Chapter I

Introduction

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Chapter 1

1. Tuberculosis

Tuberculosis (TB) remains a leading infectious killer in the world. One of the hallmarks of TB is its persistent phase of infection. One-third of the world's population is thought to be contaminated with the causative agent *Mycobacterium tuberculosis* (MTB) a gram positive bacterium, the causative agent of TB. The disease is transmitted via the respiratory route as highly infectious aerosol, whose exposure result ranges from immediate organism damage by the host's immune system to infected individuals developing active primary TB [1]. It is the leading cause of morbidity and mortality among the infectious diseases. In immunocompetent individuals the initial acute infection is controlled by the immune system, and living bacteria are restricted in a peculiar localized pulmonary structure called granuloma. However, these patients have the risk of 10% to develop active form during their life even with the absence of any cause of immunosuppression (**Figure 1.1**) [2]. There the bacteria endures indefinitely in a latent non-virulent form, and gets reactivated whenever an immunosuppressive condition occurs [3]. If importance of a disease for mankind is measured from the number of fatalities which are due to it, then tuberculosis must be considered much more important than those most feared infectious diseases, like., cholera, plague, and the statistics have shown that 1/7 of all humans die of tuberculosis.

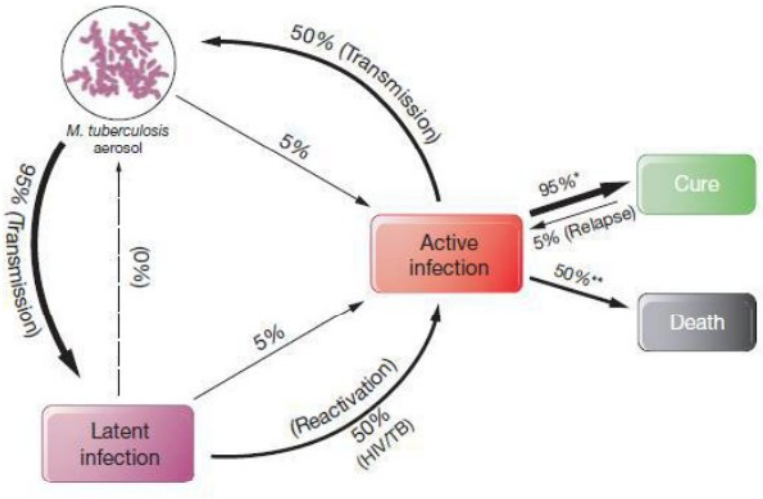


Figure 1.1: Stages of *Mycobacterium tuberculosis* infection.

Even now TB continues to claim about 2 million deaths per each year (WHO report, 2015) [4] and results in vast mortality with a huge economic burden on undeveloped countries. The standard treatment recommended by World Health Organisation (WHO) contains four drugs to be administered for six months to treat drug sensitive TB [5]. Prolonged TB treatment results in poor patient fulfilment and severe side effects arising from some of the suggested drugs. During the past years most of the drugs developed for TB were ineffective due to the emergence and spread of resistant MTB strains to these front line drugs resulting in multidrug resistant (MDR), extremely drug resistance (XDR) and totally drug resistant (TDR) strains of MTB. MDR MTB is the one at least resistant to isoniazid and one of the antibiotics like rifampicin (RMP) is called as multi drug resistance strain. XDR MTB is the MDR strain which is resistant to fluoroquinolone (FQ) and an injectable aminoglycoside is termed as an extremely drug resistant strain. The strain of MTB which is resistant to all first line and second line licensed anti-tubercular drugs is defined as totally drug resistant stain (TDR MTB) [6]. The global emergence of these MDR, XDR and TDR TB strains makes to fail greatly the control and suppression of TB.

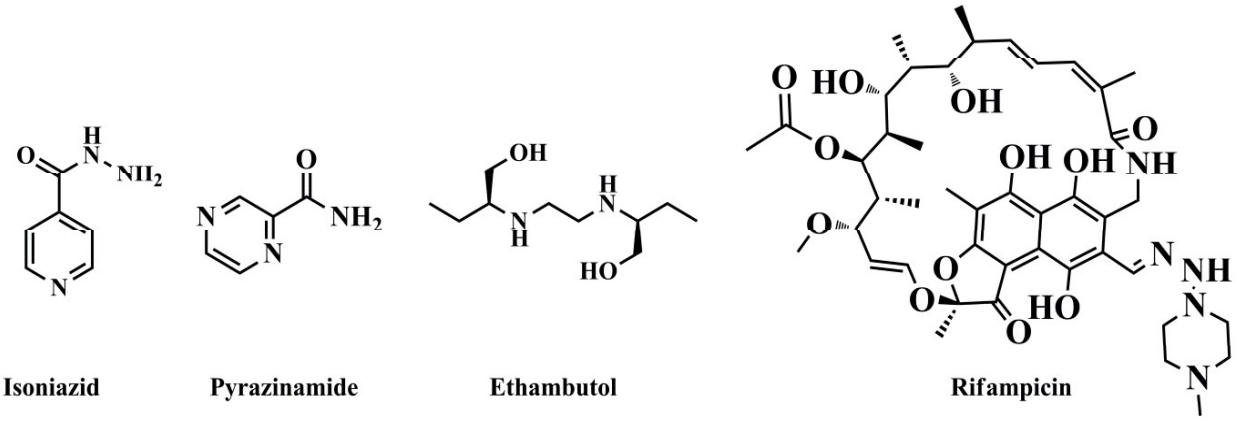


Figure 1.2: First line anti-TB drugs.

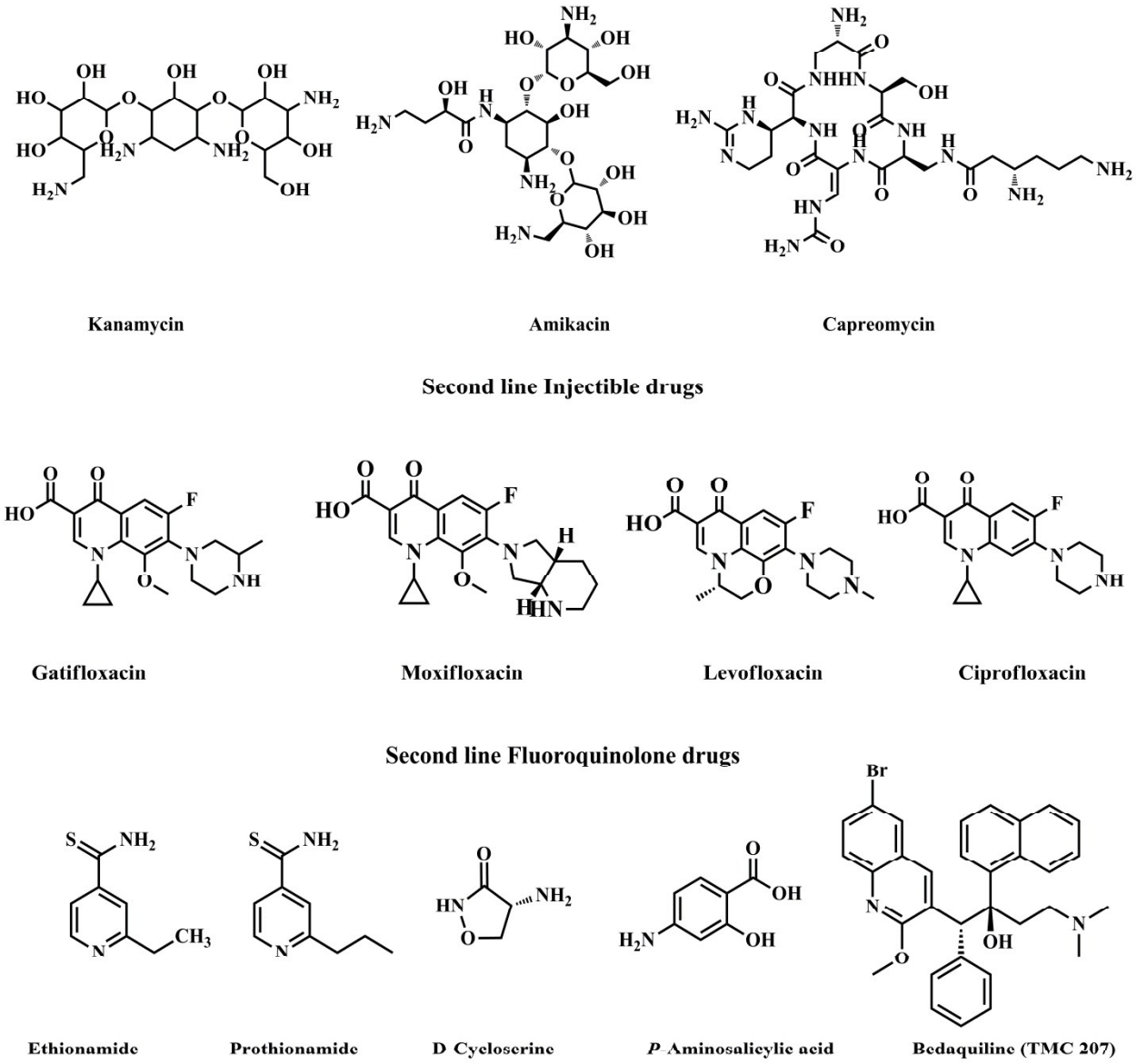


Figure 1.3: Second line anti-TB drugs.

The World Health Organization (WHO) estimated that in 2015, about 9.6 million people developed TB and 1.5 million died from the disease (4,00,000 of whom were HIV-positive), with the huge majority of these from developing parts of the world [4]. An estimated 1.2 million (12%) of the 9.6 million people who developed TB in 2014 were HIV-positive. In 2014, an estimated 4,80,000 women died as a result of TB. From 2000 to 2014, 43 million lives were

saved through effective diagnosis and treatment. Out of 9.6 million people who developed TB in 2014, more than half (58%) were in the South-East Asia and Western Pacific regions. India and China alone accounted for 23% and 10% of total cases, respectively [4]. In 2016, 87% of latent TB cases took place inside the 30 excessive TB burden international locations. Seven nations accounted for 64% of the new TB cases: India, Indonesia, China, Philippines, Pakistan, Nigeria, and South Africa. Global development depends on advances in TB prevention and care in these countries. WHO estimates that there were 600 000 new cases with resistance to rifampicin – the handiest first-line drug – of which 490 000 had MDR-TB. The MDR-TB burden in large part falls on three countries – India, China and the Russian Federation – which together account for nearly half of the global cases. About 6.2% of MDR-TB instances had XDR-TB in 2016 [4].

India is one of the highest burdens of TB. According to WHO, It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent TB rather than TB. An estimated incidence of around 2.79 million cases of TB were reported in 2016 for India including HIV TB patients. Totally, 1,78,00 males were effected. 1,47,00 MDR TB cases were identified in 2016 with including 84,000 new cases. A total of 4,23,000 people died due to TB [4].

The revised estimates are based on data from various sources including sub-national prevalence surveys and enhanced TB notification from the private sector. The Revised National Tuberculosis Control Programme notified 17.5 lakh TB patients in 2016 including both from public and private health sectors and 33,820 drug resistant TB patients are notified additionally. Major TB cases were identified in Uttar Pradesh (297,746), Maharashtra (195,139), Madhya pradesh (129,915), Gujarat (126,665), Rajasthan (106,756). In 2016 out of the total reported patients, almost one fifth of the patients were reported from the private sector. As shown below there was wide variation from state to state in respect of the proportionate reporting of TB patients from the two sectors. In Kerala the reporting was almost equal, whilst the number reported was nil in some of the northeast states [5].

This situation highlights the relative shortcomings of the current treatment strategies for TB and the limited effectiveness of public health systems; particularly in resource-poor countries where the main TB burden lies.

1.1. *Mycobacterium tuberculosis* - the etiological agent of TB

TB has plagued humans for thousands of years. It has been found in the skulls and spines of Egyptian mummies. Hippocrates who was ancient Greek physician noted that tuberculosis which at the time was called phthisis or consumption was the most widespread disease and fatal to almost everyone who became infected with it. During the 17th and 18th centuries, TB had made its way to Europe. MTB during this time was called the "White Plague" in Europe. In 1882, Robert Koch developed a staining technique which allowed him to see tubercle bacillus which identified the etiological agent. The disease was finally named tuberculosis in 1839 by J.L. Schonlein because of the numerous tubercles or holes formed in the lungs by the bacterium. *Mycobacterium tuberculosis* is an obligate intracellular pathogen which can survive up to decades in a phenotypically non-replicating state, primarily in hypoxic granulomas in the lung [7]. It has outstanding mechanisms to run away from elimination and a high degree of intrinsic resistance to most antibiotics, chemotherapeutic agents and immune eradication [8]. Mycolic acids are the hallmark of the cell envelope of MTB, which are long chain α-alkyl-β-hydroxy fatty acids, the major constituents of this protective layer, has been shown to be critical for the survival of MTB. One major problem for host defence mechanisms and therapeutic intervention is robust, mycolic acid-rich cell wall, which is unique among prokaryotes [9]. Mycolic acids are the primary constituent of the mycobacterial cell wall which contributes to the outer membrane permeability and integrity as well as virulence [10]. This contributes to the chronic nature of the disease, imposes long treatment regimens and represents a formidable obstacle for researchers [11].

1.2. Mycobacterium tuberculosis (MTB): An overview

The causative agent of TB, *Mycobacterium tuberculosis* is typically a nonmotile, rod-shaped, non-spore forming, and aerobic bacteria, classified as acid-fast bacilli. Morphologies can be observed when grown on solid media and some species exist as curved rods or shorter coccibacilli on artificial media [12]. The rods are 2-4 μM in length and 0.2-0.5 μM in width. The distinguishing characteristics of MTB are its complex cell wall and its slow generation time. Escherichia coli can replicate in approximately 20 minutes but MTB replication times are 16-20 hours. In nature, the bacterium can grow only within the cells of a host organism, but MTB can be cultured in the laboratory [13].

The MTB morphology is classified in two categories; i) which are frequently seen at exponential phase of growth that is rod, V, Y-shape, branched or buds (**Figure 1.4A**), and ii) those that are seen occasionally under stress or environmental conditions which are round, oval, ultravirus, spore like, and cell wall defiant or L-forms (**Figure 1.4B**) [14].

