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The Medicinal Chemistry of Tuberculosis Chemotherapy

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Abstract The development of effective chemotherapy for the treatment of tuberculosis (TB) began in the 1940s and has been reinvigorated recently due to concern regarding the emergence of highly drug-resistant TB strains. This chapter explores the medicinal chemistry efforts that gave rise to current frontline and second-line drugs in global use today and attempts to comprehensively summarize ongoing discovery and lead optimization programs being conducted in both the private and the public sector. TB has a large number of disease-specific considerations and constraints that introduce significant complexity in drug discovery efforts. Conceptually, the disease encompasses all the drug discovery challenges of both infectious diseases and oncology, and integrating these considerations into programs that often demand collaboration between industry and academia is both challenging and rewarding.

Contents

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1 Introduction

1.1 TB: A Global Epidemic

Tuberculosis (TB) is the second major cause of death due to an infectious disease in adults worldwide with nine million new cases and close to 1.8 million deaths annually [1]. TB is caused by *Mycobacterium tuberculosis* (MTb), an airborne pathogen transmitted among humans which infects macrophages in the lungs. Two possible outcomes follow macrophage infection: (1) the infected macrophage can be recognized by effectors of the innate immune system and eradicated; or (2) the bacilli may further multiply in the cell, ultimately leading to its destruction and the infection of new macrophages drawn to the site of infection. The second scenario may initiate T cell-mediated adaptive immunity enabling the host to eradicate the bacilli at the initial site of infection. Failure of adaptive immunity to eradicate the bacilli leads to uncontrolled growth of the organism and subsequent spread through the lymphatic system to secondary sites. These sites may be in the lung or in some cases in extra-pulmonary sites, which is manifested as clinical disease with various degrees of severity which, if not treated, kills more than 50% of patients. There is also an intermediate situation wherein adaptive immunity may be able to contain the growth of the organism by controlling its metabolism for years, even decades, until waning of immunity allows reactivation of this latent form of disease [2]. More recently, researchers have begun to suspect that latent disease may not be a single

metastable state but rather a subtle guerilla war with waxing and waning local battles on a small scale resulting in a "spectrum" of subclinical active disease [2].

Based on the global incidence of a positive response in the tuberculin skin test, which is associated with adaptive immunity, it is estimated that about two billion people are latently infected with MTb [1]. Of these two billion people, about 10% will develop active tuberculosis in their lifetime, although HIV infection dramatically increases this risk to a 10% annual risk of conversion to active TB [3]. TB is the major cause of death in HIV-infected individuals. A person with contagious pulmonary TB infects 10–15 more people on average, which created tuberculosis epidemics in the developed world until the advent of chemotherapy. Isolation of patients resulted in limited success in TB control through the establishment of sanatoria in the mid-nineteenth century. Sanatoria patients would occasionally achieve spontaneous resolution of the disease, although subsequent relapse rates were high, highlighting the chronic and dynamic nature of this infection.

Albert Calmette and Camille Guérin produced a strain of *Mycobacterium bovis* (bacille Calmette-Guérin, BCG) by serial passaging of an isolate of the related bacillus that causes TB in cattle on potato–bile–glycerin media until it was no longer virulent in laboratory animals. Vaccination started in the 1920s, but the efficacy of the vaccine varied greatly depending on factors such as geographic location and strain of BCG used for vaccination [3, 4]. Large-scale clinical trials throughout the world have shown that the vaccine protects against severe forms of TB in children but does not protect against the development of adult pulmonary TB [3]. Thus, BCG vaccination has not reduced the global incidence of TB. Disturbingly, recent data suggest that this vaccine applied now for decades on a global scale may also have accelerated the development of even more virulent forms of TB [5].

In accord with the Millennium Development Goals established by the United Nations, the World Health Organization's "Stop TB" Strategy aims to halve the prevalence of, and mortality due to, TB compared to that seen in 1990 by the year 2015 and to have reduced the incidence of new TB cases to one per million by 2050. Very few think that these goals remain realistic, given our current progress with available tools. Our only chance of achieving such progress lies in the development of better diagnostic methods and new drugs to combat both drug-sensitive and drug-resistant disease [6].

1.2 The Medical History of Current TB Chemotherapy

Effective chemotherapy for tuberculosis began in 1940s with the discovery and use of streptomycin (STR, Fig. 1; **1a**) and *para*-aminosalicylic acid (PAS, Fig. 1; **2a**) [7–9]. The first randomized controlled study of STR treatment for TB by the British Medical Research Council (BMRC) showed that streptomycin was effective in the short term but that ultimately so many patients developed STR-resistant TB and hearing loss that at 5 years, no net clinical benefit was seen [10]. Contemporaneously, PAS was found to be bacteriostatic against MTb (including STR-resistant strains) in experimental models and able to prevent the development of STR

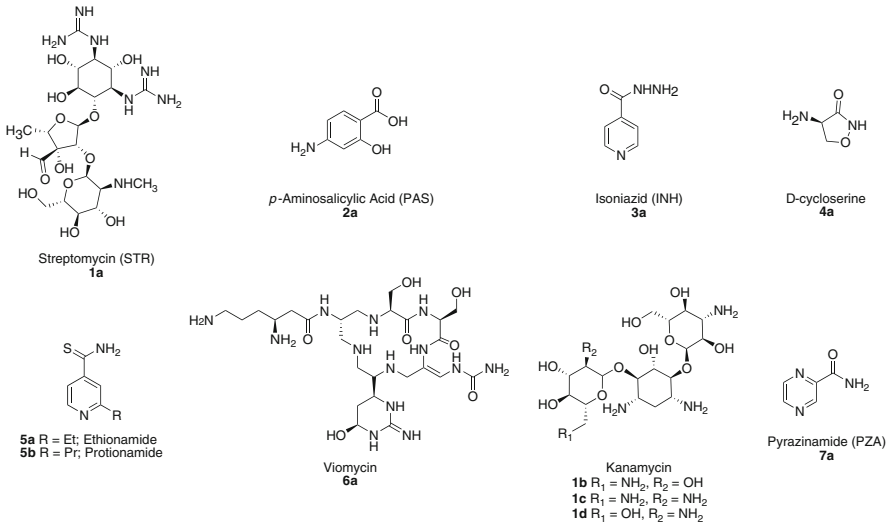


Fig. 1 TB drugs introduced in the 1940s and 1950s

resistance [11, 12]. PAS was also shown to be useful in pulmonary TB patients as monotherapy, but development of resistance occurred and patients tolerated the drug poorly, mainly due to gastrointestinal side effects and occasional hepatitis [13]. A BMRC trial in subjects with pulmonary TB found that STR with or without PAS was as effective, or even slightly more effective, than PAS alone, but that the combination with PAS greatly reduced the development of drug resistance [10]. By the end of the 1940s, the standard of care was combined therapy with STR and PAS, typically given for 12–24 months.

The 1950s were significant because of the discovery and initial use of isonizaid (INH, Fig. 1; **3a**). There were several trials to optimize treatment combinations of INH with STR and PAS. Although INH was generally well tolerated in patients, some experienced rash or hepatitis with this drug. INH treatment led to rapid improvement over the first month of therapy, but recrudescence of disease was common due to acquired resistance [9, 14, 15]. Drug combination studies showed that INH with STR was superior to INH with PAS as measured by radiographic, microbiologic, and clinical improvement. In addition, such studies showed that STR was more effective than PAS in preventing the emergence of INH resistance [9, 16]. The triple drug combination of INH, STR, and PAS was found to be better than therapy with INH and PAS combined and achieved 98% sputum culture conversion at 6 months compared to 84% with INH and PAS alone [17]. These early studies also underscored the importance of extended treatment durations with chemotherapy of less than 1 year, 1 year, or 15 months associated with an 8%, 1.4%, or 0% relapse rate, respectively [17].

Other drugs discovered during the 1950s include cycloserine (Fig. 1; **4a**) [18], ethionamide (Fig. 1; **5a**), and the closely related prothionamide (Fig. 1; **5b**) [19], viomycin (Fig. 1; **6a**) [20], kanamycin (Fig. 1; **1b-d**) [21], and pyrazinamide (PZA, Fig. 1; **7a**) [22]. At the time of their discovery, these drugs were thought to be inferior to INH, PAS, and STR and were used only in patients with disease refractory to standard therapy [23].

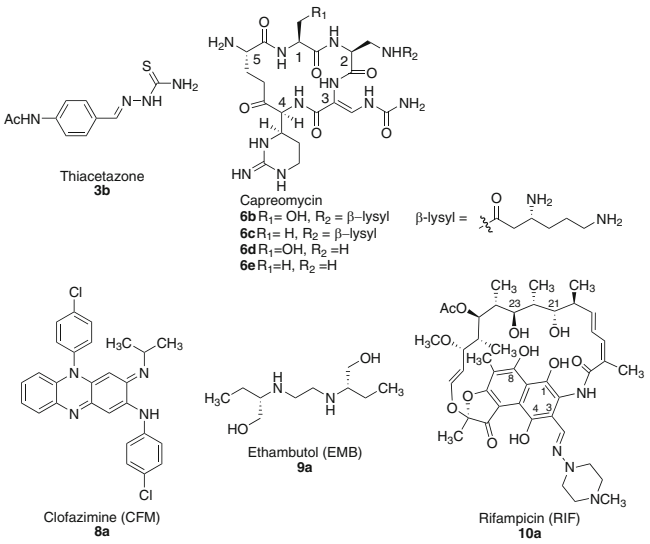


Fig. 2 TB drugs introduced in the 1960s and 1970s

In the 1960s, care shifted from sanatoria or hospitals to the home after a landmark study in Madras, India, which showed that care in the home was equally efficacious to treatment in a sanatorium or hospital [24]. Other TB drugs introduced during the 1960s include thiacetazone (Fig. 2; **3b**) [25], capreomycin (Fig. 2; **6b–e**) [26], and clofazimine (CFM, Fig. 2; **8a**)[27]. These early studies highlight one of the key problems with second-line agents that persists today: tolerability. In the words of one set of authors of these trials ". . .the patients considered the cure worse than the disease" [23]. This aspect complicated systematic clinical trials to devise an optimal regimen or to establish the relative efficacy of many of these new agents. Notably, these same agents are used in second-line therapy today, where clinicians confront the same issue. Ethambutol (EMB, Fig. 2; **9a**) supplanted PAS in the standard drug regimen since this drug was better tolerated than PAS and also allowed the treatment regimen to be shortened to 18 months [28, 29].

Rifampicin (RIF, Fig. 2; **10a**), one of the last drugs to be introduced into clinical practice, revolutionized TB therapy [30]. Landmark clinical trials in the 1970s in East Africa and Hong Kong showed that addition of RIF to the standard INH/EMB/STR or INH/STR drug regimens allowed the duration of treatment to be decreased from 18 to 9 months without increasing the relapse rate [31, 32]. Renewed interest in PZA was sparked by reports that PZA was more effective than STR in reducing organ burdens in MTb-infected mice when combined with INH [33, 34]. Clinical trials at the end of the 1970s and in the 1980s investigated the use of PZA in various combinations and treatment durations with STR, INH, RIF, EMB, and thiacetazone. PZA was instrumental in allowing shortening of TB chemotherapy to 6 months [35–37]. Although thiacetazone was initially used in chemotherapy instead of RIF due to the high cost of RIF, it was later omitted because of life-threatening

Stevens–Johnson syndrome in those with HIV coinfection. STR was also largely replaced by EMB to avoid the requirement of intravenous administration of STR. The culmination of these studies was the introduction of modern "short-course" chemotherapy for drug susceptible TB where PZA forms part of the drug regimen for the first 2 months (the "intensive phase") in combination with INH, EMB, and RIF, but then is not included in a subsequent continuation phase of 4 months of additional treatment with INH and RIF (sometimes in combination with EMB) to obtain a cure rate greater than 95% [31]. With a new, highly effective treatment regimen, the world celebrated the end of the "White Plague" and quickly turned its attention elsewhere.

One could argue, with some justification, that the resulting collapse of research efforts into developing new antitubercular agents in the 1970s and 1980s happened too soon. We still have little idea why the substitution of EMB for PAS allowed the regimen to be shortened from 24 to 18 months, a poor understanding of why adding RIF allowed the regimen to be shortened to 9 months, and no information at all as to why adding PZA allowed treatment to be further truncated to 6 months. Perhaps more importantly, the consequences of widespread global programs of drug treatment in less-controlled environments than the clinical trials supervised by the BMRC were poorly understood. In retrospect, the trial conclusions in the developed world have been borne out; widespread TB epidemics are a thing of the past, and small outbreaks in the USA or Europe are the subjects of alarmed news headlines. Meanwhile, in the developing world, the rise of drug resistance through improper drug usage, poor compliance, and lack of government commitment to eradication programs began in earnest.

1.3 The Emergence of Drug-Resistant TB

As the history of clinical development of TB drugs shows, to limit the risk of developing resistance developing to every new agent, extensive combination therapies were prescribed. The World Health Organization developed strategies to try to avoid the acquisition of resistance, primarily in the form of the "directly observed therapy, short course" (DOTS) which involved implementing a system to monitor patients' ingestion of pills and recording compliance and treatment completion [38, 39]. Central to the DOTS strategy is government commitment to TB control programs, diagnosis of smear-positive TB cases, observed treatment, ensured drug supply, and standardized reporting. While DOTS can be effective and is recommended by the World Health Organization, it is programmatically difficult and expensive. The natural sequence of events then, despite the introduction of both short-course chemotherapy and DOTS, was that treatment became marked by high relapse rates, and the 1990s marked a period of increasingly resistant TB ranging from mono- to multidrug-resistant tuberculosis (MDR-TB). The phrase "MDR-TB" was coined in the 1990s to refer specifically to isolates that had developed resistance to INH and RIF (according to conventional wisdom the two most important

drugs in determining outcome). MDR-TB developed initially by acquisition of resistance during standard treatment as a result of poor compliance or improper chemotherapy with subsequent amplification of resistant populations in treated patients. The development of MDR-TB-infected patients ultimately led to transmission of drug-resistant MTb, first within institutions and hospitals and later in the community [40–43].

Treatment of MDR-TB and higher degrees of resistance has required reintroduction of second-line drugs with unproven efficacy in untested combinations as well as the use of broad-spectrum agents developed for other indications such as fluoroquinolones that have incidental activity against MTb. The treatment of MDR-TB relies upon a backbone of an injectable agent (kanamycin, capreomycin, or amikacin; see Sect. 2.8) [21, 26] and a fluoroquinolone (see Sect. 3.4) [44–48]. The choice of injectable agent and fluoroquinolone for patient treatment is based on drug-sensitivity results from the sputum-borne strain of the patient in question and prior treatment history. Drugs from the first-line agents (EMB, PZA, INH, RIF, and STR) are administered if the strain is sensitive to any of these and combined with second-line drugs (amikacin, kanamycin, capreomycin, viomycin, enviomycin, fluoroquinolones, ethionamide, prothionamide, cycloserine, PAS) with a goal of having five active drugs based on drug-sensitivity results. In cases where extensive resistance does not allow five drugs to be selected from the first- and second-line agents, agents can be selected from non-WHO approved lists of third-line agents (rifabutin, macrolides such as clarithromycin, augmentin, imipenem, clofazimine, linezolid, thioacetazone, and thioridazine have all been reported for such cases). There is minimal data to support the use of the third-line agents [49, 50] with the exception of linezolid (see Sect. 3.3 for further discussion of oxazolidinones) where its use is limited by toxicity and expense [51, 52]. Even in the case of linezolid, the available data are anecdotal and not prospectively collected, but ongoing clinical studies are likely to provide data supporting its use [53, 54]. The duration of treatment for MDR-TB is based on data from the 1950s where 1–2 years of treatment was best at preventing relapse [55]. Treatment is divided into a 6–9-month intensive phase that includes the injectable agent followed by a continuation phase of up to 18 months for a total of 24–30 months of treatment. The injectable agent is stopped to reduce the potential for nephro- and ototoxicity associated with these agents. In the case of uncomplicated MDR-TB, cure rates of greater than 80% have been reported [56].

Following the turn of the century, treatment of MDR-TB with poorly active second- and third-line agents inevitably gave rise to the emergence of extensively drug-resistant tuberculosis (XDR-TB) which has been defined based on the loss of the two components of the MDR treatment backbone perceived to be most important, the fluoroquinolones and the injectable agents [57]. For patients with XDR-TB lucky enough to have access to drug-susceptibility testing and the full suite of second- and third line agents, cure rates now range from 30 to 75% [58–62]. Even more disturbing, there are now reports of totally drug-resistant TB (TDR-TB) for which no chemotherapeutic options remain [63, 64]. The end result of the widespread use of drugs to treat ever-increasingly resistant strains of the organism has been the looming threat of a return to the pre-chemotherapy era. As these strains have evolved, natural selection

will restore whatever fitness costs they incur by acquiring drug resistance, and, ultimately, these strains will emerge in the developed world again. The pace of our discovery efforts has been too slow; we are now approaching a situation where we will have lost all of the achievements of the past. We will need to develop entirely new regimens, and we urgently need to consider the mistakes that were made 40 years ago and devise a strategy to avoid repeating them.

1.4 Special Challenges in TB Drug Development

There are four widely accepted primary objectives for improving TB therapy: (1) shortening and simplifying the treatment for active, drug-sensitive TB, (2) improving treatment efficacy, safety, and duration for drug-resistant disease, (3) improving the safety of co-therapy for TB patients coinfected with HIV, and (4) establishing an effective therapy for latent, persistent TB. The solutions achieving each of these goals will have different features, but there are some overarching issues that complicate each area of concern.

