Chapter 5

Tuberculosis: At a Glance
5.1 INTRODUCTION

The mycobacterium, *Mycobacterium tuberculosis*, is the causative agent of tuberculosis (TB) and is responsible for the morbidity and mortality of significant population in the world and its control is a high priority task for both the developed as well as developing economies.\(^1\) This gram-positive bacteria, isolated by R. Koch in 1882, has become the single biggest killer in the world today. According to World Health Organizations

- Someone in the world is newly infected with TB bacilli every second.
- Overall, one-third of the world's population is currently infected with the TB bacillus.
- 5-10% of people who are infected with TB bacilli (but who are not infected with HIV) become sick or infectious at some time during their life. People with HIV and TB infection are much more likely to develop TB.

TB is the most common opportunistic infection among people with HIV and is a leading cause of death among people who are HIV positive. As HIV progressively destroys the immune system, there is a greater chance for a person infected with HIV developing tuberculosis. Worldwide 30% of people with HIV get ill with TB and in some countries it can be higher than 80%.

In 2004, 14.6 million people had active TB and there were 8.9 million new cases and 1.7 million deaths, mostly in developing countries. This situation has further been complicated by the spread of HIV/AIDS and it is estimated\(^3\) that between 2000 and 2020 nearly one billion people will be newly infected, 200 million people will be sick and 35 million will die from TB if proper steps are not taken to control this disease.\(^1,2\)

5.2 CELL WALL STRUCTURE OF MYCOBACTERIUM

*Mycobacterium tuberculosis* is a gram (+ve) bacteria containing peptidoglycan as an important constitutates of their cell wall. In addition to peptidoglycan, the cell wall of *Mycobacterium* contains a large amount of glycolipids, especially mycolic acids that make up approximately 60% of the cell wall. The peptidoglycan layer is linked to arabinogalactan (D-arabinose and D-galactose) that is then linked to high-molecular weight mycolic acids. The arabinogalactan/mycolic acid layer is overlaid with a layer of polypeptides and mycolic acids consisting of free lipids, glycolipids, and peptidoglycolipids. Other glycolipids include lipoarabinomannan and phosphatidyinositol.
mannosides (PIM). The peptidoglycan protects the *Mycobacterium* from osmotic lyses. The mycolic acids and other glycolipids also impede the entry of chemicals causing the organisms to grow slowly and be more resistant to chemical agents and antibacterial drugs. The cell wall of *Mycobacterium*, in its full structural and functional integrity, is essential for growth and survival in the infected host. Consequently, some of the most effective antimycobacterial drugs isoniazid and ethambutol act by inhibition of the biogenesis of the cell wall components.\(^3\,^4\)

5.3 MULTIDRUG-RESISTANT TUBERCULOSIS (MDR-TB)

MDR-TB is a form of tuberculosis that is resistant to two or more of the primary drugs used for the treatment of tuberculosis. Resistance to one or several forms of treatment occurs when the TB bacilli develops the ability to withstand antibiotic attack and transmit this ability to newly produced bacteria. Since that entire strain of bacteria inherits this capacity to resist the effects of the various treatments, resistance can spread from one person to another. Resistance has been developed against almost every first-line drug.\(^5\) The resistance against existing drugs is developed through different mechanisms such as reduced permeability of the highly hydrophobic cell envelop to many drugs, development of drug-efflux system, production of certain enzymes to inactivate the drugs (β-lactamases, aminoglycoside acyl transferase) and at the molecular level acquisition of resistance in *M. tuberculosis* because of mutational in the chromosomes.\(^6\,^7\) On an individual basis, the inadequate treatment or improper use of the anti-tuberculosis medications remains an important cause of drug-resistant tuberculosis. MDR-TB is more difficult to treat than drug-susceptible strains of TB. The success of treatment depends upon how quickly a case of TB is identified as drug resistant and whether an effective drug therapy is available.\(^8\)

5.4 CLINICALLY USED ANTITUBERCULAR DRUGS

Traditionally, the clinical management of TB has relied heavily on a limited number of drugs such as isonicotinic acid hydrazide, rifampicin, ethambutal, streptomycin, ethionamide, pyrazinamide, fluoroquinolones, etc.
Based on their efficacy against *Mycobacterium*, the drugs for TB are divided into first-line and second-line antitubercular drugs.

