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Hit discovery of *Mycobacterium tuberculosis* inosine 5′-monophosphate dehydrogenase, GuaB2, inhibitors

Niteshkumar U. Sahu[a], Vinayak Singh[b,c], Davide M. Ferraris[d], Menico Rizzi[d], Prashant S. Kharkar[a,*]

[a] *Department of Pharmaceutical Chemistry, SPP School of Pharmacy & Technology Management, SVKM's NMIMS, V.L. Mehta Road, Vile Parle (West), Mumbai 400 056, India*
[b] *Drug Discovery and Development Centre (H3D), University of Cape Town, Rondebosch 7701, Cape Town, South Africa*
[c] *South African Medical Research Council Drug Discovery and Development Research Unit, Department of Chemistry and Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa*
[d] *Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Via Bovio 6, 28100 Novara, Italy*

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ABSTRACT

Tuberculosis remains a global concern. There is an urgent need of newer antitubercular drugs due to the development of resistant forms of *Mycobacterium tuberculosis* (*Mtb*). Inosine 5′-monophosphate dehydrogenase (IMPDH), *guaB2*, of *Mtb*, required for guanine nucleotide biosynthesis, is an attractive target for drug development. In this study, we screened a focused library of 73 drug-like molecules with desirable calculated/predicted physicochemical properties, for growth inhibitory activity against drug-sensitive *Mtb*H37Rv. The eight hits and mycophenolic acid, a prototype IMPDH inhibitor, were further evaluated for activity on purified *Mtb*-GuaB2 enzyme, target selectivity using a conditional knockdown mutant of *guaB2* in *Mtb*, followed by cross-resistance to IMPDH inhibitor-resistant SRMV2.6 strain of *Mtb*, and activity on human IMPDH2 isoform. One of the hits, **13**, a 5-amidophthalide derivative, has shown growth inhibitory potential and target specificity against the *Mtb*-GuaB2 enzyme. The hit, **13**, is a promising molecule with potential for further development as an antitubercular agent.

Introduction

Tuberculosis (TB), an infectious disease caused by the bacillus *Mycobacterium tuberculosis* (*Mtb*), is the ninth leading cause of death worldwide and number one from a single infectious agent, ranking above HIV/AIDS.[1] The increased prevalence of infections caused by multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mtb*, with limited treatment choices, is a wake-up call urging the development of more effective antitubercular agents, ideally with novel mechanism(s) of action. This is not the end of the story! Emergence of totally drug-resistant (TDR)-TB in which *Mtb* is resistant to all first- and second-line antitubercular drugs has terrified the healthcare professionals.[2]

Tuberculosis, mainly being the disease of the developing or underdeveloped nations, was a neglected disease in terms of drug discovery. Bedaquiline,[3] a recently approved antitubercular drug, has shown promise in treating TB. Several drug repurposing campaigns of approved drugs[4,5] are likely to offer potential alternatives for the treatment of TB. In light of the fact that the strategies involving development of potent and target-selective enzyme inhibitors which arrested essential biochemical processes, failed miserably in whole-cell *Mtb* assays,[6] newer molecules with novel mechanism(s) of action are essential to tackle TB menace. One such pathway is purine nucleotide biosynthesis. The enzyme inosine 5′-monophosphate dehydrogenase (IMPDH, EC 1.1.1.205) catalyzes a crucial step in the biosynthesis of guanine nucleotides leading to oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) with concomitant reduction of nicotinamide adenine dinucleotide (NAD^+) to reduced nicotinamide adenine dinucleotide (NADH).[8] The XMP is further converted into guanosine 5′-monophosphate (GMP) by GMP synthase. Inhibition of IMPDH leads to depletion of guanine nucleotide pool, affecting cell division, and ultimately inhibition of cell proliferation.

