Chapter 1

Chemical approaches against tuberculosis therapy in last decade
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Chemical approaches against tuberculosis therapy in last decade

1.1 Introduction

*Mycobacterium tuberculosis* is a causative agent of tuberculosis (TB).[^1^] More than one-third of the world's population are infected with TB bacilli. After AIDS, tuberculosis is the leading cause of death among infectious diseases in the world. According to WHO 2009 fact sheet, 1.8 million people died from TB in 2008, including 500,000 people with HIV.[^2^][^3^] The vast majority of TB deaths are in the developing world, and more than 50% of all deaths occur in Asia. In 2008, 9.4 million new TB cases were observed while 36 million people were cured in DOTS programmes in between 1995-2008. The top five countries with the largest number of cases are India, China, the Russian Federation, South Africa and Bangladesh and XDR-TB has been found in 57 countries to date.[^2^][^3^]

Although TB is considered a single disease, it may be caused by several microorganisms, *Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. avium* and *M. leprae*. They are intracellular pathogens of higher vertebrates, the infection with them may lead to death in animals and humans.[^4^] Out of the above microorganisms, two groups of mycobacteria *M. tuberculosis* and *M. avium* cause a significant challenge to the clinical management of tuberculosis in HIV-infected patients and are often responsible for their deaths.[^5^] The research on *M. tuberculosis* has undergone much progress in last decade. The regimens were optimized along with the working of the directly observed therapy short course (DOTS) and much work done insights into the mechanisms of action of the antituberculosis drugs currently used.

Recently number of articles has been published which illustrates, the various classes of compounds with antitubercular activity,[^6^] antimycobacterial activity in natural products,[^7^][^11^] drug candidates and the possible biological targets.[^12^][^19^] Here a review of the drugs currently used in tuberculosis treatments with their mode of action, antitubercular drug targets, present day problems, the compounds undergoing clinical trials followed by antitubercular agents developed within last 10 years is being given.

1.2 Tuberculosis: Past, Present, Future

This year marks the 127th anniversary of Robert Koch's discovery of the tubercle bacillus *Mycobacterium tuberculosis* (*Mtb*). Exact pathological and anatomical description of the
disease appeared in the 17th century. The antibiotic era started after the Fleming’s serendipitous discovery of penicillin. The search for new antibiotics was dominated by the discovery of natural products viz the antibiotics inhibited its growth in vitro was the peptide actinomycin a streptomycete derived natural product. In 1943 aminoglycoside streptomycin was shown to inhibit Mtb in vivo in test animals and within the year it was used to treat a critically ill TB patient providing the first example of antitubercular drug therapy. In year 1944, p-aminosalicylic acid (PAS) was discovered as an anti-TB agent. The first successful clinical trial of an anti-TB agent, namely, isoniazid (isonicotinic acid hydrazide, INH), was disclosed in 1952. Further in 1950s ethambutol (EMB), discovered at Lederle Laboratories, and the natural product cycloserine, an inhibitor of cell wall biosynthesis new classes of anti-TB drug was discovered in 1952. The macrolide rifamycin, a polyketide natural product isolated in 1966 from soil bacterium Amycolatopsis mediterranei an important natural product as antiTB drug and it provided the lead for development of rifampin, a semisynthetic derivative which used as first line anti-TB drug today.

![Figure 1. First line drugs currently used for treating TB](image)

The main objectives of current anti-TB drug therapy are to kill all actively metabolising bacilli in the lungs and eliminate less actively replicating and near-dormant bacilli. So the current short-course TB therapy used the administration of first-line drugs: isoniazid (INH, 1), rifampicin (RIF, 2), pyrazinamide (PZA, 3) and ethambutol.
(EMB, 4) or streptomycin (SM, 5), (Fig. 1). Treatment with these drugs is carried out initially for two months, after which treatment continues with RIF and INH alone for four months, where any residual dormant bacilli are eliminated by RIF and any remaining RIF-resistant mutants are killed by INH.\textsuperscript{28,29} Several excellent articles have reviewed the properties of these first-line anti-TB agents.\textsuperscript{30-35} Infection by MDR-TB strains requires treatment with second-line drugs such as kanamycin (6), amikacin (7), capreomycin (8), fluoroquinolones (e.g., levofloxacin (9), p-aminosalicylate (PAS, 10), ethionamide (11), and cycloserine (12) where treatments often extend for as long 2 years (Fig. 2).

\textbf{Figure 2.} Second line drugs currently used for treatment of TB

The challenges to eradicate the tuberculosis in the future are due to factors such as latency and drug resistance. \textit{Mtb} has the ability to remain dormant or metabolically silent within host lesions for years to decades.\textsuperscript{36} The World Health Organization defines MDR-TB as resistance to RIF and INH while XDR-TB as resistance to first-line drugs RIF and INH together with resistance to second-line drugs of the fluoroquinolones and capreomycin, kanamycin or amikacin.\textsuperscript{37} XDR-TB raises concerns of a future TB epidemic with restricted treatment options and till now XDR-TB is almost untreatable. It is therefore vital that TB control is managed properly and new tools developed to prevent, treat and diagnose the disease. A number of inter-related factors have contributed to the recovery of TB including\textsuperscript{38-46}
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1. Synergy with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic

2. Poor compliance of treatment regimens and their side effects. i.e., multidrug-resistant tuberculosis (MDR-TB) extensively drug-resistant tuberculosis (XDR-TB), which is virtually incurable with existing antibiotics

3. Increased mobility and immigration of people from countries where TB is endemic

4. Increased poverty and homelessness

5. Premature dismantling of the health infrastructure for TB treatment, i.e.
   a) Limited biomarkers of drug efficacy for use in early clinical development
   b) Long doubling time of \(M.\) tuberculosis
   c) Lengthy treatment period
   d) Requisite long patient follow up time
   e) Relatively large number of patient

6. Poor funding for tuberculosis drug discovery, as this disease is more prevalent in developing and poor countries so funding issue are also on major front and the funding has fortunately been enhanced due to HIV-TB synergism.

