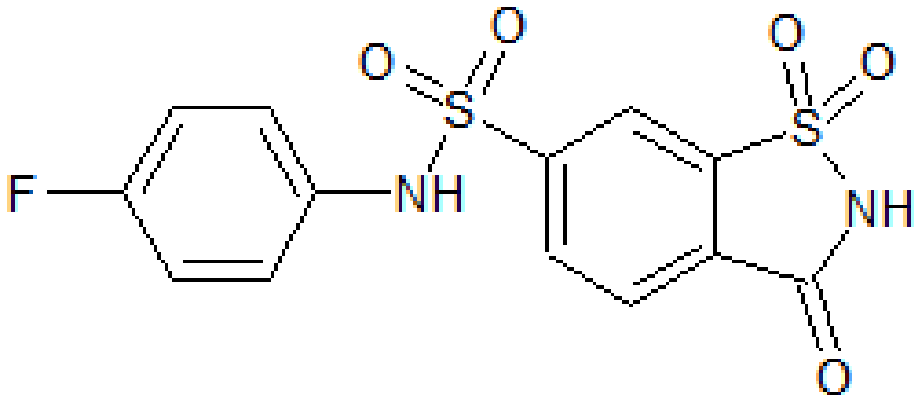


Figure 08: Mass Spectrum of Compound AMA-1

COMPOUND AMA-2



IUPAC Name: N-(4-fluorophenyl)-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide.

Molecular Formula	$C_{13}H_9FN_2O_5S_2$
Formula Weight	356.3493632
Composition	C(43.82%),H(2.55%) F(5.33%),N(7.86%),O(22.45%) S(18.00%)
Molar Refractivity	79.20 ± 0.4 cm^3
Molar Volume	210.9 ± 3.0 cm^3
Parachor	612.8 ± 6.0 cm^3
Index of Refraction	1.674 ± 0.02
Surface Tension	71.2 ± 3.0 dyne/cm

Figure 07: IR Spectrum of Compound AMA-2

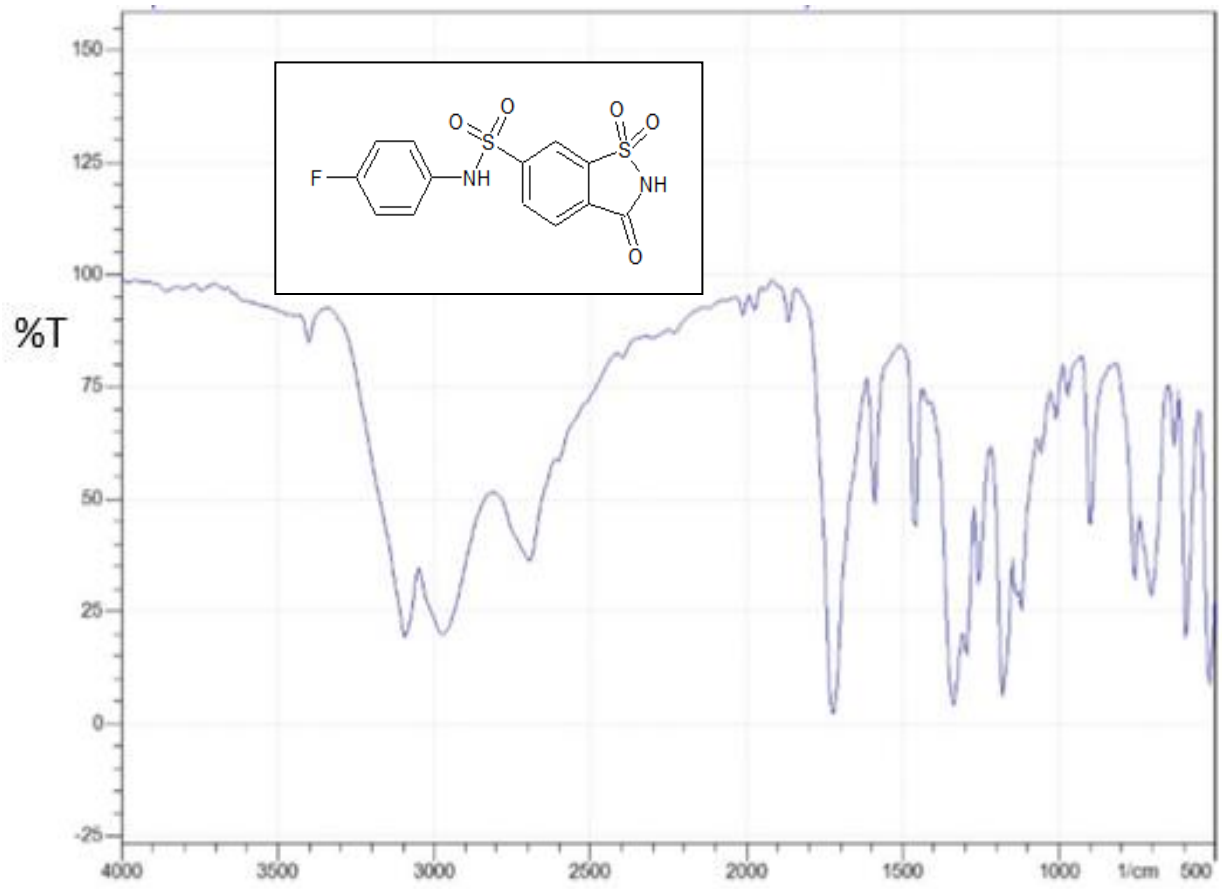


Table 11. Interpretation of IR Spectrum AMA-2

S.No	Wave Number(cm-1)	Functional Group
1	1475 and 1600	C=C(Aromatic)
2	3000	-NH(Secondary amino)
3	1720	-C=O(Keto)
4	1160 and 1340	S=O (Sulphonamide)
5	1275	C-F(Fluoro)
1	1475 and 1600	C=C(Aromatic)

Figure 08: ^{1}H NMR Spectrum of Compound AMA-2

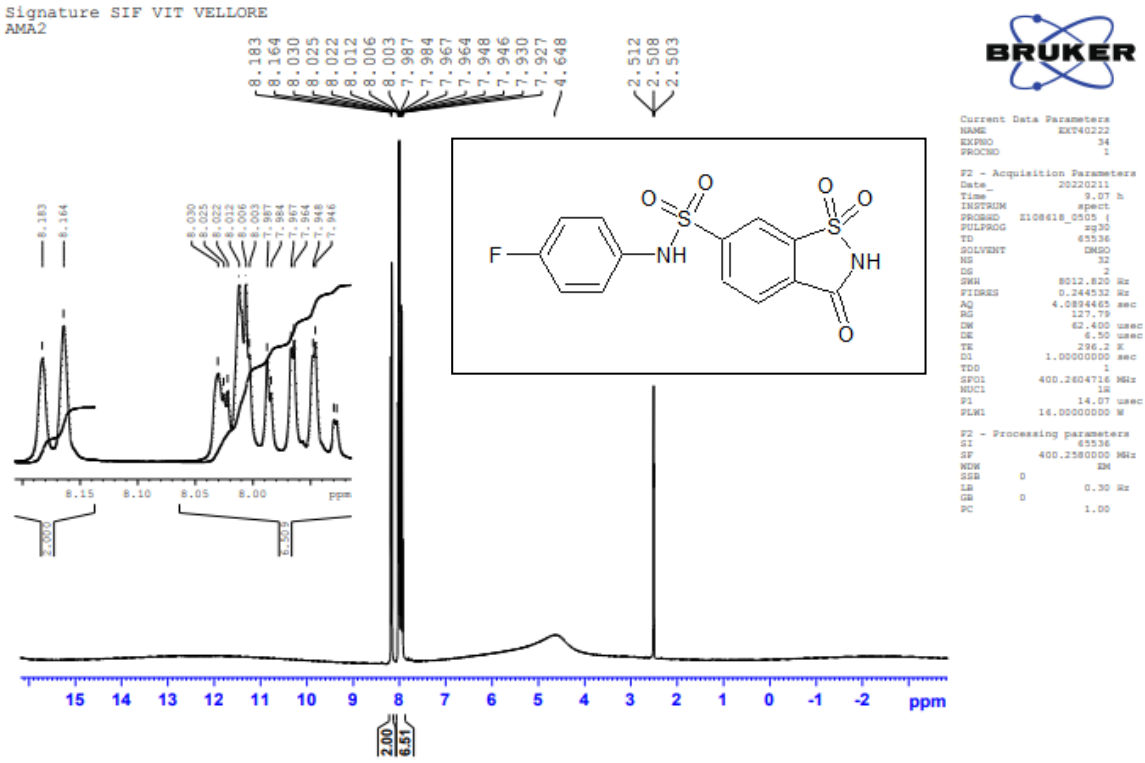


Table 12: Interpretation of 1H NMR Spectrum of Compound AMA-2

S.NO	δ VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
01.	4.648	-NH-	Singlet	2
02.	7.946 – 7.948	Ar-H	Doublet	1
03.	7.964 – 7.967	Ar-H	Doublet	1
04.	7.984 – 7.988	Ar-H	Doublet	1
05.	8.030 – 8.031	Ar-H	Doublet	1
06.	8.164 – 8.183	Ar-H	Doublet	1

Mass Spectrum of Compound AMA-2

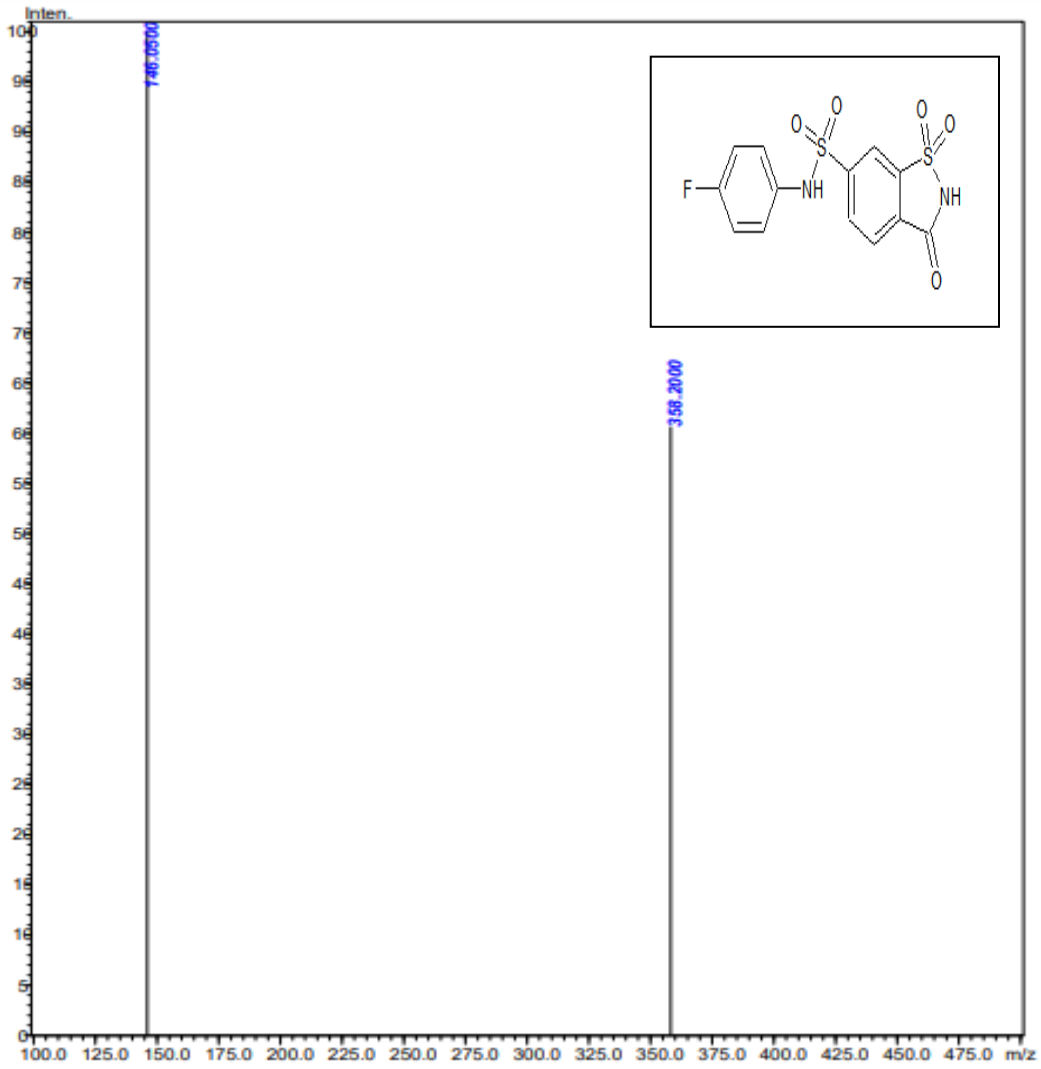
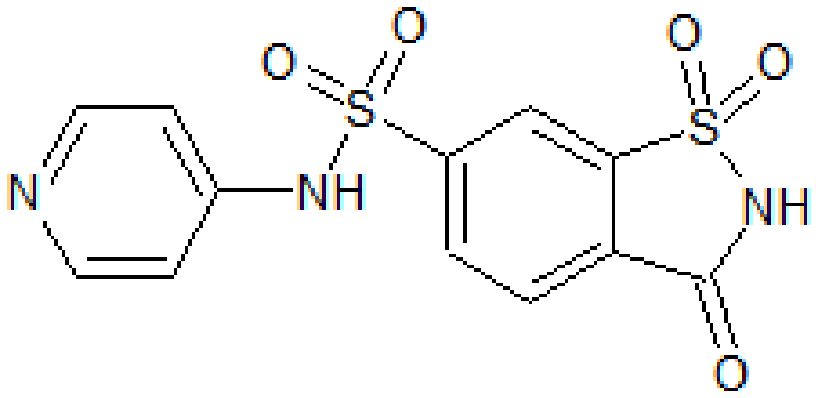


