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Review Article

Tuberculosis: New Drug Discovery Pipelines

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Abstract

The resurgence of tuberculosis from a forgotten disease to a modern and resurgent pathology is a matter of serious global concern. Development and transmission of Multi Drug- Resistant (MDR) and extensively drug-resistant (XDR) tuberculosis is an important public health problem worldwide. This review covers mainly the therapeutic limitations to treat these resistant disease forms, as well as, the current status of drug discovery and development for anti-tubercular therapy and treatment.

Keywords: Tuberculosis; Multi drug- resistant tuberculosis (MDR- TB); Extensively drug-resistant tuberculosis (XDR-TB); New drug discovery pipelines

Introduction

"Tuberculosis is a Social Disease with a Medical Aspect". By Sir William Osler (1902)

Tuberculosis (TB) is a contagious but curable infectious disease caused by the pathogen, *Mycobacterium tuberculosis* (*Mtb*) and is again becoming a major cause of mortality worldwide. The *Mtb* and its pathogenic strains cause infection mainly in the oxygen-rich macrophages of the lungs. The main causes for the resurgence of this once nearly eradicated infectious diseases are the reduction in the emphasis on TB control programs, the declined socioeconomic standards and also the emergence of immune deficiency states like, AIDS. Despite the fact that TB has been recognized for thousands of years and its etiological agent has been identified since the earliest days of medical microbiology, TB continues to loom as one of the largest infectious diseases, with enormous global burden of morbidity and mortality. TB thrives in impoverished or malnourished communities; individuals weakened by immunological deficiencies and situations where healthcare delivery is poor.

According to global tuberculosis report by World Health Organization (WHO), in 2012, an estimated 8.6 million people developed TB and 1.3 million died from the disease (including 320, 000 deaths among HIV-positive people). Nearly 20 years after the WHO declaration of TB as a global public health emergency, major progress has been made towards 2015 global targets set within the context of the Millennium Development Goals (MDGs) [1,2]. Today the absence of completely protective TB vaccine, slow development of new anti-TB drugs are issues that highlight the re-emergence of the TB crisis [3,4]. Development of multidrug-resistant (MDR) and extremely drug-resistant (XDR) strains of *Mtb* to anti-TB agents is an increasing problem worldwide [5,6].

After a gap of nearly 40 years, in December 2012, the anti-tubercular New Drug Discovery Research (NDDR) efforts have seen some success. The US Food and Drug Administration (FDA) approval of two new compounds, delamanid and bedaquiline to treat multidrug-resistant TB (MDR-TB) validates the renewed efforts to develop new, better treatments for TB after decades of stagnation. However, the euphoric mood can turn to pessimism as the road to adequate treatment for people with TB is still a long one and full of many difficulties.

Anti-mycobacterial therapy though available; drugs are often partially effective because of the impermeable nature of the mycobacterium cell wall and the propensity of *Mtb* to develop resistance to the existing drugs in TB therapy [7]. Additionally, *Mtb* has the capacity to remain viable within infected hosts for a prolonged time. Notably, MDR-TB and XDR-TB have become obstacles to the effective global TB control and have been recognized by the WHO as major challenges to be addressed in the fight against tuberculosis [8,9]. A key strategy to combat and destroy these drug resistant pathogens is the discovery of novel antitubercular agents bearing newer structural skeletons (scaffolds), acting through novel mechanisms of action and bearing no or minimal cross resistance.

The Biology of Mtb.

The common causative organism for TB, *Mycobacterium tuberculosis* (*Mtb*) is a major component of the microbiological history and is also referred as the *tubercle bacillus*.

- *Mtb* is a small, non-motile, gram-positive slow growing bacillus with a generation time of 12 to 20 hours and prolonged culture period on agar of up to 21 days [10,11].
- *Mtb* possesses a unique cell wall highly rich in lipid content, which includes mycolic acids and other glycolipids. It has an unusual waxy coating of mycolic acid on its cell surface making it impervious to gram staining therefore; it is detected by acid fast technique.
- It is highly aerobic [12,13].
- It does not produce toxins.
- It can survive for long periods under adverse conditions.

While, in humans *Mycobacterium tuberculosis* is the main cause of the infection, several other *Mycobacterium* species including *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium*

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microti, Mycobacterium canettii, Mycobacterium caprae and *Mycobacterium pinnipedii* are also known to cause the disease.

