Changing the rules of TB-drug discovery

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Abstract

The discovery of new drugs with novel targets is paramount to the continued success of tuberculosis (TB) treatment due to the increasing prevalence of antibiotic resistant infections in the TB population. *Mycobacterium tuberculosis* (*Mtb*) fumarate hydratase (fumarase) is a highly conserved essential protein which shares an active site with human fumarase, making active site inhibition equally cytotoxic for both bacteria and humans. The recent discovery of a set of new *Mtb* inhibitory compounds that target *Mtb*-fumarase by binding to a non-conserved allosteric site is a major advancement, providing further evidence to dispel the antibiotic discovery dogma that conserved proteins don't make good antibiotic targets.

Main Point, Background, Detail, Impact and Viewpoint

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis (TB) is attributed to 1.3 million deaths and 10 million new cases per annum[1] and remains one of the top 10 causes of death worldwide. The treatment strategy for drug-sensitive *Mtb* infections is a 6 month course comprising of 4 drugs, isoniazid (INH), rifampicin (RIF), pyrazinamide and ethambutol for the first two months and INH and RIF for the remaining 4 months, and this strategy has remained largely unchanged since its advent in the 1980s. Unsurprisingly, there has been a steady emergence of drug resistance to this frontline strategy, culminating in the identification of widespread multi-drug resistant TB (MDR-TB), comprising around 5% of newly identified *Mtb* infections, wherein resistance to INH and RIF is observed. More concerning still is the increasing prevalence of extensively drug-resistant TB (XDR-TB), resistant to INH, RIF and at least 3 injectable second-line drugs (8.5% of new MDR-TB cases)[1]. This clearly exemplifies the urgent need to discover new antibiotics that are able to overcome or bypass the resistance mechanisms that are becoming abundant in *Mtb* in order to halt the progression of antibiotic resistance. On page XXX of this issue, authors report the outcome of a successful structure-activity relationship screen, providing a new *Mtb* inhibitor that targets a non-conserved allosteric site in the highly conserved fumarase enzyme. Their findings provide new opportunities for TB drug discovery and a philosophical change in exclusion criteria for what makes a valuable antibiotic drug target.

Despite a recent plethora of new *Mtb* inhibitory molecules, there remains a high attrition rate, with the majority of new molecules never making it into clinical trial. This is largely due to early exclusion factors such as the identification of a target protein which is highly evolutionarily conserved. Widely, the assumption is held that a highly conserved target is unlikely to yield positive results under clinical trial due to the risk of also inhibiting the human orthologue. There are recent notable exceptions to the rule in terms of *Mtb* drug discovery,

including bedaquiline, the first new TB drug to be approved by the U.S. Food and Drug Administration (FDA) in 40 years. Bedaquiline (BDQ), a member of the diarylquinoline class of inhibitors, targets one of the most evolutionarily conserved proteins, the proton pump ATP synthase. Perhaps unsurprisingly this drug carries a number of serious "black-box warnings" from the FDA including an increased risk of death and arrhythmias due to off-target effects blocking the hERG channel and as a result is approved as a drug of last resort for MDR-TB. In spite of the relatively conserved sequence and structure of ATP synthase, BDQ has been shown to have much higher specificity for the mycobacterial c-ring of ATP synthase over other prokaryotic and eukaryotic homologues, where steric hindrance prevents a number of crucial drug-protein interactions[2]. Ultimately, this results in significantly higher IC_{50} values (>200 μM) for eurkaryotic ATP synthases, over mycobacterial homologues (20-25 nM)[2]. However, despite the limitations, this drug provides a new treatment option and consequently has been widely implemented, catalyzing the debate surrounding exclusion of antimicrobial drugs with conserved targets.

Bacterial core metabolism presents a number of alluring candidates to target chemotherapeutically, given the essentiality of the enzymes involved. However, that enthusiasm is offset by the level of evolutionary conservation with human homologues and their equal importance for organism viability. Fumarate hydratase (fumarase) catalyzes the reversible hydration and dehydration of fumarate to malate in the tricarboxylic acid (TCA) cycle, ultimately driving the production of reduced nicotinamide adenine dinucleotide (NADH) as well as the metabolic precursors of certain amino acids. Fumarase is a core enzyme in cellular metabolism and is highly conserved between prokaryotes and eukaryotes. Furthermore, with a 53% sequence homology to the human ortholog, the *Mtb* fumarase presents enough of a challenge to make it an unlikely candidate as an anti-TB drug target.

Nevertheless, these obstacles failed to deter the Hyvönen, Boshoff, Abell, Barry and Thomas laboratories to explore a hit molecule from a high-throughput screen. They previously identified *N-(5-(azepan-1-ylsulfonyl)-2-methoxyphenyl)-2-(4-oxo-3,4-dihydrophthalazin-1-yl)acetamide*, otherwise known as compound 7, from a library of just under 500,000 small molecules using a fumarase-resazurin coupled assay, followed by X-ray crystallography, which yielded fumarase inhibition at a newly discovered allosteric site. They further demonstrated that compound 7 showed no inhibition of the human homologue at much higher concentrations, confirming selectivity[3].

