International Journal of Biological Macromolecules 243 (2023) 125022

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Review

Protein targets in *Mycobacterium tuberculosis* and their inhibitors for therapeutic implications: A narrative review

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ARTICLE INFO

Keywords:
Mycobacterium tuberculosis
Protein targets
Drug development
Cell wall biosynthesis
Efflux pumps
Enzyme inhibitors

ABSTRACT

Advancement in the area of anti-tubercular drug development has been full-fledged, yet, a very less number of drug molecules have reached phase II clinical trials, and therefore "End-TB" is still a global challenge. Inhibitors to specific metabolic pathways of *Mycobacterium tuberculosis (Mtb)* gain importance in strategizing anti-tuberculosis drug discovery. The lead compounds that target DNA replication, protein synthesis, cell wall biosynthesis, bacterial virulence and energy metabolism are emerging as potential chemotherapeutic options against *Mtb* growth and survival within the host. In recent times, the *in silico* approaches have become most promising tools in the identification of suitable inhibitors for specific protein targets of *Mtb*. An update in the fundamental understanding of these inhibitors and the mechanism of interaction may bring hope to future perspectives in novel drug development and delivery approaches. This review provides a collective impression of the small molecules with potential antimycobacterial activities and their target pathways in *Mtb* such as cell wall biosynthesis, DNA replication, transcription and translation, efflux pumps, antivirulence pathways and general metabolism. The mechanism of interaction of specific inhibitor with their respective protein targets has been discussed. The comprehensive knowledge of such an impactful area of research would essentially reflect in the discovery of novel drug molecules and effective delivery approaches. This narrative review encompasses the knowledge of emerging targets and promising chemical inhibitors that could potentially translate in to the anti-TB-drug discovery.

Abbreviation: Tuberculosis, TB; World health organisation, WHO; Coronavirus disease, COVID; *Mycobacterium tuberculosis*, *Mtb*; Clusters of differentiation 4, $CD4^{+}$; Multidrug-resistant, MDR; Extensively drug-resistant, XDR; Peptidoglycan, PG; Arabinogalactan, AG; Mycolic acid, MA; Minimum Inhibitory concentration, MIC; N acetyl glucosamine, GlcNAc; N Acetyl Muramic acid, MurNAc; *N*-acetylglucosamine-1-phosphate uridyl transferase, GlmU; *N*-acetylglucosamine-1-phospha, GlcNAc-1-P; Glucosamine-1-phosphate, GlcN-1-P; UDP-*N*-acetyl glucosamine enolpyruvyl transferase, MurA; UDP-*N*-acetylenolpyruvyl-glucosamine reductase, MurB; UDP-*N*-acetyl glucosamine, UDPGlcNAc; UDP-*N*-acetylmuramate, UDP-MurNAc; Cytoplasmic protein amidase, CwlM; *N*-acetyl muramic acid hydroxylase, NamH; UDPN-glycolylmuramic acid, UDP-MurNAc/Glyc; Meso-diaminopimelic acid, mDAP; Aspartate semi-aldehyde dehydrogenase, ASADH; Dihydrodipicolinate synthase, DapA; DAP epimerase, DapF; D-alanine ligase enzyme, Ddl; D-cycloserine, DCS; Bacterial translocase 1, MurX; *N*-acetyl glucosamine transferase, MurG; Penicillin-binding proteins, PBP; L-lysyl-D-alanine, LD; D-alanyl-D-alanine, DD; D-arabino furanose, Araf; D-galacto furanose, Galf; Glcnac-1-P transferase, WecA; Rhamnosyl transferase, WbbL; Decaprenylphosphoryl-2-ketoribose, DPX; Nitrobenzothiazinone, BTZ-043; Macozinone, PBTZ169; Dinitrobenzamides, DNB; N-(2-(4-methoxyphenoxyl) ethyl)-3, 5-dinitrobenzamide, DNB1; N-(2-(benzyloxy) ethyl)-3, 5-dinitrobenzamide, DNB2; Decaprenylphosphoryl arabinose, DPA; Decaprenylphosphoribose 2′ epimerase, DprE1; Fatty acid synthases, FAS; Acyl-coa carboxylases, ACCase; Enoyl reductase, InhA; Acyl carrier protein, AcpM; Triclosan, TCN; Pretomanid, PA824; Dacb2, DD-carboxypeptidase; Resuscitation promoting factor, Rpf; Glucose-1-phosphate thymidyl transferase, RmlA; Dtdp-d glucose 4,6-dehydratase, RmlB; Dtdp-6-deoxy-d-xylo-4-hexulose 3,5-epimerase, RmlC; Dtdp-6-deoxyd-xylo-4-hexulose reductase, RmlD; Mycobacterial membrane protein Large 3, MmpL3; O-glcnac transferase, *ogt* gene; Dihydrofolate reductase, DHFR; Ribonucleotide reductases, RNR; Core domain, CD; Sigma interacting domain, SID; Zinc-binding domain, ZBD; Methionyl trna, Met-tRNAi; Initiation complex, IC; Initiation factor, IF 1-3; Elongation factors, EF-Tu, EF-TS and EFG; Release factor, RF1 and RF2; Transfer-messenger RNA, tmRNA; 2-[5-(2-{[4-(2-thienyl)-2-pyrimidinyl] sulfanyl}acetyl)- 2-thienyl]acetic acid, TPSA; N-(3-methoxyphenyl)-7-nitrobenzo[c] [1,2,5]oxadiazol-4-amine, JSF-2164; 2-(o-Tolyloxy)-5-hexylphenol, PT70; Indenedione, BRD-9942; Thymidine kinases, TK; ATP binding cassette, ABC; Resistance-nodulation-cell division, RND; Proton motive force, PMF; Phenylalanyl arginyl β-naphthylamide, PAβN; 2, 2, 2-trifluoro-1-(1, 4, 5, 6-tetrahydropyridin-3-yl) ethanone derivative, NUNL02; Isocitratelyase, ICL; 6-hydroxymethyl-7, 8-dihydropteroate synthase, DHPS; B-(1, 2, 4-Triazole-3-yl)-DL-alanine, DLA; Tryptophan synthases, TRPS; Thymidylate synthase A, ThyA; 3-Deoxy-D-arabinose-heptulosonate-7-phosphate synthase, aroG; 5-chloro–N-(pyridine-2-yl)-1, 3-thiazol-2-yl] thiophene-2-carboxamide), IMB-T130; 3-dehydrogenate synthase, DHQS.

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https://doi.org/10.1016/j.ijbiomac.2023.125022
Received 20 January 2023; Received in revised form 19 May 2023; Accepted 20 May 2023
Available online 25 May 2023
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1. Introduction

Tuberculosis (TB), a deadly disease caused by *Mycobacterium tuberculosis (Mtb)*, kills about two million people each year, yet no promising newer therapeutic options has been introduced in the past 50 years. According to WHO, Ending TB is not only a priority of public health importance, but also there is a prominent developmental challenge. The COVID-19 pandemic drastically impacted TB diagnosis and treatment, resulting in 1.5 million deaths due to TB in 2020 compared to 1.4 million in 2019 [1]. It can be attributed to the possible break down of active TB during post-COVID-19 infection when there is a significant reduction of $CD4^+$ T cells, which are otherwise a key immune defender against *Mtb* [2]. The evolution of selective drug candidates against *Mtb* with the complete understanding of its genome sequence has led to the advancement in the target-based approaches for drug discovery. However, most of the drug candidates are failed before advancing in to a stage of clinical trials and unsuccessful in translating therapeutically, which is the major disadvantage in this field. Limitations in finding suitable animal models or *in vitro* models in the drug discovery processes are still a major concern. The emergence of multidrug (MDR) and extensively drug-resistant (XDR) tuberculosis are challenges in the field though new combinations of drugs and new drug candidates in the anti-TB drugs pipeline stand in a better position than in the last decade. In TB medication, prolonged treatment duration, adverse drug reactions of some major anti-TB drugs, and the co-occurrences of morbidities such as HIV and diabetes are significant downsides. This demands a critical thinking in new drug discovery, development and validation. Global Alliances for Tuberculosis Drug Development is one of the significant leaders in developing new TB regimens globally. The mobilization of resources and improvement in understanding the catalytic mechanism of protein synthesis in *Mtb* is crucial in identifying suitable targets for the development of new therapeutic molecules. Primarily the enzyme-catalyzed reactions help in hypothesizing and formulating the different types of inhibitors depending on the target. The enzymes and the cofactors in the metabolic pathway are attractive drug targets as they exhibit significant specificity in achieving antimycobacterial activity [3]. This narrative review is a comprehension on the drug candidates and inhibitors currently reported in the anti-TB drug discovery and development pipelines, with specific emphasize on protein targets of the cell wall, nucleic acids, efflux pumps, virulence pathways and metabolic enzymes of *Mtb*.

2. Drug targets and inhibitors of *Mtb* cell wall biosynthesis

The highly complex hydrophobic cell wall is vital for the survival and pathogenesis of *Mtb*. Three covalently interlinked layers of cell wall components form a natural barricade which regulates the movement of hydrophilic and hydrophobic compounds, including drugs [4]. The inner peptidoglycan (PG) layer, middle arabinogalactan (AG) layer and outer mycolic acid (MA) layer, which in turn is overlaid with a variety of polypeptides and glycolipids, gives resistance to several drug candidates. The protein targets involved in the synthesis and metabolism of this mycolyl arabinoglycan peptidoglycan layer are very important and hence given a major focus in this review.

2.1. Peptidoglycan (PG) layer

Linear glycan chain with alternating residues of N acetyl glucosamine (GlcNAc) /N Acetyl Muramic acid (MurNAc) arranged in β 1, 4 configurations to form the PG layer. It is highly cross-linked and provides anchoring sites for other cell wall elements. The steps involved in PG biosynthesis and their respective inhibitors are illustrated in Fig. 1.

