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

Kerstin A. Wolff*, Marissa Sherman* and Liem Nguyen**
Case Western Reserve University School of Medicine, Cleveland, Ohio
United States of America

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

* Both authors contributed equally to this work.

**Correspondence: liem.nguyen@case.edu

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.

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 inhibitions *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. smegmatis* 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).

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 potentiators 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 $\alpha 5$, 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 potentiation.

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 *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 monooxygenase, encoded by *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 *M. tuberculosis* (Fraaije *et al.*, 2004). In the majority of ETH resistant *M. tuberculosis* isolates, mutations have been mapped to four principal categories: (i) mutations that alter activity of EthA, (ii) mutations in *ethR*, the gene located adjacent to *ethA*, (iii) mutations in InhA that prevent binding of the activated drug, and (iv) mutations in the *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 categories, several additional genes (*ndh*, *mshA*, and *dfrA*) might also be involved in ETH resistance. For example, mutations in *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 *ndh* mutations and ETH resistance has been demonstrated in *M. bovis* BCG and *M. smegmatis*, it has not been observed in *M. tuberculosis* (Brossier *et al.*, 2011). *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 *mshA*

mutations might be readily identified *in vitro* under ETH selection pressure, mutations in *mshA* only represent a minority among ETH resistant *M. tuberculosis* clinical isolates (Brossier *et al.*, 2011). Lastly, *dfrA* encodes the dihydrofolate reductase activity involved in folate biosynthesis. As it was suggested that dihydrofolate reductase is inhibited by INH adducts (Argyrou *et al.*, 2006), this enzyme may also be targeted by the adducts of ETH. Thus far, mutations in *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, *in trans* overexpression of the prodrug activator EthA in *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 *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 trans* overexpression of *ethR* causes strong inhibition of *ethA* expression, whereas chromosomal inactivation of *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, *M. tuberculosis* EthR was shown to cooperatively multimerize on a 55-bp operator, O_{ethR} , located within the *ethA* promoter, thereby repressing *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 α -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 $\alpha 1$, 2, and 3. The remaining six α -helices comprise the carboxy-terminus, which contains the ligand-binding site responsible for controlling the conformational changes that prevent binding of EthR to O_{ethR} . Interactions between α -helices of each monomer form a four-helix bundle resulting in dimerization of the repressor. The crystal structures also revealed a ligand cocrystallized 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 *M. smegmatis* growth (Fraaije *et al.*, 2004). Whereas benzylacetone itself did not display antimycobacterial activity, its addition to ETH used at subinhibitory concentrations (5 $\mu\text{g ml}^{-1}$) produced significant inhibition of mycobacterial growth (Frenois *et al.*, 2004).

As an intracellular pathogen, *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_{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 *M. tuberculosis* susceptibility to ETH (Weber *et al.*, 2008). *In vitro* analysis of *ethA* transcripts by quantitative real time PCR verified that 2-phenylethyl-butyrate dissociates EthR from the *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 *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 (Flipo *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 μ M) respectively allows a 10 (0.1 vs 1 μ g ml⁻¹) or 20 (0.025 vs 0.5 μ g 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 (H₄PteGlu, 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 H₄PteGlu (Figure 4B).

Folates are important metabolites indispensable for the development and propagation of all organisms. H₄PteGlu 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, pterate; Ser, serine. (B) Chemical structure of monoglutamylated tetrahydrofolate and its derivatives carrying C1 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-H₄PteGlu (Abs, 285nm) to 5,10-CH⁺-H₄PteGlu (Abs, 360 nm), catalyzed by the *M. tuberculosis* MTHFS homolog, Rv0992c, a novel determinant of antifolate resistance.

