"DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL INDOLE DERIVATIVES AS ANTI TUBERCULAR AGENTS TARGETING GLUTAMINE SYNTHETASE 1"

A Dissertation Submitted to
THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600032

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

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
KALAIYARASI.P
Reg.No : 261715702

Under the guidance of
Dr.A.JERAD SURESH M. Pharm., Ph.D., M.B.A
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DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
COLLEGE OF PHARMACY, MADRAS MEDICAL COLLEGE
CHENNAI-600 003

MAY 2019

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

1.

2.

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ACKNOWLEDGEMENT

First and foremost I thank the Almighty for all the love, grace and blessings he has bestowed upon me, for strengthening me throughout, to successfully complete this dissertation.

I consider this as an opportunity to express my gratitude to all the people ,who have been involved directly or indirectly with the successful completion of this dissertation.

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Last but not the least , I owe my heartfelt and deepest gratitude to my mother **P.Nageshwari,** my dear brother, sisters and my wellwisher C.Radha Krishnan.

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LIST OF ABBREVIATIONS

TB	Tubercle Bacillus
HIV	Human Immuno Deficiency Virus
AIDS	Acquired Immuno Deficiency Syndrome
DOTS	Directly Observed Treatment Short-Course
MDR-TB	Multi Drug Resistant tuberculosis
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
µg/ml	Microgram per milliliter
QSAR	Quantitative Structural Activity Relationship
˚C	Celsius
3D	Three Dimensional
mins	Minutes
Hrs	Hours
%	Percentage

INTRODUCTION

DEDICATED TO

MY FAMILY,

RESPECTED

TEACHERS AND

MY DEAR FRIENDS

INTRODUCTION

Tuberculosis is a chronic infectious disease, usually caused by the bacteria *Mycobacterium tuberculosis*. It is known to be the major reason for mortality of nearly two million people in each year.[1] About one third of the world's population is infected with TB and 5 to 10% of those can develop active TB in their life time[2] . TB generally affects the lungs called as pulmonary tuberculosis, but also affects other parts of the body like it can affect the bones, the nervous system or many other organ systems called as extra pulmonary tuberculosis. Basically it is characterized as pulmonary disease which occurs due to the accumulation of *Mycobacterium tuberculosis* (MTB) onto the lungs alveolar surfaces. [3] Synonyms for tuberculosis are phthisis, phthisis pulmonalis, consumption [4]. TB infection can occurs by inhaling the droplet containing *M.tuberculosis* organism by succeptible persons through cough, sneeze, spits, laughing, talks. [5]

Fig.1 ***Mycobacterium tuberculosis***[1]

NEED FOR AN ANTI-TUBERCULAR AGENT:

- To enhance the activity against MDR-TB strains.
- To reduce the toxicity.
- To shorten the duration of therapy
- To rapid the microbicidal mechanism of action

SIGNS OF TB: [5]

- Cough that lasts more than 3 weeks
- Chest pain
- Coughing up blood
- Tired
- Night sweat
- Chills, fever, loss of appetite and weight loss

TYPES OF TUBERCULOSIS: [6]

ACTIVE TB:

The TB bacteria rapidly multiply and invade different organs of the body. The person with active TB may spread TB to others through air.

LATENT TB:

In this condition the bacteria remains inactive in our body and causes no symptoms. Latent TB is non-contagious but has a chance of becoming active

MILIARY TB:

Miliay TB is characterized by wide dissemination into the human body and by the tiny size of the lesions (1-5mm)

EPIDEMIOLOGY:

- In 2011, there were 8.7 million new cases of active tuberculosis worldwide (13% of which involved co-infection with the Human Immuno Deficiency Virus [HIV]) and 1.4 million deaths, including 430,000 deaths among HIV-infected patients representing a slight decrease from peak numbers in the mid-2000s (Fig. 2). [7]
- It has been estimated that there were 310,000 incident cases of multidrug-resistant tuberculosis, caused by organisms resistant to at least isoniazid and rifampicin, among patients who were reported to have tuberculosis in 2011.
- More than 60% of these patients were in China, India, the Russian Federation, Pakistan, and South Africa.
- The absolute number of cases is highest in Asia, with India and China having the greatest burden of the disease globally.[7].
- A total of 84 countries have reported cases of extensively drug-resistant tuberculosis, a subset of multidrug-resistant tuberculosis with added resistance to all fluoroquinolones plus any of the three injectable antituberculosis drugs, kanamycin, amikacin, and capreomycin. [8-9]

TB BURDEN IN INDIA

Each year 12 lakh (1,200,000) Indians are notified (that is reported tothe RNTCP) as having newly diagnosed TB. In addition at least 2.7 lakh (270,000) Indians die. Some estimates calculate the deaths as being twice as high. [7]

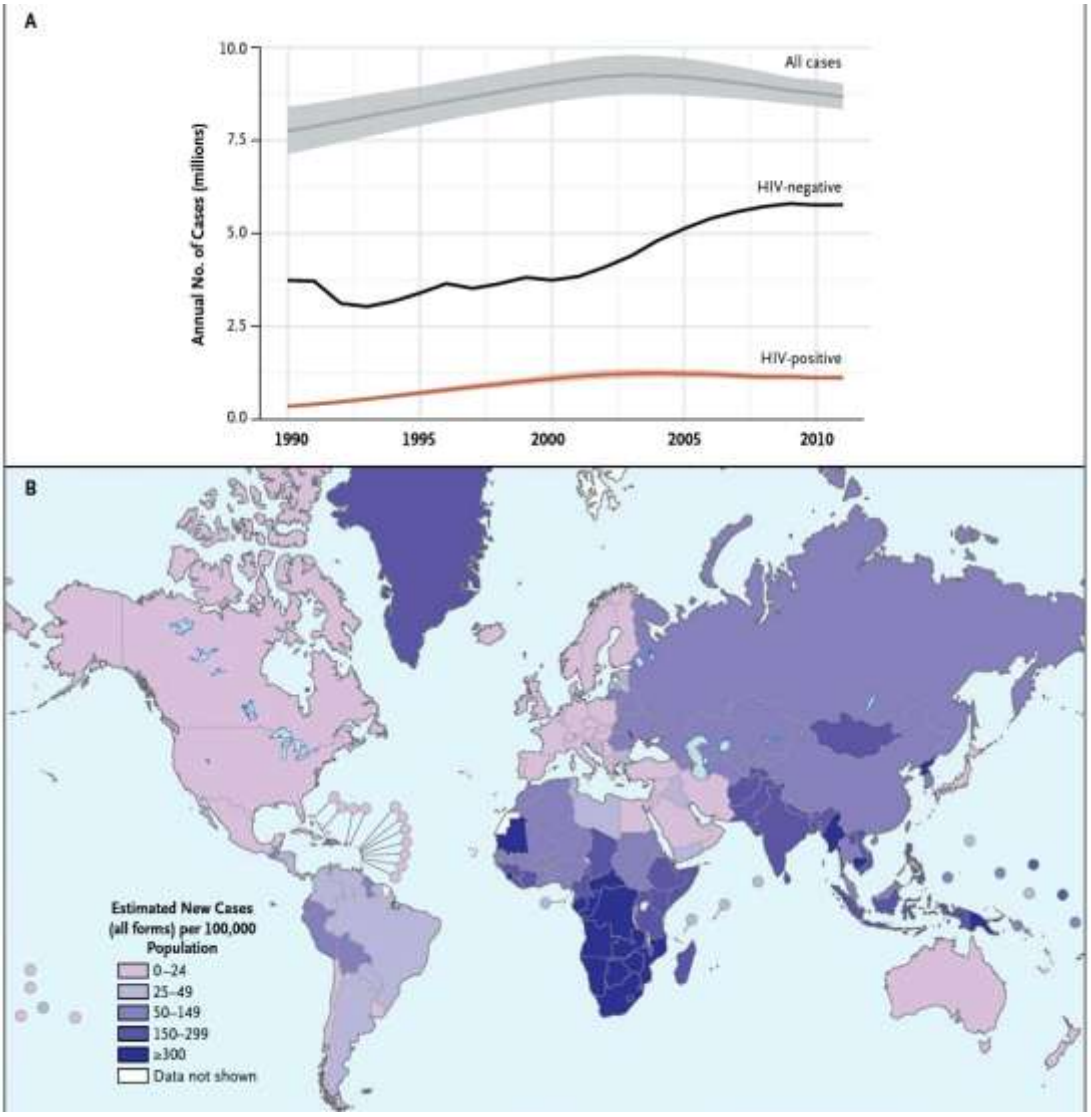


Fig.2 Global incidence of tuberculosis[31]

Panel A shows global trends in the estimated incidence of tuberculosis from 1990 to 2011 among all patients, those with human immunodeficiency virus (HIV) co-infection, and without HIV co-infection. The shading around the data curves indicates uncertainty intervals on the basis of available data. Panel B shows the estimated global incidence of tuberculosis in 2011.

MYCOBACTERIUM TUBERCULOSIS:

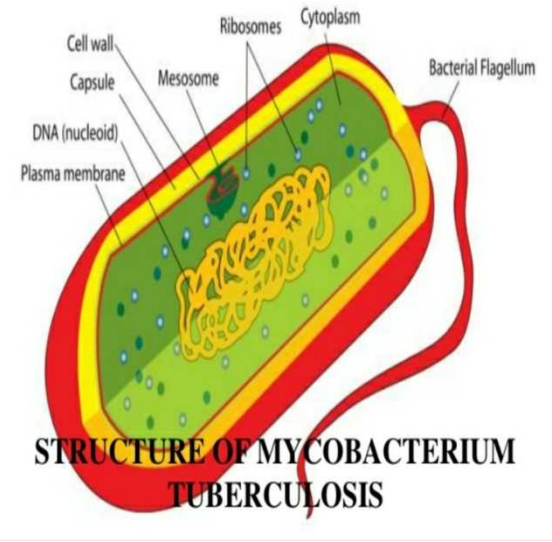


Fig.3 Structure of *Mycobacterium tuberculosis* [10]

Mycobacterium tuberculosis is a species of pathogenic bacteria in the family of Mycobacteriaceae and the causative agent of tuberculosis. It was first discovered by Robert Koch in 1882 . MTB are usually found on the well aerated upper lobes of the alveolar surfaces because they are aerobic in nature. MTB is not classified as a Gram negative or Gram positive bacteria because of a particular characteristic exhibited by its cell wall . It poses a waxy coating on its cell surface which is due to the presence of mycolic acid, which makes it insensitive to Gram staining . MTB is an intracellular parasite, and it has low generation time of 15-20 hours, which helps for its virulence factor. [10]

SCIENTIFIC CLASSIFICATION: [4]

Domain : Bacteria

Phylum : Actinobacteria

Class : Actinobacteria

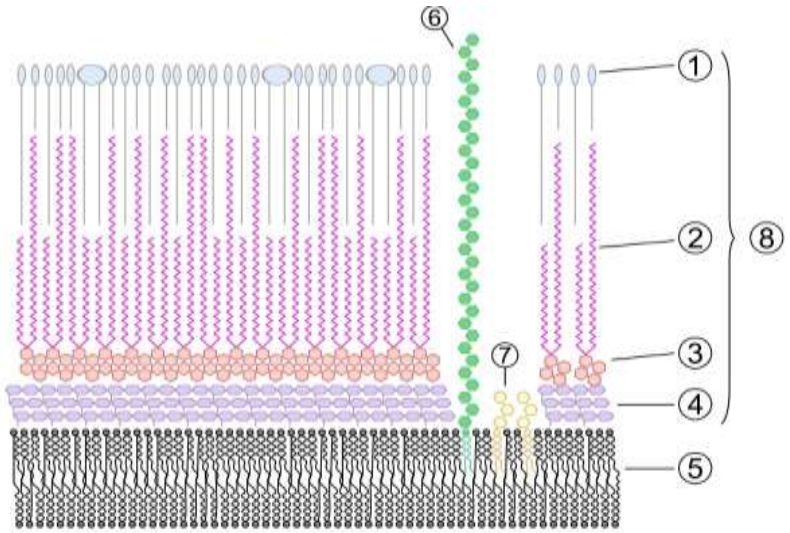
Order : Actinomycetales

Family : Mycobacteriaceae

Genus : *Mycobacterium*

Species : *M. tuberculosis*

CELL WALL OF *MYCOBACTERIUM TUBERCULOSIS:*



Mycobacterial cell wall: 1-outer lipids, 2-mycolic acid, 3-polysaccharides (arabinogalactan), 4-peptidoglycan, 5-plasma membrane, 6-lipoarabinomannan (LAM), 7-phosphatidylinositol mannoside, 8-cell wall skeleton

Fig 4. Cell wall of *mycobacterium tuberculosis* [12]

The compositional and architectural complexity of mycobacterial cell envelope distinguishes species of the Mycobacterium genus from other prokaryotes. It is the basis for many of the physiological and pathogenic features of mycobacteria and the site of susceptibility and resistance to many other anti-tubercular drugs.[11] The mycobacterial cell wall is made up of three segments, the plasma membrane, the cell wall core and outermost layer. The cell wall core is essential for viability, consists of peptidoglycan (PG) in covalent attachment via phosphoryl-N-acetylglucosaminosyl-ramnosyl linkage units with heteropolysaccharide arabinogalactan (AG). AG is in turn esterified at its non-reducing ends to long chain mycolic acids. The latter form the bulk of the inner leaflet of the outer membrane which consisting of non-covalently attached glycolipids, polysaccharides, lipoglycans and proteins.[12]

GENOME OF *MYCOBACTERIUM TUBERCULOSIS*:

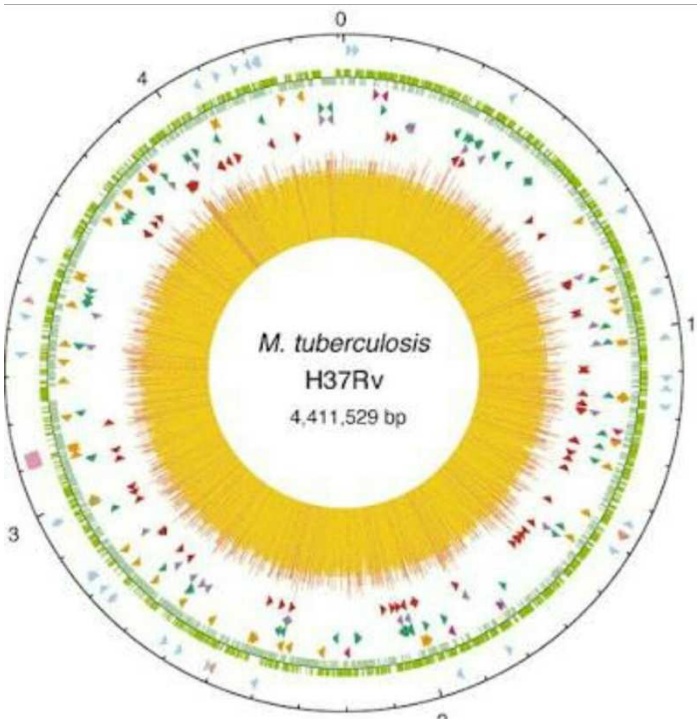


Fig.5 Genomic structure of *Mycobacterium tuberculosis* H37RV strain[13]

The genome of the H37RV strain was published in 1998. *Mycobacterium tuberculosis* has circular chromosomes containing 4,200,000 nucleotides long and the Guanine+Cytosine content of 65%.[13] The genome of M. tuberculosis was studied using the strain M. tuberculosis H37Rv [14] . Its size is 4 million base pairs, with 3,959

genes. The genome contains 250 genes involved in fatty acid metabolism, with 39 of these involved in the polyketide metabolism generating the waxy coat. About 10% of the coding capacity is taken up by the PE/PPE gene families that encode acidic, glycine-rich proteins. Nine noncoding sRNAs have been characterized in *M.tuberculosis*.[15-16]

DRUG DISCOVERY PROCESS: [17]

CADD Strategy depends upon the extent of structural information available regarding the target (enzyme/receptor) and the ligands. Direct and indirect design are the two major modeling strategies used in the drug design process. Indirect approach involves designing of drug based on comparative analysis of the structural features of known active and inactive compounds which is otherwise known as Ligand based drug design. Direct design involves the three dimensional features of the target (enzyme/receptor), otherwise known as Structure based drug design.

PREPARATION OF A TARGET STRUCTURE:

Virtual screening depends upon the amount and quality of structural information about both the target and the small molecules being docked. The first step is to evaluate the target for the presence of an appropriate binding pocket. This is done through the analysis of known target-ligand co-crystal structures or using in-silico methods to identify novel binding sites. [18-19]

X-ray crystallography or NMR techniques are used to determine the target structure experimentally. It is deposited in the PDB is the ideal starting point for docking. Based on comparative models of target proteins several successful virtual screening campaigns have been reported in the absence of experimentally determined structures. [20-21]

STRUCTURE-BASED VIRTUAL HIGH-THROUGHPUT SCREENING:

SB-vHTS selects for ligand predicted to bind a particular binding site, inhibit, or allosterically alter the protein‘s function. The key steps in SB-vHTS are: (1) preparation of the target protein and compound library for docking, (2) determining a favorable binding pose for each compound and (3) ranking the docked structures. [22]

BIOLOGICAL TARGET:

There are various biosynthetic enzymes that are essential for the survival of the Mycobacterium and are considered as potential drug targets. A comprehensive *insilico* target identification pipeline for *Mycobacterium tuberculosis* was identified and reported. It comprises a total of 451 high-confidence targets .[23]

***GLUTAMINE SYNTHETASE* 1 ENZYME:**

Glutamine Synthetase is an enzyme that plays an important role in nitrogen metabolism by catalyzing the condensation of glutamate and ammonia to form glutamine. *Glutamine synthetase* uses ammonia produced by nitrate reduction, amino acid degradation, and photorespiration. The amide group of glutamate is a nitrogen source for the synthesis of glutamine pathway metabolites. Competition between ammonium ion, influences glutamine synthesis and glutamine hydrolysis. [24]

Fig 6. Structure of *Glutamine synthetase1* enzyme

Protein name	:	*Glutamine synthetase1*
Classification	:	Ligase
Chains	:	A, B, C, D, E,F
Total structure weight	:	332264.16
Length	:	486
Gene name	:	glnA1 glnA Rv2220 MTCY190.31 MTCY427.01. [25]

MECHANISM:

GS catalyzes the ATP dependent condensation of glutamate with ammonia to yield glutamine. This mechanism takes place in two step. [26-27]

The first step is the formation of the activated intermediate glutamyl phosphate. The Mg 2+ ion coordinates the -phosphate oxygen of ATP to allow phosphoryl transfer to the carboxylate group of glutamate, yielding the intermediate (acyl phosphate). ADP and Pi do not dissociate until ammonia binds and glutamine is released. The presence of ADP causes a conformational shift in GS that stabilizes the γ-glutamyl phosphate moiety.