2.1.1. Peptidoglycan biosynthesis - cytoplasmic pathways

N-acetylglucosamine-1-phosphate uridyl transferase (GlmU), a bifunctional enzyme with acetyltransferase (C terminal) and uridyl

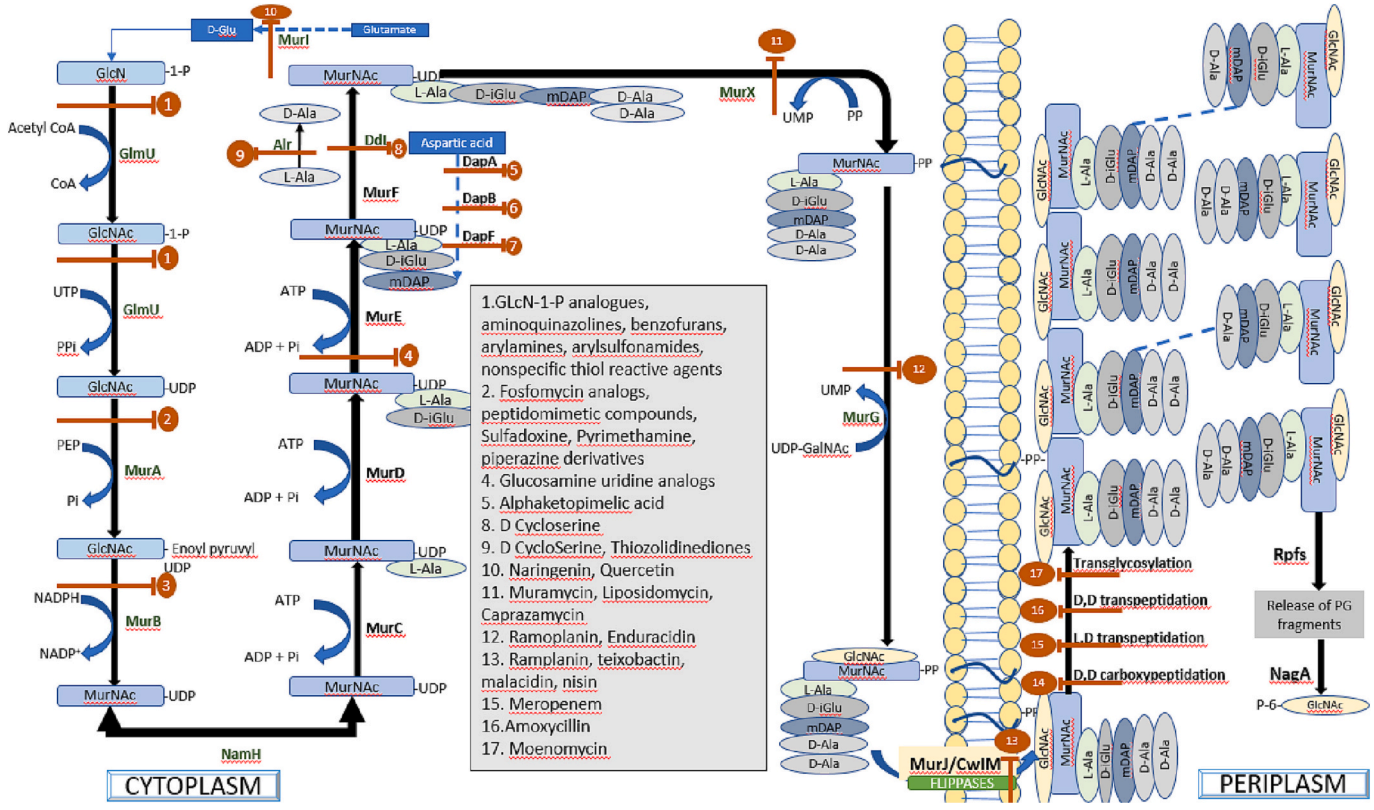

Fig. 1. Schematic representation of potential targets and their inhibitors of peptidoglycan biosynthesis in *Mtb*. The inhibitors of specific enzyme targets marked as orange ovals with respective numbers within.

transferase (N terminal domain) activity is auto-regulated by protein kinases at the acetyltransferase domain. The GlmU with Interleukin-8 accelerates the infection by means of mycobacterial attachment to neutrophils [5]. The uridyl transferase and acetyl transferase activities are crucial for mycobacterium as this enzyme uses *N*-acetylglucosamine-1-phosphate (GlcNAc-1-P) which is a common substrate in the host and *Mtb*. On the other hand, glucosamine-1-phosphate (GlcN-1-P) is absent in humans, which facilitates its potency as a drug target against *Mtb*. The GLcN-1-P analogues target the acetyltransferase domain of GlmU and also exhibit demonstrated anti-TB activities. In Table 1, few of the attractive protein targets of *Mtb* cell wall biosynthesis is presented [6].

The UDP-*N*-acetyl glucosamine enol pyruvyl transferase (MurA) and UDP-*N*-acetyl enol pyruvyl-glucosamine reductase (MurB) catalyzes the conversion of UDP-*N*-acetyl glucosamine (UDPGlcNAc) to UDP-*N*-acetylmuramate (UDP-MurNAc). A cytoplasmic protein, CwlM (a hypothetical amidase), undergoes covalent modification by phosphorylation to regulate the activity of MurA. In the presence of adequate nutrients, the CwlM gets phosphorylated and stimulate the activity of MurA by nearly 30 folds. Therefore, inhibiting *Mtb* colony formation by targeting CwIM and MurA may help in reducing the treatment duration [7]. The MurB enzyme is a potential target for drug development as it is crucial for bacterial cell viability. Further, the absence of MurB or its homologue in eukaryotes makes it a specific target. *N*-acetyl muramic acid hydroxylase (NamH) produces UDPN-glycolylmuramic acid (UDP-MurNAc/Glyc) by hydroxylation of UDP-MurNAc. The NamH imparts unique structural modifications to *Mtb* PG, which provides the ability to withstand lysozymatic degradation of the PG layer [4]. The Mur C—F pathway proteins (ATP-dependent ligases) are coded by single-copy genes and are unique to prokaryotes and are attractive therapeutic targets to prevent the growth of microorganisms [8]. The MurI, a glutamate racemase, provides the substrate (D-Glu) necessary for PG synthesis. Besides a consistently low cellular D-glutamate, the poor catalytic activity of MurI makes the enzyme highly susceptible for inhibition [9].

Meso-diaminopimelic acid (mDAP) is a core component of the PG linkages. It is synthesized through the metabolic pathway of aspartic acid which is absent in humans. This pathway is also involved in the synthesis of lysine, threonine, isoleucine and methionine. Aspartate semi-aldehyde dehydrogenase (ASADH) is a worthy drug target as it is an essential enzyme for the growth and pathogenicity of *Mtb* [10]. The alpha ketopimelic acid is another drug target in the pathway which inhibits the activity of dihydrodipicolinate synthase (DapA) [5]. Wang et al., reported an excellent lead compound, IMB-XMA0038 which could inhibit *Mtb* ASADH [10]. The DapB enzyme is an important member in the biosynthetic pathway of mDAP. The DAP epimerase (DapF) is involved in the interconversion of L-DAP and meso-DAP, belongs to the subfamily of epimerases known as pyridoxal phosphate–independent amino acid racemases in *Mtb* [11]. Choudary et al., 2020 has generated ligand based pharmacophores to block the mDAP synthesis, thereby disrupt the lysine biosynthesis [12].

The D-alanine ligase enzyme (Ddl) is required to ligate alanine residues in the peptide stem of PG back bone. The D-Cycloserine (DCS) is known to target Ddl by competitive inhibition primarily and disrupts PG synthesis by forming an irreversible adducts with isoxazole and oxime [13]. Despite the proven bactericidal activity of D-cycloserine against MDR and XDR strains of *Mtb*, the associated adverse effects limit its usage as a last option [4]. A novel competitive inhibitor of DdlA, IMB-0283 exhibits similar extent of antimycobacterial potency against both standard and clinical drug-resistant *Mtb* strains. There were 238 analogues of IMB-0283, but a little is known about their biological activity [5,14].

Table 1
Attractive protein targets of *Mtb* cell wall synthesis.

Site	Feasible targets	References
Cell wall PG	DacB2 (DD-carboxypeptidase), Resuscitation promoting factor (Rpf), (rpfA-rpfE), *Mtb*nagA	[5]
Cell wall Arabinogalactan	Glucose-1-phosphate thymidyl transferase (RmlA), dTDP-d glucose 4,6-dehydratase (RmlB), dTDP-6-deoxy-d-xylo-4-hexulose 3,5-epimerase (RmlC) and dTDP-6-deoxyd-xylo-4-hexulose reductase (RmlD)	[30]
Cell wall Mycolic Acid	MmpL3	[4]

2.1.2. Peptidoglycan biosynthesis – pathways associated with the membrane

In *Mtb,* the lipid carriers transport the cytoplasmic products of PG synthesis across the cell membrane. Lipid carrier synthesized as decaprenyl diphosphate in the cytoplasm is dephosphorylated by decaprenyl pyrophosphate phosphatase before releasing it into the periplasmic phase of the membrane [5]. Muramycin, liposidomycin, caprazamycin, sansanamycin and capuramycin are peptide scaffolds which can bind to different sites on the bacterial translocase 1 (MurX), which is involved in the synthesis of Lipid I (Fig. 1) [4,15]. *N*-acetyl glucosamine transferase (MurG) catalyzes lipid II synthesis. The GatD and MurT are binary protein complexes, and their primary function is to amidate the PG layer. The MurT-GatD complex and AsnB amidated Lipid II stimulates other PG cross-linking and hence most favorable drug targets [5]. MurJ is also involved in the translocation of Lipid II from the cytoplasm [6].

2.1.3. Peptidoglycan biosynthesis: potential targets during maturation

The lipid II further undergo polymerization in the periplasmic space to form mature PG. Penicillin-binding proteins (PBP) like transglycosylases and transpeptidases catalyze the formation of mature PG chains with D-alanyl-D-alanine (DD) cross-links in the periplasm. Tetrapeptide products formed subsequently act as substrates for L-lysyl-D-alanine (LD) trans peptidases [4]. Classical DD cross-links (4 → 3) in the mycobacterial PG can change to non-classical LD cross-links (3 → 3) due to the pressure experienced through environmental and growth conditions, resulting in resistance against these enzymes. Covalent adducts formed by LD-transpeptidases, LdtMt1, with the β-lactamase drugs result in its inhibition [14]. Emerging evidence suggests that carbapenems irreversibly inhibit L, D - transpeptidases by covalently binding to the active site of cysteine [6].

