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Expanding benzoxazole based inosine 5'-monophosphate dehydrogenase (IMPDH) inhibitor structure-activity as potential anti-tuberculosis agents

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ABSTRACT: New drugs and molecular targets are urgently needed to address the emergence and spread of drug-resistant tuberculosis. *Mycobacterium tuberculosis* (*Mtb*) inosine 5'-monophosphate dehydrogenase 2 (*Mtb*IMPDH2) is a promising yet controversial potential target. Inhibition of *Mtb*IMPDH2 blocks the biosynthesis of guanine nucleotides, but high concentrations of guanine can potentially rescue the bacteria. Herein we describe an expansion of the structure-activity relationship (SAR) for the benzoxazole series of *Mtb*IMPDH2 inhibitors and demonstrate that minimum inhibitory concentrations (MIC) of ≤ 1 μM can be achieved. The antibacterial activity of the most promising compound, **17b** (**Q151**), derives from inhibition of *Mtb*IMPDH2 as demonstrated by conditional knockdown and resistant strains. Importantly, guanine does not change the MIC of **17b**, alleviating the concern that guanine salvage can protect *Mtb in vivo*. These findings suggest that *Mtb*IMPDH2 is a vulnerable target for tuberculosis.

INTRODUCTION

The worldwide burden of tuberculosis is staggering: more than 1 million deaths and 10 million new cases occur each year (https://www.cdc.gov/tb/statistics/default.htm, accessed 5/21/2017). Approximately one third of the world's population is infected with the causative agent *Mycobacterium tuberculosis (Mtb)*, many with latent infections that can recrudesce if the patient becomes immunosuppressed. Treatment requires administration with isoniazid, rifampicin pyrazinamide and ethambutol for two months followed by an additional four months with isoniazid and rifampicin. However, almost 0.5 million cases involve *Mtb* strains that are resistant to isoniazid and rifampicin, further complicating treatment. Extensively drug resistant strains have also emerged that are resistant to at least isoniazid and rifampicin, a fluoroquinolone, and any one of the second-line injectables, capreomycin, kanamycin and amikacin. Frustratingly, as illustrated by the newly approved tuberculosis drugs bedaquiline and delamanid [1], resistance can emerge just a few years after introduction of a new therapy, underscoring the critical need for new drugs, drug targets, and drug regimens to improve tuberculosis treatment and combat the relentless evolution of resistance.

Inosine 5'-monophosphate dehydrogenase (IMPDH) is the enzyme that catalyzes the NAD^+-dependent conversion of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP). This reaction is the first and the rate limiting step in the *de novo* biosynthesis of guanine nucleotides and therefore controls the size of the guanine nucleotide pool.[2] IMPDH is an attractive target for the development of new antibiotics due to the essential role of guanine nucleotides in DNA and RNA synthesis, signal transduction, energy transfer, glycoprotein biosynthesis and many other processes involved in cell proliferation.[3] *Mtb* has three genes,

designated as *guaB1*, *guaB2* and *guaB3*, which encode IMPDH homologs. Only *guaB2* is essential, [4-6] and only *guaB2* encodes an active IMPDH (*Mtb*IMPDH2).[7] The proteins encoded by *guaB1* and *guaB3* are each missing key catalytic residues and neither display IMPDH activity [7]. The functions of these proteins are currently unknown.

*Mtb*IMPDH2 is a controversial target for tuberculosis. Several small molecule inhibitors of *Mtb*IMPDH2 have been identified that display antibacterial activity *in vitro* and in macrophages [7-13], but as yet no compounds have been reported with activity in animal models of tuberculosis. Recently, Park et al. suggested that high levels of guanine (200-2000 μM[10]) in the lungs of infected rabbits and humans can protect bacteria from *Mtb*IMPDH2 inhibitors,[10] calling into question the vulnerability of this target. However, the availability of free guanine for salvage is by no means certain since these concentrations exceed the solubility limit. More promisingly, Singh et al. constructed a conditional knockout of *guaB2* in *Mtb* and found that depletion of *Mtb*IMPDH2 is rapidly bactericidal *in vitro*.[11] They also showed that silencing of *Mtb*IMPDH2 expression prevented infection in mice [11], consistent with the lower levels of guanine found in mouse tissue (7-20 μM [10]). On the basis of these findings, Singh et al. concluded that this enzyme is indeed vulnerable.[11]

IMPDH also appears to have moonlighting functions of unknown physiological consequence, which further complicates the question of whether it is a vulnerable target. The protein contains a regulatory CBS subdomain in addition to the catalytic $(\beta/\alpha)_8$ domain. CBS domains bind nucleotides and modulate the activity of other enzymes and channels [14]. However, deletion of the CBS subdomain does not affect the activity of the catalytic domain in IMPDH [15-18]. The CBS subdomain binds ATP and/or GTP depending on the particular IMPDH [19-22]. These interactions modulate the oligomerization of tetramers to form octamers and higher order structures *in vitro*,

with modest effects on enzymatic activity [19-22]. The physiological relevance of ATP/GTP binding and these higher order structures is uncertain. Most interestingly, deletion of CBS subdomain dysregulates the purine nucleotide pools in *E. coli*, leading to the toxic accumulation of ATP and other adenine nucleotides [23, 24]. IMPDH binds nucleic acid and can act as a transcription factor in eukaryotes, and this activity is mediated by the CBS subdomain [25-27]. IMPDH also interacts with numerous other proteins in bacteria, including the ribosome, RNAP, amino acyl tRNA synthetases, penicillin binding proteins, uridylate kinase, PRPP synthetase, adenylosuccinate synthetase and DNA helicase [28, 29]. Neither the sites nor the physiological consequences of these interactions have been elucidated. Therefore it is possible that the essentiality of *guaB2* in *Mtb* derives from a moonlighting function rather than enzymatic activity. Moreover, the physiological consequences of enzyme inhibition may well be different from protein depletion, which downregulates both enzymatic and moonlighting activities [30]. More potent *Mtb*IMPDH2 inhibitors are required to resolve this conundrum and determine the vulnerability of *Mtb* infections to IMPDH inhibition [31].

We have developed triazole (**A**-series), benzimidazole (**C**-series), phthalazinone (**D**-series), 4-oxo-[1]benzopyrano[4,3-c]pyrazole (**N**-series), urea (**P**-series) and benzoxazole (**Q**-series) inhibitors of prokaryotic IMPDHs, including *Mtb*IMPDH2 [9, 32-40]. These inhibitors exploit the highly diverged cofactor binding site, which explains their selectivity for bacterial over eukaryotic orthologs of IMPDH [9, 17]. Individual members of the **A**, **D**, **P** and **Q**-series display antibacterial activity against *Mtb* as well as other pathogens [9, 41, 42]. The best **Q** compound, **3** (Figure 1A), was a potent inhibitor of *Mtb*IMPDH2 ($K_{i,app}$ = 14 nM) and displayed moderate antibacterial activity (MIC = 6.3 and 11 μM in minimal GAST/Fe and rich 7H9/ADC/Tween

media, respectively). The values of MIC increased 3 to 7-fold in the presence of guanine, suggesting that antibacterial activity resulted from the on-target inhibition of *Mtb*IMPDH2.

Herein we report an expansion of the structure-activity relationship (SAR) for the **Q**-series of *Mtb*IMPDH2 inhibitors and improved antibacterial activity. Importantly, antibacterial activity is not reduced in the presence of guanine for the most potent compounds. Nonetheless, antibacterial activity appears to derive from the inhibition of *Mtb*IMPDH2 as demonstrated by conditional knockdown and resistant strains. These findings suggest that *Mtb*IMPDH2 is indeed a vulnerable target.