Pathogenesis of TB [14]

The steps in TB pathogensis are – exposure, infection, disease and death. Infection with *Mtb* can be pulmonary or extra pulmonary (affecting other parts of the body). Pulmonary TB [15] (the most common form in developed countries) occurs in the lung and comprises almost 85% of all TB cases. The upper lung lobes are more frequently affected by tuberculosis than the lower ones.

Extra pulmonary TB [16] means tuberculosis spreads outside of the lungs. It may co-exist with pulmonary TB as well. Its sites are; lymph nodes, bones and joints (in Pott's disease of the spine), intestine, genito-urinary tract (in urogenital tuberculosis), pleura (in tuberculosis pleurisy), central nervous system (in scrofula of the neck), meninges, peritoneum, skin, etc. Though it occurs in approximately 15 to 20% of active non HIV cases, it occurs more commonly in immune-suppressed persons and young children. In those with HIV, it occurs in more than 50% of cases.

Infection of a host is initiated with the inhalation of air droplets containing a small number of the bacilli, which spread from the site of initial infection in the lung through the lymphatic system or blood to other parts of the body, as well as, the apex of the lung and the regional lymph nodes. Once in the lung, bacilli are subjected to phagocytosis by the resident macrophages of the lung, the alveolar macrophages, which in turn are activated by the appropriate stimuli can effectively, transfer the phagocytosised *Mtb* to the destructive environment of lysosomes. However, some bacilli escape lysosomal delivery and survive within the macrophage. Infected macrophages can then either remain in the lung or are disseminated to other organs in the body. As the immune defense system is sufficient to keep *Mtb* in a check in healthy individuals, only 10% of infected individuals develop TB. Any deterioration of host immunity results in a potentially life-threatening condition of the individual harboring live *Mtb*.

Symptoms & transmission of the TB infection

The early symptoms of active tuberculosis include, weight loss (consumption), fever, night sweats, fatigue and loss of appetite. At advanced stages of the disease persistent cough with blood-tinged sputum is an additional symptom. About 25% of patients may not have any symptoms or remain "asymptomatic" (Latent TB). While persons with latent TB are not infectious, they may develop the active form of the disease if their immune system is compromised. Diagnosis of active TB relies on radiology (chest X-rays), as well as microscopic examination and microbiological culture of body fluids. Diagnosis of latent TB relies on the tuberculin skin test (TST) and/or blood tests. Prevention relies on screening programs and vaccination with the bacillus Calmette–Guérin (BCG) vaccine. About 5 to 10% of non-HIV individuals, infected with tuberculosis, develop active disease during their lifetime.

TB is an airborne disease and spreads like the common cold by the circulation of aerosols containing *Mtb* in the air from the coughing, sneezing, talking or spitting of TB patients. When people with active pulmonary TB cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0 μm in diameter. Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low [17,18].

Drug therapy and its limitations

TB chemotherapy was first introduced in 1946, when Streptomycin (STR) was used to treat the disease. In most parts of the world, combination of five drugs is used to treat TB effectively namely rifampicin (RIF), isoniazid (INH), ethambutol (ETH), streptomycin (STR) and pyrazinamide (PZA). Antitubercular chemotherapy problems arise when patients develop bacterial resistance to any of these first-line drugs. However, as many of the second-line drugs; ethionamide, aminosalicylic acid (PASA), cycloserine (CYC), amikacin, kanamycin and capreomycin are very toxic they cannot be employed simultaneously (Tables 1,2).

The current chemotherapy of infectious TB involves an initial two month treatment regimen of first-line drugs comprising of either: STR, INH, RIF and PZA or INH, RIF, PZA and ETH. This is followed by treatment with INH and RIF over a four to seven months period. Current chemotherapy of latent TB involves treatment with INH for 6 - 9 months in order to prevent the development of the active form of the disease [19].

Today the difficulties faced in managing TB are due to, the long duration treatment regimens, the emergence of drug resistant *Mtb* strains and coinfection with HIV/AIDS. To overcome these, efforts are on for (a) development of long-acting drugs with extended-intervals of dosing in order to facilitate "Directly Observed Treatment Short course (DOTS)" and enhanced patient compliance, (b) prevention of MDR-TB strains by using drugs, which exhibit potent early microbicidal activity and (c) eradication of slowly metabolizing and, if possible, dormant *Mtb* population that cause relapse, using new classes of anti-TB drugs [20].

