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REVIEW ARTICLE

NEW CELL WALL BIOSYNTHESIS INHIBITORS UNDER ACTIVE DEVELOPMENT FOR TUBERCULOSIS

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CONTENTS

ABSTRACT

Tuberculosis caused by the bacterial pathogen Mycobacterium tuberculosis is a major human disease predominantly affecting the developing world. The complex lifestyle and cell wall of M. tuberculosis confer intrinsic resistance to most commonly used antibacterials, often requiring the use of specialized antituberculosis drugs for the treatment of the disease. Coinfection with human immunodeficiency virus (HIV) and tuberculosis is cause for growing worldwide concern, and the emergence of multidrug-resistant and extensively drug-resistant tuberculosis further complicates the situation. Therefore, there is a demand for new antituberculosis drugs to combat the disease. Fortunately, characteristics that impart uniqueness and complexity to the mycobacterial cell wall also make it an attractive target for drug development. The current review describes some of the cell wall inhibitors under active development for the treatment of tuberculosis.

INTRODUCTION

The scientific and technical advancements associated with the 21st century have not been able to eradicate tuberculosis (TB), which still remains a major cause of morbidity and mortality (1). Current estimates suggest that approximately 9 million people are infected annually with TB and the disease accounts for 1.5 million deaths every year (2). The most common form of short-course TB chemotherapy consists of an intensive phase of 2 months of daily dosing with isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA) and ethambutol (EMB) (or streptomycin) followed by a 4-month continuation phase of INH and RIF (3). The need for a lengthy chemotherapeutic regimen with multiple drugs, some of which are associated with a relatively high incidence of side effects, is a major reason for patient noncompliance. Common adverse effects caused by anti-TB drugs include skin, gastrointestinal and neurological reactions, whereas hepatotoxicity is a major cause for concern when INH, RIF and PZA are used (4).

Noncompliance with the TB chemotherapeutic regimen is a driving factor for the emergence of drug resistance (5), although irregular drug intake and improper treatments, such as the use of inappropriate drugs or monotherapy, are also important contributors. Multidrug-resistant TB (MDR-TB) refers to TB that has become resistant to at least RIF, as well as INH, whereas extensively drug-resistant TB (XDR-TB) is defined as MDR-TB with additional resistance to a fluoroquinolone and a second-line injectable (capreomycin, amikacin or kanamycin) (2). MDR-TB is treated by using second-line drugs such as ethionamide, capreomycin, *p*-aminosalicylic acid, fluoroquinolones, cycloserine and certain rifamycin antibiotics, along with the first-line agents to which the bacterium is still susceptible. It takes up to 2 years to treat MDR-TB, with significant drug-associated toxicity, whereas the prognosis for XDR-TB is extremely poor, with few resources left for treatment. The global incidence of MDR-TB is estimated to be at least half a million cases, although the numbers are unreliable due to lack of diagnosis in many of the countries bearing the highest burden of TB drug resistance (6).

The combination of human immunodeficiency virus (HIV) with TB infection is lethal (7), with 23% of global TB deaths attributable to HIV coinfection in 2007, sub-Saharan Africa experiencing the highest morbidity due to HIV-TB (2). The need to combine antiretroviral therapy with TB chemotherapy places further restrictions on the antitubercular drugs that can be used, due to combined toxicities of drugs and the induction of host cytochrome P450 systems that inactivate critical components of these regimens (8). Furthermore, HIV-infected individuals have a 10% annual risk of developing TB if they are latently infected with *M. tuberculosis*, whereas this figure

decreases to a 10% lifetime risk in immunocompetent individuals latently infected with this pathogen. It is estimated that up to one-third of the world's population is latently infected with *M. tuberculosis* and that these individuals are a reservoir of future TB cases. Therefore, successful TB therapy requires not only the eradication of actively growing populations of cells, but also that of the nonreplicating or slow-growing bacteria thought to characterize latent disease, in order to impact the prevalence of TB on a global scale. Moreover, with global TB-HIV coinfection rates of at least 14.8% (2), it is imperative that TB chemotherapeutic regimens be compatible with antiretroviral therapy.

Therefore, it is of tremendous importance to identify and develop drugs with reduced host toxicity, increased potency against the pathogen which can significantly shorten treatment time, and which are effective against drug-resistant isolates. Fortunately, although the overall field of antimicrobials is languishing, with very few new agents being brought into the clinic, there are a number of anti-TB drugs in the pipeline (Table I), mainly due to support from government and philanthropic organizations (9).

