Chapter II

Review of Literature

While tuberculosis (TB) is acknowledged as a historical ailment, it continues to be a major cause of death on a universal measure. Tuberculosis is responsible for more deaths caused by a single infectious agent than any other disease, surpassing both HIV and Malaria.[22] It results from a causative agent *Mycobacterium tuberculosis* as well as some genetically associated species like *M. microti, M. bovis, M. africanum, M. canettii, M. pinnipedii,* and *M. caprae* that entirely led to the formation of the *Mycobacterium* complex. *Mycobacterium tuberculosis,* often referred to as "Koch's bacillus" afterward the unprecedented contribution of "Sir Robert Koch", who was awarded the Nobel prize for medicine or physiology in the year 1905 "for his examinations and revelations corresponding to tuberculosis" is exceptionally contagious. Infection is usually acquired by inhalation along with the influx of alveolar macrophage at a remarkably small portion of 5 to 10 bacteria.[4] *Mycobacterium tuberculosis*, a member of the GC-rich gram-positive actinobacteria phylum, belongs to the suborder Corynebacterial within the order Actinomycetales. This suborder encompasses the genera *Corynebacterium* and *Nocardia*, along with *Mycobacterium tuberculosis*.[23] There are pathogenic and non-pathogenic species of the genus *Mycobacterium*. A Pathogenic *Mycobacteria* comprises "*Mycobacterium tuberculosis"* and "*Mycobacterium leprae",* disease-causing agents of leprosy, and the "*Mycobacterium avium"*-intercellular complex, a major opportunistic disease-causing agent affiliated with AIDS. Buruli ulcers are brought on by the bacterium

"Mycobacterium ulcerans", a come-out skin disease. A non-tubercular *Mycobacterium, "M. abscessus"*, is related to colonization and infection in a patient via cystic fibrosis.[4] These pathogenic organisms all have sluggish growth rates and the generation time is 18-24 hours.

Pulmonary tuberculosis, which primarily targets the lungs, is the most prevalent form of TB, often referred to as pneumonic TB. Apart from its pulmonary effects, tuberculosis can also manifest in different parts of the body, including the lymph nodes, bones, joints, pleural space, central nervous system, and intra-abdominal organs, among others. The TB population is divided into three groups based on clinical data and research on disease transmission. First is LTBI, a latent TB infection usually considered asymptomatic, chronic, or non-communicable. Another is active TB, which has symptoms, is contagious and may be diagnosed using molecular means. The third is sub-clinical TB, which is contagious and symptomless.[24] After persistent inconsequential, in the year 1993, WHO announced tuberculosis as a worldwide well-being crisis and prompted revitalization and retrospection of a few TB control programs.[25] Presently, utilizing strategies like "Sputum circle microscopy", "Xpert MTB/RIF" and "Hain-line-test" examines have empowered quickly recognizable proof of *Mycobacterium* complex (MTBC). It remains unclear whether these factors alone can confirm a drug-resistant TB diagnosis. The battle against tuberculosis has fostered collaboration among various stakeholders, including "World Health Organization", "multiple governments", "the pharmaceutical industry", "non-governmental organizations", "academic institutions", also "several product development partnerships". Notable partnership in this endeavor includes the "Global

TB Alliance", "STOP-TB Partnership", "The TB Drug Accelerator", and "The Treat TB Initiative". In the ongoing battle against tuberculosis, scientists are confronted with substantial challenges. These include "enhancing our understanding of the biology of MTB", "identifying and validating new targets for tuberculosis treatment", "formulating novel drug regiments that are well tolerated by patients with comorbidities such as HIV and diabetes", "reducing the duration of standard therapy", "enhancing the cost-effectiveness of treatments", "improving the efficiency and speed of diagnostic methods" and "developing new drugs to combat the disease". These challenges collectively underscore the multidimensional nature of the efforts required to combat tuberculosis effectively.

TB is the most powerful pathogen that affects humans, and it is the only disease, apart from HIV/AIDS, that causes a higher mortality rate due to rare pathogenic microbes. According to the Global TB Report of 2021, it is estimated that approximately 25% of the global population is infected with tuberculosis.[26] In 2021, the global count of individuals diagnosed with TB reached a total of 10.6 million, with a 95% uncertainty interval ranging from 9.9 to 11 million, which equates to a rate of 134 cases (95% UI: 125-143) per 100,000 individuals. HIV-infected people reported 6.7% of the total tuberculosis cases. In terms of geographic distribution, the maximum percentage of tuberculosis cases in 2021 were reported in Southeast Asia (45%), shadowed by Africa (23%) and the Western Pacific (18%) regions. Conversely, the Eastern Mediterranean (8.1%), the Americas (2.9%), and Europe (2.2%) recorded the lowest incidence of TB cases during that period.[26] These regional disparities emphasize the varying levels of TB burden across different parts of the world.[26,27]

After experiencing a marginal decrease for several years, the estimated number of global tuberculosis cases took a negative turn in 2021, with a 4.5% increase. The incidence of TB increased from 10.1 million (with a 95% uncertainty interval of 9.5-10.7 million) in 2020 to an estimated 10.6 million (with a 95% uncertainty interval of 9.9-11 million) in 2021.[27]

In 2020, tuberculosis resulted in the loss of 1.3 million lives who were not infected with HIV, while HIV-positive individuals contributed an additional 214,000 deaths to the total TB death toll.[26] These number indicates endemic factors like poverty and geographical superposition of contagion with HIV/AIDS. This reflects three major hardships experienced in the battle next to MTB. First, the ongoing vaccine, BCG "bacillus Calmette Guerin" doesn't dependently hinder the prevailing pattern of the disease.[4] Secondly, the indicative test for tuberculosis has been inadequate and preferably convoluted for some time. Thirdly, even though drug medicines are generally viable, they are truly challenging to adapt to in terms of consistency, span, and incidental effects.

In 1948, the first clinical experiment was conducted to treat tuberculosis. In reaction to the emergence of drug-resistant tuberculosis strains, several advanced anti-TB drugs have made significant progress and advanced clinical trials over approximately a decade.[28] In achieving the United Nations' "Millennium Development Goals" (2000-2015); the World Health Organization implemented initiatives such as "Directly Observed Treatment Short-course" (DOTS) from 2000 to 2005 and "Stop TB" from 2006 to 2015. The UN's sustainable development goals included the "End TB strategy" (2016-2035), which was designed to eliminate TB across the globe.

However, in the year 2016, close to 600,000 cases of “rifampicin-resistant tuberculosis” and roughly 490,000 cases of resistance to both “rifampicin and isoniazid” were reported. Additionally, 6% of MDR-TB cases were classified as XDR-TB due to their heightened resistance to fluoroquinolones and certain first-line injectables.[29] The new "Global Tuberculosis Report 2021”, authored by the WHO, certainly expresses that a large portion of the great TB load nations and several WHO locales are out of control to accomplish the year 2020 achievements of the End TB program. The undervaluation, under-determination, the jumble in the middle of assessed incidence and enlisting cases, and the problem with the TB drug care cascade.[30] Therefore, the exploration focussing on TB obstruction should think about the accompanying points of view: first, from the programmatic view, the techniques ought to carry outfitting drug use as well as accomplish higher attachment to the tailor-made regimens; and Second, on the ground of clinical position, the methodologies ought to contrive the surveillance and oversee techniques of the drug-resistant problem and corroborate existing or novel medications or regimens represents this hole including the ongoing development of drug-resistant toward anti to TB drugs.[31] The length and medication routine intricacy itself stay the significant difficulties in the treatment of TB, as it influences the tailor-made drug attachment as well as additionally influences harmful adverse impacts (essentially DR-TB regimens) and pediatric treatment. Additionally, the conjunction of HIV with TB further adds to the sedate medication interaction between anti-TB agents and also, as antiretroviral treatments. The aggregate medication toxicity levels of consolidated treatments additionally enhance the possibility of patients in the direction of the generation of a syndrome known as IRIS- “Immune Reconstitution Inflammatory Syndrome”. There

has been significant progress in tuberculosis research, which is much needed to search for effective, affordable, non-toxic, or minimally toxic new drugs, possibly with more rapid treatment schedules, to achieve the aim set out by the World Health Organization. Basically, "Increased research and development"-the prompt integration of the third mainstay of the End TB program is necessary to overcome current challenges with determination. Even though the compounds bedaquiline and delamanid have been endorsed by many nations, these medications are genuinely unfavourable are utilized as they were at the point when there could be no alternative option for treatment for MDR-TB.[32] Different methodologies are utilized to foster new anti-TB drugs i.e., first, novel medications focusing on novel targets and work by various operations, e.g. as diarylquinoline class; second, use of current antibodies to deal with TB, for example, ciprofloxacin; and third, adjustment of present medications or unification of novel admixtures with improved adequacy next to tuberculosis.[33] The development of new medication for TB treatment imitates a significant job of diminishing the pace of events and mortality. It is a crucial stage to arrive at overall objectives set apart by WHO. Looking for novel anti-TB compounds, attempts were made to examine the benefits and impediments of different medications that have entered clinical preliminaries, consequently working with the specialists associated with this arena to gain from ongoing research in planning inventive programs or strategies for the revelation as well as improvement of better anti-TB drugs.

In the current era, the treatment of tuberculosis comprises drugs that were discovered fifty years back. To treat drug-resistant TB, a recommended system is known as DOTS, which is commensurate treatment under direct supervision. This

system comprises four oral drugs referred to as first-line drugs including ethambutol (EMB/E), pyrazinamide (PZA/Z), isoniazid (INH/H), and rifampicin (RIF/R). Such a combination of drugs is effective for active, drug-susceptible tuberculosis, as long as the patient completes his treatment for a time of something like a half year (four months HR then two months HRZE).[4] As part of LTBI treatment, INH monotherapy for six months is generally prescribed for both adults and children in regions with both high and low TB prevalence. While INH monotherapy is a common treatment for LTBI, the WHO suggests considering other treatment options as well, these are (i) recently suggested precautionary treatment comprising of 90 days day to day mix treatment with isoniazid (INH) and rifampicin (RIF) for youngsters and teenagers (individuals below 15 year of age) in elevated tuberculosis trouble nations; (ii) a new recommended preventive treatment involves a weekly combination of isoniazid (INH) and in countries with a high burden of tuberculosis, both children and adults are being treated with rifapentine for a duration of three months and (iii) when it comes to tuberculosis, prevention in countries with low TB burden, the current recommendation is to use a 90-day weekly INH and rifapentine combination, INH monotherapy for 9 months, or a 3-4 month treatment of either RIF alone or a combination of INH and RIF.[34] The treatment strategy for tuberculosis cases susceptible to rifampicin but resistant to isoniazid (INHR-TB), a half-year regimen containing RIF, EMB, PZA, and levofloxacin is used as therapy. The structure of a lengthier multi-drug resistant TB (MDR-TB) and rifampicin-resistant TB (RIFR-TB) routine comprises each of the three medications from Group A (such as bedaquiline, linezolid, and levofloxacin/monofloxacin). When used, it is typically accompanied by the administration of at least one medication from group B such as clofazimine or a

combination of terizidone and cycloserine. The duration of treatment for extensive multi-drug resistant tuberculosis can vary (between nine to twenty months) depending on the patient's response to treatment and the medication schedule chosen. In situations where there is no history of prior exposure to second-line drugs within a timeframe of less than one month, or there is no indication of resistance to second-line drugs and fluoroquinolones, a shorter drug regimen of 9 to 12 months could be contemplated.[22] During treatment where improper treatment has been administered or treatment is not completed, the patient may potentially develop a strain of drug-resistant tuberculosis that could necessitate a treatment duration of as long as two years with primary oral medications like ZE, as well as second-line inj. aminoglycosides including "kanamycin", "amikacin", "streptomycin" or peptides including "capreomycin", "fluoroquinolones" (ofloxacin), bacteriostatic mediators including "ethionamide", "para-aminosalicylic acid" and "cycloserine".[35]

2.1. Emergence of Drug Resistance

Tuberculosis is an airborne illness and, as the greater part of diseases of this sort, doesn't spread the dynamic stage after attainment of the target. Because of its impressive capacity to adjust to the human immune system, Mtb can persist in a dormant and non-replicating state within infected individuals. This hibernation-like state allows the bacteria to evade the immune response and remain latent within the host for an indefinite period, it is then reactivated whenever there is an immunological disorder. By creating reactive nitrogen intermediates (RNIs) such as NO, NO_2, and N_20_3 preventing the fusion of phagosomes, and disrupting the MHC class II pathway, bacillus resistance is established during the dormant period.[36] The activation of

resuscitation-promoting factors, “Rpfs A to E” and the action of “peptidoglycan-hydrolyzing” enzymes play a crucial role in enabling *Mycobacteria* to transition from a dormant phase to an active state once again. On the other hand, the *“DosR”* regulon system (including *“DosR”* or Rv3133c) in Mtb helps to regulate this process.[36] Enabling *Mycobacteria* to transition between respiring and non-respiring environments and conversely deprived of loss of reasonability, together prompting dynamic and infectious tubercular infection. These striking structures of the bacteria make TB the most provoking and irresistible infection to battle and annihilate. One of the topmost reasons behind the complete eradication of TB is antibiotic resistance which has become a challenge to overcome now. According to Darwin’s theory of evolution, drug-resistant strains of *Mycobacteria* have developed the ability to overcome anti-tuberculosis drugs through “genetic mutations” and additional mechanisms, which are further strengthened by selective environmental pressures. Environmental factors can induce genetic alterations in the *M. tuberculosis* genome, leading not just to reduced efficiency of applied medications as well as increasing Mtb’s ability to survive in extreme conditions.[37] This survival mechanism can promote multidrug resistance in Mtb and expose it to bactericidal antimicrobials via radical-induced mutagenesis. In situations where “reactive oxygen species” and “free radicals” are unable to effectively eliminate the *Mycobacterial* cell, it can lead to increased cell mutagenesis and drug resistance.[37]

Drug resistance to tuberculosis can be divided into three categories. First is MDR-TB: multi-drug resistance. It relates to a type of tuberculosis resulting from *Mycobacterium* strains that have developed resistance to the key drugs typically

administered in initial treatment, namely isoniazid (INH) and rifampicin (RIF). Currently, this MDR form of TB is present in all countries as surveyed by the WHO and is no longer treated with Ist-line and requires IInd-line drugs. Extensive drug resistance is the second type of tuberculosis that is resistant to drugs, resulting from the inappropriate use or mismanagement of second-line drugs. This condition arises from *Mycobacterial* strains that exhibit resistance to the initial drugs and demonstrate further resistance to fluoroquinolones, along with certain second-line injectable medications such as “capreomycin”, “kanamycin”, and “amikacin”. TDR-TB another type of drug-resistant tuberculosis, also called extremely drug-resistant XXDR-TB, was resistant to all drugs included in both the first and second-line drug regimens.

Over the past two decades, there have been notable advancements in identifying effective antimycobacterial compounds derived from natural sources. A variety of drugs have been discovered from natural sources, such as STR and RIF. Typically, sources play a crucial role in the advancement of semi-synthetic drugs. As an example, the semi-synthetic drug RIF is derived from the natural compound rifamycin, highlighting the significance of natural sources in drug development.[37] The WHO empowers and encourages the level of a national, regional, and global search for a novel therapeutic compound for the eradication of TB in 2030.

