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Screening and Identification of New Potential Targets against *Mycobacterium tuberculosis*

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Editorial

Tuberculosis (TB), which is caused mainly by *Mycobacterium tuberculosis* (MTB), is still a serious concern worldwide due to rising number of drug resistance cases and HIV-TB co-infection [1]. In 2013, over than 9 million new cases were diagnosed and around 1.5 million deaths were caused by MTB infection. In 2010, it was estimated that 4% of new TB cases were multidrug-resistant TB (MDR-TB) [2]. The increased number of MDR-TB and extensively drug-resistance TB (XDR-TB), adverse effects of available drugs and long-term therapy justify the search for new safe and effective antitubercular drugs.

Since rifampicin discovery in the sixties, few advances were reached in anti-TB therapy. Only in 2012, the United States Food and Drug Administration (FDA) approved the drug bedaquiline to treat MDR-TB. Despite this scary scenario, in the last years the number of drug candidates to treat resistant TB has increased and compounds such as OPC-67683, SQ-109, PA-824, LL-3858 are currently under evaluation [3]. The success to identify new drug candidates is related in part to screening campaigns performed by both academia and pharmaceutical companies. Target-based assays and whole-cell screening can be used during screening for antitubercular compounds. The target-based strategy is a powerful tool that allows identifying the mode of action of compounds. This strategy also contributes to optimize the antimycobacterial activity of prototypes. However, the main limitation of this strategy includes the need of target validation since some biochemical pathway in MTB can be not essential in the disease pathogenesis [4]. In addition, activation of alternative pathways and efflux mechanisms present in the mycobacteria can decrease the efficacy of this strategy. On the other hand, whole-cell screening has shown to be a promising strategy to identify new antitubercular drug candidates. Bedaquiline and compounds such as NITD-304 and NITD-349 were discovered using this strategy. This phenotypic assay does not require knowledge about the mode of action allowing the identification of new chemical entities able to inhibit new targets or pathways in MTB. Furthermore, this strategy is useful for prodrug's identification and it allows characterizing the selectivity of the compounds against MTB. Since there is not information about the target, the optimization of compounds using this strategy can be a hard task.

Nowadays, the combination of both strategies seems to be more efficient to identify new chemical structures with different mechanism of actions. In general, anti-mycobacterial activity is first identified through phenotypic assay. After, techniques such as DNA microarrays, gene knockdown and characterization of mutated genes of compound-resistant mutants contribute to the identification of probable targets. The expression, purification and crystallization of these targets allow optimizing the activity of those compounds in order to identify a possible drug candidate [5].

Several potential targets have been identified in MTB using combined strategies. Some examples include: a) DNA gyrase (gyrB subunit) and topoisomerase I – both involved in DNA synthesis; b) MbtA – involved in the iron metabolism of MTB; c) cytochrome b subunit (QcrB) and type II NADH dehydrogenase – involved in energy generation; d) decaprenylphosphoryl-β-D-ribose 20-epimerase (DprE), fatty acid synthases (FASs) and polyketide synthases (PKSs) involved in cell wall biosynthesis; e) Mycobacterial membrane protein large (MmpL) – involved in transport of ions and organic compounds through mycobacteria membrane; f) ClpP proteins (ClpP1 and ClpP2) – involved in protein turnover [6].

During the last years, potent and selective antitubercular compounds were identified. The compound 6-chloro-2-ethyl-*N*-(4-(4-(4-(trifluoromethoxy)phenyl)piperidin-1yl)benzyl)imidazo [1,2-a] pyridine-3-carboxamide (Q203), for example, acts by inhibition of QcrB of the cytochrome bc1-aa3 complex. The MIC_{50} value against *M. tuberculosis* H37Rv was found to be 2.7 nM. Furthermore, *in vivo* studies revealed that infected mice treated with Q203 for 4 weeks (at 10 mg/Kg) have reduced the mycobacterial load at 99% in lungs [7]. Another example is the compound ((2R,3S,4R,5R)-5-(6-amino-2-phenyl-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl (2-hydroxybenzoyl)sulfamate that demonstrated MIC_{99} value of 49nM against *M. tuberculosis* H37Rv under iron deficient conditions. This compound acts by inhibition of aryl acid adenylating enzyme (AAAE), known as MbtA, involved in iron metabolism in MTB. The selectivity index using Vero cells was characterized as being superior to 2000 for this chemical structure. Despite the potent *in vitro* activity this molecule demonstrated inadequate pharmacokinetic profile with low bioavailability and short halflife [8].

During screening using whole-cell assays the compound 1-((1r,3r,5r,7r)-adamantan-2-yl)-3-(2,3,4-trifluorophenyl)urea have exhibited MIC value of 30 nM against *M. tuberculosis* (H37Rv) and it also demonstrated activity against MDR strains [9]. Using genetic approach, it was characterized through sequencing of resistant mutants the MmpL3 as target for this molecule. Afterward, the optimization of pharmacokinetic properties was performed in order to improve the water solubility and reduce the lipophilicity [10]. Another example of target identified after phenotypic assay occurred with the compound 1-(4-(tert-butyl)benzyl)-3-nitro-1H-1,2,4-triazole. Using high-throughput screening (HTS) approach the authors identified for this molecule MIC value of 500 nM. They observed that resistant mutants had presented a mutation in dprE1 gene and that the covalent inhibition of DprE was the mode of action for this compound [11].

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In summary, the development of a new platform driven by phenotypic assays, genetic approach and target-based assays seems to be promising to identify new potential targets and compounds active against *Mycobacterium tuberculosis* resistant strains [12,13]. Despite current advances in this field during the last years, challenges involving the discovery of new drugs against latent tuberculosis are still distant perspectives in the therapy.

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