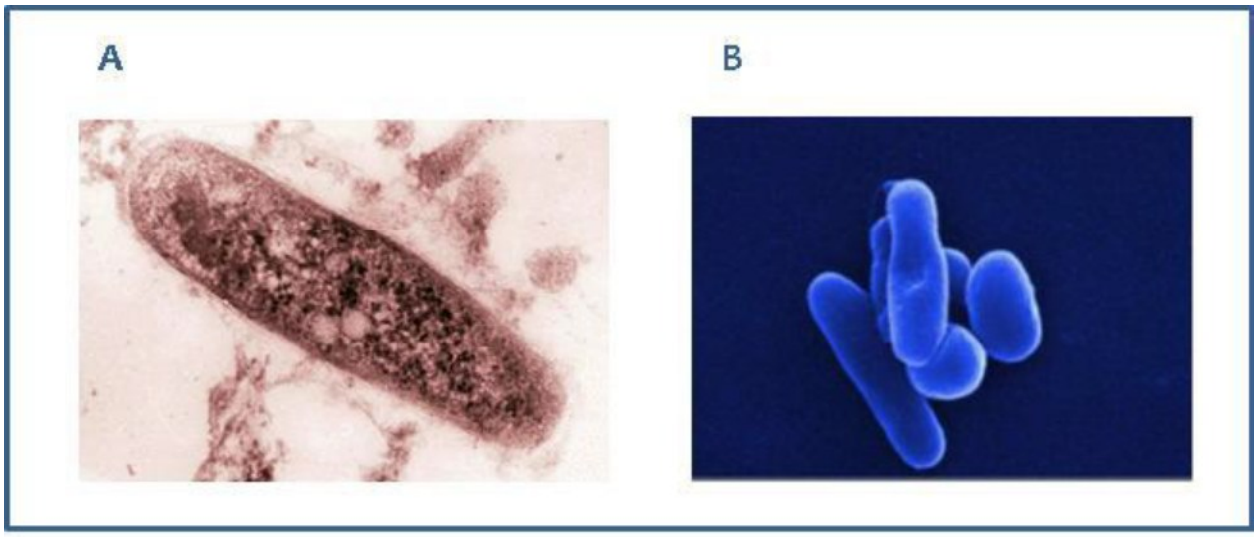


Figure 1.4: Morphological variations in MTB. (**A**) Thin section transmission electron micrograph of MTB (extracted from www.wadsworth.org/databank/mycotubr.htm); (**B**) Scanning electron microscope shows shape variation in MTB at exponential phase of growth [14].

1.3. Classification of mycobacteria

The classification of mycobacteria started in 1896 when Lehmann and Neumann proposed for the first time the genus mycobacterium which included *Mycobacterium tuberculosis* and *Mycobacterium leprae* species [15]. DNA-based molecular taxonomy, mycobacteria are classified as grampositive bacteria due to genes high similarity with other gram-positive organisms, such as Bacillus [16]. Based on the mycobacteria growth rate genus is usually separated into two major groups: i) slow-growing species including *M. tuberculosis, M. bovis* and *M. leprae;* and ii) fast-growing species such as *M. smegmatis.* Based on their epidemiological features, mycobacterium includes: i) non-pathogenic or rarely pathogenic mycobacteria, ii) strictly pathogenic mycobacteria iii) potentially pathogenic mycobacteria [17].

Table 1.1: Classification of mycobacteria according to the risk of infection.

Rare pathogens		Potential pathogens	Strict pathogens
M. smegmatis	*M. aurum*	*M. avium*	*M. tuberculosis*
M. phlei	*M. chitae*	*M. intracellulare*	*M. bovis*
M. fallax	*M. duvalii*	*M. chelonae*	*M. africanum*
M. thermoresistibile	*M. gadium*	*M. fortuitum*	*M. ulcerans*
M. parafortuitum	*M. gilvum*	*M. kansasii*	*M. microti*
M. gastri	*M. komossense*	*M. malmoense*	*M. canetti*
M. triviale	*M. lepraemurium*	*M. marinum*	*M. caprae*
M. nonchromogenicum	*M. neoaurum*	*M. scrofulaceum*	*M. pinnipedii*
M. gordonae	*M. terrae*	*M. simiae*	*M. leprae*
M. flavescens	*M. vaccae*	*M. szulgai*	
M. farcinogenes	*M. agri*	*M. xenopi*	
M. senegalense	*M. aichiense*	*M. asiaticum*	
M. paratuberculosis	*M. paratuberculosis*	*M. haemophilum*	
M. porcinum	*M. chubuense*	*M. shimoidei*	
M. diernhoferi	*M. obuense*		
M. pulveris	*M. rhodesiae*		
M. tokaiense	*M. moriokaense*		
M. poriferae			

The pathogenic species the most relevant for human health are *M. tuberculosis* and *M. leprae*, the causative agents of two of the world's oldest diseases, tuberculosis and leprosy, respectively [16].

TB causative agents *M. canettii* and *M. africanum,* were isolated from African patients. *M. bovis* demonstrates the broadest spectrum of host infection, affecting humans, domestic or wild bovines and goats. *M. microti* can also cause disease in immune compromised human patients [18-19] and *M. pinnipedii* infects seals [20].

M. kansasii, M. malmoense and *M. xenopi* represent pulmonary opportunists, while *M. marinum* is the skin pathogen infecting organism by entering through damaged or ulcerated skin. *M. ulcerans* is the causative organism of buruli (tropical) ulcer [21].

All of the species of MTB complex (MTBC), which includes *M. tuberculosis, M. canettii, M. africanum, M. microti, M. bovis, M. caprae* and *M. pinnipedii*, are known to cause TB in humans.

The *M. avium* complex (MAC) comprises *M. avium* subspecies responsible for disease in birds, but also for disseminated disease in patients with AIDS, causing systemic infections late in the

progress of AIDS, cervical lymphadenitis, and chronic lung disease in immunocompetent or non-HIV patients [22].

The *Mycobacterium fortuitum* complex includes *M. peregrinum, M. fortuitum, M. chelonae* and *M. abcessus* which are regularly responsible for abscess formation in local injection or surgical wounds and can be related with pulmonary disease [23].

1.4. The *Mycobacterium tuberculosis genome*

The genome of MTB was studied generally using the strain MTB H37Rv. The genome sequence of MTB is one of the first complete genomes to be sequenced, and was decoded in 1998 by Cole and co-workers [24]. It contains sequence of 4,411,529 bp and characteristically high guanine plus cytosine (G+C) content (65.5%). Genome analysis revealed an efficient DNA repair system with nearly 45 genes related to DNA repair mechanisms [25] and despite over 10,000 years of evolution, when 16 genetically diverse clinical strains were examined for conservation of 24 genes known to encode antigenic proteins, minimal variation was observed [26].

MTB H37Ra is the avirulent counterpart of virulent strain H37Rv and both strains are derived from their virulent parent strain H37, which was originally isolated from a 19 year-old male patient with chronic pulmonary tuberculosis by Edward R. Baldwin in 1905 [27]. H37Rv and its avirulent counterpart H37Ra strains have been widely used as reference strains for studying virulence and pathogenesis of MTB worldwide since 1940. H37Ra is used as an adjuvant to boost immunogenicity during immunization with BCG [28].

Several of the MTB genes discussed are attractive targets for healing intervention, either through drug development or through incorporation into vaccine strains. It is also previously obvious that a more complete understanding of the pathogenic strategies of this highly successful intracellular pathogen will elucidate novel features of macrophage defenses and the host immune response. In addition to this anticipated scientific dividend, the dividend of greatest immediate importance is the development of new drugs and vaccines against this deadly disease [29].

1.5. The mycobacterial cell envelope

The mycobacterial cell wall is a complex structure required for cell growth, resistance to antibiotics and virulence [30]. It is an elaborate cell envelope comprised of several layers. Each of these layers display different chemical modifications and the architecture of the cell wall is

also molded through complex regulation. High molecular weights of lipids represent the complexity of the cell wall [31]. Unusual impermeable properties of MTB cell wall are thought to be advantageous for the bacilli in stressful conditions of osmotic shock [32] and the polymers, covalently linked with peptidoglycan and trehalose dimycolate, provide a thick layer involved in MTB resistance to antibiotics and the host defense mechanisms [33].

The mycobacterial cell wall consists of an inner layer and an outer layer that surround the plasma membrane. The outer compartment consists of both lipids and proteins (capsuleprotien and capsule sugars). The inner compartment has three distinct macromolecules of peptidoglycan (PG), arabinogalactan (AG), and mycolic acids (MA) covalently linked together to form an insoluble complex referred to as the essential core of the mycobacterial cell wall [34]. (**Figure 1.5**)

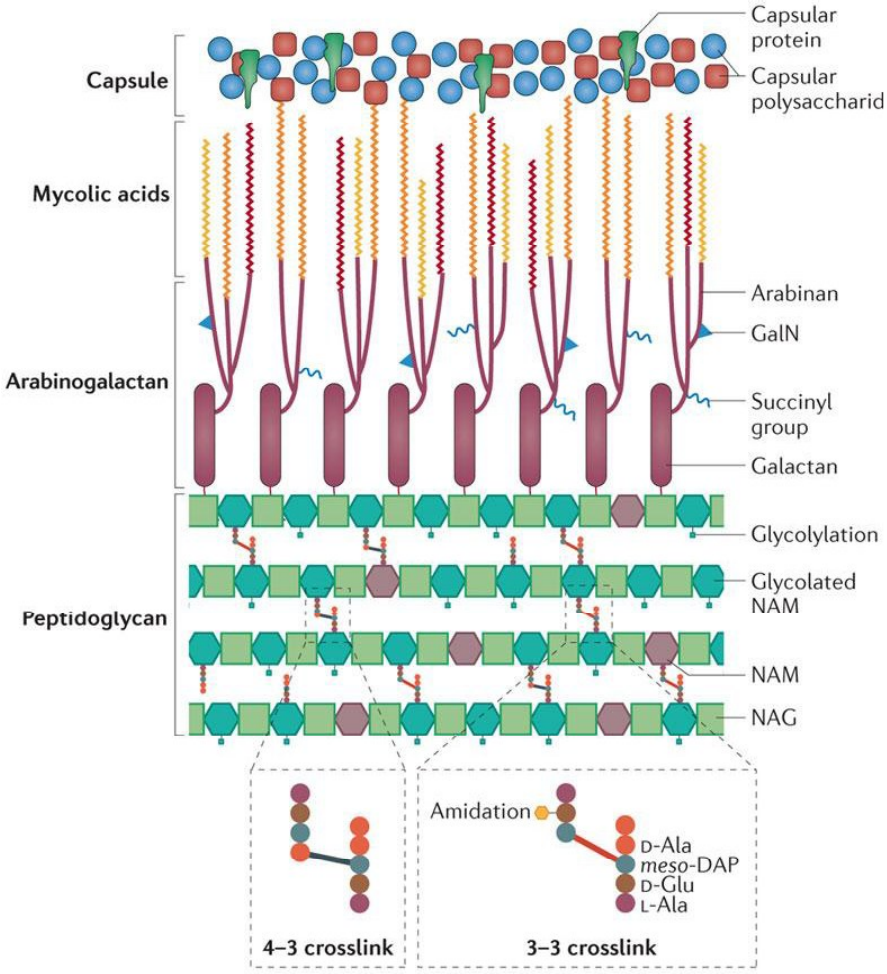


Figure 1.5: The mycobacterial cell wall.

The peptidoglycan, which forms the "backbone' of the cell wall skeleton [35]. The PG is made of peptides and glycan strands and covered with the plasma membrane. The long glycan strand typically consists of repeating *N*-acetylglucosamines (NAGs) linked to *N*-acetylmuramic acid (NAM). These strands are cross linked by peptides bound to the lactyl group on NAMs from different glycan strands [34, 36]. These peptide chains normally consist of *L*-alanyl-*D*-iso-glutaminyl-meso-diaminopimelic acid (DAP) from one strand linked to the terminal *D*-alanine residue from *L*-alanyl-*D*-iso-glutaminyl meso-DAP-*D*-alanine from a different strand. Mycobacterial PG is heavily crosslinked; up to 80% of the PG contains 'nontraditional' 3→3 peptide crosslinks instead of the 'traditional' 4→3 crosslink [37]. The highly cross-linked glycan meshwork of PG that surrounds bacteria is the primary agent that maintains bacterial shape.

Arabinogalactan (AG) forms a mycolyl-arabinogalactan-peptidoglycan (mAGP) complex. This complex is comprised of AG moiety anchored into a PG layer unique to mycobacteria and esterified at the distal end by a dense layer of long chain mycolic acids [38]. AG lacks repeating units and is instead made up of a distinct structural motif: The entire AG structure is tethered to the PG at the C-6 position of the *N*-glycolylmuramic acid by a linker unit containing a diglycosylphosphoryl bridge, α-L-Rha-(1→3)-α-D-GlcNAc-(1→P), common among only Actinomycetes [39]. Arabinan is ligated with long carbon chain mycolic acids. It forms the characteristic thick waxy lipid coat of mycobacteria is responsible for the impermeability of the cell wall and virulence [40].

Mycolic acids are long-chain fatty acids, up to 90 carbon atoms long, that are α-branched and β-hydroxylated [40]. These lipids make up 60% of the dry weight of MTB compared to 10% in most other bacteria and they are bound to the AG by esterification of a terminal pentaarabinofuranosyl [41]. MA are heterogeneous with regard to chain length, number of double bonds, cyclopropyl groups and side groups (keto-, methoxyand epoxy-groups) [40].

The biosynthetic pathway of mycolic acid involves type I and type II fatty acid synthases, FAS-I and FAS-II respectively. FAS-I catalyses the *de novo* synthesis of fatty acids from acetyl-CoA. In contrast, FAS-II is similar to systems found in bacteria, apicomplexa parasites and plants, and is composed of four dissociable enzymes that act successively and repetitively to elongate the

growing acyl chain. Acyl-primers are continually activated *via* thioester linkage to the prosthetic group of coenzyme A (CoA) for FAS-I, or of an acyl carrier protein (ACP) for FAS-II **(Figure 1.6**) [42].

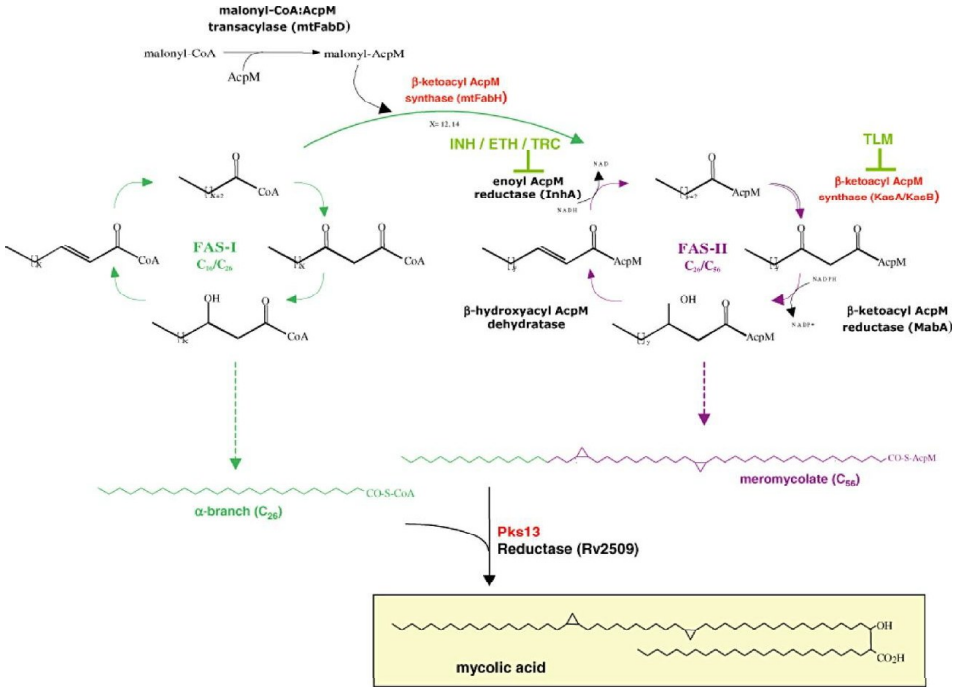


Figure 1.6: Fatty acid/mycolic acid biosynthesis in mycobacteria.