2.2. Mechanisms of Drug Resistance

Drug resistance poses a serious obstacle to the treatment of TB and is a problem for global therapeutics. Despite the effectiveness of anti-TB medications, drug-resistant Mtb has started to emerge. Various types of machinery work with the expansion of medication resistance in MTB, for example, “clonal interference”, “compensatory

development", "cell envelope impermeability", "efflux pump", "epistasis", "phenotypic drug tolerance", "target mimicry", "drug degradation", "modification", etc.[38] The inability of tuberculosis treatments to succeed can be attributed to both natural, "inherent high levels of antibiotic resistance" and external "changes" or "mutations" that cause antibiotic resistance. For instance, intrinsic resistance is observed in Mtb, which possesses a lactamase enzyme encoded in its genetic makeup, leading to resistance against the β-lactam antibiotic class.[39] Prolonged tuberculosis treatment often leads to patients developing resistance to medication regimens, which can accelerate the progression of drug-resistant Mtb to "multidrug-resistant" and "extensive drug-resistant". To address the problem of drug resistance in *Mycobacterium tuberculosis* effectively, it is of utmost importance to gain a comprehensive understanding of the underlying mechanisms driving antibiotic resistance.

2.2.1. Intrinsic drug resistance

The intricate composition of the cell wall in *Mycobacterium*, characterized by the presence of mycolic acid, results in a limited permeability that hinders the efficient uptake of therapeutic drugs or medications. Prolonged exposure to low doses of drugs to the microorganism can trigger the carrier proteins, leading to irreversible overexpression and the development of phenotypic resistance.[40] It eventually can lead to an additional genuine hereditary resistance. The presence of *β-lactamase* enzymes encoded by the organism can impede the effectiveness of β-lactam antibiotics, subsequently bringing about resistance to the drugs.[40] It has been possible for Mtb to adapt to the cytotoxicity of antibiotics and other harmful synthetic compounds due to

the evolution of several molecular components, this occurrence has resulted in a notable rise in the development of inherent drug resistance.[41]

2.2.2. Acquired drug resistance

During treatment, the chromosomal transformation of drug target genes can lead to acquired resistance in *Mycobacterium*. It can be transmitted to others and result in the expansion of resistant mutants due to various factors i.e., extended treatment, sporadic consumption of prescribed medication, drug lacking, or neediness in the low-pay section. This can ultimately lead to resistance to certain commonly used drugs, followed by cross-resistance to all medications. Insufficient medication or administering low doses of anti-TB drugs during treatment can create an opportunity for the TB bacteria to survive and select resistant strains to proliferate. Ultimately, this process can give rise to mutagenesis leading to the development of more drug-resistant strains and ultimately multi-drug resistance.[40]

2.3. Novel and Current Anti-tubercular Agents

MTB has an advanced cell wall which contributes to a remarkable lipid barrier. This hindrance works with the appearance of either acquired or inherent resistance to antibiotics.[42] To develop new and effective anti-TB medications or treatment regimens that target drug-resistant strains such as MDR, XDR, and TDR, it is important to understand how current medications work and the mechanism of bacterial resistance. The current and emerging drug used against TB inhibits various processes within the *M. tuberculosis* bacteria, including the cell wall, cell wall acids, peptidoglycan, DNA gyrase, DNA replication, protein synthesis, and more. As a result, gaining a thorough

understanding of how these drugs function and the mechanisms behind bacterial resistance is essential and becomes imperative to foster the development of efficacious treatments for tuberculosis.

At present, the majority of the anti-tubercular drugs consist of rifampicin, ethambutol, isoniazid, pyrazinamide, etc. Isoniazid was discovered in 1952. This medication came after the pioneering clinical antibiotics streptomycin and para-aminosalicylic acid. Isoniazid (INH), isonicotinic acid hydrazide, which is also recognized as a "pyridine-4-carboxy hydrazide", since its discovery, has been the most widely utilized anti-TB medication. Three different pharmaceutical corporations, "Hofmann LaRoche" (Nutley, NJ, USA), "ER Squibb", "Bayer" (Leverkusen, Germany), and Children (Princeton, NJ. USA), discovered this drug, which was demonstrated to inhibit the cell envelop of *M. tuberculosis*. Considering the significance of the cell membrane in the development of *Mycobacterium's* pathogenesis, INH was hailed as the "wonder medicine" for its ability to relieve many patients.

Isoniazid (INH) functions as a prodrug, necessitating activation by the *Mycobacterium tuberculosis* catalase-peroxidase enzyme *KatG* to form an INH-NAD complex. It inhibits the essential component of Mtb's mycolic acid biosynthesis pathway, nicotinamide adenine dinucleotide (NADH)-dependent enoyl-ACP reductase of the fatty acid synthase type II framework (encoded by *inhA* quality).[43] The inhibition of "enoyl-ACP" disrupts the synthesis of long-chain fatty acids. As a result, the accumulation of these unfinished fatty acid intermediates occurs within the bacterial cell. This accumulation interferes with critical cellular processes and leads to cell

death, which is encoded by the *inhA* quality. Clinical isolates have demonstrated that alterations in the *KatG* and *inhA* genes justify around 70% and 80% of isoniazid-resistant Mtb, respectively.[43] Being a prodrug, a mutation in the *KatG* enzyme significantly impacts how it moves. Thus, a logical approach to circumventing this isoniazid-related resistance pathway would be to design a compound that targets the *inhA* enzyme without also activating the *KatG* enzyme. For instance, triclosan inhibits the *inhA* enzyme, however, due to its poor bioavailability it has not been successfully used against TB medications.[43] Isonicotinic acid's derivative ethionamide (ETH, 2-ethylisonicotinamide), has been employed as an anti-tubercular drug starting around 1956. The drug exhibits a mechanism of action that is similar to isoniazid. One of the first antibiotics to have anti-tubercular action was "para-aminosalicylic acid" (PAS). Nonetheless, its mechanisms of action of activity are rarely explained, however, it is believed to work by preventing the synthesis of thymine.

Looking for an intense compound with a novel mechanism of the compound. Due to the evolution of the whole genomic structure of the *Mycobacterial* genome, the potential for discovering target-based drugs against TB through repurposed old drugs, newly discovered promiscuous compounds, and newly identified promising druggable sites should not be underestimated. Target-based drug discovery is a major focus of study for the expansion of anti-tuberculosis medications, and it involves choosing the targets for existing, recently approved, or novel chemical substances (NCEs) in the TB pipeline.[44]

Target-based drug development has been ongoing over the past ten years, NCEs acting against "Iso-citrate lyase" (*ICL*) inhibitors,[45] "ATP synthase",[46] "Enoyl-acyl

transporter protein (ACP) reductase" (*InhA*),[47] "Decaprenylphosphoryl-β-D ribose 2′-epimerase" (*DprE1*),[48] "Cytochrome bc1 complex" (*QcrB*),[49] "Pantothenate synthetase" (*PS*),[50] and "Cytochrome bd oxidase" were described for to assume a critical part in contradiction of both drug-resistant but also as drug-sensitive *Mycobacterial* strain. With the novel pipeline against tuberculosis, the particles like sanfetrinem, MPL-447, JSF-3285, CPZEN-45, MPL-446, and so on under beginning phase improvement or as the new chemical ingredients against *Mycobacteria*, preliminary toxicity studies have emerged.[27,51]

Nitroimidazoles class (for example, TBAJ-876 and TBAJ-587), benzothiazoles (including BTZ-043 and macrozinone), and rifamycin, fluoroquinolones such as rifapentine and moxifloxacin (4-months regimen in combination) and other drugs of the oxazolidinones class are currently in the clinical stage. Furthermore, the re-purposed drugs have unclogged another window against TB action to satisfy strong strength for the further advancement of novel medication for a resistant form of TB.[52,53] In addition, a few tiny compounds that significantly impact Mtb's energy metabolism have been discovered.[54] As may be seen from a search using Sci-Finder, the research team has been working diligently on TB all around the world. Alkaloids, terpenoids, quinones, peptides, pyrones, lactones, sterols, and their semi-synthetic form and bioactive compounds inspired by nature, often sourced from marine or plant origins, are among the potential candidates in the early stages of development as anti-tubercular drugs with limited progress in clinical trials.[55]

A few medications amalgamation have been utilized to get rid of tuberculosis and the present anti-tuberculosis drug discovery worldview has moved on the way to the

utilization of “Ligand-based drug design” mediated via “Computer-assisted drug designing”, “Structure-based drug development”, “Combinatorial science”, and so forth.[55-58]

2.4. The *Mycobacterium* Cell Wall

There has been significant discussion in recent times about the biosynthesis of the components that make up the *Mycobacterial* cell wall. The *Mycobacteria* cell wall consists of three separate constituents: peptidoglycan (PG), lipopolysaccharides (LPS) which include arabinogalactan, lipomannan, and lipoarabinomannan and an exterior layer containing mycolic acid. Beyond the *Mycobacterial* internal membrane, *Mycobacterial* TB peptidoglycan gives the cell rigidity, integrity, and shape.[7] PG is a polysaccharide comprised of “N-acetylglucosamine” and “muramic acid” residues that are substituted with amino acids that are joined together by β-(1-4) bonds. Muramic acid can be either N-acetylated or N-glycosylated.[59] The *Mur* ligases *MurC/D/E/F* are responsible for producing the “pentapeptide L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanyl-D-alanine”, which is then acylated on the “muramic acid” component by polysaccharide strands. The acylation procedure is conducted by enzymes known as D-ala: D-ala ligases, with a particular focus on *DdlA*.[59] The peptidoglycan is framed by the pentapeptides, which are cross-connected. *M. tuberculosis* exhibits two different types of cross-linkages, firstly, the penicillin-binding proteins *PonA1* and *PonA2* operate as D, D-transpeptidases, joining D-alanine and meso-diaminopimelic acid for a 3→4 linkage. To create 3→3 linkages and connect two meso-diaminopimelate residues via cross-linking, transpeptidases L, D (specifically LdtMt1 to 5) are employed.[7]

The arabinogalactan is a long polysaccharide composed of residues of the furanose-arranged sugars arabinose (*Araf*) and galactose (*Galf*). To initiate the biosynthesis of arabinogalactans, a linker must first be created that binds the AG complex to the PG. This process utilizes the residual N-glycosylated-muramic acid.[59] The linker connecting the arabinogalactan complex to peptidoglycan is created by *WecA* and takes the form of a "decaprenyl-diphosphate-N-acetylglucosamine-rhamnosyl" particle, gradually converting "N-acetylglucosamine-1-Phosphate" to "decaprenyl-phosphate" and then exchanging "L-rhamnose" with *WbbL*. The "galactofuranosyl transferases", *GlfT1*/*GlfT2* add 30 linear galactofuranosyl residues to this linker. *Araf* is incorporated into the galactan chain using decaprenylphosphoryl-D-arabinose as a catalyst, which functions as the arabinose donor. Several steps are involved in the formation of DPA, starting with "Phosphor-D-ribosyl-1-pyrophosphate" (pRpp). *UbiA* is responsible for the addition of decaprenyl, which is then transformed into DPA through the actions of *DprE1* and *DprE2*. The initial unit of *Araf* is transferred during the synthesis of galactan by the arabinofuranosyltransferase *AftA*. The arabinosyltransferases *EmbA* and *EmbB* play a role in the subsequent elongation of *Araf* to create the arabinan. The result, arabinogalactan, is a linear structure connecting extensively elongated arabinans. Mycolic acids are held in place by the arabinan in the mAGP complex, which provides stability to the structure.[59]

Mycolic acid, a crucial constituent of *Mycobacterium tuberculosis* cell wall, influences acid-fast staining, virulence, viability, and permeability. The coupling of mycolic acids to other saccharides or their presence in a free form can lead to the

production of TMM/TDM and "glucose monomycolate". Mycolic acids are typically associated with the arabinose portion of the arabinogalactan complex.

2.5. Targeting Cell Wall Biosynthesis

Among the few targets accessible, cell wall biosynthesis is an important factor that influences value potency for developing inhibitors. As inhibiting cell wall synthesis induces bacteria to disintegrate due to osmotic pressure, bactericidal inhibitors disrupt cell wall production. Due to the significantly complicated structure of the *Mycobacterial* cell wall, MTB differs from other bacteria and the existence of virulence factors. The mAGP complex is a structural attribute of the Mtb cell wall that is formed by the integration of three layers: the outermost layer of mycolic acid, the middle layer of arabinogalactan, and the innermost layer of peptidoglycan. The integration of these layers results in the formation of a complex assembly that is critical for the "persistence" and "pathogenicity" of *Mycobacteria*. The inmost layer, Peptidoglycan (PG), is distinctive to bacteria, has been studied extensively, and is well-considered a desirable target for pharmacological development. Evaluation of the main enzymatic targets and their pharmacological inhibitors might benefit from the knowledge of the biosynthetic pathways essential for cell wall synthesis in MTB.

Peptidoglycan is a macromolecule consisting of long chains of sugar molecules linked together by short peptides, and make up the mAGP complex's bottom layer. The formation of the PG layer occurs in three stages, which depend on the cellular location of the enzyme. Within the sugar polymer, *GlcNAc*: "N-acetylglucosamine" and *MurNAc*: "N-acetylmuramic acid" are organized in a β (1→4) conformation. The assemblage of peptidoglycan assembly happens in three different stages. Stage I:

involvement of cytoplasmic precursor synthesis, Stage II: involves membrane-bounded precursor translocation over the periplasm, and Stage III: involvement of "precursor polymerization" as well as peptide cross-linking.