Figure 08: Mass Spectrum of Compound AMA-2

Calculated Molecular Weight: 356.3493632g/mol

Actual Molecular Weight: 358.20000g/mol

COMPOUND AMA-3



IUPAC Name: 1,1,3-trioxo-N-(pyridin-4-yl)-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide

Molecular Formula	$C_{12}H_9N_3O_5S_2$
Formula Weight	339.34696
Composition	C(42.47%) H(2.67%) N(12.38%) O(23.57%) S(18.90%)
Molar Refractivity	76.89 ± 0.4 cm^3
Molar Volume	199.9 ± 3.0 cm^3
Parachor	601.0 ± 6.0 cm^3
Index of Refraction	1.695 ± 0.02
Surface Tension	81.6 ± 3.0 dyne/cm
Density	1.696 ± 0.06 g/cm^3
Polarizability	30.48 ± 0.5 $10^{-24}cm^3$

Figure 09: IR Spectrum of Compound AMA-3

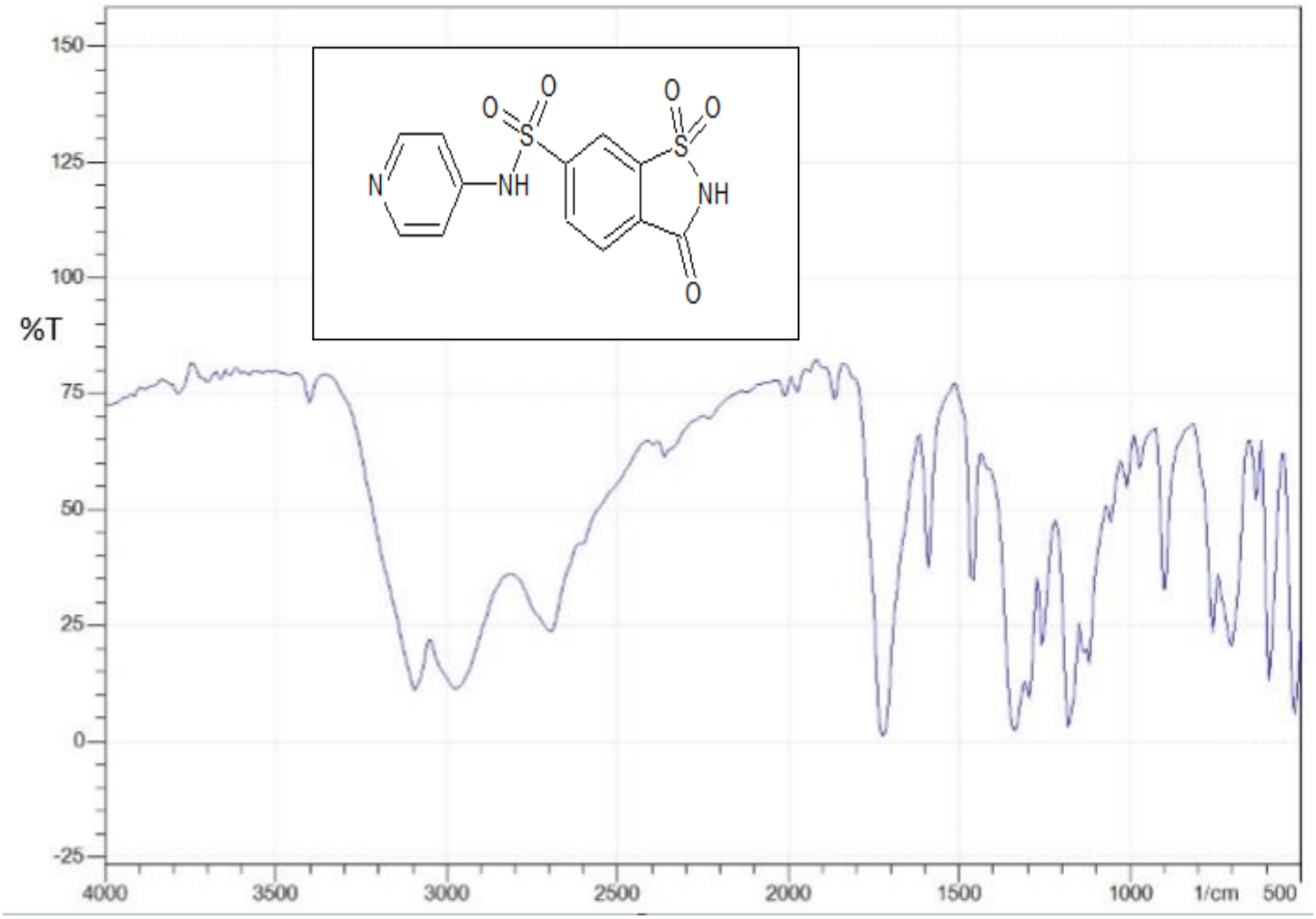


Table 13. Interpretation of IR Spectrum AMA-3

S.No	Wave Number(cm-1)	Functional Group
1	1475 and 1600	C=C(Aromatic)
2	3000	-NH(Secondary amino)
3	1270 and 1340	S=O (Sulphonamide)
4	1725	-C=O(Keto)

Figure 10: ^{1}H NMR Spectrum of Compound AMA-3

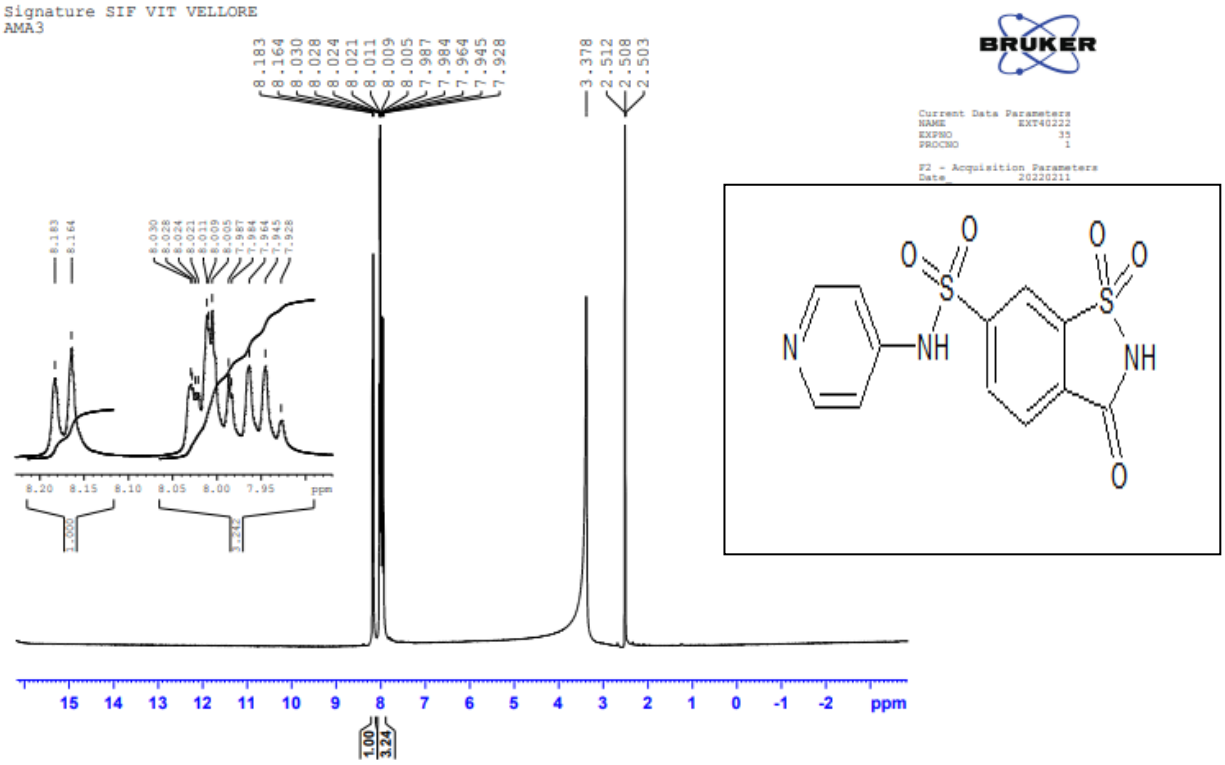


Table 14: Interpretation of 1H NMR Spectrum of Compound AMA-3

S.NO	δ VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
01.	3.378	-NH-	Singlet	2
02.	7.928 – 7.984	Ar-H	Multiplet	2
03.	8.005 – 8.011	Ar-H	Doublet	1
04.	8.021 – 8.024	Ar-H	Doublet	1
05.	8.028 – 8.030	Ar-H	Doublet	1
06.	8.164 – 8.183	Ar-H	Doublet	1

Mass Spectrum of Compound AMA-3

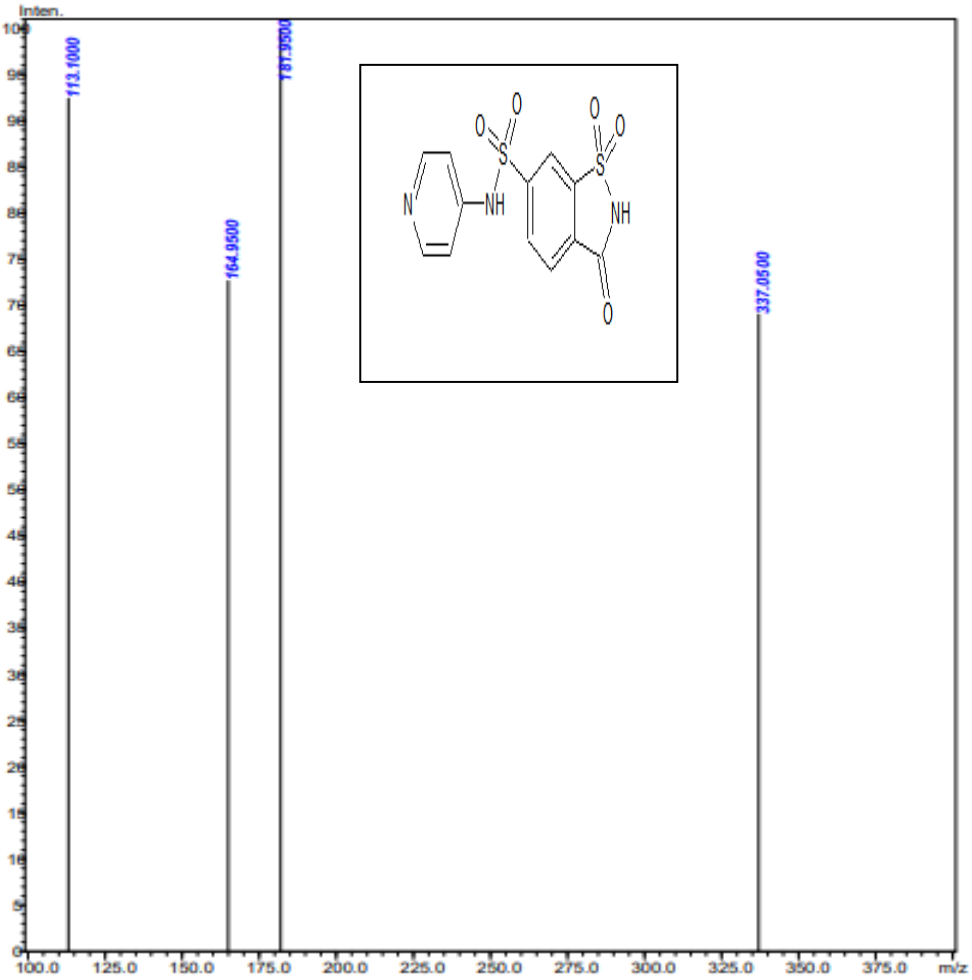
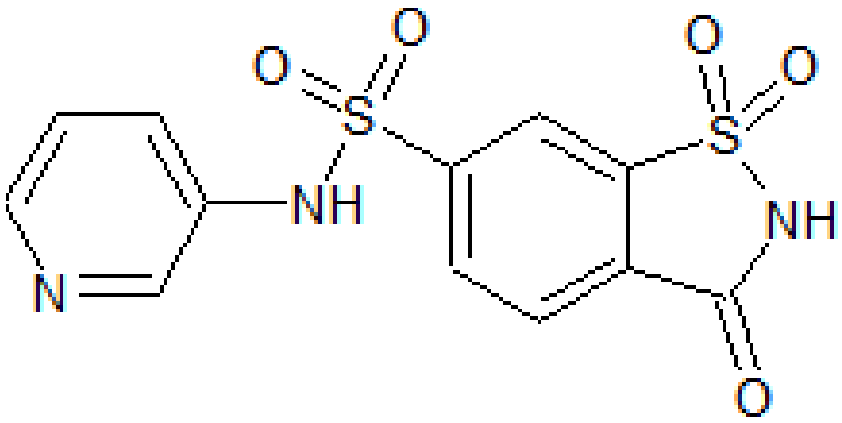