Drug resistance in tuberculosis [21-23]

TB organisms resistant to the drugs used in its treatment are widespread and occur in all countries. Drug resistance emerges as a result of inadequate treatment and once TB organisms acquire resistance they can spread from person to person in the same way as drug-sensitive TB strains. Individuals with drug-resistant TB disease can also transmit the resistant strain of the disease directly to others.

Multidrug resistant tuberculosis (MDR-TB) [21]

Multi-drug-resistant (MDR) TB refers to simultaneous resistance to at least two or more of the five first-line anti-TB drugs (INH, RIF, PZA, ETH and STR), caused by sharing of genes between different species or genera of the pathogen, generally mediated by small pieces of extra-chromosomal DNA, known as transposons or plasmids. According to WHO, about 3.6% of new TB patients in the world have MDR-TB strains. Treatment for multi-drug-resistance tuberculosis is a prolonged, less effective, costly and therapeutically poorly tolerated.

Extensively drug resistant tuberculosis (XDR-TB) [22]

Extensively-drug-resistant (XDR) TB is defined as resistance to at least INH and RIF, in addition to any fluoroquinolone and at least one of the three injectable second-line agents, i.e, capreomycin, amikacin or kanamycin. The principles used for MDR-TB and XDR-TB treatment are the same. The main difference is that XDR-TB is associated with a much higher mortality rate than MDR-TB, because of the reduced number of effective treatment options.

Totally drug-resistant tuberculosis (TDR-TB) [23]

This term has been now-a-days commonly referred to the

Table 1: Antitubercular Drugs (First line & Second line).

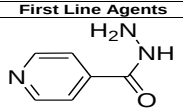
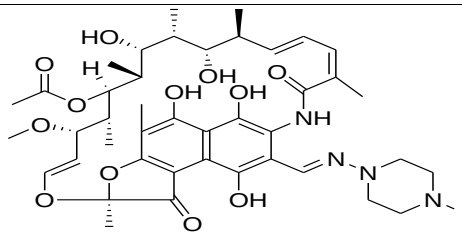
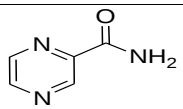
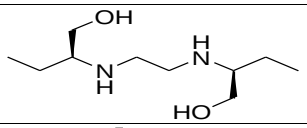
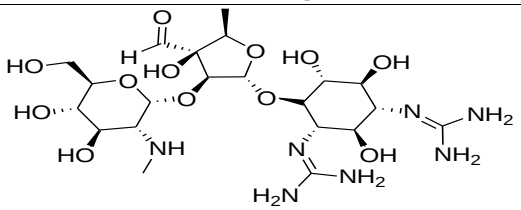
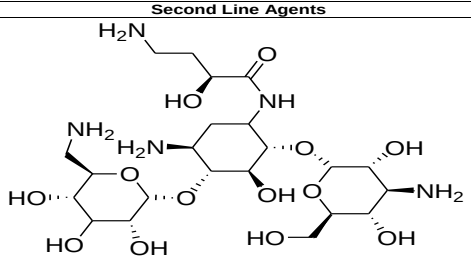
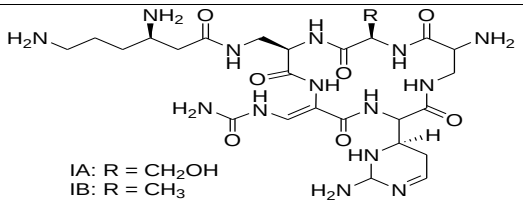
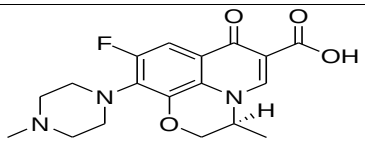
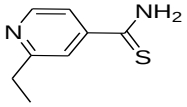
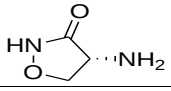
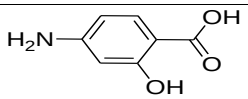
Chemical Class	Drug	Structure	Mechanism of action
First Line Agents			
Hydrazides	Isoniazid (INH)		After activation by Kat G, active moiety forms adduct with nicotinamide adenine dinucleotide and InhA, inhibiting mycolic acid synthesis thereby inhibiting cell wall synthesis
Ansamycin Antibiotic	Rifampin (RIF)		Inhibits transcription by binding to RpoB, the β subunit of DNA dependent RNA polymerase
Amides	Pyrazinamide (PZA)		Pyrazinoic acid accumulates intracellularly, acidifying Mycobacterium tuberculosis to inhibit translation. It may also inhibit ATP synthesis.
Ethylenediamines	Ethambutol (EMB)		Inhibition of cell wall synthesis Arabinofuranosyl Transferases (EmbC, EmbA and EmbB)
Aminoglycoside	Streptomycin		30S ribosome Inhibition of protein synthesis
Second Line Agents			
Aminoglycoside	Amikacin		Protein synthesis inhibition (binds to 16S subunit of rRNA)
Polypeptides	Capreomycin	 IA: R = CH_2OH IB: R = CH_3	Protein synthesis inhibition (inhibition of translocation)
Fluoroquinolones	Levofloxacin		DNA synthesis inhibition (inhibition of gyrase)
Thioamides	Ethionamide		Inhibits InhA and mycolic acid synthesis
Amino acid analogues	D-cycloserine		Inhibition of cell wall synthesis (D-Alanine:D Alanine ligase Alanine racemase)
Salicylic acid derivatives	*p*-amino salicylic acid		Thymidylate synthase inhibition and interference in iron uptake and/or folate biosynthesis

