7.24 Antimycobacterium Agents

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7.24.1 Introduction

The mycobacteria are responsible for more human disease than any other bacterial genus. There are 115 species of mycobacteria currently recognized,[1] over 30 of which are capable of causing human disease. Mycobacteria are nonmotile, acid-fast, weakly Gram-positive bacilli, in the shape of slender, straight, or slightly curved rods. They are subdivided into facultative and obligate human pathogens, and further classified based on their growth rate in culture into rapid- and slow-growing species. The most pathogenic members of the genus are *Mycobacterium tuberculosis*, the species responsible for tuberculosis (TB), *M. leprae*, the agent that causes leprosy, and *M. ulcerans*, the cause of Buruli ulcer, a devastating, necrotizing infection of the skin, highly prevalent in many tropical countries, most prominently in West Africa.

With the explosion of the AIDS epidemic over the past several decades, the global TB epidemic has grown and spread. Disease due to mycobacteria other than TB (MOTT; also known as nontuberculous mycobacteria, atypical mycobacteria, or environmental mycobacteria) has also become significantly more prevalent, as human immunodeficiency virus (HIV)-infected individuals are particularly susceptible to a number of mycobacterial infections, including, for example, infections due to *M. avium intracellulare* complex (MAC). This chapter will focus primarily on drug therapy for TB, due to the exceptional global morbidity and mortality burden of the disease – both current drugs and novel compounds under development.

7.24.2 Disease State

Worldwide, TB is the second-leading cause of death due to a single infectious agent, after AIDS. In 2003, there were an estimated 1.7 million deaths due to TB and 8.8 million new cases, representing a 1% annual growth rate globally.[2] Without modern anti-TB chemotherapy, which began with the discovery of streptomycin by Schatz, Bugie, and Waksman in 1944,[3] death rates due to TB would be significantly higher. TB appears to have been present, although relatively rare, in the prehistoric era in mammals and humans, and *M. tuberculosis* DNA has been identified in pre-Columbian mummies from Peru.[4] By the 1500s, a TB epidemic known as 'The Great White Plague' had spread through Europe. TB continued to spread and increase in incidence throughout Western Europe and North America from the 1600s to early 1800s, when approximately 25% of all deaths were due to TB. It remained a major cause of mortality throughout the first half of the twentieth century in these parts of the world, causing an estimated 110 000 deaths per year in the USA in the early 1900s, before its incidence began to decline. This decline is most convincingly attributed to a combination of improved living and sanitation standards, and perhaps the survival of a more TB-resistant human population.[5] Subsequently, the TB epidemic spread to Africa and Asia, primarily in the latter half of the twentieth century. Today, the highest morbidity and mortality burden due to TB is found in sub-Saharan Africa, parts of Asia, and the Russian Federation.[2]

Currently, one-third of the world's population, approximately 2 billion people, is estimated to be infected with *M. tuberculosis*. Although the majority (over 90%) of *M. tuberculosis*-infected individuals remain latently infected throughout their lives without experiencing any clinical symptoms or being infectious to others, this population

represents an enormous reservoir of future cases of active, transmissible disease. Among these latently infected individuals, those with intact immune systems have a 5–10% lifetime risk of developing active (clinically symptomatic) TB and becoming capable of transmitting the infection to others; those who are HIV-infected or otherwise immunocompromised have an estimated 8% per year probability of developing active TB. Latent TB infection (LTBI) is typically curable, but requires a lengthy treatment with drugs having significant rates of toxicity,[6] and therefore treatment often is not provided to these clinically asymptomatic individuals.

7.24.2.1 Human Immunodeficiency Virus–Tuberculosis

The HIV and TB epidemics not only co-exist in most parts of the world today, but are synergistic. In individuals latently infected with *M. tuberculosis*, HIV co-infection is estimated to increase the risk of progression to active TB by 50-fold on a yearly basis compared with those who are not infected with HIV. The risk in these co-infected individuals of activating their *M. tuberculosis* infections appears to correlate with their degree of suppression of cellular immunity as measured by CD4+ cell counts. Presentation of TB also correlates with CD4+ cell counts: patients with higher counts present with typical pulmonary TB, whereas those with low CD4+ cell counts tend to present with extrapulmonary TB, and more often have sputum smears and cultures negative for *M. tuberculosis*, making their TB more difficult to diagnose. Approximately, 12 million individuals are currently co-infected with HIV and *M. tuberculosis* (HIV–TB co-infected), and approximately 15% of AIDS patients globally die of TB.[7]

Treatment of TB in AIDS patients is complicated by drug–drug interactions between antiretroviral agents and anti-TB agents, particularly HIV protease inhibitors and rifamycin derivatives, especially rifampicin (rifampin in the USA). Rifampicin induces hepatic microsomal enzymes, specifically cytochromes CYP1A2, CYP2C9, CYP2C19, and CYP3A4 and P-glycoprotein (P-gp), as well as weakly inducing 2D6. Rifampicin is also a substrate for P-gp. HIV protease inhibitors such as indinavir and nelfinavir are substrates for and inhibitors of CYP3A4 and P-gp. The most significant result of these interactions is that rifampicin decreases the serum half-lives of these antiretroviral agents. Rifabutin, a rifampicin analog, can be prescribed instead to patients on indinavir or nelfinavir, but rifabutin is not entirely free of these interactions. HIV-infected patients also tend to have decreased exposures to standard TB drugs, most likely from poor absorption. As a result, effective and safe co-administration of TB treatment and HAART (highly active antiretroviral therapy) requires careful monitoring of drug serum levels and is impractical in many endemic country settings, further compromising effective treatment of HIV–TB co-infected patients.

7.24.2.2 Multidrug Resistant-Tuberculosis (MDR-TB)

Multidrug resistant TB (MDR-TB) is increasing in prevalence, and threatens the ability of standard control measures to contain the global TB epidemic.[8] In TB, drug resistance is mostly a human-made problem, resulting from inappropriate prescribing, poor treatment adherence, irregular drug supply, and/or poor drug quality. The most recent report of the World Health Organization (WHO) Global Project on Anti-Tuberculosis Drug Resistance Surveillance found drug resistance in 74 of 77 settings tested from 1999 to 2002 and in all regions of the world. The highest levels of MDR-TB among newly diagnosed cases were found in countries of the former Soviet Union, China, Ecuador, and Israel. Globally, a median of 1.1% of all newly diagnosed cases were found to be MDR (range: 0–14.2%); in previously treated cases, a median of 7% were MDR (range: 0–58%), and a median of 18.4% demonstrated resistance to at least one of the first-line TB drugs (range: 0–82%).[2] Treatment of MDR-TB is significantly more costly, toxic, and complex than treatment of drug-sensitive TB, and relies on a battery of second-line drugs.

7.24.3 Disease Basis

TB can take many forms in the human host, but the most common is pulmonary disease, in which the bacilli are inhaled from aerosols generated, for example, by coughing, forceful breathing, or sneezing by a patient with active disease, and are deposited on the alveolar surfaces of the lung in the terminal air sacs. There they are taken up by and replicate within macrophages, where they reside within a membrane-bound vacuole and inhibit maturation of the vacuole.[9–12]

The typical pattern of disease has been described as occurring in four stages.[13] The first is the implantation of inhaled bacteria in alveoli, which occurs 3–8 weeks postinhalation. It is followed by dissemination through the lymphatic circulation to the regional lymph nodes in the lung, forming the primary (or Ghon) complex. In the second stage, which occurs over the ensuing approximate 3 months, the bacteria circulate through the bloodstream

to other organs. Some patients suffer fatal disease during this stage, known as 'primary' disease. The third stage can occur at any time up to 2 years following infection, and is typically marked by inflammation of the pleural surfaces (pleurisy) and severe chest pain.[14] The fourth and final stage consists of resolution of the primary complex, and can take several years. In some cases, extrapulmonary disease can become manifest during this time. Before the HIV epidemic, approximately 5–10% of newly diagnosed cases were extrapulmonary.[15] In HIV-positive individuals, however, over 50% of cases are extrapulmonary. Because extrapulmonary disease is significantly more difficult to diagnose than pulmonary TB, these figures are estimations. As noted above, most immunocompetent, infected persons do not exhibit clinical disease, and remain latently infected throughout their lifetimes. But postprimary or 'reactivation' disease occurs in approximately 10% of these individuals at some point during their lives.

As noted above, the mycobacteria are intracellular pathogens, residing primarily within host macrophages. They have a complex and unique cell wall, which has been elucidated through research extending back to the 1960s. This research has been based on a combination of classic biochemical techniques with more modern genomics, nuclear magnetic resonance, and mass spectral analysis. The cell wall structure is composed of a peptidoglycan core covalently joined through a linker (L-Rha-D-GlcNAc-P) to galactofuran, which itself is connected to highly branched arabinofurans, which are in turn attached to mycolic acids. The last form a lipid barricade, key to many aspects of TB pathogenesis.[16] The metabolic pathways involved in synthesis of these cell wall lipids have the potential to be attractive drug targets once elucidated.

Other targets that will presumably be crucial to the development of drugs to shorten current therapy are those involved in the pathways essential to bacterial survival during the persistent state. Persistence is operationally defined here as the phenotypic bacterial state(s) that enable(s) *M. tuberculosis* to evade chemotherapy for prolonged periods, and thus is responsible for the extended duration of current TB treatment regimens. Genomic approaches have been used to identify genes and pathways transcriptionally active during persistence.[17] The pathologic hallmark of TB is the caseating granuloma. The host immune response and molecular mechanisms responsible for the formation of the caseating granuloma are not yet fully defined, but are likely to be the key, ultimately, to understanding *M. tuberculosis* persistence and latency.

7.24.4 Experimental Disease Models

Experimental disease models of TB, as in other disease areas, play a critical role in the development of effective diagnostics, vaccines, and therapeutic agents. Since the identification of *M. tuberculosis* as the etiological agent of TB,[18] many forms of in vitro and in vivo models have been developed. TB is a complicated disease involving many disease forms and stages, and the mechanism by which *M. tuberculosis* becomes persistent is still largely unknown. The validation of many of the disease models is difficult if not impossible, and the predictive value of these models continues to be the subject of debate. This section will focus on in vitro and in vivo models that are relevant to and commonly used in TB drug discovery research.

7.24.4.1 In Vitro Drug Susceptibility Models

The most commonly used in vitro model is a drug susceptibility test that measures the minimum inhibitory concentration (MIC) of a given drug or drug combination against *M. tuberculosis* in its exponential growth phase.[19] This model is performed in a rich, highly oxygenated culture medium. There have been several new variations of the susceptibility test described for rapid, high-throughput screening of drug susceptibilities.[20] Because of the rapid, high-throughput nature, the susceptibility tests are primarily used for confirming biochemical leads, developing structure–activity relationships (SARs), and evaluating the microbial susceptibility or resistance to a given drug. Organisms are generally more susceptible to drugs during the exponential phase of growth under oxygen- and nutrient-rich conditions. In an infected host, pathogens may adopt different physiological growth states, depending on the local growth conditions. In certain loci such as inside a lung granuloma, pathogens adopt a slowly replicating or nonreplicating state, and are extremely hard to eradicate. Therefore, susceptibility tests performed under nutrient- and oxygen-rich conditions may have limited value for predicting the efficacy against pathogens in an infected host.

To address the deficiency of the conventional susceptibility tests, many in vitro persistence models have been introduced.[21] The Wayne model is perhaps the most commonly used and cited persistence model: in this model, the persistent state is induced by slow oxygen depletion in capped tubes.[22] The Wayne model appears to be most useful in predicting in vivo bacterial sterilizing activity against TB. However, this model has clear limitations as a drug discovery tool due to its low throughput, long testing duration, and requirement for large amount of test compounds.

Another widely used susceptibility model is the ex vivo intracellular macrophage model.[23] This model is supported by a body of evidence demonstrating that macrophages are the predominantly infected cell type in TB[24] and may play an important role in drug persistence and latent infections.

7.24.4.2 In Vivo Models for Acute, Chronic, and Latent Infections

Animal models have served an important function in the development of therapeutic agents against TB.[25] The models vary significantly depending on a number of parameters, including animal species, bacterial strains, and routes and stages of infection. It is crucial to use models that are relevant to the issues being addressed. For a lead optimization program, the predictability, speed, throughput, and sample size are important factors to be considered. Mouse models have become the primary choice at this stage, in order to quickly screen a large number of compounds. After a drug candidate is selected, models with the best predictive value for treatment results in human disease are the best choices for studying proper dose regimens and drug combinations. In this instance, guinea pig, rabbit, or even monkey models could be considered. However, to date, the best substantiated model for this purpose is the aerosol-infected mouse model.

Various mouse models have been developed to mimic *M. tuberculosis* infections in the acute, chronic, or latent stage.[26] Compared with models in other animal species, the mouse models are better characterized and have clear advantages in the lead optimization stage of a drug discovery program. The acute infection model[27,28] can be used for quickly screening a large number of compounds and assessing some of the important pharmacokinetic/pharmacodynamic parameters, such as drug oral availability and penetration into the infection locus. Compounds with good efficacy in the acute model can then be moved into the chronic infection model,[29] to assess efficacy in the chronic phase of infection. Mouse models have played a key role in developing the current anti-TB agents. However, due to differences in the host immune response to *M. tuberculosis* infection, mouse models are not expected to reproduce the human disease in all aspects. Most notably, mice do not form caseating granulomas in response to *M. tuberculosis* infection.

The guinea pig and rabbit have also been used to develop TB models. These models may have some specific advantages over the mouse model; in particular, the lung pathology from infected guinea pigs and rabbits resembles more closely that from TB patients. Rabbits are the only nonprimate known to form caseating granulomas. These species may therefore be better than mouse models for studying persistent and latent infections. However, additional studies are clearly needed to validate this hypothesis. Both the guinea pig and rabbit models are more expensive and require a larger amount of test compounds than the mouse models. The monkey model may mimic human TB most closely of all the animal models.[30] However, economic and ethical concerns limit its value in drug development.

