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Patent developments in antimycobacterial small-molecule therapeutics

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Summary

At present, clinical management of patients infected with *M. tuberculosis* faces difficult problems, such as the worldwide emergence of multidrug-resistant tuberculosis (MDR-TB), and the increase in AIDS-associated infections. Development of new drugs with greater or distinct antimycobacterial activity than those currently used is, therefore, urgently desired. Three main strategies toward this effort are pursued: discovery of new targets, structural modification of existing antibiotics, and identification of natural resources for novel antibiotics.

Keywords drug discovery, drug leads, *Mycobacterium tuberculosis*, TB

1. Introduction

Infectious diseases remain the largest cause of death in the world today, preceding cardiovascular disease and cancer. Tuberculosis (TB) has currently the highest death toll from a single infectious agent in the world. It is a major infection in developing countries, as well as an increasing problem in developed countries. Data from 2000 indicate that TB, caused by infection with *Mycobacterium tuberculosis*, kills almost 2 million people worldwide each year [1]. According to current estimates of the World Health Organisation (WHO), one third of the world's population is infected with the bacillus and about 35 million people will die from TB in the first twenty years of the 21st century [2].

TB mainly affects lung parenchyma (pulmonary TB), but the bacillus can also penetrate other organs (extrapulmonary TB), e.g., pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges. The most important symptoms are fever, loss of weight, and a typical cough, which is accompanied with blood and sputum.

Recently, improved methods for prevention, detection, diagnosis, and treatment have largely reduced the number of people who develop TB and those dying from it. However, during the last decade, there has been an unfortunate revival of TB. The resurgence of this disease is

caused by the outbreak of MDR-TB [3] and the global HIV pandemic. Indeed, HIV and TB form a lethal combination, while each disease speeds up the progress of the other. An individual infected with HIV has a 6- to 60-fold greater risk of developing TB [1].

Standard treatment of TB, as recommended by the WHO, lasts at least 6 months and requires a combination of different antibiotics [4]. Usually, the chemotherapy is initiated with three drugs: isoniazid, rifampin, and pyrazinamide, often with the inclusion of a fourth drug such as ethambutol (or streptomycin). These agents are daily administered in combination for two months. In a continuation phase, isoniazid and rifampin are administered biweekly for four to twelve months. This standardized treatment regimen avoids failing by acquired resistance and is part of the new paradigm in TB treatment: the Directly Observed Treatment Short-course (DOTS). This strategy was developed to shorten the length of the illness, to lower the risk of death, and to prevent the development of resistant strains. It is built on 5 elements: political obligation, microscopic provision, drug supply, a monitoring system and a direct observation of the treatment.

Although single-drug therapy can inhibit the majority of organisms in an infected site, it permits and, in fact, encourages uncontrolled growth of the resistant mutants. Likewise, inadequate treatment, due to poor compliance, inappropriate regimens or irregular drug supply, leads to the development of drug resistance.

In cases of emergency (MDR-TB), the so-called second-line anti-TB drugs are applied. However, severe side effects, high costs and the fact that MDR-TB treatment requires tailoring to the individual patient and strain impede general use. Examples of second-line drugs are ethionamide, fluoroquinolones, *p*-aminosalicylic acid, cycloserine, kanamycin (a synthetic variant of streptomycin), and capreomycin.

The only vaccine currently in use is the Bacille Calmette-Guérin (BCG) vaccine, which is a live attenuated strain of *M. bovis*. The widespread use of this vaccine has not been able to significantly affect the growing pandemic of TB. Although BCG is efficacious in preventing the less common yet severe paediatric forms of TB (meningitis and systemic disease) and

adult pulmonary TB in some parts of the world, there are clearly populations in high-burden countries which do not benefit from the current vaccination regimen.

An important phase in the development of new anti-tuberculous drugs is the *in vitro* determination of a compound's capacity to inhibit the growth of *M. tuberculosis*. For that purpose, mycobacteria are cultured in artificial media containing different concentrations of the compound under investigation. The determination of the minimal inhibitory concentration (MIC) of an antibiotic against mycobacteria by observing their growth *in vitro* using culture media is the cornerstone of antimicrobial selection for treatment of TB. MIC is the lowest concentration effecting a reduction in bacterial growth of 99% relative to controls.

