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REVIEW

Protein targets for structure-based anti-*Mycobacterium tuberculosis* drug discovery

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ABSTRACT

***Mycobacterium tuberculosis*, which belongs to the genus *Mycobacterium*, is the pathogenic agent for most tuberculosis (TB). As TB remains one of the most rampant infectious diseases, causing morbidity and death with emergence of multi-drug-resistant and extensively-drug-resistant forms, it is urgent to identify new drugs with novel targets to ensure future therapeutic success. In this regards, the structural genomics of *M. tuberculosis* provides important information to identify potential targets, perform biochemical assays, determine crystal structures in complex with potential inhibitor(s), reveal the key sites/residues for biological activity, and thus validate drug targets and discover novel drugs. In this review, we will discuss the recent progress on novel targets for structure-based anti-*M. tuberculosis* drug discovery.**

KEYWORDS *Mycobacterium tuberculosis*, crystal structure, drug discovery, target

INTRODUCTION

Mycobacterium tuberculosis, an intracellular bacterial pathogen, is the causative agent of tuberculosis (TB) and accounts for latently infects in around one-third population all over the world. Infection leads to fever, loss of weight, lung pain and coughing up blood/sputum. Co-infection of HIV and *M. tuberculosis* causes both TB and HIV to progress more rapidly (Lederer et al., 1975; Kurth et al., 2009). Although several antibiotics and the 'Directly Observed Treatment, Short-course' (DOTS) (Qureshi et al., 2010) have been used to effectively reduce the burden of TB, emergence of drug-resistant and drug-sensitive TB and co-infection with HIV result in increasing incidence of TB in recent years (Bocchino et al., 2000). Therefore, it is crucial to identify novel targets to develop new approaches and agents for anti-drug-resistant and -drug-sensitive *M. tuberculosis*.

M. tuberculosis belongs to the genus *Mycobacterium* and is a slow-growing aerobic rod-shaped bacterium. Comparing to other pathogenic viruses, *M. tuberculosis* has a unique cell wall, which is proved to be a significant virulence determinant (Godreuil et al., 2007; Rivers and Mancera, 2008a, b). The *M. tuberculosis* cell wall is formed by complicated structure, with peptidoglycans, mycolic acids, arabinogalactan, lipoarabinomannan and other components. The cell wall protects *M. tuberculosis* from the attack of immune system in infected host. In addition, the genome of *M. tuberculosis* encodes a series of pathways that are unique in *M. tuberculosis* but are absent in mammalian cells. These pathways become efficient targets to selectively inhibit the growth of *M. tuberculosis* with reduced side effects. Moreover, the genomic and structural genomic studies revealed several key enzymes that are crucial for *M. tuberculosis* growth, and also presented detailed structural information of these enzymes. Current data from 'TB Structural Genomics Consortium' shows that there are over 720 available TB protein structures, among which 266 are unique in TB (http://fold.doe-mbi.ucla.edu/TB/). These data suggest the potent targets to discover and/or design anti-*M. tuberculosis* drugs with high selectivity. This review discusses the novel targets for anti-*M. tuberculosis* drug discovery, particularly those with structures and complex structures containing potential inhibitors.

TARGETS IN CELL WALL BIOSYNTHESIS

The cell wall of *M. tuberculosis* is essential for its growth and survival in infected host, and thus, contributes to the resistance to most commonly-used antibiotics and che-

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motherapeutic agents. It has been revealed that the mycobacterial cell wall is composed of three covalently linked macromolecules, peptidoglycan, arabinogalactan and mycolic acids, which is a hallmark of mycobacteria (Chatterjee, 1997; Mdluli and Spigelman, 2006). Cell wall is characterized as a preferred source of molecular targets because the biosynthetic enzymes do not have homologues in mammalian system (Chatterjee, 1997; Mdluli and Spigelman, 2006).

