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Potentiation of Available Antibiotics by Targeting Resistance – An Emerging Trend in Tuberculosis Drug Development

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1. Introduction

Mycobacterial infections are one of the leading causes of death through disease world-wide (World Health Organization, 2010), encompassing infections such as tuberculosis (TB), leprosy, Buruli ulcers, and opportunistic non-tuberculosis mycobacterial (NTM) infections in immune-compromised individuals, especially patients with acquired immune deficiency syndrome (AIDS). The World Health Organization has estimated that one third of the world’s population is currently infected with Mycobacterium tuberculosis, the causative agent of TB, although only ten percent of those infected will develop active disease (World Health Organization, 2010). Highest TB incidences are located in sub-Saharan Africa and Southeast Asia, coinciding with human immunodeficiency virus (HIV) hot spots (World Health Organization, 2010).

The extremely high level of intrinsic resistance to most antimicrobial drug classes exhibited by M. tuberculosis has left us with a very limited arsenal of useful anti-TB drugs (Nguyen & Thompson, 2006). The five available first-line drugs, isoniazid (INH), rifampicin (RIF), ethambutol (EMB), pyrazinamide, and streptomycin, are all more than sixty years old (Nguyen & Pieters, 2009, Nguyen & Thompson, 2006). Furthermore, the current standard regimen (DOTS) for TB is comprised of six to nine months of daily antibiotic treatment with a combination of four out of these five drugs, often leading to poor patient adherence and incomplete courses of treatment. The rapid rate of mutations occurring in bacteria in general, together with the frequent exposure of M. tuberculosis to sub-optimal doses of drugs, have granted ample opportunity for this pathogen to acquire additional resistance by amassing sequential mutations in drug-target encoding genes (Nguyen & Pieters, 2009, Nguyen & Thompson, 2006). Accordingly, we now face the problem of multiple drug resistant (MDR) and extensively drug resistant (XDR) M. tuberculosis strains. MDR strains exhibit resistance to at least the two most potent first-line drugs (RIF and INH). Besides RIF and INH, XDR strains are resistant to any fluoroquinolones and to at least one of the three injectable second-line drugs (capreomycin, kanamycin, and amikacin) (World Health Organization, 2006). Infections with such strains require further prolonged and aggressive treatment courses employing

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combinations of numerous second-line drugs that often exhibit toxic side effects and are expensive to administer (Dye, 2000, Nguyen & Thompson, 2006). Moreover, the spread of these infections is diminishing our already limited arsenal of effective antibiotics even further with which some XDR *M. tuberculosis* strains have become virtually untreatable with current medicines (Gandhi *et al.*, 2006, Jassal & Bishai, 2009, LoBue, 2009).

The current prevalence of drug-resistant strains poses a dire need for alternative TB therapies. Development of completely new TB drugs is both time-intensive and costly. Although a few compounds have made their way into pre-clinical or clinical stages, this approach thus far has provided us with no newly approved anti-TB drugs. On average, it takes twelve to fifteen years and US $500 million to get a new drug from the laboratory to the market (Bolten & DeGregorio, 2002). Clearly, the possibility that new resistant strains may rapidly occur and diminish the utility of a new drug after approval represents a significant risk factor for the development of anti-infective drugs. An alternative approach to this pathway is presented by the concept of “targeting resistance”. This drug potentiation approach, which uses knowledge of resistance mechanisms to (re)sensitize pathogenic bacteria to already available drugs, may become an important trend in the new era of drug development for infectious diseases. The coadministration of existing drugs and inhibitors that suppress resistance mechanisms allows ineffective drugs to (re)gain their antimicrobial activity (Wright, 2000, Wright & Sutherland, 2007) (Figure 1). In the case of *M. tuberculosis*, this approach could be used to rescue and extend the utility of current TB drugs, or make use of other available drugs that are currently inactive against the bacillus. The extended lifespan of valuable approved antibiotics of known pharmacology, toxicology, and treatment schedule, represents a unique advantage of the drug potentiation approach.

This chapter will explore recent findings that suggest several available drugs as promising candidates for resistance-targeted potentiation. Future directions regarding this approach in TB-drug development will also be discussed.

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**Fig. 1.** Concept of drug potentiation by targeting resistance. An active resistance mechanism allows survival of bacterial pathogens in the face of an antibiotic(s). A potentiator that inhibits the resistance mechanism would (re)sensitize the bacteria to the antibiotic(s), thus enhancing antibacterial activity.
2. β-Lactams

The most widely used group of antibiotics today is the β-lactams, a broad class of drugs including penicillin and penicillin derivatives, cephalosporins, monobactams and carbapenems (Figure 2 A), that target bacterial cell wall synthesis at the peptidoglycan layer (Koch, 2003, Waxman et al., 1980). Peptidoglycan is the major component of the cell wall in both Gram-positive and Gram-negative bacteria and is extensively cross-linked by penicillin-binding proteins (PBPs), lending it stability (Koch, 2003, Waxman et al., 1980). β-lactam antibiotics are cyclic amides containing a hetero-atomic ring consisting of three carbon atoms, and one nitrogen atom (Figure 2 A), mimicking certain precursors of peptidoglycan (Koch, 2003, Waxman et al., 1980). When PBPs mistakenly use β-lactams as their substrate rather than peptidoglycan precursors, the antibiotics are incorporated irreversibly into the PBP structure, inhibiting cross-linking activity (Koch, 2003, Waxman et al., 1980), leading to consequent cell lysis in hypotonic environments (Beveridge, 1999, Lee et al., 2001, Severin et al., 1997).

Although serving as the only successful clinical example of potentiation through targeting resistance, inhibitors of β-lactamases have prolonged the life of β-lactams for more than thirty years (Drawz & Bonomo, 2010). Without these potentiators, many β-lactams would have long become useless against multiple bacterial pathogens. β-lactams such as penicillin are now commonly coadministered with β-lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam that prevent degradation of β-lactams, thus sustaining bacterial susceptibility to β-lactams.

2.1 β-Lactam resistance mechanisms in *Mycobacterium tuberculosis*

In the case of mycobacteria, resistance to β-lactams involves three main components: permeability of the mycobacterial cell wall (Chambers et al., 1995, Jarlier et al., 1991, Jarlier & Nikaido, 1990, Kasik & Peacham, 1968), affinity of the drugs to their target PBPs (Chambers et al., 1995, Mukherjee et al., 1996), and degradation by β-lactamase activity (Jarlier et al., 1991, Quinting et al., 1997). In addition, since *M. tuberculosis* is an intracellular pathogen, an effective TB drug must be able to penetrate the macrophage and phagosomal membranes to reach the bacilli residing within.