RESULTS AND DISCUSSION

Design strategy. IMPDHs are a square planar homotetramers, with each monomer composed of two domains, the catalytic domain and a smaller domain containing tandem cystathione β-synthetase (CBS) motifs.[43] The physiological role of the CBS domain is unknown. Deletion of this domain does not impact enzymatic properties or tetramer formation, but does improve solubility and crystallization. The crystal structure of the CBS deletion variant of *Mtb*IMPDH2 (*Mtb*IMPDH2ΔCBS)[9] in complex with IMP and inhibitor **3** reveals that the aromatic ring of the 2,3-dichloroaniline is involved in a π- π interaction with the purine base of IMP, whereas the amide participates in an ionic-dipole interaction of the NH with the carboxylate of Glu458 and a hydrogen bond of the amide carbonyl with a water molecule (Figure 1B). The nitrogen atom of the 1,3-benzoxazole interacts with a water molecule that also engages the main chain nitrogen and oxygen atoms of His286. Moreover, the 4-pyridyl–1,3-benzoxazole moiety of **3** binds in a

hydrophobic pocket formed by Val60, Pro61, and Tyr487 and the pyridyl nitrogen atom interacts with the

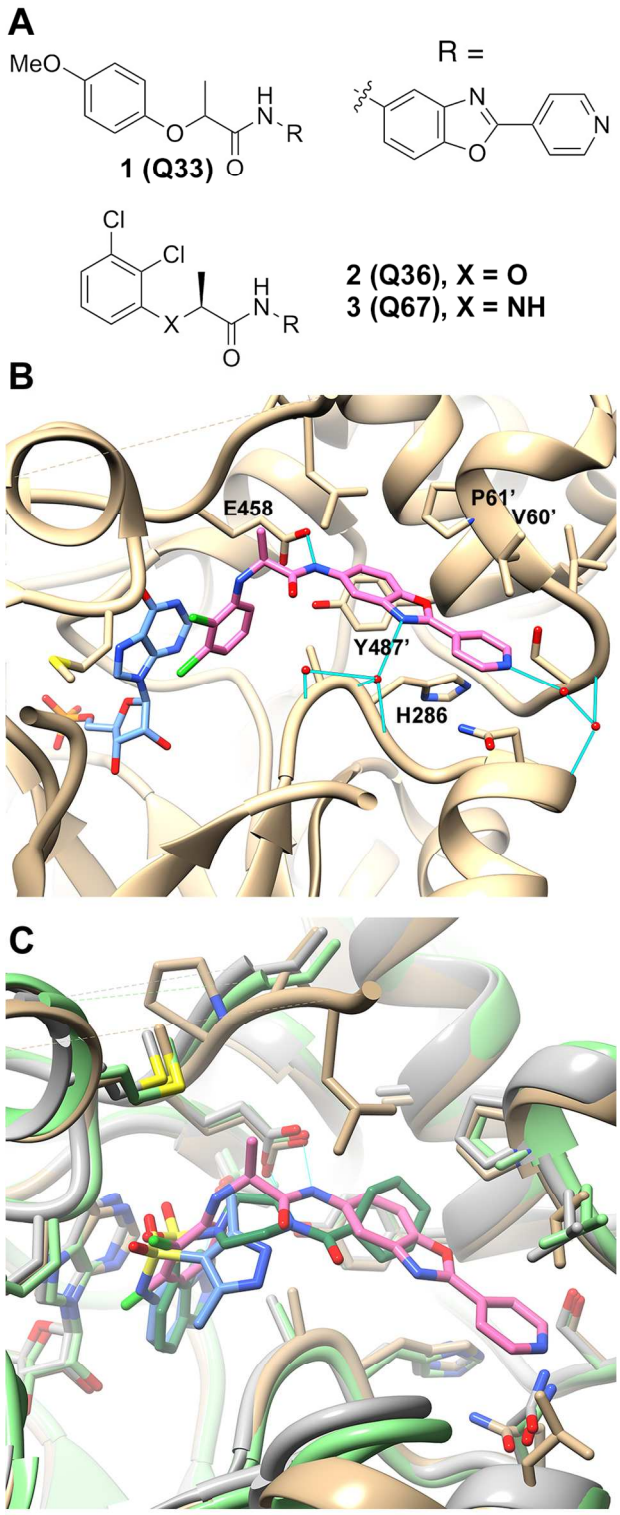


Figure 1. **Inhibitors of *Mtb*IMPDH2. A**) Structures of **1**, **2** and **3**. **B**) Crystal structure of *Mtb*IMPDH2ΔCBS •IMP•**3** (PDB 4ZQO)[9]. **3** is pink, IMP is blue, hydrogen bonds are shown in

cyan, residues from the adjacent subunit are marked with '. **C**) Crystal structures of *M. thermoresistible* IMPDH2 in complex with IMP and cyclohexyl[4-(5-isoquinolinylsulfonyl)-1-piperazinyl]methanone (VCC234718, PDB 5J5R, protein and IMP are spring green, inhibitor is forest green) [11], and N-1H-indazol-6-yl-3,5-dimethyl-1H-pyrazole-4-sulfonamide (6Q9, PDB 5K4X, protein and IMP are gray, inhibitor is blue) [10]. The structure of *Mtb*IMPDH2ΔCBS •IMP•**3** (PDB 4ZQO, protein and IMP are tan, **3** is pink)[9] is also included. This figure was produced with UCSF Chimera [44].

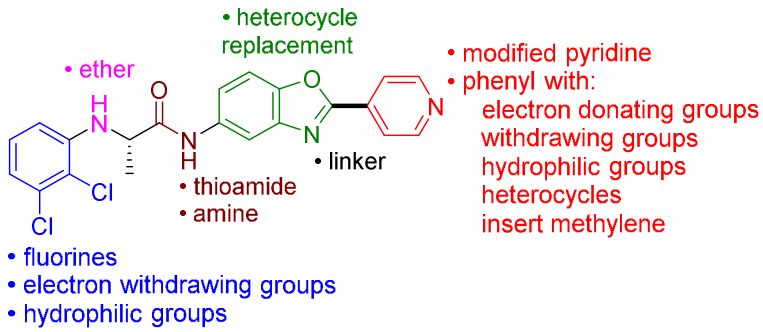


Figure 2. An overview of the SAR explored in this study.