The enzyme IMPDH, thus, is an interesting target for anticancer, antiviral, immunosuppressive and antimicrobial therapies.[9] Mycophenolic acid (MPA, **1**, Fig. 1) is an IMPDH inhibitor widely used as immunosuppressant and antiviral drug.[10] Three genes encode IMPDH in *Mtb*, namely, *guaB1* (*Rv1843c*), *guaB2* (*Rv3411c*) and *guaB3* (*Rv3410c*). However, only *guaB2* has shown the IMPDH activity.[11,12]

Chen et al. reported mycophenolic adenine dinucleotides (MAD1) as a *Mtb*IMPDH inhibitors, where the pyrophosphate linker in $\mathbf{NAD^+}$ was replaced with isosteric 1,2,3-triazole

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[*] Corresponding author.
E-mail address: prashant.kharkar@nmims.edu (P.S. Kharkar).

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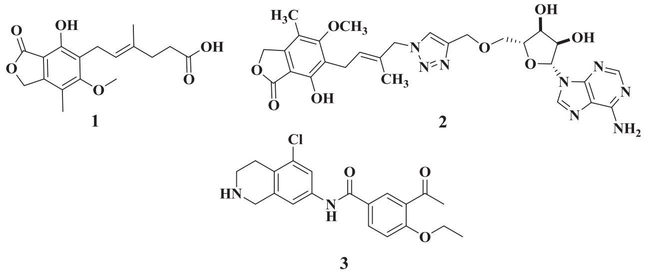


Fig. 1. Mycophenolic acid (MPA) and earlier *Mtb*IMPDH inhibitors.

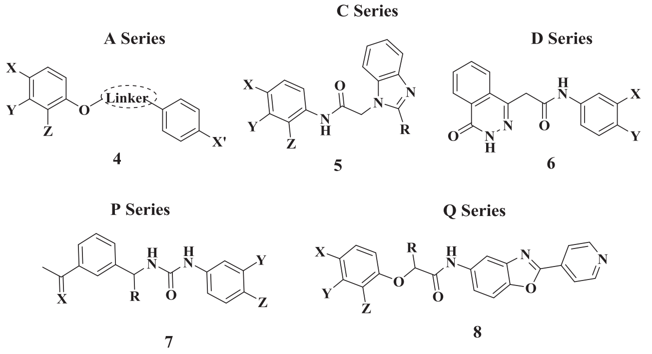


Fig. 2. Chemotypes observed in *Cp* – and bacterial IMPDH inhibitors.

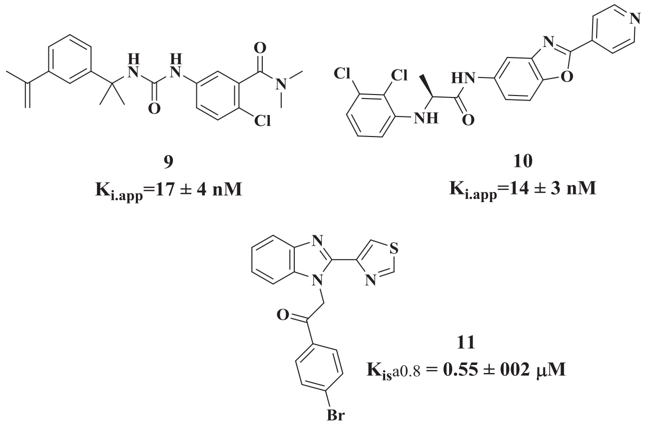


Fig. 3. Few *Mtb*IMPDH (GuaB2) inhibitors.

(**2**, $K_i^{app} = 1.5\ \mu M$).[13] Recently, several *Mtb*-GuaB2 inhibitors have been identified in a target-based high-throughput resistance-based phenotypic screen.[14] The identified hits are represented by compound **3**. In similar studies, *Cryptosporidium purvum* IMPDH (*Cp*IMPDH) selective inhibitors belonging to five chemical series (**4**–**8**, Fig. 2) were screened against *Mtb*IMPDH.[7] Few of these molecules were potent inhibitors (**9**, P series and **10**, Q series, Fig. 3).[10] Another series, 1*H*-benzo[*d*]imidazole, of *Mtb*IMPDH was reported with submicromolar inhibition constants (**11**, Fig. 3).[15]

