DESIGN, SYNTHESIS,CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL HETEROCYCLIC ANTI-TUBERCULAR AGENTS AGAINST *InhA* (Enoyl Acyl Carrier Reductase Protein)ENZYME.

A Dissertation Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI-600032

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

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY
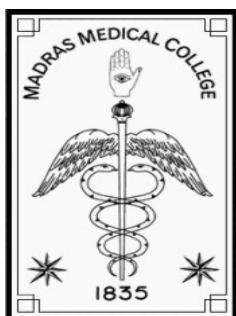
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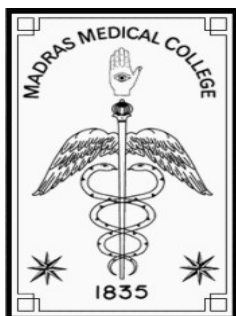### COLLEGE OF PHARMACY, MADRAS MEDICAL COLLEGE
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MAY 2019

CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL HETEROCYCLIC ANTI-TUBERCULAR AGENTS AGAINST *InhA* (Enoyl Acyl Carrier Reductase Protein) ENZYME"** submitted by the candidate bearing the Register No: **261715701** in partial fulfillment of the requirements for the award of degree of **MASTER OF PHARMACY in PHARMACEUTICAL CHEMISTRY** by the Tamil Nadu Dr. M.G.R Medical University is a bonafide work done by her during 2018 - 2019 in the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai- 600 003.

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*DEDICATED
TO
MY PARENTS,
TEACHERS
AND FRIENDS*

ACKNOWLEDGEMENT

LIST OF ABBREVIATIONS

TB	Tubercle Bacillus
HIV	Human Immuno Deficiency Syndrome
AIDS	Acquired Immuno Deficiency Syndrome
BCG	Bacilli Calmette Guerin
DOTS	Directly Observed Treatment Short-Course
MDR-TB	Multi Drug Resistant
XRD-TB	Extensively Drug Resistant-TB
LTBI	Latent Tuberculosis Infection
CADD	Computer Aided Drug Design
OSIRIS	Optical, Spectroscopic and Infrared Remote ImagingSystem
SBDD	Structure Based Drug Design
LBDD	Ligand Based Drug Design
Logp	Partition Co-Efficient
WHO	World Health Organization
MIC	Minimum Inhibitory Concentration
PDB	Protein Data Bank
TLC	Thin Layer Chromatography
IR	Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
GC-MS	Gas Chromatography-Mass Spectroscopy
REMA	Resazurin Micro Plate Assay
MABA	Micro Plate Alamar Blue Assay
NRA	Nitrate Reducates Assay
QSAR	Quantitative Structural Activity Relationship

CONTENT

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INTRODUCTION

TUBERCULOSIS:

Tuberculosis (TB), is the chronic infectious disease caused by the bacterium *Mycobacterium tuberculosis,* that generally affects the lungs (Pulmonary), but also affect other parts of the body (Extra-Pulmonary) which remains a major public health problem globally[1].According to WHO in 2017, more than 10 million people are estimated to have fallen ill with TB while 1.6 million people died of the disease[2].The introduction of the first drugs for TB treatment some 50 years ago - **Streptomycin, Para-Aminosalicylic Acid, Isoniazid** - led to optimism that the disease could be controlled if not eradicated[3].Most infections do not have symptoms, in which case it is known as latent tuberculosis. About 10% of latent infections progress to active disease which, if left untreated, kills about half of those infected. The disease is closely associated with poverty, which explains the high rates of TB in geographic areas within countries where poverty rates are high. The disease is closely associated with HIV which has been the major factor for the high rates of TB in many countries [4]

SYMPTOMS:
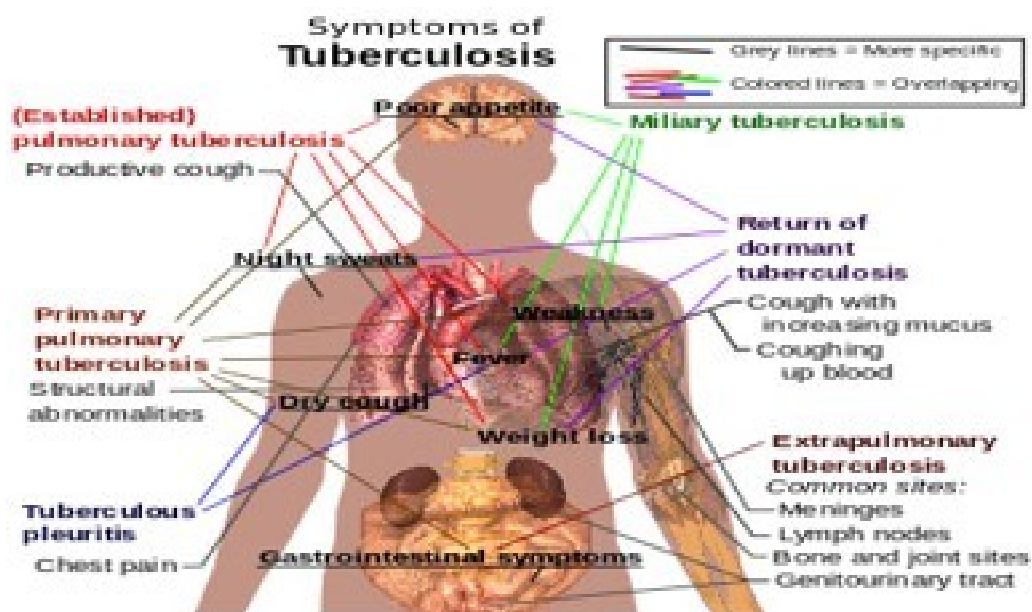
Fig1.Symptoms of Tuberculosis[4]

HISTORY:

Tuberculosis originated 150 million years ago. It is a disease of ancient past which is thought to have evolved sometime between the seventh and sixth millennia BC. German microbiologist Robert Koch announced that *Mycobacterium tuberculosis* caused TB in the year 1882[5]. Mortality rates significantly turned down from the early to mid-20th century; in spite of this, funding for research was much reduced between 1970 to 1990. The Directly Observed Treatment Short-Course (DOTS) program was introduced in 1993[6]. In 1998 the DOTS-plus program was introduced to address multidrug resistant (MDR) TB.

LONG TERM COMPLICATIONS OF PULMONARY TB :

Pulmonary TB is associated with various long term lung complications including lung scarring , bronchiectasis, Chronic Pulmonary Aspergillosis (CPA), air way stenosis and Chronic Obstructive Pulmonary Disease (COPD) and it may even be a risk factor for lung cancer.[7]

THE ETIOLOGICAL AGENT:

Mycobacterium tuberculosis is the etiologic agent of tuberculosis in Humans.The *Mycobacterium tuberculosis* complex includes strains of five species—*M. tuberculosis*, *M. canettii*, *M. africanum*, *M.microti,* and *M. bovis* and two subspecies—*M. caprae* and*M. pinnipedii* .[8-9]

Fig 2.Mycobacterium tuberculosis[8]

EPIDEMIOLOGY:

Epidemiology is the study of distribution of disease in society and the factors affecting this distribution.

The epidemiology of tuberculosis varies substantially around the world. The highest rates (100,000 or higher) are observed in sub-Saharan Africa, India, China, and the islands of Southeast Asia and Micronesia Estimates provided by USAID in 2007 for South Sudan were 228 cases per 100,000 population. In South Sudan, an estimated 18,500 people develop TB, and 5,300 die of TB annually [10].

More than 1.7 billion people are estimated to be infected with M.tuberculosis. The global incidence of TB peaked around 2003 and appears to be declining slowly with the newer drug development. According to WHO, in 2017,10 million individuals are estimated to have fallen ill with TB while 1.6 million people died of the disease.

Poverty, HIV and drug resistance are major contributors to the resurging global TB epidemic. Approximately 95% of TB cases occur in developing countries. Approximately 1 in 14 new TB cases occur in individuals who are infected with HIV; 85 percent of these cases occur in Africa. An estimated half million cases of multidrug resistant (MDR)-TB also occur annually in Africans; even higher rates of drug resistant disease occur in Eastern Europe [11]
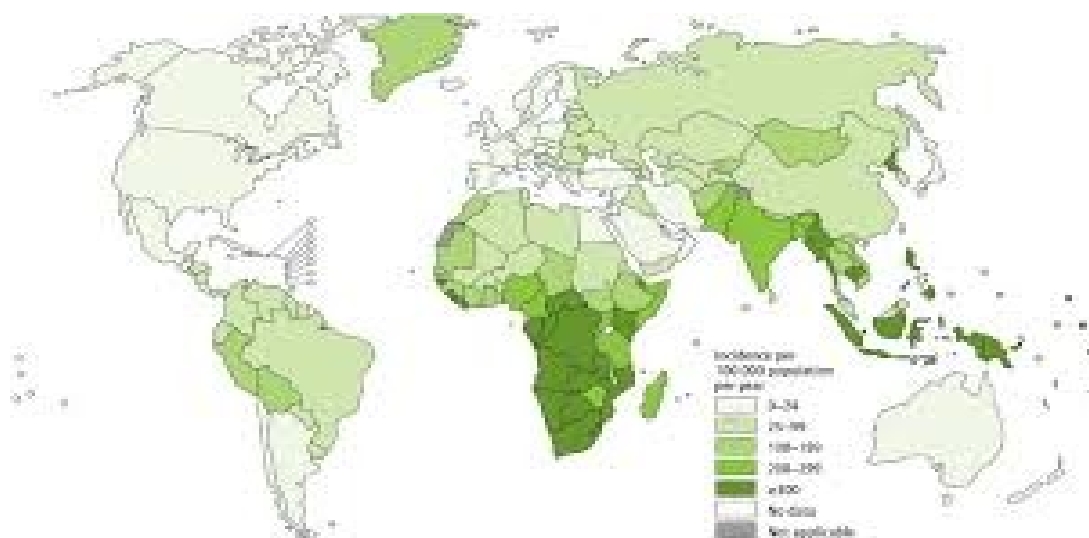
Fig 3.Epidemiology of Tuberculosis[11]

BURDEN OF TB GLOBALLY:

➢ Tuberculosis (TB) is contagious and airborne**.** It ranks alongside HIV/AIDS as a leading cause of death worldwide. 10**.4 million** People fell ill with TB in 2017, including 1.3 million People Died[12].

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

➢ In 2016, 10.4million people fell ill with TB, and 1.7 million died from the disease(including 0.4 million people with HIV)

➢ In 2016,an estimated 1 million children became ill with TB, and 250,000 children died of TB(including children with HIV associated TB).

➢ TB is the leading killer of HIV-positive people: In 2016, 40% of HIV deaths were due to TB.

➢ An estimated 53 million lives were saved through TB diagnosis and treatment between 2000 and2016.

➢ MDR-TB remains a public health crisis and a health security threat.

➢ WHO estimates that there were 60,000 new cases with resistance to Rifampicin-the most effective first line drug, of which 490000 had MR-TB.

➢ Globally, TB incidence is falling by about 2% per year.

➢ Ending the TB epidemic by 2030 is among the health targets of the Sustainable Development Goals.

DIAGNOSIS OF TUBERCULOSIS:

1. **Microbiological tests:**

2. **Immunological tests:**
   a) ALS (Antibodies from lymphocyte secretions) assay- this is an immunological assay to detect active diseases like tuberculosis, cholera, typhoid etc. [13]

   b) **Mantoux test (MT)/Tuberculin Skin Test (TST)**- positive test indicates infection by TB bacilli, doesn't exclude active disease cases from latent cases and inconclusive in BCG vaccinated people.

3. **Adenosin Deaminase Assay (ADA) test**:

ADA is an enzyme which contributes in purin metabolism and converts adenosine to inosine. ADA is essential for proliferation and differentiation of lymphoid cells, especially T cells, and helps in the maturation of monocytes to macrophages. It seems ADA is an index for cellular immunity. Activity of this enzyme increases in TB, empyema, lymphoma and other chronic inflammatory conditions like Rheumatoid Arthritis (RA). [14]

CURRENT TREATMENT AGAINST TUBERCULOSIS:

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

A Regimen of Isoniazid (INH), Rifampin (RIF), Pyrazinamide (PZA), and either Ethambutol (EMB) or Streptomycin (SM) are usually the drugs of choice for the treatment of TB.[15]

DRUG RESISTANT STRAINS:[16]

M. tuberculosis strains that are resistant to the two most potent anti-TB drugs are termed as multidrug-resistant TB (MDR-TB) strains. (I.e,Isoniazid and Rifampicin)

Extensively drug-resistant TB (XDR-TB) is a form of TB caused by bacteria that are resistant to Isoniazid and Rifampicin (i.e., MDR-TB), as well as to any fluoroquinolone and any of the second-line anti-TB injectable drugs.(I.e, Amikacin, Kanamycin, Capreomycin)

NEED FOR NEWER ANTI-TB DRUGS:[17-18]

Recent research regarding anti-tuberculosis agents is interested in:

- ➢ Shortening treatment time
- ➢ Combating MDR-TB
- ➢ Most of the work in this field focused in finding agents selective on inhibiting FAS-II system especially preventing mycolic acid biosynthesis.
- ➢ There is a need to design new drugs that are more active against slowly growing and nongrowing persistent bacilli.

> ➢ To provide more effective treatment for latent tuberculosis infection.
> ➢ New drug that would reduce both the total length of treatment and the frequency of drug administration.

MYCOBACTERIA:

Mycobacterium tuberculosis has an unusual, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which makes the cell impervious to gram staining[19]. Mycobacterium Tuberculosis is the rod-shaped,spore forming, aerobic bacterium.They are bacillary in form, at least in most phases that have attracted human microbiological attention to date.

MYCOBACTERIAL CELL WALL:

The cell wall is a major virulence factor of *Mycobacterium tuberculosis* and contributes to its intrinsic drug resistance. Cryo-electron microscopy showed that the mycobacterial cell wall lipids form an unusual outer membrane[20]. Identification of the components of the uptake and secretion machinery across this membrane is critical for understanding the physiology and pathogenicity of *Tuberculosis* and for the development of better anti-tuberculosis drugs.

Although the genome of *Tuberculosis* appears to encode over 100 putative outer membrane Proteins, only a few have been identified and characterized. The membranes contain Mycolic acid, Peptidoglycan and Arabinogalactan.
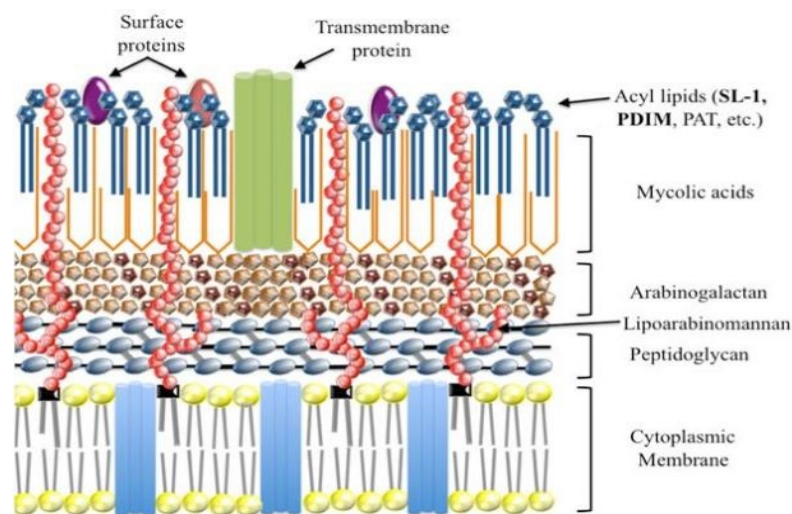
Fig 4.Mycobacterial Cell wall[20]

GENOME

Mycobacterium tuberculosis has circular chromosomes containing 4,200,000 nucleotides long. The G+C content of 65%[21]

The genome of *M. tuberculosis* was studied using the strain **M. tuberculosis H37Rv**[22]. The genome contains about 4000 genes. Genes that code for lipid metabolism are a very important part of the bacterial genome. 8% of the genome is involved in this activity.

The different species of the *Mycobacterium tuberculosis* complex show a 95-100% DNA relatedness based on studies of DNA homology, and the sequence of the 16S rRNA gene are exactly the same for all the species.
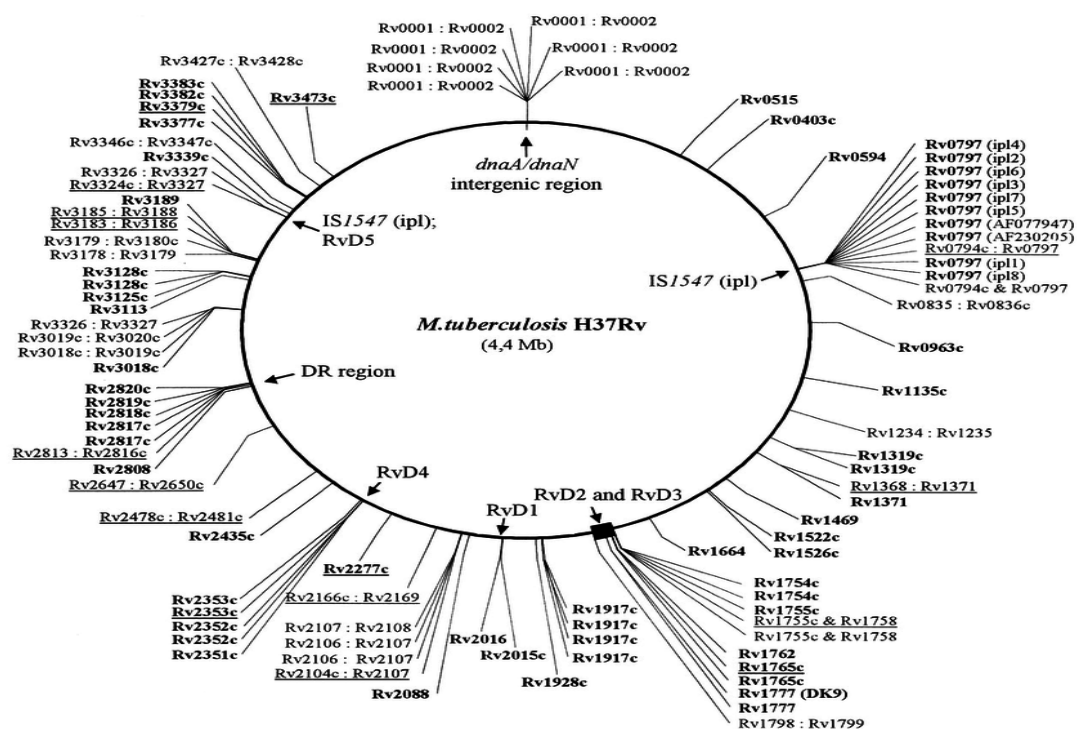
Fig 5.Genome of Mycobacterial Cell wall[21]

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

ENZYME PROFILE:

Among the most attractive molecular targets to the design of novel antibacterial agents are the Fatty Acid Synthase (FAS) pathway enzymes. The *Mycobacterium tuberculosis InhA* (MtInhA) or 2-trans-enoyl-ACP (CoA) reductase , the fourth enzyme of the type II fatty acid synthase system (FAS II), is one of the key enzymes involved in the elongation cycle of fatty acids in M. tuberculosis.

