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(RESEARCH ARTICLE)

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Review for QSAR studies and drug design of selected heterocyclic nucleus of antitubercular drugs

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Abstract

This review article explores the pressing issue of drug-resistant strains of Mycobacterium tuberculosis, which have emerged due to the widespread and uncontrolled use of antibiotics in clinical settings over several decades. In response to this challenge, various methods have been developed for synthesizing new antitubercular compounds. Among these, the fragment-based drug discovery (FBDD) approach has shown promise as an effective strategy.

One class of compounds that has exhibited significant potential in combating tuberculosis is 1,2-diazoles. The article discusses the importance of these compounds and their potential as future antitubercular drugs. Additionally, it delves into the various strategies employed in drug development, emphasizing the relevance and efficacy of FBDD.

Analytical methods play a crucial role in characterizing antitubercular antibiotics, and the article highlights liquid chromatography and voltammetry as preferred techniques for determining these compounds. The redox (oxidation/reduction) properties of antituberculars make them amenable to analysis using electrochemical methods, with voltammetry being particularly suitable.

Furthermore, the article underscores the significance of utilizing voltammetry for quantifying different categories of antibiotics in both dosage forms and human body fluids. The affordability of this method makes it particularly advantageous for developing countries, providing a cost-effective approach to monitor and assess the presence of antitubercular drugs. Overall, the review article offers valuable insights into the current strategies for addressing drug resistance in tuberculosis and highlights the potential of 1,2-diazoles as future antitubercular agents.

Keywords: QSAR; Antitubercular; Mycobacterium tuberculosis; Fragment-based drug discovery; Chronic infection

1. Introduction

Tuberculosis is a chronic infection characterized by caseous necrosis and granuloma formation. It is caused by the bacteria called ***Mycobacterium tuberculosis***. Most cases of tuberculosis involve the lungs, where the disease produces hemoptysis, or bloody sputum, along with the typical cough[1]. However, it can also affect other sites of the body known as Extrapulmonary TB. In 1882, Dr. Robert Koch discovered the organism responsible ie., Bacillus *Mycobacterium tuberculosis*. This widely-known disease is one of the diseases of stigmatization and affects one-third of the world population[2]. World Health Organization (WHO) reports that a relatively small proportion (5–10%) of the estimated 1.7 billion people infected with *M. tuberculosis* will develop TB disease during their lifetime. However, the probability of

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developing TB disease is much higher among people infected with HIV; it is also higher among people affected by risk factors such as malnutrition, diabetes, smoking, and alcohol consumption.

Figure 1 Mycobacterium tuberculosis[3]

1.1. Mycobacterium genus[4]:

- Kingdom:Bacteria
- Genus: Mycobacterium
- Phylum:Actinobacteria
- Order: Actinomycetales
- Suborder: Corynebacterineae
- Family: Mycobacteriaceae

Mycobacterium tuberculosis is a small, aerobic, nonmotile, rod-shaped bacillus, 2-4 μm in length and 0.2-0.5μm in width. The human host serves as the only natural reservoir for this bacterium, but it can be cultured in the laboratory. It divides every 16 to 20 hours, which is an extremely slow rate compared with other bacteria[5]. The cell wall of this bacterium is composed of complex lipids, such as mycolic acids that make up over half the cell envelope of the mycobacteria. Their hydrophobicity prevents the diffusion of many chemicals including drugs into the bacterium. Mycolic acids also play a major role in virulence of *M. tuberculosis*, by protecting the organism from complement fixation and damage from lysozymes and free radicals in the phagolysosomes of neutrophils. The cell wall also contains acyl glycolipids and other substances such as free lipids and sulfolipids. The high concentration of lipids in the cell wall of this bacterium has been associated with unique clinical characteristics such as impermeability to stains and dyes, resistance to many antibiotics, resistance to killing by acidic and alkaline compounds, resistance to osmotic lysis via complement deposition, resistance to lethal oxidations and survival inside macrophages.

1.2. Clinical classification of mycobacteria

According to their growth rate, the *Mycobacterium* genus is usually separated into two major groups:

- Slow-growing species including *M. tuberculosis*, *M. bovis,* and *M. leprae*.
- Fast-growing species such as *M. smegmatis.*[6]

Among the pathogenic species, the most relevant for human health are *M. tuberculosis* and *M. leprae*, are the causative agents of two of the world's oldest diseases, tuberculosis and leprosy, respectively. *M. canettii* and *M. africanum*, which

also can cause human TB, are most commonly isolated from African patients. *M. bovis* demonstrates the broadest spectrum of host infection, affecting humans, domestic or wild bovines, and goats. *M. microtia* can also cause disease in immunocompromised human patients. *M. kansasii, M. malmoense,* and *M. xenopi* represent pulmonary opportunists, while *M. marinum* is the skin pathogen infecting organisms by entering through damaged skin.

1.3. Sites of TB Disease:

1.3.1. Pulmonary Tuberculosis:

TB disease most commonly affects the lungs; this is referred to as Pulmonary TB. Patients with Pulmonary TB usually have a cough and an abnormal chest radiograph and may be infectious. Although the majority of TB cases are Pulmonary, TB can occur in almost any anatomical site.

1.3.2. Extrapulmonary Tuberculosis[7]*:*

Extrapulmonary TB isease occurs in places other than the lungs, including the larynx, the lymph nodes, the pleura, the brain, the kidneys, or the bones and joints. In HIV-infected persons, Extrapulmonary TB disease is often accompanied by Pulmonary TB.

1.3.3. Miliary Tuberculosis[8]*:*

Miliary TB occurs when tubercle bacilli enter the bloodstream and disseminate to all parts of the body, where they grow and cause disease in multiple sites. This conditionis rare but serious. “Military” refers to the radiograph appearance of millet seeds scattered throughout the lungs. It is most common in infants and children younger than 5 years of age and severely immune-compromised persons.

1.4. Pathogenisis[9,10]:

Infection occurs when a person inhales droplet nuclei containing tubercle bacilli that reach terminal alveoli in the lungs. The first step is the recognition of mycobacteria as invading pathogens, followed by activation of innate host defense responses. Cell-mediated immunity is usually developed within approximately two to eight weeks from the initial infection, followed by the initiation of adaptive immune responses. The activatedT lymphocytes, macrophages, and other immune cells form granulomas that limit further replication and spread of the tubercle bacilli.