The second step is deprotonation of ammonium, which allows ammonia to attack the intermediate from its nearby site to form glutamine. The inhibition of GS secreted by *M.tuberculosis* is sufficient to halt the growth of the bacterium, suggesting that TB-GS might be a valid target for anti-tuberculosis drug-design. The structure of TB-GS is currently being solved to aid in the design of novel inhibitors for this enzyme .

BASIC NUCLEUS :

Indole nucleus continuously drawing interest for development of newer drug moiety due to its wide range of pharmacological activities such as antibacterial , antiinflammatory , analgesic, anti-viral , antifungal, anti-tubercular, anti-depressant. [28]

Indole is a bicyclic, heterocylic ring system in which the benzene ring is fused with pyrrole ring through the α, β-position. The word Indole is derived from the words Indigo and Oleum since indole is first isolated by treatment of indigo dye with oleum. Indole nucleus is also found in many natural products such as indole alkaloids, fungal metabolites and marine natural products. Indole occurs in coal tar and in the oils of jasmine and orange blossoms.[30]

INDOLE:

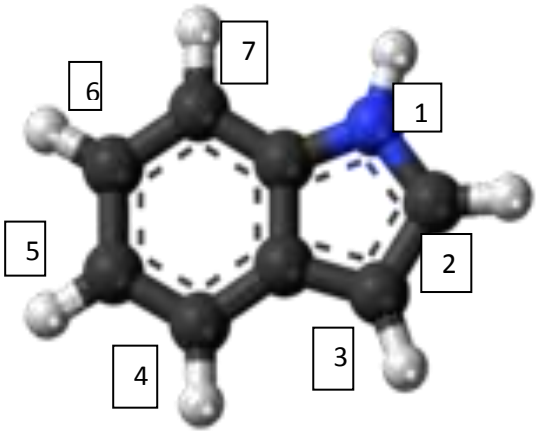


Fig.7 Structure of indole nucleus

Indole nucleus posses various medicinal activities. Insecticidal, Anti-viral activities of isatin and indole oximes and anti-inflammatory activity of indole-3-acetic acids has been reported. Oximes derivatives of 2-substituted indoles and 3-substituted indoles are reported to have Fungicidal activity. Antibacterial activity of some substituted 3-(aryl) and 3-(Heteroaryl) indoles have been reported. Indole derivatives were reported to have antioxidant activity . A new series of 1H-indole-2, 3-dione derivatives were reported for in vitro antituberculosis activity against Mycobacterium tuberculosis H37Rv [29]

REACTIVITY OF INDOLE:

- Indole can readily undergo aromatic electrophilic substitution. The C-3 position is the most nucleophilic, followed by the N and C-2 positions.
- The C-2 and C-3 bonds can often react like alkenes.
- Indole can be deprotonated at nitrogen. The resulting salts can be good nucleophiles.
- When N is substituted, C-2 can be deprotonated.
- Highly ionic salts favour N substitution.
- Softer counter ions favour C-3 substitution. [30]

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Review of literature regarding tuberculosis:

1. Alimuddin Zumla et al.,(2013) [31] reviewed the global incidence of tuberculosis and global number of cases with MDR-TB and current recommendations for tuberculosis treatment
2. Williams B.G et al.,(2010)[32] studied about Population Dynamics and Control of Tuberculosis.
3. Robert Koch (2008) [33] detailed about the history of Tuberculosis.
4. Pierpalo de colombai *et al*.,(2007) [34] has described The Global Plan to Stop TB.
5. Balabanova Y *et al*., (2006) [35] made a summary about "The Directly Observed Treatment Short-Course (DOTS) therapy.
6. Ruohonen RP *et al*., (2002) [36] Implemented the Directly Observed Treatment Short-course strategy.
7. Keane J *et al*., (1997) [37] reported that *Mycobacterium Tuberculosis* promotes Human alveolar macrophage apoptosis.

Review of literature regarding genomic aspects of *Mycobacterium Tuberculosis*:

8. Thomas R. Ioerger et al.,(2010)[38] studied about the variation among the Genomic sequences of H37Rv strains of *M.tuberculosis.* They carried whole genomic sequencing on six strains of H37Rv from different laboratories.
9. Zheng et al.,(2008) [39] determined the whole genomic sequence of attenuated *M.Tuberculosis* H37Ra, and performed comparative genomic analysis of H37Ra against its virulent counterpart H37Rv , and studied their genetic variations which is useful for understanding the pathogenesis of *M.Tuberculosis*.

Review of literature regarding the enzyme *glutamine synthetase 1:*

10. Wojciech W. Krajewski et al.(2008) [40] summarized that *glutamine synthetase* catalyzes the ligation of glutamate and ammonia to form glutamine, with the hydrolysis of ATP.

11. Andreas Burkovski (2003) [41] studied about the Ammonium assimilation and nitrogen control in M. tuberculosis with emphasis on the GSI enzyme, which has been identified as a potentially important determinant of pathogenicity.
12. David Eisenberg et al (1999) [42] studied about the highly regulated glutamine synthetase enzyme at the core of nitrogen metabolism. They studied about both bacterial and eukaryotic glutamine synthetases, with emphasis on enzymatic inhibitors.
13. Woolfolk and Stadtman (1967) [43] done the kinetic studies showed that the biosynthetic reaction catalyzed by E.coli GS is inhibited by nine end products of glutamine metabolism: serine, alanine, glycine, AMP,CTP, tryptophan, histidine, carbamoyl phosphate,and glucosamine-6-phosphate.

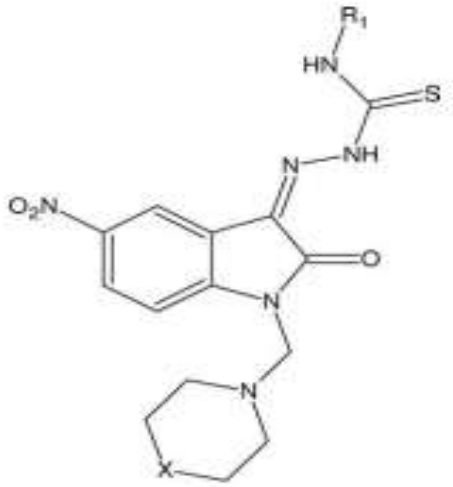
Review of literature regarding drug design:

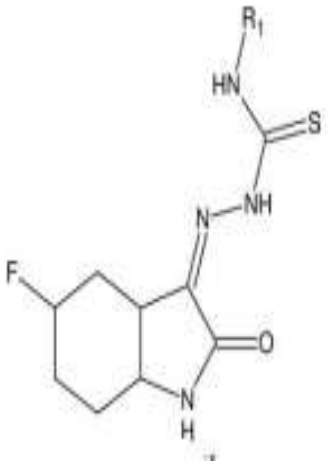
14. Pratik Swarup Das and Puja Saha (2017) [44] studied about the tools and techniques to assist in drug discovery process.
15. Wermuth C G., (2006) [45] reviewed the similarity in drugs with the importance and reflections on analogue design.
16. Laurie AT, Jackson RM (2006) [46] studied about the methods for the prediction of protein-ligand binding sites for the structure based drug design in virtual screening process.
17. Lipinski CA. et al. (2001) [47] reported the approaches to estimate the solubility and permeability in drug discovery experimentally and computationally.

Review of literature regarding indole nucleus:

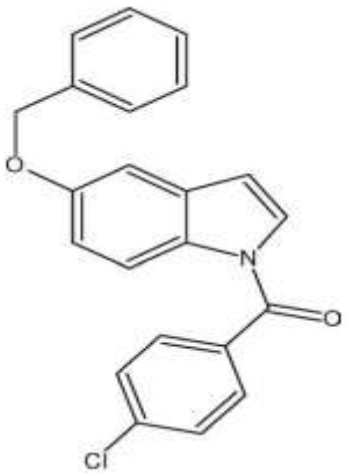
18. Amit K Singh et al(2013) [48] synthesized novel indole derivatives and evaluated for invitro anti-bacterial, anti fungal and anti-inflammatory activity.

19. Karali et al(2007) [49] synthesized a series of 1H-indole-2,3-dione derivatives and evaluated for in vitro antituberculosis activity against Mycobacterium tuberculosis H37Rv strains. Among the synthesized compounds 5-nitro-1H-indole-2,3-dione-3-thiosemicarbazones and its 1-morpholinomethyl derivatives exhibited significant inhibitory activity with MIC values 75%.

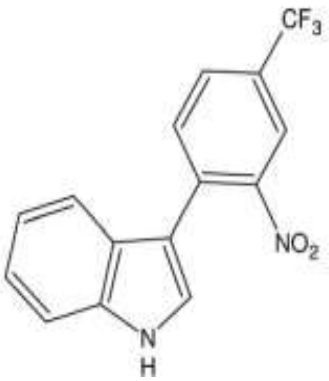




20. Li et al (2007) [50] synthesized some indole derivatives and the compounds were evaluated for their insulin sensitizing and glucose lowering effects The indole derivatives showed good anti-diabetic activity.



21. Hiari et al(2006) [51] synthesized some substituted 3-(aryl) and 3-(heteroaryl) indoles were reported to have anti bacterial activity. The most active compound was reported to be 3-(4-trifluoromethyl-2-nitrophenyl) indole

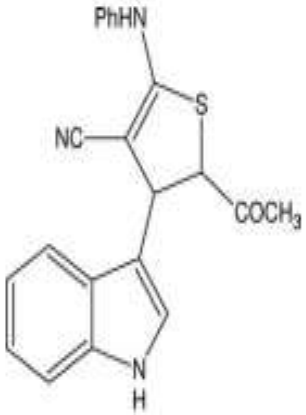


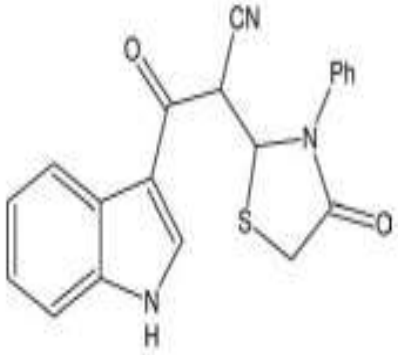
22. Hong et al (2006) [52] synthesized a series of tricyclic and tetracyclic indoles and evaluated for anticancer activity. the compounds found to exhibit highest in vitro activity against human nasopharyngeal carcinoma (HONE-1) and gastric adenocarcinoma (NUGC-3)

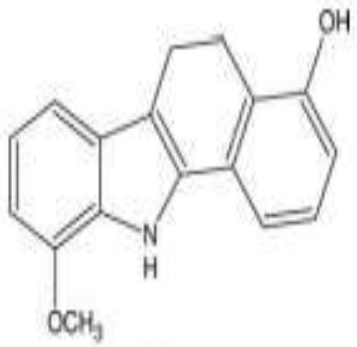
23. Enien et al (2004) [53] found that Indole-2 and 3-carboxamides having antioxidant properties.

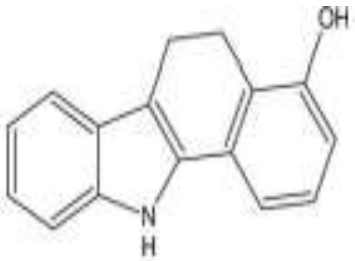
24. Abele et al (2003) [54] synthesized indole oximes were found to be exhibiting high fungicidal activity. where the oxime derivates of 2-substituted indoles demonstrated significant antifungal activity.

25. Radwan et al (1997) [55] carried out the synthesis and biological evaluation of 3-substituted indole derivatives as potential anti-inflammatory and analgesic agents. They reported 3-(3-indolyl) thiophene derivative as apotent anti-inflammatory compound whereas thiazolidine-4-one derivative exhibit analgesic activity.

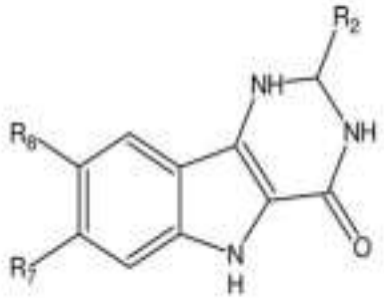








26. Merino et al(1999) [56] synthesized analogs of pyrimido [5,4-b] indoles and biologically evaluated for their possible HIV inhibitory activity.



Review of literature regarding isatin derivatives:

27. Deng et al.,(2018) [57] synthesized a series of novel heteronuclear 5-fluoroisatin dimers tethered through ethylene and examined for their in vitro anti-mycobacterial activities against Mycobacterium tuberculosis H37Rv strains and multi-drug resistant tuberculosis (MDR-TB).

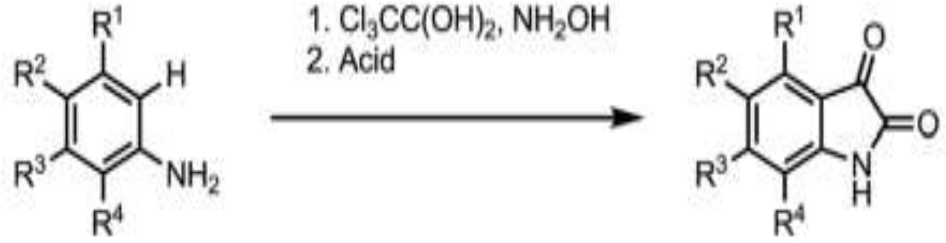
28. Parvanesh Pakravanet al.,(2013) [58] studied that Isatin derivatives has the Sability to intercalate with DNA. Among them, Isatin-3-isonicotinyl hydrazone was found to be potentially capable of intercalation with DNA.

29. Verma et al.,(2003) [59] reported that Schiff bases of N-methyl and N-acetyl isatin derivatives with different aryl amines posses potent anticonvulsant activities.

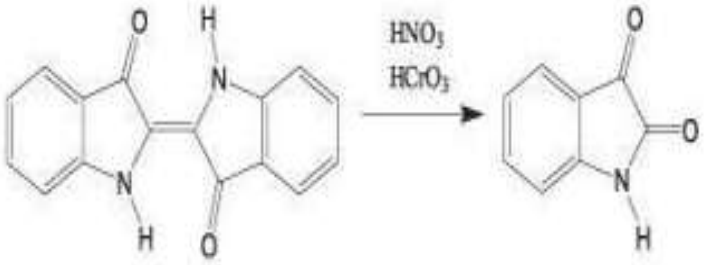
30. Garden et al.,(1998) [60] described the synthesis of N-alkylated isatins from the respective isatins using calcium hydride and alkyl halide in DMF.

31. Pinto et al., (1994) [61] detailed that the synthesis of N-alkyl indoles under mild reaction conditions using N-acetyl isatins as substrates.

32. Sandmeyer et al., (1919) [62] developed the method for the synthesis of isatin. It consists of the reaction of aniline with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate to form an isonitrosoacetanilide, which after isolation, when treated with concentrated sulfuric acid, gives isatin.



33. Erdmann et al.,(1840) [63] synthesized 1H-indole-2,3-dione (Isatin) by reaction of indigo with chromic and nitric acids.



Review of literature regarding to the evaluation of anti TB activity:

34. Anitha G. et al (2009) [64] described a rapid invitro method using Microplate Alamar Blue Assay to determine the Minimum Inhibitory Concentration for novel antimycobacterial agents.

35. Neetu kumar Taneja and Jaya Siwaswami Tyagi (2007) [65] determined the MIC for M.tuberculosis using aerobic resazurin microplate assay(REMA) and correlated with those obtained by the Colony Forming Unit assay.

36. Juan-Carlos P et al,(2002) [66] summarized the method for detecting multidrug-resistant Mycobacterium tuberculosis by using Resazurin Microtiter Assay Plate method (REMA).

AIM AND OBJECTIVE

AIM AND OBJECTIVES

AIM

The aim is to design and synthesize some novel heterocyclic analogues such as indole derivatives which will prove to be effective against *Mycobacterium tuberculosis.*

OBJECTIVES:

- Design of *Glutamine synthetase* inhibitors by docking studies using Autodock ® software
- Prediction of *Insilico* Drug likeness by Molinspiration® software.
- *Insilico* Toxicity Assessment done by OSIRIS® software.
- Laboratory synthesis of chosen compounds with top Docking Scores.
- Characterization of the synthesized compounds by
    - TLC
    - Melting point
    - IR Spectroscopy
    - H1 NMR Spectroscopy
    - LC-Mass Spectrometry.
- Evaluation of *In-vitro* anti -tubercular activity of the synthesized compounds by (MABA).

PLAN OF WORK

Design of *Glutamine synthetase 1* inhibitors by using Autodock software.

Insilico Drug likeness Prediction by Molinspiration ®

Insilico Toxicity prediction by OSIRIS ®

Laboratory synthesis of the selected compounds

Justification of purity by TLC, Melting point and Characterization by IR, NMR, LC-MS

Evaluation of *invitro* anti -tubercular activity of synthesized compounds by MABA

MATERIALS AND METHODS

MATERIALS AND METHODS

The Project is carried out in the following phases.

- Drug design by using Autodock® software.
- Synthesis of the designed molecules.
- Characterization of the synthesized molecules.
- Biological evaluation of the synthesized molecules.

DRUG DESIGN

Drug design is referred to as rational drug design, which is an inventive process of finding newer drug molecules based on the knowledge of a biological target. [67] The drug is an organic small molecule that activates or inhibits the function of a biomolecule such as protein, which in turn results in a therapeutic benefit to the patient. Drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and will bind to it. [68]

TYPES OF DRUG DESIGN

There are two major types of drug design.

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

LIGAND-BASED [69]

Ligand-based drug design (or indirect drug design) depends upon the knowledge of other molecules that bind to the biological target of interest (protein). The other molecules may be used to derive a pharmacophore model that offers 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) depends upon the 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[70-71]

The crystal structure of the enzyme was downloaded from the Protein Data Bank (An Information Portal to Biological Macromolecular Structures) (PDB id – 3zxr). The target enzyme *glutamine synthetase I* from *Mycobacterium tuberculosis*, is one of the key enzymes involved in GLUTAMINE SYNTHESIS , which is critical for the survival and growth of Mycobacterium tuberculosis.

BINDING SITE IDENTIFICATION [72-73]

Binding site identification is an important step in structure based drug design. Location of the binding site is trivial, if the structure of the target or a sufficiently similar homolog is determined in the presence of a bound ligand. However, there may be unoccupied allosteric binding sites that may be of interest. Furthermore, it may be only apoprotein (protein without ligand) structures are available and the reliable identification of unoccupied sites that have the potential to bind ligands with high affinity is non-trivial.

