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THE MAGIC BULLETS AND TUBERCULOSIS DRUG TARGETS

Ying Zhang
Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland 21205; email: yzhang@jhsph.edu

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■ **Abstract** Modern chemotherapy has played a major role in our control of tuberculosis. Yet tuberculosis still remains a leading infectious disease worldwide, largely owing to persistence of tubercle bacillus and inadequacy of the current chemotherapy. The increasing emergence of drug-resistant tuberculosis along with the HIV pandemic threatens disease control and highlights both the need to understand how our current drugs work and the need to develop new and more effective drugs. This review provides a brief historical account of tuberculosis drugs, examines the problem of current chemotherapy, discusses the targets of current tuberculosis drugs, focuses on some promising new drug candidates, and proposes a range of novel drug targets for intervention. Finally, this review addresses the problem of conventional drug screens based on inhibition of replicating bacilli and the challenge to develop drugs that target nonreplicating persistent bacilli. A new generation of drugs that target persistent bacilli is needed for more effective treatment of tuberculosis.

INTRODUCTION

Humankind's battle with tuberculosis (TB) dates back to antiquity. TB, which is caused by *Mycobacterium tuberculosis*, was a much more prevalent disease in the past than it is today, and it was responsible for the deaths of about one billion people during the last two centuries (1). Improved sanitation and living conditions significantly reduced the incidence of the disease even before the advent of chemotherapy. The introduction of TB chemotherapy in the 1950s, along with the widespread use of BCG vaccine, had a great impact on further reduction in TB incidence. However, despite these advances, TB still remains a leading infectious disease worldwide, especially in the third world countries.

M. tuberculosis is a particularly successful pathogen that latently infects about 2 billion people, about one third of world population (2). Each year, there are about 8 million new TB cases and 2 million deaths worldwide. TB is on the increase in recent years, largely owing to HIV infection, immigration, increased trade, and globalization (2). The increasing emergence of drug-resistant TB, especially

multidrug-resistant TB (MDR-TB, resistant to at least two frontline drugs such as isoniazid and rifampin), is particularly alarming. MDR-TB has already caused several fatal outbreaks (2, 3) and poses a significant threat to the treatment and control of the disease in some parts of the world, where the incidence of MDR-TB can be as high as 14% (2). The standard TB therapy is ineffective in controlling MDR-TB in high MDR-TB incidence areas (4, 5). Fifty million people have already been infected with drug-resistant TB (2). There is much concern that the TB situation may become even worse with the spread of HIV worldwide, a virus that weakens the host immune system and allows latent TB to reactivate and makes the person more susceptible to reinfection with either drug-susceptible or drug-resistant strains. The lethal combination of drug-resistant TB and HIV infection is a growing problem that presents serious challenges for effective TB control. In view of this situation, the World Health Organization (WHO) in 1993 declared TB a global emergency (6).

There is an urgent need to develop new TB drugs (7). However, no new TB drugs have been developed in about 40 years. Although TB can be cured with the current therapy, the six months needed to treat the disease is too long, and the treatment often has significant toxicity. These factors make patient compliance to therapy very difficult, and this noncompliance frequently selects for drug-resistant TB bacteria. The current TB problem clearly demonstrates the need for a re-evaluation of our knowledge of the current TB drugs and chemotherapy and the need for new and better drugs that are not only active against drug-resistant TB but also, more importantly, shorten the requirement for six months of therapy. This review provides a brief overview of the history of TB drugs and chemotherapy, discuss the targets of the current TB drugs, examine some promising drug candidates, propose potential new targets for drug development, and finally address issues of novel drug screens that target the nonreplicating persistent bacilli that currently require lengthy therapy. Several recent reviews on TB drug discovery are available (8–12).

HISTORY OF ANTITUBERCULOSIS DRUGS

The TB drugs in use today reflect their origins in two sources of antimicrobial agents, i.e., chemical origin and antibiotic origin. Albert Schatz and Selman Waksman discovered the first effective TB drug streptomycin (Figure 1) from *Streptomyces griseus* in 1944 (17), a discovery that marked the beginning of modern TB chemotherapy.

The modern chemotherapeutic treatment of TB also had its beginning in sulfa drugs developed by Domagk for the treatment of gram-positive bacterial infections (14). In 1938, Rich and Follis from Johns Hopkins University found that sulfanilamide at high doses significantly inhibited the disease pathology in experimental TB infection in guinea pigs (18) but without significant effect in treatment of human TB in tolerable doses. This finding stimulated further effort to refine sulfa

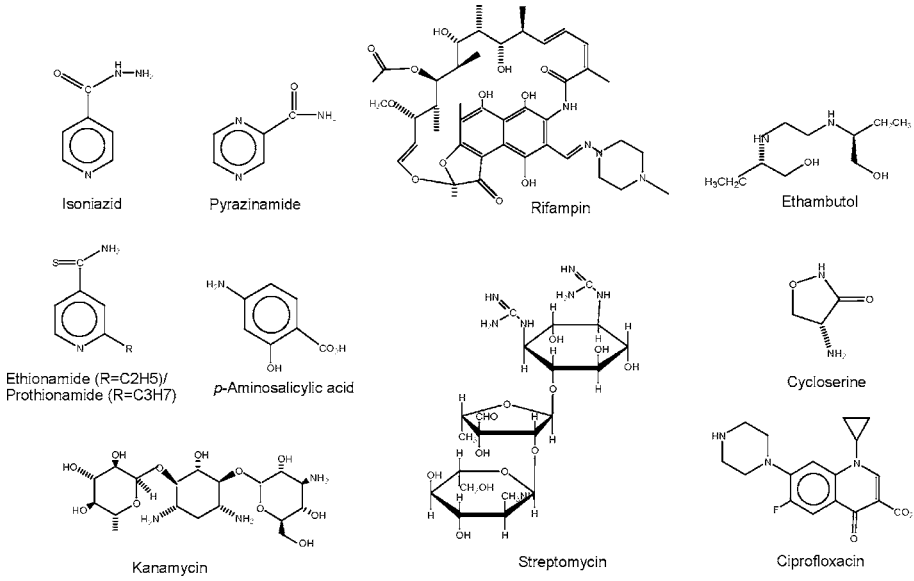

Figure 1 Structures of some commonly used TB drugs.

drugs for the treatment of TB and subsequently led to synthesis of thiosemicarbazones such as Conteben (also called amithiazone), which were more active than sulfanilamide and had definite clinical value but were not as effective as streptomycin (19). In 1946, two years after the discovery of streptomycin, Lehmann from Sweden discovered para-aminosalicylic acid (PAS) (Figure 1) as an effective TB drug (20), a discovery based on a curious observation made by Bernheim that salicylate and benzoate stimulated the oxygen consumption of tubercle bacillus (21). This was quickly followed in 1952 by the sensational discovery of the highly active TB drug isoniazid (INH) (Figure 1) simultaneously by three drug companies: Hoffman LaRoche, E.R. Squibb & Sons, and Bayer. The discovery of INH was based on the nicotinamide activity against tubercle bacilli in the animal model observed by Chorine in 1945 (22) and the reshuffling of chemical groups in the thiosemicarbazone (23–25). INH represented a major milestone in the chemotherapy of TB because it is highly active, inexpensive, and without significant side effects (26). Remarkably, the nicotinamide lead also led to the discovery of pyrazinamide (PZA) (Figure 1) in 1952 by the Lederle Research Laboratories (27) and ethionamide (ETH)/Prothionamide (PTH) (Figure 1) in 1956 (28). Ethambutol (EMB) was discovered in 1961 at Lederle on the basis of the observation that polyamines and diamines had activity against tubercle bacilli; subsequent synthesis of diamine analogs led to the identification of EMB (29). Further screening for antibiotics from soil microbes led to discovery of many other antituberculosis drugs: cycloserine (30); kanamycin (31) and its derivative amikacin; viomycin (32); capreomycin (33); and rifamycins (34) and its derivative rifampin (RIF), developed at

Dow-Lepetit Research Laboratories, Italy (35), which has been the drug of choice for treatment of TB since the 1970s. The 1950s and 60s represent a golden era of TB drug discovery. Most of the TB drugs in use today were discovered during this period, except the broad-spectrum quinolone drugs, which were developed in 1980s on the basis of the antibacterial activity of nalidixic acid discovered in the 1960s (36). Although quinolone drugs were not initially used for TB treatment, they were subsequently shown to have high activity against tubercle bacillus and were used as second-line drugs for the treatment of drug-resistant TB since the late 1980s (37, 38).