Peptidoglycan biosynthesis

Peptidoglycan consists of alternating units of *N*-acetylglucosamine and *N*-gycolylmuramic acid and forms the backbone of the mycolyl-arabinogalactan-peptidoglycan (mAGP) (Kurth et al., 2009). The side chains of peptide are attached to muramic acid, and thus, crosslinked to arabinogalactan (AG) via a phosphodiester line to the position 6 of a proportion of muramic acid residues (Lederer et al., 1975; Chatterjee, 1997). In 2005, LeMagueres and colleagues reported the 1.9-Å crystal structure of alanine racemase from *M. tuberculosis* (Alr (Mtb)) and identified a conserved entryway into the active site (PDB code: 1XFC) (LeMagueres et al., 2005) (Fig. 1). These knowledge of detailed structural investigation and related biochemical analysis made this enzyme a challenging but important target for drug design against TB; furthermore, the related studies provided valuable insights about the precise mechanism of D-cycloserine inhibition against drug-resistant TB (Feng and Barletta, 2003; LeMagueres et al., 2005).

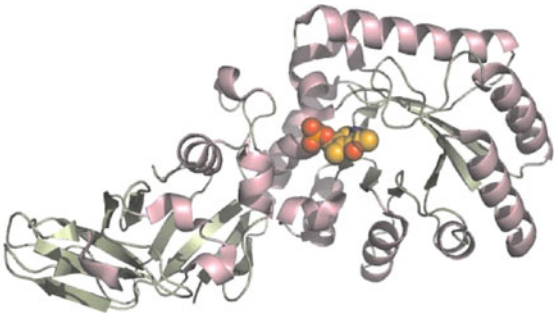


Figure 1. The crystal structure of *M. tuberculosis* alanine racemase with bound PLP.

Arabinogalactan and lipoarabinomannan biosynthesis

Arabinogalactan polymer is composed exclusively of D-galactofuranoses and D-arabinofuranoses, which are extremely rare in nature, while other crucial components of mycobacterial cell wall are known to be embedded into the framework of mAGP (Chatterjee, 1997). In addition to the essential role in mycrobacterial cell wall, lipoarabinomannan (LAM) also exhibits a wide spectrum of immunomodulatory activity (Chatterjee, 1997). These observations suggest the possibilities to target arabinogalactan and LAM biosynthesis pathways to treat TB. For example, ribosyltransferase is key to catalyze decaprenyl-phosphoryl-D-atabinose synthesis (Huang et al., 2005); UDP-galactopyranose mutase (Kremer et al., 2001), galactofuranosyl transferase (Brunger et al., 1998), dTDP-6-deoxy-L-lyxo-4-hexulose reductase (RmlD) (Nakano et al., 2000), RmlB and RmlC are also essential for mycobacterial growth (Ma et al., 2001; Li et al., 2006). These enzymes can be the potential targets for anti-*M. tuberculosis* drug discovery.

Mycolic acid biosynthesis

M. tuberculosis produces three types of mycolic acids that differ primarily in the oxygen-containing substituents at the distal portion of the meromycolate branch (Chatterjee, 1997). Mycolic acids are large, α-alkyl branched and β-hydroxylated fatty acid. They are among the major determinants for the fluidity of mycobacterial cell wall and are also related to the sensitivity of mycobacterial species to hydrophobic antibiotics (Yuan et al., 1997). Because mycolic acids generally extend from C70 to C90, the correct folding and function of essential enzymes in type II fatty acid biosynthetic (FAS-II) pathway (Mdluli and Spigelman, 2006) that is responsible for large fatty acid synthesis are crucial for mycolic acid synthesis (Chatterjee, 1997). In contrast, FAS-I pathway generally synthesizes C16–C26 fatty acid. Mycobacterial FAS-I pathway shares the similarity with that in mammalian system, while FAS-II does not. Therefore, the distinct enzymes in FAS-II can be the potential targets for drug discovery.

Polyketide synthase Pks13, which is crucial for mycobacterial growth, catalyzes the final condensation step in mycolic acid synthesis in FAS-II pathway (Raman et al., 2005; Takayama et al., 2005; Bhatt et al., 2007). However, the full length structure of Pks13 protein is still unavailable. Nevertheless, several crystal structures of Pks13 homologues or active domain provide valuable insights about the mechanism of Pks13 enzyme activity (Maier et al., 2006; Tang et al., 2006). Acyl-AMP ligase (Trivedi et al., 2004; Portevin et al., 2005), FadD32 (Portevin et al., 2005; Leger et al., 2009) and the AccD4-containing acyl-coenzyme A (CoA) carboxylase (Portevin et al., 2005), are also proved to be essential for mycobacterial growth. Moreover, FabH (PDB code: 2QO1) (Scarsdale et al., 2001; He and Reynolds, 2002; Musayev et al., 2005), MabA (Cohen-Gonsaud et al., 2002; Ducasse-Cabanot et al., 2004) and InhA (PDB code: 1ZID) (Dessen et al., 1995; Sharma et al., 1998; Nunn et al., 2002; Ducasse-Cabanot et al., 2004; Oliveira et al., 2006) have indispensible roles in mycolic acid synthesis (Fig. 2A and 2B). As expected, large amount of compounds targeting these proteins were found to inhibit mycobacteria growth. In particular, isoniazid (INH), which targets *M. tuberculosis* InhA, is used as a first-line therapeutic drug in TB treatment.