FAS-I is involved in the synthesis of C_{16} and C_{26}. The C_{16} acyl-CoA product acts as a substrate for the synthesis of meromycolic acids by FAS-II, whereas the C_{26} fatty acid constitutes the α-branch of the final mycolic acid. MtFabH has been proposed to be the link between FAS-I and FAS-II by converting C_{14}-CoA generated by FAS-I to C_{16}-AcpM, which is channelled into the FAS-II cycle. This latter comprises four enzymes which will act successively and repeatedly to ensure fatty acid elongation, ultimately leading to meromycolates (C_{56}). These enzymes are the condensing enzymes KasA and KasB, the keto-reductase MabA, an unidentified dehydratase, and the enoyl-reductase InhA. Finally, the polyketide synthase Pks13 catalyses the condensation of the α-branch and the meromycolate to produce mycolic acids. Targets for the action of activated INH, ethionamide (ETH), triclosan (TRC), or thiolactomycin (TLM) are indicated. FAS-II enzymes are labelled in black except the condensing enzymes, which are indicated in red.

The relative contribution of FAS-I and FAS-II activities in fatty acid/mycolic acid biosynthesis is represented in green and purple respectively [42].

It's interesting to note that mammals do not synthesize mycolic acids and thus compounds that antagonize the enzymes involved in mycolic acid biosynthesis are promising leads for developing novel anti-tubercular agents. Many efforts currently focus on revisiting and optimizing existing inhibitors of validated drug targets in FAS-II, particularly because it can remove much of the uncertainty surrounding new drug targets and *in vivo* clinical efficacy.

Mycolic acids give rise to important characteristics, including resistance to chemical injury, resistance to dehydration, low permeability to hydrophobic antibiotics, virulence [43].

InhA encoded by the MTB gene InhA, catalyses the final enzymatic step in the elongation cycle of the FAS-II pathway [44]. It is a NADH-dependent reductase that exhibits specificity for long chain enoyl thioester substrates, and its reduction mechanism [45].

InhA, an enoyl-ACP reductase, involved in mycolic acid synthesis, is a well-known target for front-line anti-tubercular drugs [46] such as INH [47], and ETH [44], much interest has been devoted to deciphering the chemistry and biosynthesis of mycolic acids in the alarming context of the emergence of MDR, XDR, and TDR TB. Mycolic acids are processed and matured by a cascade of enzymes [48], which results in three distinct meromycolate variants: α-meroacids, methoxy-meroacids and keto-meroacids [30]. All three variants are required for full virulence during infection and have varying levels of saturation, cyclopropanation and oxygenation [42, 43].

1.6. Tuberculosis: Drug resistance

A growing trend of drug-resistance in the TB disease is threatening the gains in global TB control. Despite the implementation and success of DOTS course, there is a steady increase in the number of patients infected with multi and extensively mycobacterial drug resistant strains [49]. Drug resistance in TB therapy is not an immediate past, as the strains of MTB that were resistant to streptomycin were experimental soon after its introduction for TB treatment in 1944 [50].

Nowadays resistance to all the available anti-tubercular drugs have been found in different constituents of the world. The most important factors causing drug resistance is incomplete and inadequate treatment methods and it emerges mostly where TB control

programmes are weak [51]. TB intrinsically resistant to many antibiotics due to the low permeability of its mycolic acid-rich waxy cell envelope, the action of efflux pumps and the presence of chromosomally encoded resistance genes [52].

K.D. Green and S. Garneau-Tsodikova reported drug resistance are in MTB due to this three factors (i) mutations or modifications of the drug targets (RMP, EMB, kanamycin (KAN), amikacin (AMK), Capromycin (CAP), and the fluoroquinolones (FQ), (ii) the inability in prodrugs activation (INH, PZA and ETH) due to mutations which leads to a loss of function, and (iii) enzymatic inactivation of the drug (KAN) [53].

TB resistant classify as a) resistant to INH and RMP as MDR TB; b) Resistance of MDR TB strains to the FQ and aminoglycosides are classed as XDR TB; c) TDR TB has been used to describe strains found resistant to all available drugs, but there is not yet an agreed definition of TDR TB; (d) RMP-resistant TB (RR TB) is also known exist [54].

Expression of clinical efficacy in drug-sensitive TB is demanding, given high success rates for existing regimens, concerns about substituting an investigational agent for the most effective agents in a regimen and difficulties in determining the effect size of the components of a combination regimen. In difference, exploring efficacy of novel treatments in the setting of drug-resistant disease may experience with the activity and the safety of new agents in drug-resistant disease may provide a stage which their sign can diversify to include drug-sensitive disease [55].

1.6.1. Multidrug-resistant TB (MDR TB)

MDR TB, also known as Vank's disease) is defined as TB where a bacterium is resistant were to least two of the most powerful first-line anti-TB drugs, INH and RMP. WHO in 2014 estimated that 300,000 had MDR TB. Globally in 2014; 123,000 patients with MDR TB or RR TB were actually notified. It was just 41% of the number of estimated cases of 300,000. There were also approximately 190,000 deaths from MDR TB. More than half of these patients were in India, China and the Russian Federation [4].

MDR TB is consequence of inappropriate use of essential anti-TB drugs. MDR TB results primarily from accumulation of mutations in individual drug target genes. The chance of resistance is very high for less effective anti-tubercular drugs such as ETH, CAP, thiacetazone, cycloserine, and viomycin; intermediate for drugs such as INH, SM, EMB, KAN, and PAS; and

lowest for RMP. In addition to accretion of mutations in the individual drug target genes, the permeability barrier imposed by the MTB cell wall can also involve to the development of low-level drug resistance. Studies addressing resistance to SM have found evidence of such a two-step mechanism for the development of drug resistance [56]. Treatment options are limited and expensive, recommended medicines are not always available, and patients experience many adverse effects from the drugs [4].

Treatment of MDR TB requires treatment with second-line drugs, usually four or more anti-TB drugs for a minimum of 6 months, and possibly extending for 18-24 months if RMP resistance has been identified in the specific strain of TB with which the patient has been infected [14]. In general, second-line drugs are less effective, more toxic and much more expensive than first-line drugs. Under ideal program conditions, MDR TB cure rates can approach 70% only [56].

1.6.2. Extensively Drug-Resistant TB (XDR TB)

XDR TB (extensively drug resistant TB) is defined as strains resistant to at least RMP and INH. This is in addition to strains being resistant to one of the FQ, as well as resistant to at least one of the second line injectable TB drugs AMK, KAN or CAP, resulting in a longer treatment course for a minimum of 18-24 months, lower cure rates, and significantly increased healthcare costs [14]. Moreover, second-line therapeutic treatment requires strict patient monitoring, supervision, counseling, and support to prevent further drug resistance that could potentially render the disease untreatable [14]. XDR TB is of special concern for persons with HIV infection or other conditions that can weaken the immune system. These persons are more likely to develop TB once they are infected, and also have a higher risk of death once they develop TB.

1.6.3. Totally drug-resistant TB (TDR TB) or extremely drug resistant TB (XXDR TB)

Researchers have recently identified the existence of the most dangerous form of TB strain reported till date. TDR TB refers to MTB clinical strains exhibiting *in vitro* resistance not only to RMP and INH, two of the main first line TB drugs, but strains that are also resistant to fluoroquinolone and to at least one of the second line injectable TB drugs (INH, RMP, SM, ethambutol, PZA, ETH, PAS, cycloserine, ofloxacin, AMK, ciprofloxacin, CAP, KAN). TDR TB, or XXDR TB, refers to strains that are resistant to all the first line drugs as well as all the

second line TB drugs. The presence of TDR TB was first observed in Italy in 2003, subsequently in Iran and India [57].

TDR TB is relatively poorly documented, as many countries do not test patient samples against a broad enough range of drugs to diagnose such a comprehensive array of resistance. The United Nations' Special Programme for Research and Training in Tropical Diseases has set up a TDR TB Specimen Bank to archive specimens of TDR TB [58].

TDR TB bacilli while designing new drugs and if it is so, whether the previously designed drugs could be effective? Last but not the least; as far as, there is no cure for TDR TB patient, hence it is not exaggeration to say that world is on danger of untreatable drug resistant TB strain. Therefore, if authorized health organization do not consider immediate action plan for such bacilli, then we may face a new outbreaks of untreatable TB [59].

Recently, Bedaquiline (TMC-207), Delamanid (OPC-67683) and Linezolid, three new drugs approved by the US-Food and Drug Administration and the European Medicines Agency, may offer therapeutic solutions for TDR TB. With more new anti-tubercular agents in the pipeline, there is hope of identifying drugs that may be bactericidal or bacteriostatic in TB treatment and challenge the TDR TB terminology [60].

1.6.4. Rifampicin-resistant TB (R TB):

If bacteria are just resistant to RMP then it is called rifampicin-resistant TB. It is also with or without resistance to other drugs includes any resistance to rifampicin, whether mono-resistance, multidrug resistance, polydrug resistance or extensive drug resistance. Both MDR TB and XDR TB are forms of RR TB [61].

1.7. TB in HIV

The risk of developing TB is estimated to be between 26 and 31 times greater in people living with HIV than among those without HIV infection. In 2014, in the new cases of TB, 1.2 million were people living with HIV and 0.4 million among people with HIV-positive died along with TB disease [4].

In patients with CD4 counts $\geq$50 cells/mm^3 who present with clinical disease of major severity, as indicated by clinical evaluation (including low Karnofsky score, low body mass index, low hemoglobin, low albumin, organ system dysfunction, or extent of disease),

antiretroviral therapy should be initiated within 2 to 4 weeks of starting TB treatment. The strength of this recommendation varies on the basis of CD4 cell count. In HIV-infected patients with documented MDR and XDR in TB, antiretroviral therapy should be initiated within 2 to 4 weeks of confirmation of TB drug resistance and initiation of second-line TB therapy [62].

It must also be noted that several of the problems associated with TB, such as resistance and HIV co-infection are highly coupled. For example, due to the high abandon rate of treatment by co-infected patients, there is a higher emergence of drug resistant strains [63]. Such opportunistic infections have a disastrous effect on the mortality rate in infected patients.

1.8. Current treatment in TB

Table 1.2: Group name and mechanism of action of first and second line anti-TB agents.

Name of the group	Drug*	Mechanism of action
First line anti-TB drugs	**Isoniazid**	Inhibition of Mycolic acid biosynthesis
	Rifampin	Inhibition of RNA synthesis
	Pyrazinamide	Disruption of electron transport across the membrane
	Ethambutol	Arabinogalactone synthesis inhibitor
Second line Injectable anti-TB drugs	Kanamycin	Protein Synthesis Inhibitor
	Amikacin	Protein Synthesis Inhibitor
	Capreomycin	Protein Synthesis Inhibitor
Second line Fluoroquinolones	Levofloxacin	Inhibition of DNA gyrase
	Gatifloxacin	Inhibition of DNA gyrase
	Ofloxacin	Inhibition of DNA gyrase
	Ciprofloxacin	Inhibition of DNA gyrase
	Moxifloxacin	Inhibition of DNA gyrase
Second line (oral bacteriostatic) anti-TB drugs	**Ethionamide**	Cell wall synthesis inhibitor
	Prothionamide	Cell wall synthesis inhibitor
	Cycloserine	Inhibition of peptidoglycan synthesis
	***p*-Aminosalicylic acid**	Inhibition of folic acid and Iron metabolism
	Bedaquiline	ATP synthetase Inhibitor

*Drugs in bold letters are FDA-approved for use in TB therapy.

1.8.1. Treatment for latent-TB infection (LTBI)

TB germs can live in our body without making you sick. This is called latent TB infection**.** People with latent TB infection do not have symptoms, and they cannot spread TB bacteria to others. LTBI testing is very mandatory for these people; close contacts of infectious TB patients, health care workers (particularly susceptible to TB exposure and infection) and frequent travellers abroad people [64]. For treating LTBI there are few regimens used based on the results of drug susceptibility testing [65]. INH nine months therapy but it is long treatment, RMP four months therapy but it is direct treatment and RMP–PZA two months therapy due to stern hepatic injury and death, this regimen was not recommended.

CDC has recommended a 12-dose regimen; the regimen is a combination of INH and RMP doses under directly observed treatment. This 12-dose regimen is very effective which reduces the required treatment for LTBI from 270 daily doses over 9 months.

1.8.2. Treatment for drug susceptible-TB

The existing TB treatment consists of isoniazid, ethambutol, RMP and PZA for two months followed by isoniazid and RMP for four months. This standard TB therapy is prolonged as patients have to take the drugs for six months and often leads to patient's non-adherence. In these circumstances an incomplete treatment results in development of drug resistance. To confront this situation, WHO promoted a program known as "Directly Observed Treatment-Short course (DOTS)", DOTS is effective in many controlled trials; few studies have evaluated its effectiveness under programmatic conditions [66]. In this type of treatment there is a direct observation by trained personnel on patients undergoing treatment. The DOTS therapy has established to be one of the most cost effective health interventions available today around the world. It has been proven to be one of the most efficient approaches to fight the global TB epidemics [4]. Effectiveness and tolerability relation of first- and second-line drugs used in TB treatment is depicted in **figure 1.7**.