2.2. Arabinogalactan (AG) layer

Arabinogalactan (AG), composed of D-arabino furanose (Araf) and D-galacto furanose (Galf), makes the middle layer of the cell wall of *Mtb*. The backbone of this layer is a linear chain of about thirty units of β-D-Galf, and from 3 separate chains of approximately 23 Araf units originate at the 8th, 10th and 12th position, respectively [4,6]. The drug targets and their inhibitors in the arabinogalactan biosynthetic pathway are illustrated in Fig. 2.

GlcNAc-1-P transferase (WecA) catalyzes the synthesis of PG linker moiety (C50-P-P-GlcNAc-L-Rha) from GlcNAc-1-P, which is the initial substrate. WecA is inhibited by CPZEN-45, a derivative of caprazamycin [6] shown to arrest the growth of dividing and non-dividing *Mtbs* in both drug sensitive- drug resistant murine models. Rhamnosyl transferase (WbbL) synthesizes PG linker moiety (C50-P-P-GlcNAc-L-Rha) from GlcNAc-1-P. The UDP Galf derivatives or iminopentitol derivatives are reported to cause inhibitory activity of WbbL and thereby results in premature galactan chain termination [6]. The UDP Galf derivatives or iminopentitol derivatives inhibit galactofuranosyl transferase enzymes (GlfTquino1 and GlfT2) and the steps involved in the arabinogalactan synthesis is presented in Fig. 2 [4,6,17].

As seen in Fig. 2, DprE1 converts DPR to decaprenylphosphoryl-2-ketoribose (DPX) and DprE2 reduces it to DPA [18]. DprE1 inhibitors can be divided into covalent and noncovalent inhibitors, of which compounds acting by covalent mode are discussed hereafter.

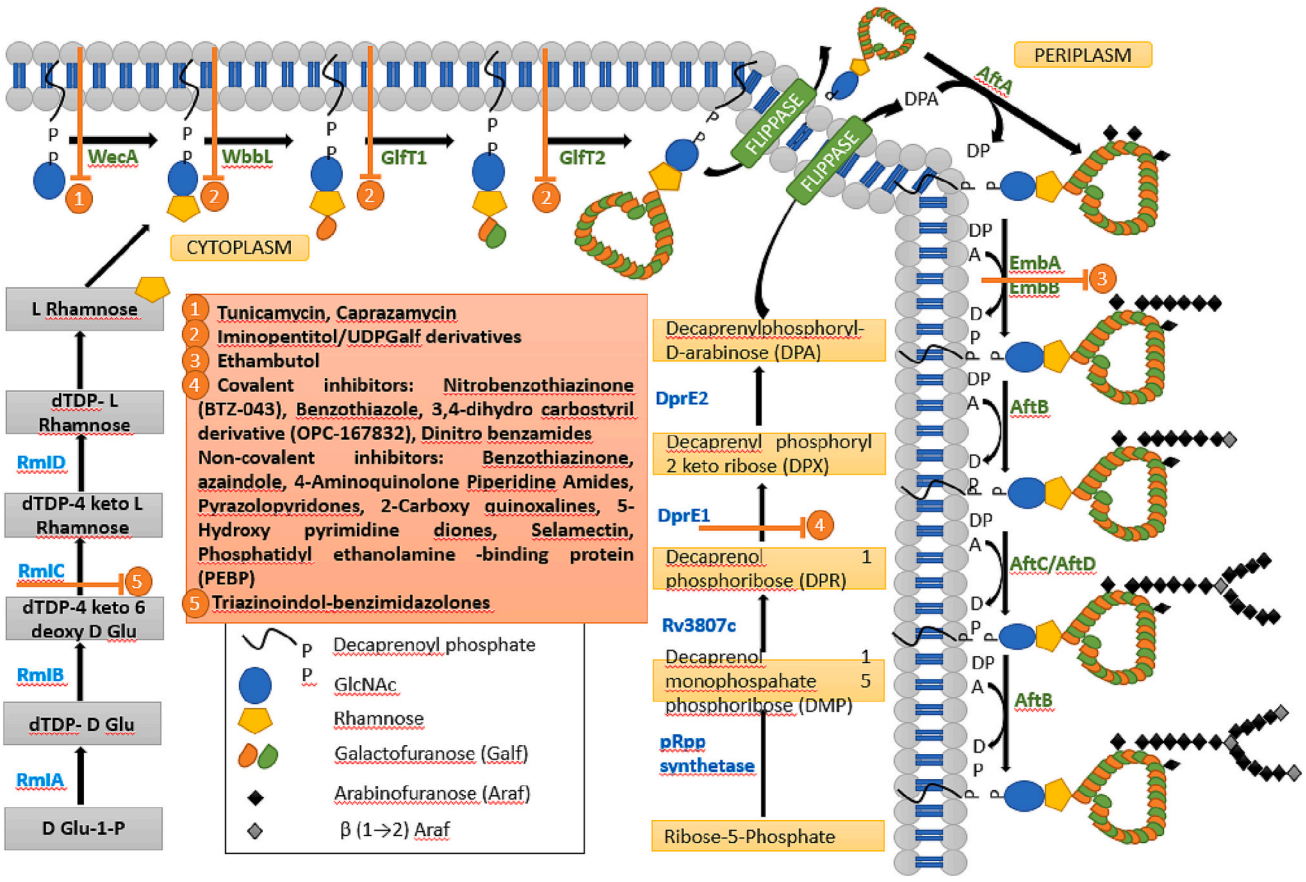

Fig. 2. Schematic representation of potential targets and their inhibitors in the arabinogalactan biosynthesis of *Mtb*. The inhibitors of specific enzyme targets in cytoplasm (blue) and periplasm (green) marked as orange ovals with respective numbers within.

Nitrobenzothiazinone (BTZ-043) inhibits replicating and non-replicating *Mtb* by acting on DprE1. The catalytic pocket of DprE1 contains $FADH_2$ generated by the oxidation of the substrate.

When compared to the standard drug regimen, OPC167832 (MIC 0.24–2 ng/mL) in combination with delamanid showed improved effectiveness *in vivo* [4]. The dinitrobenzamides (DNB) inhibit DPA synthesis, which is supported by the fact that BTZ-resistant DprE1 mutants were also resistant to DNBs [16]. Modification of DNB by the addition of nitro group in the benzene ring resulted in the formation of two compounds, namely DNB1 [N-(2-(4-methoxyphenoxyl) ethyl)-3, 5-dinitrobenzamide] and DNB2 [N-(2-(benzyloxy) ethyl)-3, 5-dinitrobenzamide]. These were found to be highly effective on MDR and XDR strains of *Mtb* [19].

Noncovalent inhibitors of DprE1 such as, benzothiazinone exhibit similar effects to BTZ under *in vitro* conditions, however the effectiveness to be proved in animal models. TBA-7371, a 4-azaindole with MIC-0.78-3.12 mM against *Mtb*, is found to have non-covalent interactions and covalent binding to DprE1. The safety, tolerability, pharmacokinetics and drug-drug interactions of TBI-7371 are studied in a phase I trial [4,20].

An extensive screening of a commercial database of AstraZeneca (320,000 compounds) resulted in the identification of aminoquinolone scaffold that showed excellent antimycobacterial properties. An increased potency was exhibited by 6-and 6, 7- fluorinated analogues of 4-aminoquinolone piperidine amide. Another stereo-selective azabicyclo octanyl ring (piperidine modification) demonstrated at least tenfold enhancement in antitubercular activity [10]. Replicating *Mtb* was sensitive to pyrazolopyridone when compared to non-replicating bacteria. Modifications of pyrazolopyridones, like methylation of secondary amine linker, the addition of phenyl group, cyclopropyl group and trifluoromethyl group, were more efficient [9]. *In vitro* experiments showed that selamectin treated with DprE1 in a loop that included Leu275 and can act as a multi-target antimycobacterial compound [21]. In addition, rhamnose is a vital component of the mycobacterial cell wall and it is absent in humans, therefore it could be a potential druggable candidate (Table 1).

2.3. Mycolic acid (MA) layer

Mycolic acid (MA) (2-alkyl, 3-hydroxy long-chain (C54 to C63) fatty acids) layer constitutes the hydrophobic outer lipid layer of the mycobacterial cell wall. This impermeable layer is the hallmark of the cell wall, and it controls the passage of molecules across it [4]. MA in *Mtb* exists as alpha (70 %), methoxy (10–15 %), and keto-mycolic acids (10–15 %) [6]. Fatty acid synthases I and II (FAS I and FAS II) are involved in the synthesis of MA. The products of FASI and FASII pathways are combined to produce the MA, which is then transported across the cell membrane [4] as illustrated in Fig. 3.

2.3.1. Alpha mycolic acid inhibitors

Acyl-CoA carboxylases (ACCase) help in the biosynthesis of MAs from acyl-CoAs. There are six subclasses of ACCase in *Mtb* (AccD1–6), among which AccD4, AccD5, and AccD6 are significant for the cell envelope lipid biosynthesis. Propionyl-CoA is the preferred substrate for AccD5 to produce methyl malonyl-CoA, which leads to the further synthesis of multi methyl-branched fatty acids. NCI-65828 ligand was found to be an effective inhibitor of AccD5 through *in silico* screening procedures. Docking studies of NCI-65828 and AccD5 showed that NCI-65828 adhere with the binding motif of acyl-CoA effectively [22].

