REVIEW

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The challenge of new drug discovery for tuberculosis

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Tuberculosis (TB) is more prevalent in the world today than at any other time in human history. *Mycobacterium tuberculosis*, the pathogen responsible for TB, uses diverse strategies to survive in a variety of host lesions and to evade immune surveillance. A key question is how robust are our approaches to discovering new TB drugs, and what measures could be taken to reduce the long and protracted clinical development of new drugs. The emergence of multi-drug-resistant strains of *M. tuberculosis* makes the discovery of new molecular scaffolds a priority, and the current situation even necessitates the re-engineering and repositioning of some old drug families to achieve effective control. Whatever the strategy used, success will depend largely on our proper understanding of the complex interactions between the pathogen and its human host. In this review, we discuss innovations in TB drug discovery and evolving strategies to bring newer agents more quickly to patients.

In 1882, Robert Koch identified *Mycobacterium tuberculosis* as the causative agent of TB, but since his discovery the global TB epidemic seems unabated; this year it is anticipated that there will be about 9.8 million new cases, more than in any other year in history[1]. This situation highlights the relative shortcomings of the current treatment strategies for TB and the limited effectiveness of public health systems, particularly in resource-poor countries where the main TB burden lies. The ease with which TB infection spreads (for example, by inhalation of a few droplet nuclei 2–5 μm in diameter containing as few as 1–3 bacilli[2]), has helped to sustain this scourge at current levels. In spite of half a century of anti-TB chemotherapy, one-third of the world's population asymptomatically still harbour a dormant or latent form of *M. tuberculosis* with a lifelong risk of disease reactivation (Fig. 1). Reactivation of latent TB, even after decades of subclinical persistence, is a high risk factor for disease development particularly in immunocompromised individuals such as those co-infected with human immunodeficiency virus (HIV), on an anti-tumour necrosis factor therapy or with diabetes[3] (Box 1). In recent years, the TB epidemic has been further fuelled by the emergence

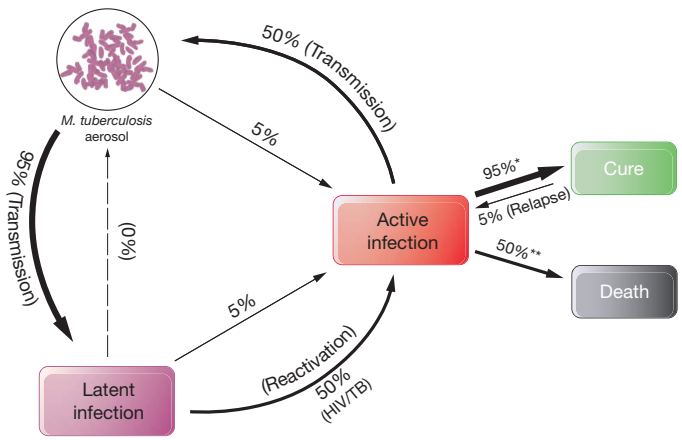

Figure 1 | Stages of *M. tuberculosis* infection. *M. tuberculosis* aerosol transmission and progression to infectious TB or non-infectious (latent) disease. A sizeable pool of latently infected people may relapse into active TB, years after their first exposure to the bacterium. Latent TB is commonly activated by immune suppression, as in the case of HIV. In cases of drug-susceptible (DS)-TB (denoted by an asterisk), 95% of patients recover upon treatment, whereas 5% relapse. If untreated (denoted by two asterisks), high mortality results.

BOX 1

Drug resistant TB and mycobacterial latency

Treatment of drug-susceptible (DS)-TB involves an initial phase of isoniazid, a rifamycin, pyrazinamide and ethambutol for the first 2 months followed by a continuation phase of isoniazid and a rifamycin for the last 4 months. Up to 95% of people with DS-TB can be cured in 6 months with this four-drug regimen.

MDR-TB is resistant to at least isoniazid and rifampicin, the two most important first-line drugs used in the treatment of TB. This may result from either primary infection with drug-resistant bacteria or may develop in the course of a patient's treatment when non-optimal treatment durations or regimens are used. Cure rates for MDR-TB are lower, typically ranging from 50% to 70%.

XDR-TB is resistant to isoniazid and rifampicin as well as any fluoroquinolone and any of the second-line anti-TB injectable drugs (amikacin, kanamycin or capreomycin). It has very high mortality rates.

Latent TB is asymptomatic and not infectious; it arises upon immune restriction of the growth of *M. tuberculosis* in hosts. Approximately 5% (higher risk if immunosuppressed; for example, with HIV) of these patients will go on to develop active disease at some stage in life. The term dormancy is used to describe latent TB disease as well as a metabolic state of non-replicative TB bacterium. Of several environmental stresses encountered by the TB bacterium in host cells, hypoxia has been shown to induce non-replicative bacterial phenotypes, leading to tolerance towards certain drugs like cell-wall inhibitors such as isoniazid; and lack of potent drug activity on these bacterial phenotypes may be responsible for prolonging the TB treatment duration[74].

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of multi- and extensively-drug-resistant (MDR-TB and XDR-TB) strains and dwindling treatment options that are decades old. The last drug with a new mechanism of action approved for TB was rifampicin (discovered in 1963). Further complicating the situation are drug–drug interactions that preclude the co-administration of some available TB drugs with certain anti-HIV treatments or other chronic disease medications, such as those used in diabetics.

To achieve global control of this epidemic, there is an urgent need for new TB drugs, which can: (1) shorten treatment duration; (2) target MDR or XDR strains; (3) simplify treatment by reducing the daily pill burden; (4) lower dosing frequency (for example, a once-weekly regimen); and (5) be co-administered with HIV medications (Table 1). The challenge of meeting the expectations of this desired target product profile complicates drug discovery efforts. Considering how few drugs from the discovery stage successfully enter the TB clinical pipeline, an increased understanding of the drug discovery hurdles should facilitate development of novel intervention strategies. The situation is further hampered by the unfavourable economics of TB drug development and the lack of proper policy incentives. In this review, we present our perspective on how to refocus discovery and development efforts and identify the underlying knowledge gaps and scientific obstacles in TB drug development. Finally, we highlight some emerging chemical scaffolds, which will hopefully fuel the TB clinical pipeline.

