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Anti-tuberculosis drug development *via* targeting the cell envelope of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis possesses a dynamic cell envelope, which consists of a peptidoglycan layer, a mycolic acid layer, and an arabinogalactan polysaccharide. This envelope possesses a highly complex and unique structure representing a barrier that protects and assists the growth of *M. tuberculosis* and allows its adaptation to the host. It regulates the immune response of the host cells, causing their damage. Therefore, the cell envelope of *M. tuberculosis* is an attractive target for vaccine and drug development. The emergence of multidrug-resistant as well as extensively drug resistant tuberculosis and co-infection with HIV prevented an effective control of this disease. Thus, the discovery and development of new drugs is a major keystone for TB treatment and control. This review mainly summarizes the development of drug enzymes involved in the biosynthesis of the cell wall in *M. tuberculosis*, and other potential drug targets in this pathway, to provide more effective strategies for the development of new drugs.

KEYWORDS

***Mycobacterium tuberculosis*, anti-tuberculosis drug, cell envelope, drug target, lead compounds**

Introduction

Tuberculosis (TB) is an infectious disease caused by *M. tuberculosis*. In 2020, approximately 9.9 million people fell ill with TB globally (World Health, 2021). Since early 2020, the COVID-19 pandemic has had a dramatic impact on the outbreak and treatment of TB (Tadolini et al., 2020). The current standard treatment for drug-sensitive TB is a combination of four first-line drugs for 6 months. However, long-term use of multiple drugs may cause adverse drug reactions, which may lead to the interruption of anti-TB treatment, and even develop into multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB; Miotto et al., 2018). The emergence of multidrug-resistant TB and co-infection with HIV prevented an effective control of the disease (Bell and Noursadeghi,

2018). Therefore, the exploration of new drug targets and new anti-TB compounds is urgently needed.

The structure of the cell envelope of *M. tuberculosis* is very different from that of Gram-negative and Gram-positive bacteria. The mycolic acids (MA) of *M. tuberculosis* is covalently attached to the peptidoglycan (PG) layer and arabinogalactan (AG) polysaccharide，the whole complex is called mAGP (Kalscheuer et al., 2019). Mycobacterial outer membrane consists of two parts: the innermost leaflet contained mainly the mycolic acids, the outermost leaflet is composed of various glycolipids, trehalose monomycolate (TMM), trehalose 6,6′-dimycolate (TDM), phospholipids, porins, and waxes. (Alderwick et al., 2007; Jankute et al., 2015; Figure 1A). The lipids represents approximately 60% of the dry weight of the cell wall, and the virulence increase when the lipid contents (non-covalently exposed glycolipids, sulfoglycolipids and some phospholipids) increase; thus, the lipid content is closely related to the virulence of the bacteria. This unique cell wall enables the survival of *M. tuberculosis* in extremely harsh environments, as well as its survival under the treatment with chemotherapeutic agents (Daffe, 2015; Bhat et al., 2017). Changing the compounds entering pathway and/or increasing its entering amount are the challenges to developing inhibitors targeting the intracellular synthesis stage of bacterial cell wall. With the comprehensive understanding of the key enzymes and extensive studies in cell wall synthesis, the prospect of cell wall as a drug target is quite brilliant. Therefore, the biosynthesis pathways of the precursors of *M. tuberculosis* cell wall and their subsequent process of transport and polymerization are effective targets for anti-TB drug development. In this mini review, we summarized the potential drugs and drug targets existing in the biosynthetic pathways of mycobacteria cell envelope.

Potential drug targets of mycolic acids

Mycolic acids (MA) are important components of the cell wall in *M. tuberculosis* (Asselineau and Lederer, 1950); its biosynthetic pathway mainly involves FAS-I (fatty acid synthase type I) and FAS-II (fatty acid synthase type II; Figure 1B). The FAS-I utilizes acetyl-CoA and malonyl-CoA to generate a butyryl-S-enzyme complex; then the butyryl group receives two carbon units from malonyl-CoA. Each round of elongation lengthens the chain by two carbons and finally synthesizes 16- or 18-carbon-containing fatty acids (Jankute et al., 2015). The FAS-II uses ACP transacylase (FabD, Rv2243; Kremer et al., 2001), β-ketoacyl-ACP synthesis III (FabH, Rv0533c; Lai and Cronan, 2003), β-ketoacyl-ACP reductase (MabA/fabG1, Rv1483; Parish et al., 2007), β-hydroxy acyl-ACP dehydratase (HadABC, Rv0635/Rv0636/Rv0637; Sacco et al., 2007; Slama et al., 2016; Farjallah et al., 2021), 2-trans-enoyl-ACP reductase (InhA, Rv1484; Prasad et al., 2021) and β-ketoacyl-ACP synthetase (KasA/B, Rv2245/ Rv2246; Lee and Engels, 2013; Vilcheze et al., 2014) for the extension of the substrate. The acyl-CoA and meroacyl-ACP are synthesized after several cycles. Meromycolyl-AMP and 2-carboxyl-acyl-CoA are generated with the catalysis of FabD32 (Rv3801c) ligase (Portevin et al., 2005; Gavalda et al., 2009) and AccD4/AccD5 (Rv3799c/Rv3280) enzymes (Portevin et al., 2005; Oh et al., 2006). The condensation of meromycolate and C-26 is manipulated by polyketide synthase 13 (Pks13, Rv3800c; Figure 1B; Portevin et al., 2004; Gavalda et al., 2009). Mycolic acids biosynthesis in *M. tuberculosis* occurs through the concerted action of more than 20 enzymes that are components of different multi-enzyme complexes. Therefore, this pathway represents an important reservoir of novel targets for the development of new drugs to cure TB, especially in the context of the emergence of drug resistance.