Efforts to develop new drugs for the treatment of TB are hampered by difficulties associated with laboratory studies of *M. tuberculosis*, i.e., mainly the long doubling time of the organism (18 to 24 h) and the need to work under stringent level-3 biosafety conditions. The growth of *M. tuberculosis* can be measured by several methods such as monitoring colony formation in solid media or turbidity in liquid media. However, the observation of the slow growing *M. tuberculosis* requires long incubation periods. Many investigators tried to circumvent the problem by performing tests in liquid media. Several indirect growth observation methods have been developed for clinical use. These include observing the production of radioactive carbon dioxide in the BACTEC460 system [5] and of oxygen in the Mycobacterium Growth Indicator Tube [6] or monitoring the bioluminescence from the Luciferase enzyme that is transduced into *M. tuberculosis* by a specially-engineered virus [7]. In the latter reporter gene assay, the ability of a compound to inhibit growth of a mycobacterial reporter strain is measured by a decrease in bioluminescence resulting from a reduction in Luciferase expression and intracellular ATP levels, both of which are requisites for enzymatic activity. Most of these methods can decrease the test period from 3-4 weeks to only 7-10 days.

Candidate anti-TB drugs must have low toxicity, because patients might need to ingest them for a long time. Primary testing for toxicity consists of incubation of the compounds with human cells, that are cultivated *in vitro* and monitored for cytopathic effects. A promising lead

compound must have the ability to inhibit the growth of the organism at low concentrations and to be toxic to human cells only at high concentrations. These characteristics are translated in a high selectivity index (SI).

The current TB drug arsenal is largely the result of development programs conducted between 1940 and 1970 and no new antitubercular drugs except rifabutin and rifapentine have entered the clinic since rifampin in the early 1970s. The resurgence of TB, the development of MDR-TB, and the discovery that the progression of TB is accelerated in HIV-positive patients underscore the importance of the development of more efficient drugs to combat this disease [8]. Unfortunately, until recently, TB has with few exceptions not been an attractive target disease for the pharmaceutical industry, as it was perceived as primarily a disease of the poor, and most major pharmaceutical companies did not expect an adequate return on investment. Also, clinical development of TB drugs is not straightforward. Efficacy trials are tedious and complex and, because few products have been newly registered during the last 30 years, current regulatory requirements are not well defined or standardized, and previous guidelines are outdated.

In the Scientific Blueprint for TB Drug Development [9], the objectives are set as follows:

- to shorten the overall duration of chemotherapy and/or reduce the number of doses,
- to improve treatment of MDR-TB, and
- to identify a better treatment for latent TB infections

It is the purpose of this review to examine the patent literature from 2002 to September 2004 on the discovery of small-molecule agents that appear useful for the treatment of TB.

Emphasis will be on new low-molecular-weight compounds. Patents covering formulations, drug delivery methods or manufacturing processes are beyond the scope of this review.

2. Derivatives of existing drugs

Using thioacetazone (1) and *p*-aminosalicylic acid (2) as templates, the University of Sciences in Philadelphia prepared some halogenated derivatives [101]. The *in vitro* evaluation of their activity was conducted against *M. tuberculosis* H37Rv. Compounds were also tested for cytotoxicity and for their capacity to inhibit growth of virulent *M. tuberculosis* in an *in vivo* aerosol mouse model. Thioacetazone is a thiosemicarbazone that is used in association with other antimycobacterial agents in the initial and continuation phases of antituberculosis treatment. Thioacetazone containing regimens are less effective than the recommended short-course regimen and are used in some developing countries to reduce drug costs. Its mechanism of action is still unknown. As most active derivative of thioacetazone emerged the 3-fluorinated derivative 3. The compound showed a MIC-value < 0.1 µg/ml and an SI > 312.5. This derivative proved also active against TB strains that are resistant to isoniazid, rifampin, ethambutol, kanamycin, and ciprofloxacin. Compared to the MIC-value of thioacetazone against *M. tuberculosis* H37Rv (> 2.0 µg/ml), this fluorinated derivative is about 20 times more effective.

FIGURE 1

p-Aminosalicylic acid (2) is known to be only active against growing bacilli. Being a mimic of *p*-aminobutyric acid (paba), it inhibits DNA synthesis. 4-Amino-5-fluorosalicilic acid (4) emerged as the most active derivative of 2. It displays a MIC-value < 3.13 µg/ml and an SI > 10. It was also tested for activity against drug resistant strains, where it shows lower but still significant activity. Only strains resistant to isoniazid are less susceptible (MIC = 25 µg/ml). Compared with the MIC of *p*-aminosalicylic acid (1.25 µg/ml), this derivative seems less active than the original lead.

