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Current Opinion in Biotechnology

Novel and revisited approaches in antituberculosis drug discovery

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The increasing prevalence of multidrug-resistant *Mycobacterium tuberculosis* (*Mtb*) necessitates the discovery and development of novel drugs against tuberculosis. In this review, we focus on two recent approaches that led to the discovery of promising antitubercular compound classes: (I) Hits derived from large compound library screens are increasingly difficult to translate into clinical application; this in turn fostered the development of innovative screening methods. (II) An alternative strategy towards high-quality hits and leads is to evaluate chemically diverse scaffolds which can be found among natural products. The so-called rekindling and repurposing approaches were particularly fruitful in the last few years.

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Current Opinion in Biotechnology 2017, **48**:94–101

This review comes from a themed issue on **Pharmaceutical biotechnology**

Edited by **Chu-Young Kim** and **Tiangang Liu**

http://dx.doi.org/10.1016/j.copbio.2017.03.023

Introduction

In 2015, tuberculosis, caused by *Mycobacterium tuberculosis* (*Mtb*) was responsible for an estimated 1.8 million deaths [1]. Control of this pandemic is threatened due to a strong increase of multidrug-resistant *Mtb* (MDR-TB) [2]. The number of MDR-TB cases in Europe has more than doubled between 2009 and 2014 [1]. This emergence of difficult to treat strains requires the development of safe drugs with new mechanism of action. Few promising drugs are currently in preclinical and clinical trials [3,4]. Resistance among recently approved drugs is already emerging leading to virtually untreatable clinical isolates [5•]. In addition, promising drugs with good *in vivo* efficacy failed in clinical trials [6]. Thus, there is an urgent need to constantly fill the drug pipeline with candidate compounds active against MDR-TB.

Screening of synthetic small molecule libraries

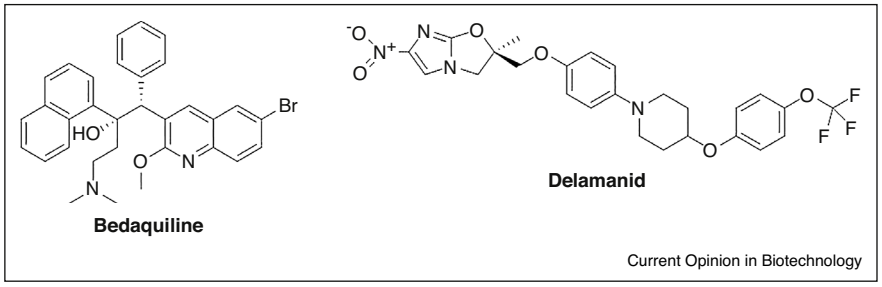
Since the advent of successful antibiotic therapy for tuberculosis, synthetic small molecules such as isoniazid continue to play a major role in the highly active combination regimen. When drug resistance against several first line drugs appeared in the late 1990s, several consortia driven efforts were undertaken to screen large small molecule libraries to identify compounds exhibiting new modes of action against *Mtb*. Outstandingly successful were relatively simple phenotypic live/dead high throughput screens performed with *Mtb* cultured in broth. Target identification of hits derived from these screens involved sequencing of resistance-associated mutations in most cases [4]. Almost all synthetic small molecules currently in clinical or preclinical trials were identified following this straightforward route including two recently Food and Drug Administration (FDA)-approved drugs: the diarylquinolone bedaquiline, an ATP-synthase inhibitor and delamanid, a nitroimidazo-oxazole targeting *Mtb* mycolic acid synthesis (Figure 1) [7–9].

A whole series of promising and structurally diverse compounds identified in these phenotypic whole cell screens were shown to target essential membrane associated proteins such as decaprenylphosphoryl-β-D-ribose oxidase (DprE1) or the transmembrane transporter MmpL3. DprE1 inhibition disrupts arabinogalactan synthesis, the corresponding polysaccharides being essential for mycobacterial cell wall integrity [10]. MmpL3 inhibitors also impact cell wall synthesis by interfering with transport of the essential cell wall component trehalose monomycolate into the periplasmic space [11••]. These molecules and their targets have been extensively reviewed elsewhere and will not be further discussed here [4,11••].