7.24.5 Clinical Trial Issues

The design of clinical trials to evaluate new TB drugs faces a number of key challenges. These include, first, that efficacy trials are of long duration – due to both the ability of the bacteria to evade at least the current chemotherapeutic agents for a ptolonged period (i.e., 6-month current treatment duration) and the lack of surrogate markers that could substitute reliably for the need to measure long-term relapse rates as a primary efficacy endpoint. The current state-of-the-art is the assessment of the efficacy of a treatment based on relapse rates in the first year or more following completion of therapy. Although the percentage of patients converting their sputum to bacteriologic negativity after 2 months of treatment is used in early clinical trials as an indicator of the ability of a regimen to shorten treatment duration, this endpoint is neither very sensitive nor rigorously validated at this time. A second major challenge for the design of TB treatment trials is that TB therapy must consist of multiple drugs used in combination to prevent development of resistance. Therefore, conventionally, one new drug under development is added to or substituted into the current regimen at a time, necessitating 6–8 years to evaluate each new drug in sequence, and therefore potentially two or more decades to test an entirely new multidrug regimen.

The design of clinical trials for new MDR-TB treatments will be even more challenging, as each patient should in theory receive an individualized treatment regimen based on the drug susceptibilities of that patient's own strain of *M. tuberculosis*. Consequently, it is difficult to devise appropriate control groups for these trials, and therefore difficult to accurately assess the efficacy of a new drug or regimen.

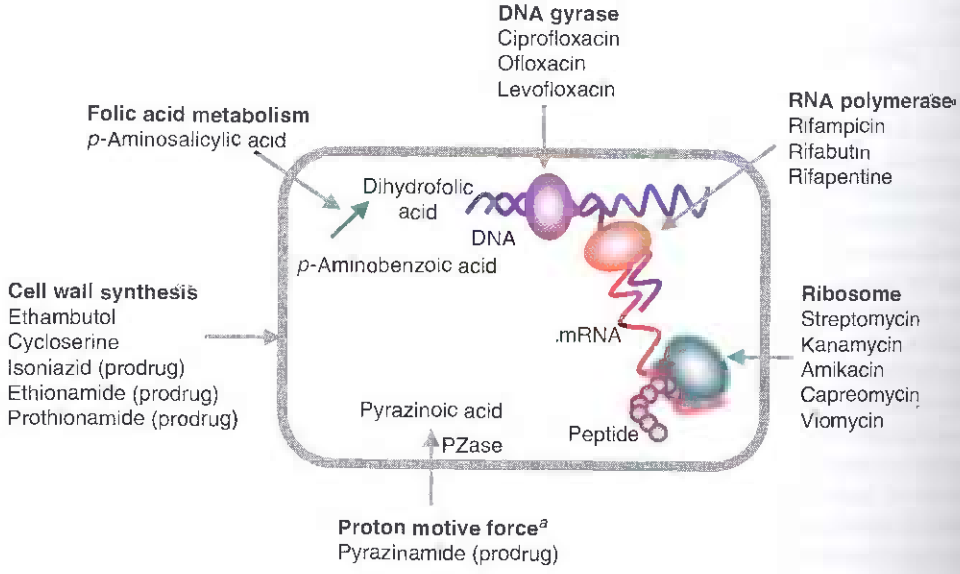
Lastly, trials of new treatments for LTBI require relatively large patient numbers and long duration times, as the endpoint is development of active disease, which can occur, as noted previously, in a small number of infected patients as well as decades after the time of infection.[31–34] Further complicating the design of these trials has been the lack of a

sufficiently specific diagnostic tool. The tuberculin skin test, the standard tool for diagnosing infection with *M. tuberculosis*, can be positive not only as a result of infection with *M. tuberculosis*, but also from bacillus Calmette–Guérin (BCG) vaccination or exposure to mycobacteria other than TB. New-generation diagnostics recently introduced and based on T cell responses to *M. tuberculosis*-specific antigens should be an improvement in this latter regard.[35–37] Another approach taken recently by some investigators to streamline the clinical evaluation of new regimens for the treatment of LTBI is to study populations at high risk of developing active disease, such as HIV-positive patients.[38–41] This approach limits the duration of follow-up needed to observe an adequate number of clinical events for achievement of statistical power.

7.24.6 Current Treatment

Currently, active TB is treated by combination therapies that consist of three or more drugs (four, most typically) selected from more than a dozen known anti-TB agents. Directly observed treatment, short course (DOTS), is considered the regimen and treatment approach of choice for active TB, and has been recommended and promulgated globally by the WHO. During the treatment, patients with active TB are typically administered isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months (the intensive phase), followed by isoniazid and rifampicin for an additional 4 months (the continuation phase). The initial intention of a combination therapy was to minimize the development of resistance to streptomycin after that drug was first introduced. More recently, it has come to be believed by many in the field that various drugs in the standard regimen act orchestrally against different populations of *M. tuberculosis*[42] Isoniazid, a cell wall synthesis inhibitor, kills actively growing bacteria rapidly, and plays a key role in eradicating the replicating population. Rifampicin, an inhibitor of RNA synthesis, is active against both replicating and slowly or nonreplicating bacteria. Pyrazinamide, presumably an inhibitor of proton motive force, appears to be active only under acidic conditions during the first 2 months of therapy. Rifampicin and pyrazinamide played a major role in shortening the duration of therapy, from more than 24 months to 6 months currently. It is reasonable to believe that the mechanism of action of each individual agent dictates the role of this agent in TB therapy. The more than one dozen anti-TB agents presently in the arsenal for the treatment and prevention of TB can be divided into six groups, based on their mechanisms of action, as show in Figure 1. The mechanisms of action for some agents are not totally defined, and therefore what is indicated in Figure 1 for these agents must be treated as hypothetical at this stage.

Streptomycin was initially used intramuscularly in combination with first-line drugs to treat active disease, but due to the inconvenience of parenteral delivery and concern about the transmission of HIV, it was later replaced by orally available ethambutol in the recommended regimen. LTBI is currently best treated by daily isoniazid for 9 months.



[a] Indicates a hypothetical mechanism

Figure 1 Schematic illustration of the sites of action for the available anti-TB agents.

Table 1 The current anti-TB agents, their available dose form, year of discovery, source, and mechanism of action

Agent	*Dose form*[a]	*Discovery*[b]	*Source*	*Mechanism*
Streptomycin	IM	1944	*Streptomyces griseus*	Inhibits ribosome/protein synthesis
p-Aminosalicyclic acid	PO	1946[d]	Synthetic	Inhibits folic acid synthesis and iron metabolism
Viomycin	IM	1951	*Streptomyces puniceus*	Inhibits ribosome/protein synthesis
Isoniazid[c]	PO/IM	1952[d]	Synthetic	Inhibits cell wall mycolic acid synthesis
Pyrazinamide[c]	PO	1952[d]	Synthetic	Inhibits proton motive force and/or cell wall synthesis
Cycloserin	PO	1955	*Streptomyces orchidaceus*	Inhibits cell wall peptidoglycan synthesis
Ethionamide[c]	PO	1956[d]	Synthetic	Inhibits cell wall mycolic acid synthesis
Prothionamide[c]	PO	1956[d]	Synthetic	Inhibits cell wall mycolic acid synthesis
Kanamycin	IM/IV	1957	*Streptomyces kanamyceticus*	Inhibits ribosome/protein synthesis
Ethambutol	PO	1961	Synthetic	Inhibits arabinosyl transferase/cell wall synthesis
Capreomycin	IM	1961	*Streptomyces capreolus*	Inhibits ribosome/protein synthesis
Rifampicin	PO/IV	1966	Semisynthetic	Inhibits RNA polymerase/RNA synthesis
Amikacin	IM/IV	1972	Semisynthetic	Inhibits ribosome/protein synthesis
Rifapentine	PO	1976	Semisynthetic	Inhibits RNA polymerase/RNA synthesis
Rifabutin	PO	1979	Semisynthetic	Inhibits RNA polymerase/RNA synthesis
Ofloxacin	PO/IV	1982	Synthetic	Inhibits DNA gyrase/translation and transcription
Ciprofloxacin	PO/IV	1983	Synthetic	Inhibits DNA gyrase/translation and transcription
Levofloxacin	PO/IV	1987	Synthetic	Inhibits DNA gyrase/translation and transcription

[a] IM, intramuscular; IV, intravascular; PO, peroral.
[b] See *Merck Index*, 13th edition.
[c] Parent compound is a prodrug.
[d] Listed as year of the first introduction for TB use.

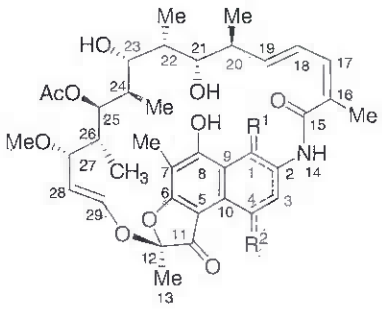
The remaining agents are used ad hoc in combinations to treat MDR-TB and infections that have failed to respond to first-line drugs. The treatment of MDR-TB infections typically requires at least 18 months of therapy. The majority of the known anti-TB agents were introduced during the antibiotic golden era between 1940 and 1960. The origins, available dose forms, years of discovery, and mechanisms of action of these anti-TB agents are summarized in Table 1.

7.24.6.1 Rifamycin Class

Rifamycins are broad-spectrum agents having a unique ansa structure (Figure 2). They were initially isolated from *Streptomyces mediterranei* in 1957.[43] The early members of the family are generally undesirable as therapeutic agents, due primarily to poor potency, low solubility, poor bioavailability, or short half-life. Structural modification of the natural rifamycins led to several derivatives that are highly potent and orally available. Currently, three semisynthetic compounds, rifampicin, rifapentine, and rifabutin, are in clinical use. Rifampicin has become the cornerstone of the current therapy, mainly responsible for reducing the treatment duration from 12 months to the current 6 months. The newer members, rifapentine and rifabutin, have demonstrated some advantages over rifampicin, including a longer half-life, a reduced potential for drug–drug interactions, and/or activity against some rifampicin-resistant strains.

7.24.6.1.1 Sites and mechanisms of action

Rifamycins are potent RNA polymerase (RNAP) inhibitors. The detailed interactions between rifampicin and RNAP have been elucidated by high-resolution crystal structure studies of the *Thermus aquaticus* core enzyme complexed with rifampicin.[44] Rifampicin binds to a deep pocket of the β subunit within the RNA channel that is about 12 Å away from the active center. The drug works by blocking the path of the elongating RNA when the transcript reaches two to three



	R^1	R^2
Rifamycin B	–OH	$-OCH_2COOH$
Rifamycin O	=O	=(1,3-dioxolan-4-on)-2-yl
Rifamycin S	=O	=O
Rifamycin SV	–OH	–OH

Figure 2 Structures of some early naturally occurring rifamycins and the rifamycin numbering system.

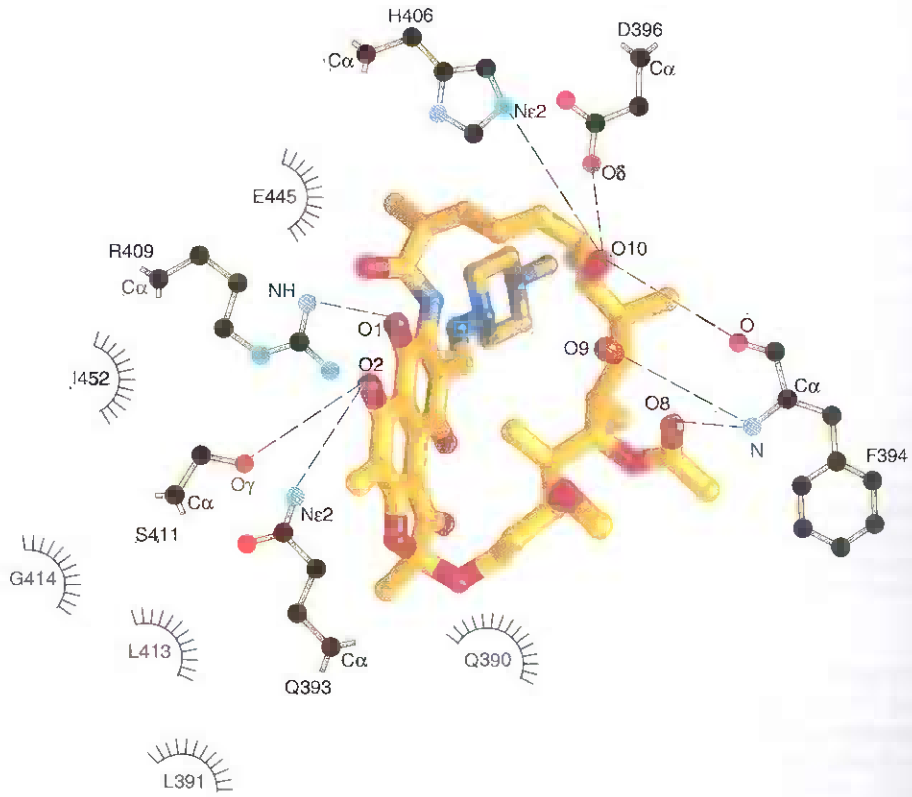


Figure 3 Detailed interactions of rifampicin and RNAP from *Thermus aquaticus*. (Reproduced from Campbell, E. A.; Korzheva, N.; Mustaev, A.; Murakami, K.; Nair, S.; Goldfarb, A.; Darst, S. A. *Cell* **2001**, *104*, 901–912, Copyright (2001), with permission from Elsevier.)

nucleotides in length. Resistance to rifamycins can occur frequently, and is mainly due to point mutations in the rifamycin-binding region of the β subunit of RNAP. A single-step mutation of one of the key residues in the binding region generally leads to high-level resistance (**Figure 3**). The most commonly observed mutations among *M. tuberculosis* clinical isolates are Q432, F433, H445, S450, and L452 residues (corresponding to Q393, F394, H406, S411, and L413 positions of *Thermus aquaticus*, respectively). The S450 and H445 mutations lead to high-level rifampicin resistance.[45]

7.24.6.1.2 Structure–activity relationships

Chemical modifications of the natural products have produced several clinically important semisynthetic rifamycins with improved pharmacological profiles. The SARs accumulated to date are highlighted in **Figure 4**.[46]

