In Search of New Cures for Tuberculosis

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Abstract: The last 10 years have seen resurgent industry activity in discovery and development of new drugs for the treatment of tuberculosis (TB), a growing widespread and devastating (more than 2 million deaths annually) bacterial infection that is of increasing concern in developing and developed nations alike. This review describes drugs currently being evaluated in the clinic for treatment of uncomplicated and drug resistant pulmonary TB, and updates the literature on 5 new drugs that entered clinical trials in the last 4 years.

INTRODUCTION

Despite a five thousand year history and decades of attempts to find a cure, TB remains the leading single-agent infectious disease killer in the world. Nearly 2 billion people, or roughly one-third of the world's population, are infected with the causative agent of TB, *Mycobacterium tuberculosis*. Yearly, nearly 9 million people advance to active and contagious pulmonary TB and 20% of these victims, more than 1.6 million people, die of their infection [1-6]. Due to the tragic synergy between TB and HIV, TB is currently the leading cause of death in HIV positive people, accounting for a third of all HIV deaths (an additional 900,000 annually) [7-9].

The current World Health Organization (WHO) recommended therapy for treating TB requires administration of four drugs (isoniazid [INH], rifampicin [RIF], pyrazinamide [PZA], and ethambutol [EMB] or streptomycin [SM], Fig. **1**) for the first two months of treatment, followed by four months of treatment with INH and RIF alone [10-12]. This six-month therapeutic regimen is often extended to 9-12 months, but even the shorter 6-month course of drugs has a dismal compliance rate (30-60%) caused by drug combination side effects and the inconvenience of daily therapy for such a long time. Poor compliance with this long drug regimen is blamed for the emergence of drug resistant TB around the world [13-15]. The WHO estimates that 50 million people today have multi-drug resistant (MDR) TB. In countries where adequate supplies of the drugs are not readily available, the prevalence of MDR-TB is as high as 50% [13-15]. In the U.S., 20% of TB isolates are already resistant to INH, one of the cornerstone drugs of TB therapy, and 2-4% of isolates are resistant to more than one drug. In the early 1990s, New York City experienced one of the worst outbreaks of MDR-TB the U.S. has ever experienced, and the city spent nearly $1 billion over two years on containment [15]. When patients are diagnosed with TB infections that are resistant to the first line TB drugs, they are prescribed second-line drugs, such as capreomycin, ethionamide [ETA], para-aminosalycilic acid, kanamycin, or cycloserine (Fig. **2**), that are less effective, require higher doses (1-10g daily), and are more toxic; treatment with second line drugs is also 10 times more expensive than uncomplicated TB and requires up to two years to effect a durable cure. In early 2006, clinicians began reporting the isolation of extreme drug resistant *M. tuberculosis* (XDR-TB) that is resistant to the two most important front-line TB drugs, RIF and INH, and also resistant to at least two classes of second-line drugs. Currently, 4% of clinical isolates from MDR-TB patients in the U.S. are XDR-TB [16].

In the middle of the twentieth century, just after the discovery of antibiotics, pharmaceutical companies were very active in the discovery and commercialization of new TB drugs. The current first-line antitubercular drugs were introduced in the 1940s (SM), 1950s (INH, PZA), 1960s (EMB), or 1970s (RIF). Except for newer rifamycins, however, no new drugs have been developed or approved for TB in the last 35 years, despite superb academic achievements in understanding the biology and genetics of *M. tuberculosis* and the now well-recognized need for more potent drugs to shorten the regimen and treat drug resistant infection. In part, this lack of pharmaceutical effort at the end of the twentieth century was a consequence of the discovery by clinicians that 4 drugs (INH, RIF, PZA, and EMB) given concurrently for up to 24 months could cure TB, thus signaling to commercial entities in the 1970s that there was no longer an unmet medical need in TB. The first seven years of the twenty-first century, however, saw resurgence in pharma activity, and we are witnessing a welcomed renaissance in TB drug discovery. Five novel drug candidates have entered clinical development in the last 4 years, and several of them have advanced to Phase 2 early efficacy studies. All drugs in clinical trials are drugs discovered in pharmaceutical or biotechnology companies, and many were developed specifically for use in TB therapy.

The purpose of this review is to provide an update on the state of the anti-TB drug pipeline and discuss those drugs that are currently in the clinic, focusing on the experimental evidence for their activity and their position and potential within a multi-drug regimen based on animal and, when available, clinical data.

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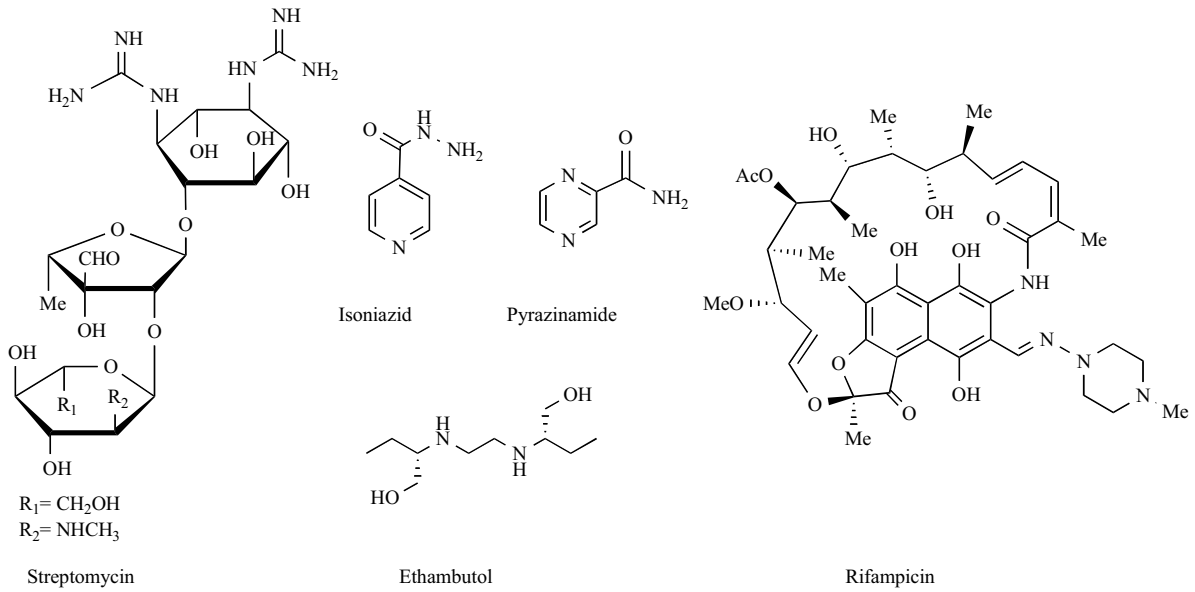


Fig. (1). Chemical structures of the first-line anti-TB drugs.

IN SEARCH OF NEW AND BETTER DRUGS AND DRUG REGIMEN(S) FOR TB

Desirable characteristics of a new anti-tubercular include most or all of the following: orally active, long acting, bactericidal, novel mechanism of action (MOA), potent against both drug sensitive and drug resistant *M. tuberculosis* and non-replicating bacteria, absence of cross-resistance or antagonistic activities with current anti-TB drugs, presence of synergistic or additive effects with current anti-TB drugs that could potentiate efficacy, large therapeutic window (or limited and dose-regulated toxicities), and inexpensive to produce. In addition, since many TB patients are co-infected with HIV, it would be helpful if a new therapeutic has no significant drug-drug interactions with anti-retrovirals used in HIV therapy.

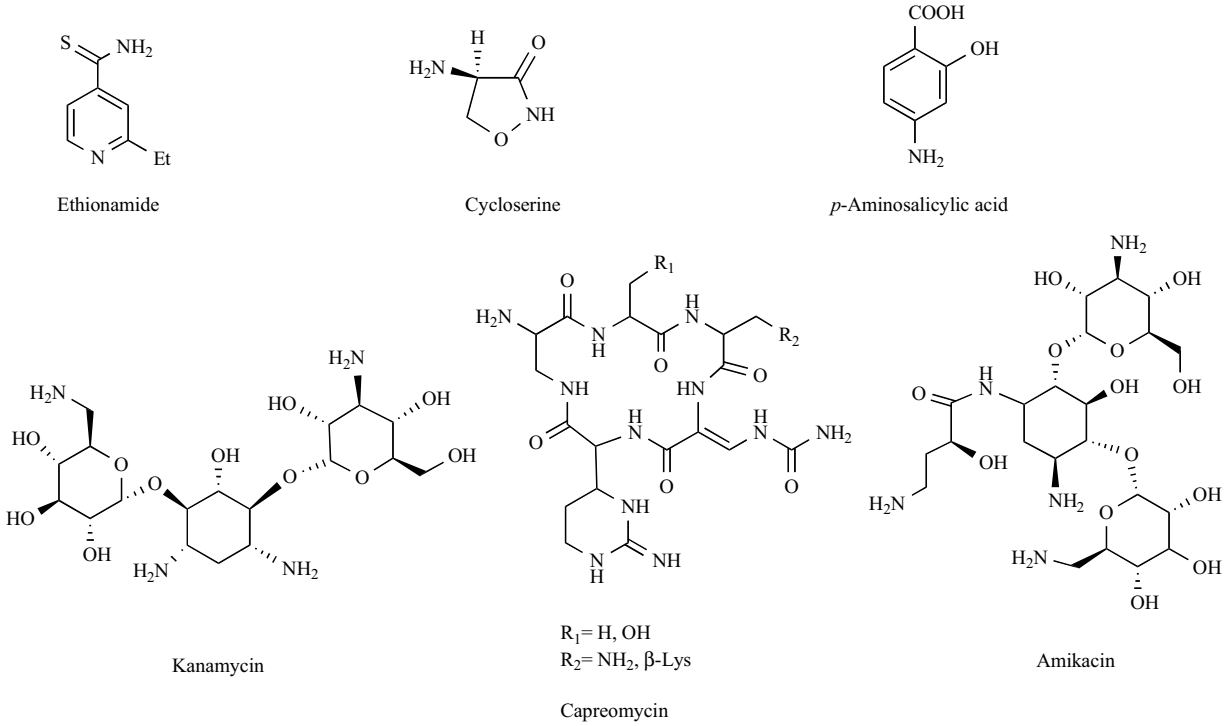


Fig. (2). Chemical structures of the second-line anti-TB drugs.