Table 2: Some Common Adverse Effects of TB drugs.

Drugs	Common Adverse Effects
Ethionamide Cycloserine PAS Fluoroquinolones Clofazimine Rifabutin	Gastrointestinal complaints
INH Rifampicin/rifabutin Ethionamide PZA PAS Fluoroquinolones	Hepatotoxicity (early symptoms are anorexia and malaise, then abdominal pain, vomiting, jaundice)
INH Ethionamide Cycloserine Linezolid Ethambutol	Peripheral neuropathy
All	Rash
Fluoroquinolones Isoniazid Cycloserine Ethionamide Ethambutol	Headache
Cycloserine	Seizures
Ethionamide, PAS	Hypothyroidism
Aminoglycosides, Capreomycin	Hearing loss, Vestibular toxicity
Cycloserine, Ethionamide, Isoniazid, Fluoroquinolones	Behavioral changes
Ethambutol, Rifabutin, Isoniazid, Linezolid	Visual changes
Aminoglycosides, Capreomycin	Renal failure Hypokalemia, Hypomagnesemia

Source:*http://www.istcweb.org/documents/ISTC%20Training%20Material%202009/ISTC%20TB%20Training%20Modules%202009/Management_drug_resistant_TB_Nov2009.ppt*

tuberculosis caused by the strains resistant to all available first line as well as second line TB drugs. This highlights the urgent need for discovery and development of new drugs.

Hence, there is an urgent need for novel drugs acting through newer mechanisms active against *Mtb* in order to circumvent these problems of prolonged drug regimens as well as resistance to existing drugs.

Hope for new drugs

Therapy for tuberculosis is a tricky situation at times since it may put forth new challenges. Therefore, therapeutic monitoring of anti-tubercular drugs over wider extents may provide important clues for reforms in the treatment of tuberculosis. Anti-TB chemotherapy is combinatorial in nature, requiring long and resource intensive clinical development to treat a disease that mostly affects resource-poor countries. Efforts are on for identifying a drug combination in which the drugs shall complement each other in (i) their ability to penetrate a wide range of lesions found in the spectrum of TB disease and (ii) their potential to kill all bacterial sub-populations present in these lesions [24,25].

Since their advent the existing first and second line anti-TB drugs have helped to cause a rapid decline of tuberculosis in many industrialized countries, leading to a global euphoric mood about its irradication. However, it has also led to a callous and indifferent approach of apathy towards new anti TB drug discovery research, for quite some years [26,27]. Further, as TB was considered to be a disease of third world countries and thus unlikely to generate suitable returns on financial investments, the pharmaceutical industry also had a rather indifferent view towards new anti-TB drug discovery research, for a very long time. This has culminated in no new discovery of any anti-TB drug for nearly 4 decades [28-30].

