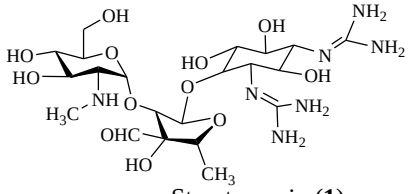
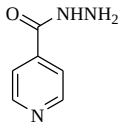
1. Introduction

Tuberculosis (TB) and humankind goes way back to sixth and seventh centuries BC. TB got intensified century by century and as years passed, experts all over the world put forth number of findings either to treat TB or adding valuable information about it. During the nineteenth century, in the year 1860, Louis Paster reported TB as airborne transmitted disease whereas Jean Antoine Villemin, a French physician, proved TB as contagious disease in 1865. Thereafter a German physician, Dr. Robert Koch identified *Mycobacterium tuberculosis* (*Mtb*) the infectious microbe causing TB in 1882 which was the stepping stone of the program to control and terminate the crisis of this lethal disease.[1] However, despite the identification of causative agent of TB, no medication was available for the treatment of TB till 1943. In the year 1944, streptomycin (**1**) was identified to kill *Mtb* and first drug to manage TB. Later on, isoniazid (**2**) was introduced that made it possible to treat TB patients at home rather than hospital or sanatoriums.[2,3]



Streptomycin (**1**)

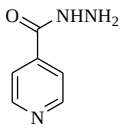


Isoniazid (**2**)

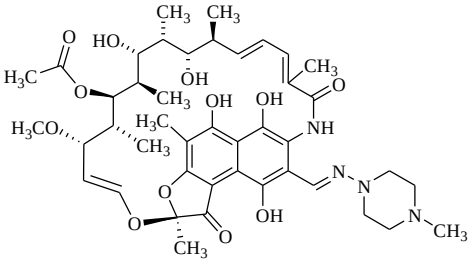
TB is the major death-causing disease all around the world especially, in the developing countries. TB was the leading cause of mortality from a single infectious agent, until the coronavirus pandemic in 2019. It is a granulomatous, contagious airborne disease proved to be fatal if not treated. TB is caused by *Mycobacterium* species and generally affects the lungs, but it can also affect other body parts as well[4]. Earlier TB was successfully controlled by a set of drugs namely, isoniazid (**2**), rifampicin (**3**), ethambutol (**4**) and pyrazinamide (**5**). These drugs are the first line agents used primarily to cure the disease. During the later years, other drugs such as fluorouinolones, ofloxacin, cycloserine (**7**) etc were introduced to aid the first-line agents.[5] These are also known as second line drugs. There are more than two dozen drugs available to treat TB (**Table 1.1**). Still, TB remains one of the major concerns to the healthcare professionals, as it is associated with development of resistance, patient's incompliance, serious side effects and long duration of treatment[6].

Development of resistance against available drugs have been the major concern for quite some time now. When the patient is non-responsive to the first line agents like, isoniazid (**2**) and rifampicin (**3**), it known as Multidrug resistance (MDR).[7] If the resistance has been developed against first line drugs and one of the second line drugs, then it's called extensive drug resistance (XDR). If the patient is non-responsive to any treatment given, then its a case of severe total drug resistance (TDR). Resistance may be caused by mutation in one or more chromosomal genes in *Mtb*.[8] There is lot of research going on the field of TB, yet only three drugs namely bedaquiline (**17**), delamanid (**19**) and pretomanid (**18**) have been approved lately by USFDA for the treatment of resistant TB (**Figure 1.1**). But, unfortunately bedaquilline resistant strains of *Mtb* have also been reported lately.[9]

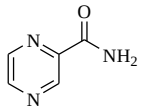
First-line anti-TB drugs



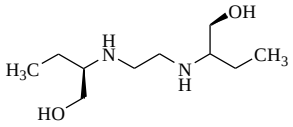
Isoniazid (**2**)



Rifampicin (**3**)

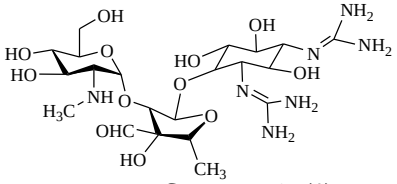


Pyrazinamide (**4**)

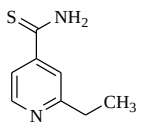


Ethambutol (**5**)

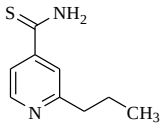
Second-line anti-TB drugs



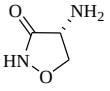
Streptomycin (**1**)



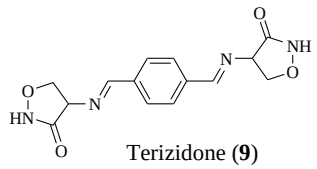
Ethionamide (**6**)



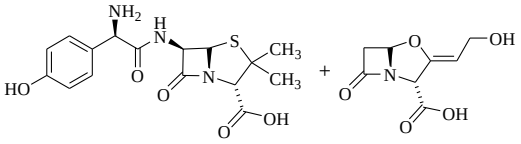
Prothionamide (**7**)



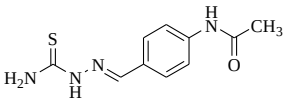
Cycloserin (**8**)



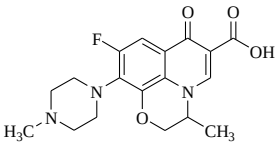
Terizidone (**9**)



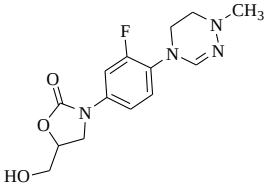
Amoxycillin (**10**) + Clavulanic acid (**11**)
(combination)



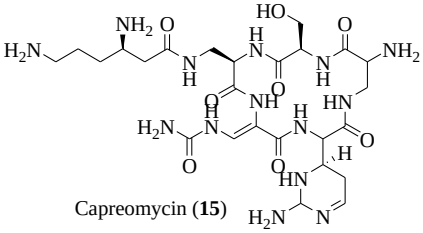
Thioacetazone (**12**)



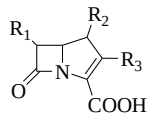
Ofloxacin (**13**)



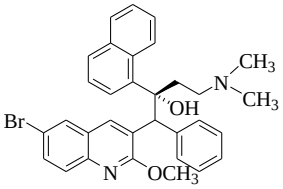
Delpazolid (**14**)



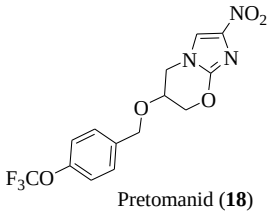
Capreomycin (**15**)



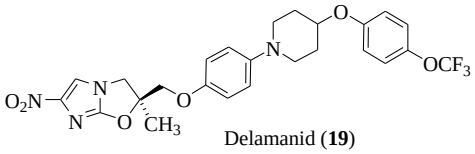
Carbapenems (**16**)



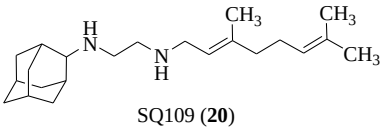
Bedaquiline (**17**)



