DESIGN, SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF SOME NOVEL 1, 3, 4-THIADIAZOLE DERIVATIVES AS ANTI-TUBERCULAR AGENTS TARGETING DECAPRENYL PHOSPHORYL BETA-D-RIBOSE 2' EPIMERASE-1"

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

Submitted by
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This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF SOME NOVEL 1, 3, 4-THIADIAZOLE DERIVATIVES AS ANTI-TUBERCULAR AGENTS TARGETING DECAPRENYL PHOSPHORYL BETA-D-RIBOSE 2'EPIMERASE-1''**submitted by the candidate bearing the Register No:**261515702** in partial fulfillment of the requirements for the award of degree of **MASTER OF PHARMACY IN PHARMACEUTICAL CHEMISTRY** by the **TamilNadu Dr. M.G.R Medical University** is a bonafide work done by her during the academic year 2016-2017 in the **Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai- 600 003**.

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ACKNOWLEDGEMENT

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

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

I would like to express my sincere thanks to, **Dr. K. Narayana Samy, M.D., D.M., Dean,** Madras Medical College, and Chennai for providing all the facilities required during the period of my academic study.

It is with great pleasure that I place on record my deep sense of gratitude and heartfelt thanks to my Project advisor, **Prof. Dr. A. Jerad Suresh M.Pharm, Ph.D., MBA**, Principal & Professor and Head, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03. This work would not have been possible without his able guidance, support and encouragement. Under his guidance I Successfully overcame many difficulties and learned a lot. His Passion for Perfection combined with eagerness and enthusiasm to the highest standards inspired and motivated me. I thank him immensely for his guidance.

I wish to thank all the teaching staff **Dr.R.Priyadarsini, M.Pharm, Ph.D., Dr.M.Sathish, M.Pharm., Ph.D., Dr.P.G.Sunitha, M.Pharm, Ph.D., and Mrs.T.Saraswathy, M.Pharm.** Assistant Professors in Pharmacy, Department of Pharmaceutical Chemistry for their gracious support and encouragement in making this work successful.

I express my thanks to all non-teaching staff members **Mr.R.Sivakumar, Mr.S.Baskar, Mrs.Maheshwari, Mrs.Mala, Mrs.K.Murugeshwari, Mrs.Geetha and Mr. Umapathy,**

Mrs.Vijaya Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03, for their assistance during my thesis work.

I sincerely thank **Mr. K.M.Noorulla and Ms. P.R.Surya,** Research Scholars for their support, valuable Suggestions and comments during my project.

I Convey my thanks to **Dr.Kishore Bhatt**, Professor and Head, Department of Microbiology, Maratha Mandals Institute of Dental Science and Research Institute, Belgaum, Karnataka and VIT for helping me to carry out the Spectral Studies in due time.

I have no words to express my hearty thanks to my dear friend's **R.Narayane, A.Menaka, N.vidhyashree, K.Bharathi, M.Madhuraj, G.Leelavathi, V.Durga, and V.Shivakumar.** And all other friends and batch mates for their constant motivation and support.

I express my special thanks to my dear friends, **V.Sundar, R.Gowtham, V.Vivek**, **A.Dinesh Kumar, and E.Ramar.** And my Sisters **S.kavitha, S.Ramya** for their kind support and motivation extended throughout my research work.

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

Finally yet importantly, I wish to express my heartfelt thanks to my beloved **Mother Mrs. S.Sasikala,** for their blessings, encouragement and wishes for the successful completion of this dissertation.

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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
DPRE1	Decaprenyl phosphoryl-beta-D-ribose 2-epimerase
CADD	Computer Aided Drug Design
QSAR	Quantitative Structural Activity Relationship
PSA	Polar Surface Area
OSIRIS	Optical, Spectroscopic and Infrared Remote Imaging System
OPLS	Optimized Potential for Liquid Stimulation
TPSA	Total Polar Surface Area
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

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INTRODUCTION

BACKGROUND:

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

In 2011, tuberculosis [TB] remained the second cause of death from infectious disease worldwide. It mainly affects the poorest countries of Africa and Southeast Asia. In 2010, according to the world health organization [WHO], TB incidence and prevalence were estimated at 8.8 and 12 million cases respectively about 1.1 million among HIV-positive people died from TB. Most importantly, one third of the world population is infected with latent infection and10% of those infected people will develop active TB in their life.

The directly observed treatment short-course [DOTS], a multiple therapy program developed by WHO is one of the most efficient weapons against the global TB epidemic. Nevertheless, the treatment success rate struggles to reach the target of 85%. Unfortunately, first –line treatment can fail due to poor compliance which leads to the emergence of multidrug resistance [MDR] strains of M.tuberculosis. The number of TB drugs in preclinical and clinical development is today higher than that during the past 40 year **(1)**

TB IN INDIA

India is the country with the highest burden of TB, with world health organization [WHO] statistics for 2014 giving an estimated incidence 2.2 million cases of TB for India out of a global incidence of 9 million. The estimated TB prevalence figure for 2014 is given as 2.5 million. It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB **(2)**

HISTORY OF TUBERCULOSIS:-

Tuberculosis (TB) is believed to have been present in humans for thousands of years. Skeletal remains show that prehistoric humans (4000 BC) had tuberculosis, and tubercular decay has been found in the spines of Egyptian mummies (3000-2400 BC).

During the 17th century, exact pathological and anatomical descriptions of tuberculosis began to appear. In 1679, Sylvius wrote his *Opera Medica*, in which he was the first to identify actual tubercles as a consistent and characteristic change in the lungs and other areas of consumptive patients. The earliest references to the infectious nature of tuberculosis also appeared in 17th century Italian medical literature**.** [(3)]

Due to the variety of its symptoms, TB was not identified as a unified disease until the 1820s, and was not named tuberculosis until 1839 by J.L. Schonlein.

In 1854, Hermann Brehmer proposed the idea that tuberculosis was indeed a curable disease. The introduction of the sanatorium cure provided the first big step toward treatment for tuberculosis. Brehmer himself was a TB patient. His doctor advised him to move to a healthier climate, so he spent some time in the Himalayas and came home cured. This experience moved him to build the first sanatorium, a place where patients could get plenty of fresh air and good nutrition. This setup became the blueprint for the subsequent development of sanatoriums.

On 24 March 1882 Robert Koch discovered the staining technique that identified Tuberculosis bacillus and thus treatment begin. In 1890 Robert Koch discovered Tuberculin a Diagnostic use when injected in to the skin. [(3, 5)]

In 1895 Roentgen Discovery of x-rays made possible a early diagnosis of Pulmonary disease. In 1908-1920 Attenuated strain of Mycobacterium calmette and Guerin were in use after that in 1943 Selman Abraham Waksman discovered Streptomycin injections dramatically recovers patients with patients**.** [(6)]

In 1950 combination of Streptomycin and Para aminosalicyclic acid were used and in 1952 a major drug called Ionized replaces Sanatorium as major treatment patients can be treated as out-patients.

CURRENT THERAPIES:

Tuberculosis [TB] is caused by mycobacterium tuberculosis that most often affect the lungs. Tuberculosis is curable and preventable. In 1882, the German physician Robert Koch isolated the bacterium. Tuberculosis is contagious and airborne disease.

In 1944, streptomycin was to treat tuberculosis [TB]. This amino glycoside interferes with protein biosynthesis through an interaction with the small 30s subunit of the ribosome. The discovery of Para amino salicylic acid in 1946 was quickly followed by the important discovery of Isoniazid [INH], as one of the most active anti-TB drugs used today. Inhibition of mycolic acids biosynthesis, one of the essential components of the mycobacterium cell wall was determined as the mechanism of action. Pyrazinamide [PZA] appeared as a potential Anti-TB drug in 1952.

The TB treatment in the 1980s was a great success as it allowed to shorten the duration of the therapy from 9 to 6 months. Ethambutol [EMB] and Rifampin [RIF], the two last derivatives used in the TB first-line treatment, were discovered during the 60s. Ethambutol is an ethylenediamine discovered in 1961, which affects the cell wall by specifically targeting the polymerization of arabinogalactan and lipoarabinomannan. Finally, Rifampin appeared as a drug of choice for TB treatment around 1970, by acting on replicating and non-replicating mycobacteria. This derivative belongs to the rifampicin family and inhibits bacterial RNA synthesis by binding to the b-subunit of the DNA-dependent polymerase.

The current standard regimen [DOTS] for TB recommended by WHO is a combination of isoniazide, rifampicin, ethambutol, and pyrazinamide for 6 months therapy. To treat MDR-TB, WHO recommended the use of second-line drugs which include amino glycosides [Kanamycin, amikacin], Capreomycin, cycloserin, para-

aminosalicylic acid, Thionamides [Ethionamide, proethionamide] and fluoroquinolones [ciprofloxacin, ofloxacin, Levofloxacin]. [1]

TYPES OF TUBERCULOSIS [9, 10]

Tuberculosis is a contagious disease; it affects almost all the important organs of the body. Clinically, tuberculosis is broadly categorized into three major categories

1. Primary tuberculosis.
2. Secondary tuberculosis.
3. Disseminated tuberculosis

Primary tuberculosis:

When tuberculosis affects a person who had never been exposed to the bacterium earlier, the condition is called primary tuberculosis. In this form of tuberculosis the source of bacterium is external.

Secondary tuberculosis:

It is also known as post primary tuberculosis. This type of tuberculosis occurs in a person who previously had TB. In primary TB, the bacterium goes into an inactive face while in secondary TB the bacterium regain its active mode and causes the symptom. Secondary tuberculosis more infectious than primary TB. Secondary TB increases the chance of the infections spread to other organs such as kidneys, heart, and brain

Disseminated tuberculosis:

Disseminated means that the tuberculosis has infected the entire body system. It is a very rare type of disease. It primarily affects the bones of spines, hips, joints and knees and even the central nervous system. It infects the CSF, the GIT, the adrenal gland, skin of the neck and even the heart.

CELL WALL:- [11]

The well - developed cell wall contains a considerable amount of a fatty acid, mycolic acid, covalent attached to the underlying peptidoglycan-bound polysaccharide arabinogalactan, providing an extraordinary lipid barrier. This barrier is responsible for many of the medically challenging physiological characteristics of tuberculosis. The composition and quality of the cell wall components affect the bacteria's virulence and growth rate.

The peptidoglycan polymer confers cell wall rigidity and just external to the bacterial cell membrane, another contributor to the permeability barrier of mycobacteria. The peptidoglycan polymer confers cell wall rigidity and is just external to the bacterial cell membrane, another contributor to the permeability barrier of mycobacteria.

Another important component of the cell wall is lipoarabinomannan, a carbohydrate structural antigen on the outside of the organism that is immunogenic and facilitates the survival of mycobacteria within macrophages. The cell wall is key to the survival of mycobacteria and a more complete understanding of the biosynthetic pathways and gene functions and the development of antibiotics to prevent formation of the cell wall are areas of great interest.

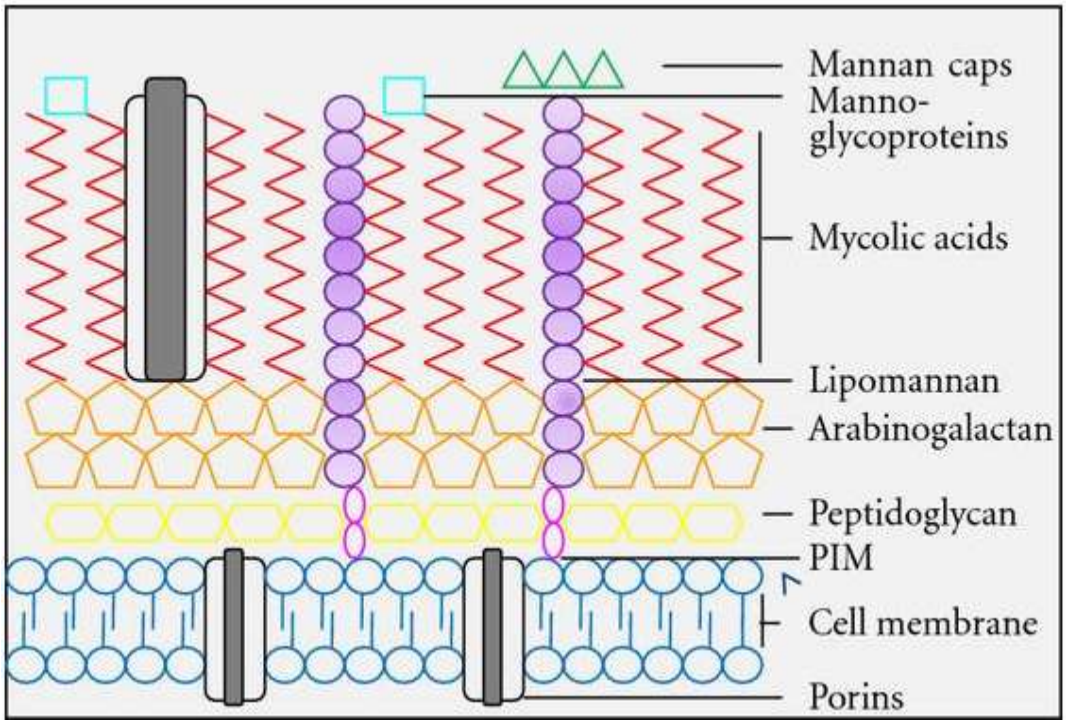


Fig No1:-Structure of cell wall

Pathogenesis of tuberculosis[12]:

In the lungs, M. tuberculosis is taken up by alveolar macrophages, but they are unable to digest the bacterium. Its cell wall prevents the fusion of the phagosome with a lysosome. Specifically, M. tuberculosis blogs the bridging molecule, early endosomal auto antigen 1(EEA1); however, this blockade does not prevent fusion of vesicle filled with nutrients. Consequently, the bacteria multiply

unchecked within the macrophages. The bacteria also carried the UreC gene, which prevents acidification of the phagosome. The bacteria also evade macrophages-killing by neutralizing reactive nitrogen intermediates. tuberculosis usually enters the alveolar passages of exposed humans in an aerosol droplet, were its first contact is thought be with resident macrophages, but it is also possible that bacteria can be initially ingested by alveolar epithelial type II pneumocystis this cell type is found in greater numbers than macrophages in alveoli, and M. tuberculosis can infect and grow in this pneumocystis *ex vivo.*

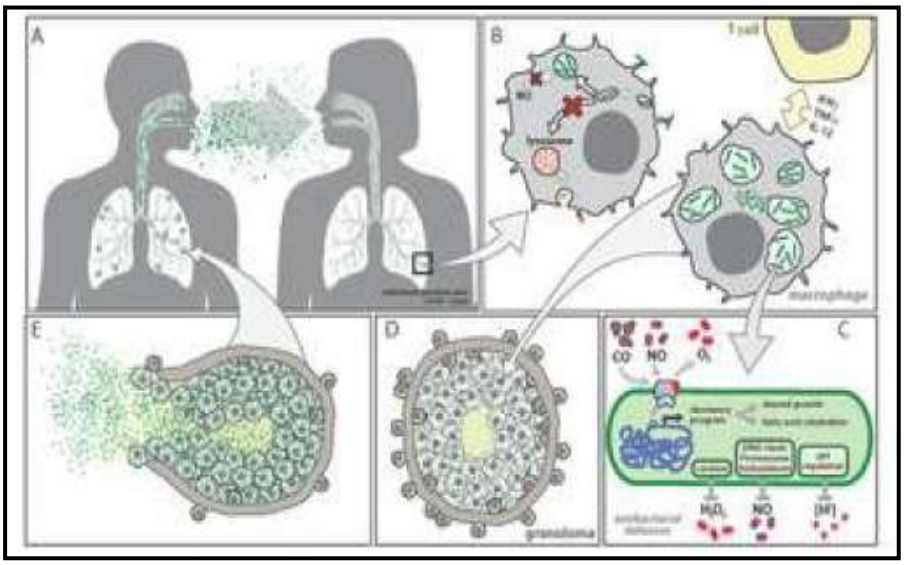


Fig No:2 Pathogenesis of M. Tuberculosis

DIAGNOSIS OF TUBERCULOSIS:-

The most common diagnostic test for TB is a skin test where a small injection of PPD tuberculin, an extract of the TB bacterium, is made just below the inside forearm. The injection site should be checked after 2-3 days, and, if a hard, red bump has swollen up to a specific size, then it is likely that TB is present. Unfortunately, the skin test is not 100 percent accurate and has been known to give incorrect positive and negative readings. However, there are other tests that are available to diagnose TB. Blood tests, chest X-rays and sputum tests can all be used to test for the presence of TB bacteria and may be used alongside a skin test. MDR-TB is more difficult to diagnose than regular. TB it is also difficult.

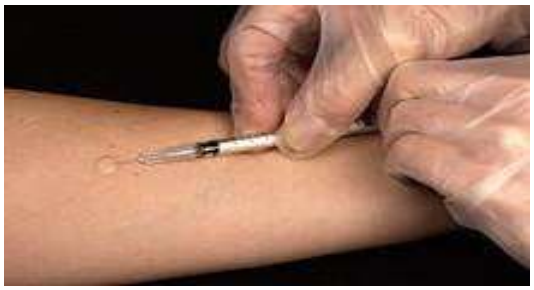


Fig No:3 Diagnosis of Tuberculosis

TREATMENTS FOR TUBERCULOSIS :-[8]

The majority of TB cases can be cured when the right medication is available and administered correctly. The precise type and length of antibiotic treatment depends on a person's age, overall health, potential resistance to drugs, whether the TB is latent or active, and the location of infection (i.e. the lungs, brain, kidneys).People with latent TB may need just one kind of TB antibiotics, whereas people with active TB (particularly MDR-TB) will often require a prescription of multiple drugs. Antibiotics are usually required to be taken for a relatively long time. The standard length of time for a course of TB antibiotics is about 6 months TB medication can be toxic to the liver, and although side effects are uncommon, when they do occur, they can be quite serious. Potential side effects should be reported to a doctor and include:

- Dark urine
- Fever
- Jaundice
- Loss of appetite
- Nausea and vomiting

It is important for any course of treatment to be completed fully, even if the TB symptoms have gone away. Any bacteria that have survived the treatment could become resistant to the medication that has been prescribed and could lead to developing MDR-TB in the future. Directly observed therapy (DOT) may be

recommended. This involves a healthcare worker administering the TB medication to ensure that the course of treatment is completed

PREVENTION OF TUBERCULOSIS:-

A few general measures can be taken to prevent the spread of active TB. Avoiding other people by not going to school or work, or sleeping in the same room as someone, will help to minimize the risk of germs from reaching anyone else. Wearing a mask, covering the mouth, and ventilating rooms can also limit the spread of bacteria.

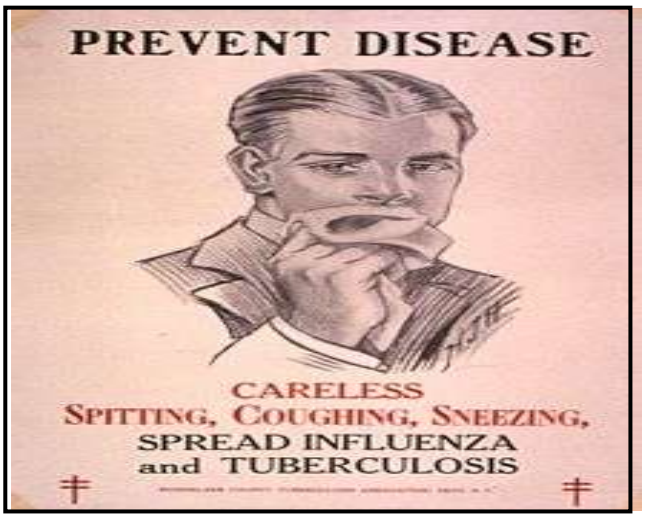


Fig No: 4 Prevention of Disease

TB VACCINATION:-

In some countries, BCG injections are given to children in order to vaccinate them against tuberculosis. It is not recommended for general use in the U.S. because it is not effective in adults, and it can adversely influence the results of skin testing diagnoses. The most important thing to do is to finish entire courses of medication when they are prescribed. MDR-TB bacteria are far deadlier than regular TB bacteria. Some cases of MDR-TB require extensive courses of chemotherapy, which can be expensive and cause severe adverse drug reactions in Patients. [12, 15]

CURRENT STATUS OF TUBERCULOSIS:-

Despite all the drugs available today, tuberculosis is still a problem in many nations. According to World Health Organization (WHO) estimates, each year, 8 million people worldwide develop active tuberculosis and nearly 2 million die. While the overall rate of new tuberculosis cases has continued to decline in the United States since national reporting began in 1953, the annual decrease in tuberculosis cases has slowed dramatically. TB continues to kill between 2 and 3 million people every year. The WHO estimates that 36 million people will die of tuberculosis by 2020 if it is not controlled**.** **(2)**

EPIDEMIOLOGY:-

Tuberculosis is estimated to affect 1.7 billion individuals worldwide, with 8 to 10 million new cases and 1.7 million deaths every year. After HIV, tuberculosis is the leading infectious cause of death in the world. Infection with HIV makes people Susceptible to rapidly progressive tuberculosis over 50 million people are infected with both HIV and M.tuberculosis. From 1985 to 1992, the number of tuberculosis cases in the United States increased by20% because of increase in disease among people with HIV, among immigrants, and among those in jail or homeless shelters. Because of increased public health efforts, the number of cases of tuberculosis has declined since 1993. Currently, there are about 16,000 new cases of active tuberculosis in the United States annually, and about 45% of these are in immigrants. **(17, 18)**

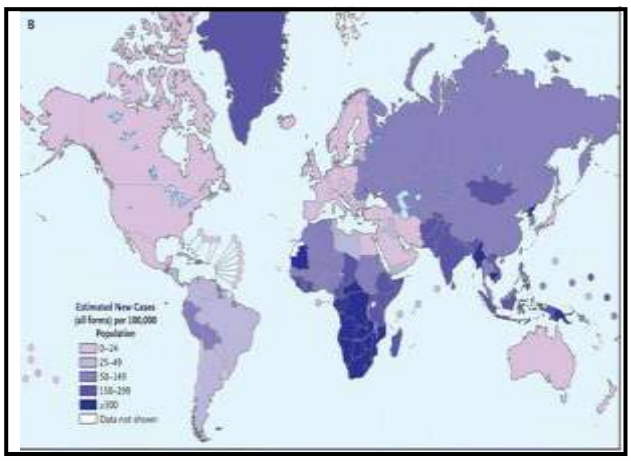


Fig No: 5 Epidemiology of Tuberculosis

MEDICINAL CHEMISTRY [22, 23]

Medicinal chemistry and pharmaceutical chemistry are disciplines at the intersection of chemistry, especially synthetic organic chemistry, and pharmacology and various other biological specialties, where they are involved with design, chemical synthesis and development for market of pharmaceutical agents, or bio-active molecules (drugs).

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

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

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

The concept of the drug-receptor interaction has undergone much modification from 1960s to 1990s. The use of computer graphics to portray drug-receptor interaction has also been a notable interaction has been a notable development of the decade.

The approaches to practice of Medicinal chemistry has developed from an empiric one involving organic synthesis of new compounds, based largely on

modification of structure of known activity, to one that is more logical and less intuitive is mostly because of advancement in molecular biology, Pharmacology, and Enzymology .[22]

ENZYME PROFILE:-

Resistance against currently used Anti tubercular therapeutics increasingly undermines efforts to contain the worldwide tuberculosis epidemic. Recently, benzothiazinone [BTZ] inhibitors have shown nanomolar potency against both drug-susceptible and multidrug-resistance strains of the tubercle bacillus. However, their proposed mode of action is lacking structural evidence. The crystal structure of the BTZ target, FAD-containing oxido-reductase Mycobacterium tuberculosis DPRE1, which is essential for viability.

Different crystal forms of ligand –free Dpre1 reveal considerable levels of structural flexibility of two surface loops that seem to govern accessibility of the active site. Structure of complexes with the BTZ- derived nitroso derivative CT325 reveal the mode of inhibitor binding.

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

Biochemical studies showed that rv3790and the neighboring gene rv3791 code for proteins that act in concert to catalyze the epimerization of decaprenylphosphoryl ribose[DPR] to decaprenylphosphoryl arabinose [DPA] a precursor for Arabian synthesis without which a complete mycobacterium cell wall cannot be produced.

DPA is the sole known donor substrate for a series of membrane-embedded Arabinosyl transferees', including the Ethambutol targets Embc, EmbA, and Emb. Essentiality of DPA supply and lack of alternative synthetic pathways position DPRE1, which is highly conserved in mycobacterium, and Dpre2 at a critical intersection of cell wall biosynthesis. A motion confirmed by transposon mutagenesis. This situation has led DPRE1 as a magic drug target**.** [27]

GLOBAL IMPORTANCE ABOUT THE TARGET:-

The global tuberculosis epidemic and emergence of drug resistance call for intensive research on new antimycobacterial agents. Recent development is focused mainly on heterocyclic molecules. In many cases, introduction of sulphur has improved antimicrobial activity; many drugs feature Sulphur heterocyclic. Thiophene derivatives and Thiadiazoles including derived ortho -condensed heterocycles have been found to have a wide range of biological activities. This review highlights the recent progress in the field with a focus on whole-cell antimycobacterial activity of the agents as well as targeting of enzymes from Mycobacterium tuberculosis. Some of the compounds have exhibited high activity with submicromolar minimum inhibitory concentrations including activity against drug-resistant strains and/or IC50 values for a range of enzymes as their targets (InhA, dehydroquinase, Pks13, carbonic anhydrates, DprE1). Mechanisms of action, toxicity, and structure-activity relationships are also discussed. Several compounds have exhibited promising in vitro and in vivo activities and safety profiles, thus constituting novel, promising leads.

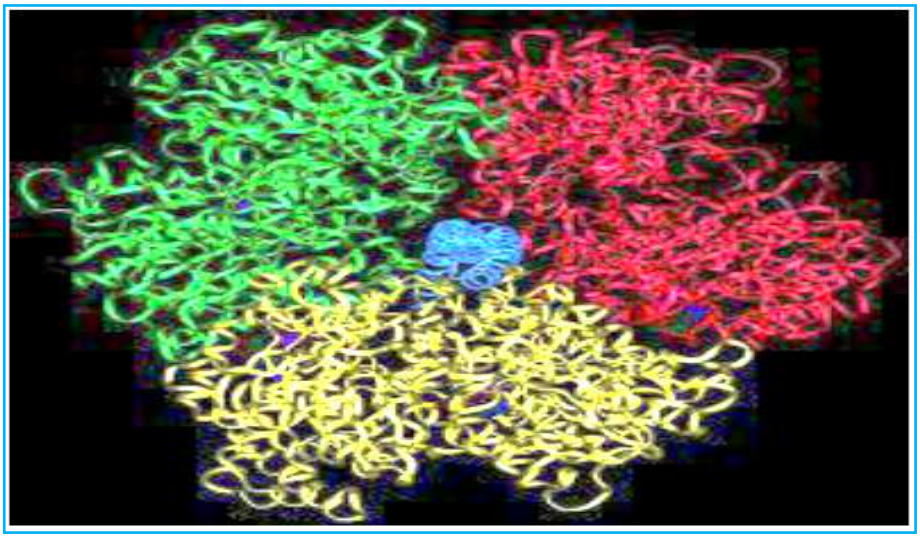


Fig NO: - 6 Decaprenylphosphoryl beta-D-ribose 2-epimerase enzyme

GENERAL ANNOTATION [20]

Gene name: DPRE1

RV number: Rv3790

Type : CDS

Function : Together dpre1 [Rv3791, catalyzes epimerization of decaprenyl phosphoryl ribose

DPR to decaprenyl phosphoryl arabinose [DPA] in Arabians synthesis.

Product: Decaprenylphosphoryl-beta-D-ribose 2-epimerase-1

Molecular mass: 50163.2

Isoelectric point: 7.769

Gene length : 1386

Protein length : 461

Location [Kb] : 4235.78

Functional category: Lipid metabolism

Proteomics: Identified in the membrane fraction of M.tuberculosis H37RV using

ID-SDS-PAGE and ULC-MS/MS [See GU et al., 2003].

Identified in the membrane fraction of M.tuberculosis H37RV using

2DLC/MS [see Mawuenyega et al., 2005].

Identified by mass spectroscopy in triton X-114 extracts of

M.tuberculosis H37RV [see Malan et al., 2010]. Identified by mass

Spectroscopy in the membrane protein fraction and whole cell lysates.

DRUG DESIGN:-

Drug discovery process involves a rapid search for a small molecule often called as lead. A lead molecule is chemical compound which possess pharmacological or biological activity. Sources of lead compounds can come from natural sources, such as plants, animals, or fungi and also from synthetic chemical libraries.

LEAD OPTIMIZATION:

Newly invented pharmacologically active moieties may have poor drug-likeness and may require lead optimization step. This step involves chemical modification of a lead in order to improve their potency, selectively towards binding site, pharmacokinetic parameters and reduced toxicity.