Emerging challenges in TB treatment

The world's two most populous countries, India and China, account for more than 50% of the world's MDR-TB cases and as such these countries are encountering a high and increasing TB disease burden[4]. The sheer size of their TB case populations results in the highest estimated numbers of MDR-TB cases (about 100,000 each) emerging annually from these two countries. Moreover, the emergence of XDR strains of *M. tuberculosis* (5.4% of MDR-TB cases are found to be XDR-TB[4]) is challenging TB treatment programmes in several other countries and even raises the possibility of a return to a situation akin to the pre-antibiotic TB era. At present, MDR-TB is treated by a combination of eight to ten drugs with therapies lasting up to 18–24 months; only four of these drugs were actually developed to treat TB[5]. Such suboptimal therapy leads to almost 30% of MDR-TB patients experiencing treatment failure[6]. The treatment options for XDR-TB are very limited as XDR-TB bacilli are resistant not only to isoniazid and rifampicin, but also to fluoroquinolones and injectables such as aminoglycosides. In addition, there are serious side effects with most MDR-TB and XDR-TB drugs, including nephrotoxicity and ototoxicity with aminoglycosides, hepatotoxicity with ethionamide and dysglycaemia with gatifloxacin[7]. Thus, the current situation necessitates the immediate identification of new scaffolds that can address emerging resistance and also demands the conduct of appropriate clinical trials as historically very few clinical studies have been performed to evaluate the efficacy of drugs in MDR-TB or XDR-TB patient groups. Improving the diagnostics with wider coverage of drug susceptibility testing will also help to address the high mortality of MDR/XDR-TB and curb the emergence of resistance.

Table 1 | Desired target product profile for a new TB drug

Desired target product profile	Biological characteristics
Treat MDR-TB and XDR-TB	New chemical class with a new mechanism of action
	Existing chemical class covering resistant isolates
	Drugs with low toxicity issues, like hepatotoxicity
Shorten treatment duration	Strongly bactericidal activity
	Good activity on latent or dormant or heterogeneous populations
	More potent and safer regimens of a novel drug and its combinations
Lower dosing frequency	Good pharmacokinetics including longer half-life and target tissue levels
	Retain potency when administered intermittently (for example, 1–3 times a week)
	Novel fixed-dose formulations and delivery technologies
Reducing pill burden	Combinations of more efficacious drugs to reduce number of pills taken
	Child-friendly formulation of newer drugs
Drug–drug interactions	No cytochrome P450 induction liabilities
	Minimal drug–drug induction particularly with antiretrovirals or oral diabetics

Each target product profile feature is accompanied by the biological characteristics needed to accomplish that respective feature.

TB accounts for about one in four of the deaths that occur among HIV-positive people[8]. Of the 9.4 million TB cases in 2009, 11–13% were HIV positive with approximately 80% of these co-infections confined to the African region[8]. The frequent co-infection of TB in HIV patients further complicates the selection of an appropriate treatment regimen because: (1) increased pill burden diminishes compliance; (2) drug–drug interactions lead to sub-therapeutic concentrations of antiretrovirals; and (3) overlapping toxic side effects increase safety concerns. The main interaction between HIV and TB anti-infectives is rifampicin-induced increased expression of the hepatic cytochrome (CYP) P450 oxidase system[9]. This CYP induction results in increased metabolism and decreased therapeutic concentrations of many co-medications such as HIV protease inhibitors[10]. Even in the presence of CYP450 inhibitors such as ritonavir, normal trough levels of various classes of protease inhibitors cannot be rescued and consequently, standard protease inhibitor regimens, whether boosted or not, cannot be given with rifampicin. The only treatments for HIV-infected TB patients with minimal drug–drug interactions are non-nucleoside-reverse-transcriptase-inhibitor (NNRTI) containing regimens. However, there are fewer options for patients with NNRTI-resistant mutations and therefore new chemistry approaches are being used to identify new rifamycins, such as rifabutin, with reduced CYP-induction properties[7]. However, the presence of ritonavir in the protease cocktail increases the serum concentration of rifabutin, thereby increasing its accompanying toxicity[11].

To discover newer rifamycin analogues with minimal interaction with HIV and other co-medications, the upfront screening of newer molecules in a CYP profiling (pregnane-X receptor) assay can be performed[12]. This receptor drives transcription of CYP genes and can identify chemical analogues with minimal interactions with drug metabolizing enzymes like CYP450. Further, availability of co-crystal structures of rifampicin with bacterial RNA polymerase[13] can help to design molecules with better drug-resistance profiles. In HIV patients harbouring MDR- or XDR-TB strains, drug–drug interaction studies are not well established, as most of these second-line TB drugs (for example, ethionamide, cycloserine, kanamycin, amikacin, capreomycin and para-amino salicylate) were discovered several decades ago[14]. Thus, there is a clear need for new studies to investigate the interaction of antiretrovirals with second-line TB drugs and with those currently in clinical development.

Confounding these issues is the association of TB with other chronic diseases such as diabetes, which is known to increase the risk of developing active TB by threefold[1]. The biological rationale for the slower response of diabetics to anti-TB drugs and for their increased risk of developing MDR-TB is poorly understood, although it is well known that cell-mediated immunity is suppressed in diabetes, which could explain higher TB rates. Attainment of bacterial culture negativity, relapse rates and mortality are significantly higher in diabetic TB patients[15] so we need to identify new TB molecules that are strongly bactericidal and have minimal drug–drug interactions with oral anti-diabetic drugs[16]. Further, diabetics tend to be heavier and more obese, which may in part lead to lower TB drug exposure[17]. Where there is a poor response to TB treatment in diabetic patients, therapeutic drug monitoring may be useful in TB management.