### 5.4.1 First line anti-TB drugs

All first-line antitubercular drug names (Fig. 1) have a standard three-letter and a single-letter abbreviation:

- Streptomycin (STM or S)
- Isoniazid (INH or H)
- Rifampin (RMP or R)
- Ethambutol (EMB or E)
- Pyrazinamide (PZA or Z)

These drugs are prescribed in combinations of two drugs for the duration of 6 to 10 months.
Isoniazid (INH, isonicotinic acid hydrazide): It is the highly potent antitubercular drug. INH is highly effective against TB bacilli, well tolerated to both adults and children and inexpensive. It is a highly specific agent, ineffective against other microorganisms. INH is bactericidal to rapidly-dividing mycobacteria, but is bacteriostatic if the *Mycobacterium* is slow-growing. The mode of action of INH involves the inhibition of mycolic acid biosynthesis in mycobacterial cell. INH is a prodrug in nature and requires the activation by bacterial catalase-peroxidase enzyme (KatG) to generate reactive oxygen radicals and reactive organic radicals. These radicals attack multiple targets i.e. mycolic acid synthesis, DNA damage, lipid peroxidation, and NAD metabolism in the TB bacillus which leads to cell death.9

Rifampine (RMP): It is a semisynthetic agent prepared from rifamycin B, an antibiotic isolated from *Streptomyces emditteranei*. It is a member of broad-spectrum antibacterial agent. RMP inhibit bacterial DNA-dependent RNA polymerase (DDRP) and is highly active against rapidly dividing intra and extracellular bacilli. Inhibition of DDRP leads to the blocking of the initiation of chain formation in RNA synthesis which leads to the bacterial cell death.10

Pyrazinamide: It is a prodrug and requires its activation to active form to stops the growth of *Mycobacterium tuberculosis*. The drug is largely bacteriostatic, but can be bactericidal on actively replicating tuberculosis bacteria. *M. tuberculosis* has the enzyme pyrazinamidase which is only active at acidic pH. Pyrazinamidase converts pyrazinamide to the active form, pyrazinoic acid which disrupted membrane energetics and inhibited membrane transport function in *Mycobacterium tuberculosis*. Mutations of the pyrazinamidase gene (pncA) are responsible for pyrazinamide resistance in *M. tuberculosis*. Pyrazinamide is only used in combination with other drugs such as isoniazid and rifampin in the treatment of TB.11

Streptomycin: Streptomycin, an aminoglycosides class of antibiotic drug was the first antibiotic remedy for tuberculosis. It is derived from the actinobacterium *Streptomycyes griseus*. Streptomycin stops bacterial growth by damaging cell membranes and inhibiting protein synthesis. Specifically, it binds to the 16S rRNA of the bacterial ribosome, which prevents the release of the growing protein (polypeptide chain). Humans have structurally different ribosomes from bacteria, thereby allowing the selectivity of this antibiotic for
bacteria. An adverse effect of this medicine is ototoxicity. It can result in permanent hearing loss.\textsuperscript{12}

\textbf{Ethambutol:} Ethambutol has bacteriostatic against actively growing TB bacilli. Its mechanism of action involves the interfering of the mycobacterial cell wall synthesis. Mycolic acids attach to the 5'-hydroxyl groups of D-arabinose residues of arabinogalactan and form mycolyl-arabinogalactan-peptidoglycan complex in the cell wall. Ethambutol disrupts the arabinogalactan synthesis and inhibits the formation of this complex which leads to increased permeability of the cell wall and cell lyses.\textsuperscript{13}