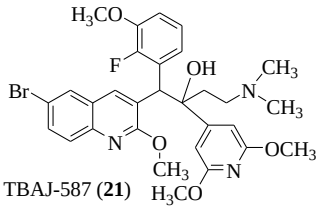
Pretomanid (**18**)



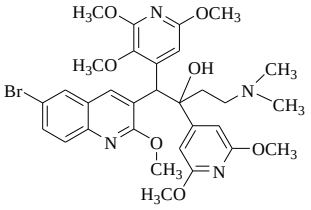
Delamanid (**19**)



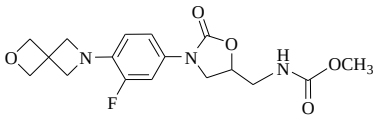
SQ109 (**20**)



TBAJ-587 (**21**)



TBAJ-876 (**22**)



TBI-223 (**23**)

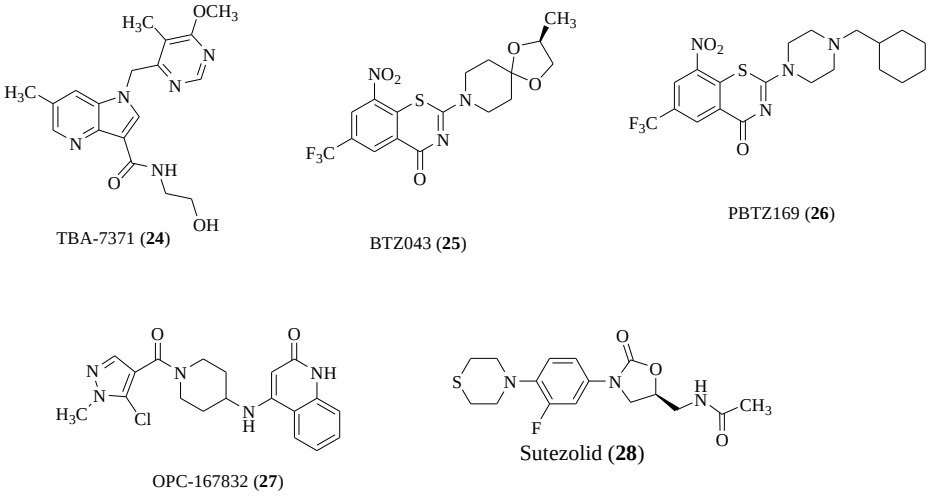


Figure 1.1: Drugs for the treatment of tuberculosis. First line agents (**2-5**), second line agents (**1, 6-19**), drugs in clinical trials (**20-28**)

1.1 Epidemiology (WHO-TB report)

The World health organization (WHO) has been publishing an annual global TB report since 1997. The WHO global TB report 2021, consists statistics from 197 countries covering more than 99% of the World's population[4]. As per this report, 10 million people developed TB in 2020. Geographically, South East Asia accounts for 43% of the total TB burden whereas Africa contributes for about 25% (**Figure 1.2**). Across the world, the highest burden is reported in men (56%) of all cases in 2020; whereas it was 33% and 11% for women and children respectively. Approximately, 1.3 million HIV negative patients died with the TB whereas 214000 deaths were reported among HIV-positive people. Further, COVID-19 has adversely affected the progress in reducing the mortality rate of TB patients[10].

Disturbingly, India has accounted for a total of 38% of the global TB deaths among HIV-negative people. Also, India has been reported among the countries with highest burden in all the three categories ie. TB cases, HIV associated TB and MDR/RR-TB.

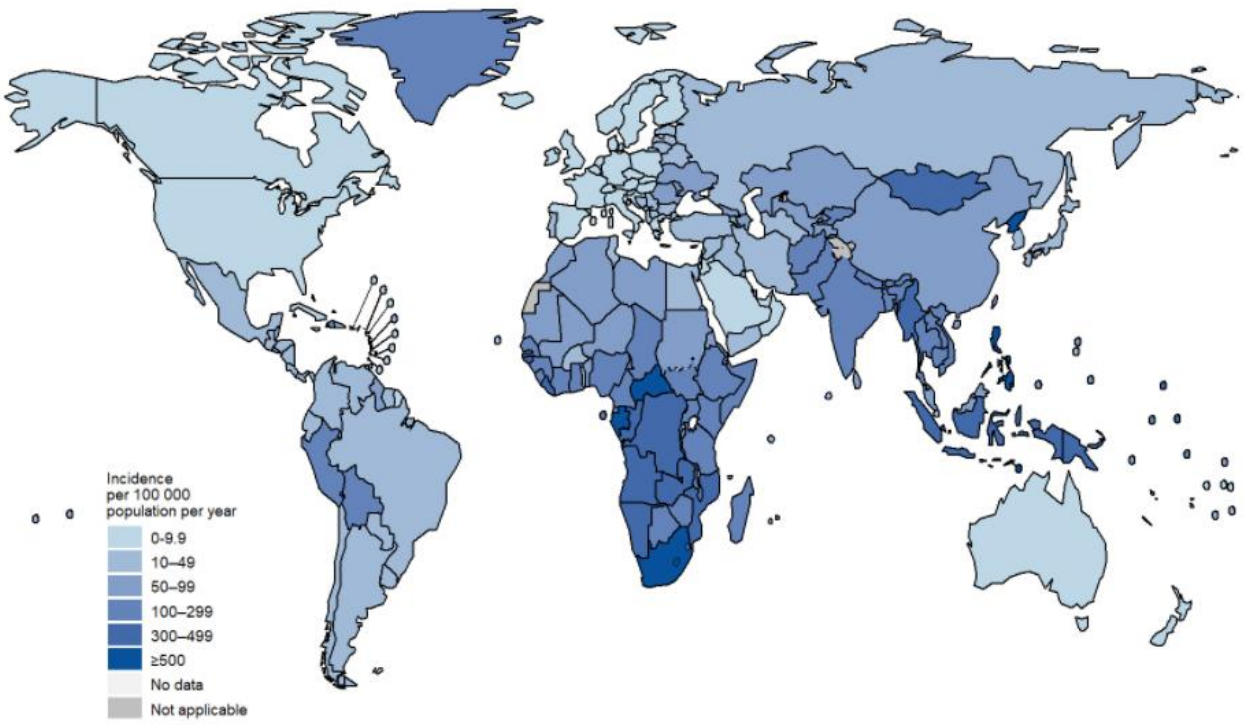


Figure 1.2: Estimated TB incidence rates 2020[4]

1.2 Pathophysiology

TB spreads *via* air transfer of microbes from an infected person to a healthy person. The microbes enter the body via air passage. Once inhaled, *Mtb* infects alveolar macrophages, which in response will impose stress on the bacterium. The bacterium replicates in macrophages, which in turn release cytokines and employ a greater number of cells towards the site of infection. The primary infection will lead to the generation of a granuloma- a bundle of pulmonary tissues, bacteria, and immune cells. This granuloma is essential for both transmission and containment of the disease (**Figure 1.3**). Generally, the infection ceases at this stage itself, resulting in a non-contagious static form of infection that may or may not progress later. In some cases, the infection progresses directly after the formation of granuloma, resulting in tissue damage, coughing, irritation, and aerosol transmission[11,12]. After infection, the following symptoms can be noticed:

- Cough with or without blood/mucous for more than three weeks
- Chest pain
- Unintentional weight loss

- Fatigue
- Fever
- Difficulty in breathing

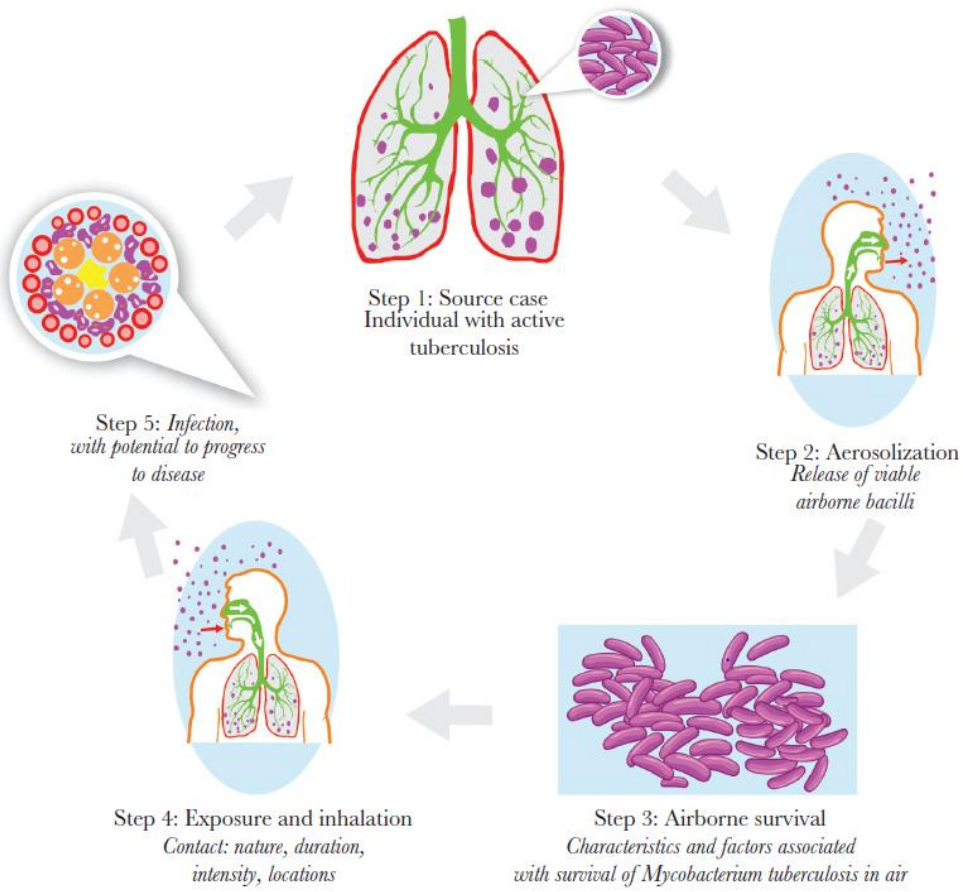


Figure 1.3: Cascade of TB transmission[12]

1.3 Mycobacterium

The causative organism of TB is a gram-positive acid-fast bacillus *Mycobacterium tuberculosis (Mtb)*, identified by Robert Koch in 1882. The bacterium is aerobic in nature and divides every 18-24 hours, slow compared to the other bacteria. The distinguishing feature of *Mtb* is the presence of complex fatty acid on the cell surface. These fatty acids act as a waxy layer on the cell surface and have the ability to mutate and thereby leading to the development of resistance[13].

Other causative Mycobacteria are:

- *M. bovis*
- *M. africanum*
- *M. microti*
- *M. avium* complex

1.4 Mycobacterial targets

Just like any other bacteria, *Mtb* is regulated by a number of enzymes that are involved in the growth, metabolism and multiplication[5,14,15]. Inhibition of these enzymes would lead to cell death. So, these enzymes are the potential targets for development of newer anti-TB agents[15]. Potential targets for *Mtb* are as follow:

1.4.1 Decaprenylphosphoryl- β -D-ribose 2'-epimerase (DprE1/E2)

The enzyme DprE is a flavoprotein that is involved in the epimerization decaprenylphoshopryl-β-D-ribose (DPR) to decaprenylphosphoryl-2-keto-β-D-erythro-pentofurnaose (DPX), that further gets converted to decaprenylphosphoryl-β-D-arabinose (DPA)[5,19]. DPA serves as the sole donor of the Araf residues in the biosynthesis of arabinan. These arabinans are essential in the synthesis of arabinogalactan and lipoarabinomannan layer of cell wall. Thus, inhibition of the enzyme DprE1 is good tactic for development of new anti-tubercular agents[20]. This enzyme has been discussed in detail in the later section.

1.4.2 Enoyl acyl carrier protein reductase (InhA)

Fatty acid synthesis is the major biosynthetic pathway for synthesis of mycolic acid. The classic anti-TB drug Isoniazid inhibits mycolic acid synthesis by inhibiting InhA. The enzyme is responsible for the important steps in fatty acid synthesis (FAS II)[16]. InhA catalyses the reduction of trans double bond present on the intermediate attached to the acyl carrier. Isoniazid is a prodrug that requires activation by an enzyme catalase peroxidase to oxidize the INH into an INH-NAD adduct[17]. This oxidized form binds to the active site of InhA via van der Waals interactions and thereby reducing the double bond to form enoyl intermediate along with protonation of C-2 to release meromycolic acid that undergoes Claisen condensation to synthesize mycolic acid.[18]

1.4.3 DNA gyrase

DNA gyrase has been a proven drug target for minimizing the duration of TB treatment. Moxifloxacin among many other fluroquinolones have been used in MDR-TB and XDR-TB. The enzyme converts positive DNA supercoils into negative supercoils, thereby helps in maintaining the topology of bacterial DNA. The enzyme is a hetero-tetrameric protein made up of two subunits gyrA and gyrB that assemble to form catalytically active A2B2 complex[21].

1.4.4 ATP synthase

ATP synthase is a crucial enzyme for energy metabolism in living cells. It helps in the generation of ATP by utilizing the energy stored in the trans-membrane electrochemical potential difference of a coupling ion[22]. ATP synthase is a membrane protein complex that consist of soluble catalytic F_1 region and a membrane embedded proton translocating F_0 region. Some pathogenic bacteria such as *Mtb* by using ATP synthase, can withstand energetically unfavorable conditions such as low oxygen or low energy in the human host, and survive in human macrophages for longer period of time. These pathogens can further down regulate their metabolism and become dormant in nature. The enzyme is essential for the obligate aerobic *Mycobacterium* genus. Bedaquiline is known to inhibit ATP synthase and is one of the few drugs approved recently by USFDA for the treatment of MDR-TB. Upon binding, Bedaquiline brings a large conformational change in the enzyme to create a tight binding pocket at the interface of subunit a and c. The crystal structure of the enzyme depicted a hook-like extension from the α-subunit to prevent the enzyme from running in reverse, thus inhibiting ATP hydrolysis and preserving energy during hypoxic conditions[23].