Its biological role includes the preferential reduction of long chain enoyl thioester substrates (e.g., containing 16 or more carbon atoms) yielding the long carbon chain of the meromycolate branch of mycolic acids (C40−60), α-branched fatty acids, the hallmark of mycobacteria.Previously, it has been shown that *InhA* is essential to the mycolic acid biosynthesis in Mycobacterium.[23]

Jmol
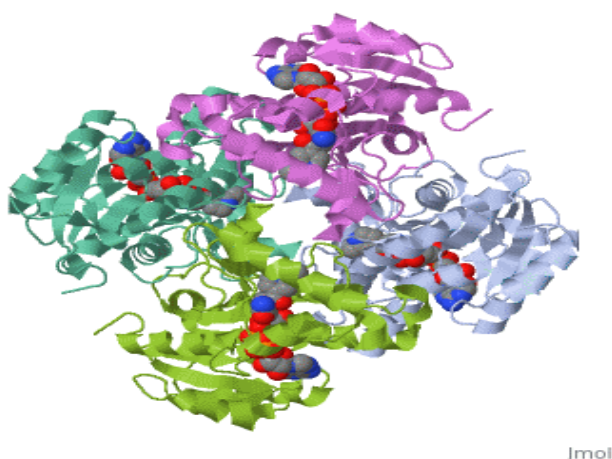
Fig 6.InhA Enzyme[23]

ENZYME NAME	:	ENOYL-ACP REDUCTASE
ORGANISM	:	Mycobacterium Tuberculosis
CLASSIFICATION	:	Oxido Reductase
POLYMER	:	1
TYPE	:	Protein
CHAINS	:	A,B
PROTEOME	:	Chromosome
FUNCTIONAL CATEGORY	:	Type II fatty acid biosynthesis pathyway

InhA, the enoyl-ACP redutase in mycobacterium tuberculosis is an attractive target for the development of novel drugs against tuberculosis, a disease that kills more than two million people each year. *InhA* is the target of the current first line drug isoniazid for the treatment of tuberculosis infections. Compounds that directly target *InhA* and do not require activations by the mycobacterial catalase –peroxidase kat G are promising candidates for treating infections caused by isoniazid –resistant strains.

However these compounds rapid reversible inhibitors of the enzyme and based on the knowledge that long drug target residence times are an important factor for in vivo drug activity,which set out to generate a slow onset inhibitor of *InhA* using structure based drug design.

2-(o-Tolyloxy)-5-hexyl phenol(PT70) is a slow,tight binding inhibitor of *InhA* with a K(1) value of 22 pm.

Crystal structure of the ternary complex between *InhA*,NAD(+),and PT70 reveals molecular details of enzyme-inhibitor recognition and supports the hypothesis that slow onset inhibition is coupled to ordering of an active site loop ,which leads to the closure of the substrate binding pocket .

BASIC NUCLEUS INFORMATION

Thiophene is a heterocyclic compound with the formula C_4H_4S. Consisting of a planar five-membered ring, it is aromatic as indicated by its extensive substitution reactions.Thiophene was discovered as a contaminant in benzene.[24]

Thiophenes are important heterocyclic compounds that are widely used as building blocks in many agrochemicals and pharmaceuticals. The benzene ring of a biologically active compound may often be replaced by a thiophene without loss of activity.[25] This is seen in examples such as the NSAID lornoxicam, the thiophene analog of piroxicam.
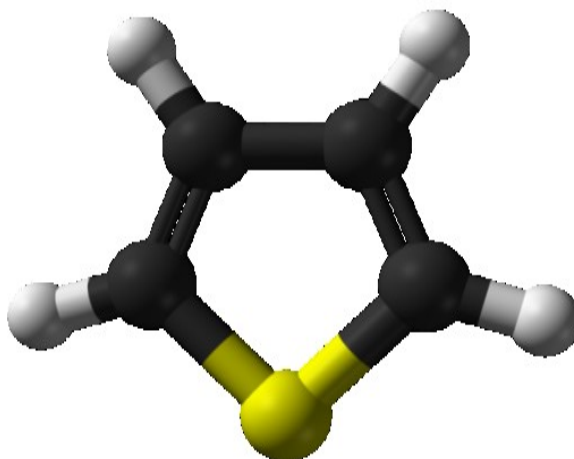
Fig 7. 3D Structure of Thiophene[24]

Thiophene can be used in the production of various kinds of dyes, perfumes, thermal shock resistant plastic, highly active solvent, stimulating hormone, insecticide, brightening agents, cosmetics and bio-activating substances and vitamins, anesthetics and antibiotics. It can also be used as the raw materials of preparing a broad spectrum anthelmintic pyrantel as well as antibacterial drugs cephalosporin I and II. Thiophene derivatives have following pharmacological activities:

 ➢ Antimicrobial activity
 ➢ Antibacterial activity
 ➢ Antifungal activity

LITERATURE REVIEW

In order to know the current status regarding the advances in TB the literature pertaining to the disease, design, synthesis, characterisation and biological evaluation were reviewed.

LITERATURE REVIEW BASED ON TUBERCULOSIS:

1. **Keane J** *et al* [26]., (1997) reported the"Mycobacterium Tuberculosis promotes Human alveolar macrophage apoptosis, Infection and immunity."

2. **James C Sacchettini et al.**[27] (2004) worked on TB drug discovery. Addressing issues of persistence and resistance by reviewing the recent developments of some of the pathways involved in a persistent infection and pathogenesis of mycobacterium

3. **De Souza M V N** *et al* [28]., (2006) studied the current status and future prospects for new therapies for Pulmonary Tuberculosis.

4. **Pierpalo de colombai**. *et al* [29]., (2007) outlined the Global Plan to Stop TB.

5. **Robert Koch** (2008) [30] outlined the history ofTuberculosis.

6. **Williams B.G** et al(2010) [31] studied about the "The Population Dynamicsand Control of Tuberculosis**.**

LITERATURE REVIEW BASED ON DRUG DESIGN:

7. **Andrew Worth. et al.** (1998) Described the Absorption, Distribution, Metabolism and Excretion (ADME) properties, which are often important in discriminating between the toxicological profiles of parent compounds and their metabolites/degradation products. [32]

8. **Lipinski CA. et al.** (2001) reported the experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings.[33]

9. **Romono T Kroemeret. et al.** (2003), explained the introduction into ligand–receptor docking. It illustrates the basic underlying concepts. [34]

10. **Lipinski CA.et al** (2004) outlined A Lead and drug-like compounds and the role of fine resolution. [35]

11. **Deepak D Borkar. e*t al. (2012)*** Performed the Design and Synthesis of p-hydroxybenzohydrazide Derivatives for their Antimycobacterial Activity[36]

12. **Ghorpade S R** *et al* [37]., (2013) studied a pharmacophore-based search which led to the identification of thiazolopyridine urea as a novel scaffold with antitubercular activity acting through inhibition of DNA Gyrase B (GyrB) ATPase.

13. **Frederick W G** *et al* [38]., (2015) outlined general principles that should be applied to ensure the building block collection's impact on drug discovery projects.


LITERATURE REVIEW BASED ON TARGET ENZYME:

14. **Banerjee et al**., 1994 reported the wild-type inhA gene of M. tuberculosis or M. smegmatis was shown to confer INH resistance and ethionamide (ETH) resistance to M. smegmatis and to Mycobacterium bovis BCG when transferred on a multicopy .Moreover, a point mutation (causing the amino acid substitution S94A) within the inhA genes of an INH-resistant M. smegmatis and an INH-resistant M. bovis mutant was shown to be sufficient to transfer INH and ETH resistance to M. smegmatis when transferred by allelic exchange within M. smegmatis. [39]

15. **Johnsson and Schultz, 1994** the inhA gene was predicted to encode an enoyl-ACP reductase of the fatty acid synthase II (FASII) system of mycobacteria. In an in vitro mycolic acid synthesis assay, KatGactivated INH inhibited the activity of purified InhA protein. [40]

16. **Heym et al., 1994., Ristow et al., 1995.,** reported the numerous groups have identified mutations from INH-resistant clinical isolates of M. tuberculosis within the promoter of inhA and the inhA protein product that are consistent with the premise that inhA encodes the target of INH and ETH in M. tuberculosis. [41]

17. **Vilcheze, C. *et al*., (2006) developed the transfer of a point mutation in Mycobacterium tuberculosis.[42]**

18. **Todd P Primm. et al**. (2007)Developed the Recent Advances in Methodologies for the Discovery of Antimycobacterial Drugs. **(43)**

19. **Argyrou, A. *et al*.,** (2007) developed new insight into the mechanism of action of and resistance to isoniazid: interaction of Mycobacterium tuberculosis enoyl-ACP reductase with INH-NADP.[44]

20. **Luckner, S.R *et al*.,** (2010) reported that Inh A, the enoyl-ACP reductase in Mycobacterium tuberculosis is an attractive target for the development of novel drugs against tuberculosis.[45]

LITERATURE REVIEW BASED ON CHEMICAL SYNTHESIS:

21. **Chairil Anwar et al., (2015)**reported a synthesis of series of chalcone deriavatives was presented by having considerable In Vitro activity against breast carcinoma cell lines T47D and colon carcinoma cell line WiDr.[46]
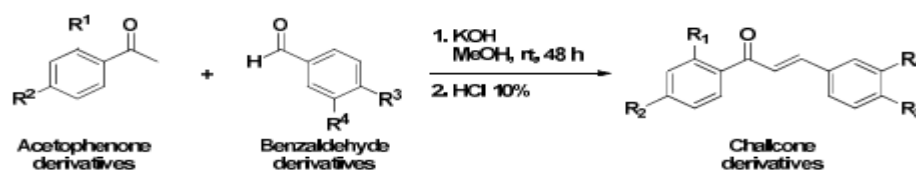
22. **Suresh et al,(2017)** synthesized a series of *(*substituted aryl aldehyde)-1-(furan-2-yl) prop-2-en-1-one chalcone derivatives.was presented by having considerable for MTFAB D, MALONYL COA - Acyl Carrier Protein Transacylase and cytotoxic activity against Tuberculosis Activity.[47]
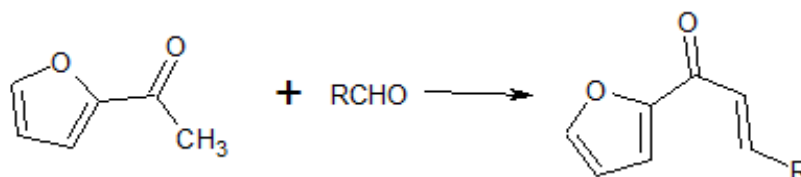
23. Hui Zhang et.al, (2018) synthesized a series of Novel chalcone derivatives was presented by having considerable growth inhibitory activity against MCF-7 and A549 Cell lines in vitro showed anti-proliferative and anti-tubulin activity.[48]

24. Romeo Romagnoli t.al, (2018) synthesized a series of chalcone like agents in which the double bonds of the enone system is embedded within a thiophene ring was presented by having an potent anti-tubulin activity.[49]

25. Qui Hy et.al. (2017) synthesized a series of novel chalcone containig shikonin derivatives was designed and evaluated for biological activities. Among them, derivative PMMB-59 was identified as a potent as a potent inhibitor of tubulin polymerization.[50]

LITERATURE REVIEW BASED ON SPECTRAL STUDIES:

26. **D.Kealey*et al*.,**(2010)Text book on Instant notes Analytical Chemistry.[51]

27. **Y.R.Sharma,*et al.,***(2008)Text book on Elemental Organic Spectroscopy.[52]

LITERATURE REVIEW BASED ON ANTI-TUBERCULAR ACTIVITY :

28. **Collins L** A *et al* [53]*.,* (1997) reported the high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium* using Microplate Alamar Blue Assay (MABA) and compared with BACTEC 460 Assay System.[53]

29. **Sephra N Rampresad. et al.** studied the various applications of Alamar Blue as an indicator. Alamar Blue is a redox indicator that is used to evaluate metabolic function and cellular health. The Alamar Blue Bioassay is being utilized to access cell viability and cytotoxicity in a range of biological and environmental system and in a number of cell types including bacteria, yeast, fungi, and protozoa.[54]

30. **Scott G Franzblau. et al.** studied MIC determination by MABA. A colorimetric, Microplate Based Alamar Blue Assay (MABA) method was used to determine the MICs of Isoniazid, Rifampin, Streptomycin and Etambutol for 34 peruvian Mycobacterium tuberculosis isolates and the H37Rv strain by using bacterial suspensions prepared directly from media. The MABA is a simpe, rapid, low cost, appropriate technology which does not require extensive instrumentation and which makes use of a nontoxic, temperature stable reagent.(55)

31. **Jose de Jesus Alba-Romero et al.** applied the Alamar Blue Assay to determine the susceptibility to anti-tuberculosis pharmaceuticals.[56]

32. **Ferreira M L** *et al* [57]*.,* (2009) synthesized six Schiff base derivatives of D-mannitol, and evaluated for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* H37Rv using the Alamar Blue susceptibility test.

33. **Prithwiraj D** *et al* [58]*.,* (2011) reported the synthesis and SAR of a series of new cinnamic derivatives. They also concluded that, many compounds exhibited sub-micromolar minimum inhibitory concentrations against *Mycobacterium tuberculosis* strain (H37Rv).

AIM AND OBJECTIVE

AIM:

To design and synthesize some novel Heterocyclic anti-tubercular agents which will prove to be effective against *Mycobacterium tuberculosis*.

OBJECTIVE:

The project directly aims to synthesis of some novel molecules which will overcome Multi-Drug Resistance Tuberculosis Disease (MDRTD).

The present study includes the following:

- Design of *InhA* (Enoyl-Acyl Carrier Protein reductase) inhibitors by docking studies using Autodock® Tools (1.5.6 version) software.
- *Insilico* Drug likeness prediction.
- *Insilico* Toxicity Assessment.
- Laboratory synthesis of those compounds with top Docking Scores.
- Characterization of the synthesized compounds by

    Infrared Spectroscopy.

    ^{1}H Nuclear Magnetic Resonance Spectroscopy.

    Melting point.

    LC-Mass Spectrometry.
- *In-vitro* anti -tubercular activity of synthesized compounds (MABA).

PLAN OF WORK

INHA Enzyme Target is chosen From Medicinal Chemistry Journals

⬇

In Silico Drug Likeness by Molinspiration

⬇

In Silico Toxicity Assessment

⬇

Synthesis

⬇

Characterization - Spectroscopy (IR, NMR, MASS)

⬇

In vitro anti-tubercular activity by MABA
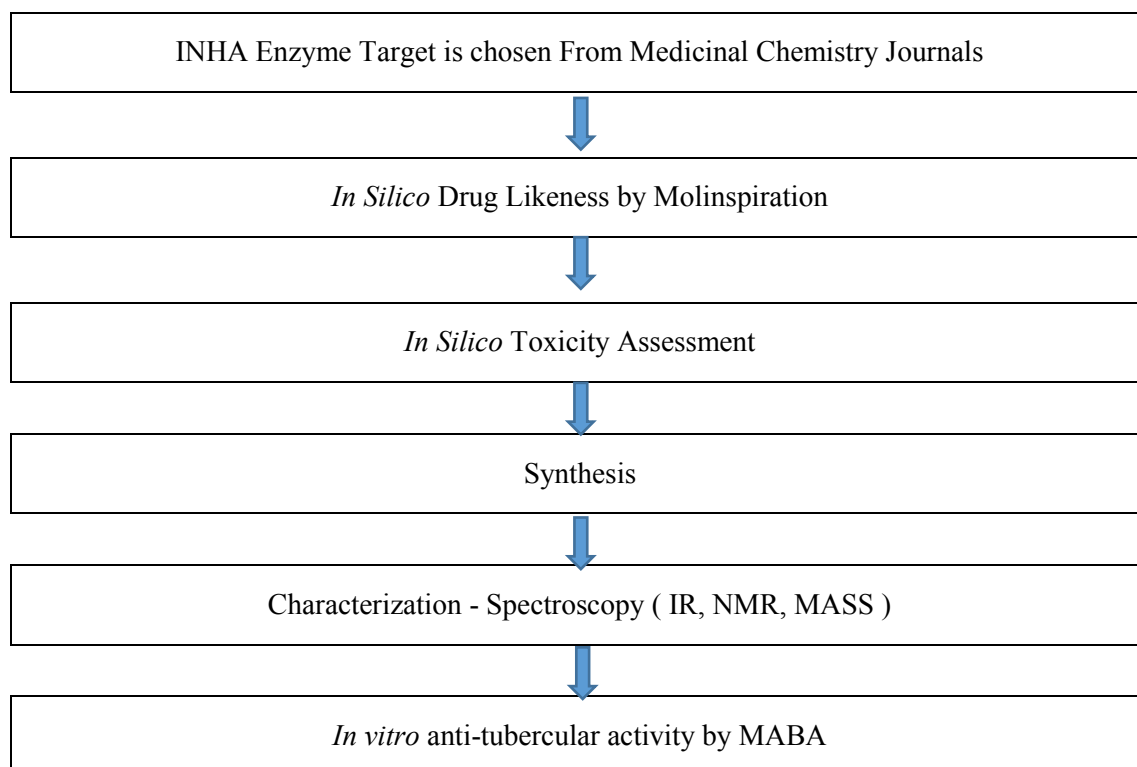
MATERIALS AND METHODS

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

> ➤ Drug design by using Autodock® Tools.
> ➤ Synthesis of the designed molecules.
> ➤ Characterization of the synthesized molecules.
> ➤ Biological evaluation of the synthesized molecules.

