

Review article

## Targets for the development of new anti-tubercular agents

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### Abstract

In the developing world it is necessary to develop novel drugs to improve the control of tuberculosis. The global resurgence of tuberculosis and rampant drug resistance has urged the importance for the development of new anti tubercular agents, which address different targets, as those of currently used. This review is on development of new target based anti tubercular agents. In this review we have also attempted to look at the current status of anti tubercular drugs to overcome the persistent drug resistant problem and shortening the TB therapy.

### Introduction

Tuberculosis (TB) is a potentially fatal contagious disease that can affect almost any part of the body but is mainly an infection of the lungs. It is caused by a bacterial microorganism, the tubercle bacillus or Mycobacterium tuberculosis. Although TB can be treated, cured, and can be prevented if persons at risk take certain drugs, scientists have never come close to wiping it out. Few diseases have caused so much distressing illness for centuries and claimed so many lives[1] tuberculosis is spread through air when people have active TB in their lungs cough spit speak or sneeze. People with latent TB do not spread the disease active infection occurs more often in people with HIV/AIDS and in those who smoke. Diagnosis of active TB is based on chest x rays, as well as microscopic examination and culture of body fluids. Diagnosis of latent TB relies on the tuberculin skin test (TST) or blood tests. Prevention of TB involves screening those at high risk, early detection and treatment of cases, and vaccination with the bacillus Calmette vaccine. Those at high risk include household, workplace, and social contacts of people with active TB. Treatment requires the use of multiple antibiotics over a long period of time. Antibiotic resistance is a growing problem with increasing rates of multiple drug-resistant tuberculosis (MDR-TB). One-third of the world's population is thought to be infected with TB. New infections occur in about 1% of the population each

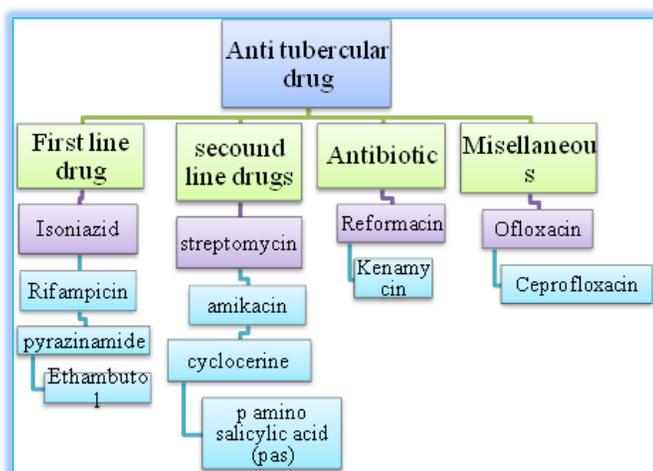
year. In 2014, there were 9.6 million cases of active TB, which resulted in 1.5 million deaths. More than 95% of deaths occurred in developing countries. The number of new cases each year has decreased since 2000. About 80% of people in many Asian and African countries test positive while 5–10% of people in the United States population tests positive by the tuberculin test. Tuberculosis has been present in humans since ancient times [2].

### History

Tuberculosis was popularly known as consumption for a long time. Scientists know it as an infection caused by M. tuberculosis. In 1882, the microbiologist Robert Koch discovered the tubercle bacillus, at a time when one of every seven deaths in Europe was caused by TB. Because antibiotics were unknown, the only means of controlling the spread of infection was to isolate patients in private sanitarium or hospitals limited to patients with TB—a practice that continues to this day in many countries. The net effect of this pattern of treatment was to separate the study of tuberculosis from mainstream medicine. Entire organizations were set up to study not only the disease as it affected individual patients, but its impact on the society as a whole. At the turn of the twentieth century, more than 80% of the populations in the United States were before age 20, and tuberculosis was the single most common cause of death. By 1938, there were more than 700 TB hospitals in this country. Tuberculosis spread

much more widely in Europe when the industrial revolution began in the late nineteenth century. The disease became widespread somewhat later in the United States, because the movement of the population to large cities made overcrowded housing so common. When streptomycin, the first antibiotic effective against *M. tuberculosis* was discovered in the early 1940s, the infection began to come under control. Although other more effective anti tuberculosis drugs were developed in the following decades, the number of cases of TB in the United States began to rise again in the mid 1980s. This upsurge was in part again a result of overcrowding and unsanitary conditions in the poor areas of large cities, prisons, and homeless shelters. Infected visitors and immigrants to the United States have also contributed to there surge of TB. An additional factor is the AIDS epidemic. AIDS patients are much more likely to develop tuberculosis because of their weakened immune systems. There still are an estimated 810 million new cases of TB each year worldwide, causing roughly 3 million deaths [3].