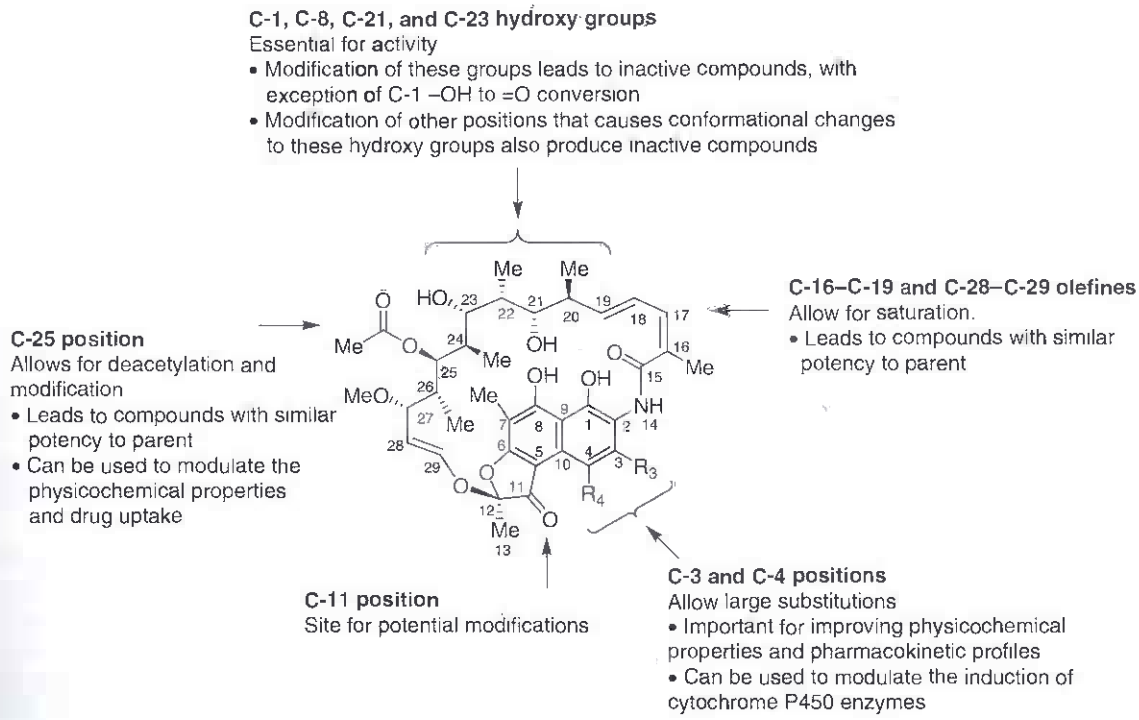


Figure 4 Important SARs of the rifamycin class.

The four hydroxy groups located at the C-1, C-8, C-21, and C-23 positions of the rifamycin scaffold are essential for antibacterial activity. Any modifications to these hydroxy groups, with the exception of the C-1 –OH to =O conversion, led to compounds with reduced activity. Other modifications that changed the conformation of these hydroxy groups also led to inactive compounds. Based on the co-crystal structure of rifampicin–RNAP, these hydroxy groups directly interact with RNAP through hydrogen bonding. Any modification that interferes with such interactions would lead to compounds with reduced binding affinity.

Saturation of one or more of the double bonds located at the C-16/C-17, C-18/C-19, and C-28/C-29 positions generally leads to compounds with equivalent or slightly reduced activity compared with their parent compounds. These modifications are not significant enough to have any major impact on potency, nor provide any advantage over the parent compounds. The C-25 deacetylation products are often observed as the metabolites of rifamycins. These C-25 hydroxy metabolites are generally as potent as their parent. Other modifications of the acetyl group also led to potent compounds with different physicochemical properties. However, any modifications to this position could be 'temporary' because the ester groups attached to this position are readily cleaved in vivo. The liability of the C-25 ester linkage has been exploited as an advantage to develop a rifamycin-based 'Trojan Horse' that brings other impermeable drug molecules into the cells.[47] Siderophore scaffolds were also attached to this position, and successfully transport rifamycins into Gram-negative bacteria.[48]

The most important rifamycin derivatives are those with modifications at the C-3 and C-4 positions. Synthetically, these positions are very accessible and easy to modify. Structurally, these positions point to the open space of the RNAP-binding pocket, and allow for significant modifications. Modification at the C-3 and C-4 positions often yields compounds with significantly improved physicochemical properties and pharmacokinetic profiles.[49] Structural modification of 3-formyl rifamycin SV led to rifampicin, a more potent and orally active compound that has become the cornerstone of modern anti-TB therapy. Further modification of the rifampicin series produced rifapentine, a cyclopentyl analog of rifampicin. Rifapentine has a longer half-life than rifamycin, and therefore can be used as intermittent therapy. Rifabutin was produced by bridging the C-3 and C-4 positions, to form a spiro structure. This molecule has the advantages relative to rifampicin of improved tissue penetration and reduced cytochrome P450 enzyme induction. Rifalazil is a newer member of the rifamycin family currently in clinical development for other indications. This compound has a benzoxazino group fused to the C3 and C4 positions, and has a long half-life of 61 h, no substantial cytochrome P450 induction, and activity against certain rifampicin-resistant bacteria.[50]

The co-crystal structure of rifampicin–RNAP provides some insights into potential positions for future modification.[44] The C-11 carbonyl group points to the open space of the rifampicin-binding pocket, and is a potential site for modulating physicochemical properties and pharmacokinetics of the drug class.

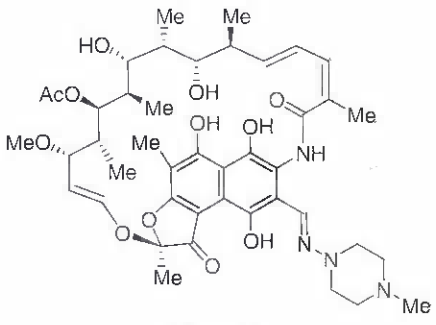
7.24.6.1.3 Comparisons of available agents within the class

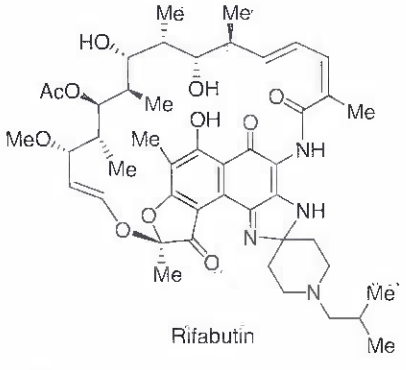
Currently, four agents in the rifamycin class, rifampicin, rifapentine, rifabutin, and rifalazil (Figure 5), are in clinical use or in clinical development. Despite the structural similarities, these agents possess very distinct characteristics. The main advantages and disadvantages of these analogs are compared in Table 2.

7.24.6.1.4 Limitations and future directions

Rifamycins are one of the few drug classes active against bacteria in both replicating and nonreplicating states, and are mainly responsible for shortening TB treatment from over 12 months to 6 months. However, the current members of the rifamycin family are optimized for activity against bacteria in the replicating state, which is not a good predictor of their efficacy for shortening therapy. By utilizing proper assays against bacteria in the drug-persistent state, it is anticipated that a better rifamycin with optimized activity against persistent *M. tuberculosis* and greater potential for shortening therapy could be developed.

In addition to several of the intrinsic safety issues associated with the rifamycin class, two specific limitations are particularly important for modern TB therapy. The first limitation is the high prevalence of rifampicin resistance among clinical isolates of *M. tuberculosis*. Although rifabutin and rifalazil are active against some rifampicin-resistant *M. tuberculosis*, cross-resistance is expected. New agents that overcome rifampicin resistance would be advantageous. Second, rifampicin is a strong inducer of several cytochrome P450 enzymes, including CYP3A4, which leads to drug–drug interactions when co-administered with antiretroviral therapy, particularly HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors. This causes difficulties for the effective management of TB-HIV co-infections. Some progress has been made in this area, with the introduction of rifabutin and rifalazil.[51] New agents that are free of drug–drug interactions would be highly desirable.





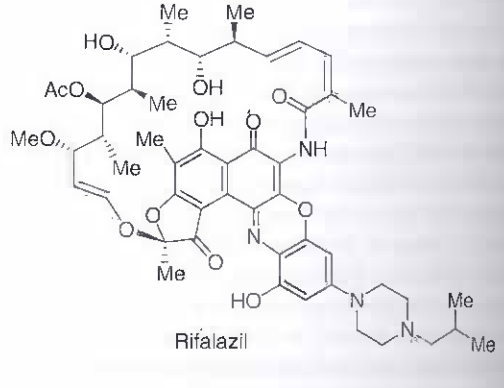


Figure 5 Structures of rifampicin, rifapentine, rifabutin, and rifalazil.

Table 2 Comparisons of rifampicin, rifapentine, rifabutin, and rifalazil as anti-TB agents

Compound (introduced)	*Key advantage(s)*	*Key disadvantage(s)*	*Current/potential use*
Rifampicin (1966)	Mainly responsible for shortening TB therapy from 12 months to 6 months	Drug–drug interactions, and drug resistance	Key component of the first-line regimen
Rifapentine (1976)	Longer half-life than rifampicin, slightly reduced cytochrome P450 induction	Cross-resistance with rifampicin, drug–drug interaction, food effects	Potential agent for intermittent therapy
Rifabutin (1979)	Higher tissue level, reduced cytochrome P450 induction and active against some rifampicin-resistant strains	Partial cross-resistance with rifampicin, low oral availability	Treatment of MAC. Potential use in TB-HIV co-infections
Rifalazil (1990)	Highly potent, no cytochrome P450 induction, longer half-life than rifapentine, active against some rifampicin-resistant strains	Partial cross-resistance with rifampicin, skin discoloration, flu-like symptoms	Potential use for intermittent therapy and TB-HIV co-infections

7.24.6.2 Isoniazid and Related Compounds

Isoniazid, also called isonicotinic acid hydrazide, is a synthetic compound first prepared in 1912. The anti-TB activity of this compound was first reported in 1952 during the search for more potent derivatives of nicotinamide, an early lead against TB. Subsequent studies also identified ethionamide and prothionamide as having potent anti-TB activities.

Isoniazid is one of the most widely used anti-TB agents, and one of the key components of first-line therapy for active disease. A 9-month isoniazid monotherapy is used for the treatment of latent infections. Isoniazid is highly effective against replicating *M. tuberculosis*, and is mainly responsible for the early reduction of the bacterial load in the initial phase of therapy. Ethionamide and prothionamide have limited applications, and are mainly used as second-line agents to treat patients who have failed to respond to first-line therapy.

7.24.6.2.1 Sites and mechanisms of action

Isoniazid, ethionamide, and prothionamide are all prodrugs. Isoniazid is activated by a catalase peroxidase enzyme (KatG). Mutations in KatG account for the majority of isoniazid resistance. The active species of isoniazid appears to have multiple cellular targets.[52] The primary target of isoniazid is believed to be InhA, a NADH-dependent enoyl acyl carrier protein reductase involved in the synthesis of mycolic acid.[53,54] The activated species, presumably an isonicotinic acyl radical, forms an adduct with the NAD radical. The resulting isonicotinic acyl-NADH adduct (INA) binds to InhA, and inhibits the synthesis of mycolic acid, an essential cell wall component of *M. tuberculosis* (Figure 6).[55]

The crystal structure formed between InhA isolated from *M. tuberculosis* and INA indicated that INA binds to InhA in a competitive fashion with NADH (Figure 7), and mutations within the NADH-binding region of InhA confer isoniazid resistance among clinical isolates.

The primary target of ethionamide is also believed to be InhA. However, this agent is activated by a different enzyme, EthA. Prothionamide is presumed to have the same mechanism of action as ethionamide due to their structural similarity. Resistance to isoniazid, ethionamide, and prothionamide is mainly due to either mutation of the activation enzymes (KatG or EthA) or their molecular target (InhA).[56] Therefore, cross-resistance between isoniazid and ethionamide/prothionamide is expected, but not common.

7.24.6.2.2 Structure–activity relationships

The discovery of isoniazid was a serendipitous event. Isoniazid was identified by pursuing derivatives of nicotinamide, an earlier anti-TB lead. Optimization of nicotinamide directed by in vitro and in vivo anti-TB assays produced two distinct drugs with very different characteristics (Figure 8), isoniazid and pyrazinamide.[57] While pyrazinamide exhibits a similar biological profile and shows cross-resistance with nicotinamide, isoniazid is biologically and mechanistically distinct from its parent. The discovery of isoniazid and pyrazinamide fully illustrated the advantages and potential risks

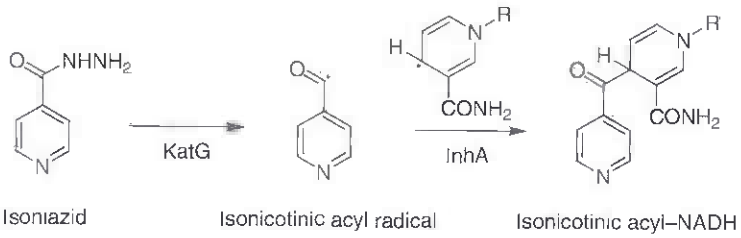


Figure 6 Activation of isoniazid and formation of isonicotinic acyl–NADH

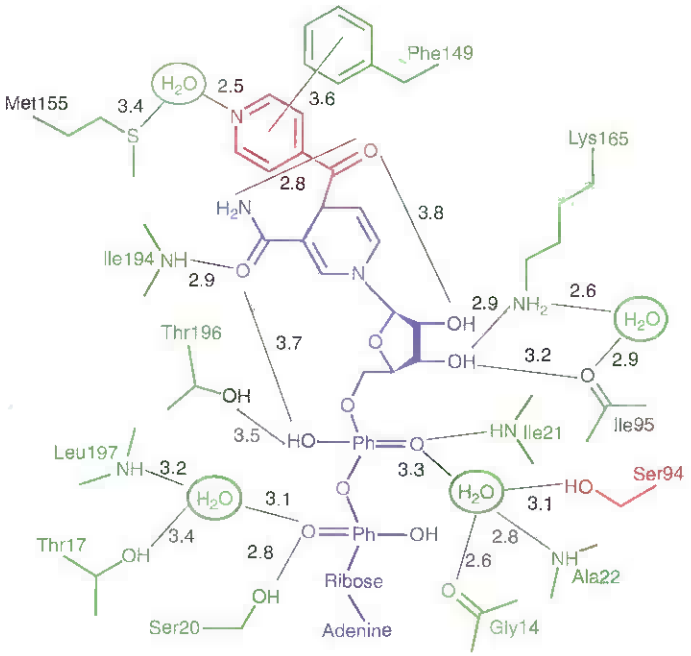


Figure 7 Structures of INA and the active site of InhA reveal key interactions based on the x-ray crystal structure (distances in angstroms). (Reprinted with permission from Rozwarski, D. A.; Grant, G. A.; Barton, D. H. R.; Jacobs, W. R.; Sacchettini, J. C. *Science* **1998**, *279*, 98–102. Copyright 1998 AAAS.)

