PART A

Chapter 1

Tuberculosis: A Review
1.1. Introduction

Tuberculosis (TB), an airborne infectious disease caused predominantly by *Mycobacterium tuberculosis* (*Mtb*), is a global health problem and a leading cause of death among adults in the developing world. According to the World Health Organization (WHO), one third of the world’s population is infected with *Mycobacterium tuberculosis* (*Mtb*). In 2008, there were an estimated about 9.4 million incident cases of TB, 11.1 million prevalent cases of TB, 1.3 million deaths from TB among HIV-negative people and an additional 0.52 million TB deaths among HIV-positive people.\(^1\) Owing to population growth, the number of new cases arising each year is increasing globally, posing a continued health and financial burden in various parts of the world, particularly Asia and Africa. When coupled with the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* (*MDR-TB*),\(^2\) the scale of the problem becomes clear, as it will inevitably become even more difficult to treat TB in the future. It is now more than a decade since the World Health Organization declared TB “a global health emergency”.\(^3\)

Treatment of tuberculosis is protracted and burdensome.\(^4\) Tuberculosis control is further complicated by the synergy between tuberculosis and HIV/AIDS\(^5,6\) and by the development of multi-drug resistant tuberculosis (MDR-TB), which can be defined as strains that are resistance to at least isoniazid and rifampicin, important first line drugs used in TB treatment.\(^7\) Another serious problem, in the context of MDR-TB, is the XDR-TB [abbreviation for extensively drug-resistant tuberculosis (TB), defined as multidrug-resistant tuberculosis plus resistance to a fluoroquinolone and an injectable second-line drug (capreomycin, kanamycin, or amikacin)], has recently emerged as a public health threat.\(^8,9\) Furthermore, common HIV/AIDS anti-retroviral therapies are not compatible with the current TB regimen because of shared drug toxicities and drug interactions, for example, as a consequence of rifampicin-induced cytochrome P\(450\) activation.\(^10,11\) This has spurred new efforts to find new anti-tuberculosis drug candidates with novel modes of action, develop pipelines for drug discovery and development and, in particular, try to find new regimens that can considerably shorten the duration of effective therapy.
which would improve patient compliance and slow down the emergence of drug resistant strains.\textsuperscript{12-17}

1.2. The biology of \textit{Mycobacterium tuberculosis}

\textit{M. tuberculosis}, the agent of human TB, was discovered in 1882 by Robert Koch and for a long time called after his name (the \textit{Koch bacillus}). \textit{Mycobacterium} genus are slow growing, aerobic Gram-positive bacteria that share the property of acid-fastness (Ziehl-Neelsen staining), due to their mycolic acid rich cell wall structure. The genus mycobacterium includes the highly pathogenic organisms that cause tuberculosis, \textit{Mycobacterium tuberculosis} (\textit{M. tuberculosis}) and sometimes \textit{M. bovis} and \textit{M. leprae} (leprosy).

Tuberculosis (TB) is an infectious disease caused spread almost exclusively by airborne transmission. Although the disease can affect any site in the body, it most often affects the lungs. When persons with pulmonary TB cough, they produce TB bacteria containing tiny droplets which can remain suspended in the air for prolong periods of time. Anyone who breathes air that contains these droplet nuclei can become infected with TB.

The cell wall of Mycobacterium species in its full structural and functional integrity is essential for its growth and survival in the infected host. In fact, some of the most effective anti-mycobacterial drugs including isoniazid and ethambutol are known to inhibit the biogenesis of cell wall dominated by covalently linked mycolic acids, arabinogalactan and peptidoglycan (AGP), the mycolic acids of which are complimented by glycolipids such as $\alpha,\alpha$-trehalose monomycolate (TMM).\textsuperscript{18} This mycolic acid based permeability barrier shields the organism from environmental stress and contributes to disease persistence and the refractoriness of \textit{M. tuberculosis} to many antibiotics. One of the most prominent macromolecular entities of mycobacterial cell wall is arabinan, a common constituent of both arabinogalactan (AG) and lipoarabinomannan (LAM). In the chemical setting of the mycolylarabinogalactan-peptidiglycan complex, AG forms an
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Integral part of cell wall proper, whereas LAM, based on a phosphatidylinositol anchor, apparently exists in a state of flux. LAM is an essential part of the cell envelope, which lacks covalent association with the cell wall core. Anchored in the cell membrane and traversing the cell wall, as well appearing as an excretory product, LAM has been implicated as a key surface molecule in host-pathogen interactions. The biosynthetic pathways leading to formation of the key mycobacterial cell wall components AG and mycolic acids, are the targets for the rational design of new anti-tubercular agents.

The determination of the complete genome sequence of M. tuberculosis\textsuperscript{19} has had a profound effect on researchers in the TB field. New approaches are being followed that depend upon the availability of this information. These include detailed comparative genomics using bioinformatics, functional genomics, proteomics, transcriptomics, and structural genomics. Combined new tools for manipulating the genome of M. tuberculosis, offers a better understanding of the complex biology of this pathogen. Recently, CSIR, India has initiated an Open Source Drug Discovery (OSDD) program under which the genome of Mycobacterium tuberculosis has been comprehensively mapped, compiled and verified.

1.3. Multidrug resistant tuberculosis (MDR-TB)

It refers to the simultaneous to at least Isoniazid (INH) and Rifampicin (RIF) with or without resistance to other drugs. Multidrug-resistance arises from the sharing of genes between different species or genera generally mediated by small pieces of extrachromosomal DNA known as transposons or plasmids.\textsuperscript{20} Some antibiotics can actually induce the transfer of these resistance genes.\textsuperscript{21} Alternatively, as with the problematic multidrug-resistant M. tuberculosis (MDR-TB) strains accumulation of multiple point mutations in the chromosomal DNA can take place.\textsuperscript{22} Contamination of some commercial antibiotic preparations with the DNA (containing the inherent resistance genes) of the organisms that produce the antibiotic has been implicated as a source of drug resistance genes. The presence of DNA encoding drug resistance in antibiotic
preparations has been proposed as a factor in the rapid development of multidrug resistance in bacteria.23