THE CURRENT TB THERAPY AND THE PROBLEM OF PERSISTERS

The current TB chemotherapy evolved from numerous experimental and clinical studies primarily conducted between the 1950s and 1970s (39). The current recommended standard TB chemotherapy, called DOTS (directly observed treatment, short-course), is a six month therapy consisting of an initial two-month phase of treatment with four drugs, INH, RIF, PZA, and EMB, followed by a continuation phase of treatment with INH and RIF for another four months (2). DOTS is currently the best TB therapy; it has a cure rate of up to 95% and is recommended by the WHO for treating every TB patient (2). However, DOTS alone may not work in areas where there is high incidence of MDR-TB (4, 5), where its cure rate is as low as 50%. In such situations, WHO recommends the use of DOTS-Plus, which is DOTS plus second-line TB drugs (see next section) for the treatment of MDR-TB and TB (2). However, treatment of MDR-TB with DOTS-Plus takes up to 24 months and is not only costly but also has significant toxicity.

Although DOTS can cure TB, the lengthy six month therapy makes patient compliance difficult, and noncompliance is a frequent source of drug-resistant strains. Although the TB chemotherapy renders a patient noninfectious a few weeks after the initiation of the therapy, the therapy has to be continued for a considerable period to prevent relapse. Compared with treatment of other bacterial infections such as *H. pylori* and pneumococcal infections, which takes no longer than one to two weeks, it is striking that treatment of TB requires at least six months. Why is the TB therapy so long? This is a fundamental problem facing TB chemotherapy and deserves some in-depth analysis. Several factors may be responsible. First, the nature of the disease pathology can influence the efficacy and duration of chemotherapy. For example, open cavities teeming with large numbers of bacilli present a particular problem for eradication of the bacilli by chemotherapy (40). Second, the phenotypic resistance in nonreplicating persisters presents a major problem for the current TB therapy. Antibiotics are active against growing bacteria but are ineffective against nongrowing bacteria. There are at least three types of nongrowing bacteria that are phenotypically resistant to antibiotics: (*a*) the stationary phase bacteria, (*b*) residual survivors or persisters not killed during antibiotic exposure when a growing culture is treated with antibiotics, and

(*c*) dormant bacteria. Although all three types of phenotypic resistance may share some common mechanism, the mechanism of phenotypic resistance in *M. tuberculosis* is unknown. There is currently considerable interest in the study of mycobacterial persistence and dormancy (41–43), with the aim to better understand the basis of this phenomenon and devise therapeutic strategies that target the persistent or dormant organisms for improved treatment of TB. Current TB drugs are mainly active against growing bacilli, and except for RIF and PZA, they are not good at killing persisters. Although RIF and PZA are important sterilizing drugs that significantly reduce the number of bacilli in lesions and play an important role in shortening the therapy from 12–18 months to 6 months, there are still other persister populations that are not killed by RIF or PZA. TB is like a "little universe" or a "Russian Doll," consisting of layer after layer of different bacterial populations within a large bacterial population. At this time, we have little knowledge about the biology of these persisters, despite significant interest in this area (41–43). The intracellular location of the bacilli could render some drugs such as streptomycin inactive. However, most drugs do penetrate the necrotic tissues (40), although they cannot effectively kill nonreplicating bacilli in the lesions. Third, host immune system may not effectively eliminate tubercle bacilli in the lesions. In many bacterial infections, small numbers of residual bacteria that remain after antibiotic therapy can be effectively mopped up by the immune system. However, in TB, it appears that the host immune system is not very effective in controlling the residual bacteria not killed by TB chemotherapy. Thus, although achieving a clinical cure, the current chemotherapy cannot achieve a bacteriological cure, i.e., the therapy cannot completely eradicate all bacilli in the lesion (40). This depressing fact underscores the need for developing better sterilizing drugs and other interventions, such as improving host immune status, as adjunct treatment for more effective therapy.

The varying types of lesions determine different metabolic status of tubercle bacilli in vivo and are the basis for diverse bacterial populations. According to Mitchison (44), tubercle bacilli in lesions consist of at least four different subpopulations: (*a*) those that are actively growing, which are killed primarily by INH [but in case of INH resistance, are killed by RIF, SM (streptomycin), or inhibited by EMB]; (*b*) those that have spurts of metabolism, which are killed by RIF; (*c*) those that are of low metabolic activity and reside in acid pH environment, which are killed by PZA; and (*d*) those that are "dormant," which are not killed by any current TB drug. A modified version of the Mitchison hypothesis is shown in Figure 2, where the speed of growth in the original Mitchison hypothesis is replaced with metabolic status.

TARGETS AND MODE OF ACTION OF CURRENT TB DRUGS

The current TB drugs can be divided into two categories: bacteristatic and bactericidal drugs. The static drugs include EMB and PAS, whereas the cidal drugs include INH, RIF, SM, and FQ (fluoroquinolones). However, the distinction

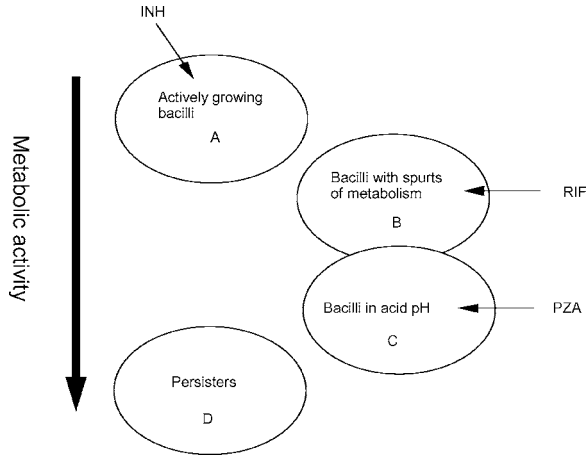

Figure 2 Special bacterial populations and TB chemotherapy.

between static and cidal drugs is only relative, because some static drugs can be cidal under some conditions (such as with higher drug concentrations, smaller inoculum, or change in bacterial physiological status). For example, PZA can show cidal activity against small numbers of nongrowing bacilli at acid pH but primarily shows static activity for growing bacilli with active metabolism (45). Cidal drugs exhibit higher activity over static drugs in reducing the number of bacilli in the lesions. The current TB drugs can also be categorized as either first-line drugs or second-line drugs. The first-line drugs include INH, RIF, PZA, EMB, and SM; the second-line drugs include kanamycin, amikacin, capreomycin, cycloserine (CS), PAS, ETH/PTH, thiacetazone, and FQ. According to their specificity, TB drugs can also be grouped as TB or mycobacteria-specific drugs such as INH, PZA, EMB, PAS, ETH, and thiacetazone, and the broad-spectrum antibiotics such as RIF, SM, kanamycin, amikacin, capreomycin, CS, and FQ. The mechanisms of action and resistance to TB-specific drugs are specific to *M. tuberculosis*, whereas mechanisms of action and resistance of the broad-spectrum drugs in *M. tuberculosis* are the same as in other bacterial species. The chemical structures and the targets of inhibition for the first-line and second-line TB drugs are shown in Figure 1 and Table 1, respectively. The mechanisms of action and resistance of TB drugs have been reviewed recently (46, 47). For the purpose of comparison with new drug targets, the mechanisms of action and resistance of the current TB drugs will be briefly reviewed here. These drugs can be grouped as cell wall synthesis inhibitors, nucleic acid synthesis inhibitors, protein synthesis inhibitors, and energy inhibitors.