RATIONAL DRUG DESIGN:-

Drug design, often referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. In the most basic sense, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling is sometimes 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.

The phrase "drug design" is to some extent a misnomer. A more accurate term is ligand design (i.e., design of a molecule that will bind tightly to its target). Although design techniques for prediction of binding affinity are reasonably successful, there are many other properties, such as bioavailability, metabolic half-

life, side effects, etc., that first must be other properties, such as bioavailability, metabolic half-life, side effects, etc., that first must be optimized before a ligand can become a safe and efficacious drug. These other characteristics are often difficult to predict with rational design techniques. Nevertheless, due to high attrition rates, especially during clinical phases of drug development, more attention is being focused early in the drug design process on selecting candidate drugs whose physicochemical properties are predicted to result in fewer complications during development and hence more likely to lead to an approved, marketed drug. Furthermore, in vitro experiments complemented with computation methods are increasingly used in early drug discovery to select compounds with more favorable ADME (absorption, distribution, metabolism, and excretion) and toxicological properties .

In contrast to traditional methods of drug discovery (known as forward pharmacology), which rely on trial-and-error testing of chemical substances on cultured cells or animals, and matching the apparent effects to treatments, rational drug design (also called reverse pharmacology) begins with a hypothesis that modulation of a specific biological target may have therapeutic value. In order for a biomolecule to be selected as a drug target, two essential pieces of information are required. The first is evidence that modulation of the target will be disease modifying. This knowledge may come from, for example, disease linkage studies that show an association between mutations in the biological target and certain disease states. The second is that the target is "druggable". This means that it is capable of binding to a small molecule activity can be modulated by the small molecule. Once a suitable target has been identified, the target is normally cloned and produced and purified. The purified protein is then used to establish a screening assay. In addition, the three-dimensional structure of the target may be determined.

The search for small molecules that bind to the target is begun by screening libraries of potential drug compounds. This may be done by using the screening assay (a "wet screen"). In addition, if the structure of the target is available, a virtual screen may be performed of candidate drugs. Ideally the candidate drug compounds should be "drug-like", that is they should possess properties that are predicted to lead to oral bioavailability, adequate chemical and metabolic stability, and minimal toxic effects.

Several methods are available to estimate druglikeness such as Lipinski's Rule of Five and a range of scoring methods such as lipophilic efficiency. Several methods for predicting drug metabolism have also been proposed in the scientific literature.

COMPUTER AIDED DRUG DESIGNING:-

The most fundamental goal in drug design is to predict whether a given molecule will bind to a target and if so how strongly. Molecular mechanics or molecular dynamics is most often used to estimate the strength of the intermolecular interaction between the small molecule and its biological target. These methods are also used to predict the conformation of the small molecule and to model conformational changes in the target that may occur when the small molecule binds to it. Semi-empirical, ab initio quantum chemistry methods, or density functional theory are often used to provide optimized parameters for the molecular mechanics calculations and also provide an estimate of the electronic properties (electrostatic potential, polarizability, etc.) of the drug candidate that will influence binding affinity.

Molecular mechanics methods may also be used to provide semi-quantitative prediction of the binding affinity. Also, knowledge-based scoring function may be used to provide binding affinity estimates. These methods use linear regression, machine learning, neural nets or other statistical techniques to derive predictive binding affinity equations by fitting experimental affinities to computationally derived interaction energies between the small molecule and the target.

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

1. Hit identification using virtual screening (structure- or ligand-based design).
2. hit-to-lead optimization of affinity and selectivity (structure-based design, QSAR, etc.).
3. Lead optimization of other pharmaceutical properties while maintaining affinity.

In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and compound 3D structure information are used for analysis. For structure-based drug design, several post-

screening analyses focusing on protein-ligand interaction have been developed for improving enrichment and effectively mining potential candidates. Consensus scoring Selecting candidates by voting of multiple scoring functions May lose the relationship between protein-ligand structural information and scoring criterion Cluster analysis Represent and cluster candidates according to protein-ligand 3D information Needs meaningful representation of protein-ligand interactions.

Ideally, the computational method will be able to predict affinity before a compound is synthesized and hence in theory only one compound needs to be synthesized, saving enormous time and cost. The reality is that present computational methods are imperfect and provide, at best, only qualitatively accurate estimates of affinity. In practice it still takes several iterations of design, synthesis, and testing before an optimal drug is discovered. Computational methods have accelerated discovery by reducing the number of iterations required and have often provided novel structures**.** [30]

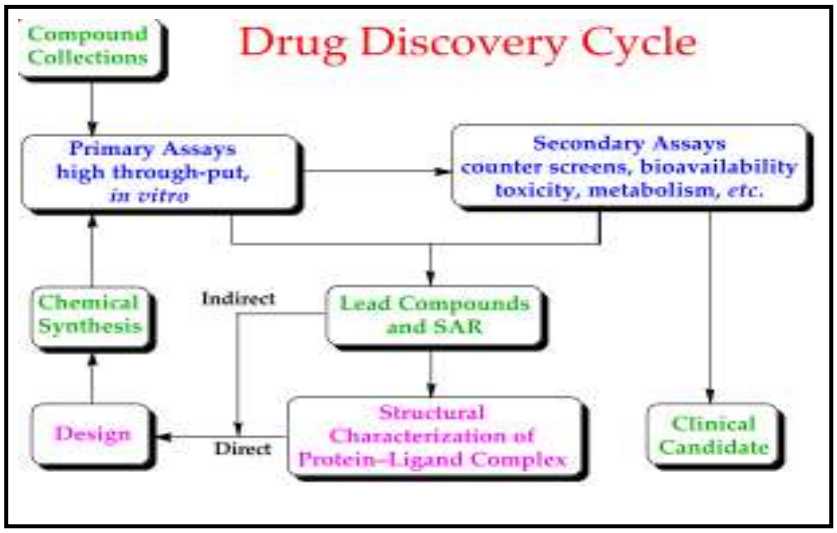


Fig NO: 7 DRUG DISCOVERY CYCLE

TYPES OF DRUG DESIGN:-

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

LIGAND BASED DRUG DESIGN:-

Ligand-based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. [30] In other words, a model of the biological target may be built based on the knowledge of what binds to it, and this model in turn may be used to design new molecular entities that interact with the target. Alternatively, a quantitative structure-activity relationship (QSAR), in which a correlation between calculated properties of molecules and their experimentally determined biological activity, may be derived. These QSAR relationships in turn may be used to predict the activity of new analog.

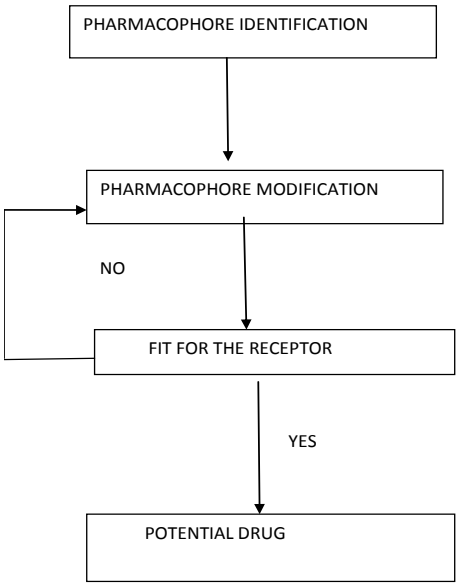


Fig No: 8Ligand based drug design

STRUCTURE BASED DRUG DESIGN:-

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

target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist. Alternatively various automated computational procedures may be used to suggest new drug candidates.

Drug discovery cycle highlighting both ligand-based (indirect) and structure-based (direct) drug design strategies. Current methods for structure-based drug design can be divided roughly into three main categories. The first method is identification of new ligands for a given receptor by searching large databases of 3D structures of small molecules to find those fitting the binding pocket of the receptor using fast approximate docking programs. This method is known as virtual screening. A second category is de novo design of new ligands. In this method, ligand molecules are built up within the constraints of the binding pocket by assembling small pieces in a stepwise manner. These pieces can be either individual atoms or molecular fragments. The key advantage of such a method is that novel structures, not contained in any database, can be suggested. a third method is the optimization of known ligands by evaluating proposed analogs within the binding cavity.

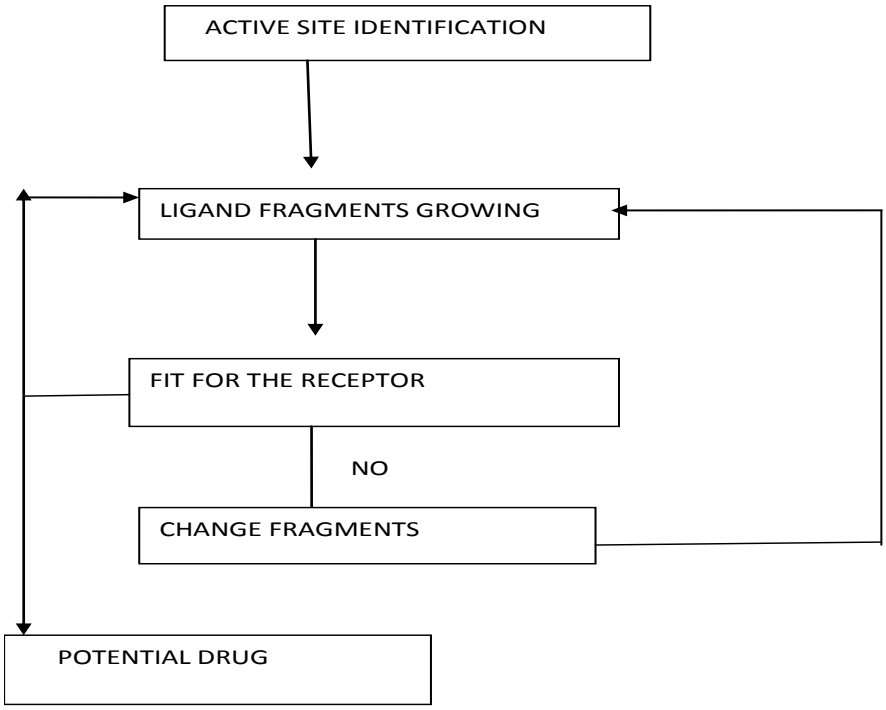


Fig NO: 9 Structure based drug design

Binding site identification:-

Binding site identification is the first step in structure based design. If the structure of the target or a sufficiently similar homolog is determined in the presence of a bound ligand, then the ligand should be observable in the structure in which case location of the binding site is trivial. However, there may be unoccupied allosteric binding sites that may be of interest. Furthermore, it may be that only apoprotein (protein without ligand) structures are available and the reliable identification of unoccupied sites that have the potential to bind ligands with high affinity is non-trivial. In brief, binding site identification usually relies on identification of concave surfaces on the protein that can accommodate drug sized molecules that also possess appropriate "hot spots" (hydrophobic surfaces, hydrogen bonding sites, etc.) that drive ligand binding.

SCORING FUNCTIONS:-

Structure-based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying the principles of molecular recognition. Selective high affinity binding to the target is generally desirable since it leads to more efficacious drugs with fewer side effects. Thus, one of the most important principles for designing or obtaining potential new ligands is to predict the binding affinity of a certain ligand to its target (and known anti targets) and use the predicted affinity as a criterion for selection.

Due to the large number of drug properties that must be simultaneously optimized during the design process, multi-objective optimization techniques are sometimes employed. Finally because of the limitations in the current methods for prediction of activity, drug design is still very much reliant on serendipity and bounded rationality.

SCREENING AND DESIGN:-

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

The structure –activity relationship [SAR] is to improve certain features of the lead compound

- Increase activity against the chosen target
- Reduce activity against unrelated targets
- Improve the drug likeness or ADME properties of molecule

This process will require several iterative screening runs, during which, it is hoped, that properties the new molecule entities will improve , and allow the favored compounds to go forward to in vitro and in vivo testing for activity in the disease model of choice. A range of parameters can be used to assess the quality of a compound, or a series of compounds, as proposed in the Lipinskis Rule of Five. Such parameters include calculated properties such as clog p to estimate liphophilicity, molecular weight, polar surface area and measured properties, such as potency, in-vitro measurement of enzymatic clearance etc. some descriptors such as ligand efficiency[LE] and lipophilic efficiency[LIPE] combine such parameters to assess drug likeness. Other methods, such as virtual high through put screening, where screening is done using computer-generated models and attempting to'' dock'' virtual libraries to a target, are also often used

DOCKING

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 complementarity to the target in terms of shape and properties such as electrostatics in order to identify the most favorable energetical pose.

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.

PROTEIN PREPARATION

It is now accepted that the old idea of the "key and lock" interaction of a ligand and its protein. Receptor is not an accurate description of most biological complexes. The ligand-protein interactions resemble more a "hand and glove" association, where both parts are flexible and adjust to complement each other induced fit. The can modify their shape and mould their complementarity. So, as to increase favorable contacts and reduced adverse interactions, maximizing the total binding free energy. It has been found that active site regions of enzymes appear to present areas of both low and high conformational stability.

RECEPTOR CONFORMATION

The three dimensional (3-D) structures of both ligand and protein are necessary for the application of docking techniques. While the manifold of conformational structures of small molecule may be relatively easy to predict, the lowest energy conformation obtained may not correspond to that of the bound ligand. Many proteins targeted for drug design do not have an experimentally determined structure and, therefore, docking studied cannot be performed directly. In some cases, computational techniques can be used to predict the 3D structure of a protein provided the structure of a closely related protein homolog is known. Homology modeling or sequence threading techniques may be used to generate models of protein structure which, although not as good as experimentally determined structures, can be used as docking targets.

ADME ANALYSIS: [24]

For a drug to be pharmacologically active and exert the action it should posses' pharmacokinetic properties like absorption, distribution, metabolism and excretion. In the field of drug research and development many failures do occur, as they do not undergo these properties satisfactorily. This has to be ruled out earlier in the process of drug discovery. Many in-vitro studies are more frequently used to evaluate ADME properties. Some computational methods [in silico tools] have been evolved to investigate the most suitable drug molecules.

Prediction of ADME related properties [25]

Absorption:

To investigate this in silico models uses simple parameters like log D [diffusion coefficient] and polar surface area are the descriptors for hydrogen bonding capacity and logp (partition coefficient) values should fall under the prescribed values as per the rule of five, which determines the absorption.

Bioavailability:

Size and shape of the molecules, lipophilicity and flexibility determines the bioavailability.

Metabolism:

Various in silico approaches are exisiting in evaluating the metabolism namely QSAR and 3D QSAR.

BASIC NUCLEUS INTRODUCTION HETEROCYCLIC CHEMISTRY:

Heterocyclic structures always are a part in the field of research and development in organic chemistry. Millions of heterocyclic structures are found to exist having special properties and biological importance. A series of thiadiazole have been synthesized using an appropriate synthetic route and characterized by elemental analysis and spectral data.

There are various types of thiadiazole rings are present:

1, 2, 4-Thiadizole

1, 3, 4-Thiadizole

1, 2, 5-Thiadizole

1, 2, 3-Thiadizole

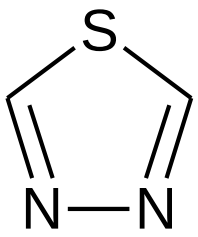
THIADIAZOLE NUCLEUS:

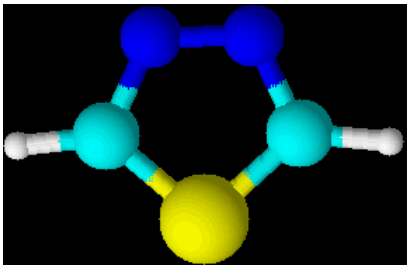
1, 3, 4-THIDIAZOLE:

Thiadiazole contains the five-membered di unsaturated ring structure having molecular structure formula C2H2N2S containing two carbon atoms, two hydrogen , two nitrogen's and one sulphur. The ending azole designates a five membered ring system with two or more heteroatom, one of which is Nitrogen. Thiadiazoles are associated with diverse biological activity probably by virtue of –N=C-S-

1,3,4- Thiadiazole moiety contain a heterocyclic nucleus in which sulfur present at position -1, and two nitrogen atom at position-3&position-4. Thiadiazole derivatives have a unique place in the field of medicinal chemistry.

Thiadiazole is a biologically identical to that of Pyrimidine and Ox diazole and given the prevalence of pyrimidine in nature, it is not surprising that thiadiazole shown significant therapeutic potential properties. The Sulfur atom of thiadiazole imparts improved liposolubility and mesoionic in nature.





1, 3, 4 Thiadiazole

This literature review shows that the thiadiazole nuclei have Various Biological activities such as

- Anti-tubercular, [39]
- Analgesic, [37]
- Anticonvulsant, [40]
- Anti-inflammatory, [37]
- Antioxidant, [41]
- Anticancer,[41]
- Anti-fungal.[38]

On view of the importance of the Thiadiazole nucleus. It was decided to design nucleus Based on the Thiadiazole nucleus. One hundred different molecules with the thiadiazole Scaffold were drawn and docked.

Thiadiazole is a versatile moiety that exhibits a wide variety of biological activities. Thiadiazole moiety acts as “hydrogen binding domain” and “two-electron donor system”. It also acts as a constrained pharmacophore. Many drugs containing thiadiazole nucleus are available in the market such as Acetazolamide, Methazolamide, Sulfamethazole,etc. Thiadiazole can act as the bio-isosteric replacement of the thiazole moiety. So it acts like third and fourth generation cephalosporin, hence can be used in antibiotic preparations.

Thiadiazole derivatives possess interesting biological activity probably conferred to them by the strong aromaticity of this ring system, which leads to great in vivo stability and generally, a lack of toxicity for higher vertebrates, including humans. When diverse functional groups that interact with biological receptors are attached to this ring, compounds possessing outstanding properties are obtained.

2. LITERATURE REVIEW

The purpose of Literature Review is to:

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

2.1 The review on following Works provided basic information about the target enzyme, DPRE1 and its function.

Sarah M. Bhatt., et al., (2012), [27] Structural basis of inhibition of Mycobacterium tuberculosis Dpre1 by benzothiazinone inhibitors.

Maria Loreto in candela, et.al, (2013), [28] reported that Dpre1, a new taxonomic marker in mycobacteria.

Manina, et al., (2010), [30] reported that Decaprenylphosphoryl-β-D-ribose 2′-epimerase from *Mycobacterium tuberculosis* is a magic drug target.

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

Donald R Ronning., et al., (2012), is targeting the mycobacterium envelope for tuberculosis drug development**.** [31]

Sarala Men on, et al., (2012), studied the Drug resistance profiles of Mycobacterium tuberculosis isolates to first line anti-tuberculous drugs**.** [32]

P.Mudassar, et al., (2011), had a Brief review on Multi Drug resistant Mycobacterium tuberculosis**.** [33]

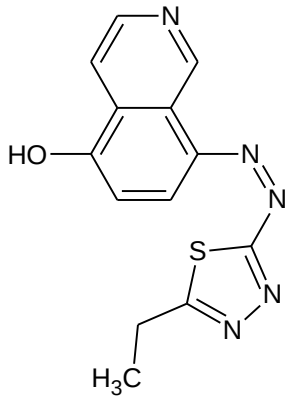
Christian Leichardt., et al., (2010), had a review on new drugs and new regimens for the treatment of tuberculosis: review of the drug development pipeline and implications for national programmes. [34]

Sarah L. Kinnings., et al., (2010), reviewed the Mycobacterium tuberculosis Drug and its Polypharmacological Implications**.** [35]

2.3 The review on following works provided ideas about the Thiadiazole Nucleus and its Biological Activity.

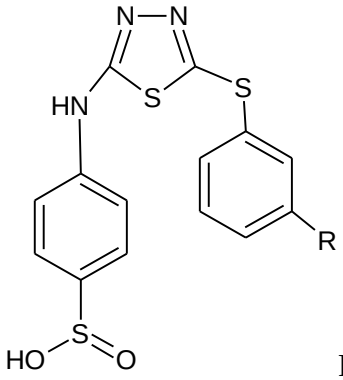
Antimicrobial activity:-

Kumar, et al., (2011), synthesized the heterocyclic azodyes derived from thiadiazole and evaluated them for antimicrobial activity. In this study 5-ethyl-1, 3, 4-thiadiazole-2-amine was synthesized by a single step reaction. A series of heterocyclic azodyes were synthesized by coupling 8-hydroxyquinoline, 2, 6-diaminopyridine, *N, N*-dimethyl aniline, 2-napthol and resorcinol with diazotized 5-ethyl-1, 3, 4-thiadiazol-2-amine in nitrosyl sulphuric acid. The synthesized compounds were also screened for biological activity and showed maximum activity**.** [(36)]



Anti inflammatory and analgesic activity:-

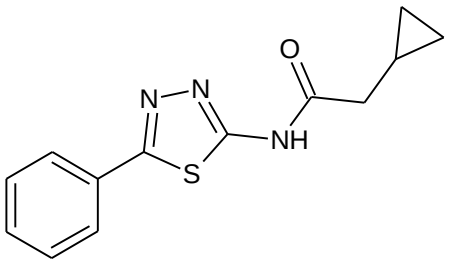
Sainy et al., (2009), synthesized a series of 2-amino-5-sulfanyl-1, 3, 4-thiadiazole derivatives and several 2-amino-5-sulfanyl-1, 3, 4-thiadiazoles and concluded that the compounds were associated with lesser degree of anti-inflammatory activity when compared to indomethacin. Only compound 4-[5-(4-Fluorophenylsulfanyl) -[1,3,4] thiadiazol-2-ylamino] benzene sulfonamide showed 65.90% inhibition of paw edema after 3 h at 56 mg/kg (body weight) dose and 66.40% protection in acetic acid induced inflammation in mice**.**[(37)]



R- Br, OH

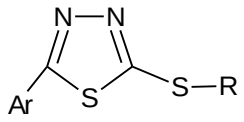
Antifungal Activity:-

Liu *et al*., (2011), reported the antibacterial and antifungal activity of 1,3,4-thiadiazoles bearing imidazo [2,1-b] thiazole moiety against *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *T. tonsurans* NCPF245 with MIC of 64, 32, and 8 μg/ml, respectively Applying QSAR study, it has been observed that positions-2 or position-3 of benzene attached with thiadiazole ring where as electron-donating and bulky group would be favorable for higher antifungal activity. On the basis of COMFA findings, and designed a compound which was found to display a good antifungal activity (79.38%). **(38)**



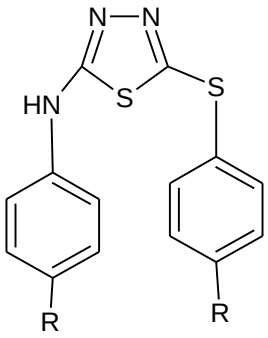
Anti tubercular activity:-

Foroumadi et al., (2002), synthesized 2-(5-nitro-2-furyl) and 2-(1-methyl- 5-nitro-lH- imidazol-2-yl)-1, 3, 4-thiadiazole derivatives and evaluated *in vitro* anti tuberculosis Activity. In this study two series of 2-(5-nitro-2-furyl) and 2-(1-methyl-5-nitro-lH-imidazol-2-yl)-5-propyl, ally and propargyl) thio-l,3,4-thiadiazoles derivatives and 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitro-lH-imidazol- 2-yl)-5-(nitro benzyl)thio-l,3,4-thiadiazole derivatives have been synthesized and evaluated against *Mycobacterium tuberculosis* .and exhibited good activity**.** **(39)**



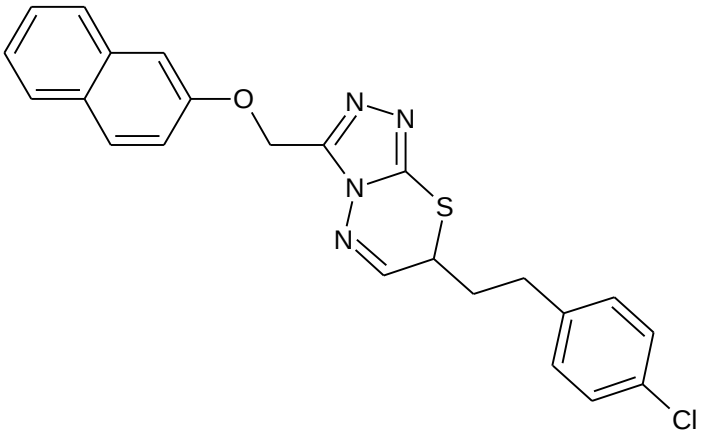
Anticonvulsant activity:-

Sharma et al., (2011), synthesized a new series of 2-amino-5-sulfanyl-1, 3, 4-thiadiazole derivatives. All compounds were screened for central nervous system activity. Exhibited significant antidepressant, anxiolytic and anticonvulsant activity when compared with the Standard drugs**.** [(40)]



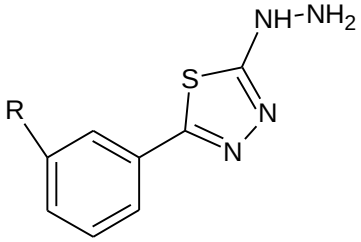
Anticancer activity and antioxidant activity:-

Dhanya et al.,(2011), synthesized a series of 6-[3-(4-chlorophenyl)-1-H-pyrazol-4-yl]-3-[(2-naphthyloxy)methyl][1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazole. Were evaluated for their in vitro cytotoxic effects against a panel of 60 human tumor cell lines. All the screened compounds showed significant in vitrocytotoxic effects against a variety of human tumor cell lines including cells derived from solid tumors such as colon, non-small cell lung, central nervous system, ovarian, melanoma, prostate and breast cancer, and also few cell lines of renal cancer and leukemia. Substitution of a formyl group at the 5- and substituted aromatic and antioxidant activity and exhibited good activities. [(41)]



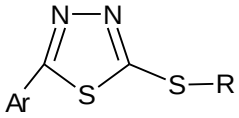
Antihypertensive activity:-

Turner et al., (1998), synthesized a series antihypertensive thiadiazole derivative and evaluated antihypertensive activity and indicated higher activity when compared with the Standard and other compounds. **(42)**

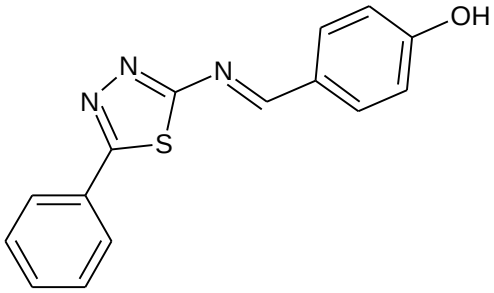


2.4 Of the Several works of Thiadiazole, few of them are enlisted here in support of their Thiadiazole Anti tubercular activity

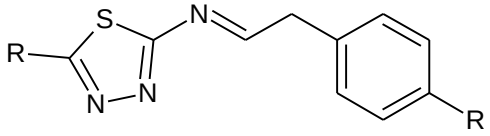
Novli M.N et al., (2013), synthesized a series of imidazo [2, 1-b] 1, 3, 4-thiadiazole derivatives and the synthesized compounds were synthesized compounds were evaluated for their invitro anti tubercular activity against M. tuberculosis H37RV strain by using Alamar Blue susceptibility test. Among the tested compounds, 2-(1-methylimidazol-2-yl)-6-(4-nitrophenyl) imidazo [2, 1-b] 1, 3, 4-thiadiazole have shown the highest inhibitory activity with MIC of 3.14µg/ml as compared to other compound. **(47)**



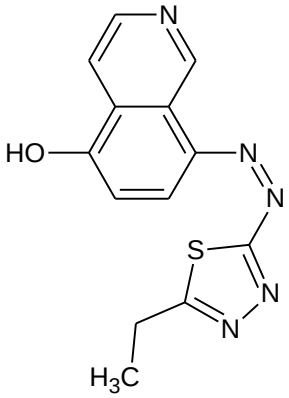
Gadad A.K et al., (2004), Evaluated 6-aryl-2-triflouromethylimidazo [2, 1-b] 1, 3, 4-thiadiazole derivatives against M. tuberculosis against H37RV strain by radiometric BACTEC and broth dilution method. It was found that 4-flouro phenyl derivative causes maximum inhibition at 6.25µg/ml concentration. All the synthesized compounds were reported to be less active than standard drug Isoniazid**.** [(44)]



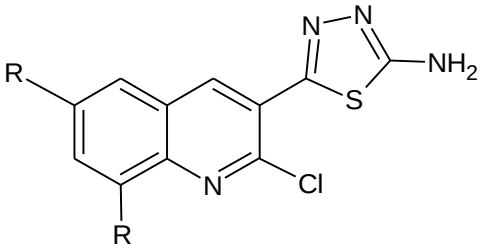
Shiradhkar M et al., (2005), synthesized a series of S-triazolo [3, 4-b] 1, 3, 4-thiadiazoles and screened for their antitubercular activity against M. tuberculosis H37RV. The final data of the MIC was compared with the standard drug Rifampicin at 0.03µg/ml concentration which showed more than 95% inhibition. Among the derivatives, nitro phenyl derivatives were shown to possess maximum activity against M. tuberculosis. [(43)]