Figure 08: Mass Spectrum of Compound AMA-3

Calculated Molecular Weight: 339.34696g/mol

Actual Molecular Weight: 337.0500g/mol

COMPOUND AMA-4



IUPAC Name: 1,1,3-trioxo-N-(pyridin-3-yl)-2,3-dihydro-

1lambda6,2-benzothiazole-6-sulfonamide

Molecular Formula	$C_{12}H_9N_3O_5S_2$
Formula Weight	339.34696
Composition	C(42.47%) H(2.67%) N(12.38%) O(23.57%) S(18.90%)
Molar Refractivity	76.89 ± 0.4 cm^3
Molar Volume	199.9 ± 3.0 cm^3
Parachor	601.0 ± 6.0 cm^3
Index of Refraction	1.695 ± 0.02
Surface Tension	81.6 ± 3.0 dyne/cm
Density	1.696 ± 0.06 g/cm^3

Figure 11: IR Spectrum of Compound AMA-4

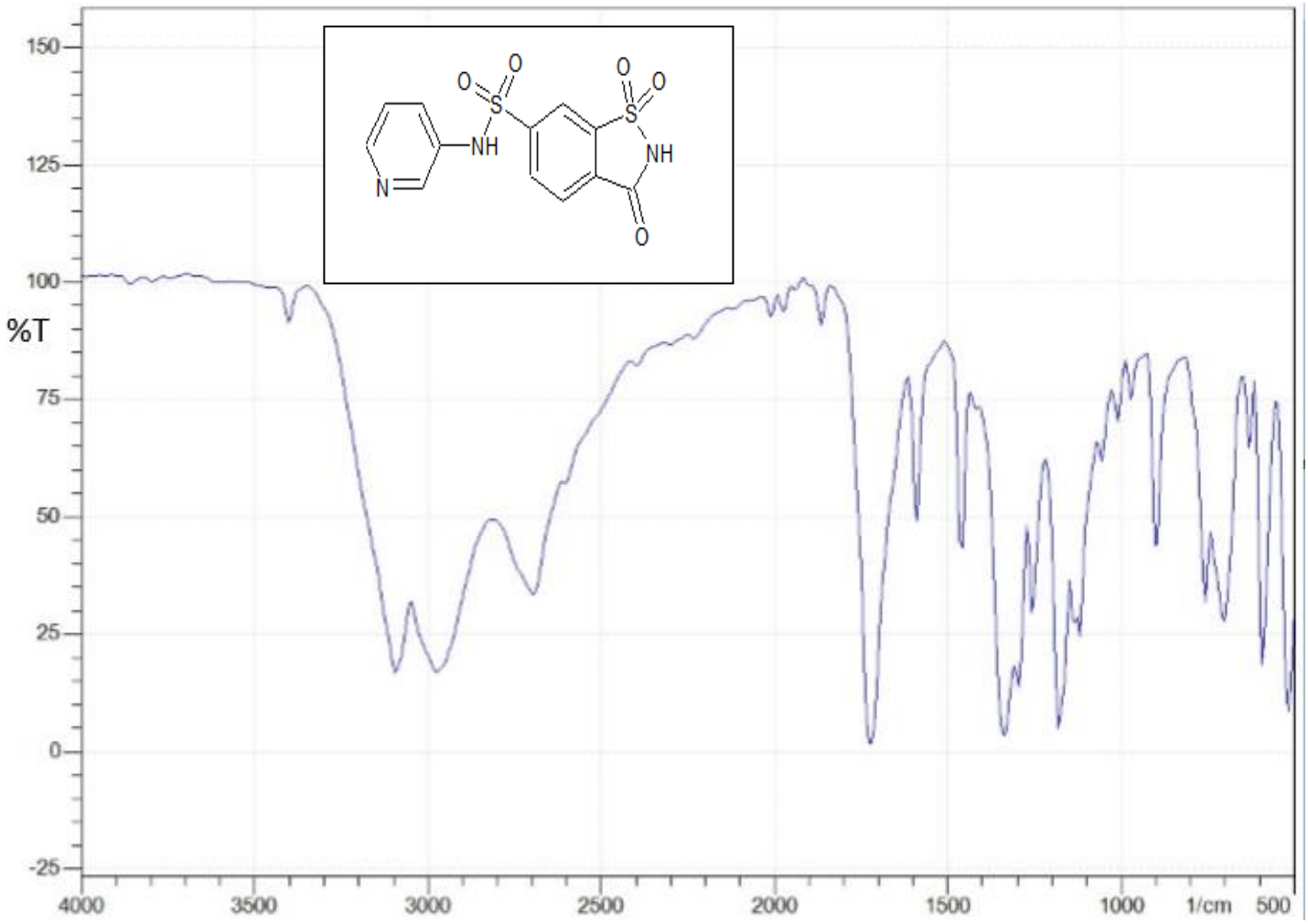


Table 15. Interpretation of IR Spectrum AMA-4

S.No	Wave Number(cm-1)	Functional Group
1	1475 and 1600	C=C(Aromatic)
2	2950	-NH(Secondary amino)
3	1728	-C=O(Keto)
4	1175 and 1340	S=O (Sulphonamide)

Figure 12: ^{1}H NMR Spectrum of Compound AMA-4

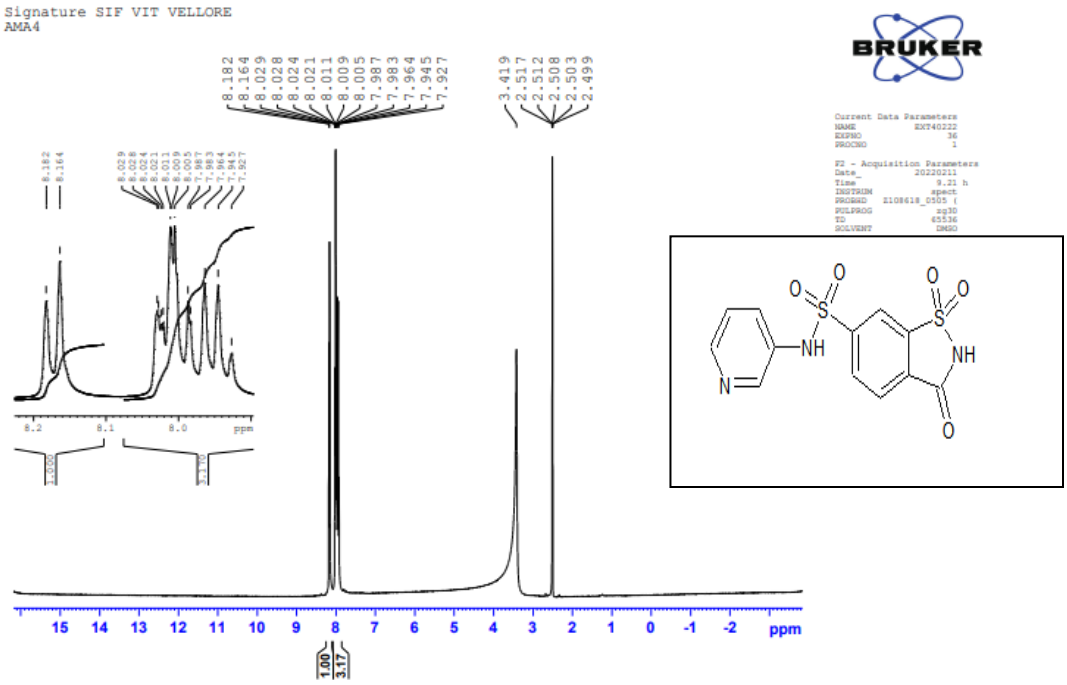


Table 16: Interpretation of 1H NMR Spectrum of Compound AMA-4

S.NO	δ VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
01.	3.419	-NH-	Singlet	2
02.	7.927 – 7.964	Ar-H	Doublet	1
03.	7.983 – 7.987	Ar-H	Doublet	1
04.	8.006 – 8.011	Ar-H	Triplet	2
05.	8.021 – 8.024	Ar-H	Doublet	1
06.	8.028 – 8.029	Ar-H	Doublet	1
07.	8.164 – 8.182	Ar-H	Doublet	1

Mass Spectrum of Compound AMA-4

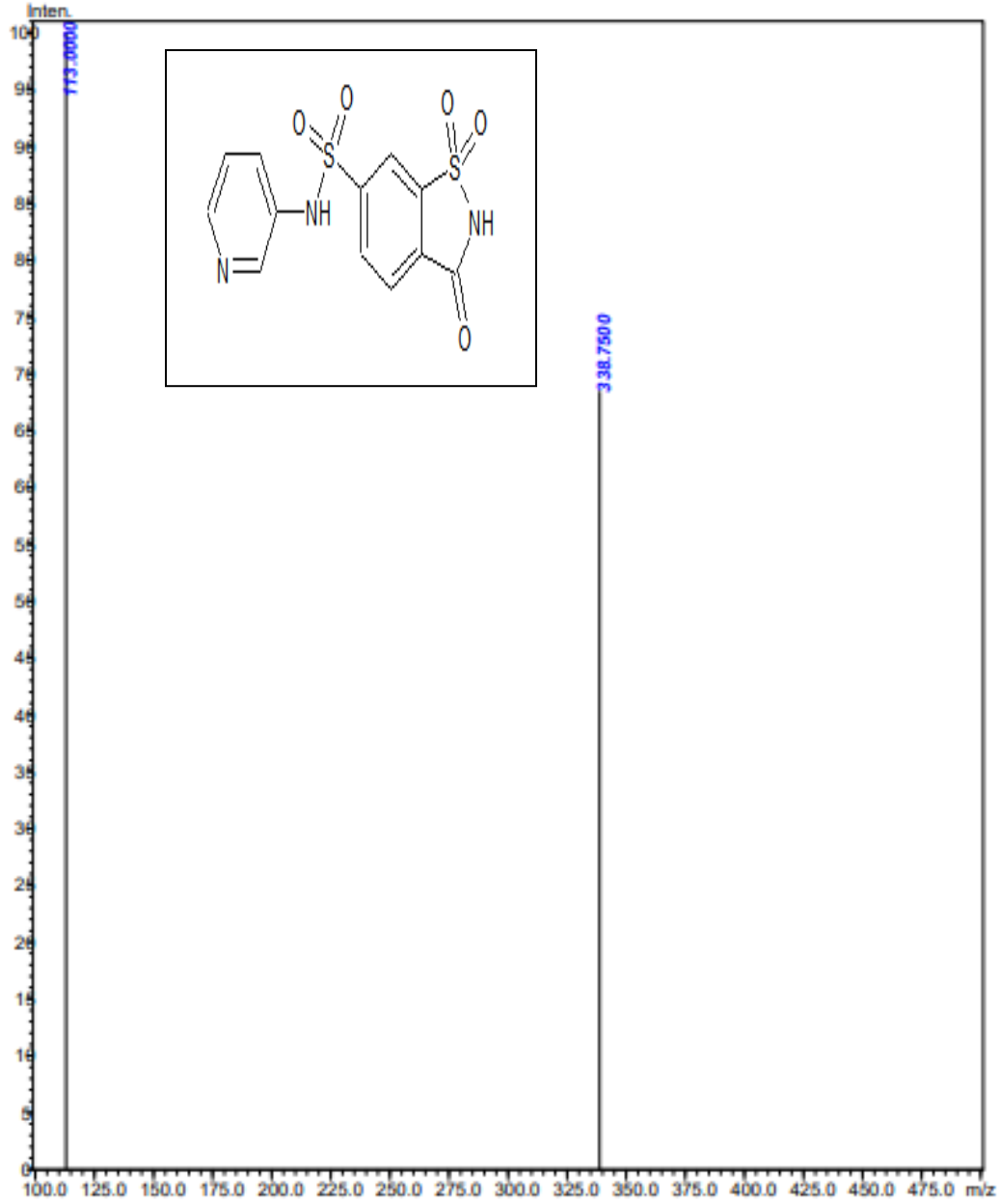
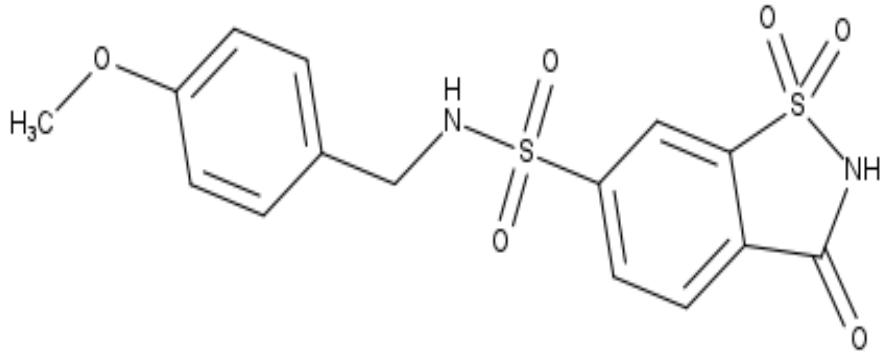