Although mycobacteria are classified as Gram-positive bacteria, their cell wall is extremely thick and multi-layered with varied hydrophobicity, posing an effective obstacle for the entry of most chemical compounds. The peptidoglycan network is covered by an arabinogalactan layer, both of which are hydrophilic and likely limit penetration of hydrophobic compounds (Brennan & Nikaido, 1995). On top of these aforementioned layers is another layer consisting of mycolic acids linked to acyl lipids, which forms a waxy, non-fluid barrier restricting transport of both hydrophobic and hydrophilic molecules (Liu et al., 1995). Penetration by diffusion of β-lactams through the mycobacterial cell wall is hundreds of times slower than that of *Escherichia coli* (Chambers et al., 1995, Kasik & Peacham, 1968). However, because of the extremely long generation time of *M. tuberculosis*, the slow rate of drug penetration is enough to allow for half-equilibration over the membrane well before the cell divides, making cell wall permeability and therefore drug penetration important but not a major determinant of β-lactam resistance (Chambers et al., 1995, Quinting et al., 1997).

As for drug target affinity, four major PBPs have been identified in *M. tuberculosis*, all of which bind β-lactams at therapeutically achievable concentrations (Chambers et al., 1995). The 49-kDa PBP from *M. smegmatis* is also sensitive to several β-lactams at similar
concentrations (Mukherjee et al., 1996). Therefore, target affinity does not significantly contribute to the mycobacterial β-lactam resistance. Cell division in M. tuberculosis is extremely slow, only occurring every 15-20 hours. This slow growth contributes both negatively and positively to drug resistance. Carbapenem antibiotics, which seem to be the most effective β-lactam with antimycobacterial activity, are relatively unstable and lose activity much faster than the mycobacterial growth rate (Watt et al., 1992). It has however been shown that a daily antibiotic regimen can compensate for loss of activity and markedly increase growth inhibitors in vitro (Watt et al., 1992).

With drug penetration and target affinity being negligible for β-lactam resistance in mycobacteria, degradation by β-lactamases constitutes the principal resistance mechanism. β-lactamase activity has been reported in all known mycobacterial species (Kasik, 1979), except the non-pathogenic M. fallax, which exhibits hypersusceptibility to β-lactams (Quinting et al., 1997). Mycobacteria, including M. tuberculosis, export β-lactamases to the cell wall via the twin-arginine translocation (Tat) pathway (McDonough et al., 2005, Voladri et al., 1998), thus disruption of the Tat transporter leads to lower β-lactamase activity in M. smegnatis culture filtrates and increased β-lactam susceptibility (McDonough et al., 2005).

The major β-lactamase in M. tuberculosis, BlaC, is a member of the Ambler Class-A β-lactamases and exhibits broad substrate specificity, catalysing hydrolysis of both cephalosporins and penicillins (Voladri et al., 1998, Wang et al., 2006). This broad substrate specificity is attributed to the large and flexible substrate-binding site of this particular β-lactamase (Wang et al., 2006). Two additional β-lactamase-like proteins, encoded by the rv0406c and rv3677c genes, have been identified to provide M. tuberculosis H37Rv with a lower β-lactamase activity (Nampoothiri et al., 2008). Expression of these proteins in E. coli confers significant resistance to β-lactam antibiotics (Nampoothiri et al., 2008).

In general, mycobacterial β-lactamases exhibit low-level activity compared to those of other pathogenic bacteria. However, because of the slow equilibration of β-lactams across the thick cell wall, this low β-lactamase activity is effective enough to provide protection to mycobacteria from β-lactam action (Jarlier et al., 1991). When M. fallax in trans expresses the β-lactamase from M. fortuitum, MICs for β-lactams increase dramatically, indicating that β-lactamase-mediated degradation is the critical contributor to β-lactam resistance in mycobacteria (Quinting et al., 1997). For most bacterial β-lactamases, β-lactams of the carbapenem subgroup are highly resistant to hydrolysis. Unfortunately, M. tuberculosis BlaC shows measurable activity with carbapenem compounds including imipenem, ertapenem, doripenem and meropenem, even though imipenem and meropenem seem somewhat more effective than other carbapenems and penicillins in antimycobacterial activity (Hugonnet & Blanchard, 2007, Tremblay et al., 2010).

Interestingly, BlaC production in M. tuberculosis is β-lactam inducible, and controlled by a regulatory network that is also present in other Gram-positive bacteria (Sala et al., 2009). The transcriptional repressor BlaI, a winged helix regulator, forms homodimers that bind DNA at specific recognition sites in the absence of β-lactam antibiotics (Sala et al., 2009). BlaC is the only β-lactamase in M. tuberculosis whose gene is among the BlaI regulon (Sala et al., 2009). Exposure to β-lactams dissociates BlaI from its DNA binding site, lifting its suppression on blaC transcription thus allowing the production of BlaC β-lactamase activity (Sala et al., 2009).
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Fig. 2. (A) Structures of β-lactams. The β-lactam ring structure constitutes the base of all β-lactams while the secondary ring structure determines the class. (B) Mechanism of reaction between β-lactamase and clavulanate. The serine residue of the reactive site of β-lactamase reacts with the carbonyl group of clavulanate, followed by breakage of the amide bond that results in acylation. The acylation step is followed by the formation of an imine and secondary ring opening. Note that the ring opening step does not occur with all β-lactamase inhibitors.

2.2 Potentiation of β-lactams in mycobacteria
Attempts to promote the utility of β-lactams to treat TB and other mycobacterial infections have been continuously explored by many laboratories. Rather than ignoring these antibiotics, their antimycobacterial activity could be potentiated by coadministration with β-lactamase inhibitors, as routinely practiced for other bacterial infections. As mycobacterial susceptibility to β-lactams is quite high in the absence of β-lactamase activity (Flores et al., 2005, Quinting et al., 1997), effective chemical inactivation of β-lactamases should similarly increase β-lactam sensitivity in these bacteria. In fact, in vitro studies first showed that three FDA-approved inhibitors, sulbactam, tazobactam, and clavulanate, effectively inhibit nitrocefin degradation by purified BlaC protein (Hugonnet & Blanchard, 2007, Tremblay et al., 2008). While sulbactam inhibits BlaC competitively and reversibly, tazobactam inhibits BlaC in a time-dependent manner with reappearing enzyme activity. Interestingly, clavulanate forms hydrolytically stable, inactive forms of the enzyme, completely and irreversibly inhibiting BlaC in a mechanism in which after acylation of clavulanate, a secondary ring-opening leads to reactive intermediates that occupy the active site of the enzyme (Figure 2 B) (Hugonnet and Blanchard 2007; Tremblay et al. 2008). Hence, clavulanate provides a potential lead for the development of effective β-lactam potentiators for TB.