Identifying new chemical scaffolds

The poor efficiency of identifying new TB drugs by screening pharmaceutical library collections has been linked to the limited chemical diversity within these collections[18]. Additionally, most TB drugs and antibacterials in general do not follow Lipinski's 'rule of 5', which defines the optimal drug-like features; whereas pharmaceutical compound collections are biased towards these properties[19]. In spite of these challenges,

the current TB pipeline (Fig. 2) is slowly expanding, although it is inadequate for the development of a completely novel regimen. A key question is: how to search for new TB drugs and where to look for them?

Novelty in screening

Advances in the identification of new TB drug targets have been driven largely by the availability of the genome sequence of *M. tuberculosis*[20], but unfortunately this approach has yet to lead to the identification of new drug candidates. Genome-derived, target-based approaches have had little success in the antibacterial therapeutic area in general[18]. The essentiality of a target for replication may be a prerequisite but it does not ensure its druggability; for many essential targets we are unable to identify specific inhibitors with drug-like properties. For example, several high-throughput screening campaigns for identifying inhibitors of isocitrate lyases, which are key glyoxylate-shunt-pathway enzymes found to be essential for mycobacterial intracellular growth and their long-term persistence in mice, were discontinued owing to lack of druggability of these targets[21]. Second, we have often failed to understand how to convert good bacterial enzyme inhibitors into a compound that can easily penetrate the highly impermeable bacterial cell wall. Without proper understanding of the entry mechanisms of antibiotics across bacterial cell walls, any medicinal chemistry approach to engineer (via chemical modifications) a 'permeability property' into an enzymatic inhibitor has proven to be quite challenging.

Over time, it has emerged that shifting the screening strategy from single-enzyme targets to phenotypic screens at a whole bacterial cell level is a much more successful strategy[18]. Such a strategy recognizes the potential holistic interactions of a drug target(s) with one or more components in a bacterial cell and defines its essentiality in a more relevant physiological space. One of the drawbacks of the whole-cell-screening approach is that upfront knowledge regarding the mechanism of action remains largely lacking, thereby preventing any input from structural biology into medicinal chemistry efforts around drug design. Another challenge of whole-cell screening is to identify the right *in vitro* growth conditions that are relevant for *in vivo* infections, as certain metabolic targets behave differently depending on the composition of the growth medium[22]. Whole-cell screening can deliver many hits, but several of these may work via non-specific mechanisms (such as detergent effects) and have cytotoxic effects. As such, the key in a whole-cell-screening campaign is to identify the 'quality hits' by certain counter-screening assays (for example, cytotoxicity across several cell lines, monitoring non-specific membrane leakage, analysing red-blood-cell haemolysis), so as to account for good selectivity and specificity.

The recent success with the whole-cell-screening approach is particularly exemplified by the identification of new TB drug candidates such as diarylquinolines (TMC207), which target ATP synthesis, and benzothiazines (BTZ043), which target essential cell-wall arabinan synthesis[23–25]. An interesting feature of both these molecules is that they target membrane-associated proteins that may be more easily accessible to drugs from the periplasmic space (that is, the target binding sites are exposed to the periplasm) and this to some extent may overcome certain issues of mycobacterial membrane permeability.

Interestingly, more refined multi-target 'pathway' screens can be initiated to search for inhibitors blocking validated metabolic or signalling pathways. In this regard, respiratory membrane vesicles of *M. tuberculosis*, which have been grown in a variety of conditions in order to simulate the host microenvironment, can be used to screen drug classes or analogues inhibiting respiratory chain components (Fig. 3). For example, such a pathway screen could monitor a drug's influence on diverse mycobacterial respiratory chain functions such as ATP synthesis, redox homeostasis and proton gradients[26]. Modulating external growth stimuli, such as the carbon source, micronutrients, or oxygen levels in such an assay, results in target respiratory proteomes that can be used to screen against functions essential during those metabolic states. For instance, ATP synthase is highly downregulated during hypoxic conditions, and its inhibition by TMC207 indicates an essential role of ATP synthesis in the generation of

