Fragment-Based Approach to Targeting Inosine-5′-monophosphate Dehydrogenase (IMPDH) from Mycobacterium tuberculosis

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Supporting Information

ABSTRACT: Tuberculosis (TB) remains a major cause of mortality worldwide, and improved treatments are needed to combat emergence of drug resistance. Inosine 5′-monophosphate dehydrogenase (IMPDH), a crucial enzyme required for de novo synthesis of guanine nucleotides, is an attractive TB drug target. Herein, we describe the identification of potent IMPDH inhibitors using fragment-based screening and structure-based design techniques. Screening of a fragment library for Mycobacterium thermoressistible (Mth) IMPDHΔCBS inhibitors identified a low affinity phenylimidazole derivative. X-ray crystallography of the Mth IMPDHΔCBS–IMP–inhibitor complex revealed that two molecules of the fragment were bound in the NAD binding pocket of IMPDH. Linking the two molecules of the fragment afforded compounds with more than 1000-fold improvement in IMPDH affinity over the initial fragment hit.

INTRODUCTION

Tuberculosis (TB) is a contagious infectious disease caused by Mycobacterium tuberculosis (Mtb), which can be transmitted through the air as droplets. The infection predominantly affects the lungs, but it can spread to other parts of the body, especially in patients with a suppressed immune system.

The World Health Organization (WHO) has estimated that nearly one-third of the world’s population is infected with Mtb, leading to 1.8 million TB deaths in 2015.1 Although there has been a slow decline in new TB cases and TB-related deaths in recent years, the emergence and spread of multidrug resistant (MDR) and extensively drug resistant (XDR) strains of Mtb has increased the threat that this disease poses for global public health. According to the WHO, approximately 480,000 cases of MDR-TB emerged in 2015, and the cure rate of those patients was only 50%.1

Current TB treatments require combinations of four first-line drugs, isoniazid, rifampicin, ethambutol, pyrazinamide, and streptomycin, which must be taken for six months or longer.2

Resistant strains are not susceptible to the standard drugs, and although MDR-TB is treatable using second-line drugs, such treatments have a number severe side effects.3 Consequently, there is an urgent need for the development of novel and more effective drugs for the treatment of drug resistant TB.

Inosine-5′-monophosphate dehydrogenase (IMPDH, E.C. 1.1.1.205) has received considerable interest in recent years as an important target enzyme for immunosuppressive,4 anti-cancer,5,6 and antiviral drugs.7 Most recently, IMPDH has emerged as a promising antimicrobial drug target.5–11

IMPDH catalyzes the first unique step in the de novo synthesis of guanine nucleotides, the oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP) with the concomitant reduction of the cofactor nicotinamide adenine dinucleotide (NAD⁺) to NADH (Figure 1).12 XMP is then
subsequently converted to guanosine 5′-monophosphate (GMP) by a GMP synthetase.13 IMPDH has been deemed essential in every pathogen analyzed to date, including Mtb, Staphylococcus aureus, and Pseudomonas aeruginosa, which are three of the most serious bacterial threats.14−16 However, this has been somewhat contradictory,17 in comparing cell versus animal work. IMPDH is an ubiquitous enzyme present in several eukaryotes, bacteria, and protozoa.18 The IMPDH reaction involves two chemical transformations. The first step of the IMPDH catalyzed reaction involves the attack of catalytic Cys on substrate IMP followed by hydride transfer to NAD+, forming the covalent enzyme intermediate E-XMP*. In the second step, E-XMP* is hydrolyzed to XMP.11 The enzyme exists in two different conformations, an open form that accommodates both the substrate and cofactor during the first step and a closed form where the active site flap moves into the NAD+-binding site for the E-XMP* hydrolysis.11

In recent years, there has been considerable effort aimed at identifying small molecule inhibitors of IMPDH as potential antitubercular agents.20−26 We have sought to develop IMPDH inhibitors using a fragment-based approach. Fragment-based drug discovery (FBDD) is now established in both industry and academia as an alternative approach to high-throughput screening for the generation of hits or chemical tools for drug targets.30 We have previously reported the discovery of several series of novel and potent inhibitors using FBDD to target Mtb.21,23−25 Previously we have reported the fragment elaboration strategies that we have applied which have included fragment growing, merging, and linking. Although fragment linking is conceptually the most appealing strategy for fragment elaboration, in practice,
this strategy can be challenging where the choice of the optimal fragment linker can be crucial.\textsuperscript{31,32}

In the fragment-based approach, biophysical techniques are usually used to identify small chemical compounds (fragments) that bind with low affinity to the drug target. X-ray crystallography is then usually employed to establish the binding mode of the fragment and to facilitate the design of elaborated fragments. The availability of high-resolution X-ray crystal structures of a truncated form of the IMPDH,\textsuperscript{21,24,25} in both the substrate-free and substrate/ligand-bound forms, makes this enzyme attractive for a fragment-based approach.

In this work, the discovery of a new class of potent nM inhibitors of IMPDH using a FBDD approach is reported. A library of 960 fragments was screened against \textit{Mth IMPDH}ΔCBS using a biochemical assay. The fragment hits from this assay were examined using X-ray crystallography, and an X-ray crystal structure of one of the fragment complexes was solved to a resolution of 1.45 Å. Examination of the X-ray crystal structure suggested a strategy of fragment-linking for optimization of this fragment hit.

### RESULTS AND DISCUSSION

**Fragment Screening.** An in-house fragment library composed of 960 fragments was screened using a biochemical assay against \textit{Mth IMPDH}ΔCBS. \textit{Mth IMPDH}, which shares 85% sequence identity with \textit{Mtb IMPDH} and is 100% identical in the active site,\textsuperscript{24,25} was chosen for the fragment screening and structural studies because it gave higher protein expression yields and better diffracting crystals than the \textit{Mtb} orthologue. IMPDH activity was monitored spectrophotometrically by measuring the formation of NADH at 340 nm. The biochemical assay was performed at a fragment concentration of 1 mM, and hits were retested in triplicate. Compound 1 (7759844) previously

| Compd | Structure | % Inhibition at 1 mM | Compd | Structure | % Inhibition at 1 mM |
|-------|-----------|---------------------|-------|-----------|---------------------|
| 1     | 92 ± 1\textsuperscript{a} | 11 | 11 | 77 ± 1 |
| 2     | 65 ± 2 | 12 | 64 ± 6 |
| 3     | 52 ± 4 | 13 | 72 ± 1 |
| 4     | 59 ± 1 | 14 | 86 ± 6 |
| 5     | 53 ± 3 | 15 | 51 ± 1 |
| 6     | 50 ± 3 | 16 | 88 ± 10 |
| 7     | 68 ± 3 | 17 | 53 ± 2 |
| 8     | 57 ± 1 | 18 | 79 ± 4 |
| 9     | 98 ± 2 | 19 | 99 ± 1 |
| 10    | 66 ± 1 | | | |

\textsuperscript{a}% Inhibition at 10 μM.
reported as IMPDH inhibitor was used as a positive control in assays (Table 1). The screen resulted in 18 hits (1.9% hit rate), where a hit was defined as a compound that gave greater than 50% inhibition at a concentration of 1 mM. A complete list of fragment hits identified is included in Table 1. A number of common scaffolds were observed, in particular phenylimidazole (fragments 2–4), aminopyrazole (fragments 5–6), and naphthol (fragments 7–11), and the remaining compounds contained a substituted phenyl or a heterocyclic five membered ring (fragments 12–19).

The IC_{50} values of six of the most active fragments were measured and the IC_{50} and ligand efficiency (LE) values of these fragments are summarized in Table 2. The fragment screen provided an array of hits with IC_{50} ranging from 325 μM to 674 μM and ligand efficiencies from 0.31 to 0.42. The inhibition constant [K_i] with respect to both substrates IMP and NAD^+ was determined by assaying various concentrations of each inhibitor with five different concentrations of substrate and a fixed saturating concentration of the cosubstrate. The inhibition data for these fragments are summarized in Table 2. All compounds yielded an uncompetitive inhibition pattern with respect to NAD^+ with K_i values ranging from 262 to 525 μM. Fragments 14, 16, and 19 yielded a mixed inhibition with respect to IMP with K_i values ranging from 126 to 398 μM, and compounds 1, 2, 11, and 18 yielded an uncompetitive inhibition with K_i values ranging from 361 to 609 μM.