2-trans-enoyl-ACP reductase

Isoniazid (INH), with the enoyl-AcpM reductase InhA being the primary target, can attack many targets in *M. tuberculosis* after oxidation by KatG (Rv1908c) (catalase-peroxidase). The isonicotinic acyl–NADH complex is created, which blocks the metabolic pathway of mycolic acids in the cell wall by inhibiting 2-trans-enoyl-ACP reductase (InhA) active site, and finally leads *M. tuberculosis* death (Vilcheze and Jacobs Jr., 2019). Ethionamide (ETH) is similar in structure to INH and is also a prodrug. It is activated by the enzyme ethA (Rv3854c, a monooxygenase), and binds NAD^+ to form an ETH-NAD adduct which inhibits the same target site as INH (Vilcheze and Jacobs Jr., 2014).

Aside adduct-forming compounds, there are many inhibitors that directly bind to InhA occupying the fatty-acyl binding site and even bi-substrate inhibitors, that bind simultaneously in both FA and cofactor-binding sites. These directly binding small molecule inhibitors include, arylamides (He et al., 2007), triclosan, diphenyl ethers derivatives (Freundlich et al., 2009; Kamsri et al., 2014), pyrrolidine carboxamide analogs (Kouassi et al., 2015), imidazopiperidine (Wall et al., 2007), and 4-hydroxy-2-pyridones (Figure 1B; Manjunatha et al., 2015; Guardia et al., 2016). The isoniazid-NAD truncated adducts are bi-substrate inhibitors of InhA, the hydrophobic substituents of isoniazid-NAD truncated adducts would be recognized by the fatty-acyl binding site of InhA, and the nicotinamide moiety interacts with the cofactor capsule (Delaine et al., 2010). These inhibitors directly bind to the InhA target without prior activation, avoiding the issues due to clinical drug resistance related to KatG (Prasad et al., 2021). A drug directly targeting InhA in a different location than the activated INH and not requiring activation by KatG may have bactericidal and sterilizing properties superior to those of INH. Since most INH resistance is mediated by mutations in katG, there should also be little or no cross-resistance between a direct inhibitor of InhA and INH. However, these compounds have some limitations. The triclosan, which has been shown associated with the low oral