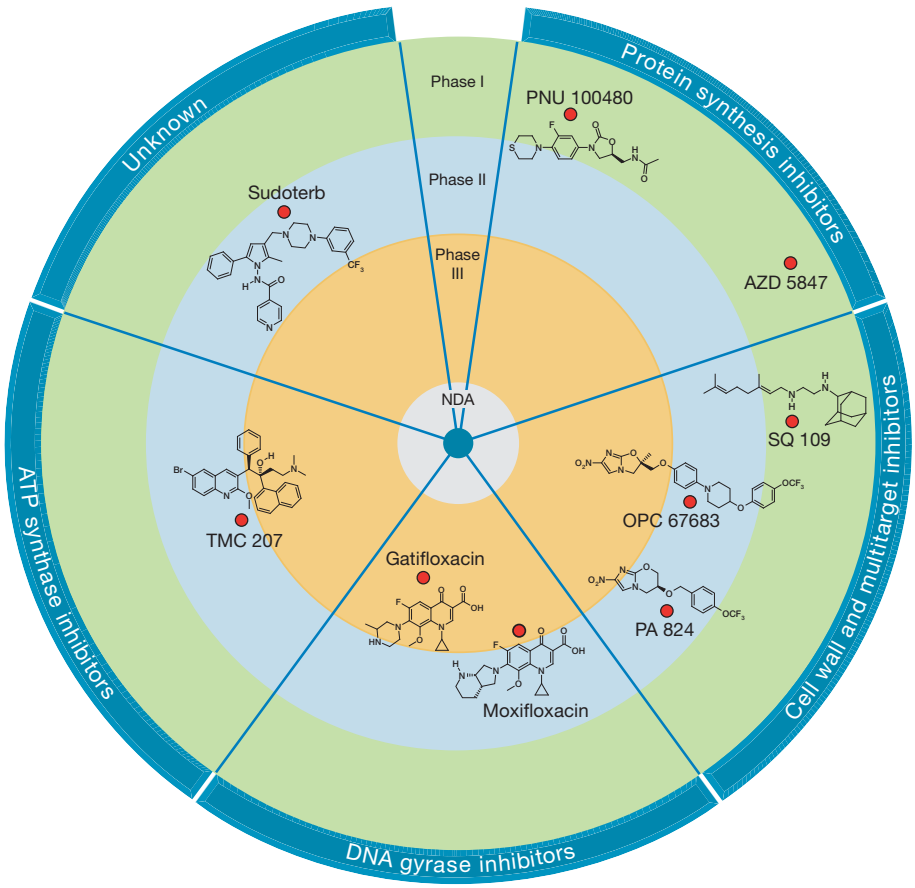

Figure 2 | A bull's-eye representation of the current clinical pipeline for TB. Each drug candidate is shown with its current clinical phase of development along with the target family. TMC207 is in phase IIb trials for MDR-TB and in phase IIa trials for DS-TB. The structure of AZD-5847 has not been disclosed. NDA, new drug application (for regulatory approval).

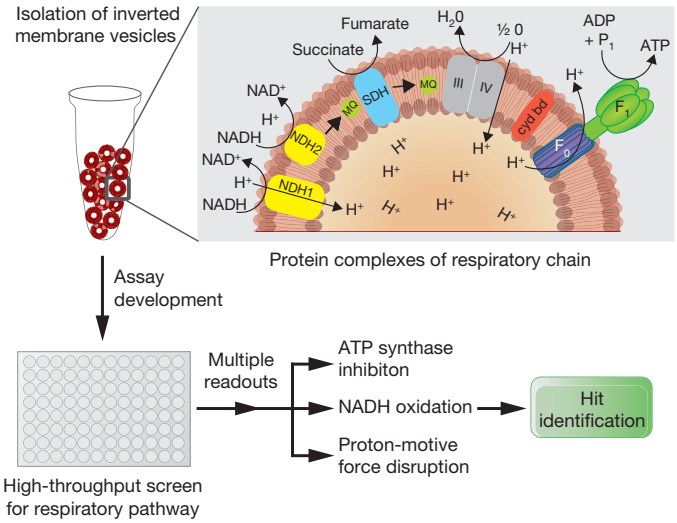

Figure 3 | Screening for mycobacterial respiratory pathway inhibitors. Schematic flowchart of a multiple-target screen for the identification of hits targeting the mycobacterial respiratory pathway. Enriched pharmaceutical compound libraries, or compound family analogues, can be used to screen inverted membrane vesicles for inhibition of NADH dehydrogenase, ATP synthase, or other targets impairing electron flow or proton-motive force. The enlargement shows the graphic view of the mycobacterial respiratory chain proteins with menaquinone (MQ) being reduced by NADH dehydrogenase or succinate dehydrogenase (SDH) and oxidized by a supercomplex consisting of complex III and IV (cytochrome bc_1 and aa_3)[29]. The transcriptional profiling in infected mice lungs during chronic phase showed downregulation of proton-pumping type-I NADH dehydrogenase (NDH1) and low-affinity aa_3-type cytochrome *c* oxidase, but upregulation of alternative target enzymes such as high-affinity cytochrome *bd* oxidase (cyd bd) and non-proton pumping NADH dehydrogenase (NDH2), which can serve as effective targets for latent or persistent infections[68]. Small molecules such as antipsychotic phenothiazine and diarylquinolines (for example, TMC207) have been shown to target NDH2 (ref. 69) and the transmembrane subunit-c of ATP synthase[24], respectively, with potent antimycobacterial activity on actively metabolizing and non-growing cells.

energy in the dormant bacteria, which may explain the potent *in vivo* sterilizing effect of the drug[27]. Dormant *M. tuberculosis* seems to be exceptionally susceptible to inhibition of respiratory chain processes such as ATP synthesis or interference with the cellular redox state[28], but it still remains to be determined if such inhibition leads to potent sterilization in human lesions with varied microenvironments. Because most TB drugs are less efficient in killing slowly replicating or dormant bacilli in the chronic phase of TB infection, a key challenge for identifying sterilizing drugs is to translate information about the chronic state mycobacterial metabolome and proteome adaptations into drug discovery screening platforms. This strategy will not only facilitate development of proper drug discovery tools that might eventually lead to a faster cure, but may also help us to understand the life cycle of mycobacteria in their host (for example, their switch to anaerobic metabolism[29]).

Engineering existing scaffolds

Many new antibiotic candidates are chemical molecules reengineered from old drug classes discovered decades ago[30]. This approach has identified new TB drugs from existing antibacterial drug classes and either involved the redesign of accessible scaffolds to improve their antimycobacterial potencies or, more directly, the repositioning of known antibacterial drugs with good antimycobacterial activity for testing in TB clinical trials (Fig. 4). During re-engineering of known scaffolds, chemical modifications are introduced into the core structure that may lead to improved bactericidal activities, better resistance profiles, safety, tolerability or superior pharmacokinetic/pharmacodynamic properties.

The modified versions of the oxazolidinones (such as linezolid, a marketed product from this class with activity against Gram-positive infections) have led to new structures such as PNU-100480 and AZD-5847 with better activity against *M. tuberculosis*[31]. These oxazolidinone TB candidates, currently in phase I studies, must address in their clinical development plan the known toxicity issues of linezolid, namely inhibition of mitochondrial protein synthesis, thrombocytopenia and myelosuppression, which has been observed in patients treated for longer than the recommended 14 days[32]. Because TB treatment can take months, safety is of paramount importance with any new tailored oxazolidinone and it will be important to monitor for bone marrow toxicity early in clinical trials. The good human pharmacokinetic profile of linezolid (for example, excellent oral bioavailability, low CYP inhibition and good distribution to lung epithelial lining fluid[33]) raises the hope that this drug class can penetrate the difficult to reach thick-walled lung cavities and lesions where TB bacilli normally hide.