One of the most pressing issues in improving TB chemotherapy involves the use of RIF (see Sect. 2.1 for further discussion of development of RIF as a chemotherapeutic agent), the most effective drug in reducing patient bacillary burdens in the first-line regimen for drug-susceptible TB. Recent studies indicate that treatment outcomes worsen with reduced durations and intermittent use of RIF [65]. Reducing RIF treatment to 1–2 months results in increased rates of relapse and acquired resistance compared to established regimens using RIF for a 6-month period. Additionally, intermittent weekly or twice weekly administrations may promote relapse and acquired resistance. Although RIF is an essential drug in first-line therapy for establishing a positive treatment outcome, use of RIF in combination with various drugs is problematic because of drug–drug interactions as a consequence of RIF's powerful induction of many cytochrome P450 (Cyp) enzymes. These enzymes metabolically inactivate other drugs thereby reducing effective serum concentrations and exposure. RIF particularly induces cytochrome P450 (CYP) 3A4, the most abundant enzyme found in the liver and the gut, which metabolizes drugs and toxins [66]. RIF is also associated with upregulation of membrane transporters (P-glycoproteins) that regulate transport of substances across membranes, which often function as cellular efflux pumps thereby limiting bioavailability of drugs [67]. HIV-coinfected patients receiving antiretrovirals whose serum levels are known to be affected by RIF induction of CYP3A4 are sometimes provided rifabutin as a substitute for RIF. Rifabutin has reduced CYP3A4 induction and thus simplifies co-therapy; however, current clinical evaluation does not fully support substitution of RIF with rifabutin [68]. Therefore, any new agent introduced for drug-susceptible disease will likely have to be introduced in combination with RIF and against a background of strong induction of CYP 3A4.

A further complication in TB drug metabolism is malabsorption [69]. Patients presenting with TB are often malnourished; weight loss is a hallmark of the disease.

Sometimes this is linked to advanced HIV disease where patients are malnourished or have diarrhea, but it is also a common complication for patients with diabetes mellitus (DM), another frequent comorbidity of TB patients [69–71].

A particular challenge in the development of new TB drugs is the heterogeneity of TB pathology [2]. TB shows not only the differences in clinical manifestation but also the underlying host and pathogen physiology, which poses particular challenges to antitubercular drug development. Human TB patients harbor a variety of granulomas resulting in different microenvironments to which the resident MTb is exposed. Thus, the metabolism of MTb in each lesion is likely to be different. The presence of discrete populations of MTb in the human host possessing different susceptibilities to antitubercular drugs might explain the combined activity of frontline chemotherapy [72]. One theory (supported by virtually no hard evidence) proposes that rapidly growing bacilli are cleared by drugs such as INH, while sporadically replicating intracellular organisms are killed more efficiently by drugs such as RIF and the more slowly dividing bacteria within acidic environments are selectively sensitive to PZA [72]. Furthermore, it is the eradication of the slowly growing and non-replicating bacilli which requires such an extended duration of chemotherapy. PZA is often put forward as a paradigm for a drug that has the highest capacity to reduce treatment duration. Importantly though, PZA has little or no in vitro activity (except under conditions where the bacteria is acid-stressed), and the precise mechanism of action of this drug remains unclear [72]. The antitubercular activity of PZA was discovered only because it was applied directly to infected mice, an impractical strategy for evaluating large numbers of compounds. Strictly in vitro, a variety of different growth conditions have been shown to result in alterations in the susceptibility of MTb to different drugs; for example, stationary phase [73, 74], anoxia [75], and nutrient deprivation [76, 77] all provide models of treatment-refractory disease, yet none of these has been validated as meaningful in predicting clinical efficacy.

Finally, although serum pharmacokinetic data are widely available for many TB drugs in use and development, TB is not a systemic bacteremia and tissue concentration studies are scarce. Drug penetration is likely to be limited due to tissue damage from disease and the loss of vasculature making primary sites of infection difficult to saturate [78]. A truly effective compound must not only be able to penetrate the bacterial cell wall, but also be able to reach the bacteria within a fibrous, necrotic, or cavitary lesion that may harbor the persistent organisms [78, 79].

2 The Development of Commonly Used First-Line and Second-Line Agents for TB Therapy

2.1 Rifamycins

The rifamycin antibacterials represent one of the most effective and widely used classes of therapeutic compounds used in modern TB treatment. The first of the rifamycins, rifampicin (RIF, Fig. 3; **10a**), was introduced into TB chemotherapy in

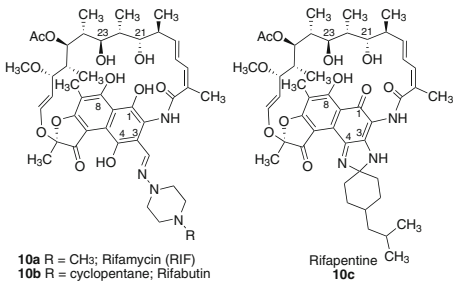


Fig. 3 Clinically used rifamycins

the 1960s after extensive structure–activity relationship (SAR) studies performed on rifamycin B, the natural product produced by *Amycolatopsis mediterranei,* from which the rifamycins were derived [80]. This isolated natural product was only active when delivered intravenously, and attaining oral bioavailability of rifampicin required a considerable effort because of the complex chemistry of this scaffold. Shortly thereafter, other rifamycin derivatives, rifabutin (Fig. 3; **10b**) and rifapentine (Fig. 3; **10c**), were developed and currently serve as alternatives to RIF. The rifamycin-derived antituberculous agents all share a general structure characterized by a naphthalene core that is spanned by a 19-atom polyketide bridge. SAR studies have established the role of the aliphatic bridge in stabilizing the overall conformation of the molecule and positioning the C(1) and C(8) phenols and the C(21) and C(23) hydroxyl groups for interaction with their bacterial target, RNA polymerase [81]. As such, modification of the phenol or hydroxyl groups in these positions abolishes antibacterial activity of the molecule. Conversely, modifications made at the C(3) and C(4) positions have been the focus of many efforts to improve the oral bioavailability of the rifamycins, since the C(3) appendages do not appear to interfere with rifamycin-RNA polymerase binding [82].

The primary mode of action of the rifamycins involves disruption of RNA transcription through binding of the drug to bacterial DNA-dependent RNA polymerase [83]. Accordingly, resistance to the rifamycins occurs primarily through point mutations acquired in the RNA polymerase β-subunit gene, *rpoB* [83]. Resistance may also occur through ADP-ribosylation of the alcohol at position C(21) [84].

The most common adverse effects associated with rifamycin therapy are mild influenza-like symptoms, hepatotoxicity, and altered liver function. Additionally, due to the furanonapthoquinone chromophore within the rifamycin structure, bodily fluids (e.g., sweat, tears, or urine) may take on an orange-red color. As discussed in Sect. 1.4, rifamycins may also have adverse interactions with other coadministered drugs, in particular antiretroviral drugs (ARDs). Of the three aforementioned rifamycins, RIF is the most potent inducer of CYP3A and rifabutin is the least [85], making it the preferred rifamycin derivate for treating HIV-TB coinfected patients [86].

2.2 *Isoniazid*

INH (Fig. 4; **3a**) is an analog developed from the antitubercular drug thiacetazone (Fig. 4; **3b**) which had been used effectively in TB patients in the 1940s but was associated with toxic side effects [87]. In an attempt to improve thiacetazone (**3b**), the phenyl ring was replaced with a pyridine ring based on the observation that nicotinamide (Fig. 4; **3c**) had a growth inhibitory effect on MTb. The isonicotinaldehyde thiosemicarbazone (Fig. 4; **3d**) proved to be more active than thiacetazone, which inspired evaluation of other intermediates in the synthesis, leading to the discovery of isonicotinic acid hydrazide (INH, **3a**) the best antitubercular agent developed to date.

Hundreds of derivatives of INH have been synthesized since its original discovery, but none improved on the activity of INH. *N*-acetyl-INH, an INH metabolite produced in humans, is inactive, although *N*-alkyl derivatives such as iproniazid (Fig. 4; **3e**) and hydrazones such as verazide (Fig. 4; **3f**) show in vivo efficacy although the active metabolite in MTb is INH which is released by in vivo hydrolysis [88–91].

The minimum inhibitory concentration (MIC) of INH is 0.2 μM against rapidly growing MTb, with lower activity against slowly growing MTb and practically no in vitro activity against anaerobically adapted bacteria [92]. INH is a prodrug that is activated by the KatG catalase to an isonicotinoyl radical that reacts with nicotinamide-containing molecules such as NAD(P) to yield acyclic isonicotinoyl-NAD(P) adducts and their cyclic hemiamidals. The INH-NAD adduct is a potent inhibitor of the NADH-dependent enoyl-ACP reductase, InhA, involved in mycolic acid biosynthesis [93–95]. Mutations in *katG* or *inhA* confer the majority of resistance, but other resistant isolates show mutations at targets that use pyrimidine nucleotides (which are structurally similar to adducts formed during INH activation) [96]. Isoniazid is well tolerated although side effects as a result of hepatic enzyme abnormalities resulting in hepatitis occur (especially in older patients). Also, peripheral neuritis can occur but is easily prevented by pyridoxine administration.

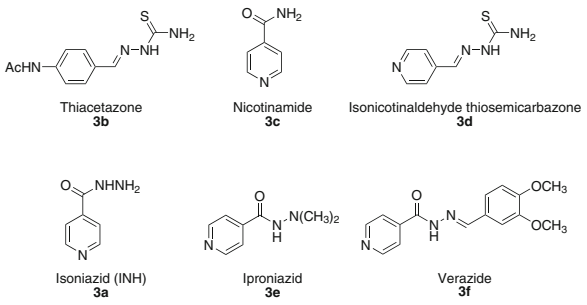


Fig. 4 Thiacetazone derivatives leading to isoniazid (INH)

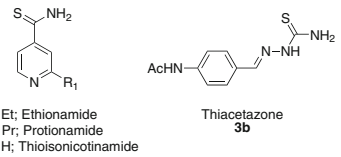


Fig. 5 Thioisonicotinamide Derivatives

2.3 *Thioisonicotinamides and Thiosemicarbazones*

The thioisonicotinamides, ethionamide (Fig. 5; **5a**) and prothionamide (**5b**) were discovered during efforts to improve on the MTb inhibitory activity of nicotinamide. Thioisonicotinamide (Fig. 5; **5c**) showed better in vivo efficacy than in vitro efficacy [97] which prompted further SAR studies on this series resulting in the observation that 2-alkyl derivatives were more active than the parent nicotinamide with 2-ethyl and 2-propyl derivatives showing the best activity. Ethionamide (**5a)** is a prodrug that is activated by *S*-oxidation by a monoxygenase (EtaA) to a 4-pyridylmethane radical intermediate that, similar to the active radical produced from INH by KatG (discussed in Sect. 2.2), reacts with NAD(P) to form a tight-binding inhibitor of InhA [98, 99]. The sulfoxide, the major metabolite produced in humans, is also active against MTb. The thioisonicotinamides have unpleasant gastrointestinal side effects.

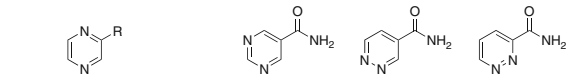
Thiacetazone (Fig. 5; **3b**) was discovered to have antitubercular activity in the 1940s and was used as an antitubercular agent despite its toxic side effects [100, 101]. Thiacetazone, similar to the thioisonicotinamides, is activated by EthA resulting in a reactive intermediate that inhibits mycolic acid oxygenation as well as cyclopropanation [102, 103]. Thiacetazone causes gastrointestinal disturbances and, particularly in HIV-infected patients, can cause severe life-threatening skin reactions known as Stevens–Johnson syndrome [104].

2.4 *Pyrazinamide*

PZA (Fig. 6; **7a**) was developed based on reports describing the antitubercular activity of vitamin B3 (niacin) [105]. It is unlikely that PZA would be discovered in modern drug discovery programs since it has no activity against MTb under normal in vitro growth conditions although it has good activity in infected animals [106, 107].

Initial SAR studies [106–109] were performed by in vivo assays of derivatives of nicotinamide (**3c**) and PZA in infected mice. The presence of a pyrazine heterocycle with a carboxamide at the C(2) position was essential for activity. Modification of the carboxamide to tetrazole, nitrile, hydrazide, or carboxylic acid (Fig. 6; **7b–e**) leads to completely inactive compounds in vivo. Substitutions on the amide nitrogen with either a methyl (Fig. 6; **7f**) or an acetyl group (Fig. 6; **7g**) were detrimental

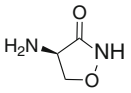
Fig. 6 Derivatives of PZA screened in the murine model of TB



7a R = $CONH_2$; Pyrazinamide (PZA)
7b R = tetrazole
7c R = CN
7d R = $CONHNH_2$
7e R = CO_2H
7f R = $CONHCH_3$
7g R = CONHAc
7h R = CO_2Pr
7i R = $CONHCH_2NEt_2$
7j R = CONH-*N*-(morpholinomethyl)
7k R = $CSNH_2$

7l **7m** **7n**

Fig. 7 Cycloserine



D-cycloserine
4a

to activity. Pyrazinoic acid (**7e**) is considered to be the active metabolite from PZA; hence, various ester derivatives (e.g., **7h**) were synthesized and found to be active in vitro but inactive in vivo probably due to premature hydrolysis or poor solubility. However, more stable aminomethylene prodrugs (**7i** and **7j**) did not show improvement in activity presumably because they were not substrates for the amidase. The thioamide (**7k**), pyrimidine nucleus (**7l**), and the pyridazine nucleus (**7m,n**) were inactive or weakly active. Thus, PZA is the minimum pharmacophore; further substitutions on the amide or changes to the pyrazine ring are detrimental to activity.

Pyrazinamide likely kills MTb by intracellular acidification following hydrolysis by MTb nicotinamidase/pyrazinamidase [110], although inhibition of fatty acid synthase has also been proposed as a mechanism [111–113]. PZA increases serum uric acid concentrations thereby causing nongouty arthralgia and, when used in combination with INH and/or RIF, often causes some hepatotoxicity.

2.5 Cycloserine

D-cycloserine (Fig. 7; **4a**) is an antibiotic produced by *Streptomyces* sp. that is currently used in second-line TB therapy [114]. The isoxazolidinone is the pharmacophore of D-cycloserine, and attempts to modify it with additional substituents have been unsuccessful since *N*-substitution prevents the tautomerization which is necessary for its activity, and replacement of the heteroatoms on the isoxazolidinone ring leads to dramatic loss of activity [115, 116]. In addition, the stereochemistry is essential since the L-isomer is inactive [116].

D-cycloserine prevents D-alanine incorporation into the bacterial cell wall peptidoglycan by forming an irreversible isoxazole-pyridoxal adduct in the enzyme

alanine racemase, which converts L-alanine to D-alanine [117]. Although the major target in MTb is alanine racemase, D-cycloserine also inhibits the D-alanine–D-alanine ligase involved in synthesis of the terminal D-alanine–D-alanine of the peptidoglycan UDP-*N*-acetylmuramyl-pentapeptide [118]. The MIC of this antibiotic against MTb is about 100 μM. Because of the side effects observed with D-cycloserine (CNS effects and hypersensitivity), it is often given at more frequent but lower doses in TB patients as second-line therapy.

2.6 *Para*-Aminosalicylic Acid

The success of early clinical trials of PAS (Fig. 8; **2a**) in TB patients [119] prompted synthesis of analogs to enhance the activity of the parent compound. These analogs showed that PAS exhibits very specific SAR [120].

The mechanism of action of PAS is not fully understood, although folate biosynthesis has been proposed as the target, since inactivation of thymidylate synthase confers resistance [121]. PAS is generally poorly tolerated in patients due to gastrointestinal disturbances often leading to discontinuation of PAS administration.

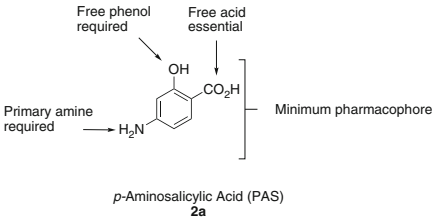


Fig. 8 SAR of *p*-aminosalicylic acid (PAS)

2.7 *Capreomycin*

Capreomycin is synthesized by *Saccharothrix mutabilis* subsp. *capreolusa* as a mixture of four related cyclic pentapeptides, capreomycins IA (Fig. 9; **6b**), IB (Fig. 9; **6c**), IIA (**6d**), and IIB (**6e**). The peptide backbone is made up of 15 unnatural

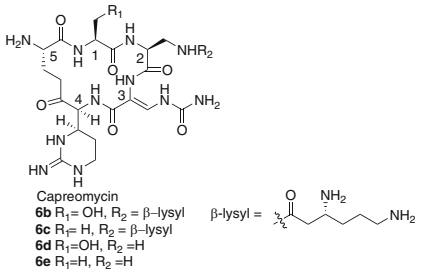


Fig. 9 Capreomycin

amino acids and either L-serine or L-alanine, known as Capreomycin A and B, respectively.

A few limited SAR studies [122, 123] have shown that both alanine and serine at position 1 have antitubercular activity, that small ureido modifications such as *N*-methyl groups (but not *N*,*N*-dimethyl) are acceptable, whereas *N*-aryl ureido substitution increases general antibacterial activity. Capreomycin inhibits protein synthesis by binding at the interface between helix 44 of the 30S subunit and helix 69 of the 50S subunit of the bacterial ribosome [124]. Like the aminoglycosides with which it is often confused, capreomycin has both nephro- and ototoxic side effects.