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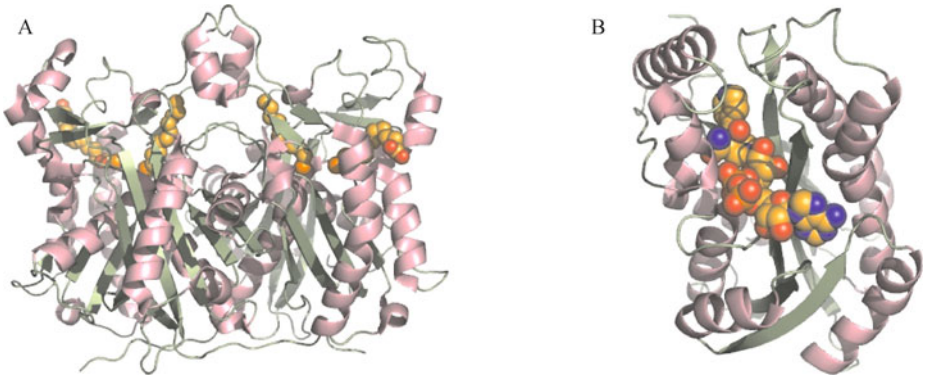


Figure 2. The crystal structure of FabH and InhA with their bound substrate or inhibitor.

TARGETS IN AMINO ACIDS AND COFACTORS BIOSYNTHESIS

Biosynthesis pathways of essential amino acids and cofactors are also crucial for the growth of *M. tuberculosis*, especially when the pathogen could not ingest enough nutrition from infected host. Therefore, the key enzymes in amino acid and cofactor biosynthesis pathways, which could be unique in mycobacteria, are believed to be effective drug targets.

The shikimate pathway, which is catalyzed by seven enzymes (AroB, AroC, AroE, AroG, AroK, AroK and AroQ), is essential for mycobacterial growth and is completely absent in mammalian cells. Therefore, the inhibitors to target the enzymes in shikimate pathway are hypersensitive. In addition, the crystallographic studies present detailed structural information and allow scientists to perform the structure-based drug design (Gourley et al., 1999; Rodriguez-Concepcion, 2004; Dias et al., 2006).

Vitamin B2 (riboflavin) and B5 (pantothenate) are also essential for *M. tuberculosis*. Therefore, the four enzymes in pantothenate biosynthesis (Pan B–E) and lumazine synthase (LS) that catalyzes vitamin B5 and B2 synthesis, are characterized as attractive targets for anti-*M. tuberculosis* drug discovery (Cole et al., 2001; Sambandamurthy et al., 2002; Visca et al., 2002). Currently, several crystal structures are available and present the possibility for highly selective and sensitive anti-*M. tuberculosis* drug design (Cole et al., 2001; Chaudhuri et al., 2003; Wang and Eisenberg, 2003, 2006; Chetnani et al., 2009).

TARGETS IN DNA METABOLISM

In *M. tuberculosis*, there are two classes of ribonucleotide reductases (RNRs), which are currently named as Class Ib and Class II, respectively, and are responsible for catalyzing the formation of deoxyribonucleotides from ribonucleotides. Class Ia is found in mammalian cells and certain bacteria, such as *E. coli* (Georgieva et al., 2008). Due to their essential roles, RNRs are characterized as the potential targets for antibacterial drug discovery. Class I enzymes are composed of