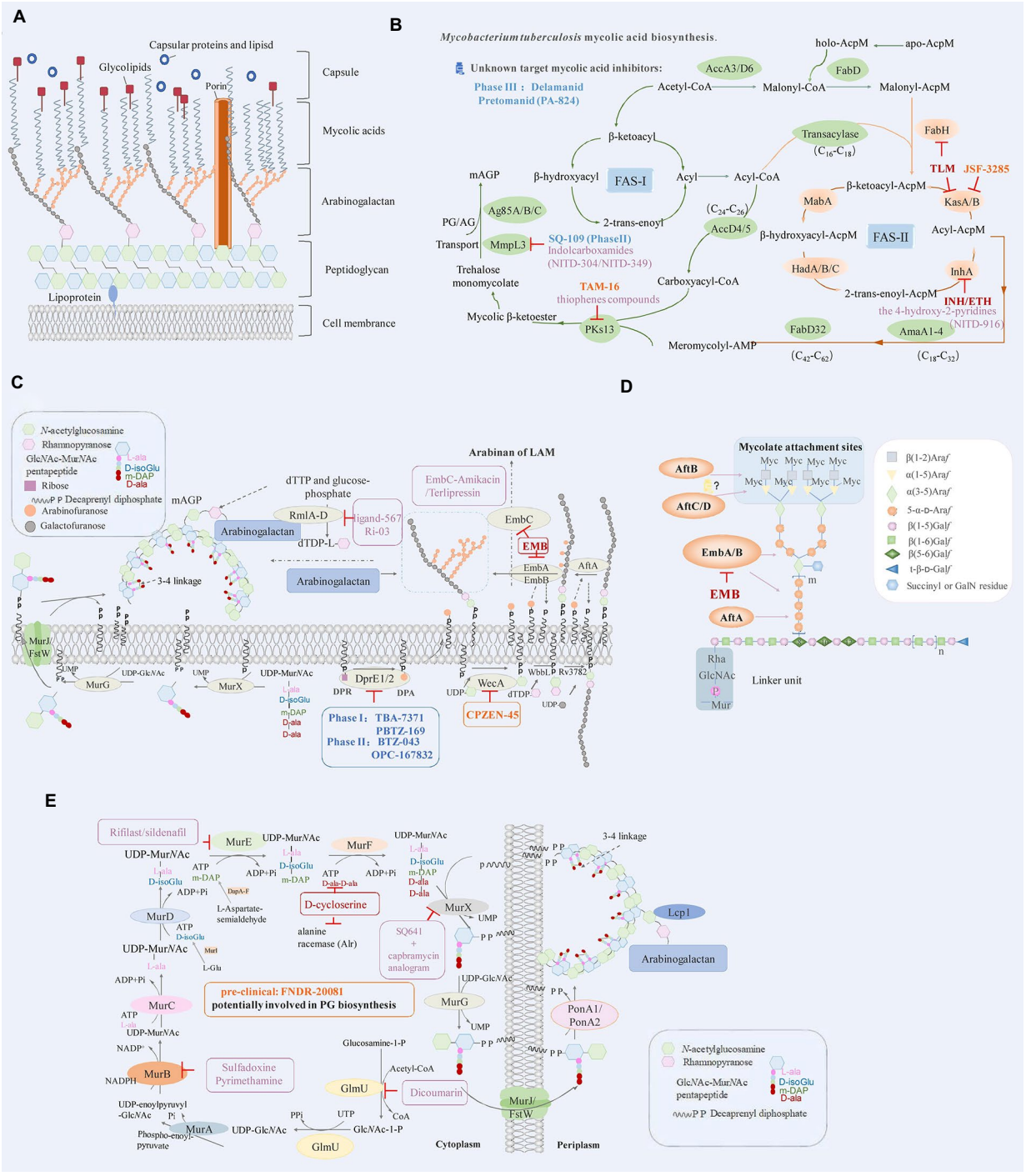


FIGURE 1
(A) Schematic representation of *Mycobacterium tuberculosis* cell envelope. **(B)** *Mycobacterium tuberculosis* mycolic acid biosynthesis. The key enzymes and drug targets of mycolic acids biosynthesis are shown as indicated. FAS-I: fatty acid synthase types I, FAS-II: fatty acid synthase types I. **(C)** Drugs targeting the decaprenylphosphoryl arabinose biosynthesis pathway. DPA, the precursor of arabinan, is synthesized by the epimerization of DPR by DprE1 and DprE2. The key enzymes and drug targets of decaprenylphosphoryl arabinose biosynthesis are shown as indicated. **(D)** Structure of mycobacterial arabinogalactan and roles of key enzymes that are responsible for its biosynthesis. **(E)** The biosynthesis pathway of peptidoglycan in *M. tuberculosis* and roles of key enzymes that are responsible for its biosynthesis. The key enzymes and drug targets of peptidoglycan biosynthesis are shown as indicated. The diagram represents the drugs (red) that are effective against *M. tuberculosis*, drugs in clinical trials (blue), pre-clinical (orange), and compounds in the discovery stage with promising development (purple).

bioavailability (Vosatka et al., 2018), the neurodevelopment impairment (Alfhili et al., 2021), gestational diabetes (Ouyang et al., 2018) and the decline of reproductive function in male mice (Priyanka et al., 2020). Future novel agent should be orally administered, well-tolerated, and with a low propensity to generate resistance.

β-ketoacyl-ACP synthase (KasA, KasB, and FabH)

The β-ketoacyl-AcpM synthase (KasA) of *M. tuberculosis* is an essential enzyme in the mycobacterial fatty acid biosynthesis (FAS-II) pathway and is a potentially promising target for antibacterial drug development. Inhibitors of KasA have been previously reported, and among them, the most significant inhibitor is thiolactomycin (TLM; Figure 1B), which can inhibit KasA, KasB, and FabH (Choi et al., 2000; Kremer et al., 2000). Many *M. tuberculosis* beta-ketoacyl-ACP synthase KasA inhibitors lack sufficient potency and/or pharmacokinetic properties. JSF-3285 (Table 1) is an indazole targeting KasA, as demonstrated by the article published in 2020 by Rutgers University (Inoyama et al., 2020). JSF-3285 is a promising preclinical candidate for TB. This study plans to identify and develop novel, small molecule inhibitors of KasA that can be used in combination with other agents to improve the therapeutic effect and to treat drug-resistant forms of TB.