2.8 Aminoglycosides

Streptomycin (STR, Fig. 10; **1a**), kanamycin (KM, Fig. 10; **1b–d**), and amikacin (AK, Fig. 10; **1e**) (Fig. 8) comprise the main aminoglycosides used in TB chemotherapy. As discussed in Sect. 1.2, the aminoglycosides are still widely used in modern TB drug regimens although mainly as second-line agents. The general structure of the aminoglycosides is characterized by an aminocyclitol ring connected to one or more amino sugars by a glycosidic linkage. The second generation aminoglycosides KM and AK were largely developed to circumvent resistance mechanisms in other bacteria, not specifically for MTb; hence, their SAR will not be discussed.

This class of antitubercular compounds primarily acts by binding to the 16S rRNA of the bacterial 30S ribosomal subunit, which interferes with protein synthesis and ultimately leads to cell death [125]. As such, resistance mechanisms observed in clinical isolates have principally been the acquisitions of mutations in the 16S rRNA gene (*rrs*) and in genes that encode for proteins that interact with the 16S rRNA in the region where the drug binds [125–129]. Alternative resistance mechanisms that have been reported include drug efflux and inactivation by aminoglycoside-modifying enzymes, but there is little evidence to suggest these are clinically relevant [130–132]. Common adverse effects associated with aminoglycoside therapy include nephro- and ototoxicity [133, 134].

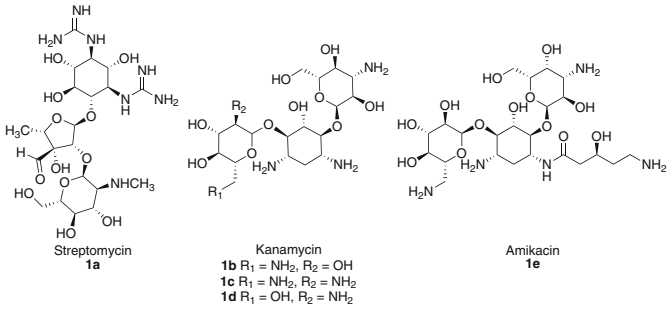


Fig. 10 Aminoglycoside antibiotics

3 Classes of Compounds in Clinical Development

3.1 Nitroimidazoles

3.1.1 History

Nitroimidazoles are a class of compounds with growing importance in the field of tuberculosis chemotherapy. Nitroimidazoles show better activity against obligate anaerobes than aerobic organisms because their bactericidal activity requires a bioreduction of the aromatic nitro group whose reduction potential lies beyond the reach of eukaryotic aerobic redox systems [135, 136]. 2-Nitroimidazole derivatives modified at the 1- and 5-positions were among the first series (Fig. 11) of this class reported to display antimycobacterial activity [137].

Further improvement in antimicrobial activity was gained by lowering the reduction potential by changing from 2-nitro- to 5-nitroimidazole derivatives. A notable example in this class is metronidazole (Fig. 12; **11a**), which was the lead compound from a screen of over 200 derivatives of azomycin (2-nitroimidazole) for antitrichomonal activity at the French pharmaceutical company Rhône-Poulenc in the mid-1950s [136, 138]. Metronidazole (**11a**), which is bactericidal against anaerobic non-replicating Mtb in vitro and in hypoxic granulomas in vivo (as well as other anaerobic bacteria and protozoa) [79, 136], has been in clinical use for four decades and is listed in the essential drug list by the WHO [139]. In 1989, Ciba Geigy India was the first to report antitubercular activity from a series of bicyclic 4- and 5-nitroimidazole [2, 1-*b*]oxazoles. Their lead compound CGI-17341

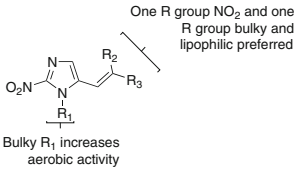


Fig. 11 Early SAR for nitroimidazoles

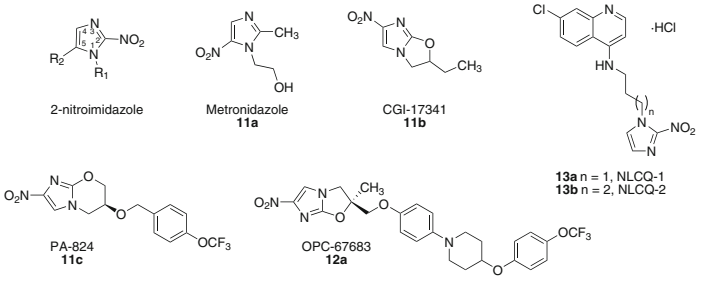


Fig. 12 Nitroimizadoles with antitubercular activity

(Fig. 12; **11b**) was active against drug susceptible and MDR-TB (MIC of 3.3 μM) [140] and showed dose dependency in a mouse model but was not further developed due to its mutagenicity in the bacterial Ames assay.

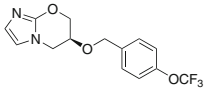
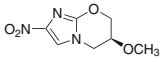
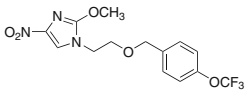
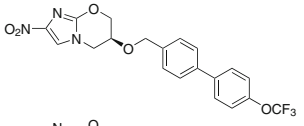
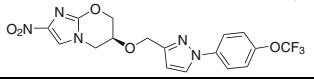
The bicyclic 5-nitroimidazole [2,1-*b*]oxazole series showed much lower activity than its 4-nitro counterpart [141]. A decade later, PA-824 (Fig. 12; **11c**), the lead compound from a series of more than 300 nitroimidazooxazine derivatives [142], and OPC-67683 (Fig. 12; **12a**), the lead compound from a series of nitroimidazooxazole derivatives [143], were discovered by PathoGenesis (now Novartis) and Otsuka Pharmaceutical Co. Ltd, respectively. Both compounds showed increased activity against MTb with potential to decrease the current treatment duration.

Most recently, two nitroimidazo-chloroquine derivatives NLCQ-1 (Fig. 12; **13a**) and NLCQ-2 (Fig. 12; **13b**), which are also prodrugs requiring bioreductive activation, have been reported to show a twofold increase in bactericidal activity against non-replicating MTb compared to PA-824 [144].

3.1.2 SAR of Nitroimidazooxazines

PA-824 shows bactericidal activity against drug susceptible (MIC range 0.04-0.8 μM) and resistant (MIC range 0.08–1.5 μM) MTb strains [142]. SAR studies show that the key features responsible for aerobic activity are the nitro group at the 4-position of the imidazole ring (Table 1, Entry 1), the conformationally rigid bicyclic system (Table 1, Entry 3) and the lipophilic tail at the 6-position of the oxazine ring (Table 1, Entries 4 and 5) [145–147]. Antitubercular activity was seen with a biaryl linker (*para* > *meta* > *ortho*), but these compounds exhibited poor solubility in most cases [146]. Heterobiaryl analogs improved solubility over biaryl linkers, and varying lengths of hydrophobic regions at the 6-position of the oxazine

Table 1 SAR of PA-824 [145–147]

Entry	Compound name	Structure	MIC against H37Rv (μM)
1	**11d**		>160
2	**11e**		>125
3	**11f**		6.25
4	**11g**		0.04
5	**11h**		0.05

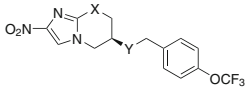
ring were well tolerated [148] indicating the presence of a large hydrophobic pocket in the active site of Rv3547 (see below for further discussion of mode of action).

The oxygen at 2-position of the nitroimidazole ring could be substituted with nitrogen or sulfur with equipotent aerobic activity, but acylation of the nitrogen, oxidation of sulfur, or replacement of oxygen by a methylene lead to decreased activity (Table 2) [148]. The *S*-enantiomer is more than 100-fold more active than the *R*-enantiomer. Replacement of the benzylic ether at the 6-position with an amine marginally increased activity and improved water solubility [148]. Overall SARs for PA-824 are summarized in Fig. 13.

3.1.3 Biology of Nitroimidazooxazines

Deazaflavin (F_{420} cofactor)-dependent nitroreductase (Ddn) Rv3547 is responsible for the reductive activation of the pro-drug PA-824 (**11c**), generating a reactive nitrogen species (likely $^{\cdot}NO$), production of which correlates with the cidal activity toward anaerobic non-replicating MTb [149, 150]. PA-824 has been shown to inhibit cell wall lipid and protein biosynthesis in a dose-dependent manner

Table 2 SAR on heteroatoms of oxazine ring [148, 149]



Entry	Compound name	X	Y	MIC against H37Rv (μM)
1	PA-824 (**11c**)	O	O	0.80
2	**11i**	CH_2	O	25
3	**11j**	NH	O	0.8
4	**11k**	NAc	O	6.25
5	**11l**	S	O	0.8
6	**11m**	SO_2	O	>100
7	**11n**	O	NH	0.31
8	**11o**	O	$NHCO_2$	0.05
9	**11p**	O	$NH(CH_2)_2$	0.08
10	**11q**	O	$NH(CH_2)_4$	0.08

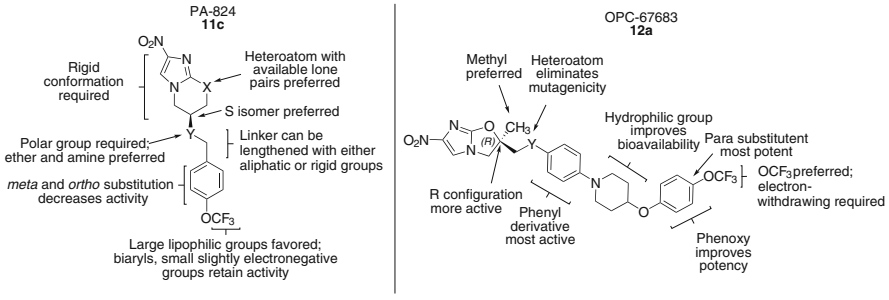


Fig. 13 SAR for PA-824 and OPC-67683 series

[142, 151]. There is poor correlation between aerobic and anaerobic activity, and transcriptional profiling analysis suggests that both respiratory inhibition and inhibition of cell wall biosynthesis are related to the activity of PA-824 [152]. Studies in mice found the daily minimal effective dose and minimal bactericidal dose of PA-824 to be 12.5 mg/kg and 100 mg/kg, respectively, and it exhibited potent bactericidal activity in both the initial and continuation phases of treatment [153]. A combination of PA-824, moxifloxacin, and PZA was shown to cure mice more rapidly than standard regimen of RIF/INH/PZA [154].

3.1.4 Clinical Use of Nitroimidazooxazines

PA-824 has oral bioavailability (subdose proportional) and a relatively long half-life (16–20 h in humans) consistent with once a day regimen [155]. Clinical studies showed that even though PA-824 inhibits excretion of creatinine at high dosage, it did not affect the glomerular filtration rate, thereby limiting concerns about nephrotoxicity [156]. It is non-mutagenic, shows no cross-resistance with current drugs, and can be coadministered with antiretroviral agents. Phase IIa clinical studies on patients with newly diagnosed, uncomplicated, smear-positive, pulmonary tuberculosis[157] ascertained PA-824 to be safe and well tolerated for 2 weeks with daily dosing varying from 200 to 1,200 mg/day in which time-frame decrease of bacillary burdens in sputum was observed [158].

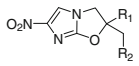
3.1.5 SAR of Nitroimidazooxazoles

OPC-67683 (Fig. 12; **12a**) shows potent antitubercular activity against both replicating and non-replicating bacteria and is equipotent against drug-resistant MTb [143, 159]. Derivatives of these 6-nitro-2,3-dihydroimidazo [2,1-*b*]oxazoles were not mutagenic in contrast to the structurally similar CGI-17341 (Fig. 12; **11b**), with heteroatoms at the side chain of 2-position contributing to the absence of mutagenicity. Addition of a methyl group at the 2-position was found to improve activity, and the absolute stereochemistry was found to be critical with the *R*-enantiomer (MIC of 180 nM) being 60-fold more active than the *S*-enantiomer (Table 3). Subsequent development of *R*-enantiomers of 6-nitro-2,3-dihydroimidazo [2,1-*b*] oxazole culminated in identification of lead compound OPC-67683 [159]. Figure 13 compares the SAR for both the oxazine and the oxazole series of antitubercular nitroimidazoles.

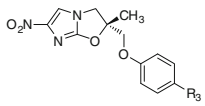
3.1.6 Biology of Nitroimidazooxazoles

OPC-67683 has an MIC of 20 nM, which is more potent than any other nitroimidazole and does not show cross-resistance with currently used antitubercular drugs. It is active against intracellular MTb in a dose-dependent fashion with OPC-67683 being superior to INH and as effective as RIF [160]. In MTb-infected mice,

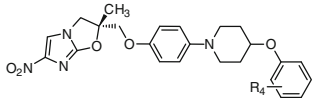
Table 3 SAR of OPC-67683 [159]



R_1	R_2	Configuration	MIC (μM)
H	OPh	Racemic	2.98
CH_3	OPh	(*R*)	0.18
CH_3	OPh	(*S*)	11.37



R_3	MIC against MTb strains (μM)		
	H37Rv	H37Rv INH resistant	H37Rv RIF resistant
H	0.18	0.18	0.18
Cl	0.08	0.04	0.02
CF_3	0.58	0.58	0.29
OCF_3	0.56	1.09	0.56
	2.18	1.09	1.09
	2.16	1.08	1.08
	2.07	1.04	0.53



R_4	MIC against MTb strains (μM)		
	H37Rv	H37Rv INH resistant	H37Rv RIF resistant
H	0.87	0.87	0.44
p-Cl	0.10	0.10	0.05
p-F	0.83	0.83	0.43
p-OCF_3	0.01	0.01	0.01
o-OCF_3	0.73	0.73	0.37
m-OCF_3	0.04	0.04	0.04

a combination of OPC-67683 (2.5 mg/kg) with RIF and PZA showed faster rate of MTb clearance from organs than a standard regimen of RIF, PZA, ETB, and INH [143]. OPC-67683 is a prodrug that is likely activated by the same nitroreductase as PA-824 (Ddn/F_{420} reductase) [141, 143]. Similar to PA-824, it is an inhibitor of methoxy- and keto-mycolic acid synthesis, which is essential for biosynthesis of the cell wall [161].

3.1.7 Clinical Use of Nitroimidazooxazoles

OPC-67683 shows a half-life in mouse plasma of 7.6 h with a C_{max} of 0.55 μM (6 h, 2.5 mg/kg) [143]. OPC-67683 is not metabolized by liver microsome enzymes,

making it suitable for coadministration with drugs that induce cytochrome P450 enzymes. It is absorbed better with a high fat diet and up to 400 mg/day can be tolerated safely without adverse side effects by healthy volunteers [162]. OPC-67683 is less effective than PA-824 in reducing sputum-borne acid-fast bacilli in the first 4 days of treatment in patients with pulmonary TB. This drug is under development and is currently in the phase II clinical trials for use against MDR-TB [163].

3.2 Diarylquinolines

3.2.1 History

The diarylquinoline TMC207 (Fig. 14; **14a**), first reported in 2005, is the first known antitubercular compound in the diarylquinoline class [164] (for additional details, see [165, 166]). Tibotec, a subsidiary of Johnson and Johnson, has reported the vast majority of research and development on TMC207, although recent efforts by Chattopadhyaya and coworkers have contributed new related compounds [167, 168]. TMC207 was one of the lead compounds discovered in a high-throughput screen for compounds with activity against *Mycobacterium smegmatis* (a nonpathogenic rapid-growing mycobacterium), which were subsequently evaluated against MTb [164].

3.2.2 SAR of TMC207

Correct absolute and relative configuration of the two stereocenters of TMC207 (Fig. 14; **14a**), which have been assigned by NMR and X-ray crystallographic analysis [169, 170], are required for activity [171, 172]. Sterically undemanding functional groups can be substituted for the bromine on the quinoline ring without significant loss of activity, although a bromine atom appears to be preferred. The naphthyl substituent can be replaced with other electron-poor aryl groups and still maintain good activity against MTb. Based on initial reports, the dimethyl-substituted tertiary amine appears to be required for activity, with the replacement of one methyl substituent with a proton or ethyl substituent resulting in a decrease in activity [171]. However, more recent reports suggest that the *N*-monodesmethyl

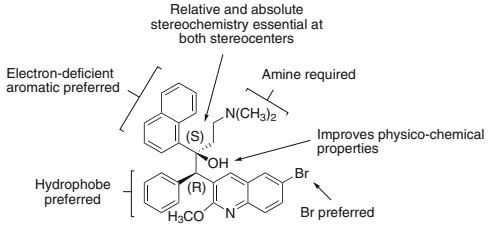


Fig. 14 SAR of TMC207

metabolite of TMC207 produced by oxidation by CYP3A4, a cytochrome P450 that is potently induced by RIF, maintains significant antitubercular activity [173].

3.2.3 Biology

TMC207 is highly specific for mycobacteria [172]. Both H37Rv and clinical isolates show MICs in the range of 54–217 nM. TMC207 targets the c subunit of ATP synthase (*atpE* gene), a mechanism of action distinct from fluoroquinolones and other quinoline derivatives [164, 174, 175]. Docking studies have suggested the tertiary amine of TMC207 serves as an arginine mimic, allowing the compound to disrupt the proton transport chain of ATP synthase [168, 176]. Point mutations in *atpE* confer resistance; these mutations occur at a rate of one in 10^7 to 10^8, similar to the bacterial mutant frequency of rifampicin resistance [164].

