ABSTRACT  Biotin is an essential cofactor for enzymes present in key metabolic pathways such as fatty acid biosynthesis, replenishment of the tricarboxylic acid cycle, and amino acid metabolism. Biotin is synthesized de novo in microorganisms, plants, and fungi, but this metabolic activity is absent in mammals, making biotin biosynthesis an attractive target for antibiotic discovery. In particular, biotin biosynthesis plays important metabolic roles as the sole source of biotin in all stages of the Mycobacterium tuberculosis life cycle due to the lack of a transporter for scavenging exogenous biotin. Biotin is intimately associated with lipid synthesis where the products form key components of the mycobacterial cell membrane that are critical for bacterial survival and pathogenesis. In this review we discuss the central role of biotin in bacterial physiology and highlight studies that demonstrate the importance of its biosynthesis for virulence. The structural biology of the known biotin synthetic enzymes is described alongside studies using structure-guided design, phenotypic screening, and fragment-based approaches to drug discovery as routes to new antituberculosis agents.

TUBERCULOSIS AND THE RISE OF ANTIBIOTIC RESISTANCE

Tuberculosis (TB) is a global pandemic that ranks alongside HIV-AIDS and malaria as the leading cause of death by infectious disease, with the highest incidence rates observed in Southeast Asian, African, and Western Pacific countries (1). In 1993 the WHO declared TB to be a global health emergency and set the Millennium Development Goal of reducing the prevalence and mortality rates to 50% of those observed in 1990 by the 2015 deadline (2). Although the rates of new TB cases and mortality have declined over the past decade and are within reach of the 2015 target, the number of TB patients and the prevalence of drug-resistant strains are rising (3). Multidrug-resistant TB (MDR-TB) must be addressed now as a public health crisis to achieve the ambitious Millennium Development Goal target of complete elimination of TB as a public health concern by 2050 (4).

The worldwide TB epidemic has been aggravated by several factors such as the rising epidemic of HIV-AIDS coinfection (5), decreasing efficacy of the BCG vaccine (6), inadequate or inefficient administration of chemotherapies, and noncompliance of treatment (7).
factors have contributed to the rise of drug-resistant Mycobacterium tuberculosis strains that are causative agents of MDR-TB (resistant to isoniazid and rifampicin) and extensively drug-resistant TB (resistant to isoniazid, rifampicin, fluoroquinolones, and second-line injectable drugs) (8, 9). In 2012, MDR-TB was found in 3.6% of new TB cases and 20% of treated TB patients. Approximately 10% of patients with MDR-TB go on to develop extensively drug-resistant TB (1). Of immense concern is the rise of totally drug-resistant TB strains, discovered in Italy (in 2007), Iran (in 2009), and India (in 2012) (10–13). This new definition has not yet been formally recognized by the WHO (3).

The development of novel TB antibiotics is a crucial component of an effective fight against this epidemic. Since rifampin was approved in 1963, the only new antibiotic to combat TB has been Sirturo (bedaquiline), which was approved by the FDA in 2012 (14). This highlights a void in antibiotic discovery over the past decade. Sirturo inhibits mycobacterial ATP synthase, which is essential for energy production. This novel mechanism of action allows for the treatment of MDR-TB when combined with other therapies (15). The prevalence of drug-resistant TB will increasingly drive demand for novel antibiotics that are not circumvented by existing resistance mechanisms (15, 16). In addition, treatments targeting latent M. tuberculosis infection are highly desirable because only rifampin and bedaquiline can address this stage of the life cycle (17). Targeting latent infection is challenging because the pathogen can invoke various mechanisms to evade host immune responses and antibiotic chemotherapy. Ideally, the desired properties of new treatments should feature (i) shortened treatment durations compared to the current 6 months minimum, (ii) unique mechanisms of action to address drug-resistant strains, (iii) good pharmacokinetics and bioavailability, (iv) reduced daily number of pills and dosing frequency to simplify treatment and increase patient compliance, (v) effectiveness for treating both active and latent phases of the tuberculci life cycle, and (vi) safe coadministration with nonrelated medications, e.g., antiviral drugs for patients with TB/HIV coinfection.

BIOTIN AND ITS ROLE IN MYCOBACTERIA

Biotin (also known as vitamin B7, vitamin H, or coenzyme R) is a water-soluble vitamin that is required for the growth and pathogenicity of M. tuberculosis. Biotin is an essential cofactor for the biotin-dependent enzymes that are involved in important metabolic pathways such as membrane lipid synthesis, replenishment of the tricarboxylic acid cycle, and amino acid metabolism (described further below) (18–22). Although biotin is essential for all living cells, only microorganisms, plants, and some fungi synthesize biotin de novo (23). Humans and other mammals lack this metabolic pathway, thereby relying on exogenous biotin obtained from dietary sources or from intestinal biotin-producing bacteria (24, 25). Biochemical and genetic studies suggest that de novo biosynthesis is the sole source of biotin in M. tuberculosis due to the lack of a high-affinity transporter through which to scavenge exogenous material. Several biotin transporters from Eubacteria and Archaea have recently been identified and characterized. The biotin transporter, BioY, which works in cooperation with an ATP-dependent energy-coupling system (BioMN), has been characterized in several bacterial species (26, 27). Alternatively, the elusive YigM transporter in Escherichia coli has finally been identified 42 years after the first reports on this transporter (28–38). A homologue of YigM, MadN, has also been identified in certain Gram-negative bacteria (28). Noteworthy genome annotation studies have failed to identify a BioY homologue in mycobacteria (26, 39). Likewise, our own nucleotide sequence analysis using online homology algorithms has not found a homologue of YigM or MadN in the M. tuberculosis genome.

Furthermore, inhibition of mycobacterial growth using the natural products amiclenomycin and actithiazic acid, which target biotin biosynthetic enzymes, strongly suggests that mycobacteria rely exclusively on de novo biotin synthesis (described in further detail later) (40–44). The inhibitory activity of amiclenomycin was not compromised unless the growth media was supplemented with high concentrations of biotin (>42 μM) (45). This concentration is at least 3 orders of magnitude higher than that normally found in human plasma (46, 47), suggesting that biotin can diffuse into mycobacterial cells at nonphysiological concentrations but is not imported via a high-affinity transporter. Hence, biotin biosynthesis is essential for M. tuberculosis, leading to the enzymes that synthesize biotin as promising drug targets for new antibiotics, and these are the subject of this review.