Inhibitors of Cell Wall Synthesis

INH INH is a prodrug that requires activation by *M. tuberculosis* catalase-peroxidase (KatG) (48) to generate a range of reactive oxygen species and reactive

Table 1 Commonly used TB drugs and their targets

Drug (year of discovery)	MIC[a] (g/ml)	Effect on bacterial cell	Mechanisms of action	Targets	Genes involved in resistance
Isoniazid (1952)	0.01–0.2	Bactericidal	Inhibition of cell wall mycolic acid synthesis and other multiple effects on DNA, lipids, carbohydrates, and NAD metabolism	Multiple targets including acyl carrier protein reductase (InhA)	*katG*[b] *inhA* *ndh*
Rifampin (1966)	0.05–0.5	Bactericidal	Inhibition of RNA synthesis	RNA polymerase β subunit	*rpoB*
Pyrazinamide (1952)	20–100 pH 5.5 or 6.0	Bacteriostatic/ bactericidal	Disruption of membrane transport and energy depletion	Membrane energy metabolism	*pncA*[b]
Ethambutol (1961)	1–5	Bacteriostatic	Inhibition of cell wall arabinogalactan synthesis	Arabinosyl transferase	*embCAB*
Streptomycin (1944)	2–8	Bactericidal	Inhibition of protein synthesis	Ribosomal S12 protein and16S rRNA	*rpsL*, *rrs*
Kanamycin (1957)	1–8	Bactericidal	Inhibition of protein synthesis	16S rRNA	*rrs*
Quinolones (1963)	0.2–4	Bactericidal	Inhibition of DNA synthesis	DNA gyrase	*gyrA* *gyrB*
Ethionamide (1956)	0.6–2.5	Bacteriostatic	Inhibition of mycolic acid synthesis	Acyl carrier protein reductase (InhA)	*inhA* *etaA/ethA*[b]
PAS (1946)	1–8	Bacteriostatic	Inhibition of folic acid and iron metabolism?	Unknown	Unknown
Cycloserine (1952)	5–20	Bacteriostatic	Inhibition of peptidoglycan synthesis	D-alanine racemase[c]	*alrA*, *Ddl*[c]

[a]MIC is based on Inderlied & Salfinger (13).
[b]KatG, PncA, and EtaA/EthA are enzymes involved in the activation of prodrugs INH, PZA, and ETH, respectively.
[c]In fast growing *M. smegmatis.*

organic radicals, which then attack multiple targets in the tubercle bacillus. The primary target of inhibition is the cell wall mycolic acid synthesis pathway (49), where enoyl ACP reductase (InhA) was identified as the target of INH inhibition (50). The active species for InhA inhibition has been found to be isonicotinic acyl radical, which reacts with NAD to form INH-NAD adduct and then inhibits the InhA enzyme (51, 52). The reactive species produced during INH activation could also cause damage to DNA, carbohydrates, and lipids (53) and inhibit NAD metabolism (54, 55). Changes in the NADH/NAD ratios caused by mutations in NAD dehydrogenase II (*ndh*) could cause resistance to INH (56, 57). The cidal activity of INH is very likely to be due to its effect on multiple targets in tubercle bacillus (47). Mutations in KatG involved in INH activation (48), in the INH target InhA (50), and Ndh II (NADH dehydrogenase II) (57) could all cause INH resistance. KatG mutation is the major mechanism of INH resistance (46, 47).

ETH/PTH ETH, structurally related to INH (Figure 1), is also a prodrug that is activated by the enzyme EtaA (a monooxygenase, also called EthA) (58, 59) and inhibits the same target InhA as INH (50) of the mycolic acid synthesis pathway. PTH (prothionamide) shares almost identical structure and activity as ETH, where the R group in ETH is C2H5 and the R group in PTH is C3H7 (Figure 1). EtaA is an FAD-containing enzyme that oxidizes ETH to the corresponding S-oxide, which is further oxidized to 2-ethyl-4-amidopyridine, presumably via the unstable oxidized sulfinic acid intermediate (60). EtaA also activates thiacetazone, thiobenzamide, and perhaps other thioamide drugs (60). Mutations in the drug-activating enzyme EtaA/EthA and the target InhA cause resistance to ETA (61).

EMB EMB [(*S*,*S*′)-2,2′(ethylenediimino)di-1-butanol] (EMB) interferes with the biosynthesis of arabinogalactan, a major polysaccharide of mycobacterial cell wall (62). It inhibits the polymerization of cell wall arabinan of arabinogalactan and of lipoarabinomannan (63) and induces accumulation of D-arabinofuranosyl-P-decaprenol, an intermediate in arabinan biosynthesis (64). Arabinosyl transferase, encoded by *embB*, an enzyme involved in synthesis of arabinogalactan, has been proposed as the target of EMB in *M. tuberculosis* (65) and *M. avium* (66). In *M. tuberculosis*, *embB* is organized into an operon with *embC* and *embA* in the order *embCAB*. *embC*, *embB*, and *embA* share more than 65% amino acid identity with each other and are predicted to encode transmembrane proteins with 12 transmembrane-spanning domains (65). Mutations in *embCAB* operon are responsible for resistance to EMB and are found in approximately 65% of clinical isolates of *M. tuberculosis* resistant to EMB (67).

CS CS inhibits the synthesis of cell wall peptidoglycan by blocking the action of D-alanine racemase (Alr) and D-alanine:D-alanine ligase (Ddl) (68, 69). Alr is involved in conversion of L-alanine to D-alanine, which then serves as a substrate for Ddl. The D-alanine racemase encoded by *alrA* from *M. smegmatis* was cloned and its overexpression in *M. smegmatis* and *M. bovis* BCG caused resistance to

cycloserine (70). Inactivation of *alrA* (71) or *ddl* (72) in *M. smegmatis* caused increased sensitivity to CS. Overexpression of Alr conferred higher resistance to CS than Ddl overexpression in *M. smegmatis*, suggesting Alr might be the primary target of CS (73). Consistent with this finding, CS also preferentially inhibited Alr over Ddl in *M. smegmatis* (73). However, the mechanism of resistance of CS in *M. tuberculosis* remains to be identified.

Inhibitors of Nucleic Acid Synthesis

RIF RIF is a broad-spectrum semisynthetic rifamycin B derivative that interferes with RNA synthesis by binding to the bacterial DNA-dependent RNA polymerase β-subunit encoded by *rpoB*. An important feature of RIF is that it is active against both actively growing and slowly metabolizing nongrowing bacilli. Its activity against the latter is thought to be involved in shortening the TB therapy from 12–18 months to 9 months (74). Mutations in a defined 81-bp region of the *rpoB* are found in about 96% of RIF-resistant *M. tuberculosis* isolates (75). Resistance to RIF could confer cross-resistance to other rifamycins such as rifabutin and rifapentine. Rifapentine, with a longer half-life and greater activity than RIF, is a new drug approved by the FDA in 1998 for treatment of TB (76). Rifapentine can reduce the frequency of drug dosage required, but it is not active against RIF-resistant *M. tuberculosis* (77).