Figure 08: Mass Spectrum of Compound AMA-4

Calculated Molecular Weight: 339.34696g/mol

Actual Molecular Weight: 338.7500g/mol

COMPOUND AMA-5



IUPAC Name: N-[(4-methoxyphenyl)methyl]-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide

Molecular Formula	:	$C_{15}H_{14}N_2O_6S_2$
Formula Weight	:	382.41146
Composition	:	C(47.11%) H(3.69%) N(7.33%) O(25.10%) (16.77%)
Molar Refractivity	:	90.09 ± 0.4 cm^3
Molar Volume	:	252.9 ± 3.0 cm^3
Parachor	:	704.1 ± 6.0 cm^3
Index of Refraction	:	1.630 ± 0.02
Surface Tension	:	60.0 ± 3.0 dyne/cm
Density	:	1.511 ± 0.06 g/cm^3
Polarizability	:	35.71 ± 0.5 $10^{-24}cm^3$

Figure 13: IR Spectrum of Compound AMA-5

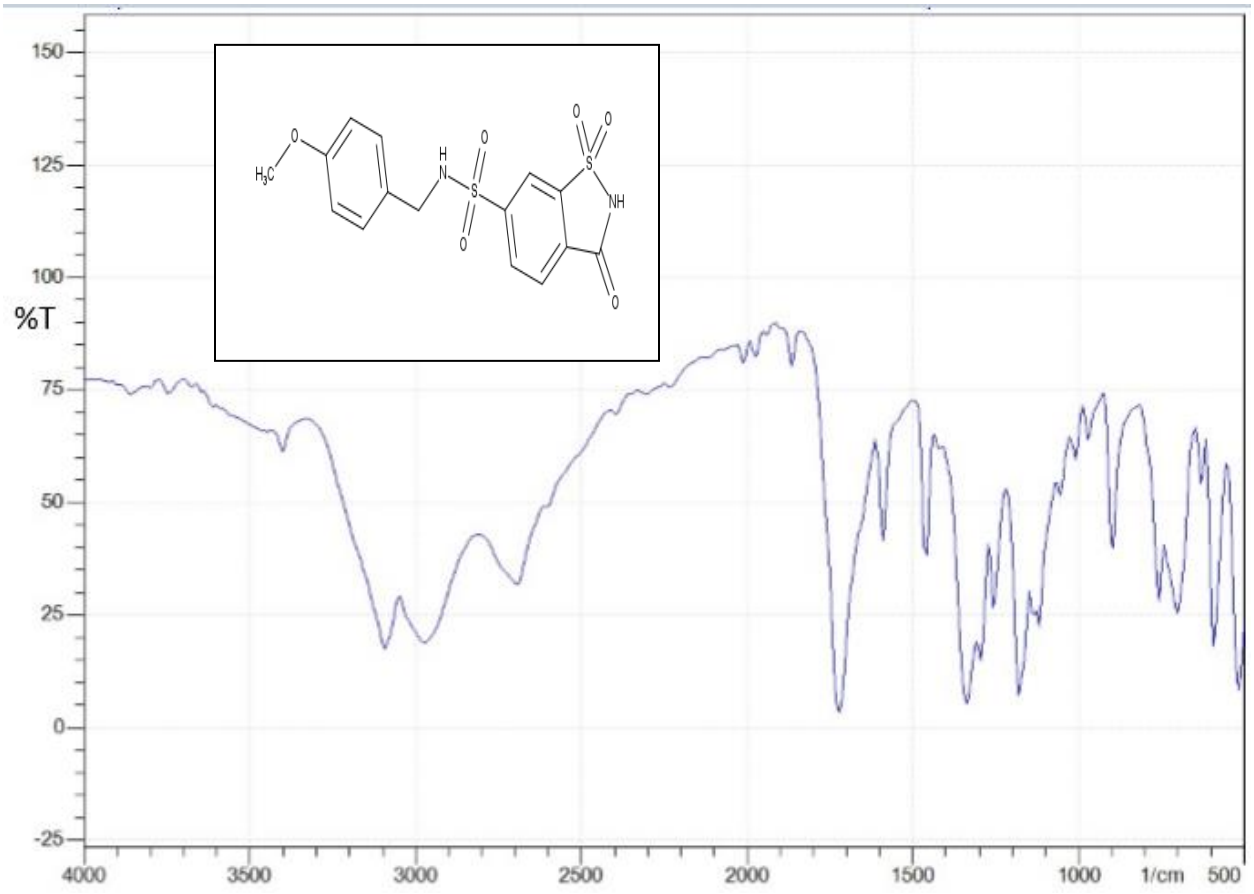


Table 17. Interpretation of IR Spectrum AMA-5

S.No	Wave Number(cm-1)	Functional Group
1	3100 and 2970	-NH(Secondary amino)
2	2670	-CH(Methylene)
3	1315 and 1170	S=O (Sulphonamide)
4	1725	-C=O(Keto)

Figure 14: ^{1}H NMR Spectrum of Compound AMA-5

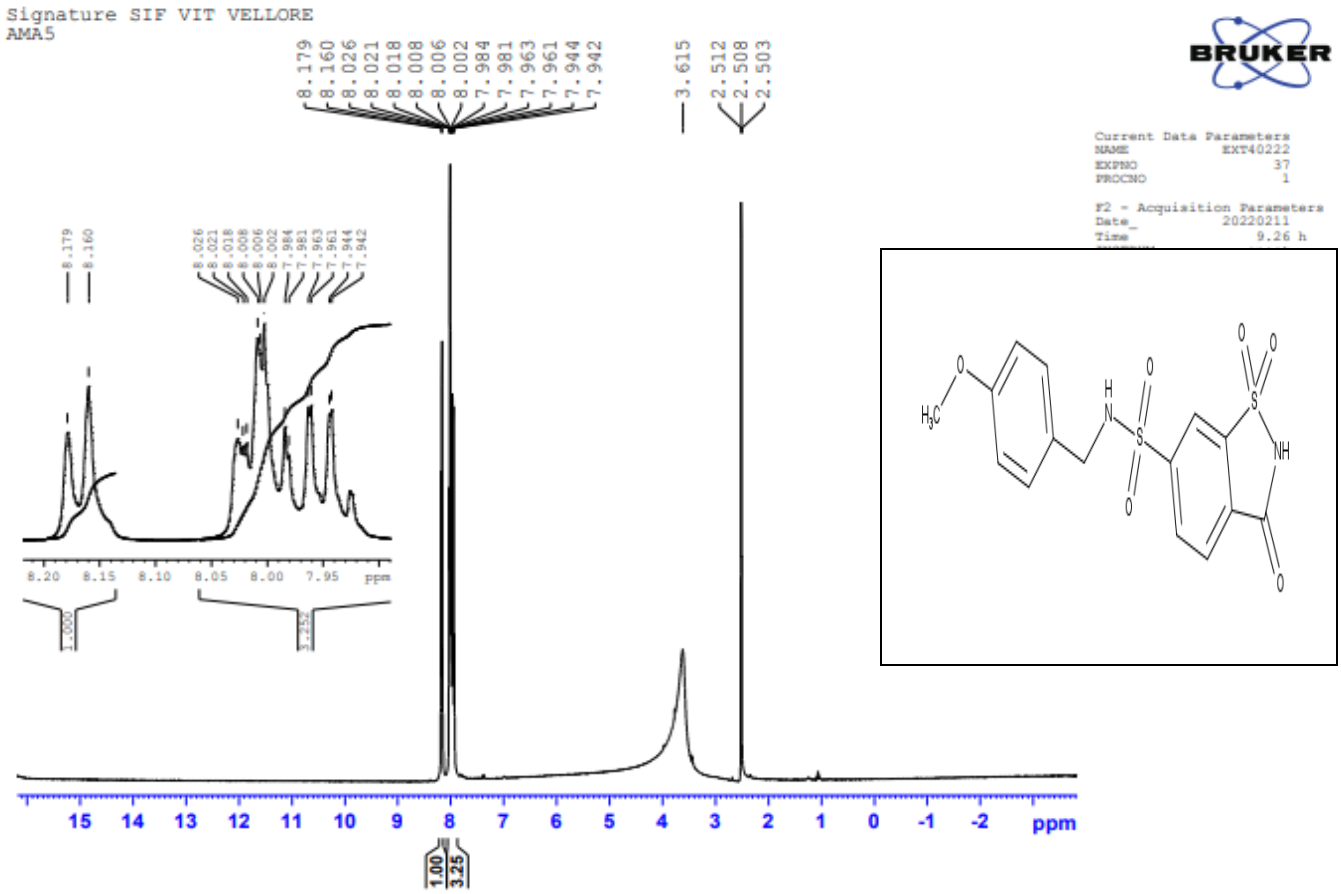


Table 18 : Interpretation of 1H NMR Spectrum of Compound AMA-5

S.NO	δ VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
01.	3.615	-NH-	Singlet	2
02.	7.942 – 7.944	Ar-H	Doublet	1
03.	7.981 – 7.984	Ar-H	Doublet	1
04.	8.002 – 8.008	Ar-H	Doublet	1
05.	8.018 – 8.026	Ar-H	Doublet	1