Using ethambutol (6), a widely used drug for the treatment of TB, as lead, a large library of ethylene diamines with a variety of amine substituents, as well as substituents in the linker region has been produced using split-and-pool procedures on a solid support [102, 10]. Initial

evaluation of the library was done by a high-throughput screening assay using bioluminescent reporter strains that produce light in response to inhibition of the cell wall synthesis [11]. A number of hits resulted from this HTS assay. Compound **6**, flanked by a 2-adamantyl and a geranyl substituent, for instance, gave a MIC-value of 0.2 μ M against *M. tuberculosis*, compared to 9 μ M for ethambutol in the same BACTEC assay [10]. This derivative proved also active against 3 MDR patient isolates that were also highly resistant to ethambutol and was tolerable in mice up to 600 mg/kg. *In vivo* assays in mice infected with *M. tuberculosis* H37Rv showed that 10 mg **6** had similar activity as 100 mg ethambutol in reducing colony-forming units (CFUs) in spleen, while the same dose had superior activity than 100 mg ethambutol in reducing CFUs in lung.

Rifamycins are potent inhibitors of prokaryotic DNA-dependent RNA Polymerase, with little activity against equivalent mammalian enzymes. By inhibiting this enzyme, RNA transcription is terminated and cell growth and replication fail. Rifampin (**7**), a semisynthetic antimicrobial drug derived from rifamycin, is a front-line drug in the treatment of TB. Rifampin is crucial in achieving sterilization by killing persisting semi-dormant bacilli. Since its introduction in the treatment of susceptible TB in the beginning of the 70s, new derivatives have been synthesized and evaluated for the treatment of TB (and non TB mycobacteria) [12]. The most promising work has been on rifapentine (**8**), a long-acting rifamycin derivative characterized by a lower MIC-value (0.06 μ g/ml versus 0.25 μ g/ml), which was approved for the treatment of TB in the US in 1998.

WO03084965 describes *N*-(3-rifamyciny)carbamates (**9**) as new substances for treating and preventing TB [103]. Compounds were tested *ex vivo*. The intracellular activity of growth inhibition in mouse macrophages, infected with *M. tuberculosis* H37Rv, was determined by counting the CFU/ml. The most active compounds (**R** = ethyl, methyl) have an 8-fold better activity than rifampin.

FIGURE 2

WO04005298 [104] highlights new derivatives of rifabutine Ia (**10**), a spiropiperidyl-rifamycine, which has been recommended for HIV-infected TB patients who cannot receive rifampin because of interactions with antiretroviral agents. Antimycobacterial activity was determined against *M. avium* 1581. While the known lead **10** displayed a MIC-value of 0.15 µg/ml, the most active newly synthesised derivatives were slightly less active. Only rifabutin IIIa (**11**) had comparable activity to **10** (MIC-value of 0.2 µg/ml).

Several patents from Hokuriku Seiyaki are related to novel series of 14-membered macrolide derivatives. Among the 1265 erythromycin analogues disclosed in WO00226753 [105], several compounds are claimed useful for preventing and/or treating TB. Analogues **13-16** showed MIC-values in the 0.10-0.20 µg/ml range against different *M. avium* and *M. intracellulare* strains. Another typical analogue **17** showed a MIC-value of 1.56 µg/ml against *M. tuberculosis* H37Rv and some other *M. tuberculosis* strains [106], while **12** exhibits the same MIC-value against *M. avium* strains [107].

3. Drugs on known targets

A patent by Janssen Pharmaceutica discloses novel 6-bromoquinoline derivatives with potent antimycobacterial activity [108]. These so-called diarylquinolines (DARQ) are structurally distinct from known broad-spectrum fluoroquinolone antibiotics. With a MIC-value of 0.01 µg/ml against *M. tuberculosis* H37Rv and *M. smegmatis* strains, one of the four possible diastereoisomers of compound **18** showed potent antituberculous activity.

Unfortunately, the absolute configurations of two stereogenic carbons were not determined.

A recent paper of Andries et al., however, attributes the activity to the (1*R*,2*S*) diastereomer (called R207910) [13]. This paper further reveals that (1*R*,2*S*)-**5** does not inhibit *M.*

tuberculosis DNA gyrase, the target for fluoroquinolones. A gene commonly affected in isolated resistant mutants encodes for atpE, a part of the F0 subunit of ATP synthase. This indicates that the atpE gene product (*i.e.*, the proton pump of *M. tuberculosis* ATP synthase)

is inhibited by R207910. This distinct target of (1*R*,2*S*)-**5** implies that there's no cross-resistance with existing anti-TB drugs. Pharmacodynamic studies in mice where one of the first-line drugs of the triple combination therapy was replaced with 25 mg/kg R207910 proved that the activity of each combination containing 207910 was significantly better than the standard regimen (e.g. culture-negative lungs after two months).