Enzymes related to the last step of mycolic acids biosynthesis

Polyketide synthase 13 (PKs13) is an essential enzyme that forms mycolic acids, and it is required for the formation of the cell wall of *M. tuberculosis*. PKs13 is involved in the last step of the mycolic acids biosynthesis pathway and has been widely studied as a drug target for TB (Figure 1B; Yu, M. et al., 2018). Aggarwal et al. (2017) used structure-guided methods to develop a lead molecule that targets the thioesterase activity of Pks13 (Figure 1B; Table 1). In another study, a series of thiophenes that kill *M. tuberculosis* were identified by targeting the N-terminal ACP_N domain of Pks13. Wilson et al. (2013) revealed that the compounds work by blocking the interaction of ACP_N with the FabD32 protein, which transfers the meromycolyl chain and is essential for the growth of mycobacteria. It can also inhibit the activity of PKs13 by blocking the TE domain of PKs13 (Ioerger et al., 2013; Zhang, W. et al., 2021). These results confirm Pks13 as a target for drugs against *M. tuberculosis* and highlight its potential in the development of new TB drugs that interfere with the critical pathway of mycolic acids synthesis. It further proves that structure-guided drug development is an effective method for producing new agents, although with limited application in the process of developing new antimycobacterial compounds.

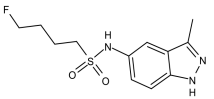
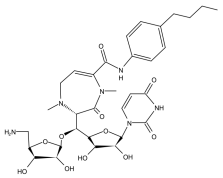
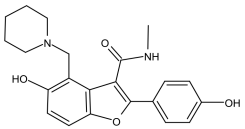
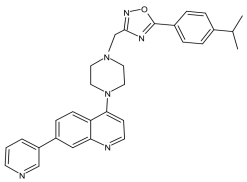
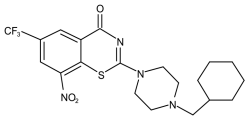
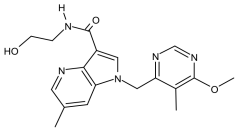
Enzymes related to mycolic acids transport

MmpL3 (Rv0206c) is an essential inner membrane protein in *M. tuberculosis* (Domenech et al., 2005). MmpL3 is responsible for the transport of mycolic acids in the form of trehalose monomycolate (TMM), the precursor of trehalose dimycolate (TDM) and mycolates bound to arabinogalactan that together forms the mycomembrane (Figure 1B). MmpL3 is critical for mycobacterial replication and viability (Belisle et al., 1997; Bhatt et al., 2005). MmpL3 has a periplasmic pore domain and a 12-helix transmembrane domain (Zhang et al., 2019), the structural data will greatly advance the development of MmpL3 inhibitors. Zhang et al. (2019) determined the crystal structure of the compound-MmpL3 and proved the direct interaction between the compound and MmpL3. Those compounds including the ethylenediamine derivative SQ109 (Table 1; Sacksteder et al., 2012; Tahlan et al., 2012; Li et al., 2014), indole-2-carboxamide ICA38 (Rao et al., 2013), adamantylurea AU1235 (Grzegorzewicz et al., 2012; McNeil et al., 2020). SQ109 is currently under Sequella's US investigational new drug (IND) and completed three Phase 1 studies in the U.S. and two Phase 2 studies in drug-sensitive TB patients in Africa, in addition to the Phase 2b-3 study in Russia (Bukhdruker et al., 2020). SQ-109 appears to be safe and well-tolerated in human studies, with mild to moderate, dose-dependent gastrointestinal discomfort being the most frequently observed adverse events (Heinrich et al., 2015).

Unknown target mycolic acids inhibitors

The new anti-TB drugs Delamanid (Table 1) producing nitric oxide, block the synthesis of mycolic acids, further damaging the stability of cell membranes (Matsumoto et al., 2006; Xavier and Lakshmanan, 2014). Preclinical studies showed that Delamanid is not genotoxic or potentially carcinogenic, and it has been approved for marketing by the State Food and Drug Administration in 2018 Clinical trials with a treatment plan containing Delamanid are underway in the United States because of the current increase in TB cases among AIDS patients (Nguyen et al., 2020). Pretomanid (PA-824), a bicyclic nitroimidazoles, is a novel anti-tuberculosis agent (Lenaerts et al., 2005). PA824 is a pro-drug that operates directly as a NO donor, it has many attractive characteristics as a potential TB therapy, most notably its novel mechanism of action. PA824 involves a dual-mode function, disrupting mycolic acids synthesis pathway for aerobic bacteria (Table 1; Tyagi et al., 2005; Manjunatha et al., 2009) and respiratory poisoning for anaerobic bacteria (Singh et al., 2008; Bahuguna and Rawat, 2020; Priyanka et al., 2020). PA-824 is converted by deazaflavin nitroreductase to nitrous oxide (NO) and other toxic products, that accumulate within bacteria and obstruct normal electron flow and homeostasis, which abrogate *M. tuberculosis* growth. The TB Alliance's new drug application (NDA) for the novel TB drug candidate pretomanid has been accepted for review by the United States Food and Drug Administration (FDA). The application was submitted for the use of pretomanid as part of a new regimen, in combination with bedaquiline and linezolid, in the treatment of extensively drug-resistant (XDR) TB, treatment intolerant multidrug-resistant (MDR) TB, and treatment non-responsive MDR-TB (Conradie et al., 2020), the effectiveness of PA824 in treating MDR/XDR TB with combinations of first-line TB medications has been confirmed.