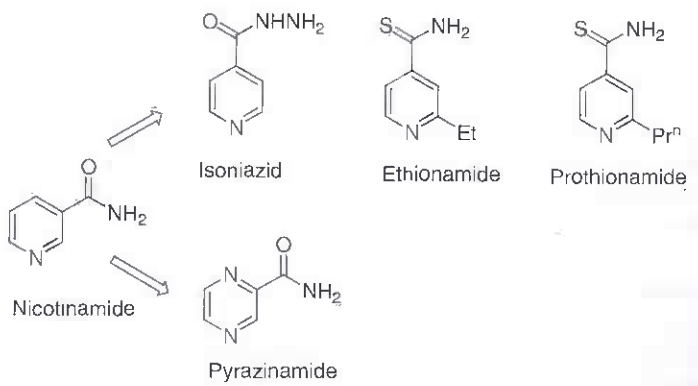


Figure 8 Structures of the early lead nicotinamide and the follow-up compounds isoniazid, ethionamide, prothionamide and pyrazinamide

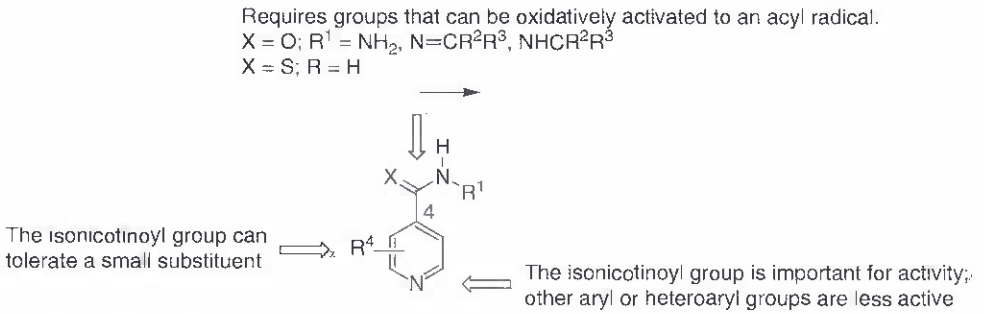


Figure 9 Highlights of the SARs of the isoniazid series.

associated with the whole-cell-based approach for lead optimization. Lead optimization solely directed by whole-cell activity can lead to compounds with 'off-target' mechanisms. In most cases, the off-target activities are nonselective and unwanted. However, in certain instances, the off-target activities can lead to serendipitous discovery of useful drugs, such as isoniazid.

Most of the medicinal chemistry on the isoniazid series was completed in the 1950s. There are two independent tracks of SARs for this drug series, one governing the activation of the prodrug and another governing the interaction of the active species with InhA. Many groups at the 4-position can be activated inside *M. tuberculosis*, and are possible prodrug structures (Figure 9).[58] After activation, the structural requirements for InhA binding appear to be very stringent. Only the isonicotinoyl core structure is substantially active among many aryl and heteroaryl groups explored.[59] The isonicotinoyl group could be substituted with lower alkyl groups while maintaining excellent activity.

7.24.6.2.3 Comparisons of available agents within the class

As a first-line drug, isoniazid is by far the most commonly used agent within the class. Due to its relatively good efficacy and safety, isoniazid has become one of the key components of modern anti-TB therapy. Ethionamide and prothionamide are mainly used as second-line drugs to treat MDR-TB and patients who cannot tolerate first-line agents. Recently, isoniazid resistance has become a major problem in many parts of the world.[8] The majority of isoniazid-resistant clinical isolates have mutations in the activating enzyme, KatG. Since ethionamide and prothionamide are activated by a different enzyme, EthA, these agents show little cross-resistance with isoniazid. Both ethionamide and prothionamide are less tolerable compared with isoniazid.[60]

7.24.6.2.4 Limitations and future directions

There are two main limitations associated with the isoniazid class: the high prevalence of drug resistance and the low efficacy against drug-persistent bacilli. In addition, the toxicity of this drug class, particularly ethionamide and prothionamide, is a significant concern.[52]

Recent work in this area mainly focused on overcoming isoniazid resistance. A new agent that directly targets InhA without the need for KatG or EthA activation could potentially be effective against the majority of isoniazid-resistant strains. InhA inhibitors that bind to a different binding site than isoniazid may be able to overcome the remaining cause of resistance – InhA mutations.

A new member of the isoniazid family that is effective against isoniazid-resistant strains and is safer than isoniazid will likely find utility in the treatment of MDR-TB. However, it is unclear whether an agent that targets cell wall biosynthesis can play a major role in shortening therapy. The definite answer to this question will not be available, of course, until such an InhA inhibitor is identified and its efficacy against persistent *M. tuberculosis* is tested in in vitro and in vivo models, and eventually by clinical trials.

7.24.6.3 Pyrazinamide and Related Compounds

Pyrazinamide is a close analog of nicotinamide, and shares the same root with isoniazid (see Figure 8). Pyrazinamide is another first-line agent, and one of the two agents that played a significant role in shortening the duration of therapy from 12 months to 6 months.[61] Pyrazinamide appears to be active only under acidic conditions. The drug is more efficacious in vivo than would be predicted by its in vitro potency.

7.24.6.3.1 Sites and mechanisms of action

Pyrazinamide is a prodrug that requires activation by pyrazinamidase (PZase). The activation product is pyrazinoic acid, which is believed to be the active species. Mutations in PZase lead to loss of PZase activity, and are mainly responsible for the development of pyrazinamide resistance. The same mutations show cross-resistance to nicotinamide, suggesting that pyrazinamide and nicotinamide are activated by the same enzyme. Pyrazinoic acid, the active form of pyrazinamide, remains sensitive to these mutants. No pyrazinoic acid-resistant mutants have been identified, and the exact target of pyrazinoic acid is unclear. Although evidence based on an experiment done with 5-chloropyrazinamide, a close analog of pyrazinamide, on *M. smegmatis* suggested that fatty acid biosynthesis I (FAS-I) is the potential target of pyrazinoic acid,[62] this hypothesis was later questioned.[63] Recently, a new mechanism of action for pyrazinamide was proposed, suggesting that disruption of the proton motive force and energy production is the basis of its antibacterial activity.[64] According to this hypothesis, pyrazinoic anion, under acidic conditions, serves as a proton carrier that transports protons from the outer membrane to the intracellular space. This process effectively depletes the proton motive force and impacts energy production. This hypothesis helps to explain some of the unusual properties of pyrazinamide, including its requirement for acidic conditions and its activity against persistent bacilli.

7.24.6.3.2 Structure–activity relationships

Optimization of pyrazinamide was performed mainly in the 1950s as a follow-up to work on isonicotinamide. The SARs of this series have been extremely hard to elucidate since the series is essentially inactive under normal culture conditions. Even under acidic conditions at pH 5.5, the potency of pyrazinamide is in the range of 16 $\mu g\,mL^{-1}$ or higher. Therefore, the SARs for pyrazinamide are mainly derived through in vivo animal studies, and the data that are available for SAR analysis are very limited. One should take extra precautions when performing a SAR analysis for a drug series whose mechanism of action is not well defined. As an example, 5-chloropyrazinamide is a structural analog of pyrazinamide, but appears to have a different mechanism of action.[63] Therefore, 5-cloropyrazinamide should not be included in the SAR analysis of the pyrazinamide series. Instead, this compound could serve as a potential lead for a new series.

As prodrugs, the pyrazinamide series also follows two independent SAR tracks, one for PZase activation and another for target interaction by the resulting acid (Figure 10). The R^1 group serves two important functions. First, the active species, pyrazinoic acid, is usually in its anionic form at physiological pH, and is not permeable or bioavailable. The R^1 group masks the acid group, and allows the drug to pass through cell membranes. Second, the killing effect of the active species is unlikely to be a selective process. The selective removal of the R^1 group by *M. tuberculosis* and other bacterial species provides the desired selectivity. In principle, any structures that can be selectively removed by mycobacteria would be a good choice for the R^1 group. Esters, in addition to amides, have therefore been explored as prodrugs.[65] The esters appeared to have a broader spectrum of activity than amides, and did not show cross-resistance with the corresponding amide, indicating that they are activated by different enzymes.[66] Information regarding the structural requirements for the aromatic group is limited, and most of the relevant work was performed many years ago, in the 1950s. In general, structures that lead to acids with a relatively lower pK_a are more active than those that produce acids with a higher pK_a. Thus, pyrazinoic acid has better activity than nicotinic acid. The pK_a of the active species can be influenced by both the structures of the aromatic system and its substitution.

7.24.6.3.3 Comparisons of available agents within the class

Pyrazinamide is the only agent currently used clinically for the treatment of TB in this class. The initial lead, nicotinamide, was abandoned before reaching clinical development due to potential antagonism with isoniazid. This

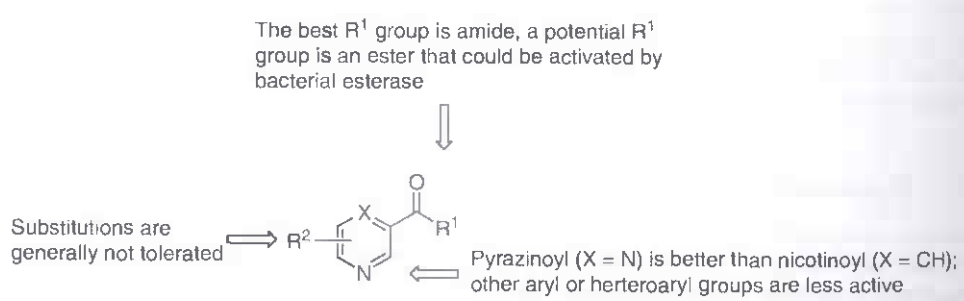


Figure 10 Highlights of the available SARs for the pyrazinamide series.

compound resurfaced recently as a potential agent that might have dual activities against both TB and HIV.[57] Pyrazinamide is more active both in vitro and in vivo than nicotinamide against *M. tuberculosis*. In addition, nicotinamide appears to show antagonism with isoniziad, while pyrazinamide does not.

7.24.6.3.4 Limitations and future directions

The most obvious limitation of pyrazinamide is, in addition to its marginal safety profile, its narrow window of activity. This agent is active only during the first 2 months of treatment and under acidic conditions. No evidence suggests that pyrizinamide has any therapeutic benefit during the continuation phase of treatment after the initial 2 months of therapy. Presumably, because pyrazinamide is only active under acidic conditions, it plays a limited role in preventing the development of resistance to co-administered agents.[67]

Due to its unique mechanism, pyrazinamide may serve as a useful tool for understanding the biology of persistence. Conversely, a better understanding of the mechanism of action of this agent may lead to new strategies to overcome persistence.

To overcome the limitations of pyrazinamide, a safer compound active against both replicating and nonreplicating bacterial populations is highly desirable. One can envision that a pyrazinamide analog with a dual mechanism of action could achieve this goal. 5-Chloropyrazinamide, a compound that appears to target both FAS-I and the proton motive force, might serve as one interesting lead.[68]

7.24.6.4 Aminoglycosides and Polypeptides

Aminoglycosides are widely used antibiotics with broad-spectrum activity. The first agent in the class, streptomycin, was isolated in the 1940s from *Streptomyces griseus*. Many additional aminoglycosides were later isolated or synthesized. Currently, three agents in the class, streptomycin, kanamycin, and amikacin, are used for the treatment of TB (**Figure 11**). Among these agents, streptomycin and kanamycin are natural products, and amikacin is a semisynthetic compound derived from kanamycin. In 1948, the first recorded, randomized, placebo-controlled trial was conducted, its purpose being to evaluate the efficacy of streptomycin as an antitubercular agent.[42] Aminoglycosides are not orally active, and have limited intracellular activity. In addition, nephrotoxicity, ototoxicity, and other adverse reactions limit their use. Aminoglycosides are used widely for the treatment of other infections, as discussed in other chapters. In this section, we will focus on the anti-TB applications of this drug class.

Polypeptides, capreomycin, and viomycin are structurally related compounds that possess similar mechanisms of action, pharmacokinetic, potency and toxicity profiles, to aminoglycosides (**Figure 12**). Capreomycin was isolated from *Streptomyces capreolus* in 1961, and viomycin, which is produced by various *Streptomyces* species, was first reported in 1951. Both compounds are parenteral agents, and possess significant nephrotoxicity and ototoxicity.