IN-SILICO APPROACH:

Target enzyme: *InhA* (Enoyl-[acyl-carrier-protein] reductase) [59]:

The target enzyme *InhA* (Enoyl-[acyl-carrier-protein] reductase) from *Mycobacterium tuberculosis*, is one of the key enzymes which is involved in the biosynthesis of mycolic acids, a major component of Mycobacterial cellwall, which is critical or the survival and growth of *Mycobacterium tuberculosis*. This target enzyme was selected from the *in-silico* target identification pipeline for *Mycobacterium tuberculosis*[60].

The crystal structure of the enzyme was downloaded from the Protein Data Bank (An Information Portal to Biological Macromolecular Structures) (PDB id – *2h9i*).

DRUG DESIGN[61]:

Rational drug design is the inventive process of finding new medications based on the knowledge of a biological target.[62] The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit for the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind toit.[63]

Drug design frequently but not necessarily relies on computer modeling techniques.This type of modeling is often referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of

the biomolecular target is known as structure based drug design. In addition to small molecules, biopharmaceuticals and especially therapeutic antibodies are an increasingly important class of drugs and computational methods for improving the affinity, selectivity, and stability of these protein-based therapeutics have also been developed.[64]

Drug design with the help of computers may be used at any of the following stages of drug discovery :[65]

Hit identification using virtual screening(structure-or ligand-base)

Hit – to - leadoptimization of affinity and selectivity(structure-based design, QSAR, etc.)

Lead optimization optimization of other pharmaceutical properties while maintaining affinity.[66]

TYPES[67]
There are two major types of drug design.
- **Ligand-based drug design**
- **Structure-based drug design**

LIGAND-BASED[68]
Ligand-based drug design (or **indirect drug design**) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.

STRUCTURE-BASED[69]
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.(42) If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.

BINDING SITE IDENTIFICATION:[70]

Binding site identification is the first step in structure based design. If the structure of the target or a sufficiency similar homolog is determined in the presence of a bound ligand, then the ligand should be observable in the structure in which case location of the binding site is trivial 71 . However, there may be occupied allosteric binding sites that may be of interest. Furthermore, it may be that only apoprotein (protein without ligand) structures are available and the reliable identification of unoccupied sites that have the potential to bind ligands with the high affinity is non-trivial.

DOCKING:[71]

Docking program is used to fit the ligand molecule into the target structure in a variety of position, conformations and orientations. Docking mode is known as pose. Each pose 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[72]:

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: $\Delta G0$ – 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 $\Delta Gionic$ – contribution from ionic interactions

$\Delta Glip$ – contribution from lipophilic interactions where is surface area of lipophilic contact between the ligand and receptor

$\Delta Grot$ – entropy penalty due to freezing a rotatable bond in the ligand bond upon binding

$\Delta Gbind = -RT \, ln \, Kd$

$$KD = \frac{[LIGAND] \, [RECEPTOR]}{[COMPLEX]}$$

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.

MOLECULAR DOCKING BY AUTODOCK®:

Autodock® 1.5.6 is an automated procedure for predicting the interaction of ligands with biomacromolecular targets.[73] 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

AutoDock® 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 will provide reproducible docking results for ligands with approximately 10 flexible bonds. The quality of any docking results 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[74].

❖ **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 **.dpf (**docking parameterfile**)** Open command prompt **[autodock4.exe –p a.dpf –l a.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 [75][76]:

Variants

In an attempt to improve the predictions of drug likeness, the rules have spawned many extensions,

1. Partition coefficient log P in −0.4 to +5.6 range
2. Molar refractivity from 40 to 130
3. Molecular weight from 180 to 500

Number of atoms from 20 to 70 (includes H-bond donors [e.g. OHs and NHs] and H-bond acceptors [e.g. Ns and Os])

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. Partition coefficient log P in −0.4 to +5.6 range
2. Molar refractivity from 40 to 130
3. Molecular weight from 180 to 500

Number of atoms from 20 to 70 (includes H-bond donors [e.g. OHs and NHs] and H-bond acceptors [e.g. Ns and Os])

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 A^2 are predicted to have good oral bioavailability[77].

IN-SILICO TOXICITY PREDICTION OSIRIS® :[78]

In silico toxicity prediction is done using **OSIRIS®** Property Explorer. It is a free software available for access in the Organic Chemistry Portal. Using this prediction tool, mutagenicity, tumerogenicity, skin irritation and reproductive effects can be calculated. 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. These fragment lists is created by rigorously shredding all compounds in the data base known to be active in a certain toxicity class. During the shredding any molecule is first cut at every rotatable bonds leading to a set of core fragments.

MOLINSPIRATION® [79]:

The designed and docked molecules are screened *in silico* using **MOLINSPIRATION®** Chemoinformatics software to evaluate drug likeness. This tool is quick and easy to use. 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 prediction of bioavailability score for the most important drug targets (GPCR ligands, Kinase inhibitors, ion channel modulators, nuclear receptors).

SYNTHETIC SCHEME:
SYNTHESIS:
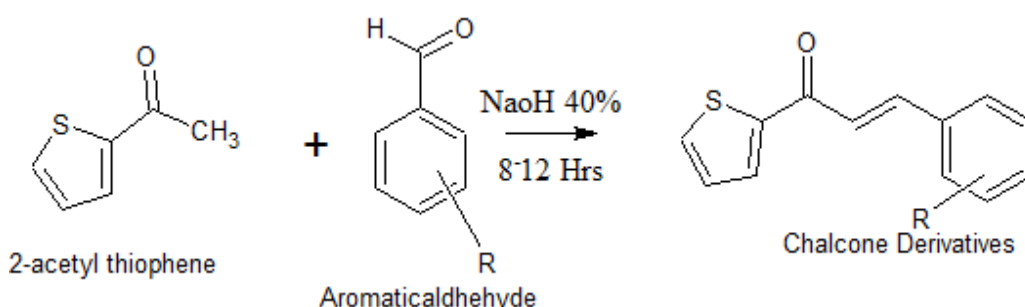
PROCEDURE:

1mmol 2-acetyl Thiophene was put into the round-bottom flask, 20ml of absolute ethanol and 5 ml of 40% sodium hydroxide was added as catalyst. 1mmol of substituted aromatic aldehyde was added. Mixture was stirred for 8-12 hrs at room temperature.

The reaction was monitored by TLC and pH was adjusted to 7. This mixture was poured into crushed ice followed by neutralization with HCl to produce precipitates. The precipitate was filtered off, washed and dried and recrystallized with absolute ethanol to give chalcone.

REACTANT PROFILE:

The reactant profile of the compounds used in the scheme is given below:

KETONE USED:

2- Acetyl Thiophene
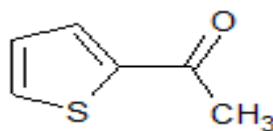
Molecular Formula   : C_6H_6OS

Molecular Weight   : 127.173g/mol

Solubility   : Ethanol

Melting Point   : 10-11℃

Boiling Point   : 214℃

ALDEHYDES USED:

Salicyaldehyde
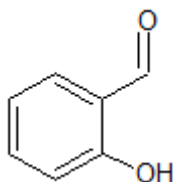
Molecular Formula   : $C_7H_6O_2$

Molecular Weight   :122.12g/mol

Solubility   : Ethanol

Melting Point   : -7℃

Boiling Point   : 196-197℃

5-NITRO VANILLIN
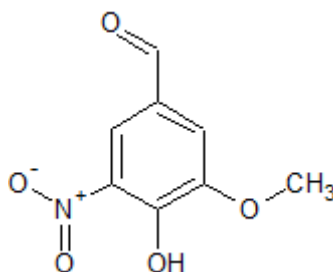
Molecular Formula   : $C_8H_7NO_5$

Molecular Weight   :197.14g/mol

Solubility   : Methanol
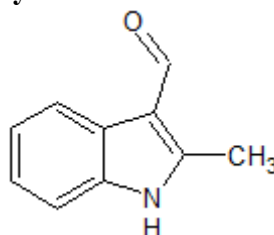
Melting Point   : 172-175℃

Cinnamaldehyde
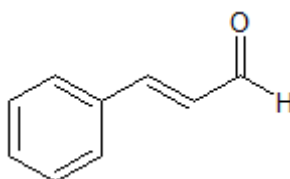
Molecular Formula   : C_9H_8O

Molecular Weight   :132.16g/mol

Solubility   : Water

Melting Point   : -7.5℃
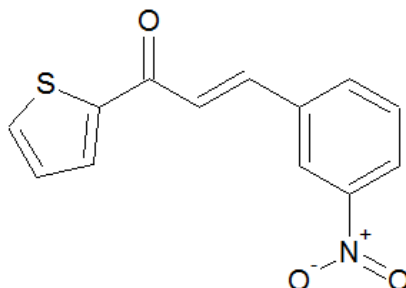
Boiling Point   : 248℃

4-BENZOYLOXY BENZALDEHYDE
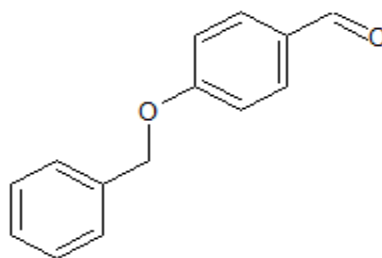
Molecular Formula   : $C_{14}H_{12}O_2$

Molecular Weight   : 212.24g/mol

Solubility   : Ethanol

Melting Point   : 71- 74℃

Boiling Point   : 248℃

2-Methyl indole 3- carboxaldehyde

Molecular Formula   : $C_{10}H_9NO$

Molecular Weight   :159.18g/mol

Solubility   : ETHANOL

Melting Point   : 200-201 ℃

3-Nitro Benzaldehyde

Molecular Formula : $C_7H_5NO_3$

Molecular Weight   : 151.12g/mol

Solubility   : Water

Melting Point   : 58℃

CHARACTERIZATION:

The purity of the synthesized compounds checked by TLC method and sharp melting point.

PHYSICAL EVALUATION:

1. The physical properties of the synthesized compounds are evaluated as follows:
   - ❖ Colour
   - ❖ Nature
   - ❖ Solubility
   - ❖ Molecular Weight
   - ❖ Molecular formula
   - ❖ Melting point
   - ❖ Boiling point.

2. Moreover the synthesized compounds are characterized by the following instrumental methods.[80]

IR SPECTROSCOPY:

Infrared spectroscopy is one of most commonly used spectroscopic technique for identification of functional groups in molecules. IR spectroscopy is an important tool in the structural elucidation of organic compounds. In IR spectroscopy finger print region is used to compare the two compounds. Infrared spectrum shows percent transmittance versus frequency expressed as wave numbers.[81]

1. 3540-3300 cm-1 N-H Stretching Vibration
2. 3670-3230 cm-1 O-H Stretching Vibration
3. 1690-1630 cm-1 C=N Stretching Vibration
4. 2975-2840 cm-1 C-H Aliphatic Stretching Vibration

INSTRUMENT USED : ABBNB 3000-PH FTIR

NMR SPECTROSCOPY [82]

Nuclear Magnetic Resonance (NMR) spectroscopy is an important analytical technique used in the structural elucidation of organic molecules. It involves the interaction of the electromagnetic radiation and the proton of an nucleus of an atom when placed in an externally applied static magnetic field. NMR spectra provide the detailed information about a molecule's structure. The chemical shift is used to predict the number of protons with refers to DMSO as standard .The NMR spectra is recorded on 300 MHZ BRUKER advance III NMR spectrometer. DMSO is used as a solvent.

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

INSTRUMENT USED : Bruker Topspin 500MHZ using DMSO-d$_6$

HYPHENATED TECHNIQUE:[83]

LC-MS:

LC-MS is a hyphenated technique, combining separation power of HPLC with the detection power of Mass Spectrometry.**Liquid chromatography–Mass spectrometry (LC-MS)** is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography - MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity. This tandem technique can be used to analyze biochemical, organic, and inorganic compounds commonly found in complex samples of environmental and biological origin. Therefore, LC-MS may be applied in a wide range of sectors including biotechnology, environment monitoring,food processing and pharmaceutical, agrochemical, and cosmetic industries.[84][85]

INSTRUMENT USED : AGILENT TECHNOLOGIES, 6230B Time of Flight(TOF).

BIOLOGICAL EVALUATION

Anti-tubercular Activity:

There are various assay methods available for the evaluation of new chemical entities against tuberculosis. They are as follows:

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

MICROPLATE ALAMAR BLUE ASSAY (MABA)[86]

The anti-microbial activities of the synthesized compounds is determined by MABA method. The organism used in the studies 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 MICs of TB drugs for strains of *Mycobacterium tuberculosis*. In this assay, the redox indicator Alamar blue monitors the reducing environment of the living cells. It turns from blue to pink in the presence of Mycobacterium growth.

Procedure:

1. The anti-mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay(MABA).
2. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

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

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

5. The final drug concentrations tested is made up to 100 to 0.2μg/ml.

6. Plates are covered and sealed with Para film and incubated at37^{O}C for five days.

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

8. A blue colour in the well is interpreted as no bacterial growth, and pink colour was scored as growth.
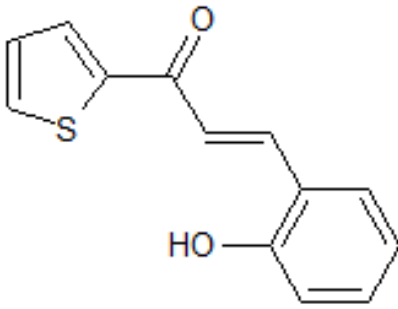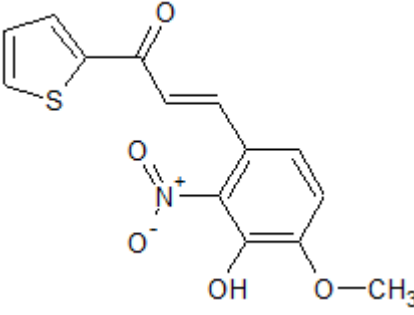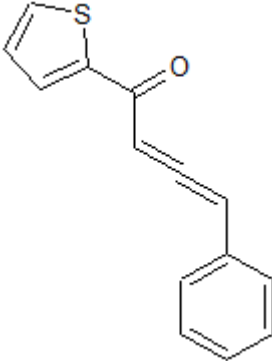
The MIC is defined as lowest drug concentration which prevents the colour change from blue to pink

RESULTS AND DISCUSSION
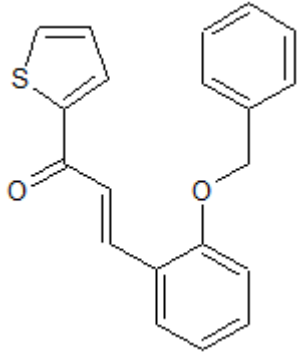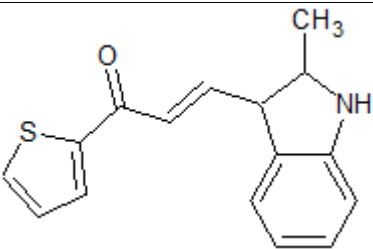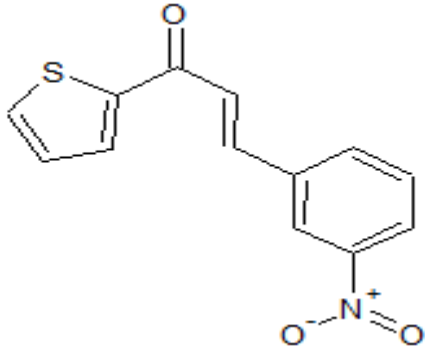
ACTIVITY PREDICTION:

   More than 100 compounds sketched using chemsketch were docked against the enzyme *InhA* using Autodock® Tools 1.5.6 software. The molecules with the good docking scores and favourable interactions were synthesized.