Host cell-based antitubercular drug discovery

The above mentioned whole cell-based high throughput assay has proven to be a feasible way to significantly increase the number of potential drug candidates targeting *Mtb*. However, to constantly fill the anti-TB drug

Figure 1



Recently approved new antitubercular agents.

pipeline, this approach requires continuous extension and diversification of compound libraries. Large campaigns virtually screening millions of compounds for their ability to kill *Mtb* in broth have taught us some lessons: First, although there are many essential proteins in *Mtb*, only a minority of these seem to be druggable. Second, several drug targets can indeed be addressed with diverse chemical scaffolds, however, several of the chemically distinct hit compounds share similar resistance-associated mutations, as shown for many MmpL3 inhibitors [11••]. Third, antibiotic drug screening in nutrient rich broth contains a bias towards selection of compounds with potent *in vitro* activity but devoid of *in vivo* activity [12]. Fourth, *in vitro* activity screens often fail to mimic the *in vivo* situation during infection and therefore, potentially useful compounds escape detection.

Scientists have tried to circumvent these obstacles by creating novel drug screening methods. One successful approach is targeting *Mtb* inside its mammalian host cell [13,14]. Though being more complex and expensive, testing drugs in the natural intracellular habitat of *Mtb* has several advantages: assay conditions are much closer to the real scenario of antibiotic treatment in humans, intracellularly the pathogen's metabolism changes dramatically opening the door for identification of novel drug targets, cytotoxic compounds are eliminated instantly in the process and the host cell can be analyzed for its ability to activate antibiotic prodrugs. One excellent example for a successful host cell-based screening campaign is the imidazopyridine amide Q203 (Figure 2), which was identified in a high content screen performed on *Mtb* infected RAW 264.7 macrophages [15,16]. Q203 was shown to target the cytochrome bc_1 B-subunit (QcrB) of the mycobacterial respiratory chain leading to massive ATP-depletion in treated bacteria thus representing a novel mode of action which explains the lack of cross-resistance of MDR-TB clinical isolates against Q203. The compound was shown to have excellent intracellular activity against *Mtb* with an IC_{50} in the picomolar range (0.28 nM). Importantly, potent intracellular activity translates very well into excellent *in vivo* activity in mice. As a result of these findings, Q203 just entered phase I clinical trials.

A recently published screening project provided an interesting proof of principle for host-cell dependent activation of antitubercular prodrugs. In this study, MRC-5 human lung fibroblasts were infected with *Mtb* after pre-incubation with 1280 FDA approved molecules of the Prestwick library. One hit compound with intracellular activity against *Mtb* was lansoprazole (Prevacid®), an over-the-counter gastric proton-pump inhibitor [17••]. Using an *ex vivo* pharmacokinetics approach it was shown that lansoprazole, chemically a benzimidazole, is converted to lansoprazole sulfide (LPZS; Figure 2) in the cytoplasm of the host cell. LPZS is a highly stable metabolite which also targets the cytochrome bc_1 complex of *Mtb*. This novel cytochrome bc_1 inhibitor shares no cross-resistance with Q203 and spares the human target of lansoprazole, the gastric H^+K^+-ATPase. These discoveries clearly show that alternative screening methods such as host cell-based drug screens are capable of finding novel hit compounds in old libraries.

Another extensive intracellular screen tested more than 300 000 synthetic small molecules and natural products for growth inhibition of mCherry-expressing *Mtb* phagocytosed by J774 macrophages [18•]. The large majority of 1390 initial hit compounds that inhibited growth inside these macrophages were conditional, meaning they were non-active in standard carbohydrate rich Middlebrook 7H9 OADC medium which is routinely used for growth of *Mtb* in laboratories. Retesting in media containing the host lipid cholesterol as the main carbon source showed activity comparable to intracellular values in more than 50 % of hit compounds. This approach identified novel mycobacterial enzymes as targets involved in cholesterol catabolism. Host fatty acids and lipids such as cholesterol are essential nutrients of *Mtb* growing inside its human host. Intracellular drug screens are thus capable of unmasking the respective enzymatic pathways as targets for antitubercular drug discovery.