Mass Spectrum of Compound AMA-5

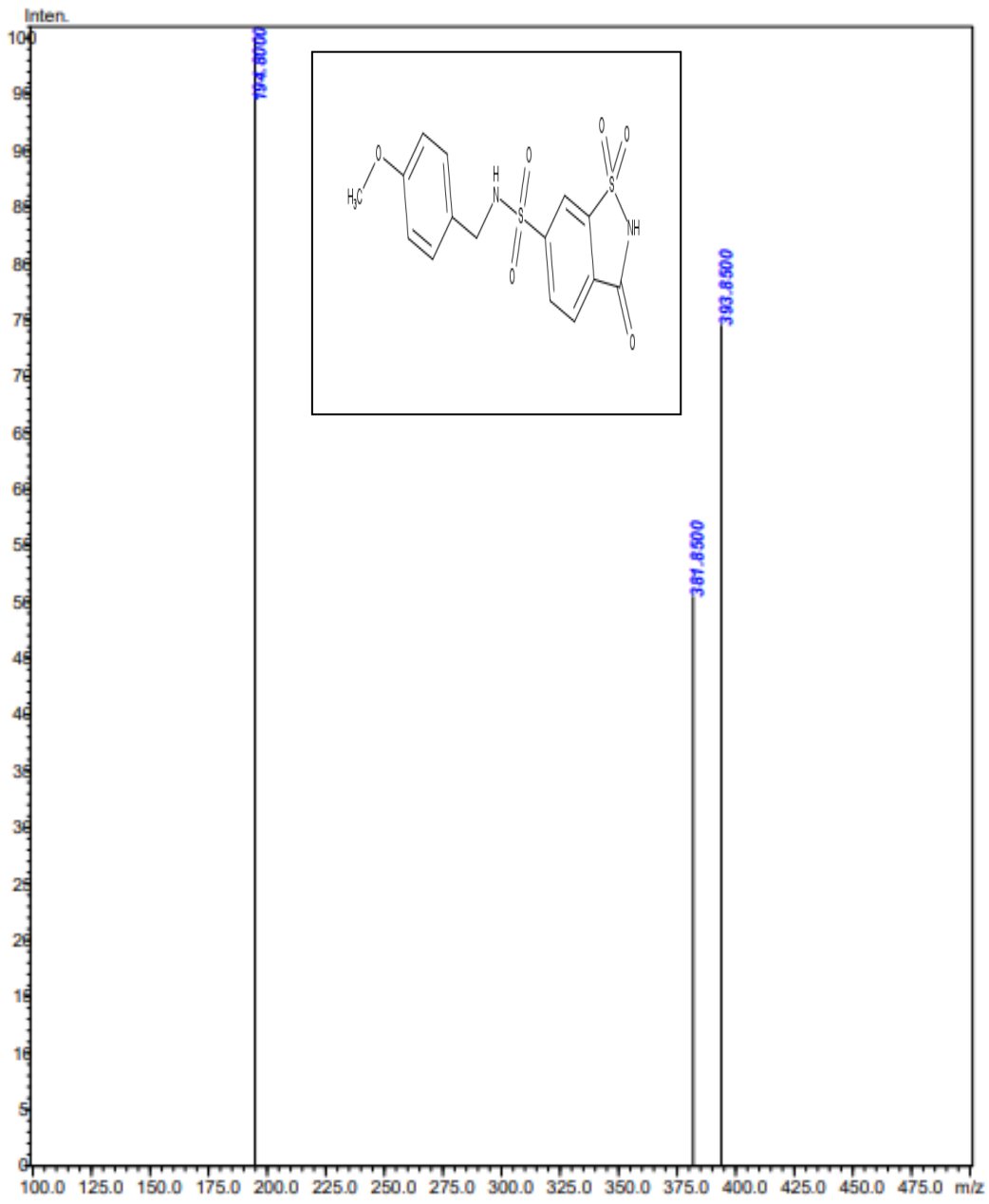
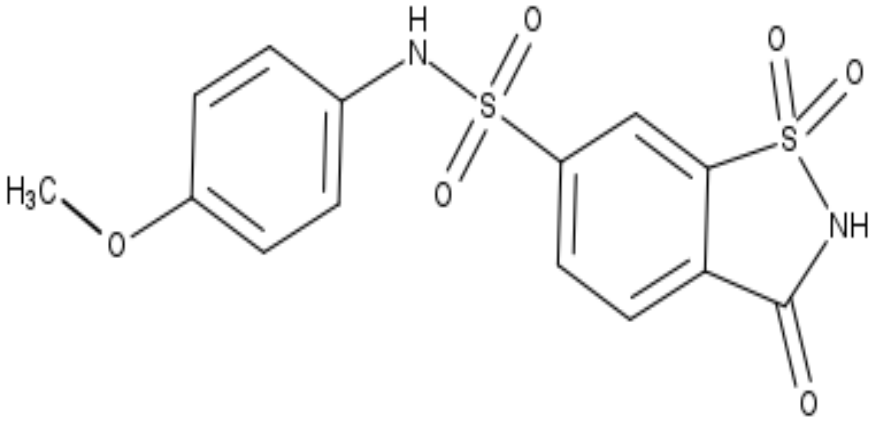


Figure 08: Mass Spectrum of Compound AMA-5

Calculated Molecular Weight: 382.41146g/mol

Actual Molecular Weight: 381.8500g/mol

COMPOUND AMA-6



IUPAC Name: N-(4-methoxyphenyl)-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide

Molecular Formula	$C_{14}H_{12}N_2O_6S_2$
Formula Weight	368.38488
Composition	C(45.65%) H(3.28%) N(7.6%)O(26%) S(17.41%)
Molar Refractivity	85.46 ± 0.4 cm^3
Molar Volume	230.7 ± 3.0 cm^3
Parachor	664.0 ± 6.0 cm^3
Index of Refraction	1.662 ± 0.02
Surface Tension	68.6 ± 3.dyne/cm
Density	1.596 ± 0.06 g/cm^3

Figure 15: IR Spectrum of Compound AMA-6

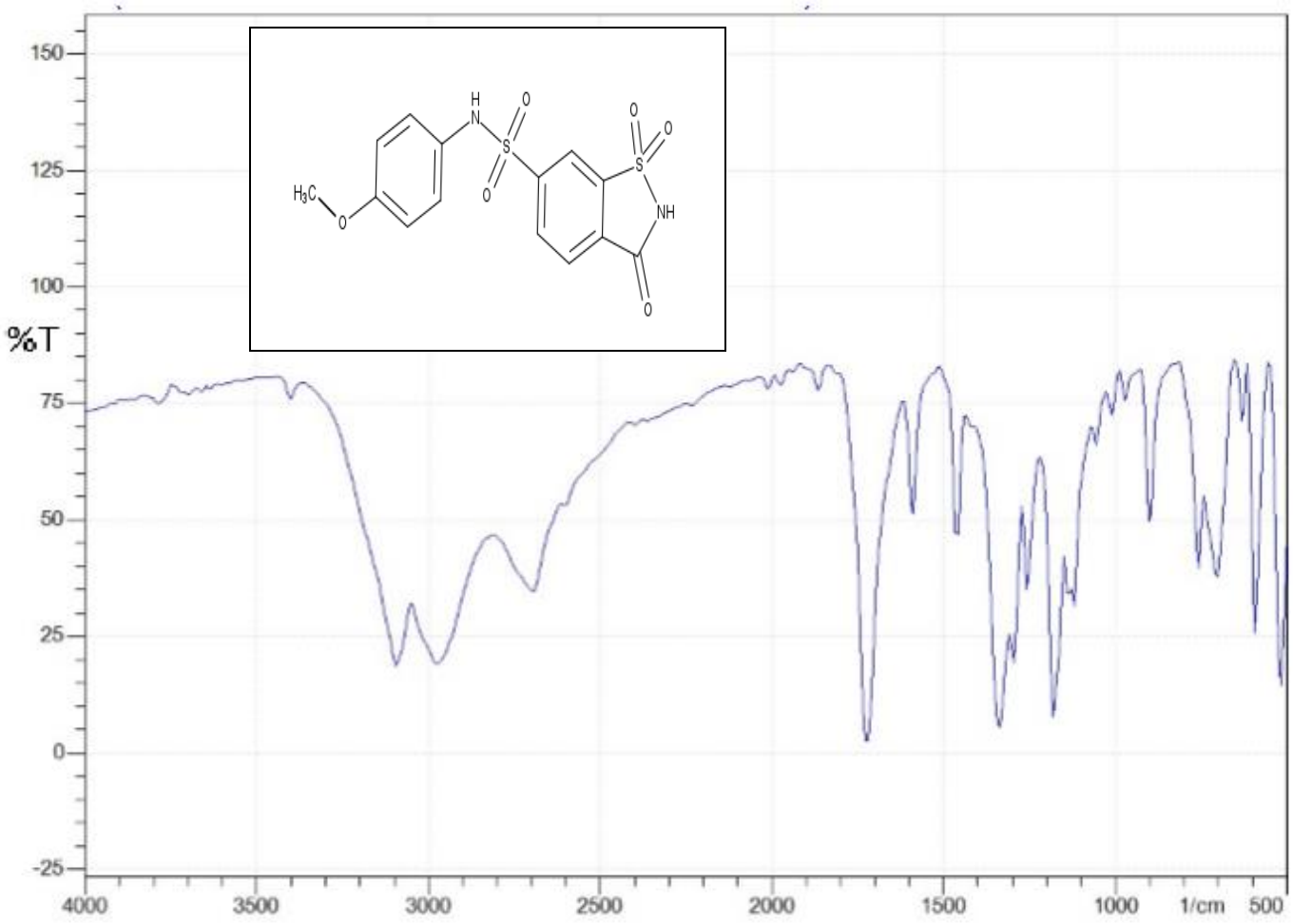


Table 19. Interpretation of IR Spectrum AMA-6

S.No	Wave Number(cm-1)	Functional Group
1	1475 and 1600	C=C(Aromatic)
2	3110 and 2985	-NH(Secondary amino)
3	1190 and 1290	S=O (Sulphonamide)
4	1725	-C=O(Keto)

Figure 16: ^{1}H NMR Spectrum of Compound AMA-6

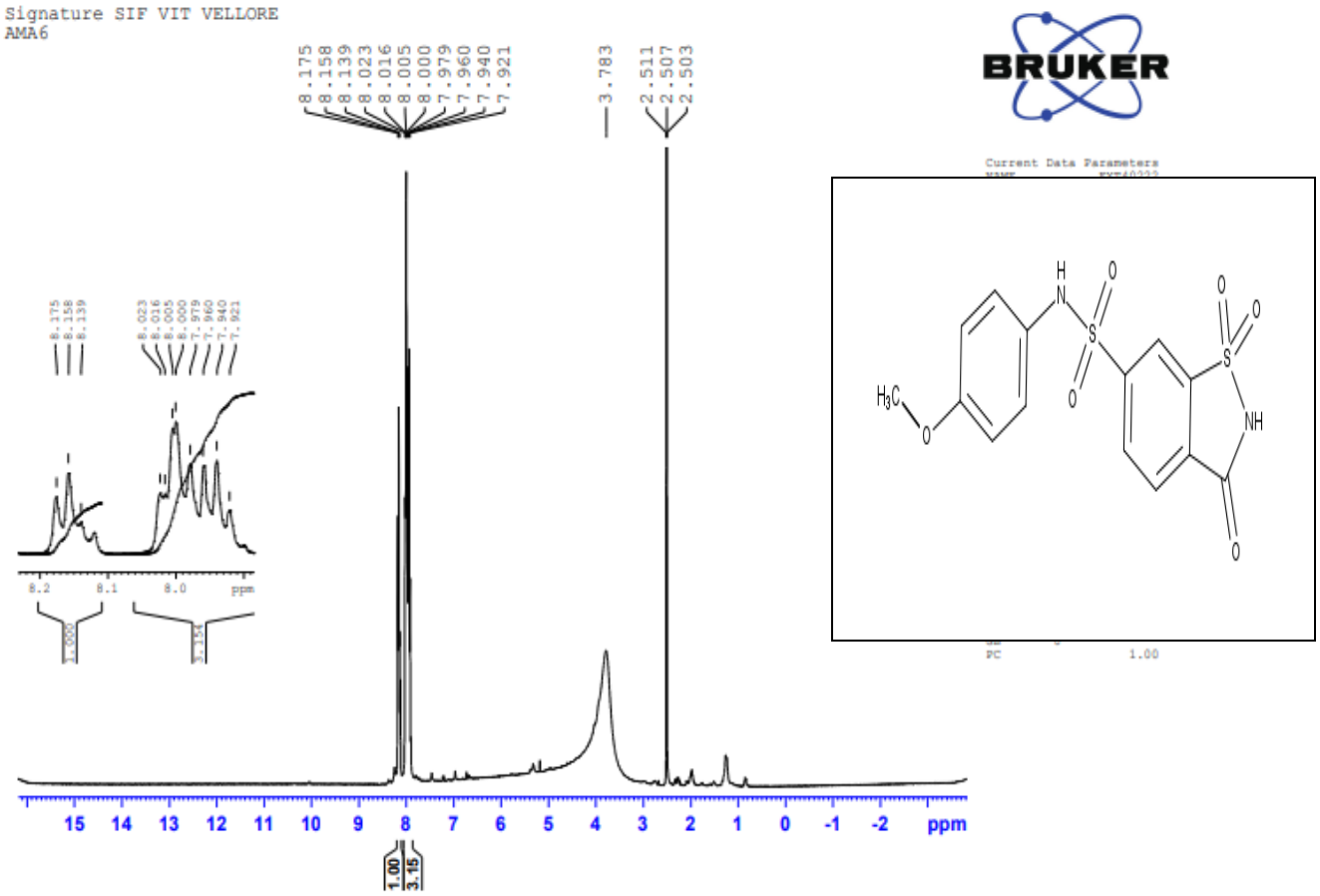


Table 20: Interpretation of 1H NMR Spectrum of Compound AMA-6

S.NO	δ VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
01.	3.783	-NH-	Singlet	2
02.	7.921 – 7.979	Ar-H	Doublet	1
03.	8.000 – 8.005	Ar-H	Doublet	1
04.	8.016 – 8.023	Ar-H	Doublet	1
05.	8.139 – 8.175	Ar-H	Doublet	1