THE *M. TUBERCULOSIS* CELL WALL

The complexity and uniqueness of the lipid-rich mycobacterial cell wall make it an attractive target for drug development (Fig. 1A). The mycobacterial cell wall consists of an inner peptidoglycan layer that is covalently linked to a second arabinogalactan layer, to which the mycolic acids are esterified. Exterior to the mycolyl–arabinogalactan–peptidoglycan (mAGP) complex is a third layer of mainly glucans, arabinans and arabinomannans. The mAGP complex is essential for the structural integrity of the mycobacterial cell wall (10).

The peptidoglycan consists of repeating units of β-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid or *N*-glycolylmuramic acid, and is located on the outer surface of the cell membrane (11). The muramic acid lactoyl groups are amidated with L-alanyl-γ-D-glutaminyl-L-*meso*-diaminopimelate-D-alanine stem tetrapeptides and L-alanyl-γ-D-glutaminyl-L-*meso*-diaminopimelate stem tripeptides (12). The stem peptides are linked either via 4→3 interpeptide bridges (between the carboxy-terminal D-Ala residue of a tetrapeptide stem linked to the amino group of a neighboring strand diaminopimelic acid residue), or via 3→3 interpeptide bridges (between the carboxy terminus of diaminopimelic acid of a tripeptide linked to the amino group of a lateral diaminopimelic acid residue) (13). These interpeptide bridges crosslink the glycan strands, with mycobacterial peptidoglycan being highly crosslinked and, at least under certain conditions, consisting of predominantly 3→3 crosslinks (14). The galactan component of the mAGP complex is attached to the *O*-6 of certain muramic acid residues of the peptidoglycan via an α-L-rhamnose-(1→3)-α-β-D-*N*-acetylglucosamine-1-phosphate linker (15). The galactan is a linear polymer of about 30 alternating β-(1→5) and β(1→6) galactofuranosyl units, which is further linked to two or three branched polymers of arabinosyl residues connected by α(1→5), α(1→3) and β(1→2) linkages (11). Some of the nonreducing termini of arabinan are esterified with mycolates at the 5-position (15), which impart the characteristic hydrophobicity to the mycobacterial cell wall (10).

In addition to the structural elements, the cell wall also contains components such as lipoarabinomannan (16) and other peripheral free lipids, including trehalose mono- and dimycolates, phthiocerol dimycocerosate, sulfolipids and phosphatidylinositol mannosides, which are involved in pathogenesis and immunomodulation (10). In addition, within this outer lipid-rich region are located the porins, transmembrane protein channels that facilitate the transport of hydrophilic solutes (17). Therefore, the different components of the mycobacterial cell wall provide ample opportunities for drug target identification/development.

CURRENTLY USED MYCOBACTERIAL CELL WALL INHIBITORS

It is noteworthy that three out of the four frontline drugs used in the treatment of TB are cell wall inhibitors: INH and EMB (Fig. 1B) inhibit mycolic acid and arabinogalactan biosynthesis, respectively,

Table I. *Details of cell wall inhibitors currently under clinical or active preclinical development.*

Compound	MIC[a] (μg/mL)	Details	Status/developer
PA-824	0.15-0.30	Mode of action is probably complex and involves NO release, inhibition of mycolic acid and protein synthesis. Prodrug, active against MDR-TB, no cross-resistance with currently used drugs and has sterilizing activity.	Phase I/Global Alliance for TB Drug Development
OPC-67683	0.006-0.012	Mode of action is probably complex and involves inhibition of mycolic acid synthesis. Prodrug, active against MDR-TB, no cross-resistance with currently used drugs and has sterilizing activity.	Phase II completed/Otsuka Frankfurt Research Institute GmbH
SQ-109	0.16-0.64	Mode of action unclear, probably a cell wall inhibitor. Active against MDR-TB and no cross-resistance with currently used drugs.	Phase Ib completed/Sequella, Inc.
SQ-609	Not reported	Mode of action unclear, probably a cell wall inhibitor. Shows prolonged activity after stopping drug administration in a murine model of TB.	Preclinical/Sequella, Inc.
SQ-641	0.5-2.0	Bacterial translocase I inhibitor. Active against MDR-TB and no observable cross-resistance with INH or RIF.	Preclinical/Sequella, Inc.