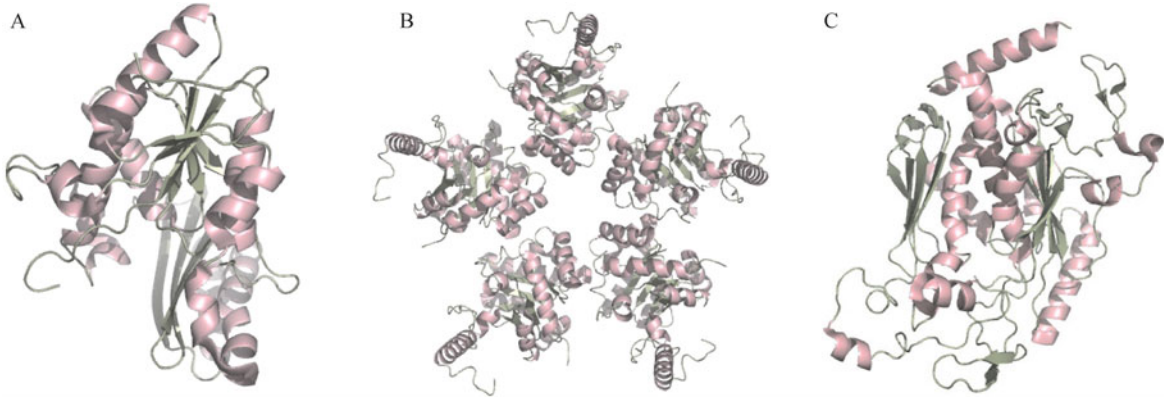


Figure 3. The crystal structures of the (A) pantothenate synthetase (PDB code: 2A88), (B) hydroxymethyltransferase (PDB code: 1OY0) and (C) chorismate synthase (PDB code: 1ZTB).

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the two homodimeric proteins, R1 and R2. Protein R1 contains substrate binding site, whereas R2 has a stable free radical on a tyrosine residue, neighboring an antiferromagnetically coupled high spin Fe(III)-Fe(III) site in each polypeptide of the R2 dimer, which is crucial for the enzyme activity (Sjoberg et al., 1978; Fontecave et al., 1992; Jordan et al., 1996). Although two genes encode RNR subunit in *M. tuberculosis*, R2-2 is generally believed to be the enzymatically active form (Meganathan, 2001; Marques et al., 2008). Uppsten and colleagues reported the crystal structure for the small subunit of *M. tuberculosis* RNR and identified the clear active center, which helps elucidate the mechanism of its activity (PDB code: 1UZR) (Uppsten et al., 2004) (Fig. 4). As the small subunit of *M. tuberculosis* RNR was reported to be hypersensitive to the class I RNR inhibitor hydroxyurea (Mowa et al., 2009), the structural knowledge provides a new direction to discover anti-*M. tuberculosis* inhibitors.

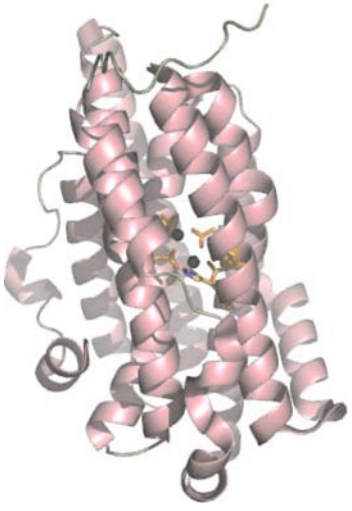


Figure 4. The crystal structure of the *M. tuberculosis* small subunit of RNR.

DNA ligase can link together two DNA strands that have double-strand break. DNA ligases are classified as either NAD+- or ATP-dependent, depending upon their specific cofactor (Vispe and Satoh, 2000). Comparing to the universal ATP-dependent ligases, NAD+-dependent ligases (LigA) are only found in certain viruses and bacteria, including *M. tuberculosis*. Srivastava and colleagues recently reported the crystal structure of *M. tuberculosis* LigA with bound AMP and provided it as a novel target for anti-*M. tuberculosis* drug discovery (Srivastava et al., 2005) (Fig. 5). Moreover, it is found that several nucleoside analogue and other compounds are effectively LigA inhibitors (Srivastava et al., 2005; Srivastava et al., 2007). In particular, glycofuranosylated diamine-based inhibitors could distinguish between the two types of ligases, and showed anti-TB activity (Gong et al., 2004).