As a complete study of *Mycobacterium* biosynthesis is still a work in progress, its chemotherapeutic potential is relatively unexplored. The introduction of novel biochemical assays will advance drug discovery efforts in this discipline.[60,61] The GlcNAc-1-P enzyme is a widespread target originating in eukaryotes, while the uridyltransferase activity of *Mycobacterial* GlmU is similar to that of human enzymes, it will be difficult to target it specifically and efficiently although GlmU acetyltransferase inhibitor TPSA was discovered,[62] "Aminoquinolines", "Arylamines", "2-Phenylbenzofurans", "Aryl sulfonamides", "Non-specific thiol-reactive agents", "GlcN-6-P" and "GlcN-1-P GlmU" correspondents as well as diterpenoids isolated from therapeutic plants have all been described as auspicious inhibitors of GlmU. TPSA and "2-amino-2,3-dideoxy-3-fluoro-α-D-glucopyranosyl phosphate" also demonstrate exceptional activity.[63]

The acetyltransferase capability of MTB's GlmU has been employed. due to the absence of GlcN-1-P in humans and has demonstrated anti-TB activity. Numerous broad-spectrum chemical inhibitors, designed to target bacterial *Mur* ligases, have been identified. The creation of a novel "one-pot assay" that integrates multiple stages for the identification of pharmacological inhibitors of "*Mur* Ligases" (A-F), coupled with the application of "computational techniques" to comprehend substrate binding, could greatly aid drug discovery endeavors.[64] Cycloserine has demonstrated

bactericidal properties against MDR and XDR-Mtb strains, its usage has been restricted due to associated toxicity.[65]

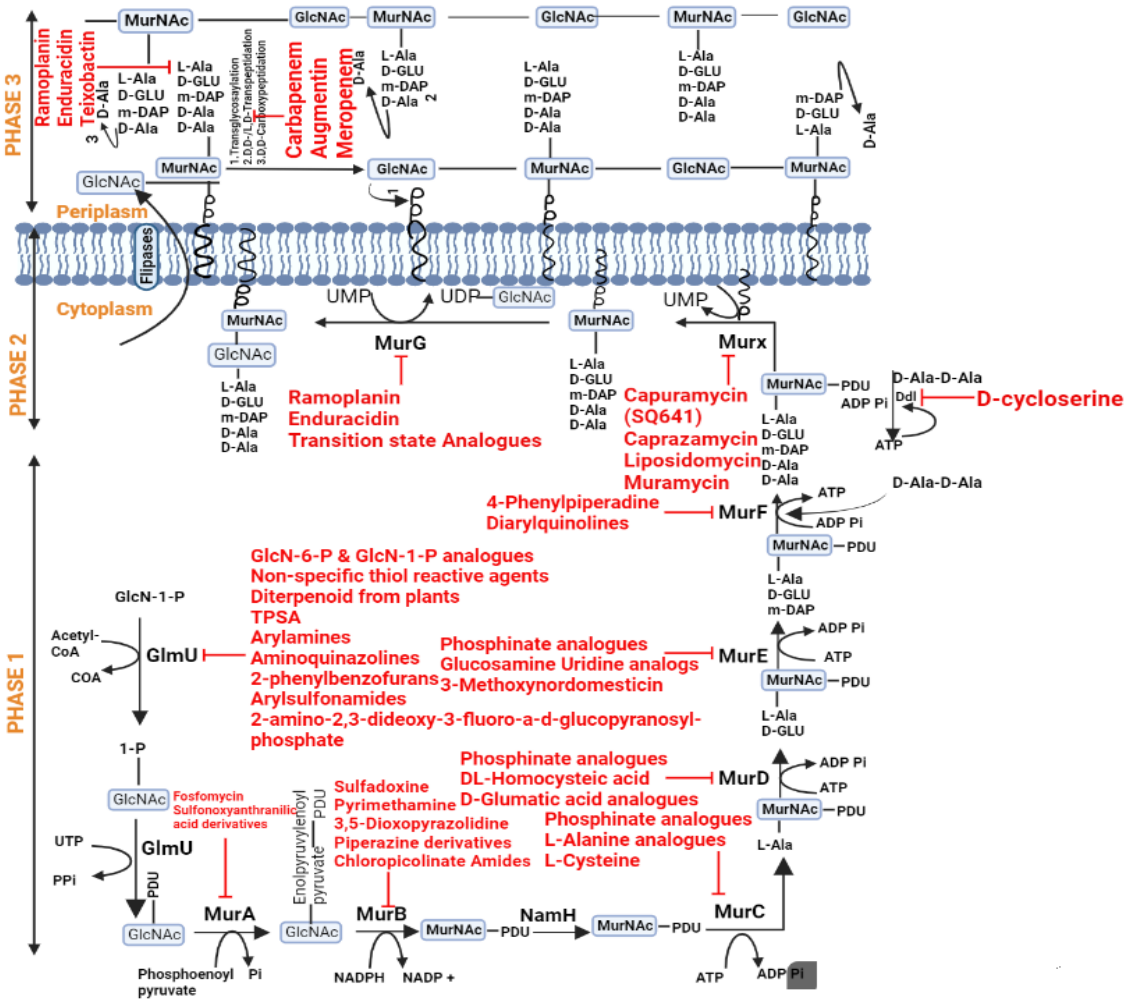


Figure 2.1. Biosynthesis of Peptidoglycan with an arrow pointing in the direction of the target, inhibitors for various enzymes in Phases I[st], II[nd], and III[rd] are displayed in the red text.

SQ641, a capuramycin-based molecule, is now in preclinical development for tuberculosis treatment. "Muramycin", "liposidomycin", "caprazamycin", and "capuramycin" are peptide scaffolds bound to nucleosides, which interact with different sites within bacterial translocase 1, *MraY*, or *MurX* (Table 2.1). The

glycosyltransferase of Mtb is chemically inhibited. Few compounds have been discovered that replicate the transition state of the enzyme process, which has restricted *MurG's* success to date.[1]

Antibiotics that bind Lipid II, i.e., ramoplanin (a lipoglycodepsipeptide), teixobactin, and enduracidin impede further transglycosylation and could be investigated as a chemical inhibitor of tuberculosis (Table 2.1).[1] Utmost β-lactam antimicrobials have traditionally been "D, D transpeptidase inhibitors", although carbapenems (for example, imipenem and meropenem) could similarly hinder L, D transpeptidases enzyme that represents about 80% of peptide linkage in *Mycobacterium's* peptidoglycan whereas surviving the hydrolytic breakdown by microbial β-lactamases (*BlaC*).[66,67] Kaushik and colleagues showed that carbapenems with RIF had a synergistic effect against drug-resistant Mtb strains.[68]

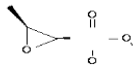
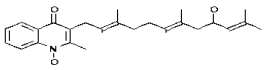
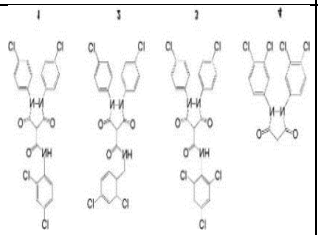
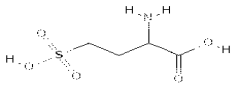
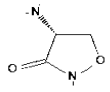
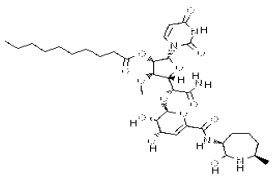
Mur enzymes play a role in peptidoglycan (PG) formation, making them crucial proteins in *M. tuberculosis*. They also have no human counterparts, making them attractive therapeutic targets. The glutamate racemase enzyme that *Murl* encodes is involved in the initial steps of PG production and is hence a viable target for therapeutic development. The interconversion of L-glutamate to D-glutamate is mediated by D-amino acid racemase or D-glutamate synthase, and L-glutamate plays a vital character as a component in the creation of the peptidoglycan layer.[2] Glutamate racemase (*MurI*) also plays an important function in the sequestration of DNA gyrase enzymes. Data suggest, that in the bacteria kingdom, glutamate racemases may be found almost everywhere and are largely preserved. Its absence in eukaryotes like humans also suggests it is an appealing target for therapeutic development.[2]

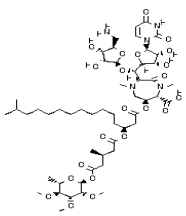
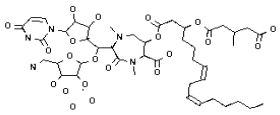
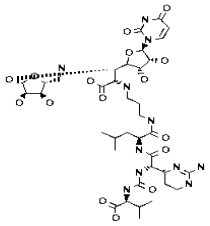
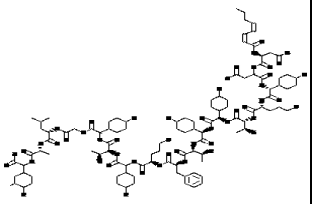
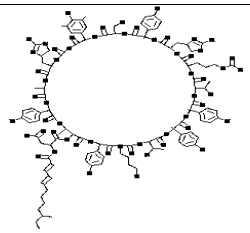
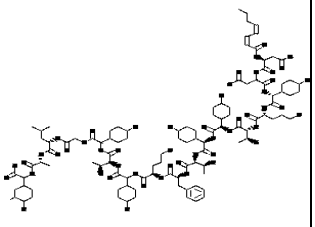
Flavonoids have been demonstrated to have effects on cytokine regulation and glutathione (GSH) enrichment,[69] detoxification process initiation, nucleic acid inhibition, and/or proteasome inhibition. In their study, Pawar and colleagues suggest that both quercetin and naringenin could serve as valuable leads in the development of anti-TB drugs.[2]

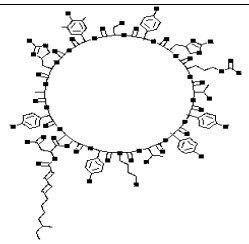
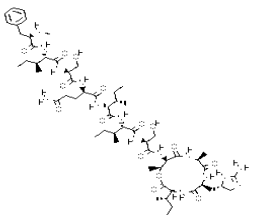
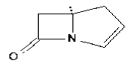
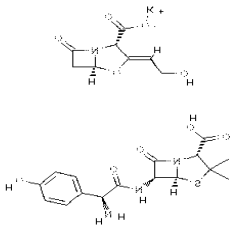
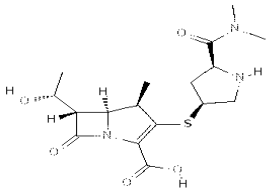
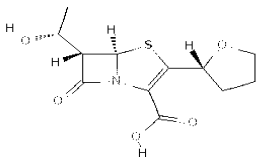
Isoniazid and ethambutol, two of the four basic drugs, both alter the biosynthesis of a crucial element of the cell wall core.[70] Furthermore, numerous TB medicines in research target the pathogen's cell-wall biosynthesis. The mAGP complex present in *Mycobacterial* cell walls creates a distinctive “lipidic” and highly “hydrophobic” barrier that shields the pathogen from the host’s immune system as well as the most commonly used antibiotics.[71] The catalyst for the synthesis of galactan should be explored as a possible candidate for innovative therapeutic formulation as polymer plays a crucial role in preserving the structural integrity of the *Mycobacterial* cell wall, highlighting its significant importance.[72]

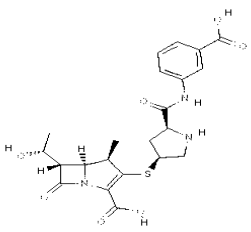
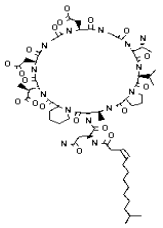
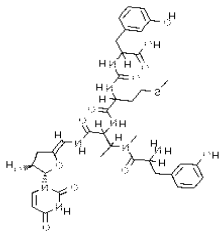
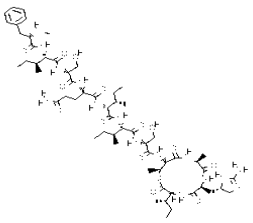
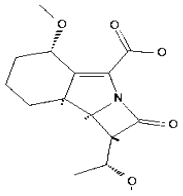
According to Pawar A. and colleagues’ research, the flavonoids caused cell wall breakdown and membrane permeabilization.[2] Quercetin and naringenin have been shown to induce dose-dependent death of *Mycobacterial* cells after 48 hours, with IC_{50} concentrations of 350M and 312M, respectively.

Table 2.1. Inhibitors targeting the Peptidoglycan biosynthesis pathway.

Compound /Inhibitor	**Chemical class**	**Structure**	**Cell wall components inhibited**	**Reference**
Fosfomycin	Phosphonic		*MurA*	[63]
Aurachin RE	Prenylated quinoline		*MurA*	[73]
3,5-dioxopyrazolidine	Pyrazolidines		*MurB*	[63]
DL-homocysteic acid	Non-proteinogenic alpha-amino acid		*MurD*	[63]
CS (D-cycloserine)	D-cycloserine		*ddlA*	[74]
SQ641	Capuramycin		*MurX*	[1,75]

Caprazamycin	Natural product		*MurX*	[1,75]
Liposidomycin	Lipopeptides		*MurX*	[1,75]
Muramycin	Nucleoside antibiotics		*MurX*	[1,75]
Ramoplanin	Glycolipodepsi peptide		*MurG*	[63]
Enduracidin	Cyclic polypeptide		*MurG*	[63]
Ramoplanin	Glycolipodepsi peptide		*L-Ala*	[1,70]

Enduracidin	Cyclic polypeptide		*L-Ala*	[1,70]
Teixobactin	Cyclodepsipeptide		*L-Ala*	[1,70]
Carbapenem	β-lactam		*D, D-/L, D-Transpeptidation*	[76,77]
Augmentin	Penicillin's		*D, D-/L, D-Transpeptidation*	[76,77]
Meropenem	Carbapenem		*D, D-/L, D-Transpeptidation*	[76,77]
Faropenem	β-lactam antibiotics		*D, D-/L, D-Transpeptidation*	[76,77]

Ertapenem	Carbapenem β-lactam		*D, D-/L, D-Transpeptidation*	[76,77]
Friulimicin B	Oligopeptides		*C55-PP*	[78]
Mureidomycin A	Peptidylnucleoside		*MurY*	[27,63]
Teixobactin	Lipo-peptide		*Lipid II*	[1]
Sanfetrinem	The third generation of cephalosporins		Inhibit peptidoglycan synthesis Exact target unknown	[59,79]
GlcN-1-p analogs	Derivatives of glucosamine		*GlmU*	[1]
Phosphinate analogs	Phosphonates			

D-Glumatic acid analogs	Amino acids	NA	*MurD*	[63]
3-Methoxynordomesticin	Coumarins	NA	*MurE*	
Phosphinate analogs	Phosphonates			
Diarylquinolines	Quinolines		*MurF*	
4-Phenylpiperadine	Piperidines			
Pacidamycins	Uridyl peptide		*MurY*	[27, 63]
Napsamycins				

2.5.1. *MurA* inhibitors

There is great potential for the *Mycobacterium* cell wall production pathway as a tuberculosis therapeutic target. One example of such an enzyme is *MurA*, which is a transferase that initiates the production of peptidoglycan. Inhibition of the *MurA* enzyme should result in a drop in *Mycobacterium* cell synthesis. *MurA* functions as a transferase enzyme, facilitating the transfer of the enol pyruvate group from "phosphoenolpyruvate" (PEP) to "UDP-N-acetylglucosamine" (UDP-GlcNAc), resulting in the formation of "UDP-GlcNAc-enol" pyruvate. This acts as a substrate for the enzyme *MurB*, which functions as a reductase, which acts on the enol pyruvate moiety in a reduction reaction that is NADPH-dependent to create UDP-Mur-NAc.

The sole antibiotic clinically approved to target *MurA*. Fosfomycin is ineffective against *Mycobacterium* because its active site does not include cysteine but rather aspartate residue.[80] Peptidomimetic substances and derivatives of "sulfonoxyanthranilic acid" have a promising repressive impact on the MTB's *MurA* enzyme.[81] Kumar P and colleagues showed that six compounds from two different libraries, three from respectively, ChemDiv: "D675-0217", "D675-0102" and "L291-0509"; Asinex: "BDG 34016655" and "BDE 26717803" and "BDE 25373574" with good pharmaceutical activity were identified. *MurA* substrate binding may be partially or completely blocked after binding with identified peptidomimetic agents. This may lead to minimal or no synthesis of downstream substrate molecules for subsequent *Mur* enzymes. These compounds are therefore effective antimycobacterial agents and, with more testing, might be employed as potential inhibitors.[81]

2.5.2. *MurB* and *MurE* inhibitors

Bacterial cell survival critically relies on the essentiality of the *MurB* enzyme but it does not have a homolog in eukaryotic cells, making it a possible target for various inhibitors. Through the application of structure-based drug discovery methods, various *MurB* inhibitors, such as "sulfadoxine", "pyrimethamine", "piperazine derivatives", and "chloropicolinate amides", have been documented. The potential inhibitors of Mtb-*MurB*, as identified in this study, are listed in Table 2.1. In MTB, *MurE* (also recognized as "UDP-n-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase") enhances m-DAP to "UDP-MurNAc-L-Ala-D-Glu". Analogs of "glucosamine uridine" inhibit the Mtb *MurE* enzyme.[82] Table 2.1. describes the compounds identified as potential Mtb-*MurE* inhibitors in this review.