BIOTIN-DEPENDENT ENZYMES

The biotin-dependent enzymes are a family of enzymes that are found throughout the living world. Because these enzymes all require the biotin cofactor, they are commonly known as the biotin-dependent enzymes.
Based on current literature and genome annotation studies, *M. tuberculosis* appears to have two classes of biotin-dependent enzymes: acyl-CoA carboxylases and pyruvate carboxylase (PC). These enzymes are positioned in key metabolic pathways that are required for mycobacterial growth and virulence, as discussed further below. All biotin-dependent enzymes function through a conserved reaction mechanism that requires biotin to bind and transfer carbon dioxide between metabolites in carboxylation, decarboxylation, and transcarboxylation reactions (48, 49). The biotin-dependent enzymes contain three highly conserved subunits required for catalysis, namely, biotin carboxylase, carboxyltransferase, and biotin carboxyl carrier protein (49–51). Biotin is covalently attached to the biotin carboxyl carrier protein that oscillates between two partial reaction sites during catalysis. At the first site biotin is carboxylated through the activity of biotin carboxylase. The biotin carboxyl carrier domain–carboxybiotin complex then translocates to the sites of the transcarboxylase, where the CO₂ is transferred to the metabolic substrate. Without the attached biotin cofactor, these enzymes are inactive and unable to fulfill the important metabolic activities that are essential for bacterial survival.

**Acyl-CoA Carboxylases (ACCs)**

Bacterial ACCs are multisubunit enzymes composed of a homodimeric biotin carboxylase, a heteroligomer carboxyltransferase, and the biotin carboxyl carrier protein (52). Mycobacterial genomes contain three *accA* and six *accD* genes that encode the two subunits of the carboxyltransferase complex (53). These subunits are believed to combine in a variety of different assemblies to produce unique acyl-CoA carboxylases that can utilize various-length short chain acyl-CoAs as substrates including acetyl-CoA, propionyl-CoA, and butyryl-CoA (22, 54). Carboxylation of these substrates provides metabolites that feed into the fatty acid synthesis and polyketide synthesis pathways, resulting in the production of structurally diverse lipids such as mycolic acids and multimethyl-branched fatty acids (21). One of the important, intrinsic features of *M. tuberculosis* is the presence of a complex lipid bilayer that is primarily composed of mycolic acid in the mycobacterial cell envelope. It is estimated that lipids account for up to 60% of the mycobacterial dry cell weight (55), and 10% of the *M. tuberculosis* genome is devoted to fatty acid biosynthesis (56), reflecting its importance for bacterial physiology and pathogenicity. The complex nature of the cell envelope greatly enhances the mycobacteria’s ability to resist chemical assault and survive hostile environments and limit its susceptibility to certain antibiotics (8, 9). Some of the surface-exposed lipids have also been found to be important virulence factors unique to *M. tuberculosis*. Targeting fatty acid synthesis represents one of the most successful approaches to combating TB, as demonstrated by the clinical efficacy of the drugs isoniazid, ethionamide, and thiacarlide (11).

Of particular relevance is the first committed step in the fatty acid synthesis pathway that is catalyzed by the biotin-dependent enzyme acetyl-CoA carboxylase. Here the carboxylation of acetyl-CoA yields malonyl-CoA, which is required for fatty acid elongation. Hence, acetyl CoA carboxylase is an important metabolic enzyme that is positioned at a crucial regulatory point for fatty acid synthesis. This is highlighted by a mutagenesis study that showed that the genes encoding the subunits of ACC, namely, *accA3, accB4*, and *accD6*, are essential for the growth of *M. tuberculosis in vitro* (57). Fatty acid synthesis has been proposed as a promising pathway to target for the development of novel agents against certain bacterial pathogens (58–60), and a recent review focuses on efforts to specifically target acetyl-CoA carboxylase (52).

**Pyruvate Carboxylase (PC)**

PC catalyzes the synthesis of oxaloacetate through the fixation of CO₂ onto pyruvate. Because oxaloacetate is one of the intermediates in the tricarboxylic acid cycle, one role for PC is to replenish this pathway (61). It has traditionally been difficult to study metabolism in intracellular pathogens such as *M. tuberculosis* due to redundant pathways and the contribution of the host cell. Hence, biotin-dependent PC has not been as well explored as the ACCs. However, advances in technology together with access to mutant strains of *M. tuberculosis* have provided new insights into metabolic adaptation and the relationship between host and pathogen. Using isotopically labeled substrates coupled with mass spectrometry has allowed the study of metabolomics of bacteria cultured inside host macrophages (62). A critical finding from these studies is that *M. tuberculosis* is capable of utilizing a variety of carbon substrates simultaneously, including CO₂ (63, 64). Moreover, macrophages are unable to fix CO₂ (62), suggesting that carbon fixation is potentially a novel target for anti-TB agents. The labeled amino acids aspartate, threonine, and methionine, all derived from the common precursor oxaloacetate, were all detected in infected macrophages that were cultured in ¹³C bicarbonate, but not in uninfected macrophages.
PC is one of three enzymes present in *M. tuberculosis* that is capable of oxaloacetate production, with phosphoenol pyruvate carboxykinase (PEPCK) and malate dehydrogenase being the other two. PEPCK-deficient strains of mycobacteria have helped establish an essential role for this enzyme in the establishment and maintenance of *M. tuberculosis* infection in a mouse model (65). PEPCK also played a key role in the synthesis of aspartate, threonine, and methionine in the $^{13}$C profiling study when a mutant *M. tuberculosis* strain was employed. However, PEPCK could only account for half of the amino acid production, suggesting a role for other enzymes in CO$_2$ fixation, such as PC. This work is important because the intracellular environment of the macrophage is hypoxic and CO$_2$ rich. Further exploration of the metabolic pathways required for survival inside cells and whole animals promises to yield new drug targets to combat TB.

**PROTEIN BIOTINYLLATION**

The covalent attachment of biotin to protein, or “protein biotinyllation”, of biotin-dependent enzymes is a biotin and ATP-dependent reaction. Protein biotinyllation is performed by biotin protein ligase (BPL; encoded by *birA*) (66). BPL is generally divided into three classes based on the divergent nature of the N-terminal domain, while the catalytic domain for catalyzing biotinyllation is highly conserved in all classes. Class I BPLs, such as those from *M. tuberculosis*, are the smallest of the three BPL classes (67). These enzymes are composed of the catalytic module that is required to catalyze protein biotinyllation (66, 68). Class II BPLs, such as those from *E. coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, contain an N-terminal region on the catalytic module that facilitates DNA binding, making these proteins truly bifunctional since they act as transcriptional repressors and catalyze protein biotinyllation (69–72). Class III BPLs, found in mammals, yeast, and insects, also possess an extended N-terminal region that permits selective binding of certain protein substrates for biotinyllation (73). Because protein biotinyllation is essential for the activity of ACCs and PC, BPL has also been proposed as a promising target for anti-TB drug development (68). This work has recently been reviewed in (74).