In addition, a phenotypic screening with activity-based protein

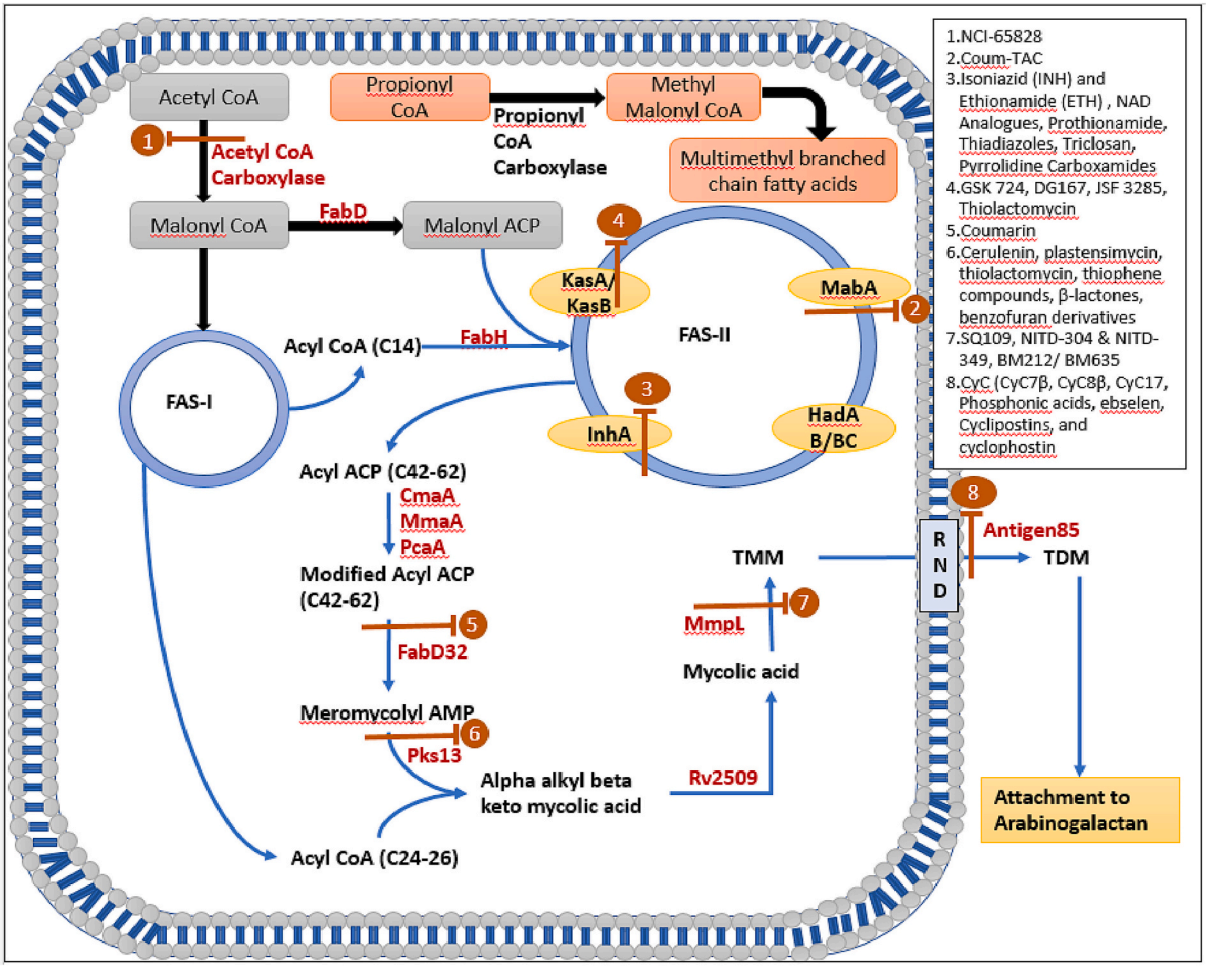

Fig. 3. Schematic representation of molecular targets and their potential inhibitors in mycolic biosynthesis of Mtb. The inhibitors of specific enzyme targets marked as orange ovals with respective numbers within.

profiling was performed to locate the serine hydrolase targets for 1, 2, 3-triazole ureas that restrict *Mtb* growth *in vitro*. Out of 200 screened compounds, 4 compound have turned out to be potential targets for inhibiting the *Mtb* growth and viability, as evident from a novel behaviour in activity-based protein profiling (ABPP) study [23].

During antibiotic stress, FabG is the major protein expressed in *Mtb,* FabG1 and FabG4 (Fig. 3) are conserved among mycobacterial species and they are known for their NADH binding sites. Triazole polyphenol hybrid compounds compete for NADH binding sites of Fab. Beta-Lactam isoniazid-based compounds bind subsequently with two NADH binding sites, thus acting as a dual inhibitor. S enantiomer of thiophene with tri substituted methane works synergistically with rifampicin. InhA inhibitors like pyridomycin can also inhibit FabG4 [24]. Fatty Acid Synthase Type II Dehydratase, HadA component of FASII, is inhibited by Coum-TAC, a novel coumarin-based molecule, through "dual covalent inhibition". The usage of Coum-TAC as an imaging probe for labelling *Mtb* also depend on this interaction [25]. In addition, an active compound, 10A25 was designed as an anti-mycobacterial lead that bind to the enoyl reductase (InhA) PDB - 4TZK and peptide deformylase PDB - 3E3U of *Mtb* [26].

Isonicotinic acid and isonicotinamide were evaluated for their anti-mycobacterial effects against the drug susceptible strain. Docking simulation using enoyl ACP reductase (InhA) with isonicotinic acid, and isonicotinamide revealed favorable ead compound NITD-916 directly blocked InhA in a dose-dependent manner and showed *in vivo* efficacy in acute and established mouse models of *Mycobacterium tuberculosis* infection. Collectively, our structural and biochemical data open up new avenues for rational structure-guided optimization of the 4-hydroxy-2-pyridone class of compounds for the treatment of MDR-TB protein-ligand interactions. The *in silico* pharmacokinetics and hepatotoxicity experiments predicted that the derivatives of isonicotinic acid were with better oral bioavailability and lesser hepatoxicity than isoniazid [27]. A novel ligand screening approach identified 4-aminoquinolines as a potential inhibitor (87 fold) of NADH-dependent enoyl-acyl carrier protein reductase (MtInhA) and an effective growth inhibitor against H37Rv (32 fold inhibition) with no genotoxicity and acute systemic toxicity in a murine model of TB [28].

Meromycolate extension acyl carrier protein (AcpM) shuttles the fatty acyl chain among the components of FAS II system. InhA (enoyl-ACP reductase) reduces trans-2-enoyl-AcpM intermediate to form C18-AcpM. The first-line anti-TB drugs isoniazid and ethionamide have to be activated by KatG or EthA, respectively, further to generate reactive oxygen species (ROS) [30]. It is been reported elsewhere, thiadiazolyl methyl thiazoles, a novel tetracyclic thiadiazole-based compound, as an inhibitor of InhA, which was further validated based on its mechanism of action and revealed its binding mode in the active site [8]. Isoniazid-resistant strains (promoter mutation at inhA) can be targeted using tetrahydro pyranyl methyl benzamides [29].

Derivatives of quinazolinone-triazole hybrids are developed using 2-aminobenzamide and carbonyls affording 2,3-dihydroquinazolin-4(1H)-ones with a promising antimicrobial activity. Researchers have also found that three first line anti-TB drugs (INH, RIF, and EMB) are found to interact synergistically with quinazolinone-triazol. They also performed molecular docking studies to identify the target compounds with the

active sites of 1DQY(antigen 85C) from Mtb and Enoyl acyl carrier protein reductase (InhA) enzymes, confirming quinazolinone-triazole hybrids framework can be useful in drug development for TB [31].

Mutations in KatG inhibit the formation of the isoniazid-NAD adduct which results in resistance to isoniazid. Therefore, drugs that can directly inhibit InhA are beneficial in preventing the development of high resistance frequencies [8]. Triclosan (TCN) is a broad-spectrum antibacterial agent which targets InhA in pre activation stage [29]. InhA forms hydrogen bonding with the A-ring of triclosan and NADP cofactor. Orthogonally configured chlorine atoms of B-ring and A-ring, makes hydrophobic interactions at InhA binding site [30]. Diphenyl ethers, a novel direct inhibitor of InhA work similarly to the mechanism of action of TCN [29]. Abdelaziz et al. 2022, have explored TCN as an inhibitor for enoyl acyl carrier protein reductase InhA enzyme in susceptible and resistant Mtb strains [32].

Pyrrolidine carboxamides are most potent direct inhibitors of InhA. *In silico* studies showed that the carbonyl group of pyrrolidine forms hydrogen bonds with the hydroxyl group of Tyr158 and nicotinamide [29]. The 4-hydroxy-2-pyridine (NITD-916) forms adducts with InhA and NADH to slow down the mycolic acid synthesis and thereby cause cell death [33]. In mouse model, NITD-916 inhibit the Mtb by blocking InhA in a dose dependent manner. This has created avenue in exploring 4-hydroxy-2-pyridone compounds in the treatment of MDR-TB [34].

The lead diazaborine, AN12855 (ethylsulfonyl0000000 benzodiazaborine), is also a direct InhA inhibitor which binds to the substrate-binding site of InhA [35–37]. SQ109 is a 1, 2-ethylene diamine that effectively inhibits drug-sensitive and resistant *Mtb*. It works along with combination regimens with reduced treatment time. Studies on the