FQ The first quinolone drug, nalidixic acid, was obtained as an impurity during the manufacture of quinine in the early 1960s (36, 78). Since then, many FQ derivatives have been synthesized and evaluated for antibacterial activity. Ciprofloxacin (Figure 1), ofloxacin, levofloxacin, and sparfloxacin are the best studied of these agents and are highly active against *M. tuberculosis* (79). FQ inhibits DNA synthesis by targeting the DNA gyrase A and B subunits. FQ drugs are now used to treat MDR-TB as second-line drugs but MDR-TB strains are becoming resistant to FQ (80). An Indian study showed some promise of oxifloxacin in combination with first-line drugs in ultra-short course of TB treatment in three months (81). Strains of *M. tuberculosis* can develop resistance to FQ by mutations in GyrA or GyrB subunit (82, 83).

Inhibitors of Protein Synthesis

SM, an aminoglycoside antibiotic, primarily interferes with protein synthesis by inhibiting initiation of mRNA translation (84), facilitating misreading of the genetic code (85) and damaging the cell membrane (86). The site of action is in the small 30S subunit of the ribosome, specifically at ribosomal protein S12 (*rpsL*) and 16S rRNA (*rrs*) in the protein synthesis (87). As in *E. coli*, mutations in *rpsL* and *rrs* are the major mechanism of SM resistance (88). Like SM, kanamycin, amikacin, viomycin, and capreomycin are inhibitors of protein synthesis through modification of ribosomal structures at the 16S rRNA (89). Mutations

at 16S rRNA position 1400 are associated with high-level resistance to kanamycin and amikacin (90–92). Cross-resistance may be observed between kanamycin and capreomycin or viomycin (90–92), but a recent study found little cross-resistance between kanamycin and amikacin (93).

Inhibition and Depletion of Membrane Energy

PZA, a structural analog of nicotinamide, is a prodrug that requires conversion to its active form, pyrazinoic acid (POA), by the PZase/nicotinamidase enzyme encoded by the *pncA* gene of *M. tuberculosis* (94). Mutation in *pncA* is a major mechanism of PZA resistance in *M. tuberculosis* (94, 95). PZA is an unconventional and paradoxical drug that has high in vivo sterilizing activity involved in shortening the TB therapy to six months (39, 74) but has no activity against the TB bacteria at normal culture conditions near neutral pH (96). PZA is active against tubercle bacilli at acid pH (97). It is more active against old cultures than young cultures (98) and also more active at low oxygen or anaerobic conditions (99). Acid pH facilitates the formation of uncharged protonated POA that permeates through the membrane easily and causes accumulation of POA and reduces membrane potential in *M. tuberculosis* (100, 101). The protonated POA brings protons into the cell and can eventually cause cytoplasmic acidification and de-energize the membrane by collapsing the proton motive force, which affects membrane transport (101). The target of PZA is thus the membrane energy metabolism. For more details about PZA, please see the review by Zhang & Mitchison (45).

PROMISING DRUG CANDIDATES

Numerous compounds have been found to have a varying degree of activity against *M. tuberculosis*. Because this is a review of potential new drug targets, it is not possible to cover all the literature on the compounds that have antimycobacterial activity. Only the promising candidates that have passed preclinical development and are close to entering clinical trials or those that are clinically used to treat other disease conditions but happen to have antituberculous activity will be discussed here. A list of the drug candidates is shown in Figure 3. For a review of natural products such as plants, fungi, and marine organisms that have significant antimycobacterial activity, please see reference (102).

New Fluoroquinolones

The new C-8-methoxy-FQ moxifloxacin (MXF) (Figure 3) and gatifloxacin with longer half-lives are more active against *M. tuberculosis*, with MIC of 0.125 and 0.06 μg/ml, than are ofloxacin and ciprofloxacin, with MIC of 2 and 4 μg/ml, respectively (103, 104). MXF was active against *M. tuberculosis* comparable to INH in a mouse model (105, 106). MXF appeared to kill a subpopulation of tubercle bacilli not killed by RIF, i.e., RIF tolerant persisters in vitro (107). A

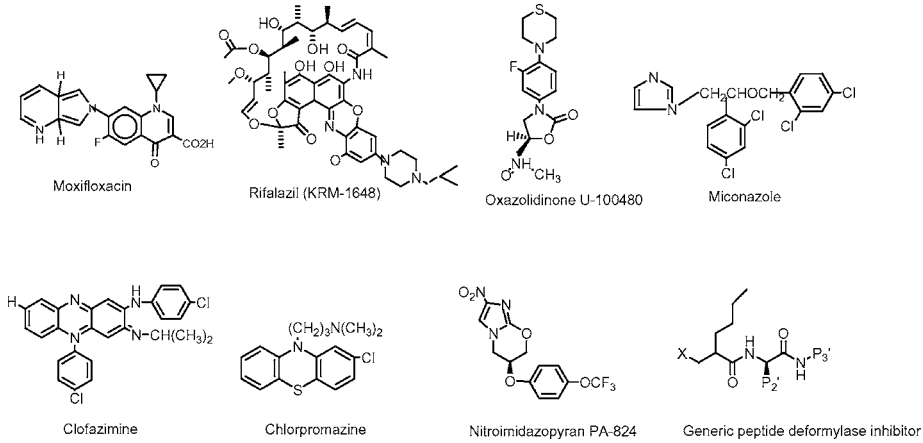

Figure 3 Structures of some promising drug candidates.

recent study showed that MXF in combination with RIF and PZA killed the bacilli more effectively than the INH +RIF +PZA in mice (108). This higher activity of MXF-RIF-PZA regimen than INH-RIF-PZA combination could be due to MXF killing a subpopulation of bacilli not killed by INH and RIF (107), or it could be due to the absence of the curious antagonism between INH and PZA (109) such that replacing INH with MXF relieved such antagonism and thus showed better sterilizing activity of MXF and PZA. The higher activity of MXF-RIF-PZA than INH-RIF-PZA has generated considerable excitement and raises the hope that MXF may replace INH in combination with RIF and PZA to shorten the TB therapy in humans. However, scientists are also concerned about the potential toxicity of MXF-RIF-PZA combination in the absence of INH as seen in the treatment of latent TB infections with RIF-PZA (110). MXF has early bactericidal activity against tubercle bacilli comparable to INH in a preliminary human study (111) and was well tolerated. Combination therapy with MXF seems to be as effective as current standard drug combinations (112). MXF and gatifloxacin are currently being evaluated in clinical treatment of TB in combination with RIF and PZA (R. Chaisson, D. Mitchison, personal communication). The highly active MXF or gatifloxacin may have the potential to be used as first-line drugs for improved treatment of TB and MDR-TB.

New Rifamycin Derivatives

Rifalazil (RLZ) (KRM1648 or benzoxazinorifamycin), a new semisynthetic rifamycin with a long half-life, is more active than RIF and rifabutin against *M. tuberculosis* both in vitro and in vivo in mice (113, 114). High-level RIF-resistant strains (MIC > 32 μg/ml) confer cross-resistance to all rifamycins; however, low-level resistant strains (MIC < 32 μg/ml) are still susceptible to new rifamycins (77, 115, 116). A preliminary safety study in humans (117) showed that RLZ produced

flu-like symptoms and transient dose-dependent decrease in white blood cell and platelet counts and did not show any better efficacy than RIF (117). Further studies are needed to more definitively assess RLZ for treatment of TB in human trials.