Molecular Docking by AUTODOCK ®

AutoDock ® 4.2.5.1 is a software for predicting the interaction of ligands with biomacromolecular targets . In any docking scheme, two conflicting requirements must be balanced: the desire for a robust. 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. The following steps are employed [74]

1. Protein preparation.
2. Ligand preparation .
3. Receptor grid generation.
4. Ligand docking (screening)

Preparation of Protein

- Read molecule from the file (allows reading of PDB coordinatefiles.)
- Edit -Charges – Compute Gasteiger (for arbitrarymolecules)
- Edit – Hydrogen –Merge non polar.
- Save as .pdbinAutoDockfolder.

Preparation of Ligand

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

Grid preparation

- Grid – Macromolecule -open (open the pdb file thet has beensavedand then save it in pdbqt extension in AutoDock folder)
- Grid – Set map types –open ligand : tools to define the atom types for the grids that will be calculated
- Grid – Grid box – launches interactive commands for setting the grid dimensions and center (Set dimension of 60: 60:60 – Center :center on macromolecule)
- File – Close savingcurrent
- Grid – Output – save as .gpf(grid parameterfile)
- Open command prompt [cdAutoDock

  cd 4.2.5.1

  autogrid4.exe –p a.gpf –l a.glg]

Preparation of Docking Parameters

- Docking –macromolecules – set rigid filename
- Docking – ligand –open
- Docking –search parameters – genetic algorithm parameters : this command open a panel for setting the parameters used by each of the search algorithms, such as temperature schedules in simulated annealing and mutation/crossover rates in genetic algorithms.
- Docking – docking parameters: opens a panel for setting the parameters used during the docking calculation, including options for the random number generator, options for the force field, step sizes taken when generating new conformations, and out put options.
- Docking- output –Lamarkian GA –save as .dpf (docking parameterfile)
- Open command prompt [autodock4.exe –p a.dpf –la.dlg]

Visualization / Interpretation of Docking

- Analysis –Docking – open .dlg (docking log file)file
- Analysis – macromolecule open
- 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 individual conformations
- Analysis – Docking – show interaction: specialized visualization to highlight interactions between the docked conformation of the ligand and the receptor.

INSILICO TOXICITY ASSESSMENT[75]

OSIRIS®

- *Insilico* toxicity Assessment for the molecules were predicted by using OSIRIS®, a JAVA based online tool.
- The tool predicts toxicity related parameters such as Mutagenicity, Tumorogenicity, Skin Irritancy and the effects on reproduction.

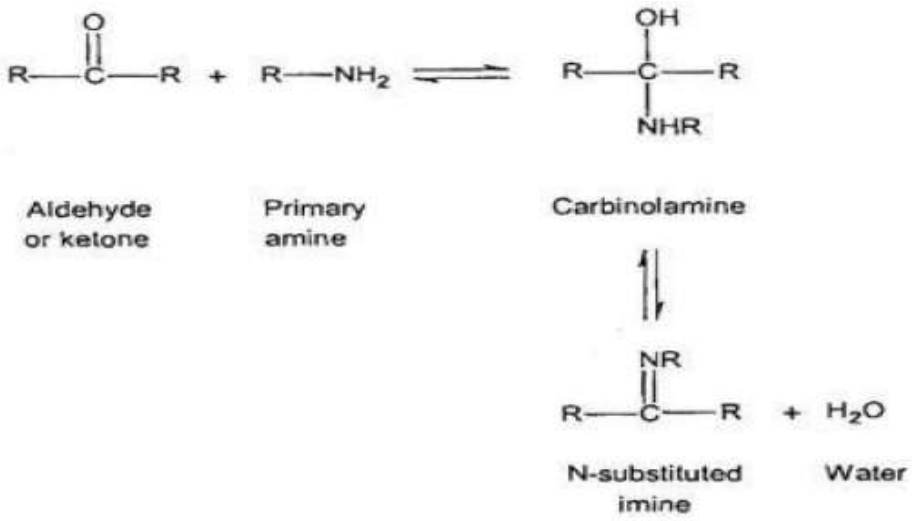
- The prediction is based the fragment contribution group present in the structure of the molecule.
- Properties with high risks of undesired effects are shown in red color. Whereas a green color indicates drug-conform behavior.

PREDICTION OF DRUG LIKENESS (MOLINSPIRATION®)

- The designed and docked molecules were screened *insilico* using MOLINSPIRATION® software to evaluate drug likeness.
- It is the online software available for the calculation of important molecular properties such as log P, polar surface area, number of hydrogen bond donors and acceptors., etc

SYNTHETIC SCHEME

The selected compounds with top docking score were selected for synthesis according to the scheme given below.



PROCEDURE

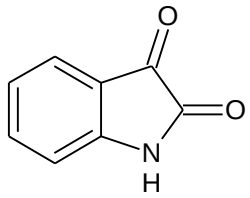
Equimolar quantities of ketone (0.01mol) and Para-substituted amine (0.01mol) are added into 20mL of absolute ethanol and 5mL of glacial acetic acid is added to it. Reaction mixture is refluxed for 24hrs at 60ºC. Completion of reaction is confirmed by TLC. The product obtained was filtered and dried. Recrystallisation is done by using ethanol.

REACTANT PROFILE

The following ketone and primary amines were used for the synthesis.

KETONES

ISATIN:



Chemical formula : $C_8H_5NO_2$

Molecular weight : 148.13g/mol

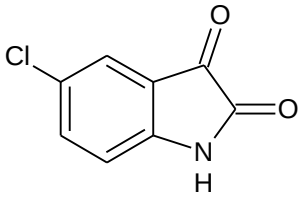
Appearance : Orange red solid

Melting point : 200°C

Solubility : dichloro methane, acetone

Boiling point : 360.3±52.0°C

5-CHLORO ISATIN:



Chemical formula : $C_8H_4NO_2Cl$

Molecular weight : 181.58 g/mol

Appearance : Yellow to Orange crystal

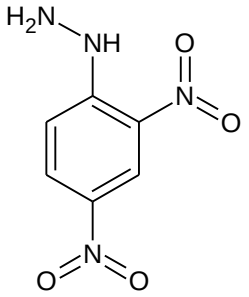
Melting point : 254-258°C

Solubility : hot water

Boiling point : 254-258°C

PRIMARY AMINES

2,4-DINITRO PHENYL HYDRAZINE



Chemical formula : $C_6H_3(NO_2)_2NHNH_2$

Molecular weight : 198.14 g/mol

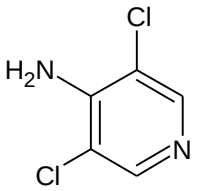
Appearance : Red to Orange crystal

Melting point : 198-202°C

Solubility : slightly soluble in water

Boiling point : 378.6±32.0°C

4-AMINO 3,5-DICHLORO PYRIDINE



Chemical formula : $C_5H_4Cl_2N_2$

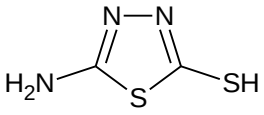
Molecular weight : 163 g/mol

Appearance : off white colour

Melting point : 159°C

Solubility : slightly soluble in methanol

5-AMINO 1,3,4-THIADIAZOLE-2-THIOL



Chemical formula : $C_2H_3S_2N_3$

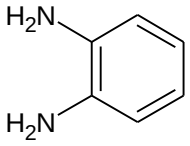
Molecular weight : 133.18 g/mol

Appearance : Cystalline white powder

Melting point : 235°C

Solubility : insoluble in water, soluble in DMSO, slightly soluble in methanol.

O-PHENYLENE DIAMINE



Chemical formula : $C_6H_4(NH_2)_2$

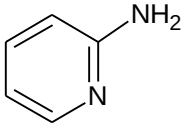
Molecular weight : 108.14 g/mol

Melting point : 102-104°C

Boiling point : 252°C

Solubility : soluble in hot water

2-AMINO PYRIDINE



Chemical formula : $C_5H_6N_2$

Molecular weight : 94.12 g/mol

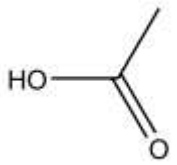
Appearance :colourless solid

Melting point : 59-60°C

Boiling point : 210°C

Solubility : slightly soluble in water.

GLACIAL ACETIC ACID:



Molecular Formula : $C_2H_4N_2$

Molecular Weight : 60.05

Boiling Point : 390.87[K]

ETHANOL:

Molecular Formula : C_2H_6O

MolecularWeight : 46.07

BoilingPoint : 337.54[K]

CHARACTERIZATION

PHYSICAL EVALUATION

1. Physical properties of the synthesized compounds are evaluated, such as
   - Color
   - Nature
   - Solubility
   - Molecular weight
   - Molecular formula
   - Melting point

2. Further the synthesized compounds are characterized by the following Spectroscopic and Spectrometric methods [76] such as
    - IR Spectroscopy by ABB MB 3000-PH FTIR spectrometer using KBr pellets.
    - ^{1}H-NMR Spectrometry by 500 MHZ BrukerTopSpin using DMSO
    - LC-MS by AGILANT technologies 6230B Time Of Flight(TOF)

IR SPECTROSCOPY [77]

The absorption of infrared radiations can be expressed in terms of wavelength or in wavenumber. The infrared region ranges from wavenumber 4000cm^{-1} to 667cm$^{-1.}$ The bonds in a molecule which are accompanied by a change in dipole movement will absorb IR radiation. Ex. C=O, N-H, O-H. The identity of an organic compound can be established from its finger print region(1400-900 cm^{-1}). Alkali metal halides such as KBr or NaCl which is transparent to IR region is used for sampling of solids.

The possible characteristic bands expected for the compounds to be synthesized are

- 3540-3300 cm-1 N-H StretchingVibration
- 3670-3230 cm-1 O-H StretchingVibration
- 0-1630 cm-1 C=N StretchingVibration
- 2975-2840 cm-1 C-H Aliphatic StretchingVibration
- 3100-3000 cm-1 C-H Aromatic StretchingVibration

NMR SPECTROMETRY [78]

NMR spectrometry involves the change in spin state of a nuclear magnetic moment when the nucleus absorbs electromagnetic radiation in a strong magnetic field. By using ^{1}H-NMR Spectroscopy we can get the following information. They are,

1. The relationship between the number of signals or peaks in the spectrum and the number of different kinds of hydrogen atoms in the molecule. Thus we can know the different kinds of environment of the hydrogen atom in the molecule.
2. The areas underneath each signal are in the same ratio as the number of hydrogen atoms causing each signal.

3. The principal signal may get split into smaller peaks, such as spin-spin splitting may be observed. The type of splitting depends upon the number of neighbouring non-equivalent protons.
4. The spacing between the peaks gives information on molecular structure and stereochemical features.

The basic peaks expected for the compounds to be synthesized are,

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

LC-MS

LC-MS is a hyphenated technique, coupling the separation power of HPLC, with the detection power of Mass spectrometry. When the sample cannot be vapourised, GC-MS cannot be performed . So we can prefer LC-MS.

EVALUATION OF ANTI TUBERCULAR ACTIVITY

MICROPLATE ALAMAR BLUE ASSAY (MABA) [79] is performed to evaluate the *invitro* anti tubercular activity.

PROCEDURE [80-81]

- *The anti mycobacterial activity of compounds were assessed against M. tuberculosis using* microplate Alamar Blue assay (MABA).
- This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
- Briefly, 200µl of sterile de-ionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
- The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilution of compounds were made directly on plate.
- The final drug concentrations tested were 100 to 0.2µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days.

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

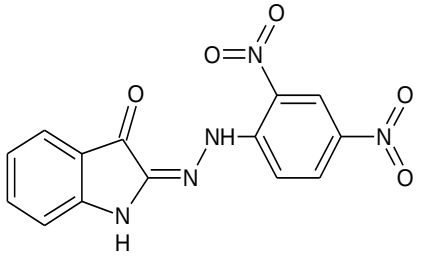
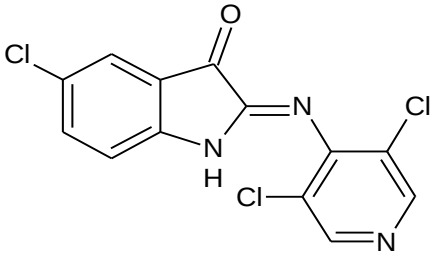
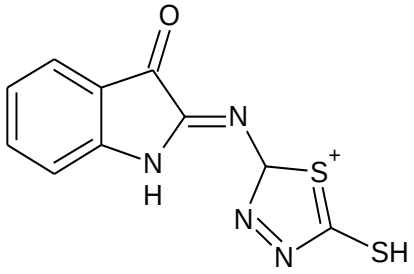
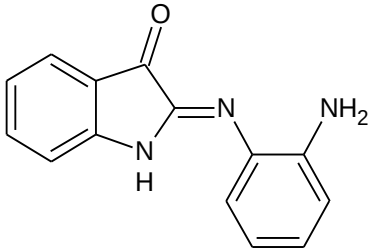
RESULTS AND DISCUSSION

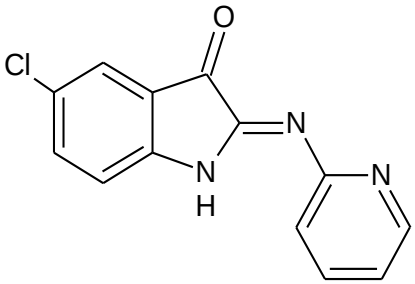
RESULTS AND DISCUSSION

ACTIVITY PREDICTION :

More than 150 compounds were docked against the enzyme Glutamine synthetase I using AUTODOCK® tools 4.2.5.1 software. The molecules with good docking score and good interactions were synthesized and characterized.

Table 1 : the selected molecules with docking score are mentioned below:

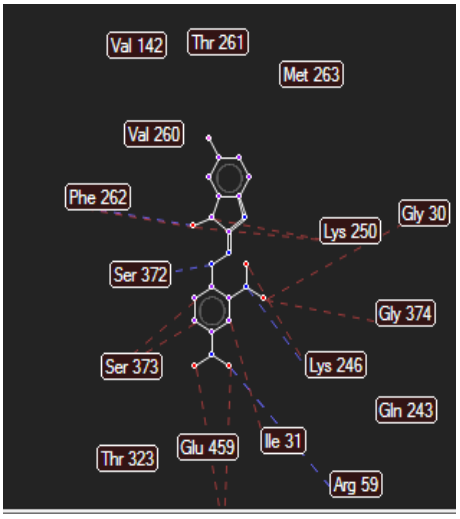
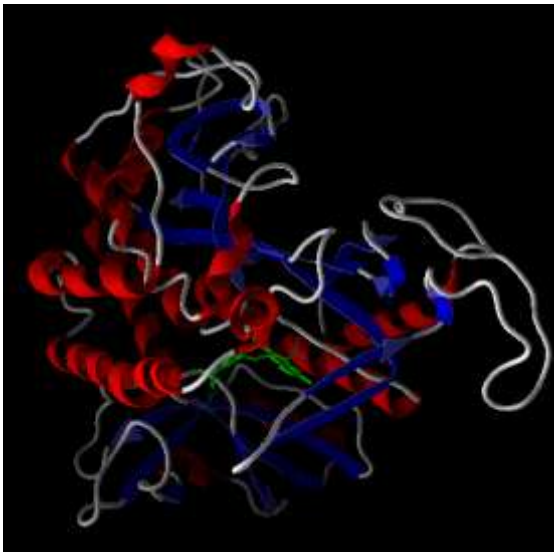
SAMPLE CODE	STRUCTURE	DOCKING SCORE
PK1		**-7.56**
PK2		**-7.1**
PK3		**-6.57**
PK4		**-5.83**

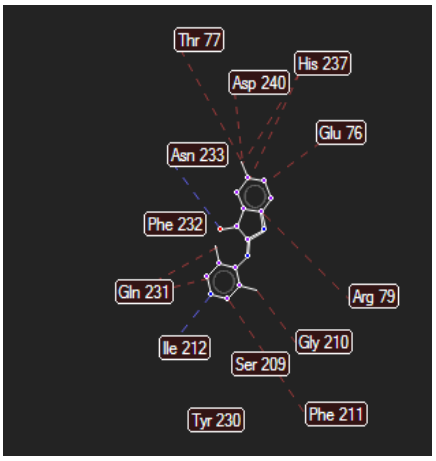
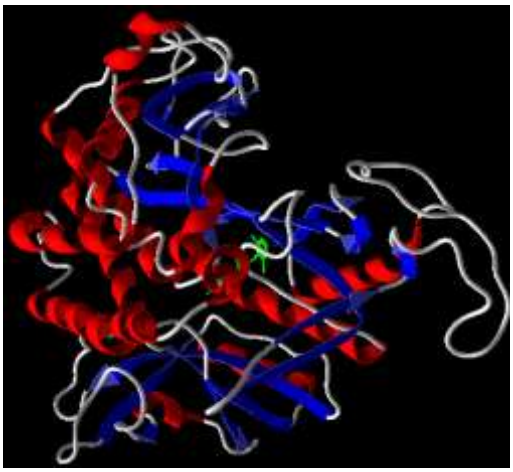
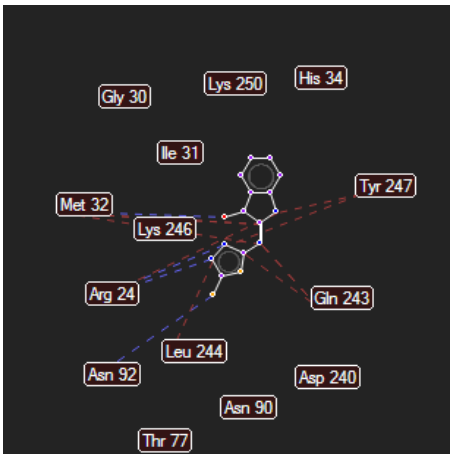
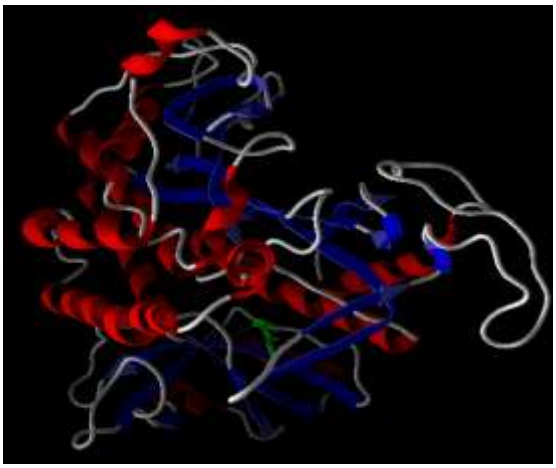
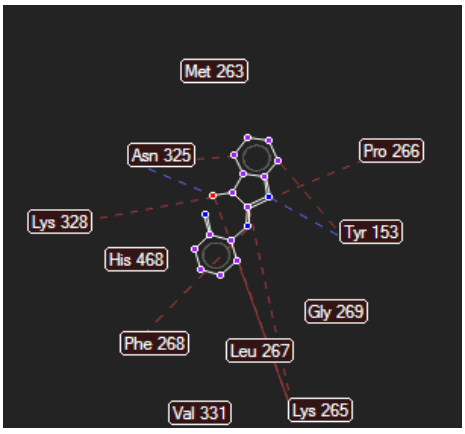
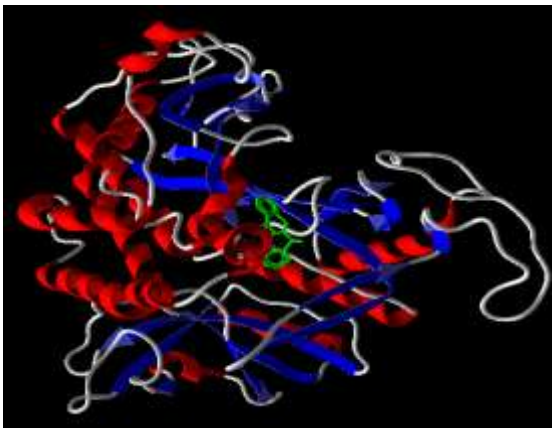
PK5		**-5.7**