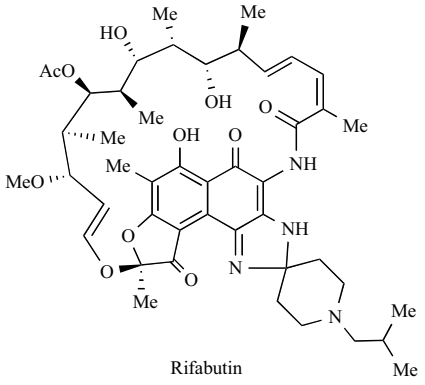
The underlying idea for development of one or more new TB drugs with these characteristics is to create a new drug regimen that is superior to standard TB therapy, with increased efficacy and decreased time to durable cure.

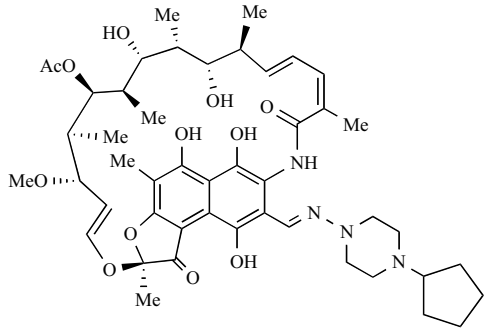
In search for new effective treatment, it is valuable to establish the regimen first in animal models of TB prior to initiating large and expensive clinical trials. Although murine TB infection does not precisely mimic human disease [17-21], mice have been used for more than 50 years to develop new TB drugs and drug regimens. There are a number of experimental protocols for inducing and treating murine TB that differ in duration, timing of chemotherapy, and surrogate markers of disease or cure, although all seem to be useful in determining drug and drug regimen efficacy with current TB drugs.

These protocols use a variety of infection systems, including intravenous high (10^7) or moderate (10^5) doses of *M. tuberculosis* colony-forming units (CFU) [22-32], or intranasal *M. tuberculosis* inoculation with high doses (10^6 -10^7 CFU) [27,33], or low-dose (50-100 CFU) aerosol-delivered bacteria [34-43]. The murine TB infection itself can be short in duration for a rapid (20-30 days) preliminary estimation of drug efficacy [44-46] or last several months for a detailed analysis of drug action on bacteria in specific organs during chronic TB infections [22, 24, 28, 34, 36]. The main criteria for determining *in vivo* potency of experimental drugs and drug regimens in these model systems are the ability to (a) reduce CFU in lungs and/or in spleens of mice infected with *M. tuberculosis* and (b) prevent TB relapse after therapy withdrawal. Although the various mouse models differ in inocula, duration, and endpoints, they have been evaluated for years with existing TB drugs and have been predictive for existing drug and drug regimen efficacy in human TB. The reality for new TB drugs, however, is that only clinical trials in TB patients will identify drug:drug interactions specific to humans and ultimately demonstrate the practical utility of a new drug or a new regimen.

1. NEW RIFAMYCINS

RIF is one of the most potent frontline TB drugs and is the cornerstone of TB therapy today. Chemical modification of RIF resulted in the development of several new compounds with increased potency *in vitro* (minimal inhibitory concentration, MIC), increased half-life in animals, and/or improved interaction with HIV drugs: Rifalazil (RFZ), Rifabutin (RFB), and Rifapentine (RFP), Fig. **3**. These newer RIF compounds have the same MOA as RIF, but have significantly improved MIC than the parent compound: RLZ MIC using BACTEC growth assay was 0.004 μg/ml compared to RIF MIC of 0.125 μg/ml [47]; BACTEC also demonstrates a RFB MIC of 0.016 μg/ml, significantly lower than RIF, but not as low as RFZ [47]. Finally, a study by Arioli *et al.* found the MIC of RFP by broth dilution in





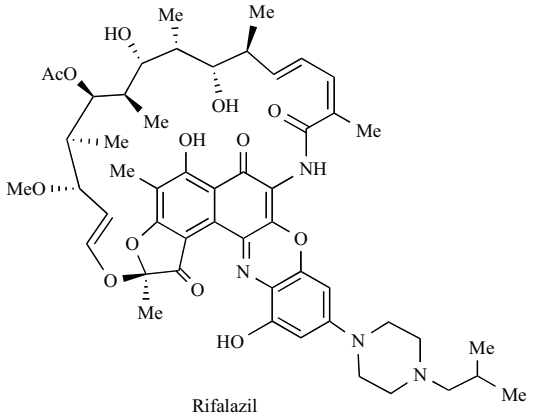


Fig. (3). Chemical structures of new rifamycins effective in treatment of *M.tuberculosis* infection.

Kirchner medium to be 0.04-0.16 ug/ml, significantly lower than the RIF MIC by this technique (0.2-1.0 μg/ml) [48]. The *in vitro* MIC data suggested that these derivatives have promise for delivering a more effective and efficient treatment of TB.

RFZ (Pathogenesis/Chiron and ActivBiotics, Inc.)

RFZ has a longer half-life than RIF: 9 hours compared to 2.5 in humans following a 25 mg dose [49]. Combined with its lower MIC, the RFZ longer half-life led to speculation that it could shorten treatment time from the current 9 months of RIF-containing regimens. In a mouse study of TB treatment with RIF+INH or RFZ+INH, replacement of RIF with RFZ reduced the time to elimination of bacteria in lungs by half, from 12 to 6 weeks of treatment. Organs of mice treated with RFZ for the full 12 weeks remained free of *M. tuberculosis* after therapy withdrawal, whereas mice receiving RIF for 12 weeks showed re-growth of residual TB bacteria as quickly as 4 weeks post therapy [50]. Another mouse study used RFZ in various combinations with PZA and EMB and compared the efficacy of these combinations to RIF and INH. Twelve weeks after daily therapy began, the mouse group treated with RFZ+PZA+EMB was free of residual cultivable *M. tuberculosis*, just like the group administered INH+RIF. The study demonstrated that RLZ combined with two weaker TB drugs was just as effective as the two most potent front-line drugs. Furthermore, and perhaps more importantly, the study found that 12 weeks after therapy was withdrawn, mice in the RFZ+EMB+PZA group showed significantly less bacterial re-growth in their lungs than the mice who were in the RIF+INH group. The investigators suggested that a therapy employing RFZ, PZA, and EMB may be more effective than the current recommended regimen [51].

Although the mouse studies were encouraging, only one study is reported for RFZ in TB-infected humans. In an early bactericidal activity (EBA) clinical trial, patients were given INH alone or INH in combination with either 10 or 25 mg of RFZ. Not surprisingly, since INH has potent activity against *M. tuberculosis* in the first few days of therapy, no additive antituberculosis activity (or decreased sputum bacillary count) over INH was demonstrated in the patients who received INH+RFZ, possibly because the RFZ doses were almost insignificant compared to the standard RIF dose (600 mg) [49,52]. Dosing of RFZ was likely limited by toxicity that has been reported for this drug. RFZ is currently being developed for a non-TB indication by the company that licensed the drug from Chiron, ActivBiotics (www.activbiotics.com).

RFB (Pfizer, Inc.)

Like the two other RIF derivatives, MIC data on RFB suggested it might be a more effective drug than RIF when administered daily. Several studies in mice showed that RFB was, in fact, more potent *in vivo* than RIF. In one study, mice treated daily for 2 weeks with RFB starting 8 weeks after TB infection eliminated cultivable TB bacteria from spleens of 4 out of 5 mice, while RIF was effective in spleens of only 1 out of 5 mice [53]. A second study using a low dose aerosol infection demonstrated that mice treated with RFB 10 days after infection had significantly lower CFU in organs than mice treated with RIF, regardless of whether the RFB dose was 5.0 mg/kg or 2.5 mg/kg [34]. Finally, a mouse study to investigate RFB potential as a prophylactic therapy to cure latent TB used *M. bovis* strain bacillus Calmette-Guerin (BCG)-vaccinated mice subsequently infected with *M. tuberculosis* to mimic a latent TB infection in humans. Vaccinated and infected mice were treated with either RFB or RIF given daily for up to 12 weeks, and organs of mice were cultured to quantify residual TB bacteria. RFB was just as effective after 6 weeks of treatment as RIF was in 12 weeks. In fact, RFB was effective even when given less frequently than daily: when RFB was administered 2 times per week, it was as effective as a daily administration of RIF [54]. In the mouse model, then, RFB could either be used to shorten treatment time or to provide a less frequent dosing compared to the currently prescribed regimen with RIF.