Oxazolidinones represent the first completely new class of synthetic antibacterial agents to achieve regulatory approval in over 30 years, as exemplified by linezolid (**19**, Zyvox®). In addition to its potent activity against Gram-positive pathogens, this class is of great interest because it exerts its antibacterial action by a mechanism distinct from other antibacterial agents. Oxazolidinones inhibit bacterial protein synthesis (translation) at a very early step. They inhibit the formation of a ribosomal initiation complex involving 30S and 50S ribosomes. Because of their unique mechanism, the oxazolidinones are not cross-resistant with any known antibiotic. Since this class was found to be endowed with promising antibacterial properties many pharmaceutical companies worldwide began research programmes in the oxazolinone area, which resulted in an impressive number of patents [14]. However, most patents are silent about antimycobacterial activity of the disclosed compounds and only a few of the disclosures present compounds that have demonstrated potential as antimycobacterials. A general feature of the oxazolidinone derivatives is that only enantiomers with a (5*S*)-acetamidomethyl configuration are known to exhibit antibacterial activity. Another feature is that almost all oxazolidinones endowed with antibacterial activity carry a phenyl ring attached to the nitrogen atom of the oxazolidinone.

Linezolid forms an attractive starting point to design anti-TB agents, since it displays a MIC-value of 0.5 µg/ml against *M. tuberculosis* and it is known that resistant bacterial mutants to linezolid arise at low frequency. Some of the potential side effects associated with extended linezolid treatment are toxic optic neuropathy and myelosuppression.

Ranbaxy disclosed novel oxazolidinone analogues related to eperezolid [109]. All analogues have a diazine moiety attached to the phenyloxazolidinone, which is further substituted by a

heterocyclic or aromatic ring. Compound **20** with a 5-nitrothien-2-yl moiety directly attached to the piperazinyl oxazolidinone core was very effective against the different *M. tuberculosis*, *M. intracellulare*, *M. avium* and *M. bovis* strains tested and MIC-values compared well with those of established anti-tuberculosis agents.

FIGURE 3

A Lupin Patent disclosed 78 novel oxazolidinones as antibiotic agents against *M. tuberculosis* [110]. Common to all claimed analogues is elongation of the acetamido moiety (as it occurs in linezolid). Among the most potent analogues are **21** and its *N*-oxide (MIC-value of 0.25 µg /ml for both compared to 0.50 µg /ml for linezolid) and the related **22** (MIC-value of 0.50 µg /ml). The latter analogue was also potent against resistant clinical isolates and showed a favourable acute toxicity profile (LD₀ > 1000 mg/kg P.O.). When administered at 12.5 or 25 mg/kg to mice infected with *M. tuberculosis* ATCC27294 5 days/week for 4 weeks, a mean log₁₀ reduction in colony forming units of 0.20 and 2.3 (in lung) and of 0.26 and 2.49 (in spleen) was observed. The *N*-oxide derivatives, which form a novel aspect of this invention, are expected to be highly water soluble.

Previously, the enzyme Glutamine Synthetase (GS) was identified as a potential antibiotic target [15,16]. In addition to its key role in nitrogen metabolism in the cell, GS appears to play an important role in cell wall biosynthesis, providing substrate for the synthesis of a major poly L-glutamate/glutamine cell wall component found exclusively in pathogenic mycobacteria. GS is one of the abundantly released proteins by *M. tuberculosis*. Interestingly, only pathogenic mycobacteria release large amounts of GS extracellularly, whereas non-pathogenic mycobacteria (e.g., *Mycobacterium smegmatis*) do not. Exposure of *M. tuberculosis* cultures to L-methionine-*SR*-sulfoximine (MSO, **23**), which irreversibly inhibits only the extracellular form of the enzyme as it is unable to cross the cell membrane, causes inhibition of bacterial growth (actually, it is L-methionine-*S*-sulfoxime, one of the four possible diastereoisomers, that accounts for the GS-inhibitory activity). The GS inhibitor is believed to affect the integrity of the cell wall. It blocks the growth of *M. tuberculosis* and *M. avium* within

human mononuclear phagocytes, the primary host cells of these pathogens, at concentrations that are non-toxic to these mammalian cells. This reflects the fact that purified *M. tuberculosis* GS is one to two orders of magnitude more sensitive to MSO than a representative mammalian GS [15]. The efficacy of MSO was measured *in vivo* in guinea pigs. One week after challenge, administration of 1.5 mg/kg.day MSO gave a reduction of the CFU with approximately 1 log, compared with control animals. This dose is the maximum tolerated dose for guinea pigs infected with *M. tuberculosis*.