Table 1: The molecules with good docking score were mentioned as below:

CODE NAME	STRUCTURE	DOCKING SCORE kcal/mol^{-1}
DKN-5		-8.07 kcal/mol^{-1}
DKN-6		-9.07 kcal/mol^{-1}
DKN-7		-7.52 kcal/mol^{-1}
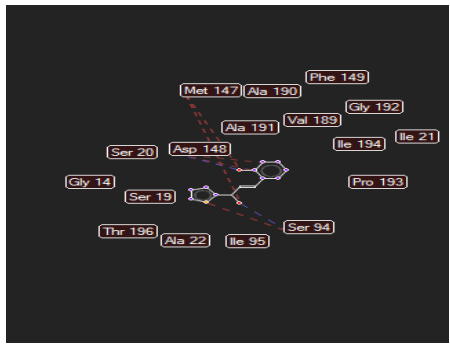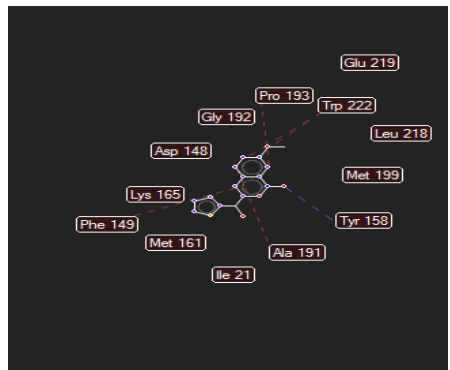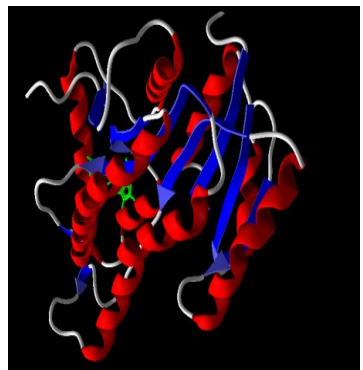
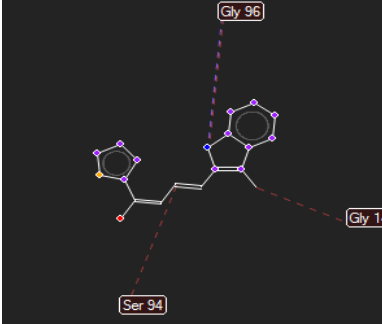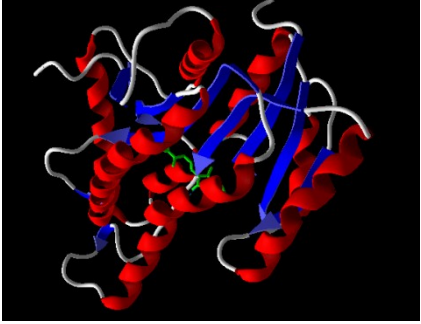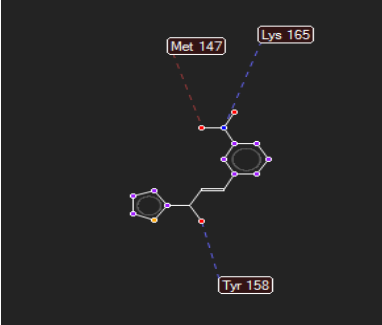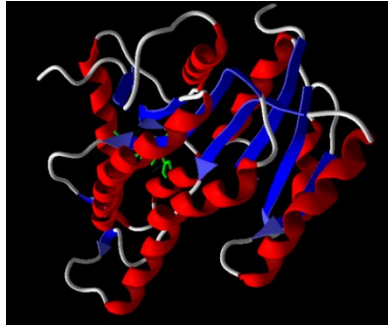| CODE NAME	STRUCTURES	DOCKING SCORE
DKN-8		-8.52 kcal/mol^{-1}
DKN-9		-9.39 kcal/mol^{-1}
DKN-10		-8.05 kcal/mol^{-1}
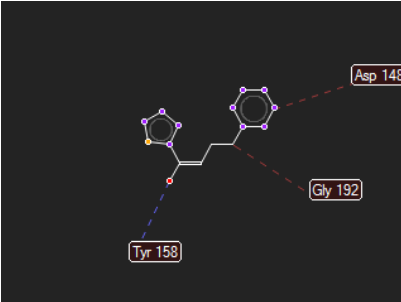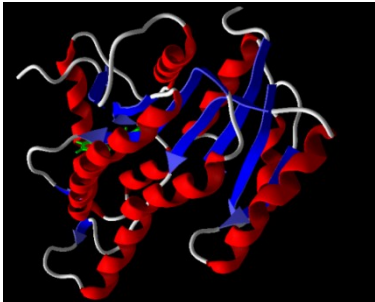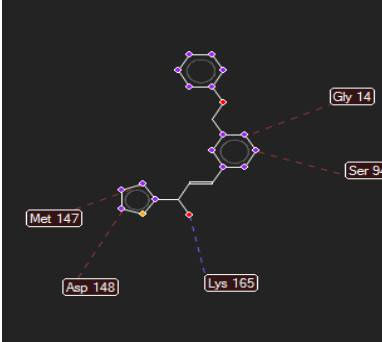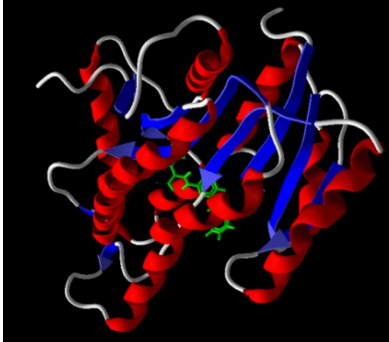
DOCKING VIEW AND INTERACTION OF THE DOCKED MOLECULES WITH THE ENZYME:

During the docking using ,AUTODOCK® Tools 1.5.6 initially performs a complete systemic search of the conformations, orientations and position of a compound in the defined binding site was concluded.Unwanted poses using scoring and energy optimization were eliminated. The best poses were selected on the basis of the scoring function and the quality of pose orientation within the active site of the Aminoacids.

Table 2: Interaction with Aminoacids

S. No	SAMPLE CODE	INTERACTIONS	SECONDARY STRUCTURE
1.	DKN-5		
2.	DKN-6		

S. No.	SAMPLE CODE	INTERACTION	SECONDARY STRUCTURE
3.	DKN-7		
4.	DKN-8		
5.	DKN-9		
6.	DKN-10		

PREDICTION OF DRUG LIKENESS:

Lipinski's rule of five was used to evaluate drug likeness or to determine if a chemical compound with certain pharmacological or biological activity has properties that would make an orally active drug .

All the novel analogues were checked for conformance to Lipinski's rule using online version of Molinspiration® software. It was used to calculate physiochemical properties of molecules such as logP, Polar Surface Area, Number of Hydrogen bond Donors and Acceptors and number of rotatable bonds.

VARIANTS:

PREDICTION OF TOXICITY (INSILICO):

Toxicity prediction was done by OSIRIS® Property explorer,the online software of Thomas Sander Actelion Pharmaceuticals Ltd, Switzerland.

The chemical structure was drawn in OSIRIS® Property explorer to show the biological property of the compound. Properties with high risks of undesired effects like mutagenicity, tumerogenicity, irritant and reproductive effect were indicated in red. Green colour was used to indicate drug conform behavior.

The following are the results of toxicity prediction for Six selected molecules based on docking score:

SAMPLE CODE : DKN -5
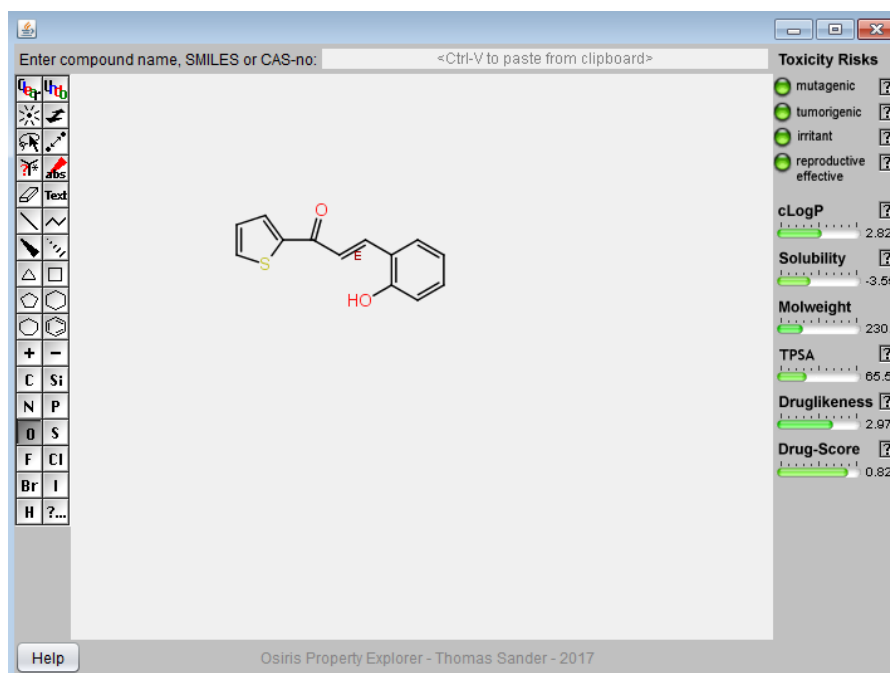
SAMPLE CODE : DKN-6

SAMPLE CODE : DKN 7

SAMPLE CODE : DKN-8

SAMPLE CODE : DKN – 9
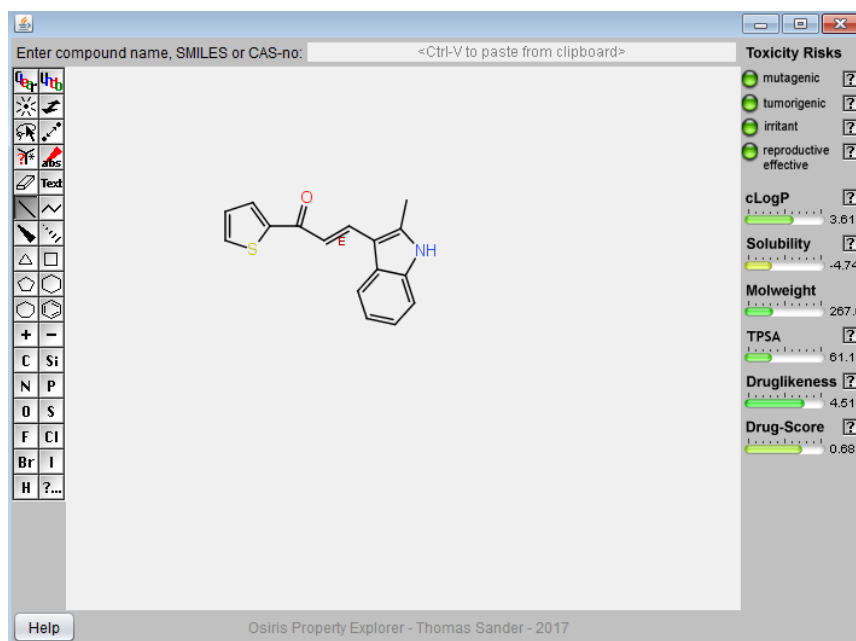
SAMPLE CODE : DKN -10
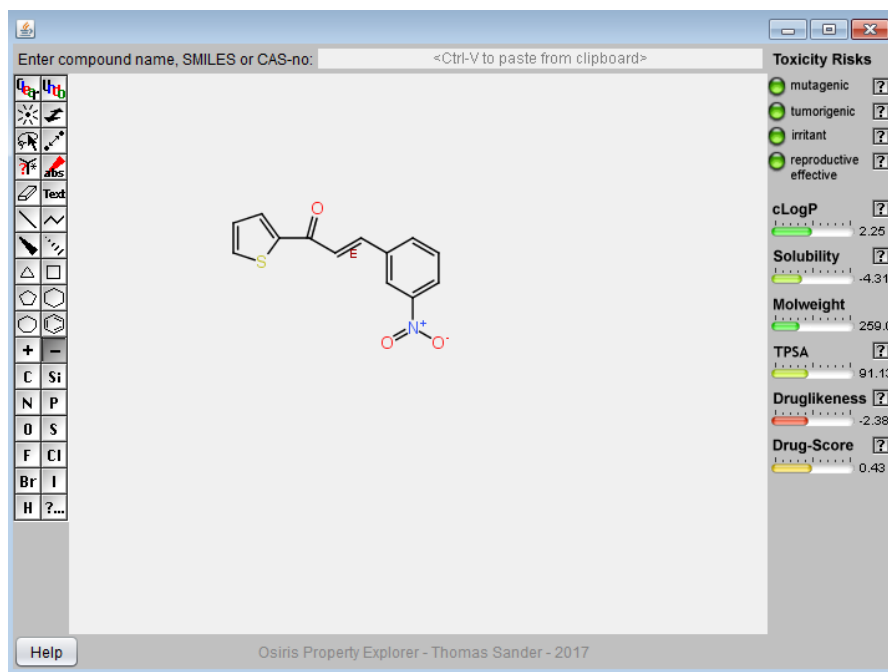
None of the compounds showed violations and thus these compounds are further subjected for synthesize and characterization.

In an attempt to improve the predictions of drug likeness,the rules have spawned many extensions.They are given below:
- ✓ Partition coefficient log P -0.4 to +5.6range
- ✓ Molar refractivity from 40 to130
- ✓ Molecular weight from 180 to500 Daltons
- ✓ Number of atoms from 20 to 70 (includes H-bond donors [eg.OHs and NHs] and H-bond aceeptors

The snapshot of the prediction for drug likeness is presented below for the chosen compounds The compounds **DKN-5,DKN-6,DKN-7,DKN-8,DKN-9,DKN-10** were in conformance with Lipinski's rule and chosen for synthesis.

SAMPLE CODE : DKN-5
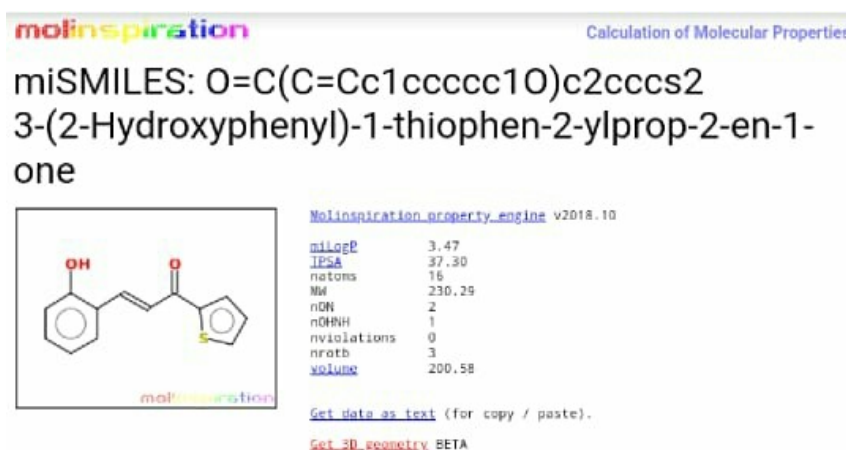
SAMPLE CODE : DKN-6

miSMILES: COc2cc(C=CC(=O)c1cccs1)cc(N(=O)=O)c2O
CID 84854263
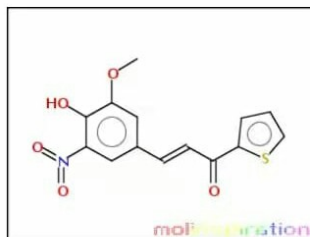
Molinspiration property engine v2016.10

miLogP	3.17
TPSA	92.36
natoms	21
MW	305.31
nON	6
nOHNH	1
nviolations	0
nrotb	5
volume	249.46

Get data as text (for copy / paste).

Get 3D geometry BETA

This was request 21 out of 1000 available this month for your site 168.235.198.198

SAMPLE CODE: DKN-7

miSMILES: O=C(C=C=Cc1ccccc1)c2cccs2
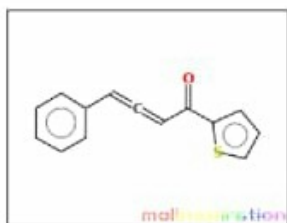
Molinspiration property engine v2018.10

miLogP	3.64
TPSA	17.07
natoms	16
MW	226.30
nON	1
nOHNH	0
nviolations	0
nrotb	3
volume	203.64

Get data as text (for copy / paste).

Get 3D geometry BETA

SAMPLE CODE : DKN – 8

miSMILES:
O=C(C=Cc1ccccc1OCc2ccccc2)c3cccs3
3-(2-Phenylmethoxyphenyl)-1-thiophen-2-ylprop-2-en-1-one

Molinspiration property engine v2018.10

miLogP	5.13
TPSA	26.30
natoms	23
MW	320.41
nON	2
nOHNH	0
nviolations	1
nrotb	6
volume	289.76

Get data as text (for copy / paste).

Get 3D geometry BETA

SAMPLE CODE : DKN -9

miSMILES:
Cc2[nH]c1ccccc1c2C=CC(=O)c3cccs3

Molinspiration property engine v2016.10

miLogP	3.90
TPSA	32.86
natoms	19
MW	267.35
nON	2
nOHNH	1
nviolations	0
nrotb	3
volume	238.10

Get data as text (for copy / paste).

Get 3D geometry BETA

SAMPLE CODE: DKN-10

miSMILES: O=C(C=Cc1cccc(N(=O)=O)c1)c2ccsc2

Molinspiration property engine v2018.10

miLogP	3.33
TPSA	62.90
natoms	18
MW	259.29
nON	4
nOHNH	0
nviolations	0
nrotb	4
volume	215.90

Get data as text (for copy / paste).

Get 3D geometry BETA

CHARACTERIZATION :

PHYSICAL EVALUATION:

Table 3: Physical Evaluation of the synthesized compounds

S. NO	SAMPLE CODE	MOLECULAR FORMULA	APPEARANCE	MELTING POINT (℃)
1.	DKN-5	$C_{13}H_{10}O_2S$	LIGHT GREEN COLOUR	124-126
2	DKN-6	$C_{14}H_{11}NO_5S$	RED COLOUR	160-162
3	DKN-7	$C_{14}H_{10}OS$	BROWN COLOUR	114-116
4	DKN-8	$C_{20}H_{16}O_2S$	CREAM WHITE COLOUR	110-112
5	DKN-9	$C_{16}H_{13}NOS$	YELLOW COLOUR	118-120
6	DKN-10	$C_{13}H_9NO_3S$	BROWN COLOUR	120-122

TLC PROFILE:

TLC was carried out on precoated plates (604 GF 254 MERCK),using a suitable solvent system The selected 6 compounds were synthesized, recrystallised and purified .The purity was determined by a single spot obtained on the TLC Plate. The Rf values were recorded accordingly.

Table 4: Rf value of the synthesized compounds

S. NO.	SAMPLE CODE	MOBILE PHASE	RF VALUE
1.	DKN-5	HEXANE:ETHYL ACETATE(6:4)	0.72
2.	DKN-6	HEXANE:ETHYL ACETATE(6:4)	0.86
3.	DKN-7	HEXANE:ETHYL ACETATE(6:4)	0.68
4.	DKN-8	HEXANE:ETHYL ACETATE(6:4)	0.75
5.	DKN-9	HEXANE:ETHYL ACETATE(6:4)	0.65
6.	DKN-10	HEXANE:ETHYL ACETATE(6:4)	0.79

Rf value of the synthesized compounds varies from the value of the reactants. Thus it is concluded that the reaction was completed.