1.3 Targets for antitubercular drugs

Many well known anti-TB drugs target were reported such as the biosynthesis of the macromolecules proteins, the nucleic acids, cell wall polymers and DNA topoisomerase which are essential for survival of bacteria. After sequencing of the genome of \(Mtb\) H37Rv in 1998\(^47\) and reannotated in 2002,\(^48\) the challenge of identifying the essential genes suitable as drugable targets for new antibiotics. The targets and mechanism of action of both existing drugs and drugs under clinical trials are presented as pictorial diagram (Fig. 3) and briefly discussed below.

1.3.1 Protein Synthesis

Targeting protein synthesis possibly will lead to drugs that prevent the growth and survival of \(M.\) tuberculosis. Most of the aminoglycosides\(^49\) act through targeting protein synthesis such as streptomycin which disrupts the protein synthesis in bacteria. The resistance against streptomycin was developed in \(M.\) tuberculosis due to mutation of 16s ribosomal subunit in the RNA molecule. Although many other inhibitors of protein
synthesis, including tetracycline, chloramphenicol, and macrolides (erythromycin), but they do not show activity against \textit{M. tuberculosis}.

1.3.2 Nucleic Acids Biosynthesis

Tetrahydrofolate and other reduced folates are required in bacteria for the biosynthesis of purines, thymidylate, panthothenate and few amino acids.\textsuperscript{50} Therefore; inhibitors of tetrahydrofolate biosynthesis pathway inhibit the growth of bacteria. The first line anti-TB drug p-amino salicylic acid has been reported to act on the tetrahydrofolate pathway as well as salicylate dependent biosynthesis of mycobactins which is required for iron transport. A detailed study of enzymes involved in tetrahydrofolate biosynthesis may lead to a rational design of new and novel anti-TB drugs.

![Diagram of biological processes](image)

**Figure 3.** Targets, mechanism of action of current and investigated drugs

1.3.3 DNA Topoisomerases

DNA topoisomerases particularly DNA gyrase, a type II topoisomerase is a promising target for inhibition of bacterial growth.\textsuperscript{51} Many biochemical reactions are influenced by DNA gyrase including ATP-dependent negative supercoiling of closed circular double stranded DNA; ATP-independent relaxation of negatively supercoiled DNA, nucleotide-dependent relaxation of negatively supercoiled DNA; formation and resolution of
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catenated DNA; resolution of knotted DNA; quinoline or calcium ion induced double stranded breakage of DNA and DNA dependent ATP hydrolysis. Biosynthesis of nucleotides has recently been reported to be a good target particularly for TB in HIV cases. Thus thymidine monophosphate kinase (dTMKase)\textsuperscript{52} has been suggested as a validated target to develop new anti-tubercular agents, particularly for the treatment of MDRTB and TB in HIV infected patients.

1.3.4 Cell Wall Biosynthesis

The cell wall of mycobacteria is the most important target to develop new drugs.\textsuperscript{53,54,55,56} The cell wall consists of a plasma membrane surrounded by a lipid and carbohydrate rich wall, which in turn is encircled by a capsule of polysaccharides, proteins and lipids.

![Figure 4. Generalized structure of mAGP complex](image)

The insoluble cell wall core which is formed after the removal of soluble proteins, lipids and carbohydrates, is chemically composed of highly cross-linked peptidoglycan, arabinogalactan (AG) and mycolic acids.\textsuperscript{57,58,59} The mycolyl-arabinogalactan peptidoglycan 13 (mAGP, Fig. 4) complex is the essence of the mycobacterial cell wall and its biosynthesis is very important in design and development of new drugs.
1.3.1.1 Biosynthesis of mycolic acids

Mycolic acids 14 control the permeability of the mycobacterial cell wall and hence are an important drug target. The chemical structure of mycolic acid is shown in figure 5. The biosynthesis of mycolic acids appears to be a discontinuous process in growing mycobacterial cells. Several steps in synthesis of mycolic acid are now understood. Several possibilities exist for the building of complete mycolic acids but recent results are favoured the condensation of C40-C60 (meromycolic) main carbon backbone and a C22-C26 fatty acid pathway. 60 The biosynthesis of mycolic acid involves four steps. 61,62,63

Step 1- The C24-C26 straight chain saturated fatty acids are formed that provide the C-1 and C-2 atoms leading to the formation of the α-alkyl branch of mycolic acid (presumably via fatty acid synthetase (FAS) I and FAS II); Step 2- The formation of meromycolic acids C40-C60, which constitute the main carbon backbone; Step 3- The introduction of distal or proximal functionalities such as double bonds, cyclopropane rings, oxygen functions etc.; Step 4- The transfer of these mycolic acids to various mycolyltransferase
processes to cellular lipids, such as glucose dimycolate and trehalose dimycolate via antigen 85 and cell wall AG.

### 1.3.1.2 Biosynthesis of Arabinogalactan

Arabinogalactan is the major polysaccharide component in cell wall of *M. tuberculosis*. AG and PG are linked together by phosphodiester link to the position 6 of approximately 10-12% of muramic acid residues. All the arabinose and galactose residues are in the furanose form peculiar in mycobacterium. The non-reducing termini of arabinan consists of a branched hexafuranosyl structure \(\beta\)-D-Araf(1-2)-\(\alpha\)-D-Araf\(2\)-3,5-\(\alpha\)-D-Araf\(1,5\)-\(\alpha\)-D-Araf; the majority of arabinan chain consists of 5-linked \(\alpha\)-D-Araf with branching introduced by 3,5-\(\alpha\)-D-Araf replaced at both branch positions with 5-\(\alpha\)-D-Araf; arabinan chains are attached to the galactan core through some of the 6-linked Galf units; the galactan region consists of linear alternating 5- and 6-linked \(\beta\)-D-Galf residues, the galactan of AG is linked to C-6 of some muramyl residues of PG via the diglycosylphosphoryl bridge, L-Rahp(1-3)-D-GlcNAc-(1-P) and mycolic acids are located in clusters of four on the terminal hexaarabinofuranosyl units, but only approximately two-thirds of these arrangements are mycolated. Recently, certain oligosaccharides consisting of 26 glycosyl residues with alkylation patterns have also been reported.