1.4.5 Maltosyl transferase (GlgE)

The biosynthesis of mycolic acid is complex process involving many enzymes and substrates. Trehalose (α-*D*-glucopyranosyl-(1→1)-α-*D*-glucopyranoside) acts as a carrier for mycolic acid during the synthesis of mycolyl arabinogalactan and trehalose-6,6'-dimycolate (TDM). Further these trehalose molecules get converts into branched α-glucan. GlgE uses M1P to generate linear α-glucans[24]. Subsequently, α-1,6 branches are introduced into the α-glucan by GlgB. It has been reported that non-availability of GlgE leads to self-poisoning in *Mtb* by accumulation of M1P phospho-sugar to a lethal concentration. Thus, GlgE can be considered as

enzyme essential for survival of the pathogen, the absence of human homologue imparts it a druggability potential[25].

1.4.6 Fatty acid synthetase (FAS I and FAS II)

The FAS is involved in the biosynthesis of mycolic acids[26]. FAS I is responsible for generation of acyl-coenzyme A (CoA) which is stretched by FAS II to generate mycolic acid precursor. These further include various enzymes which participate in step-by-step synthesis of mycolic acid and its precursors[27].

1.4.7 Beta-ketoacyl-ACP synthetase (Kas)

β-Ketoacyl-ACP synthase (KAS) enzymes is responsible for catalyzing the Claisen condensation of an acyl donor and malonyl-ACP to form a *β*-ketoacyl-ACP[28]. In *Mtb* the enzyme is subcategorized as KasA, KasB, and mtFabH. The enzyme mtFabH is responsible for catalysis of the initial condensation of acetyl-CoA with malonyl-ecACP. The former two enzyme KasA and KasB are involved in the elongation of the growing fatty acids[29].

1.4.8 *D*-Alanine-*D*-alanine ligase

D-Alanyl-*D*-alanine ligase (Ddl) belongs to the class of adenosine triphosphate (ATP) dependent enzyme and, catalyzes the ligation reaction of two molecules of *D*-alanine into a dipeptide molecule to form the precursor of peptidoglycan layer[30]. *D*-alanyl-*D*-alanine along with diphospho-*N*-acetyl muramic acid is an essential component of the intracellular peptidoglycan layer. *D*-Cycloserine (DCS), an anti-TB drug acts as Ddl inhibitor[31].

1.4.9 Mycolic acid cyclopropane synthetase (MACS)

Mycobacterial cyclopropane synthase 1 is known to be responsible for *cis*-cyclopropanation of the unsaturated mycolates[32]. The enzyme MACS acts as catalyst for the reaction of cyclopropanation of the unsaturated mycolic acids at the proximal and distal ends with the use of *S*-adenosyl-*L*-methionine as methyl donor. These chemical modifications are of great importance regarding virulence, persistence and pathogenicity of *Mtb*[33].

1.4.10 Mycobacterial membrane protein large 3 ($MmpL_3$)

MmpL3 is a mycobacterial membrane protein large that belongs to the super family of transporters known as Resistance-Nodulation-cell Division (RND)[34]. It has been reported that

Mycobacterium tuberculosis has 13 MmpL proteins with MmpL3, 4, 5, 7, 8, 10 and 11 being involved in the cell envelop synthesis. Further, it is also speculated that MmpL5 and MmpL7 are capable of effluxion anti-TB drugs such as bedaquilline. These enzymes are involved in transportation of trehalose monomycolates (TMM), sulfolipids, di- and poly-acyltrehaloses, phthiocerol dimycocerosates, mono mycolyldiacylglycerol, glycopeptidolipids and mycobactins[35–37]. In *Mtb*, MmpL3 is the one and only MmpL considered indispensable for replication and cell viability[38]. It is observed that down regulation of MmpL3 lead to stoppage of cell division and rapid cell death[39]. The function of MmpL3 is based on the proton motive force (PMF) as an energy source to drive TMM transport across the membrane. MmpL3 is responsible for transporting TMM across the cell membrane to the periplasmic space, wherein it is responsible for form TDM by acting as a mycolic acid donor to TMM, or to the arabinogalactan as part of the mAGP core. Thus, disabling the MmpL3, inhibits essential step of synthetic pathway of mycolic acid in *Mtb*[39].

1.4.11 Mur enzyme

In *Mtb*, the peptidoglycan synthesis starts in the cytoplasm with the conversion of fructose-6-phosphate to UDP-*N*-acetylglucosamine (UDP-GlcNAc), which is catalyzed by bifunctional enzyme Glm U[40,41]. The first step wherein the enol gets converted to pyruvate moiety from phosphoenolpyruvate (PEP) is catalyzed by Mur A. This PEP is transferred to UDP-GlcNAc which is later reduce by NADPH dependent oxidoreductase Mur B to D-lactate ultimately generating into UDP-*N*-acetylmuramic acid (UDP-MurNAc). The peptide stem of the monomer is synthesized by Mur C, Mur D, Mur F by catalyzing addition of *L*-Ala, *D*-Glu, mDAP, and *D*-Ala-*D*-Ala to UDP-MurNAc respectively[42].

1.4.12 *L, D*-transpeptidase

Peptidoglycan layer serves as an attractive target for anti-bacterial therapy. Furthermore, PG-crosslinking enzymes like the penicillin-binding proteins have always been the best targets for antibiotics. However, *L,D*-transpeptidase (Ldt) are have mysterious role in the PG synthesis. In the past various reports have been published about Ldts and their role in development of newer anti-TB agents[43]. Ldt is known to have a monomeric form with catalytic LDT domain that serves as conserved active site motif[44]. Additionally, it also possess substrate capping subdomain, a PG binding domain, and a large domain for N-terminal helping in interactions[45].

The enzyme is subcategorized in five classes namely $LDt_{mt1,}$ $LDt_{mt2,}$ $LDt_{mt3,}$ $LDt_{mt4,}$ and $LDt_{mt5.}$ It has been found that LDt_{mt5} plays important role in maintaining the integrity of the cell wall. Furthermore, it assists in the cross linking of peptidoglycan[46,47].

1.4.13 Alanine racemase

Alanine racemase (Alr) or *L*-alanine racemase is involved in the peptidoglycan synthesis by providing *D*-alanine as the precursor[48]. The enzyme is also involved in the metabolism of the alanine, aspartate and *D*-alanine. The Alr also racemizes *L*-alanine to *D*-alanine by using pyridoxal 5'-phosphate (PLP) thereby providing *D*-alanine for cell wall synthesis. The special feature of the enzyme that makes it the druggable target is its limited presence to the prokaryote bacteria[49].