Initial in vivo studies showed encouraging results. Treatment of MTb-infected mice exclusively with TMC207 at 25 mg/kg was as effective as triple combination therapy of RIF/INH/PZA [164]. Also in mice, the addition of TMC207 to standard MDR-TB regimens showed an improved cure rate over the standard regimen alone [177]. TMC207 acts synergistically with PZA in mice [178]. In guinea pigs, treatment with TMC207 for 6 weeks resulted in almost complete eradication of MTb bacilli from lesions [179]. Furthermore, TMC207 has also been shown to be bactericidal in vitro against non-replicating MTb, suggesting that TMC207 might prove therapeutically effective against latent tuberculosis [180].

Also, a once-weekly schedule of administration of TMC207/rifapentine/PZA tested in mice was more active than the standard regimen of RIF/INH/PZA given daily [181]. Because TMC207 has a long half-life in humans (44–64 h in plasma) [164], once-weekly tuberculosis treatments might one day be possible. However, metabolism of TMC207 is enhanced by the presence of RIF, suggesting that coadministration of these drugs might not be straightforward [182].

3.2.4 Clinical Use

In humans, C_{max} is reached in 4–5 h [164, 173, 183], and a daily dose of 400 mg administered daily for 7 days results in a C_{max} of 10 μM [183]. A steady-state concentration of 1 μM, which appears to be required for bactericidal activity [183], can be maintained with a dosing schedule of 400 mg daily for 2 weeks followed by reduced doses of 200 mg three times weekly [173]. Adverse events occurred at a low rate and side effects were considered mild to moderate [164, 173, 183].

In preliminary clinical trials, TMC207 showed significant bactericidal activity after 4 days of a 7-day trial treating previously untreated TB patients, although onset of bactericidal activity was delayed in comparison to RIF and INH [173]. In 2009, the first stage of a phase II trial testing TMC207 in combination with a standard, five-drug, second-line antituberculosis regimen in MDR patients showed that after 8 weeks of treatment, 48% of study participants receiving the TMC207 regimen converted to negative sputum culture, compared with 9% of those on the

standard regimen [183–185]. Additional trials are ongoing in MDR patients [185], and TMC207 is undergoing further development for drug-susceptible TB [186].

3.3 Oxazolidinones

3.3.1 History

Oxazolidinones are a new structural class of synthetic antibacterial drugs. Reports of structurally novel anti-infectives by DuPont (Fig. 15; **15a,b**) in the mid-1980s [187] drew the interest of researchers at the former Pharmacia and Upjohn Inc. (now Pfizer) [188–190]. Two lead compounds, eperezolid (Fig. 15; **15c**) and linezolid (LZD, Fig. 15; **15d**) [191], proved to be exceedingly effective wide-spectrum drugs, although LZD was better tolerated in clinical trials. Further development of the oxazolidinone scaffold has yielded PNU-100480 (**15e**) [191], a linezolid analog currently in phase II clinical trials [192, 193], as well as DA-7218 (Fig. 15; **15g**) and its metabolite DA-7157 (Fig. 15; **15h**), which are in preclinical development (Dong-A Pharmaceuticals, Ltd.) [194]. Ranbaxy Laboratories Limited (acquired in 2008 by Daiichi Sankyo Company) has also made contributions in the form of RBx-7644 (**15i**) and its more potent analog RBx-8700 (**15j**) [195], which are in preclinical development. Additionally, AstraZeneca has developed two oxazolidinones, AZD2563 (**15k**) [196] (discontinued at preclinical stage) and AZD5847 (structure not yet available) which is starting phase II clinical trials [197, 198].

3.3.2 Structure–Activity Relationship

Because the oxazolidinones were not developed specifically to treat TB, their SARs have been developed mostly against a number of Gram-positive and Gram-negative bacteria, and little is known about TB-specific SAR. DuPont was the first to publish

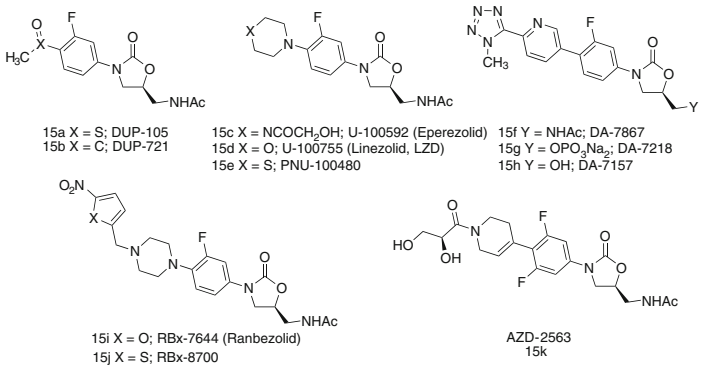


Fig. 15 Oxazolidinones

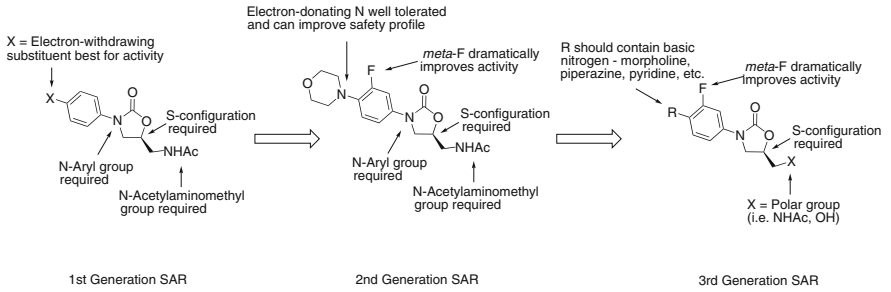


Fig. 16 Evolution of SAR in oxazolidinone class of antibacterials

Table 4 MIC of various oxazolidinone candidates against TB

Drug candidate	MIC against H37Rv (μM) [Ref.]	MIC against RIF or INH-resistant clinical isolates (μM) [Ref.]	MIC against RIF and INH-resistant clinical isolates (μM) [Ref.]
Linezolid (**15d**)	0.74 [204]; 17 [205]	1.40 [204]; 13 [205]	1.24 [204]; 2 [205]
DA-7867 (**15f**)	–	0.15 [206]	0.15 [206]
DA-7157 (**15h**)	–	–	0.25 [207]
DA-7218 (**15g**)	–	–	0.25–1 [207]
RBx-8700 (**15j**)	–	0.09 [205]	0.34

their conclusions about the structural motifs required for antibacterial activity (Fig. 16) [199–201]. These relationships were further refined during the development of eperezolid and LZD [202]. Finally, the development of the RBx and DA compounds has expanded the limits of the functional groups that display antitubercular activity [203].

Activities of the oxazolidinones against TB are shown in Table 4. LZD (Fig 15; **15d**) has an MIC against first-line susceptible TB strains of 1.55 μM [204]. For the DA class of compounds, which contain a triazole as the basic side chain, DA-7867 (Fig 15; **15f**) proved to be poorly water soluble; hence, a water-soluble prodrug DA-7128 (Fig 15; **15g**, which is metabolized to DA-7157, Fig 15; **15h**) was developed. Interestingly, against MTb, prodrug DA-7128 performed similar to its (usually more active) metabolite against MTb, giving an MIC of 0.25 μM [207].

3.3.3 Biology

The oxazolidinones inhibit bacterial protein synthesis by binding to the bacterial 23S rRNA of the 50S subunit, [208, 209] which blocks the interaction between charged tRNAs at the P site and the A site (Fig. 16) [210]. Specifically, LZD disrupts initiation of protein synthesis by inhibiting peptide bond formation between the carboxyl terminus of the *N*-formylmethionine–tRNA complex residue bound at the P site

and the amino terminus of the amino acid-tRNA bound at the A site [210]. Crystal structures show LZD bound near the A site of the 50S ribosomal subunit at the 23S rRNA in such a way that peptide bond formation should be inhibited [211].

LZD-resistant tuberculosis has been observed in both following in vitro selection [212] and clinical strains (occurring only rarely) [213] and may arise through an active efflux system [214]. Many reports have shown that LZD is effective against MDR-TB both in vitro [215, 216] (MIC < 24 μM) and in vivo [51, 217, 218].

3.3.4 Clinical Use

For DA-7867 (**15f**), oral bioavailability in rats is 70.8%, with 8.3% not absorbed and 21.8% eliminated by intestinal first-pass metabolism [194]. LZD (**15d**) has nearly 100% bioavailability (regardless of whether or not it is taken with food [219]), and its half-life of 5.40 ± 2.06 h [220] allows for a 12-h dosing schedule [221]. In healthy human subjects, steady-state plasma concentrations of 63 ± 17 μM are obtained at T_{max} of 1.03 ± 0.62 h [220]. LZD's major metabolites (Fig. 17; **15l**, **15m**) are formed via oxidation alpha to the morpholine ring heteroatoms followed by ring opening [220]. It is metabolized through hepatic oxidation (and thus should not affect drugs metabolized by cytochrome P450 enzymes); hence, doses do not have to be altered for patients with renal or hepatic impairment [221]. LZD does not show suppressed antibiotic activity when coadministered with other antibiotics [221] and even shows synergistic activity with fluoroquinolones and RIF [222]. PNU-100480 was studied in healthy volunteers (phase I clinical trials)[192, 193] and appeared to be well tolerated at doses of 1,000 mg/day [216]. Additionally, a whole blood assay against MTb showed PNU-100480 to be more effective

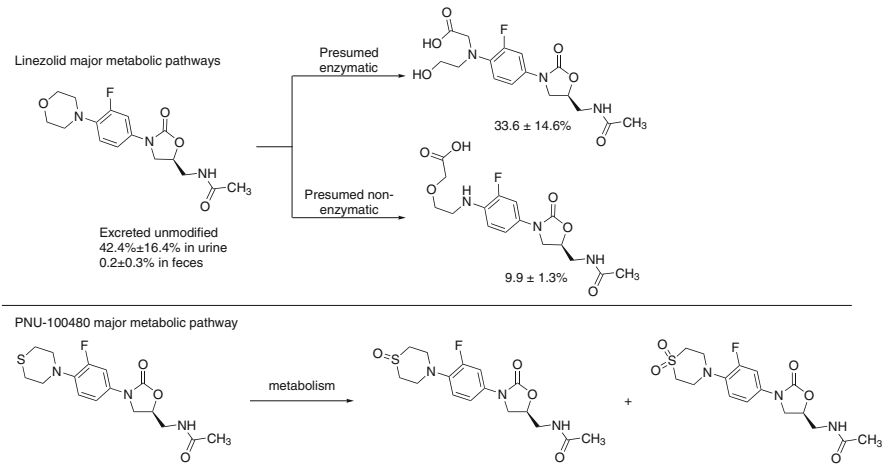


Fig. 17 Metabolism of LZD (*upper*) [220] and PNU-100480 (*lower*)

than LZD, although doses of PNU-100480 used were higher (300 mg linezolid vs 1,000 mg PNU-100480 dosed daily until steady-state plasma concentrations were achieved) [216]. PNU-100480 forms metabolites by the oxidation of the sulfur atom (Fig. 17; **15n,o**).

Side effects reported during phase III clinical trials of linezolid were generally not severe (however, the duration of exposure in such trials has been notably shorter than those used in MTb chemotherapy) [221]. More than half of the patients experienced digestive side effects (including constipation, diarrhea, vomiting, nausea), rash, headache, insomnia, or dizziness [221]. Hematological side effects including thrombocytopenia, anemia, leucopenia, or pancytopenia [221], although rare, warrant monitoring for longer treatment durations [223]. LZD can cause peripheral and optic neuropathy [221], and lactic acidosis has been reported in patients on longer treatment courses [221].

The largest (184 patients) retrospective analysis of patients empirically treated using LZD in a multidrug regimen for MDR- and XDR-TB patients in a multidrug regimen showed an overall 59% cure rate for the entire cohort, with an 87% cure rate in cases with definitive outcomes [224]. The use of LZD was also associated with a favorable outcome in a retrospective analysis of 176 XDR-TB-infected patients [62]. No prospective controlled data are available at this point although two trials are currently underway [53, 54].

3.4 Fluoroquinolones

3.4.1 History

The fluoroquinolones are a synthetic class of antibacterial drugs discovered by the Sterling-Winthrop Institute in 1962 as an impurity during synthesis of the antimalarial compound chloroquine [225]. This byproduct, nalidixic acid (Fig. 18; **16a**), was approved by the FDA in 1963 to treat Gram-negative urinary tract infections.

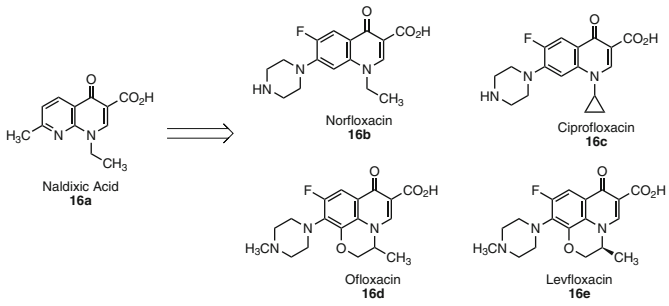


Fig. 18 First- and second-generation fluoroquinolones

However, despite its good bioavailability and straightforward synthesis, nalidixic acid has had limited clinical use due to a poor pharmacokinetic profile and narrow antibacterial spectrum [226]. Interest in the quinolones was renewed in 1980 with the discovery of the first reported antibacterial fluoroquinolone, norfloxacin (Fig. 18; **16b**), by the Dainippon Pharmaceutical Company [227]. Norfloxacin showed broad spectrum antibacterial activity 1,000-fold greater than nalidixic acid [228, 229] as well as improved pharmacokinetic properties, with a longer half-life and improved solubility [228–230]. Norfloxacin and several other second-generation fluoroquinolones such as ciprofloxacin (Fig. 18; **16c**) (first reported in 1982 by Bayer [231]), ofloxacin (Fig. 18; **16d**) (first reported in 1983 by Daiichi Pharmaceutical Co., Ltd., now Daiichi Sankyo Co., Ltd. [232]), and levofloxacin (Fig. 18; **16e**), which is the isolated *S*-isomer of racemic mixture ofloxacin (also developed by Daiichi Pharmaceutical Co., Ltd. [232]), have proven relatively safe and remain among the most frequently prescribed drugs [226].

Following the discovery of norfloxacin (**16b**), SARs for the fluoroquinolone core were studied in detail. This led to the development of a number of analogs with broader antibacterial activity, better solubility, and longer serum half-lives [226, 229]. Among the third and fourth generations of fluoroquinolones, moxifloxacin (Fig. 18; **16f**) (developed in 1991 by Bayer [233]), which has a bulky hydrophobic modification at C(7), has been the most successful. Unfortunately, several third and fourth generation agents have been restricted or withdrawn due to severe adverse effects (Fig. 19) including temafloxacin (**16g**), grepafloxacin (**16h**), trovafloxacin (**16i**), and clinafloxacin (**16j**) [226, 234, 235].

Many new fluoroquinolones are in development such as gemifloxacin (Fig. 20; **16n**), patented in 1998 by LG Life Sciences Ltd. [236], and sitafloxacin (Fig. 20; **16o**) (first reported in 1994 by Daiichi Seiyaku Co. [237]) which show activity against a panel of respiratory pathogens [229]. Sitafloxacin is currently in clinical development;

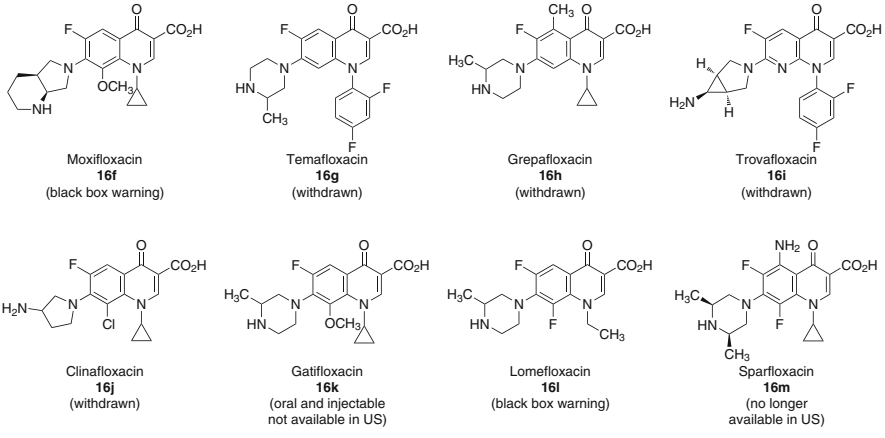


Fig. 19 Third- and fourth-generation fluoroquinolones

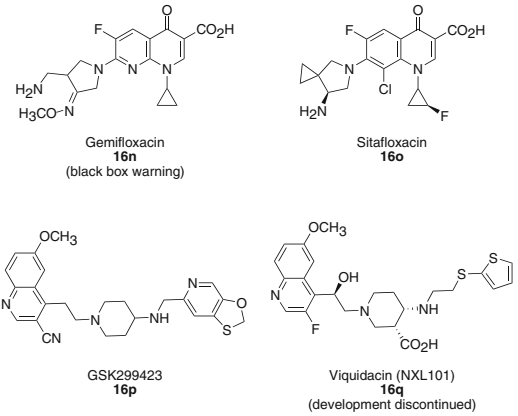


Fig. 20 New DNA gyrase inhibitors

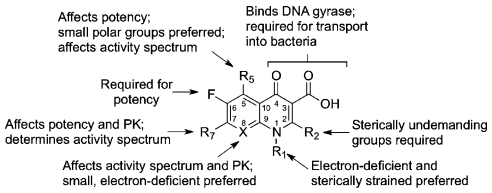


Fig. 21 Fluoroquinolone SAR (adapted from [229, 242, 243])

gemifloxacin is a clinically prescribed drug. Recently, novel bacterial topoisomerase inhibitors (NBTIs) with modes of action similar to the fluoroquinolones have been reported, including GSK 299423 (Fig. 20; **16p**) [238], NXL101 (Fig. 20; **16q**) [239], and a series of tetrahydroindazole compounds [240, 241]. While these new compounds have shown good in vitro activity against a spectrum of both Gram-positive and Gram-negative microbes including strains resistant to fluoroquinolones, it remains to be seen whether they will also exhibit activity toward MTb.