Oxazolidinones (Linezolid)

Oxazolidinones are a new class of antibiotics developed by Pharmacia which were approved by the FDA for the treatment of drug-resistant gram-positive bacterial infections (118). Oxazolidinones inhibit an early step of protein synthesis by binding to ribosomal 50S subunits, most likely within domain V of the 23S rRNA peptidyl transferase and forming a secondary interaction with the 30S subunit (118, 119). Oxazolidinones had significant activity against *M. tuberculosis* with an MIC of 2–4 μg/ml and were also active against tubercle bacilli in mice (120, 121). One derivative, PNU100480 (Figure 3) had activity against *M. tuberculosis* comparable to that of INH and RIF in a murine model (122). Recently, a series of 3-(1H-pyrrol-1-yl)-2-oxazolidinone analogues of PNU-10,0480 were synthesized and some of them were found to have significant activity against *M. avium* in vitro (123). Oxazolidinones may have promising potential for the treatment of mycobacterial infections. However, treatment of human TB with oxazolidinones has not yet been reported.

Azole Drugs

The azole drugs that are used to treat fungal infections have been shown to have activity against *M. tuberculosis* (124). The azole drugs miconazole (Figure 3) and clotrimizole were quite active against growing *M. tuberculosis* with an MIC of 2–5 μg/ml, and they were also active against stationary phase bacilli (124). The subsequent identification of cytochrome P450 homologs, a target for azole drugs, in the *M. tuberculosis* genome (125) provides an explanation for the activity of azole drugs against *M. tuberculosis* and led to studies to examine the correlation between the presence of P450 and susceptibility to azole drugs in *M. tuberculosis* (126–129). The *M. tuberculosis* cytochrome P450 enzyme has recently been crystallized and is being pursued as a target for TB drug development (130). Further in vivo studies are needed to assess whether azole drugs can be used for the treatment of TB.

Nitro-Containing Drugs

M. tuberculosis is quite susceptible to nitro-containing compounds. For example, niclosamide, furazolidone, 2-nitroimidazole, and 4-nitroimidazole are active against tubercle bacilli (124). The nitro-containing compounds are likely to be prodrugs that require activation by nitroreductases in *M. tuberculosis* to produce reactive species that can damage DNA. Nitrofuran was active against nonreplicating bacilli in the Wayne "dormancy" model (131). It is interesting to note that

nitrofuran is more active against INH-resistant bacilli (132), which is probably a reflection of the defect in KatG in INH-resistant strains such that they are more sensitive to the reactive oxygen species generated during nitrofuran activation. Some of nitro-containing compounds such as nitrofuran and furazolidone that are currently used in clinics to treat other bacterial infections should have less safety concern and could potentially be tested for the treatment of TB if proven to be active against *M. tuberculosis* in animal models.

Riminophenazine Derivatives

Clofazimine (Figure 3) is a riminophenazine derivative originally developed in the 1950s from components in lichens active against *M. tuberculosis* (133). Clofazimine is commonly used to treat leprosy in combination with dapsone and RIF, and it is also used to treat *M. avium intracellulare* infections (134). The emergence of drug-resistant TB has stimulated renewed interest in developing phenazines as TB drugs. The MIC of clofazimine and its derivative B669 for *M. tuberculosis* is 0.15–2.5 μg/ml (134). The mode of action of riminophenazines is not clear, but was proposed to induce mycobacterial phospholipase A2 activity, causing interference with bacterial potassium transport (135). However, a recent study failed to confirm this proposition (136). Clofazimine at the maximum tolerated dose of 5 mg/kg had no effect on tubercle bacilli in mice (137), but the liposomal form of clofazimine at 50 mg/kg reduced the bacterial numbers in infected organs by 2–3 logs (137). Novel tetramethylpiperidine (TMP)-substituted phenazines were found to be more active than clofazimine against *M. tuberculosis* and MDR-TB strains in vitro and also had higher activity against intracellular bacilli than clofazimine and RIF in macrophages (138). No animal studies with TMP-substituted phenazines are available.

Phenothiazines

Phenothiazines such as chlorpromazine (CPZ) (Figure 3), thioridazine, and trifluroperazine are antipsychotic drugs with antituberculosis activity (139). Phenothiazines are calmodulin antagonists and their antituberculous activity appears to correlate with the presence of a calmodulin-like protein in mycobacteria (140). Phenothiazines are also active against MDR-TB (141, 142), suggesting that they inhibit a novel target in *M. tuberculosis*. The MIC of trifluoperazine was 8–32 μg/ml in vitro (143). CPZ inhibited intracellular mycobacteria at lower concentrations 0.23–3.6 μg/ml because of its accumulation inside macrophages (144). CPZ may also enhance the effectiveness of TB drugs against intracellular mycobacteria (144). However, because of significant side effects, CPZ is not recommended for treating human TB but may be used along with other TB drugs to treat TB in psychiatric patients (139). Thioridazine, which has identical anti-TB activity as CPZ but fewer side effects, has been proposed as a candidate for human testing (139, 141).

Nitroimidazopyran (PA-824)

PA-824 (Figure 3) is a new nitroimidazole derivative developed by PathoGenesis-Chiron (145) on the basis of an earlier observation by Indian researchers that 5-nitroimidazole had good in vitro and in vivo activity against *M. tuberculosis* (146, 147). PA-824 was highly active against *M. tuberculosis* with an MIC of 0.015–0.25 μg/ml (145). PA-824 was also active against nonreplicating tubercle bacilli. PA-824 is a prodrug that is activated by F420-dependent glucose-6-phosphate dehydrogenase and a nitroreductase activity in the bacilli (145). The resulting active metabolites interfere with cell wall lipid biosynthesis by inhibiting an enzyme responsible for the oxidation of hydroxymycolic acid to ketomycolate (145). PA-824 was also active against MDR-TB strains, suggesting that it inhibits a new target in tubercle bacilli. PA-824 was as active as INH in animal models of TB infection (145). A preliminary toxicity study indicated that mice tolerated a single dose of PA-824 at 1000 mg/kg or 500 mg/kg daily for 28 days (145). However, no safety and efficacy data in humans are available. PA-824 is being jointly developed by the Global Alliance for TB Drug Development and Chiron.

Peptide Deformylase (PDF) Inhibitors

PDF is a metalloprotease enzyme essential for bacterial survival but is not vital to human cells (148). PDF is a target for a new generation of broad-spectrum antibiotics that has generated considerable recent interest. PDF inhibitor (Figure 3) NVP PDF-713 had activity against linezolid-resistant staphylococci (MIC = 0.25–2 μg/ml), *E. faecalis* (MIC = 2–4 μg/ml), *E. faecium* (MIC = 0.5–4 μg/ml), and quinupristin/dalfopristin-resistant *E. faecium* (MIC = 1–2 μg/ml) (149). The PDF inhibitor BB-3497 has recently been found to be active against *M. tuberculosis* with MIC of 0.06–2 μg/ml (150). The PDF inhibitor BB-83,698 was highly active against drug resistant *S. pneumoniae* in a mouse model (151). BB-83,698 had a favorable PK and PD profile. At 80 mg/kg, BB-83,698 had a peak concentration in lung tissue of about 62 μg/ml within 1 h (152). BB-83,698 is currently in clinical trials in Europe (153) and may have good potential as a new candidate drug for the treatment of TB.

POTENTIAL NEW DRUG TARGETS

Because of the drug-resistant TB problem, it is important to develop new drugs that inhibit novel targets that are different from those of currently used drugs. To avoid significant toxicity, the targets of inhibition should be present in bacteria but not in the human host. Although modification of existing drugs for improved half-life, bioavailability, or drug delivery may be of some use, agents obtained by this approach may have a cross-resistance problem, as seen in the new rifamycins or quinolones. Similarly, targeting existing TB drug targets for drug development (154) may be of limited value because of potential cross-resistance. New drugs that

inhibit novel targets are needed. In choosing targets for drug development, it is important that they be involved in vital aspects of bacterial growth, metabolism, and viability. These targets could include cell wall synthesis, nucleic acid biosynthesis, protein biosynthesis, and energy metabolism, resulting in either growth inhibition or death of the bacteria. Recent developments in mycobacterial molecular genetic tools such as transposon mutagenesis, signature-tagged mutagenesis, gene knockout, and gene transfer will facilitate the identification and validation of new drug targets essential for the survival and persistance of tubercle bacilli not only in vitro but also in vivo. Below is a list of potential targets whereby new drugs may be developed for improved treatment of TB.