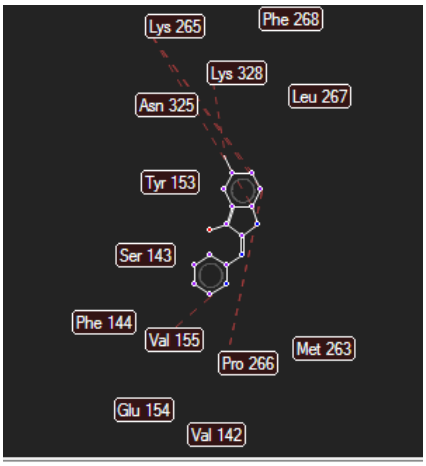
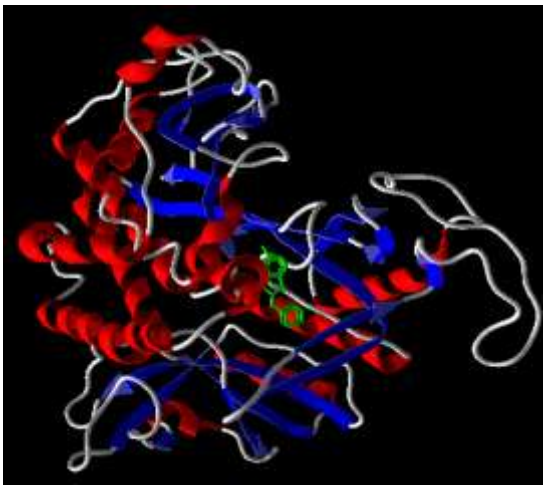
DOCKING VIEW AND INTERACTION OF THE DOCKED MOLECULES WITH THE AMINOACIDS:

AUTODOCK® 4.2.5.1 tools performs a complete systemic search of the conformations, orientations and position of a compound in the defined binding site and eliminates unwanted poses using scoring and energy optimization. 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 of the molecules with amino acids and docking view

SAMPLE CODE	INTERACTION WITH THE AMINO ACID	DOCKING VIEW
PK1		

PK2	 Thr 77 His 237 Asp 240 Glu 76 Asn 233 Phe 232 Gln 231 Arg 79 Ile 212 Gly 210 Ser 209 Tyr 230 Phe 211	
PK3	 Gly 30 Lys 250 His 34 Ile 31 Tyr 247 Met 32 Lys 246 Arg 24 Gln 243 Leu 244 Asn 92 Asp 240 Asn 90 Thr 77	
PK4	 Met 263 Asn 325 Pro 266 Lys 328 Tyr 153 His 468 Gly 269 Phe 268 Leu 267 Val 331 Lys 265	

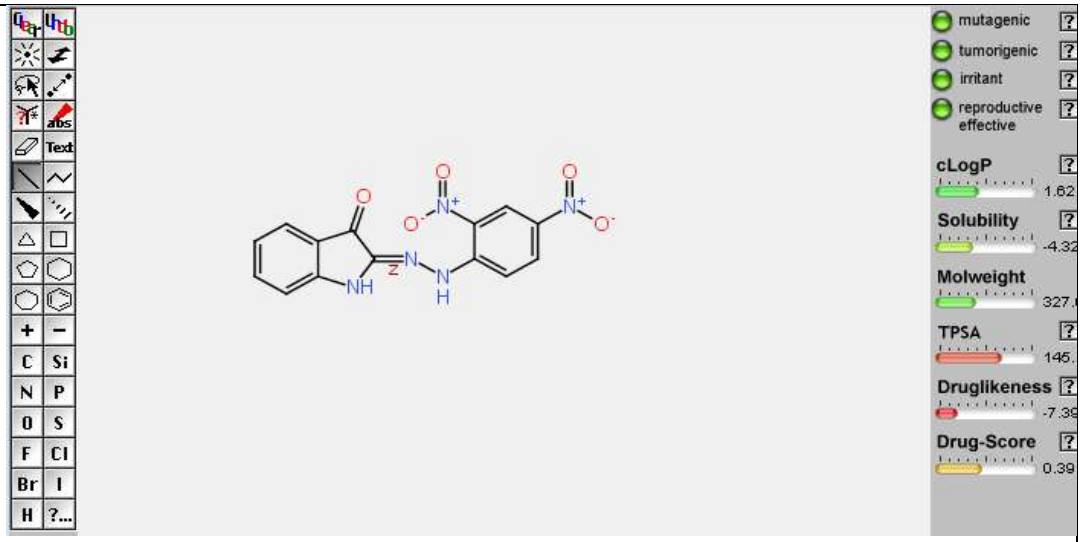
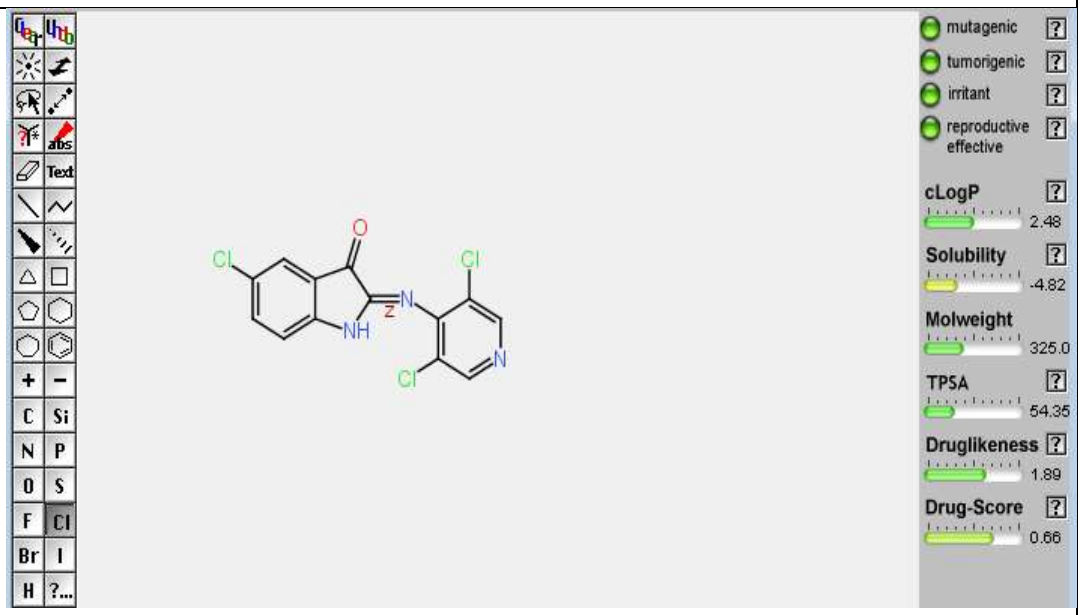
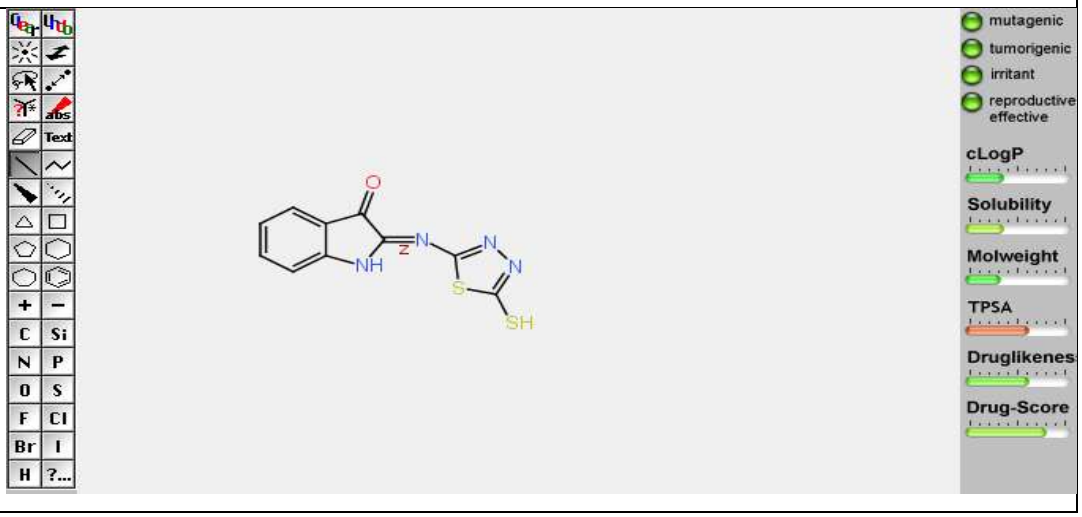
PK5		

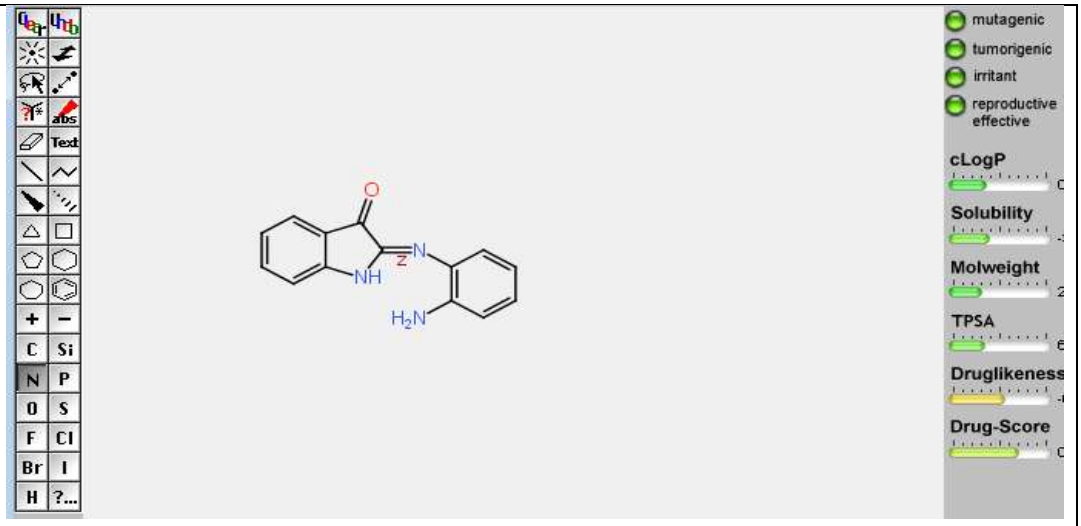
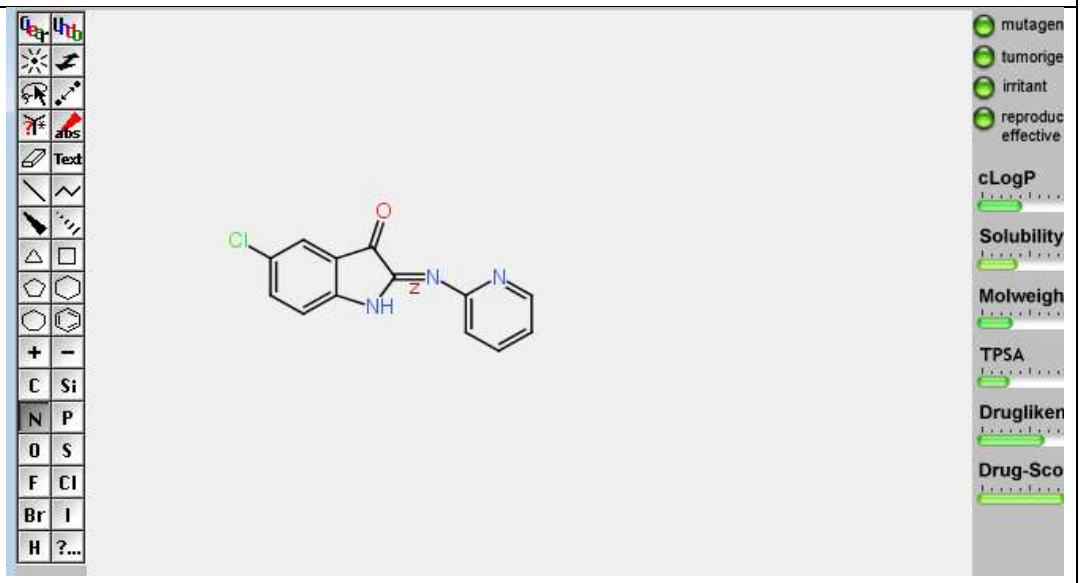
VARIANTS:

PREDICTION OF DRUG TOXICITY (INSILICO):

- *In-silico* toxicity assessment for the chosen molecules were predicted by using OSIRIS®,a JAVA based online tool.
- The tool predicts toxicity related parameters such as Mutagenicity, Tumourogenicity, Skin Irritancy and Teratogenicity apart from other toxicities.
- The prediction is based on the fragment contribution group present in the structure of the molecule.
- Properties with high risks or undesired effects are shown in red color. Green colour shows drug conform behavior.

Table 3 : The following are the results of the toxicity prediction for five selected molecules based on docking score:

PK1	
PK2	
PK3	

PK4	
PK5	

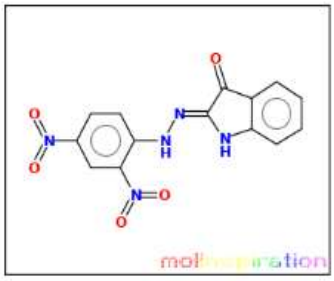
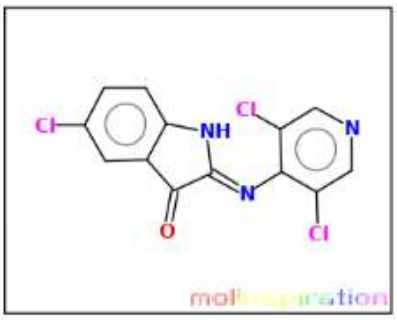
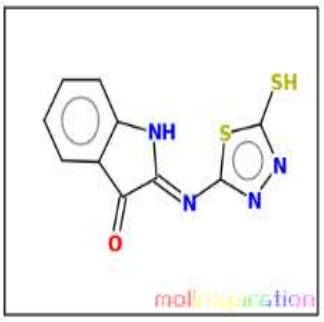
PREDICTION OF DRUGLIKENESS:

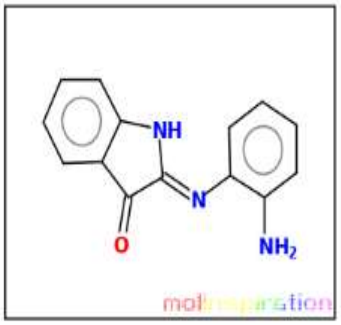
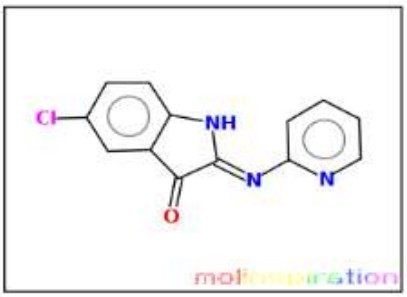
The designed molecules were screened *insilico* using MOLINSPIRATION® software to evaluate drug likeness. It is an online software available for the calculation of important molecular properties such as log P, polar surface area, number of hydrogen bond donors acceptors and number of rotatable bonds.

In an attempt to improve the predictions of drug likeness, the rules have spawned many extensions.They are given below:

- Partition coefficient log P, range from -0.4 to +5.6
- Molar refractivity from 40 to130
- Molecular weight from 180 to500 daltons.
- Number of atoms from 20 to 70
- Not more than 5 hydrogen bond donors and 10 hydrogen bond acceptors

Table 4 : results of drug likeness prediction by molinspiration

<table>
<tr>
<td>PK1</td>
<td>molinspiration
miSMILES:
O=c2c(=NNc1ccc(N(=O)=O)cc1N(=O)=O)
[nH]c3ccccc23
 
Molinspiration property engine v2018.10
miLogP 2.84
TPSA 148.90
natoms 24
MW 327.26
nON 10
nOHNH 2
nviolations 0
nrotb 4
volume 258.33</td>
</tr>
<tr>
<td>PK2</td>
<td>molinspiration
miSMILES: O=c2c(=Nc1c(Cl)cncc1Cl)
[nH]c3ccc(Cl)cc23
 
Molinspiration property engine v2018.10
miLogP 4.25
TPSA 58.12
natoms 20
MW 326.57
nON 4
nOHNH 1
nviolations 0
nrotb 1
volume 235.71</td>
</tr>
<tr>
<td>PK3</td>
<td>molinspiration
miSMILES: O=c2c(=Nc1nnc(S)s1)[nH]c3ccccc23
 
Molinspiration property engine v2018.10
miLogP 2.47
TPSA 71.01
natoms 17
MW 262.32
nON 5
nOHNH 1
nviolations 0
nrotb 1
volume 199.32</td>
</tr>
</table>

PK4	molinspiration miSMILES: Nc1ccccc1N=c3[nH]c2ccccc2c3=O  Molinspiration property engine v2018.10 miLogP 2.67 TPSA 71.25 natoms 18 MW 237.26 nON 4 nOHNH 3 nviolations 0 nrotb 1 volume 210.54
PK5	molinspiration miSMILES: O=c2c(=Nc1ccccn1)[nH]c3ccc(Cl)cc23  Molinspiration property engine v2018.10 miLogP 2.99 TPSA 58.12 natoms 18 MW 257.68 nON 4 nOHNH 1 nviolations 0 nrotb 1 volume 208.64

CHARACTERIZATION:

The selected compounds were synthesized, recrystallised and purified by using ethanol .

JUSTIFICATION OF PURITY OF SYNTHESIZED COMPOUNDS:

Melting point:

The melting points of the synthesized compound were determined by one end open capillary method. Sharp melting point indicated that the synthesized compounds were pure.