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 (H_2Pte) and (iv) glutamylation which adds one or more glutamate groups to form dihydrofolate ($H_2PteGlu$) that is reduced to form $H_4PteGlu$. The downstream folate utilization is usually called one-carbon metabolism in which different active forms of $H_4PteGlu$ 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 pteridin group, catalyzed by dihydropteroate synthase (DHPS) (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 (Libecco & 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 cyclo-ligase, 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 folinic acid (5-formyl-tetrahydrofolate, 5-CHO-H₄PteGlu) along with the reduced metabolism of 5-methyl-tetrahydrofolate (5-CH₃-H₄PteGlu), two major folate derivatives in the cell (Ogwang *et al.*, 2011). 5-CHO-H₄PteGlu is formed by the hydrolysis of 5,10-CH⁺-H₄PteGlu catalyzed by serine hydroxymethyltransferase (SHMT, Figure 4A) (Holmes & Appling, 2002, Stover & Schirch, 1990), whereas MTHFS is the only enzyme known to recycle 5-CHO-H₄PteGlu back to 5,10-CH⁺-H₄PteGlu in an irreversible, ATP-dependent reaction (Figure 4C). As a consequence of MTHMS absence in mycobacterial cells, polyglutamylated forms of 5-CHO-H₄PteGlu are elevated up to 80 fold, whereas the corresponding polyglutamylated forms of 5-CH₃-H₄PteGlu are reduced (Ogwang *et al.*, 2011). Interestingly, 5-CHO-H₄PteGlu is the only H₄PteGlu 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-H₄PteGlu 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-H₄PteGlu 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-H₄PteGlu 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-H₄PteGlu 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-H₄PteGlu, 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, Ogowang *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 *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 *et al.*, 2009, Hugonnet *et al.*, 2009, Ong *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

- Adrian, P. V. & Klugman, K. P., (1997) Mutations in the dihydrofolate reductase gene of trimethoprim-resistant isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 41: 2406-2413.
- Anguera, M. C., Suh, J. R., Ghandour, H., Nasrallah, I. M., Selhub, J. & Stover, P. J., (2003) Methenyltetrahydrofolate synthetase regulates folate turnover and accumulation. *J Biol Chem* 278: 29856-29862.
- Argyrou, A., Vetting, M. W., Aladegbami, B. & Blanchard, J. S., (2006) *Mycobacterium tuberculosis* dihydrofolate reductase is a target for isoniazid. *Nat Struct Mol Biol* 13: 408-413.
- Baca, A. M., Sirawaraporn, R., Turley, S., Sirawaraporn, W. & Hol, W. G., (2000) Crystal structure of *Mycobacterium tuberculosis* 7,8-dihydropteroate synthase in complex with pterin monophosphate: new insight into the enzymatic mechanism and sulfa-drug action. *J Mol Biol* 302: 1193-1212.

- Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Um, K. S., Wilson, T., *et al.*, (1994) *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science* 263: 227-230.
- Baulard, A. R., Betts, J. C., Engohang-Ndong, J., Quan, S., McAdam, R. A., Brennan, P. J., *et al.*, (2000) Activation of the pro-drug ethionamide is regulated in mycobacteria. *J Biol Chem* 275: 28326-28331.
- Bermingham, A. & Derrick, J. P., (2002) The folic acid biosynthesis pathway in bacteria: evaluation of potential for antibacterial drug discovery. *Bioessays* 24: 637-648.
- Bertino, J. R., (1971) Folate antagonists as chemotherapeutic agents. In: *Annals of the New York Academy of Sciences*. New York: New York Academy of Sciences, pp. 519.
- Beveridge, T. J., (1999) Structures of gram-negative cell walls and their derived membrane vesicles. *J Bacteriol* 181: 4725-4733.
- Blakley, R. L., (1969) *The biochemistry of folic acid and related pteridines*, p. 569. North-Holland Publishing Co., Amsterdam, London.
- Bolten, B. M. & DeGregorio, T., (2002) From the analyst's couch. Trends in development cycles. *Nat Rev Drug Discov* 1: 335-336.
- Brennan, P. J. & Nikaido, H., (1995) The envelope of mycobacteria. *Annu Rev Biochem* 64: 29-63.
- Brossier, F., Veziris, N., Truffot-Pernot, C., Jarlier, V. & Sougakoff, W., (2011) Molecular investigation of resistance to the antituberculous drug ethionamide in multidrug-resistant clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 55: 355-360.
- Burns, M., (1999) Management of narrow therapeutic index drugs. *J Thromb Thrombolysis* 7: 137-143.
- Chambers, H. F., Kocagoz, T., Sipit, T., Turner, J. & Hopewell, P. C., (1998) Activity of amoxicillin/clavulanate in patients with tuberculosis. *Clin Infect Dis* 26: 874-877.
- Chambers, H. F., Moreau, D., Yajko, D., Miick, C., Wagner, C., Hackbarth, C., *et al.*, (1995) Can penicillins and other beta-lactam antibiotics be used to treat tuberculosis? *Antimicrob Agents Chemother* 39: 2620-2624.
- Chambers, H. F., Turner, J., Schechter, G. F., Kawamura, M. & Hopewell, P. C., (2005) Imipenem for treatment of tuberculosis in mice and humans. *Antimicrob Agents Chemother* 49: 2816-2821.
- Date, A. A., Vitoria, M., Granich, R., Banda, M., Fox, M. Y. & Gilks, C., (2010) Implementation of co-trimoxazole prophylaxis and isoniazid preventive therapy for people living with HIV. *Bull World Health Organ* 88: 253-259.
- Dauby, N., Muylle, I., Mouchet, F., Sergysels, R. & Payen, M. C., (2011) Meropenem/Clavulanate and Linezolid Treatment for Extensively Drug-Resistant Tuberculosis. *Pediatr Infect Dis J*. [published online ahead of print]
- DeBarber, A. E., Mdluli, K., Bosman, M., Bekker, L. G. & Barry, C. E., 3rd, (2000) Ethionamide activation and sensitivity in multidrug-resistant *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* 97: 9677-9682.
- Doull, J. A., (1963) Sulfone Therapy of Leprosy. Background, Early History and Present Status. *Int J Lepr* 31: 143-160.
- Dover, L. G., Corsino, P. E., Daniels, I. R., Cocklin, S. L., Tatituri, V., Besra, G. S. & Futterer, K., (2004) Crystal structure of the TetR/CamR family repressor *Mycobacterium tuberculosis* EthR implicated in ethionamide resistance. *J Mol Biol* 340: 1095-1105.