3.4.2 Structure–Activity Relationships

While the SAR of the fluoroquinolones has not been analyzed specifically for mycobacteria, it is reasonable to assume that many of the relationships found in other types of bacteria will be applicable to MTb (Fig. 21). Modifications at N(1) control potency, with electron-poor and sterically strained cyclopropyl being optimal, followed by 2,4-difluorophenyl and *t*-butyl [242]. This substituent also controls Gram-negative and Gram-positive activities, and a 2,4-difluorophenyl group increases activity against anaerobes. The C(2) position is near the DNA gyrase-binding site, and thus a sterically undemanding hydrogen atom at R_2 is optimal [244]. The dicarbonyl moiety is required for binding to DNA gyrase and thus is critical for activity. Modifications at C(5) control in vitro potency with the most

active groups being small electron-rich groups such as -NH_2, -OH, and -CH_3 [242]. Additionally, C(5) modifications affect activity against both Gram-negative and Gram-positive organisms. The fluorine atom at C(6) (for which the class is named) enhances DNA gyrase inhibition [226, 244] and can increase the MIC of the compound 100-fold over that of other substitutions [242]. The most active substituents at C(7) have been five- and six-membered nitrogen heterocycles, with pyrrolidines increasing activity against Gram-negative bacteria and piperazines affecting potency against Gram-positive organisms. The C(8) position controls absorption and half-life, and optimal modifications for in vivo efficacy include groups that create an electron-deficient *pi* system, i.e., N, CF, and CCl [245]. Several modifications that create a N(1) to C(8) bridge have also been successful, i.e., ofloxacin (Fig. 18; **16d**) and levofloxacin (Fig. 18; **16e**), which both display significant gyrase inhibition [244].

3.4.3 Biology

The fluoroquinolones alter DNA topology and block replication by inhibiting two essential bacterial enzymes, DNA gyrase (topoisomerase II) and topoisomerase IV. DNA gyrase, encoded by *gyrA* and *gyrB*, maintains the levels of supercoiled DNA required for efficient replication and is the primary target for the fluoroquinolones in most Gram-negative bacteria [246]. Topoisomerase IV, encoded by *parC* and *parE*, is responsible for decatenation of DNA following replication and is the major target of the fluoroquinolones in many Gram-positive bacteria [229, 247]. Mycobacteria are unique in that genome sequence analyses have failed to identify DNA topoisomerase IV [229]. Thus, *gyrA* and *gyrB* are likely the only targets of the fluoroquinolones in MTb.

The MIC for numerous fluoroquinolones has been determined for both wild type (H37Rv) and clinical isolates of MTb. The MIC values against H37Rv for the clinically relevant fluoroquinolones are displayed in Table 5 and range from 0.1 to 5 μM.

3.4.4 Clinical Use

The fluoroquinolones have several pharmacokinetic features that have proven valuable in treating tuberculosis. For example, the oral bioavailability for many of the fluoroquinolones is good, ranging anywhere from 70 to 100%, with levels in

Table 5 MIC data for fluoroquinolones commonly used in treatment of MTb

Fluoroquinolone	MIC (μM)	References
Ciprofloxacin (**16c**)	1.51	[248]
Gatifloxacin (**16k**)	1.25	[249]
Levofloxacin (**16e**)	1.25	[249]
Lomefloxacin (**16l**)	5	[249]
Moxifloxacin (**16f**)	0.16	[249]
Ofloxacin (**16d**)	2.5	[249]
Sparfloxacin (**16m**)	0.08	[249]

the blood peaking soon after administration [248, 250–253]. Moreover, the fluoroquinolones are cell permeable and widely distributed throughout the body, which is important for killing intracellular bacteria and treating disseminated disease [250]. For the most part, the later generation fluoroquinolones have longer serum half-lives, but these vary extensively, from 5.37 h for ciprofloxacin to 18.3 h for sparfloxacin [245]. Finally, most fluoroquinolones are cleared via the kidneys, but liver metabolism and elimination by a combination of routes do occur for several of the compounds [250].

Generally, the fluoroquinolones are well tolerated, causing mild side effects that tend to be self-limiting and rarely require discontinuation or regimen changes [235, 250] (Fig. 22, Table 6). The most frequent adverse events reported include gastrointestinal upset, disturbances of the CNS, and skin reactions [226, 234]. A number of more serious side effects have been documented with fluoroquinolones use as well. In particular, the fluoroquinolones have been associated with tendonitis and tendon rupture due to collagen damage, which in 2008 prompted a black box warning for all currently available drugs within this class [234]. Phototoxicity due to the generation of reactive oxygen species and inflammatory responses to sunlight is also commonly reported [226].

While all fluoroquinolones may cause photosensitivity, there is considerable variation within the class due to structural differences [234]. For example, the presence of halogen atoms at C(5) or C(8) and a bulky side chain or methyl group at C(5) show the highest potential for this effect [226, 242]. Moreover, fluoroquinolones can cause QTc interval prolongation by blocking voltage-gated potassium channels, which has been

Table 6 Notable side effects of selected fluoroquinolones

Fluoroquinolone	Adverse effects	Implications	References
Ciprofloxacin (**16c**)	Tendonitis/tendon rupture	Black box warning; 2008	[234]
Clinafloxacin	Phototoxicity, hypoglycemia	Development stopped	[243]
Gatifloxacin (**16k**)	Dysglycemia	Oral and injectable formulations no longer available in USA; 2006	[234]
Grepafloxacin (**16h**)	Cardiotoxicity	Withdrawn; 1999	[226, 243]
Levofloxacin (**16e**)	Tendonitis/tendon rupture	Black box warning; 2008	[234]
Lomefloxacin (**16l**)	Phototoxicity, CNS effects	Black box warning; 2008	[234, 243]
Moxifloxacin (**16f**)	QTc interval prolongation, tendonitis/tendon rupture	Black box warning; 2008	[234]
Ofloxacin (**16d**)	Tendonitis and tendon rupture	Black box warning; 2008	[234]
Sparfloxacin (**16m**)	Phototoxicity, QTc interval prolongation	No longer available in USA	[243]
Temafloxacin (**16g**)	Severe hemolytic reactions, clotting abnormalities, renal failure	Withdrawn; 1992	[226, 243]
Trovafloxacin (**16i**)	Hepatotoxicity	Withdrawn/limited use; 1999	[226, 234]

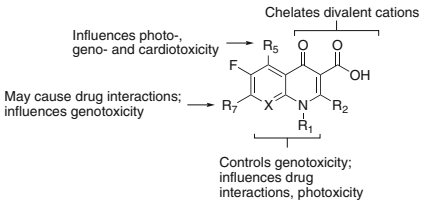


Fig. 22 Structure–toxicity relationships of the fluoroquinolones (adapted from [234, 235, 243])

associated with torsades de pointes syndrome, severe arrhythmia, cardiotoxicity, and death. However, the severity varies according to structural differences and the dose administered [226, 234]. Other adverse effects attributed to fluoroquinolone use include: hepatotoxicity, kidney and liver dysfunction, and dysglycemia [226, 235].

As discussed in Sect. 1.3, patients with MDR-TB receive one of several fluoroquinolones used as second-line agents in the treatment of TB, namely gatifloxacin, levofloxacin, moxifloxacin, or ofloxacin [254, 255]. Based on murine model studies [256–258], the most active fluoroquinolones are: moxifloxacin = gatifloxacin > levofloxacin > ofloxacin [259]. In addition to the aforementioned fluoroquinolones, several clinical studies have investigated the efficacy of sparfloxacin and lomefloxacin [260]. While sparfloxacin appears efficacious for treating MDR-TB, a role for lomefloxacin in tuberculosis therapy is unclear [260]. Based on data from the mouse studies, moxifloxacin and gatifloxacin are currently in phase III clinical trials to determine whether they can shorten duration of therapy [259, 261, 262]. Thus far, clinical trials of fluoroquinolones based on extrapolation of murine results of reduced therapy duration have failed to show similar effects in humans as in mice.

3.5 Ethylenediamines

3.5.1 History

N,*N*′-diisopropylethylenediamine (Fig. 23; **9b**)was the first compound in this series developed in early 1950s against MTb [263]. Structural modification of the lead compound led to the discovery of ethambutol (EMB, Fig. 23; **9a**) [263–266]. Despite its modest potency, EMB is a first-line drug for the treatment of TB.

3.5.2 Structure–Activity Relationship

Initial studies of structural modifications of EMB concluded that the size and nature of the alkyl group on the ethylenediamine nitrogens were critical for activity. These studies confirmed that small α-branched alkyl groups were more effective than alkyl chains branched at positions other than α and that a longer alkyl chain was detrimental to activity [264]. Alterations in the linker region of the molecule were deleterious since any lengthening, incorporation of heteroatoms, or branching of the ethylene

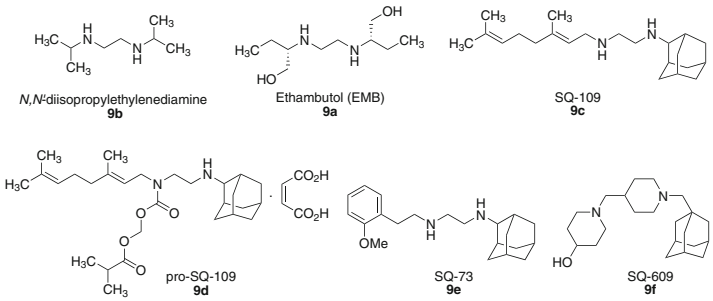


Fig. 23 Ethylenediamines

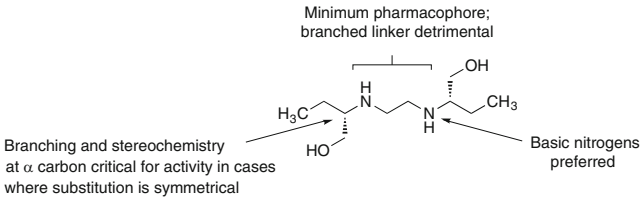


Fig. 24 SAR of ethylenediamines

linker led to reduced activity. In addition, aryldiamines and cycloalkylamines were far less effective than the parent compound. These studies confirmed that the ethylenediamine unit is the minimum pharmacophore required for antitubercular activity. Any change in the basicity of either amino group led to decreased antimycobacterial activity, with the exception of substitution of the amine with an amide that retained partial activity in some analogs [267]. Due to the lack of crystallographic information about the membrane-bound arabinosyltransferase enzyme, which is the presumed target of EMB [268, 269], a thorough study was undertaken to use combinatorial chemistry to develop a comprehensive SAR. A library of 63,238 asymmetric diamines was screened against MTb [270] of which 25 were either more effective or had comparable activity to the parent compound. The most effective compound, SQ-109 (Fig. 23; **9c**), was chosen for development based on its activity and pharmacokinetic properties. A summary of the SAR of the ethylenediamines is shown in Fig. 24.

3.5.3 Biology

Although it was initially assumed that SQ-109 (**9c**) and EMB (**9a**) would share the same arabinosyltransferase target, which catalyzes the transfer of arabinosyl residues to the cell wall arabinogalactan polymer, SQ-109 retained potency against EMB resistant strains. In addition, transcriptional profiling studies and analyses of

cell wall-linked sugar residues indicated that MTb responds differently to these compounds, suggesting that SQ-109 acts on a different target than EMB [271, 272].

3.5.4 Clinical Use

Pharmacokinetic profile of SQ-109 after a single dose administration shows C_{max} after intravenous and oral administration as 1,038 and 135 ng/mL, respectively. The $t_{1/2}$ for the drug after i.v. and oral administration were 3.5 and 5.2 h, respectively. SQ-109 displayed a large volume of distribution into various tissues. SQ-109 levels in most tissues after a single administration were significantly higher than that in blood. The highest concentration of SQ-109 was present in lung (>MIC), which was at least 120-fold (p.o.) and 180-fold (i.v.) higher than that in plasma with the next ranked tissues being spleen and kidney [273, 274]. SQ-109 is highly unstable to human microsomes as evidenced by its oxidation, epoxidation, and *N*-demethylation and has been shown to have poor oral bioavailability, presumably due to its poor solubility and first pass metabolism [275]. In a continued effort to enhance the efficacy of SQ-109, carbamate analogs (Fig. 23; **9d**), which act as prodrugs of the parent compound, have recently been synthesized. Carbamate-based esterase-sensitive drug conjugates have been used to create prodrugs of both amines and amidates [276–278]. These carbamates are stable in microsomal assays, but are substrates for plasma esterases. When administered orally, these prodrugs can bypass first pass metabolism in the liver. The bioavailability studies of the new analog **9d**, when compared with SQ-109 in a rat model, showed significant improvement [279]. After oral dosing of 13 mg/kg of SQ-109 or **9d**, bioavailability of free SQ-109 from pro-SQ-109 **9d** was 91.4% compared to 21.9% from SQ-109 [280]. This study also showed that the concentration of SQ-109 after oral administration is higher in lungs than in liver, spleen, and plasma [279], which may be beneficial for a pathogen predominantly associated with lung disease. SQ-109 has currently completed phase Ia clinical trials [280].

3.5.5 Other Diamine Derivatives

Another compound, SQ-73 (Fig. 23; **9e**), having a moderate MIC at 12.5 μM but a better therapeutic index (for macrophage toxicity) of 6.4, was studied further as this compound exhibited better activity in macrophages. In vivo studies with SQ-73 exhibited moderate tissue distribution [271]. A structurally related dipiperidine class of compounds was also recently reported, with the most effective compound from this series exhibiting an MIC of 6.25 μM against MTb [281]. After further optimization and analysis of the dipiperidine library, the compound SQ-609 (Fig. 23; **9f**) was selected as the most promising in the class. This compound has moderate in vitro cytotoxicity in cultured mammalian cells and a suitable therapeutic window. SQ-609 has shown efficacy against intracellular MTb, good aqueous solubility, and oral bioavailability. In murine studies, SQ-73 (5 mg/kg), SQ-109

(10 mg/kg), and SQ-609 (10 mg/kg) all exhibited activity similar to INH (25 mg/kg) after 3 weeks of treatment [281].

4 Series in Preclinical Development

4.1 Benzothiazinones

4.1.1 History

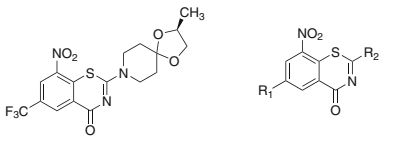
The nitro-benzothiazinone (BTZ) class was originally derived from a series of sulfur-containing heterocycles to develop antibacterial and antifungal agent [282]. BTZ-043 (Fig. 25; **17a**), the most promising compound among the benzothiazinones, shows high antitubercular activity in vitro, in macrophages, and in the murine model of chronic TB [283].

4.1.2 Structure–Activity Relationship

From SAR studies, the sulfur atom and the nitro group at positions 1 and 8, respectively, play a critical role in bactericidal activity. When the nitro is replaced with either an amine or a hydroxylamine at position 8, the resulting analogs show a 500 to 5,000-fold decreased activity [283]. More than 30 different BTZ derivatives showed MICs of less than 116 nM against MTb. Electron-withdrawing group such as CN, CF_3, and Cl at the R_1 position and 1,4-dioxa-8-azaspiro[4.5] decane groups with methyl substituents at R_2 show promising activity against MTb.

4.1.3 Biology

The BTZ class of compounds is thought to inhibit decaprenylphosphoryl-β-D-ribose 2′-epimerase, hereby preventing the conversion of decaprenylphosphoryl ribose (DPR) into decaprenylphosphoryl arabinose (DPA), which is a substrate for



BTZ-043
17a

Minimum pharmacophore

Fig. 25 Structure of BTZ-043 and its pharmacophore

the arabinosyltransferases of mycobacterial cell wall synthesis [284]. The MIC of BTZ-043 against H37Rv is 2.3 nM [283]. Despite the 100-fold better in vitro activity of BTZ-043 against MTb than frontline agents such as INH, its in vivo effect during treatment of chronically infected mice was comparable to that of INH and RIF.

In mice, BTZ-043 has a $t_{1/2}$ greater than 2 h, a C_{max} of 2 mg/mL, and an AUC of 4.6 h·mg/mL [283]. It is also relatively stable to degradation by human liver microsomes and shows less than 20% inhibition of various cytochrome P450 enzymes. BTZ-043 showed high activity against clinical isolates of MTb including MDR and XDR strains [285]. This compound is in preclinical development and will soon enter phase I clinical trials.