TLC:

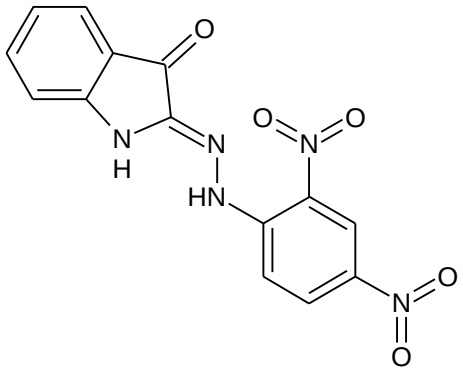
- Precoated aluminum TLC plates were used. Solutions of the reactants and products were prepared by dissolving them in methanol.
- Appearance of a single spot not corresponding to the parent compounds confirms the purity of the synthesized Compounds.
- Rf values of the synthesized compounds varies from the parent compounds indicates that the reaction was completed.

Table 5 : Rf values of the synthesized compounds:

S.NO	COMPOUND CODE	MOBILE PHASE	Rf VALUE
1	PK1	HEXANE:ETHYLACETATE(7:3)	0.63
2	PK2	HEXANE:ETHYLACETATE(7:3)	0.71
3	PK3	HEXANE:ETHYLACETATE(7:3)	0.78
4	PK4	HEXANE:ETHYLACETATE(7:3)	0.69
5	PK5	HEXANE:ETHYLACETATE(7:3)	0.75

PRODUCT PROFILE:

PK1



(2*Z*)-2-[2-(2,4-dinitrophenyl)hydrazinylidene]-1,2-dihydro-3*H*-indol-3-one

Molecular Formula	= $C_{14}H_9N_5O_5$
Formula Weight	= 327.25176
Composition	= C(51.38%) H(2.77%) N(21.40%) O(24.45%)
Molar Refractivity	= 80.75 ± 0.5 cm^3
Molar Volume	= 194.5 ± 7.0 cm^3
Parachor	= 586.3 ± 8.0 cm^3
Index of Refraction	= 1.768 ± 0.05
Surface Tension	= 82.4 ± 7.0 dyne/cm
Density	= 1.68 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 32.01 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF THE COMPOUND PK1

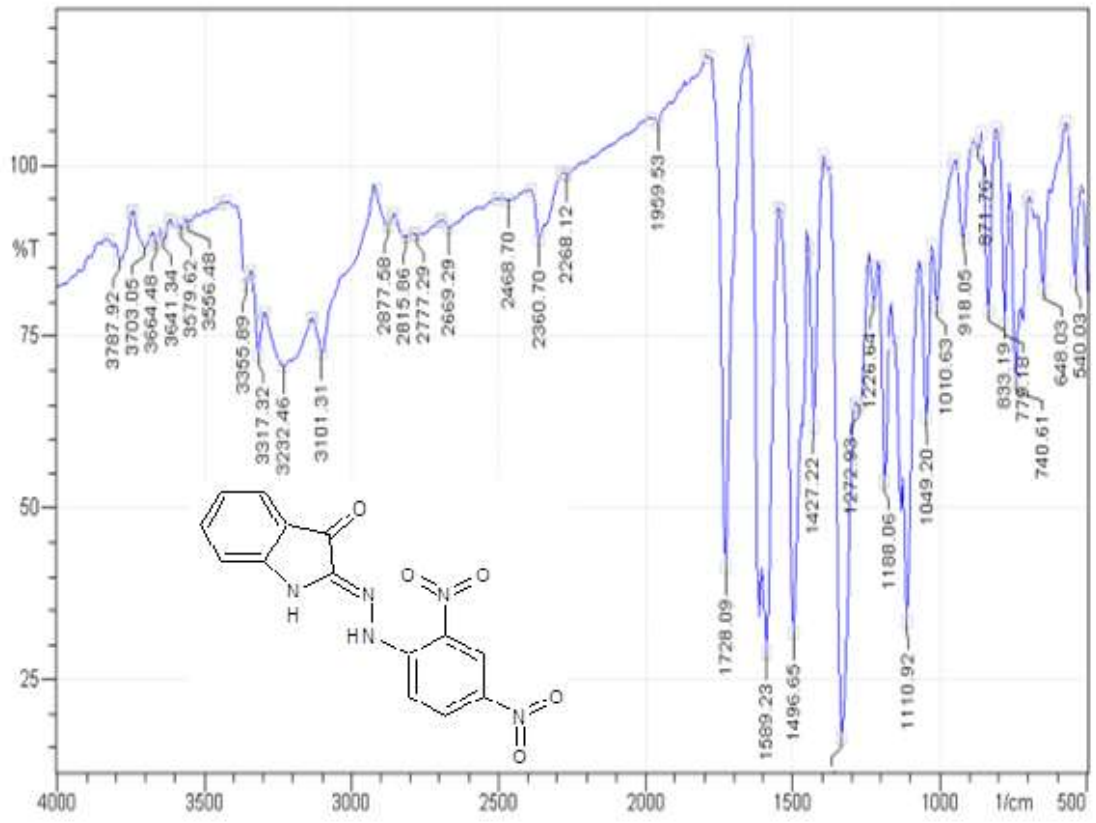
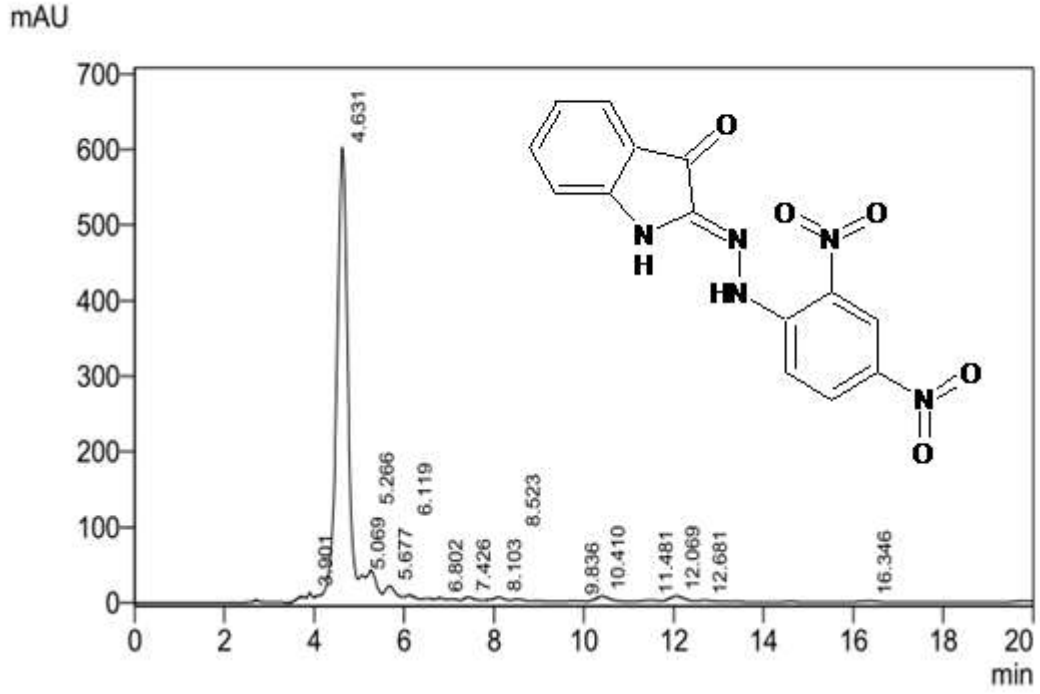
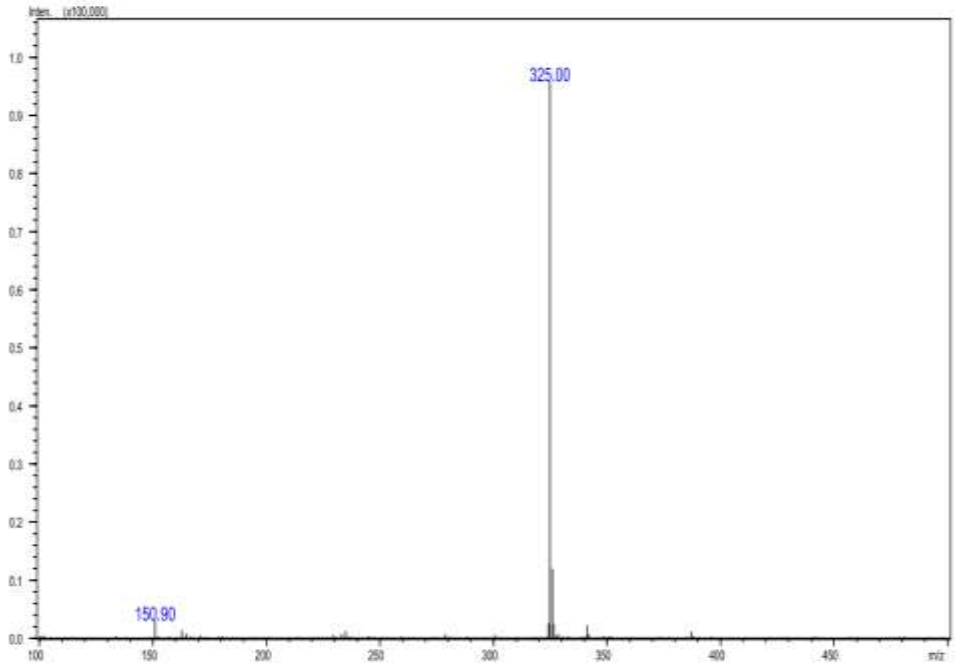


Table 6: IR interpretation of the compound PK1

S.NO	WAVE NUMBER (cm^{-1})	TYPES OF VIBRATIONS	FUNCTIONAL GROUP
1	2885	C-H Stretching	Presence of alkyl group
2	1496.65	NO_2 Stretching	Presence of aromatic nitro group
3	1728.09	C=O Stretching	Presence of keto group

LC-MS SPECTRUM OF THE COMPOUND PK1 (molecular weight:327.26)





NMR SPECTRUM OF COMPOUND PK1

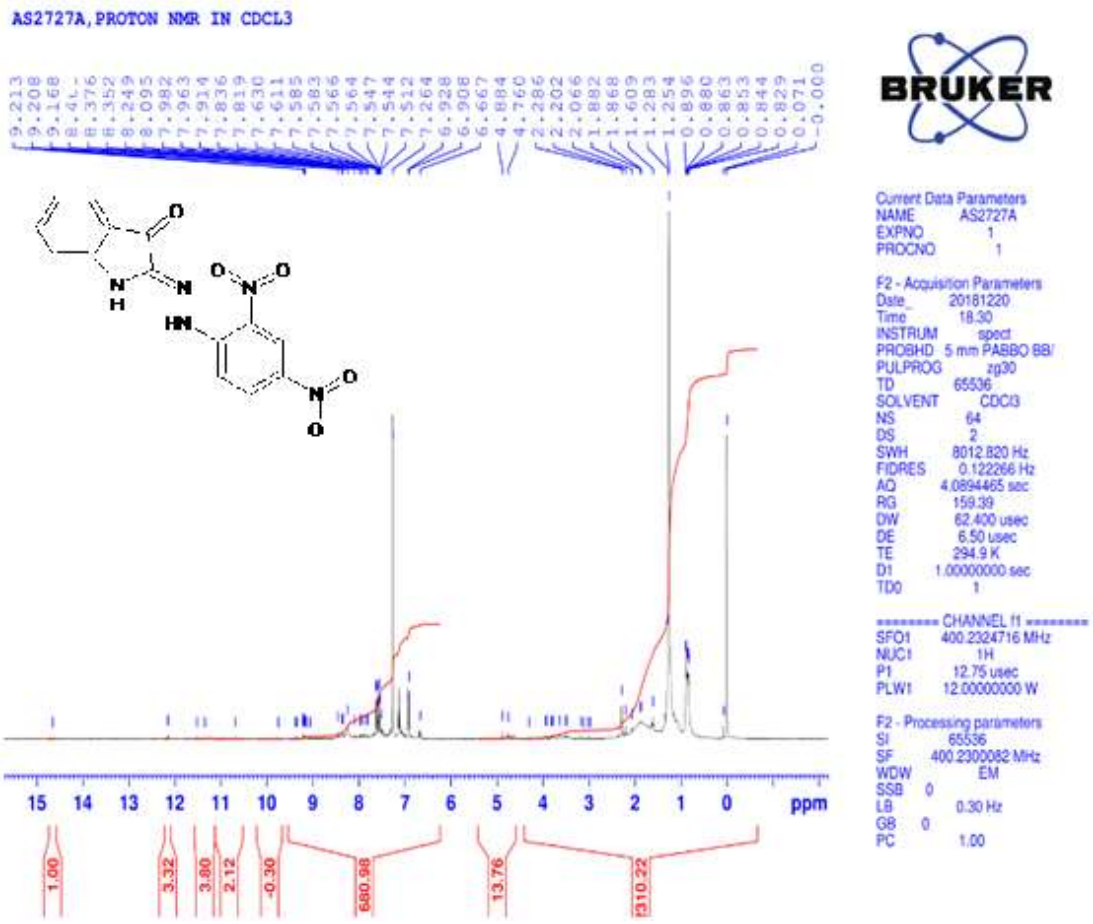
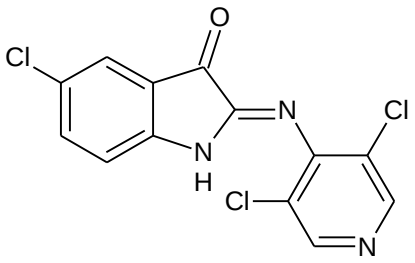


Table 7: NMR interpretation of compound PK1

S.NO	δ VALUES	TYPES OF PEAK	NUMBER OF PROTONS
1	7.6-7.8	Multiplet	Five protons
2	7.9-8.4	Multiplet	Four protons

PK2



Molecular Formula	= $C_{13}H_6Cl_3N_3O$
Formula Weight	= 326.56524
Composition	= C(47.81%) H(1.85%) Cl(32.57%) N(12.87%) O(4.90%)
Molar Refractivity	= 78.62 ± 0.5 cm^3
Molar Volume	= 195.7 ± 7.0 cm^3
Parachor	= 543.3 ± 8.0 cm^3
Index of Refraction	= 1.736 ± 0.05
Surface Tension	= 59.4 ± 7.0 dyne/cm
Density	= 1.66 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 31.16 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF THE COMPOUND PK2

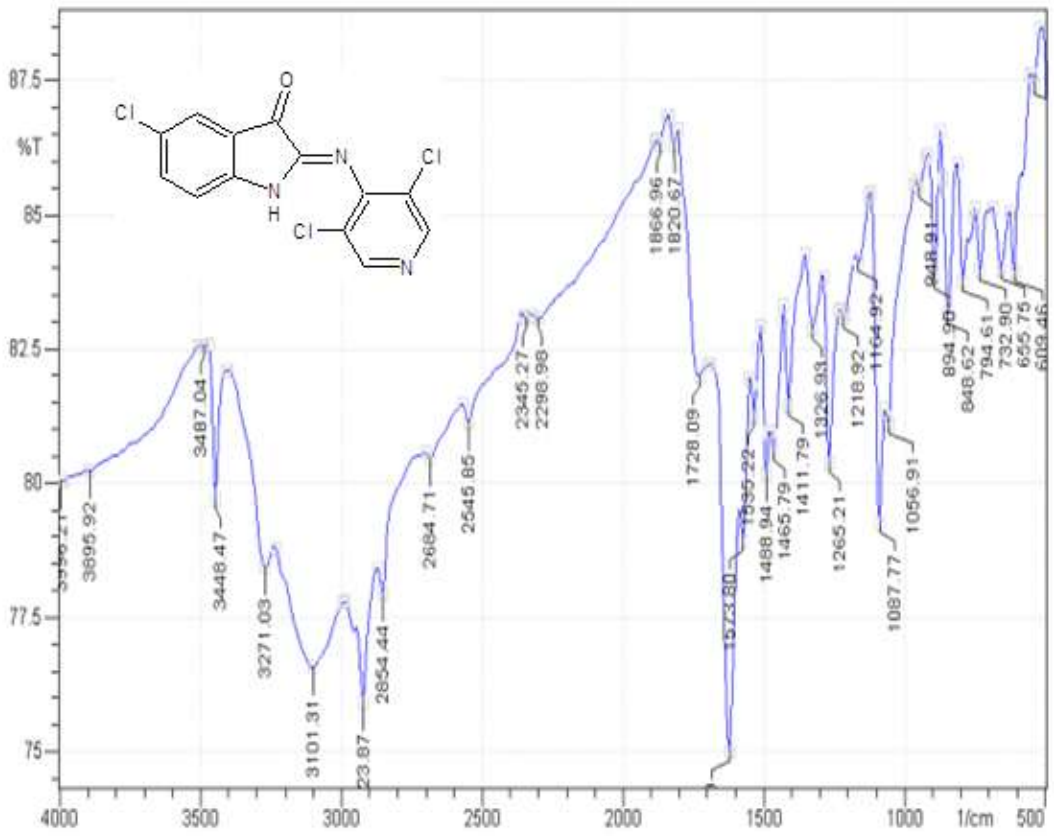
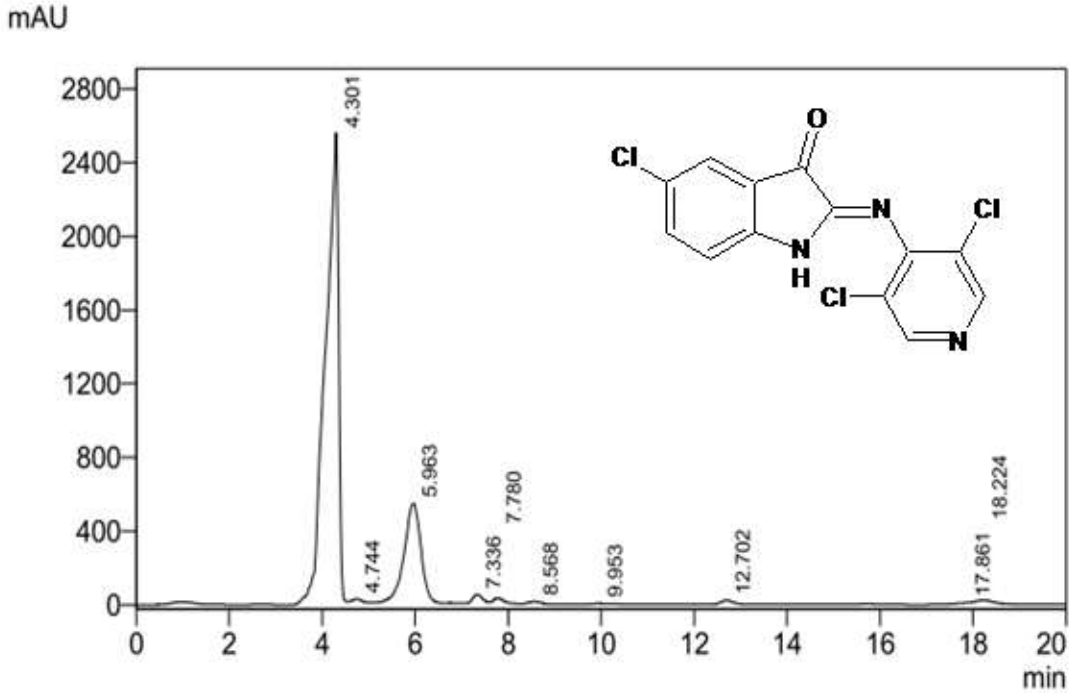
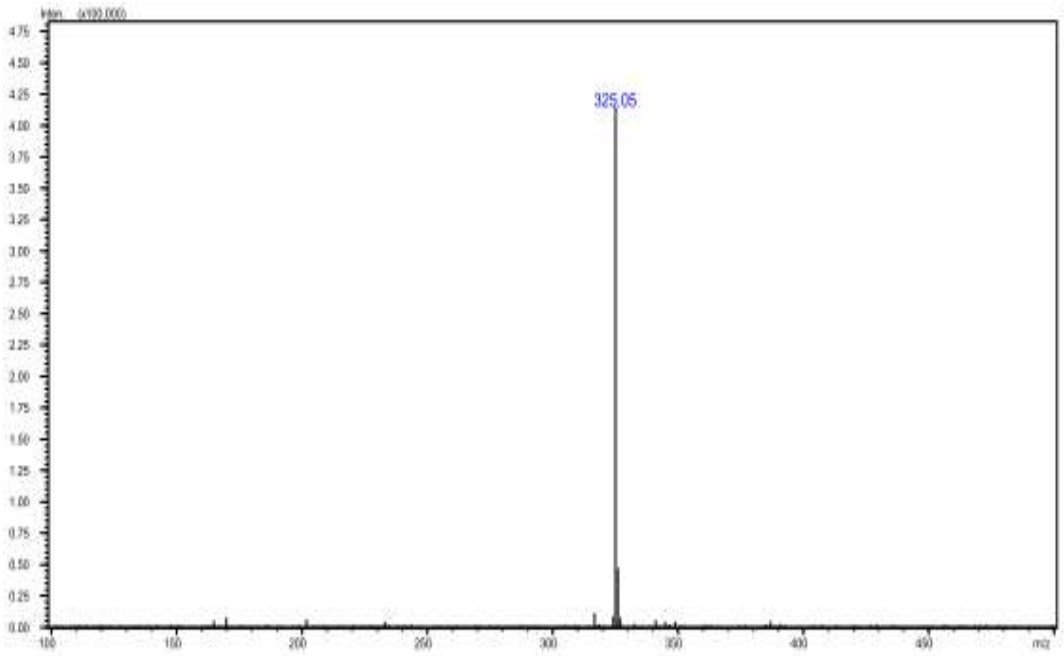