Subsequent human studies with RFB, however, did not quite live up to the promising results in mice. In one study, TB patients were administered standard therapy with RIF in one group and a second group received standard therapy, but replaced RIF with RFB. Treatment lasted 6 months. The median time to negative culture conversion from sputum was 34 days in the RIF group and 37 days in the RFB group, an indistinguishable result with RIF performing marginally better. When follow-up was done two years later to determine TB relapse rates, the two groups yielded very similar results. It should be noted, however, that the dose of RFB was 300 mg, half of the RIF dose of 600 mg [55].

Although the RFB studies in uncomplicated TB did not show sufficient differences with RIF to warrant switching to the newer rifamycin, RFB was demonstrably better and more appropriate than RIF in the treatment of TB in HIV co-infected patients. RFB-containing regimens showed faster clearance of acid-fast bacilli at 2 months [56]. Narita *et al.* [57] also demonstrated that HIV patients receiving standard HIV drugs and RFB concurrently had negative sputum at 2 months while maintaining the appropriate concentrations of protease inhibitors (PI). This finding was particularly noteworthy because RIF lowers the serum levels of PI by activating cytochrome P_{450} CYP3A4. In fact, the Centers for Disease Control and Prevention (CDC) of the U.S. now recommends replacing RIF with RFB for TB therapy of HIV infected individuals [57]. HIV infection compromises the immune system and accelerates the progression of TB, while the host immune response to the TB bacteria can exacerbate HIV replication. Thus RFB found a critical niche for itself in the treatment of TB in HIV positive patients. Additional studies will be needed to expand the use of RFB outside of the HIV community.

RFP (Sanofi Aventis)

Of all the RIF derivatives, RFP pharmokinetic properties appear to be the most promising to establish a new TB therapeutic regimen with an improved dosing schedule. In addition to its superior MIC compared to RIF, its half life in humans of 16 hours is over five times greater than RIF at 2.5 hours [52]. Even 144 hours after administration of RFP, the concentration of RFP was still above its MIC [54]. This long half life suggests that it could be used less frequently than RIF, helping to reduce the cost of daily treatment and lessen-

ing the problem of non-compliance. Its drawback, however, is that it does not appear to penetrate macrophages (*in vivo* host cell for *M. tuberculosis*) as well as its parent RIF [58]. Moreover, in the current standard TB regimen, all the other drugs (INH, EMB, PZA) are given daily, so having 1 drug of 4 given weekly actually complicates the delivery of TB therapy for patients and clinicians alike.

Initial mouse studies supported intermittent therapy with RFP: mice were treated with INH and SM twice a week plus daily RIF or weekly RFP. Bacteria in organs decreased 6.7 to 7 $\log_{10}$ CFU in both groups [59]. When used as a monotherapy in BCG vaccinated infected mice, RFP given 2 times a week was just as effective in reducing CFU in the lungs as RIF given daily. However, when RFP frequency was reduced to once a week or once every two weeks, CFU reduction was less than that seen with daily administration of RIF [54].

These initial experiments suggested that a less-than-daily drug regimen for TB treatment might be possible. Grosset *et al.* [60] tested the timing of several RFP-containing regimens and compared them to the standard daily RIF, INH, and PZA dosing. In each case, the weekly treatment with RFP-containing regimens was roughly 1 log less effective than the corresponding daily RIF group. All groups, however, demonstrated a 4 to 5 log reduction in tissue bacteria [60]. If the weekly RFP+INH regimen was preceded by a 2 month intensive phase with RIF+INH+PZA given daily, the treatment was just as successful at eliminating *M. tuberculosis* at the end of the treatment (6 months total) as the daily RIF containing regimen [25]. Unfortunately, the relapse rate of mice treated with 2 month intensive phase plus 4 months of weekly RFP+INH was worse than the daily RIF regimen [61]. Additionally, the 2 months of daily therapy would still face a compliance problem (50% of noncompliant people stop taking their drugs by 2 months). So the RFP-containing TB therapeutic regimen using the "old" TB drugs was not viewed as a great improvement over the current regimen by the clinical community.

A major breakthrough in the search for an improved regimen was discovered when RFP was paired with Moxifloxacin (MXF). MXF, a fluoroquinolone, also has a long-half life (9 hours in humans) and is bactericidal [62, 63]. Its long half life suggested that it might pair with RFP in a regimen of weekly dosing. Mice treated daily for 2 weeks with RIF+INH+PZA+MXF and then subsequently treated weekly for 5.5 months with RFP+INH+MXF were all organ culture negative at the end of treatment; the TB relapse rate was improved over INH+RFP alone, but was still 15% [64]. If the relapse rate could be improved, 5-6 months intermittent therapy with RFP+MXF would be much easier to administer to patients.

One logical idea to enhance the performance of the RFP/MXF intermittent therapy was to increase the dosage of each drug. RFP binds more tightly to protein than RIF, 97% *vs*. 85%, potentially affecting concentrations of biologically active RFP and requiring higher doses to achieve a good therapeutic range [65]. This concept was reinforced during EBA studies of RPT in humans: the optimal dose was between 900 and 1200 mg, significantly higher than the 600 mg dose currently approved by the U.S. Food and Drug Administration (FDA) [66]. In subsequent human safety trials, a dose of 900 mg was well tolerated [65]. For MXF, pharmacokinetic studies [67] indicated that a dose of 400 mg/kg in mice, not the 100 mg/kg of previous studies, is more in line with the 400 mg dose prescribed for humans [68]. When mice were dosed with RFP at 15 mg/kg (equivalent to 900 mg in humans) and MXF at 400 mg/kg, the results were very encouraging. The increased MXF dose induced organ culture negativity after 2 weeks of intensive daily therapy with RIF+INH+ PZA+MXF followed by weekly administration of RFP+ MXF for 5.5 months. More importantly, no relapses were observed 3 months after the end of therapy. This was the first demonstration that intermittent therapy could be equivalent in efficacy to the prescribed CDC and WHO regimen [69]. An initial 2 week intensive phase followed by 5.5 months of intermittent therapy would be a considerable improvement in dosing, although human trials of this regimen have not yet been conducted.

RFP is now approved in the U.S. for use in intermittent therapy with INH in the continuation phase of treatment in a limited number of tuberculosis patients. Following clinical trials in China [70], South Africa [71], and the U.S. [72], the CDC recommended treatment using RFP in certain well-defined cases: the patient is >12 years of age, has culture-positive, noncavitary pulmonary TB, is infected with isolates susceptible to RIF, INH, and PZA, has sputum smear results negative for acid-fast bacilli after the 2 month intensive phase, and is HIV negative [73]. Although the use of RFP in a small number of pulmonary TB patients is a move towards a more effective treatment regimen, it is still a very limited patient population, and the fact that a 2 month intensive phase still exists is not a significant improvement over the standard of care in regards to compliance.

RFP has also been used in the treatment of latent TB. In a clinical study in Brazil, investigators evaluated 12 weeks of RFP+INH delivered weekly and compared this regimen to 8 weeks of RIF+PZA daily (standard of care) in close contacts of patients with newly diagnosed TB. Unfortunately, the study was halted before completion because a significant number of patients in the RIF+PZA arm experienced hepatoxicity. In those contacts who completed treatment, RFP+ INH was well tolerated and provided very good protection against acquiring pulmonary TB [74].

In summary, RFL (Rifalazil) shows promise in mouse studies, but has yet to be tested in humans for efficacy against pulmonary TB; RFB (Rifabutin) appears to be a more appropriate drug than RIF for use in TB/HIV co-infected patients, and RFP (Rifapentine) could radically improve TB treatment if paired with a second drug that could be used intermittently, not daily. Intermittant treatment would not only improve patient compliance, but reduce the healthcare costs of daily directly observed therapy (DOT).

2. ACTIVE FLUOROQUINOLONES

The fluoroquinolone class of antibiotics possesses good activity against a wide spectrum of Gram-negative and Gram-positive aerobic and anaerobic bacteria and intracellular pathogens [75-78]. Many fluoroquinolones have been approved

by the FDA and EMEA and are marketed for treatment of bacterial infections. Several fluoroquinolones currently available in the marketplace, including ciprofloxacin, ofloxacin, levofloxacin, gatifloxacin (GAT), and MXF (Fig. **4**), also possess activity against *Mycobacteria,* including *M. tuberculosis* [79-90], and are sporadically prescribed for treatment of MDR TB. Fluoroquinolones act by inhibiting DNA topoisomerase IV and DNA gyrase, enzymes that control DNA topology and are vital for cellular processes that involve duplex DNA, namely replication, recombination, and transcription [91-94].