1.4. Extensively drug resistant tuberculosis (XDR-TB)

XDR-TB, defined as extensively drug-resistant tuberculosis are cases of TB disease in persons whose *M. tuberculosis* isolates were resistant to isoniazid and rifampin and at least three of the six main classes of second-line drugs (aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine and *p*-aminosalicylic acid).24 XDR-TB is related to the poor management of multidrug resistant tuberculosis cases (which in turn is the consequence of poorly managed susceptible TB).25,26 As per the new definition of XDR-TB it is defined as the MDR-TB that is resistant to quinolones and also to any one of kanamycin, capreomycin or amikacin.27 The principles of treatment of MDR-TB and for XDR-TB are the same. The main difference is that XDR-TB is associated with a much higher mortality rate than MDR-TB, because of a reduced number of effective treatment options. The epidemiology of XDR-TB is currently not well studied, but it is believed that XDR-TB does not transmit easily in healthy populations, but is capable of causing epidemics in populations which are already stricken by HIV and therefore more susceptible to TB infection.28

1.5. Transmission and pathogenesis

A microbe becomes a pathogen when its biochemical pathways, either individually or acting in concert with one another, causes disease in a host. In microbial pathogenicity, two terms encountered are infection and disease. Infection refers to the multiplication (or colonization) or the persistence of the organism within the host environment, while disease refers to the significant damage caused by the organism in the host due to the infection.

*M. tuberculosis* is an intracellular pathogenic bacterium, which has developed sophisticated mechanisms to survive inside host monocellular phagocytic cells and thus
evade the immune system. TB bacilli usually multiply first in the macrophages in the lung alveoli and alveolar ducts and in draining lymph nodes. Infected macrophages eventually get killed, progressively creating a primary tubercle. Delayed cutaneous hypersensitivity develops and together with other cellular immune reactions leads to the caseous necrosis of the primary complex. CD4+ T-cells accumulate in great numbers in the early granulomatous lesions, where they are later joined by CD8+T-cells. Bacilli eventually spread to many parts of the body such as liver, spleen, meninges, bones, kidneys and lymph nodes, where they can either be a source of over disseminated TB or, more commonly, remain dormant. CD4+ T-cells play a major role in containment of infection: progressive TB is usually associated with a Th2 T-cell response, whereas a pure Th1 response mediates protection. Th1 type cytokines, notably IFN-γ and TNF-α, are instrumental in walling off *M. tuberculosis* inside granulomatous lesions and controlling the evolution of the disease. In addition, T cells expressing a γδ T-cell receptor with specificity for small phosphorylated ligands and T-cells with specificity for glycolipids are stimulated. Individuals with deficient IFN-γ signaling suffer from rapid evolution of the disease. Treatment with anti-TNF-α antibodies readily leads to tuberculosis reactivation in patients with rheumatoid arthritis. Granulomas persist for years and efficiently contain *M. tuberculosis* in a state of dormancy, as long as the host remains immunocompetent. Occasional decline in cell-mediated immunity leads to reactivation tuberculosis, most frequently seen in adults as a pulmonary disease with infiltration or cavity in the apex of the lung. This is the most infectious form of TB.

### 1.6. Current anti-tuberculosis drugs

Currently, TB chemotherapy is made up of a cocktail of first-line drugs, isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB), given for six months. If the treatment fails as a result of bacterial drug resistance, or intolerance to one or more drugs, second-line drugs are used, such as para-aminosalicylate (PAS), kanamycin, fluoroquinolones, capreomycin, ethionamide and cycloserine, that are generally either less effective or more toxic with serious side effects.
The WHO-recommended DOTS (directly observed treatment, short course) anti-TB therapy involves the administration of four drugs: isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) or streptomycin (SM). Treatment with these so called first-line drugs is carried out initially over two months, leading to the destruction of bacteria in all growth stages, 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{36,37}

\textbf{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>
1.6.1. *Streptomycin (1943)*

![Chemical structure of Streptomycin](image)

**Streptomycin (1)**

![Chemical structure of Dihydrostreptomycin](image)

**Dihydrostreptomycin (2)**

1, an aminoglycoside antibiotic derived from *Streptomyces griseus* was the first antibiotic remedy for tuberculosis. It is made up of three components streptidine, streptose and N-methyl-L-glucosamine. Because of its poor absorbance from gastrointestinal tract it is administered intramuscularly and very occasionally by intrathecal route. It has an MIC value of 1 μg/ml with 50-60% plasma protein bound and a half life of 5-7 hr. It penetrates the inner membrane of *M. tuberculosis* and interferes with the binding of formyl-methionyl-tRNA to the 30S subunit of the ribosome. 38 Different synthetic derivatives of streptomycin (dihydrostreptomycin, 2) have been synthesized and evaluated against *M. tuberculosis* (Fig. 6) 39-41 and was found to have almost the same antibacterial activity as the parent compound. 42 Mutations in the rpsL gene of the ribosomal S12 protein of mycobacteria or base substitutions in the 16S rRNA region confers resistance to streptomycin. The toxic effects of STR are mainly manifested on peripheral, central nervous system at higher dosage and hypersensitivity reaction, streptomycin is not a popular choice for treating tuberculosis. Dihydrostreptomycin (2) once thought to be less toxic cause severe damage to eighth cranial nerve, inducing irreversible impairment of auditory function. 42
1.6.2. 4-Aminosalicylic acid (PAS, 1944)

4-Aminosalicylic acid (3), commonly known as PAS was used as an oral TB therapy reported in 1946, although it was synthesized long before.\textsuperscript{43} It is available in the form of sodium and calcium salt. p-aminosalicylic acid acts as an inhibitor of \textit{M. tuberculosis} by impairing folate synthesis. Following DOTS (directly observed therapy, short course), it is rarely used today. However, it is occasionally used in the regimens for the treatment of TB caused by MDR-TB.\textsuperscript{44} 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.\textsuperscript{45-47} It is thought to act via NF-κB (nuclear factor-kappa B) inhibition and free radical scavenging.

