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Perspective: Challenges and opportunities in TB drug discovery from phenotypic screening

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ABSTRACT

Tuberculosis poses a major global health problem and multi-drug resistant strains are increasingly prevalent. Hence there is an urgent need to discover new TB drugs. Cell based phenotypic screening represents a powerful approach to identify anti-mycobacterial compounds and elucidate novel targets. Three high throughput phenotypic screens were performed at NITD against mycobacterium. Hits were identified and chemical series selected for optimisation. This produced compounds with good in vitro anti-mycobacterial activity and pharmacokinetic properties. Some compounds displayed oral activity in mouse efficacy models of TB. Herein, we review the TB discovery efforts at NITD and share experiences in optimisation of phenotypic hits, describing challenges encountered and lessons learned. We also offer perspectives to facilitate future selection and advancement of phenotypic hits.

1. Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb), continues to pose a major global health problem and multi-drug resistant strains are becoming increasingly widespread.[1] In 2013 there were over 9 million TB cases worldwide and over 1.5 million deaths attributed to the disease. Nearly 4% of all new TB cases in 2010 were multi-drug resistant (MDR). The current first line drugs for TB (isoniazid, rifampicin, pyrazinamide and ethambutol) were discovered decades ago and are increasingly becoming less useful due to emerging resistance and synergistic interactions with HIV AIDS patients. Furthermore, effective use of these drugs require months of combination therapy, leading to issues with compliance and significant side effects. Thus, there is an urgent need to discover new TB drugs. Over the past ten years there has been a major investment by scientists from both academia and pharmaceutical companies into TB drug discovery and development.[2] High-throughput screening (HTS) has become an integral part of pharmaceutical research and fuelled drug discovery projects.[3]

Drug discovery screens can be either target or cell based/phenotypic. Each has advantages and disadvantages.[4] The power of a target based strategy includes the ability to apply molecular and chemical knowledge to investigate a specific molecular hypothesis and ability to perform HTS against the target. The main limitation is that the molecular hypothesis or target may not be relevant in the disease pathogenesis setting, thus placing a major emphasis on target validation. In contrast, the strength of a phenotypic approach is that the assay does not require knowledge of molecular mechanism of action; however in vitro HTS growth conditions should mimic disease settings. The main drawback of this approach is the challenge in optimizing hits with multi-parametric cell based activity. The advantage of a phenotypic screen is the possibility to find NCEs that inhibit new target/s or pathway/s, and the potential to find pro-drugs and other complex mechanisms of action. All currently used antibiotics were discovered using cell based phenotypic screening, highlighting the value of this approach.

2. TB drug discovery at NITD

The Novartis Institute for Tropical Diseases (NITD) in Singapore was established in 2002. The main objective of the TB program at NITD was to develop new chemical entities (NCEs) active against MDR-TB and extensively drug resistant (XDR) TB to enhance cure rates and improve patient compliance. Both molecular target screening and whole cell based phenotypic screening were pursued to identify NCEs active against Mtb. A general target-based and phenotypic cell based TB drug discovery flow-chart is illustrated in Figure 1.

Target based Mtb HTS has been pursued extensively in the last decade. During the early stages of the work at NITD, several well defined molecular targets (NAD synthase,[5] pantothenate kinase,

