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Reactive dirty fragments: implications for tuberculosis drug discovery

Pooja Gopal and Thomas Dick

Reactive multi-target fragments, old synthetic antimycobacterials that are activated inside *Mycobacterium tuberculosis* bacilli and are smaller than the usual drug-like, single-target molecules, represent critical components of current tuberculosis chemotherapies. Recent studies showed that para-aminosalicylic acid is recognized as a substrate by dihydropteroate synthase and poisons the downstream folate pathway. Pyrazinamide, a key relapse-reducing drug, is metabolized by an amidase and the reaction product interferes with trans-translation, membrane potential and other targets. However, the mechanism of action of pyrazinamide remains ill-defined and needs to be understood to rationally approach treatment shortening. The success of small dirty drugs and prodrugs suggests that fragment-based whole cell screens should be re-introduced in our current antimycobacterial drug discovery efforts.

Addresses
Antibacterial Drug Discovery Laboratory, Department of Microbiology, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore, 5 Science Drive 2, Block MD4A, Singapore 117597, Republic of Singapore

Corresponding author: Dick, Thomas (thomas_dick@nuhs.edu.sg)

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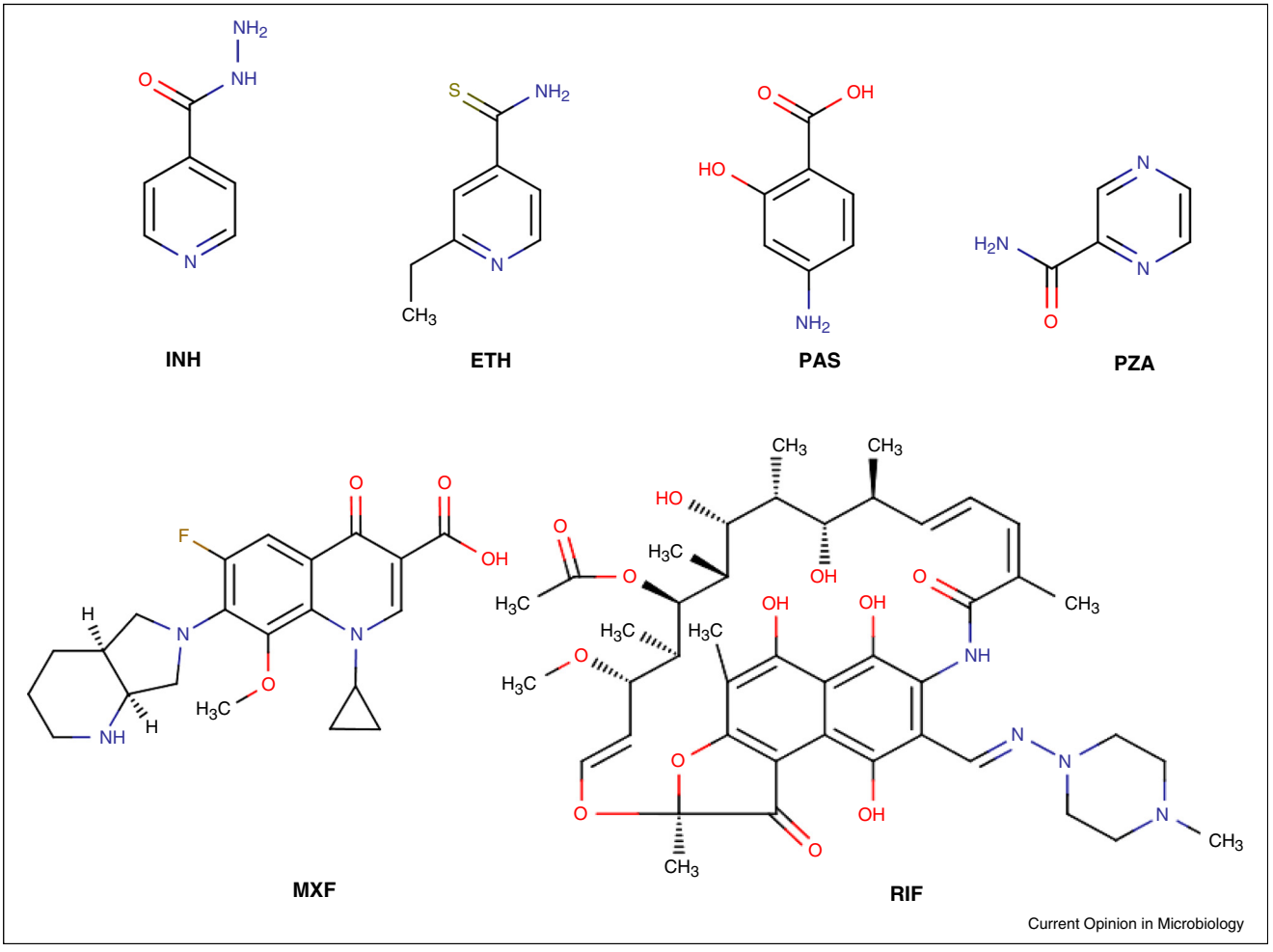
Current TB chemotherapies and drug discovery approaches