1.4.14 Arabinosyl transferase

It is an essential transferase enzyme that contributes in the polymerization of arabinogalactans. The enzyme possesses two residues arabinose and galactose, in furanose configuration[50]. Arabinogalactan synthesis starts with the configuration of a linker which will act as a medium for the complexation of arabinogalactan with peptidoglycan via *N*-glycolylated-muramic acid residues. The arabinans will also form mycolyl-arabinogalactan-peptidoglycan (mAGP) complex with mycolic acid[51,52].

1.4.15 DNA dependent RNA polymerase

DNA-dependent RNA polymerase deciphers the message carried by DNA and have important implications in the transcriptional regulation[53,54]. Transcription by RNA polymerase is the core of controlling of gene expression and serves important target for anti-microbial chemotherapy. The enzyme consists of five sub units that are responsible for transcription elongation whereas sixth subunit is important for promotor recognition and transcription initiation[54].

1.4.16 ATP phosphoribosyl transferase

The enzyme is found in many organisms including *Mtb*, *E coli*, *S typhimurium*, *C glutamicum*, *A thaliana* etc. In *Mtb* the enzyme is responsible for pentosyl group transfer in biosynthesis of histidine and thus acts a crucial enzyme in histidine biosynthetic pathway. It

catalyzes the conversion of 5-phospho-α-D-ribose-1-diphosphate and ATP to diphosphate and N-1-(5'-phosphorylribosyl)-ATP[55].

1.4.17 Fatty acid degradation protein D32

FaD32, a member of fatty acyl-AMP ligase (FAAL) family is a 35 fatty acid long enzyme. It is responsible for biosynthesis of lipids that activates and transfers the fatty acids into PKS protein to form highly diverse and complex lipids of *Mtb*. Further, fatty acid Co A ligases (FACLs) are second class of FaDs that contributes to the lipid degradation and remodeling. FaD32 catalyses mermycolic acid to load it onto *N*-terminal acyl carrier protein (ACP1) domain of the PKS13[56,57].

1.4.18 Mycothiol ligase

Mycothiol, a small molecular weight thiol analogous to glutathione, is unique to actinomycetes and responsible for maintaining intracellular redox balance and removing toxins. Mycothiol ligase acts as an essential enzyme in the biosynthesis of mycothiol by catalyzing the ATP dependent condensation of cysteine and glucosamine-inositol to yield cysteine-glucosamine-inositol. The enzyme is unique to *Mtb*, thus serves as an attractive target for chemotherapeutic use[58].

1.4.19 Methionine aminopeptidase

The step of removal of the *N*-terminal methionine from proteins and peptides depends upon class of proteases known as dinuclear metalloenzyme methionine aminopeptidase[59]. The *N*-terminal removal of methionine is a vital co-translational proteolytic process resulting in the diversity of amino-termini of proteins in organisms. The methionine aminopeptidases belong to the class of proteases. Structurally, the active site and a binuclear metal center facilitate the removal of the *N*-terminal methionine from the polypeptides[60].

1.4.20 Cytochrome bc1 complex

The enzymes of the cytochrome bc1 complex family are one of the most crucial components of all the main energy transduction systems of the cell by maintaining proton gradient[61]. All the cytochrome enzymes typically catalyze the transfer of reducing equivalents from a quinol in the lipid phase to a higher potential acceptor protein in the water phase. This electron transfer is coupled to transport $1H^+/e^-$ across the membrane[62]. The cytochrome bc1

complex is an oligomeric membrane protein complex that transfers electrons from low potential quinol to a c-type cytochrome and is involved in the energy metabolism. The complex works via a Q-cycle mechanism where the coupling to proton transfer depends on a bifurcated reaction at the Q_0-site of the complex, in which the two electrons from ubihydroquinone are passed to distinct chains[63].

1.4.21 Isocitrate lyase

The glyoxylate cycle being anaplerotic pathway of tricarboxylic acid cycle (TCA cycle), allows growth on C_2 compounds by bypassing the carbon dioxide generating steps of TCA cycle. Various microorganisms living on acetate or fatty acids as the sole carbon source employ the glyoxylate bypass for the synthesis of cellular material. The two crucial enzymes of this cycle are isocitrate lyase and malate synthase. Isocitrate lyase breaks down the isocitrate by deprotonating it into succinate and glyoxylate, which is further converted into malate by the enzyme malate synthase. The end products of the bypass can be used for gluconeogenesis and other biosynthetic process[64]. Both these enzymes are found in bacteria, and thus could be important in human, animal and plant pathogenesis.

1.4.22 Peptide Deformylase

In Mycobacteria, during the first step of protein synthesis, methionine is formylated by tRNA-formyl transferase. After emerging from the ribosome, the methionine is de-formylated by peptide deformylase[65]. After deformyaltion only this methionine can be removed from the mycobacteria by either methionine amino-peptidase A (MetAPa) or C (MetAPc). Peptide deformylase is hypothesized to be important in bacteria. PDf are vital metalloenzymes that remove the formyl group carried by the initiator methionine in Mycobacteria, chloroplast and mitochondria[66]. Although, they are crucial for cell growth, yet they are not target of any drug in use.

1.5 Treatment regimen for TB

The current regimen for TB treatment is categorized into first-line agents and second-line agents. The drugs used primarily to treat TB are known as first-line agents, namely: isoniazid (**2**), rifampicin (**3**), pyrazinamide (**4**), and ethambutol (**5**). If there is irresponsiveness or resistance towards these first-line agents then, second-line agents are used. Generally, the therapy regimen

consists of two first-line agents along with one or more second-line agents. This combination is used to increase the efficacy, reduce the side-effects and avoid resistance. Second-line agents consist of fluoroquinolones, streptomycin (**1**), cycloserine (**8**), floxacins, etc. The available drugs are discussed in the **Table 1.1.**

Table 1.1: Drugs in the current treatment regimen[5,15,67,68]

Name of the drug	Mechanism of action/ enzyme involved	Other information
	First line agents	
Isoniazid (INH) **(2)**	Upon activation, INH (**1**) generates reactive oxygen species (ROS) that attack nicotinamide adenine dinucleotide (NAD) forming covalent adduct. This adduct is responsible for inhibition of enzyme enoyl-ACP reductase (InhA). Thus, inhibiting mycolic acid synthesis	INH **(2)** is a prodrug, activated by *Mtb* catalase-peroxidase (KatG gene). Its metabolism has been linked with liver damage. Development of resistance towards INH is often associated with mutation of genes like KatG, KasA, inhA, ahpC, and ndh
Rifampicin **(3)**	Rifampicin **(3)** acts by inhibiting RNA polymerase, which catalyzes the transcription of DNA to RNA	Rifampicin **(3),** bactericidal agent that has a wide spectrum of activity. It is used in combination with another anti-mycobacterial agents
Pyrazinamide **(4)**	The exact mechanism is unknown, but it is hypothesized that it inhibits fatty acid synthesis	Pyrazinamide **(4)** is a prodrug that is activated by pyrazinamidase (PZase)/ nicotinamidase. to its active form - pyrazinoic acid (POA)
Ethambutol **(5)**	It disrupts arabinogalactan biosynthesis by inhibiting arabinosyl transferase. Inhibition of arabinogalactan synthesis also disrupts mycolyl-arabinogalactan-	Ethambutol (EMB) **(5)** has been available to treat tuberculosis (TB) since the 1960s, it provides bacteriostatic action and used in the treatment of pulmonary tuberculosis.