### Classification of Anti-tubercular Drug



### First Line Drug

#### Isoniazid

It is a pyridine ring with 2 amino group and one keto group attached to it, and also one of the cheapest anti-tubercular drug Isoniazid is a synthetic derivative from 4 pyridine carboxylic acid. The *M. tuberculosis* kat G gene encodes a dual function enzyme called catalase peroxidase, which confers sensitivity in *M. tuberculosis* to isoniazid. It is a prodrug that requires activation by mycobacterial catalase peroxidase enzyme kat G into active form, which exerts a lethal effect on intracellular targets. INH inhibits the mycolic acid biosynthesis in *M. tuberculosis* by affecting the enzyme mycolate synthase, unique for mycobacteria. A mutation within the mycobacterial *hA* gene has shown to confer resistance

to INH in *M. smegmatis* and in *M. bovis*, suggesting that in *hA* is likely target of this drug (Figure 1).

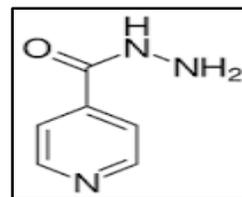


Figure 1. Structure of Isoniazid

### Ethambutol

Ethambutol (EMB) is synthetic amino alcohol (ethylene diamino-di-l-butanol) an orally effective bacteriostatic agent that is active against most strains of mycobacterium. The site of action of this drug ranges from trehalose dimycolate, mycolate and glucose metabolism to spermidine biosynthesis. The primary site of action is arabinan biosynthesis both in arabinogalactan and LAM. Activity of EMB is stereo-specific and the dextro isomer exhibited maximum anti tubercular activity (S,S form is 600 times more active than R, R) (Figure 2).

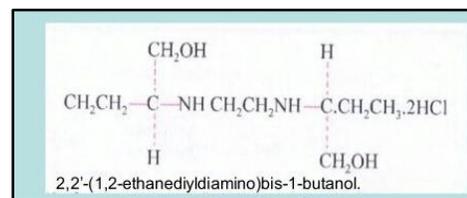


Figure 2. Structure of Ethambutol

### Second line drug

#### Streptomycin

Streptomycin is an amino glycoside antibiotic from *Streptomyces griseus* and consists of 3 structural components, streptidine, streptose and N-methyl glucosamine because of its poor absorbance in gastrointestinal tract, it is bactericidal antibiotic. It acts only on extracellular bacilli (because of poor penetration). It penetrates tubercular cavities, but does not cross to CSF, and has poor action in acidic medium. Mutations in the *rpsL* gene of ribosomal S12 protein of mycobacteria or base substitution in the 16S rRNA region confer resistance to streptomycin [4] (Figure 3).

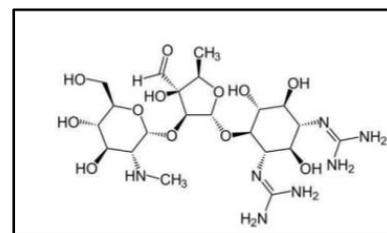


Figure 3. Structure of Streptomycin

## Antibiotic

### Amikacin and Kanamycin

Kanamycin and amikacin are aminoglycoside antibiotic. Kanamycin and amikacin exhibit their action by binding to the bacterial 30s ribosomal subunit, causing misreading of mRNA and thus leaving the bacterial unable to synthesize protein vital to its growth Kanamycin and amikacin. Although belonging to two different antibiotic families all exert their activities at the level of protein translation however amikacin causes kidney damage as well as hearing loss [5] (Figure 4) (Figure 5).