2.5.3. Alanine racemase inhibitors

This enzyme is accountable for catalyzing the process of translation of “L-alanine” to “D-alanine”, which is an indispensable step in the production of peptidoglycan, in the cytoplasm under the effect of pyridoxal phosphate. Analogs of D-alanine called “D-cycloserine” are employed as a 2nd-line TB medication (Table 2.1). By preventing the activity of “D-alanine” and “alanine racemase”, D-cycloserine prevents the production of PG.

2.5.4. L, D-transpeptidase inhibitors

D, D-transpeptidases and non-classical L, D-transpeptidases frequently collaborate to catalyze the cross-linking of the peptidoglycan (PG) structure. Carbapenems are a group of antibiotics that inhibits the enzymes “D, D-transpeptidases”, “L, D-transpeptidase”, and “D, D-carboxypeptidases”, engaged in the production and upkeep of the *Mycobacterium tuberculosis* (Mtb) cell wall. *LdtMt2*, which is suppressed by carbapenems, is thought to be a possible target for anti-tuberculosis medication and has received a lot of interest.[83] The actions of capuramycin, tunicamycin, and muramycin D2 are encouraging for developing novel inhibitors (Table 2.1).[63] *MurY* and *MurX* have been reported to be inhibited by sansanmycin and its analogs.[75]

2.5.5. *MurG* inhibitors

MurG enables the transference of “GlcNAc” from lipid-bound “UDP-GlcNAc” to “MurNAc” or “MurNGlyc” in Lipid II. A lipoglycodepsipeptide antibiotic that binds the lipid components such as ramoplanin and enduracidin prevents *MurG* action (Table 2.1).[63] Characterizing the MTB *MurJ* enzyme is essential, and further research

will contribute to the development of a new MTB *MurJ* inhibitor. While lipid II is bounded by “ramoplanin”, “teixobactin”, “malacidin”, “nisin”, “vancomycin”, and, “glycopeptides teicoplanin” binds to the “D-AlaD-Ala” terminal of lipid II, preventing its elongation.[84,85]

2.5.6. Arabinogalactan Biosynthesis

The polysaccharide backbone of arabinogalactan, forming the outermost layer of the *Mycobacterium* cell wall, consists of sugar moieties, including galactose and arabinose. The primary structure of the polysaccharide backbone is a single linear chain, *B-D-Galf*, comprising approximately thirty galactose units, and three distinct chains, *b-Araf*, each containing around thirty arabinose residues.[15] The peptidoglycan MurNGlyc layer and the mycolic layer are covalently bonded to the arabinogalactan layer through chemical connections.

The peptidoglycan linker fraction, *“C50-P-PGlcNAc-l-Rha”* is produced via the use of “GlcNAc” as the starting substrate, shadowed by intracellular modifications carried out by “GlcNAc-1-P transferase” (*WecA*) and “Rhamnosyl transferase” (*WbbL*).[1] The synthesis of the linker between the peptidoglycan (PG) and mycolic acid (MA) layers of the cell wall initiates in the cytoplasm and proceeds as a continuous process into the periplasm. The target enzyme is indicated by a red arrow for inhibitors that target arabinogalactan synthesis (Figure 2.2). The bifaceted “galactofuranosyltransferases” enzymes *GlfT1* and *GlfT2* catalyze the accumulation of galactose residues (in furanosyl form, up to 30 residues) to the PG linkage, resulting in *“C50-PP-GlcNAc-L-Rha-Galf30”*.[87] The arabinose residues necessary for the subsequent stage of arabinogalactan assembly (DPA) are provided by

"decaprenylphosphoryl-D-arabinose" (in furanose form, *Araf*).[87] DPA can be found in the periplasmic region due to various metabolic processes that begin in the cytoplasm and conclude in the periplasm. The production of "decaprenol-1-phosphoribose" (DPR) involves the consecutive catalysis of "ribose-5-phosphate" by "decaprenol-1-phosphate", "5-phosphoribosyltransferase" (*UbiA*) and "pRpp synthase" (*PrsA*) to form "decaprenol-1-monophosphate t-phosphoribose", that is then likely dephosphorylated by the Rv3807C gene product "decaprenol-1-phosphoribose" (DPR). The genes Rv3790 (*DprE1*) and Rv3791 (*DprE2*) encode the enzyme known as "decaprenylphosphoribose-20-epimerase", which facilitates the conversion of the ribose residue in DPR to arabinose through epimerization. In a two-step epimerization procedure, *DprE1* catalyzes the oxidation of DPR to "decaprenylphosphoryl-2-ketoribose" (DPX), resulting which DPR is then transformed into DPA through reduction by *DprE2*.[1]

Proteins such as "arabinofuranosyl transferase" (*AftA*) and *EmbA*/*EmbB* are responsible for catalyzing the adding *Ara(f)* remains to *"C50-P-P-GlcNAc-L-Rha-Galf30"*.[88] Hexaarabinofuranosyl (*Araf6*), a clearly defined end pattern, is created when *AftB, AftC,* and maybe *AftD* proteins come together.[1] The *Araf6* domain forms a base for binding mycolic acids. The AG is linked to the PG layer by the enzyme *Lcp1*, which completes the AG assembly.[1]

2.5.7. Inhibitors Targeting the Galactan Pathway

Ethambutol (EMB), a primary drug used to treat tuberculosis, successfully illustrates a proven method to combat TB by focusing on the synthesis of Mtb's arabinogalactan.

EMB proteins target the functioning of arabinosyl transferase, which interrupts AG assembly.

2.5.8. *WecA* inhibitors

CPZEN-45, an innovative semi-synthetic compound derived from caprazamycin found in nature and synthesized by streptomyces species, is currently undergoing preclinical testing. This nucleoside antibiotic inhibits the growth of *Mycobacterium tuberculosis* (Mtb) by targeting the *Mycobacterial WecA* enzyme. In laboratory tests, CPZEN-45 has demonstrated efficacy against both replicating and non-replicating bacteria. In mouse models, it has proven effective in managing both drug-sensitive and drug-resistant tuberculosis infections, exhibiting synergistic effects when combined with other tuberculosis drugs. To enhance inhalation delivery to humans, a co-formulated powder product was developed using spray-drying technology, combining capreomycin and CPZEN-45 (as outlined in Table 2.2).[89]

2.5.9. WbbL, GlfT1/GlfT2, and UGM inhibitors

Despite its crucial role in arabinogalactan assembly, there is currently no known chemical inhibitor of rhamnosyltransferase, *WbbL*.[1,90] The primary emphasis in efforts to identify inhibitors of *GlfT1* and *GlfT2* has centered around developing compounds that mimic transition states or substrates. The enzyme exhibits characteristics that lead to a preference for the *"UDP-D-Galp"* substrate over the *"UDP-D-Galf"* product by more than ninety percent.[15] Additionally, there is a requirement to produce *"UDP-D-Galf"*, which is commercially difficult to obtain, to observe the reverse reaction, making it challenging to establish inhibitor screening tests.

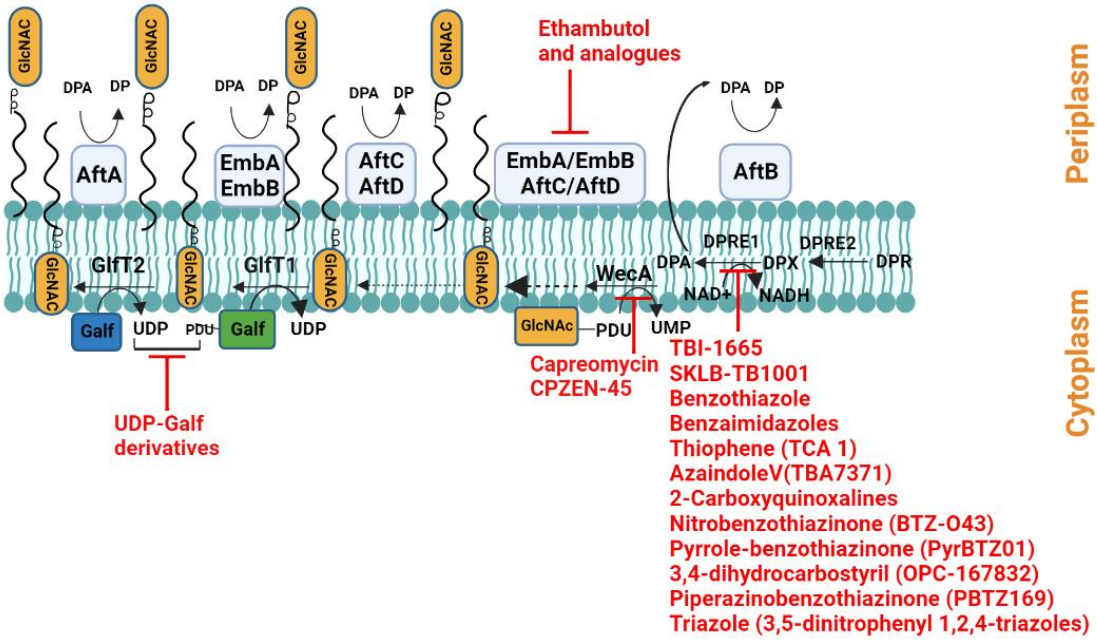
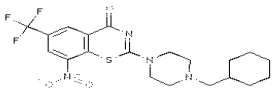
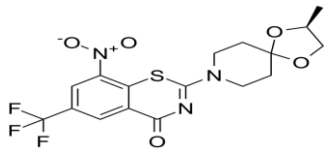
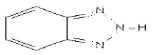
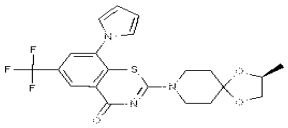
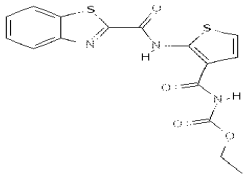
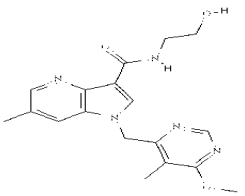
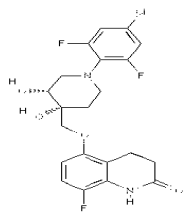
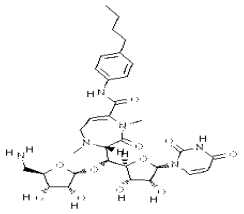
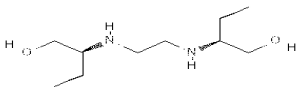


Figure 2.2. The Biosynthesis of Arabinogalactan with an arrow pointing in the direction of the target, inhibitors for several enzymes involved in the cytoplasm, and periplasm is shown in red text.

To identify potential new inhibitors of *UGM*, multiple researchers have recently conducted screenings of libraries containing natural products and related compounds. By employing dynamic combinatorial chemistry strategies, the compounds underwent optimization through chemical modification and the synthesis of novel compounds, ultimately leading to the development of molecules that showed only a slight inhibition of *Mycobacterial* development.[91,92] The strongest of these compounds, which was shown in a test that used a rough cell wall's enzyme fraction from *M. smegmatis* and *"O-alkyl-D-Galf-(16)-d-Galf"* acceptor, was the fluorinated exo-glycal analogs of *"UDP-Galf"* with and of 180 IC_{50} µM.

Table 2.2. Inhibitors targeting enzymes involved in the Arabinogalactan biosynthesis pathway.

Compound/ Inhibitor	**Chemical class**	**Structure**	**Cell wall components inhibited**	**Reference**
PBTZ169	Piperazinoben-zothiazinone		*DPrE1*	[59]
BTZ-O43	Benzothiazinone		*DPrE1*	[93]
Benzothiazole Triazole	Benzothiazinone		*DPrE1*	[94]
PyrBTZ01	Pyrrolebenzothi-azinone		Inhibit arabinogalactan synthesis	[95]
TCA 1	Thiophene		Inhibit arabinogalactan synthesis	[21]
TBA7371	AzaindoleV		Arabinogalactan LAM	[59]

OPC-167832	3,4-Dihydrocarbostyril derivatives		Arabinogalactan LAM	[1]
CPZEN-45	Caprazene Nucleoside		*WecA*	[1]
Ethambutol and analogs	Ethylene diamino di-1-butanol		*EmbA /EmbB* Inhibition of arabinogalactan synthesis	[1,4]

Recent studies have identified thiazolidinone derivatives as a promising leading candidate for *GlfT2* inhibitors, with researchers analyzing their characteristics through "Molecular docking", "3D-QSAR", and "*In silico* ADMETox" studies.[16] Since the identification of this route at the turn of the millennium, the development of new medications for tuberculosis has identified galactan biosynthesis as an interesting target. Within tuberculosis drug discovery, computational and experimental approaches offer a promising route for therapeutic innovation.[6,72,96] Additionally, *UGM*-Mtb and *GlfT2*-Mtb structures have been successfully determined, providing crucial information about the galactan biosynthetic pathway. It is important not to overlook these enzymes in contemporary efforts to discover innovative tuberculosis treatment approaches.

The presence of all three essential enzymes involved in *Mycobacterial* galactan biosynthesis is imperative for the survival of *Mycobacteria*,[86] and they all require the substrate *UDP-d-Galf*. Due to the absence of this particular form of galactose in humans, these enzymes are frequently considered potential targets for the formulation of new anti-tuberculosis drugs, and it is imperative to create a single inhibitor that targets all three enzymes, which is an interesting tactic given the possibility of a shared transition state.[15]

2.5.10. *DprE1* inhibitors

Multiple NCEs have efficiently blocked Mtb's *DprE1* enzyme's epimerase activity. These NCEs bind to *DprE1* and inhibit it either covalently or non-covalently. Benzothiazinone (PyrBTZ01),[1] "thiophene" (TCA1), "quinoxaline", "dinitro benzamide", "thiadiazoles", "azaindole", "pyrazole pyridine", "aminoquinolines" and "piperidine amides" are examples of non-covalent inhibitors. "Nitrobenzothiazoinone" (BTZ), "benzothiazole",[97] "triazoles",[95] and "nitrobenzamide"[1] are examples of covalent inhibitors (Table 2.2).