4.2 Nucleosides

4.2.1 History

Nucleoside analogs are a class of drugs typically used in the treatment of infectious diseases and cancer. The requirement for drugs that have activity against MDR-TB and XDR-TB makes the nucleoside analogs particularly attractive, since they have unique mechanisms of action from currently used antitubercular drugs. Among the nucleoside analogs currently under investigation, the capuramycin and caprazamycin classes of antibacterial antibiotics have the most potent activity [286]. Caprazamycin (Fig. 26; **18a–g**) and capuramycin (Fig. 27; **19a**) are natural products originally isolated from the culture broth of *Streptomyces griseus* 447-S3 [287] and culture broth of *Streptomyces* sp. MK730-62F2 [288] and show in vitro activity against drug-resistant MTb strains.

4.2.2 Structure–Activity Relationship

Capuramycin analog SQ-641 (Fig. 27; **19b**) has shown moderate activity against MTb. From SAR studies, the uridine unit and the protic amide are essential for

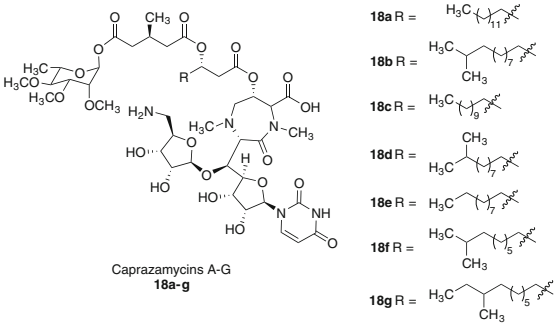


Fig. 26 Caprazamycins A–G

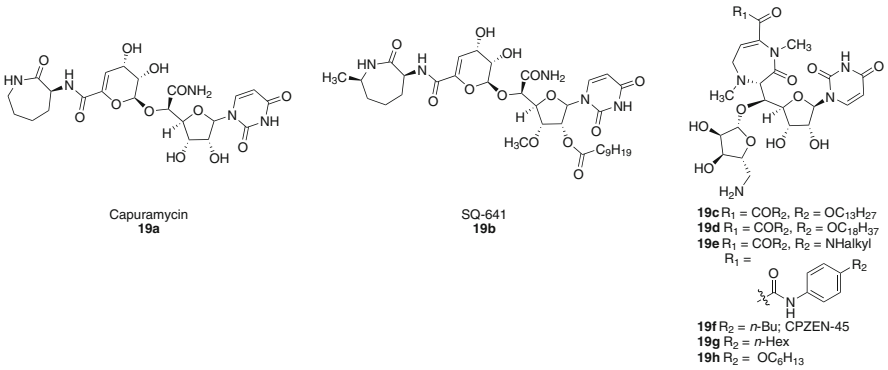


Fig. 27 Capuramycin and analogs SQ-641 and CPZEN-45

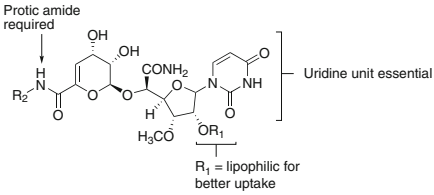


Fig. 28 SAR for capuramycins

bactericidal activity (Fig. 28) [289]. At the R_2 position in Fig. 28, lipophilic groups, including medium size alkyl chains, phenethyl, and phenyl-type substitutions, retained moderate activity but benzyl-type substitution showed decreased activity. When different lipophilic groups were placed at the R_1 position, installation of a decanoate substituent showed the largest increase in whole cell activity compared to shorter alkanoate chains, likely due to the increased lipophilicity, which (see Sect. 5.1) increases intracellular uptake into MTb.

CPZEN-45 (Fig. 27; **19f**), a caprazamycin analog, is shown in Fig. 27. SAR studies revealed that the uridine and the aminoribose are crucial for antibacterial activity [290]. Initially, installation of ester substituents at R_1 with R_2 alkyl chains showed that tridecane ($C_{13}H_{27}$, Fig. 27; **19c**) and octadecane ($C_{18}H_{37}$, Fig. 27; **19d**) esters showed equipotent activity, whereas a 21-carbon chain with unsaturation at C(18) showed decreased potency. Next, the effect of the amide substituent R_2 (**19e**) was investigated, which showed that the potency generally increased up to a 21 carbon alkyl chain and exhibited decreased potency with even longer alkyl substituents. Finally, anilinoamide substituents with *n*-butyl (CPZEN-45, Fig. 27; **19f**), *n*-hexyl (Fig. 27; **19g**), and hexyloxy (Fig. 27; **19h**) showed the most potent activity against MTb. Highly lipophilic molecules are, of course, not good candidates for lead optimization programs; thus a considerable amount of work is still required to discover better candidates from CPZEN-45 (**19f**) as leads for drug development.

4.2.3 Biology

Translocase I (encoded by *mraY*) is an essential enzyme involved in the biosynthesis of peptidoglycans, which makes it an attractive target due to its unique presence in bacteria. Caprazamycin (Fig. 26; **18a–g**) and capuramycin (Fig. 27; **19a**) inhibit Translocase I with an IC_{50} of 18 nM and 90 nM, respectively [289]. The lead compounds of the series are SQ-641 (Fig. 27; **19b**), which has an MIC of 0.67–1.35 μM against drug-susceptible MTb and 0.081–2.71 μM against MDR-TB [291], and CPZEN-45 (Fig. 27; **19e**), which has an MIC of 2.26 and 9.07 μM against drug-susceptible and MDR-TB, respectively.

SQ-641 shows promising efficacy in the murine model of TB infection and exhibits strong synergistic effects with EMB, STR, and SQ-109 (see Sect. 2.5) [286]. CPZEN-45 also exhibits no significant toxicity and a novel mechanism of action making these nucleoside compounds attractive candidates for TB drug development.

4.3 Macrolides

4.3.1 History

In the early 1950s, the first-generation prototypical macrolide, erythromycin (EM, Fig. 29; **20a**), was discovered. It is a natural antibiotic isolated from *Saccharopolyspora erythrea* [292, 293]. Erythromycin consists of a 14-membered lactone ring with two attached sugar groups: L-cladinose at the C(3) position and desosamine at the C(5) position [292, 293]. EM shows antibacterial activity against Gram-positive bacteria, but no activity has been observed against MTb [292, 293].

4.3.2 Structure–Activity Relationship

In an effort to increase potency against MTb, a series of EM analogs was synthesized with modifications at the 2, 3, 6, 9, 11, and 12 positions of the 14-membered lactone ring, as well as at the 4′ position of cladinose and the 2″ position of

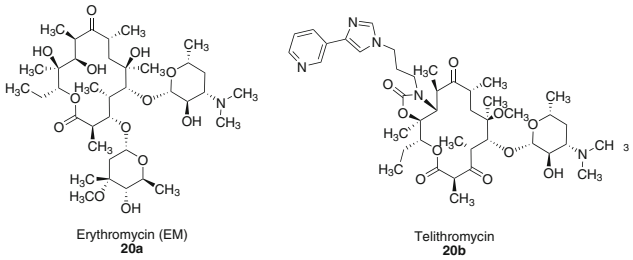


Fig. 29 Erythromycin (EM), 20a, and telithromycin, 20b

desosamine [292–294] (Fig. 30). Specific modifications on the lactone ring such as 6-substitution, 11, 12-carbamate, 11, 12-carbazate, and 9-oxime substitutions enhance potency [294]. Substitution of fluorine at position 2 in ketolides appears to improve both potency and selectivity (i.e., cytotoxicity vs activity against MTb) [293, 294]. The C(6) substituent is critical for activity of the ketolides [293], as it affords acid stability by preventing internal hemiketalization with the 3-keto group [292]. In general, ketolides are less potent than the corresponding cladinose-containing compounds for all substituents on the 6-position [293]**.** Among 9-oxime-substituted ketolides and macrolides, there is a correlation between the lipophilicity of the substituent on the 9-position (defined as calculated logP) and the potency [293], with some C(9) oximes showing submicromolar MIC against MTb [294].

The substituent at 11, 12 position appears to significantly affect potency. A variety of aryl-substituted 11, 12-carbamate and carbazate macrolides and ketolides demonstrated low or submicromolar MICs [294]. Also, the aryl substituent may be involved in determining cytotoxicity [294]. The substituted 11, 12-carbazate compounds demonstrated significant dose-dependent inhibition of MTb growth in mice, with a 10–20-fold reduction of colony forming units (CFUs) in lung tissue [294].

To further enhance lipophilicity, the 2′ and 4″-positions on desosamine and cladinose rings, respectively, have been modified via esterification, which generally improved potency and sometimes decreased CYP3A4 inhibition (more commonly in the cladinose-containing macrolides; see Sect. 4.3.3 for discussion of CYP3A4 inhibition) [293], although the substituent on the 9-position is generally more important than modifications on 2′ and 4″ positions [293].

4.3.3 Biology

Macrolides bind reversibly to the 50S subunit of 70S bacterial ribosomes, which inhibits protein synthesis [293, 295]. Although macrolides are effective for other bacterial infections, including some mycobacteria, they have not demonstrated significant efficacy against MTb [293, 294]. Ribosome methylation is the most

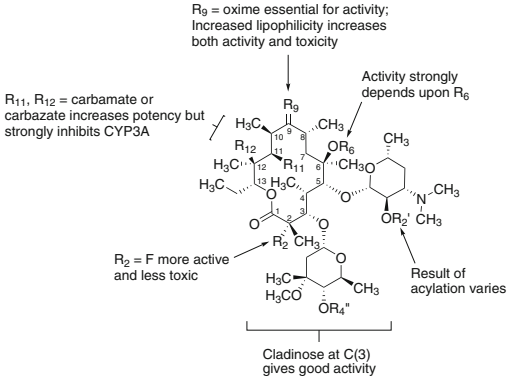


Fig. 30 General SAR for macrolides (adapted from [293])

widespread mechanism involved with macrolide resistance in MTb, and the gene *erm*MT plays an essential role [294–296]. Therefore, the current development goal for macrolides has been primarily to overcome bacterial resistances resulting from methylation of the rRNA and drug efflux [294, 295]. Furthermore, since macrolides are well-known inhibitors of CYP3A4, a cytochrome P450 enzyme, developing compounds with decreased inhibition against CYP3A4 is critical.

In addition to low efficacy against MTb, the pharmacokinetics of EM are somewhat unsatisfactory, as it is unstable to gastric acid and displays a short serum half-life (~1.4 h) [292, 293]. The second-generation macrolides such as clarithromycin and roxithromycin improved both properties [293], but clarithromycin showed only weak activity against MTb either in vitro or in vivo (200 mg/kg dose for low-dose aerosol infection mouse models), suggesting that second generation macrolides cannot be expected to offer significant antimicrobial clinical benefits for TB [293, 294]. Further improvements focused on replacement of the L-cladinose substituent, as it is associated with both drug efflux (one mechanism for development of macrolide resistance) and metabolic instability of the macrolides [293]. This third generation of macrolides replaced the cladinose ring with a ketone moiety (ketolides), leading to more metabolically stable drugs [292–294]. However, it appears that C(3) cladinose is important for antitubercular potency of macrolides, which remain more potent than either ketolides or other substituents such as 3-OH and 3-carbamoyloxy groups [293, 294]. Telithromycin (Fig. 29; **20b**), the first clinically approved ketolide, has been developed for use against respiratory pathogens but is not active against MTb [292–294].

Currently, there have been some improvements in in vitro activity with macrolides against MTb, but to date no promising drug candidate has emerged. Preclinical work in this area is ongoing.

4.4 β-Lactams

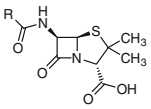
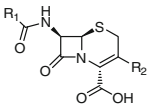
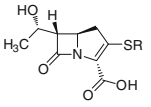
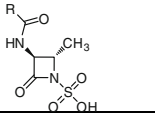
4.4.1 History

Since the discovery of 6-amino penicillanic acid (penicillin) in 1929 [297], β-lactams have been one of the most successfully used classes of antibiotics. They are irreversible inhibitors of peptidoglycan-cross-linking enzymes, D,D-transpeptidases and D,D-carboxypeptidases [298]. β-lactams are rarely used in chemotherapy of TB, however, because of the limited permeability of the mycobacterial cell envelope, expression of inactivating enzymes (β-lactamases), and involvement of β-lactam-insensitive targets in peptidoglycan transpeptidation.

4.4.2 Structure–Activity Relationship

The pharmacophore of the β-lactams is a highly reactive four-membered azetidinone ring which is generally fused to a five- or six-membered ring (Table 7). There

Table 7 Basic structures of clinically relevant β-lactams and their pharmacologic properties

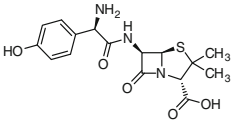
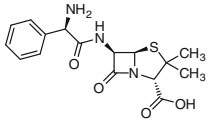
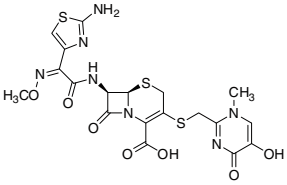
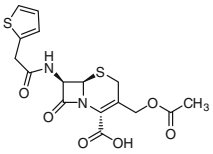
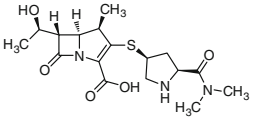
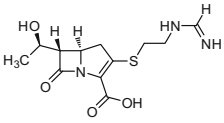
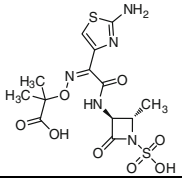
β-lactam class	Pharmacophore	Spectrum of activity	Inactivation	Adverse effects
Penams		Gram-positives	Classical β-lactamases, carbapenemases	Diarrhea, hypersensitivity, anaphylaxis, pseudomembranous colitis, yeast infections
Cephems		Gram-negatives	Extended-spectrum β-lactamases, carbapenemases, cephalosporinases	Diarrhea, hypersensitivity, pseudomembranous colitis, yeast infections
Carbapenems		Gram-positives, Gram-negatives, anaerobes	Renal dehydropeptidase, carbapenemases	Diarrhea, anaphylaxis, pseudomembranous colitis, nephrotoxicity, neurotoxicity
Mono-bactams		Select Gram-negatives	Extended-spectrum β-lactamases, carbapenemases	Diarrhea, pseudomembranous colitis, yeast infections

is an absolute requirement for the β-lactam ring and a carboxylic acid on the fused ring (or an electron withdrawing moiety such as the sulfonyl as in monobactams). An amide α to the β-lactam ring is preferred. Conformationally, this core resembles the acyl-D-alanyl-D-alanine moiety of the natural substrate. The serine nucleophile in the enzyme active site attacks the electrophilic carbonyl of the β-lactam amide leading to ring opening and irreversible acylation of the enzyme.

4.4.3 Biology

β-lactams are commonly used in combination with β-lactamase inhibitors such as clavulanic acid (CA), sulbactam, and tazobactam which themselves are β-lactams. A variety of β-lactams have been tested for in vitro efficacy against MTb (see Table 8 for examples). However, despite activity of some β-lactams against MTb in vitro, especially in the presence of β-lactamase inhibitors, none have to date shown good efficacy in vivo. Amoxicillin (Table 8; **21a**)/CA was found to be ineffective in mice [302], and imipenem (Table 8; **21f**) showed only a 16-fold reduction in bacterial burden in lungs of infected mice over 4 weeks of treatment [50]. Furthermore, only a modest decrease in viable numbers was seen in sputum of patients receiving amoxicillin(Table 8; **21a**)/CA or ampicillin(Table 8; **21b**)/sulbactam monotherapy [303]. The poor in vivo efficacy may be due to the intracellular environment of MTb, making it difficult for drugs to penetrate the phagosomal compartment. Additionally, the bacterial physiology in vivo may

Table 8 Biological activity of select β-lactams against MTb H37Rv in the presence or absence of clavulanic acid [299–301]

Compound	Structure	MIC (μM)	
		−CA	+CA
Amoxicillin (**21a**)		>250	0.5–3
Ampicillin (**21b**)		>25	13
Ceftriaxone (**21c**)		>230	7–28
Cephalothin (**21d**)		>40	25
Meropenem (**21e**)		6.5	0.8
Imipenem (**21f**)		4	0.5
Aztreonam (**21g**)		>147	>147

be different making it less responsive to β-lactam therapy, although the recent demonstration of activity of meropenem/CA against non-replicating persistent MTb has raised the possibility of use of this carbapenem against TB [299, 304].

CP-5484 (a carbapenem with activity against MRSA) [305] is currently in preclinical development for use against tuberculosis. Additionally, the meropenem/CA combination has shown potent activity against strains of MTb [299] and is currently being investigated for possible clinical use.

4.5 Rhiminophenazines

4.5.1 History

Clofazimine (CFM, Fig. 31; **8a**), a member of the riminophenazine class of compounds, was originally developed as an antitubercular drug in 1950s, but inconsistent outcomes in animal studies (effective in mouse models but less activity against in monkeys and guinea pigs) halted development [306]. Recently, CFM was reinvestigated, and several analogs of CFM that are active against MDR-TB were reported [307].

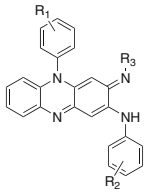
4.5.2 Structure–Activity Relationship

It has been shown that the imino group is essential for activity. Substitution with electron-withdrawing groups such as -Cl or -CF_3 at the R_1 and R_2 positions results in higher antituberculosis activity, but increased lipophilicity particularly of R_3 substituents can exacerbate the accumulation already observed with CFM in fat tissues and cells of the reticuloendothelial system [27]. Installation of a tetramethylpiperidine in the R_3 position showed higher activity than that of ethyl or isopropyl.