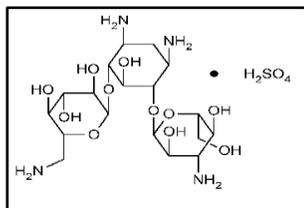


Figure 4. Structure of amikacin

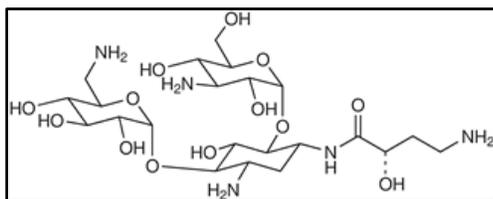


Figure 5. Structure of kanamycin

### Capreomycin & Viomycin

Capreomycin and Viomycin are cyclic peptide antibiotic used in the treatment of MDR-TB Viomycin is polypeptide antibiotic it is produced by the *actinomycete streptomycetes puniceus* that binds to RNA and inhibit prokaryotic protein synthesis and certain kinds of RNA splicing. Capreomycin is also antibiotic which is given in combination with other antibiotics to treat tuberculosis It is used for the treatment of different types of bacterial infections including M. Tuberculosis [6] (Figure 6 and 7).

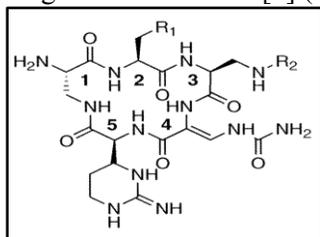


Figure 6. Structure of viomycin

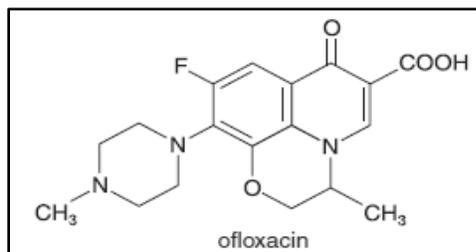


Figure 7. Structure of capreomycin

## Miscellaneous

### Ofloxacin

It comes under the antibiotic class of quinolone its site of action is Inhibition of topoisomerase (DNA gyrase) enzymes, which inhibit relaxation of supercoiled DNA and promotes breakage of double stranded DNA. Unfortunately causes adverse affects like CNS: insomnia, headache, hallucination & depression [7] (Figure 8).

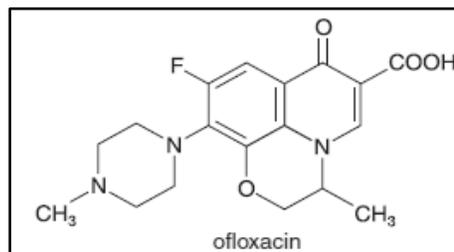


Figure 8. Structure of Ofloxacin

### Multiple Drug Resistance

**Multiple Drug Resistance:** is a manmade problem. It is costly, deadly debilitating and the biggest threat to current TB control strategies. When we say that strain of tubercle bacilli is drug resistance means that a patient yielding such organism would fail to respond to treatment with the drug concerned in normal dosage, i.e. a dosage that will cause response in patient infected with sensitive organism.

**Mono Drug Resistance TB:** disease caused by M. tuberculosis. In strains resistance to a single drug (most commonly isoniazid or streptomycin mono-resistance strains).

**Poly-Resistance TB:** disease caused by M. tuberculosis strains resistance two or more drugs, except those which are simultaneously resistance to isoniazid and rifampicin (most commonly resistance is isoniazid and streptomycin).

**Multiple Drug Resistance TB:** disease caused by M. Tuberculosis strains resistant to at least isoniazid and rifampicin.

**Extensively Drug-Resistance:** disease caused by multiple drug resistance M. tuberculosis strains with additional resistance to at least fluoroquinolones and one of the three inject able like (Kanamycin, amikacin and capreomycin) [8].

### Target for the Development of New Anti-tubercular Agents

**Target:** A cell or an organ that is affected by particular agent such as hormone or a drug [9].

In the view of persistent drug -resistant TB problem, it is important that new drugs should address different targets as those of currently used drugs including the shortening of TB therapy (Figure 9).

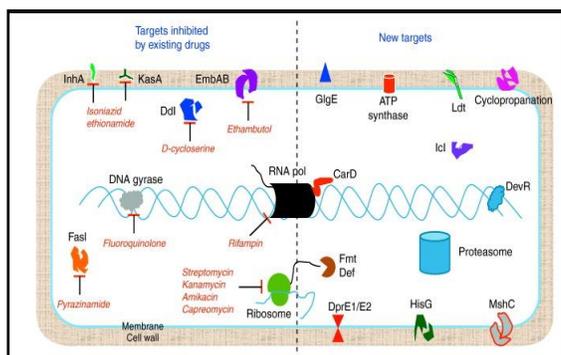


Figure 9. Targets Inhibited By Existing Drugs & new drug

### Drug Targets in Mycolic Acid Biosynthesis

*M. tuberculosis* has an extremely rigid cell wall containing mycolic acid, which are cell wall lipid components specific to mycobacterium organism such characteristics are important in virulence and persistence of MTB, since strong cell wall structure confers high resistance to bacteria to microphages antimicrobial effector molecules that are released into bacteria engulfing phagocytic vesicles and attack intraphagosomal MTB organism. Enzymes needed for synthesis are  $\beta$ -keto-acyl carrier protein synthase (kasa A BandfabH), enoyl-acyl carrier protein reductase 1,2,4 – bezothiadiazines are good targets for anti tb drug development