Mass Spectrum of Compound AMA-6

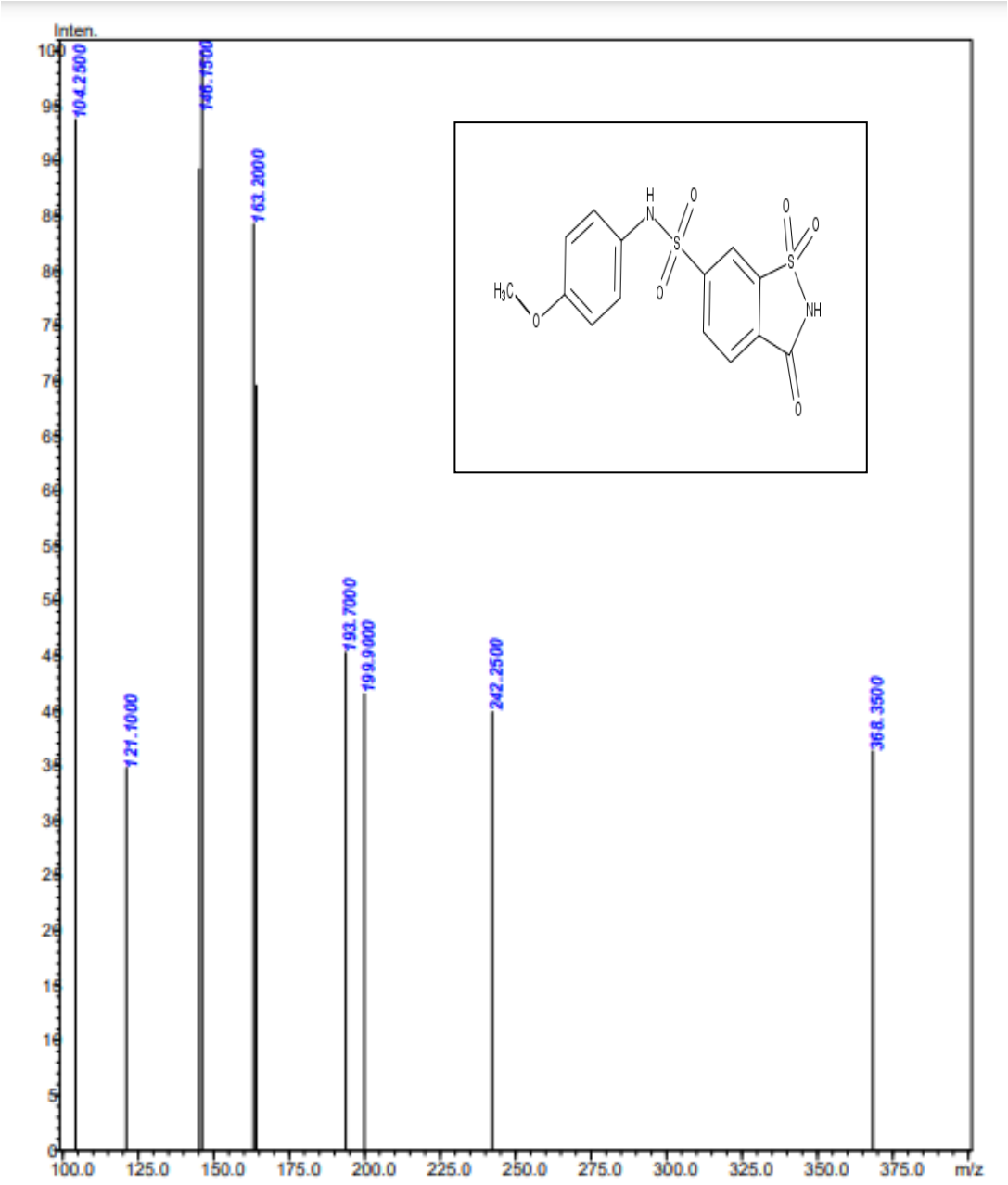
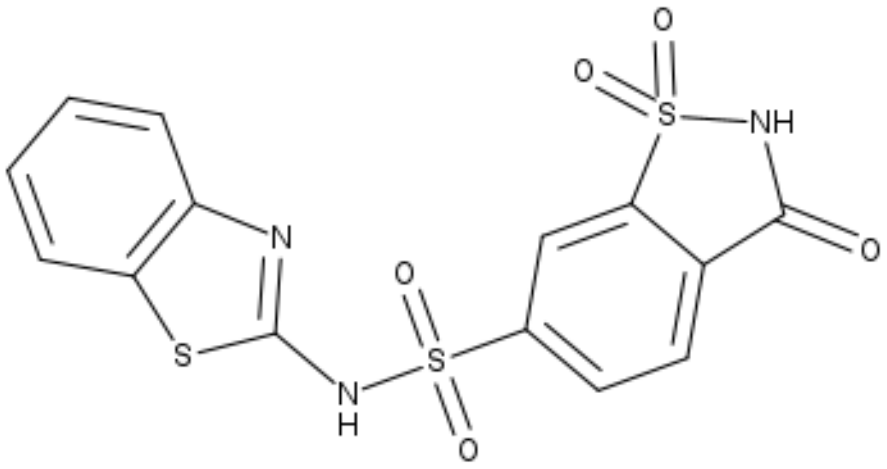


Figure 08: Mass Spectrum of Compound AMA-6

Calculated Molecular Weight: 368.38488g/mol

Actual Molecular Weight: 368.3500g/mol

COMPOUND AMA-7



IUPAC Name: N-(1,3-benzothiazol-2-yl)-1,1,3-trioxo-2,3-dihydro-1lambda6,2-benzothiazole-6-sulfonamide

Molecular Formula	$C_{14}H_9N_3O_5S_3$
Formula Weight	395.43336
Composition	C(42.52%) H(2.29%) N(10.63%) O(20.23%) S(24.33%)
Molar Refractivity	92.59 ± 0.4 cm^3
Molar Volume	223.5 ± 3.0 cm^3
Parachor	690.7 ± 6.0 cm^3
Index of Refraction	1.766 ± 0.02
Surface Tension	91.1 ± 3.0 dyne/cm
Density	1.768 ± 0.06 g/cm^3
Dielectric Constant	Not available

Figure 17: IR Spectrum of Compound AMA-7

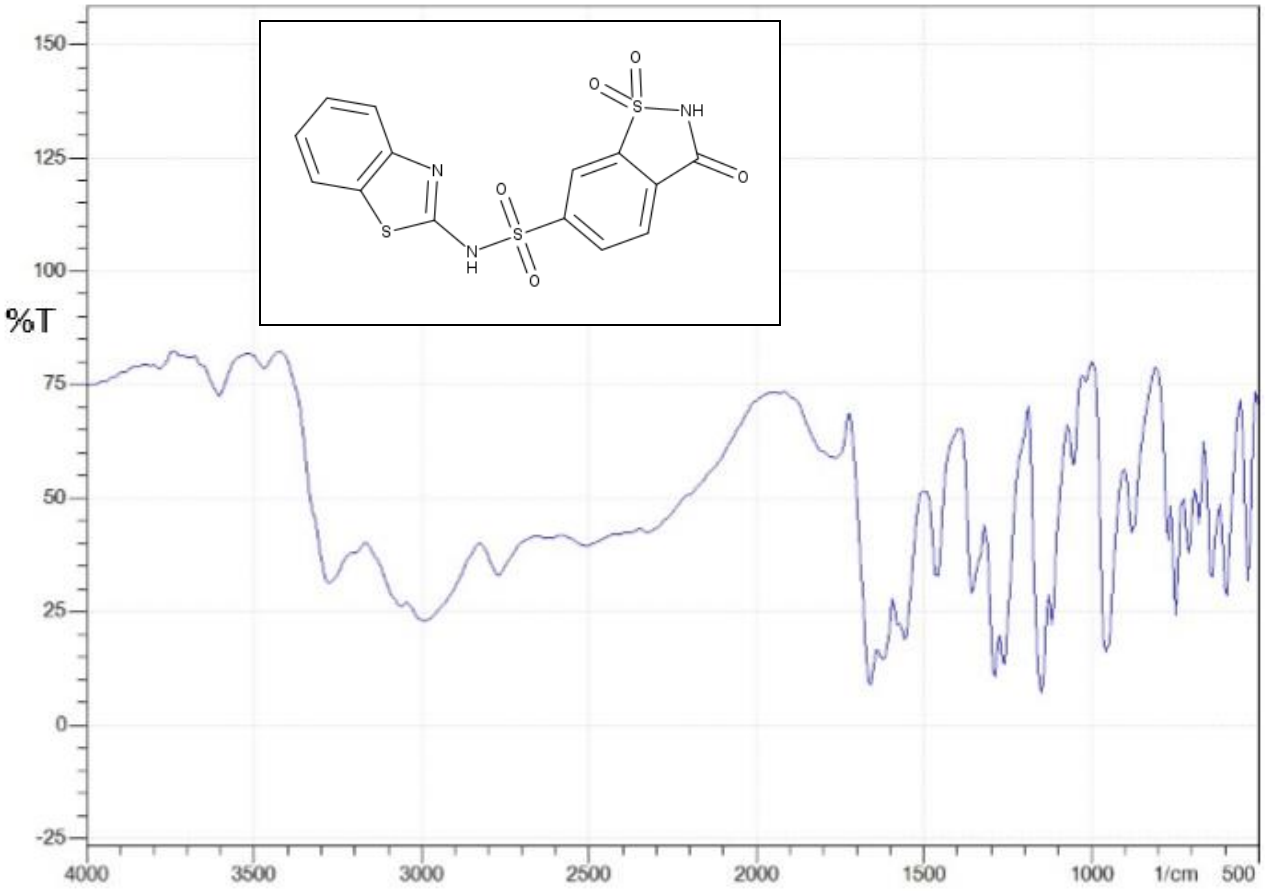


Table 21. Interpretation of IR Spectrum AMA-7

S.No	Wave Number(cm-1)	Functional Group
1	3110 and 3000	-NH(Secondary amino)
1	2790	-CH(Alkane)
2	1640	C=N (Imine)
3	1110 and 1280	S=O (Sulphonamide)
4	1725	-C=O(Keto)

PROTON NMR SPECTRUM OF AMA-7

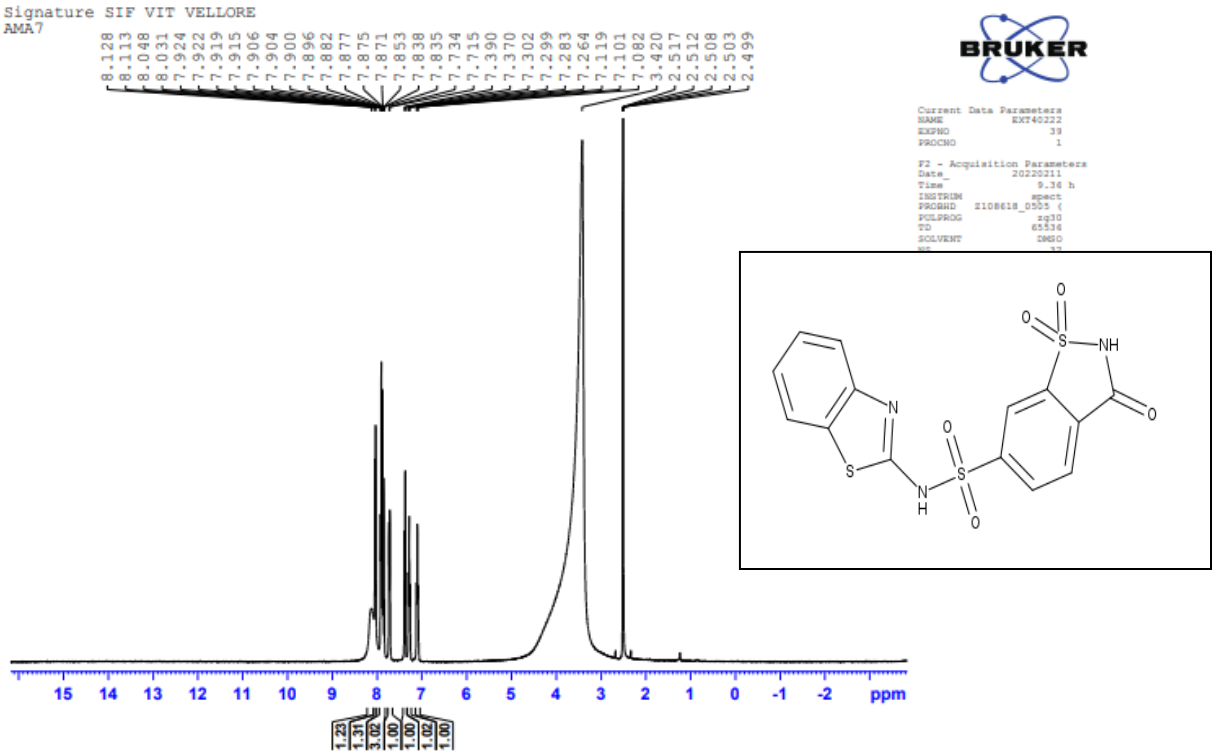


Figure 18: ^{1}H NMR Spectrum of Compound AMA-7

Table 22: Interpretation of 1H NMR Spectrum of Compound AMA-7

S.NO	δ VALUE (PPM)	NATURE OF PROTON	NATURE OF PEAK	NUMBER OF PROTON
01.	3.420	-NH-	Singlet	2
02.	7.715 – 7.734	Ar-H	Doublet	1
03.	7.835 – 7.853	Ar-H	Multiplet	1
04.	7.871 – 7.882	Ar-H	Multiplet	1
05.	7.896 – 7.900	Ar-H	Doublet	1
06.	7.904 – 7.906	Ar-H	Doublet	1