[a]Minimum inhibitory concentration (MIC) against wild-type *Mycobacterium tuberculosis* H37Rv. NO, nitric oxide; MDR-TB, multidrug-resistant tuberculosis; INH, isoniazid; RIF, rifampicin.

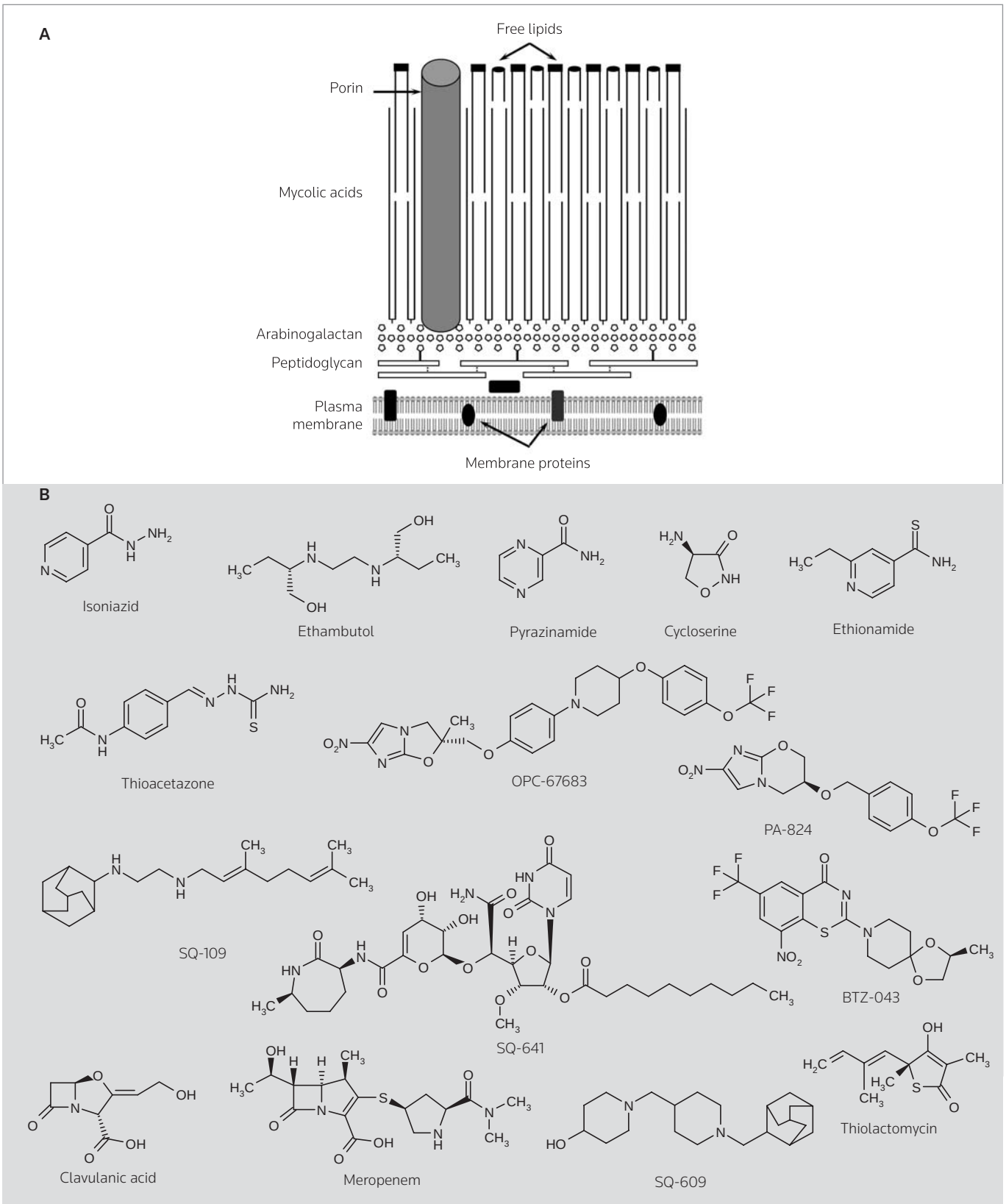

Figure 1. The *Mycobacterium tuberculosis* cell wall and inhibitors of cell wall biosynthesis currently in the pipeline. (A) Scheme of the mycobacterial cell wall showing its different components. (B) Structures of some currently used and other cell wall inhibitors in development for use as antituberculosis agents.

whereas PZA has been reported to inhibit fatty acid synthase 1, which provides 16-24-carbon fatty acids that are used in mycolic acid synthesis (18, 19).