Palkar M.B et al., (2012), synthetized a series anti tubercular activity of some 2-substituted-5, 6-diaryl substituted imidazo [2, 1-b] 1, 3, 4-thiadiazoles against M. tuberculosis H37RV strain by Micro plate Alamar Blue Assay (MABA) method using Isoniazid as the standard drug**.** [(45)]



Chitra *et al*.,(2005), synthesized a 3-heteroarylthioquinoline derivatives of 1,3,4-thiadiazole and screened there *in vitro* anti mycobacterial activity against $H_{37}Rv$ using Middle brook 7H11 agar medium supplemented with OADC by agar-dilution method. They found that the anti tubercular activity was considerably affected by various substituent like 2-methyl-1, 3, 4-thiadiazole, benzothiazole and 2-phenyl-2H-tetrazole on the 3-position of quinoline ring and it was further supported by the fact that compounds with no substitution did not show any considerable activity. Compounds **78** and **79** with chloro- add bromo-substituted aromatic ring found to be more active (MIC = 3.2–3.5 μg/ml) Analogs having methyl and methoxy groups at the C8 of quinoline nucleus showed inhibition (MIC) at 5 μg/ml). **(46)**

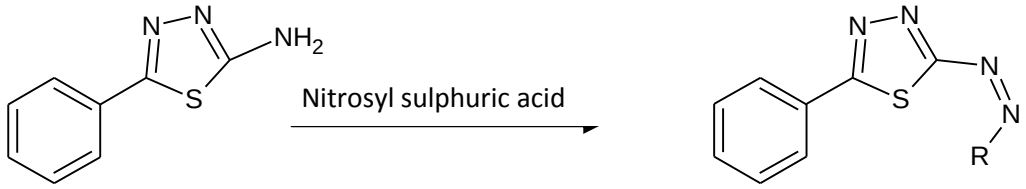


Where R- cl, Br R1- OH,

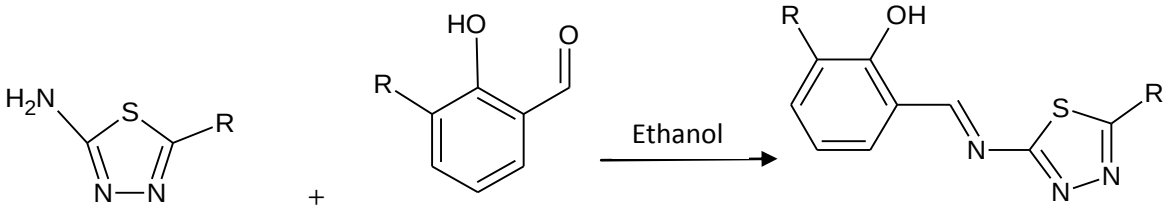
Compound 78 and 79

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

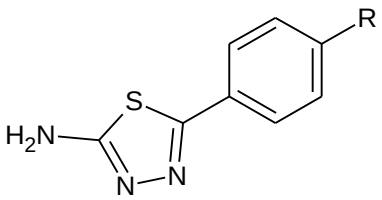
Keerthi Kumar et al., (2013), proposed synthesis of azodye incorporated 5-phenyl 1, 3, 4-thiadiazole-2-amine single step by diazotization of nitrosyl sulphuric acid followed by coupling with heterocyclic moiety. **(48)**



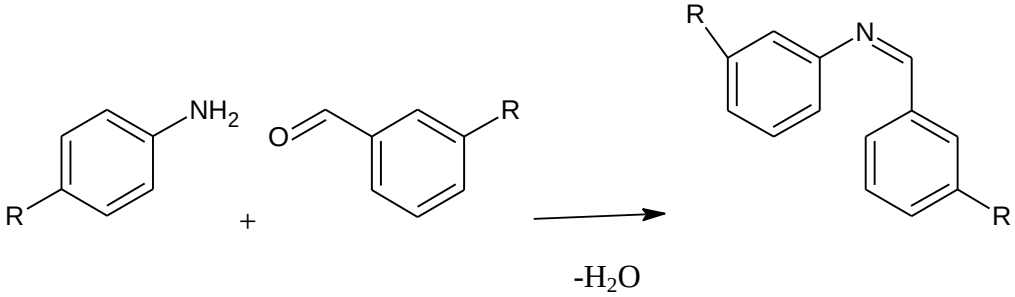
Tang, zilong et al., (2015), Presented 2-[1, 3, 4-thiadiazolyl amino methyl] phenols by one pot reaction of 2-amino -5-Alkyl [aryl] - 1, 3, 4-thiadiazole and salicylaldehyde the title compounds had moderate fungicidal activity. **(49)**



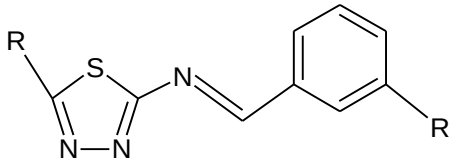
Mathew Bijo et, al. concentrated on synthesis of Schiff bases of 5-phenyl substituted 2-Amino1, 3, 4-thiadiazole as effective anthelmentics. **(50)**



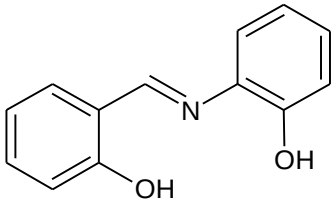
Sandeep Miglani, et al., **(2012)**, the rapid Synthesis of Schiff-bases without Solvent under microwave irradiation and their antimicrobial activity **(52)**



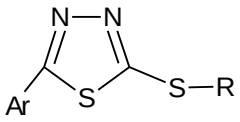
Solak N et, al.proposed synthesis of anti tuberculosis activity of 2-(aryl/alkyl amino)-5-(4-aminophenyl)-1, 3, 4-thiadiazoles and their Schiff bases. **(52)**



K. Brodowska, et al., (2014), Schiff bases- interesting range of applications in various fields of Science, and their anti microbial activity. [(53)]



Foroumadi et al., (2006), synthesized 2-(5-nitro-2-furyl) and 2-(1-methyl- 5-nitro-lH-imidazol-2-yl)-1, 3, 4-thiadiazole derivatives and evaluated *in vitro* anti tuberculosis Activity. In this study two series of 2-(5-nitro-2-furyl) and 2-(1-methyl-5-nitro-lH-imidazol-2-yl)-5-propyl, allyl and propargyl) thio-l,3,4-thiadiazoles derivatives and 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitro-lH-imidazol- 2-yl)-5-(nitro benzyl) thio-l,3,4-thiadiazole derivatives have been synthesized and evaluated against *Mycobacterium tuberculosis*



2.6The review on following works revealed the basis of Spectroscopy Study

Gurdeep R. Chatwal [2005] wrote a book on, Instrumental methods of chemical analysis. [(54)]

P.S.Kalsi Text book on Spectroscopy of organic compounds. [(55)]

Y.R.Sharma, Text book on Elemental organic Spectroscopy. [(56)]

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

Laurie AT, et.al., (2005), Q Site finder on energy based method for the prediction of protein-ligand binding site Bioinformatics**.** [58]

http; // www.modelling leads.ac.uk/q site finder/

Sanju joy, parvathy S Nair., et.al, (2006), Detailed comparison of Pro-ligdocking efficiency of GOLD, a commercial package and Argus lab, a licensable freeware [Insilco biology 6, 0053[2006**].**[60]

2.8 The review on following works revealed the basis of Alamar blue assay for evaluating the anti-mycobacterium action.

David A. J. Moore.,et al.,(2008),Inter and Intra assay reproducibility of Micro plate Alamar blue assay results for Isoniazid, Rifambicin, Ethambutol, Streptomycin, Ciprofloxin, Capromycin, drug suscepbility testing Mycobacterium tuberculosis**.**[76]

Todd P. Primm., et al [2007], Recent Advances in Methodologies for the Discovery of Antimycobacterial Drugs. [77]

Vanitha JD., et al., [2007], Evaluation of Micro plate Alamar blue assay for drug susceptibility testing of Mycobacterium avium complex isolates**.** Diagnostic Microbiology and Infectious Disease. [75]

Franzblau SG., et al., Rapid lower technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the Micro plate Alamar blue assay. Journal of Clinical Microbiology. [76]

Sephra N.Ramprasad studied the various applications of Alamar blue as an indicator. Alamar blue is an indicator that is used to evaluated metabolic function and cellular health. The Alamar blue bioassay is being utilized to access cell viability and cytotoxicity in a biological and environmental system and in a number of cell types including bacteria, yeast, fungi, and protozoa. [79]

Jose d Jesus Alba-Romero et al applied the Alamar blue assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. The results showed that the MABA test is fast and easy to apply. It is very reliable method to determining the drug susceptibility to pharmaceuticals. **(80)**

3. Aim and Objective

AIM:-

The aim of this project is to design molecules with potential anti-tubercular activity that is capable of inhibiting cell wall synthesis by inhibiting Decaprenylphosphoryl-beta-D-ribose2-epimerase-1. The designed compounds will be synthesized, characterized and evaluated for biological activity and toxicity.

OBJECTIVE:-

The compounds are designed and docked against a specific crucial target, Decaprenylphosphoryl-beta-D-ribose2epimerase-1.This is involved in the cell wall biosynthesis and Lipid metabolism. The synthesized compounds are expected to act on the same.

PLAN OF WORK

DESIGN:-

In-silico design of Decaprenyl phosphoryl – beta - D-ribose 2 epimerase-1 inhibitors.

DRUG LIKENESS:

Determination of Drug likeness

TOXICOLOGICAL PREDICTION:-

Toxicological prediction will be carried out for synthesized compounds by in-silico property explorer like OSIRIS.

SYNTHESIS:-

Novel anti-tubercular activity containing compounds are synthesized under specific conditions, which is effective against specific microbes.

CHARACTERIZATION:-

The synthesized compounds will be identified and characterized by following methods

1. Melting point.
2. TLC method
3. Infrared Spectroscopy
4. Nuclear Magnetic Resonance
5. Mass spectroscopy
6. Gas Chromatography and Mass Spectroscopy (GC-MS) and if necessary
7. Liquid Chromatography and Mass Spectroscopy (LC-MS)

BIOLOGICAL EVALUATION:-

The synthesized compounds will be screened for their anti-tubercular activity by in-vitro method called MABA.

THE PRESENT STUDY CARRIED OUT BASED ON THE FLOW CHART:

IDENTIFICATION OF LEAD COMPOUND

LEAD OPTIMIZATION

DOCKING OF THE MOLECULE TO THE TARGET PROTEIN

TOP G SCORE COMPOUNDS SELECTED

INSILICO DRUG LIKENESS AND TOXICITY RISK ASSESSMENT

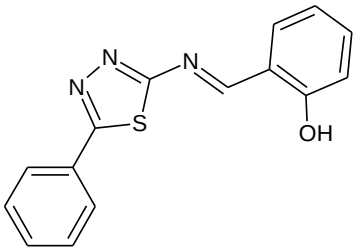
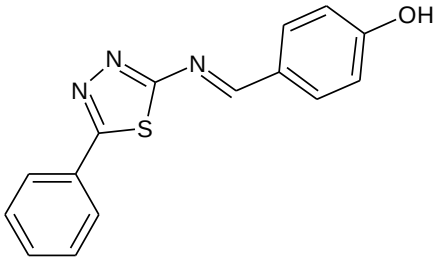
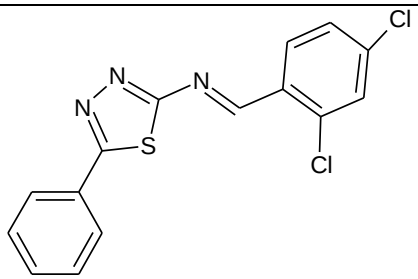
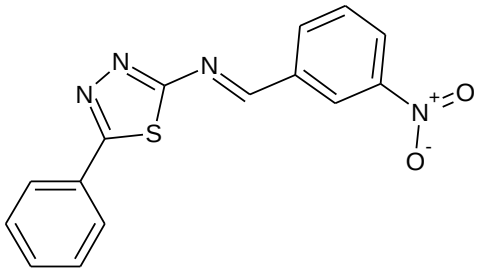
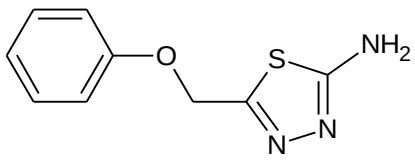
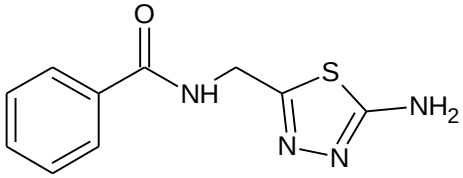
SYNTHESIS

JUSTIFICATION OF PURITY –TLC, GC

CHARACTERIZATION –SPECTROSCOPY(IR, NMR, GC-MS, LC-MS)

INVITRO ANTI TUBERCULAR ACTIVITY

LIST OF COMPOUNDS TO BE SYNTHESIZED:-

SAMPLE CODE	COMPOUNDS	IUPAC
SDK1		2-{(*E*)- [(5-phenyl-1,3,4-thiadiazol-2-yl)imino] methyl}phenol
SDK2		4-{(*E*)-[(5-phenyl-1,3,4-thiadiazol-2-yl)imino]methyl}phenol
SDK3		(*E*)-1-(2,4-dichlorophenyl)-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)methanimine
SDK5		(*E*)-1-(3-nitrophenyl)-*N*-(5-phenyl-1,3,4-thiadiazol-2-yl)methanimine
PAA		5-(phenoxy methyl)-1,3,4-thiadiazol-2-amine
HA		*N*-[(5-amino-1,3,4-thiadiazol-2-yl)methyl]benzamide

4. MATERIALS AND METHODS

DRUG DESIGN:-

A binding interaction between a small molecule ligand and an enzyme protein results in activation or inhibition of the enzyme which results in agonism or antagonism,

GLIDE DOCKING

Glide is one of the docking programs which predict the binding mode of ligand to a protein (target). It ranks the ligands via high-throughput virtual screening. Extra prediction mode (XP) was used to rank order the compounds based on the interaction with the receptors.

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

MOLECULAR DOCKING BY ARGUS LAB SOFTWARE :

Docking of ligands is carried out by Argus lab docking software. Docking allows the medicinal chemist to virtually screen a set of compounds and predict the strongest binding capacity based on various scoring function. It explores ways in which two molecules such as ligand and receptor (protein) fit together and docks to each other well. The molecule binding to a receptor inhibits its function and thus acts as drug.

Argus lab 4.0 distributed freely for windows platforms by planaria software introductory molecular modeling package with academics. Argus docking engine impl in Argus lab approximates an exhaustive search method which is similar to DO GLIDE. Flexible ligand docking is possible with Argus lab, where the ligand is desc torsion tree and grids are constructed that overlay the binding site. The accuracy of th lab docking algorithm takes into account, the key features such as the nature of the site and the number of rotatable bonds to the ligand.

Molegro molecular viewer: Molegro molecular viewer is an application which analyzing the energies and interaction of the binding site.

Q-site finder is an energy-based method for protein-ligand binding site prediction. During prediction we use the crystal structures of macromolecules (receptor) with small substrates (PDB ID)

Identifying the location of binding sites on a protein is of fundamental importance for a range of applications including molecular docking. It uses the interaction energy between the protein and a simple vanderwaals probe to locate energetically favorable binding sites.

4.1 DOCKING PROCEDURE:-[ARUGS LAB SOFTWARE 4.0]

A.PREPARATION OF PROTEIN:

STEP1:

I) Enter Protein PDB ID (4P8Y) In the Protein data bank.

ii) Go to download files and Select PDB as text file.

iii) Save the downloaded PDB text file to desktop.

STEP2:

i) Open Argus lab file ⟶ Open ⟶ Import pdb file from the desktop.

ii) 3D Structure of the Protein will appear in the workspace of Arguslab.

iii) Left Side of the Screen Shows molecular tree view.

iv) Open pdb ⟶ Open Residues ⟶ Open Misc.

v)From Misc delete the inhibitor and hetero residues, do not delete cofactor

vi) Open water press shift, select all water molecules and delete.

vii) Add hydrogen atoms.

viii) Go the calculation on toolbar ⟶ energy by UFF method ⟶ Start.

Save the prepared protein as ® agl file format in the desktop.

B. IDENTIFICATION / SELECTION OF ACTIVE SITE:-

STEP1:-

Q-Site finder was opened through online.

The Pdb format of the protein was imported.

Found all the active site and make a list out of the common amino acid residues.

STEP2:-

Residues ⟶ Open amino acids was Opened.

Controls were selected and select the amino acids which were listed from the Q-Site finder.

Make sure that all the amino acid residues listed are selected.

Right Click on the mouse ⟶ make a group from the selected residues give name ⟶ Binding site ⟶ OK.

C.PREPARATION OF LIGANDS:-

Drawn the Structure from Chem. Sketch and Save as MDL mol format.

The ligands were imported into workspace of Argus lab.

Clean geometry ⟶ Clean Hybridization. Select the ligand; right Click on the mouse ⟶ make a group from the residues ⟶ give name ligand ⟶ OK.

The ligand was selected; right click on the mouse ⟶ make a group from the residues ⟶ give name ⟶ ligand ⟶ OK.

D. DOCKING PARAMETER:

Calculation was selected from the toolbar ⟶ Dock a ligand.

Argus Dock as the Docking engine.

Dock was selected as Calculation type.

Flexible for the ligand.

Ascore as the Scoring function.

Calculation Size.

Docking was started.

Save the Docked protein Ligand Complex as Brookhaven PDB files.

E. VISUALIZATION/ INTERPRETATION OF DOCKING:

Molegro molecular Viewer will help in analyzing the energies and interaction of the binding.[60]

4.2 LIPINSKIS RULE:- [64,65,66]

Lipinski's rule of five is a rule of thumb to evaluate drug likeness, ie., or to determine if a chemical compound with a certain pharmacological or biological activity has the properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules.

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

The rule is important for the drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule. The modification of the

molecular structure often leads to drugs with higher molecular weight, more rings, more rotatable bonds, and a higher lipophilicity.

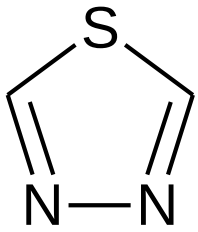
Lipinski's rule says that, an orally active drug has no more than one violation of the following criteria:

- ✓ Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- ✓ Not more than 15 rotatable bonds.
- ✓ Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- ✓ Molecular weight under 500 Daltons
- ✓ Partition coefficient of log P less than 5.

4.3 HETEROCYCLIC CHEMISTRY

Heterocyclic structures always are a part in the field of research and development in organic chemistry. Millions of heterocyclic structures are found to exist having special properties and biological importance. Among various compounds, I have chosen Thiadiazole a five membered ring containing sulfur at position 1 and Nitrogen at position 3 and 4.

THIADIAZOLE NUCLEUS



1,3,4- Thiadiazole

SYNTHETIC INVESTIGATION

The Synthesis involves following Mechanism

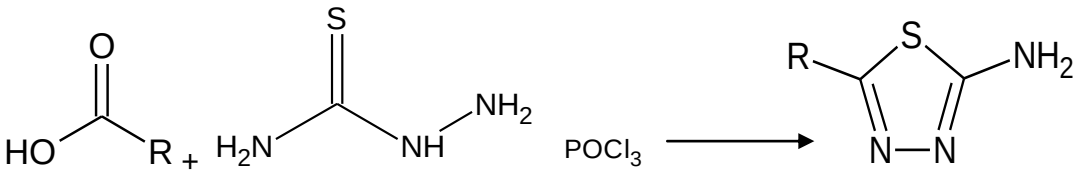
First Step Involves the Synthesis of 2-Amino 5-Phenyl 1, 3, 4-Thiadiazole Which is accomplished by **Cyclization o**f Thiosemicarbazide in Presence of Phosphorous oxy chloride / Suitable dehydrating agent. [Shmeiss et al., 2002, Sankar et al., 2011.]

Which is further converted in to imines by the treatment with various substituted Aldehydes.

Final Compounds were Synthesized by Staudinger Imines reaction of Schiff bases [Solak and Rollas 2006].

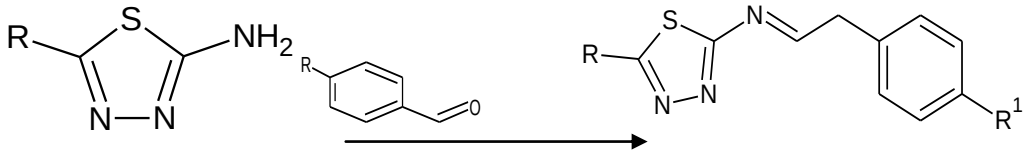
SYNTHETIC SCHEME:-

STEP1:- 2-Amino 5-substituted acid 1,3,4-thiadiazole:



Acid Thiosemicarbazide 2-amino 5-Substituted 1, 3, 4, thiadiazole

STEP2:-5[substituted]phenyl-N-[1E]-[substituted] phenyl methylene]-1,3,4 thiadiazol-2-amine:-



4-6 hours/ Ethanol Reflux

2-amino 5-Substituted 1, 3, 4, thiadiazole

STEP1:-2-Amino 5-substituted acid 1,3,4-thiadiazole:[70]

A equimolar mixture of Aromatic carboxylic acid (0.1mole) and Thiosemicarbazide (0.1mole), in $POCl_3$ (excess), was refluxed for one hour, Crushed ice (90ml) was added to the reaction mixture and again refluxed for another 4 hour, on completion of reaction TLC was monitored, cool to room temperature and filter, the filtrate was neutralized by saturated potassium hydroxide solution, filter, dried and recrystallised from suitable solvent.

STEP2: 5[substituted]phenyl-N-[1E]-[substituted] phenyl methylene]-1,3,4 thiadiazol-2-amine:[67,69]

An equimolar quantity of 2-amino 5-substituted 1,3,4-thiadiazole [0.01mole] was added to various Aldehydes [0.01mole] and dissolved in absolute ethanol the reaction mixture was refluxed for 4-6 hours on completion of reaction was monitored by TLC, cool to room temperature and pouring ice product was formed ,filter, dried and recrystallised using ethanol.

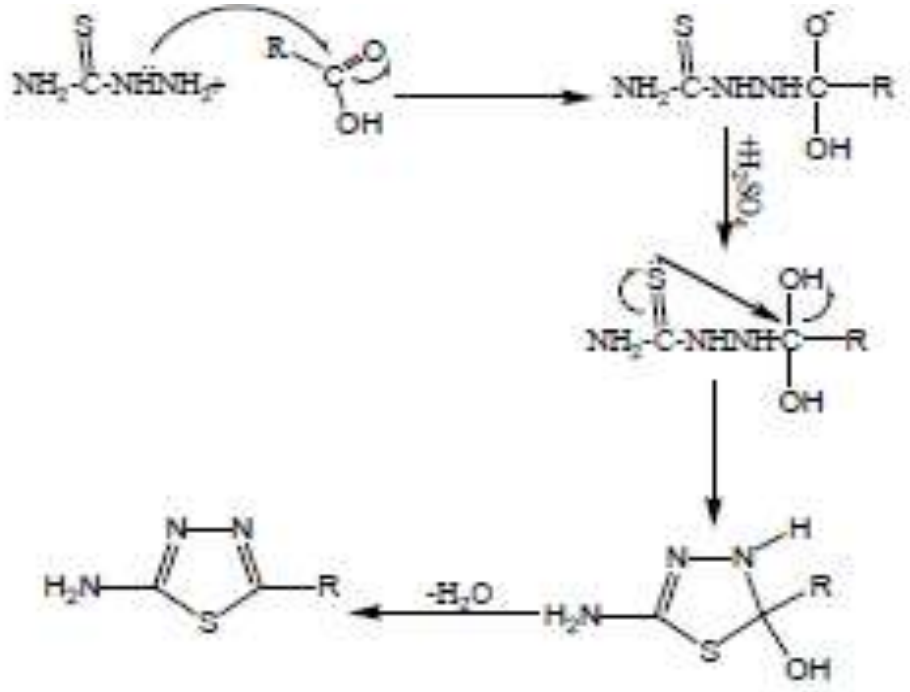
Acids used[R]:-

- Benzoic Acid
- Phenoxy Acetic Acid
- Hippuric Acid.

Aldehyde used[R1]:-

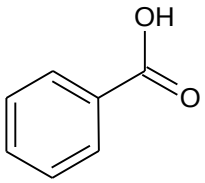
- 2-Hydroxyl Benzaldehyde
- 4- Hydroxyl Benzaldehyde
- 2,4-dichloro Benzaldehyde
- 3-Nitro Benzaldehyde

MECHANISM:



4.5 REACTANT PROFILE[73]

BENZOIC ACID:-



Synonym : Benzene Carboxylic acid

Molecular Formula : $C_7H_6O_2$

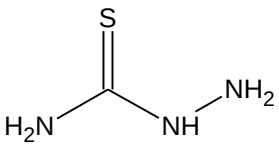
Molecular weight : 122.12

Description : White Crystalline Solid

Melting point : 171°c

Solubility : Chloroform [Slightly], Methanol [Slightly].

THIOSEMICARBAZIDE:-



Synonym: Thiocarbamoyl hydrazide

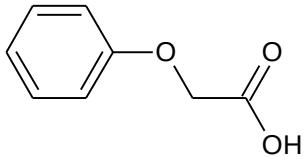
Molecular Formula: CH_5N_3S

Molecular Weight : 91.132

Description : white crystalline powder

Melting point : 180-183°c

PHENOXY ACETIC ACID:



Synonym: Glycolic acid Phenyl ether

Molecular Formula: C8H8O3

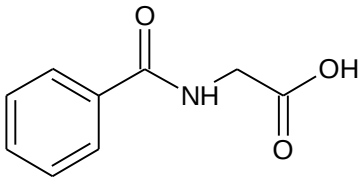
Molecular weight: 152.14

Description : Colorless needle-like crystal

Melting point: 100^{o}c

Solubility : water

HIPPURIC ACID:



Synonym : N-Benzoyl glycine

Molecular Formula: C9H9NO3

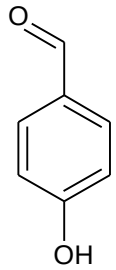
Molecular weight: 179.179

Description : white powder

Melting point: 187 to 188^{o}c

Solubility : Hot water

4-HYDROXYL BEZALDEHYDE:-



Synonym: Para hydroxyl benzaldehyde

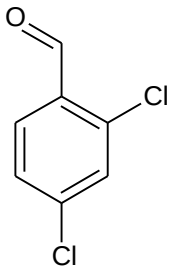
Molecular Formula: $C_7H_6O_2$

Molecular weight: 121.12

Description : Light yellowish to light brown

Melting point : 191ºc

2, 4 DICHLORO BENZALDEHYDE:-



Molecular Formula: $C_7H_4Cl_2O$

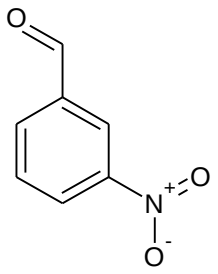
Molecular weight : 175.01

Description : white crystalline solid

Melting point : 233ºc

Solubility : Water/ Ethanol

3-NITRO BENZALDEHYDE:-



Synonym: M-Nitrobenzaldehyde

Molecular Formula: $C_7H_5NO_3$

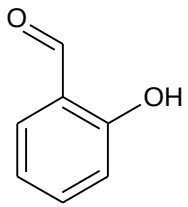
Molecular weight: 151.02

Description : Yellowish to brownish crystalline

Melting point : 55-57ºc

Solubility : Ethanol.

2-HYDROXY BENZALDEHYDE:-



Molecular Formula: $C_7H_6O_2$

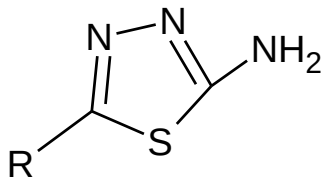
Molecular weight: 122.12

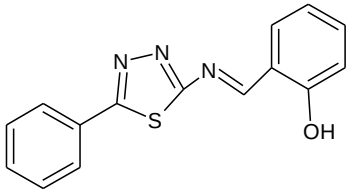
Description : Colorless or pale yellow

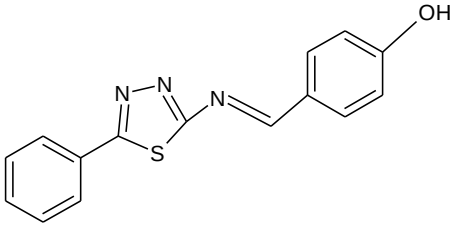
Boling Point : 196 to 197ºc

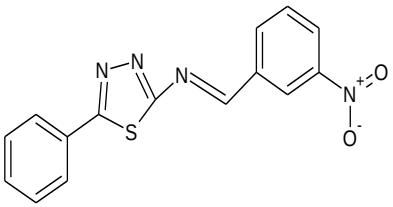
Solubility : Ethanol

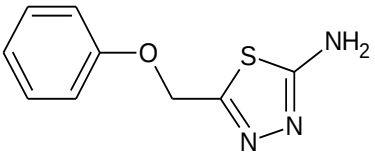
SYNTHESIS OF COMPOUNDS:-

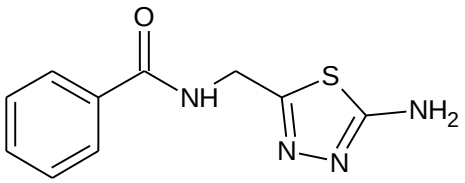


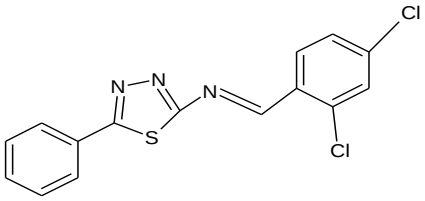












.