Mass Spectrum of Compound AMA-7

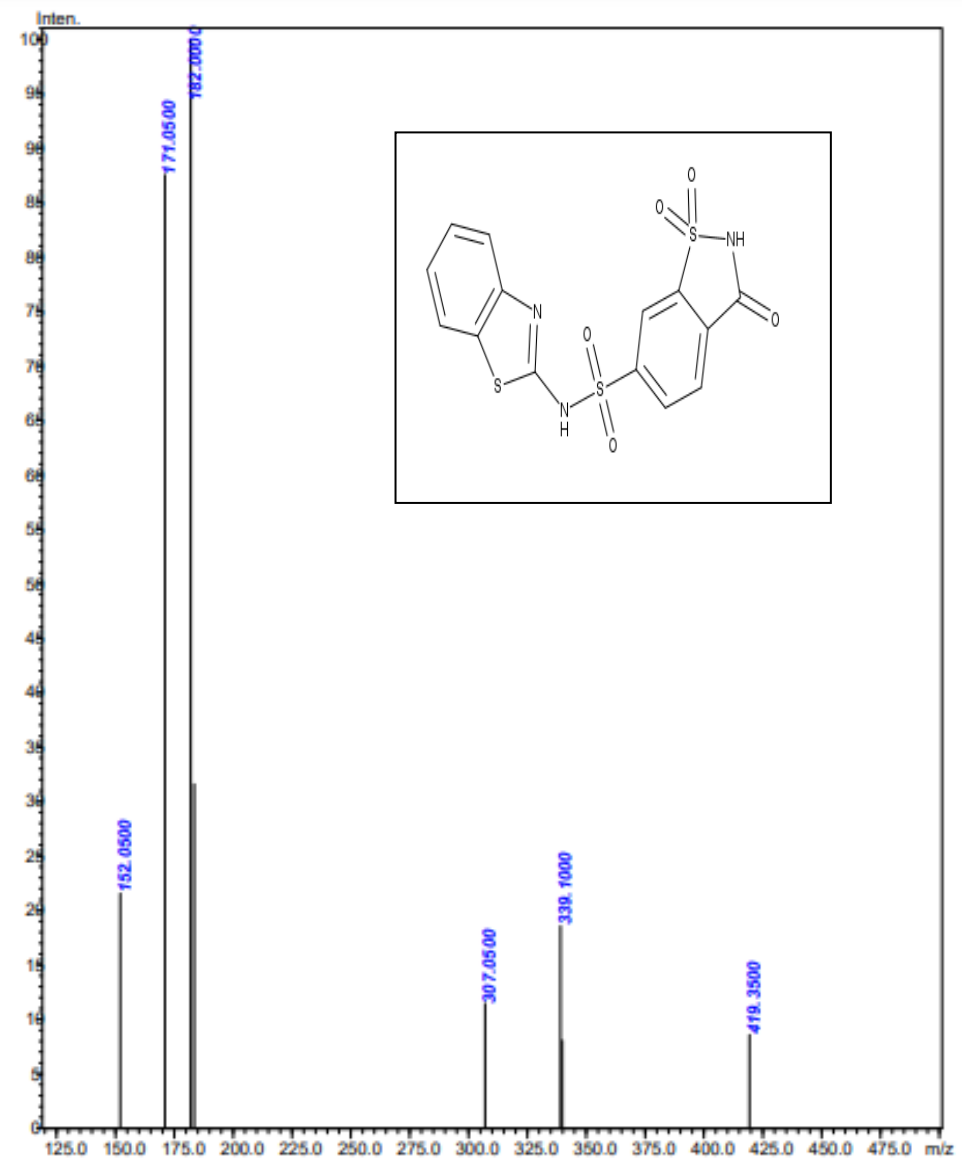


Figure 08: Mass Spectrum of Compound AMA-7

Calculated Molecular Weight: 395.43336g/mol

Actual Molecular Weight: 396.3500g/mol

- All the 5 synthesized compounds had a different Rf value from this respective reactant molecules and showed a single spot in the thin layer chromatogram.
- They exhibited a sharp melting point.
- In the IR spectrum, absorptions corresponding to the relevant functional groups were seen.
- From the NMR spectrum, numbers of protons present in the molecular structure were elucidated and the structure of the compound was confirmed.

5. RESULTS OF ANTI-TUBERCULAR ACTIVITY:

The recrystallized pure compounds were evaluated for their Anti-tubercular activity by in vitro Microplate Alamar Blue Assay (MABA). 100 – 0.8 µg/ml dilutions of the compounds were made using Dimethyl sulfoxide (DMSO).

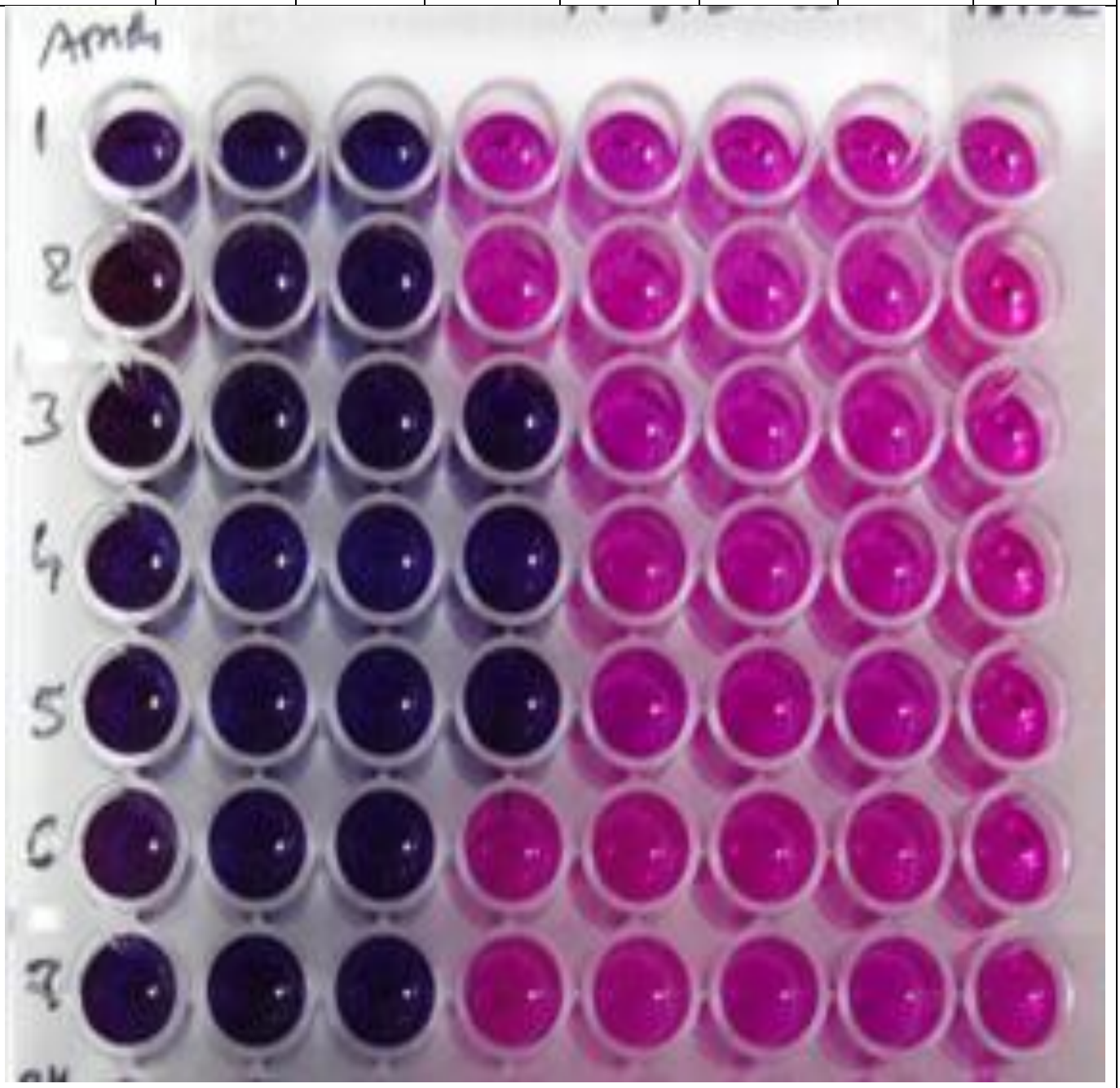
Table 23: Data of MABA results of the compounds GS1 – GS5 (100–0.8 µg/ml)

Sl No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 g/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	AMA-1	S	S	S	R	R	R	R	R
02	AMA-2	S	S	S	R	R	R	R	R
03	AMA-3	S	S	S	S	R	R	R	R
04	AMA-4	S	S	S	S	R	R	R	R
05	AMA-5	S	S	S	S	R	R	R	R
06	AMA-6	S	S	S	R	R	R	R	R
07	AMA-7	S	S	S	R	R	R	R	R

S – SENSITIVE

R – RESISTANT

Table 24: Visualization of MABA results of the compounds AMA-1 – AMA-7 (100 –0.8µg/ml)

Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
AMA-1								
AMA-2								
AMA-3								
AMA-4								
AMA-5								
AMA-6								
AMA-7								

S – Sensitive **R- Resistant**

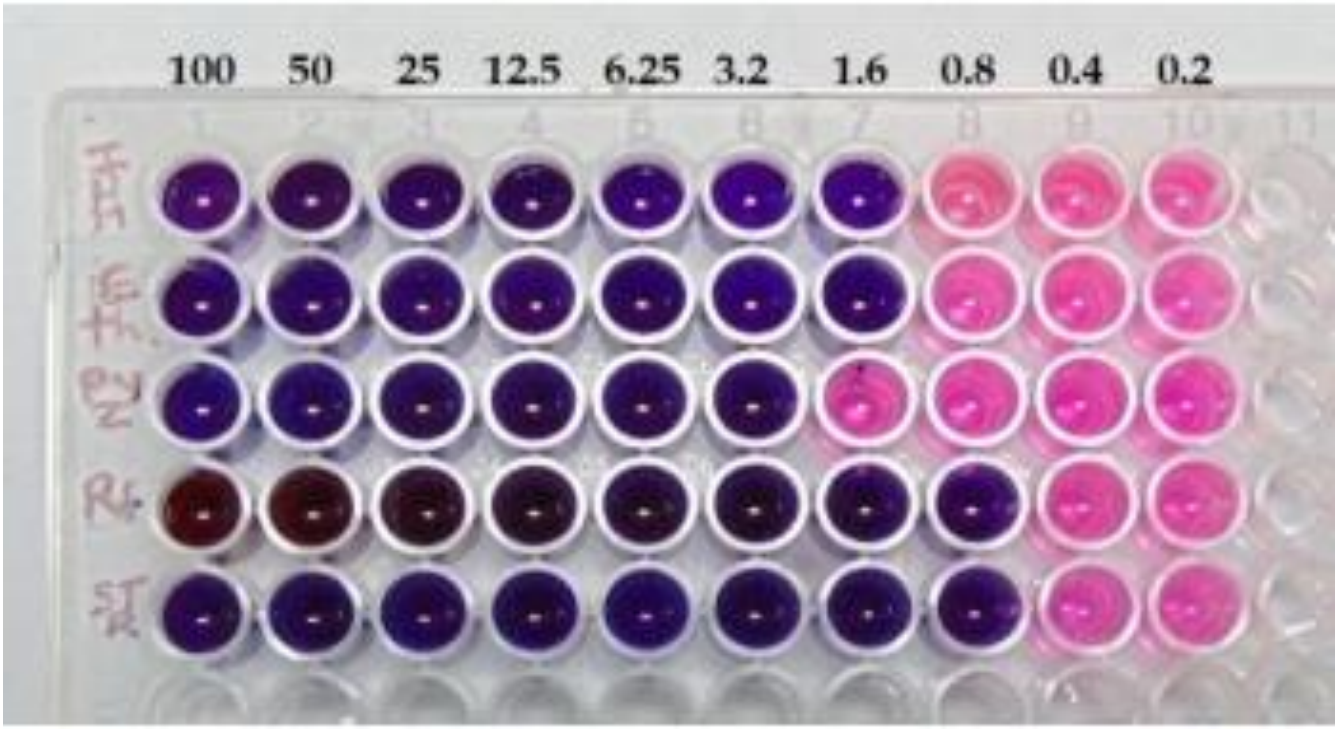
MINIMUM INHIBITORY CONCENTRATION (MIC VALUES) OF STANDARD ANTI-TUBERCULAR DRUGS:

Standard Strain used: Mycobacteria tuberculosis (Vaccine strain, H37 RV strain): ATCC No- 27294.