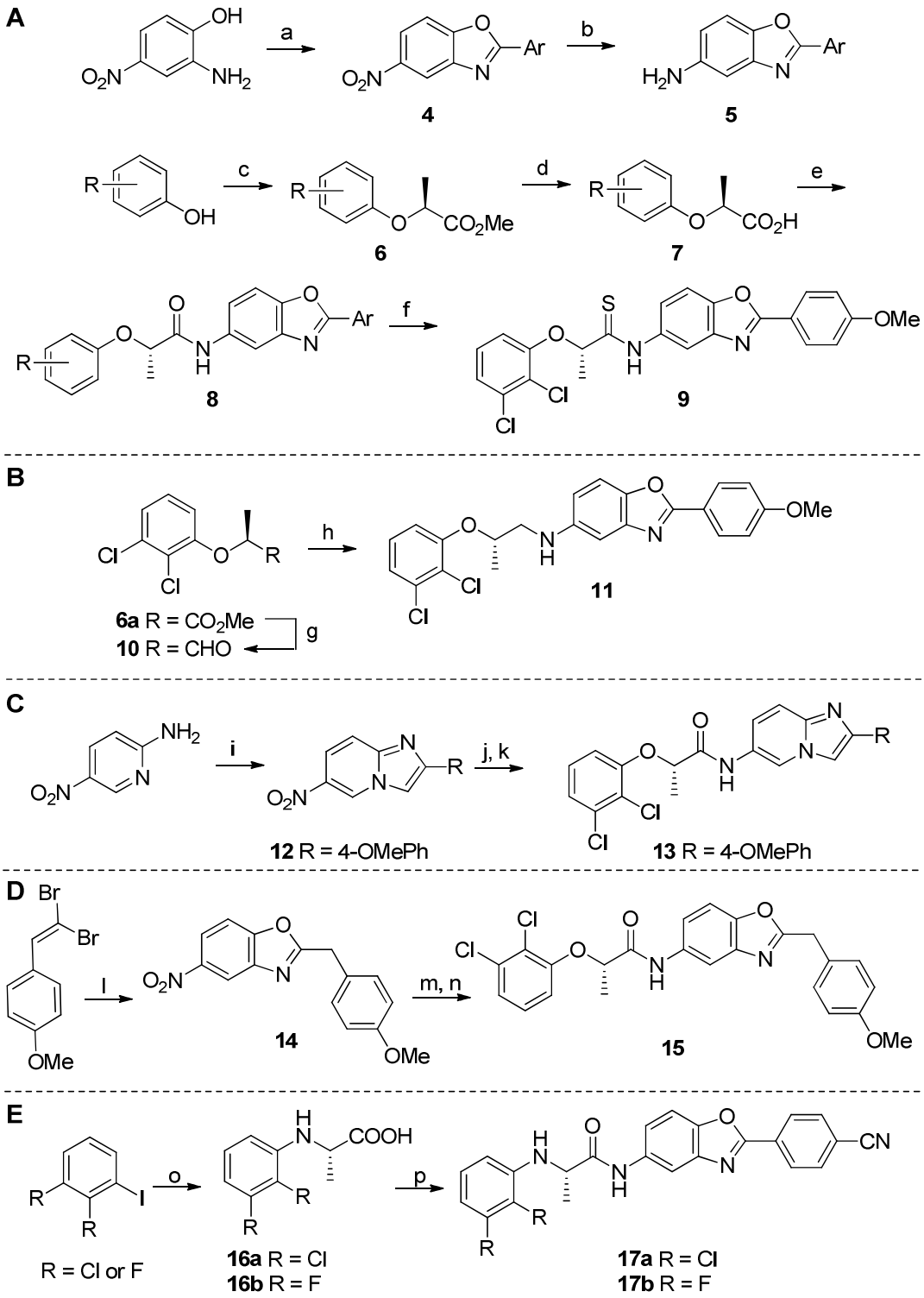
backbone carbonyl of Ser57 via a water network.[9] This pocket is not present in human IMPDHs, explaining the selectivity of the compounds for bacterial IMPDHs. Note that the two sulfonamide

inhibitors in Figure 1C do not extend into this pocket, perhaps explaining the higher affinity and selectivity of the **Q** compounds. [10, 11]

Using this information, we explored several strategies to expand the SAR of the **Q**-series (Figure 2): (1) modifications to the pyridine or replacement of the 4-pyridyl with a phenyl containing various substituents that can interact (e.g. via hydrogen bonds and ionic-dipole interactions) with the side chain of Arg290; (2) modification of the 2,3-dichloroaniline with 2,3-difluorophenyl ethers to enhance cell permeability or phenyl ethers containing hydrophilic groups capable of forming interactions with IMP and Glu458; (3) modifications of the amide group via replacement with a thioamide or amine to further explore interactions with Glu458; (4) replacement of the benzoxazole with a imidazo[1,2-a]pyridine; (5) insertion of a methylene linker between the benzoxazole and phenyl that was used as a pyridine replacement. **Chemistry.** The synthesis used to generate ether linked **Q**-series derivatives is illustrated in Scheme 1. Arylbenzoxazoles **4** were directly synthesized by oxidative cyclization methods using 2-amino-4-nitrophenol and aldehydes in the presence of activated carbon (Darco KB) under an oxygen atmosphere. The nitro group was reduced using Pd/C under 1 atm hydrogen to give 5-amine-2-arylbenzoxazoles **5**. Enantiomerically pure phenyl ethers **6** were synthesized from (+)-methyl D-lactate and the corresponding phenol using Mitsunobu reaction conditions. The esters **6** were hydrolyzed to the corresponding acids **7**, and then coupled with various 5-amine-2-arylbenzoxazoles in the presence of EDC·HCl in DMF to yield **8**.[39] Furthermore, **8** (Ar = 4-OMePh, R = 2,3-diCl) was treated with Lawesson's reagent under standard conditions to

generate thioamide **9** quantitatively. Ester **6a** was also treated with 1M DIBAL-H in DCM at -78 ºC to generate aldehyde **10**, which was subjected to reductive

Scheme 1. Synthesis of various aryl benzoxazoles **8**, thioamide **9**, amine **11**, imidazo[1,2-a]pyridine **13**, methylene linked derivative **15** and N-arylated benzoxazoles **17a-b.**[a]



[a]Reagents and conditions: (a) ArCHO, DarcoKB, O_2 (1 atm), xylene 140 ºC, 6-8 h, 70-80%; (b) 10% Pd/C, H_2 (1 atm), EtOAc:MeOH, 3 h, 85-94%; (c) PPh_3, DEAD, (+)-methyl D-lactate, THF, 0 ºC to rt, 4 h, 85-90%, (d) LiOH, THF:MeOH, 4 h, 86-91%; (e) **5**, EDC·HCl, DMF, 12 h, 62-79%. (f) Lawesson's reagent, 1,4-dioxane, reflux, 2 h, 71%; (g) 1. DIBAL-H, DCM, -78 ºC, 1h, 89%, (h) **5** (Ar = 4-OMePh), $NaBH(OAc)_3$, DCE, 1.5 h, 81%; (i) 4-OMePhC(O)CH_2Br, $NaHCO_3$, EtOH, reflux, 12 h, 88%; (j) $SnCl_2$, EtOH:EtOAc, reflux, 2 h; (k) **7** (R = 2,3-diCl), EDC·HCl, DMF, rt, 12 h, 60%; (l) 2-amino-4-nitrophenol, DABCO, NMP, 100 ºC, 24 h, 60%; (m) 10% Pd/C, H_2, MeOH:EtOAc, 2-3 h; (n) **7** (R = 2,3-diCl), EDC·HCl, DMF, rt, 12 h, 67%; (o) L-alanine, CuI, Cs_2CO_3, DMF, 90 ºC, 24 h; (p) **5** (Ar = 4-CNPh), HATU, DMF, rt, 12 h, 45-54%.

amination using **5** (Ar = 4-OMePh) and sodium triacetoxyborohydride in DCM to provide amine **11**.

The synthesis of an imidazo[1,2-a]pyridine analogue started by allowing 5-nitropyridin-2-amine to react with an α-bromoketone in the presence of $NaHCO_3$ in ethanol to give **12**. Nitro **g**roup reduction using $SnCl_2$ in ethanol followed by coupling with carboxylic acid **7** (R = 2,3-diCl) generated imidazo[1,2-a]pyridine **13**.