2 Amine 5-Substituted acids Reacts With

Step1: Benzoic acid [Intermediate for 2nd Step].

Phenoxy acetic acid

Hippuric acid.

Step2:

2-amino 5- Phenyl 1,3, 4 Thiadiazole Reacts with

2-Hydroxyl Benzaldehyde

4-Hydroxyl Benzaldehyde

2,4 Dichloro Benzaldehyde

3-Nitro Benzaldehyde.

4.6METHODS OF IDENTIFICATION:-

- Melting point
- TLC
- Infra red Spectroscopy
- Nuclear Magnetic Resonace
- Mass Spectroscopy.

The synthesized compounds were identified by using following method

Melting point:-

The melting point of the compounds is determined by the capillary tube method. The synthesized compounds were start losing their crystallinity at a particular temperature.

Thin layer chromatography:-

Pre-coated TLC plates with silica gel GF 250 are used. Samples of reactants and products are prepared with suitable solvents.

The characterization was carried out using sophisticated methods like Infra-red spectroscopy, Nuclear magnetic resonance spectroscopy and Mass spectroscopy.

Infrared Spectroscopy:- [58]

The infrared spectroscopy is one of the most powerful analytical techniques, this offers the possibility of chemical identification. The most important advantages of infrared spectroscopy over the other usual methods of structural analysis are that it provides useful information about the functional groups present in the molecule quickly. The technique is based upon the simple fact that a chemical substance shows marked selectable absorption in the infrared region. After absorbing IR radiations the molecules of a chemical compound exhibit small vibrations, giving rise to closely packed absorption bands called as IR absorption spectrum which may extend over a wide wavelength range. Various bands will be present in IR spectrum which corresponds to the characteristic functional groups and bonds present in a chemical substance. Thus an IR spectrum of a chemical compound is a fingerprint for its identification. The infrared spectrum of the prepared derivatives were taken by using Avatar 330Thermo Nicolet FT-IR spectrometer and Jasco 460 plus FT-IR spectrophotometer using potassium bromide pellet technique

Nuclear Magnetic Resonance Spectroscopy:

It is the branch of spectroscopy in which radiofrequency waves induces transitions between magnetic energy levels of nuclei of a molecule. The magnetic energy levels are created by keeping nuclei in a magnetic field. Without the magnetic field the spin states of nuclei are degenerated i. e., possess the same energy and the energy level transition is not possible. The energy level transition is possible with the application of external magnetic field which requires different Rf radiation to put them into resonance. This is a measurable phenomenon. It is a powerful tool for the investigation of nuclei structure. 1HNMR and 13CNMR Spectras of the prepared derivatives were done by using 400-MHz and 500-MHzBruker spectrometer using internal standard as tetra methyl silane. 1H and 13C NMR Spectral were taken with dimethyl sulphoxide (DMSO) as a solvent and the data of chemical shift were shown as delta values related to trimethylsilane (TM) in ppm.

Mass spectroscopy [58]

Mass spectrometer performs three essential functions. First, it subjects molecules to bombardment by a stream of more amounts of energy electrons, converting some of the molecules to ions, which are then accelerated in a field of electric. Second, the ions which are accelerated are divided according to their ratios of mass to charge in an electric or magnetic field. Finally the ions that have particular mass-to-charge ratio are detected by a device which can count the number of ions striking it. The detector's output is amplified and fed to a recorder. The trace from the recorder is a mass spectrum a graph of particles detected as a function of mass-to-charge ratio. The Mass of the synthesized compound was taken using Agilent (1100 MSD) spectrometer instrument.

HYPHENATED TECHNIQUES:-

GC-MS: - To determine the mass and also get an idea about the purity of the sample.

LC-MS: - When the sample cannot be vaporized, GC-MS cannot be performed. So LC-MS is performed.

4.7 MICROBIOLOGICAL ASSAY: [74-77]

Microbial assays or microbiological assays is a type of bioassay and are designed to analyze the compounds or substances which have effect on microorganisms. Microbiological assay is defined as the determination or estimation of concentration or potency of an antibiotic by means of measuring and comparing the area of zone of inhibition or turbidity produced by test substance with that of standard over a suitable microbe under standard conditions. So as definition says the hypothesis is that when an antibiotic is administered, there is inhibition in the growth of microbe as indicated by decrease in area of zone of microbial colony on nutrition media or decrease in turbidity due to decrease in microbial concentration.

TYPES OF MICROBIOLOGICAL ASSAY:-

REDOX BASED METHODS:

Micro plate Alamar blue assay, Resazurin Microtitre Assay, REMA, or Micro dilution Resazurin Assay, MRA. Tetrazolium Dyes, Tetrazolium Micro plate Assay, TEMA.

REPORTER GENE-BASED METHODS:

Green Fluorescent protein Micro plate Assay, GFPMA, Luciferase Assays, Beta-Galactosidase Assays.

OTHER METHODS: BACTEC 460 TB

Nitrate Reductase Assay, NRA. Disk Diffusion, Visual Micro broth, or Broth Micro dilution, Malachite Green, STC Agar, Flow cytometer.

MICROPLATE ALAMAR BLUE ASSAY:-

METHOD: Micro plate Alamar Blue Assay [MABA]

PREPARATION OF INOCULUMS: 100µl of the Middle brook 7H9 broth.

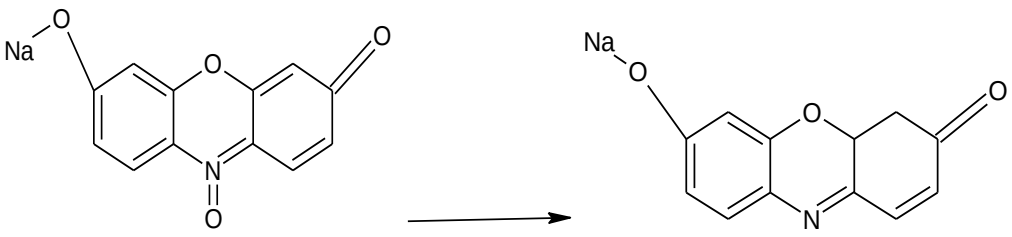
REQ UIREMENTS: 96 wells plate, Para film [all are sterilized by dry heat].

NUTRIENT MEDIUM: 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80

WORKING PROCEDURE:

Stock solutions of the synthesized compounds and standard drug used were prepared in sterile deionized water and taken in the concentration of 0.1 to 100µl/ml. 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. The 96 wells plate received 100µl of the Middle brook 7H9 broth and serial dilutions of compounds were made directly on plate. The final drug concentrations were tested were 100 to 0.2µg/ml. Plates were covered and sealed with Para film and incubated at 37°c for five days. 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. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink**.** [79, 80]

CHEMISTRY :- [78]



ADVANTAGES:-

- It involves no cell lysis.
- It is non-toxic
- It has high sensitivity and linearity.

APPLICATION: [81]

- Especially meant for studies on Mycobacterium tuberculosis.
- Used extensively in cell Viability and Cytotoxicity Studies.

4.9 IN SILICO PREDICTION: - [71, 72]

In silico toxicity prediction was done using OSIRIS Property Explorer. It is 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.

The designed and docked molecules are screened in silico using **MOLINSPIRATION** Cheminformatics 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 logP, 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).

INSILICO PREDICTION OF DRUG LIKENESS:-

Cheminformatics 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 logP, polar surface area, number of hydrogen bond donors and acceptors and others, as well as prediction of bioavailability score for the most important drug

TABLE NO: 1 LIPINSKI RULE AND SYNTHESIZED COMPOUNDS

Sample code	Logp	TPSA	Number of non-hydrogen atom	Molecular weight	No of Hydrogen bond Acceptor	No of Hydrogen bond donors	No of rule 5 Violation	No of rotable bonds	Molar volume
SDK1	3.63	58.38	20	281.34	4	1	0	3	240.54
SDK2	3.63	58.38	20	281.34	4	1	0	3	240.54
SDK3	5.39	38.15	21	334.23	3	0	0	3	259.59
SDK5	4.04	83.97	22	310.34	5	0	0	4	255.85
PAA	1.52	61.04	14	207.26	4	2	0	3	174.93
HA	1.15	80.91	15	220.2	5	3	0	2	180.53

Where,

TPSA - Total polar surface area.

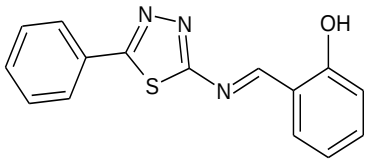
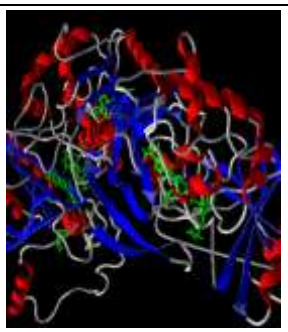
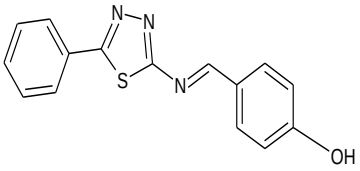
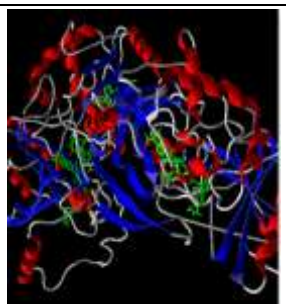
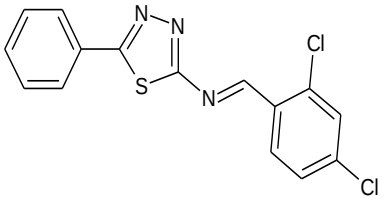
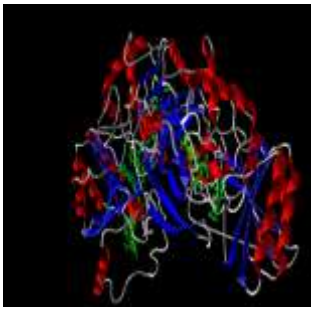
Thus the proposed compounds have satisfied all the above three filtering methods of good predictive activity, good docking scores and also drug like properties and that these molecules are accepted to be orally bioavailable.

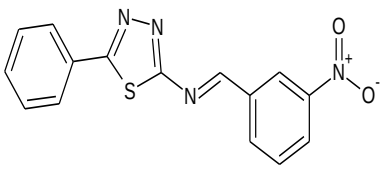
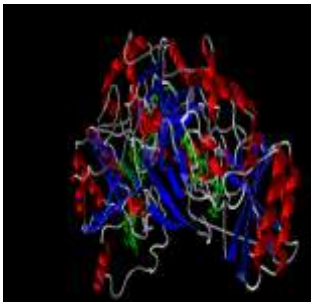
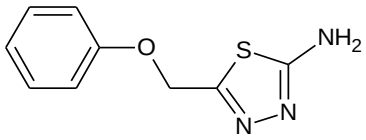
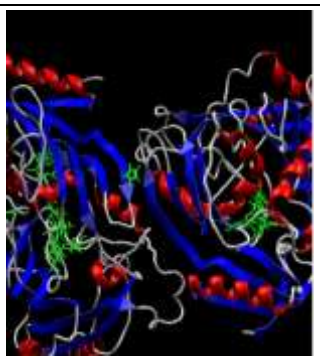
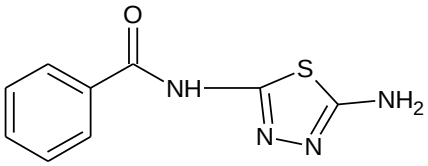
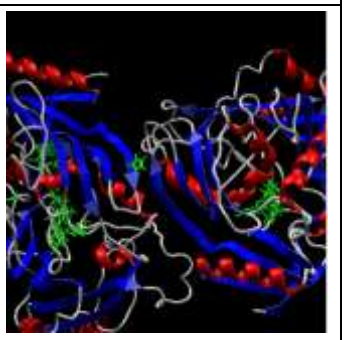
RESULTS OF DRUG DESIGN:-

DOCKING:-

Two hundred molecules, which are Sketched using Chem Sketch, were docked against the MTB enzyme. Decaprenylphosphoryl-beta-D-ribose 2'-epimerase-1 by using Argus lab 4.0.1 Software. The molecules with best docking Score and good interactions were selected and synthesized.

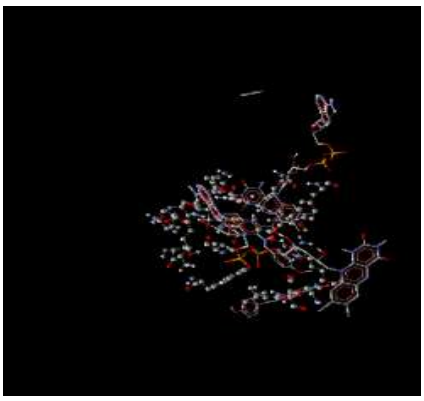
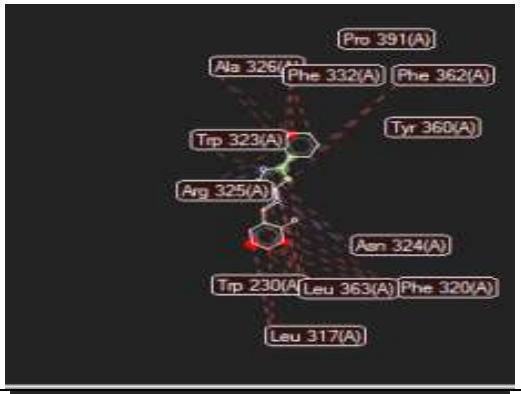
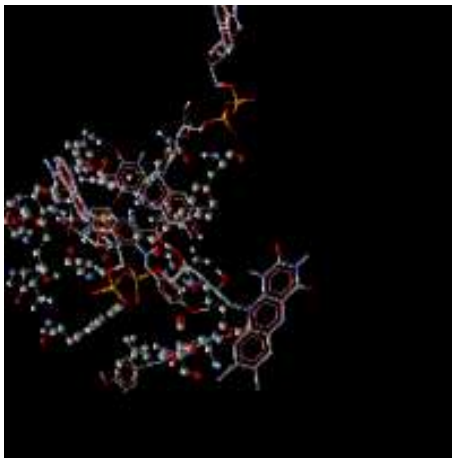
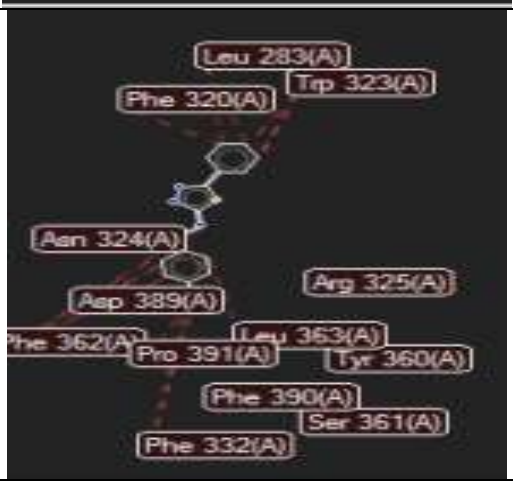
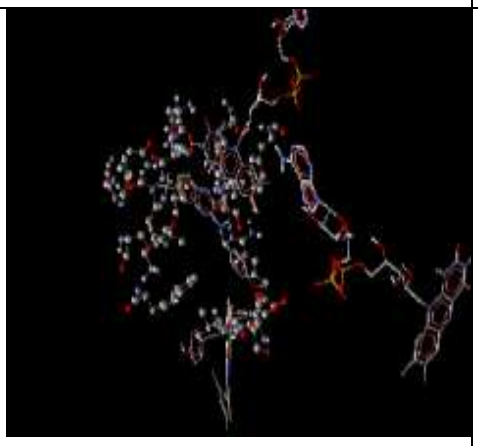
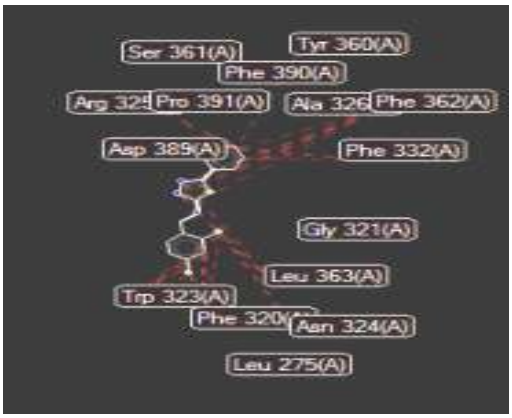
TABLE: 2 The molecules with good docking score were mentioned as below.

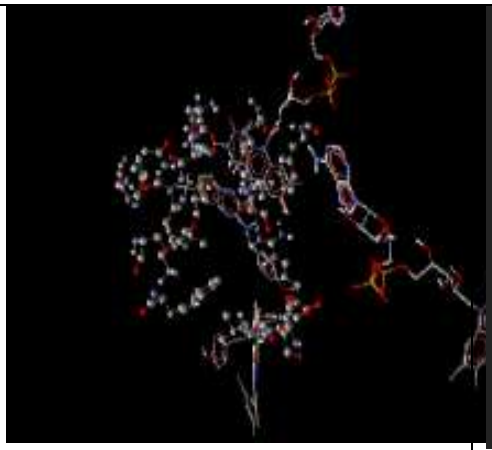
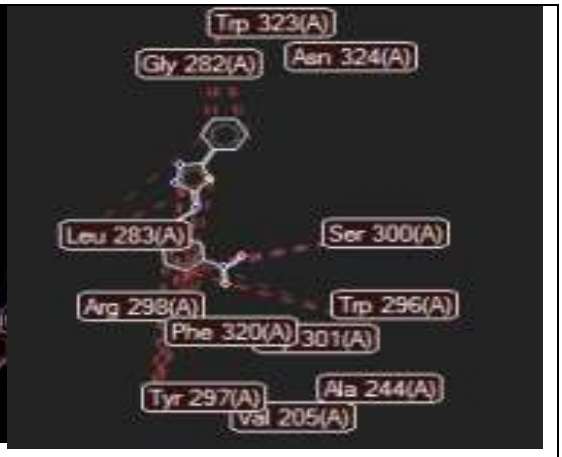
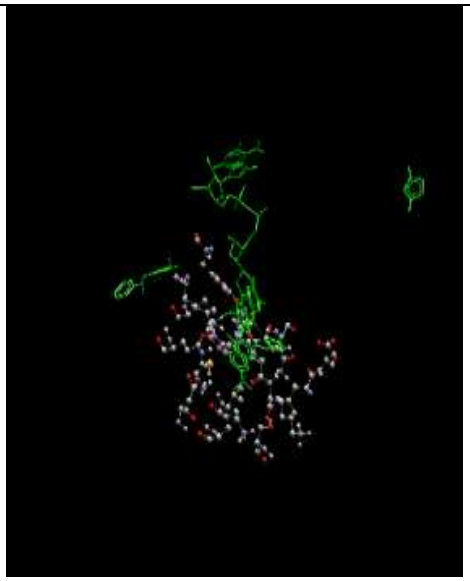
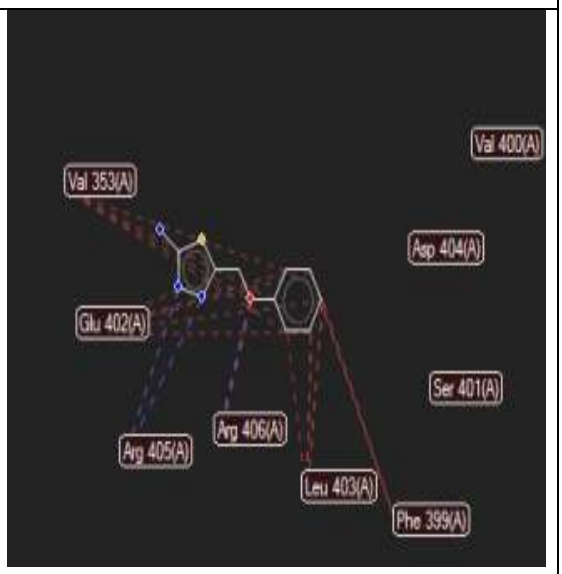
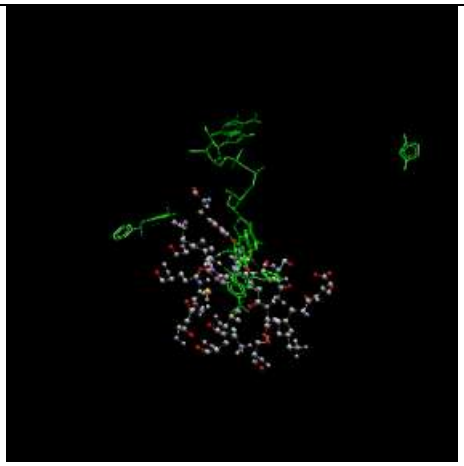
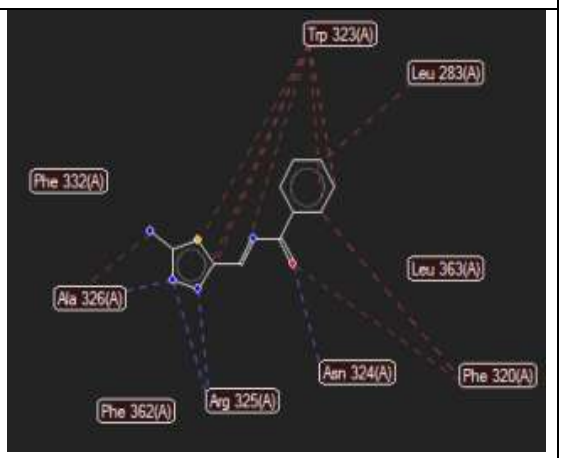
SAMPLE CODE	MOLECULES	DOCKING SCORE kcal/mole	DOCKING VIEW
SDK1		-10.54	
SDK2		-11.974	
SDK3		-11.099	

SDK5		-11.262	
PAA		-9.5781	
HA		-8.567	

INTERACTIONS OF THE DOCKED MOLECULES WITH THE ENZYME DPRE1

TABLE3:- Hydrogen bond Interactions and their Interactions with Amino acid

SAMPLE CODE	HYDROGEN BOND INTERACTION	INTERACTIONS WITH AMINOACIDS
SDK1		
SDK2		
SDK3		

SDK5		 Trp 323(A) Gly 282(A) Asn 324(A) Leu 283(A) Ser 300(A) Arg 298(A) Trp 296(A) Phe 320(A) 301(A) Tyr 297(A) Ala 244(A) Val 205(A)
PAA		 Val 400(A) Val 353(A) Asp 404(A) Glu 402(A) Ser 401(A) Arg 406(A) Arg 405(A) Leu 403(A) Phe 399(A)
HA		 Trp 323(A) Leu 283(A) Phe 332(A) Leu 363(A) Ala 326(A) Asn 324(A) Phe 320(A) Arg 325(A) Phe 362(A)

INSILICO TOXICITY PREDICTION:-

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

Molecular property prediction:-

- Toxicity prediction
- Clog P Prediction
- Solubility prediction
- Molecular weight

TABLE NO: - 4

SAMPLES	SDK1	SDK2	SDK3	SDK5	PAA	HA
MUTAGENIC	+	+	+	+	+	+
TUMORIGENIC	+	+	+	+	+	+
IRRITANT	+	+	+	+	+	+
REPRODUCTIVE EFFECT	+	+	+	+	+	+

[+] indicates absence of toxicity.

[-] indicates Presence of toxicity.