- Drawz, S. M. & Bonomo, R. A., (2010) Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev* 23: 160-201.
- Drug Information Online, (2011) Antibiotic Combo Fights Resistant TB. In: Drugs.com, Date of access: 28.02.2011, Available from: <http://www.drugs.com/news/antibiotic-combo-fights-resistant-tb-16398.html>
- Dye, C., (2000) Tuberculosis 2000-2010: control, but not elimination. *Int J Tuberc Lung Dis* 4: S146-152.
- Engohang-Ndong, J., Baillat, D., Aumercier, M., Bellefontaine, F., Besra, G. S., Locht, C. & Baulard, A. R., (2004) EthR, a repressor of the TetR/CamR family implicated in ethionamide resistance in mycobacteria, octamerizes cooperatively on its operator. *Mol Microbiol* 51: 175-188.
- Flipo, M., Desroses, M., Lecat-Guillet, N., Dirie, B., Carette, X., Leroux, F., *et al.*, (2011) Ethionamide Boosters: Synthesis, Biological Activity, and Structure-Activity Relationships of a Series of 1,2,4-Oxadiazole EthR Inhibitors. *J Med Chem* 54: 2994-3010.
- Flores, A. R., Parsons, L. M. & Pavelka, M. S., Jr., (2005) Genetic analysis of the beta-lactamases of *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* and susceptibility to beta-lactam antibiotics. *Microbiology* 151: 521-532.
- Forgacs, P., Wengenack, N. L., Hall, L., Zimmerman, S. K., Silverman, M. L. & Roberts, G. D., (2009) Tuberculosis and trimethoprim-sulfamethoxazole. *Antimicrob Agents Chemother* 53: 4789-4793.
- Fraaije, M. W., Kamerbeek, N. M., Heidekamp, A. J., Fortin, R. & Janssen, D. B., (2004) The prodrug activator EtaA from *Mycobacterium tuberculosis* is a Baeyer-Villiger monooxygenase. *J Biol Chem* 279: 3354-3360.
- Frenois, F., Baulard, A. R. & Villeret, V., (2006) Insights into mechanisms of induction and ligands recognition in the transcriptional repressor EthR from *Mycobacterium tuberculosis*. *Tuberculosis (Edinb)* 86: 110-114.
- Frenois, F., Engohang-Ndong, J., Locht, C., Baulard, A. R. & Villeret, V., (2004) Structure of EthR in a ligand bound conformation reveals therapeutic perspectives against tuberculosis. *Mol Cell* 16: 301-307.
- Gandhi, N. R., Moll, A., Sturm, A. W., Pawinski, R., Govender, T., Lalloo, U., *et al.*, (2006) Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 368: 1575-1580.
- Gangjee, A., Jain, H. D. & Kurup, S., (2007) Recent advances in classical and non-classical antifolates as antitumor and antiopportunistic infection agents: part I. *Anticancer Agents Med Chem* 7: 524-542.
- Gangjee, A., Jain, H. D. & Kurup, S., (2008) Recent advances in classical and non-classical antifolates as antitumor and antiopportunistic infection agents: Part II. *Anticancer Agents Med Chem* 8: 205-231.
- Gorlick, R., Goker, E., Trippett, T., Waltham, M., Banerjee, D. & Bertino, J. R., (1996) Intrinsic and acquired resistance to methotrexate in acute leukemia. *N Engl J Med* 335: 1041-1048.
- Goyer, A., Collakova, E., Diaz de la Garza, R., Quinlivan, E. P., Williamson, J., Gregory, J. F., 3rd, *et al.*, (2005) 5-Formyltetrahydrofolate is an inhibitory but well tolerated metabolite in *Arabidopsis* leaves. *J Biol Chem* 280: 26137-26142.