Table 8: IR interpretation of the compound PK2

S.NO	WAVE NUMBER	TYPES OF VIBRATIONS	FUNCTIONAL GROUP
1	2854.44	C-H stretching	Alkyl group
2	794.61	C-Cl stretching	Presence of C-Cl group
3	1728.09	C=O Stretching	Presence of keto group

LC-MS SPECTRUM OF THE COMPOUND PK2 (molecular weight-326.56)





NMR SPECTRUM OF THE COMPOUND PK2

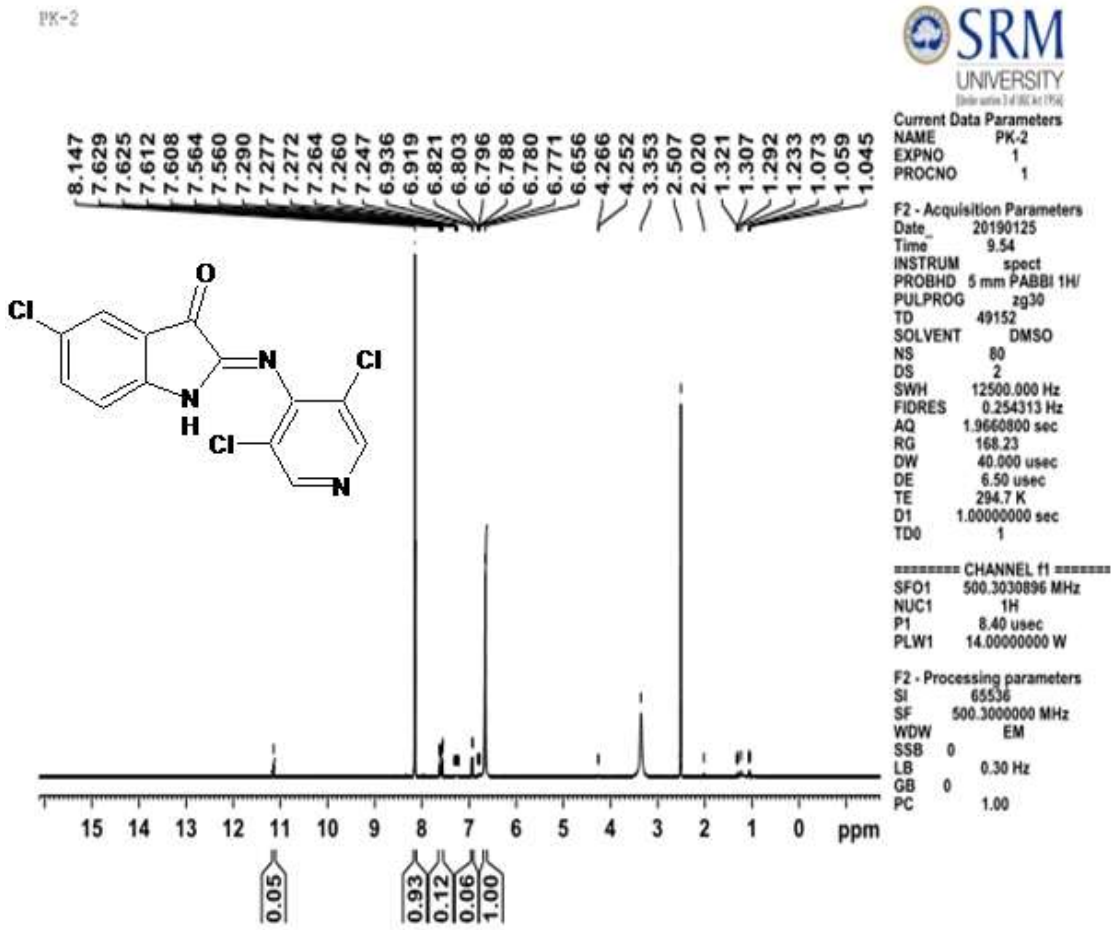
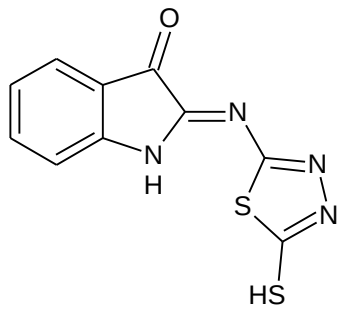


Table 9: NMR interpretation of the compound PK2

S.NO	δ VALUES	TYPES OF PEAK	NUMBER OF PROTONS
1	6.8-7.5	Multiplet	Two protons
2	3.3-4.2	Triplet	Four protons

PK3



Molecular Formula	= $C_{10}H_6N_4OS_2$
Formula Weight	= 262.31084
Composition	= C(45.79%) H(2.31%) N(21.36%) O(6.10%) S(24.45%)
Molar Refractivity	= 69.18 ± 0.5 cm^3
Molar Volume	= 147.4 ± 7.0 cm^3
Parachor	= 439.0 ± 8.0 cm^3
Index of Refraction	= 1.911 ± 0.05
Surface Tension	= 78.7 ± 7.0 dyne/cm
Density	= 1.77 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 27.42 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF COMPOUND PK3

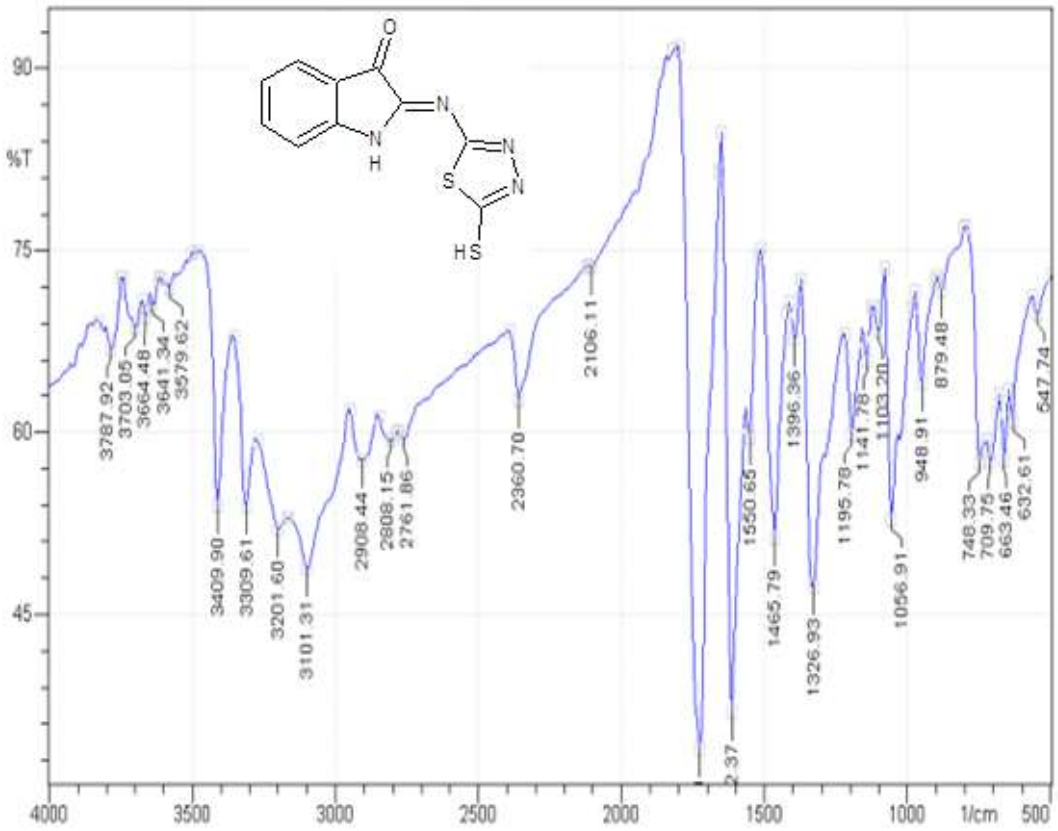
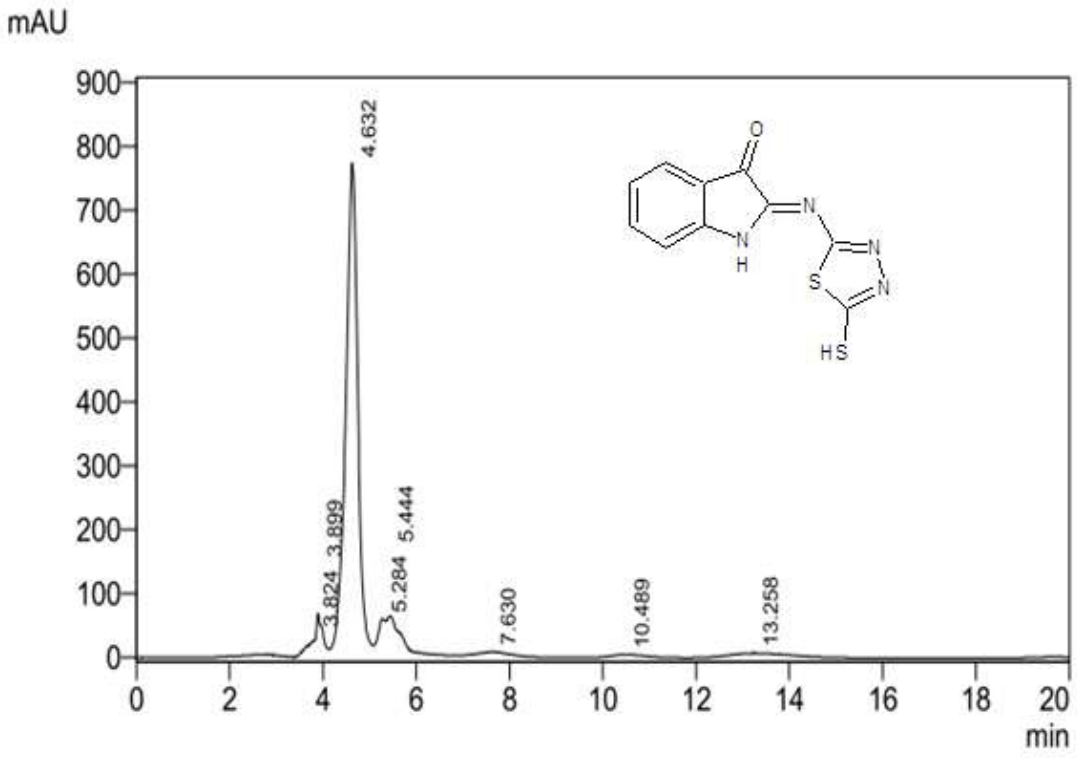
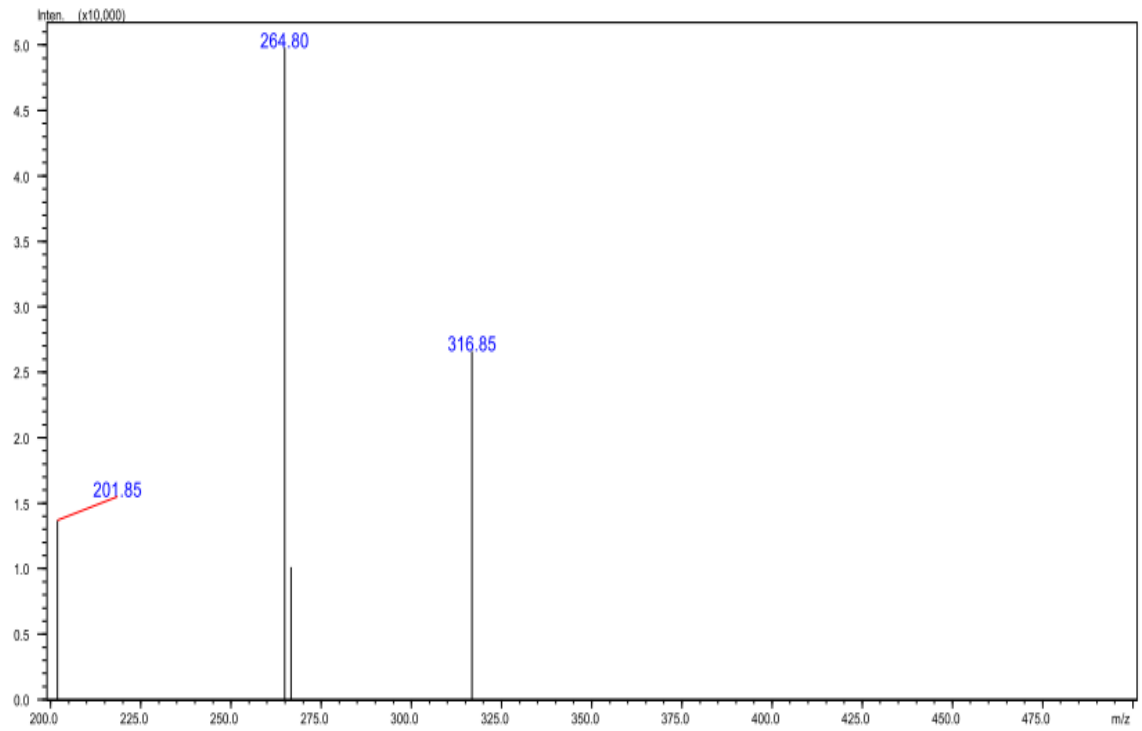


Table 10 : IR interpretation of the compound PK3

S.NO	WAVE NUMBER	TYPES OF VIBRATIONS	FUNCTIONAL GROUP
1	2908.44	C-H stretching	Alkyl group
2	1612.37	C=N stretching	Imine group
3	748.33	C-S stretching	Thiol group

LC-MS SPECTRUM OF COMPOUND PK3 (molecular weight-262.31)





NMR SPECTRUM OF THE COMPOUND PK3

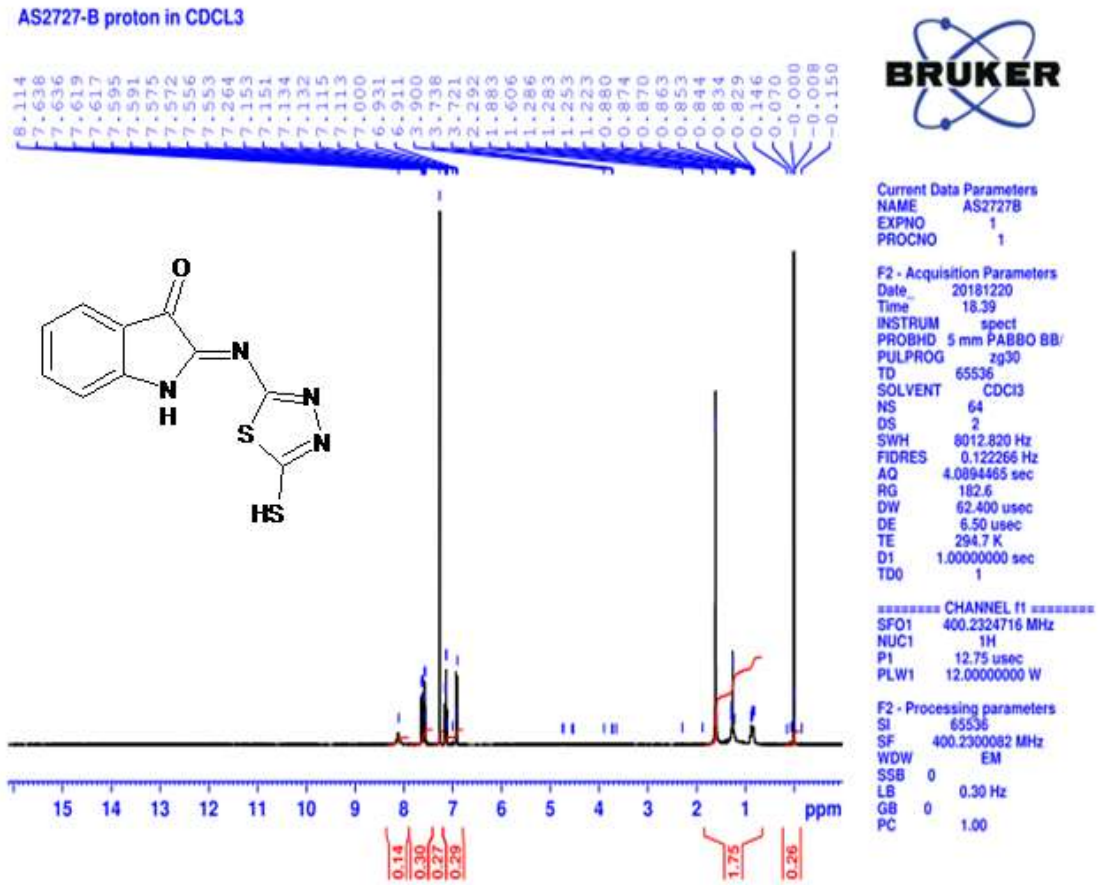
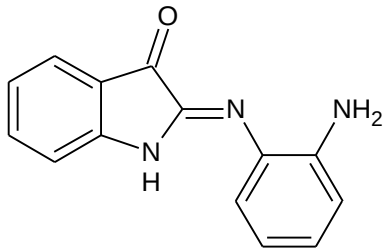