1.6.3. Isoniazid (INH, 1952)

Isoniazid (4) also known as isonicotinylhydrazine (INH) was discovered in 1952 and is a highly potent drug for the treatment of tuberculosis.\textsuperscript{48,49} It is highly selective, acts almost exclusively against the \textit{M. tuberculosis} complex (\textit{M. tuberculosis}, \textit{M. bovis}, \textit{M. africanum} and \textit{M. Microti}) and produces side effects in only 5% of patients. It is orally active and exhibits bactereostatic action on the resting bacilli and has very low MICs (0.02-0.06 μg/ml)\textsuperscript{50} against these pathogens. INH is available in tablets, syrup and injectable forms (given intramuscularly and intravenously). It inhibits the synthesis of mycolic acids (long chain α-branched β-hydroxylated fatty acids) in \textit{M. tuberculosis} by affecting the enzyme mycolate synthetase, which is unique for mycobacteria.\textsuperscript{51,52}
Isoniazid (4) is a prodrug and must be activated by bacterial catalase. INH enters the mycobacterial cell by passive diffusion. Recent developments have shown that peroxidative activation of isoniazid by the mycobacterial enzyme KatG generates isonicotinic acyl anion or radical that form adducts with NAD+ and NADP+. This complex will bind tightly to ketoenoylreductase known as InhA and prevents access of the natural enoyl-AcpM substrate. This mechanism inhibits the synthesis of mycolic acid in the mycobacterial cell wall.

1.6.4. Pyrazinamide (PZA, 1970)

Pyrazinamide (5) is a derivative of nicotinamide, is first line drug of short course tuberculosis therapy. It is active against semi dormant bacilli not affected by any other drug and is used only in combination with other drugs such as isoniazid and rifampin and shortens the therapy period to 6 months. PZA in conjunction with rifampin is a preferred treatment for latent tuberculosis. The drug has no significant bactericidal effect and is thought to act by sterilizing effect, killing persisting semi-dormant bacilli in the lungs.

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. Resistance to PZA is usually accompanied by mutation in the pncA gene responsible for the production of pyrazinamidase. Some pyrazinoic esters have also been reported to possess good anti-tubercular activities.

1.6.5. Cycloserine (1955) D-Cycloserine (6), chemically defined as D-4-amino-3-isooxazolidone, is a structural analogue of amino acid D-alanine, is derived from Strepto-
-myces orchidaceus and is active against a broad spectrum of bacteria, including *M. tuberculosis*\(^{63}\) at concentrations of 5-20 µg/ml. \(6\) is well absorbed and distributed throughout the body following oral administration. It blocks peptidoglycan biosynthesis by inhibiting the enzyme D-alanine racemase and D-alaninyl alanine synthetase,\(^ {64}\) enzyme necessary for the synthesis of UDP-muramyl-pentapeptide. 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.\(^ {65,66}\) Cycloserine (6) produces severe side effects in the central nervous system that can also generate psychotic states with suicidal tendencies and epileptic convulsion. The drug is stable in alkaline solution but is rapidly destroyed when exposed to neutral or acidic pH.

1.6.6. Ethionamide (ETH, 1966)

Ethionamide (7) is a derivative of isonicotinic acid and is bacteriostatic in nature. ETH is useful for treating drug-resistant tuberculosis, but it causes frequent toxic side effects such as anorexia, vomiting, dysgeusia, neurological reactions and reversible hepatitis. In the last six years it was confirmed that this compound is a prodrug and is oxidized by ETH A (a flavoprotein monooxygenase).\(^ {67-70}\) Oxidation of ethionamide by ETH A enzyme leads to a sulfinic acid which is likely to be further transformed to an amide and alcohol.\(^ {58}\) Moreover, it had been known that the sulfinic acid produced was as active on mycobacterial growth *in vitro* as ethionamide.\(^ {72-75}\)
1.6.7. Kanamycin (1957)

Kanamycin (8) sulfate is an aminoglycoside antibiotic and is used either as orally or intravenously. It is isolated from *Streptomyces kanamyceticus*.\(^7^6\) 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.

1.6.8. Ethambutol (EMB, 1968)

Commonly abbreviated EMB (9), is a synthetic amino alcohol (ethylene diamino-di-1-butanol).\(^7^7-7^9\) It is orally effective bacteriostatic antimycobacterial drug\(^8^0\) that is active against most strains of Mycobacterium. It disrupts arabinogalactan synthesis by inhibiting the enzyme arabinosyl transferase. Mycolic acids attach to the 5'-hydroxyl groups of D-arabinose residues of arabinogalactan and form mycolyl-arabinogalactan-peptidoglycan complex in the cell wall. Disruption of the arabinogalactan synthesis inhibits the formation of this complex and leads to increased permeability of the cell wall.\(^8^1\) Activity of EMB is stereospecific as *dextro* isomer exhibited maximum antitubercular activity (S,S form is 600 times more active than R,R). 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. The genes *embAB* of *M. avium* encode the drug target for EMB, the arabinosyl transferase responsible for the polymerization of arabinose into the arabinan
of arabinogalactan and overproduction of this ethambutol-sensitive target leads to EMB resistance. 82

1.6.9. Quinolones (1963)

Fluoroquinolines (FQ), synthetic derivatives of nalidixic acid, display broad-spectrum anti-mycobacterial activity 83-85 and it is used as part of multidrug regimens. It has good in vitro potency with MIC 1μg/ml. 86 These compounds have a good distribution throughout the body tissues and fluids following oral administration. The main effects of fluoroquinolines involve the interaction of the drugs with DNA-gyrase and DNA-topoisomerase IV. 81-88

![Chemical structures of ciprofloxacin and sparfloxacin](image)

Ciprofloxacin (10)  
Sparfloxacin (11)

R/S = Ofloxacin (12a)  
S = Levofloxacin (12b)

Derivatives recognized with activity against mycobacteria are ciprofloxacin (10), sparfloxacin (11), ofloxacin (12a), levofloxacin (12b) etc. Moxifloxacin (BAY 12-8039) is an 8-methoxyquinolone and is one of the most active quinolones against bacteria with resistance to penicillins and macrolides, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *M. tuberculosis*. 88-94

1.6.10. Rifamycin derivatives (RIF, 1966)

Rifampicin (13, INN) or rifampin (USAN) is a bactericidal antibiotic drug belongs to rifamycin group of semisynthetic antibiotics isolated from *Streptomyces mediterrani*. 95 It
is characterized by chromophoric naphthohydroquinone group spanned by a long aliphatic bridge and are potent inhibitors of prokaryotic DNA-dependent RNA polymerase, an enzyme necessary for RNA synthesis. RIF acts by inhibiting DNA-dependent RNA polymerase in bacterial cells by binding its beta-subunit resulting in the formation of a stable complex leading to suppression of transcription to RNA and subsequent translation to proteins. More specifically, the beta-subunit of this complex enzyme is the site of the action of the drug, although RIF binds only to the holoenzyme. 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 RIF may increase risk of hepatotoxicity. RIF inhibits M. tuberculosis at concentrations ranging from 0.1 to 0.2 μg/ml.