**VALIDATION OF BIOTIN BIOSYNTHESIS AS A TARGET FOR ANTI-TB DRUG DEVELOPMENT**

Biotin biosynthesis has been proposed as a promising target for the development of new antibiotics due to its intimate association with membrane lipid synthesis (58–60). As described above, the products of lipid synthesis are key components of the cell membrane that play critical roles in bacterial survival and defense. Targeting enzymes involved in membrane lipid synthesis therefore inhibits bacterial growth and virulence. This approach is clinically validated by isoniazid that targets InhA in the fatty acid synthase II system (75, 76). Importantly, screening of drugs effective against latent *M. tuberculosis* has revealed that certain lipid biosynthetic enzymes can be targeted against tuberculi at this stage of the life cycle that is challenging to treat (77, 78). These data validate biotin biosynthesis as an excellent target for the development of new anti-TB drugs that are able to combat both the active and latent stages of TB. The absence of a homologous pathway in humans and other mammals also adds to the appeal of this target for new antibiotics (18, 79).

Validation of biotin biosynthesis as a druggable anti-TB target is further supported by a number of genetic knockout studies. MMAR_2770 is the *Mycobacterium marinum* homologue of Rv1882c in *M. tuberculosis* and encodes a putative short chain dehydrogenase/reductase that is required for the early steps of the synthesis of the pimeloyl-thioester biotin precursor. Deletion of MMAR_2770 impaired the growth of *M. marinum* on blood agar unless supplemented with high concentrations of biotin (>1 μM) (80). The mutant also failed to establish an infection and colonize murine macrophages or zebra fish (80). In separate studies, disruption of *bioA* in the biotin biosynthetic operon impaired survival of *Mycobacterium smegmatis* during the stationary phase on carbon-depleted media (81, 82), while disruption of *bioF* and *bioB* debilitated the bacterial growth during and postinfection of murine macrophages (83). Likewise, genome-wide genetic screens revealed that *bioF*, *bioA*, and *bioB* are essential for virulence as shown by impeded growth of the deletion strains in murine macrophages (84). In addition, *bioF−* *M. tuberculosis* showed poor recovery from mouse lung and spleen in an infection challenge experiment (84). Together, these genetic studies further validate biotin biosynthesis as a key metabolic process during growth, infection, and survival in the latent life cycle of mycobacteria.

**THE BIOTIN BIOSYNTHETIC PATHWAY**

The biotin biosynthetic scheme can be divided into two stages: (i) synthesis of the pimelate precursor and (ii) the conserved metabolic pathway catalyzing the final four steps that yield biotin. Figure 1 shows the biotin
biosynthetic pathway and the chemical structures of the synthetic intermediates. The steps leading to the formation of a pimeloyl-thioester precursor (linked to either CoA or acyl carrier protein) are variable among biotin-producing organisms. The best-understood pathways are in *E. coli* (i.e., BioC-BioH pathway) and *B. subtilis* (i.e., Biol-BioW pathway) (reviewed in references 85, 86). The presence of BioC and BioH homologues in mycobacterial genomes suggests that *M. tuberculosis* employs the same pathway as *E. coli* (18, 80). Here, a 3-carbon malonyl-thioester (compound 1) is first methylated by the BioC-O-methyltransferase to produce methyl ester (compound 2), which serves as the precursor for two iterations of acyl chain elongation using the fatty acid synthesis pathway, producing a 7-carbon pimeloyl-acyl carrier protein (ACP) methyl ester (compound 3). The methyl group of the pimeloyl-ACP methyl ester (compound 3) is then hydrolyzed by BioH carboxylesterase to generate the pimeloyl-ACP (compound 4) (85–87). Pimeloyl-ACP, rather than pimeloyl-CoA, is believed to be the physiological intermediate required in the subsequent conserved metabolic pathway (86).

Unlike the synthesis of the pimelate precursor (compound 3), the final four reactions in the pathway that assemble the bicyclic rings of biotin are highly conserved among microorganisms and plants. Pimeloyl-ACP (compound 4) is converted to biotin by the activities of 7-keto-8-aminopelargonic acid synthase (KAPAS), 7,8-diaminopelargonic acid synthase (DAPAS), dethiobiotin synthetase (DTBS), and biotin synthase (BS), which are encoded by *bioF*, *bioA*, *bioD*, and *bioB*, respectively (18, 88–90). Among biotin-producing organisms, *Saccharomyces cerevisiae* is the only species reported that employs only the last three steps of the conserved metabolic pathway due to the presence of a 7-keto-8-aminopelargonic acid (KAPA) transporter (91, 92). Briefly, KAPAS converts pimeloyl-ACP (compound 4) to KAPA (compound 5) by using l-alanine as an amino donor and releasing ACP and CO₂ (93). At the antepenultimate step, DAPAS catalyzes the conversion of KAPA (compound 5) to 7,8-diaminopelargonic acid (DAPA) (compound 6) using S-adenosyl-l-methionine (SAM) as an amino group donor (90). Next, DTBS catalyzes closure of the ureido ring of dethiobiotin (DTB) (compound 7) from DAPA (compound 6) using CO₂ and ATP with the release of ADP and inorganic phosphate (90). Finally, closure of the thiophane ring of biotin (compound 8) by biotin synthase requires the insertion of a sulfur atom between the C6 carbon and the nonreactive methyl C9 of DTB (compound 7) (94).

**STRUCTURAL BIOLOGY OF BIOTIN BIOSYNTHETIC ENZYMES**

There has been much research activity in recent years to better understand biotin biosynthesis. This includes the determination of the X-ray crystal structures of these important biosynthetic enzymes. This data is invaluable in the rational design of inhibitors that target these enzymes. Indeed, researchers have employed structural biology to define the binding mechanism of ligands and products and to identify the chemical structures of reaction intermediates. These compounds provide starting points for medicinal chemistry. The following section highlights key structural features of the biotin biosynthesis enzymes. This is followed by a detailed overview of efforts to develop inhibitors with utility in antibacterial research. Where possible, we highlight the impact of this work for TB research.

**BioC-O-Methyltransferase (BioC)**

BioC (EC 2.1.1.197, encoded by *bioC*) is a SAM-dependent methyltransferase (86). The native form of BioC from *E. coli* and *Bacillus cereus* is monomeric, with a molecular mass of ~31 kDa (95). No crystal structure of BioC has yet been reported. The function of BioC is to generate methyl ester (compound 2) by transferring a methyl group from SAM to the free carboxyl group of a malonyl-thioester (compound 1), which is linked to either CoA or ACP (Fig. 1) (86, 95). This methylation step is essential to neutralize the negative charge of the carboxyl group prior to interaction with the extremely hydrophobic active sites of the fatty acid synthesis enzymes (95). Without BioC, the hydrophilic malonyl-thioester cannot enter fatty acid synthesis for assembling the pimelate moiety. Of note, malonyl-ACP was shown to be the preferred acceptor of methyl groups from SAM over malonyl-CoA (95) and is also an early precursor of the canonical fatty acid synthesis pathway (95). Therefore, the expression of BioC and its activity must be tightly controlled to avoid the depletion of the malonyl-ACP pool, causing impaired cell growth (95).