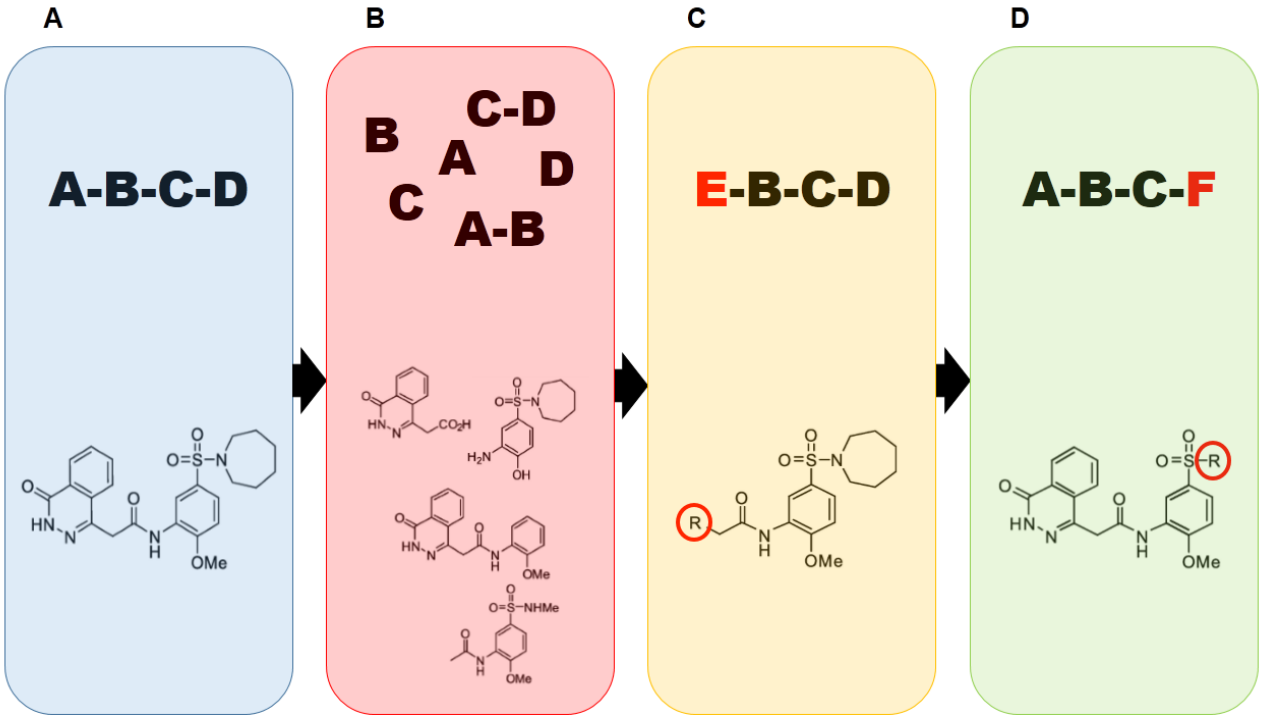

Figure 1: Workflow of *Mtb* fumarase inhibitor development. A) Complete compound 1 from HTS screen inhibits *Mtb* fumarase, but has little whole cell activity against *M. tuberculosis* H37Rv. B) Compound 1 was fragmented and screened for activity, but showed no *Mtb* fumarase inhibition. C) Derivatives of R-group A were developed and screened, showing activity against *M. tuberculosis* H37Rv, but little *Mtb* fumarase inhibition. D) Derivatives of R-group D were developed and screened, showing both activity against *M. tuberculosis* H37Rv and high inhibition of *Mtb* fumarase.

In this latest study, the lead compound, 7 has been renamed as compound 1. The team conducted hit-to-lead and defragmentation structure-activity relationship screening around compound 1 with the objective of improving the minimal inhibitory concentration (MIC) from the previously reported modest inhibitory activity at 250 μM (Figure 1A). Their initial approach involving the defragmentation of the lead compound (1) into fragment-like molecules was unyielding in terms of inhibitory activity against *Mtb* fumarase at concentrations up to 1 mM, which the authors still helpfully included in the manuscript to prevent reproduction of efforts (Figure 1B)[4]. However, their structure-activity relationship screen was much more successful, generating 5 new compounds (15a-d and 16b) with MIC ranging from 19 μM (compound 16b) to 6.3 μM (compound 15d) in Middlebrook 7H9 media supplemented with dipalmitoylphosphatidylcholine (DPPC) (Figure 1C/D)[4]. This choice of supplement is well justified as it accurately represents the *in vivo* conditions of *Mtb* growth, wherein long-chain fatty acids are catabolized to acetyl-CoA driving metabolic flux into the Krebs cycle. This is perhaps down to the increase in lipophilicity (cLogP) of the compounds from 1.6 (compound 1) to 4.1 (compounds 15c and 15d) as *Mtb*-active drugs tend to have poor solubility when cLogP >4 (for example, bedaquiline cLogP is 7.10).

The improved MICs of the new compounds from the structure-activity relationship screen somewhat impacted the inhibition of *Mtb* fumarase as percentage inhibition of *Mtb* fumarase was reduced from >90% (compound 1) to 10-38% (compounds 15a-d), therefore negating IC_{50} calculations at 50 μM (Figure 1C)[4]. However, compound 16b gave an IC_{50} of 4.0 μM (± 0.1), compared to 2.0 μM (± 0.1) for compound 1, demonstrating that it was indeed possible to maintain *Mtb* fumarase inhibitory activity whilst also producing an MIC (Figure 1D)[4]. Despite

the significant impact on the inhibitory activity of the target, the fact remains that Whitehouse *et al*. have successfully overcome the mycobacterial cell wall permeability barrier with structural modifications to their hit compound. Furthermore, they have demonstrated that despite significant homology to the human fumarase enzyme, their inhibition at an unconserved allosteric site rather than the conserved active site enables a selectivity of their compounds for *Mtb* fumarase. There is still a long road ahead for these compounds in terms of progressing them to any future clinical trial, but in our view this work cuts new ground in *Mtb* drug discovery and creates opportunities for reconsidering targets that have been previously discounted on the basis of homology.

References

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