Standard values for the Anti-Tb test which was performed.

Isoniazid – 1.6 µg/ml
Ethambutol – 1.6 µg/ml
Pyrazinamide -- 3.125µg/ml
Rifampicin – 0.8 µg/ml
Streptomycin -- 0.8µg/ml

Table 25 : Visualization of MABA results of the Standard Anti-tubercular drugs



Note:

S - Sensitive

R- Resistant

DISCUSSION

From literature review we chosen the Saccharin derivatives to evaluate the Anti-tubercular activity. The synthesized molecule AMA-1, AMA-2, AMA-3, AMA-4, AMA-5, AMA-6 and AMA-7 possesses Saccharin nucleus. According to the results of this work presence of pyridine derivatives of 3,4,5 Saccharin nucleus brings active compounds. In Saccharin nucleus 5^{th} position of sulphonamide derivative formation with 7 aromatic amine. Different aromatic amine derivatives with saccharin nucleus shows different activity. Also para-methoxy benzylamine derivative of saccharin derivative brings good compounds.

SUMMARY AND CONCLUSION

SUMMARY

- Glutamine synthetase I is a vital enzyme present in the cell wall of Mycobacterium tuberculosis H37Rv. It belongs to the Ligase family was chosen after the review of literature.
- A database of 500 molecules with high prospects of inhibiting the target Glutamine synthetase I were carefully chosen by making changes to the known hit molecules, here the saccharin nucleus was chosen.
- The designed molecules were docked against the target chosen using AutoDock 4® TOOLS 1.5.6 software.
- Seven molecules (AMA-1, AMA-2, AMA-3, AMA-4, AMA-5, AMA-6 and AMA-7) with good docking score [lower binding energy] and interactions were shortlisted for synthesis.
- The selected molecules were subjected to toxicity prediction assessment by OSIRIS® property explorer developed by Acetilon Pharmaceuticals limited which is available online. The results are color coded as green color which predicts the drug likeness and possibly better activity.
- The reaction condition were optimized, synthesized and labelled as BOB, CIN, NIB, OHB, PHB.
- The characterization of the synthesized compounds was done using TLC, Melting point Infra-red, Mass spectrometric methods [LC-MS] and Nuclear Magnetic Resonance [H1 NMR] spectroscopy methods.
- All the Synthesized compounds exhibited molecular ion peak (M+) of varying intensities.
- The final pure compounds were screened for Anti-mycobacterial activity by in vitro method called Microplate Alamar Blue Assay [MABA].
- The synthesized compounds showed sensitivity [Minimum inhibitory concentration] at 12.5mcg/ml. The standard drugs Isoniazide, Ethambutol, Pyrazinamide, rifampicin and and Streptomycin exhibited anti-mycobacterial activity at 1.6mcg/ml, 1.6mcg/ml, 3.125mcg/ml, 0.8mcg/ml and 0.8mcg/ml concentrations respectively. This indicates that the synthesized compounds are as Potent as the standard drugs.
- Based on the MABA report, Acute Oral Toxicity study were performed and observed that the administration of the synthesized molecules by oral route upto 2000mg/kg/b.w is safe.

CONCLUSION

The current research work concludes that,

- Glutamine Synthetase I is one of the critical enzymes essential for the growth and survival of Mycobacterium tuberculosis.
- Molecules designed to inhibit the target enzyme, showed better *in vitro* antitubercular activity in Microplate Alamar Blue Assay (MABA). There is a correlation between the in silico molecular docking scores and *In vitro* antitubercular activity results.
- The compounds AMA-3, AMA-4 & AMA-5 have the docking scores of -8.59, -8.68 & -8.05 Kcal/mol and were able to inhibit the growth of the organism even at the concentration of 12.5µg/ml. The Anti-tubercular activity of the compounds was found to be less superior than the Standard Anti-tubercular agents.
- The molecules were also docked against the other critical enzymes of Mycobacterium tuberculosis. The obtained docking scores showed that the molecules were able to inhibit multiple enzymes which are necessary for the survival of the organism.
- Therefore, further refinement of the molecular structure of the synthesized compounds will expected to yield promising drug candidates against the deadly disease.

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BIBLIOGRAPHY

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ANNEXURES

MADRAS MEDICAL COLLEGE, CHENNAI – 600003

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

PROCEEDINGS

PRESENT: Dr. A. JERAD SURESH, MPharm., PhD., MBA

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

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

Ref: IAEC Meeting held on 21-10-2021

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

Project Proposal ID Number	11/2021-2022
CPCSEA Registration Number	1917/GO/ReBi/2016/CPCSEA Valid till 19-9-2026
Name of the Researcher with ID Number	M. ANGURAJ 261915701
Name of the Guide	Dr. A. Jerad Suresh, MPharm., PhD, MBA.
Project Title	Design, Synthesis And Biological Evaluation Of Novel Saccharine derivatives as Anti-tubercular agents targeting Glutamine Synthetase.
Date of submission of proposal to IAEC	07-10-2021
Date of IAEC meeting	21-10-2021
Date of submission of modified proposal to IAEC	22-10-2021
Date of Approval	21-10-2021
Validity of the Approved Proposal	One Year
Number & Species of Laboratory Animals Approved	**30 Wistar Rats Approved.**

5/2/22

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai-600003

PRINCIPAL
COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

To
Dr. A. Jerad Suresh, MPharm., PhD, MBA.
Principal, Prof. & Head, Dept. of Pharmaceutical Chemistry,
College of Pharmacy,
MMC, Ch-3.

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

71st Indian Pharmaceutical Congress

Theme: Healthcare System - Role of Regulators

Certificate

This is to certify that Prof./Dr./Mr./Ms. ANGURAJ MOULISHANKAR *of* COLLEGE OF PHARMACY, MADRAS MEDICAL COLLEGE *has participated in the 71st Indian Pharmaceutical Congress held at Sri Ramachandra Institute of Higher Education and Research (DU), from 20th to 22nd December 2019, Chennai, Tamil Nadu.*

Shri. Ravi Uday Bhaskar	Dr. P.V.Vijayaraghavan	Dr. M. Dhilip Kumar	Dr. T.V. Narayana
President - 71st IPC 2019	Chairman - LOC	Secretary - LOC	General Secretary - IPCA

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Pharma vision 2030

Indian Pharmaceutical Congress Association
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ANGURAJ MOULISHANKAR

has attended the
70th Indian Pharmaceutical Congress 2018

Theme :
Pharma Vision 2030 :
Indian Pharma Industry-
A Global Leader

Venue:
Amity University, Noida

Dilip Shanghvi	B.R. Sikri	Atul Nasa	Dr Vijay Bhalla
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Hosted by :
Indian Pharmacy Graduates' Association (IPGA)

CSIR-SUMMER RESEARCH TRAINING PROGRAM (CSIR-SRTP)2020 ONLINE

CERTIFICATE

Name: ANGURAJ MOULISHANKAR **Sl. No.:** CSIR/SRTP/2020/NEIST/0527

has completed all the requirements of the CSIR-Summer Research Training Program (CSIR-SRTP) 2020 online during June to August, 2020 coordinated by CSIR-NEIST, Jorhat

DR. G. NARAHARI SASTRY **DIRECTOR** **CSIR-NORTH EAST INSTITUTE OF SCIENCE AND TECHNOLOGY**	PROF. ALOK DHAWAN **DIRECTOR** **CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH**	DR. SHEKHAR C. MANDE **DIRECTOR GENERAL, CSIR** **SECRETARY, DSIR, GOVT. OF INDIA**

Cid: 675.7196194741126

Office of the Principal Scientific Advisor to the Government of India

MoE's INNOVATION CELL (GOVERNMENT OF INDIA)

Ministry of Education Government of India

DRUG DISCOVERY HACKATHON 2020

Innovate4NewDrugs

Certificate

This Certificate is awarded to

M Anguraj

for Participating in

Drug Discovery Hackathon 2020

Prof. Vijay Raghavan	Prof. Anil Sahasrabudhe	Prof. B. Suresh	Dr. Abhay Jere
Principal Scientific Adviser Government of India	Chairman AICTE	President Pharmacy Council of India	Chief Innovation Officer Ministry of Education Innovation Cell

71st Indian Pharmaceutical Congress

Theme : Pharma Vision : Health Care System - Role of Regulators

Indian Pharmaceutical Congress Association

Certificate of Merit

This is to certify that Mr./Ms./Dr. ANGURAJ MOULISHANKAR

of COLLEGE OF PHARMACY, MADRAS MEDICAL COLLEGE

has presented a paper entitled DESIGN AND SYNTHESIS OF CHALCONES, FLAVONES AND FLAVANONES - DUAL TARGET COX-2 AS NSAID AND ANTI-CANCER and won

PDA INDIA CHAPTER BEST PAPER AWARD

in the e-Poster/ Oral Papers Presentation Sessions of 71st IPC, 2019 held at Sri Ramachandra Institute of Higher Education & Reserarch, Porur, Chennai during 20th to 22nd December 2019.

Sd/-

Mr. R.Udhay Bhaskar
President, IPCA

Dr. T.V.Narayana
Gen. Secretary, IPCA

Dr. M.Dhilip Kumar
Secretary, LOC

Prof. KPR Chowdary
Convener, SSC

One day workshop on Artificial Intelligence in Drug Discovery

Organized by
CSIR-North East Institute of Science & Technology

Sl. No. AIDD1987

Certificate of Participation

This is to certify that

Anguraj Moulishankar

has participated in the one day workshop on "Artificial Intelligence in Drug Discovery" organized by CSIR-North East Institute of Science and Technology, Jorhat on 01-09-2020.

Debabrata Das

Mr. Debabrata Das
Coordinator
CSIR-NEIST, Jorhat

Dr. G. Narahari Sastry
Director, CSIR-NEIST
Jorhat, Assam

TAMILNADU STATE COUNCIL FOR SCIENCE AND TECHNOLOGY

GOVERNMENT OF TAMILNADU

TNSCST

CERTIFICATE

This is to certify that the project entitled Design, conventional and Microwave assisted synthesis of chalcones sponsored by the Council under the sector Medical Sciences carried out and presented by Anguraj B., KMCH College of Pharmacy.

at the Seminar cum Exhibition of the **STUDENT PROJECT SCHEME (2018-2019)** held at Kalasalingam Academy of Education and Research, Krishnankoil, Srivilliputhur has won the **First Prize** for the Oral Presentation .

Chennai-600025
20.07.2019

DR.R.SRINIVASAN
Member Secretary

CBCMT

VIT
Vellore Institute of Technology
(Deemed to be University under section 3 of UGC Act, 1956)

1920-2020 Swansea University Prifysgol Abertawe

Indo-UK Virtual Conference

'Current Innovations and the Future of Therapeutic Developments'

CIFTD-2020

Certificate of Participation

This is to certify that Anguraj Moulishankar of COLLEGE OF PHARMACY, MADRAS MEDICAL COLLEGE. has participated in the Indo-UK Virtual Conference 'Current Innovations and the Future of Therapeutic Developments' CIFTD-2020, organized by Centre for Biomaterials Cellular & Molecular Theranostics (CBCMT) at Vellore Institute of Technology (VIT), Vellore, India and Swansea University, United Kingdom during 1st-3rd June, 2020.

Prof. Geetha Manivasagam
Chair, CIFTD-2020
CBCMT, VIT

Prof. Venkat Kanamarlapudi
Chair, CIFTD-2020
Swansea University, UK

Dr. Sudhagar Pitchaimuthu
Convener, CIFTD-2020
Swansea University, UK

Dr. Loganathan Rangasamy
Convener, CIFTD-2020
CBCMT, VIT

SCHRÖDINGER

JSS ACADEMY OF HIGHER EDUCATION & RESEARCH (DEEMED TO BE UNIVERSITY) MYSURU

MANIPAL ACADEMY of HIGHER EDUCATION (Deemed to be University under Section 3 of the UGC Act, 1956)

Introduction to Computational Drug Design

(Theory - Demo - Hands-on)

This is to certify that

Anguraj Moulishankar

has participated and Qualified in the Assessment Test in the above program

Co-Organized by

Schrödinger & Pharmacy Council of India

between 21st Sep - 23rd Oct 2020.

Dr B. Suresh
President
Pharmacy Council of India

Dr R. Raghu
Vice President
Schrödinger

Dr S. P. Dhanabal
Principal
JSS College of Pharmacy, Ooty

Dr C. Mallikarjuna Rao
Principal
Manipal College of Pharmaceutical Sciences, Manipal