**BioH Carboxylesterase**

As described above, the fatty acid machinery is utilized during the biosynthesis of biotin (85, 86). Thus, the carboxyl group of the pimeloyl-ACP methyl ester (compound 3 in Fig. 1) must be liberated after two cycles of carbon elongation to leave the fatty acid synthesis machinery. Indeed, the free carboxyl group is further required for protein biotinylation by BPL (48). BioH carboxylesterase (BioH; EC 3.1.1.85), encoded by *bioH*, plays an essential role in the cleavage of the methyl ester
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1. Malonyl CoA (or ACP)

2. Malonyl CoA (or ACP) methyl ester

3. Pimeloyl-ACP methyl ester

4. Pimeloyl-ACP

5. 7-keto-8-aminopelargonic acid

6. 7,8-diaminopelargonic acid (DAPA)

7. d-thiobiotin

8. d-biotin

O-methyltransferase (BioC)

FAS

Carboxylesterase (BioH)

KAPA Synthase (BioF)

DAPA Synthase (BioA)

Dethiobiotin Synthetase (BioD)

Biotin Synthetase (BioB)
BioH is active as a monomeric protein of 28 kDa (96). It belongs to the hydrolase superfamily, a group of enzymes containing a classical Ser-His-Asp catalytic triad and a pentapeptide Gly-Xaa-Ser-Xaa-Gly motif (97–99). BioH is a carboxylesterase that can employ short acyl chains as substrates (97, 100). Three crystal structures of BioH have been determined from E. coli, Shigella flexneri, and Salmonella enterica (Table 1), showing a two-domain-containing protein (Fig. 2A) (87, 97). The large N-terminal domain possesses a Rossmann fold consisting of a twisted seven-strand β-sheet in the middle sandwiched by five α-helices at both ends. Meanwhile, the small C-terminal domain consists of four α-helices. The catalytic triad in the active site is located at the interface between these two domains (87, 97). The overall structure of BioH is similar to bromoperoxidase (EC 1.11.1.10), aminopeptidase (EC 3.4.11.5), epoxide hydrolase (EC 3.3.2.3), haloalkane dehalogenase (EC3.8.1.5), and lyase (EC 4.2.1.39) (97). BioH captures the pimeloyl-ACP methyl ester (compound 3) substrate in association with an acyl carrier protein (Fig. 2A). The acyl carrier protein consists of four helices; the second helix (α2) interacts with the BioH small domain to facilitate the capture of the phosphopantetheine arm of the pimeloyl-ACP methyl ester substrate through Ser35 positioned at the N-terminus of the α2 helix (87). This acyl carrier protein-dependent complex is also found in other enzymes such as P450-BioI and castor desaturase (EC 1.14.99.6) (87, 101, 102).

Although various ACP-bound methyl ester compounds such as glutaryl (C5), adipyl (C6), suberyl (C8), and azelayl (C9) can interact with BioH, pimeloyl-ACP methyl ester (compound 3) (C7) is the preferred substrate. Its length is such that it can bind within the hydrophobic active site to reach the catalytic triad, and thus, it is hydrolyzed faster than unnatural substrates (87). Shorter carbon chains cannot span from the hydrophobic cavity to the catalytic triad, while longer alkyl chains cause steric clashes within the cavity. This suggests that the length of the substrate is critical for catalysis. The reaction mechanism of BioH has also been investigated in some detail. A recent study of E. coli BioH has revealed that the enzyme can ligate a variety of aldehydes and activated alkenes via a Baylis-Hillman reaction (103). Together, the combined knowledge of the productive carbon length of the ligand that is required for binding and the substrate promiscuity of BioH provides useful information for rational design of BioH inhibitors.

In certain BioC-containing bacteria BioH can be substituted with other biotin biosynthetic enzymes such as BioG, BioJ, and BioK to complement the growth of E. coli ΔBioH biotin auxotrophic strains on biotin-free medium (39, 104, 105). Although these enzymes share low sequence similarity to BioH, they belong to the same α,β-hydrolase family with associated esterase activity (104, 105). A recent study of BioJ demonstrated that the ΔBioJ strain of Francisella novicida had attenuated growth in minimal medium. Importantly, the replication of this mutant strain is 5-fold lower than that of wild type after infection in murine bone marrow–derived macrophages, highlighting the importance of BioH-like enzymes for bacterial virulence (104).

**7-Keto-8-Aminopelargonic Acid Synthase (KAPAS)**

KAPAS (EC 2.3.1.47), which is encoded by bioF and is also known as 8-amino-7-oxononanoate synthase, catalyzes the first step of the conserved biotin biosynthetic pathway. It is classified as a type I pyridoxal 5′-phosphate (PLP or vitamin B6)–dependent enzyme in aminotransferase subclass II (85, 89). Unusually, there are two bioF genes in the genome of M. tuberculosis: bioF and a putative bioF2, which are translated to 386 and 771 amino acid products, respectively (106). The function of an additional N-terminal extension on bioF2 is unclear, although it contains the putative conserved acetyltransferase (GNAT) domain (accession: pfam13480), similar to enzymes in the N-acetyltransferase superfamily.

KAPAS converts a pimeloyl CoA (compound 4) to 7-keto-8-aminopelargonic acid (KAPA) (compound 5) using l-alanine as an amino donor and PLP as a cofactor. Unlike the homologues from other species that utilize only l-alanine, M. tuberculosis KAPAS can use both l-alanine and d-alanine as amino donor substrates (107). The mechanism of KAPAS is similar to those of other aminotransferase enzymes where the reaction

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**FIGURE 1** Biotin biosynthetic pathway. The proposed synthesis of biotin precursors and the conserved metabolic pathway (dashed box) are shown. The atoms modified in each step are highlighted in bold text. Abbreviations: ACP, acyl carrier protein; AaaS, acyl-ACP synthetase; AMTB, S-adenosyl-2-oxo-4-methylthiobutyric acid; DOA, 5′-deoxyadenosine; FAS, fatty acid synthesis; SAM, S-adenosyl-l-methionine; SAH, S-adenosylhomocysteine. Figure adapted from Lin and Cronan (85).
**Table 1** Structural biology of biotin biosynthetic enzymes and crystallographic data for the biotin biosynthetic enzymes