MSO is not a suitable drug. First, it inhibits γ -Glutamylcysteine Synthetase (γ -GCS), which results in glutathione deficiency and mitochondrial damage, thereby causing toxicity of MSO in humans. Second, MSO is metabolized *in vivo* to potentially toxic species, including methane sulfinimide and vinylglyoxylate. Third and most importantly, MSO is a known epileptogenic agent, due to its inhibition of brain GS in mammals. Hence, analogues that are poorly transported into the brain and/or more specific for *M. tuberculosis* GS would be highly desirable. To overcome these drawbacks of MSO, several analogues of MSO were explored as described in patent WO04045539 [111]. One of the compounds claimed is α -ethyl-DL-methionine-*SR*-sulfoximine (α -Et-MSO, **24**), a specific inhibitor of GS. While the inhibitory capacity of this compound is comparable to that of MSO, α -Et-MSO is resistant to metabolism and, consequently, does not form the toxic products that are formed *in vivo* from MSO. Moreover, it does not enter the brain as readily as MSO and, accordingly, causes convulsions in mice only at higher doses.

The recently obtained high-resolution X-ray crystallographic structure of *M. tuberculosis* GS is that of a relaxed conformation that does not bind substrates or inhibitors [17]. However its close similarity with the *Salmonella typhimurium* GS structure (that has been resolved with an inhibitor in the active site) might aid additional efforts towards rational inhibitor design [18].

Gallium (a group IIIa transition metal) is known to prevent replication of intracellular pathogens. It is thought that gallium (Ga^{3+}) exerts its antibacterial activity through a novel mechanism: interference with bacterial iron uptake and metabolism through mimicry of ferric

ions (Fe^{3+}). Pathogens such as *M. tuberculosis* have developed high affinity iron-binding molecules, termed siderophores [19], to obtain iron in iron-poor environments. These molecules can scavenge iron from intracellular host binding molecules. *M. tuberculosis* produces at least two iron-binding molecules, termed exochelins and mycobactins. Replicating bacterial cells have high iron requirement, due to their need to produce Ribonucleotide Reductase (RR), a ferric ion-bearing enzyme essential for the synthesis of DNA. Gallium is chemically very similar to ferric ions and can be taken up by these cells and incorporated into RR instead of iron. As iron-free RR is non-functional, DNA cannot be synthesized and the affected cell attempting to replicate will ultimately undergo apoptosis. Intravenous infusion of gallium nitrate has significant potential side effects, such as nephrotoxicity. WO03053347 presents complexes of gallium with the ionic form of 3-hydroxy-4-pyrones (from which maltolate (**25**) is a preferred example) for oral administration that represent safer and potentially more effective alternatives to deliver gallium [112]. By a transferrin-dependent mechanism gallium could then gain entry to macrophages and be taken up by mycobacteria. Gallium maltolate (administered *per os*) proved efficacious in the treatment of guinea pigs infected with *M. tuberculosis*. Necropsies demonstrated that treated animals had strikingly less tubercles in lungs and liver relative to untreated animals. The decrease of CFUs in spleen was greater than those observed for ethambutol and slightly less than for isoniazid. Preparations of orally deliverable pharmaceutical forms of gallium maltolate for clinical evaluation in men are disclosed in this patent.

4. Agents with unspecified mechanisms of action

1'-Acetoxychavicol acetate (**26**) is a natural compound, found in some plants of the family *Zingiberaceae*. The mechanisms of action of the compound are not clear. It could inhibit the function of xanthine oxidase and NADPH oxidase. These enzymes are involved in superoxide anion production, which is one of the spontaneously occurring toxic substances in the body. It also inhibits nitric oxide synthase production. The MIC of 1'-acetoxychavicol acetate against *M. tuberculosis* H37Ra is 0.1 µg/ml (0.1 to 0.5 µg/ml to 30 clinical isolates), which is well below the toxic concentration against various mammalian cells [113].