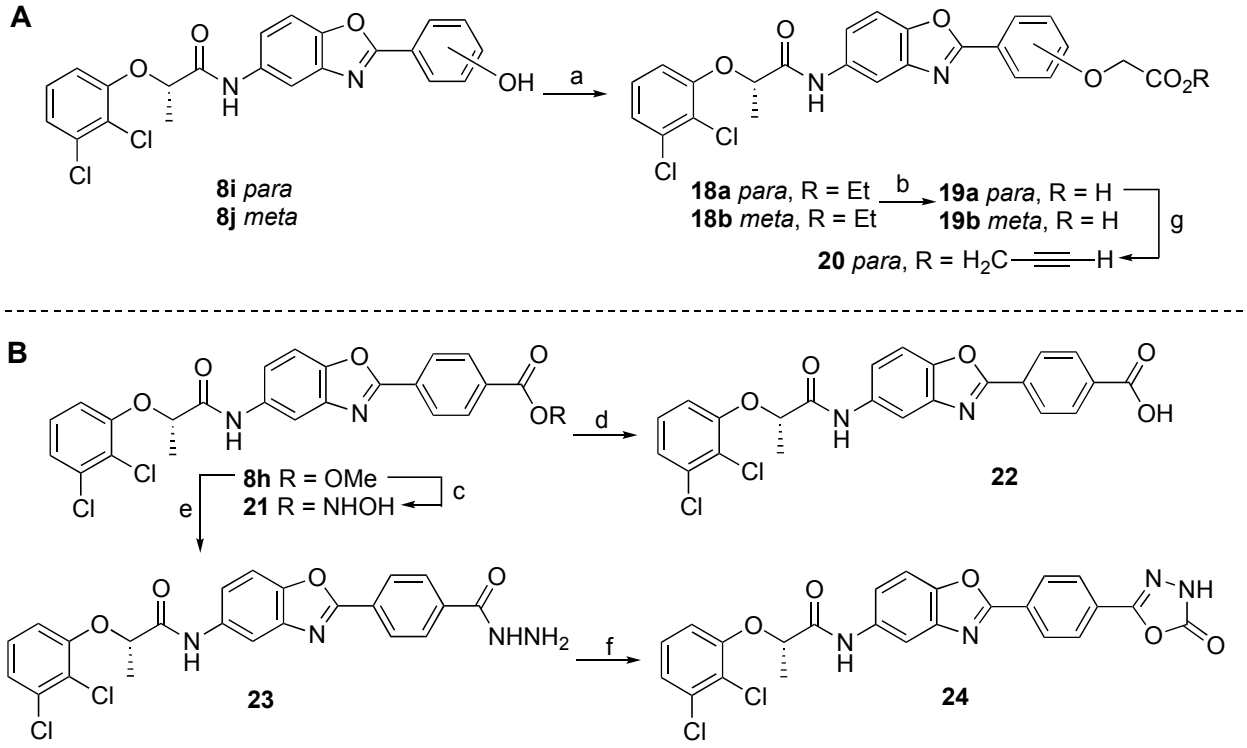
The synthesis of a derivative with a methylene linker between the benzoxazole and the benzene was undertaken. 2-Amino-4-nitrophenol was coupled with a 1,1-dibromoethylene derivative using DABCO in NMP at 100 ºC to generate **14**. The nitro group was reduced using 10% Pd/C under a hydrogen atmosphere and the resulting amine was coupled with **7** (R = 2,3-diCl) using EDC·HCl in DMF to yield **15**.

Enantiopure *N*-arylated benzoxazole derivatives were synthesized using copper catalyzed Ullman type reactions. L-alanine was treated with aryl iodides in the presence of cesium carbonate and CuI in DMF for 24 h to provide the *N*-arylated amino acids **16a-b**.[39] Attempts to couple these materials with **5** (Ar = 4-CNPh) using EDC·HCl failed. However, using HATU as the coupling reagent in DMF resulted in formation of **17a-b**.

Scheme 2 depicts the functional group transformations to various substituents attached to the benzoxazole aryl group. Compounds **8i** and **8j** were subjected to alkylation reactions with ethyl 2-bromoacetate in the presence of K_2CO_3 and DMF to give **18a** and **18b**, respectively. These compounds were hydrolyzed using LiOH in THF:MeOH (1:3) to yield **19a** and **19b**, respectively. Carboxylic acid **19a** was also converted to ester **20** using propargyl bromide and K_2CO_3 in dry DMF. Ester **8h** was also converted to an array of derivatives. For example, it was

treated with hydroxylamine hydrochloride to provide the corresponding hydroxamic acid **21**. Base catalyzed

Scheme 2. Synthesis of derivatives **18a-b**, **19a-b** and **20-24**.[a]

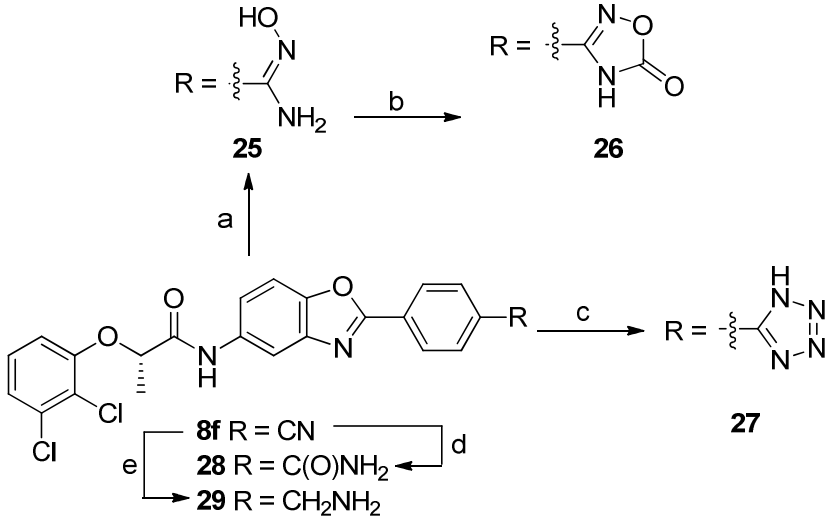


[a]Reagents and conditions: (a) $BrCH_2COOEt$, K_2CO_3, DMF, rt, 6 h, 80-83%; (b) LiOH, THF:MeOH, rt, 86-88%; (c) $NH_2OH{\cdot}HCl$, KOH, MeOH, rt, 12 h, 65%; (d) NaOH, THF:MeOH:H_2O (3:1:1), 2 h, rt, 90%; (e) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 5 h, 67%; (f) CDI, DIPEA, DMF, rt, 2 h, 66%; (g) $CH{\equiv}CCH_2Br$, K_2CO_3, DMF, rt, 6 h, 81%.

ester hydrolysis of **8h** in MeOH:THF:H_2O (3:1:1) yielded carboxylic acid **22**. Finally, ester **8h** was converted to hydrazide **23** using hydrazine hydrate in EtOH, followed by treatment with carbonyl diimidazole (CDI) in the presence of DIPEA in DMF yielding 1,3,4- oxadiazolone **24**.

Scheme 3 illustrates the synthesis of an array of derivatives via functional group transformation of **8f**. For example, **8f** was treated with hydroxylamine hydrochloride in absolute ethanol in the presence of triethylamine to afford hydroxamidine **25**, which upon treatment with CDI and DIPEA gave 1,2,4-oxadiazolone **26**. The tetrazole derivative **27** was prepared by treatment of **8f** with sodium azide in the presence of ammonium chloride in dry DMF. Primary amide **28** was generated by treating **8f** with *t*-BuOK in *t*-BuOH.[45] Finally, **8f** was treated with $NiCl_2 \cdot 6H_2O$ and $NaBH_4$ in the presence of Boc_2O in MeOH:THF to give the Boc protected primary amine, which was deprotected using trifluoroacetic acid in DCM to provide **29**.[46]

Scheme 3. Synthesis of derivatives **25-29** from **8f.**[a]