The initial steps of AG biosynthesis are the congregation of the linker unit on Pol-P using the sugar nucleotide donors TDP-Rha and UDP-GlcNAc to form polypreanol-PP-Glc-NAc-Rha followed by polymerisation of Galf using the sugar nucleotide donor UDP-Galf. EMB was shown to decrease the arabinose content of the cell wall. This drug is known to inhibit the synthesis of the arabinans in AG and lipoarabinomannan (LAM) of *M. tuberculosis*.

### 1.3.1.3 Biosynthesis of peptidoglycan

The biosynthesis of peptidoglycan has not been extensively studied in mycobacterium, it was found that the structural similarities between the mycobacterial peptidoglycan and the peptidoglycan of *Escherichia coli* (*E. coli*).
1.4 Current anti-tuberculosis drugs

Targets and mechanisms of action of current line TB drugs are summarized in Table 1. The details of molecules in clinics are mentioned below.

**Table 1.** Targets and mode of action of current TB drugs

<table>
<thead>
<tr>
<th>Drug (year of discovery)</th>
<th>Effect on bacterial cell</th>
<th>Mechanism of action</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin (1943)</td>
<td>Bacteriostatic</td>
<td>Inhibition of protein synthesis</td>
<td>Ribosomal S 12 protein of 30S rRNA</td>
</tr>
<tr>
<td>PAS (1944)</td>
<td>Bacteriostatic</td>
<td>Inhibition of folic acid &amp; iron Metabolism</td>
<td>Unknown</td>
</tr>
<tr>
<td>Isoniazid (1951)</td>
<td>Bactericidal</td>
<td>Inhibition of cell wall mycolic acid &amp; other multiple effects on DNA, lipids &amp; carbohydrate &amp; NAD metabolism</td>
<td>Primary acyl carrier protein reductase (InhA)</td>
</tr>
<tr>
<td>Pyrazinamide (1952)</td>
<td>Bactericidal</td>
<td>Disruption of membrane transport &amp; energy depletion</td>
<td>Membrane energy metabolism</td>
</tr>
<tr>
<td>Cycloserine (1952)</td>
<td>Bacteriostatic</td>
<td>Inhibition of peptidoglycan synthesis</td>
<td>D-alanine recimase</td>
</tr>
<tr>
<td>Ethionamide (1956)</td>
<td>Bacteriostatic</td>
<td>Inhibition of mycolic acid synthesis</td>
<td>Acyl carrier protein reductase (InhA)</td>
</tr>
<tr>
<td>Kanamycin (1957)</td>
<td>Bactericidal</td>
<td>Inhibition of protein synthesis</td>
<td>Ribosomal S 12 protein 16S rRNA</td>
</tr>
<tr>
<td>Ethambutol (1961)</td>
<td>Bacteriostatic/Bactericidal</td>
<td>Inhibition of cell wall arabinogalactan synthesis</td>
<td>Arabinosyl transferase</td>
</tr>
<tr>
<td>Quinolones (1963)</td>
<td>Bactericidal</td>
<td>Inhibition of DNA replication &amp; transcription</td>
<td>DNA gyrase</td>
</tr>
<tr>
<td>Rifampin (1966)</td>
<td>Bactericidal</td>
<td>Inhibition of RNA synthesis</td>
<td>RNA polymerase β subunit</td>
</tr>
</tbody>
</table>

1.4.1 Streptomycin

Streptomycin (15) was the first antibiotic remedy for tuberculosis. It is derived from the actinobacterium *Streptomyces griseus*. It consists of three structural components, streptidine, streptose and N-methyl-L-glucosamine (Fig 6). Because of its poor absorbance from gastrointestinal tract it is administered intramuscularly and very occasionally by intrathecal route. It has an MIC value of 1μg/mL with 50-60 % plasma protein bound and a half-life 5-7 hours and inhibits protein synthesis. It penetrates the inner membrane of *M. tuberculosis* and interferes with the binding of formyl-methionyl-
tRNA to the 30S subunit of the ribosome. Different synthetic derivatives of streptomycin (dihydrostreptomycin, 16) have been synthesized and evaluated against \( M. \) \( \text{tuberculosis} \) (Fig. 6).

\[
\begin{array}{c}
\text{NH} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{OH} \\
\text{OH} \\
\end{array}
\]

\[
\begin{array}{c}
\text{NH} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{OH} \\
\text{OH} \\
\end{array}
\]

\[
\begin{array}{c}
\text{NH} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{OH} \\
\text{OH} \\
\end{array}
\]

\[
\begin{array}{c}
\text{NH} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{OH} \\
\text{OH} \\
\end{array}
\]

\[
\begin{array}{c}
\text{NH} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{H}_2\text{N}-\text{C} \ 	ext{---} \\
\text{OH} \\
\text{OH} \\
\end{array}
\]

R = CHO, Streptomycin (15)
R = CH\(_2\)OH, Dihydrostreptomycin (16)

**Figure 6.**

Because of many toxic manifestations on peripheral and central nervous system at higher doses and its hypersensitivity reactions it is not a drug of popular choice. Dihydrostreptomycin once thought to be less toxic cause severe damage to eighth cranial nerve, inducing irreversible impairment of auditory function.

### 1.4.2 4-Aminosalicylic acid (PAS)

4-Aminosalicylic acid, commonly known as PAS and it was used as an oral TB therapy in 1944. It is highly specific and effective against \( M. \) \( \text{tuberculosis} \). The mode of action of this drug is still unclear but it is suggested that it interferes with the salicylate-dependent biosynthesis of the iron chelating mycobactins involved in iron assimilation. It is thought to act via NF-kB (nuclear factor-kappa B) inhibition and free radical scavenging.