	peptidoglycan (mAGP) complex, thus leading to an increase in cell wall permeability	It should not be used alone but rather in tandem with at least one other anti-TB drug such as isoniazid
	Second line agents	
Ethionamide (ETH) **(6)**, Prothionamide **(7)**	Upon activation, this drug form an adduct that lead to inhibition of InhA	Structurally the drug is thioamide, used with other second-line agents to treat MDR-TB. ETH **(6)** is activated by EthA to form oxide metabolite
Cycloserine **(8)**	It alters peptidoglycan biosynthesis	Competitively inhibits both the enzymes alanine racemase (Alr) and *D*-alanine-*D*-alanine ligase (Ddl)
Terizidone **(9)**	Inhibits *L*-alanine racemase and *D*-alanine ligase thereby inhibiting cell wall biosynthesis particularly peptidoglycan synthesis	Agent for MDR-TB that is used internationally, but not approved in the USA
Amoxycillin **(10)**, clavulanic acid (**11**)	Inactivates penicillin-binding proteins (PBPs) to inhibit cell wall synthesis	Mycobacterial β-lactamase is susceptible to clavulanic acid. It is used in the management of multidrug-resistant patients
Thioacetazone (**12**)	Inhibits mycolic acid cyclopropane synthesis, thus inhibiting cell wall synthesis	It is bacteriostatic in nature and a prodrug
Streptomycin (**1**)	A broad-spectrum antibiotic that inhibits protein synthesis by binding to ribosomes	Streptomycin is seemed to be the most bactericidal of currently available anti-TB drugs. It is still listed by the WHO as an essential anti-TB agent, and had main role in preventing resistance development and strengthening of regimens against isoniazid-resistant strains

Ofloxacin **(13)**	Targets DNA gyrase encoded by the *gyrA* and *gyrB* genes and topoisomerase topoisomerase IV encoded by *parC* and *parD* genes	Ofloxacin - a broad spectrum of antibiotic have been used with increasing frequency for the treatment of MDR-TB. It is listed as a reserve drug by the WHO
Delpazolid (**14**)	Delpazolid (**14**) inhibits protein synthesis	Oxazolidinone derivative for MDR-TB
Capreomycin (**15**)	Inhibits bacterial translocase I (TL-1) crucial for peptidoglycan synthesis	Capreomycin (**15**) is listed by the WHO as a reserve drug for the management of DR-TB
Carbapenam (**16**)	β-lactams exerts peptidoglycan lytic and synthetic functions. They inhibit *D*,*D*-transpeptidase, and non-canonical *L*,*D*-transpeptidase	Carbapenems are active against many aerobic and anaerobic gram-positive and gram-negative bacteria
	Newer agents	
Bedaquiline (BDQ) (**17**)	Inhibits ATP generation in *Mtb* by interfering with the ATP synthase activity	It is a first-in-class diarylquinoline that is highly active against *Mtb*. It was approved by the USFDA in 2012. This drug is administered for the treatment of MDR-TB
Pretomanid (**18**)	The drug acts on a multi-target mechanism. It disrupts *Mtb* cell wall lipids and simultaneously induces respiratory poisoning and inhibits protein synthesis inhibition	Bicyclic derivative of nitroimidazopyran
Delamanid (**19**)	It inhibits mycolic acid biosynthesis that specifically inhibits methoxy-mycolic and keto-mycolic acid synthesis	It's a nitro-dihydro-imidazooxazole derivative. It requires reductive activation by *Mtb*

Agents in clinical trials		
Ethylenediamine (SQ-109) (**20**)	The exact mechanism is unknown but it is believed to inhibit cell wall biosynthesis by inhibitory MmpL3	Phase 3
TBAJ-587 (**21**)	Direct inhibitor of *Mtb* ATP synthatase	Phase 1
TBAJ-876 (**22**)	Direct inhibitor of *Mtb* ATP synthatase	Phase 1
TBI-223 (**23**)	Protein synthesis inhibitor	Phase 1
TBA 7371 (**24**)	It's a reversible inhibitor of DprE1 inhibitor. Thus, inhibits arabinan synthesis of cell wall	Phase 2
Benzothiazinones BTZ043 (**25**) PBTZ169 (**26**)	They serve as irreversible inhibitor of DprE1 inhibitor	Phase 2
OPC-167832 (**27**)	DprE1 inhibition	Phase 2
Sutezolid (**28**)	Binds to the 23S ribosome and thereby block the microbial protein synthesis	Phase 2

1.6 DprE1- the most druggable target

The cell wall is the first point of contact between the bacterium and the host cell. It acts as the interface with the host, so its structure and modulation are important in the infection and treatment. The cell wall has always been a choice of target for anti-bacterial agents and *Mtb* is no different. Many existing drugs like isoniazid (**1**), cycloserine (**7**), ethambutol (**4**) etc. are known to inhibit cell wall biosynthesis. The bacterial cell wall is consisting of distinguished layers of peptidoglycan, arabinogalactan, mycolic acid, and surface lipids made up of various building blocks[5,19,69].

One such building block is Arabinogalactan (AG) that is present as the central layer of the cell wall. Branched arabinose (Araf) is linked to the galactose molecules in the galactan. Araf

molecules are transferred to the periplasm to both AG and lipoarabinomannan (LAM) from the only lipid precursor known as decaprenylphosphoryl-β-D- arabinofuranose (DPA). DPA is the only source of Araf molecules, thus its deficiency will disrupt the cell wall synthesis and integrity.

1.6.1 Mechanism of action

DPA is synthesized as the result of a two-step redox reaction wherein decaprenylphosphoryl-β-D-ribose (DPR) is oxidized to decaprenylphosphoryl-2-keto-β-D-erythro-pentofuranose (DPX) which in turn is reduced to give DPA. The whole process is catalyzed by an oxidoreductase decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE), pair of two enzymes namely Decaprenylphosphoryl-β-D-ribofuranose 2-oxidase (DprE1) and decaprenylphosphoryl-D-2-ketoerythropentose reductase (DprE2)[5,69,70]. DprE1 is FAD dependent flavoenzyme; uses FAD as an oxidant which gets reduced to $FADH_2$. The enzyme uses menaquinone to reconvert $FADH_2$ to FAD (**Figure 1.4**).