Inhibition constants of compound 2 toward full-length Mtb IMPDH were also determined. Compound 2 inhibited full-length Mtb IMPDH enzyme with a K_i value of 572 ± 14 μM with IMP as the substrate and a K_i value of 534 ± 18 μM with NAD as the substrate, which are similar to the K_i values observed for the Mtb IMPDH ΔCBS enzyme.

X-ray Structure of Compounds 1 (7759844) and 2. Compounds 1 and the six fragment hits (2, 11, 14, 16, 18, and 19) were selected for structural characterization using X-ray crystallography by soaking into preformed crystals of Mtb IMPDH ΔCBS as previously described. After molecular replacement, clear electron density was observed in the 2F_o − F_c difference map (σ = 3.0) for IMP, and in addition density was observed for one molecule of compound 1 (Figure 3A) and two molecules of compound 2 (Figure 3B), which were partially occupying the NAD^+ binding site. None of the other fragments

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**Table 2. IC_{50}, Ligand Efficiency, and Alpha K_i Values of Fragment Hits and Compound 1 (7759844) against Mtb IMPDH ΔCBS**

| Compd | IC_{50} (μM) (LE) | IMP αK_i (μM) | NAD αK_i (μM) |
|-------|------------------|---------------|---------------|
| 1     | 0.77 ± 0.06 (0.40) | 0.86 ± 0.03 (UC) | 0.55 ± 0.02 (UC) |
| 2     | 674 ± 53 (0.36)  | 609 ± 3 (UC)  | 512 ± 23 (UC)  |
| 11    | 336 ± 6 (0.39)   | 361 ± 53 (UC) | 310 ± 30 (UC)  |
| 14    | 400 ± 7 (0.42)   | 238 ± 6 (Mixed) | 262 ± 39 (UC)  |
| 16    | 433 ± 60 (0.31)  | 398 ± 81 (Mixed) | 525 ± 64 (UC)  |
| 18    | 512 ± 37 (0.37)  | 554 ± 70 (UC) | 452 ± 26 (UC)  |
| 19    | 325 ± 9 (0.37)   | 126 ± 34 (UC) | 355 ± 47 (UC)  |

*LE = (1.37 × pIC_{50})/HA, where HA means heavy atom, i.e., a non-hydrogen atom. UC: Uncompetitive inhibition.*

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**Figure 3.** X-ray crystal structure of compounds 1 and 2 bound to Mtb IMPDH ΔCBS. Ligand interactions are represented as dotted lines; hydrogen bonds are represented in red, polar interactions in orange, ionic interactions in yellow, and aromatic and π interactions in green dotted lines. Protein–ligand interactions were analyzed using Arpeggio. (a) Interactions made by 1 (green) in the X-ray crystal structure of the complex of IMPDH with IMP (orange). (b) Interactions made by 2 (yellow) in the X-ray crystal structure of the complex of IMPDH with IMP. (c) Structural alignment of the IMPDH crystal structures of 1 (green) and 2 (yellow).
Fragment 2 showed any electron density in the X-ray crystal structures. Although the kinetic studies of compounds 1 and 2 suggested that these two compounds are uncompetitive with respect to NAD⁺, the binding mode of compounds 1 and 2 closely resembles that of previously reported uncompetitive inhibitors of Mth IMPDH.²⁴,²⁵ It has been proposed that the uncompetitive mode of inhibition of IMPDH inhibitors with respect to NAD⁺ is consistent with their binding preferentially to the covalent IMPDH-XMP* intermediate after NADH has been released.²⁴,²⁵,³³

The structure of Mth IMPDH ΔCBS with compound 1 showed that the inhibitor binds in the NAD pocket in a near identical manner to our recently described IMPDH inhibitors.²⁴,²⁵ Compound 1 formed strong π interactions with the hypoxanthine group of IMP, P45′, Y471′, and polar interactions with G409, E442, P45′, and G470′. The electron density revealed two molecules of compound 2 within the NAD binding pocket of IMPDH. One molecule of compound 2 stacked with IMP, forming extensive π interactions with the hypoxanthine group of IMP. This fragment was further stabilized through polar interactions, hydrogen bonds, and π interactions to surrounding residues in the active site pocket, including A269, G318, and E442. The other molecule of compound 2 sits closer to the opening of the active site, making polar interactions with N273 and E442, and π interactions with H270 and Y471′.

A comparison of the structure of Mth IMPDH ΔCBS with compound 1 with the fragment 2 structure shows that the two molecules of 2 mimic the position of the larger inhibitor 1 (Figure 3C).

**Fragment Elaboration.** Fragment 2 was selected as the starting point for exploration because of the ease of synthetic modification and the availability of a X-ray crystal structure to guide the optimization. For chemical elaboration of 2, fragment linking as well as fragment growing were considered. As the two molecules of fragment 2 are found to bind in adjacent regions of the target protein, the fragment-linking approach was the more attractive option. However, before fragment linking, fragment 2 was further optimized with the aim of improving the binding affinity. The structures and inhibitory activities of these compounds against Mth IMPDH ΔCBS are summarized in Tables 3 and 4. The corresponding data for fragment 2 have also been included for comparative purposes.

All compounds were evaluated at a concentration of 100 μM with Mth IMPDH ΔCBS.

**Fragment Growing.** The fragment-growing strategy involves using structure-based drug design to form additional interactions by growing out from the starting fragment. Fragment 2 was modified at the 2-position of the imidazole ring to explore the introduction of various aromatic rings linked by a thioacetamide (20–22) to form π interactions with the hypoxanthine group of IMP (Figure S1). Such modifications gave compounds with improved Mth IMPDH ΔCBS inhibition. The phenyl and benzoferan derivatives (20 and 21) showed 13 and 31% inhibition, respectively. Mth IMPDH ΔCBS inhibition was shown to be sensitive to minor modifications of the phenyl substituent groups; for example, the 4-iodo substituted 22 showed 30% inhibition at 100 μM, whereas the nonsubstituted compound, 20, showed 13% inhibition at the same concentration. The effect of the removal of the 4-bromo group was investigated and compounds 23–25 were synthesized. Removal of the bromo substituent in compounds 20–22 (13–31% inhibition at 100 μM) was tolerated (23–25, 3–46% inhibition at 100 μM). The importance of the aromatic amide linked by a thioacetamide was subsequently examined. Replacing the phenyl with isopropyl (26) resulted in complete loss of activity. Substitution of the thioacetamide by a thioacetic acid also led to a complete loss in activity (compounds 27 and 28).

**Fragment Linking and SAR.** Examination of the X-ray crystal structure of the previously reported inhibitor 1 when overlaid with fragment 2 revealed that the distance between the 4-position of the phenyl ring of fragment 2 and the 2-position of the imidazole ring represents the closest approach of the molecules (Figure S1). On the basis of this structural

| Compd | Structure | % Inhibition at 100 μM |
|-------|-----------|------------------------|
| 2     | ![](image) | < 5%                   |
| 20    | ![](image) | 13 ± 3                 |
| 21    | ![](image) | 31 ± 6                 |
| 22    | ![](image) | 30 ± 3                 |
| 23    | ![](image) | < 5%                   |
| 24    | ![](image) | 36 ± 1                 |
| 25    | ![](image) | 46 ± 3                 |
| 26    | ![](image) | < 5%                   |
| 27    | ![](image) | < 5%                   |
| 28    | ![](image) | < 5%                   |

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information, three different linkers were designed to connect the two copies of the fragment 2 at these positions (compounds 29–31). Initially, a thioacetamide and urea linker moieties were examined. Compounds 29 and 30 showed 20% and 56% of Mth IMPDH ΔCBS inhibition, respectively, at 100 μM (Table 4).

Interestingly, compound 30 showed a 12-fold improvement in Mth IMPDH ΔCBS inhibitory activity with an IC$_{50}$ of 58 μM, compared to the fragment 2.