Nitroimidazoles, traditionally used to treat anaerobic bacteria and parasitic infections, represent another established scaffold for which synthetic modifications have been introduced to increase their antimycobacterial potential. An interesting feature of nitroimidazoles relates to their unique mechanism of action, mimicking host defence strategies by producing microbicidal molecules, such as nitric oxide and other reactive

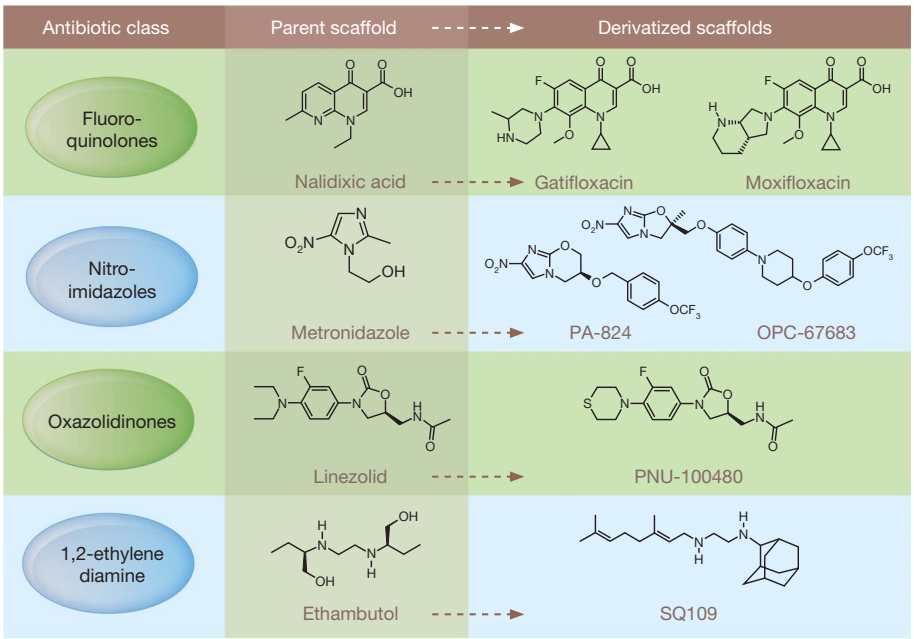

Figure 4 | Remodelling the existing antibacterial drug classes. Chemical tailoring of existing drugs or drug classes has led to the identification of new molecules with potent antimycobacterial activities. The oxazolidinones also include the recently discovered AZD-5847, the structure of which has not been disclosed yet and is therefore not listed here. SQ109, an orally active cell-wall-targeting diamine antibiotic, identified via combinatorial chemistry approaches, is currently being tested in humans.

nitrogen intermediates, which damage multiple targets including respiratory chain cytochrome oxidases[34]. The target specificity of such a mechanism of action is achieved through bioactivation of these prodrugs by flavin-dependent nitroreductases, which are absent in mammalian cells but present in *M. tuberculosis*[35]. Two candidates from this class, PA-824 and OPC-67683, are currently in clinical studies and may potentially shorten treatment duration as this mechanism of action is operational even in hypoxic induced dormant mycobacteria[34] (that are not killed by drugs such as isoniazid). This indicates that in spite of general transcriptional downregulation during mycobacterial dormancy, these nitroreductases are still sufficiently expressed.

For many years, the lack of activity of the natural or semi-synthetic β-lactams against TB was thought to be due to their poor penetration into the organism, with β-lactamase-mediated resistance only a minor confounding factor[36]. However, a recent genetic knockout of *blaC*, encoding the extended-spectrum Ambler class A β-lactamase from *M. tuberculosis*, showed improved sensitivity to β-lactams, particularly carbapenems[37]. By combining a second-generation carbapenem (meropenem) with a β-lactamase inhibitor (clavulanic acid), good *in vitro* bactericidal activity on replicating, non-replicating and resistant clinical isolates of *M. tuberculosis* was obtained[37]. Availability of structural and mechanistic knowledge around BlaC will help researchers design potent and *M.-tuberculosis*-specific inhibitors to be used in combination with classical β-lactam antibiotics. At the same time, a newer generation of broad-spectrum β-lactamase inhibitors (for example, current clinical candidates such as NXL104 (ref. 38)) should be explored for mycobacterial BlaC inhibition. Concurrently, medicinal chemistry approaches to improve the antimycobacterial activity of β-lactams, their tissue distribution and oral bioavailability, will be necessary as current drugs such as meropenem require parenteral administration[37], thereby limiting their use in more serious MDR/XDR-TB cases.

Although incremental improvements of existing scaffolds is a good strategy to fill a drug development pipeline, the increasing resistance to some of these existing drug classes, such as the fluoroquinolones[39], indicates that discovery of new chemical scaffolds is a more attractive approach. To facilitate the identification of new chemical scaffolds, a proper understanding of the physicochemical features of the existing TB drugs and analysis of their chemical space is desirable.

The physicochemical space of TB drugs

Antibacterial drugs in general occupy a unique physicochemical space that is markedly different from the space occupied by drugs in other therapeutic areas[40]. Specific physicochemical features in antibiotic drug classes are required because of the unique architecture of bacterial cell walls (especially in Gram negatives), which affects the permeability of drug molecules across these membranes. Antibacterial drugs are unique in a number of physicochemical properties, such as lower lipophilicities, higher molecular weights and increased total polar surface areas when compared to drugs for human host targets[40]. It has been proposed that screening libraries for antibacterial targets should have more polar characteristics to achieve penetration through certain bacterial cell walls[40].