Targeting Mycobacterial Persistence

DosR-Rv3133/DevR-DevS The two-component system DevR-DevS was initially identified as being preferentially expressed in virulent *M. tuberculosis* strain H37Rv over that in avirulent strain H37Ra in a subtractive hybridization analysis (155). In subsequent studies aimed at characterizing mycobacterial genes that are induced in the Wayne "dormancy" model, the same two-component system was identified by microarray analysis and named Rv3133c/Rv3132c (156). Inactivation of DosR abolished the rapid induction of hypoxia-induced gene expression (157, 158), suggesting that DosR is a key regulator in the hypoxia-induced mycobacterial "dormancy" response (158). The DosR mutant grew as well as the wild-type strain initially in a five-day incubation, but it survived significantly less well upon extended incubation up to 40 days in the Wayne model (158). A recent microarray study has found that DosR controls the expression of a 48-gene "dormancy regulon," which is induced under hypoxic conditions and by nitric oxide (NO) (159). DosR could be a good target for developing drugs against persisters.

RelA In *E. coli*, the stringent response induced by starvation is mediated by the signaling molecule hyperphosphorylated guanine (ppGpp) synthesized by RelA (ppGpp synthase I) and SpoT (ppGpp synthase II) (160). In *M. tuberculosis*, however, there is only a single RelA homolog (125). RelA mutation in *M. tuberculosis* caused significant defect in long-term survival in vitro and reduced ability to survive at anaerobic conditions, although the mutant appeared to behave as the parent strain in the initial growth phase and also survived inside macrophages (161). Mice infected with RelA mutant had impaired ability to sustain chronic infection compared with the wild-type strain H37Rv (162). Microarray analysis showed that the RelA mutant had an altered transcriptional profile with specific changes in the expression of virulence factors, cell-wall biosynthetic enzymes, heat shock proteins, and secreted antigens that may change immune recognition of the organism (162). These findings suggest that the *M. tuberculosis* RelA plays an important role in establishing persistent infection and could be a good target for drug development.

ICL (ISOCITRATE LYASE) ICL catalyzes the conversion of isocitrate to glyoxylate and succinate and is an essential enzyme for fatty acid metabolism in the glyoxylate shunt pathway. Survival of *M. tuberculosis* in the adverse in vivo environment requires utilization of C_2 substrates (generated by β-oxidation of fatty acids) as the carbon source (163). ICL was induced in the Wayne "dormancy" model (164), inside macrophages (165), and in the lesions of the human lung (166). ICL is not essential for the viability of tubercle bacilli in normal culture or in hypoxic conditions, but it is needed for long-term persistence in mice (163). The crystal structure of ICL has been determined and is being pursued as a target for structure-based drug design (167).

PcaA (PROXIMAL CYCLOPROPANATION OF ALPHA-MYCOLATES) Using a transposon mutagenesis approach based on changes in colony morphology, a gene called *pcaA* encoding a novel methyl transferase involved in the modification of mycolic acids in mycobacterial cell wall was identified (168). Although the PcaA knockout mutant grew normally in vitro and replicated in mice initially like the parent strain, the mutant was defective in persisting in mice (168) and could be a target for drug design against persistent bacilli.

Targeting Essential Genes

Essential genes are genes whose inactivation leads to nonviability or death of the bacteria. Until recently when mycobacterial molecular genetic tools (transposon mutagenesis, gene knockout and gene transfer) became available (169–171), two approaches were used to identify essential genes in *M. tuberculosis*. One approach is the random transposon mutagenesis approach, which relies on random transposon insertion into chromosomal genes followed by an analysis of the genes in which the transposon is inserted. The genes in which no transposon has been inserted are essential genes. A recent study using a transposon mutagenesis and a statistical treatment of data indicated that one third of the *M. tuberculosis* genes are likely essential genes (172). Seven gene families—aminoacyl tRNA synthases, purine ribonucleotide biosynthesis, polyketide and nonribosomal peptide synthesis, fatty acid and mycolic acid synthesis, Ser/Thr protein kinases and phosphotases, molybdopterin biosynthesis, and PE-PGRS repeats—were identified as essential genes (172). Conditionally lethal mutants, which are defective in metabolic pathways and fail to grow on minimal medium, as well as genes required for optimal in vitro growth, were also identified by transposon mutagenesis (173, 174). Another approach is to determine if a particular gene is essential by gene knockout studies. If no mutant is recovered when the gene is inactivated but the mutant can be obtained when the gene is present on a plasmid, such a gene is an essential gene. Many mycobacterial essential genes are identified this way. The targets encoded by essential genes can be good targets for drug design.

Targeting Sigma Factors

Sigma factors bind to RNA polymerase to initiate transcription. There are 13 sigma factors present in the *M. tuberculosis* genome (125). For a recent review of this topic, see Reference (175). Like other bacteria, *M. tuberculosis* has a general house-keeping sigma 70–like principal sigma factor MysA or SigA (176), as well as more specialized sigma factors such as RpoS-like sigma factor MysB (SigB), SigC, SigE, SigH, SigF, which are induced under various stress conditions (175). Increased SigA expression in *M. tuberculosis* and in transformed strains caused faster growth inside macrophages and increased virulence in mice (177). SigC, which controls the expression of virulence factors such as two-component systems *senX3-regX3*, *mtrA-mtrB* and *hspX* (alpha-crystallin homolog), is also involved in virulence (178). Expression of SigB is dependent on SigE (179) and SigH (180). SigE is involved in global gene expression, heat stress, oxidative stress, exposure to SDS, and survival in macrophages (179–181) and virulence (182, 183). SigE is regulated by SigH, which plays a central role in regulation of heat and oxidative stress responses, and *sigH* mutants are more susceptible to these stresses (180). SigF is induced in stationary phase and a variety of stress conditions such as nitrogen depletion, oxidative stress, cold shock, and anaerobic conditions (184). Mutation in SigF did not affect in vitro growth or survival in macrophages compared with the parent strain, but caused reduced virulence in mice (185). Because of their importance in mycobacterial gene transcription and their absence in the host, sigma factors could be good targets for drug design.

Targeting Virulence Factors

In recent years, scientists have become interested in developing antibacterial drugs that target virulence factors in bacterial pathogens (186). Although the idea of targeting virulence factors and two-component systems (see below) is quite attractive, it may have some potential drawbacks. For example, virulence factors may not be essential viability genes, and inhibition of virulence factors may not be lethal for the bacterial pathogen. Moreover, incomplete inhibition of virulence factors could also have problems. The most worrying aspect of this approach is that such drugs may be of little use for established infections. Although no drugs that target virulence factors have been developed so far, there is hope that such drugs may be used in conjunction with conventional antibiotics to improve treatment of bacterial infections (186). The recent developments in mycobacterial genetic tools have led to the discovery of various virulence factors in *M. tuberculosis*. For a recent review of mycobacterial virulence factors, see Reference (187). In addition, in a recent study using transposon mutagenesis, 194 genes (about 5% of genome) in the *M. tuberculosis* genome were identified as required for growth in mice (188). These virulence factors could be potential drug targets.