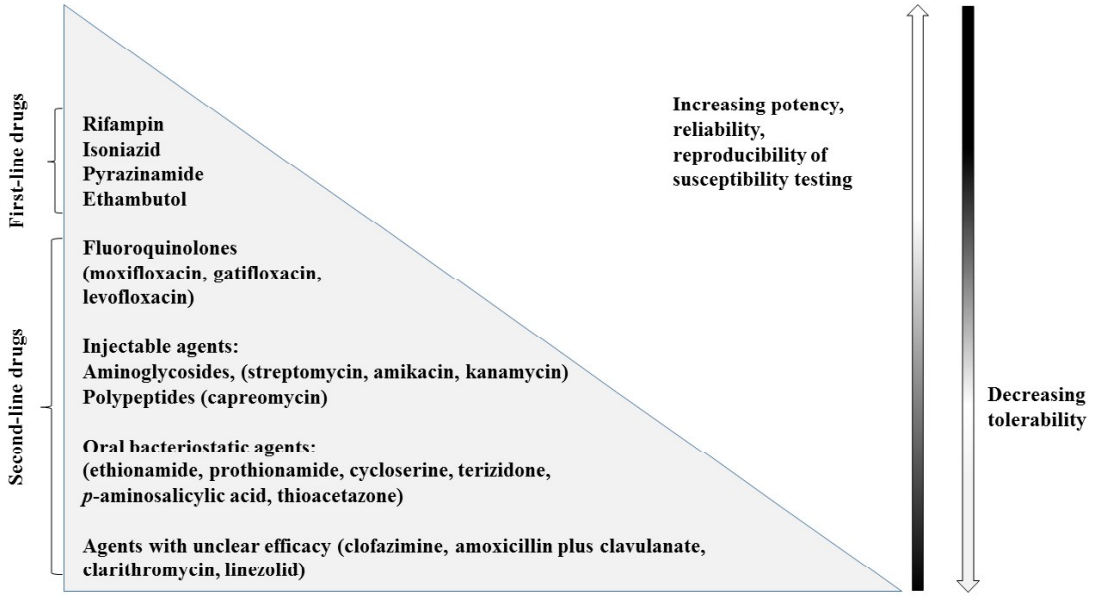


Figure 1.7: Effectiveness and tolerability relation of first- line and second-line drugs used in TB treatment.

1.8.3. Treatment for drug resistant-TB

Drug resistant TB can be cured with the suitable combination and reasonable use of available anti-tubercular drugs. The poly drug-resistant TB therapy of contagions involving (INH + EMB, INH + PZA, EMB + PZA, or INH + EMB + PZA) MTB strains entails careful clinical evaluation but can be managed with extended treatment up to 18 months by regimens containing first-line drugs, fluoroquinolones, KAN/AMK/CAP and some second-line agents. Treatment of MDR TB is lengthy, expensive, toxic, and associated with higher rates of clinical failure and disease relapse. For the treatment of MDR TB, WHO recommends the use of DOTS-Plus therapy, which includes drugs used in DOTS therapy plus second line TB drugs composition of MDR TB drug regimen; 1): a) Injectible second line drugs b) Later generation fluoroquinolone c) ETH or prothionamide (PTH) d) Cycloserine or terizidone; 2): Usage of at 4 drugs (it is unclear whether all patients with MDR TB/XDR TB should be treated with PZA); 3): Group five drugs to be used only if needed to sum up to at least four active drugs; 4): Healing for a total of 24 months with an intensive phase of 8 months; 5): Prolongation of duration of therapy should be considered based on success [67]. Bedaquiline and delamanid are recently approved by US FDA for the treatment of MDR TB in adults [4]. Bedaquiline and delamanid can be used for treatment of MDR TB in

adults when patients in serious or life-threatening conditions do not have an effective treatment regimen [68].

Drugs for treatment of XDR TB or TDR TB need to be selected stepwise on basis of safety and efficacy. New drugs (pretomanid, delamanid and bedaquiline) and novel regimens (PA-824+Moxifloxacin+ PZA and NC-003) for curing drug resistant TB are now available. The new combination 3 (NC-003) clinical trials tested the bedaquiline + PTH + PZA (BpaZ) regimen, consisting of bedaquiline, PA-824, and PZA. The two-week study found that the PZA regimen killed more than 99% of TB bacteria over the course of 14 days, and that the treatment was safe. These novel chemospheres are reducing the treatment period and cost of therapy [69, 70].
If sufferers are untreated, the analysis for people laid low with drug-resistant TB is similar to the analysis for people with drug-touchy TB (10 yr case fatality charges of about 70%). The modern-day WHO-endorsed MDR TB routine has an approximate 50% treatment price, while the cure fee in endemic settings of drastically drug-resistant TB within the absence of drugs together with bedaquiline, delamanid and linezolid is about. Thus, TB (and drug-resistant TB specially) poses a grave risk to human fitness and great of existence. High-excellent patient care, regular with the International Standards for TB Care, is essential to make sure exact consequences and hold the pleasant of lifestyles. Unfortunately, international standards are frequently not met in lots of low-income, high-burden international locations, mainly within the personal fitness quarter, which is a primary company of health care in many countries with a excessive TB incidence. Poor best of care is, therefore, a key driver of TB mortality in excessive-burden nations, and might give an explanation for the persistently excessive TB incidence in some settings. Whereas country wide programmes are responsible to country wide and worldwide authorities regarding their implementation of proper standards of care, one of the finest demanding situations in TB manipulate continues to be enticing and regulating the non-public sector. Innovative public-non-public blend procedures are required to overcome this task, together with social franchising, insurance-based totally projects, middleman corporations and provider consolidation, with a heavy emphasis on the use of records and conversation technologies.

1.9. Classification of anti-TB drugs

The available anti-TB drugs are classified based on their mechanism of action or inhibition. These are classified under following heads; i) cell wall synthesis inhibitors (INH, EMB, ETH and cycloserine), ii) nucleic acid synthesis inhibitors (rifampin and quinolones), iii) protein synthesis (SM, AMK, KAN & CAP), and iv) electron transport across the bacterial membrane or energy inhibitors. (PZA) [71].

1.9.1. Cell wall synthesis inhibitors

Cell wall synthesis inhibitors such as INH, EMB, ETH, PTH and cycloserine are used for TB treatment.

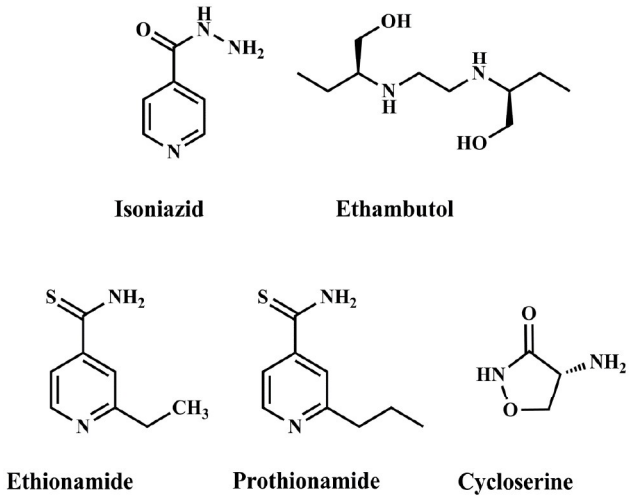


Figure 1.8: Cell wall synthesis inhibitors.

Isoniazid (INH)

Isonicotinyl hydrazide or Isonicotinic acid hydrazide or INH was introduced in 1951 for the treatment of TB and it is more potent drug than streptomycin and *p*-aminosalicylic acid. It is a prodrug activated by 'catalase peroxidase' enzyme (KatG) and active against growing tubercle bacilli, but not active against nonreplicating bacilli. The primary target of inhibition is the cell wall mycolic acid synthesis pathway [50]. KatG links the isonicotinic acyl part to NADH resulting in an isonicotinic acyl-NADH complex. This complex binds efficiently to the InhA which is an enoyl-acyl carrier protein reductase, and blocks the natural enoyl-AcpM substrate and the action of fatty acid synthase. Consequently, the inhibition of synthesis of mycolic acids is terminated [50, 71].

Recent research shows that besides InhA it also attacks DfrA ('dihyrofolate reductase' involved in DNA synthesis) [50]. Since, its wide range of usage resistance to INH has been seen more repeatedly among clinical isolates of MTB infected patients. Resistance to INH occurs due to the mutations in *KatG* gene; as a result the ability of catalase peroxidase to activate INH prodrug reduces. The Hepatitis, lupus-like syndrome, peripheral neuropathy and drug-drug interactions are major adverse reactions of isoniazid [72].

Ethambutol (EMB)

EMB ([(S,S)-2,2(ethylenediimino)di-1-butanol]) was discovered as anti-tubercular agent in 1961. EMB plays a pivotal role in the chemotherapy of drug-resistant TB, including MDR TB. It is a first line anti-tubercular drug. Ethambutol appears to target the cell wall of tubercle bacilli through interfering with arabinosyl transferases, encoded by the embCAB operon, comprised of three homologous genes, designated embC, embA, and embB, and involved in the biosynthesis of arabinogalactan and lipoarabinomannan, the key structural components of the mycobacterial cell wall. The proposed scenario of EMB action on MTB is that upon interaction with the EmbCAB proteins EMB inhibits the arabinan synthesis leading to lack of arabinan receptors for mycolic acids and accumulation of mycolic acid results in cell death [71, 73]. Resistance to EMB has repeatedly been associated with alterations in the embB gene, particularly in mutations in embCAB operon are responsible for resistance to EMB and are found in approximately 65% of clinical isolates of MTB resistant to EMB [71]. Some inconsistent reports revealed that one quarter of all EMB resistant MTB isolates do not harbour mutations in any of the above named genes, investigations suggesting further canvases are needed to explore possible mechanism of EMB resistance [72].

Ethionamide (ETH) and prothionamide (PTH)

Thioamide drugs are Ethionamide (ETH) and Prothionamide (PTH), these are generally considered second-line drugs for treatment of TB and MDR TB. ETH is structurally related to INH and as well as a prodrug that is activated by the enzyme EtaA (a monooxygenase, also called EthA) and inhibits the same target InhA as INH of the mycolic acid synthesis pathway [44]. EtaA/EthA is a flavin adenosine dinucleotide (FAD) containing enzyme that oxidises ETH to the corresponding S-oxide. Similar to INH, ETH inhibits mycolic acid synthesis by binding to the enzyme InhA. EthA to activate ETH has been convincingly demonstrated, very limited data exist on the occurrence of EthA mutations in ETH-resistant MTB clinical isolated [73]. The

existence of partially cross-resistant phenotypes has long been known. Low-level INH-resistant strains frequently display low-level ETH resistance, while high-level INH-resistant strains typically remain ETH susceptible. The structural similarity and existence of cross-resistant phenotypes suggested that these two drugs share a common molecular target [71].

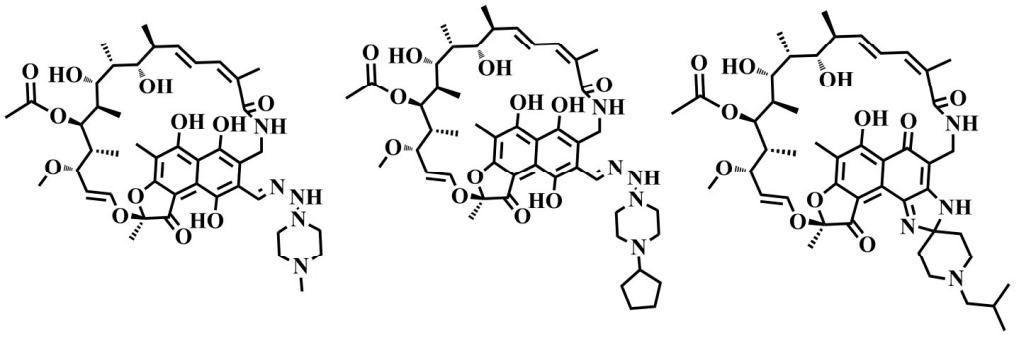
PTH shares almost identical structure and activity as ETH, where the R group in ETH is C_2H_5 and the R group in PTH is C_3H_7. It is a better tolerated and less toxic drug than its predecessor of ETH [74]. The activated PTH form adducts with nicotinamide adenine dinucleotide (NAD), which is inhibitor of the InhA enzyme in MTB. Inhibition of InhA results in inhibition of mycolic acid biosynthesis and cell lysis. Mutations in the drug-activating enzyme EtaA/EthA and the target InhA cause resistance to ETA [75].

Cycloserine (CS)

Cycloserine is a D-alanine analogue; it is classified as a second-line drug. CS is particularly used for healing MDR TB and XDR TB. CS interrupts peptidoglycan synthesis by inhibiting the enzymes D-alanine racemase (AlrA) and D-alanine:D-alanine ligase (Ddl) [76] which inhibits the synthesis of cell wall mycobacteria. Resistance to CS is due to over expression of AlrA and Ddl) [76].

1.9.2. Nucleic Acid Synthesis Inhibitors

Rifampin and other rifamycins



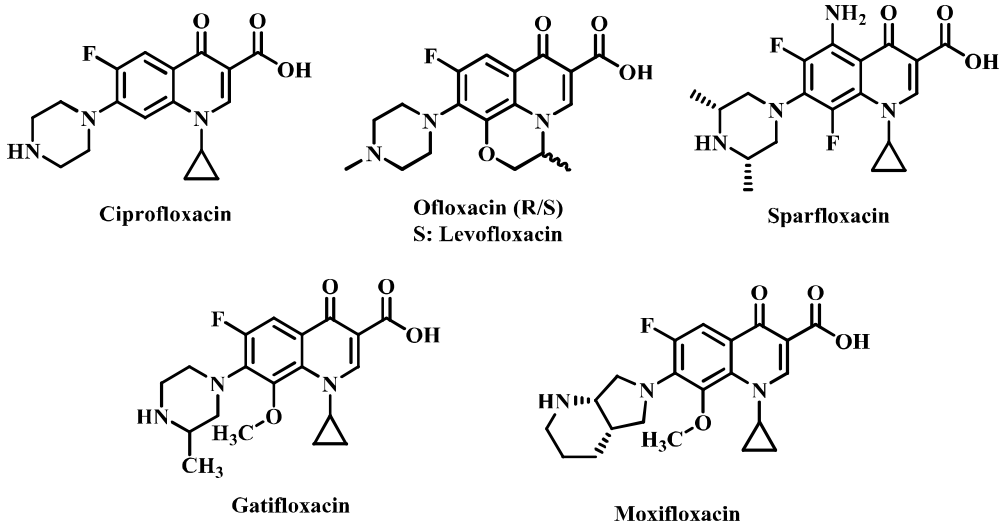
Rifampicin **Rifapentine** **Rifabutin**

The Rifamycins include Rifampin (RMP), Rifapentine, and Rifabutin. Of these, RMP is most commonly used, as first-line therapy for treatment of mycobacterial disease (including TB). The addition of rifampin to treatment regimens for TB reduces the duration of therapy needed for active disease from 12 to 6 months and reduces the duration of therapy needed for latent infection from 9 months to 2 to 3 months. RMP interfere with bacterial DNA-dependent RNA

polymerase and are potent bactericidal agents. RMP and its analogues kill actively multiplying extracellular organisms, intracellular mycobacteria, and semidormant mycobacteria in tissues [77].