1.7. Drugs currently in clinical evaluation for TB

Drug classes currently being evaluated in the clinic for their potential contribution to shortening treatment of active TB fall into two categories: (i) those already used in either first- or second-line TB treatment, or (ii) those that have completely novel mechanisms of action for TB. The former includes rifamycins, fluoroquinolones, and oxazolidinones. The latter includes nitroimidazoles, diarylquinolines, ethylene diamines, and pyrroles.
1.7.1. First- and second-line TB drug classes undergoing new clinical evaluation for a TB indication

1.7.1.1. Rifamycin derivatives

Rifamycins, in particular rifampicin (13), are currently a cornerstone of first-line TB drug treatment, are now being re-explored for use at relatively high dosages.99-101 Rifamycin derivatives, such as rifalazil (14, RLZ, also known as KRM1648 or benzoxazinorifamycin), rifabutin (15) and rifapentine (16) have been synthesized to improve antimycobacterial activity and prolong half life.

Rifapentine (16) was approved by the FDA in 1998 appears to be safe and well-tolerated at once-weekly dosing and is currently being evaluated in Phase III efficacy trials for treatment of latent tuberculosis.102 Previously, relatively high intermittent doses of rifamycins have occasionally led to toxicities, primarily a flu-like syndrome, but this appears to be less of a problem when the rifamycin is dosed daily, and also less of an
issue with rifapentine (16) than with rifampicin (13). The underlying mechanism of the rifamycin-associated flu-like syndrome has not been definitively elucidated, although it is believed to be an immunoallergic response.\textsuperscript{101,103} RLZ (14) is a new semi synthetic rifamycin derivative with a long half life, which is highly active against a range of intracellular bacteria including \textit{M. tuberculosis}, \textit{Mycobacterium avium}, \textit{Chlamydia trachomatis}, \textit{Chlamydia pneumoniae}, and \textit{Helicobacter pylori}\textsuperscript{104} and is more active than RIF or rifabutin against \textit{M. tuberculosis} in mice both \textit{in vitro} and \textit{in vivo}.\textsuperscript{105} RIF-resistant strains confer cross-resistance to all rifamycins, including RLZ,\textsuperscript{106} limiting the use of RLZ in the treatment of RIF-resistant TB.

\subsection{1.7.1.2. Fluoroquinolones}

The most efficacious of the fluoroquinolones against \textit{M. tuberculosis} appear to be the 8-methoxy fluoroquinolones, gatifloxacin (17, GATI) and moxifloxacin (18, MXF) have a longer half life and are more active against \textit{M. tuberculosis} than ofloxacin and ciprofloxacin.\textsuperscript{107-109}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{fluoroquinolones.png}
\caption{Gatifloxacin (17) and Moxifloxacin (18)}
\end{figure}

Each of these drugs is currently being evaluated in a Phase III pivotal trial for its ability to substitute for either ethambutol (GATI; MXF) or INH (MXF) in first-line TB treatment and to shorten therapy to four months from the current standard of six to nine months.\textsuperscript{110,111} Combination therapy with MXF seems to be as effective as current standard drug combinations.\textsuperscript{112}

\subsection{1.7.1.3. Oxazolidinones}

Oxazolidinones, discovered at DuPont in the 1970s and later sold to Pharmacia Upjohn, are a new class of compounds inhibits protein synthesis at an early stage by binding to
23S rRNA of the 50S ribosomal subunit. Linezolid is the first oxazolidinone to be developed and approved by the FDA to treat single- or multiple-resistant Gram-positive bacterial infections. Concerning the use of this class against tuberculosis, many active compounds were found. However; the long term use of linezolid may be plagued with forbidding side effects including anemia and peripheral neuropathy. Two novel oxazolidinones, Ranbezolid/RBx7644 and RBx8700, were active against MDR-TB and tubercle bacilli inside macrophages.

1.7.2. Drug classes with novel mechanisms of action undergoing clinical evaluation for a TB indication

1.7.2.1. Nitroimidazoles

Two novel nitroimidazoles are currently in clinical development: PA-824, a nitroimidazo-oxazine, is being developed by the Global Alliance for TB Drug Development (TB Alliance) and OPC67683, a nitro-dihydro-imidazo-oxazole, is being developed by Otsuka Pharmaceutical Company.

PA824 is highly active with MIC as low as 0.015–0.250 µg/ml against M. tuberculosis and MDR-TB and is currently undergoing Phase II clinical trials. The mechanism of action of PA-824 is two-fold, as it inhibits M.tb cell wall lipid and protein.
synthesis; however, since this drug is also active against non-replicating bacteria it appears that inhibition of cell wall biosynthesis cannot be its sole mode of action. PA-824 is, in fact, a prodrug that is metabolised by \textit{M. tb} before it can exert its effect and that may probably involve the bioreduction of its aromatic nitro group to a reactive nitro radical anion intermediate. Drug resistance has been shown to be mediated by the loss of a specific glucose-6-phosphate dehydrogenase enzyme or of its deazaflavin cofactor F420, which may provide electrons for the reductive reaction.

\textbf{OPC-67683 (22)} exhibits excellent in vitro activity against drug-susceptible and resistant \textit{M. tb}. strains and does not show cross-resistance with antituberculosis drugs, with the evidence that infrequent and low dosing may be effective. It inhibit the synthesis of mycolic acid at the stage of methoxy-mycolic and the keto-mycolic acid syntheses (like INH) but at significantly lower concentrations. Recently completed phase II clinical trials of OPC-67683 have been successful.