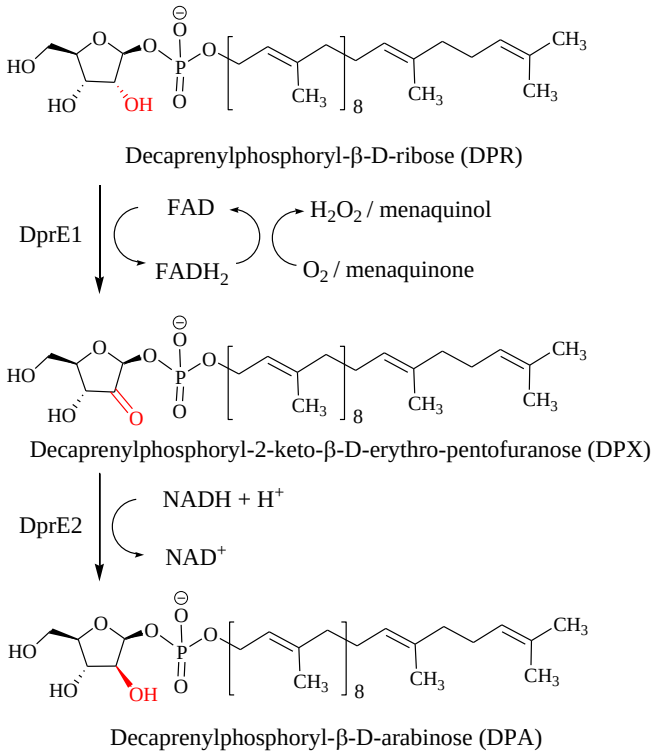


Figure 1.4: Biosynthesis of DPA by DprE1 in presence of menaquinone

1.6.2 Co-crystal structure of DprE1

The knowledge about the crystal structure of the enzyme contributed towards design and development of newer enzyme inhibitors. DprE1 has a topology somewhat similar to the vanillyl-alcohol oxidase family[19]. It has two active binding domains; FAD-binding domain with residue 7-196, 413-461 and a substrate binding domain with residue 197-412 as shown in **Figure 1.5**. Two disordered loops above the substrate binding domain interact with certain proteins of the cell membrane involved in biosynthesis of DPA. Hypothetically, the role of these loops is to keep the substrate-binding site wide open for accommodating the substrate into the domain[71,72].

1.6.3 Inhibitors

DprE1 enzyme inhibitors are sub-classified on the basis of the type of binding they form with the enzyme, viz. covalent or non-covalent binding.

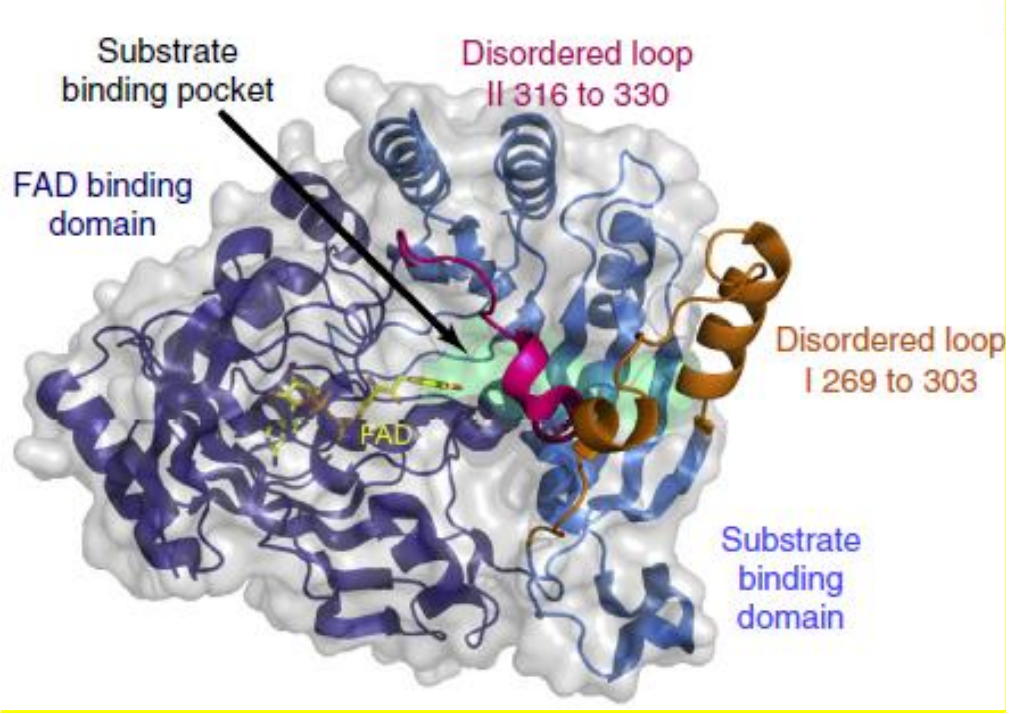


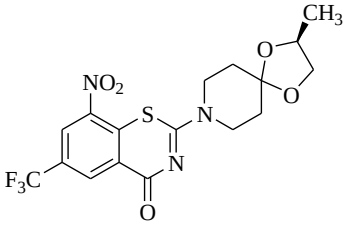
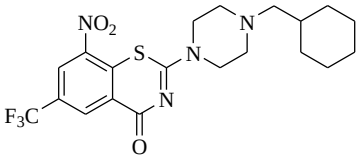
Figure 1.5: Crystal structure of DprE1 (PDB code 4p8l)[5]

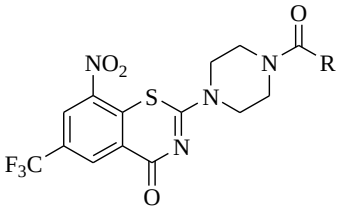
Benzothiazinones (BTZ) were the first reported covalent inhibitors of DprE1 enzyme. The compounds of BTZ series demonstrated sub-micro molar MIC values against *Mtb*. Therefore, many efforts were put forward to improvise BTZ and develop more anti-TB agents.

BTZ exerts their action by undergoing chemical change wherein the nitro group gets reduced first to nitroso then hydroxylamine and then finally to amine. It is postulated that the nitroso derivatives are the active form as they bind to the Cys387 residue of the active site via interaction with the thiol group to form an adduct, thus behaving as suicide inhibitors. Another possible mechanism suggested that the thiol group of Cysteine residue of the enzyme prompted the conversion the nitro to nitroso.[19,73]

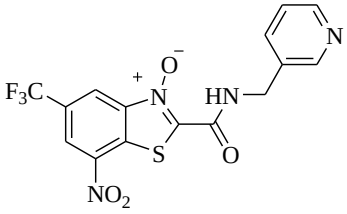
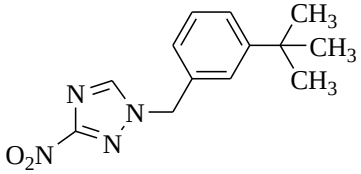
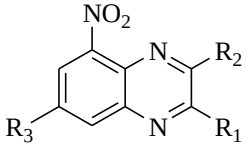
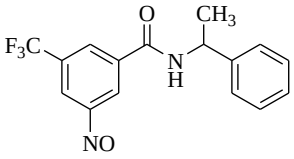
Benzothiazinones were reported to bind covalently to the enzyme until the discovery of PyrBTZ02 (**35**), which was found to have non-covalent interactions. It led to emergence of newer agents having diverse moieties such as benzothiazoles (**36**, **37**), quinoxalines (**38**), thiadiazoles (**39**), azaindoles (**24**), pyrazolopyridines (**40**), and aminoquinolones (**41**) as DprE1 inhibitors. One of the azaindole derivatives TBA7371 (**24**) acting as a non-covalent inhibitor is in clinical trials. All these compounds have opened up avenues for the development of newer derivatives that could act as DprE1 inhibitors. Some of the reported DprE1 inhibitors are summarized in **Table 1.2**.