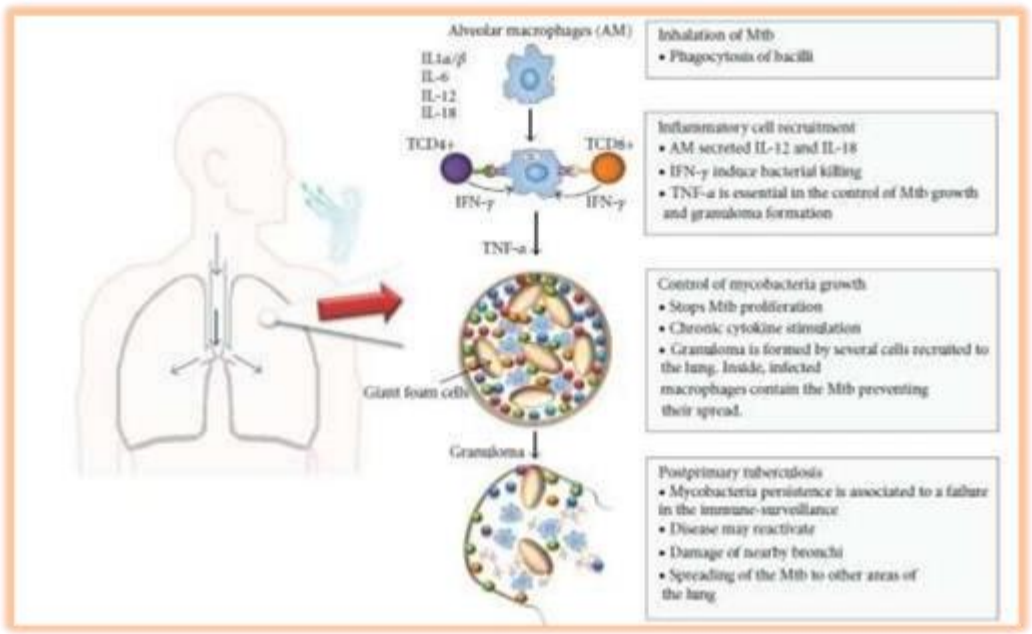


Figure 2 Pathogenesis of Mycobacterium tuberculosis[11]

1.4.1. Latent tuberculosis[12]

Latent tuberculosis (LTB), also called latent tuberculosis infection (LTBI) Latent TB infection occurs when *Mycobacterium tuberculosis* runs away from the immune system. Latent Tuberculosis infection is generally not contagious and produces no symptoms, but the bacilli may be present in the body. Individuals who have a latent infection cannot transmit infection to others. In this infection, most of the individuals do not develop active disease, but the problem occurs when latent infection becomes active. Approximately 10% of the individuals develop active Tuberculosis disease. Some of them develop active disease soon after the infection, whereas, other people develop later when their immune system becomes weak for one or the other reason. The global prevalence of LTBI broadly reflects the local prevalence of TB. Those with documented contact with a case of transmissible TB are at the highest risk of infection (up to 50% chance). In low-incidence countries, the prevalence of infection in the general population is approximately 1% among young adults but can increase to 8% in elderly adults, 5and up to 25% in adult migrants from high-incidence countries.

1.4.2. Characteristics of Latent Tuberculosis[13]*:*

- People with latent tuberculosis:
- Have no symptoms of tuberculosis
- Don't feel sick
- Can't spread tuberculosis to others
- Usually have a positive tuberculosis skin test reaction (PPD test)

In some cases, can develop active tuberculosis if they do not receive treatment forlatent tuberculosis.

1.5. Progression of TBYES

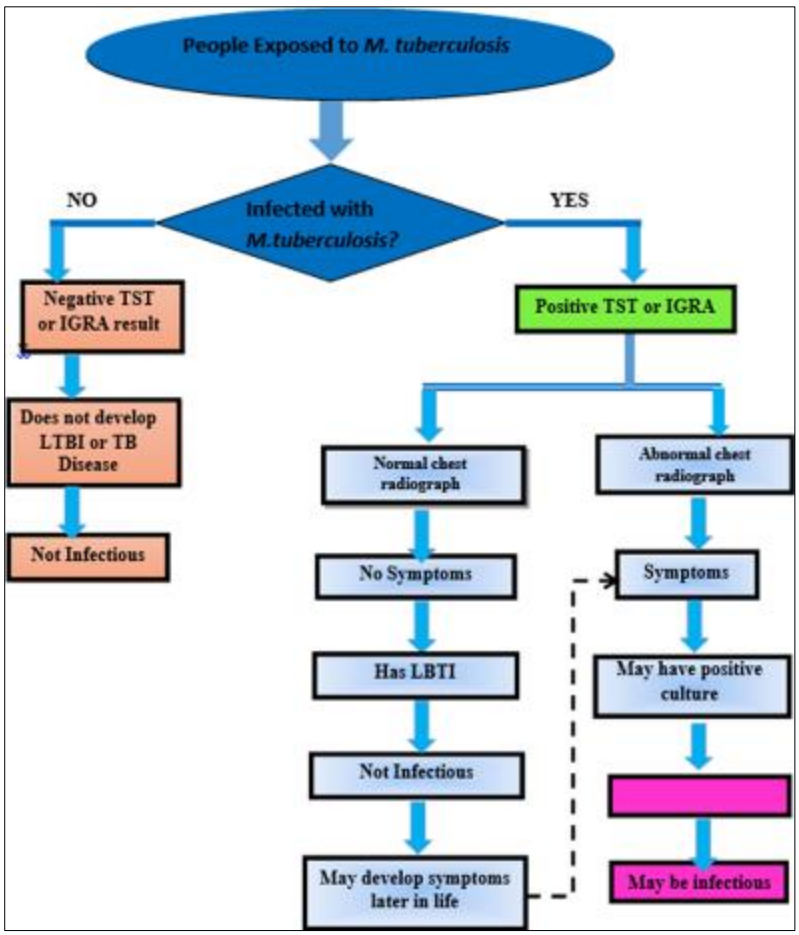


Figure 3 Progression of TB[14]

1.6. Symptoms[15]:

- Coughing with blood sputum
- Loss of appetite
- Weight loss
- Night sweats
- Fever
- Chest pain
- Urine discoloration

1.7. Diagnosis[16]:

1.7.1. Bacteriological test:

- **Ziehl-Neelsen stain,** also known as the **Acid-fast stain.** It is the first step in the diagnosis and screening of pulmonary tuberculosis.
- **Auramine-rhodamine stain** is a histological technique used to visualize acid-fast bacilli using fluorescence microscopy. Acid-fast organisms display a reddish-yellow fluorescence.
- Sputum culture test:
- Lowenstein-Jensen solid medium 4 to 18 weeks
- Liquid medium 8 to 14 days
- Agar medium 7 to 14 days

1.7.2. Tuberculin skin test:

The **Mantoux test** is the standard method of determining whether a person is infected with *Mycobacter*ium *tuberculosis*. The local skin reaction to Tuberculin Purified Protein Derivative (PPD) injected into the skin is used to assess the individual's sensitivityto tuberculin protein.

1.7.3. TB disease burden[17]:

Worldwide, tuberculosis (TB) is one of the top 10 causes of death, and the leadingcause from a single infectious agent (above HIV/AIDS); millions of people continue to fall sick with the disease each year.