### 1.4.3 Isoniazid

Isoniazid also known as isonicotinylhydrazine (INH) is the first-line antitubercular drug. It was discovered in 1951 as effective against \( M. \) \( \text{tuberculosis} \). It is a prodrug that requires activation by the mycobacterial catalase peroxidase enzyme (kat G), which confers sensitivity in \( M. \) \( \text{tuberculosis} \) to INH. It is orally active and exhibits bacteriostatic action on the resting bacilli and is highly active against the \( M. \) \( \text{tuberculosis} \) complex (\( M. \) \( \text{tuberculosis} \), \( M. \) bovis, \( M. \) atricanum and \( M. \) Microti). It has MICs (0.02-0.06 μg/mL) against these pathogens. INH enters the organism by diffusion and oxygen-dependent
active transport. INH inhibits the mycolic acid biosynthesis in mycobacterium tuberculosis by affecting an enzyme mycolate synthatase, unique for mycobacteria. A large number of compounds belonging to INH have been synthesized and evaluated against *M. tuberculosis* H$_{37}$Rv.

### 1.4.4 Pyrazinamide (PZA)

Pyrazinamide, a structural analogue of nicotinamide, is first line drug of short course tuberculosis therapy. It is used in combination with other drugs such as isoniazid and rifampin and shortens the therapy period to 6 months. Pyrazinamide in conjunction with rifampin is a preferred treatment for latent tuberculosis. The activity of PZA depends on the presence of bacterial amidase which converts PZA to pyrazinoic acid, the active antitubercular molecule and this activity is highly specific to *M. tuberculosis*. The resistance against this drug is mutation in the *pncA* gene responsible for the production of pyrazinamidase. Some pyrazinoic esters have also been reported to possess good antitubercular activities.

### 1.4.5 Cycloserine

D-Cycloserine, a structural analogue of amino acid D-alanine, possesses activity against a wide range of bacteria and inhibits *M. tuberculosis* at concentrations of 5-20 μg/mL. It blocks peptidoglycan biosynthesis by inhibiting the enzyme D-alanine racemase and D-alaninyl alanine synthetase. Microorganisms treated with cycloserine accumulate a muramic-uridine-nucleotide-peptide, which differs from that produced by mycobacteria in the absence of terminal D-alanine dipeptide. Cycloserine produces severe side effects in the central nervous system that can also generate psychotic states with suicidal tendencies and epileptic convulsion.

### 1.6.2 Rifamycin derivatives

Rifampicin (INN) or rifampin (USAN) is a bactericidal antibiotic drug of the rifamycin group. This is a group of semisynthetic derivatives of rifamycin, isolated from *Streptomyces mediterrani* with characteristic chromophoric naphthaquinone group spanned by a long aliphatic bridge which itself has very poor antimicrobial activity. It has MIC ranging from 0.1 to 0.2 μg/mL. Rifampicin inhibits DNA-dependent RNA polymerase in bacterial cells by binding its beta-subunit an enzyme necessary for RNA synthesis thus preventing transcription to RNA and subsequent translation to proteins. To
avoid rapid development of bacterial resistance rifampicin is recommended in combination with other first line agents either isoniazid or ethambutol. However, combination of INH and rifampicin may increase risk of hepatotoxicity. Rifabutins, rifapentens and other derivatives of this molecule are used as second line drugs (Fig. 7). Rifamycin derivatives, such as rifapentene (17), rifabutin (18) and rifalazil (19) (RLZ, also known as KRM1648 or benzoxazinorifamycin), have been synthesized to improve antimycobacterial activity (Fig. 7).

Rifapentene appears to be safe and well-tolerated at once-weekly dosage and it has efficacy for treatment of latent tuberculosis. RLZ is a new semisynthetic rifamycin derivative with a long half life, which is highly active against a range of intracellular bacteria including *M. tuberculosis*, *Mycobacterium avium*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, and *Helicobacter pylori*. RLZ is more active than RIF or rifabutin against *M. tuberculosis* both *in vitro* and *in vivo* in mice. RIF-resistant strains confer cross-resistance to all rifamycins, including RLZ limiting the use of RLZ in the treatment of RIF-resistant TB. The study in humans shows that although RLZ is safe at doses of 10 mg and 25 mg, a dose of >100 mg produced flu-like symptoms, decrease in white blood cell and platelet counts and did not show better efficacy than RIF.

1.4.6 Ethambutol

Ethambutol commonly abbreviated EMB, is a bacteriostatic antimycobacterial drug. It is a synthetic amino alcohol, ethylene diamino-di-1-butanol, orally effective bacteriostatic agent and active against most strains of *Mycobacterium*. It disrupts arabinogalactan synthesis by inhibiting the enzyme arabinosyl transferase. Disruption of
the arabinogalactan synthesis inhibits the formation of this complex and leads to increased permeability of the cell wall. Activity of EMB as $S,S$ (dextro) form is 600 times more than $R,R$ isomer. The exact mechanism of action of EMB is still not known completely and probability of its interference in the synthesis of proteins and nucleic acids as antimetabolite is also documented.

1.4.7 Quinolones

The first generation of the quinolones begins with the introduction of nalidixic acid in 1962. The synthetic derivatives of nalidixic acid are known to display broad-spectrum of antimycobacterial activity and it is used as part of multidrug regimens. It has good in vitro potency with MIC 1 μg/ml. The bactericidal effects of quinolones involve an interaction of the drugs with DNA-gyrase and DNA-topoisomerase IV.

![Figure 8.](image)

New quinolones are the molecules which have emerged as potent antiTB molecules. The two of them are gatifloxacin (20) and moxifloxacin (21) (Fig. 8). Their antibacterial mechanism of action of these compounds due to a dual inhibition of an ATP dependent DNA gyrase (topoisomerase II) as well as ATP dependent topoisomerase IV.

1.4.8 Kanamycin

Kanamycin sulfate is an aminoglycoside antibiotic and is used either as orally or intravenously. It is isolated from Streptomyces kanamyceticus. It affects the 30S ribosomal subunit and prevents the translation of RNA. Thus depending on the site and severity of the mutation, either a completely different protein is synthesized, or a protein similar to the one needed is synthesized, but is folded incorrectly.
1.4 Compounds under clinical trials

1.5.1 Nitroimidazopyran (PA-824) and structurally related nitroimidazo[2,1-b]oxazoles (OPC67683)

![Chemical structures of PA-824 (22) and OPC-67683 (23)](image)

Figure 9.