RMP contain an aromatic nucleus linked on both sides by an aliphatic bridge so as to easily diffuse across the MTB cell membrane due to their lipophilic profile. These have ability to inhibit bacterial DNA-dependent RNA synthesis, due to high binding affinity with RNA polymerase [71]. Resistance to RMP is a result of mutations in the *rpoB* gene, which encodes the β-subunit of RNA polymerase [77].

Fluoroquinolones (FQ)



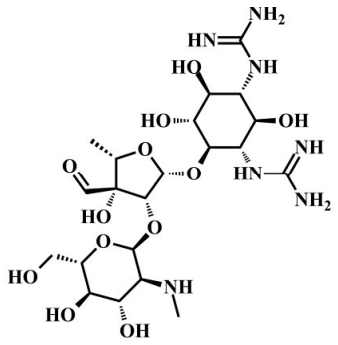
FQs were derived from quinine. Nalidixic acid, the first quinolone derivatives was introduced in 1962 by George Lesher *et al.,* discovered as a by-product of chloroquine synthesis [78]. The fluoroquinolones (moxifloxacin, gatifloxacin, sparfloxacin, levofloxacin, ofloxacin, and ciprofloxacin), possess excellent activity against MTB and are used as second-line drugs in TB treatment [71]. Most FQs are being evaluated as potential anti-TB drugs, also for their proven potential to shorten TB treatment duration. Use of FQs is one of the major strategies for TB control. They are the class of antibiotics that have potent antimicrobial activity against a wide range of gram positive and gram negative organisms. The treatment of MDR TB relies upon a backbone of an injectable agent (KAN, CAP, AMK and a fluoroquinolone namely gatifloxacin, levofloxacin, moxifloxacin, or ofloxacin. The most active fluoroquinolones are: moxifloxacin = gatifloxacin > levofloxacin > ofloxacin [79]. In addition to the aforementioned fluoroquinolones, several clinical studies have investigated the efficacy of sparfloxacin and lomefloxacin.

sparfloxacin appears effective for treating MDR TB, the role for lomefloxacin in TB therapy is unclear. Gatifloxacin and moxifloxacin are under phase III clinical evaluation aiming at better TB treatment [79].

FQs inhibits DNA synthesis by targeting the DNA gyrase A and B subunits. It blocks the movement of replication works and transcription complexes in MTB [71]. Resistance to FQs in MTB is due to mutations in the conserved quinolone resistant determining region of gyrA and gyrB involved in the interaction between the drug and DNA gyrase [71]. In addition to the bacteria gaining resistance to the FQ class of drugs, a few serious side effects such as tendonitis and tendon rupture due to collagen damage, QTc interval prolongation by blocking voltage-gated potassium channels etc associated with these classes of drugs has limited their clinical use and future progress, necessitating research into development of new antibacterial agents that lack cross-resistance mediated by mutations in the bacterial targets [80].

1.9.3. Protein synthesis Inhibitors

Streptomycin (SM)

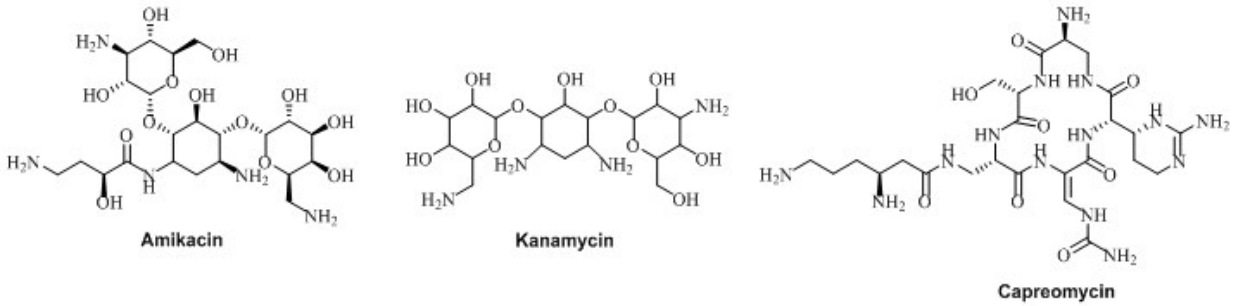


Streptomycin

Streptomycin is an antibiotic (antimycobacterial) drug, the first of a class of drugs called aminoglycosides to be discovered, and it was the first effective treatment for TB [71]. Streptomycin acts as inhibitor of protein synthesis by binding to the S12 protein of the 30S subunit of the bacterial ribosome and interfering with the binding of formyl-methionyl-tRNA to the 30S subunit of the ribosome [81]. These results in precarious ribosomal-mRNA complex, foremost to frameshift mutation and flawed protein synthesis and further to cell death. Mutations in *rpsL* and *rrs* are the major mechanism of SM resistance and it is exhibits toxic manifestations

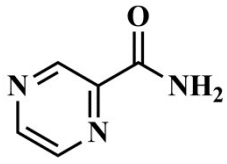
on peripheral and central nervous system at higher doses and leads to hypersensitivity reactions [81].

Amikacin, Kanamycin and Capreomycin



The aminoglycosides KAN and AMK and the macrocyclic peptide CAP are key drugs for the treatment of MDR TB as second line drugs. These are protein synthesis inhibitors. These drugs bind to 16S rRNA in the 30S small ribosomal subunit and inhibit protein synthesis. The A1401G mutation has been assorted with AMK resistance in MTB, and change in their rrs genes. CAP is a macrocyclic polypeptide, like streptomycin and KAN it modifies the ribosomal structure at 16S RNA there by inhibiting protein MTB resistant to KAN and CAP has been associated with mutations in the *rrs* gene encoding 16S rRNA [82]. Mutations at 16S rRNA position 1400 are associated with high-level resistance to KAN and AMK. Cross-resistance may be observed between KAN and CAP but a recent study found little cross-resistance between KAN and AMK [71, 82].

1.9.4. Electron transport across membrane inhibitors



Pyrazinamide

Pyrazinamide (PZA) or pyrazine-2-carboxamide is an important front-line drug for the treatment of TB; it is used in combination with additional drugs *viz.* INH and RMP for the treatment of MTB. It is still part of WHO suggested standard TB therapy. The use of PZA was first introduced in 1954 and was a great success as it resulted in shortening the duration of the TB

therapy to current 6 months than initial 9 months [83]. PZA is a prodrug that stops the growth of MTB. PZA diffuses into the granuloma of MTB, where the enzyme pyrazinamidase converts PZA to the active form pyrazinoic acid. Under acidic conditions, the pyrazinoic acid that slowly leaks out converts to the protonated conjugate acid, which is thought to diffuse easily back into the bacilli and accumulate. The net effect is that more pyrazinoic acid accumulates inside the bacillus at acid pH than at neutral pH [83]. Pyrazinoic acid was thought to inhibit the enzyme fatty acid synthase (FAS) I, which is required by the bacterium to synthesise fatty acids. PZA and its analogues inhibited the activity of purified FAS I. Pyrazinoic acid binds to the ribosomal protein S1 (RpsA) and inhibits trans-translation. This may explain the ability of the drug to kill dormant mycobacteria [84]. The PZA resistance in MTB is due to mutations in the *pncA,* furthermore to PZA resistance associated with *pncA, rpsA* or *panD* mutations, it also described that can change pncA expression, altered PZA update, or dysregulated pyrazinoic acid efflux, which creates fault in the functioning of pyrazinamidase [83, 85].

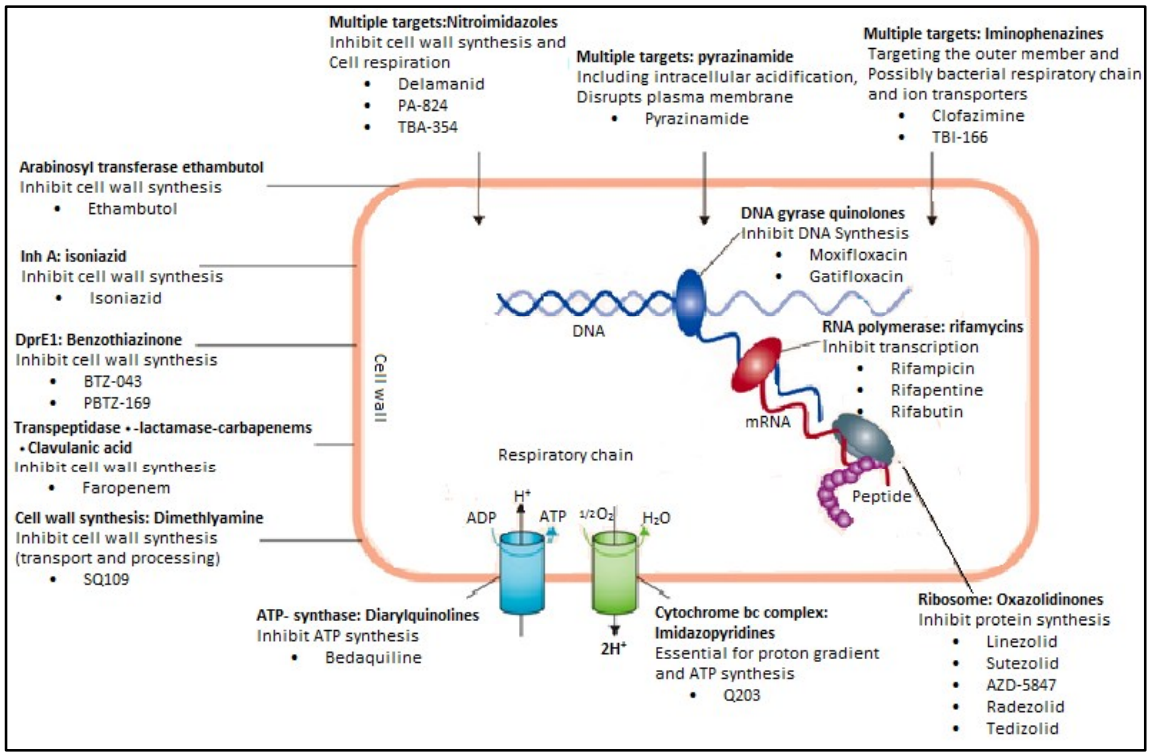


Figure 1.9: Mechanism of action of existing drugs/new anti-tubercular drugs in development.

1.10. Current emerging pipeline new anti-TB drugs

Substantial progress has been made in last 40 years and a promising portfolio of new anti-tubercular drugs is on the horizon. Some have the potential to become the cornerstone of future TB treatment. There is now recognition that new drugs to treat TB are urgently required, specifically for use in shorter treatment regimens than are possible with the current agents and which can be employed to treat multidrug-resistant and latent disease. A variety of new initiatives have been created to tackle these objectives, the most recent of which is the establishment of the so-called Global Alliance for TB Drug Development [70, 86]. The Alliance, a public/private partnership in which WHO is a partner, is a not-for-profit venture that will accelerate the discovery and development of new drugs to fight TB using a virtual operating model to outsource projects. The current decade blossoms with a promising anti-TB drug pipeline, with various potential drugs targeting diverse MTB terminating sites in several stages of drug development.

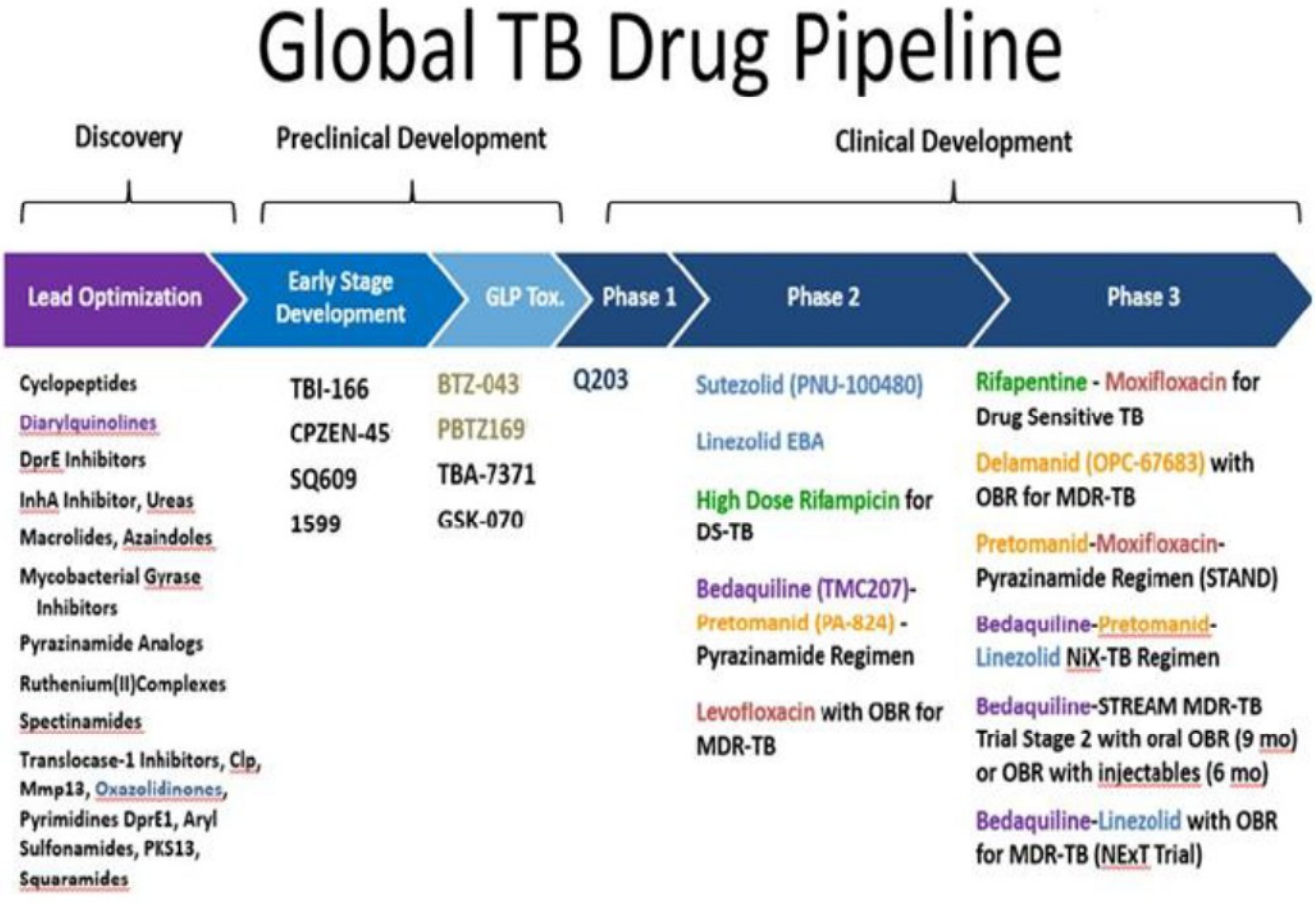


Figure 1.10: Various agents that are currently being investigated for TB therapy.