The lactate linker was then used, but all attempts to link 4-phenylimidazole with 4-(4-bromophenyl)-1H-imidazole were unsuccessful. We therefore decided to synthesize compound 31, which contains 1-methyl-4-phenyl-1H-imidazole and 4-(4-}

| Compd | Structure | % Inhibition at 100 μM | IC$_{50}$ (μM) (LE)$^{a}$ |
|-------|-----------|------------------------|--------------------------|
| 2     | ![Structure](image1) | < 5% | 674 ± 53 (0.36) |
| 29    | ![Structure](image2) | 20 ± 5 | ND$^{b}$ |
| 30    | ![Structure](image3) | 56 ± 11 | 58 ± 8 (0.21) |
| 31    | ![Structure](image4) | 99 ± 11$^{c}$ | 0.52 ± 0.004 (0.29) |
| 32    | ![Structure](image5) | 24 ± 1 | ND |
| 33    | ![Structure](image6) | 83 ± 2$^{c}$ | 2.21 ± 0.08 (0.26) |
| 34    | ![Structure](image7) | 4 ± 1$^{c}$ | ND |
| 35    | ![Structure](image8) | 97 ± 1$^{c}$ | 0.47 ± 0.03 (0.34) |

$Ligand efficiency was calculated using the equation LE = (1.37 × pIC$_{50}$)/HA, where HA means heavy atom, i.e., a non-hydrogen atom. $^{b}$ND: not determined. IC$_{50}$ values were determined for compounds that showed >50% inhibition. $^{c}$%Inhibition at 10 μM.

Table 4. Structures and Activities for Fragment 2 and Compounds 29−35 against Mth IMPDH ΔCBS
bromophenyl)-1H-imidazole linked with a lactate analogue, as in compound 1. Compound 31 (Table 4) showed markedly improved Mth IMPDH ΔCBS inhibition with a LE of 0.29 and IC₅₀ of 0.52 μM, which is 1300-fold more potent compared to the fragment 2. Although compound 31 binds in the cofactor site, the mechanism of inhibition can vary depending on its relative affinities for the E-IMP and E-XMP* complexes.⁸¹ Kinetic evaluation of compound 31 showed the mode of Mth IMPDH ΔCBS inhibition was uncompetitive with respect to both IMP and the NAD⁺ cofactor (see Figure S2, Supporting Information) with a Kᵥ value of 0.3 ± 0.02 μM with IMP as the substrate and a Kᵥ value of 0.2 ± 0.01 μM with NAD as the substrate.

Inhibition constants of compound 31 toward full-length Mtb IMPDH were also determined. Compound 31 inhibited full-length Mtb IMPDH enzyme with a Kᵥ value of 0.61 ± 0.05 μM with IMP as the substrate and a Kᵥ value of 0.39 ± 0.02 μM with NAD as the substrate. The inhibition constants were consistent with the data using the Mth IMPDH ΔCBS enzyme.

Removal of the bromo substituent in compound 29 to give compound 32 was well tolerated (Table 4). The importance of the imidazole group for the inhibitory activity against Mth IMPDH ΔCBS was confirmed by replacing the 4-(4-bromophenyl)-1H-imidazole substituent of compound 31 with 4-(4-bromophenyl)oxazole (33) which resulted in a 4-fold loss of activity (Table 4). Replacing the 4-(4-bromophenyl)-1H-imidazole of 31 by a phenyl (34) resulted in complete loss of activity (Table 4). However, the 4-iodophenyl derivative 35 demonstrated slightly improved activity (IC₅₀ = 0.47 μM, LE = 0.34) compared to compounds 31 (IC₅₀ = 0.52 μM, LE = 0.29) and 1 (IC₅₀ = 0.77 μM, LE = 0.40). It is noteworthy that LE of compound 35 was comparable to that of the original fragment hit 2 (LE = 0.36) and other reported IMPDH inhibitors.⁸⁴

The importance of the 4-bromo substituent on the phenyl ring in compound 31 was also explored (compounds 36–39, Table 5). Removal of the bromo substituent (36) resulted in a 5-fold loss in activity, whereas replacing this group with an iodine (39) or a morpholine ring (37) resulted in loss of activity. The electronic nature of the substituent in this position had little effect on inhibitory activity. For example, an electron-donating methoxy (38) retained activity comparable to that of the bromine derivative (31). Among them, imidazoles 31, 37–38, and 39 were found to be potent inhibitors of Mth IMPDH ΔCBS with IC₅₀ values ranging from 520 to 690 nM.

The (S)-isomer of 31 was found to bind preferentially (Table 5), with the racemate 31 having approximately half the Mth IMPDH ΔCBS inhibition of the (S)-isomer 31. This observation accords with the results previously reported for other series of IMPDH inhibitors.³¹,³⁵

X-ray Structure of Compound 31. From crystals soaked with compound 31, the 2Fₒ – Fᵣ difference map (σ = 3.0) revealed strong density for the inhibitor. The structure of compound 31 (Figure 4A) showed that it bound in a nearly identical manner to compounds 1 and 2 in the NAD binding pocket (Figure 4B), stacking with IMP, and maintaining the interactions with H270, N273, E442, and P45 and Y471 from the neighboring subunit. Compound 31 made additional interactions in the binding pocket, including polar interactions with D267 and N297.

Whole-Cell Activity against Mtb. The whole-cell activity of the most potent analogues 31, 33–39, and (S)-31 in vitro was determined against Mtb H37Rv (see Table S1, Supporting Information). No significant inhibition of bacterial growth was detected for any of the compounds (MIC₉₀ ≥ 50 μM) over the

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**Table 5. Structures and Activities for Compounds 31, 36–39, and (S)-31 against Mth IMPDH ΔCBS**

| Compd | Rᵢ | % Inhibition at 10 μM | IC₅₀ (μM) (LE)* |
|-------|----|----------------------|------------------|
| 31    | Br | 99 ± 1               | 0.52 ± 0.004 (0.29) |
| 36    | H  | 77 ± 3               | 2.76 ± 0.21 (0.26)    |
| 37    |     | 94 ± 1               | 0.52 ± 0.01 (0.24)    |
| 38    | OMe| 95 ± 1               | 0.64 ± 0.03 (0.27)    |
| 39    | I  | 96 ± 1               | 0.69 ± 0.06 (0.28)    |
| (S)-31| Br | 99 ± 1               | 0.27 ± 0.02 (0.30)    |

*Ligand efficiency was calculated using the equation LE = (1.37 × pIC₅₀)/HA, where HA means heavy atom, i.e., a non-hydrogen atom.

tested concentration range (0–100 μM). There are currently ongoing efforts to explain the lack of efficacy of the potent Mth IMPDH ΔCBS inhibitors described which could be caused by low membrane permeability, poor metabolic stability, and/or drug-efflux mechanisms.

Synthetic Chemistry. Compounds 20–29 and 32 were synthesized from 4-phenyl-1H-imidazole-2-thiol or 4-(4-bromophenyl)-1H-imidazole-2-thiol according to the sequence described in Scheme 1. Thioacetic derivatives 27 and 28 were prepared by treatment of 4-phenyl-1H-imidazole-2-thiol or 4-(4-bromophenyl)-1H-imidazole-2-thiol with 2-chloroacetic acid in the presence of NaOH followed by neutralization with hydrochloric acid.

Thioacetamide 26 was synthesized by amidation coupling between thioacetic derivative 28 and isopropylamine. Similarly, thioacetamides 20–25, 29, and 32 were prepared by cross-linking 4-phenyl-1H-imidazole-2-thiol or 4-(4-bromophenyl)-1H-imidazole-2-thiol with α-chloroacetamides 40–43, which were obtained by acylation of anilines with various substituents with chloroacetyl chloride.

The synthesis of urea 30 was achieved as shown in Scheme 2 by coupling amine 44 and N-(4-(1H-imidazol-4-yl)phenyl)-1H-imidazole-1-carboxamide, which was obtained from commercially available 4-(1H-imidazol-4-yl)aniline as a crude intermediate, in the presence of N,N-diisopropylethylamine. Amine 44 was synthesized from benzyl ((S)-(4-bromophenyl)-1H-imida-
zol-2-yl)methyl)carbamate, followed by the deprotection of the benzyloxycarbonyl group under acidic condition. Imidazole derivatives 31, 36–38, and 39 were prepared following the synthetic procedure outlined in Scheme 3. 2-Aminimidazoles 50–54 were synthesized according to a published microwave-assisted protocol. In brief, 2-aminoimidazoles 50–54 were prepared by reaction of the commercially available α-halo ketones and N-acetylguanidine, followed by deacetylation (Scheme 3). Acid 59 was synthesized starting with imidazole 55, which was prepared by reaction of 2-bromo-4'-hydroxyacetophenone with formamide as reported previously. The phenol 57 was synthesized by alkylation of imidazole 55, followed by deprotection of the methyl ether with BBr₃. Substituted phenol 57 was converted to the ether 58 upon treatment with methyl 2-bromopropionate in the presence of Cs₂CO₃. Enantiomerically pure phenyl ethers were synthesized by using Mitsunobu reaction conditions with ethyl D-lactate (Scheme 4).