### $\beta$ -keto-acyl carrier protein synthase

Biochemical and structural analysis of Bhatt et al revealed that  $\beta$ -keto acyl carrier protein synthase (kasB) deficient mutant synthesized with shorter chain length deletion causes the loss ketomycolic acid transcyclopropanation and drastic reduction in methoxy mycolic acid transcyclopropane synthase (cmaA2) thus resulting in colony macrophages & abolishment of cord formation. Thus, kasB is closely related virulence of MTB and therefore is attractive target for new anti TB drugs (Figure 10).

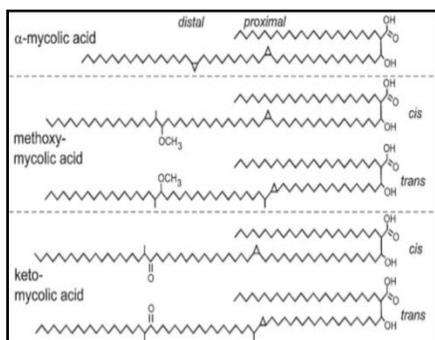


Figure 10.  $\beta$  keto acyl carrier protein synthase

### Enoyl –acyl carrier protein reductase

INH A continues to be promising drug target of MTB even today isoniazid affects the cell wall biosynthesis via alteration of MTB type II fatty acid biosynthesis (FAS-II)

pathway particularly inhibition of inhA Boyne et al recently searched for inhA inhibitors, which do not require activation of KatG, among various A–ring-modified diphenyl ether compounds. The most active compounds are 6pp and 8pp, had MIC of approximately 2 $\mu$ g/ml for MTB both 6pp and 8pp were fully active against MDR TB clinical isolates in addition Boyne et al indicate that 6pp and 8pp exhibit low toxicity and can inhibit intra macrophages MTB organism, in any cases the development of new antitubercular drugs, which are active against INH resistant MT by employing inhA protein as drug target [10].

### 1,2,4-Benzothiadiazines

The 1,2,4-benzothiadiazines system was explored by incorporating other heterocyclic rings like pyridine and pyrazine moieties. Recently some new targets such signaling kinase inhibitor has been investigated. The survival of *M. Tuberculosis* against macrophage phagocytosis relies not only on thick cell wall but also on many mycobacterial kinases and phosphatase. Which disrupt the host-cell defence mechanism against parasitism based on this kinase inhibitor benzothiofenenes (specifically inhibits pknG) and benzoquinoxalines (inhibitors of pknB, pknG and pknH) have been reported hence this intensive research on signaling [11] (Figure 11).

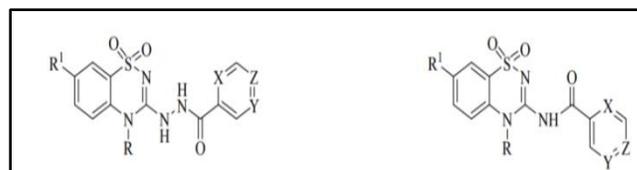


Figure 11. Structure of Pyridine & Pyrazine

### Diarylquinoline (TMC207)

Diarylquinoline (tmc207) it is formerly known as (R207910) diarylquinolones (DARQs) is structurally different from both fluoro-quinolones and other quinolone classes. It is member of new chemical class of anti-mycobacterial agents and has MIC values equal to or lower than reference compounds and reported to have a unique specificity towards mycobacterium including a typical species important in human such as MAC *M. kansasii* and the fast growers *M. fortium* and *M. abscessus* tmc exhibit good *in-vitro* activity against MTB isolates resistant to the anti-tb drugs INH, RF, streptomycin (SM), ethambutol (EMB) & pyrazinamide (PZA) (median MIC ranged from 0.01 to 0.09  $\mu$ g/ml) thus the clinical use of it this will highly targeted to treatment of the mycobacterial infections, particularly targeting the proton pump of adenosine triphosphate (ATP) synthase [11].