PRODUCT PROFILE:

CODE: DKN-5
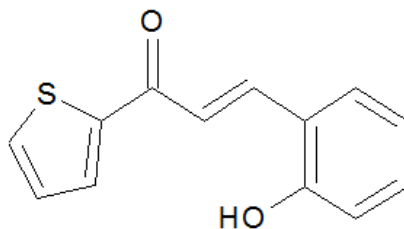
(2*E*)-3-(2-hydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one

Molecular Formula	= $C_{13}H_{10}O_2S$
Formula Weight	= 230.2823
Composition	= C(67.80%) H(4.38%) O(13.90%) S(13.92%)
Molar Refractivity	= 67.37 ± 0.3 cm^3
Molar Volume	= 177.7 ± 3.0 cm^3
Parachor	= 489.1 ± 4.0 cm^3
Index of Refraction	= 1.682 ± 0.02
Surface Tension	= 57.3 ± 3.0 dyne/cm
Density	= 1.295 ± 0.06 g/cm^3
Polarizability	= 26.70 ± 0.5 10^{-24}cm^3

MASS SPECTRUM

MOLECULAR WEIGHT:229.10g/mol
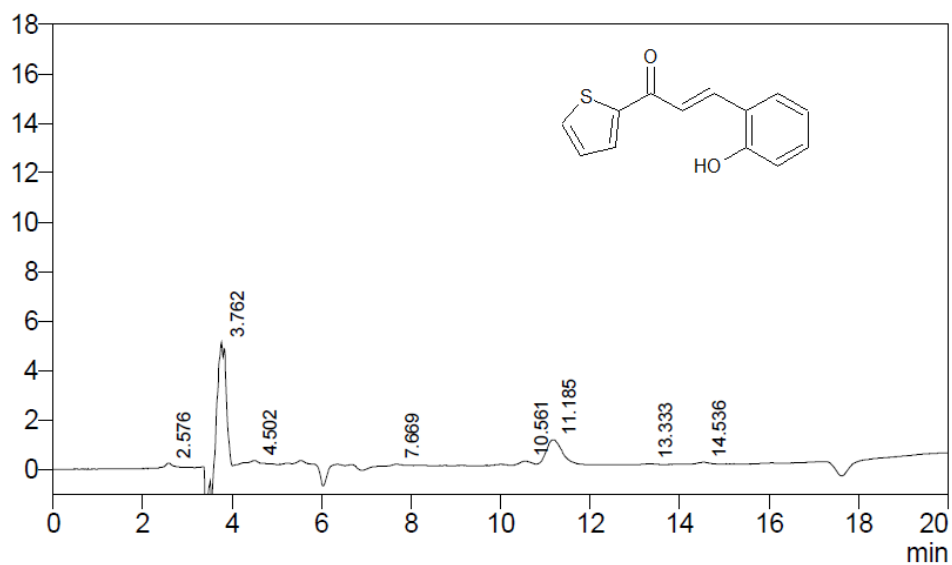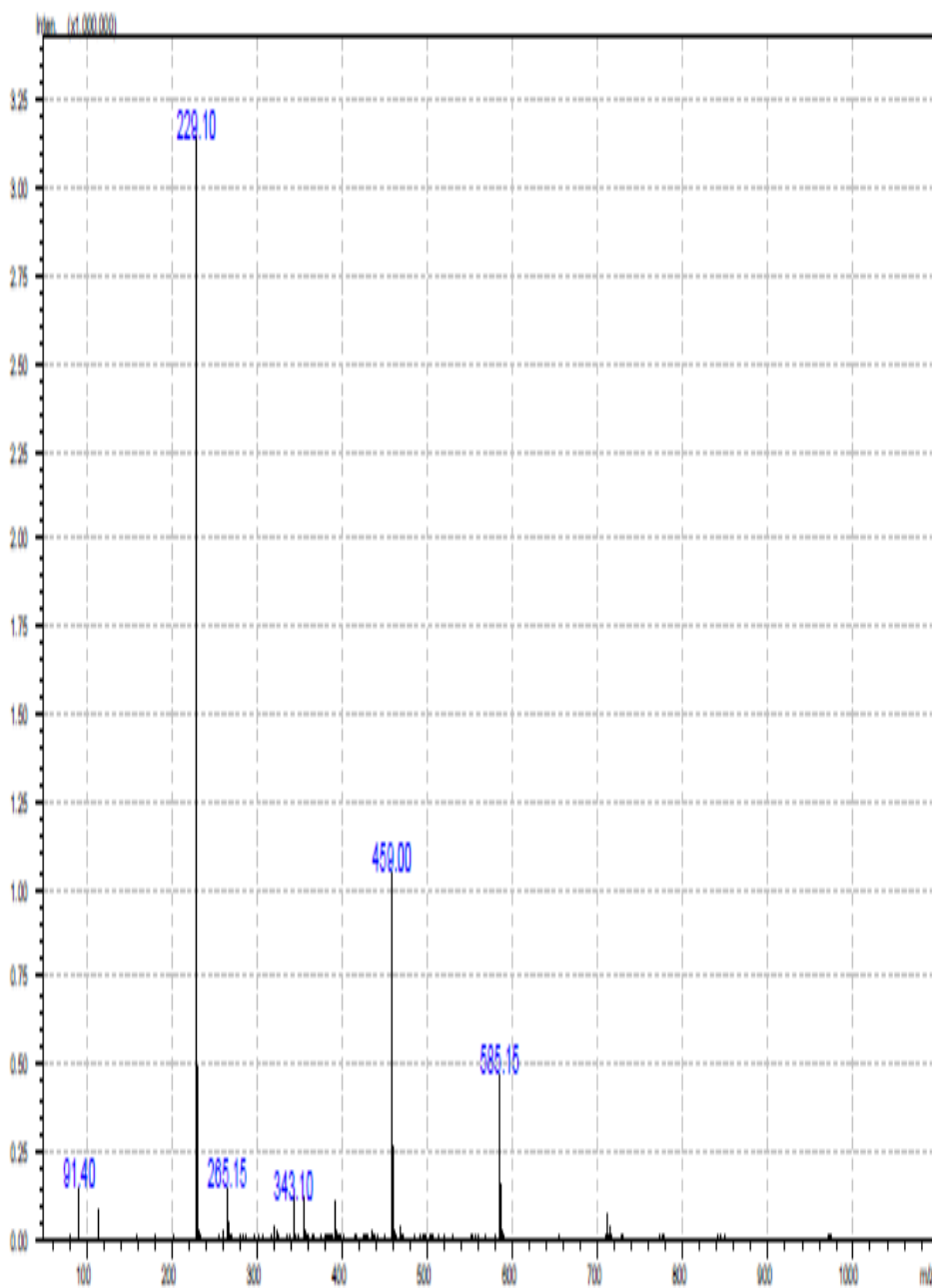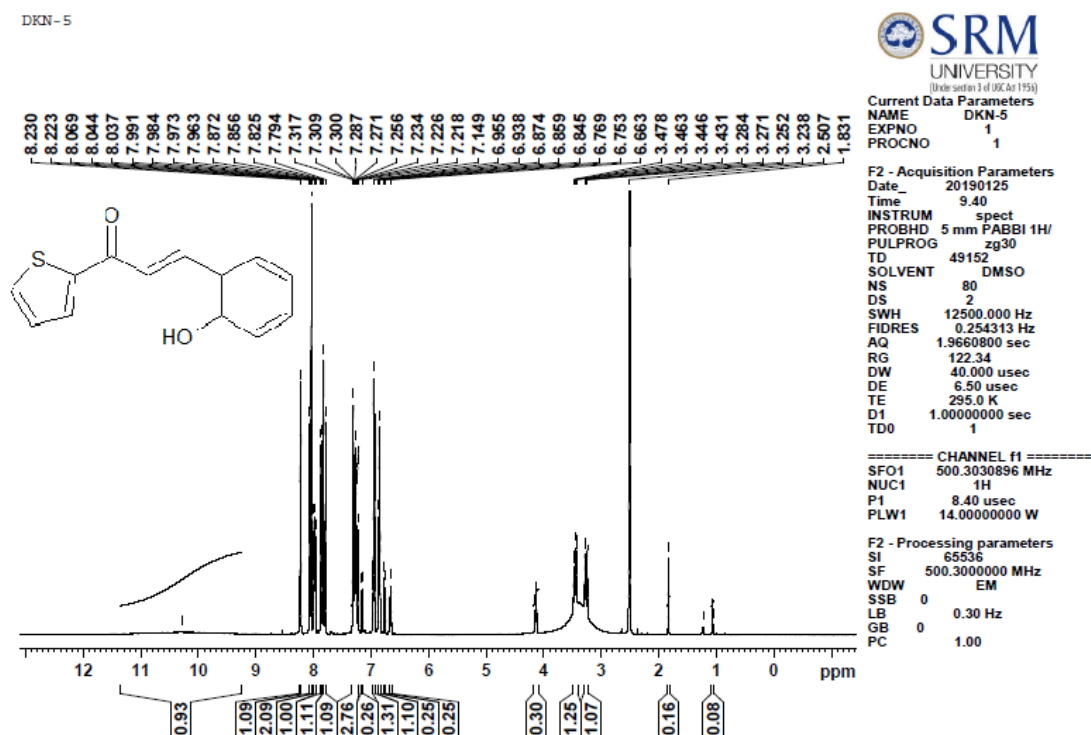
NMR SPECTROSCOPY:

Table 5: ¹H NMR INTERPRETATION OF DKN-5

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTONS
1.	2-3.4	DOUBLET	2 PROTONS
2.	6.7-7.2	TRIPLET	3 PROTONS
3.	7.2-8.2	MULTIPLET	5 PROTONS

IR SPECTROSOPY:
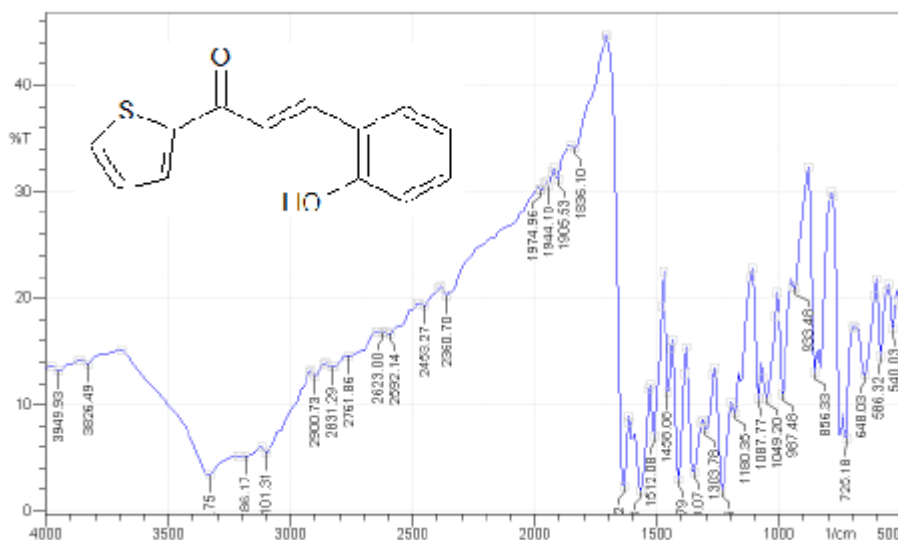
Table 6: IR Interpretation of DKN-5

S. NO	WAVE NUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUPS
1.	1712.66	C=O Stretching	Presence of Ketonic group
2.	1620.09	C=C Stretching	Presence of Alkene
3.	3695.34	C-OH Stretching	Presence of Phenolic Group

CODE :DKN-6
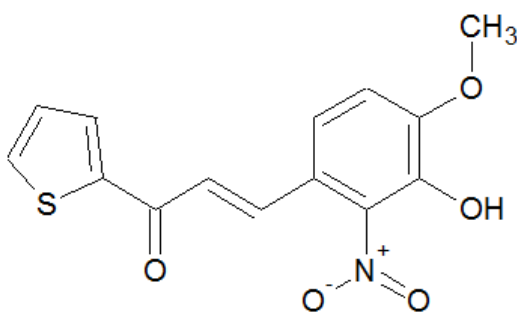
(2*E*)-3-(3-hydroxy-4-methoxy-2-nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one

Molecular Formula	= $C_{14}H_{11}NO_5S$
Formula Weight	= 305.30584
Composition	= C(55.08%) H(3.63%) N(4.59%) O(26.20%) S(10.50%)
Molar Refractivity	= 80.59 ± 0.3 cm^3
Molar Volume	= 213.5 ± 3.0 cm^3
Parachor	= 601.2 ± 4.0 cm^3
Index of Refraction	= 1.678 ± 0.02
Surface Tension	= 62.8 ± 3.0 dyne/cm
Density	= 1.429 ± 0.06 g/cm^3
Polarizability	= 31.95 ± 0.5 10^{-24} cm^3

MASS SPECTRUM:

MOLECULAR WEIGHT:304.20g/mol
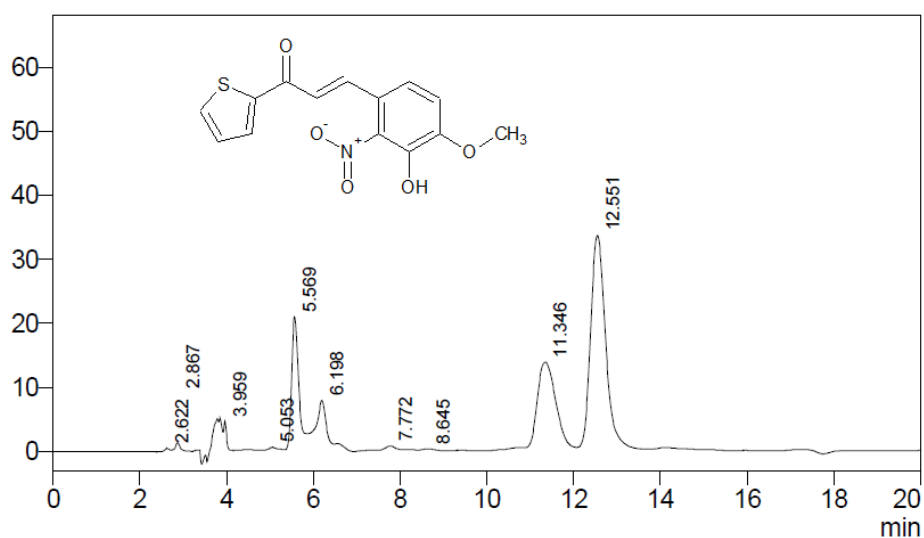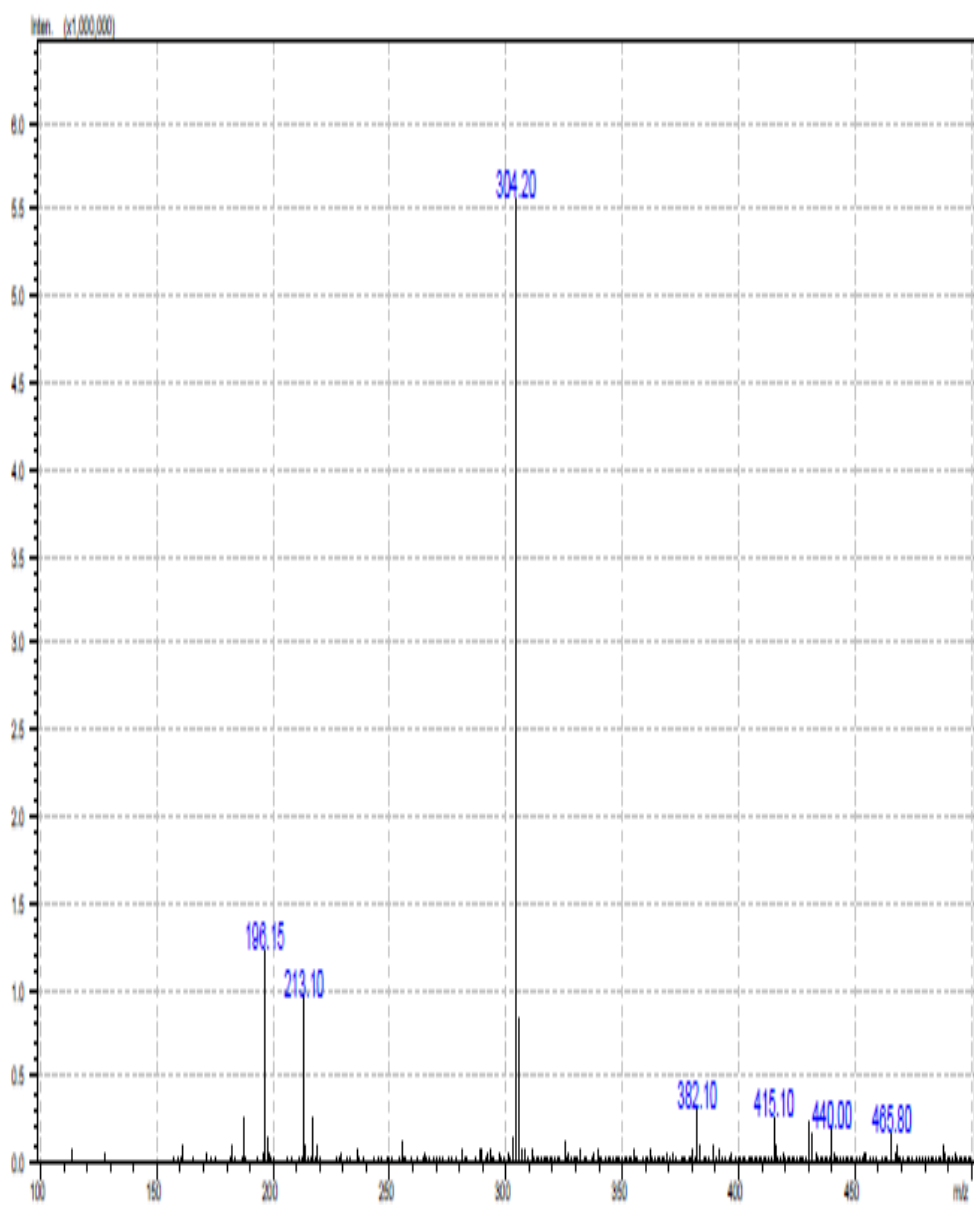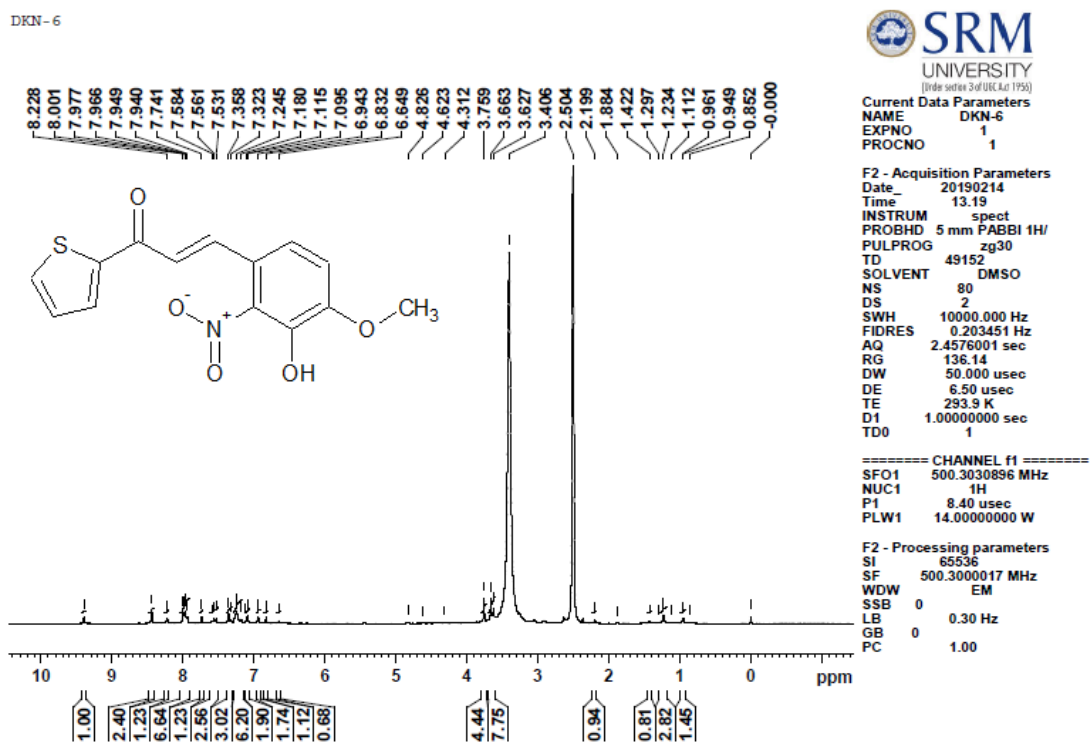
NMR SPECTROSCOPY:

Table 7: ^{1}H NMR INTERPRETATION OF DKN-6

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTON
1.	1.1-2.1	DOUBLET	2
2.	2.5-3.7	TRIPLET	3
3.	6.8-8.0	MULTIPLET	6

IR SPECTROSCOPY:
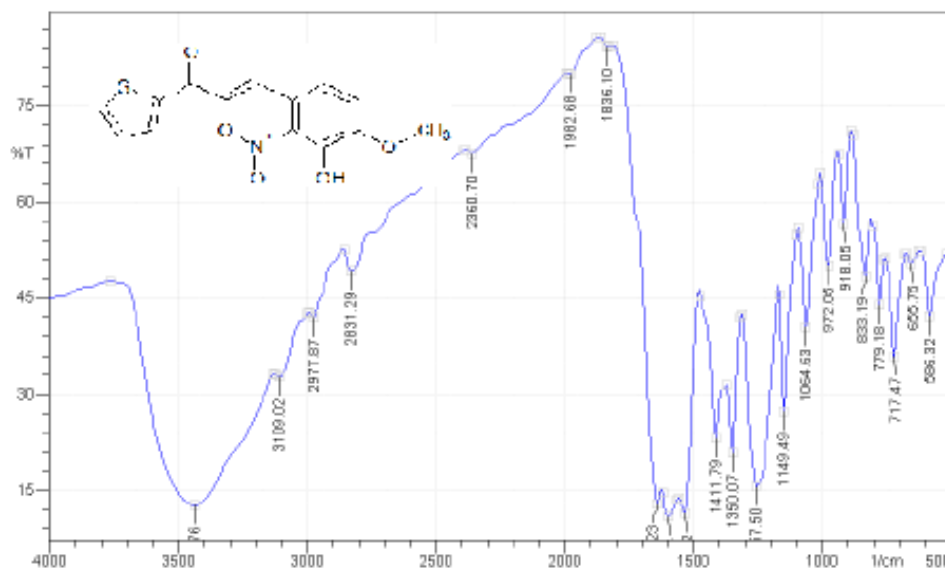
Table 8: IR Interpretation of DKN-6

S.NO	WAVE NUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUPS
1.	1712.66	C=O Stretching	Presence of Ketonic group
2.	1643.23	C=C Stretching	Presence of Alkene
3.	1566.08	NO_2 Stretching	Presence of Nitro group

DKN-7
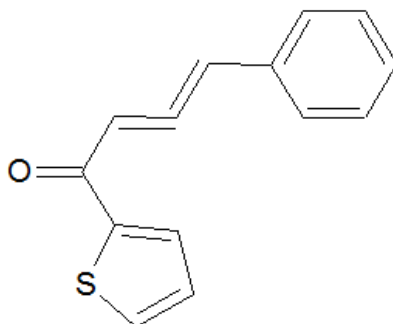
4-phenyl-1-(thiophen-2-yl)buta-2,3-dien-1-one

Molecular Formula	= $C_{14}H_{10}OS$
Formula Weight	= 226.2936
Composition	= C(74.31%) H(4.45%) O(7.07%) S(14.17%)
Molar Refractivity	= 70.10 ± 0.3 cm^3
Molar Volume	= 194.4 ± 3.0 cm^3
Parachor	= 472.3 ± 4.0 cm^3
Index of Refraction	= 1.640 ± 0.02
Surface Tension	= 34.8 ± 3.0 dyne/cm
Density	= 1.163 ± 0.06 g/cm^3
Polarizability	= 27.79 ± 0.5 10^{-24}cm^3

MASS SPECTRUM:

MOLECULAR WEIGHT:229.15g/mol
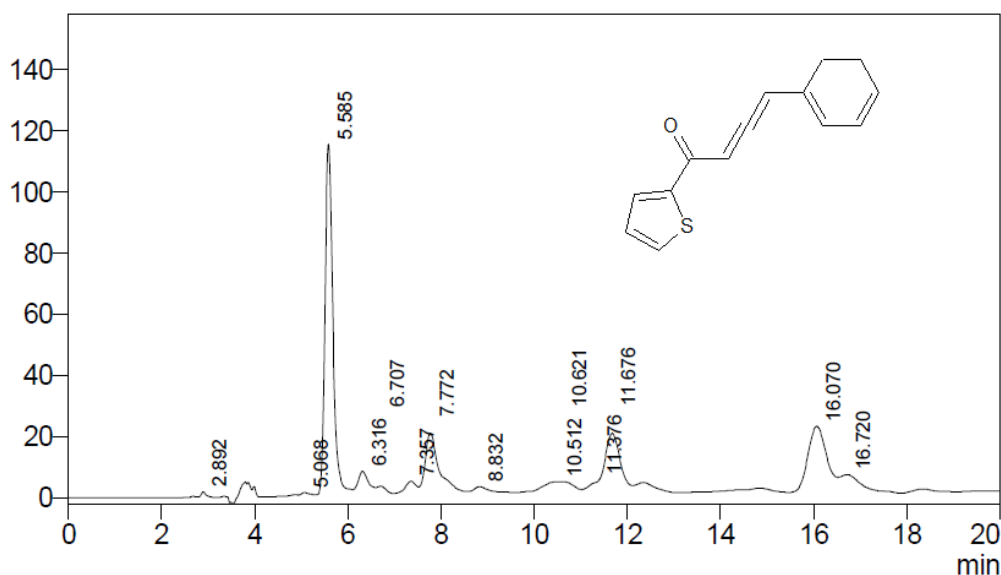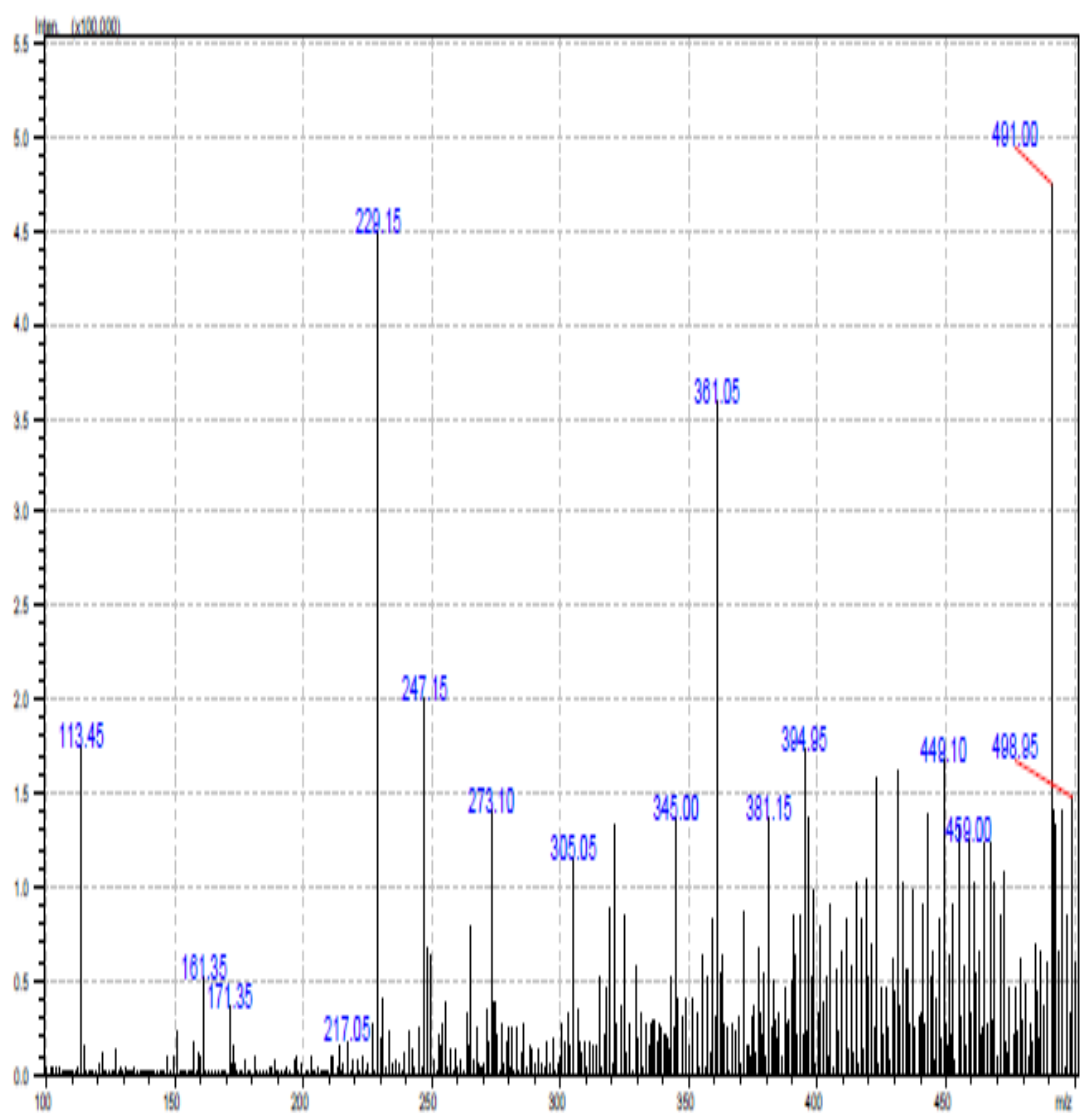
NMR SPECTROSCOPY:

Table 9: ¹H NMR INTERPRETATION OF DKN-7

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTON
1.	1.2-2.5	DOUBLET	2
2.	3.2-6.2	TRIPLET	3
3.	6.3-8.0	MULTIPLET	5

IR SPECTROSCOPY:
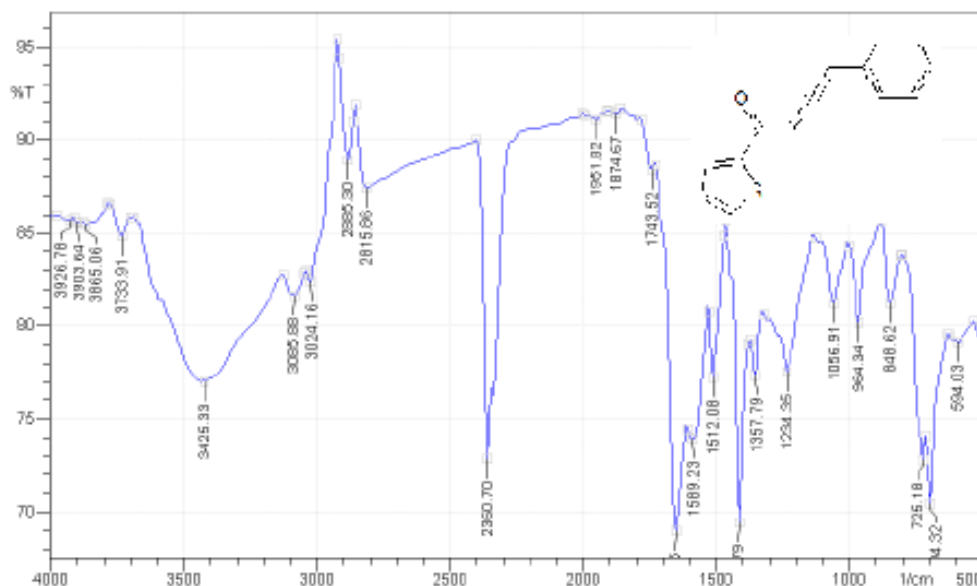
Table 10: IR Interpretation of DKN-7

S.NO	WAVE NUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUPS
1.	1743.52	C=O Stretching	Presence of Ketonic Group
2.	1650.95	C=C Stretching	Presence of Alkene
3.	3024.16	C-H Stretching	Presence of Alkane

DKN - 8
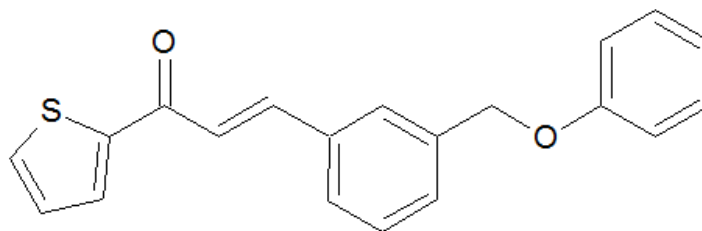
(2*E*)-3-[3-(phenoxymethyl)phenyl]-1-(thiophen-2-yl)prop-2-en-1-one

Molecular Formula	= $C_{20}H_{16}O_2S$
Formula Weight	= 320.40484
Composition	= C(74.97%) H(5.03%) O(9.99%) S(10.01%)
Molar Refractivity	= 96.65 ± 0.3 cm^3
Molar Volume	= 264.0 ± 3.0 cm^3
Parachor	= 702.9 ± 4.0 cm^3
Index of Refraction	= 1.653 ± 0.02
Surface Tension	= 50.2 ± 3.0 dyne/cm
Density	= 1.213 ± 0.06 g/cm^3
Polarizability	= 38.31 ± 0.5 10^{-24}cm^3

MASS SPECTRUM:

MOLECULARWEIGHT:319.00g/mol
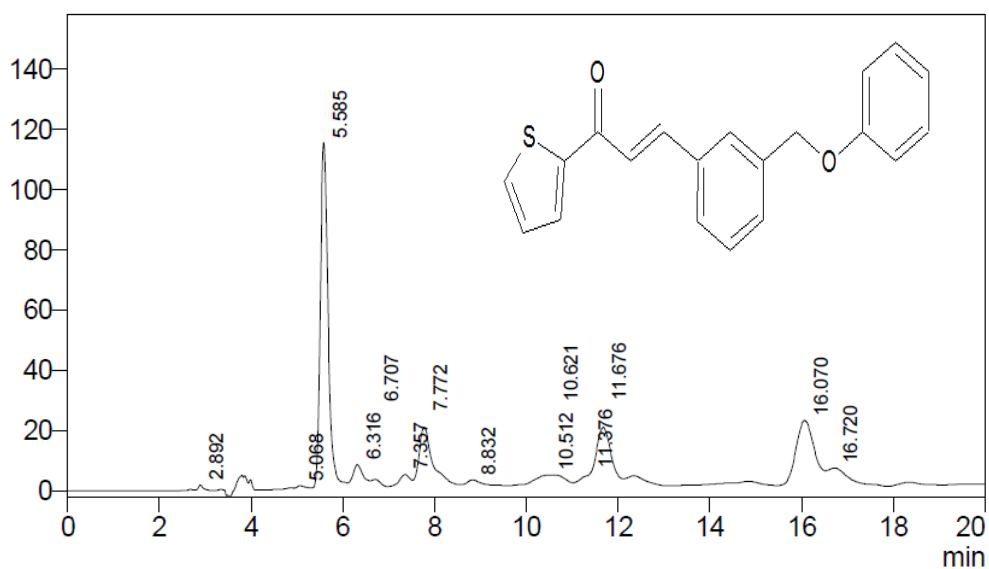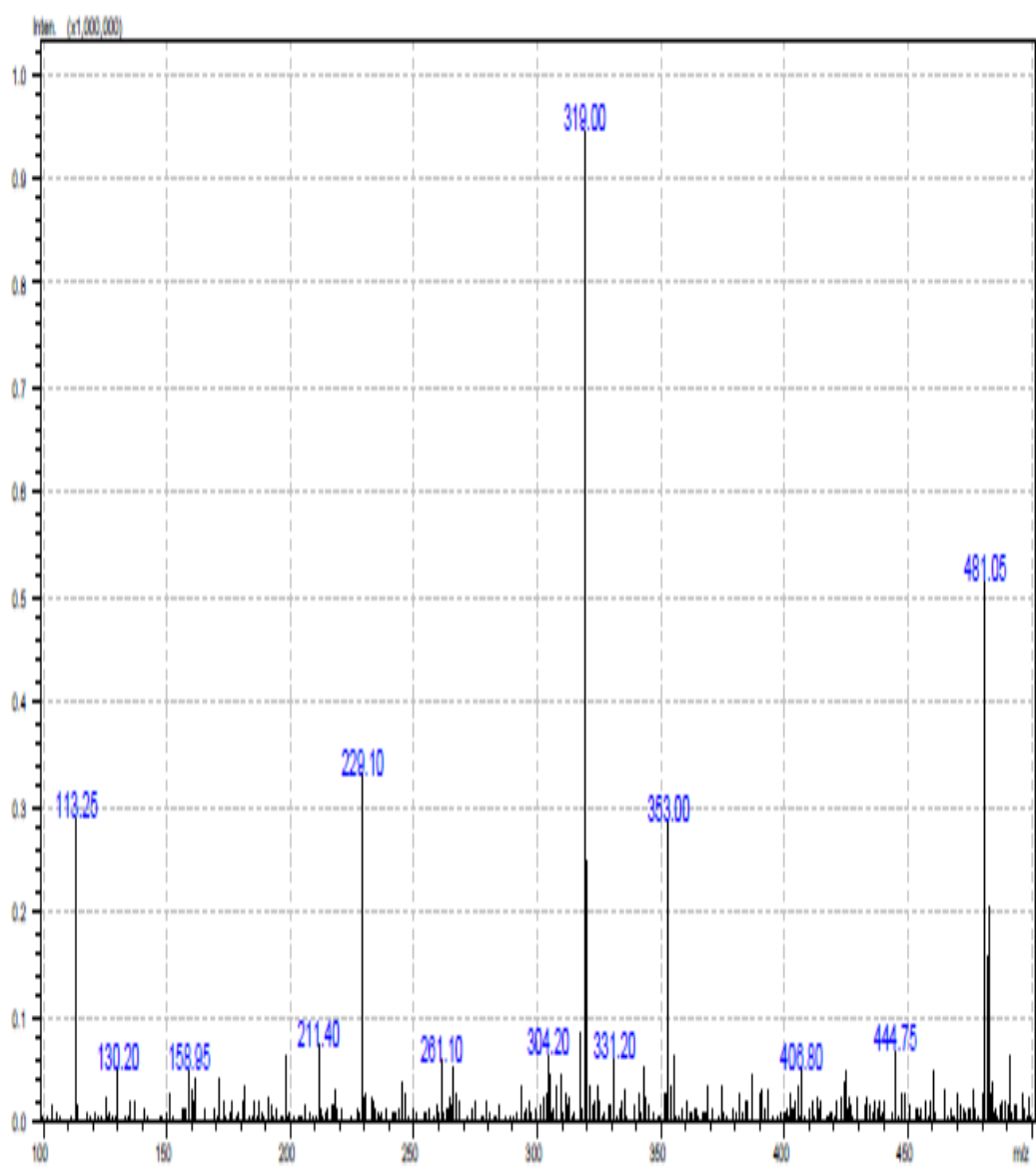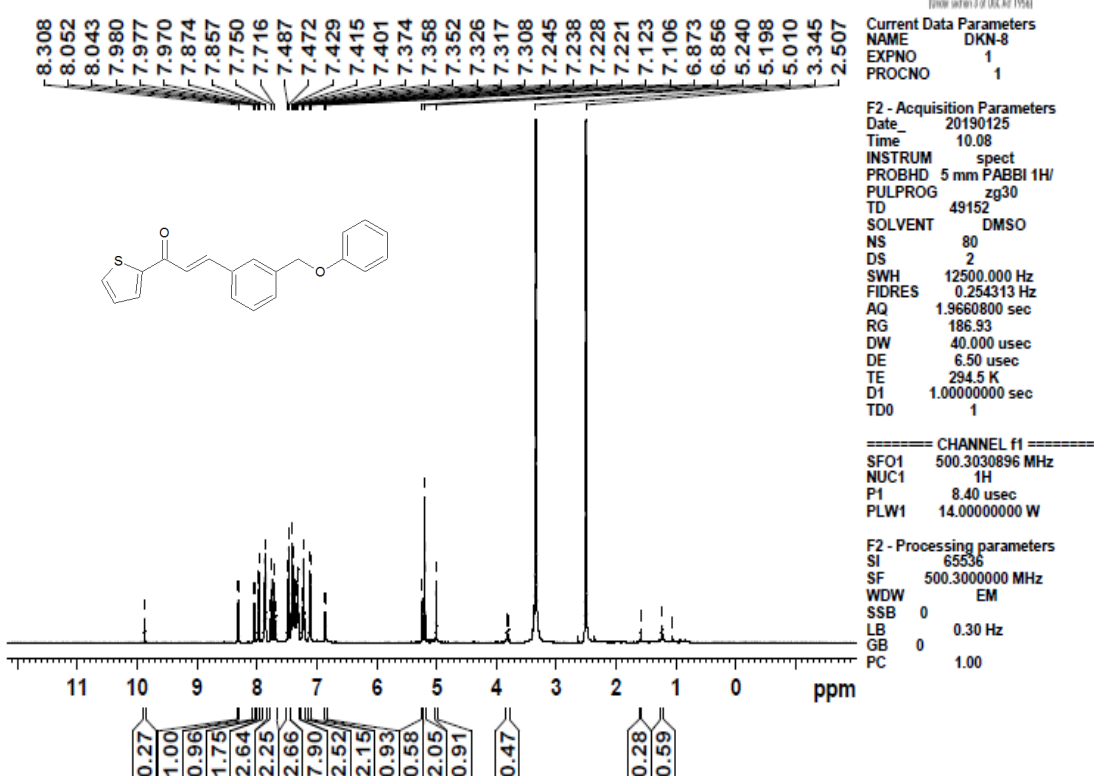
Table 11: ^{1}H NMR INTERPRETATION OF DKN-8

S. NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTON
1.	6.8-7.2	multiplet	8
2.	7.3-8.0	multiplet	8

IR SPECTROSCOPY:
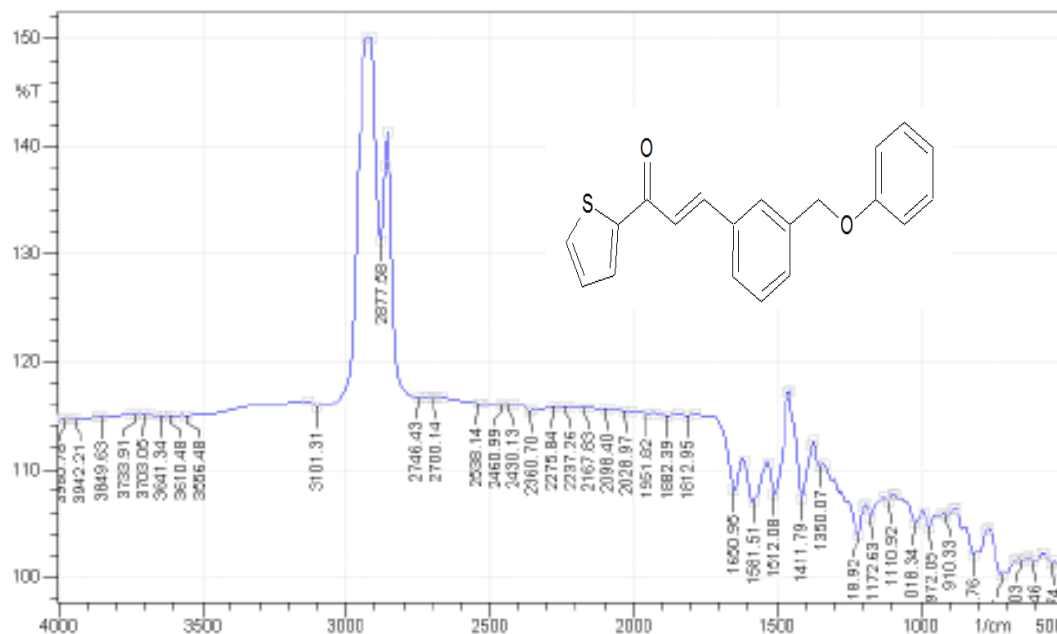
Table 12: IR Interpretation of DKN-8

S.NO.	WAVE NUMBER	TYPES OF VIBRATIONS	FUNCTIONAL GROUPS
1.	1650.95	C=C Stretching	Presence of Alkene
2.	3101.31	C-H Stretching	Presence of Alkane
3.	1812.95	C=O Stretching	Presence of ketonic group

DKN-9
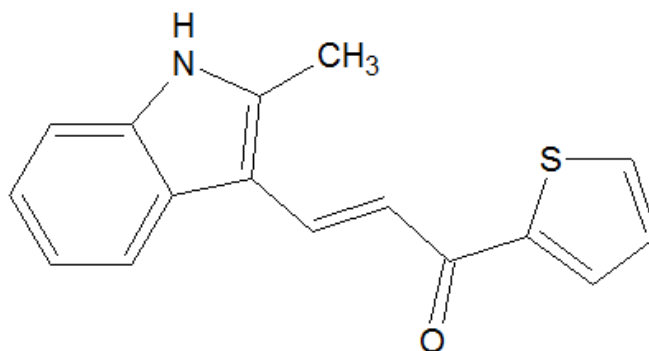
(2*E*)-3-(2-methyl-1*H*-indol-3-yl)-1-(thiophen-2-yl)prop-2-en-1-one

Molecular Formula	$= C_{16}H_{13}NOS$
Formula Weight	= 267.34552
Composition	= C(71.88%) H(4.90%) N(5.24%) O(5.98%) S(11.99%)
Molar Refractivity	$= 82.59 \pm 0.3 \ cm^3$
Molar Volume	$= 208.0 \pm 3.0 \ cm^3$
Parachor	$= 575.2 \pm 4.0 \ cm^3$
Index of Refraction	$= 1.724 \pm 0.02$
Surface Tension	$= 58.4 \pm 3.0 \ dyne/cm$
Density	$= 1.285 \pm 0.06 \ g/cm^3$
Polarizability	$= 32.74 \pm 0.5 \ 10^{-24} cm^3$

MASS SPECTROMETRY:

MOLECULAR WEIGHT:266.35g/mol

mAU

NMR SPECTROSCOPY:

AS2726A PROTON NMR IN CDCL3
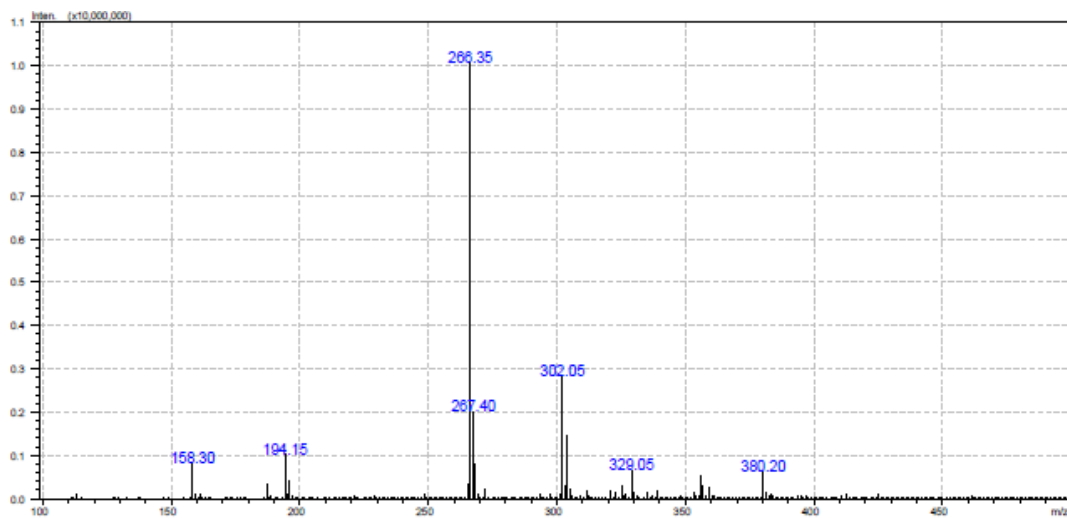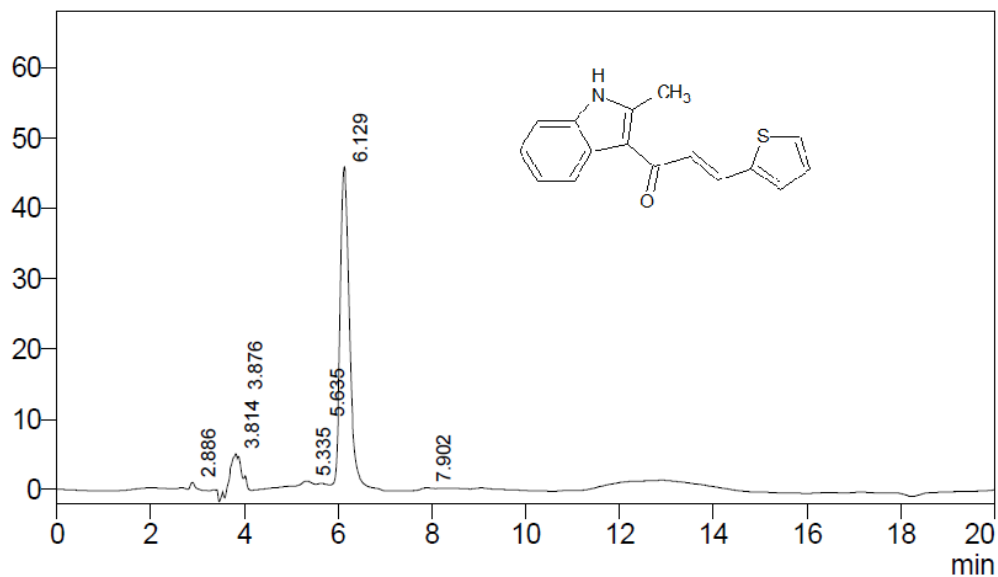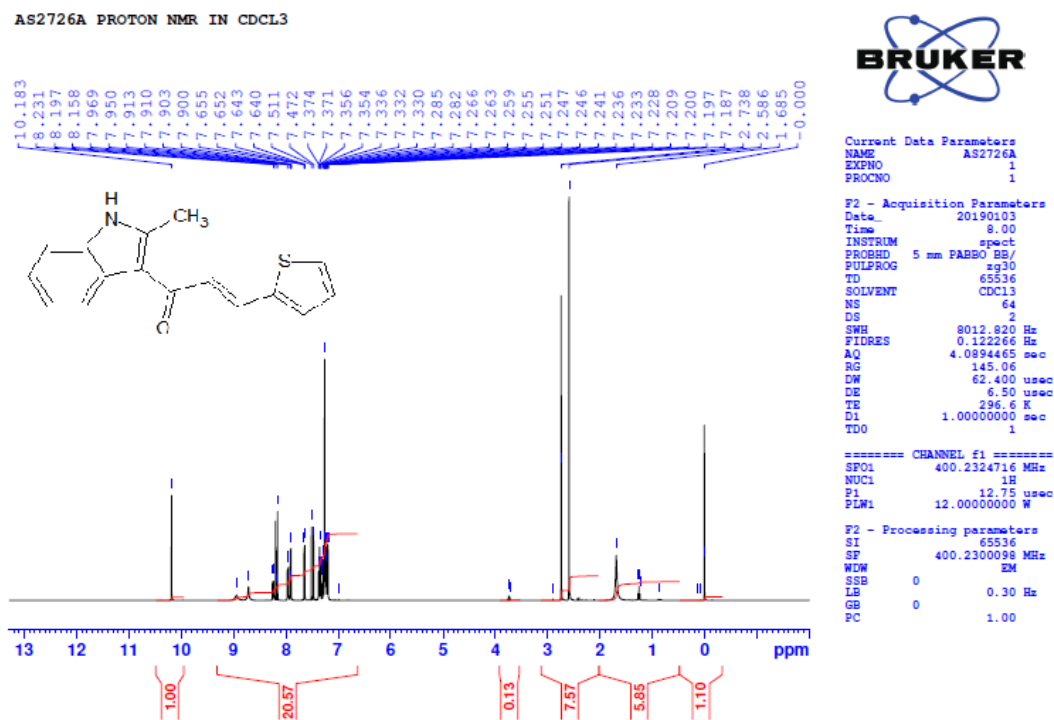
Table 13: ^{1}H NMR INTERPRETATION OF DKN-9

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTON
1.	2.5-2.7	multiplet	3
2.	7.1-7.6	multiplet	5
3.	7.9-8.2	multiplet	5

IR SPECTROSCOPY:
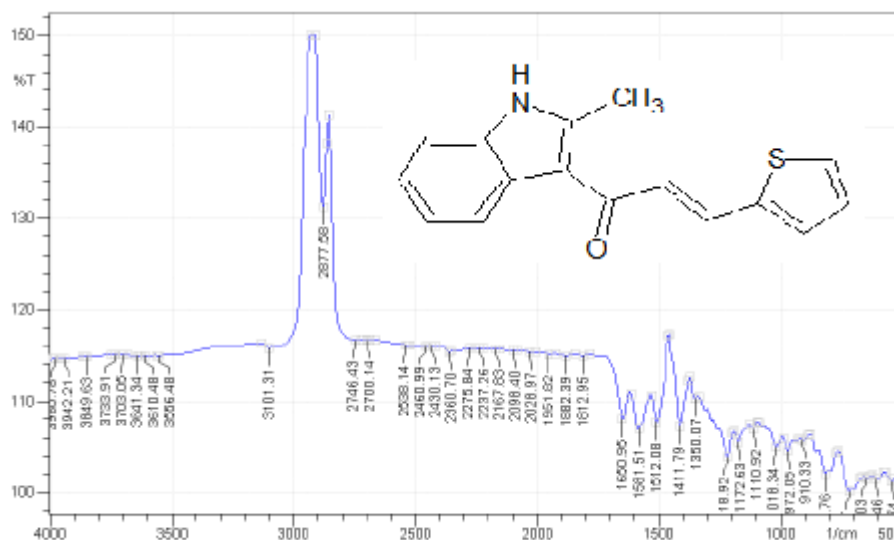
Table 14: IR Interpretation of DKN-9

S.NO	WAVE NUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUPS
1.	1743.22	C=O Stretching	Presence of Ketonic group
2.	1635.52	C=C Stretching	Presence of Alkene
3.	3363.32	-NH Stretching	Presence of Amide group