The first bicyclic nitro-bearing imidazole PA-824 (22) was reported as antitubercular agent against *M. tuberculosis*. As PA-824 is not mutagenic on the Ames test and does not seem to be metabolized by the human cytochrome P450 into potentially carcinogenic substances.\textsuperscript{118} It also has a good level of activity on resistant strains\textsuperscript{119,120} and thus, PA-824 is currently undergoing clinical trials as an antituberculosis drug (Fig. 9).\textsuperscript{118,120} Another orally active analog structurally related nitroimidazo[2,1-b]oxazoles,\textsuperscript{121,122} has been developed such as OPC-67683 (23) and under clinical trials\textsuperscript{118,123} (Fig. 9).

1.5.2 Diarylquinolines (TMC207)

![Chemical structure of TMC207 (24)](image)

Figure 10.

Diarylquinoline, TMC207 (24, Fig. 10) is a very important class of anti-mycobacterial agents.\textsuperscript{124} At least, 20 molecules of the diarylquinoline series are known to have a MIC values below 0.5 µg/mL against *M. tuberculosis* H\textsubscript{37}Rv. Antimicrobial activity was confirmed *in vivo* for three of these compounds. The most active compound of this class is TMC207 is unique in its specificity to mycobacteria. The diarylquinoline TMC207 is
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also active against MDR-TB isolates. It acts by inhibiting the ATP synthase leading to ATP depletion and pH imbalance. It has potent early bactericidal activity in the nonestablished infected murine mouse model, matching or exceeding to that of isoniazid. Diarylquinoline TMC207 has been also tested in various combination with the second line drugs e.g.; amikacin, pyrazinamide, moxifloxacin and ethionamide in mice infected with the drug susceptible *M. tuberculosis* strain H37Rv. Diarylquinoline containing regimens were more active than the current recommended regimen for MDR-TB. The amikacin-pyrazinamide-moxifloxacin, ethionamide and culture negativity of the both lungs and spleens were reached after 2 months of treatment in almost every case.\(^{125}\) The very short time (less than 3 years) duration between the patent application\(^{126}\) and the first phase I human trials of TMC207 is notable.\(^{124}\)

1.5.3 Pyrrole derivatives

Figure 11.

Pyrrole derivative BM 212\(^{127}\) (25) is moderately active against *M. tuberculosis* (MIC = 0.7 to 6.2 µg/mL) and *M. avium* (MIC 0.4 to 3.1 µg/ml). At present, after quantitative structure-activity relationships and comparative molecular field analysis isoniazid bearing analogue of BM212 (Fig. 11) was shown to be active in vivo on a murine model even infected with resistant *M. tuberculosis* strains.\(^{128,129}\) Till now no scientific results are yet publicly available, compound (26) was placed in a phase I clinical trial.

1.5.4 Ethambutol analogue (SQ109)

Figure 12.
A library of a 67,238 compounds belonging of ethambutol class was generated based on a [1,2]-ethylenediamine pharmacophore of ethambutol. The best MIC of 0.02 μM was determined for compound SQ109, (Fig. 12). The favourable pharmacological properties of SQ109 as well as its synergistic effect with other antituberculosis drugs led this molecule into clinical trial.

1.5.5 Oxazolidinone derivative (Linezolid)

Linezolid, 28 (Fig. 13) is the first oxazolidinone to be developed and approved by the FDA to treat single- or multiple-resistant bacterial infections. The mode of action of linezolid is to inhibits protein synthesis by binding to 23S rRNA and to the 50S ribosomal subunit.

1.6 New chemical entities (NCEs)

1.6.1 Thiolactomycin

Thiolactomycin, 29 (Fig. 14) is a natural product isolated from Nocardia spp. and belongs to small group of thioteric acid antibacterials. It is an unique thiolactone exhibiting antitubercular activity. This compound acts by inhibiting FAS-II of the bacteria. It has MIC of 5μg/mL but in absence of in vivo toxicology and in vitro cytotoxicity data it is difficult to judge whether these concentrations are far below the toxic concentrations or not.
1.6.3 Nitroimidazopyran and Nitroimidazofurans

The vast array of nitrated antimycobacterials reported.\textsuperscript{146,147,148,149,150,151} All of nitrated antimycobacterials probably being prodrugs to be reduced.\textsuperscript{151} Nitroimidazofuran class of molecules (nitroimidazole) are known antibacterial drugs and based on these reports nitroimidazopyrans were synthesized and evaluated against \textit{M. tuberculosis} one of such compound PA 1343 (30) recently with MIC of 0.015 μg/mL is in clinical trial (Fig. 15). The nitroimidazofuran derivative CGI 17341 (31) is potently active against MDR-\textit{Mtb} (Fig. 13) with MIC in the range of 0.08-0.3 (μg/mL). Their mechanisms of action have not been reported.

![Figure 15.](image)

1.6.4 Clofazimine and Riminophenazines

Clofazimine is effective against \textit{M. leprae} and its use in the treatment of tuberculosis has been suggested.\textsuperscript{152} Several clofazimine\textsuperscript{153,154,155} analogs 32-35 (Fig. 16) are also active \textit{in vivo} against \textit{M. tuberculosis}, \textit{M. bovis}, \textit{M. leprae} and \textit{M. avium} having a MIC value of 0.01 to 3.3 μg/ml. However, no specific mechanism of action has been established.

![Figure 16.](image)
Riminophenazines B4154 (36) and B4157 (37) (Fig. 16) are more active against *Mtb* than clofazimine (CFZ)\(^{156,157,158,159,160}\). MIC of B4154, B4157 and CFZ are 0.25, 0.12 and 1 \(\mu\)g/ml, respectively, thereby indicating a lack of cross resistance between these riminophenazines and standard resistance anti-TB drugs. Notably, both the agents cause less skin pigmentation, which is the main drawback of this group of compounds, as compared to the case of CFZ.