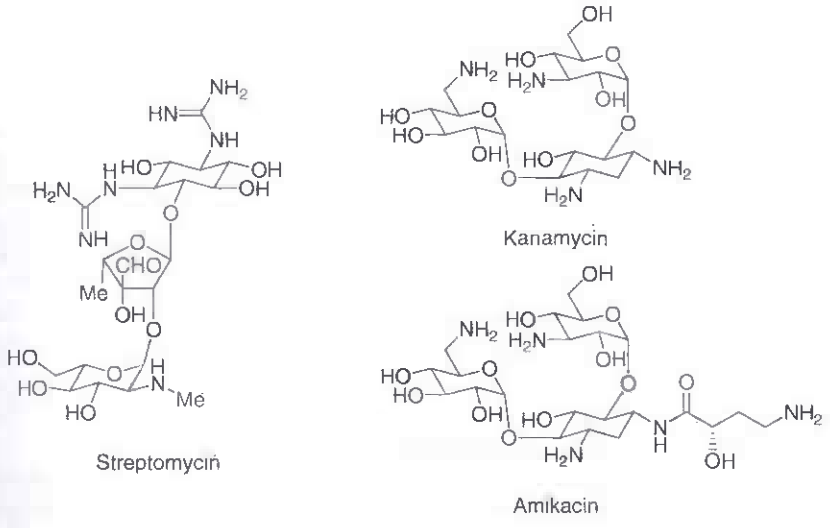
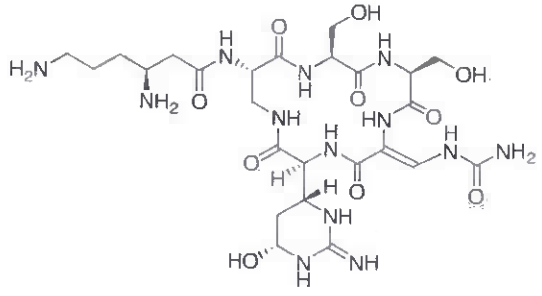
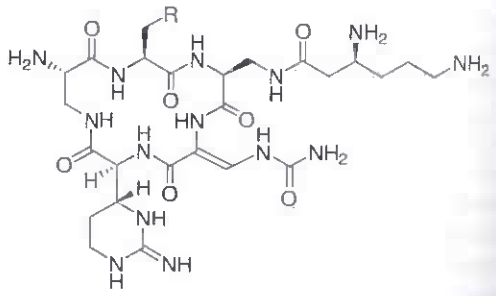


Figure 11 Structures of aminoglycosides that have been used for the treatment of TB. Amikacin is a semisynthetic compound, derived from kanamycin.



Viomycin



Capreomycin 1A: R = OH
Capreomycin 1B: R = H

Figure 12 Polypeptide anti-TB agents.

7.24.6.4.1 Sites and mechanisms of action

At physiological pH, aminoglycosides are charged molecules. They have limited permeability across the lipid bilayers of cellular membranes. Aminoglycosides are transported into the cell by an energy-dependent drug transport system that utilizes the proton gradient as its driving force.[69] The proton gradient decreases in an anaerobic environment, and, as a consequence, the uptake of aminoglycosides is reduced, leading to a reduction in antibacterial activity.[70] The aminoglycoside class, therefore, may have limited activity against drug-persistent cell populations. Once across the membrane, the drug is trapped and accumulates inside the bacteria, resulting in bactericidal events. Aminoglycosides inhibit protein synthesis by binding to the small subunit (30S) of the bacterial ribosome. High-resolution crystal structures of two aminoglycosides, streptomycin and paromomycin, complexed with the 30S ribosomal subunit from *Thermus thermophilus*, have been elucidated.[71] Based on the crystal structure, aminoglycosides bind to an area adjacent to the decoding site in the 30S subunit of the ribosome, and cause decoding errors. Streptomycin binds to four nucleotides of 16S ribosomal RNA and a single amino acid of ribosomal protein S12. Paromomycin appears to interact with a slightly different binding site in the same general region. Mutations of the S12 protein and 16S ribosomal RNA account for the majority of aminoglycoside resistance in *M. tuberculosis*. Interestingly, aminoglycoside-modifying enzymes commonly found in other bacterial species have not been found in *M. tuberculosis*.

Capreomycin and viomycin are also protein synthesis inhibitors, and appear to bind at the interface between the 30S and 50S subunits.[72] Viomycin and capreomycin resistance are largely due to methylation or mutation of ribosomes. Amikacin, kanamycin, capreomycin, and viomycin do not generally exhibit cross-resistance with streptomycin, and are currently being used for the treatment of MDR-TB.[73] There is significant genotypic overlap among the mutations responsible for resistance to amikacin, kanamycin, capreomycin, and viomycin.[74] Cross-resistance among them is, therefore expected and is commonly observed.

7.24.6.4.2 Structure–activity relationships

Currently, there are approximately a dozen naturally occurring and semisynthetic aminoglycosides available for the treatment of various bacterial infections. Among them, streptomycin possesses a unique streptidine group; all other aminoglycosides possess a 2-deoxystreptamine moiety. The SARs for this drug class are insufficiently defined, particularly against *M. tuberculosis*. As suggested by the cross-resistance data and the crystal structures of ribosomes and aminoglycosides, each aminoglycoside appears to have a slightly different binding site and mechanism of action. The promiscuous binding of this drug class has increased the difficulty of developing generalized SARs. Among all the aminoglycosides, amikacin is slightly more potent against *M. tuberculosis* than streptomycin and kanamycin. Other members of the class appear to have insufficient activity against *M. tuberculosis*. Knowledge of the SARs for the polypeptide family is totally lacking.

7.24.6.4.3 Comparisons of available agents within the class

All anti-TB agents within the aminoglycoside and polypeptide families have similar potency, pharmacokinetic and toxicity profiles, with little variation between them. Streptomycin is used as a first-line agent, and resistance to it is common among clinical isolates. Streptomycin is the least nephrotoxic aminoglycoside; however, it is highly ototoxic. Cross-resistance between streptomycin and other aminoglycosides and polypeptides is not common, and, therefore,

kanamycin, amikicin, capreomycin, and viomycin can be used as second-line agents to treat MDR-TB. One of the major differences between capreomycin and aminoglycosides is their anaerobic activity against *M. tuberculosis*. Capreomycin is active against *M. tuberculosis* under anaerobic conditions, while aminoglycosides show limited activity against this cell population.[75] Differences in drug transport may explain this difference – aminoglycosides require active uptake, which slows down substantially under anaerobic conditions, while capreomycin may be transported via a different mechanism that is still active under these conditions.

7.24.6.4.4 Limitations and future directions

There are a number of limitations for the aminoglycoside and polypeptide classes. Lack of oral availability has limited the use of these agents. Nephrotoxicity and ototoxicity are significant among members of these classes, and extra care must be taken when these drugs are administered. In addition, resistance to streptomycin is common, and further limits its use. Cross-resistance between the second-line aminoglycosides and polypeptides is common, and combinations of these agents are not recommended.

Aminoglycosides also lack activity against intracellular mycobacteria and mycobacteria in their nonreplicating state. Therefore, these agents have little role in eradicating mycobacteria after the initial phase of treatment and in shortening the duration of therapy. In this regard, the anaerobic activity of capreomycin is interesting and worth further investigation. Understanding the mechanism of the anaerobic activity of capreomycin may give us some indication how to further improve this important property.

7.24.6.5 Quinolones

Quinolones belong to one of the few classes of antimicrobial agents that are totally synthetic in origin.[76] The first quinolone, nalidixic acid, was introduced in the 1960s, and is a narrow-spectrum agent against Gram-negative organisms. The coverage of this drug class was expanded significantly by the introduction of fluoroquinolones, as second-generation agents, in the 1980s. Certain second-generation fluoroquinolones, such as ciprofloxacin and ofloxacin, are active against *M. tuberculosis*, and widely used for the treatment of MDR-TB. The anti-TB activity of the quinolone class was further improved by the introduction of third-generation agents, exemplified by moxifloxacin and gatifloxacin. The third-generation quinolones demonstrate potent sterilizing activity against *M. tuberculosis* in both the replicating and nonreplicating states. Both moxifloxacin and gatifloxacin are currently being evaluated in clinical trials as potential therapies to treat TB. A brief history of the quinolone class is illustrated in **Figure 13**.

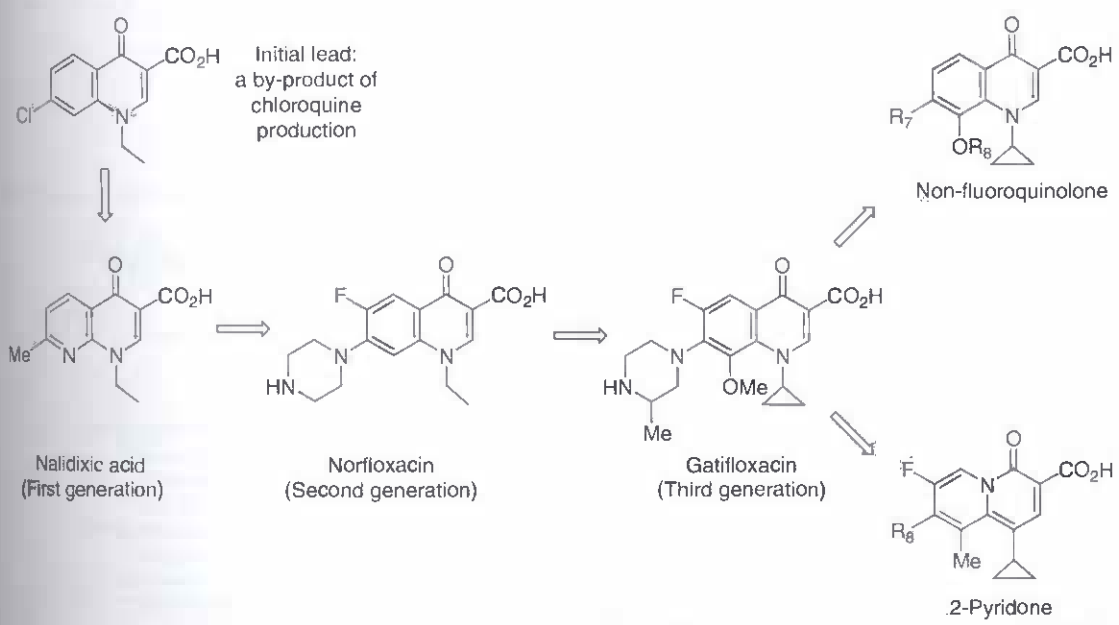


Figure 13 A summary of the quinolone class.

The safety of this drug class for long-term use has not been well defined. In addition, quinolone resistance has become a serious problem among many pathogens, such as *Escherichia coli* and *Staphylococcus aureus*, and there is concern whether relatively long-duration treatment with quinolones for TB will lead to development of resistance in commensal organisms. If quinolones become commonly used for TB, it will be important to monitor and evaluate the potential for such resistance to become a clinical problem.

7.24.6.5.1 Sites and mechanisms of action

The molecular targets of the quinolone class are DNA topoisomerases, both topoisomerase II, also known as DNA gyrase, and topoisomerase IV. DNA gyrase is essential for DNA replication, transcription, and repair, and topoisomerase IV is involved in the partitioning of chromosomal DNA during cell division. Therefore, DNA gyrase is thought to be a more important target during the nonreplicating state. Quinolones are dual-action agents against organisms that require both DNA gyrase and topoisomerase IV for viability. In *M. tuberculosis*, DNA gyrase appears to be the only type II topoisomerase present, based on genetic studies, and is likely the sole target for the quinolone class.[77] Because of the difference in molecular targets, as well as cell wall structures, quinolones (Figure 14) exhibit different SARs against *M. tuberculosis* compared with other organisms (Table 3).[78]

Quinolone resistance among *M. tuberculosis* strains is not common, and is relatively less well defined. The major resistance mechanisms, as in other bacterial species, are DNA gyrase mutations and drug efflux.

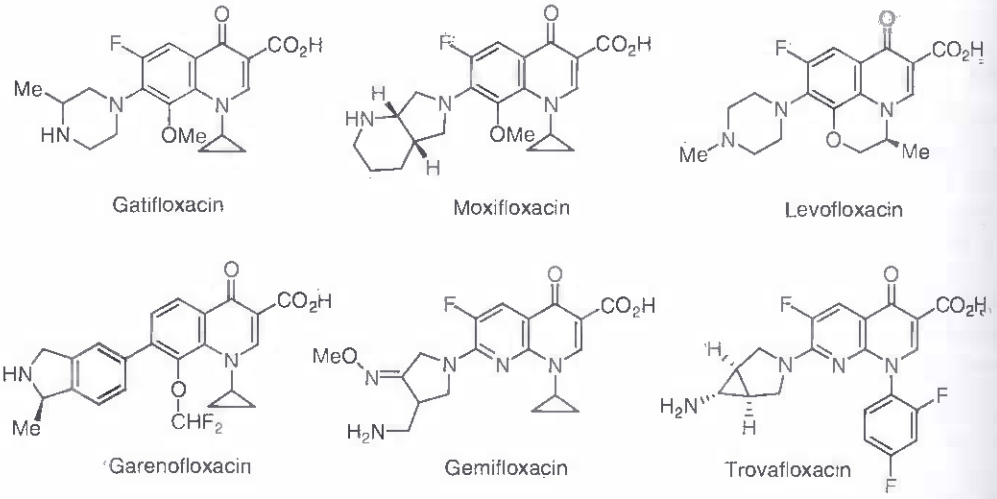


Figure 14 Structures of selected reference quinolones.

Table 3 In vitro activities of selected quinolones against *S. aureus*, *E. coli*, and *M. tuberculosis* and inhibitory (IC_{50}) and DNA cleavage (CC_{50}) activities against *M. tuberculosis* DNA gyrase

Quinolones	*S. aureus*	*E. coli*	*M. tuberculosis*	*M. tuberculosis DNA gyrase*	
	MIC ($\mu g\,mL^{-1}$)	*MIC* ($\mu g\,mL^{-1}$)	*MIC* ($\mu g\,mL^{-1}$)	IC_{50} ($\mu g\,mL^{-1}$)[a]	CC_{50} ($\mu g\,mL^{-1}$)[b]
Gatifloxacin	0.05	0.02	0.12	3	4
Moxifloxacin	0.03	0.015	0.5	4.5	4
Levofloxacin	0.12	0.008	0.5	5	12
Garenofloxacin	0.01	0.015	2	13	15
Gemifloxacin	0.01	0.13	4	11	6
Trovafloxacin	0.03	0.02	16	15	25

[a] IC_{50}: drug concentration that inhibits DNA supercoiling by 50% compared with no drug control.
[b] CC_{50}: drug concentration that induces 50% of the maximum DNA cleavage.