In 2017, TB caused an estimated 1.3 million deaths among HIV-negative people, and there were an additional 300,000 deaths from TB among HIV-positivepeople.

TB affects all countries and all age groups, but overall the best estimates for 2017 were that 90% of cases were adults (aged ≥15 years), 64% were male, 9% were people living with HIV (72% of them in Africa)

And two-thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%), and South

Africa (3%).

1.7.4. DRUG-RESISTANT TB (MDR and XDR)[18,19]:

Drug-resistant TB is caused by *M. tuberculosis* organisms that are resistant to the drugs normally used to treat the disease.

Drug-resistant TB is transmitted in the same way as drug-susceptible TB and is no more infectious than drug-susceptible TB. However, delay in the recognitionof drug resistance or prolonged periods of infectiousness may facilitate increased transmission and further development of drug resistance.

Multidrug-resistant TB (MDR TB) is caused by organisms resistant to the most effective anti-TB drugs, Isoniazid and Rifampin. These drugs are considered first-line drugs and are used to treat most persons with TB disease.

Extensively drug-resistant TB (XDR TB) is a relatively rare type of drug-resistant TB. XDR TB is resistant to Isoniazid and Rifampin, plus any Fluoroquinolone andat least one of three injectable second-line drugs (i.e., Amikacin, Kanamycin,

or Capreomycin). Because XDR TB is resistant to first-line and second-line drugs, patients are left with treatment options that are more toxic, more expensive, and much less effective.

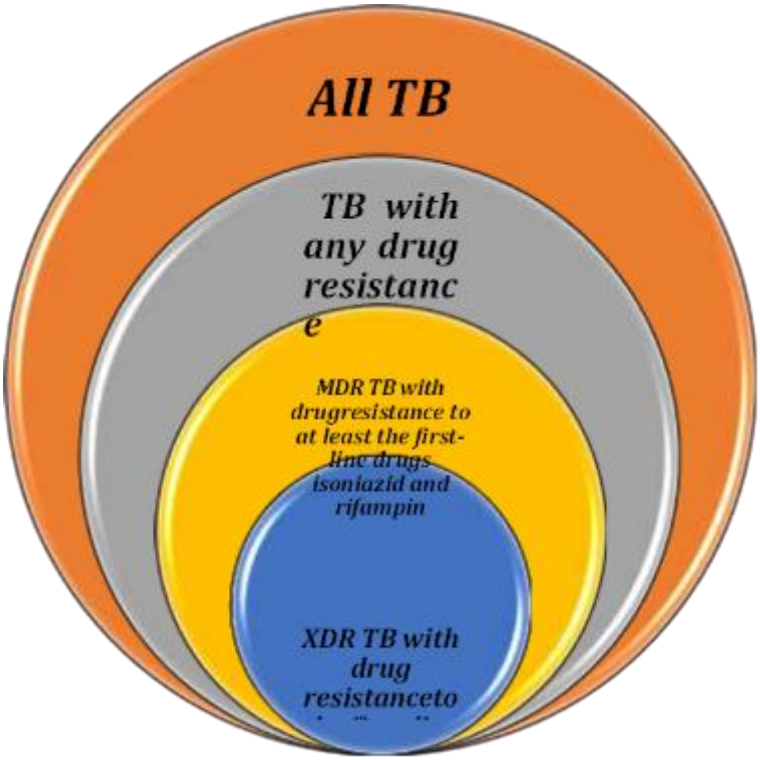


Figure 4 Drug-Resistant TB (MDR and XDR)[20]

1.8. Need for novel anti-tuberculosis agents

Several once-active Anti-tuberculosis drugs have now become inactive dueto the ever-increasing rise in drug-resistant strains of tuberculosis.

Drug-resistant TB is a major public health concern.

A growing awareness of the increasing drug resistance and a great need for therapy shortening together with killing also latent forms of *M.tb* led to the discovery of more efficient and less toxic treatment regimens.

The emergence of Multi-resistant (MDR) strains and high susceptibility of human immunodeficiency virus (HIV) infected persons to the disease forced scientists to develop novel anti-tuberculosis agents.

It is evident from these facts; that there is an ever-growing need to develop novel agents for the treatment of tuberculosis. These new agents should be potent, fast acting, have an excellent Pharmacodynamics / Pharmacokinetics profile have a high therapeutic index, and preferably have a novel mechanism of action to avoid cross-resistance with other agents.

1.8.1. Current tb research and development:

A small number of technologies emerged in 2017–2018 and several have notdemonstrated adequate performance in field evaluation studies.

There are 20 drugs in Phase I, II, or III trials for the treatment of drug-susceptibleTB, multidrug-resistant TB, or latent TB infection.

Various combination regimens with new or repurposed drugs are in Phase II orPhase III trials[21].

Twelve vaccine candidates are in clinical trials: Four in Phase I, Six in Phase II,and two in Phase III.

They include candidates to prevent the development of TB infection and disease,and candidates to help improve the outcomes of treatment for TB disease.

1.8.2. Drug discovery[22]*:*

Drug discovery is a process, which aims at identifying a compound therapeutically useful in treating and curing a disease. The process of drug discovery involves the identification of ligands, synthesis, characterization, screening, and assays for therapeutic efficacy. The phase between hit identification and lead selection is called the ***hit-to-lead phase***. The dominant and the most widely applicable technique for the identification of lead compounds is ***HTS*** an experimental screening technique based on robotingwhere large numbers of different compounds are screened in a time as short as possible and at reasonable costs.

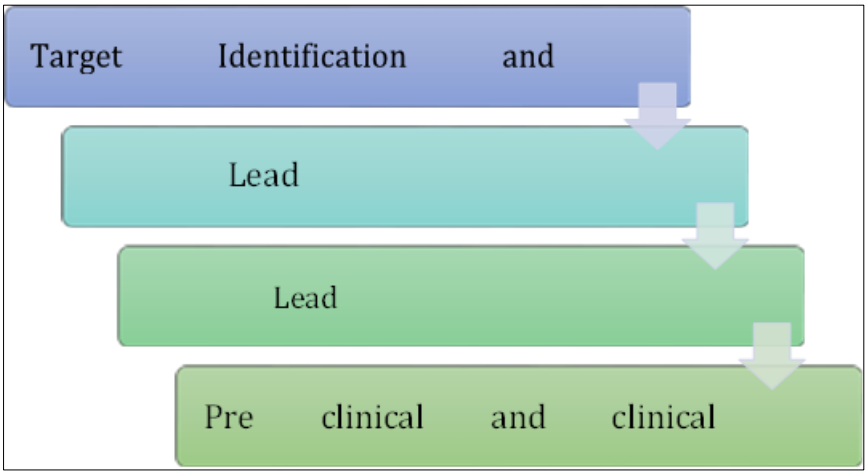


Figure 5 Drug discovery[23]

2. Computer-aided drug design in the drug discovery pipeline[24]:

CADD is capable of increasing the hit rate of novel drug compounds because it uses a much more targeted search than traditional HTS and combinatorial chemistry. It not only aims to explain the molecular basis of therapeutic activity but also to predict possible derivatives that would improve activity. CADD can be classified into twogeneral categories:

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

2.1. Structure-based drug design[25]:

It relies on the knowledge of the target protein structure to calculate interaction energies for all compounds tested. The core hypothesis of this approach is that a molecule's ability to interact with a specific protein and exert a desired biological effect depends on its ability to favorably interact with a particular binding site on that protein.