2.5.11. Covalent inhibitors of *DprE1*

Makarov and colleagues pioneered the discovery of nitrobenzothiazoinone (BTZ-043) compounds as *DprE1* inhibitors (Table 2.2).[98] These inhibitors have exhibited MICs in the nanomolar range against both replicating and non-replicating *Mycobacterium tuberculosis* (Mtb) forms.[1,98] According to crystallographic investigations, The BTZ molecule establishes a covalent linkage with the Cys387 residue located on the FAD substrate-binding domain, enabling it to interact with *DprE1*.[99] Prior research has indicated that BTZ-043 displays significant specificity for *M. tuberculosis*, showing

efficacy against both multidrug-resistant and extensively drug-resistant strains of the bacterium. Additionally, it exhibits synergistic effects when administered in conjunction with the drug bedaquiline.[1] The second-generation drug, PBTZ169 (macozinone), derived from the "piperazinobenzothiazinone" structure of BTZ-043, offers several advantages over its prototype. These advantages include a modest synthetic route, which facilitates large-scale production, and enhanced pharmacodynamics.[1] Benzothiazole, a scaffold was discovered as an Mtb growth inhibitor after screening over 100,000 molecules in whole cells. Considering the micromolar MIC and the covalent binding to the shared binding pocket, as observed with BTZ-043, the current lead compound does not possess drug-like characteristics. Therefore, lead optimization necessitates a comprehensive analysis of the structure-activity relationship (SAR).[1]

2.5.12. DprE1 Non-covalent inhibitors

A pyrrole ring was introduced to replace the nitro group at position 8 of benzothiazinone (BTZ) compounds, resulting in a compound that demonstrated *in vitro* activity similar to BTZ but was ineffective in animal models. Non-covalent *DprE1* inhibitors (Table 2.2) have been identified that exhibit potent activity in a mouse model of TB, and benzimidazoles were discovered to exist. Computational modeling of the compounds indicated potential binding configurations for benzimidazole within the active site of *DprE1*.[100] Shirude and colleagues were the first to discover that 4-azaindoles had anti-Mtb characteristics and a proclivity to bind the *DprE1* enzyme.[101]

TBA-7371 has been found to display a combination of covalent as well as non-covalent binding to *DprE1*, according to computational docking studies. OPC-167832, the US FDA has given the OPC167832 IND submission a “rapid track” designation. OP-167832 demonstrates binding affinity to the *DprE1* and effectively inhibits the development of Mtb with the MIC in the nanomolar range. In a mouse model of TB, a novel medication regimen consisting of OPC167832 and delamanid demonstrated superior efficacy compared to a standard tuberculosis medication comprising “rifampin”, “isoniazid”, “pyrazinamide”, and “ethambutol”.[102]

2.5.13. Mycolic Acid Layer Biosynthesis

The outermost stratum of the mAGP complex is composed of mycolic acid, comprising long-chain fatty acids with an unbound or bound arabinogalactan layer. It includes an "a-alkyl" (C24–C26) and "b-hydroxy" (C42–C62) chain, which are acyl derivatives of glycerol and trehalose (as depicted in Figure 2.3). This outer lipid layer's inherent hydrophobicity makes the cell wall impenetrable, preventing small hydrophilic molecules from passing through (as well as antibiotics).[103]

The biosynthesis of mycolic acid involves two interconnected enzyme pathways, namely "fatty acid synthase I" (FAS I) and "fatty acid synthase II" (FAS II).[1] FAS I is responsible for synthesizing short-chain fatty acids (up to C24) as fatty acyl CoA, utilizing a catalytically active oligo-domain. Following its generation by FAS I, the short-chain fatty acyl CoA undergoes further elongation through FAS II pathways, ultimately resulting in the formation of the β-hydroxy (C42 to C62) branch of mycolate. A β-ketoacyl ACP synthase known as *FabH* catalyzes the conversion of the FAS I by-product "C14-CoA" to "C16-AcpM”.[103] The FabD enzyme transfers an acyl

group from holo "AcpM" to "malonyl-CoA", leading to the production of malonyl-AcpM. Further conversion of *"C16-AcpM"* to *"C18-AcpM"* is achieved through the action of enzymes such as *"MabA"*, which reduces *"β-ketoacyl-AcpM"*, *"HadAB/BC"* and "*inhA"*, which dehydrate and completely saturate the aliphatic chain attached to *"AcpM"*. The β-ketoacyl synthases *"KasA"* and *"KasB"* play a role in redirecting the saturated "C18-*AcpM"* back to the initial site of the FAS II pathway. They catalyze the condensation of "C18-*AcpM"* with "Malonyl-*AcpM"*. In each cycle of the FAS II process, two carbons are added to the aliphatic chain on the *"AcpM"* assembly, resulting in the formation of a completely saturated acylated "β-hydroxy" (C42-C62) chain, referred to as "C42-C62-*AcpM"*.

The FAS II by-product undergoes several steps to produce mycolic acid. Initially, it is activated by the fatty acyl-AMP ligase *"FabD"* and subsequently binds to the fatty acyl CoA, a by-product of FAS I, in the presence of the enzyme *"Pks13"*. The resultant compound is then reduced by "Rv2509" to generate mycolic acid. RND family efflux pumps are employed to transport mycolic acids across the cell membrane. Mycolates produced in the cytoplasm are converted into trehalose monomycolate (TMM). Following this, TMM is transported out by RND pumps known as Mycobacterial membrane protein enormous (*MmpL*). After the efflux of TMM by *MmpL*, the mycolate on the displaced TMM is linked to arabinogalactan by the "Mycolyl-transferase Antigen 85 complex", completing the assembly of the mycolyl-arabinogalactan-peptidoglycan (mAGP) complex. TMM is also transformed into other non-membrane-bound mycolates, such as trehalose dimycolates, by the Antigen 85 complex.

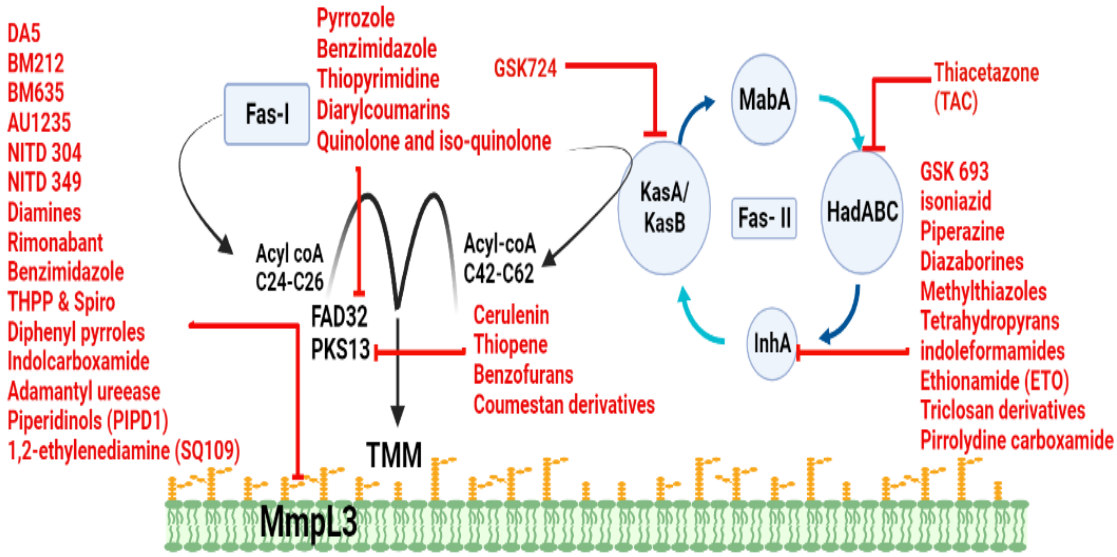


Figure 2.3. Biosynthesis of Mycolic Acid with an arrow pointing in the direction of the target, inhibitors for several targeted enzymes are shown in red text.

2.5.14. Mycolic Acid Targets and Their Inhibitors

Mycobacterial membrane protein large belongs to a group of transporters called the "resistance-nodulation-division" (RND) family, which are found in *Mycobacteria*, has been found in twelve forms, the principal function of which is metabolite transportation across the cytoplasmic membrane. The pathway for biosynthesis of mycolic acid constitutes the outermost layer of the cell wall (Figure 2.3). Compounds that inhibit MA biosynthesis are typically represented using red fond and a red projectile representing the target enzyme (Figure 2.3). *MmpL3* is an important variation and a promising novel target for TB medication development. *MmpL3* facilitates the transport of synthesized mycolates, specifically trehalose monomycolate (TMM), across the membrane.[104,105]

Rimonabant, a CB 1 receptor antagonist, appears to have a similar binding pocket to that of “SQ109” and “AU1235”. This outcome is reinforced by prior investigation[106] which revealed “rimonabant” had a 54 mM MIC and could limit the development of Mtb. The “cell-based inhibition assay” revealed the that *M. smegmatis* strain with the added overexpression of the *MmpL3* gene on a plasmid may partially reverse the inhibitory effect. These findings confirm that *MmpL3* is the rimonabant’s primary target.[9] It has been documented that various novel chemical families, including “indole-2-carboxamides”, “pyrroles”, “benzimidazoles”, “spirocycles”, “piperidinol”, “benzothiazole amides”, and “adamantly urea”, act as inhibitors of *MmpL3* (as detailed in Table 2.3).[10]

2.5.15. NITD 304 and NITD 349

A class of NCE called Indolcarboxamides binds to *MmpL3* in bacteria and inhibits Mtb growth.[1] The leading compounds in this category, developed by the Novartis Institute for Tropical Diseases, namely NITD-304 and NITD-349 (as listed in Table 2.3), have shown effectiveness against both acute and chronic infections in mouse models. Furthermore, they have demonstrated safety in preclinical tests. These compounds have proven efficacy against both drug-susceptible *Mycobacterium tuberculosis* (DS-Mtb) and drug-resistant Mtb (DR-Mtb) strains.

2.5.16. SQ109

A medication for tuberculosis by Sequella Inc has three probable modes of act: “inhibition of *MmpL3*”, “dissipation of proton motive force”, and “inhibition of menaquinone biosynthesis”. The inhibition of SQ109 has been shown to increase the

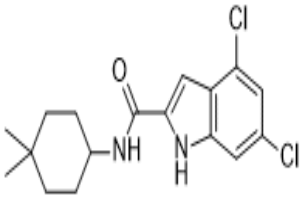
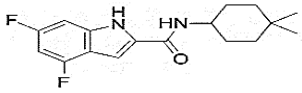
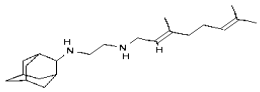
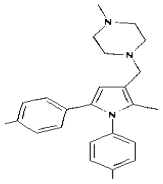
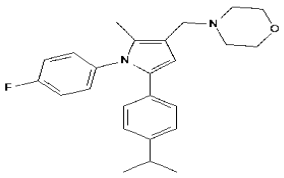
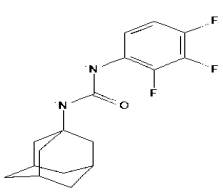
levels of TMM while decreasing the levels of TDM, this implies that SQ109 interferes with the translocation of TMM through the membrane.

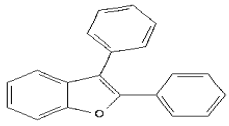
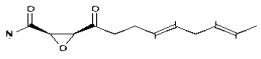
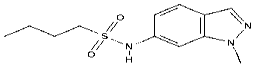
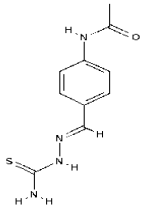
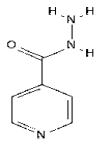
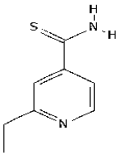
Additionally, the discovery of an *MmpL3* gene mutation in resistant mutants provides further evidence that *MmpL3* assists as a potential target for SQ109. The phase I clinical trials "NCT01358162" and "NCT01585636" were shown in the United States. The phase II trial "NCT01785186" was conducted in South Africa. Phase 2b-3 studies are currently ongoing in Russia. Studies involving SQ109 in combination with other drugs have yielded encouraging results, indicating that the drug is well-tolerated safe, and effective.

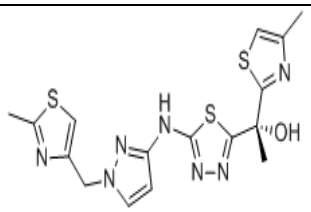
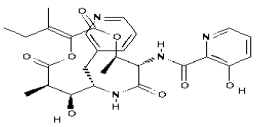
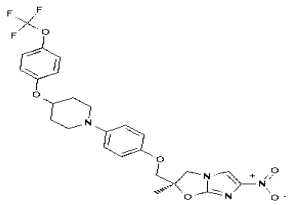
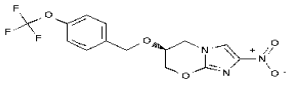
2.5.17. BM212 /BM635

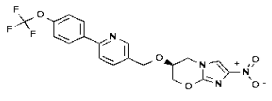
The scaffold featuring a 1,3,5-trisubstituted pyrazole motif specifically interacts with *MmpL3*, leading to the discovery of two hits, namely "BM212" also "BM635", through the optimization of the structure-activity relationship (SAR). "BM212" as well as "BM635" have demonstrated noteworthy antitubercular activity and possess pharmaceutically favorable attributes (Table 2.3).[110]

Table 2.3. Inhibitors targeting the Mycolic Acid biosynthesis pathway's enzymes.