The syntheses of 2-acylamino oxazole 33 and amides 34–35 were achieved as shown in Scheme 5. 2-Acylamino oxazole derivative 33 was obtained by coupling the acid chloride derivative 60 with 2-aminooxazole derivative 61, which was prepared by reaction of 2,4'-dibromoacetophenone with urea. Similarly, amides 34–35 were prepared by coupling the corresponding anilines with the acid chloride derivative 60.

CONCLUSIONS

FBDD has emerged as a robust approach to identify small molecules that bind to a wide range of therapeutic targets. Fragment elaboration strategies have resulted in the development of a number of compounds that have progressed into clinical trials. Within the area of TB drug discovery, a number of HTS and phenotypic screens have been performed during the past decade. Although HTS identified a number of leads that show high potency in vitro, the translation to an in vivo effect has proven challenging.
This study illustrates the successful application of a fragment-based approach followed by fragment optimization to obtain nanomolar affinity ligands of IMPDH. A library of 960 fragments were screened against *Mth* IMPDH ΔCBS, and from the screen the phenylimidazole fragment hit 2 (IC\textsubscript{50} = 674 μM) was identified. Kinetic experiments showed that 2 was an uncompetitive inhibitor of *Mth* IMPDH ΔCBS with respect to NAD\textsuperscript{+} and IMP. Two molecules of the fragment 2 were shown to bind at the NAD binding site of the enzyme. The X-ray crystal structure also revealed that one molecule of fragment 2 makes π interactions with IMP and the other molecule sits closer to the opening of the active site, making polar interactions with N273 and E442 and π interactions with H270 and Y471\textsuperscript{′}. This provides potential for further optimization of fragment 2. To explore better the possibilities given by fragment 2, fragment-linking and fragment-growing strategies were employed, resulting in low micromolar to nanomolar affinity compounds. Among them, compounds 31, 35, and 37−39 were the most potent IMDPH inhibitors of the series described in this work with IC\textsubscript{50} values between 0.47 and 0.69 μM, which represent >1000-fold improvement in *Mth* IMPDH ΔCBS potency over the initial fragment hit. Compound 31 was shown to bind at the NAD binding site of the enzyme, and the X-ray crystal structure also revealed that it makes π interactions with IMP, maintaining the interactions with H270, N273, E442, and P45 and Y471 from the neighboring subunit. Moreover, compound 31 made additional interactions in the binding pocket, including polar interactions with D267 and N297. A comparison of this structure with the fragment 2 structure shows that the two molecules of 2 mimic the position of the larger inhibitor 31. This is the first example of...
utilizing the fragment-based approach specifically to identify new potent inhibitors of IMPDH. Further structural optimization to improve the cellular activity of these analogues is ongoing with the aim of developing novel classes of anti-TB agents.

### EXPERIMENTAL SECTION

#### 1. Chemistry

1.1. General Experimental Methods. Solvents were distilled prior to use and dried by standard methods. Unless otherwise stated, $^1$H and $^{13}$C NMR spectra were obtained in CDCl$_3$, MeOD, or DMSO-$d_6$ solutions using either a Bruker 400 MHz AVANCE III HD Smart Probe, 400 MHz QNP cryoprobe, or 500 MHz DCH cryoprobe spectrometer. Chemical shifts (δ) are given in ppm relative to the residual solvent peak (CDCl$_3$: $^1$H, δ = 7.26 ppm; $^{13}$C, δ = 77.16 ppm), and the coupling constants (J) are reported in hertz (Hz). Optical rotations were measured on a PerkinElmer Polarimeter 343 at 589 nm ($\lambda$= 589 nm), and specific rotations are reported in $^{10}$ deg cm$^2$ g$^{-1}$. Microwave reactions were performed using a Biotage Initiator system under reaction conditions as indicated for each individual reaction.

Reactions were monitored by TLC and LCMS to determine consumption of starting materials. Flash column chromatography was performed using an Isolera Spektra One/Four purifying cation system and the appropriately sized Biotage SNAP column containing KP-silica gel applicable.

Liquid chromatography mass spectrometry (LCMS) was carried out using a Waters Acquity H-class coupled to a Waters SQ Mass Spectrometer detector. Samples were detected using a Waters Acquity TUV detector. Liquid chromatography mass spectrometry (LCMS) was carried out using an Ultra Performance Liquid Chromatographic system (UPLC) Waters Acquity H-class coupled to a Waters SQ Mass Spectrometer detector. Samples were detected using a Waters Acquity TUV detector at 2 wavelengths (254 and 280 nm). Samples were run using an Acquity UPLC HSS column and a flow rate of 0.8 mL/min. The eluent consisted of 0.1% formic acid in water (A) and acetonitrile (B); gradient, from 95% A to 5% A over a period of 4 or 7 min.

All final compounds had a purity greater than 95% by LCMS analysis.

General Method A: Synthesis of Thiocarbamates 20–25, 29, and 32. To a solution of the 2-chloroacetamide derivative (0.17 mmol) in MeOH (8 mL) was added 4-(4-bromophenyl)-1H-imidazole-2-thiol or 4-phenyl-1H-imidazole-2-thiol (0.17 mmol), followed by a solution of NaOH (0.67 mmol) in H$_2$O (2.5 mL). The reaction mixture was stirred at 70 °C for 3 h. After cooling to rt, the solvents were removed in vacuo and the resulting residue was taken up in 30 mL of EtOAc and washed with H$_2$O (15 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL) and the combined organic layers were washed with brine and dried over MgSO$_4$. Filtration and evaporation afforded crude products, which were purified as indicated below.

General Method B: Synthesis of Thioacetamides 27 and 28. A solution of 4-phenyl-1H-imidazole-2-thiol or 4-(4-bromophenyl)-1H-imidazole-2-thiol (2.80 mmol) and NaOH (5.0 mmol) in EtOH (5 mL) was reflux for 1 h. After cooling to rt, a solution of 2-chloroacetic acid (2.80 mmol) in EtOH (2 mL) was added. The reaction was stirred at reflux for an additional 3 h and then cooled to 0 °C. The reaction mixture was diluted with cold water (5 mL) and acidified with 1 M HCl. The precipitated product was collected by filtration and washed with DCM (2 × 2 mL).

General Method C: Synthesis of Compounds 31, 33, and 36–39. A mixture of acid 59 (0.20 mmol) and SOCl$_2$ (2 mL) was heated at 80 °C for 2 h. The solvent was removed under reduced pressure to give the acid chloride 60 as a white solid. The resulting solid was immediately dissolved in anhydrous DCM, and the resulting solution was added slowly dropwise at 0 °C to a solution of the corresponding substituted 2-aminoimidazoles (0.20 mmol) and TEA (0.80 mmol) in anhydrous DCM (5 mL). The reaction mixture was stirred at 40 °C for 36 h and then diluted with DCM (20 mL) and washed with saturated aqueous NaHCO$_3$. The aqueous phase layer was then extracted with DCM (2 × 20 mL), and the combined organic layers were dried over MgSO$_4$ and filtered, and the solvent was removed under reduced pressure to afford a yellow oil, which was purified by flash chromatography, eluting with the solvent system specified.

General Method D: Synthesis of Amides 34–35. A mixture of acid 59 (0.20 mmol) and SOCl$_2$ (2 mL) was heated at 80 °C for 2 h. The solvent was removed under reduced pressure to give 60 as a white solid. The resulting solid was immediately dissolved in anhydrous DCM, and the resulting solution was added slowly dropwise at 0 °C to a solution of the corresponding aniline (0.20 mmol) and triethylamine (0.80 mmol) in anhydrous DCM (5 mL). The reaction mixture was stirred at rt for 4 h and then diluted with DCM (20 mL) and washed with saturated aqueous NaHCO$_3$. The aqueous phase layer was then extracted with DCM (2 × 20 mL), and the combined organic layers were dried over MgSO$_4$ and filtered, and the solvent was removed under reduced pressure to afford a yellow oil, which was purified by flash chromatography, eluting with the solvent system specified.

General Method E: Synthesis of α-Chloroacetamides 40–43. Et$_3$N (4.77 mmol) followed by a solution of chloroacetyl chloride (4.77 mmol) in DCM (3 mL) were added to a stirred solution of the corresponding aniline (4.38 mmol) in DCM (5 mL) at rt. The reaction mixture was stirred at rt for 2–4 h. The reaction was then diluted with DCM (20 mL) and washed with saturated aqueous NaHCO$_3$, 1 M HCl, and brine and dried over anhydrous MgSO$_4$, and the solvent was removed under reduced pressure. Compound 43 was purified by flash chromatography eluting with the solvent system specified, although other analogues were used in subsequent reactions without further purification.