Eight drugs and combinations are in Phase I, Phase II or Phase III trials for the treatment of drug-susceptible, MDR TB or LTBI.

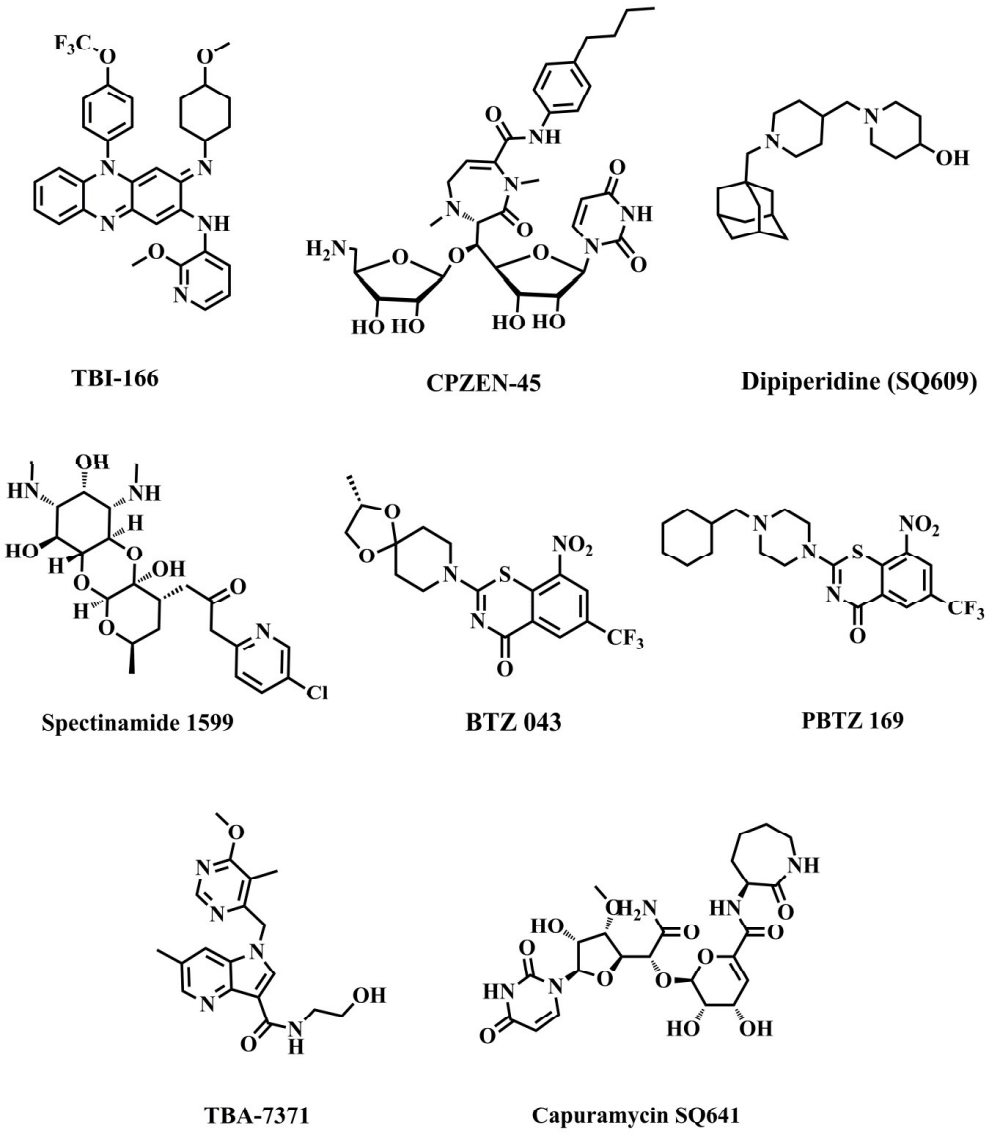


Figure 1.11: Structure of anti-TB agents under preclinical development.

1.10.1. Q203

Q203 is the first in new class of amide imidazopyridine compounds. The rapid inhibition of ATP synthesis at low concentration strongly suggests that the inhibition of cytochrome bc1 activity is the primary mode of action of Q203, which is a bacterial enzyme complex needed for respiration. Q203 causes a rapid depletion of intracellular ATP at an IC50 of 1.1 nM and interrupts ATP homeostasis in dormant Mtb at an IC50 of 10 nM. Both of these values are better

than bedaquiline's measures, and they explain Q203's excellent killing profile in chronic Mtb infection models. Q203 did not inhibit any P450 isoforms and was not a substrate or inhibitor of P-gP efflux, which indicated a low risk of drug–drug interactions. It is found that Q203, being a new chemical entity is able to inhibit MDR TB and XDR TB [87]. In phase I dose-escalation study to evaluate safety, tolerability and pharmacokinetics of single and multiple doses of Q203 in healthy volunteers started in March 2016. Though it should be noted that like bedaquiline, Q203 is a highly lipophilic drug, with very high serum protein binding. The Phase I clinical trial (clinicaltrials.gov identifier: NCT02530710) enrolling healthy patients is a dose-escalation study starting at 100 mg dosing that will be adjusted based on PK analysis.

1.10.2. Sutezolid

Sutezolid (PNU-100480, PF-02341272) is an oxazolidinone antibiotic currently in development as a treatment for XDR TB. It has safety profile than linezolid. Its activity and pharmacokinetic data shows that sutezolid converts into sulfone and sulfoxide metabolites, the sulfoxide metabolite is more active and reaches four times higher in concentration than parent compound [88]. It inhibits protein synthesis by the ribosomal initiation complex [89]. Mouse model studies showed that addition of sutezolid to current first line TB drugs improved the bactericidal activity. It also gave better results when used in combination with moxifloxacin and PZA. Sutezolid was safe and well--tolerated at doses up to 1200 mg daily for up to 14 days, or 600 mg twice daily for up to 28 days. 4 A Phase 2 trial demonstrated that sutezolid has significant early bactericidal activity and may have clinical efficacy in humans in a larger Phase 2 trial. The addition of sutezolid to the standard TB treatment regimen leads to significantly improved efficacy. In vivo studies in the chronic mouse model of TB demonstrated that the addition of sutezolid to the standard TB regimen has the potential to significantly shorten treatment. It not only reduced the numbers of bacteria in the lungs more quickly, but also led to a relapse- free cure with a shorter duration of treatment. Pfizer recently completed a phase IIa, open-label, early bactericidal activity and whole-blood activity study. This study of adults with pulmonary DS-TB compared two experimental arms one with sutezolid twice daily at 600 mg, the other with sutezolid once daily at 1,200 mg with Rifafou. These outputs suggest that sutezolid has the potential to reduce the treatment duration in both drug susceptible and drug resistant TB [88].

1.10.3. SQ109

SQ109 1,2-ethylenediamine, is an analogue of ethambutol. It was discoverednby screening a combinatorial library of more than 63,000 compounds with anwhole bacterium high throughput screen. Unlike EMB, SQ109 has different mechanism of action and belongs to the classes of cell wall inhibitors. The drug is active against both drug-susceptible and drug-resistant TB by targeting MmpL3 in MTB and specifically inhibiting the protein synthesis [90, 91]. In clinical trials study to determine safety, tolerability, pharmacokinetics and bacteriological effect of different doses of SQ109 alone and in combination with RMP was administered over 14 days. Several in vivo research in the chronic mouse version of TB the use of combinations of SQ109 and popular anti-TB tablets display both higher efficacy and shorter time to obtain the equal discount in MTB as preferred remedy with ethambutol. In studies in which SQ109 replaced EMB in the trendy first line treatment routine, no or few micro organism have been cultured from lungs of mice treated for 2 months, suggesting that SQ109 effects in a extra fast cure. It revealed no adverse drug-drug interactions, good activity against drug susceptible and drug resistant TB was observed. The *in vitro* bacterial mutation rate for SQ109 is very low which could limit the development of drug resistance to SQ109. *In vitro*, it has some synergic effects with bedaquiline and favourable interactions with sutezolid [92]. 82 unfavourable occasions, of which fifty six% were gastrointestinal occasions One affected person died during the 14 day comply with-up duration because of big hemoptysis. This turned into deemed unrelated to have a look at drug by way of the investigator. No different serious negative activities (SAEs). There had been no ECG-associated treatment discontinuations. There became no prolongation of QTcB or QTcF past 500ms, or an growth of more than 60ms in comparison to baseline. Safety/Tolerability of SQ109 in TB patients. It‘s principal facet effect is nausea, which is more reported within the 300mg dose There have been no systematic increases in QT in the SQ109 groups Steady kingdom seems to be reached at ~day 7; the induction of CYP2C19 through Rif can be conquer with 300mg SQ109 SQ109 had no bactericidal effect in humans over 14 days; RIF had a 1-log effect in human beings over 14 days. Mouse modeling statistics suggest that: - EBA statistics in people mimics that seen in mouse - SQ109 results are obvious the longer the drug is taken [92].

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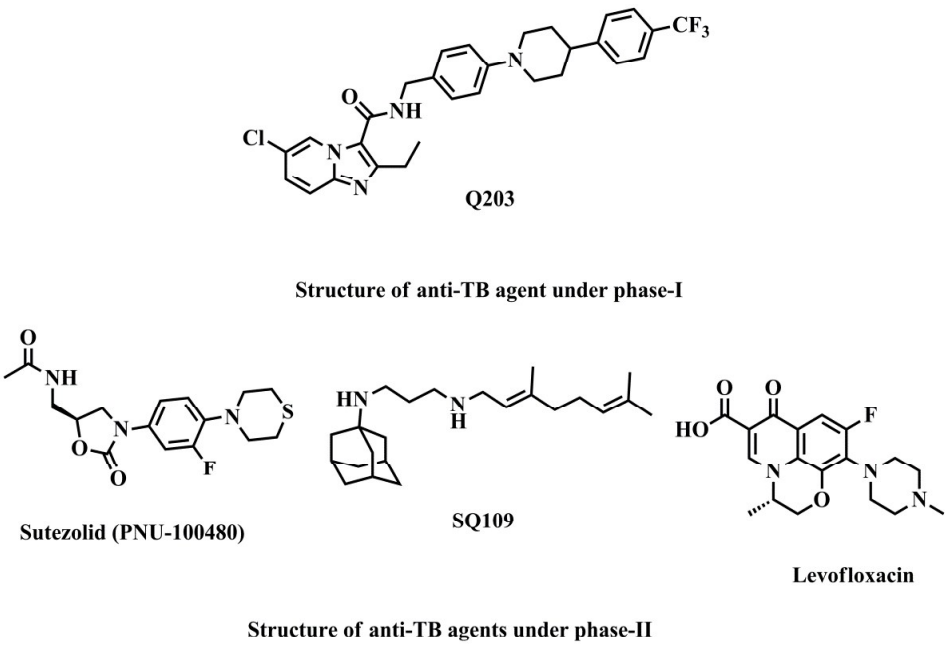

Figure 1.12: Structure of anti-TB agents under phase-I & II clinical trials

1.10.4. Levofloxacin (LEV)

LEV is higher-generation fluoroquinolone antibiotic. It inhibits the two type II topoisomerase enzymes, namely DNA gyrase and topoisomerase IV. Topoisomerase IV is necessary to separate DNA that has been replicated (doubled) prior to bacterial cell division. With the DNA not being separated, the process is stopped, and the bacterium cannot divide. DNA gyrase, on the other hand, is responsible for supercoiling the DNA, so that it will fit in the newly formed cells. Both mechanisms amount to killing the bacterium. In this way, LEV acts as a bactericide [92]. LEV and moxifloxacin imposed no additional hepatotoxicity on patients with drug-induced liver injury (DILI) secondary to first-line anti-TB therapy. These two drugs could be safely prescribed while waiting for liver function normalization. No cases of DILI occurred among patients during the follow-up period. LEV and moxifloxacin were safe for long-term use [93]. Phase-II studies determined the LEV dose and exposure that achieves the greatest reduction in MTB burden with acceptable tolerability by studying 100 adults with smear- and culture-positive pulmonary MDR TB at sites in Peru and South Africa [93]. While its side outcomes are generally mild to slight, serious reactions to levofloxacin now and again occur.

Prominent among those are side effects that became the challenge of a black box warning by means of the FDA in 2016. An FDA protection evaluation has shown that fluoroquinolones when used systemically (i.E. Pills, drugs, and injectable) are associated with disabling and probably permanent severe side outcomes that can occur together. These facet results can involve the tendons, muscle tissue, joints, nerves, and important anxious device. Such injuries, such as tendon rupture, have been determined up to 6 months after cessation of remedy; the elderly, transplant patients, and people with a present day or historic corticosteroid use are at elevated risk. A precise evaluate of threat factors for fluoroquinolone-related tendon rupture has been posted; superior age, concurrent treatment with corticosteroids, and better doses of fluoroquinolone appear like the maximum crucial chance factors. The U.S. Label for levofloxacin additionally includes a black field warning for the exacerbation of the signs of the neurological disorder myasthenia gravis.

1.10.5. Delamanid

The Delamanid (OPC-67683) (Deltyba) is a nitroimidazo-oxazole derivative; it is a new anti-TB drug which exhibits potent *in vitro* and *in vivo* anti-TB activity against drug-susceptible and drug-resistant strains of MTB. The new drugs delamanid and bedaquiline are increasingly used to treat MDR-TB & XDR-TB. Its early bactericidal activity is approved in the EU and Japan for the treatment of MDR-TB, when administered in combination with an optimized background regimen [94].

Delamanid is currently being tested in a Phase III clinical trial, as an addition to an optimized background regimen (OBR) for the treatment of MDR-TB. The trial is comparison six months of treatment with delamanid plus the OBR with a placebo plus OBR. Delamanid was well tolerated, QT prolongation was more frequently reported in patients receiving delamanid against those receiving placebo. Based on the available evidence, WHO recommends the use if delamanid at the dose of 100 mg twice daily for 6 months, added to OBR in adults, when pharmacovigilance is in place and informed consent ensured. As a result delamanid has favorable safety profile compared to existing second-line drugs [4, 93, 94].

Delamanid primarily inhibit synthesis of methoxy-mycolic and keto-mycolic acid, which are components of the mycobacterial cell wall; unlike INH, the drug does not inhibit alpha-mycolic acid but it has no action against gram-negative or gram-positive bacteria and this is

clinically beneficial as its restriction prevent the generation of resistance. Delamanid is a prodrug that requires metabolic activation for anti-TB activity to be exerted. Reactive intermediates in the metabolic pathway of the bicyclic nitroimidazoles may provide additional mechanisms of action, including interruption of cellular respiration. Activation of delamanid is mediated via the mycobacterial F420 coenzyme system [94, 95].