Whereas data obtained from the aforementioned in vitro studies are promising, drugs used for TB must be able to penetrate the mycobacterial cell wall in order to exert their activity. A later in vitro study showed that meropenem/clavulanate combination is very effective in killing both aerobically and anaerobically grown M. tuberculosis (Hugonnet et al., 2009).
More importantly, the drug combination is also effective against thirteen tested XDR *M. tuberculosis* strains (Hugonnet et al., 2009). Furthermore, studies using mouse peritoneal macrophages infected with *M. tuberculosis* indicated that penetration through neither host macrophage nor phagosomal membranes appears to be a problem for β–lactams and/or β–lactamase inhibitors (Chambers et al., 1995, Prabhakaran et al., 1999). Significant reduction of mycobacterial counts in mouse macrophages upon treatment with various combinations of β–lactams and β–lactamase inhibitors within clinically achievable doses has been demonstrated (Chambers et al., 1995, Prabhakaran et al., 1999). Encouraging results were also reported in animal models. Although less effective than the first-line anti-TB drug INH, imipenem significantly reduced *M. tuberculosis* counts in the lungs and spleens of infected mice (Chambers et al., 2005). As a result, imipenem doubled the survival rate of infected mice (35% mortality vs. 70%). While only a very few cases in which TB patients treated with β–lactams in conjunction with or without potentiaters have been reported, the results were always promising. One study using imipenem alone in MDR-TB patients with poor predicted outcomes achieved a 70% cure rate (Chambers et al., 2005). Another study showed that treatment of TB patients with an amoxicillin/clavulanate combination significantly reduced *M. tuberculosis* counts with early bactericidal activity comparable to patients treated with the frontline drug INH (Chambers et al., 1998). Importantly, a case report recently described the successful recovery of an advanced XDR-TB patient treated with meropenem/clavulanate in conjunction with other drugs (Dauby et al., 2011).

In summary, evidence obtained from numerous studies performed in vitro, in animals, and in humans, all support that β–lactams potentiated by β–lactamase inhibitors could provide an effective addition to the treatment of drug resistant TB.

### 2.3 Future perspectives

Much work remains to be done in potentiating β–lactams for the treatment of mycobacterial infections. More existing carbapenems should be tested in combination with various β–lactamase inhibitors. With crystal structures and kinetic data now available (Hugonnet & Blanchard, 2007, Hugonnet et al., 2009, Tremblay et al., 2010, Tremblay et al., 2008), rational design or high throughput screening should be done to identify better inhibitors that specifically target BlaC or the other β–lactamases of *M. tuberculosis*. Similarly, sensitization of *M. tuberculosis* to β–lactams could be achieved by preventing dissociation of BlaI from its binding site on the *blaC* promoter, thereby repressing the expression of this major β–lactamase in the face of β–lactam exposure. In other bacteria, BlaI homologs are inactivated by proteolytic cleavage at a highly conserved Asparagine-Phenylalanine bond located in helix α5, which is also present in *M. tuberculosis* BlaI (Sala et al., 2009). Although it has not been identified yet, the most likely candidate for the inactivating protease is predicted to be Rv1845c, a zinc metalloprotease encoded by a gene located adjacent to *blaI* (Sala et al., 2009). If proteolysis of BlaI could be prevented by targeting Rv1845c with protease inhibitors, the BlaI-mediated repression of *blaC* could be promoted to render the bacilli more susceptible to β–lactams. Similarly, inhibition of β–lactamase translocation by targeting the Tat transpoter system may also represent a novel strategy for β–lactam potentitiation.

Since very promising results have been obtained with *in vitro* studies of MDR and XDR *M. tuberculosis* using β–lactamase inhibitors, more comprehensive and well-structured clinical trials with human MDR and XDR TB need to be done in order to affirm the efficacy of these
agents for TB treatment. Currently, a phase II clinical trial in 100 TB patients utilizing meropenem potentiated by clavulanate is being planned in South Korea (Drug Information Online, 2011, Science Centric, 2009). In addition, frequency of dosing will need to be determined to improve and maintain effective doses over longer periods of time. One of the major obstacles to the effective use of β-lactams in long course regimens is that currently used carbapenems have to be administered intravenously, leading to high costs of treatment due to necessary supervision by health care professionals as well as complicating patient compliance over the entire course of treatment.

3. Ethionamide

Ethionamide (ETH, 2-ethylthioisonicotinamide, or Trecator SC, Figure 3 A) is an important component of most current drug regimens used in the treatment of MDR-TB. While an effective drug against more than 80% of MDR-TB clinical strains, ETH has a low therapeutic index, or margin of safety, characterized by a narrow therapeutic effective concentration range (Sood & Panchagnula, 2003, Zimmerman et al., 1984). In other words, it is more difficult to prescribe ETH treatment doses that ensure effective treatment outcomes and yet avoid toxic side effects (Burns, 1999). The lowest dose of ETH required to inhibit \textit{M. tuberculosis} growth has been shown to elicit adverse side effects such as hepatitis and gastrointestinal ailments (Flipo et al., 2011). Discovered in 1956, ETH is a structural thioamide analogue of INH and must be metabolically activated in order to form adducts with nicotinamide adenine dinucleotide (NAD). The ETH-NAD adducts subsequently inhibit InhA, the NADH-dependent enoyl-ACP reductase of the fatty acid biosynthesis type II system, allowing ETH to exert its activity against the synthesis of mycolic acids, the major component of the tubercle bacilli cell wall (Brossier et al., 2011, Morlock et al., 2003, Vannelli et al., 2002, Vilcheze et al., 2008, Zhang, 2005).