Ofloxacin, Ciprofloxacin and Levofloxacin

Two hundred and seventy-six strains of *Mycobacteria* were tested for susceptibility to ciprofloxacin, and most strains of *M. tuberculosis, M. fortuitum, M. kansasii, M. marinum* and *M. xenopi* were sensitive at MIC of 0.78–1.56 μg/ml [79]. Thirty-five clinical isolates of *M. tuberculosis*, 24 susceptible and 11 resistant to conventional primary antitubercular drugs, were tested against six new quinolones and were susceptible to both ciprofloxacin or ofloxacin at 1.0-2 μg/ml. [80]. A favorable MIC of ofloxacin (0.63–1.25 μg/ml) was demonstrated by Yew W. *et al.* [83] for 147 isolates (92% of strains tested). In fact, ofloxacin has *in vitro* activity against *M. tuberculosis* equal to RIF [81].

Levofloxacin, the *S*-enantiomer of ofloxacin (Fig. **4**), exhibited 2-fold greater inhibitory and bactericidal activities than ofloxacin against either extracellular or intracellular tubercle bacilli [95]. Of 135 isolates of *M. tuberculosis* tested, 134 were susceptible to levofloxacin, with an MIC less than or equal to 1.0 μ/ml [97]. MIC of levofloxacin against *M. tuberculosis* and *M. intracellulare* inside murine peritoneal macrophages was 2 to 4 time lower than that of ofloxacin [98]. Levofloxacin, ciprofloxacin and ofloxacin showed equal *in vitro* activity (average MIC_{90} 1 μg/ml) against two hundred isolates of *M. tuberculosis* [85]. The *in vitro* activity of the three most active fluoroquinolones were similar, with levofloxacin having better activity against intracellular *M. tuberculosis*.

Because the three active fluoroquinolones are approved for other indications and are available to physicians, several have been used for treatment of patients with MDR-TB [96, 99, 100]. Yew reported that 81% of patients with MDR pulmonary TB were cured with ofloxacin/levofloxacin-containing regimens [86]. Treatment of 124 patients with primary uncomplicated pulmonary TB showed that ofloxacin is as useful as EMB when combined with INH and RIF [101]. To date there are no reports of cross-resistance or antagonism of the fluoroquinolones with other classes of antimycobacterial drugs.

GAT (Gatifloxacin, Generic)

Among fluoroquinolones, GAT (Fig. **4**), approved for use in other bacterial infections, is one of the most active against *M. tuberculosis,* with MIC_{50} 0.03–0.12 μg/ml [63, 99, 102, 103].

Animal data for GAT activity *in vivo* is also impressive. Four week treatment of *M. tuberculosis*-infected mice with GAT at 300 mg/kg is more effective than therapy with INH at 25 mg/kg [33], but at lower doses GAT was less effective in this timeframe. A 6-month monotherapy of *M. tuberculosis*-infected C57BL/6 mice with GAT reduced bacteria in lungs by 3.9 $\log_{10}$ CFU, which was similar to efficacy of INH at 25 mg/kg [40]. Alvirez-Freites reported that GAT alone and in combination with EMB has similar efficacy *in vivo,* reducing bacteria in lungs of mice infected with *M. tuberculosis* from 7.6 $\log_{10}$ CFU in early infected controls to 3.4 $\log_{10}$ CFU for GAT alone or 3.3 $\log_{10}$ CFU for GAT+EMB. Addition of GAT to INH+RIF in combination therapy did not improve drug efficacy over INH+RIF alone, but combining GAT at 100 mg/kg with the second-line drug ETA did enhance bacterial clearance more than a full $\log_{10}$ CFU: GAT alone reduced lung bacteria from 8.0 $\log_{10}$ CFU in infected untreated controls to 4.2 $\log_{10}$ CFU, but residual lung bacteria in GAT+ETA-treated mice were only 2.9 $\log_{10}$ CFU [27]. In addition, incorporating PZA into the mixture of GAT+ETA actually resulted in no cultivable bacteria in

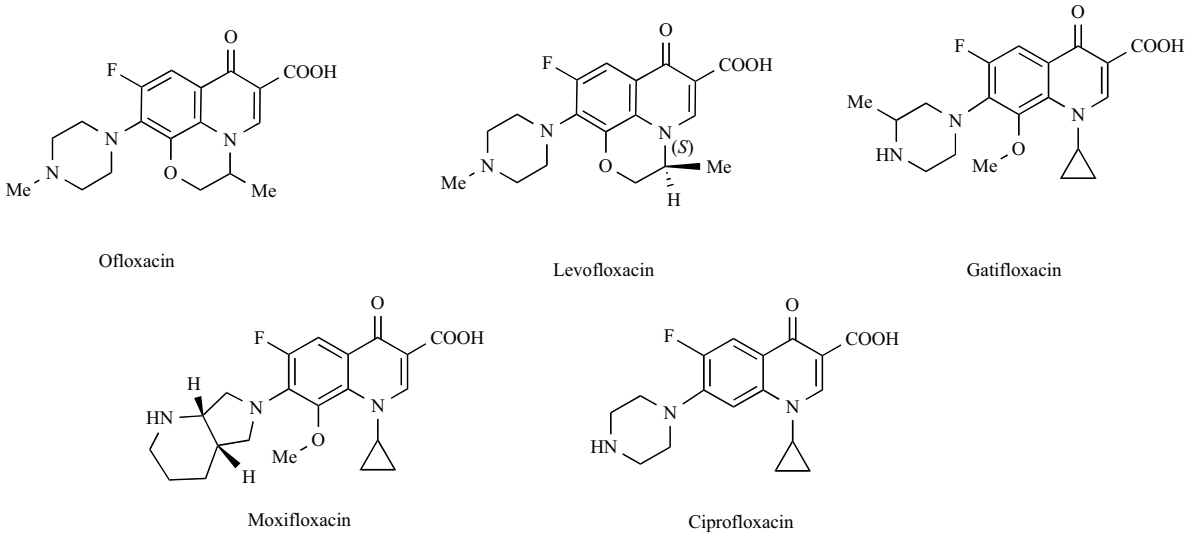


Fig. (4). Chemical structures of fluoroquinolones with activity against *M. tuberculosis*.

lungs of mice treated with this combination for either 8 or 12 weeks [33]. Mice monitored for reactivation TB after combination GAT+ETA+PZA therapy for 12 weeks remained free of bacteria for at least 2 months after therapy withdrawal.

Currently, GAT is undergoing a Phase III clinical trial through the EU clinical trials group funded in part by WHO TDR. The trial is to evaluate whether the GAT-containing multidrug regimen can shorten the standard TB treatment to four months. This multi-center study has an expected enrollment of 2070 recently diagnosed pulmonary TB patients and is being conducted in Benin, Kenya, Guinea, Senegal, and South Africa. Results are not yet available.

MXF (Bayer and Global Alliance for TB Drug Development, GATB)

MXF (Fig. **4**) is also approved for other bacterial disease indications. Like GAT, MXF is very effective against *M. tuberculosis in vitro*, with MIC_{50} range of 0.06–0.5 μg/ml [63, 99, 102, 103], and *in vivo* in experimental animals. Monotherapy with MXF for 6 months reduced lung bacteria by 3.2 log_{10} CFU compared to bacteria in lungs of control untreated mice [40]. In an earlier study [27], MXF at 100 mg/kg was almost as effective as INH at 25 mg/kg: after 4 weeks of treatment, MXF was able to reduce CFU in lungs of mice from 8.9 log_{10} CFU in infected untreated control mice to 4.8 log_{10} CFU (a 4-log reduction), while INH treatment resulted in 4.2 log_{10} CFU (nearly a 5-log reduction). In a dose study, Yoshimatsu demonstrated that MXF at 400 mg/kg has increased efficacy for treatment of BALB/c mice infected i.v with *M. tuberculosis* over INH at 25 mg/kg: a 5.2 log_{10} CFU reduction was accomplished by MXF, while INH reduced lung bacteria a log less, a 4.2 log_{10} CFU reduction [28]. Combining MXF (100 mg/kg) with INH+RIF was slightly more effective than INH+RIF therapy alone, but addition of MXF to PZA+EMB+ETA greatly improved the drug combination efficacy, reducing residual lung bacteria by 1.5 log_{10} CFU over the combination without MXF.

Treatment of aerosol *M. tuberculosis*-infected BALB/c mice with drug combinations for 6 months [39] showed that a regimen consisting of 2 months treatment with RIF+MXF (100 mg/kg)+PZA followed by 4 month treatment with RIF+MXF eliminated bacteria in lungs by month 3 after therapy initiation, whereas the standard TB therapy took 5 months, and fewer relapses occurred in the MXF-based regimen [39]. MXF could successfully substitute for INH during both the initial and continuation phases of TB treatment. MXF also appears to target a subpopulation of *M. tuberculosis* in mice that are not killed by RIF [105].