### Current status drug diarylquinoline

**Bedaquiline:** Bedaquiline is sold under the brand name sirturo its mechanism of action is it blocks the proton

pump of ATP synthase of mycobacteria ATP production is required for cellular energy production and its loss leads to cell death even in dormant and non replicating mycobacteria bedaquiline is bactericidal (Figure 12).

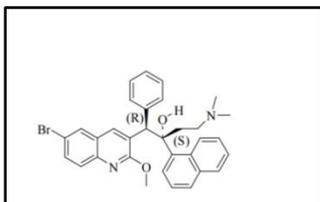


Figure 12. Structure of Bedaquiline

### Nitroimidazopyrans

A series of bicyclic nitroimidazole (NAP) have recently been reported to possess anti-tubercular activity. It is highly effective against MDR-TB and exhibit bactericidal action against dormant and MTB although its target in MTB is has not been elucidated (presumably biosynthesis of mycolic acids and nucleic acids) [11].

### Current status drug for nitroimidazopyrans

**Pretomanid (PA-824):** It is effective against both replicating and latent *M. tuberculosis* cells with MIC ranging from 0.015 to 0.25 µg/ml. The mode of action of this class of compound is by mechanism dependent on *M. tuberculosis* F420 cofactor, inhibition of protein synthesis and cell wall lipids (Figure 13)[13].

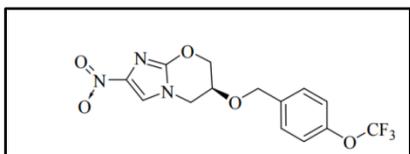


Figure 13. Structure of Pretomanid (PA-824)

### Nitroimidaoxazoles

Recently a Japanese company succeeded in remarkably improving the pharmaceutical characteristics of 6-nitro-2,3-dihydroimidazo[4,5-b]pyridine, structural analogues of bicyclic nitroimidazole. A nitroimidazole derivative is found to possess highly potent activity against TB, including MDR-TB at a concentration MIC range 0.006 - 0.024 µg/ml [11].

### Current status drug for nitroimidaoxazoles

**Delamanid (QPC-67683):** It is prodrug needs to be activated by *M. tuberculosis* and shows more potent anti-tuberculosis activity its mechanism of action is inhibition of the synthesis of methoxy and keto-mycolic acids[14] (Figure 14).

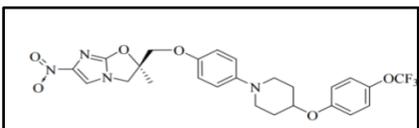


Figure 14. Structure of Delamanid (QPC-67683)

### Oxazolidinones

Oxazolidinones are totally synthetic, orally active antibacterial agents developed by dupont they are bacterial protein synthesis inhibitors, with inhibition uniquely in inhibition phase of protein synthesis [11].

### Current status for oxazolidinones

**Linezolid:** Linezolid is an antibiotic used for the treatment of infections caused by gram positive bacteria that are resistant to other bacteria. Its mode of action is it binds to ribosomal 50 s subunit and thus inhibits an early step in protein synthesis[15] (Figure 15) (Figure 16).

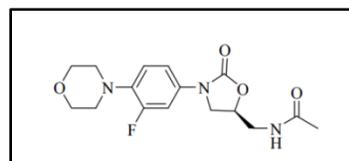


Figure 15. Structure of Linezolid

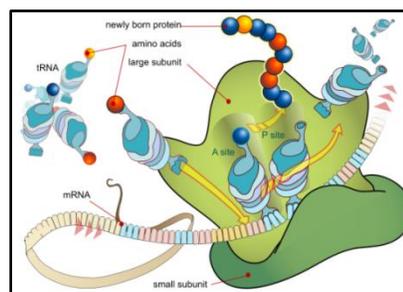


Figure 16. structure of mRNA translation

### Conclusion

In spite the availability of the BCG vaccine and some chemotherapeutic agents. Tb remains a leading infectious killer worldwide. This is mainly due to lack of new drugs in the market, particularly against spread of multi drug resistant (MDR) and extensively drug resistant (XDR) strains. In recent years, efforts are being made to develop new molecules based on different scaffolds that act on number of drug targets. Hence new molecules that are active against cell wall targets could provide valuable therapeutic options for therapeutic application. Some promising agents including nitroimidazole compounds (PA824) (QPC 67683) and diarylquinoline derivative TMC207 are currently under clinical study.

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