Compound /Inhibitor	Chemical class	Structure	Cell wall components inhibited	Reference
NITD 304	Indolcarboxamides		*MmpL3*	[108]
NITD 349	Indolcarboxamides		*MmpL3*	[108]
SQ109	1,2 Ethylenediamine		*MmpL3*	[109]
BM212	1,5-Diarylpyrrole derivatives		*MmpL3*	[110]
BM635	1,5-Diarylpyrrole derivatives		*MmpL3*	[110]
AU1235	Adamantyl urea derivatives		*MmpL3*	[1]

Benzofurans	Amphetamine and phenylethylamine		*Pks13*	[111]
Cerulenin	Oxirane carboxylic acids and derivatives		*Pks13*	[111]
GSK-724	Indazole sulfonamide		*KasA/KasB* β-ketoacyl-ACP synthase	[112]
TAC	Thiacetazone		*HadABC* Inhibit cyclopropanation of cell wall mycolic acid	[59]
Isoniazid	Isonicotinyl - hydrazide		*inhA*	[59]
ETO Ethionamide	Nicotinamide derivative (thioamide)		*inhA* Disrupt cell wall biosynthesis	[59]

GSK-693	Thiadiazole		*inhA*	[59,109]
Pyridomycin	Natural products		*inhA*	[4]
Delamanid	Nitroimidazole-s		Inhibition of methoxy and keto mycolic acid Exact target unknown	[113]
Pretomanid/ PA-824	Nitroimidazole-s		Cell wall synthesis inhibition and causing respiratory poisoning through nitric oxide (NO) release.	[113]

TBA354	Nitroimidazole-s		Inducing respiratory poisoning through nitric oxide	[24]

2.5.18. *KasA* inhibitors

A synergistic collaboration between academic institutions and the pharmaceutical industry resulted in the sighting of "GSK 724" (also known as DG167/GSK3011724A) (Table 2.3). This compound has shown promising anti-tubercular activity. There is an expectation for the leading optimization of GSK 724, which possesses a distinct two-fold binding mechanism to the identical target and displays collaborative effects when co-administered with isoniazid, to happen soon.[114]

2.5.19. *InhA* inhibitors

Isoniazid and ethionamide, key tuberculosis medications, inhibit enoyl-ACP reductase (InhA) in the FAS II pathway of mycolic acid synthesis, with prodrugs activated by *KatG* or *EthA* generating reactive oxygen species (ROS) to impair *InhA*. The Orchid consortium develops non-prodrug chemical inhibitors, such as thiadiazoles (GSK693), targeting *InhA*, alongside other reported inhibitors like "tetrahydropyrans", "diaryl ethers" (e.g., triclosan derivatives PT070, PT119), "methyl thiazoles", "diazaborine", "pyrrolidine carboxamides", and "piperazine indole formamides" (as listed in Table 2.3).[115]

2.5.20. *Pks13* inhibitors

Indole II has been identified as a compound targeting the large polyketide synthase *Pks13* (rv3800c), essential in mycolic acid biosynthesis, a crucial component of the Mtb cell wall. *Pks13,* previously recognized as a target for thiophenes and benzofurans,[116] is affected by benzofurans obstructing the active site of the C-terminal thioesterase domain, while thiophenes impede the loading of fatty acyl-AMP onto the N-terminal domain.[117]

2.5.21. OPC-67683 (Delamanid) and PA-824 (Pretomanid)

Delamanid is a medication listed in Table 2.3 that was licensed by the European Medicine Agency in 2014 and is the second TB medication to get regulatory approval in the last 50 years. Delamanid was developed by Otsuka Pharmaceutical as a first-in-class bicyclic nitroimidazole drug specifically designed to treat MDR-Mtb. OPC-67683 has demonstrated high effectiveness in animal models, with minimal toxicity, and has shown efficacy against non-replicating, replicating as well as intracellular Mtb at concentrations ranging from $1.22x10^{-5}$ to $6.44x10^{-6}$ during preclinical development. Studies involving human subjects have shown that delamanid is a safe and effective treatment for pulmonary MDR-Mtb. However, the potential for prolongation of the QTcF (corrected QT interval using Fridericia's formula) interval is a primary area of concern. A Phase III clinical trial (NCT01424670) demonstrated that the inclusion of delamanid in an optimized background regimen (OBR) did not yield any additional benefits.[113] Another study found that when administered with a delamanid-containing prescription, 84.2 percent of the patients were cured.[118]

The BPaL treatment, which incorporates the medication PA-824, has received recent approval from the US FDA for the treatment of patients with extensively drug-resistant and multidrug-resistant tuberculosis. In the Phase III clinical trial, known as Nix-TB, 89% of the initial seventy-five XDR-Mtb patients experienced recovery within six months of treatment and follow-up.

PA-824, a nitroimidazole compound with robust effectiveness against both replicating and non-replicating Mtb, was initially reported in the year 2000. While its precise target under aerobic conditions remains unclear, it is hypothesized to inhibit the synthesis of ketomycolate from hydroxymycolate. However, its mechanism of action in hypoxia is thought to be the production of reactive nitrogen species (RNS) leading to cellular respiratory toxicity.

2.5.22. Inhibiting DNA Replication and Protein Synthesis

Several drugs specifically target “DNA replication” and “protein synthesis” in Mtb. “Fluoroquinolones” target “DNA unwinding” and “replication”, while “rifampin” targets “transcription”. Additionally, “streptomycin”, “kanamycin” and “capreomycin” target translation in Mtb. These drugs have demonstrated effectiveness in tuberculosis treatment and are commonly used in combination therapy. These drugs are well tested but by no means an exhausted chemotherapeutic approach.

2.5.23. DNA replication

To ensure the transfer of genetic material to its progeny, Mtb needs to maintain consistent DNA replication and seamless cellular division. Anti-TB drugs have been developed targeting various proteins included in this crucial cellular mechanism. One

of the most important protein targets is *Mycobacterial* “DNA gyrase”, determined by *gyrA* and *gyrB,* which is a type II topoisomerase. DNA gyrase's catalytic activity involves unwinding DNA during replication.[80] Biological inhibitors that target DNA *gyrA* or RNAP using allosteric modulation are characterized by a low frequency of resistance or a lower tendency to produce cross-resistance.[119,120]

Despite the effectiveness of the thiophene series in targeting the unique binding pocket and distinct mechanism of action of certain proteins, which allosterically targets DNA *gyrA* but is damaging. Given the challenges in advancing thiophene-based compounds to clinical trials, the allosteric drug-binding site of DNA gyrase has potential druggability. Fluoroquinolones, following DNA cleavage, inhibit DNA gyrase, disrupting DNA replication and inducing permanent double-strand DNA breaks. Moxifloxacin is anticipated to exhibit a sterilizing effect *in vivo* since it kills equally replicating and anaerobic non-proliferating *M. tuberculosis*. The lack of success in shortening the tuberculosis treatment course in a phase III trial with the antibiotic moxifloxacin does not necessarily negate the desirability of DNA gyrase as a target for anti-TB drugs.[121] The target of modified griselymicin has been confirmed to be *DnaN*, a crucial subunit of DNA polymerase III. In a mouse model of TB, this natural substance proved efficacious and extremely active against *M. tuberculosis* replication. Additionally, the possibility that *DnaN* is involved in DNA repair pathways can be used to explain griselymicin’s ability to kill bacteria in non-replicating cultures.[121]

2.5.24. DNA transcription

The RNA polymerase in *Mycobacterium tuberculosis* plays a vital part in both the initiation as well as elongation phases of transcription. The Mtb RNAP comprises a dominant core composed of five subunits, along with at least one sigma subunit. Rifampicin, one of the primary tuberculosis medications, functions by attaching to the beta subunit (determined by *rpoB*) of the RNAP. This binding effectively inhibits RNA elongation.[1] RIF resistance is caused by certain mutations in the *rpoB* gene, which produce altered physical conformation of the B-subunit and reduced binding affinity for RIF.[1]

Na-aroyl-N-aryl-phenylalaninamides are another class of chemicals that bind Mtb RNAP (AAPs). These AAP compounds exhibit antimycobacterial properties.[120,122] The AAP series of drugs bind to a different site on RNAP, specifically the N-terminus of the bride helix, compared to the RIF binding site on the b-subunit. These drugs, including pseudouridimycin, which also targets RNA polymerase (RNAP) allosterically, provide benefits such as no cross-resistance with rifampin (RIF), a synergistic effect when combined with RIF, and a reduced likelihood of RIF-resistant strains emerging when used in conjunction with RIF. The uniqueness of RNAP as a target, with prokaryotes and eukaryotes not sharing this enzyme, underscores its specificity. Despite being targeted by various commercially available drugs, RNAP remains underutilized.

2.5.25. Protein synthesis (RNA translation)

Bacterial ribosomes are responsible for translating RNA into proteins. Ribosomes are comprised of deuce major subunits, the minor 30S, and the major 50S, with important

translational processes taking place at the intersection between the two. The incoming tRNA is lodged in the interface ribosomal regions, which have multiple obligatory sites including “A-site” (aminoacyl-site), “P-site” (peptidyl-site), and “E-site” (exit-side). GSK 656 inhibits “Leucyl-tRNA synthetase” (*LeuRS*) and is a member of a novel class of Oxaboroles (Table 2.4). It has shown significant efficacy in both acute and chronic animal models, along with strong potency *in vitro* studies.[123]

2.5.26. Targeting Energy Metabolism

MTB generates adenosine triphosphate (ATP) by utilizing two interconnected metabolic pathways including “substrate-level phosphorylation” and “oxidative phosphorylation” (Figure 2.4). The reason behind Mtb’s reliance on both of these pathways is due to its greater basal energy requirements in comparison to other bacteria. Mtb requires oxidative phosphorylation, according to mutagenetic research. There are numerous therapeutic compounds currently in the TB drug development channel that target both the “substrate-level phosphorylation” as well as “oxidative phosphorylation” metabolic pathways.[124]

2.5.27. Substate level oxidation

Under aerobic conditions, the tricarboxylic acid cycle is initiated by the generation of acetyl-CoA through the metabolic breakdown of carbon sources such as carbohydrates and fatty acids. Acetyl CoA is then transformed by the use of enzymes into citrate, isocitrate, and lastly carbon dioxide. Isocitrate is transformed by isocitrate lyase into glyoxylate and succinate in the glyoxylate shunt, which is used by Mtb to save carbon (2 isoforms, *ICL1* and *ICL2*).

Enzymatic reactions convert the glyoxylate that was produced into malate, restarting the TCA cycle. Finally, carbon sources are converted to carbon dioxide through substrate-level glycolysis, which is accompanied by a reduction in NAD (oxidized to NADH) (reduced). Oxidative phosphorylation, the following metabolic process, is triggered by a reduced form of NADH that pumps electrons into the electron transport chain (ETC).[1]

2.5.28. Oxidative phosphorylation pathway

Energy metabolism, specifically the "oxidative phosphorylation" (OxPhos) pathway, is a new target field that is gaining traction. In 2012, the FDA approved bedaquiline as a medication specifically designed for the treatment of Mtb infections. This marked the first clinical validation of targeting the *Mycobacterial* F1F0 *ATP synthase* as a viable route for DR-TB treatment.[125]

The nitroimidazoles Pretomanid (PA-824) and delamanid (Deltyba; DEL) then received regulatory clearance. It has been demonstrated that PA-824 kills Mtb which is not actively reproducing by blocking respiratory cytochromes and triggering the liberation of NO: nitric oxide. Telacebac (Q203) is currently undergoing phase II clinical trials and is designed to target the "cytochrome bcc-aa3" complex, the main terminal oxidase of Mtb.[126]

By focusing on specific components of *Mycobacteria* without affecting their human counterparts, researchers have been able to reduce safety concerns associated with drug development. Although specific pathways are highly conserved between prokaryotes and eukaryotes, there are unique features and differences that can be leveraged for targeted drug development.

There are several reasons why this pathway is considered a promising therapeutic target space from a biological perspective. In numerous bacteria, substrate-level phosphorylation can generate sufficient energy for reproduction, while *Mycobacterium tuberculosis* (Mtb) relies on the more energetically efficient oxidative phosphorylation (OxPhos) to sustain its growth.[124] One possible reason for the inability of *Mycobacteria* to effectively ferment is the absence of an "NADH-dependent lactate dehydrogenase" enzyme.[127]

The ETC transfers electrons from central metabolism electron donors to oxygen during the OxPhos process. During the transfer process, the "proton-pumping components" of the ETC conserve the energy released, which results in the generation of an "electrochemical gradient" in the form of a "PMF". The stored energy within the electrochemical gradient is harnessed by *ATP synthase* to generate ATP as protons flow back down the gradient. For the sustainability of non-replicating Mtb, a continuous supply of PMF and ATP is essential. This is because a constant supply of PMF, as well as ATP, is vital for the persistence of non-replicating Mtb.[125]

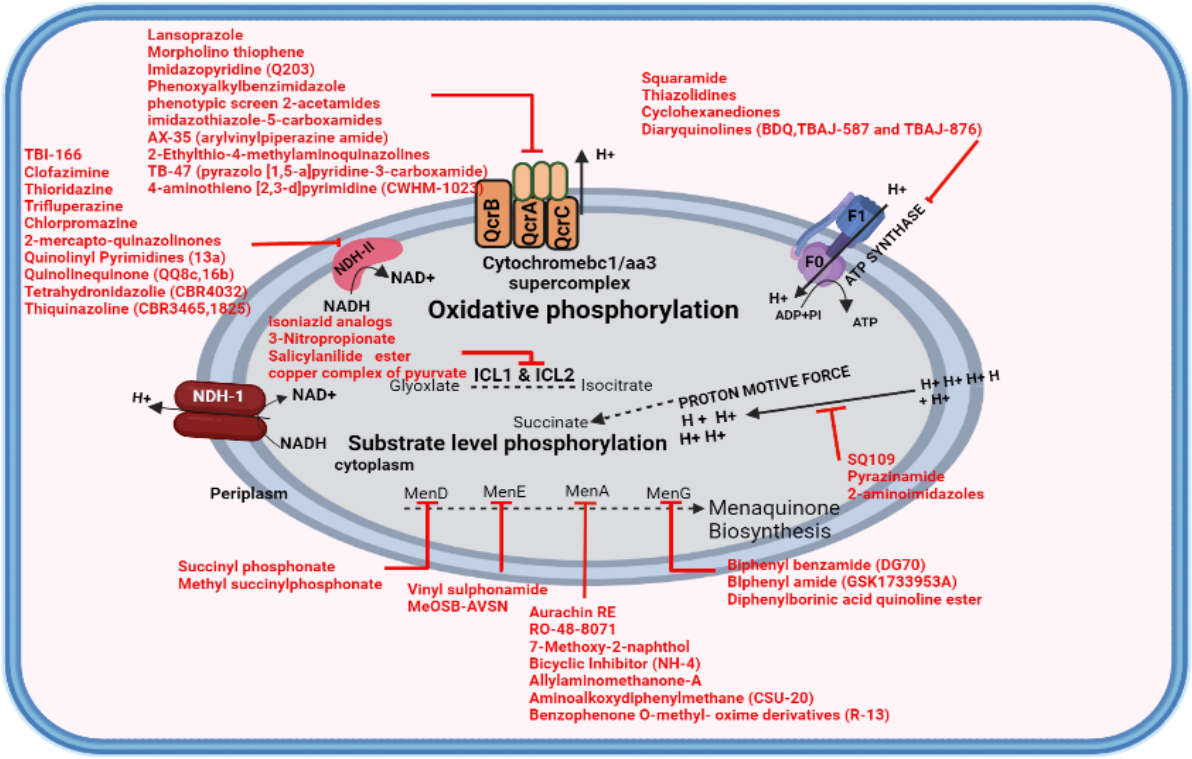


Figure 2.4. Oxidative phosphorylation and Substrate-level phosphorylation are two interconnected mechanisms used by Mtb to produce ATP. An arrow pointing in the direction of the target, and inhibitor for various enzymes involved are shown in red text.