Fig. SDK1

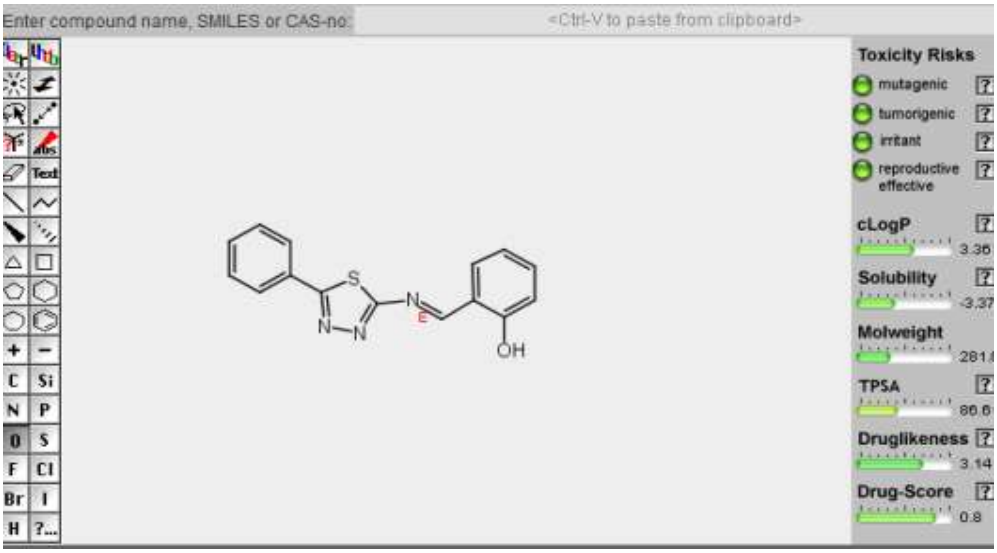


Fig.SDK2

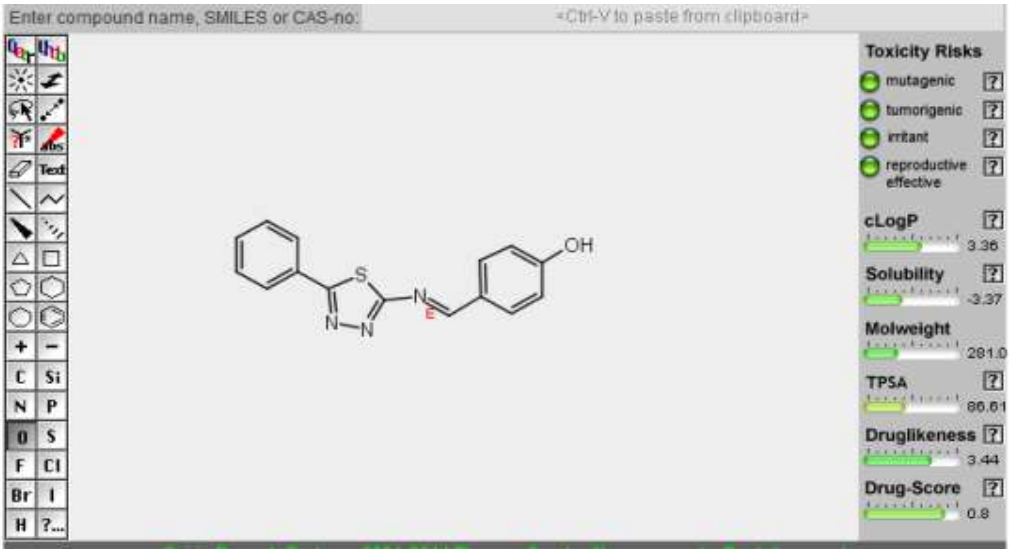


Fig. SDK3

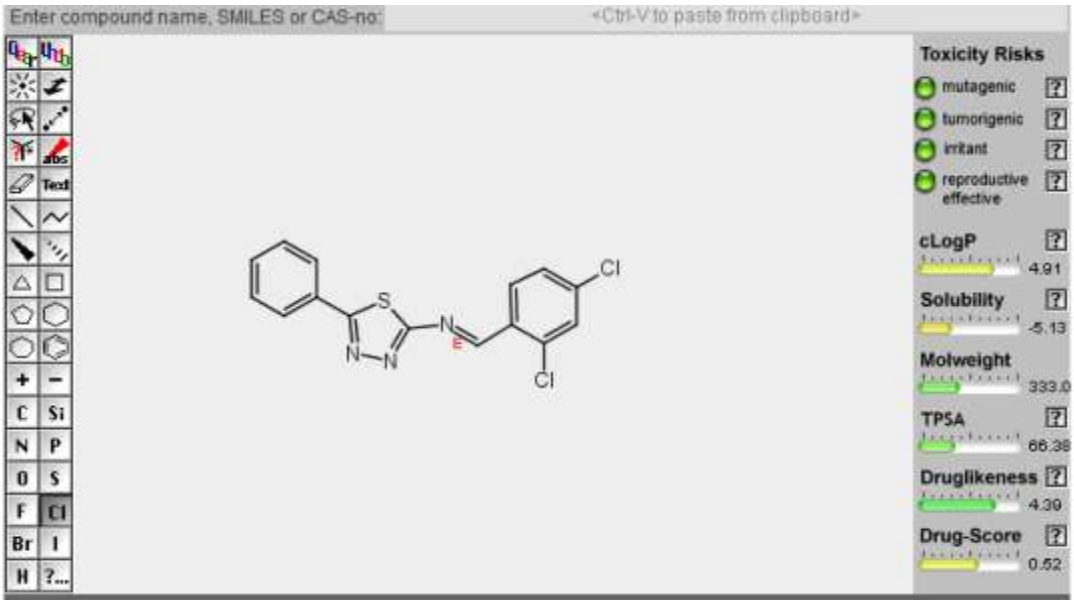


Fig. SDK5

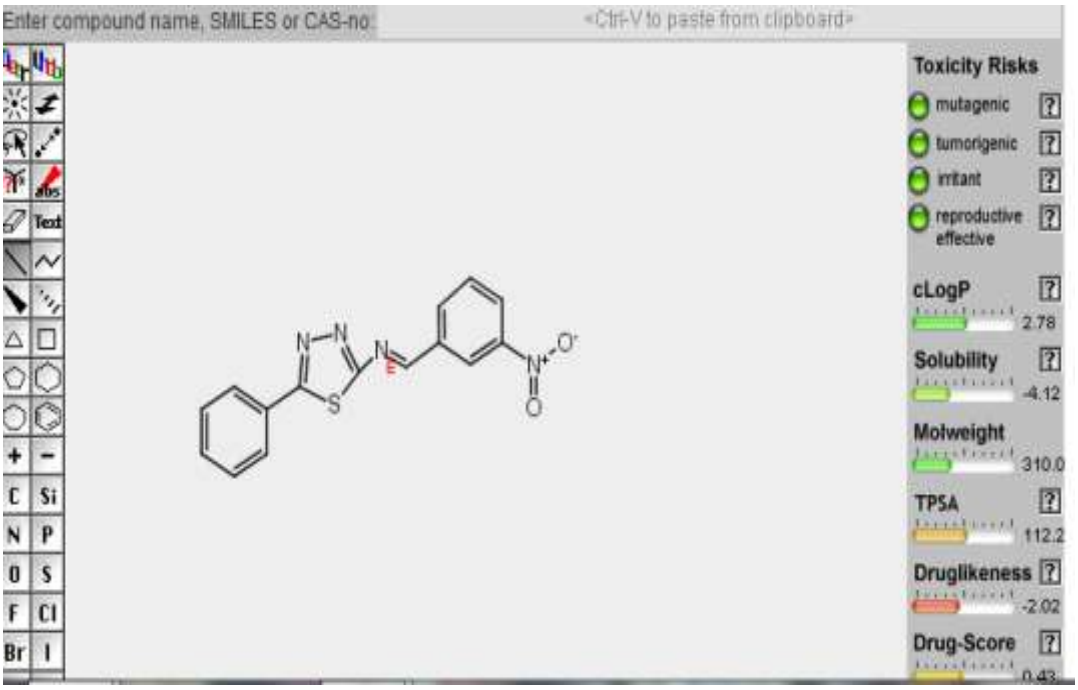


Fig. PAA

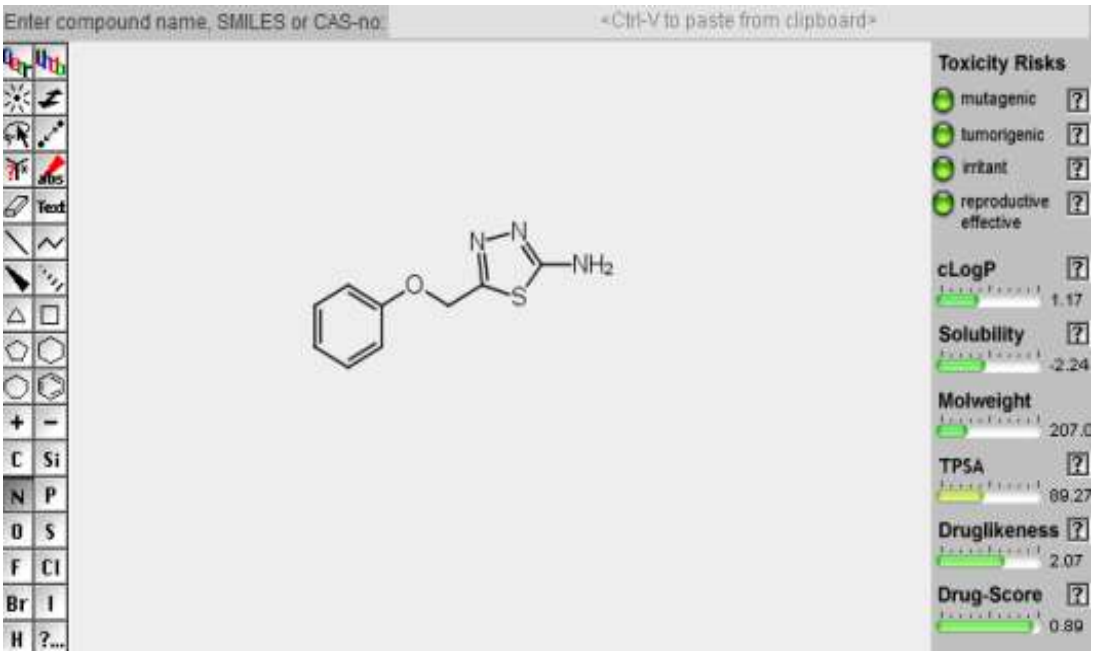
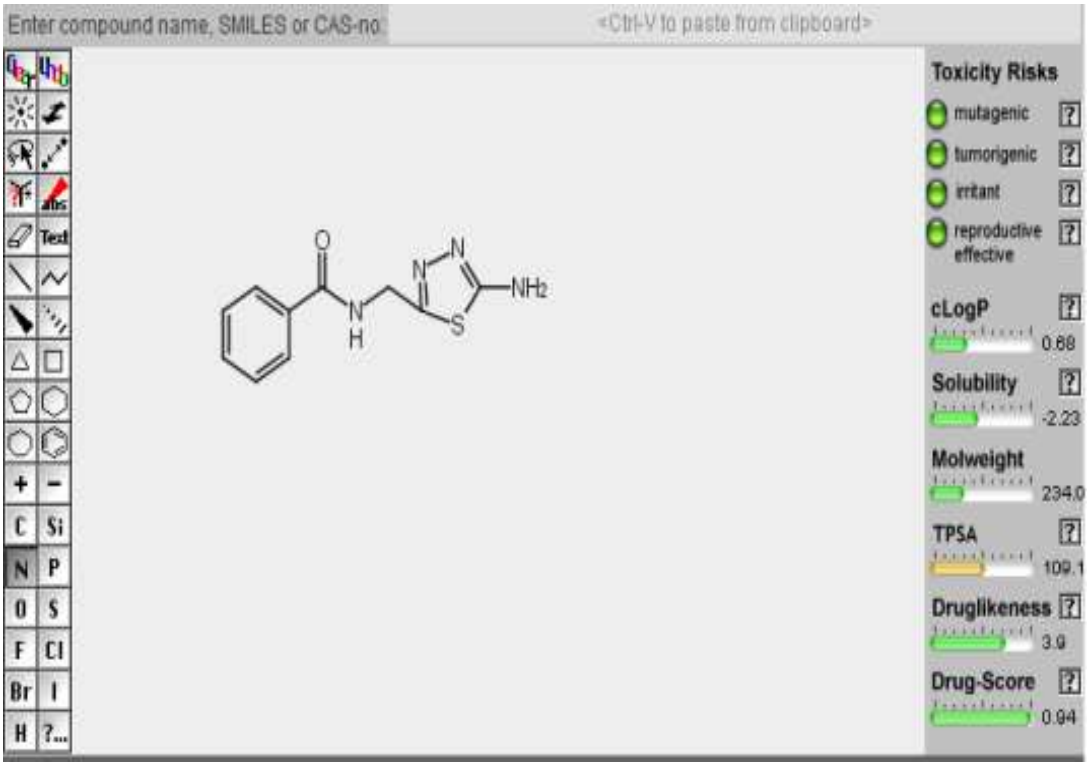


Fig. HA



RESULTS OF SYNTHETIC EFFORTS:-

The Synthetis was Performed by the conventional method. The reaction was carried out by using 0.1 mole of Benzoic acid of substituted aromatic aldehyde and ethanol acts as catalyst the mixture was refluxed. This leads to the formation of thiadiazole nucleus.

TLC PROFILE:-

Thin layer chromatography (TLC) was done on silica gel pre coated plates (604GF 254Merck) with a suitable solvent system. The Rf values were recorded accordingly. This technique is widely employed for the identification of the organic compounds with characteristic Rf values. This method is also applied to determine the progress of the reaction, to examine the purity of the end product. After the development of chromatogram, the spots were detected by placing the plate in UV chamber. In all the synthesized derivatives, single spots were seen, indicating the purity of the compound.

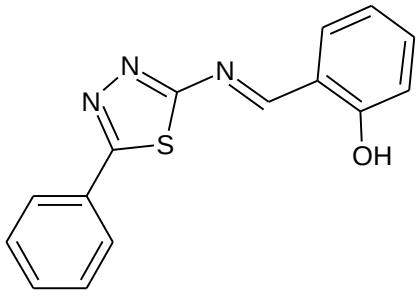
TABLE NO: 5

RESULTS OF SYNTHETIC EFFORTS:-

Compound code	Molecular weight	Melting point	Rf value	Percentage yield
SDK1	281.33	222	0.42	81%
SDK2	281.33	222	0.37	83%
SDK3	334.22	230	0.38	80%
SDK5	310.33	228	0.35	77%
HA	207.25	148	0.28	70%
PAA	219.25	151	0.32	68%

RESULTS OF SCHEMES:-

COMPOUND CODE: - SDK1



Physicochemical Properties of 2-{(*E*)-[(5-phenyl-1, 3, 4-thiadiazol-2-yl) imino] methyl} phenol

Molecular Formula: $C_{15}H_{11}N_3OS$

Formula weight : 281.33234

Composition : C [64.04%], H [3.94%], N [14.94%], O [5.69%], S [11.40%].

Appearance : Yellow in Color

Solubility : Soluble in Ethanol, Methanol.

Molar Refractivity: 81.85+ 0.5cm

Molar Volume : 214.4+7.0cm

Parachor : 583.5+8.0cm

Index of Refraction: 1.688+ 0.55

Surface Tension : 54.7+7.0dyne/cm

Density : 1.31+0.1g/cm

Polarizability : 32.44+0.5cm

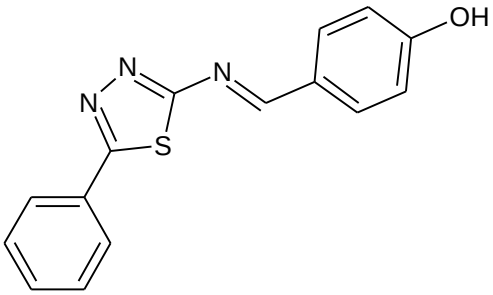
Monoisotopic Mass: 281.0622Da

Nominal Mass : 281Da

Average Mass: 281.3323Da

SAMPLE CODE: SDK2

Physicochemical properties of 4-{(*E*)-[(5-phenyl-1, 3, 4-thiadiazol-2-yl) imino] methyl} Phenol



Molecular Formula: $C_{15}H_{11}N_3OS$

Formula weight : 281.33234

Composition : C [64.04%], H [3.94%], N [14.94%], O[5.69%],S[11.40%].

Appearance : Yellow in Color

Melting point : 222ºC

Solubility : Soluble in Ethanol, Methanol.

Molar Refractivity: 81.85+ 0.5cm

Molar Volume : 214.4+7.0cm

Parachor : 583.5+8.0cm

Index of Refraction: 1.688+ 0.55

Surface Tension : 54.7+7.0dyne/cm

Density : 1.31+0.1g/cm

Polarizability : 32.44+0.5cm

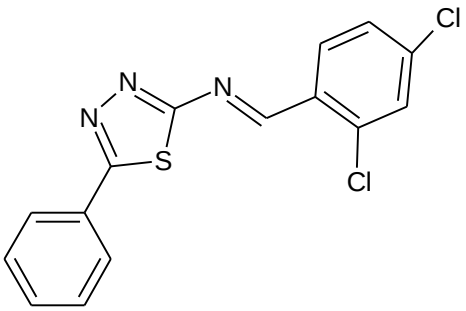
Monoisotopic Mass: 281.0622Da

Nominal Mass: 281Da

Average Mass: 281.3323Da

SAMPLE CODE: SDK3

Physicochemical properties of (*E*)-1-(2, 4 – dichlorophenyl) -*N*-(5-phenyl-1, 3, 4-thiadiazol-2-yl) methanimine



Molecular Formula: $C_{15}H_9Cl_2N_3S$

Formula weight : 334.22

Composition : C (53.90%), H (2.71%), C (21.22%), N(12.57%), S(9.59%).

Appearance : Yellow in Color

Melting point : 230ºC

Solubility : Soluble in Ethanol, Methanol.

Molar Refractivity: 90.20± 0.5cm^3

Molar Volume : 235.8± 7.0cm^3

Parachor : 635.5± 8.0cm^3

Index of Refraction: 1.60± 0.05cm^3

Surface Tension : 52.7 ± 7.0 dyne/ cm

Density : 1.41± 0.1 g/cm^3

Polarizability : 35.72± 0.5 10-24cm^3

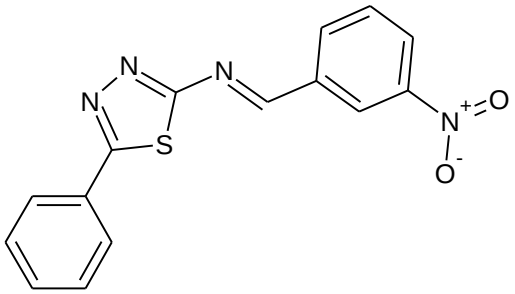
Monoisotopic Mass: 332.983 Da

Nominal Mass: 333Da

Average Mass: 334.2231Da

SAMPLE CODE: SDK5

Physico chemical properties of (*E*)-1-(3-nitrophenyl)-*N*-(5-phenyl-1, 3, 4-thiadiazol-2-yl) methanimine



Molecular Formula: $C_{15}H_{10}N_4O_2S$

Formula weight : 310.3305

Composition : C (58.05%), H (3.25%),N(18.05%),O(10.31%),S(10.33%)

Melting point : 228ºC

Solubility : Soluble in Ethanol, Methanol.

Molar Refractivity: 86.66±0.5cm^3

Molar Volume : 222.5±7.0cm^3

Parachor : 623.3±8.0cm^3

Index of Refraction: 1.706±0.05

Surface Tension : 61.5±7.0dyne/cm

Density : 1.39±0.1g/cm^3

Polarizability : 34.35±0.5 10-24cm^3

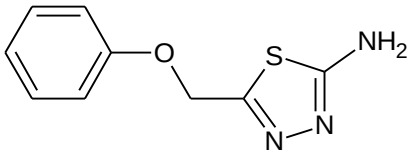
Monoisotopic Mass: 310.052466 Da

Nominal Mass: 310 Da

Average Mass: 310.3305 Da

SAMPLE CODE: PAA

Physicochemical Properties of 5-(Phenoxy methyl)-1, 3, 4- thiadiazol-2-amine



Molecular Formula: $C_9H_9N_3OS$

Formula weight : 207.25226

Composition : C (52.16%), H (4.38%), N (20.27%), O (7.72%), S (15.47%).

Melting point : 148^{o}c

Solubility : Soluble in Ethanol, Methanol.

Molar Refractivity: 56.22±0.3cm^3

Molar Volume : 152.3±3.0cm^3

Parachor : 4.33.3±4.0cm^3

Index of Refraction: 1.659±0.02

Surface Tension : 6.54±3.0dyne/cm

Density : 1.360±0.06g/cm^3

Polarizability : 22.28±0.5 10-24cm^3

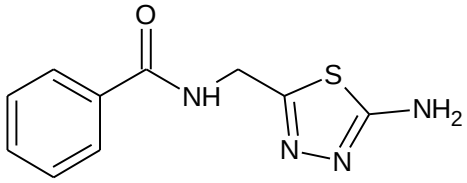
Monoisotopic Mass: 207.0466Da

Nominal Mass: 207Da

Average Mass: 207.2523Da

SAMPLE CODE: HA

Physicochemical properties of N-(5-amino-1, 3, 4-thiadiazol-2-yl) benzamide



Molecular Formula: $C_9H_8N_4OS$

Formula weight : 220.25

Composition : C (49.08%), H (3.66%), N (25.44%), O (7.26%), S (14.56%).

Melting point : 151ºC

Solubility : Soluble in Ethanol, Methanol.

Molar Refractivity: 59.63±0.3 cm^3

Molar Volume : 146.5±3.0 cm^3

Parachor : 444.1± 4.0cm^3

Index of Refraction: 1.749± 0.02

Surface Tension : 84.4±3.0 dyne/ cm

Density : 1.503± 0.06 g/cm^3

Polarizability : 23.63±0.5 10-24 cm^3

Monoisotopic Mass: 220.04 Da

Nominal Mass: 220 Da

Average Mass: 220.251 Da

CHARACTERIZATION:

The newly synthesized compounds were characterized by

- IR
- NMR
- GC-MS, LC-MS

IR SPECTROSCOPY:-

The Samples were prepared by the KBr Pellet technique and Spectrum obtained from ABB [MB 3000] Spectrophotometer

The Spectra were examined for the absence of the functional group region of parent compound and examined for presence of the Vibrational absorption band for the new functional group.

Our reaction involves reaction between Aldehyde and amine to yield Schiff bases.

The absorption bands for aldehyde are

- 2800-2700cm^{-1}
- 1700-1750 cm^{-1}

The absorption band for amine 3400-3600cm^{-1}

The absorption of newly Synthesized –C=N is 1650-1600cm^{-1}.

IR ABSORPTION BAND:-

The infrared spectroscopy is one of the most powerful analytical techniques; this offers the possibility of chemical identification. The most important advantages of infrared spectroscopy over the other usual methods of structural analysis are that it provides useful information about the functional groups present in the molecule quickly.

TABLE NO: - 6

ABSORPTION BAND	SDK1	SDK2	SDK3	SDK5	PAA	HA
NH Stretching	×	×	×	×	✓	✓
CH Stretching	✓	✓	✓	✓	✓	✓
OH Stretching	✓	✓	×	×	×	×
C=N Stretching	✓	✓	✓	✓	×	×
C-C Stretching	✓	✓	✓	✓	✓	✓
C-S-C bending	✓	✓	✓	✓	×	×

The presence of stretching and or bending for the new functional group and the absence of the stretching and or bending motion for the parent functional groups undergoing the change is indicative of the successful formation of the expected compound.

SAMPLE CODE: - SDK1

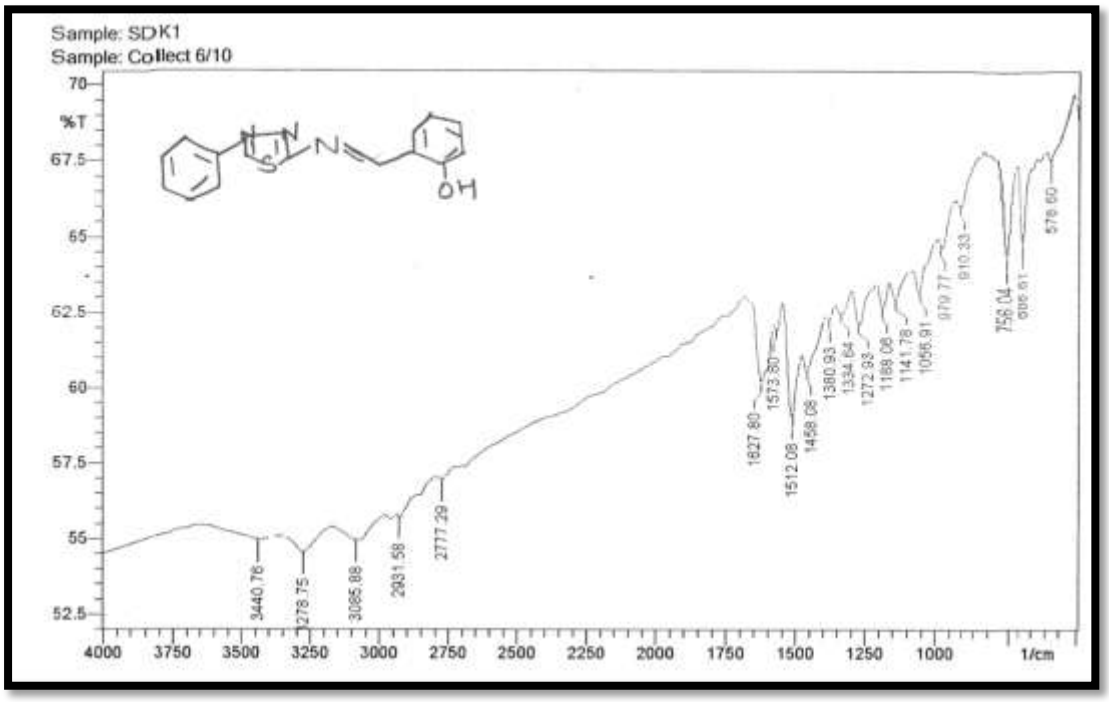


TABLE NO: 7

S.NO	FUNCTIONAL GROUP REGION	WAVE NUMBER(cm^{-1})
1.	C-H Stretching	3085.88 cm^{-1}
2.	Aliphatic C-H Stretching	2931.88 cm^{-1}
3.	OH Stretching	3440.78 cm^{-1}
4.	C=N Stretching	1627.80 cm^{-1}
5.	C-S-C Bending	756.04 cm^{-1}

SAMPLE CODE: SDK2

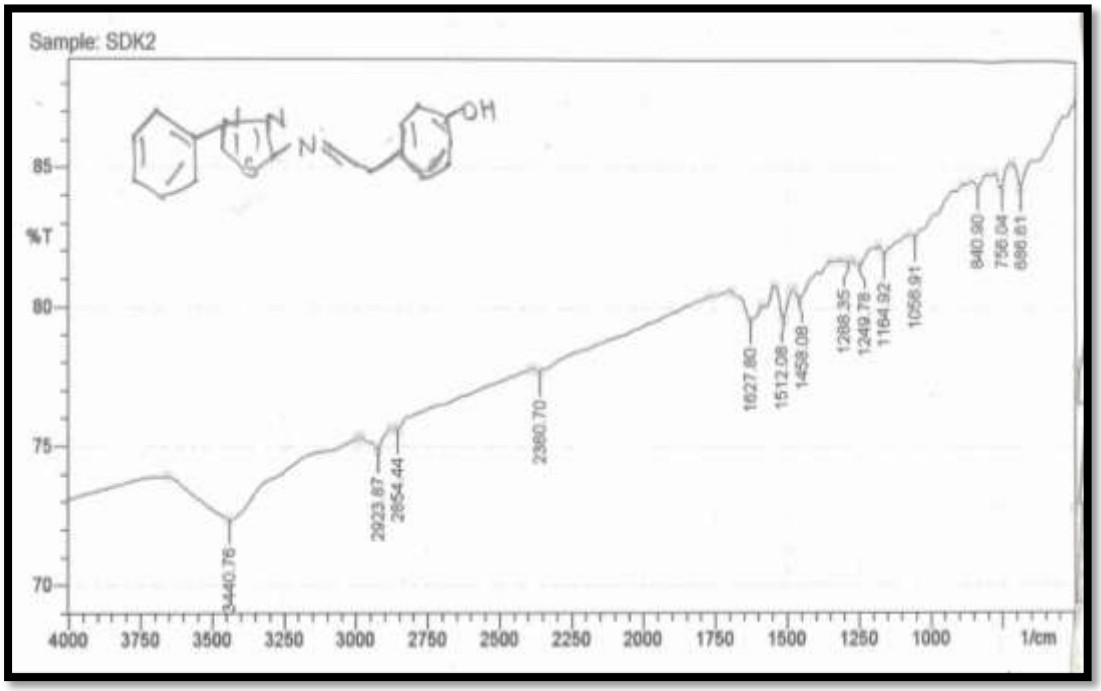


TABLE NO: 8

S.NO	FUNCTIONAL GROUP REGION	WAVE NUMBER(cm^{-1})
1.	Aromatic C-H Stretching	2923.87 cm^{-1}
2.	Aliphatic C-H Stretching	2854.44 cm^{-1}
2.	OH Stretching	3440.76 cm^{-1}
3.	C=N Stretching	1627.80 cm^{-1}
4.	C-S-C Bending	756.04 cm^{-1}

SAMPLE CODE: SDK3

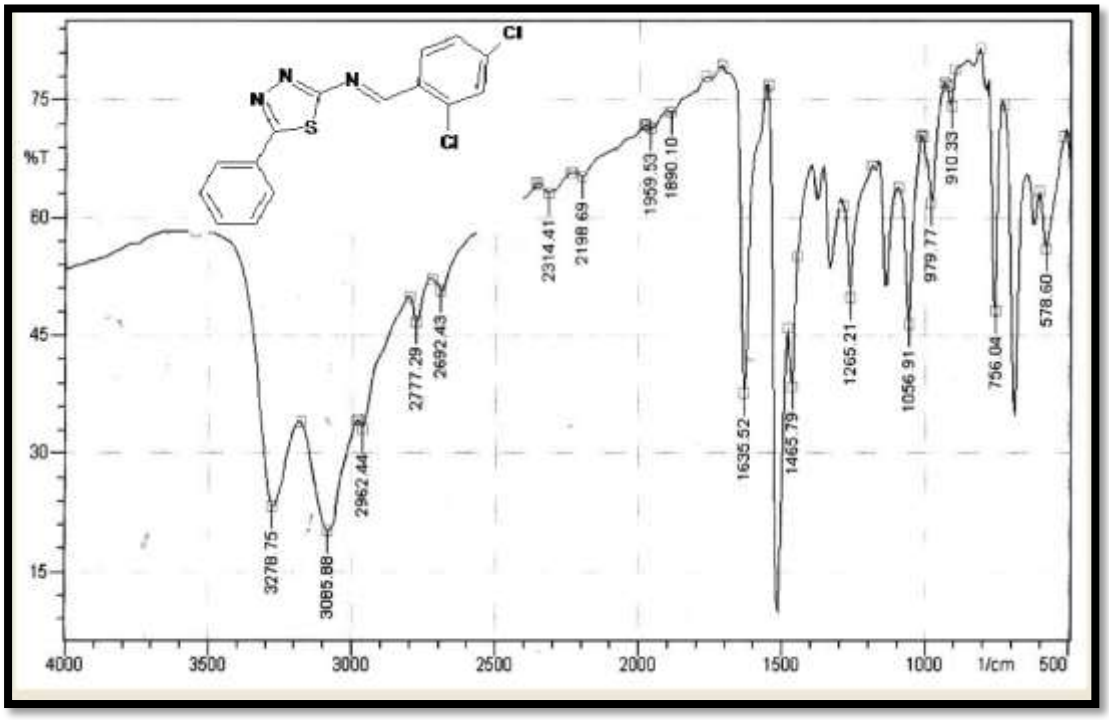


TABLE NO: 9

S.NO	FUNCTIONAL GROUP REGION	WAVE NUMBER(cm^{-1})
1.	Aromatic C-H Stretching	3O85.88cm^{-1}
2.	Aliphatic C-H Stretching	2962.44 cm^{-1}
3.	C=N Stretching	1635.52 cm^{-1}
4.	C-S-C Bending	756.04 cm^{-1}
5.	C=C Stretching	1610.33 cm^{-1}