\subsection*{1.7.2.2. Diarylquinolines (DARQ's)}

A series of diarylquinolines (DARQ's) has been developed by the Johnson and Johnson group that exhibits potent \textit{in vitro} activity against \textit{M. tb}. Modification of diarylquinolines led to the identification of diarylquinoline \textbf{R207910 (23)} (also known as \textit{J compound} and \textit{TMC207}) as the most potent, with minimum inhibitory concentration (MIC) of 0.003 \( \mu g/ml \) for \textit{M. smegmatis} and 0.030 \( \mu g/ml \) for \textit{M. tuberculosis}.

\begin{center}
\textbf{R207910/TMC207 (23)}
\end{center}

\textbf{TMC207 (23)} exhibits excellent activity against drug susceptible, MDR and XDR \textit{M. tb} strains, with no cross-resistance to current first-line drugs. Mutant strains of \textit{M. tuberculosis} generated by treatment with R207910 remained sensitive to the major
clinical drugs, suggesting a unique mechanism of action.\textsuperscript{131} Evaluation of compound 23 in combination with various clinical drugs showed that the use of 23 alone appears to be at least as effective as a combination of RIF, INH and PZA and more effective than RIF alone in mouse models.\textsuperscript{131}

It acts by blocking the function of an essential membrane-bound enzyme that makes adenosine triphosphate (ATP). The two mutations affect the membrane-spanning \( \alpha \) helices of the ATP synthase c subunit and may restrict binding of R207910 (23) to the enzyme. Although biochemical confirmation is now required, it is possible that the drug impedes assembly of the mobile disk or interferes with its rotational properties, leading to inadequate synthesis of ATP.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{atp-synthase.png}
\caption{ATP-synthase}
\end{figure}

Structure of ATP synthase: ATP synthase is a biological rotary motor made up of two major structural domains, F0 and F1 (Fig. 1).\textsuperscript{134} F1 domain is composed of nine subunits (\( \alpha_3, \beta_3, \gamma, \delta, \varepsilon \)) and is located in the cytoplasm; the F0 domain spans the cytoplasmic membrane, includes one a subunit, two b subunits, and 9 to 12 c subunits arranged in a symmetrical disk. The F0 and F1 domains are linked by central stalks (subunits \( \gamma \) and \( \varepsilon \))
and peripheral stalks (subunits b and δ). The proton-motive force fuels the rotation of the transmembrane disk and the central stalk, which in turn modulates the nucleotide affinity in the catalytic β subunit, leading to the production of ATP. The c subunit has a hairpin structure with two α helices and a short connecting loop.

**Mode of action:** TMC207 (23) acts by inhibiting Mycobacterium membrane-bound ATP synthase. This unique mechanism of action offers great potential as there is little similarity between the mycobacterial and human proteins encoded by the atpE gene that codes for the c subunit of ATP synthase,\(^{135}\) which has been identified as the specific target of TMC207.\(^{136}\) A number of mutations (I66M and A63P) have been identified in the c subunit of TMC207-resistant strains\(^{135,137}\) near the glutamate residue E61, which is involved in proton transport and is necessary for the synthesis of ATP.\(^{138}\)

![Figure 2. Mode of action of TMC207](image)

Molecular modelling studies of *M. tb* ATP synthase have characterized the binding site of TMC207 and suggested its probable mechanism of action.\(^{138}\) Normally, the sidechain of Arg-186 in the a-subunit adopts an extended conformation that reaches towards Glu-61 in the c-subunit to transfer a proton. This event leads to a conformational change in the c-subunit, making Arg-186 adopt a compact conformation.
and initiating a 308° rotation of the c-subunit. It is believed that the molecular mechanism of action of TMC207 is to mimic the side chain of Arg-186.\textsuperscript{138} TMC207 adopts a folded conformation in solution before binding owing to intramolecular hydrogen bonding,\textsuperscript{139} but this is lost upon entering the binding site, and is being compensated for by the creation of new hydrogen bonds with Glu-61, as shown in Fig.2. The lack of a cavity large enough to accommodate the bulky dimethyl amino group of TMC207 prevents the necessary rotation required for proton transfer, blocking ATP production.

Several potent quinoline-based anti-TB compounds, bearing an isoxazole containing side-chain have been identified. The most potent compounds, 24 and 25, exhibited submicromolar activity against the replicating bacteria (R-TB), with minimum inhibitory concentrations (MICs) of 0.77 and 0.95 μM, respectively.\textsuperscript{140} Also, 24 and 25 were shown to retain their anti-TB activity against rifampin, isoniazid, and streptomycin resistant \textit{Mtb} strains.

A series of 4-quinolylhydrazones was synthesized and tested \textit{in vitro} against \textit{Mycobacterium tuberculosis}, most showed 100% inhibitory activity at a concentration of 6.25 μg/ml. The most potent compound 26 exhibited 100% inhibition at concentration of 2.6 μg/ml.\textsuperscript{141}
A new series of 20 quinoline derivatives possessing triazolo, ureido and thioureido substituents was synthesized.\textsuperscript{142} Compounds 27, 28 and 29 inhibited \textit{Mycobacterium tuberculosis} H37Rv up to 96\%, 98\% and 94\% respectively, at a fixed concentration of 6.25 \( \mu \)g/ml.

Several 3-benzyl-6-bromo-2-methoxy-quinolines derivatives; 30, 31, 32 and 33 have shown 92\textendash{}100\% growth inhibition of mycobacterial activity, with minimum inhibitory concentration (MIC) of 6.25 \( \mu \)g/ml. Molecular modelling and docking studies on well-known diarylquinoline antitubercular drug R207910 (23) showed the presence of phenyl, naphthyl and halogen moieties seems critical.\textsuperscript{143}

![Chemical structures](image)

1.2.7.3. Ethylene diamines

From the structure of ethambutol (9), a modern chemical approach was undertaken leading to the synthesis of many chemical libraries of diaminated analogues.\textsuperscript{144\textendash{}146} Remarkably, SQ109 (34) turned out to be a very efficient antimycobacterial, also effective on MDR strains. Moreover, the favorable pharmacological properties of compound 32\textsuperscript{147} as well as a synergistic effect with other antituberculosis drugs\textsuperscript{148} should lead to clinical trials which were scheduled to start in 2006.
The exact mechanism of action of 34 is not known, although it is believed to target cell wall synthesis in a different manner to EMB as SQ109 is active against EMB-resistant strains, suggesting the existence of a different specific target or activation pathway.\textsuperscript{149}

1.7.2.4. Pyrroles

Pyrrole derivative BM 212\textsuperscript{150} (35) is moderately active against \textit{M. tuberculosis} (MIC = 0.7 to 6.2 µg/ml) and \textit{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 was shown to be active \textit{in vivo} on a murine model even infected with resistant \textit{M. tuberculosis} strains.\textsuperscript{151,152} Till now no scientific results are yet publicly available, compound (35) was placed in a phase I clinical trial.