TABLE 1 Molecules in various stages of drug discovery, their targets, chemical structure, efficacy against *Mycobacterium tuberculosis* and some other information.

Target	Development phase	Drug	Chemical structure	Chemical Class	Compounds activity
KasA	Pre-Clinical(Non-GLP)	JSF-3285		Indazole	MIC of H37Rv: 0.2 μM; hERG inhibition (IC_{50} > 50 μM); Vero CC_{50}:170 μM; C_{lung}/C_{plasma} at 5 h: 0.75 (Inoyama et al., 2020)
WecA	Pre-Clinical(Non-GLP)	CPZEN-45		Caprazene nucleoside	MIC of H37Rv: 1.56 μg/mL; MIC of MDR-Mtb: 6.25 μg/mL; poor solubility and low bioavailability (Ishizaki et al., 2013; Salomon et al., 2013)
Pks-13	Lead Optimization	TAM-16		Benzofuran	MIC of Mtb include MDR/XDR-Mtb: (0.05–0.42 μM); has excellent pharmacological and safety profiles; Oral bioavailability (F): 28%; CL_{int}: Mouse <0.5, Human <0.5 ml/min/g liver (Aggarwal et al., 2017)
peptidoglycan biosynthesis	Pre-Clinical(GLP)	FNDR-20081		Oxadiazole-piperazine-quinoline	No toxicity to THP-1 and HepG2 cells, (cytotoxicity >64 μg/mL); CYP 3A4 inhibition: IC_{50}>25 μM; unstable moderate in HLM and poor in MLM (Kaur et al., 2021)
DprE1	Phase I	PBTZ-169		Benzothiazinones	MIC<0.19–0.3 μg/mL; lower cytotoxi-city and better efficacy; The Phase II clinical trial of PBTZ-169 was terminated very slow enrollment (Makarov et al., 2014)
		TBA-7371		Azaindole	MIC of Mtb: 0.78–3.12 μM; inhibits DprE1 with an IC50 value of 10 nM; Recruiting for Phase II (Shirude et al., 2013; Chatterji et al., 2014)

(Continued)

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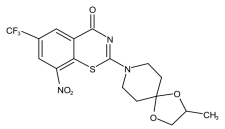
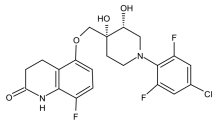
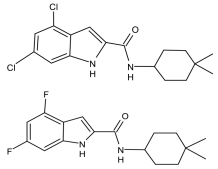
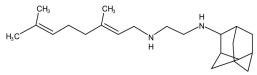
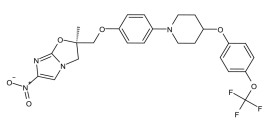
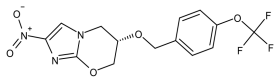
TABLE 1 (Continued)

Target	Development phase	Drug	Chemical structure	Chemical Class	Compounds activity
	Phase II	BTZ-043		Benzothiazinones	MIC of Mtb: 1 μg/mL; more cytotoxic than INH; Recruiting for Phase III clinical trials (Makarov et al., 2009)
		OPC-167832		3,4-dihydrocarbostyril derivative	MIC of Mtb, MDR/XDR-Mtb: 0.24-2 μg/mL; Phase Ib/IIa clinical trials to evaluate its safety and efficacy in TB patients (Hariguchi et al., 2020)
MmpL3	Lead Optimization	NITD-304/ NITD-349		Indolcarboxamides	MIC of H37Rv: 0.008 μg/mL and 0.016 μg/mL; good oral bioavailability (53 and 37%); CC_{50}: HepG2>20 μM, THP-1>20 μM; hERG binding and patch clamp IC_{50} were > 30 μM (a low risk of cardiotoxicity; Rao et al., 2013; Li et al., 2017)
	PhaseII	SQ-109		1,2-ethylenediamine	MIC of Mtb include MDR/XDR-Mtb:0.20–0.78 μg/mL; See the text section for other specific information (Sacksteder et al., 2012)
Mycolic acids biosynthesis	Phase III	Delamanid		Nitrodihydro imidazooxazole	MIC of Mtb: 0.006–0.024 μg/mL; it does not affect the activity of liver drug enzymes; a combination regimen of delamanid, linezolid, levofloxacin, and pyrazinamide for the treatment of fluoroquinolone-sensitive MDR-TB (Matsumoto et al., 2006; Xavier and Lakshmanan, 2014)
		Pretomanid (PA-824)		Bicyclic nitroimidazole	MIC of Mtb: 0.031–0.531 μg/mL; combination regimens (BPaL) and (BPaMZ) more effective against drug-resistant TB (Lenaerts et al., 2005; Singh et al., 2008; Burki, 2019; Xu et al., 2019)

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In conclusion, the key enzymes in the mycolic acids synthesis pathway can represent an important approach in the discovery of anti-TB drugs.