Efficacy of MXF compared to INH was confirmed in EBA clinical studies in humans: after 5 days of monotherapy, the EBA calculated on the basis of residual bacteria in sputum was 0.209 and 0.273 log_{10} CFU for INH and MXF, respectively [106]. MXF has activity similar to rifampin in human subjects with pulmonary TB [107]. A comparison EBA clinical trial to assess bactericidal activity of MFX (400 mg), GAT (400 mg) and levofloxacin (1000 mg) in comparison with INH (300 mg), all drugs delivered daily, was conducted by Tuberculosis Research Unit (TBRU) Clinical Trials Component at the Case Western Reserve University. Bactericidal activity was estimated by measuring the decline in bacilli during the first 2 days (EBA) and the last 5 days of monotherapy. The ranking order (better to worse) for EBA activity expressed as log_{10} CFU/ml/day was INH (0.67)> Levofloxicin (0.45)>GAT (0.35)>MXF (0.33), although all had identifiable EBA activity (see http://www.case.edu/affil/tbru/trials.htm).

Clinical trials by the TB Trials Consortium (TBTC, study #27) of CDC in collaboration GATB revealed that substitution of MXF for EMB in treatment of TB patients did not ultimately affect 2-month sputum culture status, but did show a difference in bacterial numbers at earlier time points [108]. Another clinical study with a similar design (MXF in place of EMB) is ongoing in Brazil, and results are not currently available. INH and MXF actually antagonize each other: having both INH and MXF in a regimen worsens treatment efficacy in human subjects [109]. Based on the animal data and human EBA studies, a new Phase II clinical trial conducted by CDC, GATB, and Bayer (TBTC study #28) where MFX replaces INH, has been initiated, with an expected enrollment of 410 patients.

Both MXF and GAT have dose-related toxicities, and their position within a new TB regimen for uncomplicated pulmonary TB has yet to be determined. For MDR-TB, however, these fluoroquinolones have clear potential to add to the drugs available for clinicians and patients.

There is growing concern over the emerging resistance to fluoroquinolones [110,111,112] that may limit their role in TB therapy. These effective and readily-available drugs have been increasingly prescribed to TB patients and a growing number of resistant TB cases have been reported. For example, from 1995 to 2003, *M. tuberculosis* resistance to ciprofloxacin, ofloxacin and levofloxacin increased from 7% to 20% in Taiwan [113].

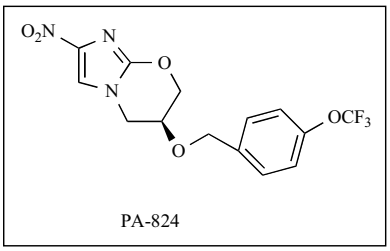
Like many bacteria, *M. tuberculosis* develops resistance to drugs used singly or in non-optimal concentrations [118]. After only eight-week monotherapy of mice with MXF, resistant *M. tuberculosis* mutants were isolated from all surviving mice [114]. Fluoroquinolone resistance is mainly due to mutations in the *gyrA* [115] and *gyrB* [116] genes. A second mechanism is mediated by mycobacterial *mfpA*-encoded protein, a member of the "pentapeptide repeat" family of bacterial proteins. The protein binds DNA gyrase, preventing formation of the DNA–DNA-gyrase complex that is a target for fluoroquinolones [117]. Ginsburg *et al.* sequenced the MXF-resistant isolates, however, and identified the resistance mechanism of these isolates as the quinolone resistance-determining regions (QRDRs) of *gyrA* and *gyrB* [114]. The mutants were resistant to fluoroquinolines as a class.

Flouroquinolones have good potential to contribute to TB chemotherapy, improving treatment or shortening treatment time. To determine their actual utility, and prevent indiscriminate induction of resistance, they should be prescribed only after the appropriate dose and multi-drug combinations are established in clinical trials, and never as monotherapy, as resistance is a class phenomenon. With ongoing clinical trials of GAT and MFX, we should be able to place these

fluoroquinolones in the context of an appropriate indication and appropriate treatment regimen in the next few years.

3. PA-824 (PATHOGENESIS/CHIRON AND GATB)

PA-824 (Fig. **5**) was discovered from a set of 328 substituted bicyclic nitroimidazoles synthesized based on the structure of orally active CGI-17341 [119,120]. High potency of the compound against *M. tuberculosis in vitro* and *in vivo* coupled with a novel MOA engendered great interest in the TB community, and GATB, who licensed the drug from Chiron in 2002, launched an expansive development program to bring this drug into the clinic.



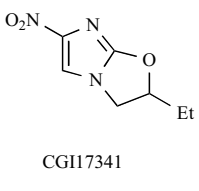


Fig. (5). Chemical structures of the drug candidate PA-824 (Pathogenesis/Chiron and GATB) and the parent compound CGI17341.

PA-824 exhibits activity against both drug-susceptible and drug-resistant strains of *M. tuberculosis,* with MIC ranging from 0.015-0.25 μg/ml, and high overall specificity for *Mycobacteria* (*M. avium*, *M. smegmatis, M .chelonae, M. fortium*). Notably, it has bactericidal activity against both replicating bacteria and nonreplicating bacilli *in vitro* [119], suggesting that it might be useful for treatment of active TB and latent disease. PA-824 has a novel MOA and inhibits both protein production and cell wall lipid biosynthesis. Like other compounds in the nitroimidazole class (metronidazole and CGI-1734) PA-824 is a prodrug that requires bacterial activation by reducing its nitro group: activation likely involves a F420-dependent mechanism [119,121]. Unlike metronidazole, however, PA-824 lacks experimental animal model mutagenicity commonly associated with nitroimidazoles [119], although mutagenicity in humans will be evaluated in a clinical setting [121,122].

PA-824 is orally active in both mouse and guinea pig models of *M. tuberculosis* infection and has activity comparable to that of INH [42, 43, 119]. In the guinea pig TB model, oral administration of PA-824 at 40 mg/kg resulted in comparable statistically significant reductions of CFU in lung and spleen as INH at 25 mg/kg. In mice, studies by Grosset *et al.* [42] demonstrate that the minimal effective dose of PA-824 is 12.5 mg/kg and the minimal bactericidal dose is 100 mg/kg. Two-month daily monotherapy of mice with PA-824 initiated at day 20 of *M. tuberculosis* infection prevents death of infected mice and reduces bacteria in both lungs and spleens by greater than 3 $\log_{10}$ CFU. PA-824 monotherapy also eliminates bacilli that remain after the 2-month intensive phase treatment: PA-824 at 100 mg/kg for an additional 4 months was superior in potency to INH at 25 mg/kg or MXF at 100 mg/kg, and approached the activity of the recommended RIF+INH 4-month continuation phase therapy.

During the initial intensive phase of therapy, PA-824 alone is slightly less effective in clearing bacteria from lungs and spleens than INH alone, and combining PA-824 with INH doesn't reduce CFU further. The combination does, however, substantially decrease the proportion of drug-resistant mutants to either drug in the lungs.

Nuermburger *et al.* [43] methodically tested several PA-824-containing multidrug regimens in a murine model of TB, replacing PA-824 for RIF or INH, with or without PZA, during both intensive (2 month) and continuation (4 month) therapy. The effectiveness of each 6-month treatment regimen was based on (1) determination of CFU counts remaining in lung at 2, 4, and 6 month time points and (2) relapse rates 3 months after treatment cessation. To eliminate the possibility of adverse pharmacological interactions, serum concentrations of RIF, INH, and PZA in the presence and absence of PA-824, as well as PA-824 by itself, were also determined: there were no significant drug-drug interactions between PA-824 and any of the front-line TB drugs. Despite the bactericidal activity of PA-824 alone, its administration in combination with other drugs did not lead to significant improvements over the standard regimen. Incorporating PA-824 into the standard regimen in either the intensive phase or the continuation phase did not change the CFU counts in organs at any of the three time points, but the PA-824-containing regimens had a higher relapse rate. Substitution of RIF and PZA with PA-824 was worse, and mice receiving the combination RIF+INH+PA824 for 6 months straight were mostly culture negative at the end of treatment, but the relapse rate was 75%. In fact, all regimens containing PA-824 had higher relapse rates than standard therapy, no matter which drug PA-824 replaced.

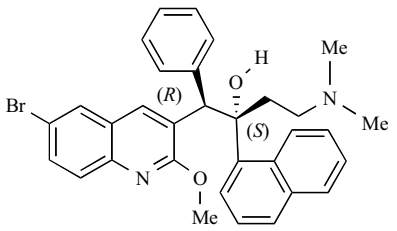
These results in the murine model of TB were disappointing, since monotherapy of mice with PA-824 showed such good results. However, while murine models have been predictive for activity of the "old" TB drugs and drug regimens, no one is exactly sure whether they will be predictive of the new drugs with new MOA. Thus, GATB began clinical development of PA-824 in 2005.

PA-824 has been evaluated in several human Phase I clinical trials to determine safety, tolerability, pharmacokinetics, food effect, and ADME (absorption, distribution, metabolism, and excretion) properties of single and multiple doses of the drug in healthy male and female volunteers. A Phase II EBA study of PA-824 in adults with sputum smear-positive, pulmonary TB is expected to start in 2007 [123].

4. DIARYLQUINOLINE R207910/TMC207 (TIBOTEC/JOHNSON&JOHNSON)

The existence of R207910 (now called TMC207, Fig. **6**), was first described in 2004 at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) meeting Late-Breaker Session, where background information and Phase 1 human safety data was described for this drug that had been quietly in development for over 7 years. Since then, a number of papers have been published that give us a good picture of this interesting new TB drug candidate.