However, since the 80's the disease has been undergoing resurgence, driven by substantial changes in social, medical and economic factors. Thus, a dramatic increase in immuno-suppressed individuals, mainly due to AIDS (and also due to cancer chemotherapy and organ-transplant practices to some extent), coupled with increasing urbanization and poverty in developing countries, has compromised primary health care structures and led to large increase in TB incidence. The occurrence of multi drug-resistant form of TB, concomitant with the resurgence of its regular form have exposed the vulnerability and limitations of the current drugs in the armoury of mankind [31].

New drug discovery pipelines

Though a variety of molecules comprise the discovery pipelines of anti-TB drug research of many pharma research organizations and companies, including the state/government funded ones, globally, only the molecules in advanced phases of clinical trials in six chemical classes are discussed, herein.

Quinolone derivatives: Fluoroquinolones, currently one of the backbones of MDR-TB treatment, continue to be explored for their potential to shorten treatment for drug-sensitive TB. Many

quinolone carboxylic acid derivatives have been investigated and found to be a potentially active as anti-TB compounds. Of which few are discussed below. Some of these molecules are in advanced phases of clinical trials. A summary of drugs for treatment of TB in pipeline is summarized (Table 3). Currently, there is little resistance to fluoroquinolones among patients with newly diagnosed TB, but resistance is increasing during the re-treatment of the disease and therefore the widespread use of this class for other indications raises concerns about emerging resistance against it.

Moxifloxacin & Gatifloxacin [32] Being in Phase III clinical trials, both moxifloxacin and gatifloxacin are the most advanced compounds under development for TB. They are both 8-methoxyquinolones originally developed as broad-spectrum antibiotics and now being repurposed as anti-TB agents. Fluoroquinolones target gyrase and topoisomerase IV in most bacteria but in *Mtb* it is assumed they solely target gyrase since there is no evidence of topoisomerase IV present in *Mtb*. Other quinolones, such as ciprofloxacin and ofloxacin, have been used as second-line TB drugs, but moxifloxacin and gatifloxacin are more potent *in vitro* than these older quinolones. Currently they both are in Phase III clinical trials. Moxifloxacin is being investigated by a large number of organizations while Gatifloxacin is sponsored by Oflotub consortium. The potential adverse effects that have been reported for these drugs are dysglycemia with Gatifloxacin and QT prolongation with Moxifloxacin. In some regions of the world, there is a fairly high incidence of quinolone resistance, (e.g, in India), and use of either of these compounds in TB regimens might prove to be limited in such circumstances. DC-159a a 8-methoxyfluoroquinolone, possessing three chiral centers, was originally developed as a broad-spectrum antibiotic. However, its potential use in TB patients was investigated by Daiichi-Sankyo. It was shown to be fourfold more potent *in vitro* than moxifloxacin and gatifloxacin against *Mtb*. In mouse efficacy studies, it was shown to be more potent than moxifloxacin in the rapidly growing phase as well as in the non- (or slow-) replicating phase. The results seem to warrant its study as part of drug combinations in animal models of TB [33]. The mechanism of action of DC-159a is still under investigation, but as other quinolone derivatives, DC-159a probably affects GyrA activity, which plays important roles in DNA replication. This compound is still in the preclinical development stage [34].

Diarylquinolines

Bedaquiline: (Brand name Sirturo, also known as TMC207). Bedaquiline, the first new TB drug from a new drug class to receive approval in over four decades, has advanced little since its FDA approval in 2012. Bedaquiline (TMC207) is a lead compound in the diarylquinoline series originally discovered by scientists at Janssen Pharmaceutica and is currently undergoing Phase II clinical development for MDR-TB indications. TMC207 was discovered via whole-cell assay screening of approximately 70,000 compounds from Johnson & Johnson's (Janssen's parent company) corporate library against the surrogate organism, *Mycobacterium smegmatis* and it is currently clinically developed by Tibotec, a subsidiary of Johnson & Johnson in collaboration with the TB alliance [35]. TMC207 inhibits the proton transfer chain of the mycobacterial ATP synthase [36,37]. TMC207 is not active on human mitochondrial ATP synthase [38]. Its mode of action is unique i.e it inhibits *Mtb* ATP synthesis, by interacting with subunit c of its ATP synthase. Subunit c forms an oligomeric structure AtpE of the transmembrane portion of ATP synthase (F_0). This compound is effective against dormant *Mtb* organisms, even though ATP biosynthesis is significantly down-regulated in these bacteria.