<table>
<thead>
<tr>
<th>Protein</th>
<th>Ligands</th>
<th>Organism</th>
<th>PDB</th>
<th>Reference</th>
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(continued)
TABLE 1 Structural biology of biotin biosynthetic enzymes and crystallographic data for the biotin biosynthetic enzymes
(continued)

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Abbreviations: PDB, Protein Data Bank; ACP, acyl carrier protein; KAPAS, 7-keto-8-aminopelargonic acid synthase; KAPA, 7-keto-8-aminopelargonic acid; DAPAS, 7,8-diaminopelargonic acid synthase; PLP, pyridoxal 5’-phosphate; DTBS, dethiobiotin synthetase; BS, biotin synthase; SAM, S-adenosyl-L-methionine; DAPA-PO₄⁷⁻, 7-(phosphonoxyacarbonylamino), 8-aminopelargonic acid; DTB, dethiobiotin.

proceeds through several intermediates, namely, an alanine-bound external aldimine complex, a quinonoid intermediate, a lysine-bound internal aldimine complex, and a 3-oxoacid aldimine complex. This leads to DAPA (compound 6) production via the addition of two carbons (C₈, C₉) and a nitrogen (N₈) atom onto the pimeloyl-ACP substrate (compound 4) (Fig. 1) (85).

Currently, the structure of KAPAS from M. tuberculosis has not been determined. However, available structures of the E. coli and Francisella tularensis orthologues determined in the apo and holo forms suggest that KAPAS is active as a homodimer (Table 1) (108–110). Each subunit assembles into three domains: (i) a small N-terminal domain composed of a three-stranded β-sheet, (ii) a large central domain containing a seven-stranded β-sheet, and (iii) a small C-terminal domain consisting of a four-stranded β-sheet with α-helices (89, 108) (Fig. 2B). The C₄’ atom of PLP cofactor is covalently attached to the ε-amino group of a conserved Lys236 (numbering in E. coli) in the active site, positioning the PLP molecule at the interface between subunits (108, 110). Apart from Lys236, active site residues His133, Ser179, Asp204, His207, and Thr233 are also important for making direct contact with PLP (110, 111). These residues are highly conserved in all species, as well as other enzymes in the α-oxoamine synthase family (111). A Mg²⁺ ion is essential for coordination with the O3 and hydroxyl groups of Ser179, the N8’ of the KAPA intermediate, and two water molecules. A conformational change within the C-terminus upon KAPA binding results in movement of Arg21 from the N-terminal domain, allowing these two arginine residues to H-bond with the carboxyl group of KAPA (110). In addition to H-bonding, the ligand is stabilized via hydrophobic interactions between the methylene chain of KAPA and Val79 from the subunit that interacts with PLP and with Tyr264 and Ile263 from the partner subunit. This molecular detail will provide powerful information for future structure-guided drug design.

7,8-Diaminopelargonic Acid Synthase (DAPAS)
DAPAS (EC 2.6.1.62), encoded by bioA, is also known as adenosylmethionine-8-amino-7-oxononanoate aminotransferase. DAPAS is analogous to KAPAS because both belong to the same subclass of the aminotransferase family (89). Briefly, DAPAS catalyzes the attachment of a nitrogen atom derived from SAM to KAPA (compound 5), which becomes the N7 moiety of biotin in DAPA (compound 6) (Fig. 1). The aminotransferase reaction of DAPAS is similar to KAPAS in that it proceeds through two partial reactions that require a PLP cofactor and an amino group donor. In contrast to KAPAS, which utilizes L-alanine, here SAM serves as the amino group donor (89, 112, 113). The first partial reaction catalyzes an aldime from DAPAS and PLP that then interacts with SAM to generate the subsequent quinonoid, ketamine, and then PMP (113). Following the second partial reaction, PMP interacts with KAPA substrate generating an intermediate in the reverse direction of the first partial reaction, i.e., ketamine, quinonoid, and then aldime intermediates, before the release of the product DAPA and recycled PLP (107, 113). Interestingly, although the KAPA substrate has an amino group, it cannot serve as an amino donor in the DAPAS reaction (112). The interaction of DAPAS in the first partial reaction with PLP and KAPA, instead of SAM, is unproductive. This is explained by the crystal structure of DAPAS in complex with KAPA, which shows that the substrate N8 amino group in the
substrate is orientated away from C4’ carbon of the PLP cofactor, thus preventing transamination and formation of the aldimine intermediate (112). This structural data demonstrated specificity for the amino acid donor and revealed the important role of the C4’ carbon of the PLP cofactor for transamination. This finding paved the way to design inhibitors of DAPAS based on chemical analogues of the reaction intermediates such as the aldimine.

Crystal structures of DAPAS have been reported from *M. tuberculosis*, *E. coli*, *Arabidopsis thaliana*, *Chromobacterium violaceum*, and *B. subtilis* (Table 1) (43, 90, 112–118). The crystal structure of *M. tuberculosis* DAPAS in complex with PLP cofactor and KAPA substrate (Protein Data Bank [PDB] 4CXQ) showed that the active form of DAPAS is a homodimer with a molecular mass of ∼46 kDa per monomer (Fig. 2C) (114). While there are a number of X-ray structures of *M. tuberculosis* DAPAS, most of the mechanistic studies of DAPAS have been performed with the *E. coli* orthologue. As with other aminotransferases, a conserved lysine (Lys283 in *M. tuberculosis* DAPA) in the active site is essential for catalysis by forming an interaction with PLP. Upon the binding of KAPA, a conformational change repositions Arg400 such that it can H-bond with the carboxyl group of KAPA (90, 112, 114). The equivalent arginine is also important for bonding to the carboxyl group of DAPA, as shown by the 180-fold increase of *K*ₘ for DAPA in the R391A mutant (numbering in *E. coli*) compared to wild type (113). The amino group of KAPA H-bonds to the hydroxyl group of Tyr157, the carbonyl oxygen of Gly316, and the phosphate group of PLP with an additional hydrophobic interaction with the aromatic ring of Trp64 (114). Currently, no crystal structure of DAPAS bound to SAM is available. However, SAM is proposed to bind the enzyme at the KAPA binding site. Based on mutagenesis studies of *E. coli* DAPAS, the R253A mutant increased the *K*ₘ for SAM by >3-fold higher than wild type, indicating that this conserved residue is important for SAM binding (118). These key residues provide useful structural information required for ligand binding and can be used in rational drug design.