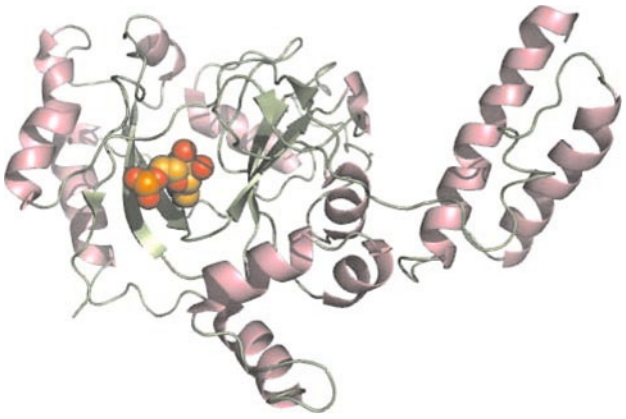


Figure 5. The crystal structure of *M. tuberculosis* LigA with bound ATP.

Thymidine kinase (TK), also known as a phosphotransferase, is found in most living cells. There are two forms of TKs in mammalian cells (TK1 and TK2), while several bacteria express their own TKs. As TKs have key functions in DNA synthesis, and therefore, in cell division, they are the targets of many anti-bacteria drugs. Recently, Li de la Sierra and colleagues reported the crystal structure of the *M. tuberculosis* TMP kinase (PDB code: 1G3U) (Li de la Sierra et al., 2001). They also identified a magnesium-binding site and a characteristic LID region. Comparing to the homologues in *E. coli* and yeast, the binding site of TMP in *M. tuberculosis* shows distinct differences. The observations about the interaction network, involving highly conserved side-chains of the protein, the magnesium ion, a sulphate ion and TMP itself, present *M. tuberculosis* TK as a good target to design selective inhibitors (Li de la Sierra et al., 2001) (Fig. 6). Based on this work, a serial of *M. tuberculosis* TK inhibitors were identified (Fioravanti et al., 2003; Haouz et al., 2003; Vanheusden et al., 2003, 2004), among which 30-azidodeoxythymidine monophosphate is a special one—it is a competitive inhibitor of *M. tuberculosis* TK, but is a substrate for TKs in human and other species (Fioravanti et al., 2005).

TARGETS IN GLYOXYLATE BYPASS

Glyoxylate shunt pathway is a carbon assimilatory pathway that allows the net synthesis of C4 dicarboxylic acids from C2 compounds. The first step of glyoxylate shunt is catalyzed by isocitrate lyase (ICL). There are two *M. tuberculosis* ICL proteins, a smaller one ICL1 and a larger one ICL2, which will cause complete impairment of *M. tuberculosis* replication and rapid elimination from infected lungs (Munoz-Elias and McKinney, 2005; Gould et al., 2006; Munoz-Elias et al., 2006). The structure of *M. tuberculosis* ICL was reported by Sharma and colleagues, and presents the precise inhibitory mechanism of the potent anti-*M. tuberculosis*

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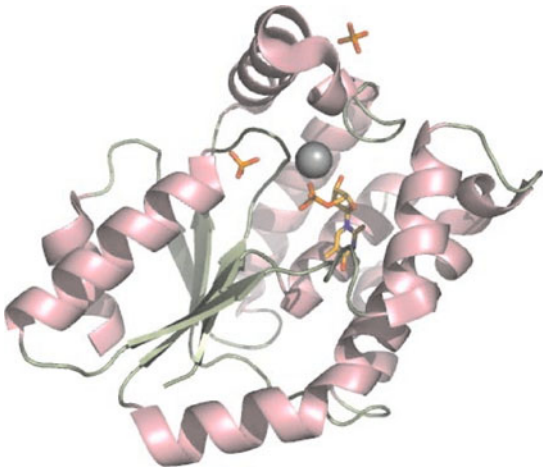


Figure 6. The crystal structure of *M. tuberculosis* LigA with bound magnesium ion, a sulphate ion mimicking and TMP.

3-nitropropionate and 3-bromopyruvate (Sharma et al., 2000) (Fig. 7).