Deciphering the mode of action of INH has been intriguing (20). Detailed mechanistic studies have shown that INH is a prodrug that is activated by the *M. tuberculosis* multifunctional catalase-peroxidase (KatG) (21). The INH adducts formed in the process of activation are potent inhibitors of the InhA enzyme (22, 23), an enoyl-ACP reductase that is involved in mycolic acid biosynthesis (24). In addition, it has been reported that the active metabolite of INH targets KasA, a β-ketoacyl-ACP synthase, also involved in mycolic acid biosynthesis (25). Other suggested mechanisms include the production of toxic nitric oxide (NO) during the KatG-catalyzed activation reaction of INH (26) and the ability of INH to form adducts with $NADP^+$ which inhibit dihydrofolate reductase (27), although inhibition of mycolic acid biosynthesis is accepted as the primary mode of action of INH (20). The most common mutations that lead to INH resistance in clinical isolates reside in *katG* (28, 29) or *inhA* (30).

EMB has been used in the treatment of TB since 1961, but the molecular basis of its action remained elusive for a considerable time and is still a topic of debate (31). Based on work conducted on other mycobacteria, it is currently accepted that EMB inhibits arabinogalactan biosynthesis (32) by blocking the activity of the EmbA and EmbB proteins encoded by the *embCAB* operon (33), which are believed to be integral membrane arabinosyltransferases involved in the polymerization of arabinogalactan (EmbA and B) (34) and lipoarabinomannan (EmbC) (35), respectively. It has been shown that EMB does not block the synthesis of decaprenylphosphoryl arabinose, the donor of arabinan in mycobacteria (36). Therefore, EMB might exert its effect by inhibition of either the arabinan acceptor or the arabinosyltransferases involved in the process (31). Furthermore, EmbB is thought to be the primary target of EMB, since most clinical *M. tuberculosis* isolates resistant to EMB carry a mutation in the *embB* gene (37), but recent evidence questions the exact function of the Emb proteins, and consequently, the mode of action of EMB in the inhibition of arabinogalactan biosynthesis (31). Therefore, further work is warranted to decipher the mode of action of currently used cell wall inhibitors and those under active development, some of which are discussed in this review.

Among the second-line agents commonly used in the treatment of MDR-TB, two are known cell wall inhibitors. Cycloserine (Fig. 1B), an analogue of D-alanine, inhibits two key enzymes involved in the formation of the peptide component of peptidoglycan on the cytoplasmic face of the cell membrane. Cycloserine is bacteriostatic, depending on the concentration used, and exerts its effect by inhibiting L-alanine racemase (catalyzing the conversion of L-alanine to D-alanine) (38) and D-alanylalanine synthetase, which incorporates D-alanine into the L-alanyl-D-*iso*-glutaminyl-*meso*-diaminopimelic-acid-D-alanine-D-alanine pentapeptide (39), which is required for crosslinking and therefore for formation of the bacterial cell wall. Under laboratory conditions, it has been shown that overexpression of L-alanine racemase in other mycobacteria can confer cycloserine resistance (40), although there is no genetic information regarding its target(s) in *M. tuberculosis* or clinically isolated strains resistant to cycloserine.

Ethionamide, or 2-ethylthioisonicotinamide (ETA; Fig. 1B), is a second-line drug that inhibits mycolic acid biosynthesis through its action on the InhA protein that is also an INH target (41). Like INH, ETA is also a prodrug, but it is not activated by KatG. Instead, ETA is activated by a flavin monooxygenase (EthA), leading to the formation of an *S*-oxide metabolite (42). It was also reported that ETA formed covalent adducts with nicotinamide adenine dinucleotide (NAD), which could bind and inhibit InhA (43). The expression of *ethA* is repressed at the transcriptional level by the TetR-like transcriptional regulator EthR and mutants that overexpress EthA are sensitive to ETA, whereas mutants overexpressing EthR or which have altered EthA are resistant (4).

Thioacetazone (TAC; Fig. 1B) is another second-line drug that is also activated by EthA (45). Recently it was shown that TAC interferes with the biosynthesis of certain mycolic acids by inhibiting cyclopropane mycolic acid synthases, which catalyze the key cyclopropanation step during mycolic acid biosynthesis (46). TAC is not a commonly used second-line agent due to associated side effects, especially in patients coinfected with HIV, and due to its lower efficacy compared to other drugs, in part due to metabolism by a flavin-containing monooxygenase (47), although it is still employed in some developing countries for the treatment of TB due to its availability and low cost.