The most common aspect consequences are nausea, vomiting and dizziness. These might also affect as many as a 3rd of all sufferers. There is likewise a critical aspect impact referred to as QT prolongation. QT prolongation is an alteration of the electrical pastime of the heart. It can cause a life-threatening abnormality of the heart rhythm. Anxiety, pins & needles, and shaking are other vital facet effects [94].

1.10.6. Pretomanid (PA-824)

Pretomanid (PA-824) is an investigational anti-TB drug. PA-824 is a bicyclic nitroimidazole-identical molecule with a very complex mechanism of action. PA-824 was developed at Pathogenesis Corporation and later transferred to the TB Alliance, where it is currently undergoing Phase III clinical trials. New regimens based on nitroimidazole novel agents are required in order to shorten or abridge the treatment of both drug-susceptible and drug-resistant forms of TB [4]. Now it is in phase-II trials and has shown significant early bactericidal activity alone and in combination with the newly approved agent bedaquiline or with pyrazinamide and in phase-III trials with pyrazinamide and moxifloxacin. PA-824 also shows promise for MDR TB patients who are sensitive to the drugs in the regimen, reducing treatment from 2 years to 4 months and costing just a fraction of the current MDR TB treatment. Additionally, PaMZ regimen can be administered in a fixed dose for all patients, and will therefore be simpler for health systems to deliver and patients to use [93].

PA-824 is also a prodrug like INH and requires the activation of aromatic nitro group by F420-dependent mechanism. It inhibits both protein and lipid synthesis but does not affect nucleic acid synthesis. It undergoes nitro reduction producing highly reactive intermediates which then reacts with multiple targets inside the bacterial cell. PA-824 has been observed to kill bacteria in two distinct mechanisms: a) by interfering with the synthesis of ketomycolate which is an essential component of the mycobacterial cell wall, and b) by acting as a nitric oxide donor and causing respiratory poising [95]. Pretomanid recently was shown to be safe, well tolerated, and

efficacious at doses of 100–200 mg daily in a dose-ranging study among drug-sensitive, sputum smear positive, adult pulmonary TB patients.

1.10.7. Rifapentine

Rifapentine (also known as cyclopentyl rifampicin and Priftin) is a medication recommended by the WHO as a first-line treatment for TB and approved by the U.S. FDA as a treatment for pulmonary TB in 1998 [4, 77]. Like other RMP, rifapentine inhibits bacterial DNA-dependent RNA polymerase. It kills TB bacteria by inhibiting bacterial RNA polymerase, which is the enzyme responsible for transcribing DNA into RNA (RNA is subsequently used to make bacterial proteins). By disrupting the bacterial RNA polymerase only, rifapentine eliminates TB bacteria while leaving human RNA polymerase unaffected. Rifapentine has a long half-life in serum and is therefore administered less frequently. Its half-life is 5 times that of RMP [71, 77]. Rifapentine should be given with isoniazid during the continuation phase of the treatment of drug-susceptible pulmonary TB after an intensive phase that consists of at least rifampin (or rifabutin), INH, PZA, and ethambutol administered for two months [77]. The 6-month regimen that included weekly administration of high-dose rifapentine and moxifloxacin was as effective as the control regimen. Rifapentine is presently in phase-III trials with moxifloxacin for the treatment of drug-susceptible TB [4]. Rifapentine was shown to be safe and effective in HIV negative patients, which was the basis for the current Centers for Disease Control and Prevention recommendation for using rifapentine and isoniazid in selected patients during the continuation phase of therapy. Common side effects consist of low neutrophil counts inside the blood, multiplied liver enzymes, and white blood cells inside the urine. Serious facet effects may additionally consist of liver issues or Clostridium difficile related diarrhea. It is uncertain if use all through being pregnant is safe. Rifapentine is inside the rifamycin circle of relatives of drugs and works with the aid of blockading DNA-structured RNA polymerase [77].

1.10.8. Moxifloxacin

Moxifloxacin is a fourth-generation synthetic FQ antibacterial. Moxifloxacin is an important option for the treatment of MDR TB. A retrospective analysis showed that levofloxacin and moxifloxacin showed equivalent efficacy for treating MDR-TB. Moxifloxacin can be replaced with gatifloxacin, the FQ used in the earlier studies of shorter MDR TB regimens. Moxifloxacin

is a key component of the experimental arms of a trial that is intended both to shorten treatment from 6 to 4 months for patients with susceptible disease and to provide an all-oral 6-month treatment for patients with MDR-TB. This provides a significant advantage over the standard-of-care MDR-TB regimen that includes an injectable antibiotic for at least 3 months [71]. Rapid evaluation of moxifloxacin in TB study to determine whether the replacement of either INH or EMB with moxifloxacin would provide effective TB treatment in 4 months, as compared with the standard 6 months regimen. In phase-III clinical trials used with moxifloxacin with refapentine for the drug-susceptible TB. Pretomanid + Moxifloxacin + Pyrazinamide was tested in the Phase-IIa NC-001 trial, in which it killed TB bacteria faster when compared with the current TB regimen, as well as other experimental regimens over the first two weeks of treatment. It was subsequently tested in NC-002, in which it met its primary endpoint after eight weeks treatment. PaMZ has the potential to cure both TB and some forms of MDR TB in 4 months, drastically improving treatment [4, 71].

Moxifloxacin causes higher QT prolongation than LEV, knowing LEV has comparable efficacy is very useful in designing regimens with other QT-prolonging drugs, such as bedaquiline and delamanid. Moxifloxacin inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell replication [77, 90]. Rare but severe unfavorable outcomes that could occur due to moxifloxacin remedy consist of irreversible peripheral neuropathy, spontaneous tendon rupture and tendonitis, hepatitis, psychiatric consequences (hallucinations, melancholy), torsades de pointes, Stevens-Johnson syndrome and Clostridium difficile-related ailment and photosensitivity/phototoxicity reactions [71].

1.10.9. Bedaquiline

Bedaquiline (trade name Sirturo, code names TMC207 and R207910) is new class of diarylquinoline drug. Although the drug is active against many different bacteria, it has been registered specifically for the treatment of MDR-TB. It was discovered by a team led by Koen Andries at Janssen Pharmaceutica. Bedaquiline was approved on 28th December 2012 by the US FDA, and a drug of novel class to be approved over 40 years for treatment of MDR TB as part of combination therapy for adults with pulmonary TB [95]. In February 2016 it was announced that bedaquiline is to be made available in India. The drug will be available as part of second line

treatment for patients suffering from MDR-TB and XDR-TB [95]. Phase-III trial to investigate the safety and efficacy of bedaquiline when used in combination shortened MDR TB regimens of 9 and 6 months duration. By the end of 2014, 43 countries reported having used bedaquiline to treat patients as part of efforts to expand access to treatment for MDR TB [4]. Bedaquiline have to now not be co-administered with different tablets that are strong inducers or inhibitors of CYP3A4, the hepatic enzyme responsible for oxidative metabolism of the drug. Co-management with rifampin, a strong CYP3A4 inducer, results in a 52% decrease within the AUC of the drug. This reduces the exposure of the body to the drug and reduces the antibacterial impact. Co-management with ketoconazole, a robust CYP3A4 inhibitor, outcomes in a 22% increase in the AUC, and potentially an increase in the price of adverse results experienced [95].

Bedaquiline specifically inhibits the mycobacterium ATP synthetase as compared to mitochondrial ATP synthetase. ATP synthase is a critical enzyme in the ATP synthesis of MTB. Binding of bedaquiline to the oligomeric and proteolipic subunit-c of mycobacterial ATP synthase leads to inhibition of ATP synthesis, which subsequently results in bacterial death [95]. Bedaquiline can affect the heart's electrical activity causing prolongation of the QT interval, which could lead to an abnormal and potentially fatal heart rhythm. Accordingly, the FDA has approved bedaquiline as part of combination therapy to treat adults with MDR pulmonary TB when other alternatives are not available. The FDA also granted fast-track designation, priority review and orphan-product designation to bedaquiline [95]. Bedaquiline has been stated to disturb the characteristic of the heart and liver specifically. Interactions with different drugs, in particular lopinavir and efavirenz (used in the remedy of HIV), ketoconazole, as well as other capsules used inside the remedy of MDR-TB (eg moxifloxacin, clofazimine) may be expected. More deaths have been pronounced among sufferers taking bedaquiline for the duration of the research completed to research the drug, even though it is not clean whether or not this became due to the drug. For all these motives, it is critical that patients are closely monitored and that unfavourable activities are systematically stated ("energetic pharmacovigilance"), specially the ones which can be critical and existence-threatening. Clinical tracking of signs, performance of special assessments at appropriate periods, and engagement of the patient to document untoward effects of remedy to the appropriate pharmacovigilance organization are the cornerstones for the powerful control of side results in a timely style [95].

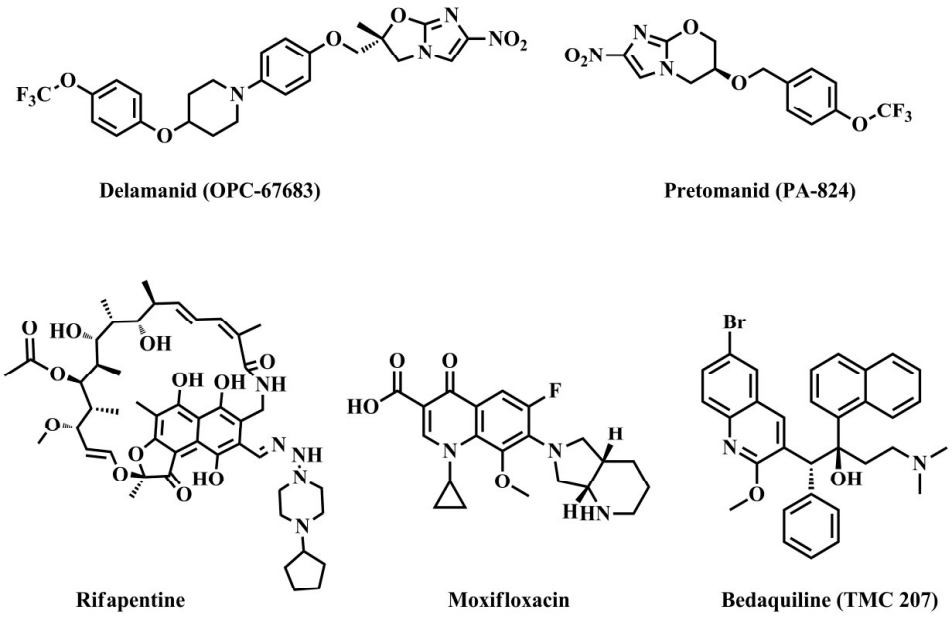

Figure 1.13: Structure of anti-TB agents under phase-III clinical trials.

1.11. Limitation of current drugs

A major adverse reaction of antituberculosis drugs, which results in discontinuation of that drug, has several implications. There may be considerable morbidity, even mortality, particularly with drug-induced hepatitis. More severe than side effects, life threatening, change in dosage of drug, discontinuation of drug, additional treatment or hospitalizations these are toxicities [96].

The length of therapy makes patient compliance difficult, most of the TB drugs available now a days are inactive against persist bacilli expect for RIF and PZA. However, there are still persist bacterial populations that are not killed by any available TB drugs. Based on these, there is a need to design drugs that are more than active against slowly growing or non-growing persistent bacilli [97].

1.12. Molecular Modification

Molecular modification is chemical alteration of a known and previously characterized lead compound for the purpose of enhancing its usefulness as a drug. This may perhaps enhance its

specificity for a particular target site, increase its potency, improve its rate and extent of absorption, and modify to advantage its time course in the body, reduce its toxicity, change its physical or chemical properties to provide desired features.

In molecular modification three approaches have been generally been explored: prodrug approach, bioisosterism and molecular hybridization [96].

1.12.1. Prodrug approach

Prodrug definition was introduced by Albert in 1958 which define prodrug as "any compound that undergoes biotransformation prior to exhibiting its pharmacological effects" [97]. Then Haper in 1959 proposed the term as latentiation. Drug latentiation is the chemical modification of a biologically active compound to form a new compound, which *in vivo* will liberate the parent compound.

A prodrug is a medication or compound that, after administration, is metabolized (i.e., converted within the body) into a pharmacologically active drug. Prodrugs are bioreversible derivatives of drug molecules which undergo chemical transformation or enzymatic conversion *in vivo* to release the active parent drug which shows desired pharmacologic effect. In both drug discovery and development, prodrugs have become an established tool for enhancing biopharmaceutical, physiochemical, or pharmacokinetic properties of therapeutic agents. The use of a prodrug is widely encouraged to optimize absorption, distribution, metabolism, and excretion (ADME) processes [97].

In general the prodrugs could be classified into two main classes: carrier prodrugs and bioprecursors. Carrier prodrugs are designed using labile linkage between a carrier group (ester or amide) and an active compound. These are classified as;

Bipartate: if the drug is directly attached to the carrier group

Tripartate: if there is a linker group between the drug and the carrier moiety

Mutual prodrug: here two drugs are linked together and synergistic to each other

These types of prodrugs have greatly modified lipophilicity due to the attached carrier group. The active drug is released by hydrolytic cleavage either chemically or enzymatically.

Advantages of carrier prodrugs are increasing absorption and chemical stability, injection site pain relief, elimination of unpleasant taste, decreasing metabolic inactivation etc.

Bioprecursor prodrugs rely on oxidative or reductive activation reactions unlike the hydrolytic activation of carrier-linked prodrugs. They metabolize into a new compound that may itself be active or further metabolized to an active metabolite. Bioprecursor is metabolized by molecular modification into either an active form or into an intermediate that will be farther metabolized. Bioprecursor does contain any carrier group. Several examples of drugs available in the market used this strategy such as sulindac, acyclovir, losartan among others [98]. The prodrug is usually inactive or less active than parental drug.

1.12.2. Bioisosterism

The notion of isosterism was introduced in 1919 by Langmuir. Compared the physical properties, chemical behavior and reactivity of various molecules possessing atoms or groups with the samenumber of valence electrons, i.e isoelectronic. Examples of various atoms and molecules; C=O and N=N; CO_2 and NO_2; N=N=N and $N=C=O^-$ [99].

Bioisosteres are substituents or groups with similar physical or chemical properties which produce broadly similar biological properties to a chemical compound. In biologically active molecule the replacement of an atom or group of atoms by another one presenting the same physicochemical properties [99].

In 1970, Burger classified and subdivided bioisosteres into two broad categories according to the degree of electronic and steric factors i.e., classic and non-classic.

The classical bioisosteres are subdivided into: a) monovalent atoms or groups; b) divalent atoms or groups; c) trivalent atoms or groups; tetravalent atoms and e) ring equivalents (**Table 1.3**)

Table 1.3: Classic bioisosteres, classifications, their atoms and groups.