### 1.6.5 Chlorpromazine and Phenothiazine

![Figure 17](image)

Chlorpromazine (38) and thioridazine (39)\(^{161,162}\) (Fig. 17) possess significant antimycobacterial activity. Amaral *et al.* reported\(^{163,164}\) antitubercular activity in phenothiazine. These compounds are inhibitors of the type II NADH:menaquinone oxidoreductase. The enzyme was inhibited in all strains of *Mtb* regardless of whether they were drug-susceptible or multidrug or polydrug resistant.\(^{163,164}\) The MIC of phenothiazines was found to be 4 to 32 \(\mu\)g/ml.

### 1.6.6 Oxazolidinones

![Figure 18](image)

Oxazolidinones\(^{165,166,167,168,169,170,171}\) was discovered by Dupont in 1970. Oxazolidinone derivatives, eperezolid (40) and PNU-100480 (41) (Fig. 18) are active against *Mtb*, moderately active against *M. fortuitum* and *Mycobacterium chelonae*, and exhibit good...
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therapeutic efficacy against *Mtb* infection in mice. These molecules inhibit protein
synthesis by binding to 23S rRNA and to the 50 s ribosomal subunit.

### 1.6.7 Ethambutol analogue

![Chemical structures of ethambutol analogues](image)

**Figure 19.**

A 67,238 compounds library was generated based on a [1,2]-ethylenediamine
pharmacophore of ethambutol. Several ethambutol analogues (42-44) (Fig. 19) were
tested against tuberculosis and displayed good *in vitro* activities against *M.
tuberculosis*. In recent years several new ethylenediamine pharmacophore based
compounds were evaluated for their antitubercular activities.

### 1.6.8 Macrolides

![Chemical structures of macrolides](image)

**Figure 20.**

The latest generation of macrolides was designed to overcome bacterial resistances
resulting from methylation of the rRNA. Erythromycin (45) is 14 membered macrolide
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consisting of a macrocyclic lactone ring attached to two sugar residues. Newer derivatives differ from the parent erythromycin in the size and/or substitution pattern of the lactose ring and include; roxithromycin (46), carithromycin (47), azithromycin (48), rokitamycin (49) and spiramycin (50) (Fig. 20). Many macrolides targeting the 50S ribosomal subunit causing dissociation of peptidyl-tRNA from ribosomes and inhibition of bacterial protein synthesis.

1.6.9 Galactopyranosyl amino alcohols

Figure 21.

A dimeric hybrid of a galactopyranosyl ethambutol analogue (51), (Fig. 21) display potent in vitro activity with MIC 1.56 μg/mL for M. tuberculosis. However, on progression into a murine model, toxicity was observed at dosage levels (50 mg/kg per day) that offered no significant protection against M. tuberculosis infection. The target of this compound is to inhibit mycobacterial cell wall biosynthesis.

1.6.10 Pleuromutilins

Figure 22.

This class of antibiotics, tiamulin and valdemulin are used in veterinary medicine. They interfere with protein synthesis by binding to the 50S ribosomal subunit and therefore inhibit the peptide bond formation. Pleuromutilins have been also shown to inhibit
the growth of *M. tuberculosis* in vitro. Recently antimycobacteril activity reported in compound 52 (Fig. 22). 188

**1.6.11 Alkyl-sulfinyl amides**

![Chemical structure](image)

**Figure 23.**

Alkyl sulfinyl amides inhibit β-ketoacyl synthase (KAS), one of the accessory fatty acid synthases peculiar to mycobacteria. The compound 53 show good MIC (0.75 µg/ml), but its selectivity toward mycobacterium is not known 189 (Fig. 23).

**1.6.12 SRI-3072**

The compound SRI-3072 (54) 190 belongs pyridopteridine class of compound (Fig. 24) was found to be active against *M. tuberculosis* with MIC 0.15 µg/ml. the target of this compound was found to be an inhibitor of bacterial tubulin polymerase homologue (FtsZ).

![Chemical structure](image)

**Figure 24.**

This provides an attractive hit, with clear scope for further diversification that may help in identifying analogues with more favorable physicochemical profiles.

**1.6.13 5-Amino-furanoside derivatives**

![Chemical structure](image)

**TH-17196**

21
This compound 55 (Fig. 25) was found in vitro activity \textit{M. tuberculosis} with MIC 3.12 \( \mu \text{g/mL} \).\textsuperscript{191} The one of the target of this compound is D-alanine racemase, a cytoplasmic enzyme responsible for the conversion of L-alanine to D-alanine, a key building block in peptidoglycan biosynthesis.

1.6.14 Sulfometuron methyl

Sulfometuron methyl 56\textsuperscript{192} (Fig. 26) is a commercially available herbicide which inhibit the branched chain aminoacid biosynthesis. The compound shows good activity against \textit{M. tuberculosis} (MIC 0.3 to 1.8 \( \mu \text{g/mL} \)), with no overt toxicity at 500 mg/kg.

1.6.15 9-Benzylpurines

Several 9-benzylpurines\textsuperscript{193} 57-60 (Fig. 27) have been found to exhibit very good inhibitory activity against \textit{M. tuberculosis} with the MIC as low as 0.78 \( \mu \text{g/mL} \) of the leading compound (57). Extensive structure activity relationship was reported for a series of substituted purines.\textsuperscript{194,195,196} The MIC values of all the other three compounds 58, 59 and 60 were 1.56 \( \mu \text{g/mL} \), 0.78 \( \mu \text{g/mL} \), 0.25-2.56 \( \mu \text{g/mL} \) respectively.
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1.6.16 Quinoxaline 1,4-dioxides (LVTZ)

The leading compound LVTZ (61)\textsuperscript{197} (Fig. 28) belongs to quinoxaline 1,4-dioxides class of compound shows very good selectivity and activity against \textit{M. tuberculosis} infected macrophage model with MIC 0.1 μg/ml. Favorable physicochemical profiles and ease of synthesis make these attractive hits, although their genotoxicity is a concern, especially if this proves to be a mechanism-based property.