PA-824

In the 1970s, a series of radiosensitizing bicyclic nitroimidazofuran compounds were analyzed for cancer radiotherapy, some of which also showed anti-TB activity in subsequent screens (48). These compounds were found to be mutagenic and were not pursued further, but precedence was set for the use of bicyclic nitroimidazoles as potential anti-TB agents (49). Pathogenesis Corp. synthesized and analyzed a series of 328 hexacyclic bicyclic nitroimidazo[2,1-*b*]-oxazines, of which the lead compound PA-824 (Fig. 1B) was found to be the most active of the series in a murine model of *M. tuberculosis* infection (50). PA-824 was also found to be active against nonreplicating bacilli and drug-resistant strains (51), with no cross-resistance to currently used drugs, and was highly specific in its activity against the *M. tuberculosis* complex (50). In addition, PA-824 showed oral activity at levels comparable to INH in the murine and guinea pig models of TB, and it was also active against intracellular bacilli in macrophages and against tubercle bacilli that persist in mouse tissues after 2 months of treatment with INH, RIF and PZA (52).

PA-824 is a prodrug that undergoes specific bioreductive activation in *M. tuberculosis* by a deazaflavin (F_{420})-dependent nitroreductase (Ddn), which requires reduced F_{420} provided by the glucose-6-phosphate dehydrogenase encoded by *fgd1* (50). Therefore, mutants defective in F_{420} biosynthesis (53, 54), F_{420} reduction ($FGD1^-$) or Ddn are all resistant to PA-824 (55). It is thought that reactive intermediates produced during nitroreduction somehow independently interfere with protein synthesis and lipid metabolism, as PA-824 exposure leads to the accumulation of hydroxymycolate and the loss of ketomycolate biosynthesis, with an observable inhibition of protein synthesis (50). Therefore, one of the targets of PA-824 could reside in the mycolic acid biosynthetic pathway, but due to the complexity associated with the action of reactive intermediates, it is clear that PA-824 affects multiple aspects of *M. tuberculosi*s cell biology. Recently, it was shown that nitroreduction of PA-824 leads to the production of reactive nitrogen species, including toxic NO that inhibits respiratory processes, which is one of the reasons for its

activity against nonreplicating bacteria under hypoxic conditions (56), again alluding to a more complex mechanism of action. PA-824 is not metabolized by the major human P450 isozymes, which bodes well in terms of drug–drug interactions and for use in combination with antiretroviral therapy. PA-824 is currently in phase I clinical trials to assess its early bactericidal activity (57).

OPC-67683

Like PA-824, OPC-67683 (Fig. 1B) is based on the parent nitroimidazofuran compound, which displayed considerable genotoxicity. Work done at Otsuka Pharmaceuticals on 2-substituted 6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazoles demonstrated that approximately 95% of the compounds tested were mutagenic, whereas this percentage was reduced to 16% upon the introduction of heteroatoms into the oxazole ring (58). One such compound, OPC-67683, was nonmutagenic and was active in in vitro and in vivo models of TB and against drug-resistant strains, with no observable cross-resistance with currently used agents. In addition, OPC-67683 displayed concentration-dependent activity against intracellular *M. tuberculosi*s, which was comparable to RIF and superior to INH, and it showed partial in vitro synergy with RIF, EMB and INH. In the murine model, when administered along with INH and RIF, OPC-67683 resulted in a reduction in treatment time of 2 months in comparison to the traditional INH, EMB, PZA and RIF regimen, with similar results (59).

Like PA-824 and INH, OPC-67683 is a prodrug that undergoes nitroreduction by Ddn to give the desnitroimidazooxazole metabolite, and it is thought that the active species may be an intermediate in the reaction. Resistance to OPC-67683 is normally attributed to loss of Ddn function. OPC-67683 inhibits the biosynthesis of methoxy- and ketomycolic acids at much lower concentrations than INH, but not that of α-mycolic acids, whereas INH inhibits the biosynthesis of all three. Therefore, some of the targets of OPC-67683 might be involved in mycolic acid biosynthesis, although additional targets cannot be ruled out. It has also been shown that in vitro OPC-67683 is not metabolized by the major P450 enzymes from humans or animals, making it a potential candidate for combined antiretroviral/TB therapy (59). OPC-67683 completed phase II clinical trials in 2008 (60).