- Green, J., Nichols, B. & Matthews, R., (1996) Folate biosynthesis, reduction, and polyglutamylation. In: *Escherichia coli* and *Salmonella typhimurium* - Cellular and Molecular Biology. F. Neidhardt, R. Curtiss III, J. Ingraham, E. Lin, J. K. Low, B. Magasanik, W. Reznikoff, M. Riley, M. Schaechter & H. Umbarger (eds). Washington, DC: ASM Press, pp. 665-673.
- Grim, S. A., Rapp, R. P., Martin, C. A. & Evans, M. E., (2005) Trimethoprim-sulfamethoxazole as a viable treatment option for infections caused by methicillin-resistant *Staphylococcus aureus*. *Pharmacotherapy* 25: 253-264.
- Hansen, S., Lewis, K. & Vulic, M., (2008) Role of global regulators and nucleotide metabolism in antibiotic tolerance in *Escherichia coli*. *Antimicrob Agents Chemother* 52: 2718-2726.
- Holmes, W. B. & Appling, D. R., (2002) Cloning and characterization of methenyltetrahydrofolate synthetase from *Saccharomyces cerevisiae*. *J Biol Chem* 277: 20205-20213.
- Hugonnet, J. E. & Blanchard, J. S., (2007) Irreversible inhibition of the *Mycobacterium tuberculosis* beta-lactamase by clavulanate. *Biochemistry* 46: 11998-12004.
- Hugonnet, J. E., Tremblay, L. W., Boshoff, H. I., Barry, C. E., 3rd & Blanchard, J. S., (2009) Meropenem-clavulanate is effective against extensively drug-resistant *Mycobacterium tuberculosis*. *Science* 323: 1215-1218.
- Jarlier, V., Gutmann, L. & Nikaido, H., (1991) Interplay of cell wall barrier and beta-lactamase activity determines high resistance to beta-lactam antibiotics in *Mycobacterium chelonae*. *Antimicrob Agents Chemother* 35: 1937-1939.
- Jarlier, V. & Nikaido, H., (1990) Permeability barrier to hydrophilic solutes in *Mycobacterium chelonae*. *J Bacteriol* 172: 1418-1423.
- Jassal, M. & Bishai, W. R., (2009) Extensively drug-resistant tuberculosis. *Lancet Infect Dis* 9: 19-30.
- Kai, M., Matsuoka, M., Nakata, N., Maeda, S., Gidoh, M., Maeda, Y., *et al.*, (1999) Diaminodiphenylsulfone resistance of *Mycobacterium leprae* due to mutations in the dihydropteroate synthase gene. *FEMS Microbiol Lett* 177: 231-235.
- Kasik, J. E., (1979) Mycobacterial Beta-Lactamases. In: *Beta-Lactamases*. J. M. T. Hamilton-Miller & J. T. Smith (eds). 0123215501, London: Academic Press, pp. 500.
- Kasik, J. E. & Peacham, L., (1968) Properties of beta-lactamases produced by three species of mycobacteria. *Biochem J* 107: 675-682.
- Koch, A. L., (2003) Bacterial wall as target for attack: past, present, and future research. *Clin Microbiol Rev* 16: 673-687.
- Koser, C. U., Summers, D. K. & Archer, J. A., (2010) Role of the dihydrofolate reductase DfrA (Rv2763c) in trimethoprim-sulfamethoxazole (co-trimoxazole) resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 54: 4951-4952; author reply 4952.
- Kruschwitz, H. L., McDonald, D., Cossins, E. A. & Schirch, V., (1994) 5-Formyltetrahydropteroylpolyglutamates are the major folate derivatives in *Neurospora crassa* conidiospores. *J Biol Chem* 269: 28757-28763.
- Lee, W., McDonough, M. A., Kotra, L., Li, Z. H., Silvaggi, N. R., Takeda, Y., *et al.*, (2001) A 1.2-A snapshot of the final step of bacterial cell wall biosynthesis. *Proc Natl Acad Sci U S A* 98: 1427-1431.