Table 11 : NMR interpretation of the compound PK3

S.NO	δ VALUES	TYPES OF PEAK	NUMBER OF PROTONS
1	6.9-7.5	Doublet	Five protons
2	7.6-8.1	Multiplet	One proton

PK4



(2*Z*)-2-[(2-aminophenyl)imino]-1,2-dihydro-3*H*-indol-3-one

Molecular Formula	= $C_{14}H_{11}N_3O$
Formula Weight	= 237.25664
Composition	= C(70.87%) H(4.67%) N(17.71%) O(6.74%)
Molar Refractivity	= 68.29 ± 0.5 cm^3
Molar Volume	= 175.9 ± 7.0 cm^3
Parachor	= 482.8 ± 8.0 cm^3
Index of Refraction	= 1.703 ± 0.05
Surface Tension	= 56.7 ± 7.0 dyne/cm
Density	= 1.34 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 27.07 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF COMPOUND PK4

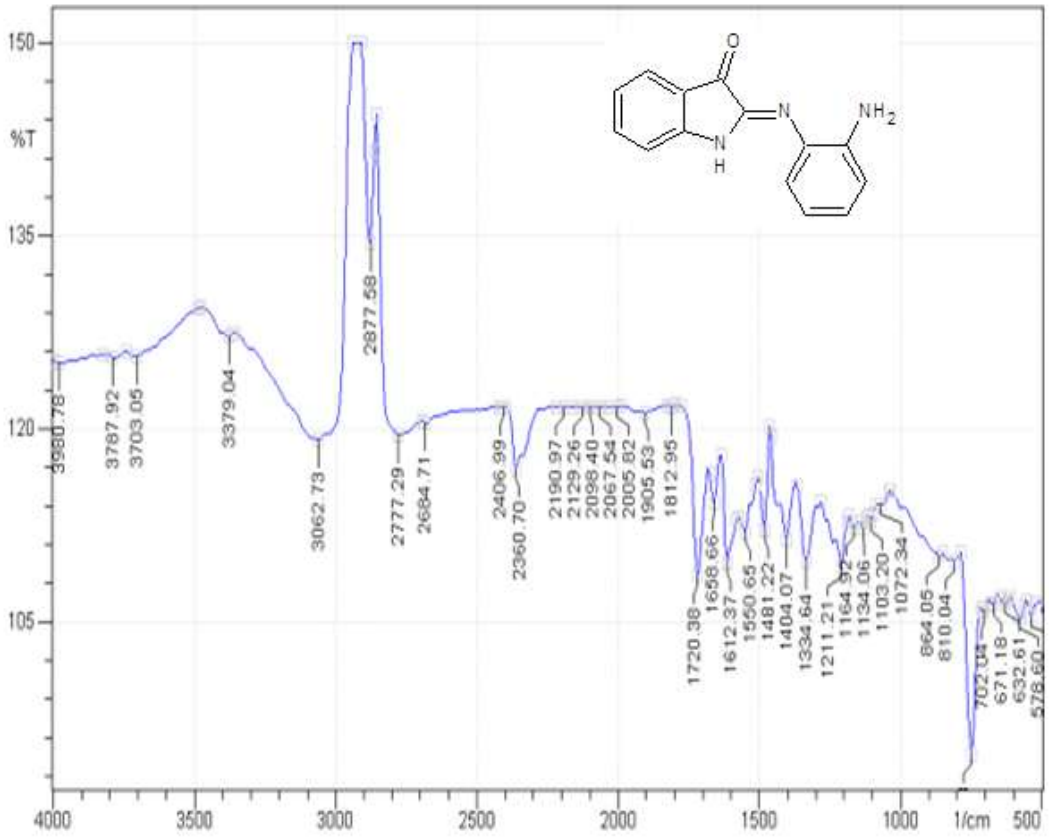
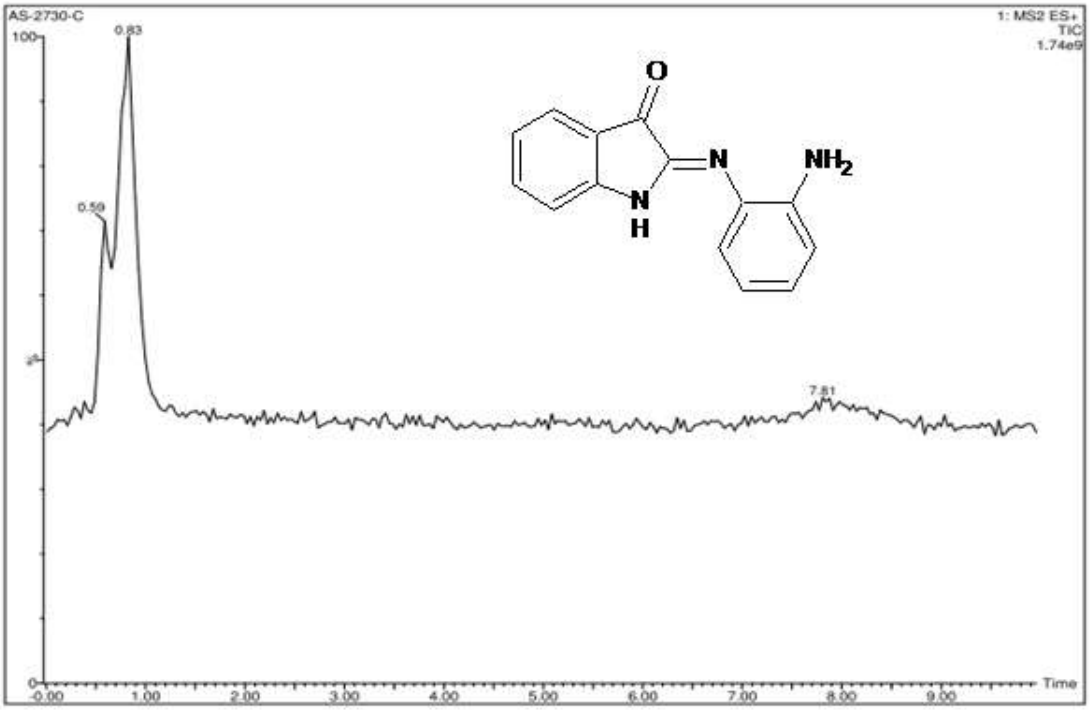
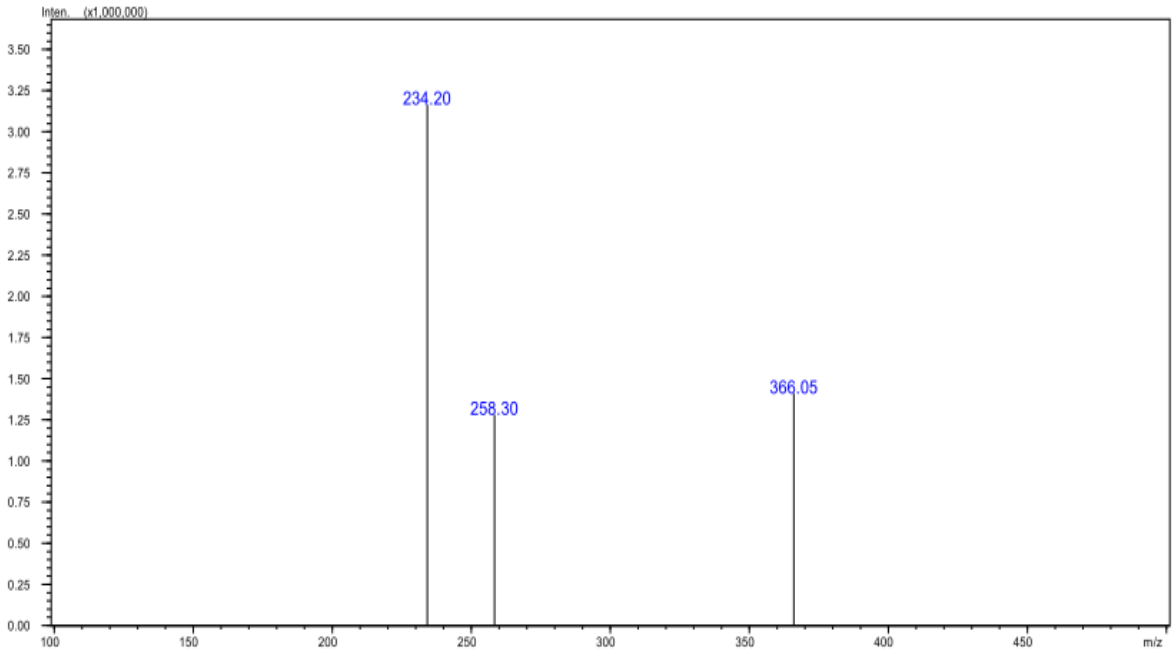


Table 12: IR interpretation of the compound PK4

S.NO	WAVE NUMBER	TYPES OF VIBRATIONS	FUNCTIONAL GROUP
1	3062.73	C-H stretching	Alkene group
2	1612.37	C=N stretching	Imine group
3	1720.38	C=O Stretching	Presence of keto group

LC-MS SPECTRUM OF COMPOUND PK4(molecular weight - 237.25)





NMR SPECTRUM OF COMPOUND PK4

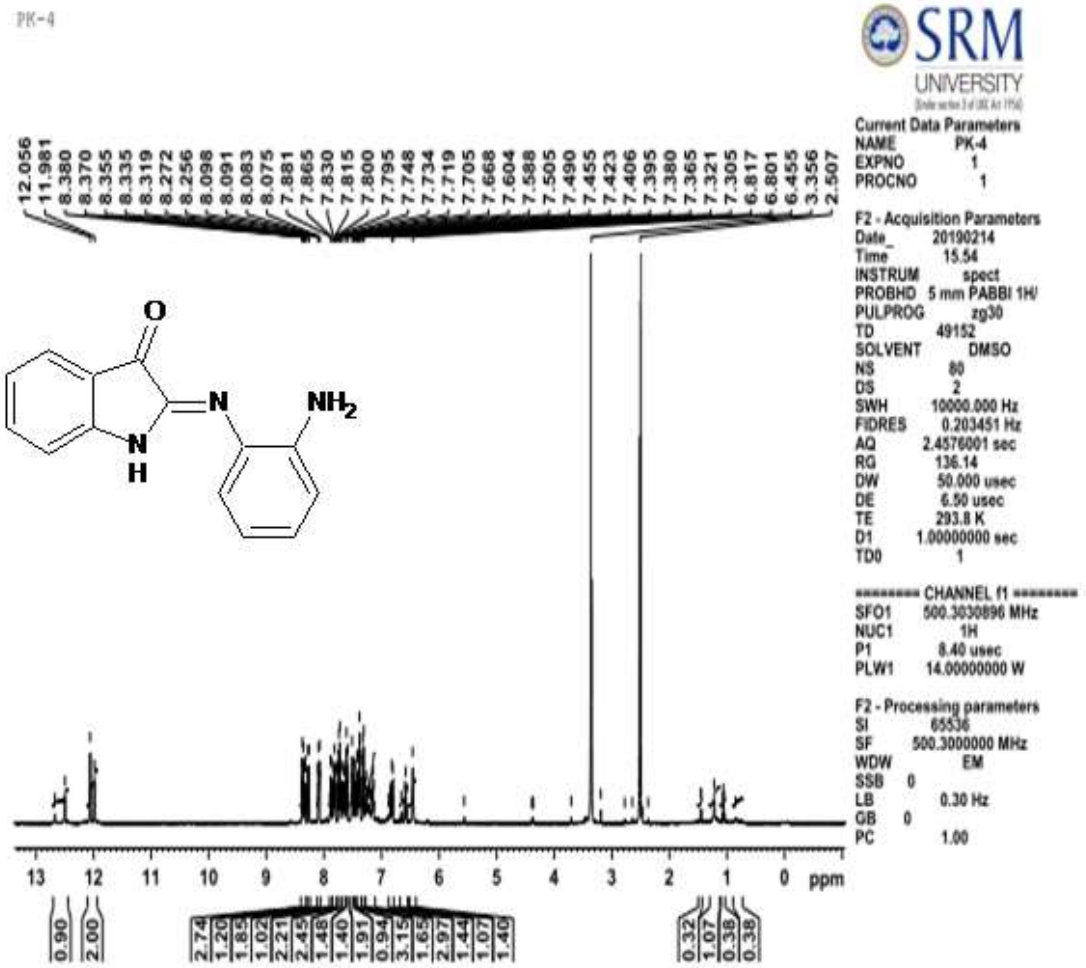
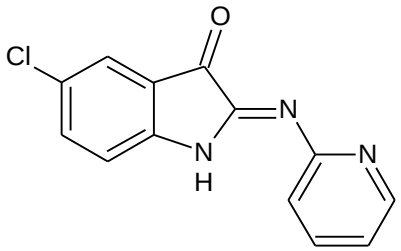


Table 13: NMR interpretation of the compound PK4

S.NO	δ VALUES	TYPES OF PEAK	NUMBER OF PROTONS
1	6.8-8.3	Triplet	Nine protons
2	2.5-3.3	Multiplet	Two protons

PK5



(2*Z*)-5-chloro-2-(pyridin-2-ylimino)-1,2-dihydro-3*H*-indol-3-one

Molecular Formula	= $C_{13}H_8ClN_3O$
Formula Weight	= 257.67512
Composition	=C(60.60%)H(3.13%)Cl(13.76%)N(16.31%) O(6.21%)
Molar Refractivity	= 69.42 ± 0.5 cm^3
Molar Volume	= 177.0 ± 7.0 cm^3
Parachor	= 485.6 ± 8.0 cm^3
Index of Refraction	= 1.712 ± 0.05
Surface Tension	= 56.5 ± 7.0 dyne/cm
Density	= 1.45 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	=27.52 ± 0.5 $10^{-24}cm^3$

IR SPECTRUM OF COMPOUND PK5

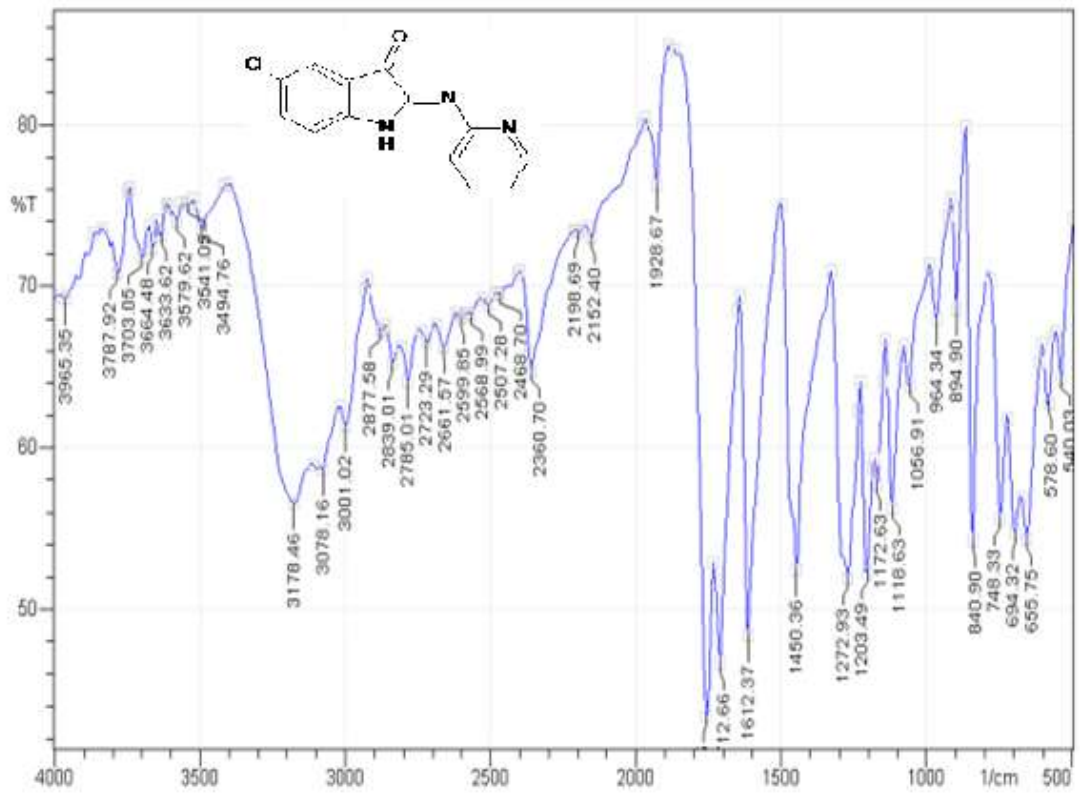
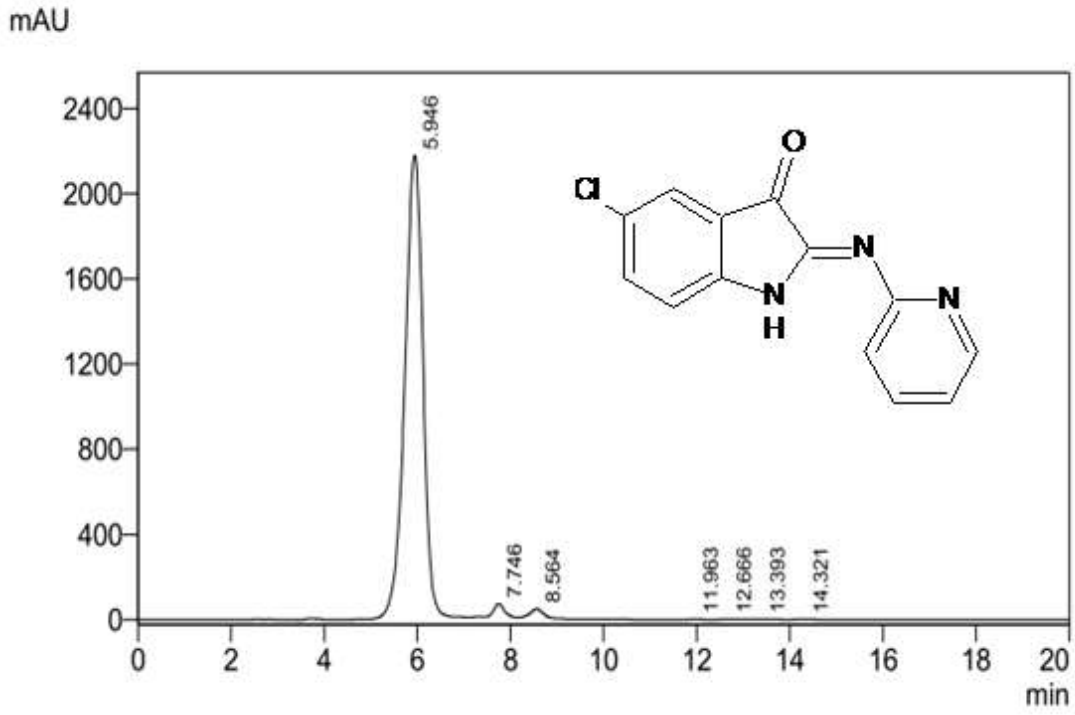
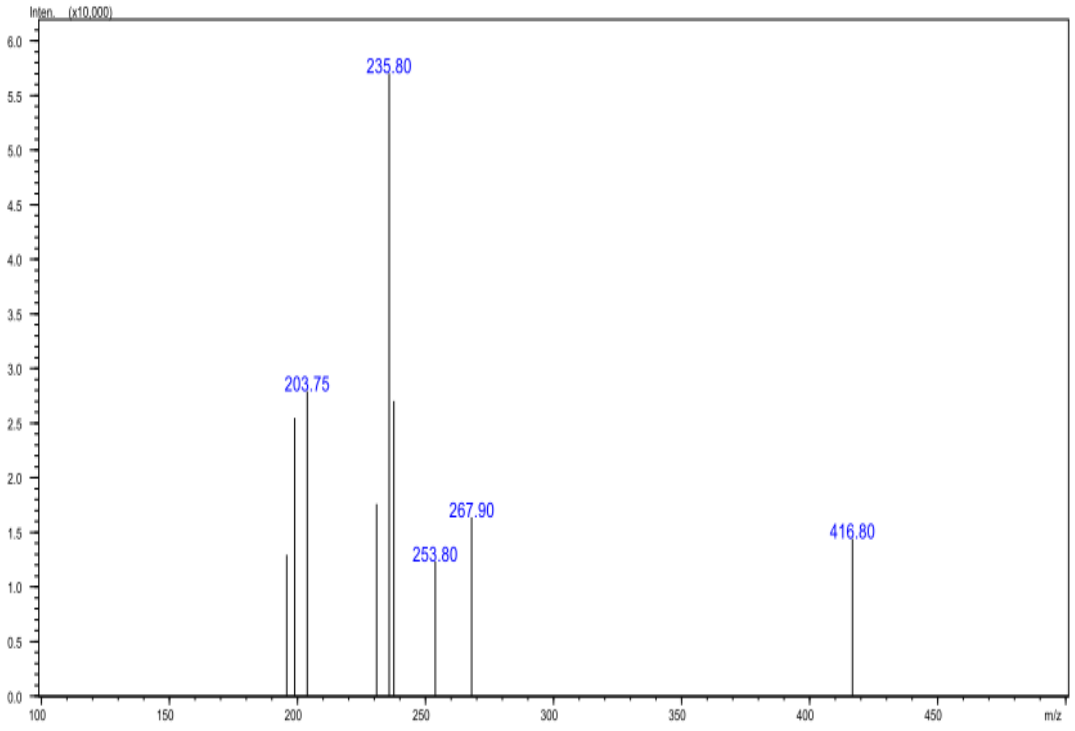