SQ-109

Researchers at the National Institutes of Health (Bethesda, MD, USA) in collaboration with Sequella, Inc. synthesized and screened a library of approximately 63,000 1,2-diamine compounds based on the core structure of EMB for anti-TB activity in an assay designed to detect inhibition of cell wall biosynthesis (61, 62). Of the identified compounds, the lead SQ-109 (Fig. 1B) displayed in vitro antimycobacterial activity superior to EMB and was active against drug-resistant isolates, without exhibiting any cross-resistance to currently used agents (62). SQ-109 was effective in inhibiting the growth of 99% of intracellular bacteria at its minimum inhibitory concentration (MIC), which is comparable to INH but superior to EMB. Depending on the concentrations used, SQ-109 showed in vitro synergy with INH and RIF, additive effects with streptomycin and borderline additive effects with EMB (63). Furthermore, SQ-109 was 100-fold more potent than EMB in the murine model when administered orally (62).

Since SQ-109 is active against EMB-resistant strains (62), it must have a different mode of action and should be classified as a novel agent rather than as an EMB analogue. From the initial screen and transcriptional profiling studies (64), it is known that SQ-109 is a cell wall biosynthesis inhibitor. The extremely low frequency of spontaneous SQ-109 resistance observed in *M. tuberculosis* suggests that either it has more than one target or that it might exert its effect via a different mechanism not requiring a specific target protein (65). SQ-109 is extensively metabolized by the human cytochrome P450 enzymes CYP2D6 and CYP2C19 (66), and therefore its compatibility with antiretroviral therapies warrants further investigation. SQ-109 recently completed phase Ib clinical trials (67).

SQ-641

In 2003, Daiichi Sankyo screened a library of semisynthetic analogues of capuramycin (68), a complex nucleoside antibiotic produced by *Streptomyces griseus* 446-S3 (69). From the library, SQ-641 (formerly known as RS-118641; Fig. 1B) showed good activity against many mycobacteria and was licensed to Sequella for further development (70). There were no significant differences in the susceptibility of non-MDR and MDR strains to SQ-641 and it lowered *M. intracellulare* loads in the lungs when administered intranasally (71). In addition, SQ-641 demonstrated in vitro synergy with EMB, streptomycin and SQ-109 against *M. tuberculosis* and induced bactericidal effects faster than any other anti-TB drug, including INH and RIF (72). One of the downsides of SQ-641 is its low oral bioavailability and low water solubility (71). To circumvent the problem of oral bioavailability, a recent report described an alternate strategy for delivery in mice using SQ-641 dissolved in α-tocopheryl–polyethylene glycol 1000 succinate (TPGS) or incorporated into TPGS-micelles, which are water-soluble (73).

As the parent compound capuramycin is an inhibitor of the bacterial phospho-*N*-acetylmuramoyl-pentapeptide-transferase (translocase I), an enzyme involved in peptidoglycan synthesis, it can be inferred that SQ-641 acts through the same mechanism (70). Translocase I catalyzes the transfer of phospho-*N*-acetylmuramic acid-pentapeptide to the lipid carrier undecaprenyl phosphate (lipid I), generating undecaprenyl-P-P-*N*-acetylmuramic acid-pentapeptide on the inner surface of the cell membrane (74). This initiates a series of reactions on lipid I-bound substrates before they are flipped to the outer surface of the cell membrane for polymerization, to eventually form peptidoglycan. Capuramycin and its analogues inhibit the first reaction in this series, further demonstrating the utility of peptidoglycan biosynthesis inhibitors in anti-TB therapy. SQ-641 is currently in the preclinical stage of development (67).

BTZ-043

The nitrobenzothiazinone (BTZ) family of compounds was shown to be highly active against mycobacteria, and the lead compound 2-(2-methyl-1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-8-nitro-6-(trifluoromethyl)-4*H*-1,3-benzothiazin-4-one (BTZ-038) was subjected to structure–activity relationship and optimization studies that led to the isolation of BTZ-043 (Fig. 1B). BTZ-043 is considerably more