FIGURE 4

Pleuromutilin (**27**) is a tricyclic diterpenoid and a naturally occurring antibiotic substance produced by the basidiomycetes *Pleurotus mutilus* and *P. passeckerianus*. A number of further pleuromutilin derivatives have been claimed by Sandoz [114]. The most active derivatives, e.g. Valnemulin (**28**), displayed MIC-values in the range of 0.5 to 8 µg/ml depending on the *M. tuberculosis* strain considered.

By using the existing lead BM212 [20], researchers at Lupin have discovered novel substituted pyrroles endowed with antimycobacterial activity [115]. Compared to the plethora of pyrrole derivatives reported earlier and reviewed in this patent, several of the ca. 90 disclosed derivatives possess higher antimycobacterial activity against clinically sensitive as well as resistant strains. The MIC against *M. tuberculosis* 27294 of **29**, the most active derivative, is 0.125 µg/ml. When assayed *in vivo* in Swiss albino mice that were challenged with the same strain, **29** compared favourably (on a mg/kg basis) with isoniazid in reducing CFUs in lung and spleen, while its LD₀ was > 2000 mg/kg, compared to a reported LD₅₀ of isoniazid of 139 mg/kg in mice.

US6268393 contains disclosures on the synthesis and the antimycobacterial activity of calanolide analogues [116]. (+)-Calanolide A (**30**), a known anti-HIV agent which was

originally isolated from the rain forest tree *Calophyllum lanigerum*, was moderately active (MIC-value of 3.13 µg/ml against *M. tuberculosis* H37Rv).

In WO03042186, Medac revealed dithiocarbamate derivatives endowed with activity against different Mycobacterium species. The most active agent is 4-dimethylamino-6-tetramethylenedithiocarbamoyl-5-nitropyrimidine (**31**) with a MIC-value of 3.12 µg/ml against *M. tuberculosis* [117].

5. Expert opinion

With the emergence of resistant forms of *M. tuberculosis*, it has become essential to develop novel antibiotics. Although many new compounds are becoming available for fighting a number of infectious diseases, TB has a thin portfolio of new compounds currently in the discovery pipeline with near-term clinical potential. This could partly be due to the complexity of the research involved and partly due to business considerations.

The complete genome sequence of the *M. tuberculosis* laboratory strain H37Rv as revealed in 1998 [21], should be a key starting point to identify new viable drug targets and to increase our knowledge of virulence genes. Efforts should be made to prioritise potential targets and generate further validation evidence (e.g., by gene disruption). Ideally, validated targets should be restricted to mycobacterial systems, e.g. enzymes involved in lipid metabolism or cell wall biosynthesis. Also, those targets that will contribute most to current TB control efforts must be chosen, particularly those that allow shortening the time course of therapy.

Currently, many bacterial gene products have already been identified, but relatively few are properly validated [22]. In some cases, lead inhibitors have been discovered for these target enzymes. However, this trend is not commonly translated into new leads with potent *in vitro* activity. A prerequisite to turn a potent enzyme inhibitor into a useful antibiotic is the ability to reach its molecular target, generally within the organism, in which the cell wall of the mycobacterium presents a major obstacle. One notable exception is Glutamine Synthetase, a validated target enzyme, which is exported by pathogenic mycobacteria. Since inhibitors do not have to cross the formidable barrier presented by the lipid-rich bacterial cell wall to inhibit this target (and probably also other extracellular proteins), this looks promising for developing new antibiotics.

It is striking that the majority of compounds with anti-mycobacterial potential disclosed in recent patents are the result of development efforts aimed at finding broad-spectrum antibacterial agents or at redesigning and optimising existing anti-tuberculars. Although some

scepticism exists against the co-development of broad-spectrum antibacterials for TB [23], several members of the oxazolidinone class show promising preliminary results against TB in *in vitro*, animal, and off-label studies.

An important exception to this trend is R207910, which is proved to inhibit a previously unaddressed target (proton pump of ATP synthase) and has a unique spectrum of potent and selective mycobacterial activity [13]. Extensive studies have demonstrated that it is equally effective against MDR TB strains as to antibiotic-susceptible strains [13]. Since this quinoline has both early and late bactericidal activity, it is believed to be a promising new TB drug candidate.

Remarkably, no new claims on fluoroquinolone antibiotics for TB infections have been reported in the period considered. For over a decade, significant advance in TB treatment has arisen from development of fluoroquinolone broad-spectrum antibiotics (e.g., ciprofloxacin, ofloxacin, and levofloxacin). These drugs have joined the armamentarium of antituberculous agents, i.e. as preferred second-line drugs for MDR-TB.