**Dethiobiocitin Synthetase (DTBS)**

DTBS (EC 6.3.3.3) is encoded by *bioD*. DTBS catalyzes the penultimate step of the biotin biosynthetic pathway to produce dethiobiocitin (DTB) (compound 7) from DAPA (compound 6) using ATP and CO₂ (Fig. 1) (89). The DTBS reaction proceeds through three discrete steps: (i) formation of N7-DAPA carbamate, (ii) formation of carboxamidophosphoric acid anhydride, and (iii) closure of the ureido ring of DTB with the release of inorganic phosphate and ADP (119). Following the formation of carboxamidophosphoric acid anhydride, a tetrahedral intermediate is proposed to form before proceeding to the last step and closure of the ureido ring with the release of inorganic phosphate. However, this mechanism has not yet been verified by crystallographic studies (119, 120).

The structures of DTBS have been reported from *M. tuberculosis*, *E. coli*, *Helicobacter pylori*, and *F. tularensis* either in apo-form or in complex with ligands such as ATP, CTP, or DAPA (Table 1) (90, 119–125). Active DTBS is a homodimer with a molecular mass of ∼46 kDa (Fig. 2D). The two active sites are placed at the interface between the two subunits in antiparallel directions 25 Å apart (124). Each subunit folds

---

**FIGURE 2** Structures of biotin biosynthetic enzymes. (A) The crystal structure of BioH S82A from *Shigella flexneri* is shown (gray ribbon) in complex with pimeloyl-ACP methyl ester (in purple) and an acyl carrier protein partner (in blue) (PDB 4ETW). Residues in the catalytic triad, namely, Ser82, Asp207, and His235 (in green), are located at the interface between the two domains. (B) One subunit of the *Escherichia coli* KAPAS homodimer is shown in complex with KAPA-PLP aldimine intermediate (shown in pink connected to blue, respectively) (PDB 1DJ9). The Mg²⁺ ion is shown in green. (C) The homodimer of DAPAS formed by two subunits, chain A (in gray) and chain B (in green). The enzyme was crystallized in complex with PLP cofactor (in blue) and KAPA substrate (in pink) (PDB 4CXQ). (D) The homodimer of DTBS is formed by two subunits: chain A (in gray) and chain B (in green). The structure of the mycobacterial enzyme has been reported in complex with DAPA carbamate (PDB 3FMF) or CTP (PDB 4WOP). Two active sites are located at the interface between the subunits where each active site contains two adjacent binding pockets of DAPA carbamate (in red) and CTP (in yellow). (E) The crystal structure of BS was determined in complex with SAM (in orange) and DTB (in blue) (PDB 1R30). Each subunit, chain A (in gray) and chain B (in green), of the homodimer folds as a triosephosphate isomerase type (α/β)₉ barrel with extensions on the N- and C-terminal ends. BS contains one [4Fe–4S] and one [2Fe–2S] per monomer as highlighted in yellow.
into a single α/β globular domain consisting of a seven-stranded parallel β sheet in the core surrounded by helices, similar to certain GTP-dependent enzymes such as adenylsucinate synthase and p21ras (120, 124). Each active site contains adjacent binding pockets for DAPA and ATP substrates. The DAPA pocket is located in the dimer interface and is formed by amino acids from the two subunits, whereas the ATP pocket is composed of residues present within each of the individual monomers. Four amino acid residues (including Lys37, Thr41 from the first subunit, Leu146, and Asn147 from the partner subunit) (numbering in M. tuberculosis) are required for DAPA binding (124). Mutagenesis studies performed on E. coli DTBS revealed that mutation of Lys37 dramatically reduced $k_{cat}$ to less than 0.9% of wild type and increased the $K_m$ for DAPA by more than 100-fold (126). Moreover, the phosphate-binding loop (P-loop; Gly8-Xaa9-Xaa10-Thr11-Xaa12-Xaa13-Gly14-Lys15-Thr16; numbering in M. tuberculosis) is crucial for H-bonding with the three phosphate groups of ATP. Binding of ATP to E. coli DTBS induces conformational changes in the phosphate-binding loop (120). In particular, the replacement of Thr11 with valine results in a 24,000-fold increase in the $K_m$ for ATP, while Lys15 is a critical residue for both catalysis and ATP binding (K15Q 0.01% of wild type $k_{cat}$ and 1,800-fold higher $K_m$ than wild type, respectively) (120, 126). These data suggest key residues for enzyme–ligand interaction that are useful for structure-based drug design.

**Biotin Synthase (BS)**

BS (EC 2.8.1.6), encoded by bioB, catalyzes the final step of the biotin biosynthetic pathway. Here a sulfur atom is inserted between the methyl carbons (C6 and C9) of DTB (compound 7), thus creating the thioane ring and generating biotin (compound 8) (Fig. 1). BS belongs to a radical SAM (or AdoMet) superfamily that uses SAM for radical generation. BS contains three cysteine in a conserved eight-residue sequence motif (C-xxx-C-xx-C) that is necessary for binding of the [4Fe-4S]$^{2+/1+}$ cluster (127, 128). Only one structure of BS has been resolved, that from E. coli in complex with both SAM and DTB (Table 1) (94). Each subunit of the homodimer folds into a triosephosphate isomerase type (α/β)$_8$ barrel flanked with two helices at the N-terminus and non-structured C-terminal region (Fig. 2E).

Two iron clusters are present within the protein; [2Fe-2S] is located deep within a barrel, while [4Fe-4S]$^{2+/1+}$ is positioned at the C-terminal end of the barrel. These iron-sulfur clusters serve different functions in BS. The [4Fe-4S]$^{2+/1+}$ cluster is involved in the extraction of a hydrogen atom from the methyl and methylene groups of DTB. The cluster is initially in the [4Fe-4S]$^{1+}$ state from the electron transfer system containing NADPH, flavodoxin, and ferredoxin (127, 129). The reduced [4Fe-4S]$^{1+}$ cluster catalyzes the reductive cleavage of SAM to generate a 5'-deoxyadenosyl radical and methionine. This radical can then extract one proton from each of C6 and C9 of the DTB substrate (127). As a result, this first half reaction requires two SAM equivalents per molecule of biotin formed. The dimer of BS has a single functional active site with a 2:1 stoichiometry of SAM:DTB per BS dimer (127, 130). In the second half reaction, the [2Fe-2S] cluster has been proposed to close the thiophane ring of biotin by donating a sulfur atom (130, 131). Thus, BS itself appears to act as a substrate in vitro rather than as an enzyme by producing less than one molecule of biotin per molecule of BS protein (0.3 to 0.4 biotin equivalents/protein monomer) (132). In vivo, BS has an extremely modest catalysis rate of 10 to 60 turnovers per monomer, suggesting that a cellular mechanism exists to repair the [2Fe-2S] cluster (133, 134). A mitochondrial matrix protein, Isa2, has been proposed to play a role in the regeneration of the [2Fe-2S] cluster. BS is inactive in the Isa mutant strain of S. cerevisiae, leading to the failure of cell growth in minimum media or DTB supplementing media unless biotin is supplied (133).