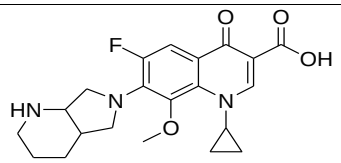
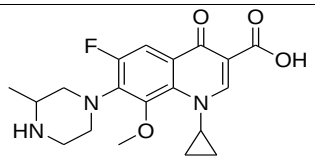
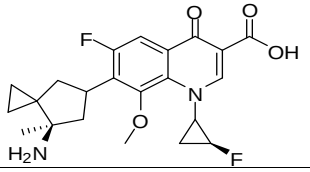
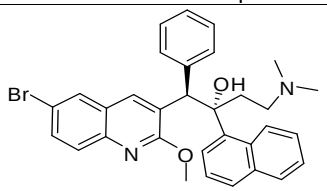
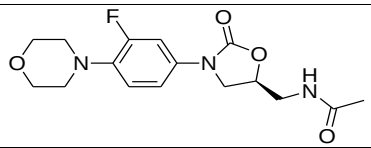
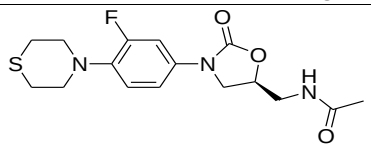
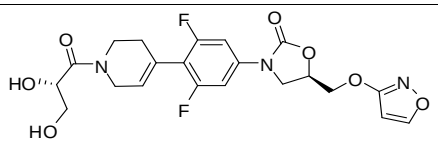
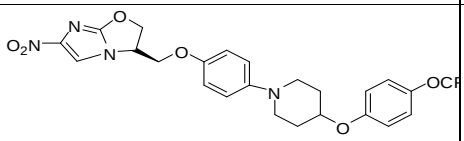
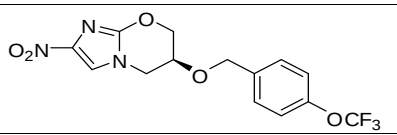
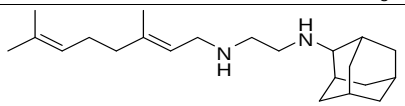
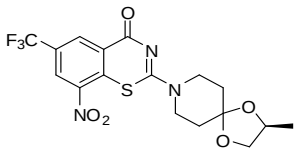
Oxazolidinones: As additional drugs are urgently needed to accompany bedaquiline and delamanid, researchers and clinicians are increasingly interested in three drugs: linezolid—a drug approved for other bacterial infections and used off-label to treat difficult cases of drug-resistant TB and its new chemical relatives, sutezolid and AZD5847. Yet data to support linezolid's clinical efficacy and safety remain limited. The oxazolidinones are a promising new class of synthetic antimicrobial agents with a unique mechanism of action in inhibiting protein synthesis. They display bacteriostatic activity against many important human pathogens including drug-resistant microbes.

Linezolid [39–42] was approved in 2000 to treat drug-resistant, gram-positive bacteria. It was introduced in the USA. It represents the oxazolidinone class of antibiotics, is one of only two new antibiotics introduced into the market in the past 40 years (the other being daptomycin, representing lipopeptide antibiotics), with the main target pathogens being Gram-positive bacteria including MRSA and vancomycin-resistant Enterococci). This compound has MIC of 0.3 to 1.25 mg/mL against *Mtb*. It has also been shown that linezolid is active against *Mtb in vitro* and in animal models. Its target is the *Mtb* ribosome and it exhibits potent activity against MDR-TB clinical isolates. It binds to the 23S RNA in the 50S ribosomal subunit and limits the growth of bacteria by disrupting its production of proteins in the first step of the synthesis by inhibiting formation of the initiation complex. Linezolid has been used for MDR-TB (and XDR-TB) and additional Phase IIb clinical trials are completed. Linezolid treatment course lasts upto 28 days.

PNU-100480 (Sutezolid) by Pfizer, was one of the earliest oxazolidinone antibiotics synthesized, exhibiting increased antimycobacterial activity compared with linezolid, but its potential as anti-TB agent has been recognized and pursued recently. *In vitro* and mouse models suggest that sutezolid may be more active than linezolid against TB. As linezolid has serious side effects, including optic and peripheral neuropathy and anemia, safer oxazolidinones are needed. The use of linezolid is limited by adverse effects that occur with long term administrations. Therefore, new analogues showing identical or better *in vivo* activities and a better therapeutic index would be useful. The development of PNU-100480 (Sutezolid), a close structural analogue of linezolid was initiated [43]. Sutezolid is of great interest to the TB research community, Pfizer has only completed two phase I and one phase IIa clinical trials, in addition to preclinical work.