The screening assay that led to identification of R207910 from diverse chemical libraries was growth inhibition of *M.*



TMC207

Fig. (6). Chemical structure of the drug candidate R207910/ TMC207 (Tibotec/Johnson & Johnson).

smegmatis in a whole-cell assay, which assesses activity on multiple targets, followed by screening of a selected series on *M. tuberculosis* to determine MIC. R207910 was identified as the most potent for *M. tuberculosis* in the diarylquinoline class: it is a (*R,S*)-diastereomer with lateral dimethylaminethyl substituent [29,124]. R207910 exhibits good *in vitro* activity against laboratory strains and both drug susceptible and drug resistant clinical isolates of *M. tuberculosis,* with MIC values ranging from 0.03 to 0.12 μg/ml. No cross-resistance with first-line anti-TB drugs is observed. Moreover, R207910 is highly specific to *Mycobacteria* and inhibits growth of *M. avium*, *M. smegmatis, M. chelonae, M. fortium*, *M. bovis, M. kansasii, M. ulcerans* [29].

Quinoline R207910 has a novel MOA, distinct from all other anti-TB drugs available commercially or in development: it affects the proton pump for ATP synthase and is unlike quinolones, whose target is DNA gyrase [29, 21]. Characterization of *M. tuberculosis* mutants resistant to R207910 demonstrate that the *atpE* gene encoding subunit *c* of the ATP synthase is responsible for resistance and may be the target of the compound [29, 125, 126]. The *atpE* gene region of R207910 resistance shows a high degree of homology for different mycobacterial species, but has limited homology with human ATP proteins [29, 126]. The targeting by R207910 of the quite-specific *Mycobacteria* ATP synthase may explain its high specificity and the lack of activity against other bacterial ATP systems. R207910 could also be a prodrug that requires activation by a *Mycobacteria*-specific enzyme [125].

PK studies in mice show that oral administration of R207910 at 6.25 mg/kg gives a C_{max} of 0.5 μg/ml after 1 hour, exceeding the drug MIC by several fold, with the following distribution in tissues over an 8 hour period: lung>liver>kidney>spleen>heart>plasma. Twenty-five mg/kg C_{max} was 1.1-1.3 μg/ml within 2-3 hours. Area under the curve (AUC) was 5 and 19.4 μg/ml respectively, for 6.25 and 25 mg/kg, and AUC correlates better with dose than C_{max} values, reflecting limitations in the rate of absorption at higher drug concentrations. R207910 has a very long half-life: 47 and 58.6 hours in plasma for dosages of 6.25 and 25 mg/kg. In tissue, half-life ranges from 28 to 92 hours.

The long half-life and good tissue penetration resulted in a bactericidal effect of a single oral 100 mg/kg R207910 dose that lasted 8 days [29]. The minimum effective dose (6.5 mg/kg) was very close to the minimum bactericidal dose (12.5 mg/kg), suggesting that drug effects were time-dependent rather than concentration-dependent. Monotherapy with 12.5 and 25 mg/kg R207910 demonstrates better clearance of TB bacteria than INH at 25 mg/kg and is as active as standard therapy using RIF+INH+PZA. Substitution of R207910 for any of the first-line TB drugs significantly decreases bacteria CFU in tissues compared to standard therapy, and a 2 month treatment with RIF+PZA+R207910 or INH+PZA+R207910 actually renders lungs of all mice in treated groups *M. tuberculosis* culture negative [29]. Moreover, R207910 combined with second-line TB drugs, amakacin (AMK) and ETA, or MXF, was significantly more potent than any of second-line drug combinations without R207910 [32], and inclusion of both R207910 and PZA to AMK, MXF and ETA shortened the time for total clearance of bacteria in lungs and spleen of mice from 9 months to 2 [127]. R207910 and PZA work especially well together, either alone or with other first-line TB drugs, clearing bacteria in lungs by 2 months for the pair and having slightly less efficacy in the presence of INH or RIF [128]. Overall, mouse data with R207910 suggest that R207910-containing regimens could shorten duration of chemotherapy, treat MDR-TB, or increase compliance by lowering the dose frequency.

R207910 has been transferred to Tibotec (Johnson & Johnson), for clinical development. It is now referred to as TMC207. The dose-escalation Phase Ia safety trials and multiple-ascen-ding-dose Phase 1b study of TMC207 in humans showed good tolerability, linear PK profile, and long terminal elimination half-life. In addition, these studies showed that TMC207 is metabolized by CYP3A4 (which may limit use of TMC207 in TB/HIV patients receiving anti-retrovirals) and co-administration of TMC207 with RIF lowers TMC207 blood levels by 50%. However, TMC207 does not affect RIF levels. Overall, 189 human subjects were treated with TMC207 the Phase 1 safety trials, with no serious adverse events related to TMC207 [129].

TMC207 has also been tested in a multicenter Phase IIa one-week EBA study in 75 adult TB patients. The drug was administered as monotherapy at 25, 100, or 400mg daily, followed by the standard of care 6-month regimen; efficacy of TMC207 was compared to that of one-week monotherapy with RIF (600 mg daily) and INH (300 mg daily). Only the 400 mg TMC207 dose showed EBA at 7 days, but activity was lower than either RIF or INH. The study also revealed that steady-state levels of CFU were not reached within 7 days, and suggested that early TMC207 drug activity might better be studied over 14 days, not 7 days. The antibacterial time-dependence (rather than concentration dependence) observed in mice supports this idea [29]. No safety alerts in TB patients were observed, however, and PK profiles were comparable to those of healthy subjects.

In summary, the diarylquinoline TMC207, with a unique MOA, may ultimately find a place in uncomplicated pulmonary TB in regimens that do not contain RIF (which decreases TMC207 concentration 50%), and will, if its safety profile continues to look as it does today, certainly contribute to the drug armamentarium for MDR-TB.

5. NITROIMIDAZOLE OPC-67683 (OTSUKA PHARMACEUTICALS)

OPC-67683 (Fig. **7**) was first described in public at the ICAAC meeting in 2005. It was the lead compound in a series of 6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazoles synthesized based on the 5-nitroimidazole pharmacophore of CGI17341 (Fig. **5**), the compound that had promising *in vitro* and *in vivo* activity against *M. tuberculosis* but was highly mutagenic [130]. Bicyclic nitroimidazopyran PA-824 discussed earlier in this review (Fig. **5**) derived from the same parent compound.

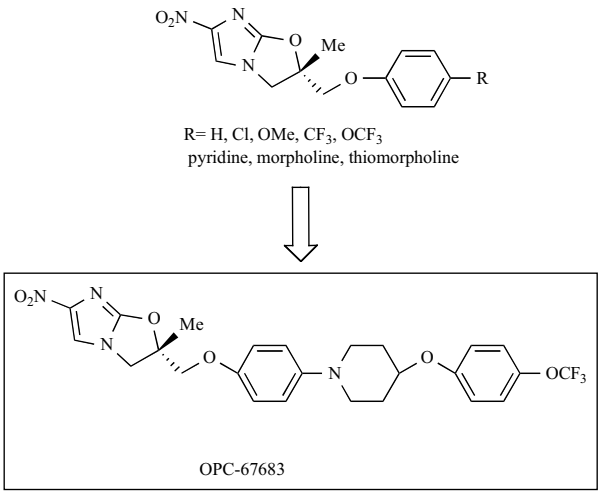


Fig. (7). Chemical structure of the drug candidate OPC-67683 (Otsuka Pharmaceuticals) and a series of substituted 6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazoles.

To further enhance antitubercular activity and eliminate mutagenicity, researchers at Otsuka in Japan designed and synthesized a series of optically active 6-nitro-2,3-dihydroimidazo[2,1-*b*]oxazoles with various phenoxymethyl or methyl groups at the 2-position. Within this set of compounds, (*R*)-enantiomers exhibited lower MIC values. Further efforts led to identification of several compounds with better *in vitro* antitubercular activity. OPC-67683 was the most efficacious *in vivo* and was selected for preclinical development [131]. MIC values of OPC-67683 against laboratory and clinical strains of *M. tuberculosis*, including drug-resistant strains, are within a very narrow range of 0.006-0.012 μg/ml, exceeding the potency of all known anti-TB drugs available commercially or in development. No cross-resistance or antagonistic activity with any of currently used first-line anti-TB drugs has been observed [132].

OPC-67683 primary MOA is the inhibition of methoxy- and keto-mycolic acid synthesis, with an IC_{50} of 0.021-0.035 μg/ml, and it has a secondary activity against *Mycobacteria* α-mycolic acid synthesis at drug concentrations 10 times higher, >0.25 μg/ml [132]. OPC-67863 is active against *M. tuberculosis* in human macrophages: after a 4 hour exposure to drug at 0.1 μg/ml, intracellular killing activity was similar to that of RIF at 3 μg/ml, and superior to INH and PA-824 [132]. Like PA-824, OPC-67683 is a prodrug that requires metabolic activation for its antimicrobial activities [121], but the active compound has not yet been identified. The only isolated and identified metabolite, desnitro-imidazo-oxazole, is not active against *M. tuberculosis* [132].