Steps Involved in Structure-based Drug Design

- Identification of drug target
- Determination of target structure
- Identification of binding site
- Computational drug design methods
- Evaluation of potential lead candidate

2.2. Ligand-based drug design

It exploits the knowledge of known active and inactive molecules through chemical similarity searches or the construction of predictive Quantitative Structure-Activity Relation (QSAR) models. The overall goal is to represent these compounds in such a way that the physicochemical properties most important for their desired interactions are retained, whereas extraneous information not relevant to the interactions is discarded.

2.2.1. Molecular docking[26]:

Molecular Docking is the technique which envisages the "preferred orientation of one molecule to a second when bound to each other to form a stable complex in three dimensional spaces". The success of a docking program depends on two components: the **Search algorithm** and the **Scoring function.**

2.2.2. *search algorithm*

The search algorithm finds different conformations for the ligand. Systemic searches explore all possible binding modes between the ligands and receptors. However, this takesa huge amount of computational time, especially for large flexible ligands. The amount of conformational space explored and the computational time required for the search mustbe balanced.

2.2.3. Scoring functions:

Scoring aims to quantify the free energy associated with protein and ligand in the formation of the protein-ligand interactions. They are used to rank the different conformations obtained by the search algorithm. The score in the empirical scoring function is derived from the individual energy contributions of each component involved in intermolecular interactions.

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

Where:

ΔG_0– empirically derived offset that in part corresponds to the overall loss oftranslational and rotational entropy of the ligand upon binding.

ΔG_{hb}– contribution from hydrogen bonding. ΔG_{ionic} – contribution from ionicinteractions. ΔG_{lip} – contribution from lipophilic interactions.

$|A_{lipo}|$ is the surface area of lipophilic contact between the ligand and receptor.

2.2.4. Biological target[27]

Various biosynthetic enzymes are essential for the survival of the Mycobacterium and are considered potential drug targets. Some of the target enzymes are,

- *Mycobacterium tuberculosis InhA*, the enoyl acyl carrier protein reductase
- *Mycobacterium tuberculosis* Glutamine synthetase 1
- *Mycobacterium tuberculosis* Thymidylate Kinase
- *Mycobacterium tuberculosis* Protein Kinase G
- *Mycobacterium tuberculosis* Gyrase Type IIA Topoisomerase
- *Mycobacterium tuberculosis* L, D Transpeptidase 2.

3. Literature review

3.1. Review of study about Tuberculosis:

Manishakotadiya *et al.,* (2018)[37], reported that the Advances in TB drug development over the past decade are leading to the development of enhanced MDR-TB treatments with simple and short regimens. Additionally, efforts mustbe made to reduce the development of resistance to these valuable new TB drugs during treatment

Cheng-Yu Kuo *et al.,*(2017)[38], reported that almost no resistance to the tested second- line Antituberculosis drugs among non-MDR-Mtbs. Anti-tuberculosis regimen with Pyrazinamide, Ethambutol, Fluoroquinolone, Kanamycin, Cycloserine, and p-Aminosalicylic acid can be empirically used for newly diagnosed MDR-TB cases.

Cucunawangsih *et al.,* (2015)[39], confirmed that drug resistance, including MDR, observed against all first-line TB drugs was a real threat in the management of TB infection in Indonesia. The resistance pattern identified in this study could assist clinicians in providing appropriate treatment regimen to TB patients and improve their clinical outcome.

Padmanesan Narasimhan *et al.,* (2013)[40], summarized that the risk ofprogression from exposure to the tuberculosis bacilli to the development of active disease. Exogenous factors play a key role in accentuating the progression from exposure to infection among which the bacillary load in the sputum and the proximity of an individual to an infectious TB case are key factors. Similarly endogenous factors lead in progression from infection to active TB disease.

Marcos Abdo Arbex*et al.,* (2010)[41], described the mechanisms by which the interactions among the antituberculosis drugs used in the basic regimen can cause drug induced hepatitis, and discussed about the alternatives in this situation.

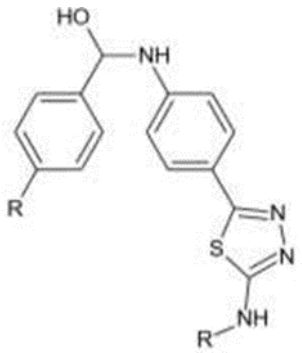
3.2. Review of study about Enoyl acyl carrier protein reductase (*InhA*) enzyme:

Denise A. Rozwarski *et al.,* (1999)[42], reported the Crystal Structure of the Mycobacterium tuberculosis Enoyl-ACP Reductase, *InhA*, in Complex with NAD^+ and a C16 Fatty Acyl Substrate.

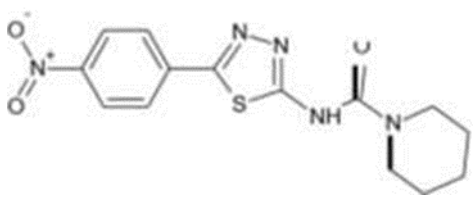
Helmut Berglert*et al.,* (1994)[43], reported that the EnvM protein was purifiedfrom an overproducing *Escherichia coli* strain. It showed NADH-dependent enoyl-acyl carrier protein (ACP) reductase activity using both crotonyl-ACP and crotonyl-CoA as substrates. It wasconcluded that EnvM is the NADH-dependent enoyl-ACP reductase of *E. coli* and we propose to rename the corresponding gene fabI.

Review of study about 1, 3, 4-Thiadiazole derivatives:

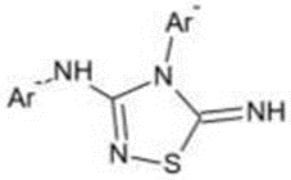
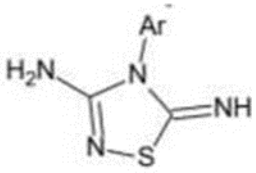
Sevgi et al., (2010)[44] were synthesized 5-[-4-(4fluorobezoylolamino) phenyl]-2- subsitutedamino-1,3,4-thiadiazole 27 and evaluate the cytotoxic activity.