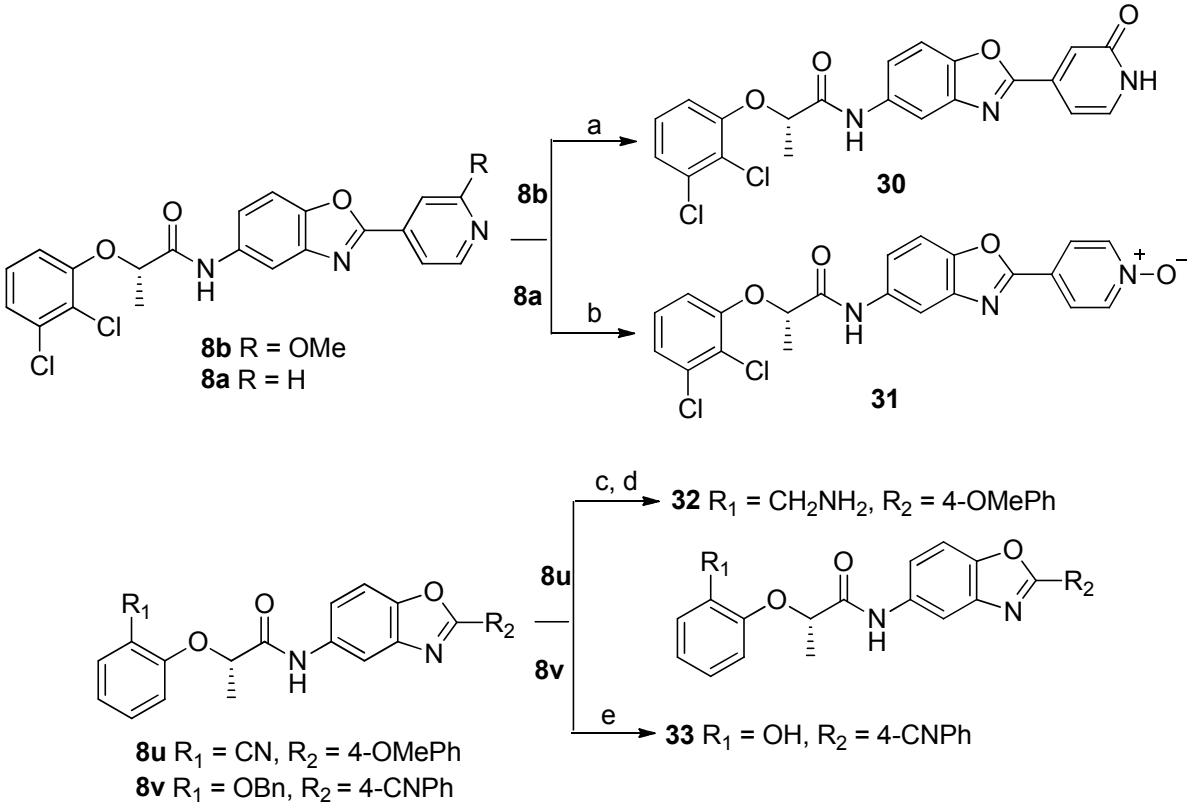


[a]Reagents and conditions: (a) $NH_2OH \cdot HCl$, Et_3N, EtOH, reflux, 4 h, 82%; (b) CDI, DIPEA, DMF, rt, 2 h, 61%; (c) NaN_3, NH_4Cl, DMF, 100 ºC, 6 h, 62%; (d) *t*-BuOK, *t*-BuOH, 12 h, 54%; (e) 1. $NiCl_2 \cdot 6H_2O$, $NaBH_4$, Boc_2O, MeOH:THF, rt, 2 h, 2. TFA, DCM, rt, 4 h, 67%.

The synthesis of 2-pyridone derivative **30** was carried out by treating **8b** with LiCl and p-toluenesulfonic acid in anhydrous methanol (Scheme 4). The pyridyl of **8a** was oxidized using *m*-CPBA in DCM to give the corresponding pyridine N-oxide **31**. The nitrile in **8u** was reduced using $NiCl_2 \cdot 6H_2O$ and $NaBH_4$ then protected as the corresponding t-butyl carbamate. Removal of the protecting group using trifluoroacetic acid in DCM provided amine **32**. The benzyl ether of **8v** was removed by hydrogenolysis in presence of Pd/C under a hydrogen atmosphere to yield **33**.

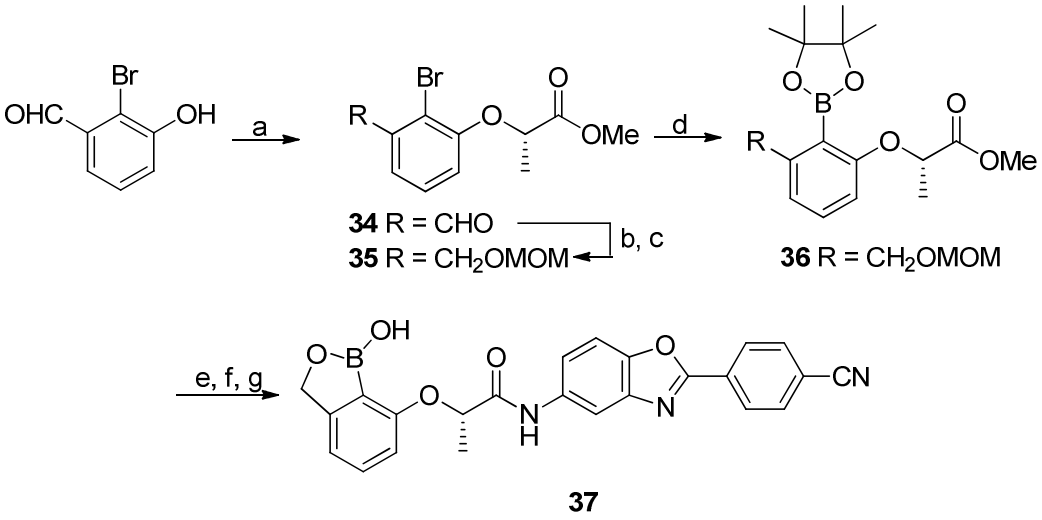
Finally, the synthesis of a benzoxaborole analogue is depicted in Scheme 5. A Mitsunobu reaction between 2-bromo-3-hydroxybenzaldehyde and (+)-methyl D-lactate was carried out in the presence of PPh_3 and DEAD in THF to give **34**. This aldehyde was treated with $NaBH_4$ in ethanol to generate the primary alcohol, which was protected as the corresponding methoxymethyl ether **35** using methoxymethyl chloride (MOMCl) and DIPEA in DCM. Boronylation of the aryl bromide was carried out using bis(pinacolato)diboron in the presence of KOAc and a catalytic amount of $Pd(Ph_3P)_2Cl_2$ in 1,4-dioxane to give **36**.[47] Ester hydrolysis followed by amine coupling with **5** (Ar = 4-CNPh) in the presence of EDC·HCl in DMF and then treatment with 6N HCl in THF gave benzoxaborole **37**.

Scheme 4. Synthesis of derivatives **30-33.**[a]



[a]Reagents and conditions: (a) LiCl, p-TSA, DMF, 120 ºC, 2 h, 77%; (b) *m*-CPBA, DCM, rt, 12 h, 74%; (c) $NiCl_2 \cdot H_2O$, $NaBH_4$, and then Boc_2O, THF, MeOH, rt, 4 h; (d) TFA, DCM, 68%; (e) 10% Pd/C, H_2, MeOH:EtOAc, rt, 3 h, 90%.

Scheme 5. Synthesis of benzoxaborole **37.**[a]



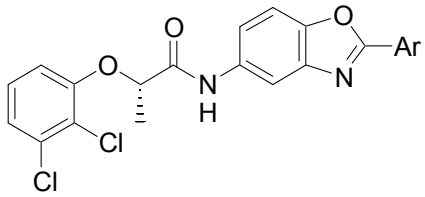
[a]Reagents and conditions: (a) (+)-Methyl D-lactate, DEAD, PPh_3, THF, 0 ºC, 2 h, 83%; (b) $NaBH_4$, EtOH, rt, 1 h; (c) MOMCl, DIPEA, DCM, rt, 12 h, 72%; (d) Pin_2B_2, $Pd(Ph_3P)_2Cl_2$, KOAc, 1,4-dioxane, 95 ºC, 12 h, 64%; (e) LiOH, MeOH:THF, 4 h, 0 ºC; (f) **5** (Ar = 4-CNPh), EDC·HCl, DMF, rt, 12 h; (g) 4N HCl, THF, 4 h, 28%.

Evaluation of *Mtb*IMPDH2 inhibition. We previously described the *Mtb*IMPDH2ΔCBS inhibition and antibacterial activity of benzoxazole (**Q**) based inhibitors.[39] The most promising compounds, **2** and **3** (Figure 1A), had $K_{i,app}$ values of 76 nM and 14 nM, and MIC values of 16 μM and 12 μM, respectively. *Mtb*IMPDH2ΔCBS was expressed and purified as previously described and the enzymatic activity was assayed by monitoring the production of NADH at sub-saturating NAD^+ (3 x K_m) and saturating IMP concentrations and 20-50 nM enzyme.[9] The $K_{i,app}$values reported herein were determined from the average of two independent experiments, unless otherwise noted. We also evaluated inhibition of the host enzymes *h*IMPDH2 and *h*GMPR2.