- Libecco, J. A. & Powell, K. R., (2004) Trimethoprim/sulfamethoxazole: clinical update. *Pediatr Rev* 25: 375-380.
- Liu, J., Rosenberg, E. Y. & Nikaido, H., (1995) Fluidity of the lipid domain of cell wall from *Mycobacterium chelonae*. *Proc Natl Acad Sci U S A* 92: 11254-11258.
- LoBue, P., (2009) Extensively drug-resistant tuberculosis. *Curr Opin Infect Dis* 22: 167-173.
- McDonough, J. A., Hacker, K. E., Flores, A. R., Pavelka, M. S., Jr. & Braunstein, M., (2005) The twin-arginine translocation pathway of *Mycobacterium smegmatis* is functional and required for the export of mycobacterial beta-lactamases. *J Bacteriol* 187: 7667-7679.
- Morlock, G. P., Metchock, B., Sikes, D., Crawford, J. T. & Cooksey, R. C., (2003) *ethA*, *inhA*, and *katG* loci of ethionamide-resistant clinical *Mycobacterium tuberculosis* isolates. *Antimicrob Agents Chemother* 47: 3799-3805.
- Mukherjee, T., Basu, D., Mahapatra, S., Goffin, C., van Beeumen, J. & Basu, J., (1996) Biochemical characterization of the 49 kDa penicillin-binding protein of *Mycobacterium smegmatis*. *Biochem J* 320 (Pt 1): 197-200.
- Nampoothiri, K. M., Rubex, R., Patel, A. K., Narayanan, S. S., Krishna, S., Das, S. M. & Pandey, A., (2008) Molecular cloning, overexpression and biochemical characterization of hypothetical beta-lactamases of *Mycobacterium tuberculosis* H37Rv. *J Appl Microbiol* 105: 59-67.
- Nguyen, L. & Pieters, J., (2005) The Trojan horse: survival tactics of pathogenic mycobacteria in macrophages. *Trends Cell Biol* 15: 269-276.
- Nguyen, L. & Pieters, J., (2009) Mycobacterial subversion of chemotherapeutic reagents and host defense tactics: challenges in tuberculosis drug development. *Annu Rev Pharmacol Toxicol* 49: 427-453.
- Nguyen, L. & Thompson, C. J., (2006) Foundations of antibiotic resistance in bacterial physiology: the mycobacterial paradigm. *Trends Microbiol* 14: 304-312.
- Nichols, R. J., Sen, S., Choo, Y. J., Beltrao, P., Zietek, M., Chaba, R., *et al.*, (2011) Phenotypic landscape of a bacterial cell. *Cell* 144: 143-156.
- Ogwang, S., Nguyen, H. T., Sherman, M., Bajaksouzian, S., Jacobs, M. R., Boom, W. H., *et al.*, (2011) Bacterial conversion of folinic acid is required for antifolate resistance. *J Biol Chem* 286: 15377-15390.
- Ong, W., Sievers, A. & Leslie, D. E., (2010) *Mycobacterium tuberculosis* and sulfamethoxazole susceptibility. *Antimicrob Agents Chemother* 54: 2748; author reply 2748-2749.
- Prabhakaran, K., Harris, E. B. & Randhawa, B., (1999) Bactericidal action of ampicillin/sulbactam against intracellular mycobacteria. *Int J Antimicrob Agents* 13: 133-135.
- Proctor, R. A., (2008) Role of folate antagonists in the treatment of methicillin-resistant *Staphylococcus aureus* infection. *Clin Infect Dis* 46: 584-593.
- Quinting, B., Reyrat, J. M., Monnaie, D., Amicosante, G., Pelicic, V., Gicquel, B., *et al.*, (1997) Contribution of beta-lactamase production to the resistance of mycobacteria to beta-lactam antibiotics. *FEBS Lett* 406: 275-278.
- Rengarajan, J., Sassetti, C. M., Naroditskaya, V., Sloutsky, A., Bloom, B. R. & Rubin, E. J., (2004) The folate pathway is a target for resistance to the drug para-aminosalicylic acid (PAS) in mycobacteria. *Mol Microbiol* 53: 275-282.