Like other SAM-dependent enzymes, three conserved cysteine residues in the C-xxx-C-xx-C sequence motif contribute to ligand binding to BS. Indeed, the replacement of Cys53, Cys57, and Cys60 with alanine was shown to abolish the SAM reduction activity of E. coli BS in the [4Fe-4S]$^{2+/1+}$ cluster (135, 136). In addition, Cys97, Cys128, and Cys188 that do not belong to the motif and a conserved Arg260 are essential for binding of the [2Fe-2S] cluster (136, 137). These identified key residues provide structural information for further use in structure-guided drug design.**

**INHIBITORS OF BIOTIN BIOSYNTHETIC ENZYMES**

A number of studies have investigated inhibitors of biotin biosynthetic enzymes for antibacterial drug discovery. For most of these studies, enzyme inhibitors have been designed using the chemical structures of the known substrates, reaction intermediates, or products. While most studies have characterized the **in vitro** properties of these compounds as enzyme inhibitors, the antibacterial activities of only a select few have been reported (see below). Like other SAM-dependent
methyltransferases, BioC is inhibited by chemical analogues of the SAM substrate (compound 9 in Fig. 3). Demethylated SAM, namely, S-adenosylhomocysteine (compound 10), is the product of the methyl transfer reaction catalyzed by BioC. S-adenosylhomocysteine inhibited BioC in a concentration-dependent manner, with 1 μM of S-adenosylhomocysteine reducing ~40% of the *E. coli* BioC activity (95). Sinefungin (compound 11), a natural antibiotic isolated from *Streptomyces griseolus*, has also been shown to inhibit BioC (95, 138). Sinefungin is a steric and electrostatic mimic of SAM but has greater potency than S-adenosylhomocysteine (138). It reduced ~60% of the enzyme activity at 0.1 μM and completely abolished activity at 1 μM (95).

The inhibition of KAPAS has also been pursued using chemical analogues of either substrate, reaction intermediate, or products. The L-alanine substrate (compound 12) analogue L-trifluoroalanine (compound 13) (Fig. 4) is a slow suicide inhibitor of *E. coli* KAPAS (t₁/₂ ~ 20 min) that covalently binds to the active site lysine and forms an irreversible 2-(pyridoximine phosphate) acetyl protein addict (109). D-alanine (compound 14), which is the enantiomer of the native L-alanine substrate, was found to competitively inhibit the *E. coli* KAPAS with a *Kᵢ* of 0.59 mM (92). In contrast, D-alanine is not a competitive inhibitor for *M. tuberculosis* KAPAS because the enzyme can utilize both L- and D-configurations (107). Interestingly, the product of the D-alanine-utilized reaction, D-KAPA (compound 15) (compare L-KAPA compound 5), was found to inhibit

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**FIGURE 3** Chemical structures of BioC substrate and inhibitors. (A) S-adenosyl L-methionine substrate. (B) S-adenosylhomocysteine product of BioC reaction. (C) Sinefungin.

**FIGURE 4** Chemical structures of KAPAS substrate, reaction intermediate, and inhibitors. (A) L-alanine. (B) L-trifluoroalanine. (C) D-alanine. (D) D-KAPA. (E) The aldimine reaction intermediate. (F) (±)-8-amino-7-oxo-8-phosphononanoic acid. (G) 4-carboxybutyl (1-amino-1-carboxyethyl) phosphate. (H) 2-amino-3-hydroxy-2-methylnonanoic acid. Abbreviation: Pyr, pyrimidine ring of the PLP cofactor.
KAPAS ($K_i = 115 \, \mu M$) (107). Several analogues of the aldimine intermediate (compound 16), such as (±)-8-amino-7-oxo-8-phosphononanoic acid (compound 17), 4-carboxybutyl-(1-amino-1-carboxyethyl)-phosphonate (compound 18), and 2-amino-3-hydroxy-2-methylnonanoic acid (compound 19) are also competitive inhibitors of $E. \ coli$ KAPAS with respect to $L$-alanine, with $K_i$ values of 7, 68, and 80 mM, respectively (92).

Amiclenomycin (Fig. 5), another suicide inhibitor isolated from $Streptomyces$ spp., showed inhibitory activity against DAPAS from $E. \ coli$ and $M. \ tuberculosis$ ($K_i = 2 \, \mu M$ and $12 \, \mu M$, respectively) (41–43, 139–142). The crystal structures of $E. \ coli$ DAPAS in complex with amiclenomycin suggest that the inhibition of the enzyme is stereoselective because the cis-isomer (compound 20) (PDB 1MLY) is active, while the trans-isomer (compound 21) (PDB 1MLZ) causes a steric hindrance at the active site that results in a significantly less potent inhibitor (43). The design of amiclenomycin analogs revealed that the cis-configuration, but not the amino acid moiety, is essential for inhibitory activity (44, 141). In addition to KAPAS, as mentioned above, D-KAPA (compound 15) (or [R]-KAPA) also showed inhibitory activity against $M. \ tuberculosis$ DAPAS, with a $K_i$ of 5.9 mM (107, 143). 8-amino-7-oxooctanoic acid (compound 22), an achiral analog of the KAPA substrate, inhibited DAPAS, with a $K_i$ of 4.2 μM (143).

To inhibit DTBS, a series of DAPA, DAPA carbamate, and ATP mimics have been rationally designed. For the purpose of herbicide development, a total of 54 compounds, such as phosphonic acid (compound 23) (Fig. 6), were synthesized and tested for inhibitory activity against $E. \ coli$ DTBS, but none showed submillimolar inhibition constants (144). In a separate study using available X-ray data of ATP bound to $E. \ coli$ DTBS, a pharmacophore was proposed and employed to design an inhibitor that targeted the ATP binding pocket. Consequently, 6-hydroxypyrimidin-4(3H)-one (compound 24) was synthesized and shown to have a $K_i$ of 11 mM (145).

Finally, a number of BS inhibitors have also been reported. Actithiazic acid (compound 25) (Fig. 7) (also known as acidomycin) isolated from $Actinomycetales$ virginaiae and $Streptomyces$ spp. inhibited BS from $E. \ coli$ ($K_i = 0.45 \, \mu M$) and $M. \ tuberculosis$ (no record of $K_i$; see the antibacterial activity discussed later) (146–149). α-methylthiobiocin (compound 26) and α-methylbiotin (compound 27), isolated from $Streptomyces lydicus$, were also shown to inhibit the $E. \ coli$ BS activity ($K_i = 1.1 \, \mu M$ for α-methylthiobiocin) (146, 150, 151).