3.1 Ethionamide resistance mechanisms in mycobacteria

ETH activation requires an NADPH-specific FAD-containing monoxygenase, encoded by \textit{ethA}, which oxidizes ETH to form the covalent ETH-NAD adducts. The active ETH-NAD adducts tightly bind to and inhibit InhA activity (Figure 3 A) (Brossier et al., 2011, DeBarber et al., 2000, Frenois et al., 2004, Morlock et al., 2003, Wang et al., 2007). EthA was shown to catalyze the conversion of ketones to esters, suggesting its physiological function in mycolic acid metabolism of \textit{M. tuberculosis} (Fraaije et al., 2004). In the majority of ETH resistant \textit{M. tuberculosis} isolates, mutations have been mapped to four principal catagories: (i) mutations that alter activity of EthA, (ii) mutations in \textit{ethR}, the gene located adjacent to \textit{ethA}, (iii) mutations in InhA that prevent binding of the activated drug, and (iv) mutations in the \textit{inhA} promoter region that lead to InhA overexpression, (Banerjee et al., 1994, Baulard et al., 2000, Brossier et al., 2011, DeBarber et al., 2000, Morlock et al., 2003). Besides these four main catagories, several additional genes (\textit{ndh}, \textit{mshA}, and \textit{dfrA}) might also be involved in ETH resistance. For example, mutations in \textit{ndh}, which encodes a NADH dehydrogenase, may result in an increased intracellular concentration of NADH that competitively inhibits the binding of ETH-NAD adducts to InhA (Vilcheze et al., 2005). While the connection of \textit{ndh} mutations and ETH resistance has been demonstrated in \textit{M. bovis} BCG and \textit{M. smegmatis}, it has not been observed in \textit{M. tuberculosis} (Brossier et al., 2011). \textit{mshA} encodes a glycosyltransferase involved in the biosynthesis of mycothiol that may enhance the ETH activation by EthA (Brossier et al., 2011, Vilcheze et al., 2008, Xu et al., 2011). Whereas \textit{mshA}
mutations might be readily identified in vitro under ETH selection pressure, mutations in
\textit{mshA} only represent a minority among ETH resistant \textit{M. tuberculosis} clinical isolates
(Brossier et al., 2011). Lastly, \textit{dfrA} encodes the dihydrofolate reductase activity involved in
folate biosynthesis. As it was suggested that dihydrofolate reductase is inhibited by INH
adducts (Argyrous et al., 2006), this enzyme may also be targeted by the adducts of ETH.
Thus far, mutations in \textit{dfrA} have not been identified among ETH resistant clinical isolates
(Brossier et al., 2011).

In summary, the reduced EthA-mediated activation of ETH represents the principal
molecular mechanism contributing to ETH resistance. Indeed, \textit{in trans} overexpression of the
prodrug activator EthA in \textit{M. smegmatis} leads to increased ETH sensitivity and inhibition of
mycolic acid synthesis (Morlock et al., 2003, Willand et al., 2009) whereas attempts to
overexpress EthA in \textit{M. tuberculosis} have been unsuccessful (DeBarber et al., 2000, Morlock et
al., 2003). In recent studies, it has been clarified that the production of EthA is negatively
controlled by the transcriptional regulator EthR, encoded by an adjacent gene (Figure 3B)
(Baulard et al., 2000, Morlock et al., 2003). In \textit{in trans} overexpression of EthR causes strong
inhibition of ethA expression, whereas chromosomal inactivation of \textit{ethR} stimulates ETH
hypersensitivity (Dover et al., 2004, Engohang-Ndong et al., 2004). Furthermore,
electrophoretic mobility shift assays and DNA footprinting analysis indicate direct
interaction of EthR with the ethA promoter (Dover et al., 2004, Engohang-Ndong et al., 2004).
EthR is a member of the TetR/CamR family of repressors that is suggested to sterically
inhibit the interaction between RNA polymerase and the affected promoter (Engohang-
Ndong et al., 2004, Frenois et al., 2004, Willand et al., 2009). In fact, \textit{M. tuberculosis} EthR was
shown to cooperatively multimerize on a 55-bp operator, \textit{O_{\text{ethR}}}, located within the ethA
promotor, thereby repressing \textit{ethA} expression (Frenois et al., 2004, Vannelli et al., 2002,
Weber et al., 2008).

Similar to other TetR/CamR repressors, recent X-ray crystallographic structures revealed
that EthR exists as a homodimer organized by two functional domains, each composed of
nine $\alpha$-helices (Dover et al., 2004, Frenois et al., 2004, Willand et al., 2009). The amino
terminus of each DNA binding domain consists of a classical helix-turn-helix motif formed by $\alpha_1$, $\alpha_2$, and $\alpha_3$. The remaining six $\alpha$-helices comprise the carboxy-terminus, which contains the
ligand-binding site responsible for controlling the conformational changes that prevent
binding of EthR to \textit{O_{\text{ethR}}}. Interactions between $\alpha$-helices of each monomer form a four-helix
bundle resulting in dimerization of the repressor. The crystal structures also revealed a
ligand cocry stallized with EthR (Frenois et al., 2006, Frenois et al., 2004). This ligand,
hexadecyl octanoate (HexOc), occupies the hydrophobic tunnel of each monomer by means of
hydrophobic interactions and hydrogen bonds (Willand et al., 2009). In the presence of
HexOc, the distance between the two DNA binding domains in the EthR structure is
augmented by 18 Å. As a result, the conformational change impairs the ability of EthR to
bind to its operator (Frenois et al., 2006, Frenois et al., 2004, Willand et al., 2009). The ligand-
binding domain, embedded in the core domain of each monomer, is characterized as a
narrow hydrophobic tunnel rich in aromatic residues (Dover et al., 2004, Willand et al., 2009).

More recently, two ETH resistant isolates expressing two unique mutations in EthR,
Phenylalanine 110 changed to Leucine and Alanine 95 changed to Threonine, further
illuminated the derepression mechanism of EthR. Both Phenylalanine 110 (located within
the $\alpha_5$ helix) and Alanine 95 (located within the vicinity of helices $\alpha_4$ and $\alpha_5$) contribute to
the ligand-binding domain (Brossier et al., 2011). Based on this wealth of knowledge, recent
efforts are being made to develop compounds that could potentially interfere with EthR repressor function. Such inhibitors could therefore potentiate the antimycobacterial efficacy of ETH and possibly reduce its adverse side effects by allowing lower prescribed doses (Flipo et al., 2011).

Fig. 3. Ethionamide activation and potentiation. (A) Model of ETH activation by EthA. ETH is first oxidized by EthA to a corresponding thioamide S-oxide that is further oxidized to form the final cytotoxic species. Although the latter oxidation steps remain unclear, it is postulated that thioamide S-oxide is converted to an imidoyl radical (right), which attacks NAD+. Following hydrolysis and release of the amine group, the final ETH-NAD adducts are formed. Alternatively, the amidoyl anion (left) can serve as the intermediate before the NAD attack. Scheme redrawn from (Wang et al., 2007). (B) Potentiation of ETH by targeting EthR. Binding of inhibitors releases EthR from its interaction with the ethA promoter. This allows for derepression of EthA expression, which is responsible for converting ETH to its active form ETH-NAD. The activated drug then binds to InhA and inhibits its activity in mycolate biosynthesis. EthR inhibitors could thereby function as ETH potentiators.