potent than the parent compound, is active against MDR-TB and XDR-TB, and is more effective than INH or RIF against intracellular *M. tuberculosis*. BTZ-043 showed excellent activity in the murine model of TB in a time-of-treatment rather than dose-dependent manner, with no observable toxicity issues. Through target overexpression and the isolation of spontaneous mutants resistant to BTZ-043, its target was identified as the membrane-associated protein DprE1 (Rv3790), which along with DprE2 (Rv3791) catalyzes the epimerization of decaprenylphosphoryl ribose to decaprenylphosphoryl arabinose, with the two proteins functioning as a decaprenylphosphoryl-β-D-ribose oxidase and a decaprenylphosphoryl-D-2-keto-*erythro*-pentose reductase, respectively (75). Decaprenylphosphoryl arabinose functions as the sole arabinan donor in mycobacteria during arabinogalactan biosynthesis. Therefore, inhibition of DprE1 by BTZ-043 abolishes arabinogalactan biosynthesis and leads to cell death. BTZ-043 is possibly the most recent anti-TB agent to emerge and is in preclinical development.

ANTI-TB ACTIVITY OF CURRENTLY APPROVED DRUGS FOR OTHER INFECTIONS

Recently there has been renewed interest in investigating the use of β-lactams in the treatment of TB as drugs that inhibit bacterial D,D-transpeptidases, which catalyze the final step in peptidoglycan crosslinking. *M. tuberculosis* is naturally resistant to many β-lactams, probably due to decreased cell wall permeability and a chromosomally encoded Ambler class A β-lactamase, BlaC, which hydrolyzes penicillins, cephalosporins and, to some extent, carbapenems (76). In 2007 it was reported that BlaC could be inhibited by the conventional β-lactamase inhibitor clavulanic acid (an oxazolidine-type β-lactam; Fig. 1B), which irreversibly binds to the protein and restores β-lactam susceptibility in *M. tuberculosis* (77). Initial studies indicated that some β-lactams bound to the major penicillin-binding proteins of *M. tuberculosis* and that these drugs demonstrated some activity against whole cells of this pathogen (78). In addition, certain β-lactams had modest activity in a mouse model and even in TB patients (79). The combination of meropenem (a carbapenem; Fig. 1B) and clavulanic acid showed in vitro activity against *M. tuberculosis* under both aerobic and hypoxic conditions, and was active against many XDR-TB strains (80). Since both of these compounds are Food and Drug Administration (FDA)-approved drugs, a considerable amount of clinical data is already available on their bioavailability, pharmacokinetics, pharmacodyamics and side effects. One possible caveat to the use of β-lactams is their poor intracellular activity (81). These antibiotics generally do not achieve sufficiently high intracellular concentrations to achieve bactericidal levels. Thus, the in vivo efficacy of β-lactams may be limited by the extent of intracellular parasitism of *M. tuberculosis*. One could hypothesize that β-lactams would be less effective in mice than in humans due to the extensive residence of *M. tuberculosis* in host phagocytic cells in the former, with more extracellular disease in certain types of granulomas in human patients (82). Therefore, it will be interesting to see how the meropenem/clavulanic acid combination fares as a treatment for TB. Clinical trials for meropenem/clavulanic acid are planned for the end of 2009 in South Africa to evaluate anti-TB activity in patients coinfected with HIV, with the goal of extending the study to MDR-TB and XDR-TB cases in the future.

COMPOUNDS IN THE PIPELINE WITH POSSIBLE CELL WALL-INHIBITORY ACTIVITY

In addition to the agents described above, there are a number of leads in various stages of development with either unclear modes of action or limited information in the public domain. The dipiperidine SQ-609 (Fig. 1B) is currently undergoing preclinical toxicological and pharmacological testing and is thought to be a cell wall inhibitor, although its exact mode of action is not known (67). In a study conducted in 2004 it was found that SQ-609 and another related compound, SQ-615, could prevent weight loss when administered to infected mice, and they had sustained effects on drug withdrawal, with SQ-609 being similar to RIF in its post-treatment activity (83).

The nitrofuranylamides are another class of compounds that were identified in a screen for inhibition of UDP-galactopyranose mutase (Glf) (84), a flavin-dependent enzyme that catalyzes the conversion of UDP-galactopyranose to UDP-galactofuranose, which is used in the biosynthesis of arabinogalactan (85). Compounds developed in this study showed better MIC values against whole cells compared to their Glf IC_{50} values, suggesting that they have additional targets in *M. tuberculosis* (84). Some lead compounds from follow-up studies showed good activity under hypoxic and aerobic conditions against *M. tuberculosis,* with little host cytotoxicity when analyzed in vitro (86), but their mode of action is probably complex due to the involvement of multiple targets.