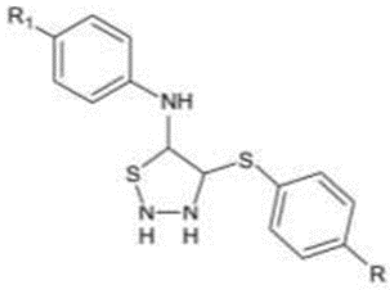
Pattan et al., (2009)[45] have been introduced the synthesis of various thiadiazolederivatives compounds and evaluated for anti diabetic activity.



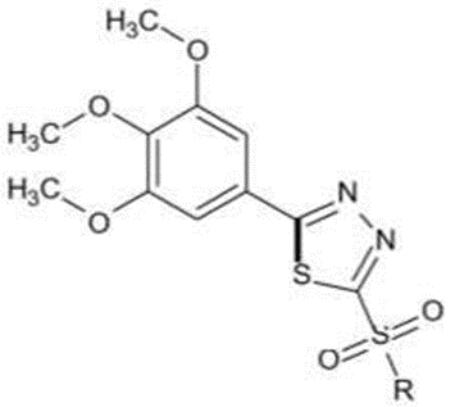
Gupta *et al.,* (2008)[46] synthesized a series of 3-aryl amino/amino-4-aryl-5-imino- D2- 1,2,4-thiadiazoline . The Anticonvulsant activity of all the synthesized compounds was evaluated against maximal electroshock induced seizures (MES) and subcutaneous Pentylenetetrazole (ScPTZ) induced seizure models in mice.

Sharma *et al.,* (2008)[47] synthesized a new series of selective COX-2 inhibitors with 2-amino-5-sulfanyl-1,3,4-thiadiazole. These compound were selective inhibitiors of COX-2 and potentiated the activity of COX-1 enzyme. The presence of Sulphonamide group is a required pharmacophore for selective inhibition of COX-2 enzyme.

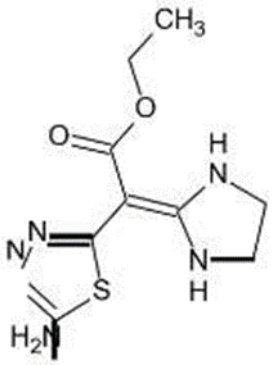


Jun-Chen *et al.,* (2007)[48] introduced a series of 5-(3,4,5-trimethoxyphenyl)-2- sulfonyl-1,3,4-thiadiazole derivatives which possess higher Antifungal activities against three kind of fungi *Gibberellazeae, Botrytis cinerea,* and *Sclerotiniasclerotiorum*.



Swamy *et al.*, (2006)[49] synthesized a series of 4,6-disubstituted 1,2,4-triazolo- 1,3,4- thiadiazole derivatives tested for *in-vitro* Antimicrobial activity against*Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens*etc. and found themto be active with these compounds having maximum activity. The presence of chloro substituent enhances the activity of the compound.

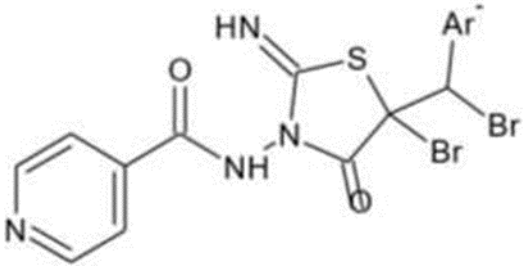
Shafiee et al., (2005)[50] A series of 2-(5-nitro-2-furyl) and 2-(5-nitro-2thienyl)-5- substituted-1,3,4-thiadiazole derivatives were synthesized. The most activecompound was found to be active with an IC_{50} 0.1 μM against Leishmania major promastigotes.



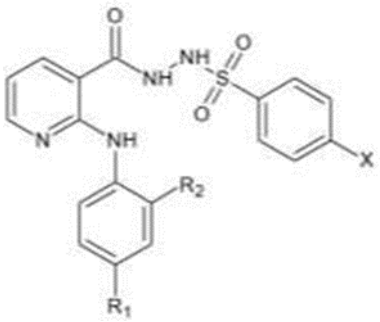
Review of study about Pyridine derivatives:

Pramod *et al.,* (2018)[51], synthesized a novel derivatives of pyridine containing azetidinone derivatives in simple two-step facile procedure.

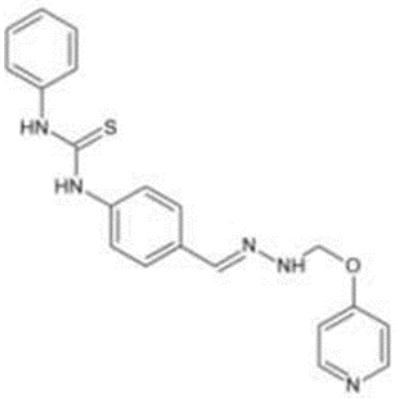
Mishra *et al.,* (2007)[52] have synthesized novel 2-Imino-3-(4'-carboxamido pyridyl)-5-arylidine-4-thiazolidinones as antimicrobial agents.



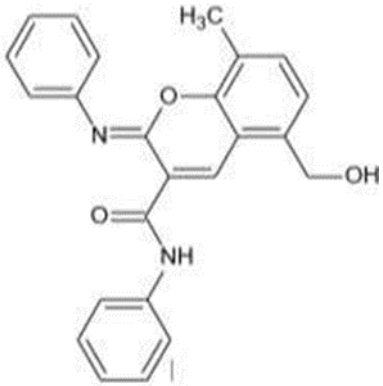
Kamal *et al.,* (2007)[53] synthesized novel 2-Anilino substituted nicotinyl arylsulfonylhydrazides have synthesized and reported as potential anticancer andantibacterial agents.



Sriram *et al.,* (2006)[54] have synthesized some 1-[(4-substituedphenyl]-3-(4-{1- [(pyridine-4-carbonyl)hydrazono]ethyl}phenyl)thiourea and studied their *in- vitro*antitubercular activity.



Zhuravel *et al.,* (2005)[55], synthesized 5-hydroxymethyl-8-methyl-2-(-arylimino)- pyrano[2,3-c]pyridine-3-(-aryl)-carboxamides and studied their antimicrobial activity.

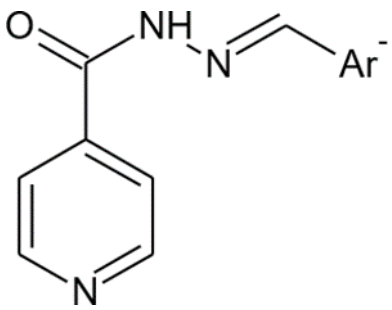


Reviews of study about the Schiff base derivatives:

Joginder Kumar *et al.,* (2017)[56] summarized that Schiff-base exhibited versatile pharmacological activity, had shown potent antitumor effects against variety of human cell lines and *in-vivo* animal models. Structure activity relationship of Schiff-base containing derivatives indicated that Schiff-base moiety essential for the pharmacologicalactivity. These results revealed that Schiff-base is an essential pharmacophore for the anticonvulsant activity.