\subsection*{5.4.2 Second line anti-TB drugs.} There are six classes of second-line drugs used for the treatment of TB (Fig. 2). A drug may be classed as second-line for one of two possible reasons: it may be less effective than the first-line drugs or it may have toxic side-effects. The second-line drugs for TB includes:\textsuperscript{14,15}

- Aminoglycosides: e.g. amikacin, kanamycin
- Polypeptides: e.g. capreomycin, viomycin, enviomycin
- Fluoroquinolones: e.g. ciprofloxacin, moxifloxacin
- Thioamides: e.g. ethionamide, prothionamide
- Cycloserine (the only antibiotic in its class)
- \textit{p}-Aminosalicylic acid (PAS).

\textbf{Aminoglycosides:} Amikacin and kanamycin are aminoglycosides class of antibiotics used in the treatment of tuberculosis. Their mode of action is similar to streptomycin. These antibiotics show their bactericidal action by damaging mycobacterial cell membranes and inhibition of protein synthesis.

\textbf{Polypeptides:} Capreomycin and viomycin are polypeptide antibiotic used in the treatment of tuberculosis. Viomycin is produced by the actinomycete \textit{S. puniceus}. It binds to RNA and inhibits bacterial protein synthesis and certain forms of RNA splicing. Little is known about capreomycin's exact mechanism of action. It is proposed that capreomycin binds to components in the bacterial cell which result in the production of abnormal proteins. These proteins are necessary for the bacteria's survival. Therefore the production of these abnormal proteins is ultimately fatal to the bacteria.


![Chemical structures of second-line anti-TB drugs](image)

**Fig. 2.** The structures of second-line anti-TB drugs

**Fluoroquinolones:** The fluoroquinolones are a family of broad-spectrum antibiotics. The fluoroquinolones are bactericidal drugs, actively kill bacteria. They inhibit the bacterial DNA gyrase or the topoisomerase II enzyme, thereby inhibiting DNA replication and transcription. Fluoroquinolones can enter cells easily and therefore are often used in the treatment of tuberculosis.
**Thioamides**: Ethinamate is bacteriostatic against *M. tuberculosis*. Ethionamide, like prothionamide and pyrazinamide, is a nicotinic acid derivative. It is proposed that it undergoes intracellular modification and acts in a similar manner to isoniazid.

**Cycloserine**: Cycloserine is an oral broad spectrum antibiotic effective against tuberculosis, by inhibiting cell wall synthesis of TB bacilli at early stage of peptidoglycan synthesis.

**p-Aminosalicylic acid**: p-Aminosalicylic acid was introduced to clinical use in 1948. It is the second antibiotic found to be effective in the treatment of tuberculosis. It is used in the treatment of multidrug-resistant tuberculosis in combinations of other agents.

### 5.5. TARGETS FOR ANTI-TUBERCULAR DRUGS

The targeting of cell wall components of mycobacteria is remained an attractive strategy for development of new drug for TB. Various antitubercular agents have been developed to interfere with the functioning and integrity of cell wall structure of mycobacteria. Apart from this various intracellular enzymes and metabolites have been exploited to develop new antitubercular drug. The summery of various targets for TB and their inhibitors is presented in Table 1.

### 5.6. NEW LEADS FOR TB

There is no drug approved for TB for the last 35 year. Various prototypes of molecules were synthesized and evaluated for their antimycobacterial activity but these are failed due to either their cytotoxicity or inefficiency against MDR-TB. There is continuous search for development of new molecule for the effective treatment of TB. The lead molecular structures for development new antitubercular agents are given in Table 2.
Table 1. Validated targets for mycobacteria and their inhibitors