Monovalent	Divalent	Trivalent	Tetravalent	Ring equivalents
-OH, $-NH_2$, $-CH_3$, -OR	$-CH_2-$	=CH-	=C=	Sulfadiazine in Pyrimidine ring
-F, -Cl, -Br, -I, -SH, $-PH_2$	-O-	=N-	=Si=	Sulfamethoxazole in

				Isoxazole ring
$-SiCH_3$, -SR	-S-	=P-	$=N^+=$	Piroxicam Benzene ring
	-Se-	=As-	$=As^+=$	Tenoxicam in Thiophene ring
	-Te-	=Sb-	$=Sb^+=$	
			$=P^+=$	

The non-classical bioisosteres do not obey the steric and electronic definition of classical isosteres. Further they do not have the same number of atoms of the substituent or moiety replaced. But retain the focus on providing similar sterics and electronic profile to the original functional group. Non-classical bioisosteres are much more dependent on the specific binding needs of the ligand in question and may substitute a linear functional group for a cyclic moiety, an alkyl group for a complex heteroatom moiety, or other changes that go far beyond a simple atom-for-atom switch. Non-classical bioisosteres we could cite: functional groups, noncyclic or cyclic and retroisosterism [100].

Table 1.4: Non-classical bioisosteres classifications of their atoms and groups.

-CO-	-COOH	$-SO_2NH_2$	-H	-CONH-	-COOR	$-CONH_2$
$-CO_2$	$-SO_3H$	$-PO(OH)NH_2$	-F	-NHCO-	-ROCO-	$-CSNH_2$
$-SO_2-$	-tetrazole		-OH		-catechol	
$-SO_2NR-$	$-SO_2NHR$		$-CH_2OH$			
-CON-	$-SO_2NH_2$				-benzimidazole	
-CH(CN)-	-3-hydroxy isoxazole		$-NHCONH_2$			C_4H_4S
R-S-R	-2-hydroxy chromones		$-NHCSNH_2$			$-C_5H_4N$

(R-O-R')	=N-		$-C_6H_5$
-RN(CN)-	-C(CN)=R'	-NHC(CHNO$_2$)NH$_2$ -NHC(CHCN)NH$_2$	$-C_4H_4NH$
-halide			
	$-CF_3$		
	-CN		
	-N(CN)$_2$		
	-C(CN)$_3$		

Bioisosterism represents an approach used by the medicinal chemist for the rational modification of lead compounds into safer and more clinically effective agents. It has significant value in drug design and lead optimization process as it may enhance the desired biological or physical properties of a compound, reduce toxicity and also alter the metabolism of the lead. Bioisosteric replacement is not simple replacement with another isostere but they are firstly analyzed by structural, solubility and electronic parameters to obtain molecules having similar biological activity. Bioisosteresim applications applied in aminopyrine to prpopylphenazone; cholesterol to diazacholesterol; tolrestat to oxotolrestat; sulfadiazine to sulfamethoxazole; piroxicam to tenoxicam and nimesulide to flosulide [99, 100].

Bioisosterism strategy has been used to discovery new compounds to treat TB. One example is the class of FQs. The FQs derivatives gatifloxacin and moxifloxacin were derivatized scaffolds from the parent nalidixic acid using the bioisosterism as molecular modification. The uses of FQs occur mainly in patients with MDR TB. The most active quinolones for the treatment of TB are: ciprofloxacin, sparfloxacin, ofloxacin, moxifloxacin and LEV. Studies comparing the bactericidal activity of various FQs against MTB in the latent and exponential growth phases demonstrated that most promising drugs are moxifloxacin and LEV [100].

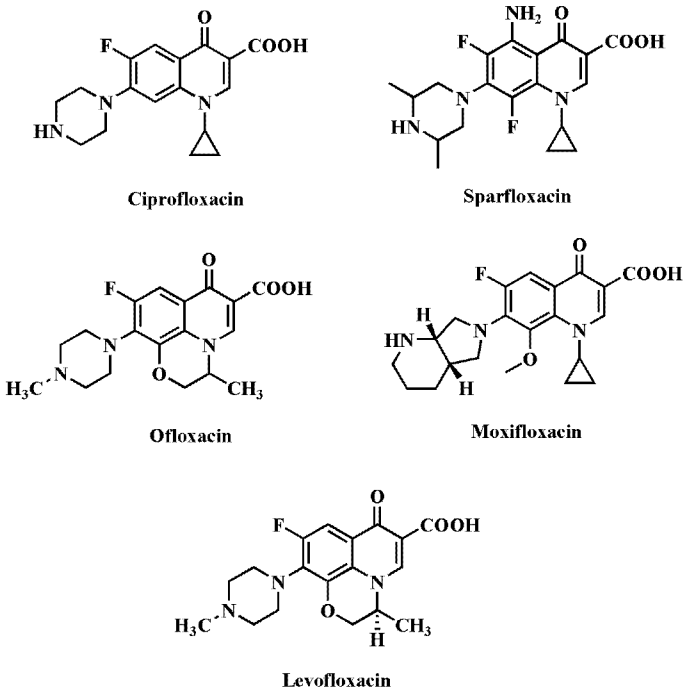

Figure 1.14: FQ drugs were obtained using bioisosteric replacement.

Linezolid is oxazolidinone drug belonging to antibacterial agents. The drug has been evaluated in the treatment of MDR TB showing interesting results [100]. Based on this interesting result, some oxazolidinone biosisoteres have been developed. Sutezolid is an analogue of linezolid in clinical trial phase-II to be used in TB treatment. Other linezolid derivatives such as radezolid and torelozid are obtained by isosteric replacement [100].

The metronidazole scaffold was used to design PA-824 and OPC-67683 using bioisosterism and molecular hybridization, the second line drug ethambutol was used as scaffold to develop the compound SQ109 using bioisosterism and molecular hybridization [100].

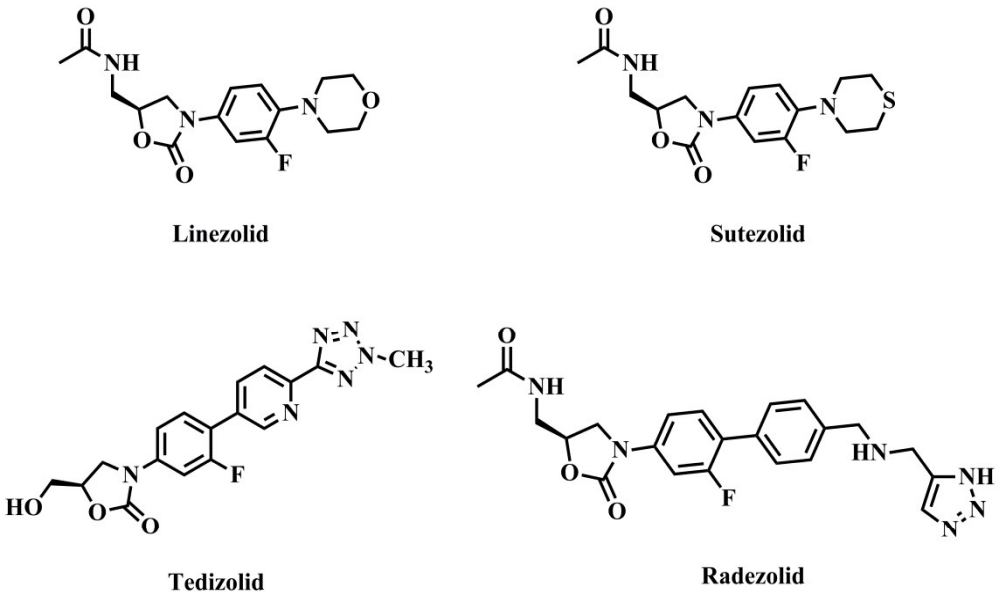

Figure 1.15: Chemical structure of linezolid bioisosteric derivatives

1.12.3. Molecular hybridization (MH)

Molecular hybridization is a structural modification strategy useful in the design of new optimized ligands and prototypes with new molecular architectures composed of two or more known bioactive derivatives, through the adequate fusion of these sub-unities. The molecular hybridization strategy is particularly interesting for the development of new prototypes for the treatment of physicopathologies where treatment is restricted to few commercial drugs or in cases when bioactive compounds are discovered but present high toxicity or pharmacokinetic and pharmacodynamic restrictions [101].

Structural requirements, ligand-protein interaction mode, site ligandreceptor interactions and quantitative structure-activity relationships, which tends to become faster and more efficient the development of new drugs [102]. On the other hand, if the degree of template-hybrid homology is either low or inexistent, the discovery of new lead-compounds should be made by massive screening of the generated chemical library.

The advantage of using MH is to activate different targets by a single molecule, thereby increasing therapeutic efficacy as well as to improve the bioavailability profile.

MH strategy shows the drug A interacts only with the receptor **A**. The drug B interacts only with the receptor **B**. It is prohibitive the interaction between drug **A** and receptor **B** (and vice versa) is

prohibited but is possible to design compounds that can interact with both receptors contributing synergically for a desired effect [103].

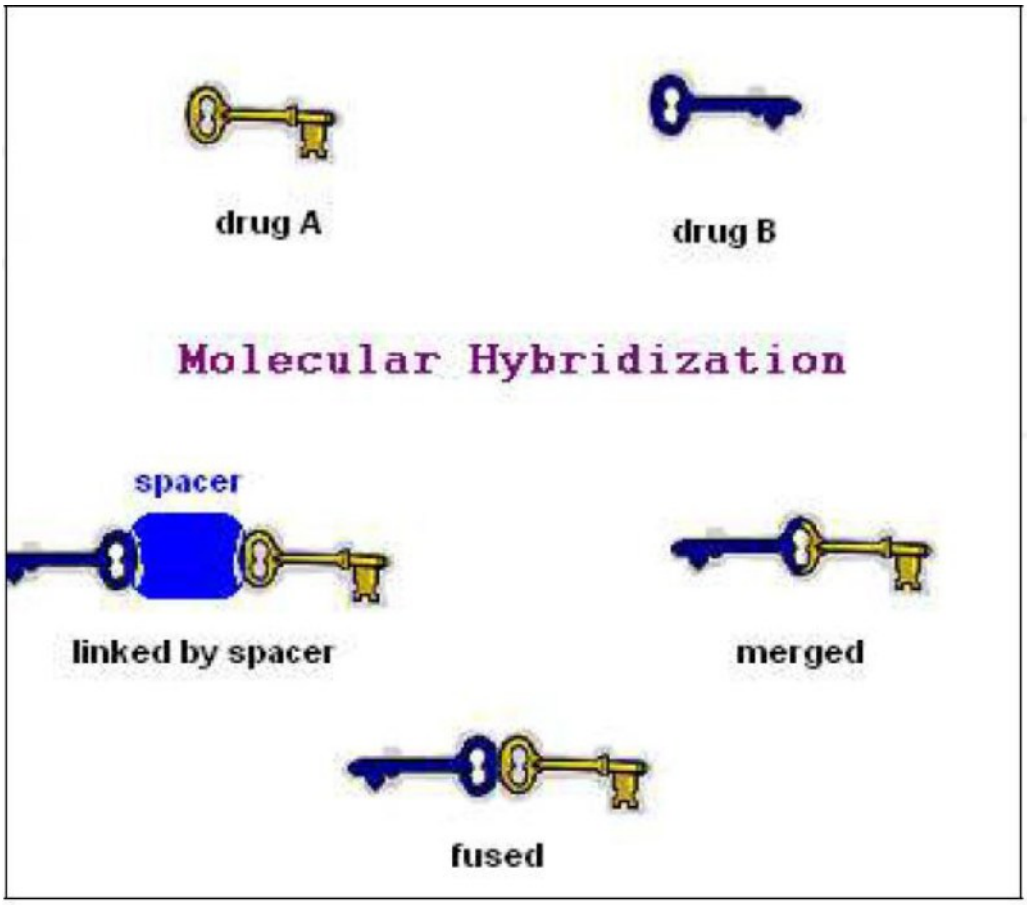

Figure 1.16: Different hybrid compounds obtained by molecular hybridization.

The drug design of hybrid compound must judge three different conditions: a) the desired subunits are linked by a spacer agent; b) both subunits are linked without spacer agent and they are fused; c) the desired activities are merged in a new structure. These different conditions are in order to design a new drug [100, 102].

MH strategy has been used in TB drug discovery to increase the efficacy and reduce drug resistance. Imrarovský and co-workers combined through MH the scaffold of three anti-TB drugs: INH, PZA and ciprofloxacin. The novel compounds showed great activity against MTB [102].

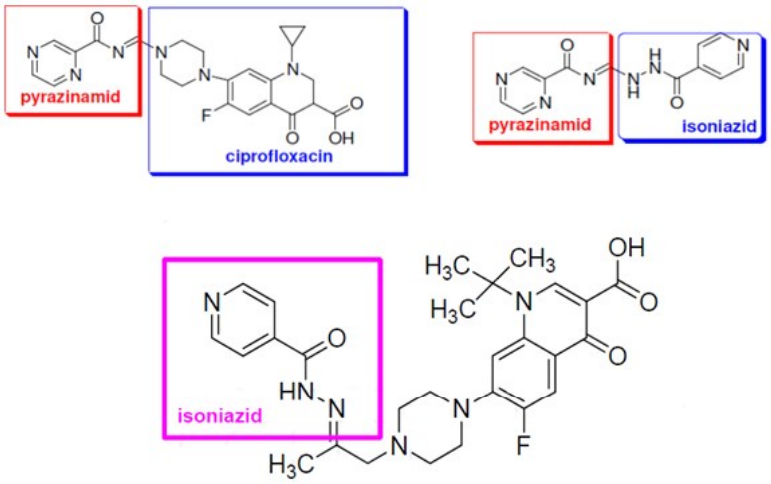

Figure 1.17: Molecular hybridization between FQ and INH.

Another example, Torres and co-workers reported the antitubercular activity from the new quinoxaline-1,4-di-*N*-oxide derivates obtained by molecular hybridization with the first line drug isoniazid [103].

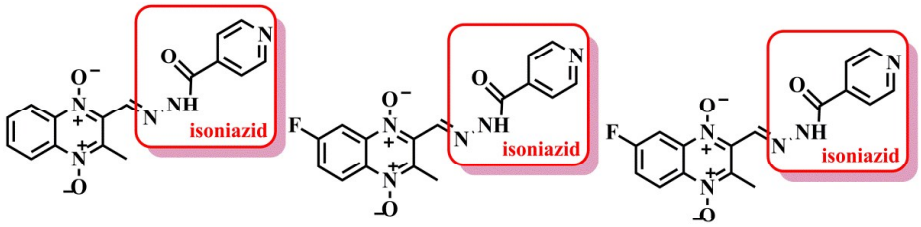

Figure 1.18: Quinoxaline-1,4-di-*N*-oxide molecular hybridization with INH.

1.13. References

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