We studied 14 different physicochemical features, including molecular weight, lipophilicity and polar surface area of first- and second-line TB drugs, and compared these properties to known marketed non-antibacterial compounds (1,663) identified from the Prous Integrity database (http://integrity.prous.com) (details in Fig. 5). A mathematical tool called principal component analysis (PCA) was used to study the relationships between various physicochemical properties[41] and to identify regions of physicochemical space required to achieve antimycobacterial activity. A two-dimensional graph indicates that TB drugs actually occupy a broad chemical space and do not fall into any defined chemical area. As expected, natural-product-based molecules such as rifamycins and aminoglycosides occupy a peripheral region of the plotted area, whereas fluoroquinolones, having more 'drug-like' features, are located among the drug bulk (Fig. 5). With no defined optimal physicochemical space for TB drugs, chemistry for the discovery of new scaffolds should be less restricted and more diverse. At the same time, the wide scatter within the PCA plot may reflect to some extent the fact that most TB drugs were discovered several decades ago without much consideration of optimal physicochemical and other drug-like features.

Although antibacterial agents are generally quite polar, water-soluble molecules, the question is whether TB medicinal chemistry should try to engineer the physicochemical characteristics of newer molecules or screening libraries towards a common parameter such as polarity. In light of this question it is worth considering that TMC207, even with its lipophilic nature (logD at pH 6.0 is 5.14), has potent bactericidal activities. Therefore, biasing our library screens towards compounds with a particular physicochemical parameter could actually be detrimental and decrease the diversity of our screening campaigns and chemistry. Nevertheless, a detailed understanding of the influence of polarity on drug penetration into the highly impermeable mycobacterium cell wall (for example, *Mycobacterium smegmatis* is about 20 times less permeable than *Escherichia coli*[42]) may guide us to improved permeability. An important question is how the unique mycobacterial membrane architecture, with its high lipid content, influences drug uptake and efflux compared to the cell walls of other Gram-positive and Gram-negative bacteria.

Targeting host–pathogen signalling pathways

Subversion of host-cell signalling pathways is one of the strategies used by pathogenic mycobacteria to survive long term in host cells[43]. As such, targeting the key signalling molecules, either bacterial- or host-derived, may lead to new antimycobacterial therapies.

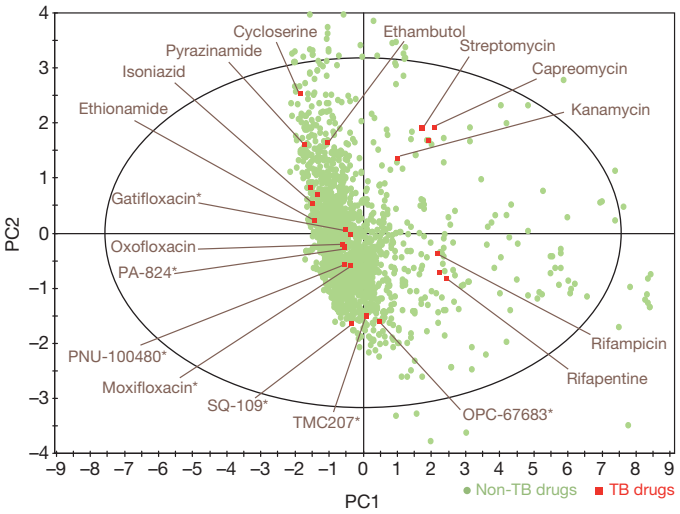

Figure 5 | Two-dimensional representation of chemical space of the anti-TB drugs. To understand the physicochemical space occupied by TB compounds, we studied 14 different physicochemical features of TB drugs and compared these properties to 1,663 marketed non-antibacterial unique compounds identified from the Prous Integrity database. The physicochemical parameters calculated using the Molecular Operating Environment software (MOE)[70] were: log of the octanol/water partition coefficient (lipophilicity evaluated by SlogP), molecular weight, number of hydrogen bond donors and hydrogen bond acceptors, number of rotatable bonds, topological polar surface area, solubility, atomic polarizabilities, absolute atomic polarizabilities, connectivity topological index, density, radius, petitjean (diameter − radius)/diameter) and molecular refractivity. A PCA analysis, a mathematical procedure that transforms a number of (possibly) correlated variables into a number of uncorrelated variables called principal components, was done using a software program (SIMCA-P+12; Umetrics)[71]. A PCA analysis was carried out using the 14 calculated properties mentioned earlier and variation in the properties between the compounds is mapped onto two axes, principal components PC1 and PC2, which contain most of the variance (in this case 70% for PC1 and PC2 combined). The axes are linear combinations of the original 14 properties and each data point in the two-dimensional graph corresponds to one compound. The complete graph shows the chemical space occupied by the different compounds. This figure indicates that TB drugs are widely distributed within the chemical space.

On the basis of the knowledge that *M. tuberculosis* has 11 serine/threonine kinases and several other ATP- (or GTP-)using enzymes, researchers have screened enriched kinase libraries for inhibition of mycobacterial growth *in vitro* or in macrophages, with limited success[44]. However, a recent kinase library screen in *E. coli* led to the identification of a pyridopyrimidine scaffold as a competitive inhibitor of the ATP-binding site of acetyl-coenzyme-A carboxylase[45]. For mycobacteria, the search for kinase inhibitors with potent *in vitro* bactericidal activity has not been successful, although chemical optimization towards the uniquely conserved ATP-binding pockets of protein kinase G did identify a new chemical scaffold, tetra-hydro-benzothiophene (AX20017), but with activity restricted to infected macrophages[46] (Fig. 6). A related concept within bacterial research is to identify inhibitors of bacterial virulence factors or host targets that can modulate pathogen survival inside the infected cells. Recently, it was revealed that the innate immune response within macrophages can be modulated by specifically inhibiting the mycobacterial tyrosine phosphatase (mptpB), which blocks host ERK1/2 and P38 signalling and promotes intramacrophage survival of mycobacteria[47] (Fig. 6). At the same time, genome-wide RNA interference screening has identified key host kinase networks and an autophagic/xenophagic machinery that is severely inhibited on mycobacterial infection[48]. This research showed that pharmacological activation of the xenophagic pathway in infected macrophages by certain drugs led to the killing of intracellular mycobacteria. However, in the absence of any *in vivo* validation and also any extracellular bactericidal activity, such drugs, if proven to be clinically efficacious, would probably be used in an adjunctive therapy along with a direct antibacterial agent. It is not known if the intracellular dwelling of mycobacteria contributes to its prolonged treatment duration and whether strategies targeting host-cell factors will lead to better bactericidal activity and shorter treatment time in patients.