It is thought that inhibiting the OxPhos pathway is a useful technique for eliminating non-replicating subpopulations and reducing future treatment times. Furthermore, because drug efflux pumps rely on energy to actively transport pharmaceuticals extracellularly, disruption in PMF and ATP levels can potentially impact their function. Despite the recognized significance of efflux pump activities in *Mycobacterium tuberculosis* (Mtb) drug sensitivity,[125] disrupting the OxPhos pathway could offer an indirect means to overcome drug resistance mediated by efflux pumps. Johnson and his team initially identified the efflux pump EfpA/RV2846c inhibitor BRD-8000 and subsequently improved its efficacy. This validation of BRD-8000 for the previously uncharacterized and vital efflux pump *EfpA/RV2846c* underscores its

potential as a promising therapeutic target.[128] Along with the previously mentioned targets, new research has focused on “tryptophan synthase”, “β-ketoacyl-ACP-synthase-I”, “biotin protein ligase” and “leucyl-tRNA synthetase”.[121]

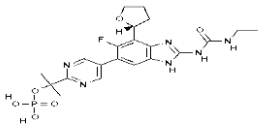
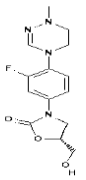
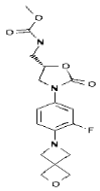
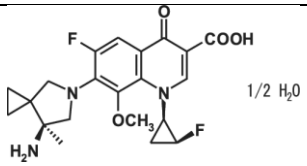
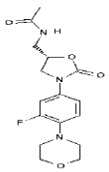
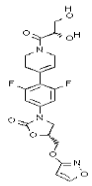
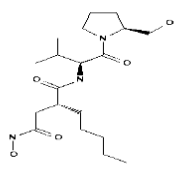
2.5.29. Targeting the Oxphos Pathway’s Components

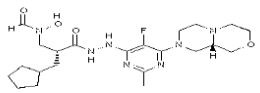
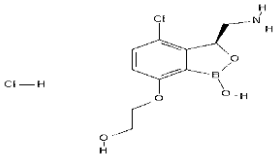
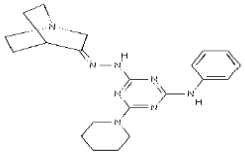
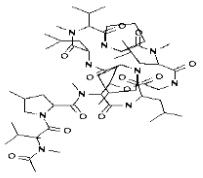
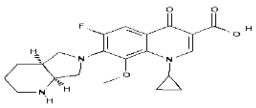
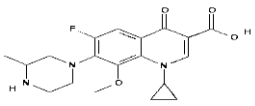
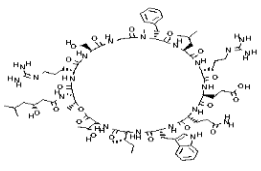
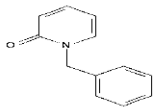
2.5.30. NADH dehydrogenases

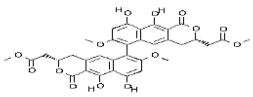
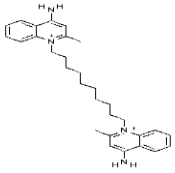
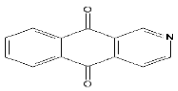
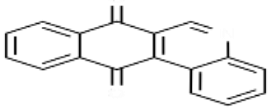
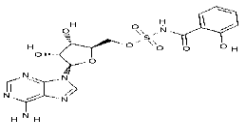
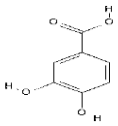
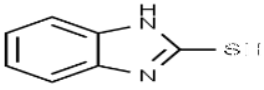
NADH dehydrogenases use MK as an electron carrier to maintain the energization of the *Mycobacterial* respiratory chain. *NDH-1* isn't required for *Mycobacterial* growth or survival.[59] Currently, there are no ongoing studies focused on developing drugs that inhibit *Mycobacterial NDH-1*.[125] Mammalian genomes lack NDH-II (Ndh and NdhA) which renders it a promising target for drug development in the quest for novel anti-TB treatments. *Mycobacteria* have been found to produce several inhibitors, such as “quinolinyl pyrimidines”, “phenothiazines”, and “2-mercapto-quinazolinones”. Table 2.5 provides structural details of the NADH dehydrogenase inhibitors mentioned in this review.

NDH-2 may potentially influence the bactericidal effect of clofazimine (CFZ), an inhibitor employed in leprosy treatment, as it stimulates the production of ROS. This connection highlights the potential impact of NDH-2 on CFZ’s efficacy against Mtb. Previously used to treat *M. leprae*, the riminophenazine antibiotic clofazimine (lamprene) is now utilized to treat Mtb (Table 2.5).

Table 2.4. Inhibitors targeting Energy Metabolism and DNA replication.

Compound /Inhibitor	**Chemical class**	**Structure**	**Cell wall components inhibited**	**Reference**
SPR720	Aminobenzimid-azole		*GyraseB*	[129]
Delpazoid (LCB01-0371)	Oxazolidinone		Targeting DNA replication and inhibits Protein synthesis	[1,130]
TBI-223	Oxazolidinone		Aiming DNA replication and inhibiting Protein synthesis	[1,130]
DC-159a	Fluoroquinolones		DNA replication	[131]
Linezolid	Oxazolidinone-s		Protein synthesis inhibition	[132]
AZD5847	Oxazolidinone-s		Protein synthesis inhibition	[132]
Actinonin	Type B peptide deformylase		Protein synthesis inhibition	[27,133]

LBM-415	Peptide deformylase		Inhibition of Protein synthesis	[27,133]
GSK-3036656 (GSK-656)	Oxaboroles		Leucyl tRNA synthetase rRNA	[134]
ATB107	Aminoglycosides		*TrpC*	[73]
Griselimycin	Fluoroquinolone		*DnaN*	[127]
Moxifloxacin	Fluoroquinolone		DNA gyrase and topoisomerases	[73]
Gatifloxacin	Fluoroquinolone		DNA gyrase and topoisomerases	[135,136]
Lysocin E	Non-ribosomal peptides		Targeting vitamin K synthesis and Cell membrane synthesis	[78,137]
Benzyl Pyridinone	Pyridones		*FabI* Fatty acid synthesis	[78]

Viriditoxin	Secondary metabolites		*FtsZ* Cell division target	[78]
Dequalinum	Quinolone derivative		*MshC*	[138,139]
Benzo[g]isoquinoline-5,10-diones	Isoquinolinedi-ones		*Mtr*	[138,139]
Benzo[J]phenanthridine-7,12-diones	Phenanthridine-diones		*Mtr* Bacillary redox homeostasis	[138,139]
Salicyl-AMS	Salicylamides		*MbtA*	[73,135]
Dihydroxybenzoate	Hydroxybenzoic acid derivatives		*Mbtl*	[73,135]
Benzimadazole-2-thione	Benzimidazoles		Mycobactin synthesis	[73,135]
Nα-aroyl-N-aryl-phenylalaninamides (AAPs)	Amides	NA	RNA polymerase Targeting DNA replication and Protein synthesis	[130]

GSK-1322322	Peptide deformylase	NA	Protein synthesis inhibition	[27, 133]
BRD4592	Azetidine derivative		*TrpAB* Inhibiting Aminoacid biosynthesis	[73,140]
Fluorinated anthranilates	Aromatic carboxylic acid		Tryptophan synthesis	[73]

Clinical investigations conducted in Bangladesh to treat drug-resistant tuberculosis have shown that the addition of clofazimine resulted in an advanced cure proportion (up to 84.2%) and a shorter treatment period. In contrast, a Chinese study found that using CLF in medication regimens was not beneficial. In the case of clofazimine for the treatment of DR-Mtb, further research is needed to better understand its potential benefits and risks in different patient populations and medication regimens.[141] Conducting a thorough SAR investigation to discover a second-generation "riminophenazine", such as TBI-166, that has higher potency, better ADME properties, and improved safety aspects may be a solution to overcome the contemporary limitations of clofazimine as an effective anti-tubercular agent.[142] High-throughput screening (HTS) was employed to recognize amalgams that inhibit particular enzymes tangled in generating ATP, and it identified two classes of chemicals that target the NADH dehydrogenase: Thioquinazoline (TQZ) and

tetrahydro indazole (THI)[143] (Table 2.5). Mercaptoquinonazolinone-type compounds were discovered to have low micromolar (MIC = 0.3 uM) antimycobacterial action as type II NADH dehydrogenase pathway inhibitors.[144]

2.5.31. Cyt-bcc-aa3 complex inhibitors

Various chemically distinct scaffolds that bind to the "Qp site" of the *QcrB* subunit, also acknowledged as the "stigmatellin pocket", are used to target the Mtb cytochrome bcc oxidase. These scaffolds are discovered using different methods and have unique properties. *QcrB* has two quinol catalytic sites, one of which is the Qp site. Quinone-based mimics can indirectly affect bacterial ATP synthesis by chemically inactivating the Mtb cytochrome "bcc-aa3" super complex. Imidazopyridine (IP) based drugs are among the most effective inhibitors (Table 2.5). The promiscuous character of this target, like other membrane-associated targets, has been linked to its location in the bacterial membrane.[145] This section discusses the chemical families that have been reported to exhibit inhibition of the Cyt-bcc-aa3 terminal oxidase (Table 2.5). Drugs that inhibit the bd oxidase and cyt-bcc-aa3 complexes are discussed in this review. Recently, the *QcrB* inhibitor drug candidate Q203 (also known as Telacebac and originated from Qurient Co., Ltd.) met its chief termini in a Phase IIA experimental trial (NCT03563599). Q203 was found to be the most improved structure upon evaluating compounds for their antimicrobial activity against Mtb within macrophages.[146,147] Resistant mutants of Q203 that occur spontaneously in the cytochrome b-subunit of the bc1 complex typically have a single amino acid mutation (T313A) encrypted by the *QcrB* gene.[148] EBA, safety, tolerability, and pharmacokinetics are all being studied with Q203. This potential new medication

candidate's clinical trial data is eagerly anticipated. A combination of “Q203”, “BDQ” also “CLF” has shown synergy *in vitro* and against intracellular *Mycobacterium*, suggesting the potential for the formulation of a novel treatment regimen.[149] A scaffold-hoping tactic was used to create TB47, to increase draggability and potency.[121] The compound targets the respiratory cytochrome bc1 complex’s QcrB subunit[150] and shares structural similarities with the imidazopyridine amide molecule Q203. TB47 exhibits promising characteristics as an oral drug due to its favourable attributes, including the absence of toxicity in human cell lines and rat models, as well as its substantial oral bioavailability in rats.[151]

Spectinamides are brand-new, semi-synthetic spectinomycin derivatives that have outstanding pharmacological profiles, have a limited range of activity against, TB, and are only effective in hypoxic and non-replicating environments.[121] Spectinomycin, which is chemically related to aminoglycosides, acts by binding to a distinct site of “helix 34” of the 16S ribosomal subunit, thereby inhibiting ribosome translocation. In numerous murine TB models, Spectinamides are efficacious and more critically have demonstrated a strong synergy with the currently available anti-TB medications.[152]

Lansoprazole, a gastric proton pump inhibitor, was identified as a potential hit (as documented in Table 2.5) during the high-throughput screening of FDA-approved drugs against Mtb in MRC-5 lung fibroblast cells. “Phenoxyalkylbenzimidazole”,[153] “2-(qquinoline4-aryloxy) acetamides” (QOAs), and “imidazo [4,5-c]” are among the chemical scaffolds being investigated for efficacy against Mtb's *QcrB*. Imidazo [1,2-a] pyridine-type compounds, pyridine-3-carboxamides, and arylvinylpiperazine

amides[125] are types of pyridine-3-carboxamides. Moreover, morpholino thiophene is also identified as a *QcrB* inhibitor.[154]

2.5.32. Inhibitors of Cyt-bd oxidase

Aurachin D is a type of chemical compound, that belongs to the Aurachin quinone family, and it is effective in inhibiting the activity of the *E. coli* Cyt-bd oxidase enzyme[125] (Table 2.5). When tested against *M. smegmatis* membrane vesicles, Aurachin D, and Aurachin quinone analogs were found to reduce oxygen consumption in a dose-dependent manner by up to 50%. When tested against membrane vesicles lacking *QcrCAB*, the consumption of oxygen was further reduced by 90%, indicating that Aurachin D may also target Cyt-bd in *Mycobacteria*. The compound "Aurachin D" did not show any antimicrobial activity against replicating *M. smegmatis* or *M. tuberculosis* when tested alone.[147]

Despite demonstrating no activity against replating *M. smeg* or *M. tuberculosis* when used alone, its combination with Q202 yielded noteworthy outcomes. The combination resulted in a ten-fold decrease in Q203's MIC against Mtb and enhanced bactericidal efficacy, leading to over a 2-log reduction in colony-forming units of Mtb. This indicates that Aurachin D has the potential to enhance the effectiveness of Q203.[147] Though, given its structural relationship with MK, Aurachin D must be proven to be a particular Cyt-bd inhibitor in *Mycobacteria*. Additionally, it is important to highlight that Aurachin D might also interact with other respiratory complexes. Furthermore, it is noteworthy that certain small compounds have shown the ability to improve the bactericidal effectiveness of QcrB inhibitors deprived of directly aiming the Cyt-bd proteins. This observation holds particular significance.

2.5.33. Menaquinone Biosynthesis Inhibitors

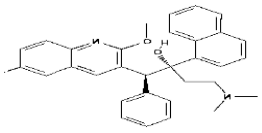
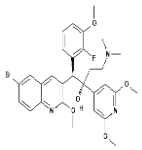
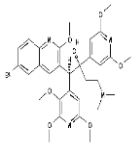
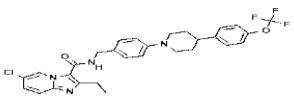
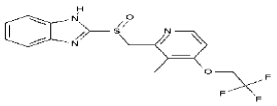
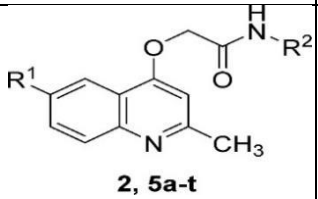
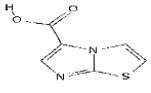
The remarkable importance of this pathway makes it a compelling candidate for the development of anti-TB drugs. Menaquinone, the exclusive redox cofactor of its kind, plays a crucial role in facilitating electron transfer to terminal reductases in gram-positive bacteria, including *Mycobacterium tuberculosis* (Mtb).

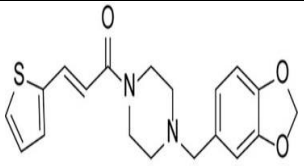
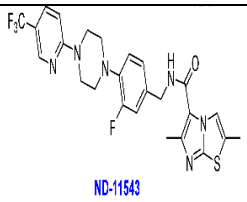
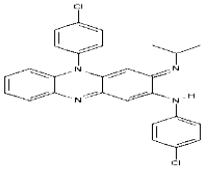
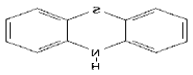
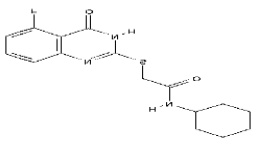
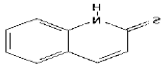
The Mtb's *Men* enzymes are attractive targets for chemotherapy. Targeting Mtb's menaquinone production hasn't shown any clinically significant results despite the obvious benefits. Kurosu and colleagues discovered that “alkylamino-methanone” grounded compounds bind to *MenA* and show a chemical response against Mtb.[155] An alternate chemical scaffold “7-methoxy-2-naphthol”, has been employed to showcase its binding with *MenA* and its whole-cell activity against Mtb.

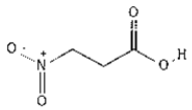
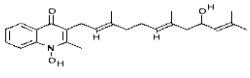
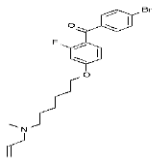
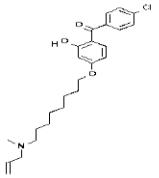
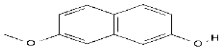
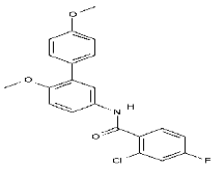
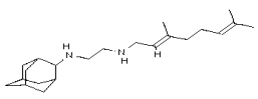
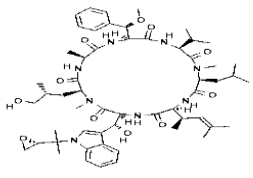
The compound DG70, which belongs to the biphenyl amide-based class, was discovered as a promising candidate with activity against *MenG*, an enzyme downstream in the menaquinone biosynthesis pathway of Mtb. Table 2.5 may contain additional information regarding these compounds.