Mycobacterium tuberculosis remains the most deadly bacterial pathogen globally [1]. Tuberculosis (TB) is treated with a combination therapy consisting of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol for 2 months, followed by 4 months of treatment with INH and RIF. This lengthy regimen has of course implementation and compliance issues, and this in turn fuels development of drug resistant disease [2,3]. Multi-drug resistant TB, defined as being resistant to INH and RIF, is treated with less potent and more toxic second line drugs, fluoroquinolones and injectables (aminoglycosides and capreomycin), and requires at least 2 years of therapy. Additional drugs include ethionamide (ETH) and para-aminosalicylic (PAS) [4]. New drugs with new mechanism of action are urgently needed to shorten the lengthy treatment regimen of drug susceptible TB and to improve the poor cure rates of drug resistant disease [5].

Current drug discovery strategies are largely based on Paul Ehrlich's magic bullet 'one drug–one target' concept. This facilitates lead optimization as it allows simple structure activity relationships and the use of target-compound co-structure guided design [6]. A few years ago, after a decade of biochemical high throughput screening against genetically validated targets, the anti-mycobacterial discovery field largely moved back to phenotypic whole cell screens to identify compounds with antimicrobial activity first, then deconvolute the target for lead optimization [7]. This strategic shift is due to the large scale failure of target-based approaches: translating biochemical enzyme inhibitors into whole cell active antimicrobials turned out to be far more difficult than anticipated [8]. A key issue is the mycobacterial cell envelope, representing a formidable permeability barrier [9]. Target based approaches also fail to capture prodrugs, which are critical components of anti-TB regimens. It is to note that both target-first and compound-first avenues use the same concept: identify single-target, high affinity (nM) binders, which are non-reactive to avoid side effects. Other recent approaches do not start with screening of compound libraries, but make use of existing antibacterials that either do not work against *M. tuberculosis*, or are not used for treatment of TB. These include elegant remodelling of antibiotics, such as spectinomycin [10], to make them stay inside the bacillus (prevent efflux), and re-purposing of old drugs, such as clofazimine [11]. High attrition rates, that is, limited success in all approaches, call for a multipronged 'leaving no stone unturned' strategy [12]. Can we find additional hit-finding avenues at the bottom of the barrel, approaches that we have overlooked or not fully utilized so far?

Some of the key TB drugs, discovered in the middle of the past century by whole cell or animal model screening, are dirty fragments: they hit multiple targets and their molecular weights are in the range of 100–300 g/mol (Figure 1). The fragments are metabolized inside the tubercle bacillus and only then, after being 'activated', exert their antimicrobial activity [13,14]. This type of mechanism of action (MoA), polypharmacology, and physicochemical properties, 'extra' small and reactive, is at odds with main stream antibacterial drug discovery: attractive leads for medicinal chemistry should inhibit

Figure 1



Structures of TB fragment drugs. Isoniazid [INH, MW 137], ethionamide [ETH, MW 166], para-aminosalicylic acid [PAS, MW 153] and pyrazinamide [PZA, MW 123] in comparison with a more standard-sized synthetic drug, moxifloxacin [MXF, MW 402], and a huge natural product, rifampicin [RIF, MW 823]. MW: molecular weights in g/mol.

a single target (to facilitate lead optimization), have a decent size (to bind a target with high affinity), and should not be reactive (to avoid side effects) [15].

Can we learn something from these old TB fragment drugs? Should we start screening again for these types of compounds to identify leads for the discovery of new TB drugs? Here we give an update on some recent developments in our understanding of the MoA of some of these unusual antibiotics, revealing new concepts and targets. Then we zoom into a critical sterilizing (relapse-preventing) TB drug: pyrazinamide (PZA). We argue that the MoA of this metabolized fragment drug remains an enigma, despite some recent progress, and that it is important to identify its targets if we are to eliminate persisters and substantially shorten TB therapy. We review some key findings from the past few years. However, in the case of PZA, we need to go back to the early 1900s, for some apparently forgotten papers. We propose that including, or re-introducing, fragment-based whole cell screens against replicating and dormant bacteria in our current approaches to TB drug discovery will deliver the next generation of TB drugs.

MoA of reactive TB fragment drugs and why it is useful to understand them

Reactive multi-target fragment drugs, unusually small antimycobacterials that are activated inside the bacilli, represent critical components of TB chemotherapies (Figure 1). INH is the key bactericidal (i.e. sputum count-reducing) fragment drug in the first line regimen [16]. The compound is oxidized by the bacterial catalase-peroxidase KatG [17], and its reactive metabolite forms adducts with NAD(P). The enoyl acyl carrier protein reductase InhA, required for synthesis of outer-membrane mycolic acids, appears to be its major target [18]. Additional