In vivo screens and preclinical validation

Animal models that mimic various metabolic stages of human infection have proven to be extremely important for TB drug discovery as some functions deemed to be essential *in vitro* (such as mycobacterial glycolysis) are not essential *in vivo*[49]. However, no animal model is perfect as each model only incompletely reproduces different aspects of human disease. The mouse model is considered imperfect because certain elements of human disease pathogenesis such as organized granulomas, caseous necrosis and hypoxia are not replicated[3]. In the absence of these features, the challenging question is whether the mycobacterial metabolic repertoire present in mice is less heterogeneous than in humans? Despite not exactly replicating the host-tissue microenvironment, the mouse model has served as a cost-effective tool to assess the bactericidal and sterilizing potencies of individual drugs and drug combinations[50].

The mouse model was recently also used to identify bacterial targets that impair or enhance mycobacterial persistence upon treatment with isoniazid[51]. Such an approach illustrates the potential role of mouse model screens for identifying factors responsible for drug tolerance, which could be easily missed in regular *in vitro* screens. At the same time, a key feature of mouse models that is not properly understood is to what extent the route of administration of TB inocula determines the relapse rates upon drug withdrawal[52]. Alternative animals such as guinea pigs, rabbits and even cynomolgus monkeys have been used as preclinical models as they mimic TB disease pathogenesis better than mice with features such as hypoxic lesions and solid necrotic granulomas. Although guinea pigs do not acquire TB naturally, it was the first TB infection model to be used in 1944, when the efficacy of streptomycin was tested in just four animals before treating patients[53]. We still need rigorous studies comparing the bactericidal and sterilizing efficacy of different drug regimens in different animal models infected by different routes of infection, to enhance our ability to predict treatment outcomes in clinical trials and to validate the models themselves. Only after we have shown for several drug classes that animal model studies are congruent with efficacy seen in clinical trials will we achieve confidence in their predictive power.

Advances in imaging technologies that can map, in real time, the response of individual granulomas to drug treatment will facilitate our understanding of TB pathogenesis and may also help in developing better models to assess relapses. Live imaging tools were recently used to reveal the initial events leading to granuloma formation in a zebrafish model upon infection with *Mycobacterium marinum*[54] and, in another instance, the lungs of patients with pulmonary TB were imaged to study the progression of disease after two months of chemotherapy[55].

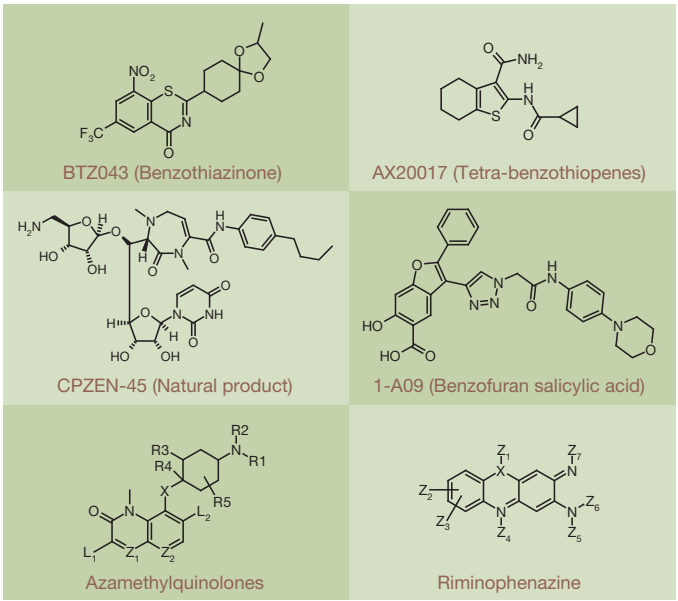

Figure 6 | Representative underexplored and new chemical scaffolds. Some of the chemical structures shown are mentioned in the text. CPZEN-45, a streptomyces-derived natural product, is a semi-synthetic nucleoside antibiotic from the caprazamycin family with TB activity[72]. Re-engineering of riminophenazine's chemical scaffold can lead to interesting energy metabolism inhibitors with the potential to kill non-replicating bacilli. Azamethylquinolones have demonstrated activity on mycobacteria and further chemical optimization may lead to interesting lead candidates hopefully with better resistance profiles[73].

Evolving science of TB clinical development

A challenge in the clinical development of new TB therapies is the lack of specific biomarkers or surrogate endpoints that are sensitive and specific enough to reliably predict success or failure early in the course of treatment. Historically, clinical development of TB drugs has relied heavily on early bactericidal activity (EBA) trials, which measured reduction in bacterial load in the sputum of patients within 2–5 days of treatment[56]. The EBA kinetics of newer TB drugs such as TMC207 do not seem to follow the fast bactericidal activity observed with isoniazid and rifampicin and therefore studies with treatment durations of less than one week may be less informative for experimental agents showing a delayed bactericidal response[57]. Such delayed responses may be due to time-dependent killing, or a killing mechanism that requires depletion of energy reserves, or to physicochemical properties of the drug that delay its distribution to the bacilli in target sites. On the other hand, EBA studies of more than 2 weeks using monotherapy may be considered unethical because of the likelihood of the emergence of resistance.