Initially, modifications of the pyridyl of **2** were evaluated (Table 1). Pyridine *N*-oxide **31** demonstrated comparable activity to the parent compound **2**. Addition of a methoxy to the pyridyl **8b** and **8c** resulted in 5-fold more inhibition than **2**. However, changing pyridyl to 2-pyridone (**30**) reduced inhibitory activity by 3-fold. Replacing pyridyl with phenyl having both electron donating groups (EDG) and electron withdrawing groups (EWG) was evaluated. Compounds **8d** (4-OMe), **8e** (4-OCF_3) and **8f** (4-CN) resulted in 7-fold increase in inhibitory activity compared to **2**. Fluorine (**8g**) and ester (**8h**) substituents were also tolerated. Replacing the pyridyl with a 4-hydroxy phenyl (**8i**) resulted in a 2-fold increase in inhibitory activity, whereas the 3-hydroxy (**8j)** and 2-hydroxy (**8k)** derivatives showed slightly decreased potency. Given the improved activityof **8d**, the effect of other ethers was examined. For example, **18a** and propargyl ester **20** shows a ~12-fold increase in activity compared to **2**, while the corresponding carboxylic acid (**19a**) and tetrazole (**27**) were moderately less potent. Translocating the alkoxy from the *para* to *meta* position resulted in a 4-fold loss in activity (**18b** and **19b**). Compounds containing hydrophilic substituents at the 4-position, such as hydroxamic acid **21,** carboxylic

acid **22** and aminomethyl **29**, were found to be much less active. Replacing the carboxylate with hydrazide **23**, 1,3,4-oxadiazolone **24**,

Table 1. *Mtb*IMPDH2ΔCBS Inhibition of Benzoxazole Derivatives Containing Various Aryl Substituents. a. Values are the average and range of at least two independent determinations. * Single determination. b. Values from Makowska-Grzyska *et al* [9].



Cmpd	Ar	$K_{i,app}$ (nM) [a]		
		*Mtb*IMPDH2	hIMPDH2	hGMPR2
1 [b]	See Figure 1A	150 ± 50	>5000	>5000
2 (e.g. **8a**) [b]	See Figure 1A	76 ± 27	>5000	>5000
3 [b]	See Figure 1A	14 ± 3	>5000	>5000
30	4-pyridin-2-one	220 ± 6	>5000	>5000
31	4-Py-N-oxide	70 ± 19	>5000	>5000
8b	4-(2-OMe)-Py	12 ± 0.1	>5000	>5000
8c	3-(6-OMe)-Py	20 ± 4	>5000	>5000
8d	4-OMe-Ph	6.9 ± 3.9	>5000	>5000
8e	4-OCF_3-Ph	4.3 ± 0.2	>5000	>5000
8f	4-CN-Ph	12 ± 3	2300	>5000
8g	4-F-Ph	35 ± 7	>5000	>5000
8h	4-$PhCO_2Me$	96 ± 43	>5000	>5000
8i	4-OH-Ph	50 ± 17	>5000	>5000
8j	3-OH-Ph	85 ± 30	>5000	>5000

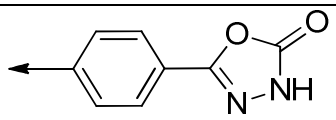
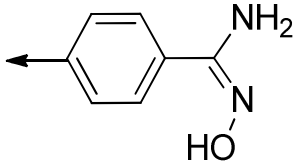
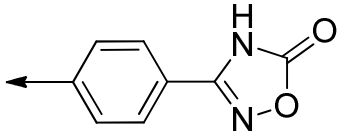
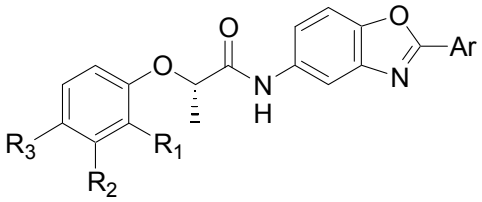
8k	2-OH-Ph	96 ± 21	>5000	>5000
18a	4-Ph-OCH_2CO_2Et	6.6 ± 0.4	>5000	>5000
20	4-Ph-$OCH_2CO_2CH_2CCH$	12 ± 2	>5000	>5000
19a	4-Ph-OCH_2CO_2H	18 ± 3	>5000	>5000
18b	3-Ph-OCH_2CO_2Et	23 ± 1	>5000	>5000
19b	3-Ph-OCH_2CO_2H	55 ± 10	>5000	>5000
21	4-Ph-C(O)NHOH	630 ± 210	>5000	>5000
22	4-$PhCO_2H$	360 ± 140	>5000	>5000
23	4-Ph-$C(O)NHNH_2$	49 ± 6	>5000	>5000
24		2.3 ± 1.0	2900	>5000
25		39 ± 12	>5000	>5000
26		46 ± 12	>5000	>5000
27	4-Ph-tetrazole	51 ± 2	>5000	>5000
28	4-Ph-$C(O)NH_2$	64 ± 26	>5000	>5000
29	4-Ph-CH_2NH_2	240 ± 26	>5000	>5000

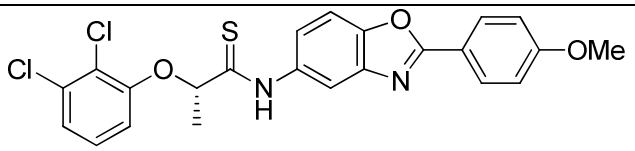
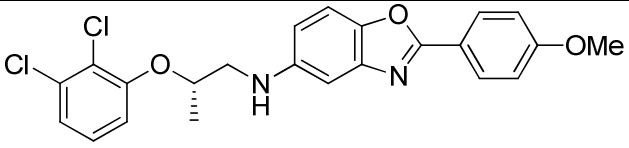
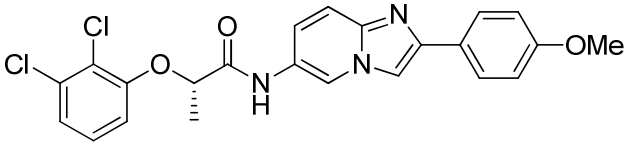
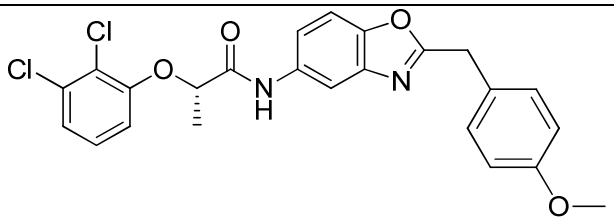
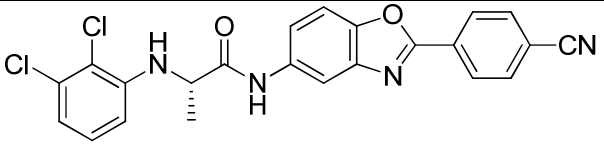
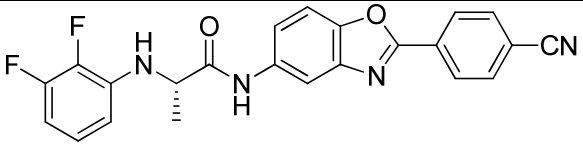
Table 2. *Mtb*IMPDH2ΔCBS Inhibition of Benzoxazole Derivatives Containing Various Phenyl Ether Substituents. a. Values are the average and range of at least two independent determinations. * Single determination, error of the fit is listed.



