Chapter I

Introduction

Mycobacterium tuberculosis is the primary causative agent of tuberculosis (TB), which is still a serious and ongoing worldwide health concern. As one of the most persistent and harmful infectious illnesses, tuberculosis has affected humanity for generations.[1] While there has been notable advancement in mitigating the global impact of tuberculosis in recent times, it is still a powerful opponent that can cause great harm regardless of a person's location, gender, or socioeconomic class. Millions of people worldwide suffer from tuberculosis (TB) each year, which is most prevalent in areas with poor access to healthcare. Drug-resistant strains have increased the risk, complicating and raising the expense of therapy. To effectively combat TB, advancing healthcare, creating better medications and diagnostic tools, and collaborating internationally are imperative.[2,3] It is vital to stop the spread of TB by early detection and infection control.[4] Despite our advancements, TB continues to be a serious issue that necessitates a focused, all-encompassing strategy to save lives and lessen its global impact.[4] To address the worldwide TB issue and create a comprehensive plan to eradicate tuberculosis by 2030, the United Nations (UN) held a pivotal meeting. To accomplish a year without TB, the UN acknowledged the urgent need for increased international cooperation. One-third of all deaths linked to antimicrobial resistance are caused by tuberculosis, which is still one of the top causes of mortality in the world and is particularly lethal for those with HIV.[1] An estimated 10.6 million cases of TB were reported in 2021, a 4.5% increase from 2020 and reversing a long-term downward trend. Following two decades of consistent annual decreases, the

tuberculosis (TB) incidence rate experienced an uptick of approximately 3.6% in the year 2021. These numbers demonstrate how urgently the world must take action to fight TB.[5]

According to estimates from 2021, there is a serious TB problem in Southeast Asia and Africa. Of the 82% of TB-related deaths among non-HIV people, 36% were attributable to India alone. In addition, Africa and Southeast Asia also had the greatest rate of TB mortality among those with and without HIV, with India accounting for 32% of the global total.[5] Given its enormous population and varied healthcare system, India is a good example of the global TB issue. This circumstance demonstrates the complicated problems that TB poses, such as the rise of drug-resistant strains. The battle against tuberculosis (TB) is growing increasingly challenging with the surge in cases of extensively drug-resistant (XDR) and multi-drug-resistant (MDR) TB. The potential escalation of total drug-resistant (TDR) TB further emphasizes the urgency of addressing this issue.[5] The emergence of drug-resistant strains, such as multi-drug-resistant (MDR-TB), extensively drug-resistant (XDR-TB), and potentially total drug-resistant (TDR-TB) cases, has undermined the effectiveness of conventional antibiotic treatments, highlighting the immediate need for the implementation of innovative strategies. These efforts include the creation of novel, targeted antibiotics, shorter and more efficient treatment plans, enhanced drug susceptibility testing, vaccine campaigns, stepped-up public health initiatives, and extensive patient support, all of which are intended to address this urgent global health issue. Therefore, researchers are increasingly looking for alternate sources of medicine due to rising resistance, unfavorable side effects, and the high expense of currently used medicines.[2] In this

regard, compounds from different repositories have been heavily utilized for their therapeutic actions that target the MTB cellular machinery.[3,5] It can be difficult to identify targets that are appropriate for this strategy. Based on a review of current TB targets, significant factors that must be taken into account when choosing a target were highlighted, which can assist in prioritizing among the approximately four thousand gene products existing in MTB. They propose some essential characteristics of an "ideal drug target", including its necessity for existence underneath various biological conditions. *Mycobacterium tuberculosis* is encountered during infection because of its limited innate mutability, ability to act as a "chokepoint" via interfering with some cellular processes, or its necessity for latency.[6] Moreover, an ideal target must be "druggable" and tests must be available to determine the enzyme's catalytic action and degree of inhibition as well as if the drug continues to operate as intended in whole cells.[6]

Over the past ten years, there has been a deliberate and sustained effort to add new target/inhibitor combos to the pipeline of anti-TB medications. Recently, considerable literature has grown around the theme of targeting the machinery of *Mycobacterium tuberculosis* indicating urgency in identifying potential targets and their novel inhibitors.[3-5] Present endeavors are concentrated on the discovery of particular compounds with the ability to target the enzymes responsible for cell wall biosynthesis in *Mycobacterium tuberculosis*. Cell wall biosynthesis remains the target with the greatest therapeutic value and efficacy among the few available choices. Inhibitors that target cell wall biosynthesis are bactericidal as they make bacteria more susceptible to osmotic pressure-induced cell wall breakage.[2]