<table>
<thead>
<tr>
<th>Target</th>
<th>Inhibitors</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycolic acid biosynthesis</td>
<td>Isoniazid</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Thiolactomycin</td>
<td>17</td>
</tr>
<tr>
<td>Folic acid biosynthesis</td>
<td>Trimethoprim</td>
<td>18</td>
</tr>
<tr>
<td>Arabinan biosynthesis</td>
<td>Ethambutol</td>
<td>19</td>
</tr>
<tr>
<td>DNA synthesis</td>
<td>azidodeoxythymidine monophosphate</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Fluoroquinolones</td>
<td>21</td>
</tr>
<tr>
<td>ATP synthesis</td>
<td>R207910</td>
<td>22</td>
</tr>
</tbody>
</table>
Riboflavin biosynthesis and lumazine synthase

Purinetrione inhibitors

Peptidoglycan biosynthesis

D-cycloserine

Terpenoid biosynthesis

Fosmidomycin

Tuberculosis: At a Glance
Table 2. New leads for the development of antitubercular drugs

<table>
<thead>
<tr>
<th>Lead</th>
<th>Structures</th>
<th>Mode of action</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td>Fluoroquinolones</td>
<td><img src="image1" alt="FluoroquinolonesStructure" /></td>
<td>DNA gyrase inhibitors</td>
<td>21</td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td><img src="image2" alt="OxazolidinonesStructure" /></td>
<td>protein synthesis inhibitors</td>
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<td>Nitroimidazopyran</td>
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<td>Inhibition of cell wall lipid synthesis</td>
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<tr>
<td>Phenazine Derivatives</td>
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<td>27</td>
</tr>
<tr>
<td>Phenothiazines</td>
<td><img src="image5" alt="PhenothiazinesStructure" /></td>
<td>NA</td>
<td>28</td>
</tr>
<tr>
<td>Acyclis sugars</td>
<td><img src="image6" alt="AcyclisSugarsStructure" /></td>
<td>Interfere with cell wall synthesis</td>
<td>29,30</td>
</tr>
<tr>
<td>Class</td>
<td>Molecular Structure</td>
<td>Pharmacological Action</td>
<td>Reference</td>
</tr>
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<tr>
<td>Aminosugars</td>
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<td>Interferes with cell wall synthesis</td>
<td>31</td>
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<tr>
<td>Nitrofuranyl amides</td>
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<td>3-Dehydroshikimic Acid</td>
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<td>Diamino based analogues</td>
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<td>Thiosemicarbazones</td>
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<td>Cyclis sugars</td>
<td><img src="image" alt="Cyclis sugars" /></td>
<td>Interferes with cell wall synthesis</td>
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</table>
5.7. PRESENT QSAR STUDY

In the present QSAR (Quantitative Structure-Activity Relationship) studies we have selected three different leads associated with antitubercular activity i.e. functionalized alkenols (functionalized heptenols/ octenols, acyclic sugars), C-3 alkyl/ arylalkyl-2,3-dideoxy hexenopyranosides (cyclic sugars) and substituted nitrofuranyl amide (NFA) analogues.

The ‘functionalised alkenols’ and ‘C-3 alkyl and arylalky 2,3-dideoxy hexenopyranosides’ are developed in our laboratory as a part of our laboratory’s ongoing new drug development program on the design of antitubercular agents. These sugar templates are considered as a potential source of new molecular scaffolds with strategically positioned functional groups that will selectively interact and communicate with the complementary groups / sites of the pathogen’s cell-wall structure and receptor(s) therein. To the best of our knowledge, these compounds were first of their class to show this kind of activity. The QSAR study on ‘functionalised alkenols’ and ‘C-3 alkyl/ arylalkyl-2,3-dideoxy hexenopyranosides’ have been carried out using Combinatorial Protocol in Multiple Linear Regression (CP-MLR) approach (Chapter 6).

The substituted nitrofuranyl amide analogues developed by Tangallapally et al. possess significant activity against mycobacteria and are described as novel antitubercular agents. The QSAR study on nitrofuranyl amide analogues has been carried out by Partial Least Square (PLS) method (Chapter 7). All these classes of compounds have been studied using various classes of descriptors obtained from Dragon software.
5.8 REFERENCES