Cmpd	R_1	R_2	R_3	Ar	$K_{i,app}$ [a](nM)		
					*Mtb*IMPDH2	hIMPDH2	hGMPR2
8l	F	F	H	4-Py	55 ± 9	>5000	>5000
8m	F	F	H	4-OMe-Ph	19 ± 2	>5000	>5000
8n	F	F	H	4-OH-Ph	121 ± 21	>5000	>5000
8o	F	F	H	3-(6-OMe)-Py	42 ± 17	>5000	>5000
8p	F	F	H	4-CN-Ph	7.2 ± 2.5	2000	>5000
8q	F	F	H	4-F-Ph	40 ± 3	>5000	>5000
8r	F	F	F	4-CN-Ph	22 ± 11	>5000	>5000

8s	CN	H	H	4-Py	76 ± 35	>5000	>5000
8t	CN	F	H	4-Py	40 ± 2	>5000	>5000
8u	CN	H	H	4-OMePh	33 ± 5	>5000	>5000
8v	OBn	H	H	4-CN-Ph	43 ± 12	>5000	>5000
32	CH_2NH_2	H	H	4-OMe-Ph	23 ± 9	>5000	>5000
33	OH	H	H	4-CN-Ph	36 ± 12	>5000	>5000
37	$B(OH)OCH_2$		H	4-CN-Ph	370 ± 70 *	>5000	>5000

Table 3. *Mtb*IMPDH2ΔCBS Inhibition of Other Benzoxazole Derivatives. a. Values are the average and range of at least two independent determinations. * Single determination.

Cmpd	Structure	$K_{i,app}$ [a](nM)		
		*Mtb*IMPDH2	hIMPDH2	hGMPR2
9		27 ± 21	>5000	>5000
11		1290 ± 250*	n.a.	>5000
13		160 ± 4	>5000	>5000
15		100 ± 40	>5000	>5000
17a		9 ± 4	3300	>5000
17b		18 ± 3	>5000	>5000

hydroxamidine **25**, 1,2,3-oxadiazolone **26** and primary amide **28** showed 2 to 7-fold increase in activity.

Previous work on the benzoxazole series had established that substitution at the 2- and 3-positions of aryl ether were important for inhibition of both *Cryptosporidium parvum* IMPDH and *Mtb*IMPDH2ΔCBS.[39,9] Several 2,3-dichlorophenyl compounds (**2**, **8c**, **8d**, **8f**, **8g** and **8i**) were compared to the corresponding 2,3-difluorophenyl analogues (**8l**, **8o**, **8m**, **8p**, **8p** and **8n**). Three of these compounds (**8l**, **8p** and **8q**) displayed similar activity whereas the others were 2 to 4-fold less active. Adding another substitution on the 4-position (**8r**) resulted in slightly reduced activity. Replacement of the 2-chlorophenyl with a 2-cyanophenyl (**8s** and **8t**) was well tolerated. In addition, compounds with 2-cyano (**8u**), 2-aminomethyl (**32**), and 2-hydroxy (**33**), and 2-benzyloxy (**8v**) substituents on the phenyl ether demonstrated *Mtb*IMPDH2ΔCBS $K_{i,app}$ values < 45 nM. However, the benzoxaborole (**37**) was not as well tolerated.

Several other areas of the **Q**-series were examined (Table 3). For example, the amide was replaced with a thioamide (**9**), which demonstrated a modest loss of activity. This decrease in potency is likely due a loss of a hydrogen bond to water, while retaining the ionic-dipole interaction between the thioamide NH and the carboxylate of Glu458. Furthermore, replacement of the amide with an amine (**11**) was not tolerated. In addition, activity was significantly decreased when the benzoxazole was replaced with an imidazo[1,2-a]pyridine (**13**). Adding a methylene between the phenyl and benzoxazole (**15**) also reduced activity by a factor of 6 compared to **8d**. However, replacing the aryl ether with aniline (**17a** and **17b**) resulted in increased inhibitory activity.

Selectivity of inhibition. The cofactor binding sites are widely diverged in bacterial and eukaryotic IMPDHs, and the *Mtb*IMPDH2 inhibitors exploit this divergence [9, 17]. Only four

compounds, **8f**, **8p**, **17a** and **24**, modestly inhibited human IMPDH2, and in these cases selectivity ranged from a factor of 200 to 1000. No inhibition of human IMPDH2 was observed for the other compounds (maximum concentration tested was 5 μM). GMP reductase (GMPR) is closely related to IMPDH, and catalyzes a similar reaction, the reduction of GMP by NADPH to produce IMP, $NADP^+$ and ammonia [48]. The adenosine site of human GMPR2 contains the Ala-Tyr motif that characterizes the inhibitor binding site of *Mtb*IMPDH2. However, none of the compounds inhibited human GMPR2 (maximum concentration tested was 5 μM). These experiments demonstrate that compounds selectively inhibit bacterial IMPDHs and do not affect related host enzymes.

Evaluation of antibacterial activity. Antibacterial activity was determined for *Mtb*IMPDH2ΔCBS inhibitors with $K_{i,app} \leq 40$ nM and selected additional compounds by monitoring the growth of *Mtb* H37Rv (ATCC 27294) after one week (Table 4). Since *in vitro* antibiotic efficacy can vary unpredictably with growth conditions,[49] antibacterial activity was assessed in both GAST/Fe and 7H9/ADC/Tween media, both of which lack purines (e.g. –Gua). Two compounds, **18a** and **20**, displayed MICs less than 1 μM in both media. An additional 8 compounds, **8f**, **8l**, **8m**, **8p**, **8u**, **17a**, **17b** and **18b**, displayed MIC ≤ 5 μM in both media. Six of these compounds retained antibacterial activity over two weeks: **8l**, **8u**, **17a**, **17b**, **18a** and **20**. Two compounds (**21** and **22**) were active in GAST/Fe medium (MIC ≤ 5 μM) but considerably less effective in 7H9/ADC/Tween medium. These compounds also retained activity over two weeks.

Figure 3 shows the dependence of antibacterial activity on inhibition of *Mtb*IMPDH2ΔCBS for the **Q** compounds described above as well as those reported previously [9]. Uptake and metabolism

also play important roles in antibacterial efficacy, so it is not surprising that some potent *Mtb*IMPDH2ΔCBS inhibitors fail to display antibacterial activity. Nonetheless, more potent

Table 4. Antitubercular activity of *Mtb*IMPDH2 inhibitors against *Mtb* H37Rv. The number of independent determinations is shown in parentheses. a. MICs determined after 1 week in culture. b. Values from Makowska-Grzyska et al [9]. +Gua, 200 μM guanine.

Compound	GAST/Fe MIC (μM) [a]		7H9/ADC/Tween MIC (μM) [a]	
	-Gua	+Gua	-Gua	+Gua
1 [b]	5.3 ± 0.9 (3)	24 ± 16 (3)	10 ± 2 (3)	21 ± 4 (3)
2 (e.g. **8a**) [b]	9.4 ± 3.0 (2)	37 (1)	16 ± 6 (3)	28 ± 9 (2)
3 [b]	6.7 ± 3 (2)	50 (1)	12 ± 6 (3)	35 ± 15 (2)
8b	6.3 ± 0.0 (2)	6.3 ± 0.0 (2)	3.5 ± 1.2 (2)	14 ± 5 (2)
8c	7 ± 2 (3)	9 ± 7 (3)	6 ± 3 (3)	> 25 (3)
8d	>50 (2)	>50 (2)	>50 (2)	>50 (2)
8e	>50 (1)	>50 (1)	>50 (2)	>50 (2)
8f	4 ± 1 (3)	5 ± 1 (3)	3 ± 1 (4)	6 ± 1 (4)

8g	>50 (2)	>50 (2)	>50 (2)	>50 (2)
8l	4 ± 2 (2)	6.3 ± 0 (2)	1.9 ± 0.5 (2)	4 ± 3 (2)
8m	5 ± 2 (3)	8 ± 4 (3)	4 ± 4 (4)	14 ± 7 (4)
8n	6 (1)	19 (1)	7 ± 3 (2)	>30 (2)
8o	6 ± 5 (2)	8 ± 7 (2)	>50 (5)	>50 (5)
8p	3 ± 1 (3)	6.3 ± 0 (2)	1.5 ± 0.8 (6)	4 ± 3 (6)
8q	>50 (1)	>50 (2)	>50 (2)	>50 (2)
8r	>50 (2)	>50 (2)	>50 (5)	>50 (5)
8t	6 ± 4 (2)	11 ± 2 (2)	4 ± 1 (2)	8 ± 2 (2)
8u	3.1 ± 0.0 (2)	6.3 ± 0.0 (2)	1.2 ± 0.0 (2)	4.7 ± 0.0 (2)
9	37 (1)	>50 (1)	>50 (2)	>50 (2)
17a	4 ± 3	13 ± 9	3 ± 1	6 ± 1