1.6.17 Quinoline derivative

Several quinoline derivatives were reported with significant antitubercular activities.\textsuperscript{198,199,200,201} For example 4-quinolylhydrazones, 62\textsuperscript{202} (Fig. 29) the structural hybrids of isoniazid and quinolones showed marked antitubercular activity with very good MIC 0.78 μg/ml but poor selectivity for mycobacteria.
This activity translated well across the series into an *M. tuberculosis* infected macrophage model, with some compounds showing up to a 10-fold increase in activity.

### 1.6.18 Thiazoline and benzothiazole analogue

The antitubercular activity in thiazoline class of compounds has been reported recently. The most potent compound (Fig. 30) shows good MICs 0.3 µg/mL. Some SAR was delineated; for example, replacing the thiazoline with a thiazolidinone substantially reduced activity.

The chemistry and biology of a series of potent 5-(2-methylbenzothiazol-5-yloxymethyl) isoxazole-3-carboxamide derivatives, led to potent antitubercular active against...
replicating *Mycobacterium tuberculosis* H37Rv. The most potent compound 64 (Fig. 31) was found to inhibit *Mtb* growth with MIC values of 1.4 μM.

### 1.6.19 Pyrrole derivatives

![Pyrrole derivatives](image)

**Figure 32.**

Several new pyrrole derivatives\(^{205}\) have been reported as moderate active against *Mtb* and *M. avium*. At present, quantitative structure-activity relationships and comparative molecular field analysis are currently under way for various pyrrole derivatives with MICs of 0.5 to >250 μg/ml. It has recently been found that the thiomorpholine introduction in BM 212 molecule improved its antimycobacterial activity.\(^{206,207,208}\) Four compounds of this series 65, 66, 67 and 68 had MIC between 1 and 2 μg/mL (Fig. 32).

### 1.6.20 Capuramycin analogs

![Capuramycin analogs](image)

**Figure 33.**

Capuramycin\(^{209}\) and Capuramycin analogs, which possess an inhibitory activity against phospho-MurNac-pentapeptide translocase and inhibit the peptidoglycan assembly of mycobacterial organisms, were reported to exhibit potent antimycobacterial activities *in vitro* and *in vivo*. *In vivo* experiments using a murine experimental TB model, other capuramycin analogs, RS-112997 (69) (Fig. 33) and RS-124922, the MIC\(_{50s}\) against
MDR-MTB strains of which were 16 and 4\(\mu\)g/mL, respectively, exhibited appreciable levels of therapeutic efficacy.

### 1.6.21 Pyrimidine and purine nucleoside analogues

![Chemical structures](image)

Figure 34.

Thymidine analogues (Fig. 34) were prepared and they inhibit the \textit{M. tuberculosis} thymidine kinase.\textsuperscript{210-217} Bicyclic thymine 70 is a weak inhibitor of \textit{M. bovis} growth.\textsuperscript{217} Dodecynyl bearing cytidine analogues 71 or the acetylated derivative 72 are the most effective for inhibition of mycobacterial growth.\textsuperscript{217} Purine nucleoside analogues (Fig. 34) were tested for their activity against \textit{M. tuberculosis} and 2-methyladenine 73 is a good inhibitor of \textit{Mtb} growth. The target this is associated with DNA synthesis.\textsuperscript{218} Adenosine analogue 74 was inhibiting adenosine kinase.

### 1.6.22 Trimethoprim and triazine

![Chemical structures](image)

Figure 35.

The antibacterial trimethoprim\textsuperscript{219} (75) and epiroprim\textsuperscript{220} (76) are poorly effective on mycobacteria and many analogues were synthesized and compound good active against
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*M. avium* (Fig. 35). Triazine (77)\(^{221}\) was having inhibitory activity against mycobacterial growth and its mode of action to inhibit dihydrofolate reductase.

1.6.23 Arylcyclopropylmethanol

![Figure 36.](image)

In our group a series of cyclopropylphenylmethanone and cyclopropylphenylmethanol were synthesized and most of them possessed very good *in vitro* activity against both sensitive and drug resistant *M. tuberculosis*.\(^{222}\) The most active compounds of the series was found to be cyclopropyl[4-(4-methoxybenzylxyloxy)phenyl]methanol, 78 (Fig. 36). It has MIC 3.12 µg/mL against *M. tuberculosis* H37Rv and has moderate *in vivo* activity.

1.6.24 Tetrahydroindazole based compounds

![Figure 37.](image)

Very recently a novel class of tetrahydroindazole based compounds were reported as potent and unique inhibitors of *Mt b*.\(^{223}\) Compounds 79, 80 and 81 (Fig. 37) exhibited activity range against *Mycobacterium tuberculosis* (R-TB), with MICs of 1.7, 1.9, and 1.9 µM respectively.

1.6.25 Piperidin-4-ones derivatives

Several piperidinone derivatives were reported as potent antitubercular agents.\(^{224,225,226,227}\) 4-(4-Fluorophenyl)-5-phenylpyrrolo(spiro[2.3'']oxindole)spiro[3.3']-1'-methyl-5'-(4-fluorophenylmethylidene) piperidin-4'-one (82) (Fig. 38) was found to
be active \textit{in vitro} with a MIC value of 0.07 \mu M against \textit{Mtb} and was 5.1 and 67.2 times more potent than isoniazid and ciprofloxacin, respectively.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure38.png}
\caption{Figure 38.}
\end{figure}

\textit{In vivo}, compound 82 decreased the bacterial load in lung and spleen tissues with 1.30 and 3.73-log 10 protections respectively and was considered to be promising in reducing bacterial count in lung and spleen tissues.

\subsection*{1.6.26 1,4-Dihydropyridine derivatives}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure39.png}
\caption{Figure 39.}
\end{figure}

1,4-Dihydropyridine based compounds have shown significant anti-tubercular activity.\textsuperscript{228,229,230} The most potent compound 83 (Fig. 39) exhibits comparable anti-tubercular activity with MIC = 1 \mu mol/mL \textit{in vitro} screen. However no \textit{in vivo} data is available.