AZD5847 [44,45] by Astra Zeneca, was originally intended as a broad-spectrum antibiotic, but has now been repurposed as an anti-TB agent. It is well recognized that with linezolid, treatment periods longer than 14 days may result in hematological adverse effects and since treatment for TB is considerably longer than 14 days, the degree and severity of this off-target activity with the next-generation oxazolidinone agents will be the key for their development. The compound has completed phase IIa trials is likely to be advancing to next phase.

Table 3: Antitubercular Drugs in Pipeline.

Class	Name	Structure	Mechanism of action	Status
Quinolones	Moxifloxacin		DNA synthesis inhibition (inhibition of gyrase)	Phase III
	Gatifloxacin		DNA synthesis inhibition (inhibition of gyrase)	Phase III
	DC-159a		DNA synthesis inhibition (inhibition of gyrase)	Preclinical
Diarylquinolines	Bedaquiline TMC207 (Sirturo)		Inhibits energy production by targeting ATP (subunit c of ATP synthase)	Phase IIb/ III
Oxazolidinones	Linezolid		Protein synthesis inhibition (translation) by inhibiting the initiation step at the ribosome.	Phase IIb
	PNU 100480 (Sutezolid)			Phase IIa
	AZD5847			Phase IIa
Nitroimidazoles	Delamanid (OPC-67683, Deltyba)		Destabilizes the bacterial cell membrane by blocking the synthesis of mycolic acids; poisons the bacterial cell by releasing nitric oxide when metabolized.	Phase III
	PA-824			Phase III
Ethylenediamines	SQ 109		Cell wall biosynthesis inhibition. possibly by targeting the MmpL3 protein; *in vitro* activity has yet to be confirmed in humans	Phase IIb/ III
Benzothiazinones	BTZ 043		Inhibition of cell wall biosynthesis (inhibition of decaprenyl-phosphoryl-β-D-ribose-2'-epimerase)	Preclinical

Nitroimidazoles

Delamanid (Brand name Deltyba, formely known as OPC-67683) an Otsuka's compound, a nitroimidazole, shows great promise in treating MDR-TB. Delamanid became the second new drug to receive regulatory approval to treat MDR-TB in Europe in 2014. Delamanid (OPC-67683) is currently under development by Otsuka Pharmaceutical Company [46]. This compound contains a fused oxazoline ring instead of an oxazine ring. After treatment with *M. bovis*, *des*-nitro-imidazooxazole was obtained [47]. It inhibits methoxy-mycolic and keto-mycolic acid biosyntheses with corresponding IC_{50}values equal to 0.036 mg/mL and 0.021 mg/mL. A large number of analogues have been prepared by varying the tail portion of the molecule. OPC-67683 is 4 to 16 times more potent than another analog, PA-824 *in vitro* and it does not show cross-resistance with other first-line TB drugs. It is currently in Phase III clinical trials for MDR-TB patients. No data exist on whether bedaquiline and delamanid can be used together safely to improve TB treatment regimens. Delamanid appears generally safe, although it does cause mild-to-moderate QT prolongation. Delamanid is being tested for administration twice daily at 100 mg for the first two months of treatment, and once daily at 200 mg for the following four months. Delamanid's pediatric formulation of small, dissolvable tablets is complete and a pediatric study has begun in the Philippines.