1.8. New chemical entities

1.8.1. Triclosan analogues

Triclosan\textsuperscript{153} (36) is a large spectrum inhibitor of the FabI, the bacterial NADH-dependent enoyl acyl carrier protein reductases, including, although with lesser strength.\textsuperscript{154-156} The
inhibition takes place via the occurrence of a ternary complex between FabI, NAD and triclosan.\textsuperscript{157-160} The octyl-bearing analogues 37 turned out to be 200-fold more active on an enzyme inhibition model than triclosan (36).\textsuperscript{161} However, another structurally very close from 35, hexachlorophene (38), may act on some bacteria through a different mechanism of action.\textsuperscript{162}

\textbf{1.8.2. Thiolactomycin and analogues}

Thiolactomycin (39) is a natural product isolated from \textit{Nocardia} spp.\textsuperscript{163} which belongs to a family of thiolactone-containing antibiotics.\textsuperscript{164-169} This compound acts by inhibiting the condensing fatty acid synthase type II enzymes\textsuperscript{170,171} (FabH, Kas A and Kas B in the case of \textit{M. tuberculosis}).\textsuperscript{172-173} It has high MIC of 5 \( \mu \text{g/ml} \), but, in the absence of \textit{in vivo} toxicology and \textit{in vitro} cytotoxicity data, it is difficult to judge whether these concentrations are far below the toxic concentrations.\textsuperscript{174} The cyclic \( \beta \)-carbonyl structure of 39 was suggested to mimic the thiomalonate portion of the malonyl-acyl carrier protein.\textsuperscript{175}

\textbf{Other natural compounds} inhibiting the FAS systems are the irreversible inhibitor cerulenin (40a),\textsuperscript{176} the remotely related exomethylenebearing compound C75
(40b) and the recently reported platensimycin (41).\textsuperscript{177,178} However, the first two are probably of a lesser interest from a chemotherapeutic point of view\textsuperscript{179} and no assay of platensimycin (41) on M. tuberculosis growth has been reported yet.  

**CS-biphenyl thiolactomycin analogues** gave a significant enhancement in *in vitro* activity against \(\text{mtFabH}\). Analogue 42 (5-(4-methoxycarbonyl-biphenyl-4-ylmethyl)-4-hydroxy-3,5-dimethyl-5H-thiophen-2-one) gave an IC\(_{50}\) value of 3 \(\mu\)M compared to the parent drug thiolactomycin (75 \(\mu\)M) against \(\text{mtFabH}\). The biological analysis of the library reaffirms the requirement for a linear \(\pi\)-rich system containing hydrogen bond accepting substituents attached to the \textit{para}-position of the CS biphenyl analogue to generate compounds with enhanced activity.\textsuperscript{180}

**1.8.3. \textit{N}-octanesulfonylacetamide (OSA)**

The \textit{N}-octanesulfonylacetamide (43, OSA) was originally designed to inhibit \(\beta\)-keto-acyl-carrier protein synthases of mycobacteria by mimicking one of the transition states arising from the condensation reaction.\textsuperscript{181,182} However, it appears that this compound...
probably acts on a different biochemical target. Nearly all species and strains tested (Mycobacterium tuberculosis H37Rv, Mycobacterium avium complex (MAC), Mycobacterium bovis BCG, Mycobacterium kansasii, and others) including isoniazid and multidrug resistant isolates of M. tuberculosis, were susceptible to OSA, with MICs ranging from 6.25 to 12.5 μg/ml.

1.8.4. **Chromene and chromane derivatives**

Antimycobacterial activity of the chromene (44), chromane (45) and its analogue was reported. Both compounds meet the criteria required for further tuberculosis drug development, with MICs measured on M. tuberculosis below 6.25 μg/ml and selectivity indexes above 10.

1.8.5. **Pyrazinamide analogues**
Pyrazinamide (5) is not active in vitro on many *Mycobacterium* strains, whereas pyrazinoic acid (46), which can result from a pyrazinamidase- catalysed hydrolysis of 5, is active *in vitro* on most. Structure activity relationship studies reported antimycobacterial properties for 5-chloropyrazinamide (47), esters of the pyrazinoic acid (46) or related derivatives. In another approach, the carboxylic moiety of compound 46 was replaced by isosteres of a carboxylic group as for the tetrazole-containing prodrug 48. The quinoxaline prodrug 49 and its pyrazine homologue were also reported to be active on *M. tuberculosis* growth.

### 1.8.6. Pyrrole derivative

Antimycobacterial and antifungal activities of many pyrrole derivatives, have been reported. Design and synthesis of new analogues of **BM212** with various substituents with different substitution patterns were added to both positions 1 and 5 of the pyrrole nucleus were evaluated for activity toward *Mycobacterium tuberculosis*. The best compound (1-(4-fluorophenyl)-2-methyl-3-(thiomorpholin-4-yl)methyl-5-(4-ethylphenyl)-1H-pyrrole, 50) possessed a MIC of 0.25 µg/ml (better than BM212 and streptomycin) and a very high protection index (256), better than BM212, isoniazid, and streptomycin (6, 128, and 128, respectively).

Also, 5-diarylpyrrole derivative 51 showed the same MIC (0.25 µg/ml) value toward MTB previously found for the corresponding 2-methyl analogue (40) and a PI value significantly improved (>512 versus 256) because of the very low cytotoxicity (MNTD>128 µg/ml). Compound 2b proved also to be very active against both MTB H37Rv and MTB rifampicin-resistant strains, being 0.25 µg/ml both the MIC values.
1.8.7. **Thiocarboxyl containing anti-tubercular drugs**

Isoxyl/thiocarlide (52), thiocarboxyl-containing antituberculosis drug, has been used clinically in the past before being supplanted by better drugs. This compound has also a history of cross resistance with ethionamide (7) or thiacetazone which was confirmed more recently. Structurally related biaryl urea (53) is an inhibitor of a Δ5-desaturase in rodents. It is noteworthy that remotely related aryl urea have also been reported recently for its antibacterial activity.