3.2 Potentiation of ethionamide in mycobacteria
Since ligand binding was shown to affect EthR function in repressing ethA expression, and increase susceptibility of M. tuberculosis to ETH (Flipo et al., 2011, Frenois et al., 2006, Frenois et al., 2004, Willand et al., 2009), much interest has been invested in determining whether synthetic compounds could be utilized to regulate DNA-binding activity of EthR (Weber et al., 2008, Willand et al., 2009). Since the DNA-binding domain of EthR is able to
accommodate a hydrophobic ester such as HexOc, initial attempts were made using several ketones to assay their ability to function as EthR ligands as well as to increase mycobacterial ETH sensitivity (Frenois et al., 2004). In vitro experiments demonstrated synergy of benzylacetone and ETH on \textit{M. smegmatis} growth (Fraaije et al., 2004). Whereas benzylacetone itself did not display antimycobacterial activity, its addition to ETH used at subinhibitory concentrations (5 µg ml\(^{-1}\)) produced significant inhibition of mycobacterial growth (Frenois et al., 2004).

As an intracellular pathogen, \textit{M. tuberculosis} resides within phagosomal compartments of host macrophages (Nguyen & Pieters, 2005). Therefore, EthR inhibitors not only have to specifically target the repressor but must be able to reach the macrophages’ cytosol (Weber et al., 2008). To screen for drug-like ETH potentiators, an EthR-based reporter system was first developed by the Fussenegger group (Weber et al., 2008). This elegant mammalian-based system allows for assessment of not only specificity and bioavailability of tested molecules, but also their cytotoxicity to the host cell. A library of hydrophilic esters, the primary products of EthA-catalyzed Baeyer-Villiger oxidation of ETH, was synthesized and tested for their ability to release EthR from its O\textsubscript{ethR} operator within a mammalian cell, using the therein described reporter system. A licensed food additive, 2-phenylethyl-butyrate, was found to effectively regulate EthR activity as well as to increase \textit{M. tuberculosis} susceptibility to ETH (Weber et al., 2008).

In vitro analysis of \textit{ethA} transcripts by quantitative real time PCR verified that 2-phenylethyl-butyrate dissociates EthR from the \textit{ethA} promoter in a dose-dependent manner. To assess bioavailability, the reporter system was transfected into human embryonic kidney (HEK) cells that were subsequently implanted into mice. In this animal model, orally administered 2-phenylethyl-butyrate effectively reached the target cells to activate the reporter gene. Most importantly, 2-phenylethyl-butyrate displayed synergistic effects with ETH on the growth inhibitory activity against pathogenic mycobacteria (Weber et al., 2008).

From previous analyses of ligand-binding EthR crystal structures (Dover et al., 2004, Frenois et al., 2006, Frenois et al., 2004), the hydrophobic interactions and hydrogen-bonding properties of the amphiphilic binding cavity was utilized to design a pharmacophore model as a means of isolating moderately lipophilic compounds that could potentially interfere with the repressor function of EthR (Willand et al., 2009). The novel pharmacophore model was designed as a low-molecular weight structure consisting of two hydrophobic ends connected by a 4-6 Å linker. This would, in turn, allow for hydrogen bonding interactions with the tunnel’s uncharged polar surface formed by Asparagine 179 and Asparagine 176 side-chains. From a library of drug-like compounds, 131 compounds fitting the pharmacophore model were selected and analyzed for properties relevant for drug development such as molecular weight, rotatable bonds, polar surface area, hydrogen bond donors and acceptors, etc. (Willand et al., 2009). Surface plasmon resonance and co-crystallization assays emphasized several compounds with the ability to inhibit EthR-DNA interaction. Using this approach, BDM14500, a lead compound comprised of a 1,2,4-oxadiazole linker, was identified to inhibit EthR-DNA interaction by more than 50%. More importantly, inhibition activity of ETH on \textit{M. tuberculosis} growth is significantly boosted by BDM14500 (Willand et al., 2009). The primary data obtained from studies of BDM14500 allowed further development of improved EthR ligands. Two thiophen-2-yl-1,2,4-oxadiazole analogs of BDM14500, BDM31343 and BDM31381, were synthesized and subjected to surface plasmon resonance, co-crystallization, and ETH potentiation assays (Filpo et al., 2011, Willand et al., 2009). Kinetic analysis showed that BDM31343 and BDM31381 inhibit
the interaction of EthR and O_{ethR} with IC_{50} values in the nanomolar to micromolar range, indicating their potentially high efficacy. Indeed, in *M. bovis* BCG culture, BDM31381 treatment results in a 35-fold increase in the level of *ethA* mRNA. It is suggested that the efficacy of BDM31381 resides in its ability to form an energetically favorable orientation by generating a new hydrogen bond between the carbonyl of the ligand and the carboxamide of the Asparagine 179 side chain. In fact, MIC assays later confirmed that BDM31343 and BDM31381 are both more effective potentiators of ETH activity (Flipo *et al.*, 2011, Willand *et al.*, 2009). The addition of BDM31343 or BDM31381 (25 µM) respectively allows a 10 (0.1 vs 1 µg ml^{-1}) or 20 (0.025 vs 0.5 µg ml^{-1}) fold reduction in ETH concentration yet retains identical *M. tuberculosis* growth inhibition activity. In other words, BDM31343 and BDM31381 are able to potentiate ETH antimycobacterial activity by factors of 10 and 20, respectively (Willand *et al.*, 2009).

In vivo, mice infected with *M. tuberculosis* were treated for 3 weeks with ETH alone or in conjunction with BDM31343 or BDM31381. Following treatment, the mycobacterial load in mouse lungs was quantified. Whereas the BDM31381/ETH combination had only a minor effect on bacterial load compared to control mice treated with ETH alone, the combination of BDM31343 with ETH resulted in a significant decrease of *M. tuberculosis* load with three times more efficiency than with ETH treatment alone. TB treatment with reduced ETH dosages by combining it with BDM31343 may thus allow for efficient elimination of the bacillus without severe side effects (Willand *et al.*, 2009).

### 3.3 Summary and future perspectives

Through the implementation of strategies including X-ray crystallography, pharmacophore modeling (Willand *et al.*, 2009), and synthetic mammalian gene circuits (Weber *et al.*, 2008), effective potentiators of ETH have been identified. While further *in vitro* and *in vivo* analyses of these compounds will need to be performed, it is expected that such potential molecules will boost activity and allow ETH to be reconsidered as a first-line anti-TB antibiotic (Weber *et al.*, 2008, Willand *et al.*, 2009). In addition, because ETH and INH inhibit the same target, InhA (Banerjee *et al.*, 1994), ETH potentiation might create an exponential boost for the anti-TB activity of INH and hence their combination. As the attrition rate of the developmental process is enormous (Bolten & DeGregorio, 2002), much work remains to be done in preclinical and clinical development and product approval stages in order to bring this concept to the clinics. Regardless of this risky process, the results obtained from these studies have showcased the potential of this approach in improving the efficacy of existing TB-drugs, thus extending their lifespan in TB treatment. Similar studies with other TB-drugs need to be encouraged, which will not only help to better understand their mechanisms of action and resistance, but also reveal further targets for the drug potentiation approach.