Targeting Two-Component Systems

Because of the important role of two-component systems in controlling bacterial virulence genes, scientists are interested in developing inhibitors that target these systems (189–194). Several series of inhibitors have been found from chemical library screens, including salicylanilides (190), diaryltriazole analogs (195), bisphenols, cyclohexenes, benzoxazines, and triphenylalkyl derivatives (192). However, most of these agents suffer from poor selectivity, excessive protein binding, or limited bioavailability (191, 194). Researchers are pursuing alternate strategies to identify inhibitors with more desirable properties; these strategies include design of substrate-based inhibitors, generation of combinatorial libraries, and isolation of natural products(192). The conserved domains of response regulators of different two-component systems offer a common site of attack by inhibitors (194).

M. tuberculosis has 11 two-component system homologs in the genome (125). Many of these homologs have now been characterized: MtrA-MtrB (197), SenX3-RegX3 (198), the DevR (DosR)-DevS (158), PrrA-PrrB (199), MprA-MprB (200), and PhoP/PhoR (201). Inactivation of the *mtrA* component of *mtrA-mtrB* of *M. tuberculosis* H37Rv was possible only in the presence of plasmid-borne functional *mtrA*, suggesting that this response regulator is essential for *M. tuberculosis* viability (200). Inactivation of either *senX3* or *regX3* caused attenuation of virulence in mice (202, 203). DevR (DosR)-DevS was found to be expressed to higher levels in virulent strain H37Rv than in avirulent strain H37Ra (204). Inactivation of DosR (205), *mprA* (200), and *phoP* (201) caused attenuated virulence in animal studies. These studies suggest that two-component systems in *M. tuberculosis* could be important drug targets.

Targeting Cell Wall Synthesis

Because several TB drugs such as INH, ETH, and EMB target mycobacterial cell wall synthesis, enzymes involved in this pathway have been preferred targets in drug development efforts. KasA and KasB, β-ketoacyl-acyl-carrier protein synthases, have been examined as potential targets for drug development. Thiolactomycin (TLM) targets KasA and KasB that belong to the fatty-acid synthase type II (FASII) system involved in fatty acid and mycolic acid biosynthesis (206, 207). TLM was also active against an MDR-TB clinical isolate. Several TLM derivatives were found to be more potent than TLM in vitro in the fatty acid and mycolic acid biosynthesis assays and against *M. tuberculosis* (208). No TLM-resistant mutants of *M. bovis* BCG could be isolated, which could be a consequence of TLM inhibiting multiple enzymes of fatty acid synthesis in mycobacteria (207). Because TLM inhibits the FASII enzyme in different bacterial species, it could be developed into a broad-spectrum antibiotic for treating different bacterial infections including TB. Cerulenin inhibits KasA involved in mycolic acid synthesis with an MIC of 1.5–12.5 μg/ml against *M. tuberculosis* (209, 210). N-octanesulfonylacetamide (OSA), an inhibitor of fatty acid and mycolic acid biosynthesis, was active against *M. tuberculosis* and also MDR-TB strains with an MIC of 6.25–12.5 μg/ml (211).

These inhibitors of fatty acid and mycolic acid synthesis could be good candidates for further development. However, drugs that target cell wall synthesis are likely to be active mainly against growing bacilli but not against persisters, and they may not be able to shorten the lengthy therapy (212).

Targeting Unique Physiology of *M. tuberculosis*

Tubercle bacillus is generally thought to be a tough organism equipped with a thick waxy cell envelope that provides a permeability barrier to a variety of agents and many antibiotics that are effective against other bacterial pathogens. However, recent studies have revealed that contrary to common beliefs, *M. tuberculosis* has some surprising weaknesses that may be exploited in designing drugs against this pathogen.

First, *M. tuberculosis* has a deficiency in efflux of POA. *M. tuberculosis* is uniquely susceptible to PZA, whereas other mycobacteria and bacteria are naturally resistant to it (100). The unique susceptibility to PZA is at least partly due to a deficient POA efflux mechanism that allows POA to be increasingly accumulated inside *M. tuberculosis* at acid pH (100). In contrast, naturally PZA-resistant *M. smegmatis* and other bacteria such as *E. coli* have a highly active POA efflux mechanism that does not allow accumulation of POA even at acid pH (100). The *M. tuberculosis* POA efflux is at least 100 times slower than that of *M. smegmatis* (100). Besides deficient POA efflux, *M. tuberculosis* appears to be defective in the efflux of other compounds such as weak acids (Y. Zhang, unpublished data). New TB drugs may be designed that take advantage of the deficient efflux mechanism in *M. tuberculosis*.

Another defect is the poor ability of *M. tuberculosis* to maintain its energy status. During our study of the mechanism of action of PZA, we found that in addition to weak acid POA, *M. tuberculosis* is also more susceptible to many other weak acids than other bacteria such as *M. smegmatis* or *E. coli* (213). This unique weak acid susceptibility of *M. tuberculosis* seems to be related to its deficient ability to maintain membrane potential and pH gradient (213), presumably caused by its slow metabolism. It will be interesting to determine if weak acids or their precursors can be developed into TB drugs.

A third defect of *M. tuberculosis* is its deficient ability to cope with endogenously generated reactive species. Studying the mechanisms of action of IHH, researchers found that *M. tuberculosis* appears to be deficient in oxidative defense and highly susceptible to endogenously produced oxygen radicals generated by KatG-mediated INH activation (26). The unique susceptibility of *M. tuberculosis* to INH is probably due to a combination of defective OxyR (214, 215) and poor ability to remove or antagonize toxic reactive oxygen species and organic radicals that have accumulated (26). In addition, *M. tuberculosis* appears to be particularly susceptible to endogenously produced reactive nitrogen intermediates. For example, niclosamide (124), nitroimidazopyran PA-824 (145), and nitrofurans (131), which presumably generate reactive nitrogen during their activation, are quite active against *M. tuberculosis*, especially nongrowing bacilli. It will be interesting

to see if compounds that generate reactive oxygen or nitrogen species inside bacilli could be designed as TB drugs.

TB Genomics and Drug Targets

The first bacterial genome was sequenced by Fleischmann and colleagues at The Institute for Genomic Research (TIGR) in 1995 (216). So far, more than 100 bacterial genomes have been sequenced (www.tigr.org). As bacterial genome sequences become available, there is increasing interest in developing new antibacterial agents using genomics-based approaches (217–220). The available genome sequence information, along with molecular genetic tools, allows researchers to identify common essential targets among different bacterial species. The common targets can then be overexpressed for biochemical assays in drug screens or structure determination, to be used in the drug design. So far, however, no company has been successful in developing a drug using a genomics approach. The availability of the *M. tuberculosis* genome sequence (125) opens up a new opportunity to understand the biology of the organism and provides a range of potential drug targets (221). The recent developments in microarray technology (222), signature-tag mutagenesis (223), mycobacterial transposon mutagenesis (169), and gene knock-out technology (170, 224) provide important tools to identify new drug targets. Microarray has been used to identify *M. tuberculosis* genes that are induced by INH and ETH (225), and by INH, TLM, and triclosan (226). Microarray was also used to identify genes that are switched on in the Wayne "dormancy" model under hypoxic and nitric oxide stress conditions (156, 159), a discovery that led to the identification of a 48-gene "dormancy regulon" controlled by DosR (159). A proteomic approach was used to identify potential proteins that are induced in starvation as an in vitro model of persistence (227). Two unique *M. tuberculosis* proteins with homology to each other were identified: Rv2557 and Rv2558 (227). Rv2557 was also induced inside granulomatous lesions in the human lung (166). Genes identified by microarray analysis or proteins identified by a proteomic approach should be further validated as potential drug targets by gene knockout and in vivo testing in mice before they are selected as targets for drug development.