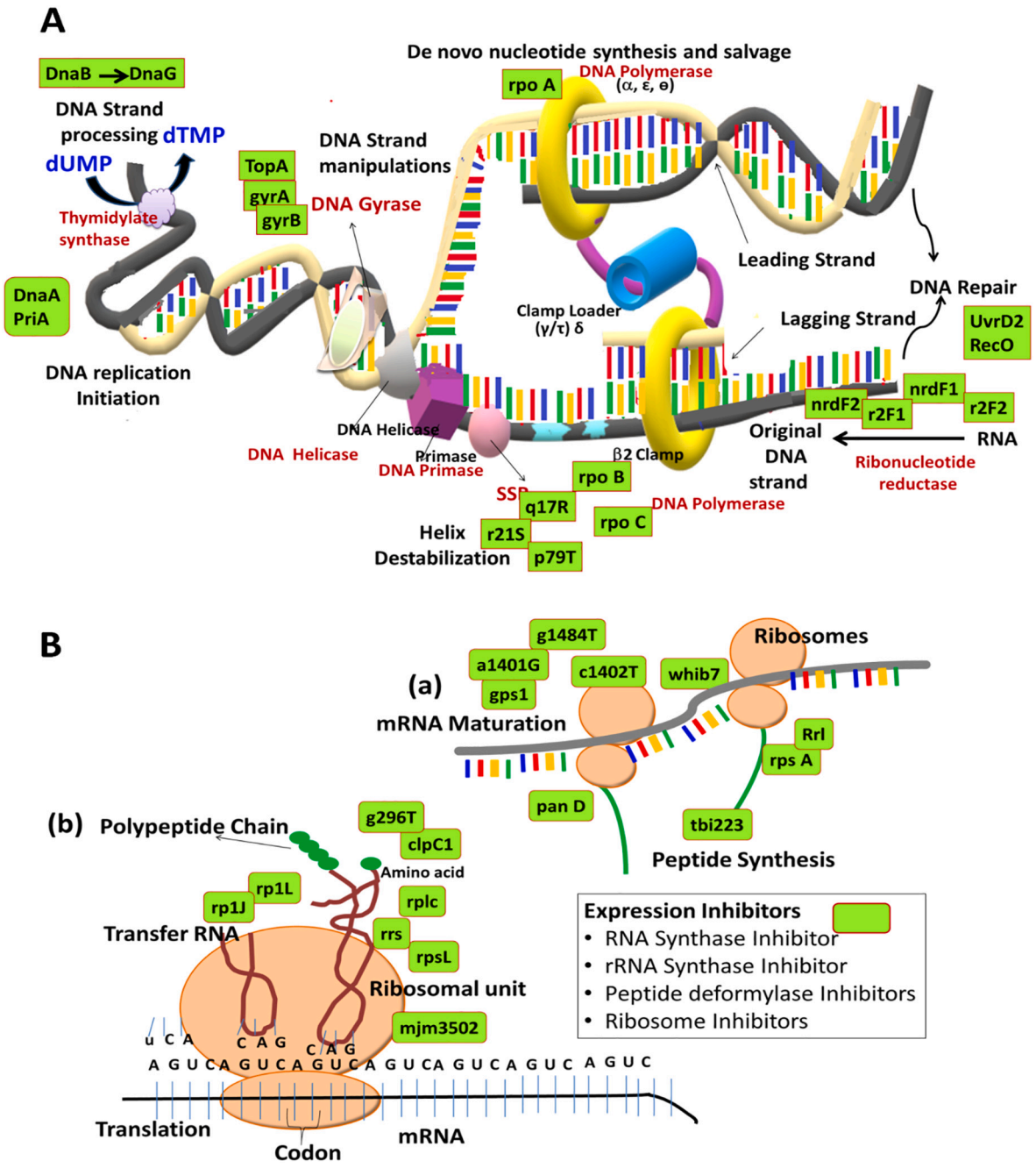

Fig. 4. Drug targets and inhibitors of *Mtb* in DNA replication and protein synthesis. [The expression vectors are represented in green rectangle boxes] A) Nucleotide synthesis in *Mtb;* B (a) mRNA maturation and (b) Translation with polypeptide subunit.

mechanism of action revealed that MmpL3 is one of the targets of SQ109 and is presumed to accept protons, thereby dissipating the proton gradient [39].

Antigen 85 converts trehalose monomycolate to trehalose dimycolate before being attached to the arabinogalactan layer. The three essential homologs of the antigen 85 complex are Ag85A, Ag85B, and Ag85C. Another vital function of Ag85C is to inhibit phagolysosome formation, thereby evading the host immune response. Mutations in Ag85C resulted in a 40 % reduction of mycolic acid production in the cell wall. Cyclipostins and cyclophostin analogues (CyC7β, CyC8β, and CyC17), phosphonic acids, ebselen, and cyclophilin are some compounds which inhibit Ag85C [6].

2.3.2. Methoxy and keto mycolic acid inhibitors

Delamanid is a bicyclic nitroimidazole compound, approved by the European Medical Agency shown to inhibit the formation of methoxy and keto mycolic acid. Clinical trials have proved its efficiency against pulmonary MDR-TB with a good safety profile, except for the extension of QTcF interval in electrocardiogram [4]. Pretomanid (PA824) is a nitro imidazole scaffold inhibiting the synthesis of keto mycolic acid from hydroxyl mycolate in aerobic conditions and induces cellular respiratory poisoning with the help of reactive nitrogen species under hypoxia. US FDA has approved BPaL (Bedaquiline, Pretomanid, and Linezolid) drug regimen to treat patients with MDR or XDR TB [4].

3. Inhibitors of DNA metabolism and protein synthesis in *Mtb*

Mtb genome encodes 4.4 × 10^4 base pairs estimated with gene density of about 91 % and an average length of about 1002 bases per gene. The intricacies in mechanisms of genetic alterations and drug resistance in *Mtb* helps to explore new drug target and lead to anti-TB drug discovery [40]. Most drug targets are proteins from DNA metabolism, which provide essential biochemical and structural functions (Fig. 4). In the microbial species, the chromosomal replication are found to be conserved in all species leaving a few variations in the mycobacterium [41].

3.1. Target and inhibitors in DNA replication systems of Mtb

In the case of DNA replication in *Mtb*, the chemotherapeutic approach has not been extensively examined except for fluoroquinolones which are responsible for DNA unwinding [42]. In mycobacterium species, DnaA and DnaB remain crucial for unwinding the double helical strand during the cell cycle. The presence of oriC and the insertion of transposable elements (IS6110) act as a marker for Restriction Fragment Length Polymorphism fingerprinting similar to *E. coli* sp. [43]. The non-functional DnaC (helicase loader) and DnaX (possess θ, χ, or ψ subunits) are responsible for the STOP codon leading to ribosomal leakage. The clamp loader subunit that connects SSB on the lagging strand (Okazaki fragments) is a suitable target for antimycobacterial activity. The fulfilment of DnaG primase leads to short RNA primers, further for the activation of Polymerase III in the transcription process. An important gene involved in the *Mtb* gene repair mechanism is the *ogt* gene which repairs the alkylated DNA [44]. This gene alters the O6-alkyl group in the C-terminal domain in the active site, promoting degradation of the protein. Inhibition of such repair mechanisms leads to genomic instability in the case of *Mtb*.

Anti-folate drugs such as plant polyphenols used to inhibit Dihydrofolate reductase (DHFR) were found to be a promising drug target in *Mtb* [45]. Polyphenols such as catechin, epigallocatechin gallate, and curcumin have shown to be the best anti-folate agents *in vitro* [46]. In *Mtb*, the ribonucleotide reductases (RNRs) are the targets for biocidal activity [47,48] and R2-2 is the enzymatically active form of RNR [49,50]. Hydroxyurea was found to create hypersensitive reactions to the RNR complex, and the finding has helped in developing new inhibitors from derivatives of hydro urea and its complexes [51].

Nitro-BTZ enters the catalytic pocket and the nitro group gets reduced by $FADH_2$ to the nitroso group, forming a covalent bond with Cys387 residue [13]. BTZ-043 such as PBTZ169 (Macozinone- a piperazino benzothiazinone), is proven to be active against both MDR and XDR-*Mtb* strains, and it is now in Phase II clinical trials with acceptable toxicity and safety profile with better pharmacodynamics (MIC <0.19 ng/mL) [52].

This mechanism of action of the antimycobacterial effects of PBTZ169 is similar to nitro-BTZ. US FDA has sanctioned a fast-track approval to 3, 4-dihydrocarbostyril derivative (OPC-167832), and it is now in Phase I/II clinical trials with drug-sensitive pulmonary TB patients. More recently, PBTZ169 is been identified as potential inhibitor of ecaprenylphospho-β-d-ribofuranose 2-oxidase. Further, BM212 and its derivatives were identified as potential inhibitors of mycobacterial membrane protein, Large 3 and benzofuran TAM16, which is necessary for polyketide synthase (Pks13) [53].

3.2. Targets and inhibitors of transcription process

Mtb transcription initiation complexes such as Rpo are facilitated by transcription factors such as CarD and RbpA (Fig. 4). CarD is the crucial mycobacterial protein which controls the rRNA transcription by binding to the beta subunit of RNA polymerase (RNAP). In *Mtb,* loss of CarD due to the increased oxidative stress and DNA damage results in rRNA transcription failure [54]. This factor, CarD, interferes throughout the DNA bubble formation and does not cause RNAP recruitment at a promoter for unwinding. The RbpA is a transcription activator with the C terminal region known to bind with the core domain (CD) and sigma interacting domain (SID). This transcription initiation complex with core domain forms RbpA-CD and acts as a motif with β subunit called the zinc-binding domain (ZBD) [55]. Hence, this RbpA interferes the core RNAP to increase its affinity for σ (A) domain and accelerate the competent promoter complexes, a novel target for inhibition of *Mtb* bacterial growth. These domains are placed on RNAP with an intervening base linker near the DNA backbone to start the transcription bubble. The mycobacterial RNA polymerase (RNAP) possesses a central core that takes part in transcription commencement and RNA elongation. The mycobacterial RNAP remains a target for many inhibitors, among which the rpoB gene is the target for rifampicin [56]. The rpoB gene combines with the beta-subunit of the RNAP, inhibiting the elongation of messenger RNA and, thereby, its transcription [57,58]. Specific mutations in the rpoB gene lead to repression in the rifampicin's binding affinity, creating resistance against *Mtb*.

The potential inhibition towards *Mtb* can also be achieved through mutation in the transcriptional regulator gene such as Rv0678 by MmpL5. These mutated gene act as targets for Clofazimine (a riminophenazine compound) and Bedaquiline, a diarylquinoline [59,60].

3.3. Targets and inhibitors in Mtb translation and protein synthesis

In protein synthesis, structure and function of mycobacterial ribosomes play a vital role in the translation regulation of cells. *Mtb* ribosomal proteins S14, S18, L28, and L33 are replaced with their non-zinc binding paralogues as target in low-zinc conditions [61].

In the process of translation, polypeptide chain are formed from the mRNA codons which is initiated by AUG start codon and decoded by the methionyl tRNA (Met-tRNA), followed by elongation step, a regulatory node for translation. As a rate-limiting step, mRNA recognition with the start codon results in Initiation complex (IC) placement with tRNA at the P site. The initiation factor (IF 1-3) facilitates the C-terminus extension to the elongation factors (EF-Tu, EF-TS and EFG) [62]. As a result of GTP hydrolysis, the carbonyl carbon of the P-site binds with the peptidyl moiety of the A-site of tRNA. Hydrolysis leads to translocation by EFR-G from the A site to the P site leading to EF-Tu-GTP. The extension leads to ribosomal A-site using stop codons.