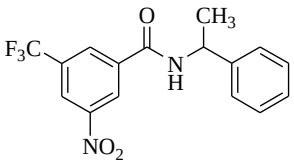
Table 1.2: Some established DprE1 inhibitors

S. No.	Chemical class	Compound	Potency
		Covalent inhibitors	
1	**Benzothiazinones**[19,73]	 BTZ043 (**25**)	MIC: 1ng/ml
		 PBTZ169 (**26**)	MIC: 0.0131 µM



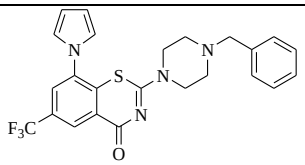
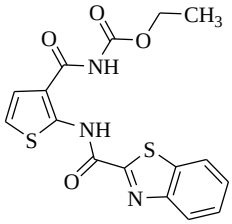
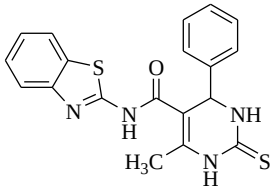
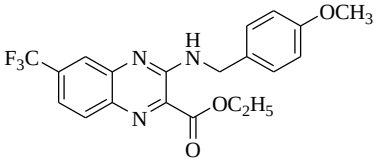
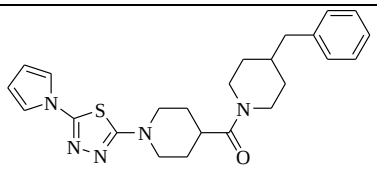
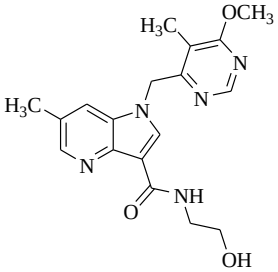
(**29**)

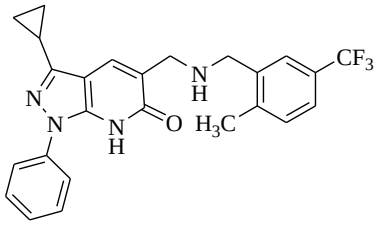
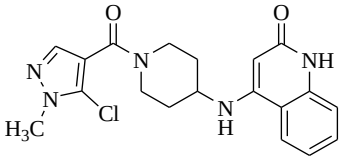
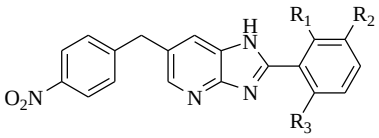
2	**Benzothiazole *N*-oxides**[74]	 8-BTO (**30**)	MIC: 0.026 μM
3	**Triazoles**[75]	 (**31**)	IC_{90} : 0.5 μM
4	**Quinoxalines**[76]	 (**32**)	MIC in the range of 0.75 - 6.25 μM
5	**Nitrobenzamides**[69]	 CT325 (**33**)	



CT319 (**34**)

Non-covalent inhibitors

6	**Benzothiazinones**[77]	 PyrBTZ02 (**35**)	MIC: 0.16 μg/mL
7	**Benzothiazoles**[78,79]	 TCA1 (**36**)	MIC: 0.08
		 (**37**)	MIC: 0.08 μM
8	**Quinoxalines**[80]	 Ty21c (**38**)	IC_{50}: 6.1 μM
9	**Thiadiazoles**[81]	 (**39**)	IC_{50}: 0.054 μM
10	**Azaindoles**[82–84]	 TBA-7371 (**24**)	MIC: 0.39 μM

11	**Pyrazolopyridines**[85]	 (**40**)	IC_{50}: 0.04 μM
12	**Aminoquinolines**[86]	 (**41**)	
13	**Imidazopyridine**[87]	 (**42**)	MIC: 0.5-0.8 μM

1.6.4 Covalent v/s non-covalent DprE1 inhibitors

DprE1 inhibitory activity can be obtained by both covalent and non-covalent binding. Agents from both these categories have been reported with good potency and lower toxicity. To understand the superiority of one over the other, its importance to assess the mechanisms involved. It is clear that, the covalent inhibitors exert their activity by binding to the Cys387 residue of the enzyme. So, any mutation on related to Cys387 will lead to inactivation of the drug or resistance.[88]

On the other hand, non-covalent inhibitors bind in reversible manner, that may lead to inefficient inhibition or development of resistance by incrementing the bacterial load. Yet, compounds from both the categories have found their way into clinical trials suggesting their potentiality.[89]

1.6.5 Dpre1 inhibitors in clinical trials

Two benzothiazinones, BTZ043 (**25**) and PBTZ-169 (**26**) are currently under phase II clinical trials. BTZ043 (**25**) was the first compound to be found as a covalent inhibitor. Later, its piperazinobenzothiazinone derivative PBTZ-169 (**26**) (Macozinone, MCZ) was developed with

no chiral centers and exerted better pharmacodynamics, and easier synthesis. The drug shows synergistic effects with other anti-TB agents. Another DprE1 inhibitor, TBA-7371 (**24**) is in phase II clinical trials. It is being tested with sutezolid and the combination is under phase I trial. Because of the lack of any pre-existing resistance or cross-resistance, TBA-7371 (**24**) can be seen as the potential agent for the treatment of drug resistance cases. Yet another agent, OPC-167832 (**27**) is also in a clinical trial[90,91].

1.6.6 Development of resistance

The development of resistance is one of the major concerns regarding tuberculosis. Thus, it is important to assess the risk of development of resistance towards DprE1 inhibitors. Various research groups have studied the possibility of resistance in DprE1. Foo *et al.*[88] studied resistance to the BTZ in *Mtb*. The authors reported the involvement of C387 residue of the enzyme in the development of resistance towards BTZ. The C387 served as the site of mutation wherein five different amino acids namely, glycine, alanine, arginine, serine, and threonine were substituted. Further, it was claimed that the mutations with threonine, arginine and serine had greater impact than glycine and alanine. These mutations on C387 are associated with the covalent inhibitors, while mutations at Ty38C residue are associated with resistance towards non-covalent inhibitors.

Another group of researchers, Warrier *et al.*[92] studied the resistance associated with genes *i.e.*, rv0560c, rv0558, and rv0559c by overexpressing these genes. Their results indicated that rv0560c is the gene responsible for s-adenosyl-L-methionine-dependent methyltransferase, an enzyme that methylates the inhibitors and reduces their activity.

1.7 References:

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