- Roje, S., Janave, M. T., Ziemak, M. J. & Hanson, A. D., (2002) Cloning and characterization of mitochondrial 5-formyltetrahydrofolate cycloligase from higher plants. *J Biol Chem* 277: 42748-42754.
- Sala, C., Haouz, A., Saul, F. A., Miras, I., Rosenkrands, I., Alzari, P. M. & Cole, S. T., (2009) Genome-wide regulon and crystal structure of BlaI (Rv1846c) from *Mycobacterium tuberculosis*. *Mol Microbiol* 71: 1102-1116.
- Science Centric, (2009) Antibiotic combination defeats extensively drug-resistant TB. In: ScienceCentric.com, Date of access: 28.02.2011, Available from: <http://www.sciencecentric.com/news/09022702-antibiotic-combination-defeats-extensively-drug-resistant-tb.html>
- Selhub, J., (2002) Folate, vitamin B12 and vitamin B6 and one carbon metabolism. *J Nutr Health Aging* 6: 39-42.
- Severin, A., Severina, E. & Tomasz, A., (1997) Abnormal physiological properties and altered cell wall composition in *Streptococcus pneumoniae* grown in the presence of clavulanic acid. *Antimicrob Agents Chemother* 41: 504-510.
- Shin, Y. S., Kim, E. S., Watson, J. E. & Stokstad, E. L., (1975) Studies of folic acid compounds in nature. IV. Folic acid compounds in soybeans and cow milk. *Can J Biochem* 53: 338-343.
- Sood, A. & Panchagnula, R., (2003) Design of controlled release delivery systems using a modified pharmacokinetic approach: a case study for drugs having a short elimination half-life and a narrow therapeutic index. *Int J Pharm* 261: 27-41.
- Stover, P. & Schirch, V., (1990) Serine hydroxymethyltransferase catalyzes the hydrolysis of 5,10-methenyltetrahydrofolate to 5-formyltetrahydrofolate. *J Biol Chem* 265: 14227-14233.
- Stover, P. & Schirch, V., (1991) 5-Formyltetrahydrofolate polyglutamates are slow tight binding inhibitors of serine hydroxymethyltransferase. *J Biol Chem* 266: 1543-1550.
- Stover, P. & Schirch, V., (1993) The metabolic role of leucovorin. *Trends Biochem Sci* 18: 102-106.
- Suling, W. J., Reynolds, R. C., Barrow, E. W., Wilson, L. N., Piper, J. R. & Barrow, W. W., (1998) Susceptibilities of *Mycobacterium tuberculosis* and *Mycobacterium avium* complex to lipophilic deazapteridine derivatives, inhibitors of dihydrofolate reductase. *J Antimicrob Chemother* 42: 811-815.
- Tremblay, L. W., Fan, F. & Blanchard, J. S., (2010) Biochemical and structural characterization of *Mycobacterium tuberculosis* beta-lactamase with the carbapenems ertapenem and doripenem. *Biochemistry* 49: 3766-3773.
- Tremblay, L. W., Hugonnet, J. E. & Blanchard, J. S., (2008) Structure of the covalent adduct formed between *Mycobacterium tuberculosis* beta-lactamase and clavulanate. *Biochemistry* 47: 5312-5316.
- Vannelli, T. A., Dykman, A. & Ortiz de Montellano, P. R., (2002) The antituberculosis drug ethionamide is activated by a flavoprotein monooxygenase. *J Biol Chem* 277: 12824-12829.
- Vilcheze, C., Av-Gay, Y., Attarian, R., Liu, Z., Hazbon, M. H., Colangeli, R., et al., (2008) Mycothiol biosynthesis is essential for ethionamide susceptibility in *Mycobacterium tuberculosis*. *Mol Microbiol* 69: 1316-1329.