Biosynthesis and potential targets of arabinogalactan and lipoarabinomannan

LAM and AG are the two major mycobacterial cell wall (lipo) polysaccharides, which contain a structurally similar arabinan domain that is highly branched. AG is synthesized on a decaprenyl phosphate (C_{50}-P) lipid carrier and then attached to peptidoglycan and mycolic acids (Jankute et al., 2012). The phosphatidyl-myo-inositol mannosidase (PIMs), lipomannan (LM) and LAM are essential for the survival and pathogenicity of *M. tuberculosis* (Torrelles et al., 2009); the enzymes involved in the biosynthesis of AG and LAM could be the ideal targets for anti-TB drug discovery.

Arabinosyltransferase (EmbA/B/C, Rv3794/ Rv3795/Rv3793)

Arabinosyltransferase (Emb) is a key enzyme responsible for the biosynthesis of AG and LAM. EmbC is mainly responsible for the synthesis of LAM and associates with ethambutol (EMB) resistance (Zhang et al., 2020). Ethambutol competitively inhibits the substrate to bind EmbB and EmbC subunits. *M. tuberculosis* has difficulties in developing drug resistance without affecting its complex cell wall construction (Figure 1C). The above research enabled the optimization in the use of ethambutol and the development of new drugs targeting Emb proteins (Jankute et al., 2012; Tan et al., 2020a). Singh et al. (2019) analyzed the US Food and Drug Administration (FDA) library for EmbC, and selected drugs with higher binding affinity to EmbC, such as Terlipressin and Amikacin. The analysis of the size and shape of minimal energy landscape area shows greater stability of the EmbC-Terlipressin complex than the other drugs. It demonstrates the EmbC binding potential of the drug Terlipressin and Amikacin (Figure 1C), further underlining the urgency to develop new and improved treatments, with the reconsideration of existing drugs representing a potential route shortcut.

Enzymes related to dTDP-L-rhamnose synthesis (RmlA/B/C/D)

The rhamnose-GlcNAc disaccharide (RmlA-D, Rv0334/ Rv3464/Rv3465/Rv3266c) linker is fundamental for the structural integrity of the mycobacterial cell wall (Li et al., 2006; Qu et al., 2021). *M. tuberculosis* encode four RmlA-D enzymes involved in the synthesis of the donor dTDP-L-rhamnose beginning with dTTP and glucose-phosphate (Dhaked et al., 2019). Van et al., used a biological layer interference method to identify inhibitory lead compounds that bind to RmlB and RmlC and determined that Ri03 affects the viability of streptococcus and mycobacteria (Figure 1C). Thus, it can be used as a lead compound in the development of a new class of antibiotics against the biosynthesis of dTDP-L-rhamnose in pathogenic bacteria (van der Beek et al., 2019). Ravichandran et al. (2020) used the Super Natural-II database with AutoDock4.0 to perform a virtual screening of the RmlD protein and found a potential inhibitor of RmlD, thus inhibiting the synthesis of the cell wall in *M. tuberculosis*. The above studies prove that RmlB-D are potential new anti-TB drug targets, although no mature drug is available yet, and much research still needs to be performed.

Key enzymes of arabinose donor (DprE1, DprE2)

DprE1 (Rv3790) and DprE2 (Rv3791) are key enzymes in the biosynthesis of arabinose donors (DPA; Figure 1C; Bhutani et al., 2015). Benzothiazolinones (BTZ) target DprE1, and BTZ043 (Table 1; phase II drug) is the most effective among these inhibitors, showing high potency (MIC of 1 ng/ml) against *M. tuberculosis* H37Rv, multidrug-resistant and extensively resistant *M. tuberculosis* isolates (Makarov et al., 2009; Trefzer et al., 2010; Zhang et al., 2018). PBTZ169 (Table 1; phase I drug) was optimized from BTZ043 by pharmacochemistry and exhibits an increased efficacy and safety (Makarov et al., 2014; Shi et al., 2018). Subsequently, many compounds with different scaffold structures inhibit enzymes in covalent or non-covalent way (Shirude et al., 2013). For example, TBA7371 (Azaindole derivative), which is a non-covalent inhibitor of DprE1 inhibitors, completed phase I clinical trials (Table 1; Chatterji et al., 2014; Zhang et al., 2018; Robertson et al., 2021; Figure 1C). Another phenotypic screening effort around the carbostyril core results in the discovery of OPC-167832. OPC-167832 in regimens combined with delamanid showed superior efficacy to a standard RHZE regimen (rifampicin + INH + pyrazinamide + ethambutol) in mice. However, trials need to compare the proportion of subjects with favorable outcomes in each experimental treatment arm with OPC-167832, delamanid and bedaquiline versus patients receiving a standard RHZE regimen (Table 1; Hariguchi et al., 2020). These results confirm DprE1 as a target for drug against *M. tuberculosis*, since it interferes with the critical pathway of mycolic acids synthesis.