SAMPLE CODE:-SDK7

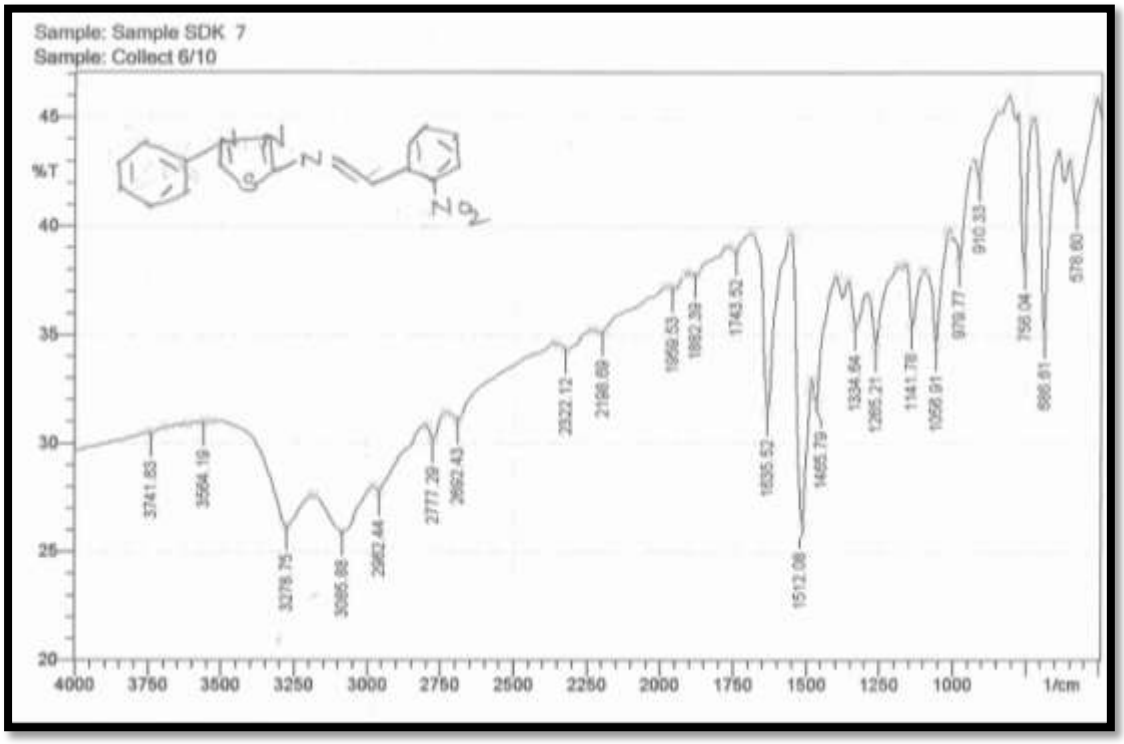


TABLE NO: 10

S.NO	FUNCTIONAL GROUP REGION	WAVE NUMBER(cm^{-1})
1.	Aromatic C-H Stretching	3O85.88cm^{-1}
2.	Aliphatic C-H Stretching	2962.44 cm^{-1}
3.	C=N Stretching	1635.52 cm^{-1}
4.	C-S-C Bending	756.04 cm^{-1}
5.	N=O Stretching	1512.08 cm^{-1}

SAMPLE CODE: - PAA

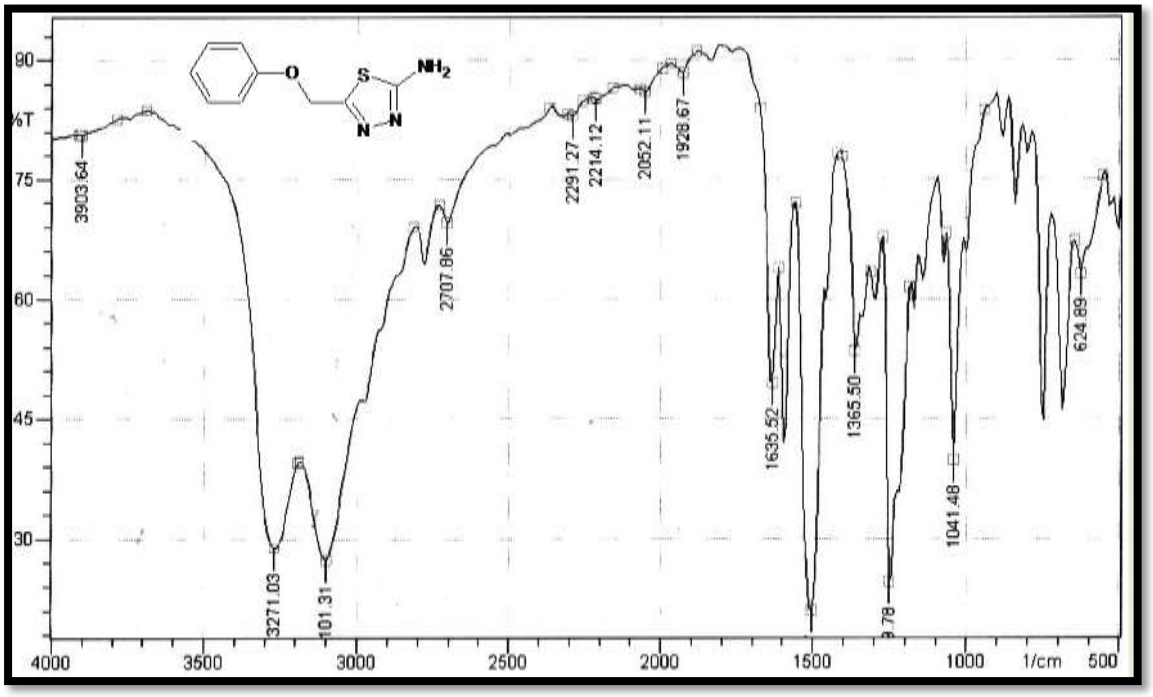


TABLE NO: 11

S.NO	FUNCTIONAL GROUP REGION	WAVE NUMBER(cm^{-1})
1.	Aromatic C-H Stretching	3105.88cm^{-1}
2.	Aliphatic C-H Stretching	2707.86 cm^{-1}
3.	N-H Stretching	3271.03cm^{-1}
4.	C-S-C Bending	624.04 cm^{-1}

SAMPLE CODE: HA

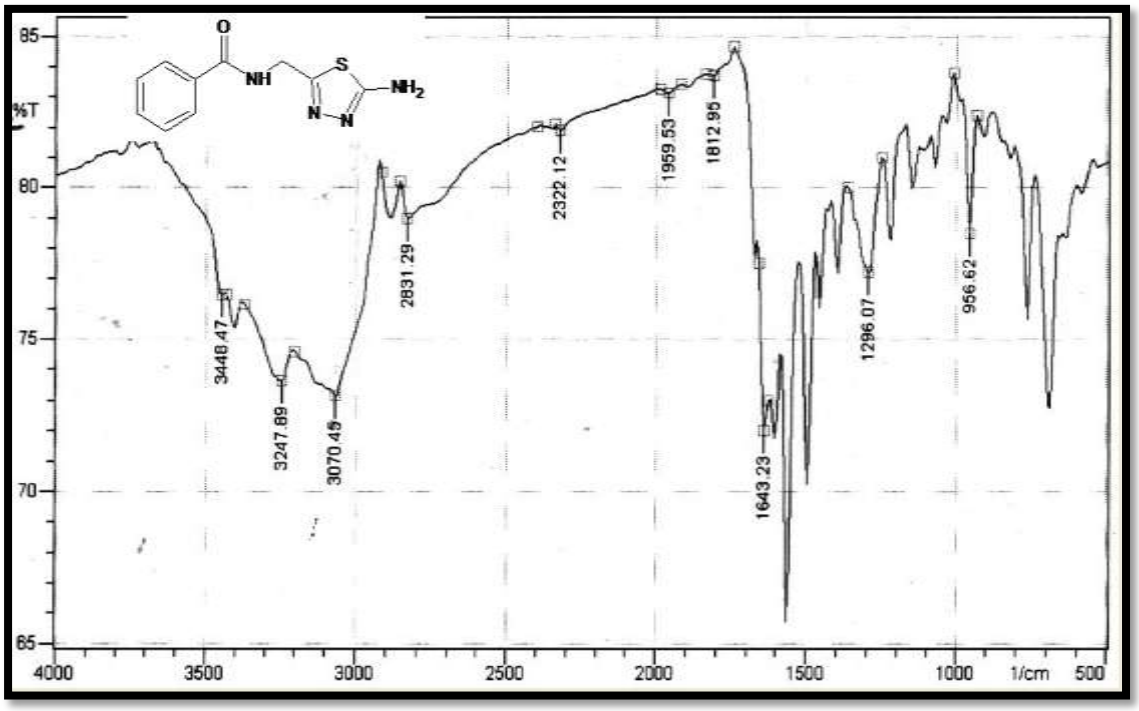


TABLE NO: 12

S.NO	FUNCTIONAL GROUP REGION	WAVE NUMBER(cm^{-1})
1.	Aromatic C-H Stretching	3070.88cm^{-1}
2.	Aliphatic C-H Stretching	2831.86 cm^{-1}
3.	N-Stretching	3247.03cm^{-1}
4.	C-S-C Bending	954.04 cm^{-1}
5.	C=O Stretching	1643.23 cm^{-1}

NMR SPECTROSCOPY:

It is a powerful tool for the investigation of nuclei structure. 1HNMR Spectra's of the prepared derivatives were done by using 400-MHz and 500-MHzBruker spectrometer using internal standard as tetra methyl silane.

^{1} H NMR SPECTRAL DATA OF THE SYNTHESIZED COMPOINDS:

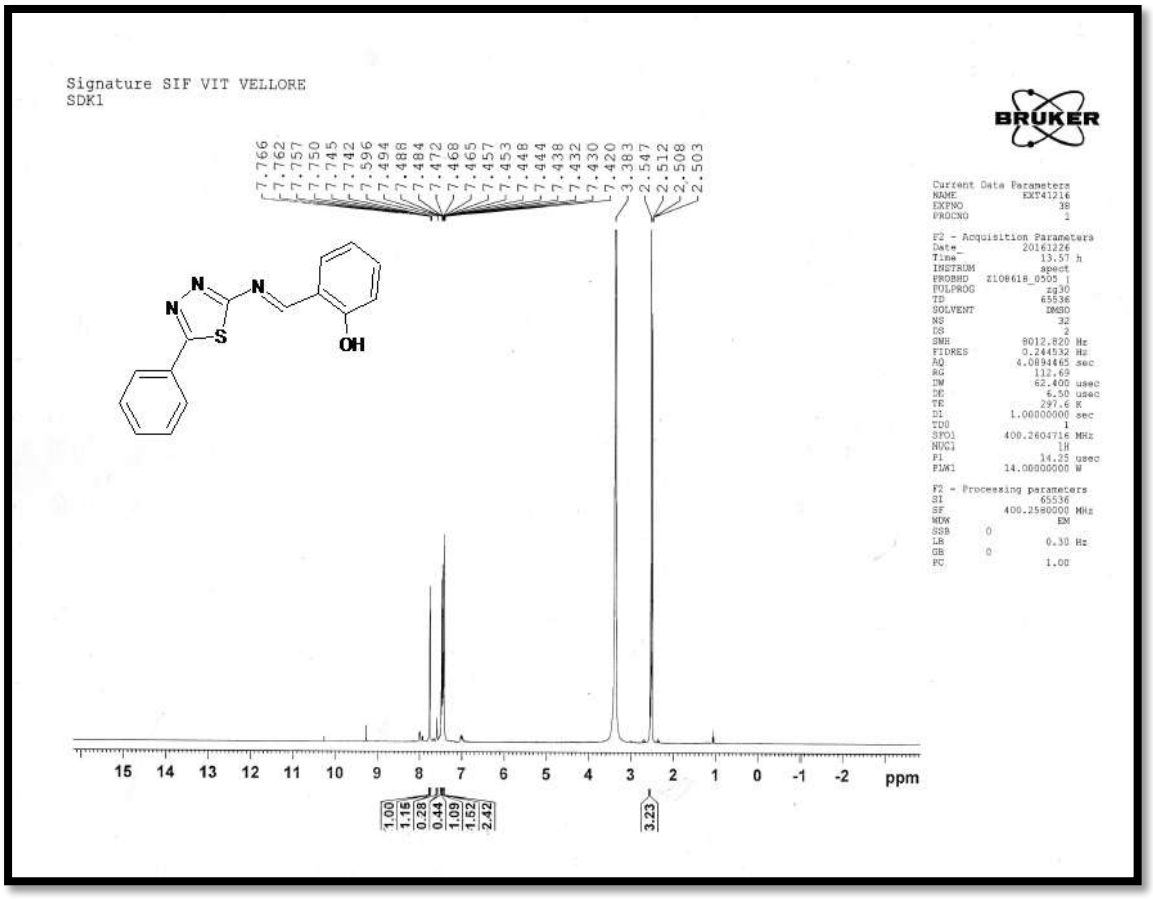


TABLE NO:-13

S.NO	DELTA VALUE	TYPE OF PEAK	NUMBER OF PROTON
1.	7.42-7.484	Multiplet	6 protons
2.	7.596	Doublet	2Protons
3.	7.74-7.766	Triplet	3Protons

SAMPLE CODE: SDK2

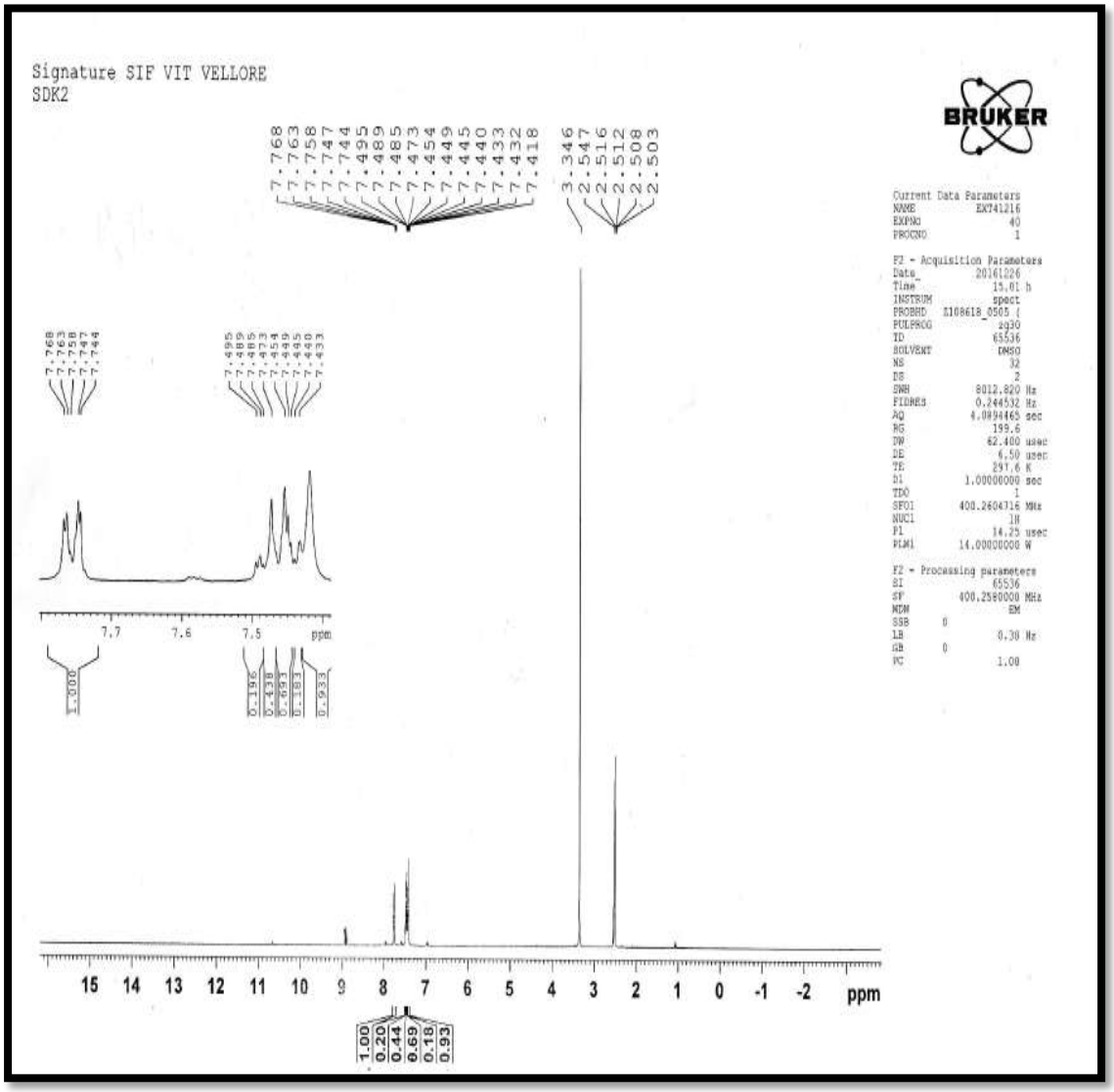


TABLE NO: 14

S.NO	DELTA VALUE	TYPE OF PEAK	NUMBER OF PROTON
1.	7.42-7.484	Multiplet	6 protons
2.	7.596	Singlet	1Protons
3.	7.74-7.766	Triplet	3Protons

SAMPLE CODE: SDK3

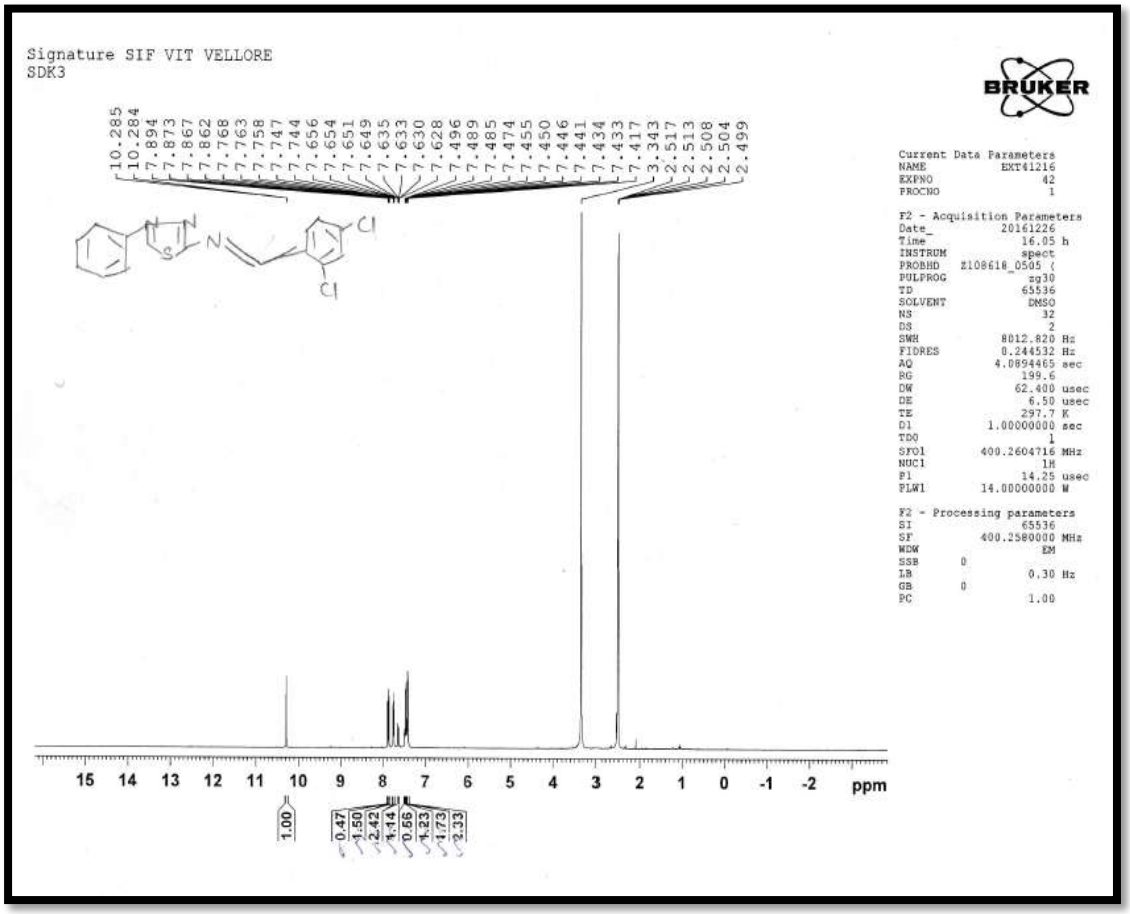
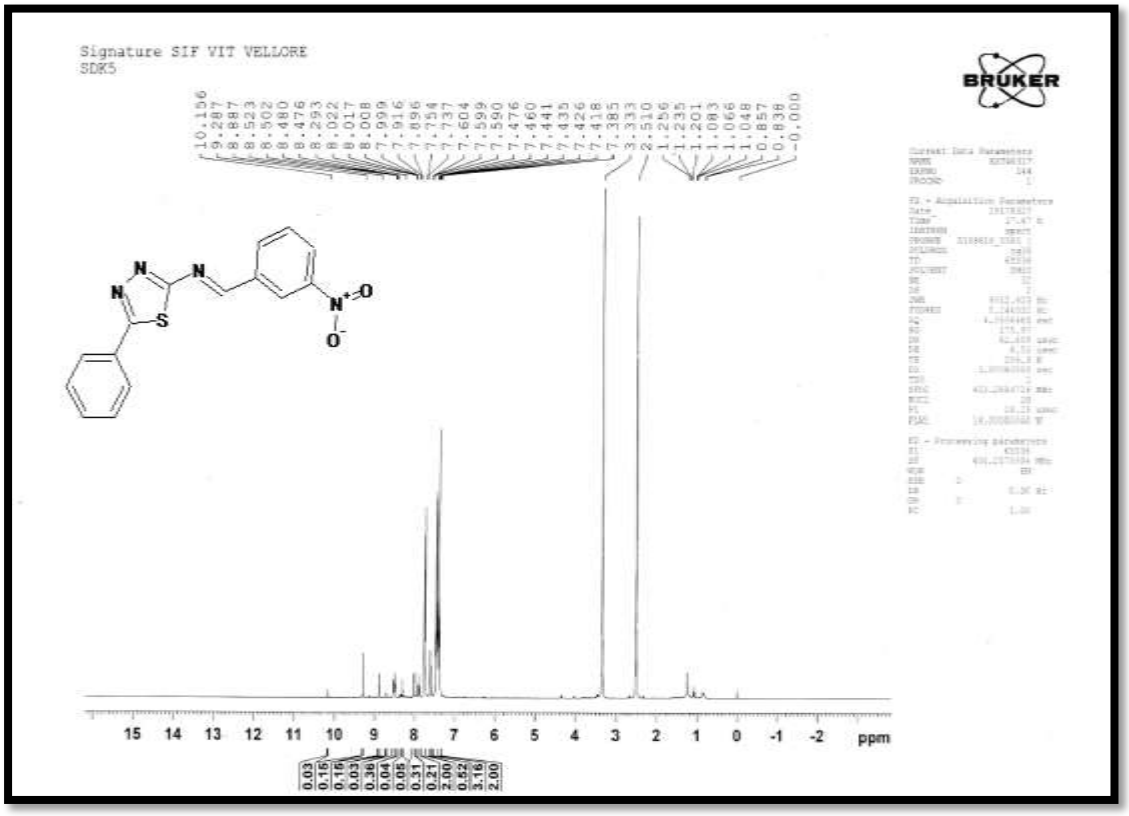


TABLE NO: 15

S.NO	DELTA VALUE	TYPE OF PEAK	NUMBER OF PROTON
1.	10.284-10.285	Singlet	1Protons
2.	7.417-7.496	Quarteret	4 Protons
3.	7.628-7.654	Doublet	1 Protons
4.	7.744-7.768	Doublet	1 Protons
5.	7.862-7.894	Doublet	2 Protons

SAMPLE CODE: SDK5



TABLENO: 16

S.NO	DELTA VALUE	TYPE OF PEAK	NUMBER OF PROTON
1.	7.207-7.229	Singlet	1 Protons
2.	7.409-7.490	Multiplet	5 Protons
3.	7.593-7.620	Singlet	1 Protons
4.	7.870-7.958	Doublet	2 Protons
5.	8.011-8.035	Singlet	1 Protons

SAMPLE CODE: PAA

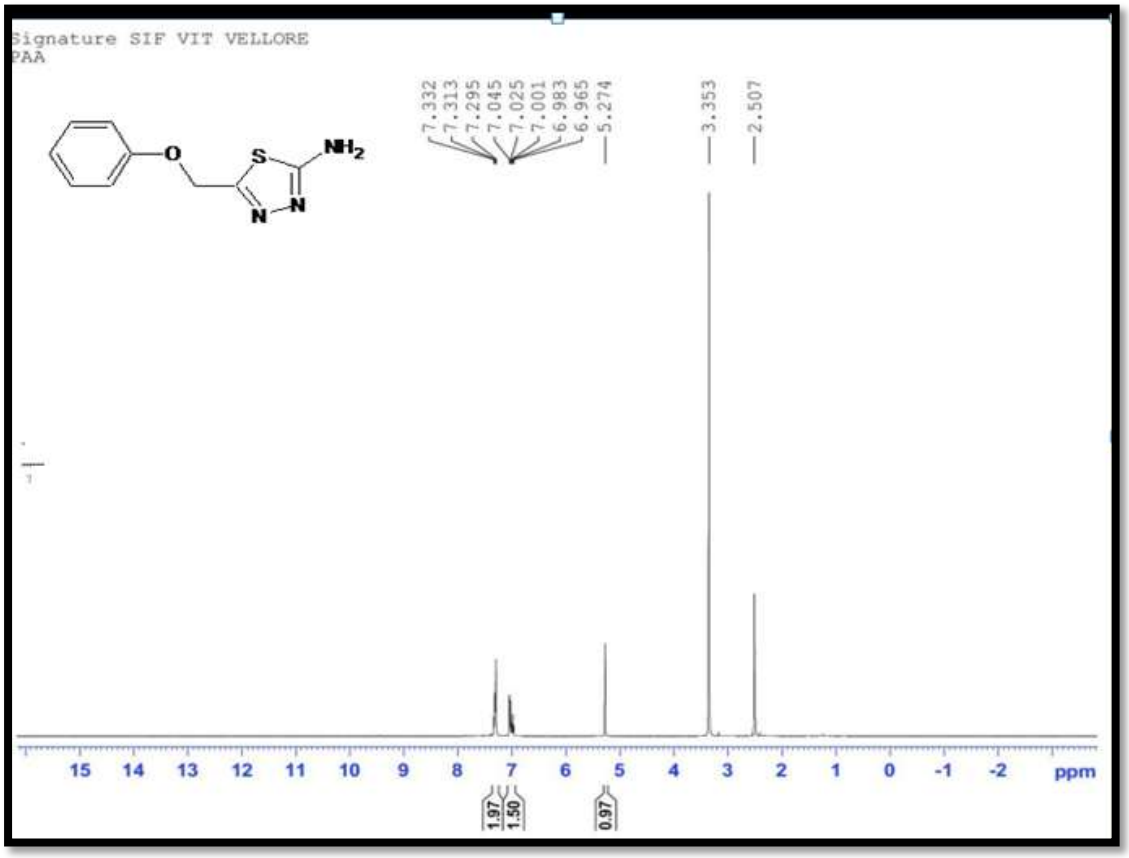


TABLE NO: 17

S.NO	DELTA VALUE	TYPE OF PEAK	NUMBER OF PROTON
1.	6.965-7.045	Doublet	2
2.	7.295-7.332	Doublet	2

SAMPLE CODE: HA

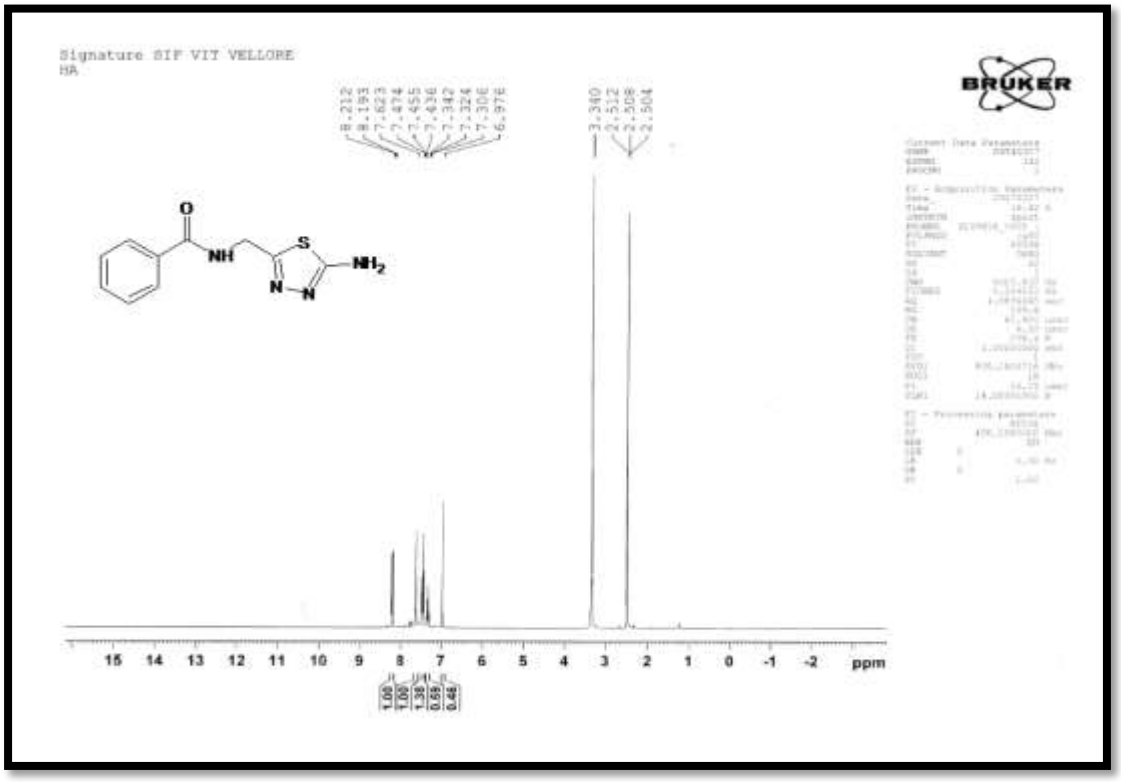


TABLE NO: - 18

S.NO	DELTA VALUE	TYPE OF PEAK	NUMBER OF PROTON
1.	6.976-7.324	Singlet	1
2.	7.436-7.474	Triplet	3
3.	7.623-8.193	Doublet	2

MASS SPECTROMETRY:-

Mass Spectrometry is to determine the molecular weight of the Synthesized Compounds.