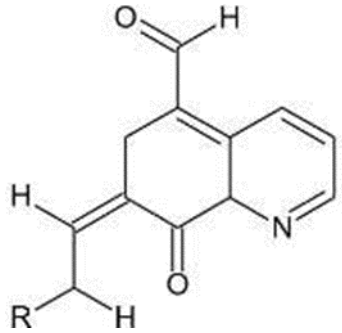
AnuKajal *et al.,* (2013)[57], attempt to reviewed all the biological activities reported for Schiff bases in the current literature with an update of recent research findings. This review reflects the contribution of Schiff bases to the design and development of novel lead having potential biological activities with fewer side effects.

B. Thomas *et al.,* (2011)[58] synthesized Schiff bases of isonicotinoyl hydrazone, N'-[(1Z)-(substituted aromatic) methylidene] pyridine-4-carbohydrazides. Synthesized compounds were evaluated for *in vivo* antidepressant and nootropic activities. The results revealed that the test compounds substituted with nitro, halogen, and dimethoxy groups exhibited significant antidepressant and nootropic activities. N'-[(1Z)- (2,5dimethoxyphenyl) methylidene]pyridine-4- carbohydrazide was found to exhibit the highest antidepressant activity.



K. S. Kumar *et al.,* (2010)[59] synthesized a series of 3-(benzylideneamino)-2 phenyl quinazoline-4(3H)-one, and evaluated for their cytotoxicity and antiviral activity. Compounds having 2-hydroxy substitution showed better antiviral activity.

K. V. Sashidhara *et al.,* (2009)[60] synthesized a series of novel keto-enamine Schiff bases, derived from 8-hydroxyquinoline was synthesized and subjected for *in-vitro* antioxidant, *in-vivo* antidyslipidemic, and postheparinlipolytic activity.



Reviews of study about the biological evaluation of Anti tubercularactivity by MABA:

Sephra N.Ramprasad *et al.,* (2012) studied the various applications of Alamar blue as an indicator. The Alamar blue bioassay being utilized to access cellviability and cytotoxicity in a biological and environmental system and in anumber of cell types including bacteria, yeast, fungi, and protozoa.

Tannaz Birdi *et al.,* (2012)[61] Assessed anti-M. tuberculosis activity of five Indianmedicinal plants such as Acetone, ethanol and aqueous extracts (prepared sequentially) of Acoruscalamus L. (rhizome), Andrographis paniculate. (leaf), Ocimum sanctum L. (leaf), Piper nigrum L. (seed) and Pueraria tuberosa DC. (tuber) were tested at 1, 10 and 100 µg/ml using the Microplate Alamar Blue Assay. which have been reported in traditional literature for various uses including respiratory ailments.

Melby Mendoza-Aguilar *et al.,* (2012)[62] concluded that the MABA appears to bea useful model for the selection of drugs that are effective for the treatment of

murine leprosy. To further validate our results, a broader study involving the use of novel anti- mycobacterial agents and other agents, including particular anti- tuberculosis drugs, should be performed.

Jose d Jesus Alba-Romero *et al.,* (2011)[63] applied the Alamar blue assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. It is a reliable method todetermining the drug susceptibility to pharmaceuticals.

Collins L A *et al.,* (1997)[64] reported the high-throughput screening of compounds against Mycobacterium tuberculosis and Mycobacterium avium using Microplate AlamarBlue Assay (MABA) and compared with BACTEC 460 Assay System.

3.3. Review of study about Spectroscopy:

Devi Thamizhanban *et al.,* (2016)[65] reviewed the highlights of a variety of analytical hyphenated analytical techniques and the role of the instrumentation, analytical methods in assessing the quality of the drugs

Patel Jayvadan *et al.,* (2010)[66] reviewed the techniques from the coupling of a separation technique and an on-line spectroscopic detection technology.

3.4. Review of study about Drug Design:

Surabhi *et al.,* (2018**)**[67] overviewed about the Computer aided drug design and development of a new drug.

Ram BabuTripathi *et al.,* (2016)[68] reviewed about the *In-silico* expectations of pharmaceutical industry to design of new drug molecules.

Ntie Kang F *et al.,* (2015)[69] reported an *in silico* evaluation of the ADMET profile of the Streptome DB database. An assessment of the "drug-likeness" and pharmacokinetic profile of >2,400 compounds of natural origin, currently available in the recently published Streptome DB database was also reported.

Salla Virtanen *et al.,* (2013)[70] developed a novel method for virtual screeningthat employs the negative image of the binding site. They were shown that the VS results by this method are often better compared to docking and ligand-based VS.

3.4.1. Aim

- To study the docking studies drug design and docking software.
- To study the series of 1,2 diazole derivatives

3.4.2. Plan of work

- Study of docking software
- Prepare the series of 1,2-diazole derivatives

4. Docking studies drug design:[(71)]

Drug design, sometimes referred to as **rational drug design** or simply **rational design**, is the inventive process of finding new medications based on the knowledge of a biological target.[(72)] 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 complementaryin shape and charge to the biomolecular target with which they interact and therefore will bind to it.

Drug design frequently but not necessarily relies oncomputer modeling techniques.[(73)] This type of modeling is often referred to as computer- aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure - based drug design.[(39)] In addition to small molecules, biopharmaceuticals and especially therapeutic antibodies are an increasingly important class of drugs and computational methodsfor improving the affinity, selectivity, and stability of these protein-based therapeutics have also been developed.[(74)]

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

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

4.1. TYPES

There are two major types of drug design. The first is referred to as

ligand-based drug design and the second, **structure-based drug design**.[(75)]

4.1.1. Ligand-based

Ligand-based drug design (or **indirect drug design**) relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive a pharmacophore model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.[76]

4.1.2. Structure-based

Structure-based drug design (or **direct drug design**) relies on knowledge of the three dimensional structureof the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy.[77] 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.[78]

4.1.3. Binding Site Identification

Binding site identification is the first step in structure based design.([79],[80] 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 sit es that have the potential to bind ligands with high affinity is non- trivial.

4.1.4. Scoring functions

Structure-based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying the principles of molecular recognition. Selective high affinity binding to the target is generally desirable since it leads to more efficacious drugs with fewer side effects. Thus, one of the most important principles for designing or obtaining potential new ligands is to predict the binding affinity of a certain ligand to its target (and known antitargets) and use the predicted affinity as a criterion for selection.[81]

One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Bohm.[82],[83] This empirical scoring function took the form:

Where:

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

ΔG_0 – empirically derived offset that in part corresponds to the overall lossof translational and rotational entropy of the ligand upon binding.