### 4. Antifolates

Folate is a generic name referring to a large group of chemically similar B vitamins that are essential for the existence of cells in all kingdoms of life. Whereas the synthetic form, widely used as a nutritional supplement, is called folic acid or vitamin B9 (pteroylmonoglutamic acid, PteGlu), most naturally occurring folate forms are derived from the reduced molecule tetrahydrofolate (H_{4}PteGlu, Figure 4). All of these compounds are comprised of three molecular components: a two-ring pteridine nucleus, a para-aminobenzoic acid (pABA) group, and one or more glutamate residues attached via amide linkages. These molecules vary by the C1 groups attached to the N-5 or/and N-10 positions of H_{4}PteGlu (Figure 4B).
Folates are important metabolites indispensable for the development and propagation of all organisms. H$_4$PteGlu derivatives are required in reactions that involve the transfer of one-carbon units (C1 reactions, Figure 4A). These reactions are essential for the biosynthesis of purines, thymidine, glycine, panthotenate, methionine, and formyl-methionyl-tRNA, the initiator of protein synthesis in bacteria (Blakley, 1969, Green et al., 1996, Selhub, 2002). Because these molecules are required for the synthesis of the building blocks of macromolecules such as nucleic acids and proteins, folate deficiency hinders cell division and consequently results in cell death. In addition, lack of folate derivatives also leads to defects in the recycling of homocysteine (Hcy, Figure 4) and S-adenosine methionine (SAM), which result in elevated homocysteine concentration (homocysteinemia) and reduced cellular methylation activities, respectively. Folates are particularly important during periods of rapid cell division and growth (Blakley, 1969, Green et al., 1996).

![Fig. 4. Folate metabolism and antagonism in bacteria. (A) Simplified interconversions of folate derivatives in de novo folate synthesis and one-carbon metabolic network. DHFS, dihydrofolate synthase; Gly, glycine; Met, methionine; MS, methionine synthase; Pte, pteroate; Ser, serine. (B) Chemical structure of monoglutamylated tetrahydrofolate and its derivatives carrying CI groups at various levels of oxidation attached to N-5 or/and N-10. Redrawn from (Waller et al., 2010). (C) Scanning spectrophotometric analysis of MTHFS reaction, which converts 5-CHO-H$_4$PteGlu (Abs, 285nm) to 5,10-CH$_3$-H$_4$PteGlu (Abs, 360 nm), catalyzed by the M. tuberculosis MTHFS homolog, Rv0992c, a novel determinant of antifolate resistance.](www.intechopen.com)
Folate metabolism is generally divided into two stages: biosynthesis (upstream) and utilization (downstream) (Figure 4A). The upstream de novo folate biosynthesis involves: (i) pterin branch synthesizing the pteridine group from guanosine triphosphate (GTP), (ii) synthesis of pABA from chorismate, (iii) condensation of pteridine and pABA to form dihydropteroate (H₂Pte) and (iv) glutamylation which adds one or more glutamate groups to form dihydrofolate (H₂PteGlu) that is reduced to form H₄PteGlu. The downstream folate utilization is usually called one-carbon metabolism in which different active forms of H₄PteGlu participate in distinct reactions donating or accepting one-carbon units for the formation of purines, thymidine, glycine, panthotenate, methionine, and formyl-methionyl tRNA (Figure 4).

Because of the vital role of folates in multiple metabolic processes of the cell, folate antagonism has been used successfully in chemotherapeutic treatments of multiple diseases including cancers, malaria, psoriasis, rheumatoid arthritis, graft-versus-host disease, and bacterial infections (Bertino, 1971, Gorlick et al., 1996, Vinetz, 2010). Folate antagonists (antifolates or antifols) have been used extensively for the treatment of infectious diseases from the late 1930s till 1960s, but their use has declined because of the emergence of resistant strains, their cytotoxicity, and most importantly the introduction of more effective drugs (Bertino, 1971, Libecco & Powell, 2004). Nevertheless, combination therapies using trimethoprim and sulfonamides to create synergistic effects are still used effectively today to treat some infectious diseases such as urinary tract infection, Pneumocystis jiroveci pneumonia, shigellosis, and for prophylaxis against recurrent and drug-resistant infections (Grim et al., 2005, Libecco & Powell, 2004, Proctor, 2008). The absence of enzymes required for a complete de novo folate biosynthesis in humans and other mammals makes this pathway an attractive and potential target for the development of novel antimicrobial agents (Bermingham & Derrick, 2002). Whereas proteins participating in folate metabolism are well known, most current folate antagonists are thought to act on either the biosynthesis or the reduction of folate (Bermingham & Derrick, 2002, Gangjee et al., 2007, 2008). Whereas trimethoprim and folate analogs such as methotrexate inhibit the reduction step through inhibition of dihydrofolate reductases (DHFR), sulfonamides and sulfone drugs are pABA analogs that outcompete pABA in the condensation with the pteredin group, catalyzed by dihydropteroate synthase (DHP5) (Bermingham & Derrick, 2002, Gangjee et al., 2007, 2008).