OPC-67683 orally administered to mice at 2.5 mg/kg has a lower plasma concentration (0.297 μg/ml), but longer half-life (7.6 hours) than any of the front-line TB drugs. PK studies in mice, rats, and dogs all demonstrate good absorption after oral administration, with distribution to tissues in the following rank order: liver>kidney>lung>heart>cerebellum>spleen>plasma. Bioavailability in experimental animals is 35-60%, and its concentration in lungs is 3-7 times higher than in plasma [133].

In vivo efficacy of OPC-67683 as monotherapy was evaluated in a short-term mouse model using BALB/c nude mice infected with 10^4 CFU *M. tuberculosis*. Ten day of dosing with OPC-67683 at 0.313 mg/kg reduced bacteria in lungs by 1 $\log_{10}$ CFU, and 0.625 mg/kg reduced bacteria to only 5% of CFU in lungs of control mice [132]. When therapy was initiated 28 days after infection in a chronic TB mouse model, OPC-67683 reduced CFU in lungs in a dose-dependent manner and, at 40 mg/kg, was more active than RIF or INH at 20 mg/kg [132].

In additional murine studies in the chronic TB model, OPC-67683 was combined with RIF and PZA for 2 months and with PZA only for an additional 2 months. By the end of treatment (4 months total), no bacteria were isolated from organs of treated mice. In contrast, organs (lungs and spleen) from 4 of 5 mice given standard TB therapy (2 months of RIF+INH+EMB+PZA and then 4 months of INH+RIF) had significant bacterial growth at the end of therapy (6 months) [132]. These studies suggest that OPC-67683 could shorten TB therapy when used with one or more standard TB drugs.

OPC-67683 is being developed clinically as an addition to the current 4-drug standard regimen (total 5 drugs in intensive phase TB treatment). At present time, OPC-67683 has completed a number of Phase I clinical trials and is undergoing a Phase II EBA trial in South Africa (with expected enrollment of 54 subjects) to evaluate the safety, efficacy and pharmacokinetics of OPC-67683 in patients with uncomplicated, smear-positive pulmonary TB (ClinicalTrials.gov identifier NCT00401271). OPC-67683 will be administered as monotherapy at four oral doses of 100mg, 200mg, 300mg and 400mg once daily for 14 consecutive days. The trial is sponsored by Otsuka Frankfurt Research Institute GmbH [129,134]. The trial is expected to be completed in the second half of 2007.

6. DIAMINE SQ109 (SEQUELLA, INC. AND NATIONAL INSTITUTES OF HEALTH, NIH)

SQ109 (Fig. **8**) was discovered at Sequella by screening a diverse library of 63,238 1,2-ethylenediamines for activity against *M. tuberculosis* using a high-throughput bioluminescence-based screening assay developed in collaboration with investigators at the NIH (recombinant *M. tuberculosis* produces light in response to cell wall-active inhibitors such as EMB, INH, and ETA) and confirming MIC in a broth microdilution assay [135]. Sixty-nine of the most potent compounds were sequentially tested in a variety of *in vitro* and *in*

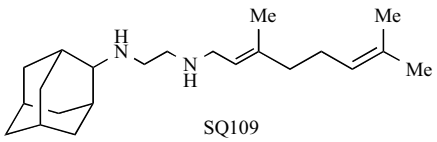


Fig. (8). Chemical structure of the drug candidate SQ109 (Sequella, Inc. and NIH).

vivo assays designed to weed out unsuitable or undrugable compounds, and SQ109 was selected as best in class from this compound series [31].

SQ109 has low cytotoxicity for cultured mammalian cells, high efficiency in killing *M. tuberculosis* within infected macrophages (99% at MIC) and in experimental animals, and a drug-like pharmacokinetic and pharmacodynamic profile [31,136,137,139]. More importantly, SQ109 primary MOA for activity against *M. tuberculosis* differs from the other cell-wall active TB drugs, including EMB (another active diamine) [141,142]: by microarray analysis, different genes are up- and down-regulated in SQ109-treated *M. tuberculosis* when compared to bacteria treated with other drugs [142]. Thus, SQ109 is a unique and very potent new drug in the broader class of diamine antibiotics.

SQ109 has high specificity for *Mycobacteria* (*M. tuberculosis, M. bovis, M. fortuitum),* with a tight MIC range (0.16-0.63 μg/ml) against laboratory strains and clinical isolates of *M. tuberculosis* that are drug sensitive, drug resistant (including EMB^r), MDR, and XDR. By the Luria Delbruck fluctuation analysis, SQ109 has a very low mutational frequency in *M. tuberculosis:* $2.55x10^{-11}$. In the same experiment, the mutational frequency of RIF was $1.9x10^{-9}$ (literature values for RIF mutation rate are $2.25x10^{-10}$) [138]. As monotherapy, SQ109 demonstrates equivalent *in vivo* activity in mice at doses of 1 and 10 mg/kg as that of EMB at 100 mg/kg [31,137].

One of the unique features of SQ109 is its pharmacological profile. Classic pharmacokinetic studies suggest that SQ109 has low oral bioavailability based on serum concentrations, 4%. At the same time, its *in vivo* tissue distribution profile shows a rapid and broad distribution into various tissues (V_{ss} =11,826 ml/kg in mice), preferably into lungs and greatly exceeding its MIC in this organ. The tissue distribution after oral administration is lung>spleen>kidney>liver >heart >plasma.

The peak concentration of SQ109 in lung is at least 50-fold (po) and 180-fold (iv) higher than in plasma of mice [136,137]. Although SQ109 preferentially locates in lungs and spleens, no statistically significant accumulation of SQ109 is observed in these tissues over 28-day repeat administration of 10 mg/kg, the efficacious dose in mice: drug concentrations in lung and spleen, while oscillating over the course of daily administration, maintain a level well above the MIC for the entire time and provide substantial pharmacological effect. These data suggest that SQ109 achieves a durable and effective drug concentration at several important sites of *M. tuberculosis* infection.

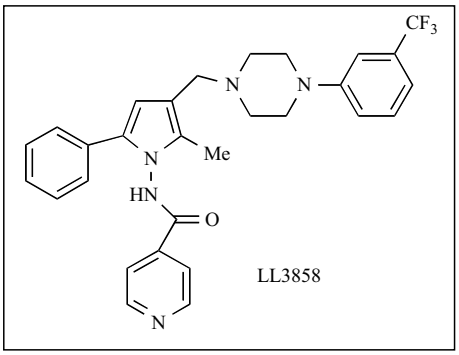
SQ109 has synergistic interactions *in vitro* and *in vivo* with two of the most important TB drugs in use today, RIF and INH. In inhibition of *M. tuberculosis* growth *in vitro*, SQ109 at 0.5 MIC demonstrates strong synergy with both 0.5 MIC INH and as low as 0.1 MIC RIF. SQ109 in the presence of SM shows additive activity but neither synergy nor additive effects with EMB or PZA. The synergy between SQ109 and RIF can also be demonstrated with RIF^r mutants of *M. tuberculosis*, and small amounts of SQ109 significantly lower the MIC of RIF against these drug-resistant strains [140].

These *in vitro* synergy data are supported by *in vivo* studies of drug combinations in drug screening and chronic mouse models of TB [143-146]. In the screening assay, using weight loss as an indicator of TB severity, the drug combinations SQ109+INH and SQ109+RIF are more effective in preventing TB-induced weight loss than any of the drugs alone, and similar effects are not seen with EMB or PZA, alone or with SQ109 [144,145]. Several *in vivo* studies in the chronic mouse model of TB using combinations of SQ109 and standard anti-TB drugs in two-, three-, and four-drug regimens for intensive phase TB therapy (2 months) demonstrate both better efficacy (fewer remaining CFU in tissues) and shorter time to achieve the same level of CFU as standard therapy. Substitution of EMB (100 mg/kg) with SQ109 (10 mg/kg) in a regimen containing RIF and INH results in better clearance of tissue bacteria and 25-30% decrease in time to standard of care effects, whether or not PZA is included in the regimen, or whether analysis is done at 1 or 2 months. In the studies using INH+RIF+SQ109+PZA, no or few (<20) bacteria were cultured from lungs of mice treated for 2 months, while control mice had more than 10^7 bacteria recovered, and standard of care mice had over 10^2 bacteria recovered [143,146]. These results suggest that combination treatment of pulmonary TB with SQ109 in a regimen with the other TB drugs, INH, RIF and PZA, may provided a better and faster therapeutic effect than the current standard of care.

The first-in-man clinical evaluation of SQ109 began in late 2006. The Phase 1 safety program includes two prospective single center, double-blind, randomized, placebo-controlled dose escalation studies to assess the safety and pharmacokinetics (and effects of feeding) of SQ109 in single (Phase 1a) and multiple dose (Phase 1b) regimens. Results are expected for the Phase 1a studies in early 2007.