“Reagent and conditions: (a) urea, CH$_3$CN, 80 °C, 78%; (b) TEA, DCM, rt (for 34 and 35) or 40 °C (for 33), 64–76%.
General Method F: Synthesis of Substituted N-(1H-imidazol-2-yl)acetamides 45–49. A mixture of the corresponding 2-bromocacetophene derivative (0.38 mmol) and acetylguanidine (1.13 mmol) in anhydrous acetonitrile (3 mL) was heated at 100 °C using microwave irradiation for 15 min. The solvent was removed, and the residue was taken in H2O (3 mL) and filtered, and the solid was washed with H2O (2 mL) and DCM (2 mL). The solid obtained was used in the next step without further purification.

General Method G: Synthesis of Substituted 2-Aminoimidazoles 50–54. To a solution of the corresponding substituted N-(1H-imidazol-2-yl)acetamides (0.31 mmol) in a 1:1 v/v mixture of MeOH and H2O (2.4 mL) was added concentrated H2SO4 (0.6 mL), and the reaction mixture was heated at 100 °C under microwave irradiation for 15–30 min. The reaction mixture was concentrated, and the resulting residue was resuspended in H2O (5 mL), and a saturated aqueous Na2CO3 was added until pH 8. The product was extracted into EtOAc (3 × 40 mL). The combined organic fractions were dried over MgSO4, and the solvent was removed under reduced pressure. The resulting solid was used in the next reaction without further purification.

2-(4-(4-Bromophenyl)-1H-imidazol-2-yl)thio)-N-phenylacetamide (20). Compound 40 (29 mg, 0.17 mmol) was reacted with 4-(4-bromophenyl)-1H-imidazol-2-thiol (43 mg, 0.17 mmol) according to general method A. Purification by flash chromatography (1–20% v/v MeOH in DCM) afforded 20 (39 mg, 15 mmol, 89% yield) as a white solid. 1H NMR (400 MHz, MeOD) δ 7.62 (2H), 7.56–7.59 (m, 5H), 7.36–7.17 (m, 2H), 7.13–7.00 (m, 1H), 3.82 (s, 2H) ppm. 13C NMR (100 MHz, MeOD) δ 169.6, 141.3, 139.6, 132.8, 129.8, 129.5, 124.4, 121.1, 112.1, 116.7, 39.6 ppm. LCMS (ESI−)/m/z: 435.0 [M−H]−, retention time 2.20 min (100%). HRMS (ESI+) m/z calculated for C21H18BrN3O3 [M + H]+: 438.0319. Found: 438.0312. N-Benzofuran-5-yl)-2-(4-(4-bromophenyl)-1H-imidazol-2-yl)thio)acetamide (21). Compound 41 (36 mg, 0.17 mmol) was reacted with 4-(4-bromophenyl)-1H-imidazol-2-thiol (43 mg, 0.17 mmol) according to general method A. Purification by flash chromatography (1–20% v/v MeOH in DCM) afforded 21 (70 mg, 0.16 mmol, 96% yield) as a white solid. 1H NMR (400 MHz, MeOD) δ 7.62 (2H), 7.56–7.59 (m, 5H), 7.36–7.17 (m, 2H), 7.13–7.00 (m, 1H), 3.82 (s, 2H) ppm. 13C NMR (100 MHz, MeOD) δ 169.6, 141.3, 139.6, 132.8, 129.8, 127.5, 125.5, 121.4, 121.2, 112.1, 116.7, 39.6 ppm. LCMS (ESI−)/m/z: 388.0 [M−H]−, retention time 2.20 min (100%). HRMS (ESI+) m/z calculated for C21H18BrN3O3 [M + H]+: 391.0319. Found: 391.0312.

2-(4-Bromophenyl)-1H-imidazol-2-thiol (48 mg, 0.25 mmol) was reacted with 4-(4-bromophenyl)-1H-imidazol-2-thiol (43 mg, 0.17 mmol) according to general method A. Purification by flash chromatography (1–20% v/v MeOH in DCM) afforded 28 (42 mg, 0.12 mmol, 80% yield) as a white solid. 1H NMR (500 MHz, MeOD) δ 7.87 (d, J = 2.1 Hz, 1H), 7.72 (d, J = 2.2 Hz, 1H), 7.68 (d, J = 7.7 Hz, 2H), 7.46 (br s, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.37–7.29 (m, 3H), 7.23 (t, J = 7.4 Hz, 1H), 6.76 (d, J = 2.2, 0.9 Hz, 1H), 3.84 (s, 2H) ppm. 13C NMR (125 MHz, MeOD) δ 169.6, 153.5, 147.4, 140.9, 134.7, 129.1, 128.2, 125.8, 118.9, 114.2, 112.1, 107.7, 50.5 ppm. LCMS (ESI−)/m/z: 310.0 [M−H]−, retention time 1.81 min (100%). HRMS (ESI−)/m/z: m/z calculated for C11H13NO2S [M + H]+: 313.0496. Found: 313.0507.

1H NMR (500 MHz, MeOD) δ 7.68 (d, J = 2.1 Hz, 1H), 7.72 (d, J = 2.2 Hz, 1H), 7.68 (d, J = 7.7 Hz, 2H), 7.46 (br s, 1H), 7.40 (d, J = 8.8 Hz, 1H), 7.37–7.29 (m, 3H), 7.23 (t, J = 7.4 Hz, 1H), 6.76 (d, J = 2.2, 0.9 Hz, 1H), 3.84 (s, 2H) ppm. 13C NMR (125 MHz, MeOD) δ 169.6, 153.5, 147.4, 140.9, 134.7, 129.1, 128.2, 125.8, 123.0, 88.1, 39.8 ppm. LCMS (ESI−)/m/z: 433.9 [M−H]−, retention time 2.13 min (98%). HRMS (ESI−)/m/z: m/z calculated for C11H13NO2S [M + H]+: 436.9981. Found: 433.9989.

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(76 mg, 0.47 mmol). The mixture was stirred at rt overnight, and then it was filtered. The filter was washed with THF (2 × 3 mL) and the resulting solid (30 mg, 0.12 mmol) was dissolved in DMF (3 mL), and 44 (42 mg, 0.12 mmol) and N,N-dissopropylethylamine (74 µL, 0.48 mmol) were added. The reaction mixture was stirred at rt for 14 h, and then it was poured into water, extracted with EtOAc, dried over Na2SO4, and concentrated. After cooling to 0 °C a 1:5 v/v mixture of MeOH and DCM (12 mL) was added and the suspended solid was collected by filtration and dried at vacuum to yield 30 (35 mg, 0.08 mmol, 26% yield) as a white solid. 1H NMR (500 MHz, DMSO-d6) δ 12.03 (bs, 2H), 8.64 (bs, 1H), 7.73−7.68 (m, 2H), 7.64−7.59 (m, 2H), 7.57 (d, J = 1.9 Hz, 1H), 7.52−7.47 (m, 2H), 7.47−7.39 (m, 2H), 7.39−7.32 (m, 2H), 6.58 (brs, 1H), 4.33 (d, J = 5.5 Hz, 2H) ppm. 13C NMR (125 MHz, DMSO-d6) δ 155.5, 146.9, 140.6, 138.9, 138.9, 135.4, 134.1, 131.7, 128.8, 126.6, 125.1, 125.0, 119.0, 118.4, 118.2, 113.7, 11.37, 37.6 ppm. LCMS (ESI−) m/z 457.0 [M − H]+, retention time 5.07 min (97%). HRMS (ESI+): m/z calculated for C20H14BrN4O3 [M + H]+: 437.0725. Found: 437.0725.