- Vilcheze, C., Weisbrod, T. R., Chen, B., Kremer, L., Hazbon, M. H., Wang, F., *et al.*, (2005) Altered NADH/NAD⁺ ratio mediates coresistance to isoniazid and ethionamide in mycobacteria. *Antimicrob Agents Chemother* 49: 708-720.
- Vinetz, J. M., (2010) Intermittent preventive treatment for malaria in sub-saharan African: a halfway technology or a critical intervention? *Am J Trop Med Hyg* 82: 755-756.
- Voladri, R. K., Lakey, D. L., Hennigan, S. H., Menzies, B. E., Edwards, K. M. & Kernodle, D. S., (1998) Recombinant expression and characterization of the major beta-lactamase of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 42: 1375-1381.
- Volpato, J. P. & Pelletier, J. N., (2009) Mutational 'hot-spots' in mammalian, bacterial and protozoal dihydrofolate reductases associated with antifolate resistance: sequence and structural comparison. *Drug Resist Updat* 12: 28-41.
- Waller, J. C., Alvarez, S., Naponelli, V., Lara-Nunez, A., Blaby, I. K., Da Silva, V., *et al.*, (2010) A role for tetrahydrofolates in the metabolism of iron-sulfur clusters in all domains of life. *Proc Natl Acad Sci U S A* 107: 10412-10417.
- Wang, F., Cassidy, C. & Sacchettini, J. C., (2006) Crystal structure and activity studies of the *Mycobacterium tuberculosis* beta-lactamase reveal its critical role in resistance to beta-lactam antibiotics. *Antimicrob Agents Chemother* 50: 2762-2771.
- Wang, F., Langley, R., Gulten, G., Dover, L. G., Besra, G. S., Jacobs, W. R., Jr. & Sacchettini, J. C., (2007) Mechanism of thioamide drug action against tuberculosis and leprosy. *J Exp Med* 204: 73-78.
- Watt, B., Edwards, J. R., Rayner, A., Grindey, A. J. & Harris, G., (1992) *In vitro* activity of meropenem and imipenem against mycobacteria: development of a daily antibiotic dosing schedule. *Tuber Lung Dis* 73: 134-136.
- Waxman, D. J., Yocum, R. R. & Strominger, J. L., (1980) Penicillins and cephalosporins are active site-directed acylating agents: evidence in support of the substrate analogue hypothesis. *Philos Trans R Soc Lond B Biol Sci* 289: 257-271.
- Weber, W., Schoenmakers, R., Keller, B., Gitzinger, M., Grau, T., Daoud-El Baba, M., *et al.*, (2008) A synthetic mammalian gene circuit reveals antituberculosis compounds. *Proc Natl Acad Sci U S A* 105: 9994-9998.
- Willand, N., Dirie, B., Carette, X., Bifani, P., Singhal, A., Desroses, M., *et al.*, (2009) Synthetic EthR inhibitors boost antituberculous activity of ethionamide. *Nat Med* 15: 537-544.
- World Health Organization, (2006) Questions : XDR-TB. In: www.who.int, Date of access: 26.02.2011, Available from: <http://www.who.int/tb/challenges/xdr/faqs/en/index.html>
- World Health Organization, (2010) Tuberculosis Fact Sheet. In: www.who.int Date of access: 26.02.2011, Available from: <http://www.who.int/mediacentre/factsheets/fs104/en/index.html>
- Wright, G. D., (2000) Resisting resistance: new chemical strategies for battling superbugs. *Chem Biol* 7: R127-132.
- Wright, G. D. & Sutherland, A. D., (2007) New strategies for combating multidrug-resistant bacteria. *Trends Mol Med* 13: 260-267.
- Xu, X., Vilcheze, C., Av-Gay, Y., Gomez-Velasco, A. & Jacobs, W. R., Jr., (2011) Precise null deletion mutations of the mycothiol synthetic genes reveal their role in isoniazid and ethionamide resistance in *Mycobacterium smegmatis*. *Antimicrob Agents Chemother* 55: 3133-3139.

- Young, L. S., (2009) Reconsidering some approved antimicrobial agents for tuberculosis. *Antimicrob Agents Chemother* 53: 4577-4579.
- Zhang, Y., (2005) The magic bullets and tuberculosis drug targets. *Annu Rev Pharmacol Toxicol* 45: 529-564.
- Zimmerman, T. J., Kooner, K. S., Kandarakis, A. S. & Ziegler, L. P., (1984) Improving the therapeutic index of topically applied ocular drugs. *Arch Ophthalmol* 102: 551-553.

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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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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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