Very often, patents rely on *in vitro* experiments to evaluate the potential of the considered compounds as anti-tuberculars. However, it should be noted that an antimicrobial compound active against *M. tuberculosis in vitro* does not necessarily indicate *in vivo* activity. To be gifted with *in vivo* activity, an antibiotic must penetrate the host cell and reach the organism within its unique intracellular compartment, a specialized, membrane-bound phagosome [24]. Therefore, it is desirable to assess the *in vivo* potential of *in vitro* leads as early as possible in a drug evaluation strategy. In such *in vivo* assays factors as drug absorption, distribution, metabolism, and clearance also become pertinent.

In addition, it is important to distinguish targets that are likely to be essential for *in vitro* survival from those that may be necessary for persistence of the organism (and may not be expressed in *in vitro* culture). While the former may yield drug candidates that might be useful tools to overcome MDR-TB, the latter could be useful to identify drugs that might shorten the duration of the treatment. Indeed, an underrated issue in the treatment of TB is

the killing of the majority of bacilli that reside in cavities (extracellular) [25]. These are believed to be composed of an important sub-population of slowly metabolising or dormant organisms that are phenotypically resistant (tolerant) to drug action and might cause relapse. Nowadays, new leads are not routinely tested for sterilising activity until at a late stage of development. *In vitro* tests based on conditions likely to be found in the extracellular bacilli of cavities, have recently been developed [25]. These tests, which are open to automation, are able to grade current drugs according to their capacity to sterilize bacilli in the lesions of TB. Novel drugs that are able to interfere with bacterial survival under non-replicating conditions are highly desirable, since they shorten treatment. Several nitroimidazoles are believed to have unique potential to shorten the course of TB therapy by exerting a bactericidal effect on non-replicating bacilli [26]. However, no new developments in this area have been claimed recently.

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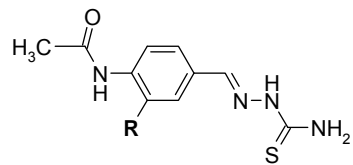
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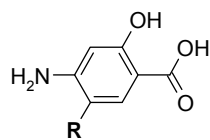
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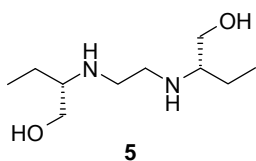
FIGURE 1