4.5.3 Biology

B-4157 (Fig. 32; **8b**) exhibited promising in vitro activity against H37Rv and MDR strains of MTb with MIC range of $\leq$114 nM to 228 nM. Tetramethylpiperidine-substituted riminophenazines (such as B-4169, Fig. 32; **8c**) showed MICs of 42.4–169 nM [308]. A generalized membrane disrupting effect, interference with potassium transport, and generation of reactive oxygen intermediates have been suggested as the mechanism of action for riminophenazines [27, 309, 310], but detailed information is still not clear. The very low mutation frequency suggests that rhiminophenazines may affect multiple aspects of metabolism [27].

The 2,2,6,6-tetramethylpiperidine-substituted riminophenazines such as B4169 have superior activity to CFM against MTb growing in macrophages and are also



8a R_1 = 4-Cl, R_2 = 4-Cl, R_3 = ^{i}Pr; Clofazimine (CFM)
8b R_1 = 4-CF_3, R_2 = 4-CF_3, R_3 = Et; B4157
8c R_1 = 3,4,5-Cl R_2 = 3,4,5-Cl, R_3 = 4-(2,2,6,6-tetramethylpiperidine); B4169

Fig. 31 Antitubercular rhiminophenazines

less toxic in animal models [308]. The B-4157 analog has similar activity to CFM in infected mice, and at a dose of 20 mg/kg was as effective as similar doses of RIF or INH in long-term monotherapy in infected animals [307]. The in vivo potency may be due to the long half-life of CFM in tissues (>70 days after repeated dosing of human patients) [27]. The absoption, distribution, metabolism, and excretion (ADME) of CFM analogs have not yet been reported. CFM is 45–62% orally bioavailable in humans, reaches serum concentrations of 0.7–1 mg/mL, and is metabolized by the liver through dehalogenation or deamination followed by glucuronidation or by hydroxylation along with glucuronidation [27]. Riminophenazines are currently in the preclinical development state, but their high potency makes them attractive drug candidates.

4.6 Pyrroles

4.6.1 History

Naturally occurring pyrrolnitrin (Fig. 32; **22a**) and its analogs were tested against MTb, and the most effective exhibited an MIC of 3.9 μM [311]. However, most of the compounds from this series were cytotoxic, presumably because of the nitro group. Structural optimization of pyrrolnitrin and other azole analogs led to the discovery of the more potent pyrrole, BM-212 (Fig. 33; **22b**), exhibiting MIC values of 1.68 μM against MTb [312]. BM-212 (**22b**) was also found to be effective against strains resistant to EMB, INH, amikacin, STR, RIF, and rifabutin, as well as against MTb growing within a human monocyte cell line.

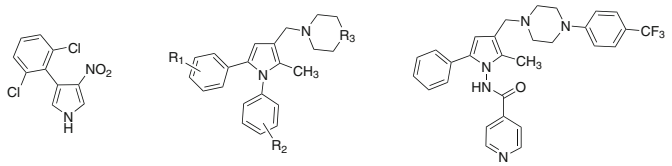


Fig. 32 Pyrrole-based antitubercular compounds

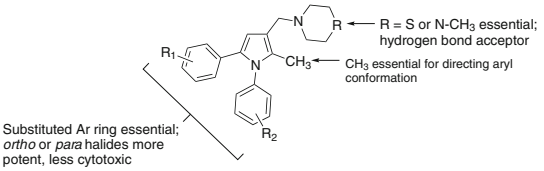


Fig. 33 Pyrrole SAR

4.6.2 Structure–Activity Relationship

Using BM-212 (**22b**) as a lead compound, systematic structural optimization led to the discovery of improved analogs, with similar or better activity in the range of 0.5–2 μM and an improved therapeutic index (ratio of cytotoxicity to in vitro activity against MTb) of 16–160 [313–316].

Based on whole cell biological activity, SARs could be deduced, with aromatic groups at N(1) and C(5) and a methyl group at C(2) as essential features (Fig. 33). Additionally, methylene-linked thiomorpholine or *N*-methylpiperazine substituents at C(3) act as hydrogen bond acceptors to improve activity (Fig. 34; **22b–g**) [316, 317]. Thus, a 1,2,3,5-tetrasubstituted pyrrole is the pharmacophore essential for antitubercular activity (Fig. 32). The 2-methyl group is not involved in any pharmacophoric interaction but influences the conformation of the substituents at positions 1 and 3 of the pyrrole ring [316, 317].

4.6.3 Biology

Another pyrrole analog, LL-3858 (Fig. 34; **22h**) (first reported by Lupin Limited in 2004), a pyrrole derivative, also complies with this pharmacophore model and is currently in phase IIa clinical trials in India. This compound has exhibited MIC values in the range of 0.05–0.1 μM, against MTb. LL-3858 (**22h**) has been reported to sterilize the lungs and spleen of infected mice after 12 weeks of treatment, none of which relapsed after 2 months of therapy termination [318].

4.7 Deazapteridines

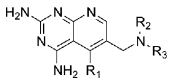
4.7.1 History

Tetrahydrofolate (reduced dihydrofolate) is a key cofactor for the synthesis of many biomolecules, and inhibition of dihydrofolate reductase (DHFR) leads to cell death [319]. Bacterial DHFR is sufficiently different from human DHFR to serve as a novel drug target [320]. To this end, the deazapteridines were designed as inhibitors of mycobacterial DHFR by researchers at the Southern Research Institute [321].

4.7.2 Structure–Activity Relationship

MIC assays against MTb and cytotoxicity assays using Vero cells were used to compare selectivity for mycobacterial DHFR. Based on the limited number of structures reported, it appears that a smaller R_1 group is better tolerated (compounds SRI-8117 vs SRI-8922 or SRI-8229 vs SRI-8911; Table 9), and either a secondary or tertiary amine is tolerated (compounds SRI-8710 vs SRI-8117 and SRI-8687 vs SRI-8686) [321]. 2,5-substituted electron-rich aromatics are preferred (data not shown). Modeling studies of these small molecules binding to MTb DHFR

Table 9 MIC vs MTb and IC_{50} vs Vero cells [321]



Compound name	R_1	R_2	R_3	MIC vs H37Rv (μM)	IC_{50} vs Vero cells (μM)
SRI-8117 (**23a**)	CH_3	2,5-$(CH_3O)_2$Ph	H	37	2,106
SRI-8922 (**23b**)	CH_2CH_3	2,5-$(CH_3O)_2$Ph	H	>35	ND
SRI-8710 (**23c**)	CH_3	2,5-$(CH_3O)_2$Ph	CH_3	8.8	200
SRI-8686 (**23d**)	CH_3	2,5-$(CH_3CH_2O)_2$Ph	H	>34	ND
SRI-8687 (**23e**)	CH_3	2,5-$(CH_3CH_2O)_2$Ph	CH_3	>32	ND
SRI-8202 (**23f**)	CH_3	2-CH_3-5-CH_3OPh	H	19	1,421
SRI-8229 (**23g**)	CH_3	2-CH_3O-5-CH_3Ph	H	19	231
SRI-8911 (**23h**)	CH_2CH_3	2-CH_3O-5-CH_3Ph	H	>37	ND
SRI-8228 (**23i**)	CH_3	2-CH_3O-5-CF_3OPh	H	4.0	2.6

suggest that the 2-ethoxy or 2-methoxy group acts as a hydrogen bond donor [322, 323]. Additionally, modeling shows that for human DHFR, the cleft to which the deazapteridines bind is lined with hydrophobic residues, whereas the analogous MTb DHFR cleft is larger and more accessible to solvent [322, 323].

4.7.3 Biology

Subsequent publications refined the pharmacophore using members of the *Mycobacterium avium* complex (MAC), which established that SRI-8686 had the highest IC_{50} ratio for MAC DHFR vs human DHFR (0.84 nM vs 2,300 nM, a 2,700-fold selectivity). SRI-8117 showed similar selectivity (1.1 nM vs 1,000 nM, 900-fold selectivity) [324]. Other MTb DHFR inhibitors are in early stages of development [325]. Although the MIC assays suggest that this series may be worth developing, the on-target effect of these compounds in MTb still needs to be verified.

5 Critical Issues in TB Drug Development

5.1 Cell Penetration

The complex, lipid-rich envelope of MTb acts as a permeation barrier to a broad range of therapeutic agents and has likely contributed to both the fitness and the success of the pathogen (Fig. 33). The plasma membrane (PM) forms the innermost region of the cell envelope and is a typical lipid bilayer, structurally and functionally similar to the PM of other eubacteria. External to the PM is the peptidoglycan sacculus. This contains repeating units of *N*-acetylglucosamine and

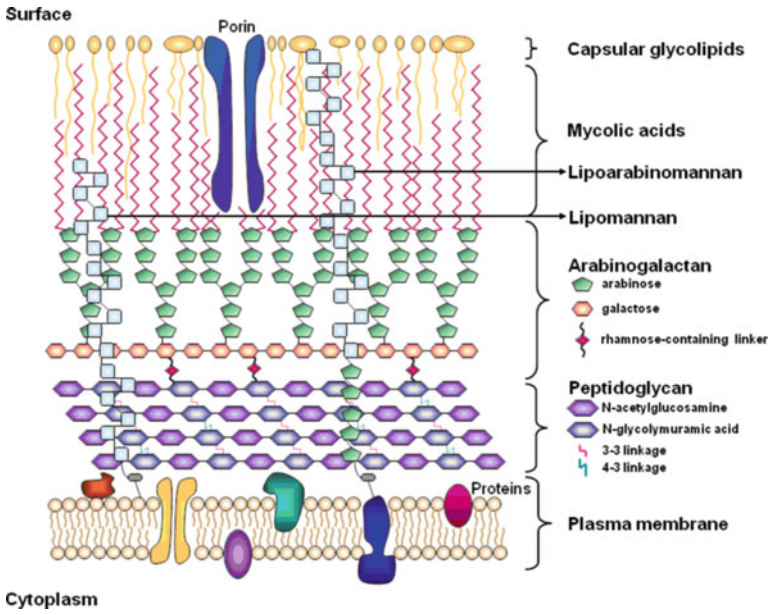


Fig. 34 Schematic of the mycobacterial cell envelope

N-glycolymuramic acid with stem peptides joined mostly through 3–3 cross-links rather than the 4–3 linkages commonly found in other bacteria [326, 327].

Peptidoglycan serves as a scaffold for arabinogalactan, a polymer of D-arabinose and D-galactose, which are covalently attached by β-1,5 linkages [328]. The arabinogalactan chain bridges between peptidoglycan and a thick layer of mycolic acids where the galactose portion of the polymer is connected to peptidoglycan by a unique glycosyl–phosphoryl bridge, and the arabinose moieties are ester-linked to four mycolic acid residues [329, 330]. The mycolic acids that are largely responsible for the impermeability of the mycobacterial cell wall consist of branched 2-alkyl-3-hydroxy fatty acids 70–90 carbon atoms in length [330]. In contrast to other bacteria, the MTb membrane also contains a number of lipids with unusual structures including: phosphatidylinositol mannosides, lipomannans and lipoarabinomannans, trehalose-6,6′-dimycolate, sulfolipids, phthicerol dimycocerosates, and phenolic glycolipids [331]. A loosely attached capsule defines the outermost layer of MTb and is composed primarily of glucans, arabinomannans, and mannans, with a small number of lipids and proteins decorating the structure as well.

Historically, the intrinsic resistance of mycobacteria to antibiotics has been attributed to the low permeability of the cell wall. For example, perturbations in the cell envelope caused by detergents, mutations, or through inhibition of cell wall polymer biosynthesis increases the susceptibility of mycobacteria to various classes of antibiotics, including aminoglycosides, EMB, and RIF [332–335]. Moreover, data indicate that the outer membrane of *Mycobacterium chelonei* is 1,000-fold less

permeable to hydrophilic molecules than *Escherichia coli* and tenfold less permeable than the notoriously impermeable *Pseudomonas aeruginosa* [331, 336, 337]. Instead, hydrophilic molecules such as the cephalosporins likely penetrate the envelope using water-filled porins located on the outer leaflet of the cell wall [338]. While typical lipid bilayers are highly permeable to lipophilic molecules, permeability is inversely correlated with membrane fluidity [331]. When a cell wall is erected from lipids containing long, hydrocarbon chains with few double bonds or cyclopropane groups, the result is membrane rigidity [331, 339]. The mycolic acids within the MTb cell wall are unique in this regard, and accordingly, the inner leaflet displays extremely low fluidity [340]. In addition, lipids with more than one fatty acid chain attached to a single head group, similar to the mycolyl-arabinogalactan found in the cell wall of MTb, decrease membrane fluidity further [331]. Data predict that the lipophilic antibiotics such as fluoroquinolones, macrolides, rifamycins, and tetracyclines do penetrate the bacteria, but this likely occurs via the lipid bilayer rather than the inefficient porins found on the outer leaflet [331]. In support of this model, the more hydrophobic agents within an antibacterial class tend to be more effective against mycobacteria [331].

5.2 Animal Models for Evaluation

As most vertebrates can be infected with a mycobacterial pathogen, it is no surprise that there are a wide range of TB animal models that to different extents recapitulate the characteristics of human disease. A summary of some of the current experimental animal models of TB chemotherapy, their typical uses, and the comparative compound requirement for use in drug efficacy studies are shown in Table 10 and briefly summarized here.

Zebrafish infected with *Mycobacterium marinum* are gaining popularity as a model of TB as the costs and space requirements are quite modest and experimental work with infected fish or their embryos does not require the biological safely laboratory level 3 containment of any work with virulent strains of the MTb complex (*M. africanum, M. bovis, M. tuberculosis*, and *M. caprae*). *M. marinum* is a natural pathogen of fish that causes necrotizing lesions within a nearly transparent host where the progress of infection can be imaged with high resolution microscopy [341] allowing real-time data collection. This model is contributing to our understanding of TB pathogenesis and may become useful for drug screening in the near future [342, 343, 361].

The inbred mouse has been used most extensively in TB studies and can be infected by a variety of routes including i.v., intranasal inoculation, and by aerosol exposure. Many strains of mice, each with different genetic backgrounds for investigating certain immunological parameters, can be reliably maintained for many months in a state of chronic infection, and used for a variety of different readouts (Table 10). While the mouse model is often used in studies of MTb strains

Table 10 Characteristics of experimental animal models of tuberculosis chemotherapy

Model	Pathology	Utility: limitations	Compound requirement (g) [mass of animal]	References
Zebrafish	Necrotizing granuloma in embryo	Early granuloma formation, high-throughput: non-pharmacodynamic	0.025 [100 mL dish]	[341–343]
Mice	Pulmonary pneumonia and aggregates of leukocytes, spleen, and liver permissive for growth	Standard model for drug efficacy: very limited pathology	0.35 [25 g]	[79, 344–347]
Rats	Similar to mice	Accepted PK/PD model: little additional information cf. mice	4.6 [0.3 kg]	[348, 349]
Guinea pigs	Pulmonary, splenic, and liver lesions; 50% solid, 50% necrotizing granulomas	Small size with more similarity to human pathology: non-cavitating	12.6 [0.9 kg]	[79, 350–353]
Rabbits	Limited infection with Mtb strains; pulmonary solid and necrotizing lesions, cavities; extensive secondary lesions (*M. bovis* only)	Pathology including pulmonary, CNS system, ocular sites: highly sensitive to GI distress from many agents	40 [3.5 kg]	[354–357]
Nonhuman primates	Diverse presentation of lesions in multiple organs	Pathology accurately reproduces human, PK similar: expensive, dosing can be difficult	56 [5 kg]	[358–360]

to define the contribution of various mycobacterial genes to virulence or in studies of immunological responses to MTb infection, it does not reproduce the lung pathology observed in the human [290, 344]. However, the poor sterilizing activity of INH and the treatment-shortening effect of treating with INH, RIF, and PZA containing regimens in the mouse model are superficially similar to their efficacy in human studies; hence, it is argued that the mouse model is predictive of human relapse rates [345]. Since the mouse does not develop the latent disease that characterizes the spectrum of human TB but rather develops a chronic disease marked by very high bacterial burdens and progressive destruction of lung tissue, it has been argued that this animal model is unsuitable for testing drugs under development for latent disease [362]. The development of the gamma interferon gene-disrupted C57BL6 mouse has shortened initial in vivo drug evaluation to about 2 weeks, but may only reflect drug bioavailability as the host immune system is crippled and unable to control bacterial replication, resulting in fulminant disseminated infection [363]. The discovery of the *sst1* susceptibility locus in the C3HeB/FeJ mouse has provided an immune competent mouse model (C3H.B6-*sst1* mice) with lesions demonstrating central necrosis which more closely resemble those observed in diseased human lungs [346]. Experiments to benchmark standard tuberculosis chemotherapy studies to determine relapse rates in this model are underway [364]. The "Cornell" model, where chemotherapy is used to completely sterilize mouse tissues of MTb bacilli, or studies where a combination of BCG vaccination and drug treatment are used to sterilize tissues require extended durations of chemotherapy (>8 months) and have been argued to reflect the length and sterilizing activity of TB drug regimens in humans [365–367]. Results of these studies have been used to determine optimal drug combinations and drug exposures in human clinical trials to identify treatment-shortening regimens for human clinical trials [368–370].