STRUCTURE-BASED DRUG DESIGN

Structure-based drug design and combinatorial chemistry represent potentially powerful and promising approaches for drug design. In the case of designing antituberculous compounds, selection of targets usually involves identifying enzymes in pathways essential for the organism but not present or less important in the human host. The number of three-dimensional structures of *M. tuberculosis* proteins has been increasing rapidly in recent years. This increase reflects an awareness of the need for new targets for design of new antituberculosis drugs. The *Mycobacterium tuberculosis* Structural Genomics Consortium (http://www.doe-mbi.ucla.edu/TB/), consisting of 70 laboratories in 12 countries, was established in 2000 and has contributed a significant number of structures of

M. tuberculosis proteins (228). This consortium aims to crystallize 400 proteins in five years. A list of 3D structures can be found in the Protein Data Bank (http://www.rcsb.org/pdb/) and also in http://www.doe-mbi.ucla.edu/TB/EDIT/tb_structures_in_pdb.php?format=html. Many of these targets have not yet been validated as essential, and the structure-based drug design is only meaningful on bacterial targets that have proven to be essential (see above). A list of crystal structures of mycobacterial enzymes with relevant properties as potential drug targets have been recently reviewed (8) and will not be recounted here.

DRUG SCREENS

Because of the problem of drug-resistant TB and the need to shorten the lengthy TB chemotherapy, there is currently a great deal of interest in TB drug development (7, 8, 11). NIH supports some antimycobacterial drug discovery research through the NIAID Division of AIDS Opportunistic Infections Branch. The NIH-sponsored consortium consists of in vitro screening facilities at the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) at Southern Research Institute and at Hansen Disease Center (Baton Rogue), and at an animal testing facility at Colorado State University. GlaxoSmithKline also has a program called Action TB for TB drug discovery research. A private organization, the Global Alliance for TB Drug Development, was recently established to facilitate TB drug development (http://www.tballiance.org) and aims to have at least one TB drug registered by 2010 (229).

Both whole cell screens and cell-free target-based screens are used for antimicrobial drug discovery. The target-based screen is a relatively recent invention and has so far been generally disappointing (233), except the recent development of peptide deformylase inhibitors which represents the first success of the target-based approach (148, 151, 152). However, all current TB drugs, with the exception of PZA, were identified by in vitro whole cell screens. The current NIAID-sponsored TB drug development effort is primarily based on screening of compounds active against growing bacilli using AlarMar Blue redox dye in a 96-well microtiter plate format. About 70,000 compounds have been screened so far (R. Reynolds, personal communication), and data for about 50,000 compounds were recently published (230), where 11% (5251) had high activity against *M. tuberculosis* in vitro. Of these, 53 were tested in vivo, and 9 were found to significantly reduce bacterial numbers in the lungs of infected mice. A luciferase-reporter mycobacterial strain has also been used for screening more than 62,000 EMB analogs generated by combinatorial chemistry for more active compounds (231). Twenty-six compounds were identified; *N*-Geranyl-*N*′-(2-adamantyl)ethane-1,2-diamine (Compound 109), the most active of these diamines, was 14- to 35-fold more active than EMB (231). Further development is required to assess its in vivo activity. A green fluorescent protein based screening system utilizing acetamidase gene promoter was recently established for high throughput antimycobacterial compound screen (232). The combinatorial chemistry can be applied to generate diverse compounds for screens in both whole cell and target-based screens.

Although the whole cell screens are useful for TB drug development, we must recognize the potential problem of developing drugs active against growing tubercle bacilli: drugs only active against growing bacilli are not going to be very useful for killing nonreplicating persisters, which are the biggest stumbling block for a more effective therapy. Although sterilizing drugs that can kill persisters and shorten the TB therapy are desperately needed, it is not clear how this objective can be effectively achieved. There is no good in vitro correlate of high sterilizing activity against persisters in vivo. That is, we cannot infer from the MIC whether the drug is going to be active against persistent bacilli or have high sterilizing activity. Low MIC does not mean the drug will have good sterilizing activity against persistent bacilli in vivo. INH is a wonderful drug that is highly active against growing tubercle bacilli with a very low MIC of 0.02–0.06 μg/ml, but has no activity against nonreplicating bacilli and therefore cannot effectively sterilize the lesions (235). In contrast to INH, PZA is a paradoxical drug that has poor in vitro activity against growing tubercle bacilli with a high MIC of 50–100 μg/ml at pH 5.5–6.0 and is completely inactive against tubercle bacilli at normal culture conditions near neutral pH, which is commonly used for whole cell MIC-based screens. Unlike common antibiotics which are active against growing bacteria with no activity against nonreplicating bacteria, PZA is exactly the opposite and is more active against nonreplicating old bacilli (98) and under hypoxic conditions (99). It is these properties that are responsible for its high sterilizing activity in vivo and its ability to shorten the therapy from 9–12 months to 6 months. PZA was discovered by a serendipitous observation in 1940s that nicotinamide had activity against mycobacteria in animal models; subsequent synthesis of nicotinamide analogs and direct screen in mice without MIC testing identified PZA as the most active agent in vivo (45). In a sense, we should feel fortunate that we have the wonderful sterilizing drug PZA, which would have been missed altogether had the conventional MIC-based screens been used. As we can see, the MIC-based approach does not work here! If there is any lesson to be learned from the PZA story, it is that we cannot use the MIC-based screens to identify drugs that have high sterilizing activity against persisters.

To identify drugs that effectively kill nongrowing persisters and shorten the therapy, we must design new and unconventional screens that mimic the persisters in vivo, such as using nonreplicating bacilli at low oxygen and acid pH in the screen, a process that is more challenging. There are different persistence models that can potentially be used for screening for sterilizing drugs (41, 212). Stationary phase bacilli, old and starved bacilli, and persisting bacilli after drug treatment can all be used in such screens. Synergy screens with different agents should also be considered. Because of our limited understanding of mycobacterial persisters, it is difficult to judge if one model is better than another. However, testing in animals will show which in vitro persistence model is more relevant to the goal of shortening the therapy. In addition, potential targets involved in persistence (see above) could also be selected for target-based screens.

CONCLUDING REMARKS

The development of modern TB chemotherapy is indeed a remarkable achievement of modern medicine and represents a major milestone in humankind's fight against TB. Yet despite the availability of TB chemotherapy and the BCG vaccine, TB is still a leading infectious disease worldwide. Along with the socio-economic and host factors that underlie this problem, a fundamental problem that hinders more effective TB control is the tenacious ability of *M. tuberculosis* to persist in the host and to develop drug resistance, often as a consequence of poor compliance to lengthy therapy. Novel screens targeting persisters are needed but such screens are challenging. PZA represents a prototype model drug that can shorten TB therapy, and improved understanding of PZA should help us to design drugs that are more active against persisters. Although having another new drug like INH that only kills growing bacilli may be useful for treating drug-resistant TB, it is unlikely to improve the current TB therapy. The development of new sterilizing drugs that target persisters and shorten the TB therapy must be a top priority. This represents a paradigm shift from previous approaches, which focused on just finding another drug, to beating mycobacterial persistence. In the big picture, we must recognize that better control of TB extends beyond better chemotherapy; it requires a multifaceted approach, including improved socio-economic conditions and nutrition, better management of adverse psychological factors, and improved host immunity as adjunct treatment (41). The recent developments in mycobacterial genetic tools and TB genomics, new technology of combinatorial chemistry and high throughput screening, structure-based drug design, and improved understanding of the unique biology of tubercle bacillus provide an exciting opportunity to discover new "Magic Bullets" that kill persisters and shorten the current TB treatment from six months to a few weeks. A new era of TB chemotherapy will arrive when these new "Magic Bullets" are identified.

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