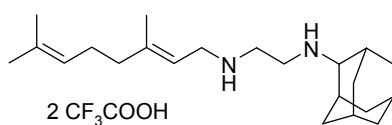
1 Thioacetazone, R = H
3 R = F



2 *p*-Aminosalicylic acid, R = H
4 R = F



5



6

FIGURE 2

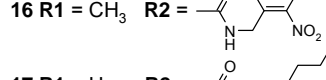
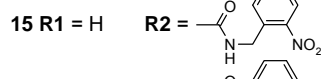
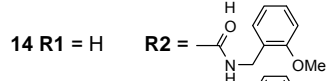
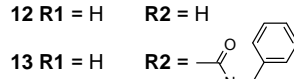
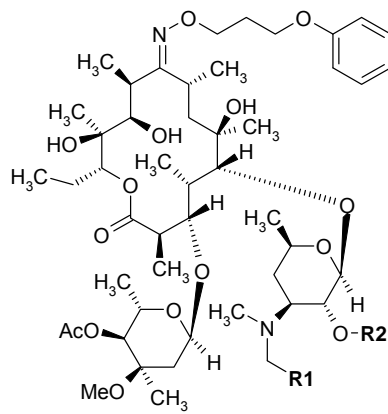
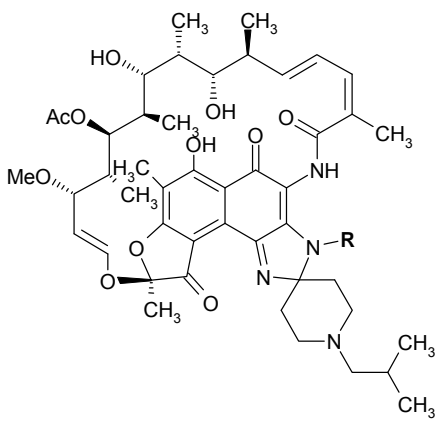
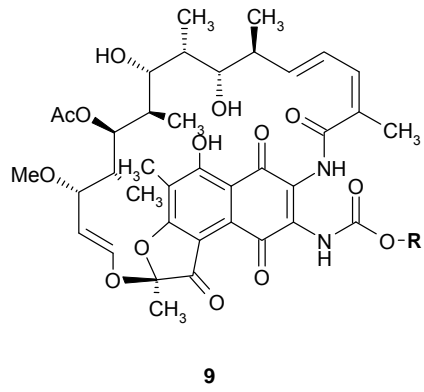
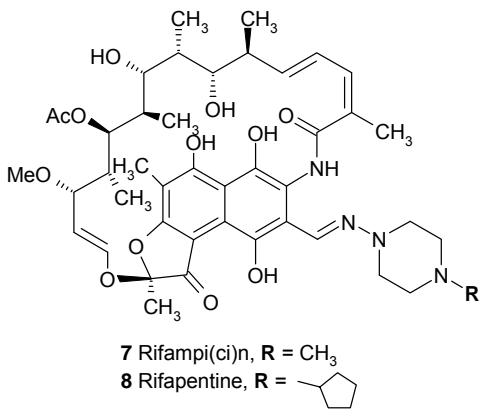
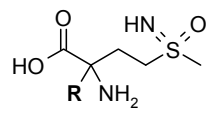
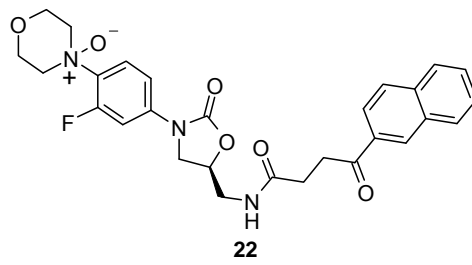
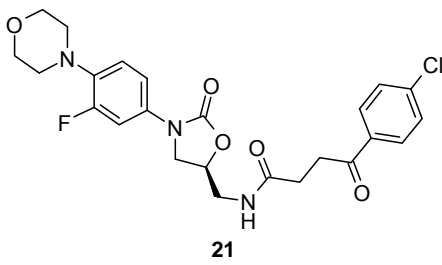
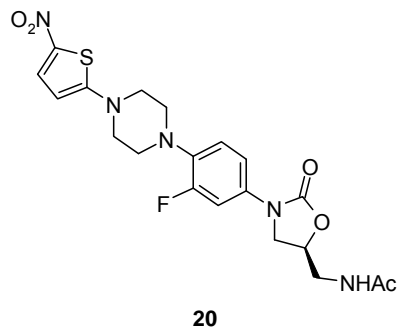
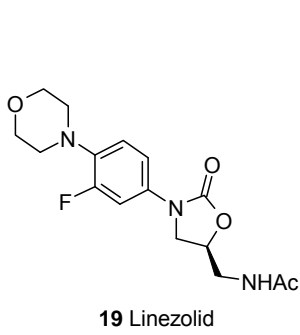
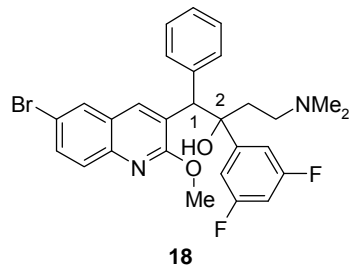
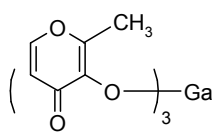


FIGURE 3

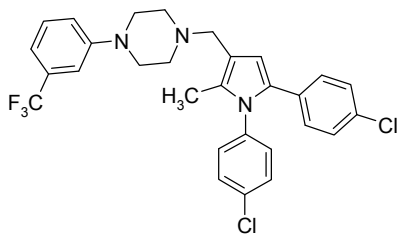
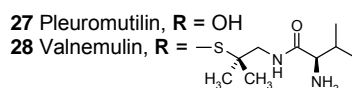
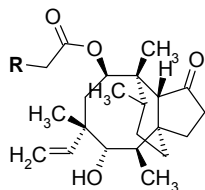
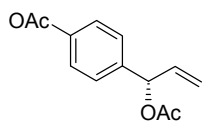


23 MSO, R = H
24 α -Et-MSO, R = Et

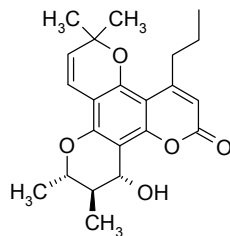


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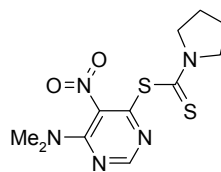
FIGURE 4



29



30 (+)-Calanolide A



31