MTB stands apart from other bacteria due to the intricate structure of its cell wall and the presence of virulence factors. The cell wall is encompassed by the mAGP-mycolyl-arabinogalactan-peptidoglycan complex, which consists of three distinct layers within the *Mycobacterial* cell wall.[7] *MmpLs* (Mycobacterial membrane protein large), which are essential for transporting polymers, lipids, and immunomodulators as well as extruding therapeutic drugs, have gained significant recognition as a pivotal therapeutic drug target in recent times.[8,9] *MmpL3* plays a crucial role in facilitating the translocation of mycolic acids, specifically in the form of trehalose monomycolate (TMM), from the cytoplasm to the cell membrane, serving as a vital function in this process. This transporter inhibits a crucial step in the mycolic acid synthesis pathway of *Mycobacteria*. Consequently, it presents a potential avenue for the creation of anti-tubercular medications.[9] As *MmpL3* inhibitors, several molecules have been discovered such as ethylenediamine, indolcarboxamides, diphenyl pyrroles, adamantyl-ureas, spirocycles, 1,5-diphenyl pyrroles, piperidinol,[10] benzimidazoles, and HC 2091.[11] Recent experiments involving spheroplasts demonstrated the direct inhibitory action of BM212, the foremost substance discovered to target *MmpL3* protein, whereas the postulated mechanism for the other molecules is the dissipation of PMF-proton motive force.[12] The initiation of clinical research on *MmpL3* inhibitors highlights the significance of this protein as a therapeutic target for the development of new tuberculosis (TB) therapies. Nevertheless, it is important to highlight that comprehensive structural data for the complete *MmpL* family is currently scarce.

MmpL3's crystal structure both by itself and in combination with other substances such as ICA38, AU1235, rimonabant, and SQ109 provided the first insight

into the processes of inhibition.[11] Remarkably, the transmembrane region of *MmpL3* houses a shared binding pocket among all while having diverse topologies.[11] They have a significant impact on the transmembrane helices (TMH) bundle because they break the hydrogen bonding connection that lies among dual preserved regions of "Asp-Tyr" pairs there, which blocks the PMF that propels substrate translocation.[11] The diamine SQ109 has successfully finished phase 2b-3 of clinical trials, making it one of these pharmacophores. Indole carboxamides, pyrroles, and THPPs are among the compounds that exhibit favorable pharmacokinetics properties and demonstrate significant efficacy in mouse models of infection.[13] Both *Mycobacterial* growth and intracellular survival require *MmpL3*.[12] Surprisingly, its broad versatility can be explained by the fact that different *MmpL3* inhibitors dissipate the PMF required for TMM, a potential indirect method, in addition to the fact that it is important for tubercle bacilli.[12]

Arabinogalactan, the central layer, and the main cell wall polysaccharide are composed of furanose (*f*)-ring-shaped galactose and arabinose sugar residues (*Galf*). Arabinogalactan is connected to peptidoglycan through a singular linker unit.[14] The formation of the linear galactan chain involves the coordinated action of bifaceted galactofuranosyltransferases (*GlfT1* and *GlfT2*). Gal*f* is initially moved by *GlfT1* from "UDP-Gal*f*" to the C-4 location of "L-Rha" and creates "C_{50}-P-P-GlcNAc-L-Rha-Galf_2" by first adding another Gal*f* deposit to the C-5 location of the prime Gal*f*.[14]

GlfT2 sequentially adds Gal*f* deposits from discontinuous β-(1→5) and β-(1→6) glycosidic connections towards the developing galactan chain. *In vivo*, around thirty

Gal*f* remains are present in the galactan chains, creating "C_{50}-P-P-GlcNAc-L-Rha-Galf_{30}" but it is still unclear how the chain length is determined.

An *in silico* target identification program justifies the choice of *GlfT2* as a potential target.[14] For activity against *GlfT2* enzymes, a few initial investigations utilizing UDP-Gal*f* or iminopentitol offshoots are documented.[1] Researchers have looked into UDP-Gal*f* offshoots through changes to the C-5 and C-6 regions as potential inhibitors of these enzymes, which result in an early end of the galactan chain.[14] A prospective finest contender thiazolidinone derivative, linked to the succession evaluated by way of UGM inhibitors, which proved to pan assay interference compounds (PAINS),[15] was identified in the most recent effort to treasure *GlfT2* inhibitors and to examine their characteristics using *in silico*-ADMETox, 3D-QSAR, and molecular docking investigations.[16]

Peptidoglycan (PG), the innermost layer, is unique to bacteria, has undergone substantial study, and is often regarded as a prime candidate for pharmaceutical development.[20] Prokaryotic cells only have bacterial peptidoglycan, which is heavily cross-linked. Peptidoglycan production enzymes are crucial for the survival of bacterial cells. Peptidoglycan requires more than ten synthetic changes, each carried out by a different enzyme.[1] The bottommost stratum of the mAGP complex consists of peptidoglycan, a polymer composed of sugar chains interconnected by short peptides.