\subsection*{1.6.27 Chromene and chromane derivatives}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure40.png}
\caption{Figure 40.}
\end{figure}
Further the antimycobacterial activity of the chromene (84), chromane (85) and its analogue was reported (Fig. 40).\textsuperscript{231,232,233} Both compounds meet the criteria required for further tuberculosis drug development, with MICs measured on \textit{M. tuberculosis} below 6.25 \textmu g/ml and selectivity indexes above 10.

\textbf{1.6.28 Isoxazoline derivative}

![Isoxazoline derivatives](image)

\textbf{Figure 41.}

The anti-tubercular activity of isoxazoline linked nitrofurans compounds 86-91 (Fig. 41) have been reported recently by Tangallapally \textit{et al.}\textsuperscript{234} Further Barbachyn \textit{et al}\textsuperscript{235} have described very good \textit{in vivo} efficacy in analogue of phenylisoxazoline 91 with MIC as low as 0.5 \textmu g/mL (Fig. 41).

\textbf{1.6.29 Thiadiazine thiones}

![Thiadiazine thiones](image)

\textbf{Figure 42.}

In our group the potent \textit{in vitro} and moderate \textit{in vivo} antitubercular activities have been reported in thiadiazine thiones recently.\textsuperscript{236} One of the compound 92 has shown potent \textit{in vitro} anti-tubercular activity against \textit{M. tuberculosis} H37Rv even in resistant strains and also protected mice marginally in experimental TB (Fig. 42).

\textbf{1.6.30 Antitubercular activities in natural products}
Four different natural product series (Fig. 43) were reported for their antimycobacterial properties. The basiliskamide A (93)\(^{237}\) and quinolone (94)\(^{238}\) share a lipophilic character and the pamamycin- (95)\(^{239}\) or the lydiamycin A (96)\(^{240}\) a macrocyclic structure.

\[ \text{Figure 43.} \]

**1.6.31 Salicylanilide carbamates**

Salicylanilide carbamates derivatives (97) exhibited very good in vitro activity against *Mycobacterium tuberculosis*, *Mycobacterium kansasii* and *Mycobacterium avium* and in particular against five multidrug resistant strains with MIC values between 0.5–2 \( \text{Imol/L} \) (Fig. 44).\(^{241}\)

\[ \text{Figure 44.} \]

**1.6.32 Dipepiridine derivatives**

A novel series \(^{242}\) of dipepiridine (98, 99 and 100, Fig. 45), structurally unrelated to any existing antitubercular drugs exhibited MIC values as low as 7.8 \( \mu \text{M} \), the ability to induce promoter Rv0341 activated in response to cell wall biosynthesis inhibition,
relatively low nonspecific cellular toxicity in the range of 30-162 μM, and log P values less than 4 (Fig. 45).

![Figure 45.](image)

**1.6.33 Spiro-pyrrolothiazoles**

Few of the spiroheterocycles displayed good in vitro antimycobacterial activity against *MTB* and MDRTB. The antimycobacterial potency of these spiro heterocycles renders them valid leads for synthesizing new heterocycles endowed with enhanced activity.

![Figure 46.](image)

Compound 101 was found to be the most active with a minimum inhibitory concentration (MIC) of 0.6 lM against *MTB* and MDR-TB (Fig. 46).

**1.6.34 Hybrids of cinnamic acids and guanylhydrazones**

Phenylacrylamide derivative incorporating cinnamic acid and guanylhydrazones were evaluated against *M. tuberculosis* H37Rv using resazurin microtitre plate assay (REMA). Compound 102 showed MIC of 6.49 μM along with good safety profile of >50-fold in VERO cell line (Fig. 47).
In addition to above molecules other class of molecules that have been evaluated for antitubercular activities are sugar triazole, imidazole derivatives, glycosyl ureas, tetrahydroacridines, bis(benzylidene)-cycloalkanones, substituted phenylmethyl- and pyridylmethyl amines, dibenzo[b,f]oxepins, 2aryl-3-(1H-Azol-1-yl)-1H-indole derivatives, S-alkylisothiosemicarbazone derivatives, thioridazine derivatives, 4-(coumarinyl)-4-thiazolin-2-one-benzylidene-hydrazones, cyclohexadiene, N-(2-naphthyl)glycin hydrazide analogous, isoxazoles and cynopyridines, benzoyl thiazole-2-carbamates, thiosemicarbazones, dithiocarbamates, succinamides, diarylsuccinamides, glutaconyl-thiosemicarbazides, 2,2-dithio-bis (benzamides), triazoles, 2-azetidinones, pyrimidines, pyrazine carboxylic acid, pyrazines, a-oxo-ketene dithioacetals, spirothiazolidinones, isonicotinoyl hydrazones, S-alkylisothiosemicarbazone, thiosemicarbazone, hydrazinecarboxamides, pyrazolines, 4-aryl hydrazono-2- pyrazoline-5-ones, N-alkyl-1,2-dihydro-2-thioxo-3-pyridine carothioamides, 2-benzylthiopyridine-4-carbothioamides, 2,5- disubstituted 1,3,4-oxadiazoles, sydnones, pyrazinoyl heterocycles, pyrazinoic acid hydrazide, azetidinone, pyrazine carboxylic acids isosteres, ureas and thioureas, thiosemicarbazones, hdantoins, and hydrazones.

**1.7 Conclusion and perspectives**

Tuberculosis (TB) is presenting new challenges as a global public health problem, especially due to HIV co-infection, multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis*. The reappearance of TB has driven an increased interest in understanding the mechanisms of drug action and drug resistance, which could provide a significant contribution in the development of new antimicrobials. Modern molecular and genetic tools have become available in
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understanding the pathogenesis of, *M. Tuberculosis* viz, targeted mutagenesis, and array based analysis of mutant libraries, techniques for conditional gene silencing. This has led to remarkable improvements in the knowledge and understanding of the fundamental biology and physiology of *M. Tuberculosis*. Today in vast amount of screenings of available chemical libraries on many identified targets.293,294

This review has summarized the global disease burden of TB, brief history of antitubercular drug discovery and developments. Existing drugs, drugs under clinical trials and their possible targets have also been addressed. Promising leading chemical entities has been discussed.

1.8 References

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