4.1 Folate antagonism in chemotherapies of mycobacterial infections and antifolate resistance

The essentiality of folate-mediated one-carbon metabolism in fundamental metabolic and cellular processes has been recognized since the 1940s. Almost immediately after folates had been identified as essential metabolic cofactors, antifolate drugs that interfere with the folate pathway were developed and found to be effective antimicrobial and antineoplastic agents. As seen with other antibiotics, acquired resistance to antifolates in pathogenic bacteria also occurred rapidly following their introduction. These resistant forms are typically caused by mutations that alter either expression levels or protein structures of the targeted enzymes (Bertino, 1971, Libecco & Powell, 2004). DHFR can acquire resistance through point mutations of active-site residues, thus altering its affinity for trimethoprim (Adrian & Klugman, 1997, Volpato & Pelletier, 2009). While clinical resistant strains frequently show a diversity of mutations, residues that are most important for trimethoprim affinity are highly conserved among the isolates (Adrian & Klugman, 1997). For example, a point mutation in the DHFR gene that changes a conserved Isoleucine residue (Isoleucine 94 in M. tuberculosis
DHFR) to Leucine, confers 50-fold higher trimethoprim resistance in *Streptococcus pneumoniae* (Adrian & Klugman, 1997). This mutation is commonly found in DHFR from mammalian, parasitic and bacterial resistant isolates (Volpato & Pelletier, 2009). For sulfonamide and sulfone drugs, single point mutations at the Serine 53 or Proline 55 residues within DHPS are found in resistant isolates of *M. leprae* (Baca et al., 2000, Kai et al., 1999). The two affected residues are located in the drug binding region of *M. tuberculosis* DHPS and are highly conserved throughout bacteria and protozoa (Baca et al., 2000). Combined mutations in DHFR and DHPS encoding genes have been known to confer resistance to all available antifolates (Bermingham & Derrick, 2002, Gangjee et al., 2007, 2008). It is important to note that most current knowledge of trimethoprim and sulfonamide resistance comes from studies of bacteria distantly related to *M. tuberculosis*, and very limited information on mechanisms involved in antifolate resistance is available for mycobacterial species. With the increasing use of antifolates (Date et al., 2010), a better understanding of antifolate resistance mechanisms in *M. tuberculosis* is urgently needed (Koser et al., 2010).

Although much remains unknown about resistance mechanisms, antifolate drugs have been used to treat mycobacterial infections. For example, PAS (p-aminosalicylic acid) is currently used as a second-line drug for TB (Rengarajan et al., 2004); the sulfone drug Dapsone has been used in monodrug regimens to treat leprosy for many decades (Doull, 1963). Interestingly, a recent study suggested that the frontline TB-drug INH may also target folate metabolism through the inhibitory action of its adducts on DHFR (Argyrou et al., 2006). In addition, recent *in vitro* studies and a case report proposed that antifolate combinations such as those of co-trimoxazole (trimethoprim plus sulfamethoxazole) might be effective against TB, thus renewing much interest in the exploitation of antifolates to treat MDR and XDR-TB (Forgacs et al., 2009, Ong et al., 2010, Young, 2009). *M. tuberculosis* clinical strains isolated from TB patients were shown to be widely susceptible to clinically achievable concentrations of co-trimoxazole (Forgacs et al., 2009), or sulfamethoxazole alone (Ong et al., 2010). Importantly, the World Health Organization has recently called for widespread use of co-trimoxazole in the prophylactic treatment of HIV-AIDS patients to prevent opportunistic infections (Date et al., 2010). While this practice shows promise, it is likely to expose infectious agents, including *M. tuberculosis*, to antifolates more frequently, which could lead to selection of resistant strains, thus shortening the lifespan of this powerful family of drugs (Vinetz, 2010). As in the case of β-lactams, strategies for potentiation of antifolates should be readily available to counterattack upcoming resistant strains, thereby extending their utility for TB treatment.

### 4.2 Potentiation of antifolates in mycobacteria

A method for boosting antifolate efficacy by utilizing combinations of drugs that target individual steps in folate biosynthesis is already in place. Trimethoprim is commonly coadministered with sulfonamides, for example sulfamethoxazole in the co-trimoxazole combination, to achieve synergy (Libeco & Powell, 2004) (Figure 4). However, in many cases including that of *M. tuberculosis*, the synergistic effect of trimethoprim on sulfonamides remains questioned and inconclusive (Forgacs et al., 2009, Ong et al., 2010, Suling et al., 1998). In addition, bacterial strains resistant to both trimethoprim and sulfonamides have readily been isolated (Bermingham & Derrick, 2002, Gangjee et al., 2007, 2008). Therefore, novel potentiation approaches targeting resistance mechanisms might be...
more effective in both potentiating available antifolates and preventing the emergence of resistant strains.

A recent study aimed at targeting intrinsic antifolate resistance in mycobacteria might reveal valuable targets for such resistance-targeted potentiation approaches (Ogwang et al., 2011). To identify novel antifolate resistance determinants, a genetic screen was first employed using a saturated transposon-insertion library of M. smegmatis. These mutants are systematically tested for increased antifolate susceptibility, followed by chemical complementation using folate derivatives of both the de novo synthesis and the one-carbon interconversion network. This chemogenomic profiling approach allows for identification of novel determinants previously unknown to function in mycobacterial intrinsic antifolate resistance (Ogwang et al., 2011). Using this non-bias screen, the genome-wide collection of antifolate resistance determinants in mycobacteria (mycobacterial antifolate resistome) was found to be composed of fifty resistance determinants (unpublished data).

A novel determinant identified from this screen was further characterized in a recent report (Ogwang et al., 2011). The M. smegmatis mutant presented in this report exhibits hypersusceptibility to several combinations of trimethoprim/sulfonamides tested (Ogwang et al., 2011). For example, its MIC to trimethoprim/sulfachloropyridazine is 64 fold lower than that of the parental M. smegmatis strain. The transposon insertion was mapped to a gene encoding a hypothetical protein with low homologies to 5,10-methenyl-tetrahydrofolate synthases (MTHFS, also called 5-formyl-tetrahydrofolate cycloligase, EC.6.3.3.2) from other organisms, including the prototype MTHFS first described in humans (Ogwang et al., 2011). Cross-species in trans expression of the human MTHFS was shown to restore antifolate resistance to the M. smegmatis mutant. A series of genetic knockout and complementation studies indicated that the disrupted gene encodes a MTHFS activity required for mycobacterial intrinsic antifolate resistance (Ogwang et al., 2011).