Arabinofuranosyltransferase (AftA, AftB, AftC, AftD)

The AftA (Rv3792), AftB (Rv3805c), and AftC (Rv2673) genes encode for the enzyme arabinofuranosyltransferase (ArafT), which is responsible for the polymerization of the arabinofuranyl (Araf) residues of DPA into the arabinose components AG and

LAM (Figure 1D; Giri et al., 2019). Kolly et al. (2015) explored the transcription of the GtrA (Rv3789) protein and its adjacent genes and found that AftA prime the transfer of the first arabinose residue to the galactose chain. AftA is co-expressed with GtrA, DprE1 and DprE2. AftC in *M. tuberculosis* is an α-1,3 arabinosyltransferase involved in the branching of α-1,5 linear arabinan of both l LAM and AG (Zhang et al., 2011). AftD (Rv0236c) is the largest glycosyltransferase in the genome of *M. tuberculosis*. Glycan array analysis shows that AftD binds to complex arabinose glycans (Figure 1D; Tan et al., 2020b). All the identified arabinosyltransferases are essential in the growth of *M. tuberculosis* and are potential targets of new anti-TB drugs.

Biosynthesis and potential targets of peptidoglycan

The biosynthesis of PG is a highly coordinated process composed of three stages of sequential reactions. In the first stage, UDP-GlcNAc is synthesized by the acetylation and uridylation of essential enzyme GlmU (Rv1018c) beginning with the fructose-1-phosphate (Figure 1E). In the second stage, the synthesis of UDP-MurNAc-pentapeptide is catalyzed by the MurA-F (Rv1315/Rv0482/Rv2152c/Rv2155c/Rv2158c/Rv2157c) ligase pathway (Rani and Khan, 2016). Starting from UDP-GlcNAc, the enzyme MurA adds phosphoenol pyruvate to form UDP-enoylpyr uvyl-GlcNAc, which is in turn transformed in UDP-MurNAc upon reduction of the enoylpyruvyl moiety to a lactoyl ether moiety with NADPH as an electron hydrogen donor catalyzed by MurB (Figure 1E; Squeglia et al., 2018). Besides, the MurX (Rv2156c) transfers the nucleotide glycopentapeptide to the decallyl phosphate to form the first membrane-bound peptidoglycan precursor (lipid I; Siricilla et al., 2014). Subsequently, the β (1 → 4) bond is formed between GlcNAc of UDP-GlcNAc and MurNAc/Glyc of lipid I by the MurG (Rv2153c) enzyme, thus forming lipid II (Zhang, L. et al., 2021). An enzyme with transglycosylase and transpeptidase activity is necessary for the final stage of peptidoglycan synthesis (Raghavendra et al., 2018). Finally, the Lcp1 enzyme links the arabinogalactan complex to the peptidoglycan (Alderwick et al., 2015). Mur ligases enable the development of cell walls through the cytoplasmic to periplasmic biosynthesis (Sangshetti et al., 2017; Figure 1E). Thus, the Mur ligases may be an ideal target for the discovery of new antibiotics/anti-TB drugs.