In the second phase of clinical development, experimental drugs are administered over 8 weeks, on top of a standardized regimen, to estimate the effect of the drug(s) being tested on time to sputum conversion (positive to negative mycobacterial growth in patient sputum samples). In this setting, strong bactericidal activity and safety is a prerequisite for the further extension of therapy to more than 6–12 months for drug-susceptible (DS-)TB and 12–24 months for MDR-TB. A lengthy follow-up period (up to 2 years after the end of treatment) is needed to access the primary clinical endpoint of sterilization as measured by relapse

rates. The absence of any other validated surrogate endpoints or biomarkers immensely extends the clinical development timelines and this is preventing any rapid progress in the field and at the same time substantially increasing the costs of running these trials. Sputum conversion to negativity after 2 or 6 months of treatment for DS- or MDR-TB, respectively, may give an early indication of microbiological sterilization[58,59]. Although this sputum conversion rate probably represents the best surrogate marker for estimating the sterilizing efficacy of a regimen, it has been recently observed in mice that bactericidal potency of a regimen does not necessarily predict its sterilizing potency[50] and, as such, culture status after 2 or 6 months of therapy still needs further validation, particularly in diverse patient populations[60].

One of the factors that might explain the discrepancy between bactericidal and sterilizing activities is the heterogeneous nature of the bacterial population in patients with differential growth rates. Recent clinical trial data confirmed the increased sensitivity of liquid cultures (for example, mycobacteria growth indicator tube) to detect sputum TB bacilli, as compared to solid cultures[61,62], clearly indicating that even sputum samples may contain different subpopulations of TB bacilli with different growth kinetics. It remains to be seen to what extent cultivation of sputum samples on liquid cultures will allow a more accurate estimation of the sterilizing activity of a regimen.

To accelerate TB drug development and reduce the long clinical development path, research into non-sputum biomarkers, such as bacterial DNA sequences in urine samples or host-derived markers, such as toll-like receptor activation, should be prioritized. The recently identified interferon-inducible blood transcriptional TB signature, which correlates with the extent of disease in active TB and diminishes upon treatment, has great potential as a diagnostic and prognostic tool[63]. This TB signature was also observed in a subset of 10–20% of patients with latent TB and may identify those individuals who will develop active disease, and thereby facilitate targeted preventative therapy. Such biomarkers need further validation to determine if they are sufficiently sensitive and specific to allow monitoring of therapy responses in adults with active TB, or in individuals who are at risk of TB reactivation, or in children with active TB but who often do not excrete mycobacteria in their sputum[60].

Drug combination trials and standardization of regimens

At present, the global TB development pipeline has nine candidates, but a key issue is how to develop them concomitantly in combination trials to identify the best regimen in the shortest period of time. In this regard, a recent initiative (Critical Path to New TB Regimens (CPTR)), involving several pharmaceutical companies and nongovernmental organizations, aspires to the development of new regimens of investigational drugs with existing TB drugs or drug candidates to avoid developing each drug sequentially and thereby shortening the development timelines that might otherwise spread over decades[64]. The CPTR approach will undoubtedly lead to improved efficiencies, but only if we can identify drugs that share similar or non-interfering pharmacokinetic features, synergistic or simply additive mechanisms of action, and non-overlapping toxicity profiles. For instance, some TB drugs in clinical development (moxifloxacin) have cardiovascular risks (prolonged QT intervals), and combining them with another drug with a similar liability will raise safety concerns. Even drugs with different mechanisms of action may interact synergistically or antagonistically with each other, and may even induce cross-resistance by common efflux mechanisms.

Reassessment and time for acceleration

Recent research into the pathogenesis of *M. tuberculosis* has led to the identification of a range of bacterial pathogenic mechanisms that permit it to escape certain host-control measures[65]. To counteract this we need innovative tools including newer drugs, vaccines, and improved diagnostics and biomarkers. The ultimate goal of the TB drug discovery effort is to eradicate both active and latent disease, possibly within a few weeks, like other more common bacterial infections. However, there are tremendous challenges to achieving this goal considering our lack of understanding of how to target heterogeneous *M. tuberculosis* populations using a single drug or a drug combination. In this regard, it is helpful to consider that TB in humans is a disease of subpopulations, with each population requiring a different drug or therapeutic approach. At the same time, it still remains to be determined to what extent *M. tuberculosis* persisters, which are phenotypically and stochastically antibiotic resistant[66], determine relapse rates following a drug's withdrawal. Any future drug discovery efforts should address the questions of how the goal of shortening the treatment durations can be linked to drug activity on latent or persistent bacterial populations. We still do not know if disrupting their energized membranes or targeting key anaerobic respiratory components such as those involved in energy generation can effectively kill these persisters.

At present, drug treatment developments show some promise owing to a renewed interest from pharmaceutical companies in researching new drugs, coupled with effective support from governmental and non-governmental organizations. The TB vaccine pipeline is also showing progress, with seven vaccine candidates currently in clinical development including candidates being evaluated in paediatric populations[67]. However, we still need more drugs and vaccines to move from discovery into the development pipeline because of the high rate of drug attrition in clinical development and the potential for post-approval failures. Importantly, we also need more drugs from different classes so as to enable the creation of successful drug regimens, and realize the World Health Organization's and United Nations millennium development goal of halting the incidence, prevalence and death rates associated with TB by 2015 and eliminating the disease altogether by 2050[8]. However, any new drug or vaccine for TB will fail to make a significant impact if it is not accompanied by proper support from local healthcare systems. And finally, a key societal and economic challenge will be to ensure the proper access of these drugs or vaccines to the patients most in need in resource-poor countries.

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