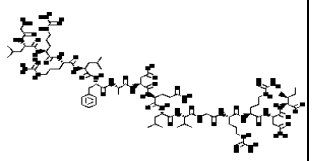
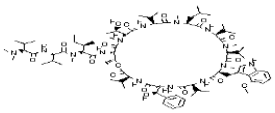
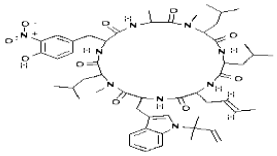
The potential advancement of TB drug development through recent findings that aim to inhibit menaquinone production remains uncertain.[156] *“MenA”, “MenB”, “MenG”, “MenD”,* and *“MenE”* chemical inhibitors have so far been demonstrated to limit Mtb growth. Table 2.5 lists the inhibitors that have been reviewed for their ability to target menaquinone (MK) biosynthesis.

Table 2.5. Targeting Oxidative phosphorylation and proteolysis.

Compound / Inhibitor	**Chemical class**	**Structure**	**Cell wall components inhibited**	**Reference**
BDQ	Diaryquinolines		*ATP synthase* c-subunit (Rv1305) and other targets are unknown	[1,27,78]
TBAJ-587 and TBAJ-876	2nd generation Diary quinolines	 	*ATP synthase* c-subunit (Rv1305) and other targets are unknown	[1,27,78]
Q203 (Telacebac)	Imidazopyridine amide		Cytochrome b subunit of the cytochrome bc1 complex	[1]
Lansoprazole	Prevacid		Cytochrome b subunit of the cytochrome bc1 complex (Rv2196)	[148]
2-(Quinolin-4-yloxy) acetamides	Acrylamides	 **2, 5a-t**	Cytochrome b subunit of the cytochrome bc1 complex	[1]
Imidazole (2,1-b) thiazole-5-carboxamides	Thiazole containing heterocycles		Cytochrome b subunit of the cytochrome	[157]

			bc1 complex (Rv2196)	
AX-35	Arylvinylpiperazine amide		*QcrB*	[158]
ND-11543	Organic compound		*QcrB*	[159]
Clofazimine	Lamprene		*NAD-II*	[142]
Phenothiazines	Phenothiazines		*NAD-II*	[142]
CBR3465	Thiquinazoline		*NAD-II*	[142]
2-Mercapto-quinazolinones	Quinazolines		*NAD-II*	[142]

3-Nitropropionate	Carboxylic acid		*ICL-I* and *ICL-II*	[1]
Aurachin RE	Prenylated quinoline		*MenA*	[73]
RO-48-8071	Oxazolidinones		*MenA*	[149]
Allylaminomethanone-A	Ketones		*MenA*	[149]
7-Methoxy-2-naphthol	Aromatic compound		*MenA*	[160]
DG-70 (GSK1733953A)	Biphenyl amide		*MenG*	[1,125,156]
SQ109	Ethylenediamine		Proton Motive Force	[1,73]
Cyclomarin A	Cyclic peptides		*ClpC* Inhibitor targeting proteolysis	[1,161]

Lassomycin	Cyclic tridecapeptide		*ClpC* Inhibitor targeting proteolysis	[1,161]
Ecumicin	Cyclic tridecapeptide		*ClpC* Inhibitor targeting proteolysis	[1,161]
Rufomycin	Cyclic Heptapeptides		*ClpC* Inhibitor targeting proteolysis	[1,161]
Squaramides	Vinylogous amides	NA	*ATP synthase* c-subunit (Rv1305) and other targets are unknown	[1,27,78]
Tetrahydronidazolie	Nitroimidazoles		*NAD-II*	[142]
Salicylanilide ester	Ester		*ICL-I* and *ICL-II*	[1]
Copper complex of pyruvate isoniazid analogs	NA			
Bioisosteres sulphamate and Sulphamide	Vinyl sulphonamide MeOSB-AVSN		*MenE*	[125]

Vinyl sulphonamide group	Sulphonamides	NA		
Diphenylborinic acid quinoline ester	Boronic acid ester		*MenG*	[1,125,156]

2.5.34. F1F0 *ATP synthase* inhibitors

Addressing the F1F0 *ATP synthase* has gained considerable attention as a promising treatment strategy for Mtb. The endorsement of BDQ by the US FDA for the treatment of MDR-TB adds substantial credibility to this therapeutic approach. BDQ, classified as a diarylquinoline, disrupts ATP production by binding to the α and €-subunits of the *ATP synthase*, impeding the displacement of the c-rotational subunit during ATP catalysis. Extensive research has been conducted to elucidate its mechanism of action in *Mycobacteria*.[162] Interrupting is essential for bacterial survival throughout both active and dormant growth stages. Unfortunately, BDQ medication resistance has already evolved in MDR-TB patients, with numerous cases being reported.[163] Mutants that were resistant to BDQ were developed, and these mutants had mutations in the gene responsible for encoding *AtpE*. These mutations were predominantly identified in the F0 region of the *ATP synthase* enzyme. Researchers are looking for better diarylquinoline analogs because BDQ therapy causes cardiotoxicity (QT prolongation) and resistance to development.[164] Targeting energy metabolism is an attractive approach for developing new anti-TB drugs, as it is a non-traditional target compared to the current TB drugs. As evidenced by the licensing of BDQ as a

tuberculosis treatment, the efficacy of the BDQ was very specific for *Mycobacterial ATP synthase* and energy metabolism inhibition may shorten the length of the course of treatment.[165]

Despite the advantages of BDQ mentioned above, its current approved use is limited to MDR-Mtb patients who have failed all other treatment options. There are several drawbacks associated with BDQ, including the risk of cardiotoxicity due to perpetuation of the QT interval with Fridericia's correction, the potential risk of detrimental drug interactions due to the presence of CYP450 inducers and inhibitors like rifamycin and anti-retroviral, coupled with an unusually extended half-life.

Given the potential negative effects of BDQ in humans, presently, extensive efforts are directed toward exploring the chemical realm of diarylquinolines to identify advanced analogs that exhibit equivalent or superior efficacy, while offering enhanced safety profiles. In this regard, two 2nd generation diarylquinolines, "TBAJ-587" also "TBAJ-876" are currently being investigated in early-stage research settings[78,166] (Table 2.5). In terms of IC_{50} values, the next-generation *ATP synthase* inhibitors, "TBAJ-587" and "TBAJ-876", demonstrate sophisticated values compared to BDQ. Specifically, "TBAJ-587" has an IC_{50} value of 13 mM, while "TBAJ-876" exhibits IC_{50} values exceeding 30 mM. There is a significant difference of 1.6mM between them.[166] Multiple high-throughput screenings, both target-based and phenotypic, have been conducted to identify new chemical inhibitors that target the *ATP synthase* of *Mycobacteria*. Among these screenings, compounds based on squalamine have exhibited encouraging outcomes in inhibiting *ATP synthase* in a mouse model of

tuberculosis infection. Currently, these squalamine-based compounds are undergoing lead optimization phases to further enhance their efficacy and properties.[167]

2.5.35. PMF inhibitors

Several compounds are known to act on the proton motive force (PMF) in bacteria, for instance, “Rotenone” inhibits the primary proton pump, while protonophores like “carbonyl cyanide m-chlorophenyl hydrazone” (CCCP) facilitate proton transfer across the membrane. Pyrazinoic acid, which is part of the first-line anti-TB drug regimen, has been shown to target PMF as well[168] (Table 2.5).

Pyrazinoic acid is the main ingredient of the Ist-line TB medications, PZA, which has been shown to reduce PMF and ATP levels in *Mycobacterium bovis* BCG. The TB drugs “SQ109”, “BDQ”, and “CFZ”, in addition to their intended enzyme targets, also function as uncouplers with multiple targets.[125] The therapeutic potential of disrupting *Mycobacterial* PMF is a significant and essential feature of the ETC. However, to develop effective drugs, it is necessary to identify compounds that specifically target the disruption of *Mycobacterial* PMF.

Compounds that deplete ATP and alter PMF could assist in speeding up TB treatment.[124] SQ109 (produced by Sequella Inc) is thought to have antimycobacterial activity via three mechanisms: “dissipation of PMF”, “disruption of cell wall assembly via the *MmpL* transporter protein”, and “suppression of menaquinone production”.

2.5.36. Inhibitors of isocitrate lyase

Within the oxygen-deprived environment of host macrophages, Mtb including persister cells, can employ the glyoxylate shunt as an energy-saving mechanism. The

vital enzyme of this pathway, “isocitrate lyase” (*ICL*), is essential for Mtb, as evidenced by a double knockout mutant (*DIcl1* and *Dlcl2*) that was incapable of metabolizing native lipids and infected mice. Some minor compounds, including “itaconate”, “itaconic anhydride”, “3-bromopyruvate”, “oxalate”, “malate”, and “3-nitopropionate”, that bear structural similarities to common *ICL* metabolites, have demonstrated antimycobacterial activity, although their cytotoxicity is notable.[1] “Salicylanilide compounds”, “benzanilide derivatives”, “pthalazinyl derivatives”, and several “copper complexes of pyruvate-isoniazid” with *ICL* action (Table 2.5) and enhanced cytotoxicity were discovered in various HTS studies.[1] To develop inhibitors with improved safety profiles, a profound understanding of the target space is essential, which involves expanding the knowledge of the underlying mechanisms.

2.5.37. Respiratory poisoning

The role of NO is significant in the innate immune response to intracellular infections like Mtb, as described in the study.[125] “PA-824” and “DEL” produced NO when the deazaflavin-dependent nitro reductase *Ddn* (rv3547) of Mtb was activated. Bicyclic nitroimidazoles like PA-824 and DEL are approved as part of a drug combination to treat DR-TB. Both medications work effectively against replicating and non-replicating Mtb. However, Swain and colleagues proved that the antibacterial action of “PA-824” is due to the production of des-nitroimidazole metabolites in anaerobic settings, which is linked to the release of NO.[37] PA-824 and DEL prevent the synthesis of mycolic acid in aerobic conditions. A study analyzing the transcriptome of Mtb cells preserved with DEL and PA-824 discovered that the antibacterial action of these medicines in *Mycobacteria* is reliant on NO poisoning.[169]

Efforts are currently being conducted to find novel inhibitors for ETC components. To date, it has been successful in finding new Mtb inhibitors by focusing on the *Mycobacterial* OxPhos pathway. Several potential targets for pharmacological intervention remain undiscovered because they bear similarities to eukaryotic components or exhibit specific bacterial responses to environmental or stress-related conditions. One such example is the *"Cyt-bd"* protein. To successfully target the diverse metabolic pathways exploited by Mtb, it is crucial to obtain a comprehensive understanding of the intricate and interconnected processes of energy metabolism. For the creation of energy metabolism inhibitors, a clearer comprehension of ETC modulation under the host physiological circumstances faced by Mtb will be highly helpful.

2.5.38. Targeting proteolysis and its inhibitors

The ability of *Mycobacterium tuberculosis* (MTB) to persist within host cells relies on its capacity to withstand the host cell's assaults on its protein apparatus through two fundamental cellular processes: "Proteostasis" and "Proteolysis". The act of keeping cellular proteins folded in their natural morphologies is known as proteostasis in Mtb and the proteolysis that breaks down damaged, excessively expressed, or dysfunctional proteins.[1] The approach of chemically aiming the enzymes engaged in maintaining the "proteostasis" and "proteolysis" of Mtb is a novel therapeutic approach.[170,171] Biochemical studies suggest that *"ClpP"* forms a complex with the chaperone proteins *"ClpC"* (encrypted by rv3596c) or *"ClpX"* (encrypted by rv2457c) which belong to the "AAA+ATPase" family. This complex contains a purposeful channel with fourteen serine proteolytic positions, each consisting of a trio

of “Ser-His-Asp”, that make up the proteolytic machinery of Mtb’s *ClpP*.[172] The two *ClpP* heptamers, *ClpP1* and *ClpP2*, that make up this core channel are layered one on top of the other. Chemically modifying *ClpP/ClpC* or *ClpP/ClpX* complexes of Mtb has three potential outcomes: inhibition, activation, and uncoupling of *“ClpC”* or *“ClpX's”* chaperon function from *“ClpP1P2's”* proteolysis process.

Actinomycetes-derived cyclic peptides, such as lassomycin, cyclomarin, rufomycin, and ecumicin are derived from the “*Lentzea kentuckyensis*” species (IO0009804). It is like ecumicin and is expected to promote *ClpC* activity, resulting in *ClpP1P2* decoupling. Cyclomarin A is a type of cyclic heptapeptide molecule that is derived from *Streptomyces* species “CNB:982”. It has been shown to inhibit the growth of Mtb by binding to the N-terminal region of the *ClpC* protein in the bacteria. Rufomycin, also known as lllamycin and originally Takeda Pharmaceuticals reported rufomycin activity in 1960.

Choules and colleagues recently demonstrated the activity of rufomycin against *M. tuberculosis* and *M. abscesses*.[158] It is isolated from a specific strain of *Streptomyces* species known as MJM3502. Ecumicin derived from the *Nonomuraea* species (MJM5123), is the sole cyclic peptide that has therapeutic effects in a mouse model of acute tuberculosis infection. The potency of “ecumicin” highlighted the importance of *ClpC in vivo*. The chemical alteration generated by cyclomarin A triggers *ClpC's* ATPases, resulting in unrestrained *ClpP1P2* proteolysis and cell death.

2.5.39. Summary and Future Perspective

Tuberculosis (TB) remains a significant global health, social, and economic burden, Underlining the urgent demand for novel TB drugs with distinctive mechanisms of action against *Mycobacterium tuberculosis* (Mtb) due to the rising threat of drug-resistant TB. Despite recent advancements in chemotherapy, TB elimination remains a major challenge on a global scale. The identification of new TB targets and inhibitors is crucial, particularly those that are essential in the context of host infection and amenable to pharmacological inhibition. Comprehensive information on the regulation of tuberculosis targets is vigorous, considering the potential influence of other medications in a treatment regimen on target expression or function. Such an approach is anticipated to enhance treatment efficacy, minimize resistance development, and potentially shorten the duration of therapy. Inhibitors of *Mycobacterial* cell wall production is an essential component of TB treatment, and enzymes participating in cell wall formation have been recognized as potential therapeutic targets for antimycobacterial drugs. The use of whole-cell phenotypic screening and target-based development techniques has resulted in the discovery of new groups of drug candidates with excellent antimycobacterial activity, giving hope for the development of novel medications to address the problem of drug resistance. To accomplish a comprehensive control plan against MTB, the scientific community should work to combine the results of many techniques, fill in any gaps in an approach, pursue newer treatment options, and consistently interact with the social-political establishment.