ΔG_{hb} – contribution from hydrogen bonding

ΔG_{ionic} – contribution from ionic interactions

ΔG_{lip} – contribution from lipophilic interactions where $|A_{lipo}|$ is surface areaof lipophilic contact between the ligand and receptor

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

Figure 1 [83]

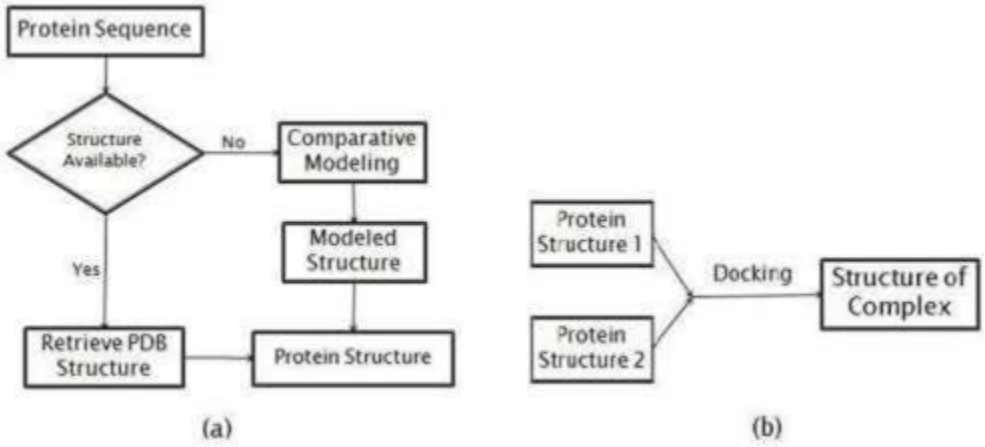


Figure 6 Protein Structure Modeling

4.1.5. Steps involved in docking [85], [86], [87]

- Docking is done by using ARGUS LAB Software
- Protein preparation.
- Selection of active site (Q-Site finder).
- Ligand Preparation.
- Docking Procedure.
- Visualization / Interpretation of Docking.

4.1.6. Protein preparation

- Step: 1
- Protein (pdb) ID is entered in the protein data bank. (1KPI)
- I clicked the download files and select pdb as text file.
- Saved the downloaded pdb (text file) to the desktop.
- Step: 2
- After I Opened Argus lab file Open Imported pdb file from thedesktop.
- 3D Structure of the protein will appeared in the workspace of Argus lab.
- Left side of the screen shows molecular tree view.
- Open pdb Open 'residues' Open 'misc'
- From 'Misc' delete the inhibitor andhetero residues [Note: Do notdelete Co-factor]
- Then I Opened water press shift, selected all water molecules and deleted.
- Added hydrogen atoms.
- Go to Calculation on the toolbar energy by UFF method start.
- Saved the prepared protein as *.agl file format in the desktop.

4.1.7. Q-SITE FINDER

- Step: 1
- Open Q-Site finder through online.
- Upload / Import the PDB format of the Protein
- Find all the active site and make a list out of the common amino acidresidues.
- Step: 2
- Open residues open Amino acids.
- Press control and select the amino acid Which were listed from the Q- Site finder.
- Make sure that all amino acid residues listed are selected.
- Right click on the mouse make a group from the selected residues give nameBinding site Ok.

4.1.8. Ligand preparation

- Draw the structure from Chem sketch and save as MDL Mol format.
- Imported the ligand into workspace of Argus lab.

- Cleaned Geometry, Cleaned Hybridisation.
- I Selected the ligand, Right click on the mouse Make a group from theresidues give name ligand Ok.
- DOCKING PROCEDURE
- Selected the set up a Dock Ligand calculation from the toolbar.
- Argus Dock as the Docking Engine.
- Dock was selected as calculation type.
- Flexible for the scoring function.
- Calculation size.
- Start docking.
- Saved the Docked protein Ligand complex as Brookhaven pdb files(*.pdb)

4.1.9. Visualization / interpretation of docking

- **Molegro Molecular viewer** will help in analysing the energies and interactionof the binding.
- View Secondary Structure view.
- View Hydrogen bond interaction.
- Ligand map Interaction overlay.

4.1.10. Toxicity prediction

All the data set molecules were subjected to the toxicity risk assessment by using Osiris program, which is available online. The OSIRIS property Explorer shown in this page is an **integral** part of Actelion's in house substance registration system. It allows drawing chemical structures and also calculates various drug relevant properties whenever a structure is valid.

Prediction results are color coded in which the red color shows high risks with undesired effects like mutagenicity or a poor intestinal absorption and green color indicates drug-conform behavior. [88]

Molecular property prediction includes

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

4.1.11. Lipinski's rule of five (89), (90)

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

The rule was formulated by Christopher A.Lipinski in 1997. The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active.

Lipinski's rule states that, in general, an orally active drug has no more thanone violation of the following criteria:

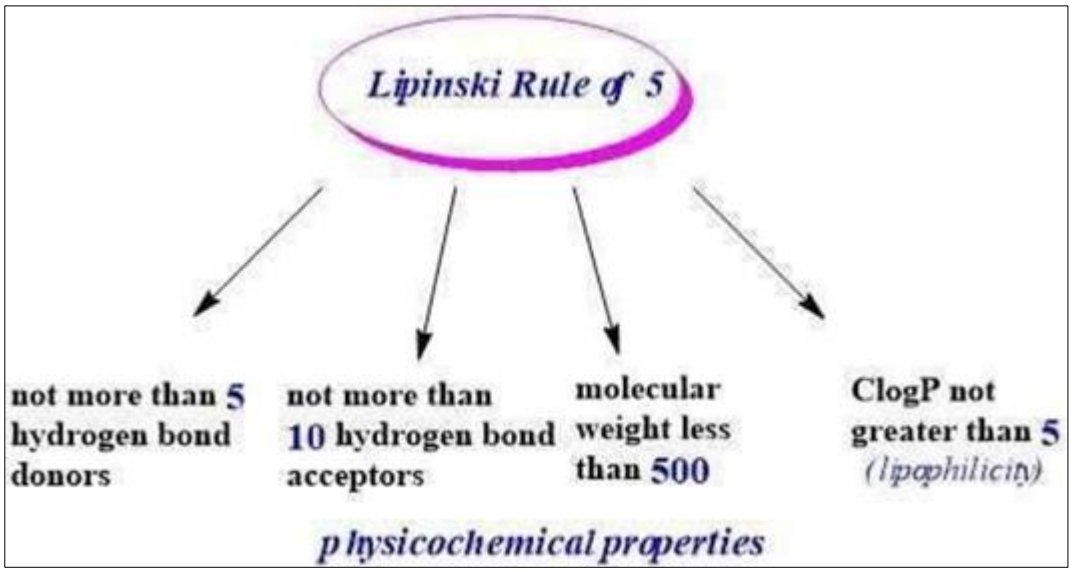


Figure 7 3D Structure of 1,2-diazole

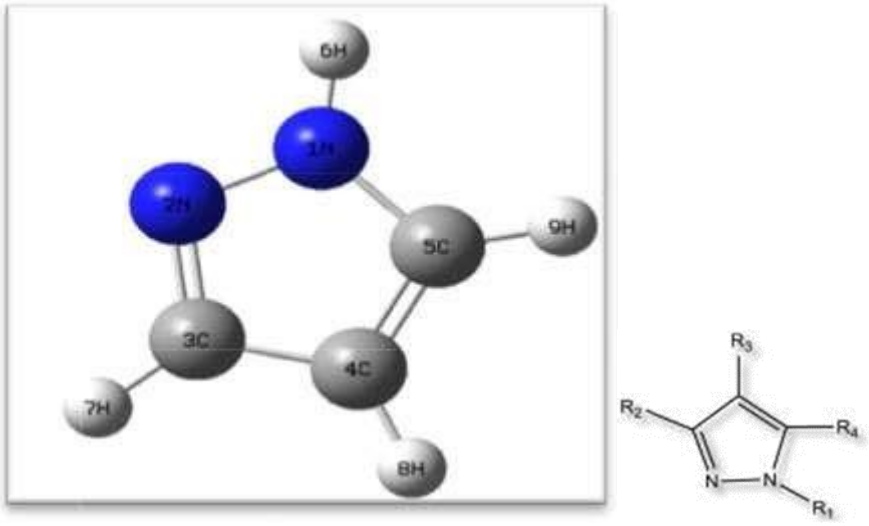


Figure 8 Structures of the 1, 2-diazole derivative series.

Table 1 Structural activity relationship for various compound

S.n	Compound position			
	R1	R2	R3	R4
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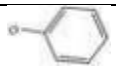
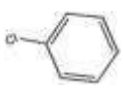
2				

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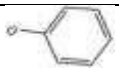
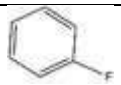
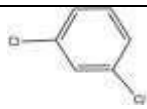
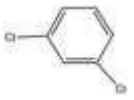
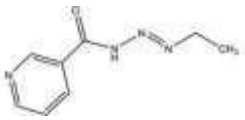
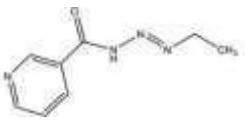
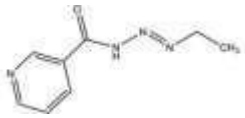
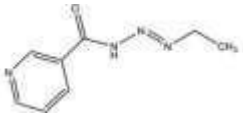
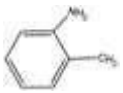
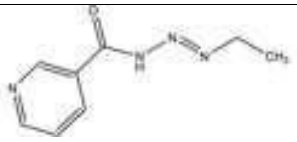
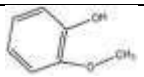
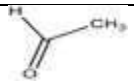
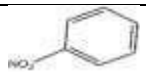
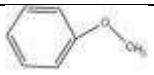
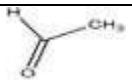
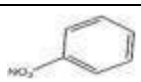
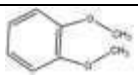
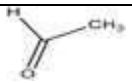
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
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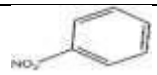
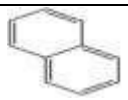
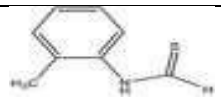
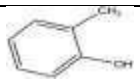
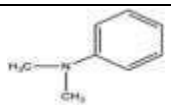
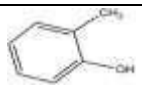
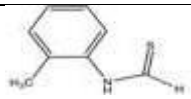
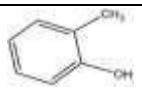
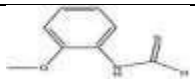
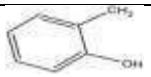
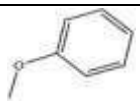
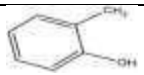
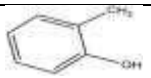
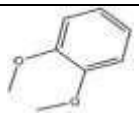
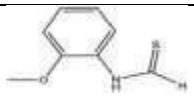
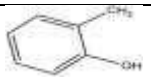
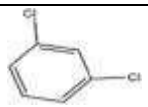
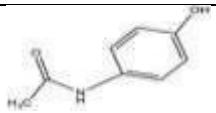
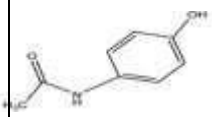
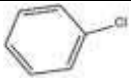
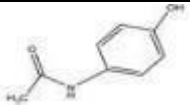
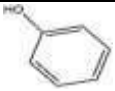
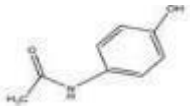
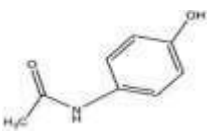
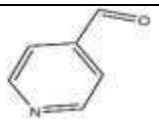
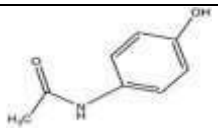
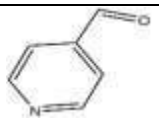
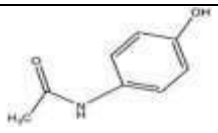
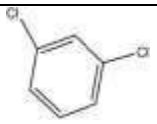
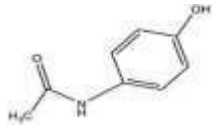
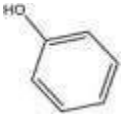
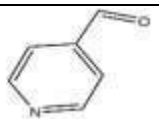
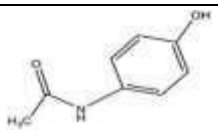
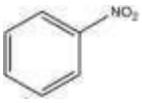
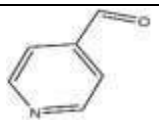
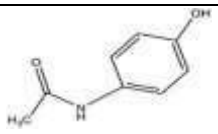
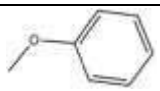
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				

29				
30				
31				
32				
33				

5. conclusion

Decades of widespread and uncontrolled application of antibiotics in clinical has resulted in the emergence of drug resistant strains of M. tuberculosis. To face drug resistance, several methods are developed for the synthesis of new antitubercular compounds. Among many strategies that are actually used in the drug development, fragment-based drug discovery (FBDD) approach has emerged as a promising strategy. 1,2-diazoles are more promising antitubercular drugs in future. Many analytical methods are used to characterize antitubercular antibiotics. Liquid chromatography and voltammetry are mostly preferred for the determination of antitubercular compounds. The redox (oxidation/reduction) properties of antituberculars make them analyzable by electrochemical methods. The quantification of the categories of antibiotics by voltammetry in both dosages forms and human body fluids seems to be the cheapest for developing countries.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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