N-(4-(Bromophenyl)-1H-imidazol-2-yl)-2-(4-(1-methyl-1H-imidazol-4-yl)phenyloxy)propanamide (35). 4-(Bromophenyl)-1H-imidazol-2-amine 50 (48 mg, 0.20 mmol) was reacted with the acid chloride 60 (53 mg, 0.20 mmol) and Et3N (112 µL, 0.80 mmol) according to general method C. The crude product was purified by flash chromatography (0−20% v/v MeOH in DCM) to give compound 35 as a pink solid (62 mg, 0.14 mmol, 68% yield). 1H NMR (400 MHz, CDCl3) δ 8.28 (bs, 1H), 7.77−7.69 (m, 2H), 7.69−7.57 (m, 2H), 7.47 (d, J = 1.4 Hz, 1H), 7.41−7.31 (m, 2H), 7.11 (d, J = 1.3 Hz, 1H), 7.03−6.91 (m, 2H), 4.80 (q, J = 6.7 Hz, 1H), 3.73 (s, 3H), 1.67 (d, J = 6.8 Hz, 3H) ppm. 13C NMR (100 MHz, CDCl3) δ 170.4, 155.5, 141.7, 137.9, 136.9, 129.0, 126.3, 126.0, 121.8, 116.0, 115.3, 78.9, 75.6, 33.5, 18.6 ppm. LCMS (ESI+) m/z 448.2 [M + H]+, retention time 2.47 min (96%). HRMS (ESI+): m/z calculated for C23H21N4O4 [M + H]+: 448.0522. Found: 448.0517.

2-(1-(Methyl-1H-imidazol-4-yl)phenyloxy)-N-(4-(1-methyl-1H-imidazol-2-yl)thio)acetamide (32). Compound 43 (40 mg, 0.17 mmol) was reacted with 4-(bromophenyl)-1H-imidazol-2-thiol (30 mg, 0.17 mmol) according to general method C. Purification by flash chromatography (1−20% v/v MeOH in DCM) afforded 32 (41 mg, 0.11 mmol, 65% yield) as a white solid. 1H NMR (400 MHz, CDCl3) δ 12.46 (bs, 2H), 10.42 (bs, 1H), 7.76 (bs, 2H), 7.68 (d, J = 8.2 Hz, 4H), 7.56 (d, J = 8.5 Hz, 2H), 7.52 (s, 1H), 7.34 (bs, 2H), 7.19 (brs, 1H), 3.99 (s, 2H) ppm. 13C NMR (125 MHz, CDCl3) δ 167.0, 141.7, 139.9, 137.7, 136.2, 134.6, 128.9, 126.7, 125.2, 124.6, 119.7, 115.9, 39.5 ppm. LCMS (ESI−) m/z 466.0 [M − H]+, retention time 4.08 min (100%). HRMS (ESI−): m/z calculated for C22H20BrN4O2 [M − H]+: 466.0879. Found: 466.0879.

2-(1-(Methyl-1H-imidazol-4-yl)phenyloxy)-N-(4-(4-morpholinophenyl)-1H-imidazol-2-yl)propanamide (37). 4-(Morpholinophenyl)-1H-imidazol-2-amine 52 (40 mg, 0.16 mmol) was reacted with the acid chloride 60 (43 mg, 0.16 mmol) and Et3N (90 µL, 0.65 mmol) according to general method C. The crude product was purified by flash chromatography (3−15% v/v MeOH in DCM) to give compound 37 as a white solid (51 mg, 0.11 mmol, 67% yield). 1H NMR (400 MHz, CDCl3) δ 10.74 (bs, 1H), 7.71 (d, J = 8.8 Hz, 2H), 7.54 (bs, 2H), 7.47 (d, J = 1.4 Hz, 1H), 7.11 (d, J = 1.4 Hz, 1H), 7.03−6.88 (m, 3H), 4.90 (q, J = 6.7 Hz, 1H), 4.06−3.83 (m, 4H), 3.73 (s, 3H), 2.77−2.70 (m, 4H), 1.09 (d, J = 6.8 Hz, 3H) ppm. 13C NMR (125 MHz, CDCl3) δ 171.5, 157.1, 153.0, 150.3, 141.8, 140.1, 137.8, 137.4, 128.9, 126.3, 126.9, 124.6, 115.9, 115.3, 74.4, 66.9, 49.3, 33.6, 18.7 ppm. LCMS (ESI+): m/z 473.2 [M + H]+, retention time 4.44 min (100%). HRMS (ESI+): m/z calculated for C23H22N5O3 [M + H]+: 473.1773. Found: 473.1867.

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method C. The crude product was purified by flash chromotography (1−20% v/v MeOH in DCM) to give compound 39 as a yellow solid (50 mg, 0.10 mmol, 61% yield). H NMR (500 MHz, CDCl3) δ 10.75 (br s, 1H), 9.42 (br s, 1H), 7.69 (t, J = 8.3 Hz, 4H), 7.53−7.33 (m, 3H), 7.10 (d, J = 8.3 Hz, 2H), 6.95 (d, J = 8.9 Hz, 2H), 4.88 (q, J = 6.7 Hz, 1H), 3.71 (s, 1H), 1.66 (d, J = 6.7 Hz, 3H) ppm.13C NMR (125 MHz, CDCl3) δ 171.5, 155.2, 141.7, 138.0, 137.7, 129.1, 126.5, 123.0, 115.8, 115.3, 108.1, 91.8, 74.5, 13.5, 18.5 ppm. LCMS (ESI+: m/z) calculated for C12H12N5O2 [M+H]+: 219.1142 [M+H]+, retention time 4.61 min (95%). HRMS (ESI+): m/z calculated for C12H10N4O2 [M+H]+: 219.0740. Found: 219.0740.

2-Chloro-N-phenylacetamide (40). Aniline (400 mL, 4.33 mmol) was reacted with chloroacetyl chloride (380 mL, 4.77 mmol) and Et3N (670 mL, 4.77 mmol) according to general method E, and the crude product was further purified. Compound 40 was obtained (720 mg, 2.44 mmol, 96% yield) as a green solid and was used without further purification. H NMR (400 MHz, CDCl3) δ 8.27 (br s, 1H), 7.20 (d, J = 7.7 Hz, 2H), 3.79 (t, J = 8.9 Hz, 2H), 2.10 (t, J = 7.4 Hz, 1H), 4.22 (s, 2H) ppm.13C NMR (100 MHz, CDCl3) δ 163.9, 136.8, 129.3, 125.4, 120.2, 14.3 ppm. LCMS (ESI+): m/z = 170.1 (M+H)+, retention time 1.58 min (100%). HRMS (ESI+): m/z calculated for C6H6ClNO [M+H]+: 170.0373. Found: 170.0366. NMR data is in accordance with literature values.39

N-(Benzofuran-5-yl)-2-chloroacetamide (41). Benzofuran-5-amine (140 mg, 1.05 mmol) was reacted with chloroacetyl chloride (93 μL, 1.16 mmol) and Et3N (162 μL, 1.16 mmol) according to general method E, and the crude product was further purified by flash chromatography (10−100% v/v EtOAc in petroleum ether) to give compound 42 as a brown solid (600 mg, 2.03 mmol, 99% yield) as a green solid and was used without further purification.1H NMR (500 MHz, CDCl3) δ 8.09 (br s, 1H), 7.94 (d, J = 2.2 Hz, 1H), 7.64 (d, J = 2.2 Hz, 1H), 7.47 (dd, J = 8.8, 0.8 Hz, 1H), 7.32 (dd, J = 8.8, 2.2 Hz, 1H), 6.76 (dd, J = 2.2, 0.9 Hz, 1H), 4.23 (s, 2H) ppm.13C NMR (100 MHz, CDCl3) δ 163.9, 152.4, 146.1, 131.9, 127.9, 117.7, 113.3, 111.6, 106.8, 43.0 ppm. LCMS (ESI+): m/z = 210.1 (M+H)+, retention time 1.71 min (100%). HRMS (ESI+): m/z calculated for C9H7ClNO [M+H]+: 210.0322. Found: 210.0319.