Further, termination is facilitated by release factor (RF1 and RF2)

subjected to the PXT motif and SPF motif for UAA/UAG and UAA/UGA, direct to peptidyl-tRNA hydrolase domain (PTH) [63]. Ribosome recycling factor (RRF) extends protein synthesis using conjugation with EF-G and IF3. These translation factors are studied by the functional complexes leading to developing new drug targets.

The shikimate pathway plays a significant role in synthesizing amino acids (Fig. 5). Capreomycin, as a cyclic peptide antibiotic effectively used as a potential inhibitor of ribosomal proteins, L12 and L10, which are responsible for protein synthesis. Capreomycin, as an inhibitor, resists the elongation factor G-dependent GTPase activity and ribosome-mediated protein synthesis. The inhibitor capreomycin perturbs the deacetylated tRNA leaving behind the empty A site leading to the next amino-acylated tRNA. Thiostrepton inhibits the translocation by interfering with elongation factors (EF-G and EF-Tu) binding and GTP hydrolysis [64]. Yuan Lin (2014) reported that the MIC of capreomycin reflects the over-expression of L12 and L10, making it a possible target for capreomycin class of inhibitors [65]. *M. tuberculosis* ClpB has been characterized by Prashant singh et al., 2020, through high throughput malachite green-based screening assay and identified lead compounds with ClpB ATPase activity in a competitive manner [66].

ClpC1 (848-amino acids) protein is an excellent target for pyrazinamide, while mutation leads to resistance to the G296T gene. The new cyclic peptide antibiotics such as cyclosarin A, lassomycin (Q17R, R21S, and P79T) [63,67] and ecumicin (L92S or F or L96P sites) [68] bind at the different sites of the ClpC1. Moreover, gene Rv2783c (bifunctional enzyme) is a potential target involved in the metabolism of RNA, single-stranded DNA and signal transducer (ppGpp) [69]. The acyldepsi peptides are shown to be inhibitors which could effectively deregulate the ClpP protease [70]. In RNA translation, the rpsL gene (375-bp) encodes 30S ribosomal protein, and rrs gene (1537 bp) encodes 16S rRNA, respectively [71,72]. Streptomycin, an aminoglycoside which is shown to be effective in hindering the translation of protein synthesis. The functioning of ribosomes is retarded by the interaction of streptomycin in formyl-methionyl-tRNA, where the rpsL gene and rrs

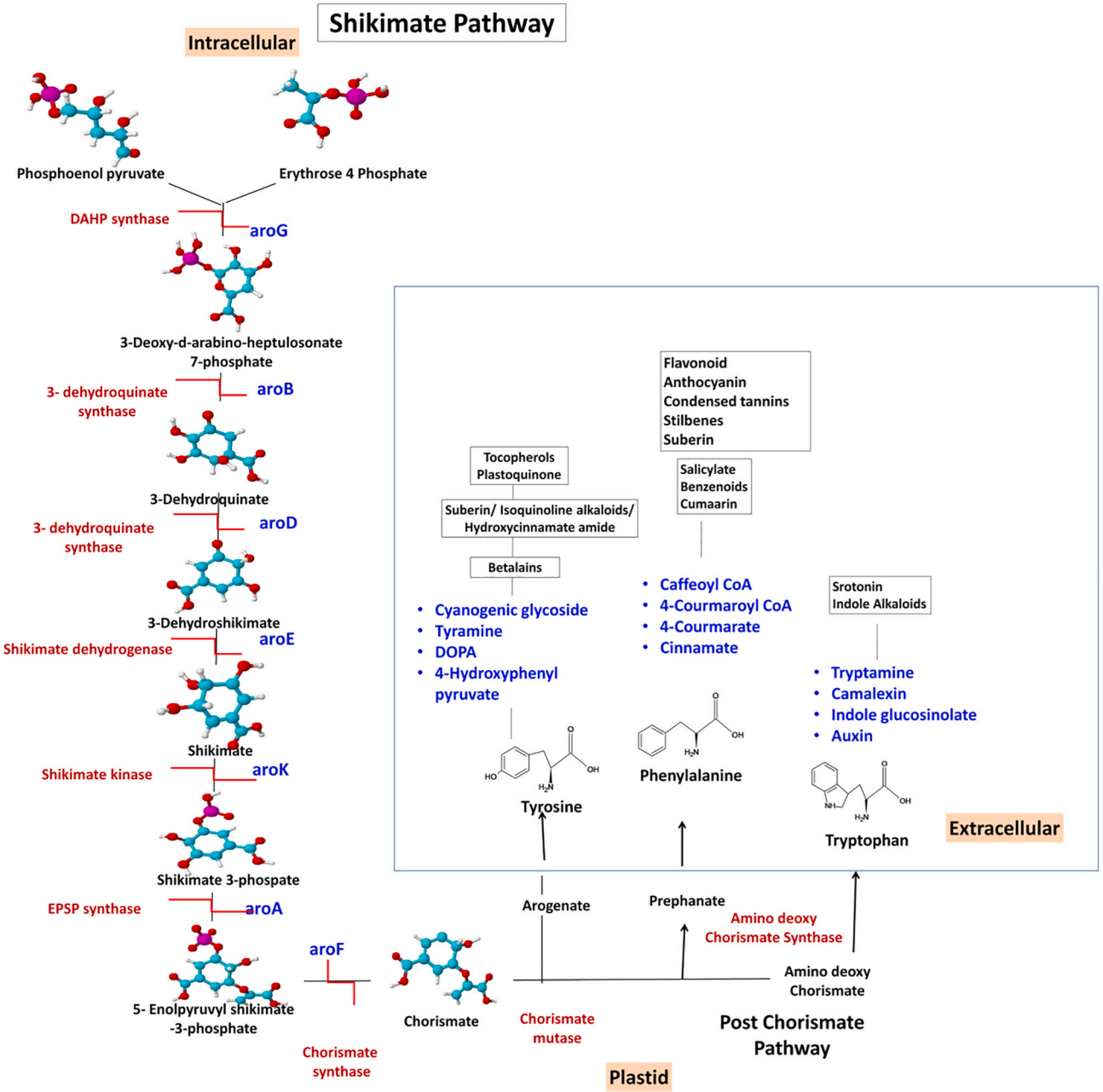

Fig. 5. Shikimate pathway with enzyme targets and respective inhibitors in both intracellular and extracellular spaces.

gene replaces the essential amino acids in 43 and 88 positions, respectively and there is a formation of streptomycin resistance [69]. Similarly, 7-methyl guanosine methyl transferase is specific for the 16S rRNA encoded gidB gene (675 bp), while its A80P mutation leads to resistance [73].

C1402T or G1484T genes inhibit *Mtb* strains using interaction with capreomycin, kanamycin and viomycin [74]. Mutation of C1402T or G1484T genes leads to resistance in the rrs gene (1400, 1401, and 1483 bp) [71]. Thiophene-based inhibitors specifically bind to DNA gyrase and induce cross-resistance to fluoroquinolones. These compounds act as a drug molecules binding allosterically with DNA gyrase enzyme; one such drug is SPR719, an amino benzimidazole [4]. In addition, Aubry et al., in 2004, explained the catalyzing efficiency of fluoroquinolones in the supercoiling of DNA [75]. The targeting sites such as Ala-74, Gly-88, Ala-90, Ser-91, and Asp-94 of gyrA act as interaction sites of fluoroquinolone, while mutation leads to its resistance [76]. GyrB inhibitors such as SPR-720 and SPR-750, act as potential drugs showing antimycobacterial effects. In addition, this leads to the development and optimization of moxifloxacin (MOX), levofloxacin and gatifloxacin for targeting DNA Gyrase. Gene pncA (561-bp) forms pyrazinoic acid in mRNA translation and trans-translation using transfer-messenger RNA (tmRNA). The PSA gene is responsible for the disruption of the trans-translation in *Mtb* [77]. In parallel, a mutation in the pncA gene due to the deletion of alanine at 438 bp (C-terminus) of rpsA or its overexpression leads to resistance against pyrazinamide. Ribosomal peptidyl transferase enzyme encoded by rplC gene (654 bp) of 50S ribosomal L3 protein, rrl gene (3138 bp) encodes 23S ribosomal RNA. Other potential targets and inhibitors are listed in Table 2.

4. Efflux pump inhibitors

Among the five families of transport systems that operates in *Mtb,* the ATP binding cassette (ABC) superfamily is the primary active transporter. The major facilitator superfamily, the small multidrug resistance family, the resistance-nodulation-cell division superfamily (RND) and the multi antimicrobial extrusion protein family are the secondary active transporters which use proton motive force (PMF) for transportation [95]. Being strictly aerobic, *Mtb* uses PMF-driven pumps rather than ABC family pumps. Efflux pump inhibitors are increasingly used as adjuvant therapy in tuberculosis. Genes that code for the important efflux pumps in *Mtb* are mmpL7, p55, efpA, MMR, Rv1258c and Rv2459. Thioridazine, one of the significant phenothiazines, inhibits the potassium efflux pump of *Mtb* which leads to accumulation of potassium in the microbe. Entrapped potassium activates vacuolar ATPase and some dormant hydrolases, which destroy the organism.

The action of thioridazine on efflux pumps renders the bacterium susceptible to otherwise resistant antibiotics thus making thioridazine an excellent option for treating XDR TB [96]. On the other hand, arrhythmia and “torsade de pointes” are some of this drug's with significantly less common side effects [97]. Rv1258c is one of the putative efflux proteins in *Mtb*. Piperine, an alkaloid which is a trans-trans isomer of 1-piperoyl-piperidine, is found to inhibit clinically overexpressed Rv1258c. It also augmented the antimycobacterial activity and post-antibiotic effect of rifampicin when used in combination [98]. Berberine, an amphipathic alkaloid with structural similarity to piperine, augments the effect of co-administered antibiotics. The mechanism of action is mainly attributed to the competitive inhibition of the efflux pump by berberine and antibiotics [99].