7.24.6.5.2 Structure–activity relationships

Quinolones have been extensively optimized against many common pathogens, and a significant amount of SAR knowledge against these pathogens is available.[76] The general SARs and structure–toxicity relationships (STRs) of the quinolone class are summarized in Figure 15.[79]

The SARs against mycobacteria are much less well defined, and most of these studies are primarily focused on activity against *M. avium*, *M. fortuitum*, or *M. smegmatis*. Positions that have significant impact on antimycobacterial activity are the N-1, C-7, and C-8 positions. At the N-1 position, the order of potency from high to low is *t*-butyl, cyclopropyl, 2,4-difluorophenyl, ethyl/cyclobutyl, and isopropyl. At the C-7 position, piperizine and pyrrolidine appear to have similar activity.[80] The contribution of the C-8 group is dependent on the structure at the N-1 position. When the N-1 substituent is a cyclopropyl group, the order of potency for the C-8 group from high to low is C–OMe, C–Br, C–Cl, C–F/C–H/C–OEt, N, and C–CF3. When the N-1 group is *t*-butyl, N is better than C–H.[81] For *M. tuberculosis*, the SAR information is extremely limited, but indicates that the potency of the quinolone class against *M. tuberculosis* is mainly driven by DNA gyrase interactions, as evidenced by a good correlation between DNA gyrase inhibitory activity and MICs.[78,82,83] Similarly to other antimycobacterial SARs, structures at the N-1, C-7, and C-8 positions are important for anti-TB activity. A potent anti-*M. tuberculosis* quinolone generally has the following structural characteristics: a cyclopropyl group at the N-1 position, a pyrrolidine or piperazine group at the C-7 position, and an F, Cl, or OMe group at the C-8 position.

7.24.6.5.3 Comparisons of available agents within the class

The second generation of quinolones, ciprofloxacin, ofloxacin, and levofloxacin, are currently in use as second-line TB drugs. These agents are slightly less active than rifampicin and isoniazid against *M. tuberculosis*. Resistance to fluoroquinolones is rare among clinical isolates of *M. tuberculosis*, so these agents are often useful for the treatment of MDR-TB.

Third-generation fluoroquinolones, moxifloxacin and gatifloxacin, are more potent than the second-generation agents. As more potent DNA gyrase inhibitors, moxifloxacin and gatifloxacin demonstrate significantly better sterilizing activity against persistent and rifampicin-tolerant *M. tuberculosis*.[84] Preclinical and limited clinical data indicate that regimens containing these agents could potentially shorten the current 6-month therapy.[85–87] Currently, clinical studies are ongoing to evaluate the potential of moxifloxacin and gatifloxacin as components of first-line anti-TB therapy.

7.24.6.5.4 Limitations and future directions

Despite widespread use of quinolones in second-line treatment of TB, none of the available agents are truly optimized for activity against *M. tuberculosis*. The SAR divergence against *M. tuberculosis* and other pathogens (Table 3) illustrates

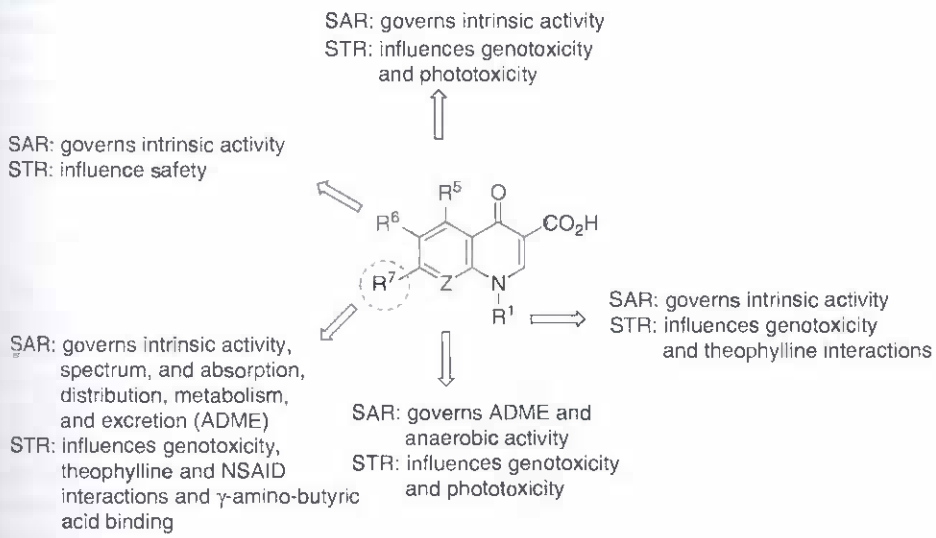


Figure 15 Highlights of the SAR and STR relationships for the quinolone class.[79]

the need for a dedicated lead optimization program focusing on activity against *M. tuberculosis*. Quinolones optimized against *M. tuberculosis* would hold great potential for shortening the duration of therapy. For the treatment of TB, quinolones are generally given for a long period and in combination with other drugs. The long-term safety and potential for drug–drug interactions of this class have not been well characterized. Development of resistance to quinolones is a concern, as discussed in Section 7.24.6.5 above, due to the high incidence of quinolone resistance among many other pathogens, although the frequency in *M. tuberculosis* is still quite low.

There are two new series of DNA gyrase inhibitors on the horizon: non-fluoroquinolones (NFQs) and 2-pyridones or quinolizinones. Members of the NFQ series were advanced to clinical development recently for other indications. This series has a potency profile similar to that of the third-generation agents, and possesses a better safety/tolerability profile. The 2-pyridones are potent DNA gyrase inhibitors, and more potent against Gram-positive and anaerobic organisms than the third-generation compounds. Both the NFQ and 2-pyridone series are potential leads for further optimization against *M. tuberculosis*.

7.24.6.6 Miscellaneous Agents: Ethambutol, Cycloserine, and *p*-Aminosalicylic Acid (PAS)

7.24.6.6.1 Ethambutol

Ethambutol (*N,N'*-bis(1-hydroxymethylpropyl)ethylenediamine) is a derivative of *N,N'*-diisopropylethylenediamine, the initial lead identified by random screening.[88] Ethambutol is a narrow-spectrum bacteriostatic agent against *M. tuberculosis*, and has low activity against nonreplicating organisms. It contributes little, if any, to the shortening of TB therapy. The main function of ethambutol is to prevent the emergence of resistance to other agents in the combination therapy.

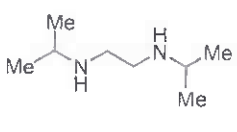
The precise mechanisms of action and resistance to ethambutol are not fully defined. The primary targets of ethambutol appear to be the arabinosyltransferase enzymes encoded by the *emb*A and *emb*B genes, which are involved in cell wall assembly.[89]

The interaction of ethambutol with its molecular target appears to be very stereospecific – only one of the four enatiomers, (*S,S*)-ethambutol, is active against *M. tuberculosis* (Figure 16). Further optimization of this series by combinatorial synthesis produced a new compound, SQ-109, a highly lipophilic compound that appears to have a different mechanism of action from ethambutol.[90]

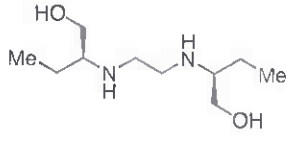
The major limitation of ethambutol and the ethylene diamine series with respect to TB treatment is their limited role in shortening therapy. This limitation could be mechanism based, and further optimization of potency may not address the problem. Another cautionary note is that optimization of the series should be done with careful monitoring of whole-cell activity in parallel to enzymatic activity, to avoid unwelcome off-target effects.

7.24.6.6.2 Cycloserine

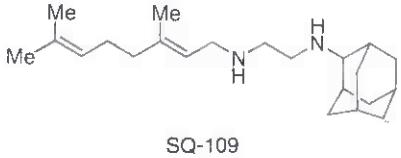
Cycloserine is a natural product, initially isolated from *Streptomyces orchidaceus* in the 1950s.[91] This compound is a broad-spectrum agent, active against both Gram-positive and Gram-negative organisms. Cycloserine is only marginally active against *M. tuberculosis*, and is primarily used for the treatment of MDR-TB. The use of cycloserine is limited due to its weak activity and frequent adverse reactions.



N,N'-diisopropyl ethylenediamine



Ethambutol

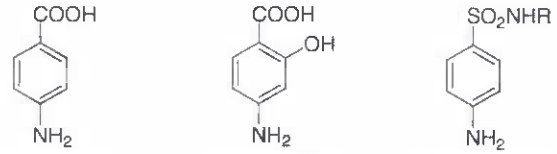


SQ-109

Figure 16 Structures of key ethylenediamine derivatives.

D-Alanine D-Cycloserine

Figure 17 Structures of D-alanine and D-cycloserine.



p-Aminobenzoic acid *p*-Aminosalicylic acid Sulfonamide

Figure 18 Structures of *p*-aminobenzoic acid, PAS, and sulfonamides.

The primary target of cycloserine is D-alanine racemase, an enzyme responsible for the interconversion of alanine enantiomers, and essential for the synthesis of the bacterial cell wall.[92] Cycloserine is believed to act as a structural analog of D-alanine, and covalently binds to the reactive center of the enzyme (Figure 17). The high-resolution crystal structure of D-alanine racemase from *M. tuberculosis*, which recently became available, should help to define further the mechanism of action of this agent.[93]

The main limitation of cycloserine, besides its relatively weak activity, is its toxicity profile. In addition, there is little evidence to suggest that this agent would play any role in eradicating drug-persistent bacterial populations. Without significant improvement in potency and safety, this series will eventually be replaced by other agents that are more potent and safer to use.

7.24.6.6.3 *p*-Aminosalicylic acid

p-Aminosalicylic acid (PAS), a synthetic compound, has been known for more than 100 years. Its anti-TB activity was first reported in the 1940s during the early era of antibiotic discovery.[94] The use of PAS has declined significantly because more potent and safer agents have become available. Currently, PAS is used only for the treatment of MDR-TB, when susceptibility to this agent is known or expected.

PAS is considered a structural analog of *p*-aminobenzoic acid, and inhibits the synthesis of folic acid (Figure 18). Different from sulfonamides in spectrum, PAS is only active against *M. tuberculosis*.

PAS has very limited utility for the treatment of TB due to its weak efficacy and low tolerability. Inhibition of folic acid synthesis generally results in a bacteriostatic effect that subsides during the persistent phase of growth. Although a safer and more efficacious agent in this class could be useful as second-line therapy, there is little evidence to suggest that even more potent inhibition of folic acid biosynthesis would have significant effect on the duration of TB treatment.

7.24.7 Unmet Medical Needs

Although the current regimen for treating active TB (2 months of isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by 4 months of isoniazid and rifampicin) is efficacious when properly prescribed and adhered to, its long duration and complexity directly contribute to the development of drug resistance and hamper the global public health community's ability to effectively control the TB epidemic, particularly in settings of high HIV prevalence. Key priorities for TB drug development are: (1) shorter, simpler regimens for effective treatment of active TB; (2) regimens that can be easily and safely administered simultaneously with highly active antiretroviral therapy (i.e., that do not demonstrate significant drug–drug interactions with commonly used antiretroviral agents); (3) regimens that are safe and efficacious against MDR strains of *M. tuberculosis*; and (4), a safe, short prophylactic regimen for use against LTBI. This last priority, in conjunction with an effective vaccine to prevent infection, would ultimately have the greatest impact in eliminating TB as a public health problem – by removing the currently vast reservoir of future patients with

active disease. Improved treatment for LTBI also poses the greatest inherent challenge – to understand the underlying mechanisms of latent infection well enough to identify key molecular targets for new drugs and to identify surrogate markers to streamline clinical trials of novel preventive therapies.

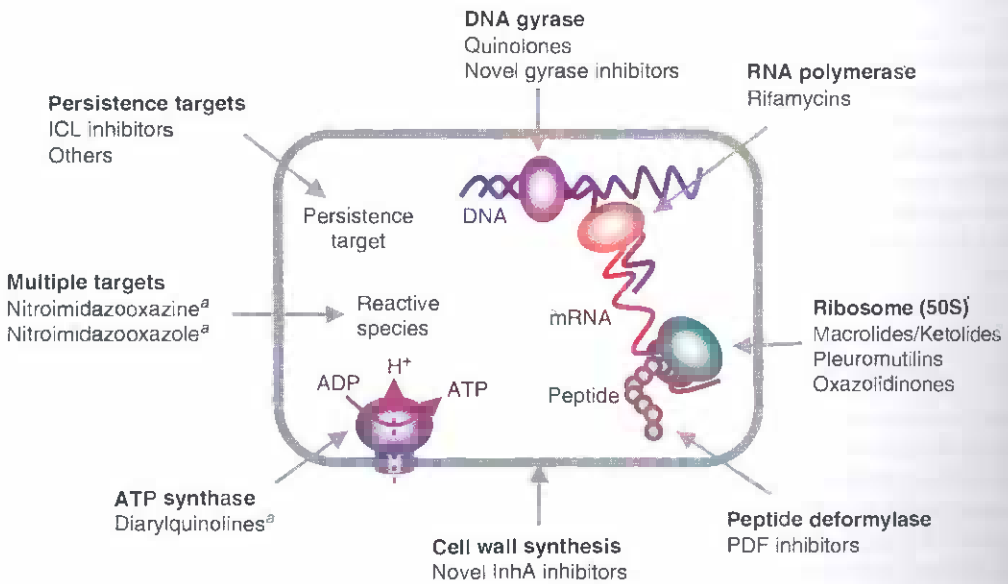
7.24.8 New Research Areas

The last new drug to be incorporated in the current standard anti-TB regimen, rifampicin, was introduced about 40 years ago. Since then, very few new agents for TB have been developed because of the lack of market opportunities. Recently, however, we have observed a surge of research and development activities in the TB therapeutic area due to heightened attention to and increased resources for this urgent, global, public health need. There are several new chemical series that are currently being optimized against *M. tuberculosis*. These new series are generally from one of three sources: (1) existing anti-TB agents, (2) known antibiotic classes not yet approved for TB treatment, and (3) novel chemical series. Examples of these three groups are shown in **Table 4**. Key factors to be considered when prioritizing the development of new agents for TB are their ability to overcome drug persistence (to shorten therapy), their potential effectiveness in treating MDR-TB, and their appropriateness (ease of use and safety) for HIV co-infected patients.