Absence of MTHFS enzymatic activity results in the inability to metabolize folic acid (5-formyl-tetrahydrofolate, 5-CHO-H\textsubscript{4}PteGlu) along with the reduced metabolism of 5-methyl-tetrahydrofolate (5-CH\textsubscript{3}-H\textsubscript{4}PteGlu), two major folate derivatives in the cell (Ogwang et al., 2011). 5-CHO-H\textsubscript{4}PteGlu is formed by the hydrolysis of 5,10-CH\textsuperscript{+}-H\textsubscript{4}PteGlu catalyzed by serine hydroxymethyltransferase (SHMT, Figure 4A) (Holmes & Appling, 2002, Stover & Schirch, 1990), whereas MTHFS is the only enzyme known to recycle 5-CHO-H\textsubscript{4}PteGlu back to 5,10-CH\textsuperscript{+}-H\textsubscript{4}PteGlu in an irreversible, ATP-dependent reaction (Figure 4C). As a consequence of MTHMS absence in mycobacterial cells, polyglutamylated forms of 5-CHO-H\textsubscript{4}PteGlu are elevated up to 80 fold, whereas the corresponding polyglutamylated forms of 5-CH\textsubscript{3}-H\textsubscript{4}PteGlu are reduced (Ogwang et al., 2011). Interestingly, 5-CHO-H\textsubscript{4}PteGlu is the only H\textsubscript{4}PteGlu derivative whose biological function remains largely unknown (Stover & Schirch, 1993). Although it is well known chemically and widely used as a medical agent, 5-CHO-H\textsubscript{4}PteGlu does not appear to function as a cofactor in any of the one-carbon metabolic reactions thus far known (Stover & Schirch, 1993). Because 5-CHO-H\textsubscript{4}PteGlu is known as the most stable form of reduced folate species in nature, and its presence is increased in plant seeds and fungal spores, it was suggested that it might function as a folate storage form required for these dormant states of life (Kruschwitz et al., 1994, Shin et al., 1975, Stover & Schirch, 1993). In mammals and yeasts, 5-CHO-H\textsubscript{4}PteGlu comprises 3-10% of total folate, whereas its presence may account for up to 50% of total folate in plant mitochondria during photorespiration when the glycine to serine flux is accelerated (Goyer et al., 2005, Roje et al., 2002). In vitro, 5-CHO-H\textsubscript{4}PteGlu is also a potential inhibitor of SHMT and other enzymes of the one-carbon metabolism, thus it may potentially serve to regulate these metabolic...
reactions (Roje et al., 2002, Stover & Schirch, 1991). Deletion of MTHFS in Arabidopsis leads to a 2-8-fold increased accumulation of total 5-CHO-H$_4$PteGlu, 46-fold accumulation of glycine, reduced growth and delayed flowering (Goyer et al., 2005). In human cells, overexpression of MTHFS lowers folate levels and increases folate turnover, suggesting that MTHFS may also function as a folate-degrading enzyme (Anguera et al., 2003). The role of MTHFS in intrinsic antifolate resistance was found not only in mycobacteria but also in E. coli, a Gram-negative bacterium (Nichols et al., 2011, Ogwang et al., 2011), suggesting that this determinant functions ubiquitously among bacteria. Indeed, further work confirmed that rv0992c, the gene that encodes the MTHFS homolog in M. tuberculosis, is also required for antifolate resistance via its MTHFS enzymatic activity (Figure 4C). Pharmaceutical inactivation of MTHFS activity is therefore expected to sensitize M. tuberculosis to classical antifolates, including those current TB-drugs that happen to target folate pathways (PAS, INH, etc.). This intervention may also allow for reduction of effective therapeutic doses, thereby minimizing the cytotoxicity of classical antifolates which has been an issue for their widespread use in the clinics. Work is underway to identify specific inhibitors of M. tuberculosis MTHFS by rational design and high throughput screening, as well as to characterize their antifolate potentiation activity against M. tuberculosis.

4.3 Future perspectives

Fundamental studies of molecular mechanisms conferring both acquired and intrinsic antifolate resistance in M. tuberculosis and related mycobacteria should be further conducted. Knowledge obtained from these studies will be essential for strategic implementations of antifolate use for TB, and will reveal valid targets for the resistance-targeted potentiation of classical antifolates.

A potential problem for the development of MTHFS inhibitors might be their nonspecific inhibition towards human MTHFS. However, the low homologies of MTHFS proteins indicate the possibility to identify species-specific inhibitors. Trimethoprim, which specifically inhibits bacterial DHFR but not the human counterpart, represents an encouraging example for such possibilities. Interestingly, a recent work showed that ygfA, the gene that encodes the MTHFS homolog in E. coli, is required for the formation of drug persisters during antibiotic treatments (Hansen et al., 2008). Although it remains to be characterized if the function of ygfA in antibiotic persister formation is related to its MTHFS activity, a similar role for M. tuberculosis rv0992c during TB latent infection is under investigation.

Although in vitro studies and a case report suggested that co-trimoxazole could be used for TB treatment (Forgacs et al., 2009, Ong et al., 2010, Young, 2009), more comprehensive well-designed trials with TB patients should be done to evaluate the efficacy of this antifolate combination. These trials should also address if these drugs may help to shorten the current TB regimens. In addition, new combinations using co-trimoxazole and PAS and/or INH should be tested against M. tuberculosis both in vitro and in patients.

5. Conclusions and future prospects

The primary goal of this chapter is to assess an emerging approach in TB drug development that uses knowledge of resistance mechanisms to sensitize M. tuberculosis to available, approved antibiotics (Figure 1) (Wright, 2000, Wright & Sutherland, 2007). Specific inhibitors that suppress resistance mechanisms would boost the efficacy of current anti-TB
drugs, or potentiate the antimycobacterial activity of currently non-TB antibiotics, thus making use of drugs that are already available but have never been used for TB treatment before. Proofs of concept have been made in recent years to demonstrate the feasibility of this approach in potentiating the antimycobacterial activity of important antibiotics such as β-lactams, ethionamide, and antifolates. It is anticipated that this trend will become increasingly important in the future of drug development, not only for TB but any disease treated by chemotherapies. As the rate of drug resistance expansion appears far beyond that of the current drug developmental process, it is logical that such sustainable approaches should be promoted to improve the utility and protection of those effective agents. Besides targeting antibiotic resistance mechanisms, currently approved drugs should be tested systematically against \textit{M. tuberculosis}, especially drug resistant strains. Recent work showed that many antibiotics that had been thought to be inactive against TB might be effective as chemotherapeutic agents for the disease (Forgacs \textit{et al.}, 2009, Hugonnet \textit{et al.}, 2009, Ong \textit{et al.}, 2010). In addition, drug-drug interactions among current combinatorial regimens for TB need to be further investigated. Most of the antibiotic combinations developed thus far are mainly aimed at minimizing the development of resistance, but disregard possible synergistic or antagonistic effects. Future drug combinations that minimize antagonistic effects but maximize synergy among the drugs used may not only reduce harmful clinical doses but also shorten treatment schedules, which would help to prevent the evolution and spread of antibiotic resistance.

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7. References


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Potentiation of Available Antibiotics by Targeting Resistance - An Emerging Trend in Tuberculosis Drug Development


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This book represents a case study based overview of many different aspects of drug development, ranging from target identification and characterization to chemical optimization for efficacy and safety, as well as bioproduction of natural products utilizing for example lichen. In the last section, special aspects of the formal drug development process are discussed. Since drug development is a highly complex multidisciplinary process, case studies are an excellent tool to obtain insight in this field. While each chapter gives specific insight and may be read as an independent source of information, the whole book represents a unique collection of different facets giving insight in the complexity of drug development.

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