2-Chloro-N-(4-iodophenyl)acetamide (42). 4-Iodoaniline (500 mg, 2.28 mmol) was reacted with chloroacetyl chloride (200 μL, 2.51 mmol) and Et3N (350 μL, 2.51 mmol) according to general method E. The crude product was purified by flash chromatography (10−100% v/v EtOAc in petroleum ether) to give compound 43 as a brown solid (600 mg, 2.03 mmol, 99% yield). H NMR (400 MHz, CDCl3) δ 8.32−8.11 (br s, 1H), 7.69 (d, J = 8.8 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H), 4.20 (s, 2H) ppm.13C NMR (100 MHz, CDCl3) δ 163.8, 138.1, 136.4, 121.8, 88.7, 42.9 ppm. LCMS (ESI+): m/z = 293.7 [M−H]+, retention time 2.38 min (100%). HRMS (ESI−): m/z calculated for C9H7ClNO [M−H]−: 293.9183. Found: 293.9187. NMR data is in accordance with literature values.40

N-(4-(1H-imidazol-4-yl)phenyl)-2-chloroacetamide (43). 4-(1H-Imidazol-4-yl)aniline (200 mg, 1.25 mmol) was reacted with chloroacetyl chloride (200 μL, 2.51 mmol) and Et3N (350 μL, 2.51 mmol) according to general method E. The crude product was purified by flash chromatography (3−20% v/v MeOH in DCM) to give compound 44 as a yellow solid (70 mg, 0.11 mmol, 89% yield).1H NMR (400 MHz, DMSO-d6) δ 10.43 (br s, 1H), 9.83 (br s, 1H), 7.98 (br s, 1H), 7.77−7.65 (m, 2H), 7.70−7.47 (m, 3H), 4.27 (s, 2H) ppm. LCMS (ESI+): m/z = 326.2 [M+H]+, retention time 1.15 min (100%). HRMS (ESI+): m/z calculated for C9H8ClN2O [M+H]+: 326.0591. Found: 326.0590.

5-(4-Bromophenyl)-1H-imidazol-2-ylmethylamine Hydrochloride (44). 4 M HCl (2 mL) was added slowly to a stirred solution of benzyl (5-(4-bromophenyl)-1H-imidazol-2-yl)methylcarbamate (105 mg, 0.26 mmol) in dioxane (1 mL). The mixture was stirred at 100 °C for 4 h, and then the solvents were removed under reduced pressure to give the desired product as a white solid (86 mg, 0.26 mmol, 100% yield).1H NMR (500 MHz, MeOD) δ 8.00 (s, 1H), 7.75−7.67 (m, 4H), 4.55 (s, 2H) ppm.13C NMR (125 MHz, MeOD) δ 140.8, 136.0, 133.7, 128.4, 127.6, 124.7, 117.8, 111.4, 34.8 ppm. LCMS (ESI+): m/z = 250.1 [M+H]+, retention time 2.13 min (100%). HRMS (ESI+): m/z calculated for C7H6BrN3 [M+H]+: 249.9980. Found: 249.9987.
4-(4-Morpholinophenyl)-1H-imidazol-2-amine (52). Following general method G, from N-(4-(4-morpholinophenyl)-1H-imidazol-2-yl)acetamide 47 (85 mg, 0.30 mmol) was obtained 52 (60 mg, 0.24 mmol, 82% yield) as a brown solid. 1H NMR (400 MHz, MeOD) δ 7.47 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.8 Hz, 2H), 7.68 (s, 2H), 6.78 (s, 1H), 3.91–3.64 (m, 4H), 3.19–3.02 (m, 4H) ppm. 13C NMR (125 MHz, MeOD) δ 149.9, 149.3, 125.0, 124.5, 115.8, 113.0, 66.6, 64.9 ppm. LCMS (ESI+): m/z 245.1 [M+H]+, retention time 1.50 min (100%). HRMS (ESI+): m/z calculated for C11H12N3O [M+H]+: 245.1074. Found: 245.1070.

4-(4-Methoxyphenyl)-1H-imidazol-2-amine (53). Following general method G, from N-(4-(4-(methoxyphenyl)-1H-imidazol-2-yl)acetamide 48 (83 mg, 0.36 mmol) was obtained 53 (65 mg, 0.34 mmol, 95% yield) as a brown solid. 1H NMR (500 MHz, MeOD) δ 7.54–7.39 (m, 2H), 7.04–6.85 (m, 2H), 6.78 (s, 1H), 3.81 (s, 3H) ppm. 13C NMR (125 MHz, MeOD) δ 158.3, 150.0, 125.9, 125.0, 124.8, 113.6, 109.8, 54.3 ppm. LCMS (ESI+): m/z 190.2 [M+H]+, retention time 1.61 min (100%). HRMS (ESI+): m/z calculated for C11H10N2O [M+H]+: 190.0980. Found: 190.0979.

1H-imidazol-2-amine (54). Following general method G, from N-(4-(4-methoxyphenyl)-1H-imidazol-2-yl)acetamide 49 (150 mg, 0.46 mmol) was obtained 54 (103 mg, 0.36 mmol, 79% yield) as a red solid. 1H NMR (500 MHz, DMSO-d6) δ 7.98 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 7.03 (s, 1H), 5.35 (br s, 2H) ppm. 13C NMR (125 MHz, DMSO-d6) δ 150.8, 137.4, 136.4, 133.4, 126.0, 111.1, 90.2 ppm. LCMS (ESI+): m/z 286.1 [M+H]+, retention time 2.09 min (100%). HRMS (ESI+): m/z calculated for C11H10N2O [M+H]+: 285.9841. Found: 285.9846.

(4H-Imidazol-1-yl)acetophenone (55). 2-Bromo-1-(4-hydroxyphenyl)-ethan-1-one (1.0 g, 4.67 mmol) was dissolved in formamide (5 mL) and quenched with MeOH (4 mL). The solvents were then removed for 10 min and 2 h at rt. The reaction mixture was then cooled to 0 °C for 5 h. After cooling to rt, the reaction was diluted with water (80 mL) and extracted with EtOAc (2 × 80 mL). The combined organic layers were washed with brine (3 × 100 mL), dried over MgSO4, and filtered under reduced pressure, and the crude product was redissolved in anhydrous DCM (10 mL) and quenched with MeOH (3 mL). The solvents were then removed. The residue was dissolved in anhydrous acetonitrile (5 mL) and heated at 80 °C for 40 min. The reaction mixture was allowed to cool to rt, the solvents were removed. The mixture was then diluted with H2O (5 mL) and acidified to pH 7–8 using 1 N HCl. The mixture was cooled, and the resulting precipitate was collected by filtration, washed with water (2 × 3 mL) and petroleum ether (2 × 3 mL), and dried in vacuo to afford acid 55 (0.33 g, 1.33 mmol, 65% yield) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 7.69–7.55 (m, 3H), 7.46 (d, J = 1.3 Hz, 1H), 6.84 (d, J = 8.8 Hz, 2H), 4.81 (q, J = 6.8 Hz, 1H), 3.66 (s, 3H), 1.49 (d, J = 6.7 Hz, 3H). 13C NMR (100 MHz, DMSO-d6) δ 173.3, 156.1, 140.4, 138.2, 127.8, 125.4, 115.8, 114.8, 71.7, 33.1, 18.4. LCMS (ESI+): m/z 247.2 [M+H]+, retention time 1.43 min (100%). HRMS (ESI+): m/z calculated for C9H7N3O3 [M+H]+: 247.1083. Found: 247.1080.

2-(4-(1-Methyl-1H-imidazol-4-yl)phenoxy)propanoic Acid (59). Methyl 2-(4-((1-methyl-1H-imidazol-4-yl)phenoxy)propanoate S8 (0.53 g, 2.04 mmol) was dissolved in a 2:1 v/v mixture of THF and H2O (7.5 mL), and then NaOH (0.16 g, 4.08 mmol) was added. The reaction mixture was heated at 80 °C for 40 min. After the reaction mixture was allowed to cool to rt, the solvents were removed. The mixture was then diluted with H2O (5 mL) and acidified to pH 7–8 using 1 N HCl. The mixture was cooled, and the resulting precipitate was collected by filtration, washed with water (2 × 3 mL) and petroleum ether (2 × 3 mL), and dried in vacuo to afford acid 59 (0.33 g, 1.33 mmol, 65% yield) as a white solid. 1H NMR (400 MHz, DMSO-d6) δ 7.53 (m, 3H), 7.50 (m, 2H). 13C NMR (100 MHz, DMSO-d6) δ 173.3, 156.1, 140.4, 138.2, 127.8, 125.4, 115.8, 114.8, 71.7, 33.1, 18.4. LCMS (ESI+): m/z 247.2 [M+H]+, retention time 1.43 min (100%). HRMS (ESI+): m/z calculated for C9H7N3O3 [M+H]+: 247.1083. Found: 247.1080.