Figure 19 illustrates the molecular targets of the new drug series that are currently under investigation. Different from the existing agents, the target pathways of the new series are believed to be more essential mechanistically to the

Table 4 Examples of new drug series that are currently under investigation

Group 1: existing anti-TB class	*Group 2: known antibiotic class not yet approved for TB*	*Group 3: novel drug class*
Rifamycins	Macrolides/ketolides	Nitroimidazoles
Ethambutol series	Pleuromutilins	Diarylquinolines
	Oxazolidinones	Novel InhA inhibitors[a]
	Quinolones	Novel ICL inhibitors
	PDF inhibitors	Novel gyrase inhibitors



[a] The site of action is not completely defined

Figure 19 Schematic illustration of the sites of action of potential new anti-TB agents currently under investigation.

nonreplicating state, and therefore have a better chance to shorten treatment duration. Compounds derived from the existing anti-TB agents and known antibiotic classes have been discussed elsewhere. This section will focus primarily on two novel compound series, nitroimidazoles and diarylquinolines. Other novel series will be discussed briefly.

7.24.8.1 Nitroimidazole Class: PA-824 and OPC-67683

The structural and mechanistic ancestor of the current nitroimidazoles is a natural product called azomycin (2-nitroimidazole), isolated from streptomycete in the 1950s (Figure 20). Structural modification of azomycin led to the introduction of metronidazole and several other first-generation compounds.[95] Metronidazole is widely used for the treatment of protozoan and anaerobic infections. Although active against *M. tuberculosis* under anaerobic conditions, metronidazole is inactive against *M. tuberculosis* under aerobic conditions. The compound appeared to be mutagenic based on Ames tests, and carcinogenic in mice and rats. CGI-17341 is a second-generation nitroimidazole with a 4-nitroimidazooxazole structure. This compound showed potent activity against *M. tuberculosis* under both anaerobic and aerobic conditions. However, the development of CGI-17341 was abandoned due to the mutagenic potential of the compound.

Further development of the nitroimidzole series led to the discovery of PA-824, a 4-nitroimidazooxazine compound with a larger substituent attached to the oxazine ring.[96] PA-824 possesses all the beneficial attributes of CGI-17341, with activity against *M. tuberculosis* under both aerobic and anaerobic conditions. This compound is active against MDR-TB strains in vitro, indicating a novel mechanism of action. More importantly, PA-824 is not mutagenic, based on various in vitro and in vivo studies. OPC-67683 is a more recent analog of CGI-17341, with the same 4-nitroimidazooxazole scaffold as CGI-17341 but with a larger substituent linked to a quaternary carbon of the oxazole ring.[97] OPC-67683 showed even better in vitro and in vivo potency than PA-824 against *M. tuberculosis*. This compound also appears not to be mutagenic.[98]

7.24.8.1.1 Sites and mechanisms of action

Nitroimidazoles are prodrugs requiring bioreduction of the nitro group for antimycobacterial activity; however, the exact targets of the bioreductive species are unknown. Most researchers believe that the bioreductive species, which are highly reactive, damage various intracellular targets, leading to cell death. This killing mechanism is nonselective and does not discriminate between eukaryotic and prokaryotic cells. The demonstrated selectivity of the nitroimidazole class must therefore come from a selective drug activation process. It is known that *M. tuberculosis* and many other anaerobic organisms possess certain electron transport systems with a reduction potential that is low enough to reduce 4-nitroimidazoles.

As noted previously, PA-824 and OPC-67683 are active under both aerobic and anaerobic conditions. The activation mechanism of these agents may be different under these different conditions. Evidence suggests that under aerobic conditions, PA-824 is activated by an F420-dependent glucose-6-phosphate dehydrogenase (Fgd). Mutations in the gene encoding the F420 enzyme (*fgd*) are responsible for some instances of PA-824 resistance identified in vitro. Under anaerobic conditions, however, nitroimidazoles are potentially activated by a different reduction system. This phenomenon has been observed in other bacterial systems. For example, *Helicobacter pylori* mutants that are resistant to metronidazole under microaerophilic conditions demonstrate restored drug sensitivity when they are tested under anaerobic conditions.[99] Nitrofuran-resistant mutants of *E. coli* also have restored drug sensitivity under anaerobic

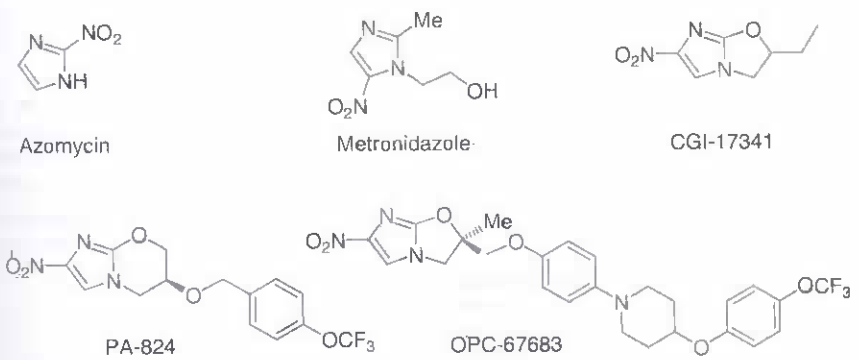


Figure 20 Structures of important nitroimidazoles.

conditions.[100] These studies suggest that the activation systems utilized under anaerobic conditions differ substantially from those utilized under aerobic conditions.

Preliminary studies suggested that PA-824 inhibits both protein and lipid synthesis but does not affect nucleic acid synthesis. Cell treatment with PA-824 results in accumulation of hydroxymycolic acid, with a concomitant reduction in ketomycolic acids, suggesting inhibition of an enzyme responsible for the oxidation of hydroxymycolate to ketomycolate. PA-824 did not exhibit cross-resistance with other therapeutic agents when tested against MDR clinical isolates. The frequency of resistance development is relatively low (10^{-7}), and appears to be due mainly to mutation of drug activation enzymes.

7.24.8.1.2 Structure–activity relationships

As to be expected for such a new series, the SARs for the nitroimidazole class are insufficient and incomplete. Figure 21 summarizes the preliminary SARs and STRs for this series. It appears that the 4-nitroimidazole core is essential for antibacterial activity, which is consistent with the mechanism of action discussed above. The nitroaromatic group is also believed to be the source for mutagenicity. The substitution on the oxazine or oxazole ring is critical to many properties, including potency, physicochemical properties, pharmacokinetic profiles, and mutagenicity. Substitution with a larger group generally reduces or totally removes the potential for mutagenicity. The absolute stereochemistry of the substituent group is important for the 4-nitroimidazo-oxazine series (the (*S*) configuration is more potent than the (*R*) configuration) but has no effect on the 4-nitroimidazo-oxazole series.

7.24.8.1.3 Comparisons of compounds within the class

There are two compounds, PA-824 and OPC-67683, within the nitroimidazole class that are currently under investigation as anti-TB agents. Preclinical data indicate that OPC-67683 is the more active compound both in vitro and in vivo. However, therapeutic indices based on human clinical trials are not yet available for these agents.

7.24.8.1.3.1 PA-824

PA-824 is a potent compound against a variety of drug-sensitive and MDR-TB isolates, with MICs in the range of $\leqslant 0.015$ to $0.25\,\mu g\,mL^{-1}$ The activity is highly selective, with potent activity only against BCG and *M. tuberculosis* among the mycobacterial species tested, and without significant activity against a broad range of Gram-positive and Gram-negative bacteria (with the exception of *Helicbacter pylori* and some anaerobes). Perhaps of even greater significance is the finding in anaerobic culture that PA-824 has activity against nonreplicating bacilli, indicating its potential for activity against persisting organisms and therefore for shortening the treatment duration. Longer term mouse studies with PA-824 at $50\,mg\,kg^{-1}\,day^{-1}$ demonstrated a reduction in the bacillary lung burden similar to that of isoniazid at $25\,mg\,kg^{-1}\,day^{-1}$ with all PA-824-treated mice surviving while all untreated control animals died by day 35. In a guinea pig aerosol infection model, daily oral administration of PA-824 at $37\,mg\,kg^{-1}\,day^{-1}$ for 35 days also produced reductions of *M. tuberculosis* counts in lungs and spleens comparable to those produced by isoniazid.

When tested in the Ames assay, both with and without S9 activation, PA-824, unlike CGI-17341, demonstrated no evidence of mutagenicity. Chromosomal aberration, mouse micronucleus, and mouse lymphoma tests have all been negative, demonstrating lack of genotoxic potential. PA-824 neither inhibits nor is metabolized by major cytochrome

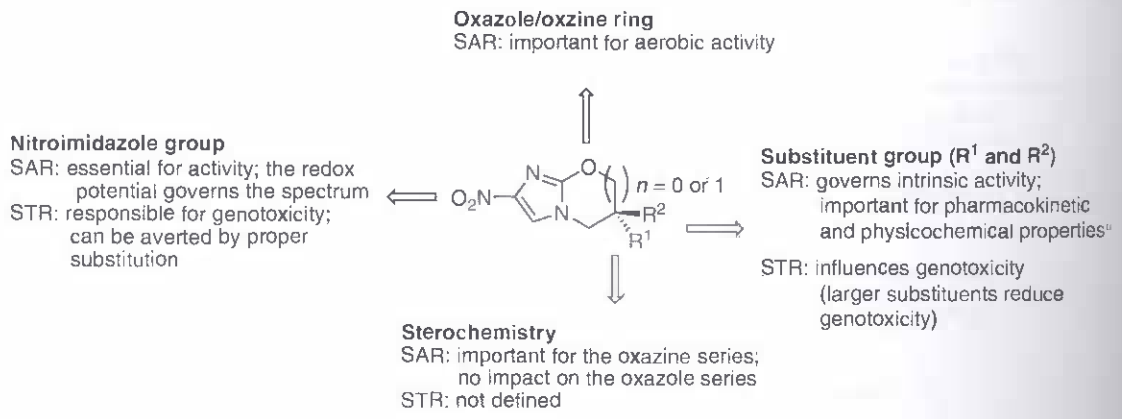


Figure 21 Highlights of preliminary SAR and STR relationships of the nitroimidazole class.

P450 enzyme isoforms in vitro, importantly indicating a low potential for drug–drug interactions, including with presently used AIDS antiretrovirals. Pharmacokinetic studies of PA-824 in the rat indicate excellent tissue penetration. Total exposure in various tissues as measured by AUC (area under the curve) is three- to eightfold higher than that in plasma.

7.24.8.1.3.2 OPC-67683

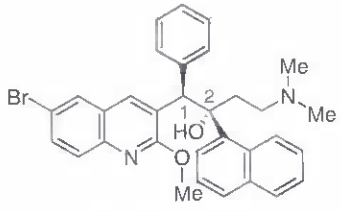
The 4-nitroimidazo-oxazole OPC-67683 has potent in vitro antimicrobial activity against *M. tuberculosis*. MICs against multiple clinically isolated *M. tuberculosis* strains range from 0.006 to 0.024 $\mu g\,mL^{-1}$. As with PA-824, OPC-67683 shows no cross-resistance with any of the currently used first-line TB drugs. There are also no indications from preclinical testing of mutagenicity or potential for cytochrome P450 enzyme-mediated drug–drug interactions. Based on their relatively similar chemical structure, it is likely that the mechanisms of action of PA-824 and OPC-67683 will prove to be the same. In a chronic infection mouse model, the efficacy of OPC-67683 is superior to that of the currently used TB drugs. In these experiments, the effective plasma concentration was 0.100 $\mu g\,mL^{-1}$, which was achieved with an oral dose of 0.625 $mg\,kg^{-1}$ confirming the remarkable in vivo potency of this compound. In nonclinical in vitro and in vivo studies, OPC-67683 in combination with various first-line TB drugs shows synergistic, additive, or no appreciable interaction, but does not demonstrate any evidence of antagonistic activity.

7.24.8.1.4 Limitations and future directions

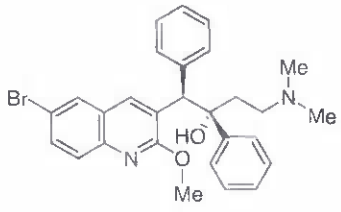
The nitroimidazole class holds great potential for addressing several key issues in TB therapy. For the reasons stated earlier, these agents could have a major impact on TB treatment by shortening therapy, and safely and efficaciously treating both MDR-TB and HIV–TB co-infections. However, the 4-nitroimidazole series appears to have divergent SARs for aerobic and anaerobic activities. Neither PA-824 nor OPC-67683 has been optimized against persistent *M. tuberculosis* under anaerobic conditions, and SARs against this important population of *M. tuberculosis* are totally lacking. Therefore, optimization of this drug class against nonreplicating *M. tuberculosis* is an important future direction. It will also be important to gain a better understanding of the mechanisms underlying the mutagenicity of some members and their relationship to chemical structures. The risk of a nitroimidazole for inducing mutagenicity needs to be further addressed.

7.24.8.2 Diarylquinolines

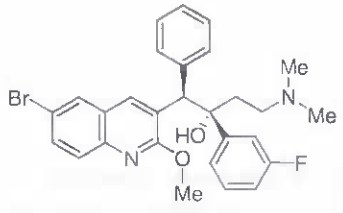
Diarylquinolines belong to a novel class of anti-TB agents initially identified by a whole-cell-based screen against *M. smegmatis*. Structural modification of the initial lead compound led to the discovery of TMC-207 (previously known as R207910, Figure 22), which is currently under clinical development.[101] TMC-207 possesses a novel mechanism of action, and is highly potent against both drug-susceptible and MDR *M. tuberculosis*. Preclinical animal studies indicate that the compound has the potential to shorten the duration of TB therapy.



TMC-207 (R207910)



R126470



R207319

Figure 22 Structures of TMC-207 and analogs.

7.24.8.2.1 Sites and mechanisms of action

Based on gene sequences of drug-resistant mutants, the mechanism of action of TMC-207 has been postulated to be inhibition of the proton pump of ATP synthase. Point mutations that conferred resistance to TMC-207 were identified in both *M. tuberculosis* and *M. smegmatis*. In three independent mutants, the only gene commonly affected encoded AtpE, a part of the F0 subunit of ATP synthase. Further transformation studies confirmed the importance of the *atpE* gene in the mechanistic pathway of TMC-207. Consistent with having a novel mechanism of action, TMC-207 showed potent activity against *M. tuberculosis* isolates resistant to a variety of anti-TB agents.

7.24.8.2.2 Structure–activity relationships

Information regarding the SARs for this new drug class is very limited. The diarylquinioline series has two chiral centers and four enantiomers. It appears that only one enantiomer, (*1R,2S*), is active against *M. tuberculosis*, clearly indicating that the drug-binding site on the molecular target (likely ATP synthase) is highly stereospecific.