HYPHENATED TECHNIQUES:-

GC-MS: - To determine the mass and also get an idea about the purity of the sample.

LC-MS: - When the sample cannot be vaporized, GC-MS cannot be performed. So LC-MS is performed.

MASS SPECTRA DATA OF THE SYNTHESISED COMPOUNDS:-

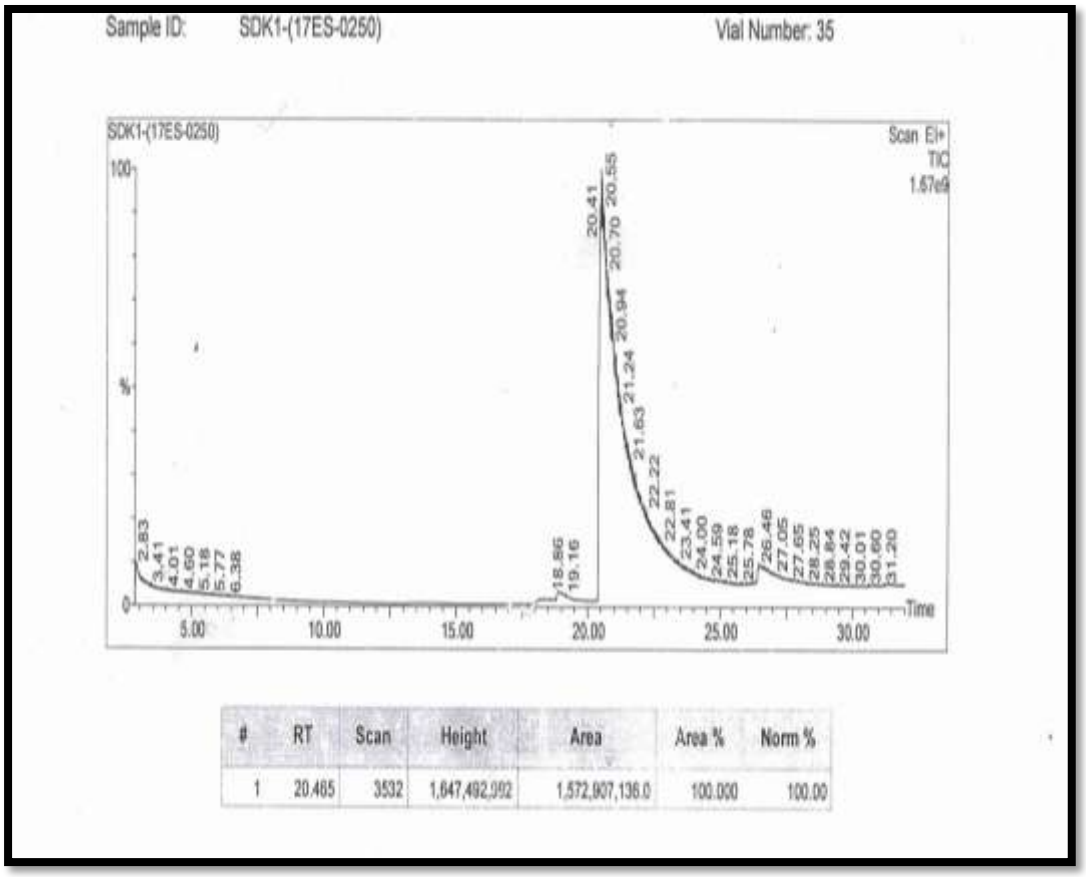
TABLE NO:-19

COMPOUND CODE	MASS SPECTRA DATA OF THE SYNTHESISED COMPOUNDS	MOLECULAR WEIGHT
SDK1	281.25	281.25
SDK2	281.25	281.25
SDK3	334.42	334.42
SDK5	310.34	309.34
PAA	207.12	207.12
HA	219.03	218.03

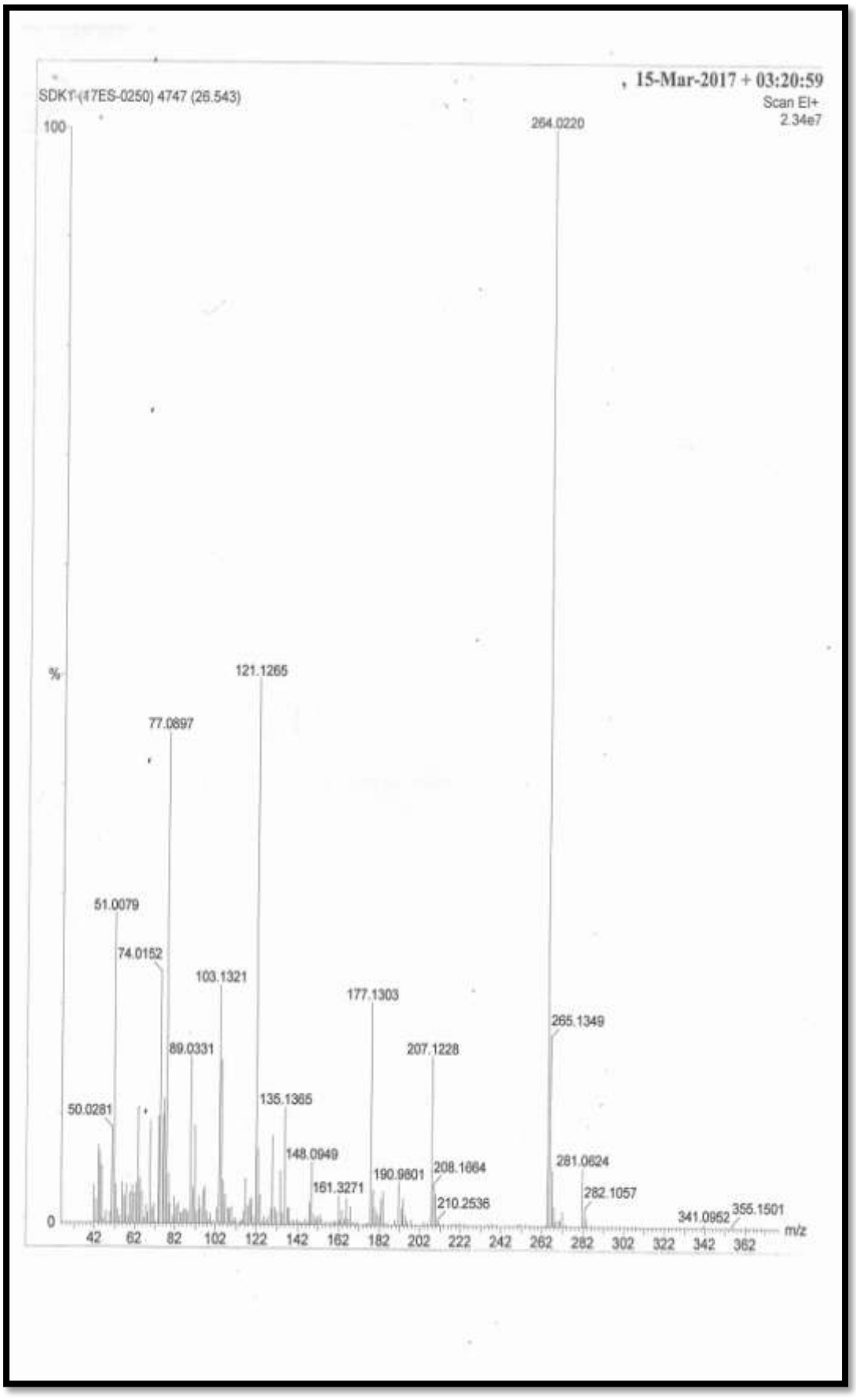
GC-MS

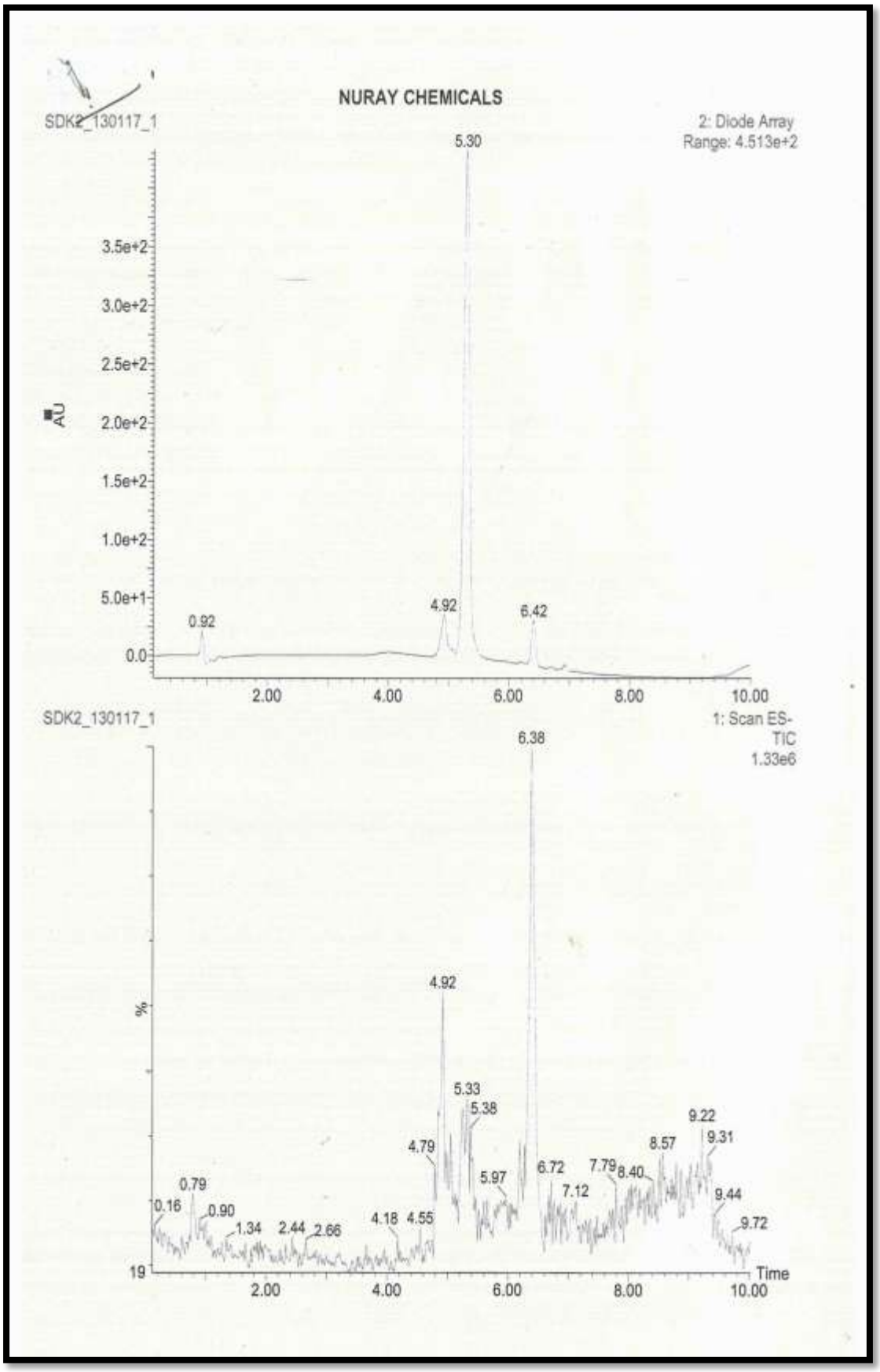
SAMPLE CODE: SDK1

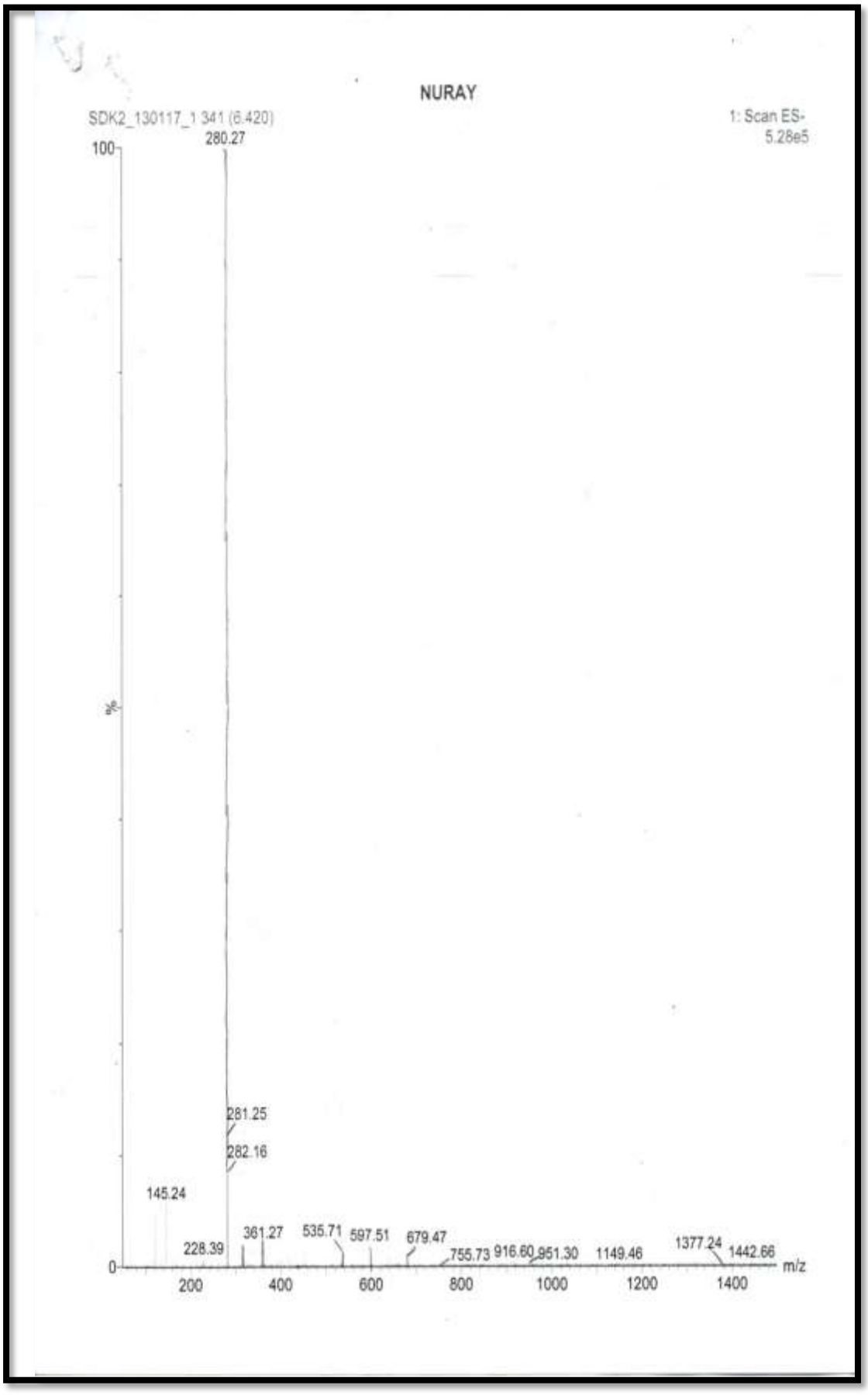
Fig.22 GC-MS Spectral data of the sample SDK1



#	RT	Scan	Height	Area	Area %	Norm %
1	20.465	3532	1,647,492,992	1,572,907,136.0	100.000	100.00


SDKT-(17ES-0250) 4747 (26.543)
, 15-Mar-2017 + 03:20:59
Scan EI+
2.34e7
100
%
0
264.0220
121.1265
77.0897
51.0079
74.0152
103.1321
177.1303
265.1349
89.0331
207.1228
50.0281
135.1365
148.0949
190.9801
208.1664
281.0624
161.3271
282.1057
210.2536
341.0952
355.1501
m/z
42 62 82 102 122 142 162 182 202 222 242 262 282 302 322 342 362

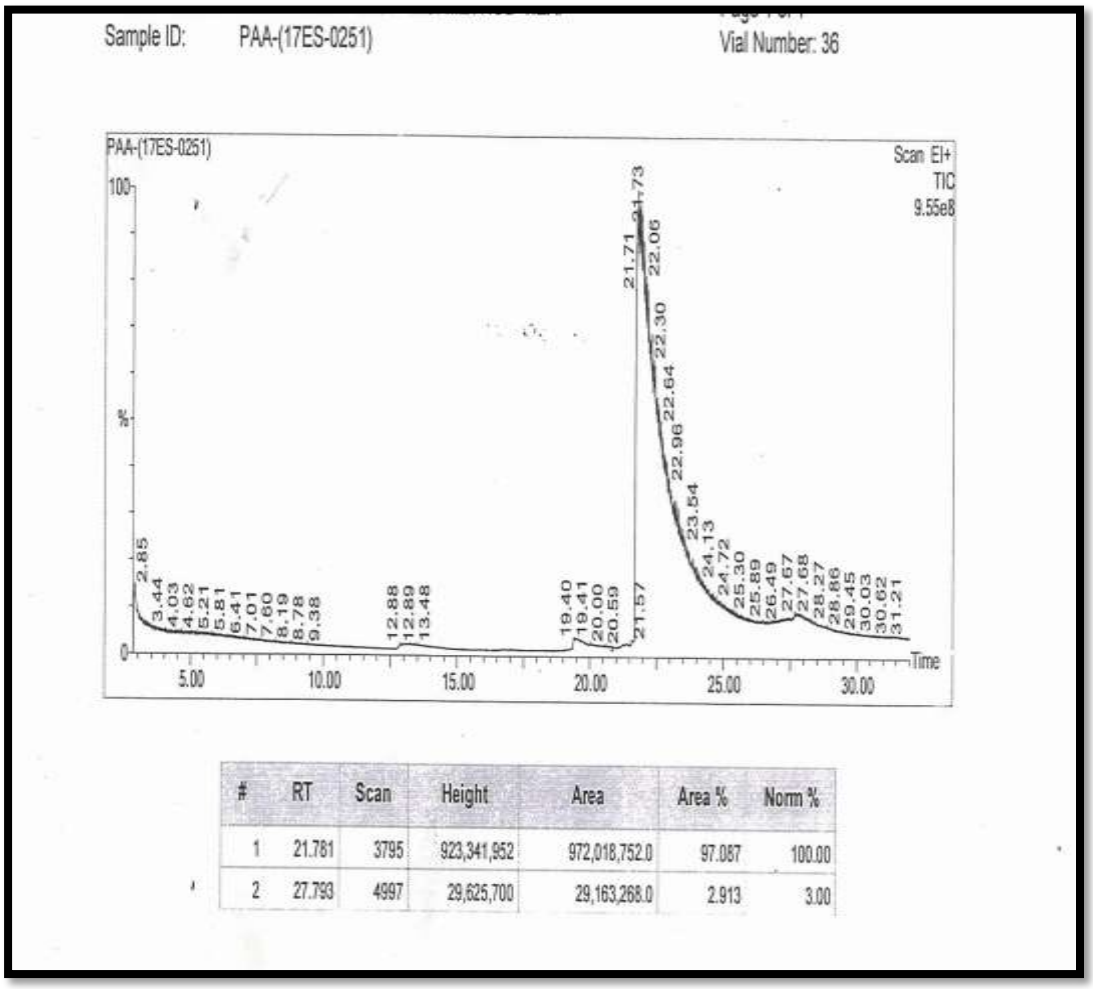

NURAY CHEMICALS
SDK2_130117_1
2: Diode Array
Range: 4.513e+2
5.30
3.5e+2
3.0e+2
2.5e+2
2.0e+2
1.5e+2
1.0e+2
5.0e+1
0.0
AU
0.92
4.92
6.42
2.00
4.00
6.00
8.00
10.00
SDK2_130117_1
1: Scan ES-
TIC
1.33e6
6.38
4.92
5.33
5.38
9.22
9.31
8.57
4.79
5.97
6.72
7.79
8.40
0.79
7.12
9.44
0.16
0.90
1.34
2.44
2.66
4.18
4.55
9.72
%
19
Time
2.00
4.00
6.00
8.00
10.00


NURAY
SDK2_130117_1 341 (6.420)
1: Scan ES-
5.28e5
100
280.27
%
0
281.25
282.16
145.24
228.39
361.27
535.71
597.51
679.47
755.73
916.60
951.30
1149.46
1377.24
1442.66
m/z
200
400
600
800
1000
1200
1400

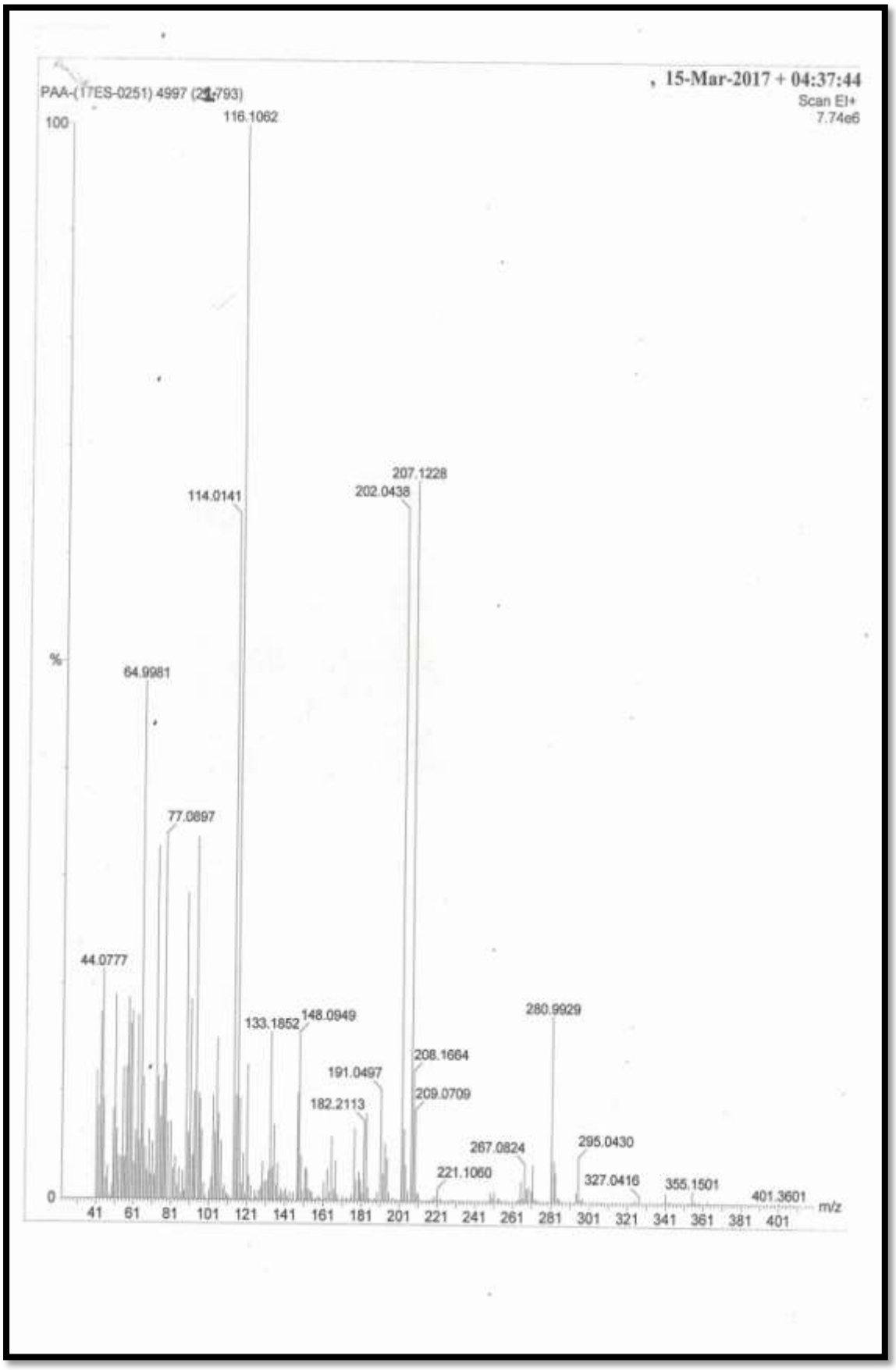
GC-MS

SAMPLE CODE: PAA

Fig.23 GC-MS Spectral data of Sample PAA

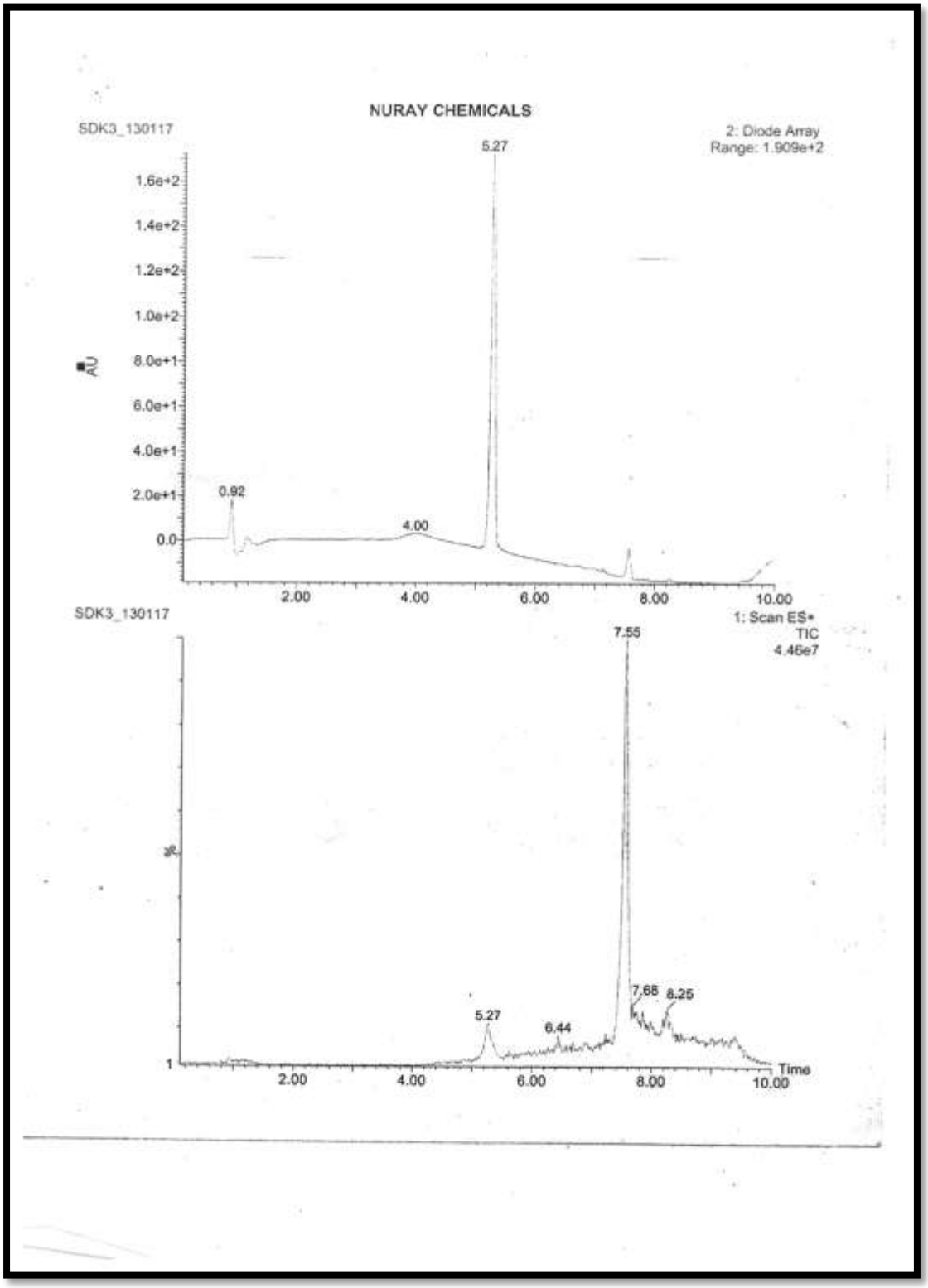


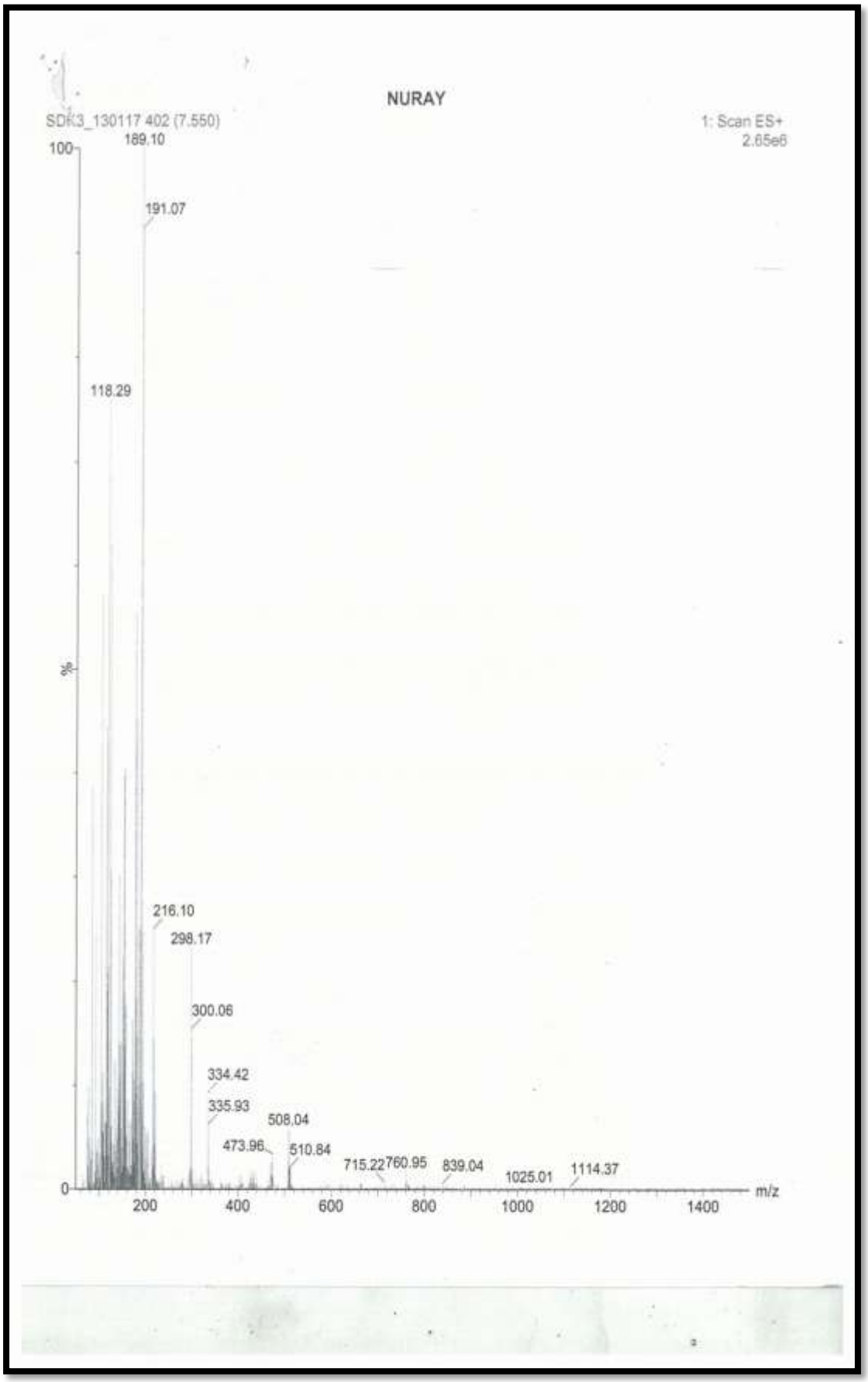
#	RT	Scan	Height	Area	Area %	Norm %
1	21.781	3795	923,341,952	972,018,752.0	97.087	100.00
2	27.793	4997	29,625,700	29,163,268.0	2.913	3.00