Reserpine is yet another alkaloid which is a calcium channel antagonist, yet it has anti-psychotic properties, it is rarely used for anti-TB treatment [99]. Phenylalanyl arginyl β-naphthylamide (PAβN), an RND-type efflux pump inhibitor, demonstrated its ability to reduce the MIC of pyrazolone class of compounds. Inhibition of efflux activity and membrane destabilization action constitutes the mechanism of action of PAβN. Organosilicon (SILA) compounds are known inhibitors of multidrug-resistant efflux pumps of cancer cells. The *in vitro* results suggests that SILA 421 is an effective inhibitor of the efflux pumps of multidrug-resistant mycobacteria, hence it is considered as an adjuvant while treating MDR/XDR tuberculosis [100]. One major limitation of using efflux pump inhibitors is their toxicity profile. Research on efflux inhibitors led to the experimentation on hybrid molecules in which combination of known efflux pump inhibitor and Triazole were tested. Such molecules were suitable growth inhibitors as well as efflux pump inhibitors. With an acceptable cytotoxicity profile, they synergized well with the first-line and second-line anti-TB drugs and ethidium bromide [101]. Another promising prototype as an adjuvant for tuberculosis treatment is a tetra hydro pyridine derivative. 2, 2, 2-trifluoro-1-(1, 4, 5, 6-tetrahydropyridin-3-yl) ethanone derivative (NUNL02) acts as a putative efflux pump inhibitor which deprives the essential nutrients to kill the bacteria. It also works synergistically with rifampicin, as an efficient drug against MDR TB [102].

A study in an experimental TB model with bioequivalent doses of anti-TB drugs showed that verapamil blocks the efflux pumps of *Mtb* in a calcium-independent manner. Treatment with verapamil aid in the rapid clearance of *Mtb* from TB affected murine lungs. When combined with standard drugs, verapamil has not only accelerated the bactericidal activity of rifampicin but also achieved durable sterilization [103,104]. Other ion channel inhibitors like chlorpromazine, thioridazine, flupenthixol and haloperidol turned out to be less efficient when compared to verapamil [105]. Tetra dines an alkaloid with similar activity as that of verapamil. Tetra dine reduced the MIC of isoniazid and ethambutol in clinical *Mtb* strains. Spectinamide and p-glycoprotein transport inhibitors are other efflux pump inhibitors of *Mtb*.

5. Inhibitors of general metabolism in *Mtb*

5.1. Carbohydrate metabolism

Isocitratelyase (ICL) is an essential metabolic enzyme of *Mtb* in its dormant stage [106]. *In-vitro* studies on log and starve-phased cultures of *Mtb* confirmed 1-cyclopropyl-7-(3,5-dimethyl-4- (3-nitropropanoyl) piperazine-1-yl) -6-fluoro- 8-methoxy-4 -oxo-1,4- dihydroquinoline −3-carboxylic acid to be the most potent inhibitor of ICL of *Mtb*, the binding activities were further confirmed by docking studies [107]. After screening a collection of 124 mannich-base compounds, one compound (Ydcm67) showed excellent inhibitory activity against ICL [108]. Itaconate, a metabolic intermediate was demonstrated as a covalent inhibitor of ICL through conjugate addition at catalytic cysteine residue [109].

5.2. Amino acid metabolism

Alpha-isopropyl amine synthase is an enzyme in the leucine biosynthetic pathway, alpha -isopropyl malate synthase catalyzes the condensation of alpha-ketoisovalerate and acetyl coenzyme A to form alpha-isopropyl malate. Amino acid substitutions at the domain interface of a-IPMS affects substrate and allosteric inhibitor binding [110]. The 6-hydroxymethyl-7, 8-dihydropteroate synthase (DHPS) is a pivotal enzyme in the folate biosynthetic pathway. It is been reported through *in silico* docking approaches that the tryptophan-lysine dipeptide has a higher efficiency than sulfa drugs in inhibiting DHPS, hence, it can be a promising lead compound for further studies on drug discovery [111]. Indole- 3-glycerol phosphate synthase (IGPS) belongs to the tryptophan biosynthetic pathway. Site-directed mutagenesis studies revealed that ATB107 is a potent competitive inhibitor of the enzyme substrate with little toxicity [112]. The B-(1, 2, 4-triazole-3-yl)-DL-alanine (DLA) exhibited inhibitory effect on protein metabolic pathway of enzyme imidazole glycerol- phosphate dehydratase *in-vitro* [113]. The tryptophan biosynthetic pathway has been a crucial target for the survival of bacteria. The tryptophan synthases (TRPS) catalyze the metabolism of tryptophan by the process of indole-3-glycerol phosphate conversion, hence it could be a suitable target against *Mtb*. The 4-(((5-(3-colour

Table 2
Potential protein targets and their inhibitors of pathways of cell wall biosynthesis and DNA transcription in *Mtb*.

Site	Enzyme	Inhibitors	MIC	Reference
Cell wall peptido glycan	GlmU	Aminoquinazolines 2-phenylbenzofurans Arylamines Arylsulfonamides, Nonspecific thiol reactive agents Diterpenoids TPSA Amino2,3-dideoxy-3-fluoro-α-D-glucopyranosyl phosphate	5.1–11.9 μM	[6]
	GlmU	TPSA	66.5 μM	[6]
	GlmS	Anticapsin, AzaserineandAlbizzin 2-Amino-2-Deoxy-D-glucitol-6P		[7]
	MurA	Fosfomycinanalogs Peptidomimetic compounds Sulfonoxyanthranilic acid derivatives		[6]
	MurB	Sulfadoxine Pyrimethamine Piperazine derivatives Chloropicolinate amides		[6]
	MurE	Glucosamine uridine analogs acarbose		[8]
	MurI	Naringenin Quercetin (flavonoids)		[9]
	Alr	D-Cycloserine Thiazolidinediones		[5,6]
	MurX	Muramycin LiposidomycinCaprazamycin Sansanamycin Capuramycin	3 mg/mL	[15]
	MurG	Ramoplanin Enduracidin		[5]
	MurJ	Humimycin Beta-lactam potentiators		[6]
	Transglycosylase	Ramoplanin Enduracidin Teixobactin, Moenomycin		[4]
Cell wall arabinogalactan	DprE1 (covalent)	Triazoles, nitrobenzamide Benzoquinoxaline 2-carboxyquinoxaline Dinitrobenzamide	1.56 mg/mL	[6,38,78]
	DprE1 (non-covalent)	Thiophene(TCA1) Quinoxaline dinitrobenzamide Thiadiazole, 1,2,4 triazole 1,3 BTZ azide Benzothiazolyl pyrimidine 5 carboxamide 5-hydroxy pyrimidinediones and benzothiazole-thiophene	1 ng/mL	[6,38,78]
	RmlC	Triazinoindol-benzimidazolones,		[6]
Mycolic acid	InhA	JSF-2164, BRD-9942, Pyridomycin, Pyrroles, Acetamides, Aryl amides, Tetrahydropyran derivatives and Triazole		[6,37,79–81]
	KasA	Indazole sulphonamides (GSK 724, DG 167, JSF3285)		[12]
	KasB and FabH	Thiolactomycin (TLM), Platensimycin, Cerulenin		[9]
	FabD32	Coumarin and 2-quinolone derivative of coumarin		[6]
	Pks13	Pentafluorophenyl, Cerulenin, Plastensimycin, Thiolactomycin, Thiophene Compounds, B-Lactones, Benzofuran Derivatives Coumestan Compounds		[82]
	MmpL3	Indolcarboxamides like NITD-304 and NITD-349, BM212/ BM635 (1,3,5-trisubstituted pyrazoles),		[5]
DNA transcription	RNAP	Pseudouridimycin, Corallopyronin, Na-Aroyl-N-Aryl-Phenylalaninamides		[83,84]
	ClpC1 protease	Heptapeptides isolated from the *Streptomyces atratus strain* MJM3502, Rufomycin I		[85,86]
	ATP-dependent and NAD + -dependent ligases (Lig A)	Glycofuranosylated diamine		[87]
	Thymidine kinases (TK)	3- azidodeoxy thymidine mono phosphate		[88]
	rRNA	Delpazolid		[89]

(*continued on next page*)

Table 2 (*continued*)

Site	Enzyme	Inhibitors	MIC	Reference
	Aminoacyl-tRNA and Leucyl-tRNA synthase	Oxaboroles (GSK3036656)		[90,91]
	23S rRNA	Sutezolid,		[92,93]
		Sutezolid in combination with BDQ, Delaminid, and MOX, Oxazolidinone		[94]
		(LZD and SZD, TBI-223)		

–phenyl)-isoxazl-3-yl)-formylamino)-methyl)-benzamide has been found to resist the *Mtb* by binding to the alpha subunit of TRPS [114]. The antimycobacterial effect is predominantly attributed to the hydrophobicity of the benzamide derivative. The benzamide derivatives hold the active site of α submit in the TRPS binding pocket of *Mtb* and thereby deliver 100 % growth inhibition at 25 μg/mL and bactericidal activity at 6 μg/mL, respectively. In addition, the benzamide derivatives have exhibited a good predictive binding score with TRPS which is confirmed through an *in silico* model [115]. On other hand, coenzyme A (CoA) and acyl carrier protein (ACP) synthesis are stimulated by the pantothenates synthetase, which is absent in mammals. The gene panC is encoded for pantothenates synthetase is responsible for condensation of D-pantoate and beta-alanine to form pantothenate in *Mtb*. 3-biphenyl-4-cyanopyrrole-2-carboxylic acid, act as a chemotherapeutic agent to exhibit inhibitory effect on pantothenates, thereby inhibiting *Mtb* [116].

5.3. Nucleic acid metabolism

Thymidylate synthase A encoded by ThyA (792 bp) plays a significant role in deoxy uridine mono phosphate conversion to deoxy thymidine mono phosphate [117], in this process, the tetrahydrofolate act as a methyl donor. It is been reported that the ThyA gene as a potential target while on mutation, leading to resistance for para-aminosalicylic acid [118]. Specifically, *Mtb* IMPDH homologs possess guaB1, guaB2, and guaB3 genes. Among these, guaB2 (Rv3411c) gene products are required for the NAD + -dependent dehydrogenation and hydrolysis of inosine 5-monophosphate to xanthine 5-monophosphate. The inhibitor interacts with guaB2 and results in the inhibition of hydrolysis of inosine 5-monophosphate. The shikimate pathway catalyzed by seven enzymes (aroA, aroB, aroD, aroE, aroG, aroK and aroF) is essential for mycobacterial growth, as illustrated in Fig. 5. The inhibitors targeting 3-Deoxy-D-arabinose-heptulosonate-7-phosphate synthase (aroG) may terminate the conversion process and result in the starvation of *Mtb*. IMB-T130 ((5-chloro–N-(pyridine-2-yl)-1, 3-thiazol-2-yl] thiophene-2-carboxamide)) is a multi-target compound which inhibits the catalytic activity of 3-dehydrogenate synthase (DHQS) enzyme. Site-directed mutagenesis analysis confirmed the inhibitory action of IMB-T130 by binding to the active center of DHQS. Inhibitor derived from the extracts of *Sutherlandia frutescens* specifically bind to the shikimate kinase, may lead to changes in the conformation in the loop. In this pathway, Arg117, Arg136 and Arg58 are the arginine residues that bind electrostatically and act as a determinant factor for S3P [119].