Targeting GlmU, MurA-F

GlmU is a bifunctional enzyme with glucosamine-1-phosphate acetyltransferase activity and N-acetylglucosamine-1-phosphate uridine transferase activity considered as a potential drug target (Agarwal et al., 2021). Kolly et al. (2015); Han et al. (2019) screened many compounds from different natural products against *M. tuberculosis* by DTNB colorimetry. The results showed that dicoumarin has a good inhibitory effect on GlmU acetyltransferase (Figure 1E). MurA-F represent important transferase/ligase in the steps of peptidoglycan biosynthesis. The amide ligases MurC, MurD, MurE and MurF are characterized by the same catalytic function because they possess similar amino acid regions and preserved comparable structural properties (Rani and Khan, 2016). Kumar et al. (2020) analyzed two peptide compound libraries (Asinex and ChemDiv) based on the structure of MurA, and they found that four compounds have acceptable pharmacokinetic properties. After screening FDA-approved drugs from two repositories, they found that sulfadoxine and pyrimethamine have stable interaction with MurB, while rifilast and sildenafil have the most reliable interaction with MurE (Figure 1E; Rani et al., 2020). D-cycloserine is a structural analog of D-alanine, it can competitively inhibit two essential enzymes in the synthesis of peptidoglycan: alanine racemase (Alr) and D-Ala: D-Ala ligase, which are involved in pentapeptide core formation (Figure 1E; Prosser and de Carvalho, 2013). Cycloserine is a broad-spectrum antibiotic introduced in 1952 and was recommended by the World Health Organization (WHO) to be administered to MDR-TB patients. Due to fewer patients with drug-resistant disease have been reported than for some other second-line antituberculosis drugs (Caminero et al., 2010; Yu, X. et al., 2018). A capbramycin analogram kills nonreplicating *M. tuberculosis* at low concentrations, with strong synergistic effects with SQ641 (a MurX inhibitor; Figure 1E; Siricilla et al., 2015).

Targeting β-lactamase

β-lactam (Bla) antibiotics inhibit the transpeptidase activity of penicillin-binding proteins (PBPs) to block the cross-linking of peptidoglycans (Hugonnet and Blanchard, 2007). However, BlaC (Rv2068c, β-lactamase) in *M. tuberculosis* is a broad-spectrum hydrolase that renders ineffective the vast majority of relevant β-lactam compounds currently in use (Lu et al., 2020). Therefore, the development of inhibitors that bind and inhibit BlaC but cannot be hydrolyzed by BlaC is urgently needed. Some studies showed that meropenem in combination with clavulanic acid (a BlaC inhibitor) enhance its activity to effectively kill non-replicating *M. tuberculosis* (Hugonnet and Blanchard, 2007; Tiberi et al., 2016; van Rijn et al., 2019). Tolerability of intravenous meropenem with amoxicillin-clavulanate was poor at all phase II clinical trial doses, which maybe an obstacle of meropenem in second-line regimens (De Jager et al., 2022).

The structure of *M. tuberculosis* peptidoglycan is atypical in that it is mainly a 3 → 3 cross-link formed by the L,D-transpeptidase (Ldts; Lavollay et al., 2008; Gupta et al., 2010; Dubee et al., 2012). Penicillin and cephalosporin classes of β-lactams cannot inhibit L,D- transpeptidase function; however, carbapenems (eg. meropenem and imipenem) inactivate its function (Soroka et al., 2015; Lopez Quezada et al., 2020). Avibactam is another β-lactamase inhibitor based on a diazabicyclooctane (DBO) scaffold containing a cyclic urea rather

than a β-lactam ring (Edoo et al., 2018). Ceftazidime was first marketed almost 40 years ago and has no activity against *M. tuberculosis*. However, Ceftazidime-avibactam has remarkable sterilizing effect at clinically achievable concentrations (Deshpande et al., 2017). Edoo et al. (2018) optimized the diazabicyclooctane (DBO) scaffold of avibactam (a β-lactamase inhibitor) and found that DBO 15a (DBO azide derivative) inhibited the LdtMt2 effectively. It was shown that optimization of avibactam to inhibit Ldt is an attractive strategy to obtain drugs with selective activity against Mycobacterium.

Potential drugs in pre-clinical phase

Currently, several potential drugs are under discovery and in a pre-clinical phase, in addition to Delamanid and PA-824, which are in clinical phase III. Some of the drugs in pre-clinical studies targeting the synthesis of cell wall are JSF-3285, CPZEN-45, TAM-16, and Indolcarboxamides (NITD-304, NITD-349). These drugs target common components such as PKs-13, WecA, KasA, and MmpL3 (Table 1). The TB drug development pipeline is promising in researching new drugs targeting DprE1 such as BTZ-043, PBTZ-169, TBA7371, and SQ109 (Table 1). The safety, tolerability, and efficacy of these drugs on healthy individuals and TB patients is under evaluation.

Conclusion

TB remains to be one of the leading causes of morbidity and mortality illness throughout the world. With the increasing number of multidrug-resistant and extensively drug-resistant TB worldwide, more effective drugs need to be developed to shorten the treatment time. However, the discovery of new drugs requires a better understanding of the virulence factors of *M. tuberculosis* in the host. The cell wall of *M. tuberculosis* plays an important role in the long-term infection and virulence of this pathogen. The biosynthetic pathways of the cell wall components reveal valuable drug targets (essential for the growth of *M. tuberculosis* and lack of their homologs in mammalian systems) that are the basis of present drugs and/or have the potential for new anti-TB drugs discovery.

Author contributions

XX wrote the original draft. JZ reviewed and edited the manuscript with help from CG and LP. ZH and BD contributed the schematic diagram. JZ and CW provided the conceptualization and fundings. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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