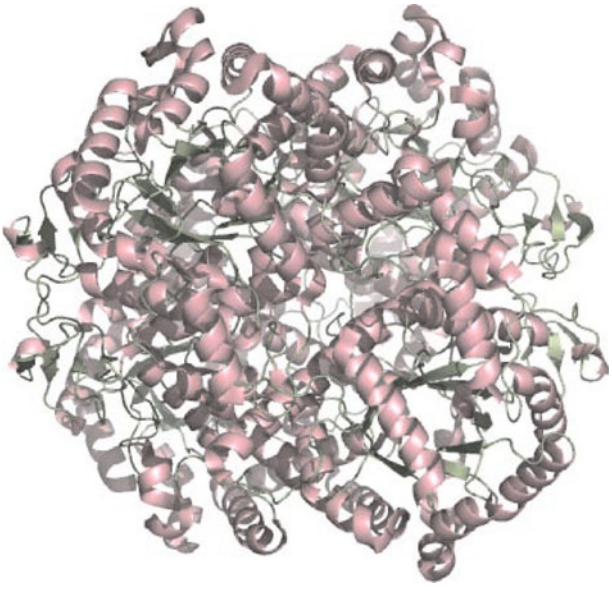


Figure 7. The crystal structure of *M. tuberculosis* ICL (PDB code: 1F8M).

TARGETS IN REGULATORY PROTEINS

The regulatory proteins, such as ArgP (Zhou et al.), GlnD and GlnE (McCoy et al., 2007; Carroll et al., 2008), and IdeR (Rodriguez-Concepcion, 2004) could act as transcription factors, regulators for other proteins, and cofactors, etc, and are essential for *M. tuberculosis* growth. Inhibition of regulatory proteins would have additive effects of disrupting a whole network of *M. tuberculosis* lifecycle and thus prevent the growth in infected host. However, the reported structural investigation is still very limited till now, and current drug discovery strategy to target these proteins is mainly based on biochemical assay.

PERSPECTIVES

Currently, several novel targets that are involved in the crucial steps in *M. tuberculosis* lifecycle and could be important for anti-*M. tuberculosis* drug discovery have been reported. Although some effective and highly selective inhibitors for these targets were identified or designed, the precise mechanism remains unclear at molecular level.

ATP biosynthesis is one of the most crucial bioreactions in *M. tuberculosis* lifecycle. Impaired function of ATP synthase may lead to ATP depletion and imbalance in pH homeostasis, both causing decreased survival (Deckers-Hebestreit and Altendorf, 1996; Hasan et al., 2006). Recently, an ATP synthase inhibitor (R207910), which targets F0 subunit of ATP synthase, atpE, was reported to contain high potency on suppressing both drug-sensitive and drug-resistant *M. tuberculosis in vitro* (Andries et al., 2005). In addition, the comparison of ATP synthase sequences from different species further supports the rationale to consider the specificity of antibacterial spectrum and the safety profile when targeting ATP synthase (Andries et al., 2005).

Menaquinone is reported to be essential for *M. tuberculosis* growth (Weinstein et al., 2005; Yano et al., 2006; Teh et al., 2007). Because menaquinone biosythesis pathway is absent in humans, it becomes an interesting target for anti-*M. tuberculosis* drug discovery. Although menaquinone biosythesis is extensively studied in *E. coil* and is relevant to some other enzymes, such as MenA–F (Meganathan, 2001), only type-II NADH-menaquinone oxidoreductase (NDH-2) is a potential target for structure-based drug discovery. However, there is only one reported structure of NDH-2 homologue, which is from *Pyrococcus furiosus Pfu-1140779-001* (PDB code: 1XHC). To elucidate the atomic coordinate of *M. tuberculosis* NDH-2 is an important future direction for anti-*M. tuberculosis* drug discovery.

In summary, the novel targets for developing anti-*M. tuberculosis* drugs shed the light for treating drug-sensitive and drug-resistant *M. tuberculosis*. Further knowledge is required to reveal the atomic coordinate and complexes with inhibitors or substrates of these novel targets in *M. tuberculosis* lifecycle. This could be helpful to understand the precise inhibitory mechanism and to discover and design novel compounds to treat TB.

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ABBREVIATIONS

AG, arabinogalactan; CoA, acyl-coenzyme A; DOTS, Directly Observed Treatment, Short-course; FAS-II, type II fatty acid biosynthetic; ICL, isocitrate lyase; INH, isoniazid; LAM, ipoarabinomannan; LigA, NAD+-dependent ligases; LS, lumazine synthase; mAGP, mycolyl-arabinogalactan-peptidoglycan; NDH-2, NADH-menaquinone oxidoreductase; RmID, dTDP-6-deoxy-L-lyxo-4-hexulose reductase; RNRs, ribonucleotide reductases; TK, Thymidine kinase

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