There are many reported studies of screens for inhibitors of enzymes involved in cell wall biosynthetic processes, including inhibitors of the RmlB, C and D enzymes, which are involved in dTP-rhamnose formation, which is critical for the synthesis of the linker between peptidoglycan and arabinogalactan (87); glycomimetic inhibitors of the rhamnosyltransferase involved in forming the peptidoglycan–arabinogalactan link (88); methyl 5-*S*-alkyl-5-thio-D-arabinofuranosides as inhibitors of the antigen 85 complex, the mycolyl transferases for the terminal hexa-arabinosyl residues of arabinogalactan, as well as trehalose dimycolate (89); iminosugar inhibitors of UDP-galactofuranose transferase, which is important for arabinogalactan synthesis (90); *n*-octyl-5-(α-D-arabinofuranosyl)-β-D-galactofuranoside sugars as inhibitors of the various glycosyl transferases involved in arabinogalactan synthesis (91); as well as a variety of inhibitors of mycolic acid biosynthesis.

Noteworthy among the latter are the various analogues that have been synthesized based on the core structure of thiolactomycin (TLM; Fig. 1B). The antibiotic TLM is an inhibitor of the β-ketoacyl-ACP synthase enzymes, which in mycobacteria results in the inhibition of mycolic acid biosynthesis (92). TLM is an attractive lead for drug development in that it largely conforms to Lipinski's rule of 5, is orally bioavailable and is nontoxic in mice. Several groups have tried to optimize the relatively poor whole-cell activity of TLM while maintaining potency against the β-ketoacyl-ACP synthase (93-97), but most of these studies yielded only minor improvements in activity against the target or against whole cells, and in the case where submicromolar activity was found against whole cells, activity against the target was lost (97). In addition, there are some reports describing new INH derivatives with enhanced activity and which are active against INH-resistant strains (98, 99). For example, isoniazid-related isonicotinoylhydrazones are more active than INH in the intracel-

lular TB-infected macrophage model (100), although the exact mode of action of these compounds has not been verified. Therefore, the development of analogues of currently used anti-TB drugs still seems to be an active area of research.

CONCLUSIONS

Currently, much emphasis is being placed on the development of drugs for the treatment of latent TB and there is little evidence that cell wall biosynthesis inhibitors will be useful in such cases. The drug of choice for the treatment of suspected latent TB is INH, which targets mycolic acid biosynthesis (101, 102). The efficacy of INH in preventing reactivation of TB is paradoxical in that INH has no effect against nonreplicating bacilli in in vitro models of latent disease. It is possible that INH targets bacteria that are emerging from a state of nonreplicating persistence. Alternatively, the bacteria that populate latent lesions in infected individuals may be in a state of constant metabolic turnover, including remodeling of cell wall components (103). In this respect, it is known that *M. tuberculosis* in latently infected individuals is not completely quiescent, as seen from the proliferative activity of host immune cells in human tissue distal to the latent lesion (104). Intriguingly, *M. tuberculosis* residing in chronically infected mouse tissues, often used as an animal model of latent disease, is not in a state of nonreplicating persistence, as calculated from the in vivo loss of an unstable plasmid in dividing cells in the absence of antibiotic selection (105). Despite this, INH has very poor efficacy in chronically infected mice, and latent disease in humans is unlikely to have much in common with the chronically infected murine model. Predicting the therapeutic effect of cell wall inhibitors for human latent disease would require testing in animal models which more closely mimic the human counterpart. It is likely that the turnover and de novo synthesis of mycobacterial cell wall components in latently infected humans are going to be low, such that a lengthy duration of treatment would be required before any effect on reactivation of the disease would be detected.

Presently there are several drugs in the pipeline that target cell wall biosynthetic processes. The majority of these target novel enzymatic reactions and are thus unlikely to show cross-resistance to current antitubercular agents, implying their utility in the treatment of drug-resistant TB. Moreover, the need to develop drugs that are compatible with antiretroviral therapies has meant that many inhibitors are profiled at an early stage for their ability to elicit metabolism by P450 systems, providing early cues as to the suitability of these leads for further drug development. Of these, the nitrobenzothiazinone BTZ-043 displays the lowest MIC against *M. tuberculosis in vitro* (1 ng/mL) as well as in vivo (< 10 ng/mL) (75), raising hopes for a drug with the potential to shorten the chemotherapy of TB in combination with other drugs.

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DISCLOSURE

The authors state no conflicts of interest.

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