R126470 and R207319 (Figure 22) are two close analogs of TMC-207 that have anti-TB activity in vivo.[101] R126470 has the same configuration as TMC-207, and a phenyl moiety, instead of a naphthyl group, at the C-2 position. This compound showed a bacteriostatic effect in a mouse model. R207319 has a 3-fluorophenyl group at the C-2 position, and showed weak bactericidal activity in the mouse model. TMC-207 was significantly more efficacious than the other two analogs in the same mouse model. These results clearly indicate the importance of the C-2 moiety.

7.24.8.2.3 Comparisons of compounds within the class

TMC-207 is the only agent in the class that is currently under clinical development. This compound is active in vitro against both drug-susceptible and drug-resistant strains of *M. tuberculosis* (MIC = 0.06 μg mL^{-1}). Strains tested included those resistant to a wide variety of commonly used drugs, including isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, and the fluoroquinolones. While TMC-207 is active in vitro against other mycobacteria, including *M. smegmatis*, *M. kansasii*, *M. bovis*, *M. avium*, and *M. fortuitum*, the compound is not active against a variety of Gram-positive and Gram-negative organisms such as *Nocardia asteroides*, *E. coli*, *S. aureus*, *Enterococcus faecium*, or *Haemophilus influenzae*. Of note, two resistant *M. smegmatis* isolates were not cross-resistant to a wide range of antibiotics, including the fluoroquinolones.

Pharmacokinetic studies in mice have shown rapid absorption, with extensive tissue distribution in liver, kidney, heart, spleen, and lung. The half-lives ranged from 28.1 to 92 h in tissues and from 43.7 to 64 h in plasma. One contribution to the relatively long half-life appears to be slow redistribution from tissue compartments.

TMC-207 has also demonstrated significant in vivo activity in mouse models of both established and nonestablished infections. In the nonestablished disease model, mice were treated for 4 weeks, beginning the day after inoculation. In this setting, a once-weekly dose of 12.5 mg kg^{-1} was almost as efficacious as 6.5 mg kg^{-1} given five times per week. At 12.5 and 25 mg kg^{-1}, TMC-207 was more efficacious than isoniazid at 25 mg kg^{-1}. In the established disease model, treatment was begun 12–14 days after inoculation. In combination studies in the mouse, the substitution of TMC-207 for any of the three commonly used drugs (isoniazid, rifampicin, or pyrazinamide) had greater efficacy than the standard regimen of isoniazid, rifampicin, and pyrazinamide. The combination of either TMC-207, isoniazid, and pyrazinamide, or TMC-207, rifampicin, and pyrazinamide, resulted in negative spleen and lung cultures after 8 weeks of therapy, while the standard therapy (rifampicin, isoniazid, and pyrazinamide) led to positive cultures both in lungs (0.97 log CFU) and spleen (1.91 log CFU).

TMC-207 has also been tested in Phase I pharmacokinetic and safety studies in healthy volunteers. Single oral administration of TMC-207 at doses ranging from 10 to 700 mg revealed the drug to be well absorbed, with peak serum concentrations at approximately 5 h post-dose. The pharmacokinetics were dose-proportional over the range studied. A multiple ascending dose study (once-daily doses of TMC-207 at 50, 150, and 400 mg day^{-1} for 14 days) was then performed in healthy volunteers. Accumulation was observed with a doubling of the AUC on the 14th day, compared with day 1. Of note, the average observed AUCs were greater than those that achieved optimal activity in an established infection in the mouse. Safety evaluations revealed only mild or moderate adverse events, with the majority considered only possibly related to the study drug.

7.24.8.2.4 Limitations and future directions

The diarylquinoline TMC-207 represents a novel drug class with excellent potential for the treatment of TB, particularly in shortening therapy and treating MDR-TB. As with any other new series, the safety and efficacy of the compound need to be evaluated thoroughly in clinical trials. A back-up program that focuses on addressing potential

safety issues identified during clinical development is important. The diarylquinoline was identified and optimized based on activity against replicating bacteria. SARs for activity against bacteria in the nonreplicating state need to be further elucidated.

7.24.8.3 Other Novel Classes: Pyrroles, Oxazolidinones, and Macrolides

7.24.8.3.1 Pyrroles

Another class of compounds being investigated for TB therapy is the pyrroles. First described in 1998 as having good antimycobacterial activity, the most potent compound was designated BM212 (**Figure 23**).[102] MICs for BM212 ranged between 0.7 and 1.5 $\mu g\,mL^{-1}$ against several strains of *M. tuberculosis*. The MICs for strains resistant to the commonly used antitubercular drugs were similar to those for sensitive strains, indicating the compound most likely has a novel mechanism of action. However, no mechanism of action has yet been elucidated for this class of compound. Some non-TB mycobacterial strains also appeared to be sensitive to BM212, albeit with MICs higher than those for *M. tuberculosis*.

A novel pyrrole compound, LL3858, is currently in Phase I clinical development for TB in India.[103] This compound has sub-micromolar MICs, and has been reported to have significant efficacy in a mouse model of TB. In combination with currently used anti-TB drugs, LL3858 sterilized lungs and spleens of infected animals in a shorter timeframe than conventional therapy.

7.24.8.3.2 Macrolides

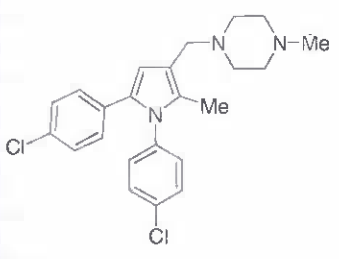
Macrolides, a well-known antibiotic class, are initially isolated from *Streptomyces erythreus* in the 1950s. Macrolides are potent inhibitors of protein synthesis, via binding to the 50S ribosomal subunit of bacteria at the peptidyl transferase center formed by 23S rRNA. Studies have suggested they also block the formation of the 50S subunit in growing cells. Macrolides could therefore add a novel mechanism of action to TB combination therapy, and thereby also hold out the promise of being equally effective against MDR-TB and drug-sensitive TB. The macrolides, known to be orally active, have also proven to be safe and well tolerated when used for non-TB indications. Key for TB treatment, the macrolides tend to exhibit high levels of intracellular activity and extensive distribution into the lungs. Macrolides have already proven to be clinically useful in the treatment of other mycobacterial diseases including MAC and leprosy.

The major challenge for this class is its weak activity against *M. tuberculosis*, which needs to be further optimized. In addition, several members of the macrolide class are known inhibitors of cytochrome P450 enzymes associated with drug–drug interactions. This class of antibiotics is currently undergoing optimization for potential TB therapy.[104]

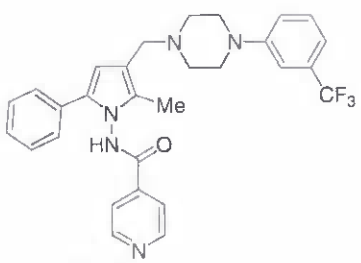
7.24.8.3.3 Oxazolidinones

The oxazolidinones, a relatively new class of antimicrobial agents, exert their antimicrobial effect by inhibiting protein synthesis through binding to the 70S ribosomal initiation complex. They have a relatively broad spectrum of activity, including anaerobic and Gram-positive aerobic bacteria as well as mycobacteria.[105] The first and thus far only oxazolidinone to be approved is linezolid. Although not approved for use in TB, linezolid has in vitro activity against *M. tuberculosis*. Of the oxazolidinones that have been evaluated for their in vitro activity, the most active compound appears to be PNU-100480 (**Figure 24**), which demonstrates potency similar to that of isoniazid or rifampicin.[105]

Because of the clinical availability of linezolid, it has been used anecdotally in patients with MDR-TB, and demonstrates biological activity, as evidenced by sputum culture conversion.[106] However, with relatively long-term use in MDR-TB patients, there are emerging reports of peripheral and optic neuropathy.[107]

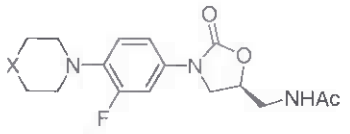


BM212



LL3858

Figure 23 Structures of important pyrrole compounds.



Linezolid: X = O
PNU-100480: X = S

Figure 24 Structures of important oxazolidinones.

Even though the oxazolidinones have the potential to be a useful addition to the armamentarium of anti-TB drugs, there has never been a truly concerted effort to optimize their activity against *M. tuberculosis*. In the meantime, the neuropathic side effects of linezolid, emerging with its long-term use, will require careful monitoring as this drug becomes more commonly used in the treatment of MDR-TB. A lead optimization program focusing on improving efficacy and safety of this drug class would be an important development.

7.24.8.4 Novel Drug Targets for Persistence

All the current anti-TB agents were identified by their ability to kill or inhibit *M. tuberculosis* in the exponential phase of growth in vitro, with the exception of pyrazinamide, whose antimycobacterial activity was discovered directly in vivo in a mouse model. Most of these agents are highly potent against replicating *M. tuberculosis* but have limited activity against *M. tuberculosis* in anaerobic or nutrient-depleted conditions in their nonreplicating state. This phenomenon is largely due to the fact that in the nonreplicating state, the drug targets of these agents are inactive, and inhibiting theses enzymes therefore has limited impact on the viability of the bacilli. Recently, significant progress has been made in identifying *M. tuberculosis* factors believed to play an essential role in the ability of *M. tuberculosis* to adopt and/or maintain a persistent state. Some of these factors are believed to be suitable drug targets for therapeutic interventions.[108,109] Drugs targeting these enzymes are likely to have a significant impact on cell viability in the persistent state, and therefore may shorten the duration of therapy.

There is significant disagreement, however, regarding how persistence is induced and maintained in the host. Various in vitro conditions have been used to mimic the host environment and to identify putative persistence factors, including oxygen depletion, nutrient depletion, and nitric oxide stress.[110] Whole-genome microarray experiments conducted under various stress conditions suggest that gene expression patterns under these various conditions have little overlap.[111] The lack of correlation among gene expression patterns under different in vitro stress conditions further illustrates the high risks involved with this approach. Without a clear understanding of the clinical relevance of these models, research should first focus on the small numbers of genes that are commonly expressed under different conditions. This group of genes may provide a better chance to identify true persistence factors. Another caveat relevant to this approach is that these putative persistence targets are often not essential under the normal growth conditions used to assay drug susceptibility. Compounds that inhibit such targets will have to be tested under special conditions or directly in vivo. This requirement adds significantly to the challenge of the lead optimization process. Currently, most programs focused on agents active against persistent *M. tuberculosis* are in the target validation or lead identification stages, the very early steps of a drug discovery program.

7.24.9 Conclusion

While current, standard, TB drugs can be effective, they necessitate lengthy and complex treatment regimens, lessening rates of patient adherence. This lack of compliance in turn has created a significant drug resistance problem and hampered control of the global TB epidemic. The lack of strong market opportunities has hindered the development of new TB drugs until very recently. Currently, increased resources and public attention have led to a resurgence in TB drug research and development activities, leading to optimism that shorter, simpler, and safer treatment regimens will be developed and registered in the foreseeable future. Medicinal chemistry is playing, and will continue to play, a key role in this endeavor.

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Biographies

Zhenkun Ma is the head of research at the Global Alliance for TB Drug Development. An experienced researcher in drug discovery, he was formerly the director of chemistry at Cumbre Inc., a Dallas-based biotechnology firm specializing in antibacterial drug discovery research. Prior to his experience at Cumbre, he worked at Abbott Laboratories for 8 years, where, as a member of the prestigious Volwiler Society, he led antibacterial programs and oversaw the medicinal chemistry for macrolide and other antibacterial programs. In 1997, he received the Chairman's Award for the discovery of cethromycin, a novel ketolide antibiotic currently in late-stage clinical development. A native of northeastern China, he studied chemistry and organic chemistry at Beijing University, and holds a PhD in organic chemistry from the University of Connecticut, Storrs. The holder of more than 40 US patents and patent applications, Ma has also authored and co-authored more than 80 peer-reviewed articles and meeting presentations.

Ann M Ginsberg has been the head of clinical development for the Global Alliance for TB Drug Development since June 2004. A highly regarded tuberculosis expert, and a former director for project management at Merck & Co., Inc., she brings 15 years of experience at the US National Institutes of Health, starting in the National Cancer Institute as a medical staff fellow and resident in anatomic pathology. She subsequently joined the National Institute of Diabetes, Digestive and Kidney Diseases as a senior staff fellow in the Laboratory of Cellular and Developmental Biology. In 1995, she joined the National Institute for Allergy and Infectious Diseases as a program officer for tuberculosis, leprosy, and other mycobacterial diseases, and was appointed chief of the respiratory diseases branch in 2000. Trained as a molecular biologist and Board Certified Anatomic Pathologist, Ginsberg holds a BA from Harvard University, an MD from Columbia University, and a PhD from Washington University. She has authored numerous scientific publications, and has received several prominent awards, including the US Department of Health and Human Services Secretary's Award for Distinguished Service in 2000. Currently a member of the Board of Directors of the Aeras Global TB Vaccine Foundation, she has served on multiple global health committees.

Melvin Spigelman is the director of research and development for the Global Alliance for TB Drug Development. A highly regarded expert in domestic and international drug research and development, Spigelman spent a decade managing drug R&D at Knoll Pharmaceuticals (a division of BASF Pharma). As the vice president of R&D at Knoll, Spigelman directed clinical development and supervised R&D activities from basic discovery to regulatory approval. As part of Knoll's senior R&D management team, he established global R&D processes, oversaw a marked increase in US regulatory filings and approvals, and supervised joint R&D programs with pharmaceutical companies. Starting as a director of oncology and immunology in 1989, Spigelman became the vice president of R&D at Knoll until its acquisition by Abbott Laboratories in 2001. He received his undergraduate degree from Brown University and his medical degree from the Mt. Sinai School of Medicine, where he specialized in internal medicine and neoplastic diseases. Spigelman holds board certifications from the American Board of Internal Medicine, the American Board's Subspecialty Board of Medical Oncology, and the American Board of Preventive Medicine, and was the recipient of the American Cancer Society Clinical Oncology Career Development Award (1985–88).

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