1.8.8. **Thiazolidinones**

Modest antimycobacterial thiazolidinones, such as 54, was reported, targeting the synthetic pathway for the biosynthesis of deoxythymidine-diphosphate-rhamnose, which is the providing cofactor for rhamnose incorporation into mycobacterial cell. The structures are reminiscent of the series of antimycobacterial arylidenehydantoins or other antibacterials such as the benzylidenethiazolidinedione (55) and some thio-analogues, for which no mechanism of action has been reported yet.
1.8.9. Capuramycin and analogues

Analogues of capuramycin (56) as well as functionalized caprazamycins\textsuperscript{215} are studied for their inhibition of \textit{M. tuberculosis} growth.\textsuperscript{216-218} The structure–activity relationship studies led, in one avenue, to the removal of the azepanone moiety and its replacement with aromatic group lead with the most active compounds, such as (RS-118641, 57), and intranasal assay on a mice model led to the conclusion that human trials should be considered.\textsuperscript{219}

1.8.10. Pyrazolinediones

Pyrazolinediones, such as 58, were investigated and were found active on Gram-positive and Gram-negative bacteria.\textsuperscript{220,221}

1.8.11. Clofazimine

Clofazimine (59) and other riminophenazines is effective against \textit{M. Leprae} and its use in the treatment of tuberculosis has been suggested.\textsuperscript{222} However, no specific mechanism of action has been established. Several clofazimine\textsuperscript{223-225} analogs 60-63 are also active in
vivo against *M. tuberculosis*, *M. bovis*, *M. leprae* and *M. avium* having a MIC value of 0.01 to 3.3 µg/ml.

![Clofazimine (59)](image)

1.8.12. Pleuromutilin analogues

The pleuromutilin class of antibiotics, tiamulin and valdemulin are used in veterinary medicine.\(^\text{226}\) They interfere with protein synthesis by binding to the 50S ribosomal subunit and therefore inhibit the peptide bond formation.\(^\text{227,228}\) *N*-benzoyl carbamate derivative \(^\text{64}\)\(^\text{229}\) and the analogue SB-264128 \(^\text{65}\) were found to be active on bacterial strains resistant to the older pleuromutilin derivative valdemulin.\(^\text{230}\)

![64](image)

![SB-264128 (65)](image)

1.8.13. Carboxylic indole derivative

A recent patent describes carboxylic indole derivatives, such as compound \(^\text{66}\), which turned out to be inhibitors of *M.tb* RNA polymerase and thus useful for treating TB.\(^\text{231}\)
1.8.14. Fluoroquinolones

Clinafloxacin (67), had withdrawn from advanced clinical assessment because of unacceptable side effects (hypoglycaemia; phototoxicity). More recently, the compound 68 bearing a remarkable side chain has been patented and turns out to be at least 10-fold more active, on a panel of bacteria, than some of the currently used quinolones. A related compound (if not 68) is undergoing preclinical trials as an antituberculosis drug under the name DW-224.

1.8.15. Folate analogues

The dihydrofolate reductase inhibitors have been reviewed recently. Compound 69 is a good inhibitor of Mycobacterium avium complex growth with a quite high selectivity of action on mycobacteria versus human dihydrofolate reductase. Surprisingly, a related series of analogues, such as 70, are inhibiting M. tuberculosis growth in vitro but do not inhibit mycobacterial dihydrofolate reductase. Triazines such as 71 were also reported for their inhibition of dihydrofolate reductase and of mycobacterial growth in vitro.
1.8.16. Imidazoles

Azole class of antifungal, such as econazole (72) or clotrimazole (73) were found to inhibit the growth of mycobacteria.\textsuperscript{240-242} It is likely that their target in mycobacterium is the P450 mono-oxygenase\textsuperscript{241,242} and that the imidazole moiety is binding the iron of these haem-containing enzymes.\textsuperscript{243} Compound 74 featuring the 2,4-dichloro and 4-chlorophenyl pattern has a slightly better activity than compound 72 or 73.\textsuperscript{244}

Ring-substituted-1H-imidazole-4-carboxylic acid ethyl esters have demonstrated a new class of anti-tuberculosis agents exhibited good anti-tuberculosis activities against
both drug-sensitive and drug-resistant strains of *M. tuberculosis*. The most effective analogues, esters 75 and 76 produced highest efficacy and exhibited >90% inhibition at a concentration of 6.25 µg/ml (MIC < 6.25 µg/ml).^{245}

**1.8.17. Pyrimidine and purine nucleoside analogues**

Thymidine analogues were prepared and they inhibit the *M. tuberculosis* thymidine kinase.\textsuperscript{246-253} Bicyclic thymine 77 is a weak inhibitor of *M. bovis* growth.\textsuperscript{253} dodecynyl bearing cytidine analogues 78 or the acetylated derivative 79 are the most effective for inhibition of mycobacterial growth.\textsuperscript{253} Purine nucleoside analogues (Fig. 34) were tested for their activity against *M. tuberculosis* and 2-methyladenine 80 is a good inhibitor of *Mtb* growth. The target this is associated with DNA synthesis.\textsuperscript{254} Adenosine analogue 81 was inhibiting adenosine kinase.

\[
\text{\includegraphics[width=\textwidth]{chemical_structures.png}}
\]

3′-Bromo analogues of pyrimidine nucleosides as a new class of potent inhibitors were evaluated for *M.tb*. 3′-bromo-3′-deoxy-arabinofuranosylthymine (82)\textsuperscript{255} was the most effective antituberculosis agent in the in vitro assays against wild-type *M. tuberculosis* strain (H37Ra) (MIC\textsubscript{50}=1 µg/ml) as well as drug-resistant (H37Rv)
(rifampicin-resistant and isoniazid-resistant) strains of *M. tuberculosis* (MIC$_{50}$=1-2 μg/ml).