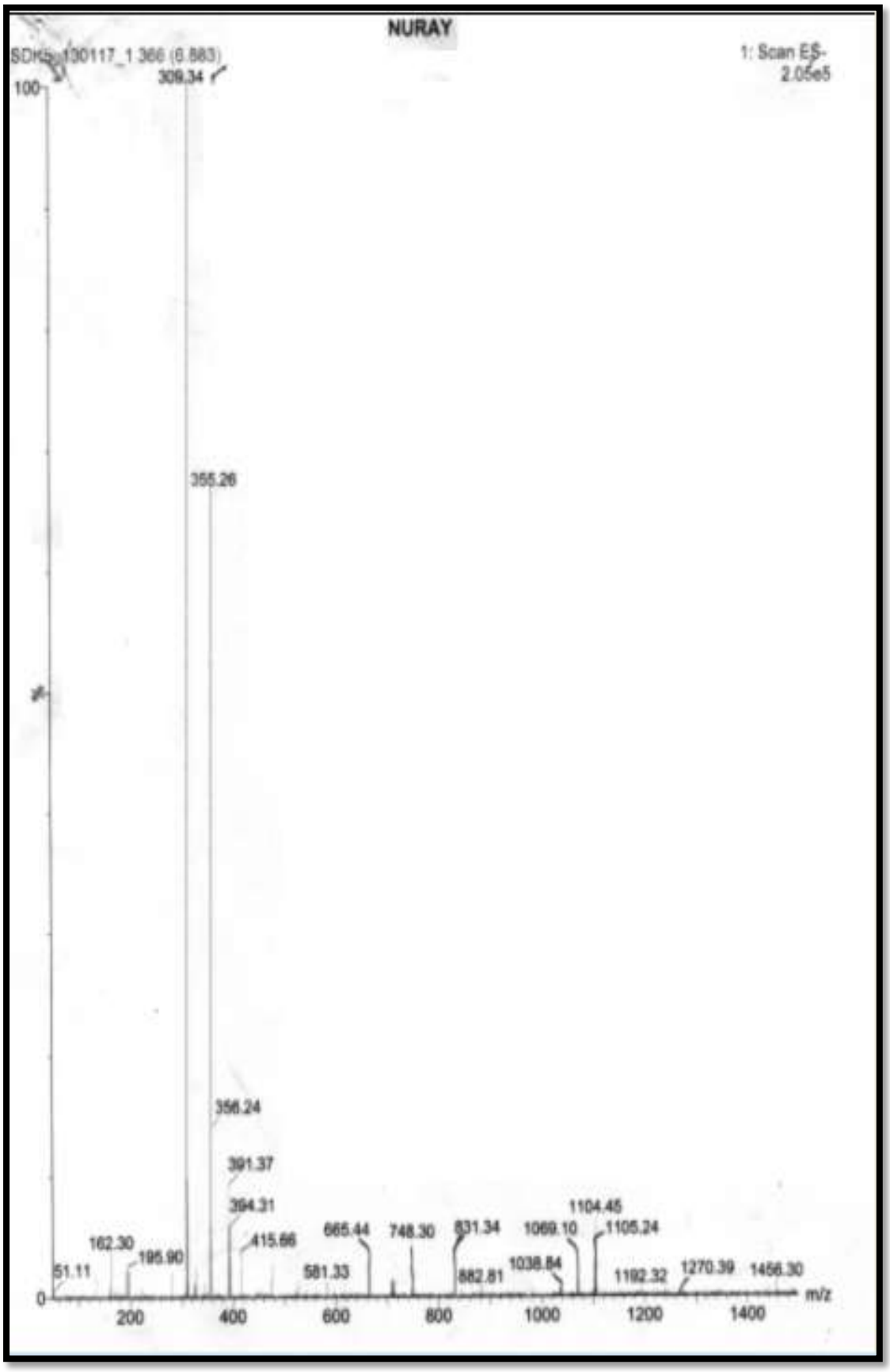

PAA-(17ES-0251) 4997 (24.793)
, 15-Mar-2017 + 04:37:44
Scan EI+
7.74e6
100
%
0
116.1062
114.0141
207.1228
202.0438
64.9981
77.0897
44.0777
133.1852
148.0949
280.9929
208.1664
191.0497
209.0709
182.2113
267.0824
295.0430
221.1060
327.0416
355.1501
401.3601
41 61 81 101 121 141 161 181 201 221 241 261 281 301 321 341 361 381 401
m/z

LC-MS

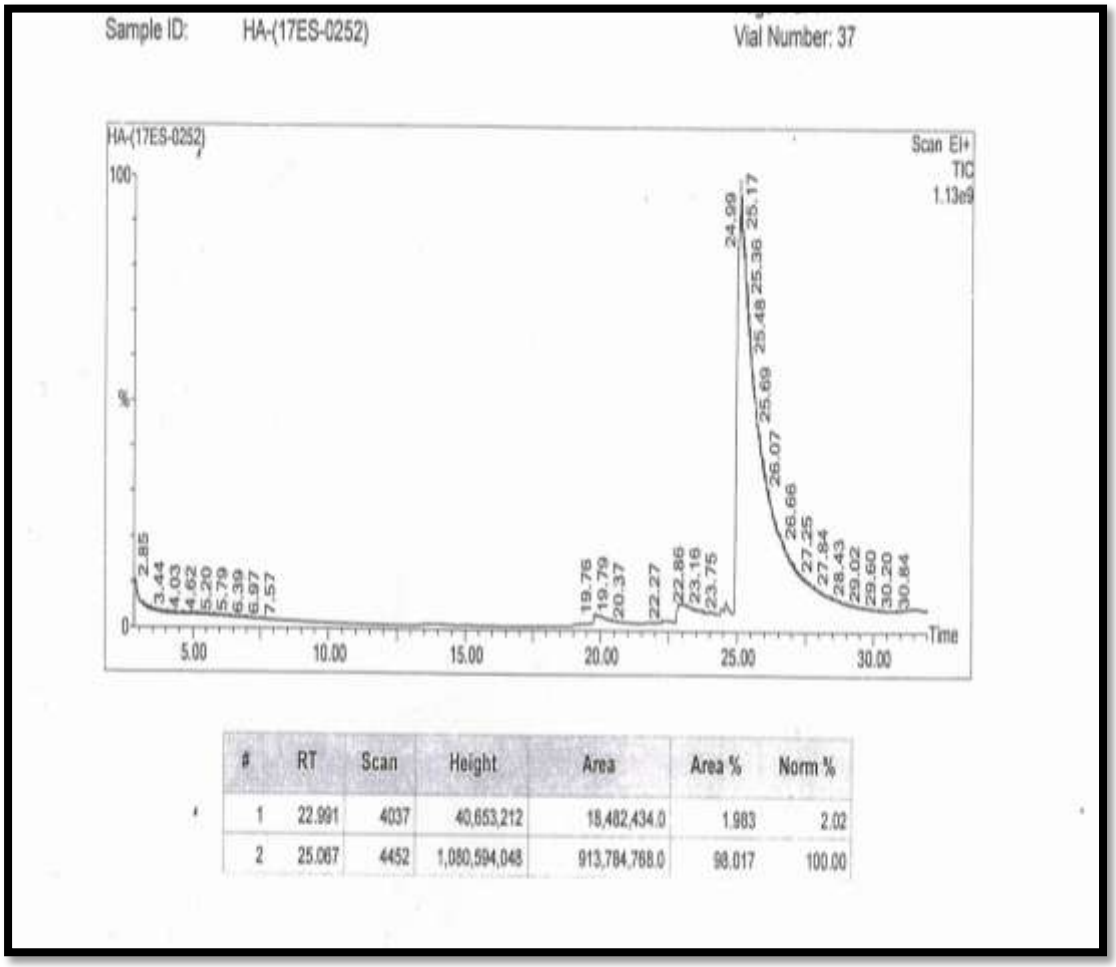
SAMPLE CODE: SDK3



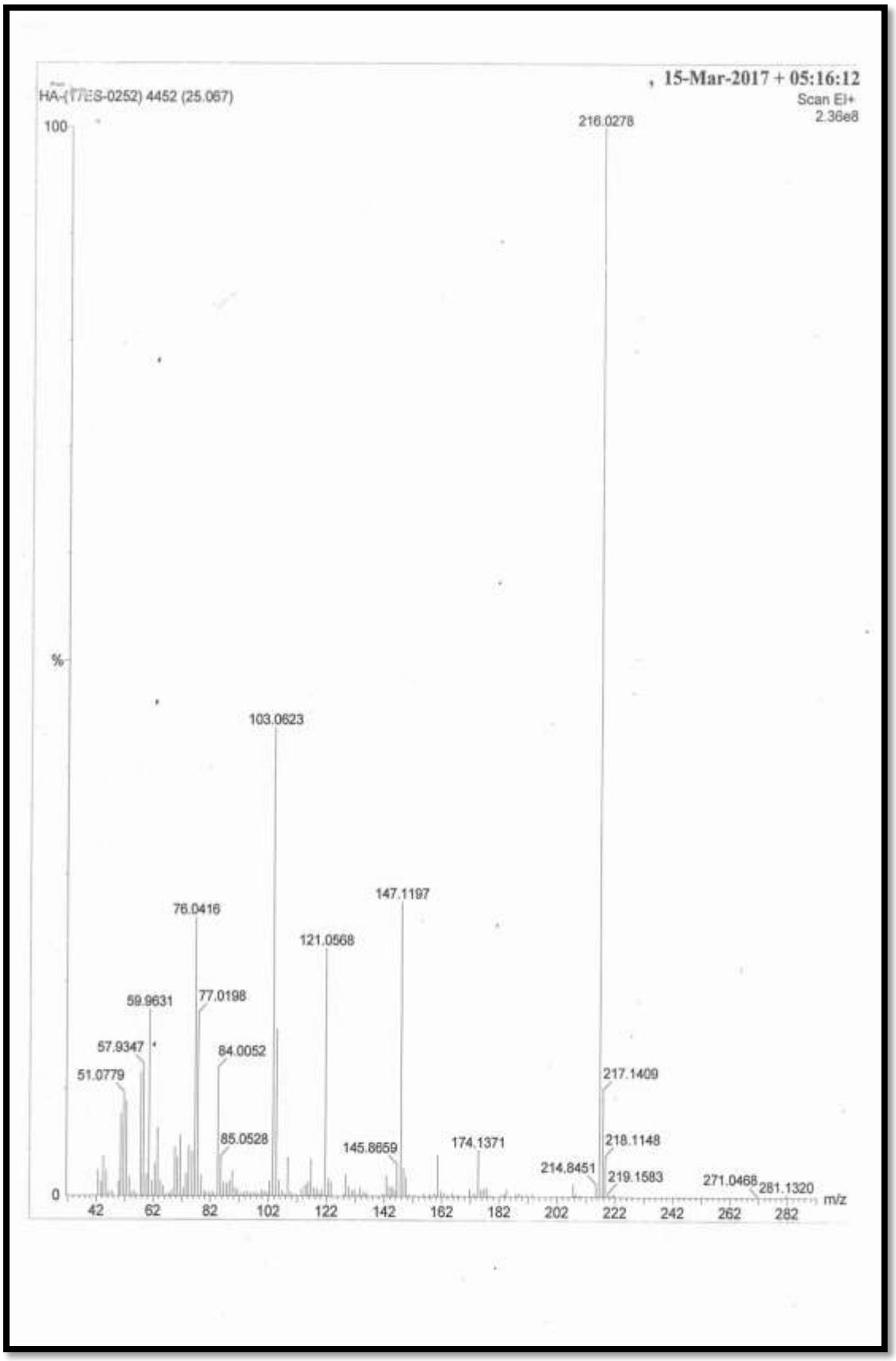

NURAY
SDK3_130117 402 (7.550)
1: Scan ES+
2.65e6
100
%
0
189.10
191.07
118.29
216.10
298.17
300.06
334.42
335.93
508.04
473.96
510.84
715.22
760.95
839.04
1025.01
1114.37
200
400
600
800
1000
1200
1400
m/z


NURAY
SDKB_130117_1 366 (6.883)
1: Scan ES-
2.05e5
100
309.34
355.26
356.24
391.37
394.31
415.66
665.44
748.30
831.34
1069.10
1104.45
1105.24
162.30
195.90
51.11
581.33
882.81
1038.84
1192.32
1270.39
1456.30
0
m/z
200
400
600
800
1000
1200
1400

SAMPLE CODE: HA



#	RT	Scan	Height	Area	Area %	Norm %
1	22.991	4037	40,653,212	18,482,434.0	1.983	2.02
2	25.067	4452	1,080,594,048	913,784,768.0	98.017	100.00


, 15-Mar-2017 + 05:16:12
HA-(TTES-0252) 4452 (25.067)
Scan EI+
2.36e8
100
%
0
216.0278
103.0623
147.1197
76.0416
121.0568
59.9631
77.0198
57.9347
84.0052
51.0779
217.1409
85.0528
145.8659
174.1371
218.1148
214.8451
219.1583
271.0468
281.1320
m/z
42
62
82
102
122
142
162
182
202
222
242
262
282

BIOLOGICAL SCREENING:-

The Synthesized compounds were screened for their in-vitro anti mycobacterial activity by means of Alamar blue assay. The compounds were tested in the concentration range of 100 to 0.8µg/ml against M.tuberculosis H37RV Strain grown in Middle brook 7H9 broth in 96 well titre plates. Pyrazinamide-3.125µg/ml and Streptomycin-6.25µg/ml were used as standards for comparison. A blue color in the well was interpreted as no bacterial growth so it is termed as sensitive, and pink color was scored as growth and is referred as resistant. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink

TABLE NO: 20 DOCKING SCORE WITH MIC VALUE

COMPOUND	DOCKING SCORE(kcal/mol)	MIC VALUE Mcg/ml
SDK3	--12.73	3.12
SDK5	-11.32	3.12
SDK2	-10.82	6.25
SDK1	-10.82	6.25
HA	-9.146	12.5
PAA	-8.537	12.5

MABA REPORT OF THE SYNTHESISED COMPOUNDS:

All the synthesized compounds showed anti-mycobacterial activity in a varying degree against the organism tested. The organism tested was susceptible to all the synthesized compounds and the minimum inhibitory concentration for the compounds varied between 6.25 and 3.125 mcg/ml. The data pertaining to these observations are presented in the table. Inhibition was compared using as Standard. Pyrazinamide- 3.125 mcg/ml Streptomycin- 6.25 mcg/ml Ciprofloxacin- 3.125mcg/ml

TABLE NO:-21

SAMPLE CODE	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
SDK1	S	S	S	S	**S**	R	R	R
SDK2	S	S	S	S	**S**	R	R	R
SDK3	S	S	S	S	S	**S**	R	R
SDK5	S	S	S	S	S	**S**	R	R
HA	S	S	S	**S**	R	R	R	R
PAA	S	S	S	**S**	R	R	R	R

NOTE:

S- Sensitive

R- Resistant

Strain used: - M. tuberculosis [H37RV]: ATCC NO-27294.

Among the synthesized compounds, compound- **SDK1** gave docking Score **of -10.82** and exhibit the activity at **6.25** mcg/ml and compound- **SDK2 gave** docking Score of **-10.87** and exhibit the activity at **6.25** mcg/ml **and SDK3** gave docking Score of -**12.73** and exhibit the activity at **3.12mcg/ml** and also compound SDK5 gave docking Score of **-11.32** and exhibit the activity at **3.12mcg/ml.**

Fig SYNTHESIZED COMPOUNDS PHOTOGRAPH

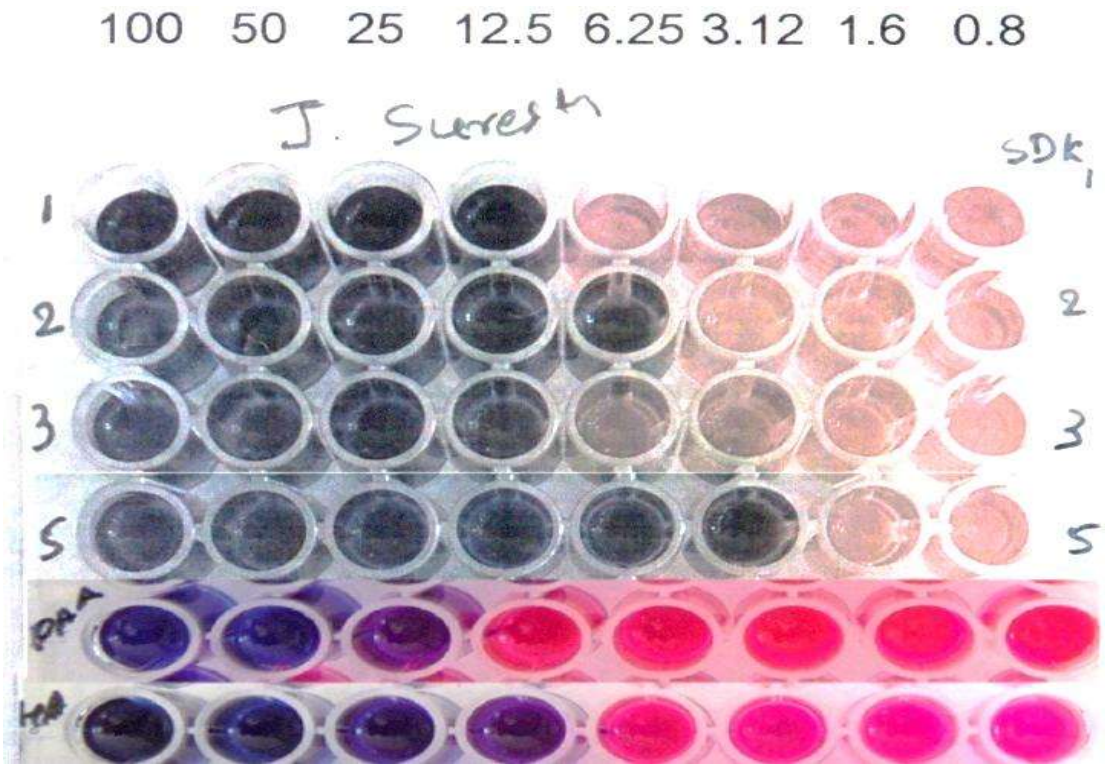
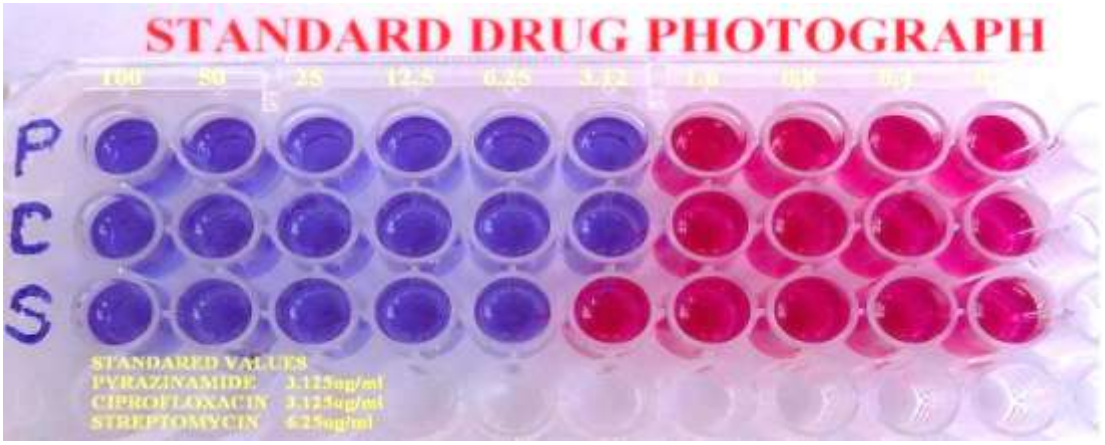


Fig STANDARD DRUG PHOTOGRAPH



COMPARATIVE STUDY OF DOCKING SCORE FOR DIFFERENT TARGET:-

Nearly 100 molecules were sketched using chem sketch, and the molecules were docked against the MTB enzyme using Argus Lab 4.0.1 software. The following molecules were docked against different targets and the molecules with best docking score and good interaction were selected and synthesised.

The molecules were also docked against the following five targets

1. Glutamine Synthetase I
2. Methoxy Mycolic acid Synthase II
3. Cyclopropane Mycolic Acid Synthase II
4. L, D-Transpeptidase
5. Decaprenyl Phosphoryl-b-d-Ribose2'-Epimerase I (DprEI)

TABLE NO: 22 Argus Lab Software (4.0.1)

S.NO	NAME OF THE ENZYMES	DOCKING SCORE					
		SDK1	SDK2	SDK3	SDK5	PAA	HA
1.	DecaprenylPhosphory Beta-D-ribose-2 Epimerase	-10.43	-10.83	-11.69	-11.34	-8.57	-9.54
2.	Cyclopropane Mycolic acid Synthase-II	-10.32	-10.23	-11.45	-11.34	-8.65	-8.32
3.	Methoxy Mycolic acid Synthase-II	-10.87	-10.56	-11.93	-10.54	-9.43	-8.34
4.	Glutamine Synthetase-I	-10.78	-10.76	-11.87	-11.54	-9.54	-8.54
5.	L&D Transpeptidase	-10.56	-10.34	-11.54	-11.75	-9.87	-8.75

It is concluded that Decaprenylphosporyl-beta-D ribose-2 epimerase has best Docking score among different targets. So the fine tuning the structures of these compounds will yield molecules with better anti mycobacterial activity. Against the Pathogen.

DISCUSSION

i) The following compounds have best docking score against specific targets and were synthesized in an appropriate manner . The purity of the compounds were determined, They exhibited sharp melting point and single spot obtained in the TLC.

ii) Of the six compounds, five of them were obtained at 98% purity. It was confirmed by GC-MS analysis (obtaining a single peak) and molecular weight also obtained at ± 1 variation. Then the functional group determination was obtained from FT-IR. It was confirmed by obtaining specific absorption band in the spectra. .

iii) The biological evaluation of the compounds is denoted that the specific organism was sensitive at 3.12 and 3.12mcg/ml and showed better activity compared to standard drugs.

iv) The toxicity of the compounds also showed that all the 6 compounds are non-toxic.

SUMMARY

- Decaprenylphosphoryl-beta-D-ribose 2-epimerase a critical enzyme for the growth of Mycobacterium tuberculosis was chosen for our study after review of literature. It belongs to the Oxidoreductase family.
- A database of 200 molecules with high prospects of inhibiting the target Dpre1 were carefully chosen by making changes to the known hit molecules, here the thiadiazole nucleus was chosen.
- Selected molecules were designed and docked against Dpre1 using Argus lab® software.
- Six molecules with good docking score [lower binding energy] and interactions were shortlisted for synthesis. Reaction conditions were optimized.
- The selected molecules were subjected to toxicity prediction assessment by OSIRIS® property explorer developed by Acetilon Pharmaceuticals limited which is available online. The results are color coded as green color which predicts the drug likeness and possibly better activity.
- The molecules were labelled as SDK1, SDK2, SDK3, SDK5, PAA, HA, and were synthesized with satisfactory yield.
- The purity of the synthesized compounds was ensured by repeated recrystallization. Further the compounds were evaluated by TLC and Melting point determination.
- The characterization of the synthesized compounds was done using Infra-red, Nuclear Magnetic Resonance [H1 NMR] and Mass spectrometric methods [LC-MS, GC-MS].
- All the Synthesized compounds exhibited molecular ion peak (M^+) of varying intensities.
- The final pure compounds were screened for Anti-mycobacterial activity by in vitro method called Micro plate Alamar Blue Assay [MABA].
- The synthesized compounds showed sensitivity [Minimum inhibitory concentration] at 3.12mcg/ml. The standard drugs Pyrazinamide, Streptomycin, Ciprofloxacin exhibited anti mycobacterial activity at 3.125mcg/ml, 6.25mcg/ml, and 3.125mcg/ml concentrations respectively. This indicates that the synthesized compounds are as Potent as the standard drugs.

CONCLUSION

All the compounds gave Docking score between -8.73 to 11.37 kcal/mol Pyrazinamide gave docking score 11.55kcal/mol for 4P8Y, Streptomycin gave docking score of 10.87kcal/mol for 4P8Y and Ciprofloxacin gave docking score of 11.25kcal/mol for 4P8Y. There is a correlation between the score and activities of all the compounds which were tested and compared with the standard drugs. This goes to prove that Decaprenyl phosphoryl beta-D-ribose 2' epimerase-1' (PDBID: 4P8Y) is a critical enzyme for anti-mycobacterial activity. So the fine tuning the structures of these compounds will yield molecules with better anti mycobacterial activity. Further structural modifications of the synthesized compounds will aid in the development of potential molecules against the tuberculosis pathogen.

FUTURE SCOPE OF THE STUDY:-

The Synthesized compounds should have significant anti-tubercular activity in MABA assay method. Hence the anti-tubercular study would deserve for further investigations of *in-vivo* toxicity and *in-vivo* anti-tubercular studies.

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