5.4. Lipid metabolism

Studies in mouse models have shown that rofumilast, an FDA-approved Type IV Phosphodiesterase (PDE) inhibitor, reduced the lung bacillary burden when co-administered with isoniazid [120]. Subbian et al. studied the inhibitory action of CC-11050, a cyclopropane carboxamide, in the experimental modal of pulmonary tuberculosis and reported that it could be used as an efficient PDE inhibitor [121].

It is been reported that AEBSF (4-(2-aminoethyl) benzene sulfonyl fluoride) interacts with the PafA residue of serine 119 (S119), a prokaryotic Ubiquitin-like protein ligase in Pup-proteasome system (PPS) of *Mtb*. The mutation in S119 results in maximum inhibitory effect on *Mtb* PafA, in turn led to the discovery of novel potential targets for *Mtb* [122]. The N-(5-(azepan-1-ylsulfonyl)-2-methoxy-)-2-(4-oxo-3, 4-dihydro phthalazine-1-yl) acetamide exhibits a unique binding mode at the allosteric site of fumarate hydratase. Irrespective of the presence of a homologous enzyme in the host, this compound exhibits selective inhibition [123]. Ethoxzolamide inhibits the PhoPR regulon of carbonic anhydrase and reduces the virulence of *Mtb* [124]. Aluciferase-based (Protein Kinase G) PknG kinase assay identified PknG inhibitors like AZD7762 (3-(carbamoylamino)-5-(3-fluorophenyl)-N-[(3*S*)- piperidin-3-yl] thiophene-2-carboxamide), R406 ((6-[[5-fluoro-2-[(3,4,5-trimethoxyphenyl) amino]-4-pyrimidinyl] amino]-2,2-dimethyl-2H-pyrido [3,2-b]-1,4-oxazin-3(4H)-one)), R406-free base (R406f) and CYC116 (4-methyl-5-[2-(4-morpholinophenylamino) pyrimidin-4-yl] thiazol-2-amine) [125]. Others important inhibitors that are involved in the *Mtb* metabolic pathways are listed in Table 3.

Table 3
Potential targets and their respective inhibitors of general metabolic pathways in *Mtb*.

Target	Inhibitor	MIC	Reference
ICL	*Manilkara zapota*	12.5	[106]
	Morinda citrifolia	mg/mL	
	Vitex negundo	12.5	
	Momordica charantia	mg/mL	
	Zingiberofficinale	0.78	
	Illiciumverum	mg/mL	
	Phthalazinyl derivatives	0.39	
	5-Nitro-2-furoic acid hydrazones	mg/mL	
	with furan-2-carbaldehyde	0.05–1	
	5-Nitro-2,6-dioxohexahydro-4-pyrimidinecarboxamides	mg/mL	
	Isatinylthio semicarbazone derivatives	2.65 μM	
	Chelerythrin extract		
	3-Nitropropionamide derivatives		
	Pyruvate-isoniazid analogue with their copper complex,		
	Thiobenzanilide salicilanilide derivatives and some heptapeptides		
CH_2 Kinase Inhibitor	DDUG (SML0781)	1.65 μM	[123]
Alpha-glucosidase inhibitor	Acarbose,	ND	[92]
	Miglitol,		
	Thiazolidinediones,		
	Pioglitazone		
Aspartate semi aldehyde dehydrogenase inhibitor	IMB-XMA0038	0.5 μg /mL	[10]
Ccytochrome b subunit of complex III	pyrazolopyridinecarboxyamides,	0.016	[124]
	TB47	μg/mL	
ATP Synthase	Diarylquinolines,	0.03	[125]
	R207910	mg/L	
ATP Synthase	Bedaquiline	0.25	[126,127]
		mg/L	[128,129]
ATP Synthase	TBAJ-587 and TBAJ-876	0.016	[4,130]
	(second generation diaryl quinolones)	mg/L	
ATP Synthase	Squaramide	0.5 μM	[4]
Cytochrome bc1-aa3 super complex	Q203 (trade name Telacebec)	0.016	[130]
	(ClinicalTrials.gov – NCT03563599)	mg/L	

5.5. Antivirulence promoting drugs

Above all, in recent time's antivirulence drugs found to be popular among researchers. The antivirulence drugs are used to inhibit the occurrence of drug resistance due to virulence factors. One of the most important virulence factors is SapM, a secreted virulence factor from *Mtb* [131]. L-ascorbic acid and 2-phospho-L-ascorbic are used as potent inhibitor of SapM which act against the catalytic activity of phosphatase. Apart from this, most of the inhibitors share trihydroxy-benzene moiety with uncompetitive inhibition. In parallel, virulence factor AcpA secreted by *Francisella tularensis* possess same catalytic activity of SapM. ESAT-6 secretion system 1 (ESX-1), is also a virulence factor which act as a potential drug target of *Mtb* [132]. In order to impede ESX-1, an inhibitor made of serine protease domain in Mycosin-1 protease MycP1, cleaves substrates such as EspB, EsxA, EsxB, EspA and EspR, thereby target the *Mtb* and open up an unconventional avenue for TB drug discovery [133].

MptpB phosphatase is a virulence factor secreted by the *Mtb* to counter balance mycobacterial clearance. Response of MptpB is critical for *Mtb* to modulate the bactericidal immune responses exhibited by the host immune cells. It is also reported that MptpB inhibition could accelerate the antimycobacterial effect of first-line drugs. Isoxazole-based MptpB inhibitors have been identified with higher specificity, excellent pharmacokinetics profile, oral bioavailability, and *in vivo* efficacy in experimental models of tuberculosis [134,135]. Zinc metallopeptidase (Zmp1), another virulence factor of *Mtb* and its substrate specificity, has been confirmed through peptide library screening. Zmp1 and related M13 metalloproteases, such as neprilysin and endothelin-converting enzyme-1, have been reported to possess the mechanisms of substrate binding and selectivity [136]. Chorismate isochorismate has been replicated by benzoate-based inhibitors for MbtI-catalyzed reaction [137]. Salicylic acid act as substrate for MbtA and catalyzes N-terminal thiolation domain of MbtB. Mycobactin scaffold has been prepared by the modification and functionalization of MbtB, MbtE, MbtC, MbtD, MbtF, MbtK and MbtG enzymes, respectively [138]. The knowledge on antivirulence activity of these enzymes and their responsive inhibitors open a new arena in antimycobacterial treatment protocols.

6. Future scope and perspectives

The TB control program faces many challenges globally as well as in India, few among are insufficient primary health-care sanitation, lack of awareness, inadequacy monitoring of first-line and second-line anti-TB drugs usage, patients compliance *etc.* Among all, the emerging multidrug-resistant TB (MDR-TB) and total drug resistant TB (TDR-TB) are severe threat to the TB eradication programs. Newer drug discovery approaches targeting multiple pathways of *Mtb* and preventing the occurrence of drug resistance are the need of the hour. In recent times for the clinical management of TB, numerous small molecule inhibitors have been developed; among them, peptides are more attractive candidates as they exhibit greater affinity and specificity towards functional targets of *Mtb*. In this current review, the structural information and the chemical moieties on potential protein targets and inhibitors pertinent to the pathways of cell wall biosynthesis, nucleic acid metabolisms, efflux pumps, metabolic pathways and antivirulence targets of *Mtb* is been discussed. The collective knowledge reveals the key sites for the validation of the potential drug targets and novel lead molecules that could effectively inhibit the *Mtb* growth. Newer technologies are developed to overcome the instability of peptides under physiological conditions and their poor membrane permeability property. Few of them are to prevent rapid enzymatic degradation of these peptides in the systemic circulation and to increase the stability. Despite these attempts to establish the antimycobacterial effect using high throughput screening, the mismatch in the host microenvironment and the *in vitro* screening conditions have become a major hurdle for the failure of these molecules at preclinical stage itself. To tackle these problem whole cell-based arrays, *in vitro* cell culture models were adopted. The intracellular survival of *Mtb* in the host is one of the critical factors which need to be studied. Molecular technologies to identify functional genomics may aid in overcoming this and to develop newer drug discovery targets. There is much of a scope for discovering and designing naturally derived protein inhibitors. The unraveling of the protein inhibitors in each biosynthetic/mechanistic pathways may help to regulate the protein synthesis, and the inhibition of processes such as cell digestion, growth, differentiation, immunological defense *etc.*, in *Mtb*. Filling the gaps between the studies on bioactive molecules and adequate high throughput screening can open up the possibility of natural compounds as effective TB drug molecules. The triumph of the BPal (Bedaquiline, Pretomanid, and Linezolid) drug regimen gives a ray of hope in the clinical management of TB possibly for the reduction of treatment duration, pill burden and adverse effects significantly. Compared with classical antibiotics, peptide inhibitors have advantages such as lower production costs, better bioavailability and numerous choices on preparation and administration methods. At the end of the day the research approaches that could translate the in-house findings effectively to the bench-side applications are necessary to achieve the possibility of "End TB" as a global reality.

CRediT authorship contribution statement

S Souparnika: Idea and Conceptualization, Methodology, Writing – Original draft preparation. **N Usharani:** Idea and Conceptualization, Methodology, Writing – Original draft preparation. **N Saravanan**: Idea and Conceptualization, Mentoring, Writing – Review, and Editing.

Declaration of competing interest

The authors have no conflict of interest to declare.

Data availability

Data will be made available on request.

Acknowledgement

Authors acknowledge ICMR-NIRT for the support and the manuscript has been approved by NIRT-RIC with the ID 22-044.

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