![Chemical structures](image)

Novel anilinopyrimidine analogues have been found to have micro molar activity against *Mycobacterium tuberculosis*. This could potentially generate new lead compounds in the fight against multi-drug resistant tuberculosis. Compound 83 showed activity with MIC at 3.12 mg/ml against *M. tb*.\textsuperscript{256}

### 1.8.18. β-lactam bearing compound

![Amoxicillin](image)

Amoxicillin (84), β-lactam bearing compound analogous to penicillin found to have an antibacterial property. The main mechanism of action is the inhibition of the transpeptidase that cross links the peptide side chains making the peptidoglycan. On the other hand, the, so far, weak activity of this class of compounds have been found on mycobacteria.\textsuperscript{257,258}

### 1.8.19. Heteroarylenamines

The marine alkaloid ascididemin\textsuperscript{259-261} and the synthetic precursor enamine (85)\textsuperscript{262} exhibit antitubercular activity.\textsuperscript{263} Several enamine-containing analogues showed
moderate to good inhibitory activity against *Mycobacterium tuberculosis* H37Rv (*Mtb*) with one analogue (86) demonstrating acceptable toxic selectivity (MIC 0.39 µg/ml, SI 15).264

![Chemical structures of compounds 85 and 86](image)

1.8.20. 5,6-dimethoxy-1-oxo-2,5-dihydro-1H-2-indenyl-5,4-substituted phenyl methanone analogues

![Chemical structure of compound 87](image)

The compound (87) was found to be the most promising compounds active against *M. tuberculosis* H37Rv and isoniazid (INH) resistant *M. tuberculosis* with Minimum inhibitory concentration 0.10 and 0.10 µM among a series of substituted 5,6-dimethoxy-1-oxo-2,5-dihydro-1H-2-indenyl-5,4-substituted phenyl methanone analogues.265

1.8.21. 3-(1H-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480

The thiomorpholine analogue of linezolid, PNU-100480 (88) showed an interesting antimycobacterial activity.266,267 New 3-(1H-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-100480 exhibited a fairly good activity against *Mycobacterium avium* complex (MAC). Compounds 89 and 90 exhibited an interesting activity against MAC, comparable to that of PNU-100480 (MIC50=2.0 µM and MIC50=1.4 µM, respectively).268
1.8.22. WR99210 analogues

WR99210 (91) has been most intensively studied as an inhibitor of the Dihydrofolate Reductase, a key enzyme in folate utilization in the malaria parasite *Plasmodium falciparum*. *Saccharomyces cerevisiae* screen identified WR99210 analogues, 92-94 out of 19 compounds that inhibit *Mycobacterium tuberculosis* DHFR. The triazine DHFR WR99210 has shown be a potent inhibitor of *M. tuberculosis* and *Mycobacterium bovis* BCG growth *in vitro* and that resistance to WR99210 occurred less frequently than resistance to either rifampin or isoniazid. These studies suggest that compounds of this class are excellent potential leads for further development of drugs effective against *M. tuberculosis*.

1.8.23. abeo-sterols

A series of 3β-hydroxy steroid analogues possessing a contracted cyclopentane B-ring were prepared based on the initial activity screening of a recently reported naturally
occurring marine 5(6 →7) abeo-sterol against *Mycobacterium tuberculosis*. All of the novel ring B abeo-sterols 95 synthesized showed good inhibitory activity thus representing novel scaffold for the development of new antitubercular agents.

1.8.24. Pyrazole analogues

Two series of pyrazole derivatives were synthesized by parallel solution-phase synthesis and were assayed as inhibitors of *Mycobacterium tuberculosis* (MTB). One of these compounds 96 showed high activity against MTB (MIC = 4 μg/ml). The newly synthesized pyrazoles were also computationally investigated to analyze their fit properties to the pharmacophoric model.

1.8.25. Thymidine and Thymidine-5′-O-monophosphate Analogues

Recently, *Mycobacterium tuberculosis* thymidine monophosphate kinase (TMPKmt) was put forward as an attractive target for the design of a novel class of antituberculosis agents. The affinity of a series of 2′, 3′- and 5-modified thymidine analogues 97 for *Mycobacterium tuberculosis* thymidine monophosphate kinase (TMPKmt) was
evaluated. The affinities of several non-phosphorylated analogues are in the same order of magnitude as those of their phosphorylated congeners. However, in view of drug delivery problems associated with phosphorylated compounds, these ‘free’ nucleosides seem more promising leads in the search of TMPKmt inhibitors as novel anti-tuberculosis agents.\(^\text{276}\)

### 1.8.26. Nitroimidazopyran and Nitroimidazofurans
All of nitrated antimycobacterial compounds probably being prodrugs to be reduced.\(^\text{277}\) Nitroimidazofuran class of molecules (nitroimidazole) are known antibacterial drugs and based on these reports nitroimidazopyrans were synthesized and evaluated against \(\text{Mtb}\).

One of such compound PA 1343 (98) recently with MIC of 0.015 μg/ml is in clinical trial. The nitroimidazofuran derivative CGI 17341 (99) is potently active against MDR-\(\text{Mtb}\) with MIC in the range of 0.08-0.3 (μg/ml). Their mechanisms of action have not been reported.

### 1.8.27. Tetrahydroindazole based compounds

Very recently a novel class of tetrahydroindazole based compounds were reported as potent and unique inhibitors of \(\text{Mtb}\).\(^\text{278}\) Compounds 100, 101 and 102 exhibited activity
range against *Mycobacterium tuberculosis* (R-TB), with MICs of 1.7, 1.9, and 1.9 μM respectively.

\[
\text{[Chemical structure image]}
\]

### 1.8.28. Dipepiridine derivatives

A novel series\(^{279}\) of dipepiridine (103, 104 and 105), structurally unrelated to any existing antitubercular drugs exhibited MIC values as low as 7.8 μM, 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.

### 1.8.29. Salicylanilide carbamates

\[
\text{[Chemical structure image]}
\]

\(R_1 = 3\text{-Cl, 3,4-Dichloro, 4-Cl}\)

\(R_2 = \text{ethyl, butyl, pentyln hexyl, heptyl, octyl, nonyl, decyl, undecyl}\)
Salicylanilide carbamates derivatives 106 exhibited very good in vitro activity against M.tb, Mycobacterium kansasii and Mycobacterium avium and in particular against five multidrug resistant strains with MIC values between 0.5-2 μmol/mL.

1.9. Conclusion

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 TB drugs currently in use were developed 40 years ago and there is a great need for new, shorter treatment regimens. In the long term, the availability of more new drugs should play an important role in reducing the global TB burden. Current research involves to find better and more effective drugs that reduce time of treatment, reduce toxicity associated with drugs and provide backup measures in case of drug resistance. The approaches include: chemical modification of existing drugs (such as rifampin, fluoroquinolones and macrolides); the identification of drug targets (such as persistence genes) using microarray analysis and molecular biology tools; structure based drug design and in vitro and in vivo screening to identify new drugs; evaluation of novel drug combinations; and the order of drugs given in treatment.

This review has summarized the global disease burden of TB, existing drugs, drugs under clinical trials and their possible targets. Promising leading chemical entities has has also been addressed.
1.10. References


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