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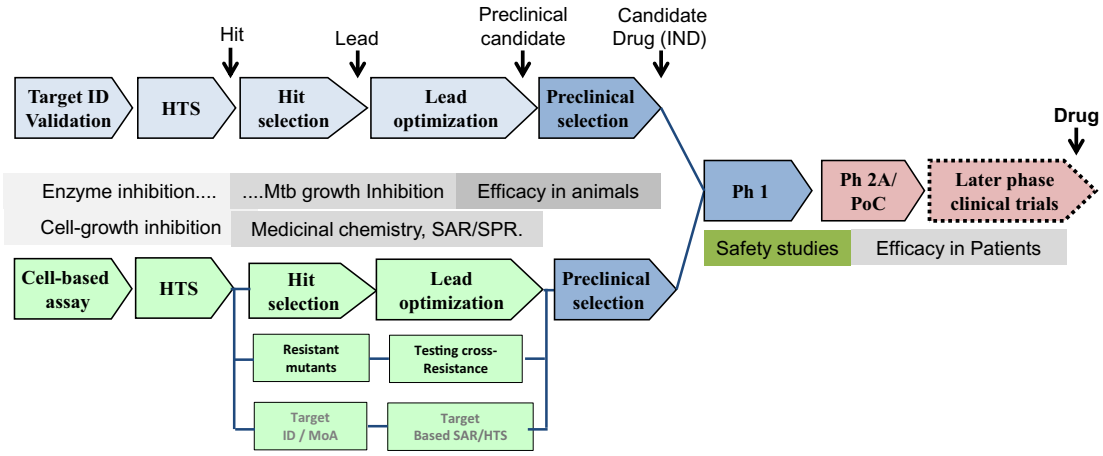
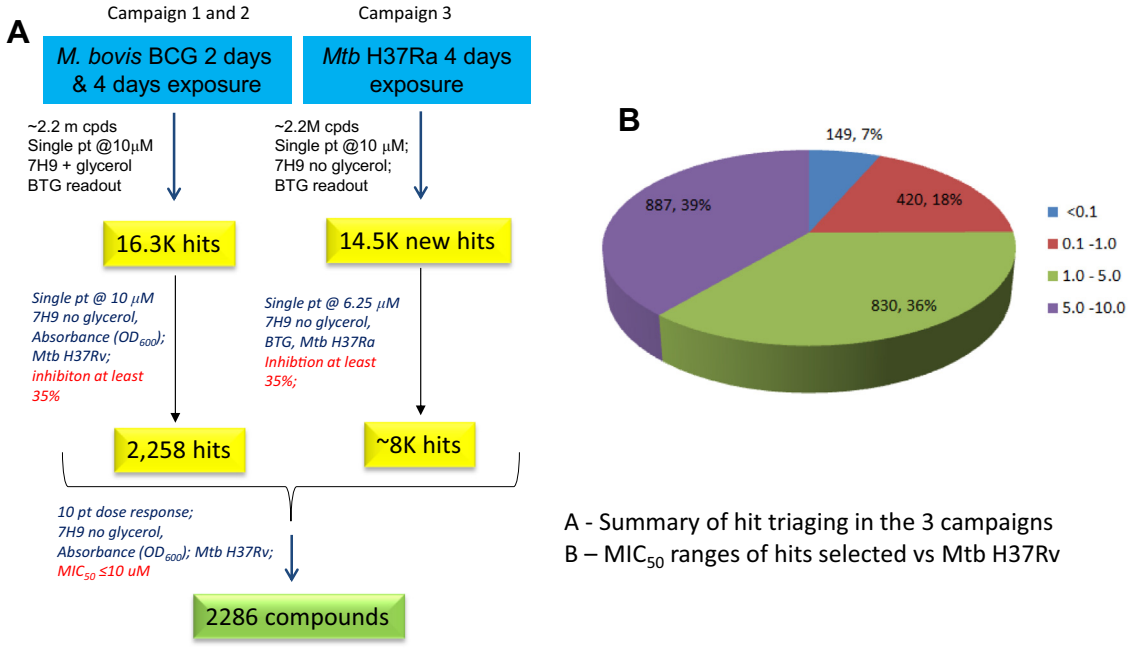


Figure 1. A typical molecular target-based and cell-based TB drug discovery cascade. (SAR, structure–activity relationship; SPR, structure–activity relationship; HTS, high through-put screen; PoC, proof-of-concept in patients.)

Table 1
Target based biochemical screens pursued at NITD

Target enzyme (Rv number)	Library size	Hits	Description/issues
Peptide deformylase[6] (Rv0429c, PDF)	Antibacterial PDF focussed library	~200	Bacteriostatic Limited in vivo mouse efficacy
Dihydropteroate synthase (Rv3608c, DHPS)	1.3 million	3071	Coupled enzyme assay Lack of cellular activity
Dihydrofolate reductase (Rv2763c, DHFR)	Focussed library	100	Coupled enzyme assay Low selectivity vs human DHFR Low permeability
Pantothenate kinase (Rv1092c, PanK)	1.3 million	~800	Lack of cellular activity
NAD (+) synthetase (Rv2438c, NadE)	2.2 million	300	Lack of tractable hits

dihydropteroate synthase, dihydrofolate reductase, peptide deformylase[6]) were screened against the Novartis compound collection or with focused libraries.[7] However, these efforts (summarised in Table 1), did not produce advanced leads. A few possible reasons for the lack of success with the target based strategy are: lack of chemical validation of the target, failure to find tractable hits, inability to translate enzyme to cellular activity, etc.[8] In the wider anti-bacterial field, target based approaches have also shown limited success and phenotypic screens identified more first-in-class molecules.[9,10] Recent work highlighted the underlying complexity of bactericidal activity by antibiotics and suggested discrete mechanisms distal to the actual molecular target leading to bacterial



A - Summary of hit triaging in the 3 campaigns
B – MIC_{50} ranges of hits selected vs Mtb H37Rv

Figure 2. The 3 HTS phenotypic screening campaigns and distribution of Mtb hits.