Table 14: IR interpretation of the compound PK5

S.NO	WAVE NUMBER	TYPES OF VIBRATIONS	FUNCTIONAL GROUP
1	2877.58	C-H stretching	Alkyl group
2	1612.37	C=N stretching	Imine group
3	748.39	C-Cl stretching	Presence of C-Cl group

LC-MS SPECTRUM OF COMPOUND PK5





NMR SPECTRUM OF COMPOUND PK5

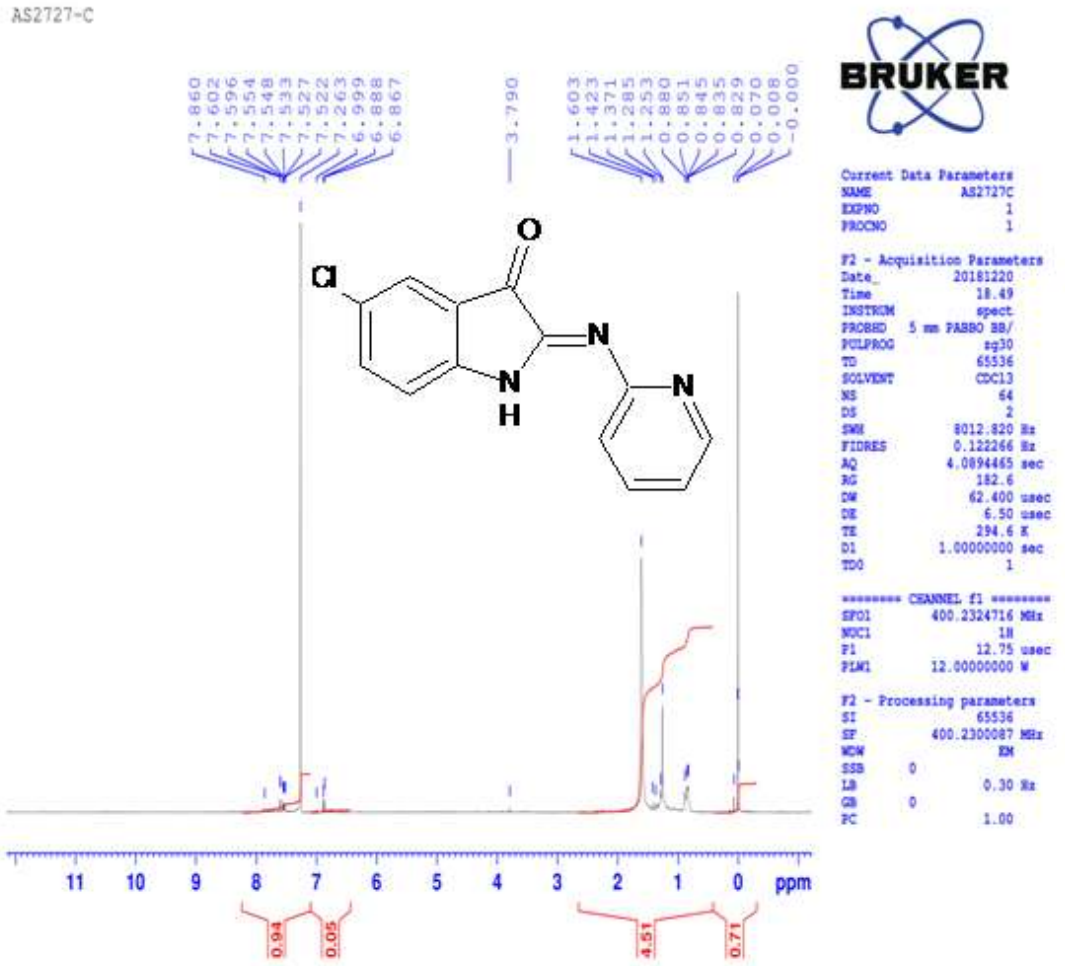


Table 15 : NMR interpretation of the compound PK5

S.NO	δ VALUES	TYPES OF PEAK	NUMBER OF PROTONS
1	6.8-7.8	Triplet	Six protons
2	3.7	Multiplet	Two protons

Table 16 : physical data of the synthesized compounds:

S.NO	COMPOUD NAME	MOLECULAR FOUMULA	MOLECULAR WEIGHT	% YIELD	MELTING POINT
1	PK1	$C_{14}H_9N_5O_5$	327.25	85	164-166°C
2	PK2	$C_{13}H_6Cl_3N_3O$	326.56	80	154-156°C
3	PK3	$C_{10}H_6N_4OS_2$	262.31	72	158-160°C
4	PK4	$C_{14}H_{11}N_3O$	237.25	80	180-182°C
5	PK5	$C_{13}H_8ClN_3O$	257.67	70	210-212°C

IR SPECTROSCOPY:

- The absence of old functionl group of the parent compound and the presence of new functional group of the synthesized compounds indicates the reaction is completed.
- All the synthesized compounds showed presence of the Imine formation. All these compounds show the absence of the stretching/bending for the reactant functional groups.

H ^{1} NMR SPECTROMETRY:

- The number of signals present in the NMR spectrum indicates the set of equivalent protons present in the molecule.
- Shielded protons shows upfield effect, and they are directed towards the signal of TMS. Shielded protons have lower δ values.
- Deshielded protons shows downfield effect, and they are directed away from the signal of TMS. Deshielded protons have higher δ values. Ex: OH, NO_2,F,Cl,Br containing protons have deshielding effect.

LC-MS:

Liquid Chromatography – Mass Spectrometry is used to determine the purity and mass of the synthesized to compounds.

Table 17 : Molecular weight determined by mass spectrometry:

S.NO	SAMPLE CODE	ACTUAL MASS	CALCULATED MASS
1	PK1	327.25	325
2	PK2	326.56	325.05
3	PK3	262.31	264.80
4	PK4	237.25	234.20
5	PK5	257.67	253.80

BIOLOGICAL EVALUATION:

MICROPLATE ALAMAR BLUE ASSAY [MABA]:

1. The compounds were evaluated for their invitro antitubercular activity by the MABA method.

2. Strains used: Mycobacterium tuberculosis H37RV strains.

3. The standard drugs used are:

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

The results for the standard drugs is presented below

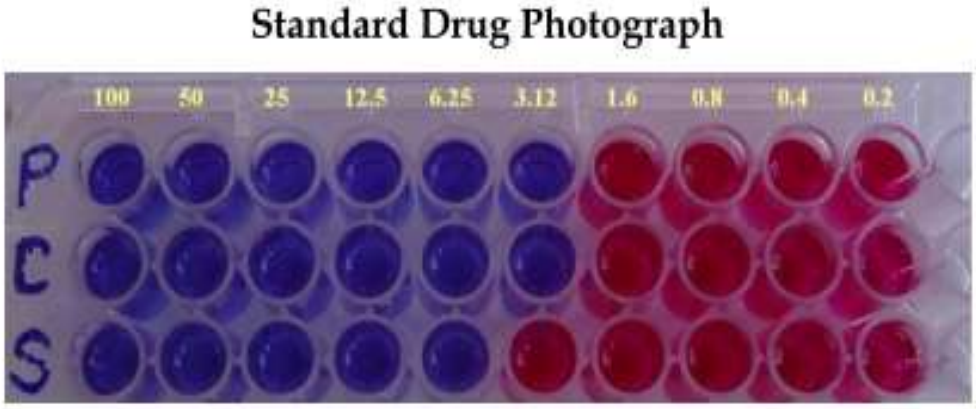


Fig 8. standard drug photograph (MABA)

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	PK1								
2	PK2								
3	PK3								
4	PK4								
5	PK5								

Fig 9: MABA photograph for synthesized compounds

Table 18 : The MABA report of the synthesized compounds were tabulated below:

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	PK1	S	S	R	R	R	R	R	R
2	PK2	S	S	S	S	S	S	S	R
3	PK3	S	S	R	R	R	R	R	R
4	PK4	S	S	S	S	S	S	S	R
ss5	PK5	S	S	R	R	R	R	R	R

Table 19 : Comparative study of docking score with MABA report:

Compound name	**Docking score Kcal/mol**	**MABA (biological activity) (µg/ml)**
PK1	-7.56	50
PK2	-7.1	1.6
PK3	-6.57	50
PK4	-5.83	1.6
PK5	-6.39	50
PYRAZINAMIDE	-4.69	3.125
CIPROFLOXACIN	-6.45	3.125

SUMMARY AND CONCLUSION

SUMMARY

- Glutamine synthetase I enzyme is the critical enzyme for the survival and growth for Mycobacterium tuberculosis is chosen as the potential drug target.

- Molecules were designed and docked against Glutamine synthetase I enzyme 3ZXR protein using Autodock® 4.2.1 software.

- Molecules with good docking score were screened for insilico toxicity by using OSIRIS® software and evaluation of drug likeness by MOLINSPIRATION software.

- The selected compounds were synthesized and labeled as PK1, PK2, PK3, PK4, PK5.

- Purity of the synthesized compounds were justified by its sharp melting point and TLC.

- Further the synthesized compounds were characterized by IR, LC-MS, H^1NMR.

- Biological evaluation is done by MICROPLATE ALAMAR BLUE ASSAY

- Compounds PK2 and PK4 showed activity at 1.6 µg/ml concentrations which are comparable to the activity of the standard drugs Pyrazinamide, Streptomycin, Ciprofloxacin which are active at 3.125µg/ml,6.25µg/ml,3.125µg/ml concentrations.

CONCLUSION

- A series of Schiff bases of isatin derivatives were designed, docked, synthesized and evaluated against Glutamine synthetase 1 enzyme which is critical for the survival and growth of MTB.
- The Minimum Inhibitory Concentration of the synthesized compounds ranges from 50-1.6µg/ml.
- The work concludes that the Compounds Compounds PK2 and PK4 showed activity at 1.6 µg/ml concentrations which are comparable to the activity of the standard drugs Pyrazinamide, Streptomycin, Ciprofloxacin which are active at 3.125µg/ml,6.25µg/ml,3.125µg/ml concentrations.
- Further, structural refinement of the synthesized compounds is expected to yield promising molecules against the pathogen MTB.

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ANNEXURE

INDIAN PHARMACEUTICAL ASSOCIATION

NATIONAL CONVENTION 2017 - 18

Theme: Pharma Vision 2030: Planning the Future

Certificate of Participation

This is to certify that

Dr./Prof./Mr./Ms. K.KALAIYARASI . . P

participated in the National Convention 2017 - 18 held at B. S. Abdur Rahman Crescent Institute of Science & Technology, Chennai, 10th - 11th February 2018

Dr. S. Manivannan	J. Jayaseelan	T. Sathish
Chairman - LOC	IPA Convention Co-ordinator - LOC	Hon. Secretary - LOC

"Skill and Will to Make and Serve Quality Pill"

69th IPC 2017

CHANDIGARH

22nd - 24th December, 2017

PHARMA VISION 2020

PHARMACISTS FOR A HEALTHY INDIA

Certificate

This is to certify that

Prof./Dr./Mr./Ms. KALAIYARASI. P

has participated as Delegate / Volunteer

in the 69th Indian Pharmaceutical Congress

held at Chitkara University, Rajpura from December 22nd to 24th, 2017.

Dr. Mahesh Burande	Dr. Shailendra Saraf	Dr. Dhirender Kaushik	Dr. Ashish Baldi
President - IPCA	Chairman - LOC	Organizing Secretary	Chairman, Registration Committee - LOC

Organised by : Indian Pharmaceutical Congress Association (IPCA)

Hosted by : Association of Pharmaceutical Teachers of India (APTI)

Workshop on

INDUSTRIAL PERSPECTIVES IN ANALYTICAL TECHNIQUES & TRAINING ON CHEMOMETRICS SOFTWARE

Conducted by

Department of Pharmaceutical Analysis
C.L. BAID METHA COLLEGE OF PHARMACY
Thoraipakkam , Chennai - 600 097.

Co - Sponsored by

The Tamil Nadu Dr. M.G.R. Medical University, Chennai

15th & 16th September 2017

CERTIFICATE

15 CME Credit Points

This is to certify that ~~Dr~~/~~Mr~~/Ms. KALAIYARASI. P

has participated as ~~Resource Person~~ /~~Chair Person~~ / Delegate in the Workshop

on **"Industrial Perspectives in Analytical Techniques and Training on Chemometrics Software"** on 15th and 16th September 2017.

CNN

Dr. C.N. Nalini
Organizing Secretary

Dr. Grace Rathnam
Convenor

ARULMIGU KALASALINGAM COLLEGE OF PHARMACY

in association with

THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY

Certificate

This is to certify that Mr./Ms./Prof./Dr. P. KALAIYARASI *of* _____ *has actively participated in the International Seminar on* **"3D - Approaches in Combinatorial Chemistry : Challenges and Perspectives"** *organized by* ***Department of Pharmaceutical Chemistry*** *on 28.02.2018 at Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamil Nadu.*

5 credit points awarded by The Tamil Nadu Dr.M.G.R. Medical University, Chennai.

Dr.R.Rajapandi	Dr.J.Amutha Iswarya Devi	Dr.N.Venkateshan	Dr.S.Shasi Anand	Dr.K.Sridharan
Scientific Advisor	Organizing Secretary	Convenor	Secretary	Chairman

DEPARTMENT OF PHARMACOGNOSY
SRI RAMACHANDRA COLLEGE OF PHARMACY
SRI RAMACHANDRA UNIVERSITY
(Declared under section 3 of the UGC Act, 1956)
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Porur, Chennai - 600 116.

in association with
Society for Ethnopharmacology, Chennai Chapter

CERTIFICATE

This is to certify that Dr./~~Mr.~~/Ms.....P. Kalaiyarasi.....
*has participated as a Resource person / Delegate in the Pre conference workshop on "**Recent Advances in Analytical Techniques - Drugs & Pharmaceuticals**" held on 27th June 2017, organized by Sri Ramachandra University, Porur, Chennai.*

This carries 2 Credits.

D. Chamundeeswari
Dr. D. CHAMUNDEESWARI
Principal &
Co-ordinator, SFE - Chennai Chapter

K.V. Somasundaram
Dr. K.V. SOMASUNDARAM
Dean of Faculties

ARULMIGU KALASALINGAM COLLEGE OF PHARMACY

The Seminar is sponsored & accredited by

THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY

Certificate

This is to certify that Mr./Ms./Prof./Dr. KALAIYARASI. P *of* _____ *has actively participated as a Delegate in the National Seminar on* **"Impact of Intellectual Property Rights in Formulation and Development"** *organized by* ***Department of Pharmaceutics*** *on 23.02.2019 at* ***Arulmigu Kalasalingam College of Pharmacy,*** *Krishnankoil, Tamil Nadu.*

5 credit points awarded by The Tamil Nadu Dr.M.G.R. Medical University, Chennai.

Dr.R.Ramprasad	Dr.S.R.Senthilkumar	Dr.N.Venkateshan	Dr.S.Shasi Anand	Dr.K.Sridharan
Organizing Secretary	Convener	President - LOC	Secretary	Chairman