Careful examination of the common structural features of the lead molecules (**3**, **9**, **10** and **11**, Figs. 1 and 3), the authors adopted a pharmacophore-based design strategy for GuaB2 inhibitors – two aromatic moieties connected with a linker (Fig. 4). Indeed, many human IMPDH2 (*h*IMPDH2) inhibitors possessed similar features and the nature of the linker was shown to be crucial in modulating potency and selectivity, if any.[16,17] Learning from previous experience with IMPDH inhibitors, a subset of our in-house library (#60) matching the pharmacophore criteria along with few compounds similar to MPA (#12) were initially screened for *anti-Mtb* activity in drug-sensitive *Mtb* H37Rv strain, followed by evaluation of the hits (#8) on two derivatives of *Mtb H37Rv*: (i) for target selectivity – *guaB2-B3* Tet-OFF *attB*::*guaB3*, a conditional knockdown mutant (cKD, *guaB2* Tet-OFF) in which *Mtb*IMPDH levels are depleted by transcriptional silencing of the IMPDH-encoding gene, *guaB2* and (ii) for cross-resistance – SRMV2.6 this strain expresses the mutant *Mtb*IMPDH *Y487C*, which is resistant to an isoquinoline sulfonamide *Mtb*IMPDH2 inhibitor.[6] Here, we report the synthesis and biological testing of the hits discovered in whole cell-based and target (*Mtb*IMPDH, GuaB2)-specific assays.

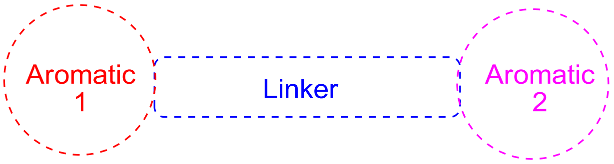
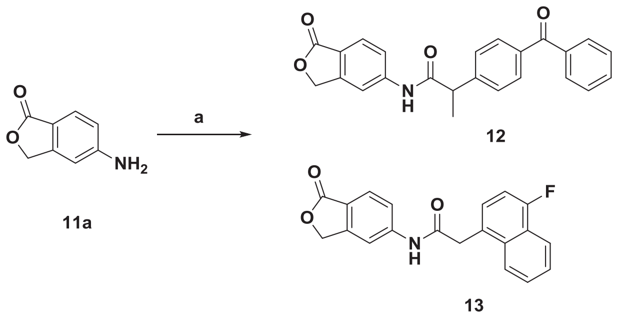


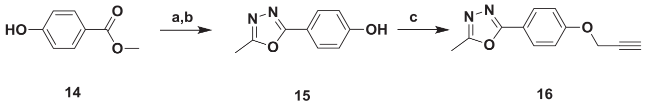
Fig. 4. Pharmacophoric features for *Mtb*IMPDH (GuaB2) inhibitors.

Methods

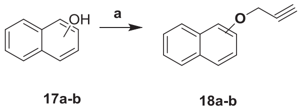
Compounds **12** and **13** were synthesized by reacting 5-aminophthalide (**11a**) and substituted acid chlorides, in presence of pyridine (Scheme 1). The synthesis of 1,2,3-triazole derivatives proceeded in two steps as described in Schemes 2–4. First, intermediate **15** was synthesized using **14**, hydrazine hydrate and triethyl orthoacetate. Further **15**, α- and β-naphthols (**17a** and **17b**, respectively) were alkylated with propargyl chloride in presence of anhydrous K_2CO_3 and DMF to yield **16** and **18a**–**b** (Schemes 2 and 3). Substituted benzyl azides (**20a**–**e**, Scheme 3) prepared from corresponding benzyl bromides (**19a**–**e**), were further reacted with



Scheme 1. [a]Synthesis of arylalkyl amides **12**–**13** [a]*Reagents and conditions.* a. substituted acid chloride, pyridine, THF, 0 °C to RT, overnight.



Scheme 2. [a]Synthesis of 1,2,3-triazole analogs [a]*Reagents and conditions.* a. NH_2-$NH_2{\cdot}H_2O$, EtOH, Reflux; b. triethyl orthoacetate; c. propargyl chloride, K_2CO_3, DMF, 12 h.



Scheme 3. [a]Synthesis of 1- and 2-(prop-2-ynyloxy)naphthalene. [a]*Reagents and conditions.* a. Propargyl chloride, K_2CO_3, DMF, 12 h.