Actual data substantiating the predictive ability of the mouse model to determine the length of treatment are very sparse. Anatomically, one major predictor of relapse in patients is the presence of cavities, a pathologic feature not represented in any mouse model [371]. Recent trials of shorter therapies based on substituting moxifloxacin for various components of the standard frontline regimen have failed to recapitulate the therapy-shortening observed in mice models [372]. Unfortunately, this evidence seems to be widely disregarded in the rush to introduce new regimens based exclusively on their comparative efficacy in mice.

A larger rodent, the Wistar or Sprague Drawley rat, is often used in medical research and is widely accepted as a model for toxicology studies. The rat model allows dual comparison of toxicology and efficacy in the same animal and, as a result, has been used in several TB drug development studies. In one of these studies, RIF was reported to give a good dose–response curve for bacillary clearance, but other TB chemotherapeutics gave less encouraging results, [348] suggesting that this model may not be a suitable model for evaluation of antitubercular drugs.

The guinea pig, used by Robert Koch in the late 1800s to demonstrate that the MTb bacillus was the etiological agent of TB, was one of the earliest animal models in mycobacterial research. Even with very low aerosolized infectious doses, these

animals experience rapid progression to granulomatous non-cavitary disease that is ultimately fatal [350]. Because of its extreme susceptibility to MTb infection, the guinea pig is used extensively in vaccine studies, but to a lesser extent in drug efficacy studies because of its relatively large size [352, 353]. Due to its high susceptibility to MTb infection, it is also gaining popularity for virulence testing of gene knockout mutants of MTb, since large differences in organ burdens are generally observed with attenuated strains of MTb in the guinea pig [179, 351]. Recently, the sterilizing activity of a species-specific, human-equivalent dosage of a INH, RIF, and PZA regimen given in a 2-week intensive phase followed by a 6-month continuation phase of biweekly dosing (the so-called Denver regimen) was tested in both the guinea pig and the BALB/c mouse [369]. The guinea pigs were found to respond to treatment more quickly and have lower relapse rates in the 6 months following treatment as compared to similarly treated mice, although the guinea pigs experienced gastrointestinal toxicities of unknown origin making the model more challenging.

The New Zealand White rabbit, a relatively resistant animal to TB infection, has often been used to study the development of lung pathology including necrotizing lesions and cavities after either i.v. or aerosol infection with *M. bovis*, MTb, and even *M. avium* strains [354, 357, 373, 374]. It has been used for studies of extrapulmonary dissemination and/or growth of MTb especially in studies of pathogenesis of the central nervous system as a model of human TB meningitis [355, 375]. It is also used for testing indwelling venous lines and ports containing drugs which are technically not feasible on smaller animals such as mice, but less often used for oral drug efficacy studies due to the large amount of compound needed for efficacy testing in rabbits (see Table 10) [376]. On the other hand, it is particularly advantageous that oral dosing and PK/PD blood collection are possible without anesthesia in this species. It has been reported that latent disease can be achieved in the rabbit model with certain MTb strains although additional validation is required [377].

Nonhuman primates (NHP), especially cynomolgus and rhesus macaques, have a long history in TB research for both vaccine and drug testing, but the advent of more restrictive laboratory practices and the requirement for BSL-3 housing has made the model prohibitively expensive and thus less utilized [344, 378]. Like other species, aerosol infection or direct installation of the bacilli into the lung is the usual route of infection. These monkeys reproduce the spectrum of disease observed in humans including pulmonary, extrapulmonary, and latent tuberculosis infection (LTBI) as well as many of the different types of granulomas observed in human patients [379]. Low dose infection of the cynomolgus macaque is associated with the induction of LTBI with roughly 60% of animals showing no signs of disease after skin test conversion [359]. The genetic similarity between NHP and humans has allowed use of the same immunological reagents such as TNF-α blockers to elicit reactivation disease from LTBI and other reagents to query host immune function for vaccine studies as those that have been used in human clinical trials [380]. A disadvantage with these relatively large NHPs is the amount of GMP or minimally GLP compound needed (Table 10) and the requirement for anesthesia

for most manipulations including dosing to achieve reliable administration in drug studies. For these and other regulatory reasons, NHP work is usually reserved for proof of concept studies to establish the link between findings in prior (lower) animal studies and anticipated outcome in humans or as the final stage in a preclinical drug development pipeline before seeking approval for investigational studies in humans.

5.3 Pharmacological Models for Antitubercular Drugs

An evaluation of the pharmacological properties of new TB drugs is essential for effective treatment and overall cure of disease. Currently, most TB drugs are evaluated through preclinical animal models to establish appropriate dosing levels that promote optimal bacterial killing with limited toxicity. In vivo efficacy for TB drugs is not solely dependent on plasma concentration but more significantly dependent on tissue concentrations near and within lesions [78]. These concentrations must remain above MIC levels for an effective period of time to eradicate bacilli (described as Time > MIC). Lower levels are associated with the development of resistance and relapse of disease. However, due to the duration of treatment, maintaining high levels of drugs in combination often poses severe issues of toxicities and tolerabilities for the patient [381]. Thus, pharmacological evaluations for TB drugs depend on optimizing treatment to an often narrowly constrained therapeutic window.

Effective therapy with any drug is dependent on the relationship between pharmacokinetic and pharmacodynamics (PK/PD) parameters [382, 383]. Pharmacokinetics (PK) defines the ADME properties of the compound, while pharmacodynamics (PD) reveals the correlation between the serum concentration and the biological effect, efficacy or toxicity. For most drugs, the primary measurement under evaluation is plasma concentration. However, it is more relevant to assess drug levels within the infected lesion. Many efforts have attempted to define key parameters for TB drugs such as C_{max}/MIC (ratio of peak serum drug levels to MIC), AUC > MIC (overall drug exposure over the dosing interval must be greater than MIC), and Time > MIC (time period at which the drug remains in the blood must be greater than MIC per dosing interval) which are all established from plasma concentrations [78, 384]. Typically, high C_{max}/MIC ratios can offer sterilizing activity as well as limit adaptive resistance or the selection of resistant subpopulations whereas for TB, AUC > MIC and T > MIC are thought to be most relevant for both to maintain long-term exposure above MIC with limited dosing. These parameters are typically measured from blood, yet it is presumed that the primary driving factor for efficacy in TB therapy involves lesion penetration at effective concentrations [78, 290]. It is this factor that will eradicate persistent bacilli and circumvent the development of resistance.

There are several confounding factors that play a role in antitubercular drug efficacy in vivo. As with most drugs, stability and bioavailability with limited metabolism are important. Delivery to the site of infection at active concentrations increases the overall efficacy of any drug. However, active pulmonary tuberculosis is a chronic complex disease with a diverse spectrum of lesions within the lung. Predominantly caseous lesions are central to the "life cycle" of a pulmonary TB infection, as they eventually erode into air passages to allow bacilli to reach blood vessels and permit dissemination. These granulomas are generally poorly vascularized, hypoxic, lipid-rich, and often fortified with fibrous tissue forming an impenetrable fortress for the TB bacilli [290]. The ability of a drug to penetrate these lesions and kill bacilli is most critical to eradicate the bacteria and prevent disseminated or extensive disease.

Currently, there are various models designed to assess the pharmacological activity of TB drugs. Traditional models focus on determining drug levels in the blood from preclinical and clinical animal and human evaluation [385]. These studies are used to optimize dosing and evaluate tolerance. Dose fractionation models in animals can determine relevant PK indices with a strong correlation with PD effects. This type of experiment can elucidate important information for clinical development and optimal dosing strategies to prioritize compounds through drug development [258, 386, 387]. Recent in vitro models have been designed to mimic human PK (half-lives and dosing schedules) to assess the development of resistance [388]. The data obtained from these models help to identify drug-exposure breakpoints required for maximal bactericidal activity and the suppression of drug resistance. Current lesion penetration studies involving tissues from animals and human resections are providing important information in regard to drug levels found in the various lesion types enabling a better understanding to drug efficacy and therapeutic response [290].

Finally, the use of therapeutic drug monitoring (TDM) has been a useful tool for assessing drug levels during treatment in the clinical setting [71]. The use of TDM in tuberculosis treatment can allow physicians the ability to adjust dosing to provide an efficacious therapeutic concentration throughout the extensive duration of treatment [71]. Patients who most benefit are those with complications which may alter drug exposure, such as those on co-therapy for HIV for which there are known drug–drug interactions, those with diabetes mellitus with typical delayed absorption or malabsorption concerns, those with renal failure undergoing dialysis, and those experiencing hepatic dysfunction (see Sect. 1.4) [69]. TDM in combination with bacteriological and clinical data can be a useful tool to assess treatment and ensure as successful outcome [71].

Understanding pharmacological activities of TB drugs is essential not only for addressing drug levels for effective sterilizing activity and optimizing dosing strategies, but more importantly, they are also useful in limiting the development of acquired resistance. PK/PD for TB agents is relevant to understanding important phenomena associated with TB. Efforts are ongoing to develop PK/PD analyses which will effectively predict success or failure of new antituberculosis drugs and combination regimens.

5.4 Clinical Development Methodologies

The goal of TB chemotherapy is to cure clinical symptoms, prevent the development of resistance, and to prevent relapse. Since TB treatment entails 6–24 months of chemotherapy depending on drug susceptibility patterns, early markers that predict durable cure would greatly facilitate and speed the evaluation of new drugs. Currently, it requires 2 years of follow-up after termination of chemotherapy to capture more than 90% of relapses, thus necessitating trials lasting up to 4 years during evaluation of new therapies. The FDA recognizes the need to develop new drugs for TB and the associated need to look for early predictors of durable cure, making regulatory approval processes for such trials logistically easier.

Biomarkers that predict durable cure could include pathogen-specific measurements, determination of host responses to the pathogen or nonspecific disease-associated responses, and imaging. Unfortunately, no biomarker of any nature has been validated as of this time. Microbiologic markers traditionally used to evaluate TB chemotherapy include: 2-month culture conversion (in which sputum samples taken at regular intervals during chemotherapy are evaluated for eradication of culturable MTb at two months posttreatment), days to positivity (the time required to obtain mycobacterial growth from sputum in liquid culture), and serial counts of CFUs of MTb from sputum samples on agar combined with scoring acid-fast bacilli in sputum during treatment. Two-month culture positivity as surrogate marker for relapse was first recognized and used as a surrogate endpoint to predict treatment efficacy during the BMRC studies where culture negativity at 2 months was associated with cure and lack of subsequent relapse and is widely used for current evaluation of TB chemotherapeutic trials [389–392].

While other culture-dependent methods show promise, they do not always predict 2-month culture conversion and there is no data to correlate findings with risk of relapse [390, 393]. In a large ongoing clinical trial (REMoxTB), where moxifloxacin is evaluated as a replacement for INH or EMB, various culture-dependent and -independent methods will be assessed against the primary endpoint of relapse within 2 years of treatment termination, which may give some insight into the utility of other biomarkers [394].

Culture-dependent methods suffer from their long turnaround time, often several weeks, due to the slow growth of mycobacteria such as MTb. Potential culture-independent pathogen-derived biomarkers include detection of MTb DNA [395], lipoarabinomannan [396, 397] in urine, MTb mRNA in sputum [393], MTb characteristic volatile organic compounds in breath [398], and several other pathogen-derived biomarkers currently under evaluation.

Host biomarkers of disease include interferon-gamma release assays in response to MTb antigens [399, 400], measurements of non-MTb-specific host responses such as C-reactive protein, serum interleukin-2, neopterin and procalcitonin which still require a definitive TB diagnosis by other means [401–403]. These may especially be useful as clinical trial endpoints in smear-negative,

paucibacillary, extra-pulmonary, and pediatric TB where microbiologic culture is less reliable.

Imaging biomarkers such as high resolution computed tomography (HRCT) have been shown to be helpful in diagnosing active TB and distinguishing it from latent disease [404], and may be useful in detecting the early stages of disease in TB contacts [405, 406] but is also used to evaluate treatment response. Positron emission tomography (PET) using 2-fluorodeoxyglucose has been used to visualize inflammatory regions in pulmonary mycobacteriosis caused by MTb and *M. avium* but is even less discriminating than HRCT in distinguishing between TB and nontuberculous mycobacterial infections [407].

Traditionally, clinical trials for new TB drugs include a 7–14-day assessment of early bactericidal activity (EBA) of a drug given as monotherapy (often at different doses). Daily sputum microbiology is performed and bacterial counts quantified. Unfortunately, reproducibility of such microbiological assays is questionable, and EBA rarely gives a definitive answer. In most cases, ambiguous EBA results are ignored and a longer study (2 months or more depending on the available safety and toxicity data) of the new drug or placebo in combination with current TB drugs followed by completion of chemotherapy with regular extended phase regimens. These studies are followed up with expensive phase III trials that give the new drug as part of combined therapy (6–24 months) with prolonged follow-up after treatment completion [369]. The caveat to using EBA as a predictor of successful treatment is that drugs such as RIF and PZA, which have a poor EBA but have good activity against MTb populations in certain lesions, would score as poor drugs [408]. PZA is the essential drug that allowed the shortening of treatment of drug-sensitive TB from 9 to 6 months [409], indicating that EBA studies may not be a good way to cull ineffective drugs.

Phase II trials typically evaluate sputum conversion at 2 months as a predictor of durable cure (cure without relapse), but the utility of 2-month culture conversion as a biomarker was derived from studies of drug-sensitive TB and may not apply to MDR-TB. In addition, evaluation of new drugs is typically compared to the standard of care, which can achieve a 95% cure rate in clinical settings. Thus, the sample size of such a trial must be large to detect either non-inferiority or superiority of new agents. With MDR- and XDR-TB, the cure rates are much lower, which may make it easier to detect the effect of the investigational drug, but such study populations are much more heterogeneous due to different degrees and spectra of drug susceptibility results, different background drug regimens, and a wide range of disease severity and treatment history all of which may confound results. Some of these problems can be dealt with by randomized stratification of strong covariates, for example by stratifying for fluoroquinolone sensitivity [259].

In summary, EBA studies, while having the advantage of seeing the effect of a single drug on MTb in lesions that are the source of sputum-borne bacilli, are clearly not a useful predictor of drug efficacy. Studies of combination therapy using 2-month culture conversion as an end point have the advantage of a partially validated end point with a weak correlation for drug-sensitive disease but should be interpreted with caution in drug-resistant disease. Imaging modalities such as

HRCT and PET are attractive endpoints that do not require culture and evaluated disease at the relevant site of infection but, like host-derived biomarkers, require validation for predicting treatment efficacy.

6 Concluding Remarks

TB drug discovery remains a challenging enterprise at every level. There is an urgent need to validate criteria for lead compounds with promise to reduce treatment duration, treat drug-resistant disease, and provide utility for prophylaxis of subclinical disease. Poorly validated in vitro assays, poorly validated in vivo assays, and a severe lack of predictive animal models all compromise efforts to improve the quality of the TB drug pipeline. Existing SAR around series explored in the 1950s and 1960s provides some starting points, but the chemistry of most of these series is either intractable or linked to enzymatic activation of prodrug and is therefore difficult to optimize systematically. As in most anti-infectives, target-based strategies have failed to lead to viable candidates, and in most cases the reason for this failure has been unexplained. In the current climate, it is no surprise that the momentum is toward improving whole cell screening and attempting to identify leads with novel targets. Biological studies working toward more predictive assays are in progress and very promising. For example, the promising use of titratable promoter elements to systematically knock down genes and mimic the pharmacological action of a drug can truly validate drug action and directly address "target vulnerability" by quantitating how much a target needs to be inhibited to affect cell death [410]. Current efforts to understand the "systems biology" of MTb also have the potential to reshape the landscape and improve our ability to select targets.

The nitroimidazoles and diarylquinolines, series developed specifically for TB, provide some reason for optimism and perhaps a sort of loose road map for how to approach the problems. Both series suffer from critical limitations including extremely poor water solubility and potential hERG problems. In general, one serious problem that persists in new drug discovery programs for TB lies in the lack of broad application of preclinical ADME and toxicology studies in lead optimization. The academic sector lacks an appreciation of the role of such studies and lacks resources dedicated to performing them. Both series may well fail because of these considerations, but at a minimum it has made clinical development programs for these two new classes considerably more complex than if these issues had been addressed systematically in lead optimization. Candidate selection in most cases continues to happen predominantly by MIC and mouse activity, and it is doubtful that the situation will improve more than incrementally without more sophistication in lead optimization programs. Nonetheless, there are ongoing phase II studies with these agents, and it is crucial that clinical development of these compounds is paired with meaningful attempts to understand the relationship of the preclinical studies that led to selection of the candidates with the ultimate clinical properties of these agents. Through such efforts, in vitro and in vivo assays

(not to mention phase I and II trials) could perhaps be optimized to be more predictive, and these could be used to guide both backup programs for the existing agents and optimization efforts for new series not yet in play.

Funding sources for TB drug discovery have also expanded dramatically over the last 10 years and now include significant efforts from private foundations (such as the Bill and Melinda Gates' Foundation through its TB Drug Accelerator Program) as well as public institutions such as the US National Institutes of Health and the European Union's Framework Programmes. Many of the activities being funded are taking place at the interface of academic laboratories and commercial pharmaceutical companies using a wide variety of models for cooperation. Although this is precisely where such programs appear to be best placed, there has been a rather steep learning curve as these two cultures are brought together with competing needs. Nonetheless, there is significant cause for excitement at the number and quality of programs that are currently operating. If we can successfully leverage the strengths of both academia and industry, there is hope that we will be able to raise the victory flag in earnest in the long struggle against TB.

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