7. PYROLE LL 3858 AND COMPOSITION LL 3848 (LUPIN LTD.)

In the 1990s, Deidda *et al.* [147] and Biava *et al.* [148] reported the promising antimicrobial activity of substituted pyrroles [147,148]. In these studies, compound BM212 (Fig. **9**) had an MIC of 0.7 μg/ml, moderate toxicity against Vero cells, and no significant cross-resistance with INH, RIF, EMB, or SM.[10] Later, a series of novel compounds based on a pyrrole fragment with significantly improved antitubercular activity was synthesized at Lupin Ltd [150]. The lead compound LL 3858 (Fig. **9**) from this new series demonstrated an MIC range of 0.125-0.25 μg/ml against laboratory strains and clinical isolates of drug-susceptible and drug-resistant *M. tuberculosis* [151,152] and synergy with RIF in *in vitro* assays [151]. Pharmacokinetic studies of orally-delivered LL 3858 in mice and dogs shows that it is rapidly



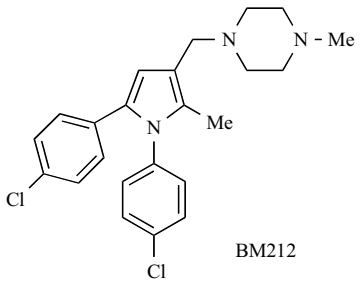


Fig. (9). Chemical structures of the drug candidate LL3858 (Lupin, Ltd.) and the parent compound BM212.

absorbed, reaching levels in serum above the MIC and with a better half-life and C_{max} than those reported for INH [151]. Toxicity studies in mice demonstrate an LD_{50} for oral compound of 700 mg/kg [151].

In vivo efficacy of LL 3858 was evaluated at 12.5 mg/kg and 25 mg/kg. Mice were infected with a sublethal dose of *M. tuberculosis* and treated with LL 3858 for 4, 8 and 12 weeks. LL 3858 administered orally significantly reduced CFU counts after 4 and 8 weeks of therapy, and no bacteria were recovered from tissues of 2 of 6 mice at 12 weeks of treatment. As monotherapy, LL 3858 was more effective than INH [151].

Lupin is also developing a fixed-dose drug combination of LL3858 with the first-line anti-TB drugs called LL3848. In the animal studies, the LL3848 was superior to standard of care (no recovery of bacteria from tissues), and no relapses were observed. In this study, treatment with existing drugs alone decreased CFU counts, but did not achieve complete bacterial clearance [153].

The single drug LL3858 and drug combination LL3848 have both completed registration-directed preclinical toxicity and are currently being evaluated in human Phase I clinical trials. At this time, there are no published articles on the results of clinical evaluation of either.

SUMMARY

This overview of the existing pipeline of drugs and drug candidates for treatment of TB demonstrates that there are a number of interesting compounds in development, all with unique properties and great potential to ultimately provide faster and more effective treatments for TB patients. Table **1** is a synopsis of *in vitro* and *in vivo* efficacy data for each of the featured compounds, along with the results and current status of clinical trials (where such data are available). Within the last ten years, in addition to the development of new drug regimens based on existing and approved rifamycins and fluoroquinolones, there has been considerable progress in TB drug discovery. We have witnessed and partici-

Table 1. Characteristic *In Vitro* and *In Vivo* Properties of Antitubercular Agents in Development and Their Clinical Trial Status

Name	Distinctive properties	Efficacy in mouse models of TB	Results of the clinical trials and current status
Rifalazil (RFZ)	• MIC 0.004 μg/ml • Inhibits RNA synthesis of *M. tuberculosis*	• Replacement for RIF in multidrug combinations leads to faster cure and lower relapse rate*	• Phase 2 EBA study: no anti-TB activity observed, possibly due to insufficient dosing • Is being developed for non-TB indications
Rifabutin (RFB)	• MIC 0.016 μg/ml • Minimal (compared to other rifamycins) interference with antiretrovirals • Inhibits RNA synthesis of *M. tuberculosis*	• Superior potency compared to RIF: replacement of RIF in multidrug combinations leads to faster cure and lower relapse rates* • Effective in intermittent therapy and treatment of latent TB infection	• No improvement over RIF in duration of treatment or relapse rates • Recommended to replace RIF for treatment of HIV positive TB patients
Rifapentine (RFP)	• MIC 0.04-0.16 μg/ml • Long half-life (16 h, 6 times that of RIF') • Inhibits RNA synthesis of *M. tuberculosis*	• No advantage in replacing RIF in standard regimen • Major improvement in outcome and treatment schedule when combined with MXF: RIF+INH+PZA+MXF (2 weeks daily) followed by RPT+MXF (5.5 months *weekly*) leads to culture negativity and improved relapse rate as determine 3 months after therapy completion*	• Approved for intermittent therapy with INH in continuation phase for uncomplicated TB • Phase 2 trial for latent TB (terminated early due to toxicity of comparator regimen): 3 months of weekly RFP+INH provided good protection from TB progression to active disease in patient contacts

(Table 1. Contd….)

Name	Distinctive properties	Efficacy in mouse models of TB	Results of the clinical trials and current status
Ofloxacin and Levofloxacin	• MIC_{90} 1 μg/ml • No cross-resistance or antagonism with other anti-TB drugs • Act on DNA topoisomerase IV and DNA gyrase of *M. tuberculosis*		• Used in treatment of MDR TB • Phase 2 EBA study: levofloxacin at 1000 mg daily is close in efficacy to INH (300 mg)
Gatifloxacin (GAT)	• MIC_{50} 0.03-0.12 μg/ml • No cross-resistance or antagonism with other antimycobacterial drugs • Acts on DNA topoisomerase IV and DNA gyrase of *M.tuberculosis*	• No improvement in combination with INH+RIF • Effective with ETA and PZA: 8 weeks of GAT+ETA+PZA leads to sterilization with no relapse (at 2 months)	• Phase 2 EBA trial: less effective than INH • Phase 3 trials for pulmonary TB to evaluate GAT-containing multidrug regimens *in progress*
Moxifloxacin (MXF)	• MIC_{50} 0.06-0.5 μg/ml • No cross-resistance or antagonism with other antimycobacterial drugs • Long half-life (9 h, plasma) • Bactericidal • Acts on DNA topoisomerase IV and DNA gyrase of *M.tuberculosis*	• Slight improvement in combination with INH+RIF • Effective in combination with PZA+EMB+ETA • Might replace INH: 2 months RIF+MXF+PZA followed by 4 months RIF+MXF leads to sterilization and lower relapse rate* • Effective with RFP	• Phase 2 EBA study: less effective than INH • Two Phase 2 trials to substitute MXF for EMB: no improvement* • Phase 2 trial to replace MXF for INH *in progress*
PA-824	• MICs - 0.015-0.25 μg/ml • Active against MDR TB and non-replicating bacteria • Inhibits both protein production and cell wall lipid biosynthesis of *M.tuberculosis*	• RIF+PA824+PZA (2 months) followed by RIF+PA824 (2 months) leads to sterilization; no advantage in relapse rate*	• Four Phase 1 single-dose and multiple dose trials in healthy volunteers complete: no serious adverse events reported • Phase 2 EBA study expected to start in 2007
TMC207 (R207910)	• MIC 0.03-0.12 μg/ml • High specificity for *Mycobacteria* • Active against MDR TB • Long half-lives in plasma and tissues • Synergy with PZA • Affects proton pump of ATP synthase of *M.tuberculosis*	• 2 months therapy with: Rif+PZA+R207910 or INH+PZA+R207910 or AMK+MXF+PZA+ R207910 or AMK+ETH+MXF+PZA+ R207910 or R207910+PZA leads to sterilization	• Phase 1 trials of single-dose and multiple doses in healthy volunteers: no serious adverse events reported • Phase 2a EBA study complete: less effective than RIF or INH (assessed over 7 days)
OPC-67683	• MIC 0.006-0.012 μg/ml • No cross-resistance or antagonistic activity with first-line anti-TB drugs • Active against MDR TB • Long half-life (7.6 hrs)	• OPC+ RIF+ PZA (2 month) followed by OPC+RIF (2 month) leads to complete culture negativity	• Phase 1 trials completed • Phase 2 EBA study *enrolling*
SQ109	• MIC 0.16-0.32 μg/ml • Active against MDR-TB • Synergy with RIF and INH; additive effect with SM • rapid distribution into tissues, preferably into lungs	• Effective replacement for EMB: 2 months of RIF+INH+SQ109+PZA is superior to standard regimen and leads to sterilization in some mice	• Dose escalation Phase 1a trial *in progress*
LL3858	• MIC_{90} 0.125-0.25 μg/ml • *in vitro* synergy with RIF	• More effective than INH; 3 month treatment with LL3858 as monotherapy leads to culture negativity in some mice	• Phase 1 clinical trials in progress
Composition LL3848	• Combination of LL3858 with the 1st line anti-TB drugs)	• Superior to standard regimen	• Phase 1 clinical trials in progress

* In comparison with standard of care TB therapy regimen.

pated in moving novel drug candidates from discovery into the clinic. With a little luck and careful clinical evaluation, a new and more effective drug regimen will be identified in a few years. It is important to sustain this pharma activity, however, so that the public health and TB patients can benefit from the innovation that will occur in the future. The message is not that we have sufficient numbers of drugs in the pipeline, but that a constant search for new and improved drugs is essential to keep one step ahead of *M. tuberculosis*, a crafty bacterium with a long history of circumventing drug action, one at a time. There is no room for complacency with a pathogen that has been successful for 5000 years, and is being helped in its pathology by the growing HIV epidemic.

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Received: 06 February, 2007 Revised: 07 February, 2007 Accepted: 07 February, 2007