The antibacterial activities for a select few of the above inhibitors that target DAPAS and BS have been investigated against several strains of mycobacteria. Amiclenomycin (compound 20) and actithiazic acid (compound 25) inhibit growth of $M. \ smegmatis$ with MICs of 12.5 and 0.4 μg/ml, respectively, but failed to reduce the bacterial burden in a murine model of infection (42, 142, 146, 148, 149). Meanwhile, α-methylthiobiocin (compound 26) and α-methylbiotin (compound 27) were found to effectively inhibit $Mycobacterium fortuitum$, $M. \ smegmatis$, $Mycobacterium avium$, $Mycobacterium phlei$, and $Mycobacterium salmoniphilum$, with MIC values of 0.8 to 80 μg/ml and 12.5 to 200 μg/ml, respectively (150). While the detailed structural information about these biosynthetic enzymes is very valuable, the application of structure-based rational-design strategies has so far resulted in

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**FIGURE 5** Chemical structures of DAPAS inhibitors. (A) Cis-amiclenomycin. (B) Trans-amiclenomycin. (C) 8-amino-7-oxooctanoic acid. (D) MAC13772. (E) Aryl hydrazine.
compounds with inhibition constants in the micromolar to millimolar range and weak antibiotic activity. This suggests that new approaches are now required.

**FUTURE DIRECTIONS FOR DRUG SCREENING**

**Phenotypic Screening**

After a decade of target-based high-throughput screening campaigns to discover new classes of antibiotics, there has been a reversal back toward using the phenotypic, whole-cell screens that researchers favored during the golden era of antibiotic discovery (152-154). This is highlighted by the recent identification of a novel depsipeptide antibiotic isolated from soil-borne bacteria (155). By culturing the bacteria in their natural habitat, the researchers avoided the need to culture the microorganisms under laboratory conditions, where many species fail to grow. Indeed, identifying the appropriate conditions that mimic the natural environment in which bacteria are found is important for antibiotic susceptibility. Often the microniches that pathogenic bacteria colonize are deficient in nutrients, causing the bacteria to adapt their metabolic activities to support their requirement for various micronutrients, including biotin. This should be considered when performing whole-cell-based screens. Recently, a new inhibitor of biotin biosynthesis was identified using differential susceptibility under varying growth conditions (65). A primary screen was performed on a library of 30,000 compounds for molecules that inhibited growth of *E. coli* on limited nutrient media. The hits were subsequently reassayed using a defined medium supplemented with a mixture of amino acids, purines, pyrimidines, and vitamins. Those compounds that showed differential antibacterial susceptibility under nutrient-limited conditions, but not the deplete media, were selected for further characterization. Through this approach compound MAC13772 (compound 28) (Fig. 5) was identified as an inhibitor of biotin biosynthesis with antibacterial activity in the nutrient-limited broth (MIC 8 μg/ml) but not in media supplemented with 2 nM biotin. The antibacterial activity was reversed when media were supplemented with biotin, desthiobiotin, or DAPA, but not KAPA. Thus, the mechanism of action for this compound was proposed to be through inhibition of DAPAS and was subsequently confirmed through biochemical analysis. Similar consideration should be given to the growth conditions when screening for new antimycobacterials, given the hypoxic and nutrient-poor intracellular environments that the bacteria naturally colonize in human macrophages.

![Chemical structures of BS inhibitors.](#)

**FIGURE 7** Chemical structures of BS inhibitors. (A) Actithiazic acid. (B) α-methyldethiobiotin. (C) α-methylbiotin.

![Chemical structures of DTBS inhibitor.](#)

**FIGURE 6** Chemical structures of DTBS inhibitor. (A) A phosphate-based mimic of DAPA carbamate. (B) 6-hydroxy-pyrimidin-4(3H)-one (also known as 6-HP4).
Fragment-Based Drug Discovery (FBDD)

FBDD has become a powerful approach for early-stage hit discovery over the past decade (156–159). This technique aims to identify small starting structures that can be optimized into drug-like compounds. FBDD has enjoyed success in drug discovery with one FDA approved drug (Zelboraf, also known as Vemurafenib), which is approved for melanoma treatment, and more than 10 compounds in clinical trials for treating leukemia, myeloma, coronary artery disease, chronic obstructive pulmonary disease, diabetes, and bacterial skin infections (160–162). Conceptually, FBDD is the discovery of small fragments that can bind to specific target sites. Fragments are defined by a “rule of three” with low molecular weight <300 Da, less than three hydrogen bond donors and acceptors, and cLogP < 3 (163). Once fragment hits have been identified, larger lead molecules with higher affinity can be created by growing or modifying the chemical structure of one fragment or by linking or merging two adjacent fragments (164–167). Fragments are preferable as starting points for hit to lead development, rather than larger (molecular weight ≤ 500) drug-like compounds obtained from conventional high-throughput screenings. First, the low molecular mass often results in fragments that have high ligand efficiency, where the binding affinity is calculated relative to the number of heavy atoms in the ligand (167–170). Second, fragment libraries can be chemically diverse such that they can probe chemical space more effectively than larger compounds (160, 171, 172). Last, fragments with less complexity can bind to various sites of the protein target such that screening a fragment library often leads to hit rates as high as 5 to 10% (160).

While FBDD has been emphasized in several fields of drug discovery, its application in the field of antibiotic discovery is still underutilized. The previous large-scale failure of hit identification from natural compound libraries, which have limited permeability through the mycobacterial cell envelope, restricts success in a target-based high throughput screening approach (153). However, a recent study of synthetic TB drugs and prodrugs suggests that many of the successful compounds that are reactive inside mycobacteria can be considered fragment-like (i.e., molecular weight ≤ 300), such as isoniazid, ethionamide, para-aminosalicylic acid, and pyrazinamide (173). Indeed, being a smaller compound with moderate lipophilicity (clogP < 3) seems to be a positive feature for penetration through the complex mycobacterial cell envelope (173). There is only one recent report of an inhibitor of M. tuberculosis DAPAS identified by FBDD, namely, aryl hydrazine (compound 29) (Fig. 5) (114). It competitively inhibited the enzyme with respect to SAM (Ki = 10.4 μM). The crystal structure (PDB 4MQP) revealed that the aryl hydrazine forms a reversible covalent adduct with the PLP cofactor bound to DAPAS. These successes suggest that fragment-based screening could be an excellent way to enhance current anti-TB drug discovery efforts, and using these methods to target the biotin biosynthesis pathway in M. tuberculosis is a particularly exciting and novel approach to finding new anti-TB agents.

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