PA-824 like delamanid is a nitroimidazole. The TB Alliance is developing PA-824 for both drug-sensitive and drug-resistant TB in its novel combination studies [48]. It is currently in Phase III clinical trials conducted by the TB Alliance and following 14-day dosing in drug sensitive TB patients, PA-824 (at dose levels of 200, 600, 1000 and 1200 mg/day) demonstrated bactericidal activity similar to that demonstrated by the standard first-line drug combination. In terms of SAR, the nitro group is essential for anti-TB activity. In addition to the oxazine ring, other six-membered heterocycles were prepared but the anti-TB activity remains essentially only with an oxazine ring and, to a lesser degree, with a thiazine ring. A diverse range of 'tail' variations have been synthesized and some of them exhibit increased *in vitro* and *in vivo* potency, and are currently in the preclinical stage of development. Two-electron reduction actually takes place not at the nitro group but at the imidazole ring and it has been hypothesized that the resulting intermediates generate reactive nitrogen species, including nitric oxide [49,50]. Under anaerobic conditions, a correlation between the amount of *des*-nitro intermediates and cell killing suggests this as the bactericidal mechanism under these conditions. However, under aerobic conditions the exact mechanism is not known although inhibition of mycolic acid biosynthesis may be involved. The apparently multiple mode of action for this class of compounds render optimization of their anti- TB activity rather challenging. At present, a combination therapy including PA-824, moxifloxacin and pyrazinamide is being evaluated in TB patients [51,52].

Ethylenediamines (Ethambutol analogues)

Ethambutol, one of the main drugs used in TB-treatment regimens presumably, interferes with construction of the arabinogalactan layer of the mycobacterial cell wall. It's simple and symmetrical chemical structure offers an attractive and symmetrical *N,N'* -disubstituted ethylenediamine scaffold for parallel and combinational high throughput synthesis of many of its analogs [53].

SQ109 is an ethylenediamine analogue of ethambutol prepared through combinatorial chemistry. SQ109 appears to act by inhibiting the MmpL3 protein where as ethambutol is known to inhibit the synthesis of arabinogalactan, which is a component of the *Mtb* cell wall. Though, SQ109 is implicated in affecting cell wall biosynthesis, but it is also active against ethambutol-resistant strains and its precise mode of action has not been elucidated and is potentially novel. SQ109 is shown to over produce the ATP-dependent DNA/RNA helicase and to reduce the production of the β-ketoacyl-acyl carrier protein synthase which may explain its action on mycobacterial cell wall synthesis. It produces a remarkable synergy *in vitro* and in animal models when combined with rifampin, isoniazid, or TMC207 [54–57]. Relatively low bioavailability might be a problem [58], but Phase IIb/ Phase III studies have been completed, successfully.

Benzothiazinones

BTZ043 is a new class of sulphur containing heterocycle belonging to the benzothiazinones (BTZ) which has been recently described as a potent antimycobacterial agent [59]. The structure activity relationships study showed that sulphur atom and one or two nitro groups on the aromatic structure are required to inhibit bacterial growth *in vitro*. Compound, BTZ038, has been found to be the most active of the series. Although still in the preclinical stage, BZT043 is an extremely potent new class of antimycobacterial agent (MIC against *Mtb* = 0.004 μg/ ml) [60]. *In vivo* this compound at 37.5 mg/kg reduced the bacterial burden by 1 and 2 logs in the lungs and spleen, respectively. Subsequently by sequencing the mutant strains, its target was determined to be the enzyme decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1), which is involved in the biosynthesis of cell-wall component, arabinogalactan. The proposed mechanism involves reduction of the nitro group to a nitroso residue, which reacts with the cysteine group of DprE1.

Future Directions

As many second-line anti-TB drugs show adverse effects **(Table 2),** an improved TB vaccine regimen is essential to achieving effective global TB control. A number of vaccines are now in clinical trials. However, their further progression to advanced stages of these trials also depends upon the further development of field sites. According to a track report by WHO, globally by 2012, the TB mortality rate had been reduced by 45% since 1990. The target to reduce deaths globally by 50% by 2015 is within reach. Five priority actions required to accelerate progress towards 2015 targets are;

- Reach the missed cases
- Address MDR-TB as a public health crisis
- Accelerate the response to TB/HIV
- Increase financing to close all resource gaps
- Ensure rapid uptake of innovations

Conclusion

The control of TB in humans is heavily reliant on short course chemotherapy. However, this approach is increasingly challenged by widespread multi- and extensively drug resistant strains of *Mtb*. New druggable targets and novel leads are required for TB drug discovery to develop compounds with greater potency that is less prone to

acquired drug resistance. TB drug development has undoubtedly advanced, but progress is slow, the number of new compounds limited, knowledge insufficient to dramatically improve cure rates, reduce treatment duration and make treatment more tolerable. This review highlights mycobacterial pathogenesis, the problem of resistant forms of the disease, current anti-TB drug research and development pipeline.

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