(5)-2-(4-(1-Methyl-1H-imidazol-4-yl)phenoxy)propanoic Acid (IS-59). To a solution of S7 (212 mg, 1.21 mmol) in anhydrous THF (5 mL) was added ethyl β-lactate (215 mg, 1.82 mmol). After the reaction mixture was cooled to 0 °C, PPh3 (478 mg, 1.82 mmol) and DEAD (286 μL, 1.82 mmol) were added, and the reaction mixture was stirred for 16 h at rt. The reaction mixture was poured into ice-water (400 mL) and extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous MgSO4, and concentrated. The crude product was purified by flash chromatography (0–15% v/v MeOH in DCM) to give ethyl S)-2-(4-(1-methyl-1H-imidazol-4-yl)phenoxy)propanoate as an orange solid. LCMS (ESI+): m/z 275.3 [M+H]+, retention time 2.06 min (94%). Ethyl (S)-2-(4-(1-methyl-1H-imidazol-4-yl)phenoxy)propanoate (200 mg, 0.73 mmol) was dissolved in a 2:1 v/v mixture of THF and H2O (7.5 mL), and then NaOH (58 mg, 1.46 mmol) was added. The reaction mixture was stirred for 1 h, and the solvents were removed. The residue was dissolved in EtOAc (40 mL) and then extracted with H2O (40 mL). The aqueous layer was acidified to pH 7–8 using 1 N HCl. The mixture was cooled, and the resulting precipitate was collected by filtration, washed with water (2 × 3 mL) and petroleum ether (2 × 3 mL), and dried in vacuo to afford acid (S)-59 (100 mg, 0.40 mmol, 56% yield) as a white solid. 1H NMR (400 MHz, MeOD) δ 7.68 (s, 1H), 7.59–7.42 (4, 1H) ppm. 13C NMR (125 MHz, MeOD) δ 2819. DOI: 10.1101/tacs.jnmedchem.700122 J Med Chem. 2018, 61, 2806–2826.
163.9, 139.8, 132.7, 132.1, 128.8, 127.9, 122.1 ppm. LCMS (ESI+) m/z 238.8 [M + H]+, retention time 1.95 min (100%). HRMS (ESI+): m/z calculated for C₉H₁₀BrN₂O [M + H]+: 238.9820. Found: 238.9816.

2. Enzyme Assay. The activity of Mth IMPDH ΔCBS was determined using a plate reader by monitoring the production of NADH in absorbance at 340 nm and corrected for noncatalyzed chemical reactions in the absence of Mth IMPDH ΔCBS. All the measurements were done in the assay buffer (50 mM Tris-HCl pH 8.1, 1 mM DTT, 1 mM EDTA, and 100 mM KCl) at 37 °C with 20 nM Mth IMPDH ΔCBS, 2.8 mM NAD+, and 1 mM IMP in a total of 150 μL volume in a 96 well plate-based format, and data were collected for 32 min. The reaction was initiated by the addition of the substrate, IMP, at a concentration of 1 mM. All reactions were performed in triplicate. Prior to reaction initiation, the compounds were preincubated in a buffer with enzyme for 5 min. The inhibitors were dissolved in DMSO-d₄ and diluted to a final concentration of 1% v/v in experimental reactions.

IC₅₀ values were calculated by plotting the percentage of inhibition against the logarithm of inhibitor concentration, and dose–response curves were fitted using Prism software (GraphPad).

The Kᵢ value for NAD⁺ was determined at a constant saturating IMP concentration (1 mM) and five different concentrations of NAD⁺ (0.35, 0.70, 1.0, 1.4, and 2.8 mM) in the presence of increasing concentrations of inhibitor. The value of Kᵢ for IMP was determined at fixed saturating concentration of NAD⁺ (2.8 mM) and different concentrations of IMP (0.12, 0.18, 0.25, 0.5, and 1 mM) and inhibitor.

The initial velocities at various inhibitor concentrations were determined based on the slope in the linear part of each reaction containing the inhibitor and the uninhibited reaction. To determine the inhibition constant (Kᵢ values), the initial rate data versus substrate concentration at different inhibitor concentrations were fit using Prism software (GraphPad) to equations for uncompetitive or mixed inhibition. For each inhibitor concentration, the reciprocal of enzyme reaction velocity versus the reciprocal of substrate concentration was plotted in a Lineweaver–Burk plot to determine the pattern of inhibition.

3. Protein Purification, Crystallization, and Data Collection of Mth IMPDH ΔCBS. Mth IMPDH was expressed, purified, and crystallized as previously described. Briefly, hexahistidine tagged Mth IMPDH ΔCBS in pHat2 was expressed overnight in BL21 DE3 (NEB) cells at 18 °C by addition of 500 μM IPTG. Cells were lysed in 50 mM Hepes, pH 8.0, 500 mM NaCl, 5% glycerol, 10 mM β-mercaptoethanol, and 20 mM imidazole, and the recombinant protein purified using a Hi-Trap IMAC FF column (GE Healthcare) charged with nickel and an elution gradient of up to 300 mM imidazole. The hexahistidine tag was cleaved by TEV protease, and the purified Mth IMPDH ΔCBS was obtained by negative nickel gravity-flow purification and size exclusion chromatography on a Superdex 200 gel filtration column equilibrated in 20 mM Hepes pH 8.0, 500 mM NaCl, 5% glycerol, and 1 mM TCEP step. The recombinant Mth IMPDH ΔCBS was then concentrated to 12.5 mg/mL for crystallization.

Mth IMPDH ΔCBS protein crystallized in 1 μL + 1 μL hanging drops with 100 mM sodium acetate, pH 5.5, 200 mM calcium chloride, and 8–14% isopropanol. Crystals were soaked overnight in drops of well solution + 5 μM IMP and either 5 mM Fragment 2 or Compound 1 or 1 mM Compound 31. Crystals were cryoprotected by passing through drops containing well solution + 25% glycerol and flash-frozen in liquid nitrogen. Data were collected from the crystals at Diamond Light Source beamline.

4. Structure Solution, Ligand Fitting, and Refinement. Data were processed using XDS and Pointless (CCP4). To solve the structure, molecular replacement was performed with Phenix Phaser using a previously solved IMP-bound Mth IMPDH ΔCBS structure as a probe (PDB IDs: 5J5H, 5KX5, 5K4Z). Refinement was performed using Phenix.refine and manually in Coot. IMP and the inhibitors were sequentially fitted into the density using the LigandFit function of Phenix, and the structures were manually refined further using Coot.

Information regarding the crystallographic statistics can be found in Table S2. Protein–ligand interactions were analyzed using Arpeggio.

5. Drug Susceptibility Testing against Mtb. An Alamar Blue fluorescence-based broth microdilution assay was used to assess the minimum inhibitory concentration (MIC) of compounds against Mtb H37Rv, as described previously. Briefly, Mtb H37Rv was grown in standard Middlebrook 7H9 broth (BD) supplemented with OADC (BD), 0.2% glycerol, and 0.05% Tween-80 to midexponential phase. Compounds dissolved in DMSO (1%) were tested in clear-bottomed, round-well 96-well microtiter plates at eight different concentrations using the standard anti-TB drugs, rifampin and isoniazid, as positive controls. An inoculum of ~10⁶ bacteria was added to each well, and the plates were incubated at 37 °C for 7 d. On day 7, 10 μL of Alamar Blue (Invitrogen) was added to each well, and plates were further incubated at 37 °C for 24 h. The fluorescence (excitation 544 nm; emission 590 nm) was measured in a FLUOstar OPTIMA plate reader (BMG LABTECH, Offenback, Germany). Data were normalized to the minimum and maximum inhibition controls to generate a dose response curve (% inhibition) from which the MICₙ₀ was determined.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b01622.

1H, 13C, and 2D NMR spectra of all new compounds, supplementary Figures S1–S2, whole-cell activity of compounds against M. tuberculosis H37Rv (Table S1), and X-ray data collection and refinement statistics (Table S2) (PDF)

Molecular formula strings data file for all chemical structures mentioned in the manuscript (CSV)

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Notes
The authors declare no competing financial interest. Additional data related to this publication is available at the University of Cambridge data repository: https://doi.org/10.17863/CAM.20512. Structures have been deposited in the Protein Data Bank (PDB codes SOU1 for IMPDH:IMP:Compound 1; SOU2 for IMPDH:IMP:Compound 2 and SOU3 for IMPDH:IMP:Compound 31 complex structures). Authors will release the atomic coordinates and experimental data upon article publication.

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Tuberculosis; Mycobacterium tuberculosis, Mt bacteria, multidrug resistant; XDR, extensively drug resistant; IMPDH, inosine-5′-monophosphate dehydrogenase; IMP, inosine 5′-monophosphate; CBS, cystathionine β-synthase; XMP, xanthine monophosphate; NAD+, nicotinamide adenine dinucleotide; Mth, Mycobacterium thermoresistible; MIC, minimal inhibitory concentration; WHO, World Health Organization.

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