MTB PG assembly is divided into three stages. Stage I is the synthesis of the cytoplasmic precursor. In stage II of the process, the membrane-bound precursor

undergoes migration within the periplasmic space, and during stage III, the cytoplasmic precursor is polymerized and subsequently cross-linked through peptide bonds.[1] Within the cytoplasm, the *Mur* enzymes facilitate the assembly of "uridine diphosphate-N-acetylmuramyl pentapeptide" as part of the peptidoglycan biosynthetic pathway. In bacteria, the *Mur* enzymes exhibit a high degree of conservation and possess unique characteristics. They play a crucial role in facilitating the development of "UDP-MurNAc-pentapeptide" through a two-step process. The initial stage in this process is facilitated by the enzyme *MurA*.

After the initial step, the conversion of the enol pyruvate group into a lactoyl moiety is performed by the enzyme *MurB*. This enzymatic process utilizes NADPH as a cofactor, ultimately leading to the synthesis of UDP-MurNAc.[14,17] Several *MurB* inhibitors including pyrimethamine, sulfadoxine, chloropicolinate amides, piperazine derivatives, and 3,5-dioxopyrazolidines have been acknowledged using "structure-based drug discovery" techniques.[1,9] The insights gained from this knowledge can serve as a guiding framework for future endeavors in structure-based drug design.[14-18]

TB eradication is still an international challenge. Finding prospective TB targets that are both essential during host infection and amenable to pharmacological inhibition is therefore essential in addition to the scientific efforts stated above. Three enzymes, first from the mycolic acid biosynthesis, a second from the arabinogalactan biosynthesis, and a third from the peptidoglycan biosynthesis pathway utilized for discovering novel inhibitors as enzymes from these pathways are attractive targets due to their moonlighting properties and essential for *Mycobacterium* survival. Moreover, very few or no inhibitors are reported against them.[1,14] If their production is disrupted

by inhibiting the targeted enzymes, *Mycobacterium* cell synthesis should be inadequate resulting in the blockage of subsequent processing.[15]

There has been an effort to identify inhibitors that are more potent, less likely to result in resistance and reduce the course of treatment. These inhibitors may have non-canonical targets, either bind to several geographic areas of the same target or shut off all potential biological pathways. Our goal is to inspire modern target-based strategies, which have previously been effectively used to produce effective inhibitors against certain *M. tuberculosis* enzymes.[6]

The current imperative revolves around the identification of potential targets within *Mycobacterium tuberculosis* and the development of novel inhibitors capable of disrupting the essential processes of this pathogen. This endeavor necessitates a comprehensive understanding of the bacterium's biology, its interactions with the host, and its mechanisms of drug resistance. Successful identification and authentication of these targets hold the promise of innovative drug development, breathing new life into our fight against drug-resistant TB.

Our research into identifying potential targets and their inhibitors in *Mycobacterium tuberculosis* transcends scientific curiosity; it is an ethical obligation. Millions of lives hang in the balance, and it is incumbent upon the global community to unite in this endeavor. The consequences of inaction are severe: the unchecked spread of drug-resistant TB strains threatens to unravel the progress achieved in TB control, imperiling vulnerable populations.

In this context, attention is turned to three enzymes crucial for *Mycobacterium* survival: those involved in mycolic acid biosynthesis, arabinogalactan biosynthesis, and peptidoglycan biosynthesis pathways. These enzymes, aside from their primary roles, possess moonlighting properties, rendering them attractive targets for novel inhibitors. Surprisingly, there exists a scarcity of reported inhibitors against these pivotal enzymes. The disruption of their production, through targeted enzyme inhibition, could render *Mycobacterium* cells unable to synthesize essential components, ultimately leading to a blockade of subsequent processing pathways.

Research Gap

- Tuberculosis is a major global health concern, and the emergence of drug-resistant strains has created a need for new therapeutic approaches.
- Many enzymes in *Mycobacterium tuberculosis* have been explored as potential drug targets, but there is a gap in knowledge regarding the inhibition of these specific enzymes involved in cell wall biosynthesis.

Hypothesis

- Identifying and characterizing potential inhibitors for these specific enzymes can lead to the development of novel anti-tuberculosis drugs.
- These enzymes are vital for the structural integrity and viability of *M. tuberculosis.*
- Inhibiting these enzymes may disrupt cell wall biosynthesis, leading to the bacterium's death or debilitation.

- Targeting these enzymes with specific inhibitors could lead to a novel class of drugs effective against drug-resistant strains and potentially shorten treatment duration.
- This research aims to address the existing knowledge gap and contribute to the global effort to combat tuberculosis.

Aims and Objectives

Given the lack of research regarding novel inhibitors against *Mycobacterium's* biosynthesis pathways, this study considers the implication of a target-based approach and experimental validation in the development of novel inhibitors against fast drug-resistant tuberculosis.

1. Identification of potential targets in *Mycobacterium tuberculosis.*
2. Virtual screening of inhibitors/ small molecules from different chemical libraries for the evaluation of the anti-tuberculosis activity.
3. *In-vitro* validation of inhibitors/small molecules having potential anti-tuberculosis activity.
4. By investigating possible inhibitors that target the *Mycobacterium* machinery of cell wall production pathways, this study seeks to add to this expanding field of study.