SAMPLE CODE: DKN-10
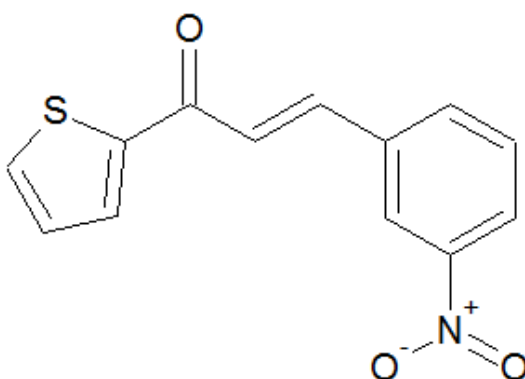
(2*E*)-3-(3-nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one

Molecular Formula	= $C_{13}H_9NO_3S$
Formula Weight	= 259.28046
Composition	= C(60.22%) H(3.50%) N(5.40%) O(18.51%) S(12.37%)
Molar Refractivity	= 72.03 ± 0.3 cm^3
Molar Volume	= 191.1 ± 3.0 cm^3
Parachor	= 529.6 ± 4.0 cm^3
Index of Refraction	= 1.677 ± 0.02
Surface Tension	= 58.9 ± 3.0 dyne/cm
Density	= 1.356 ± 0.06 g/cm^3
Polarizability	= 28.55 ± 0.5 10^{-24}cm^3

MASS SPECTRUM

MOLECULAR WEIGHT:260.00g/mol

DKN 10   14.lcd
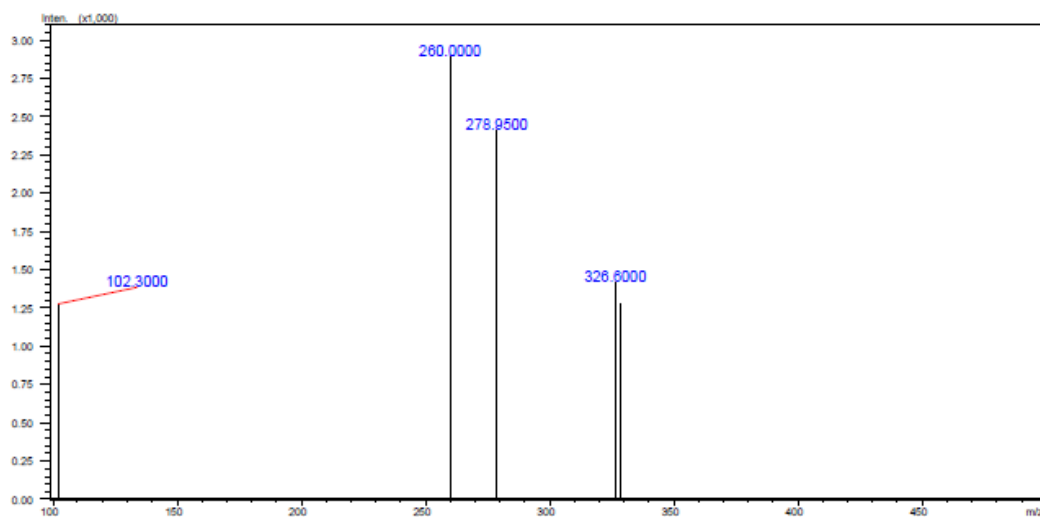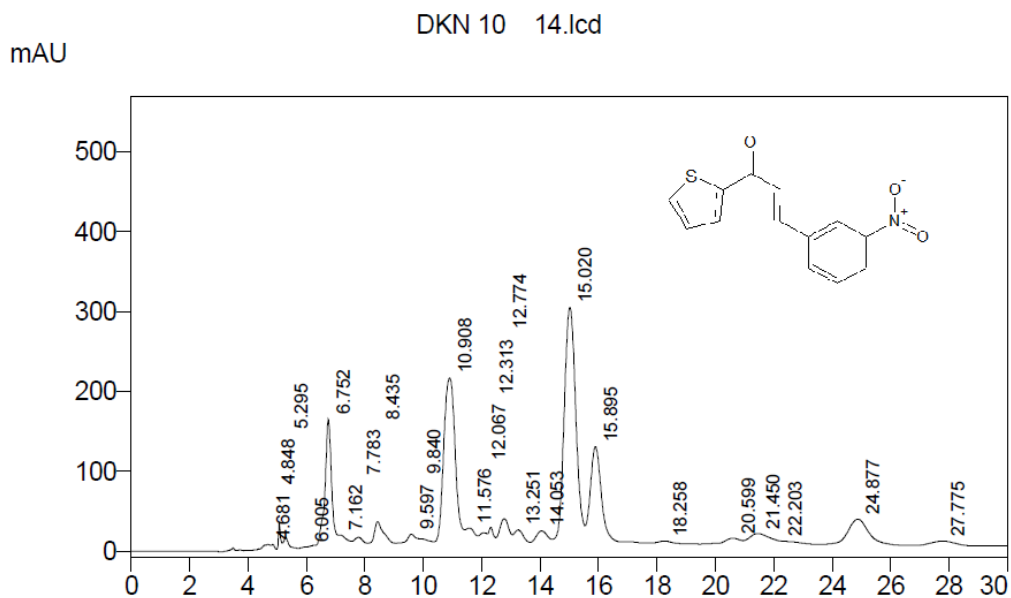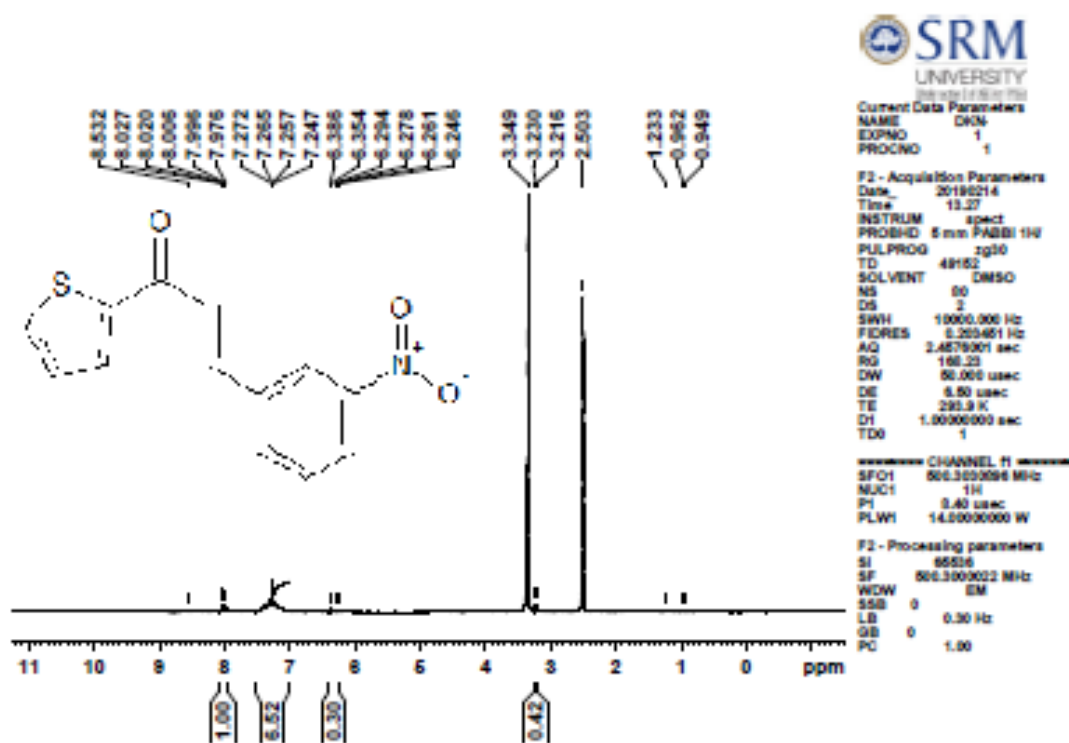
NMR SPECTROSCOPY:

Table 15: ^{1}H NMR INTERPRETATION OF DKN-10

S.NO	δ VALUE	NATURE OF PEAK	NUMBER OF PROTON
1.	2.5-3.2	singlet	2
2.	6.2-7.2	multiplet	5

IR SPECTROSCOPY:
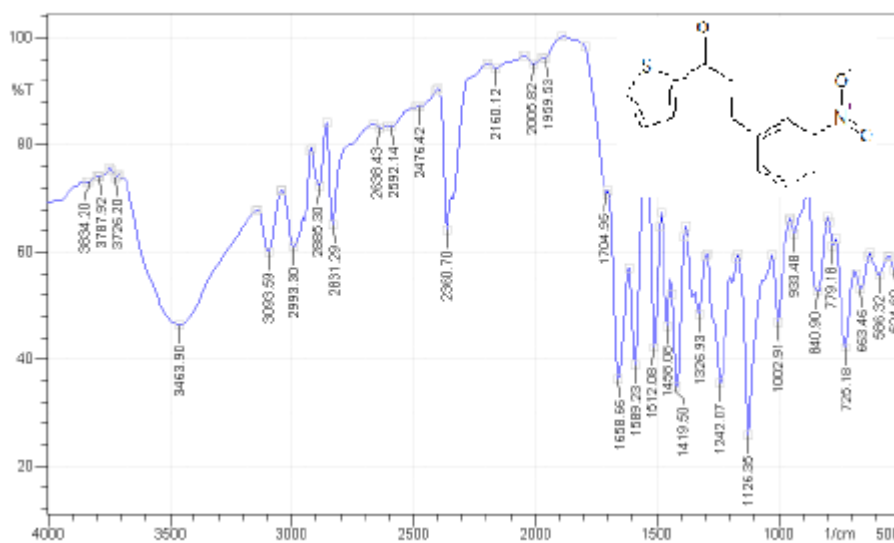
Table 16: IR Interpretation of DKN-10

S.NO	WAVE NUMBER (Cm^{-1})	TYPES OF VIBRATION	FUNCTIONAL GROUPS
1.	1583.23	NO$_2$ stretching	Presence of NO$_2$ group
2.	1658.55	C=C stretching	Presence of alkene
3.	3093.59	CH stretching	Presence of alkane

IR SPECTROSCOPY :

The IR spectrum of the synthesized compounds were inspected for presence of Stretching and Bending of the new functional groups and absence of the functional group which induced the changes in the chemical reaction.

All the compounds showed presence of the enone formation.Also these compounds showed the absence of the stretching/bending for the parent functional groups which underwent the reaction.

The stretching found in the reactant compounds were not found in the IR spectrum of the synthesized compounds.

H^{1}NMR SPECTRUM:

The number of signals in the NMR spectrum denotes the number of the set of equivalent protons in a molecule. The positions of the signals help us to know the nature of protons viz, aromatic, hetero aromatic, aliphatic ,vinyl C-H groups. Two of the synthesized compounds contain peaks from 6.8 to 8.7δ denoting the presence of aromatic protons and hetroaromatic protons between(7.1-9.0 δ).

LIQUID CHROMATOGRAPHY :

LC - MS was used to determine the purity of the compounds by looking for the additional peaks in a sample that should not be present in the case of a pure compound . Based on the report of LC-MS it is clear that all the compounds were formed as the m+1 peak or m-1 peak and the correct molecular ion peak were present.

Table 17:Molecular Weight Determination by Mass Spectra

S.NO	SAMPLE NAME	ACTUAL MASS	CALCULATED MASS
1.	DKN-5	229.10	230.29
2.	DKN-6	304.20	305.31
3.	DKN-7	229.15	226.30
4.	DKN-8	319.00	320.42
5.	DKN-9	266.35	267.35
6.	DKN-10	260.00	259.28

All the synthesized compounds exhibited molecular ion peak (m±1) of varying intensities which establishes the molecular weight corresponding to the compounds.

IN-VITRO ANTI-TUBERCULAR ACTIVITY(BIOLOGICAL EVALUATION) MICROPLATE ALAMAR BLUE ASSAY(MABA)

The MABA test is to determine the activity of the synthesized compounds at different concentration

❖ Strain used: M.tuberculosis (H37Rvstrain).

❖ Here are the standard values for the Anti-Tb test which was performed are given below

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

Table 18: Biological Evaluation of the synthesized compound

S. No	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
1.	DKN-5	S	S	R	R	R	R	R	R
2.	DKN-6	S	S	S	R	R	R	R	R
3.	DKN-7	S	S	R	R	R	R	R	R
4.	DKN-8	S	S	R	R	R	R	R	R
5.	DKN-9	S	S	R	R	R	R	R	R
6.	DKN-10	S	S	R	R	R	R	R	R

NOTE : S-SENSITIVE R- RESISTANT

The compound DKN-6 are found to have sensitivity at 25 µg/ml. The other four compounds are found to have sensitivity at 50 µg/ml.

STANDARD DRUG PHOTOGRAPH
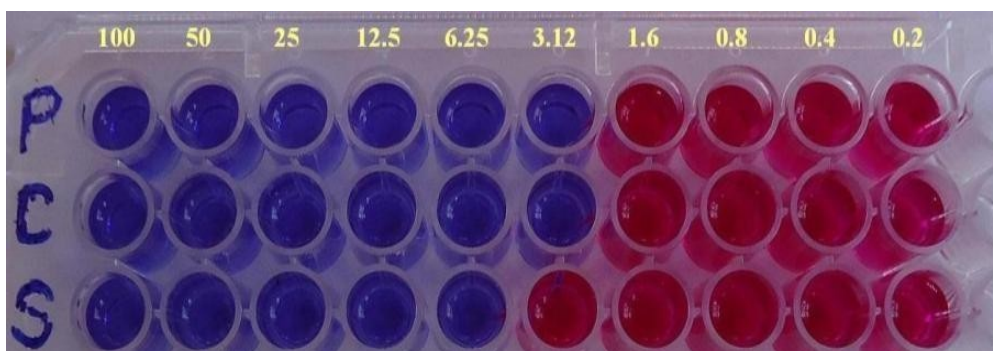
MABA reports for the synthesized compounds are listed below in the table:

Table 19: MABA reports for synthesized compounds

S. N O	NAM E	100 g/ml	50 g/ml	25 g/ml	12. 5 g/ml	6.25 g/ml	3.12 g/ml	1.6 g/ml	0.8 g/ml
1.	**DKN-5**								
2.	**DKN-6**								
3.	**DKN-7**								
4.	**DKN-8**								
5.	**DKN-9**								
6.	**DKN10**								

SUMMARY AND DISCUSSION

1. *InhA*, a critical enzyme for the cell wall synthesis of *Mycobacterium tuberculosis,* was chosen for the study after review of literature.

2. Candidate molecules were designed and docked against protein using Autodock® Tools 1.5.6 software.

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

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

5. Compounds were synthesized by conventional method and labeled as DKN-5, DKN-6, DKN-7, DKN-8, DKN-9, DKN-10.

6. Purity of the synthesized compounds was ensured by repeated recrystallization with ethanol.

7. Further the compounds were evaluated by TLC and Melting point determination.

8. The characterization of the synthesized compounds was done using Infra-red (IR), Nuclear Magnetic Resonance (H1 NMR) and Mass spectrometric method,(LC-MS).

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

10. The synthesized compounds were active at 25-50μg/ml, which were compared to the known anti-TB drugs: Pyrazinamide - 3.125μg/ml, Ciprofloxacin - 3.125μg/ml and Streptomycin - 6.25μg/ml.

CONCLUSION

- ✓ Work Concludes that the synthesized molecules are effective in inhibiting the target enzyme *InhA* , which is important for the growth of *Mycobacterium Tuberculosis* Cell wall.

- ✓ All the six compounds gave Docking score between -7.52 to -9.39kcal/mol

- ✓ They exhibited the better Docking score than the standard Anti-TB drugs like Pyrazinamide 4.41kcal/mol by using AUTODOCK$^{®}$ Software.

- ✓ Further structural refinement to the structure of the synthesized compounds is expected to yeild promising molecules against the pathogen *Mycobacterium Tuberculosis*

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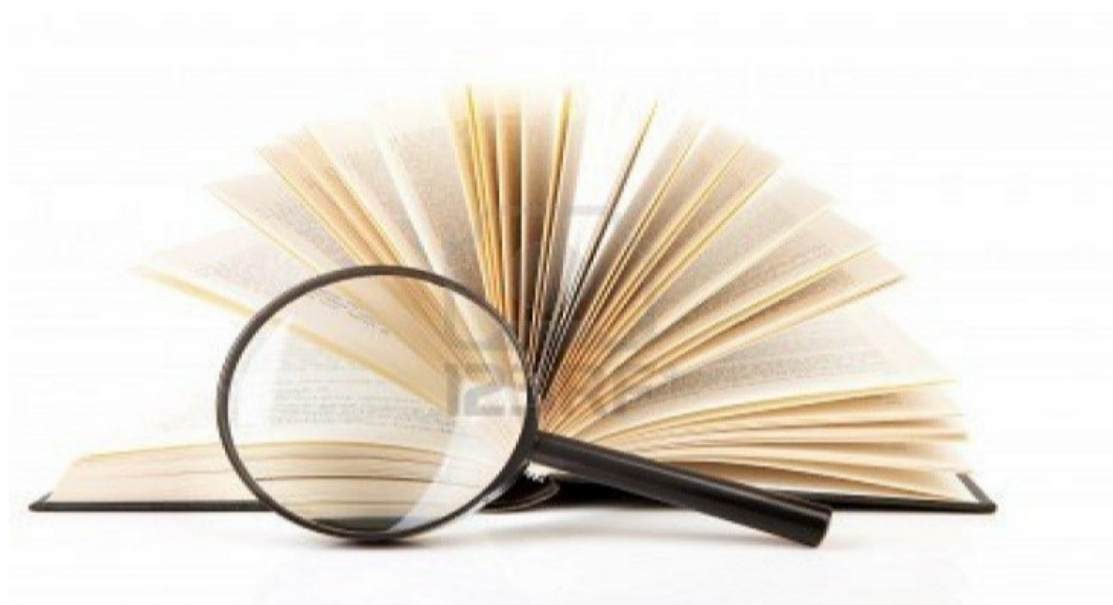
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Aim & Objective

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Annexure