	(2)	(2)	(2)	(2)
17b	2 ± 1 (2)	6 ± 0 (2)	1.0 ± 0.3 (2)	1.4 ± 0.3 (2)
18a	0.4 ± 0.0 (2)	0.5 ± 0.1 (2)	0.2 ± 0.1 (2)	0.23 ± 0.16 (2)
18b	0.9 ± 0.2 (2)	1.4 ± 0.2 (2)	1.6 ± 0.0 (2)	1.6 ± 0.0 (2)
19a	2 ± 1 (4)	4 ± 2 (4)	9 ± 4 (5)	20 ± 10 (5)
20	0.4 ± 0.0 (2)	1.4 ± 0.2 (2)	0.2 ± 0 (2)	0.4 ± 0 (2)
21	3 ± 1 (4)	6 ± 1 (4)	30 ± 10 (5)	>50 (5)
22	4 ± 2 (2)	5 ± 0 (2)	>25 (2)	>50 (2)
24	>50 (2)	>50 (2)	>50 (2)	>50 (2)
25	25 (1)	25 (1)	>50 (2)	>50 (2)
29	11 ± 2 (2)	11 ± 2 (2)	25 ± 12 (2)	25 ± 12 (2)
32	9.4 ± 3.1	16 ± 3	5.5. ± 0.8	9.4 ± 0.0

	(2)	(2)	(2)	(2)
33	22 ± 3 (2)	25 ± 0.0 (2)	9.4 ± 0.0 (2)	22 ± 3 (2)

enzyme inhibition is generally associated with greater antibacterial activity, as expected if antibacterial activity derived from inhibition of *Mtb*IMPDH2.

Many bacteria have the ability to salvage guanine or guanosine, and thus overcome inhibition of IMPDH. *Mtb* can salvage guanine, but not guanosine, and previously reported IMPDH inhibitors were much less effective in the presence of high guanine concentrations [9-11]. For example, the MIC values of indazole sulfonamide inhibitors of *Mtb*IMPDH2 increased more than 25-fold in 100-125 μM guanine [10, 11]. Smaller increases were observed for previously reported **Q** compounds [9]. However, the MIC of only one of the new **Q** compounds, **8n**, increased substantially in the presence of guanine in both media. Intriguingly, this compound was one of the weaker inhibitors of *Mtb*IMPDH2 (K_{iapp} = 121 nM). The MIC of one additional compound, **8c** ($K_{i,app}$ = 20 nM), increased in the presence of guanine in 7H9/ADC/Tween medium but not in GAST/Fe. The failure of guanine to decrease the antibacterial activity of most **Q** compounds would usually suggest that antibacterial activity derives from the engagement of another target. Alternatively, guanine salvage may not be sufficient to support growth in the presence of potent *Mtb*IMPDH2 inhibition. It is also possible that the **Q** inhibitors stabilize/disrupt a protein complex, thereby perturbing a moonlighting activity of *Mtb*IMPDH2 in addition to enzyme activity.

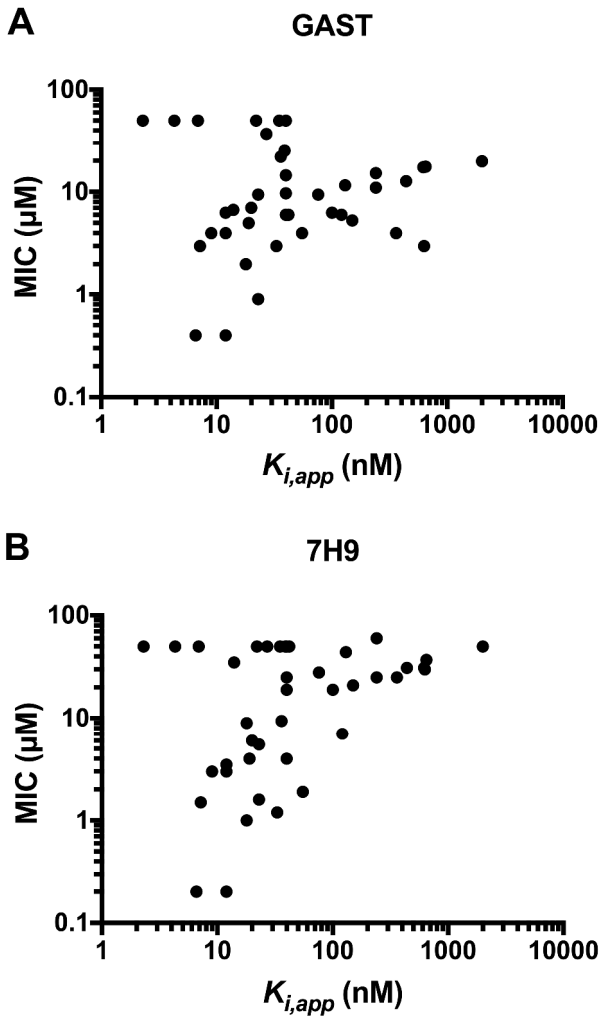


Figure 3. Correlation between antibacterial activity and enzyme inhibition for **Q** series compounds. Values from Table 4 and Makowska-Grzyska et al [9]. *Mtb* H37Rv cultured in **A**) GAST medium and **B**) 7H9 medium.

We performed two experiments to further address the question of whether antibacterial activity derived from on-target inhibition of *Mtb*IMPDH2. First, we evaluated the effect of *Mtb*IMPDH2 depletion on the antibacterial activity of four *Mtb*IMPDH2 inhibitors, including one compound with guanine-dependent antibacterial activity (**1**)[9], and three compounds with guanine-independent antibacterial activity (**17b**, **18a** and **22**; note that **22** is only active in GAST/Fe). The downregulation of *Mtb*IMPDH2 was achieved using strain *guaB2* cKD, in which *guaB2* expression is suppressed by anhydrotetracycline (ATc).[11] The antibacterial activities of **1**, **17b**, **18a**, and **22** against the wild-type strain were not affected by the addition of ATc (Table 5). In contrast, treatment with ATc hypersensitized the *guaB2* cKD strain to all four compounds (Figure 4), suggesting that antibacterial activity derives primarily from the inhibition of *Mtb*IMPDH2.

The antibacterial activity of **1**, **17b**, **18a**, and **22** was also assessed against *Mtb* strain SRMV2.6. This strain expresses the mutant *Mtb*IMPDH2/Y487C, which is resistant to an isoquinoline sulfonamide inhibitor [11]. As noted above, Tyr487 interacts with the benzoxazole group, so the substitution of Cys is expected to disrupt the binding of all the **Q** inhibitors. SRMV2.6 was resistant to **1** and **17b** (Table 5), further confirming that the antibacterial activity of these compounds resulted from on-target inhibition of *Mtb*IMPDH2. Interestingly, however, SRMV2.6 remained sensitive to **18a** and **22**.

We measured the inhibition of recombinant *Mtb*IMPDH2/Y487C to determine if the Y487C mutation decreased the affinity of all the **Q** compounds as expected (Table 5). The values of $K_{i,app}$ for **1** and **17b** were increased by more than 300-fold and 60-fold, respectively, which can account for the resistance of strain SRMV2.6. These observations further confirm that the

antibacterial activity of **1** and **17b** derive from inhibition of *Mtb*IMPDH2. The values of $K_{i,app}$ for **18a** and **22**

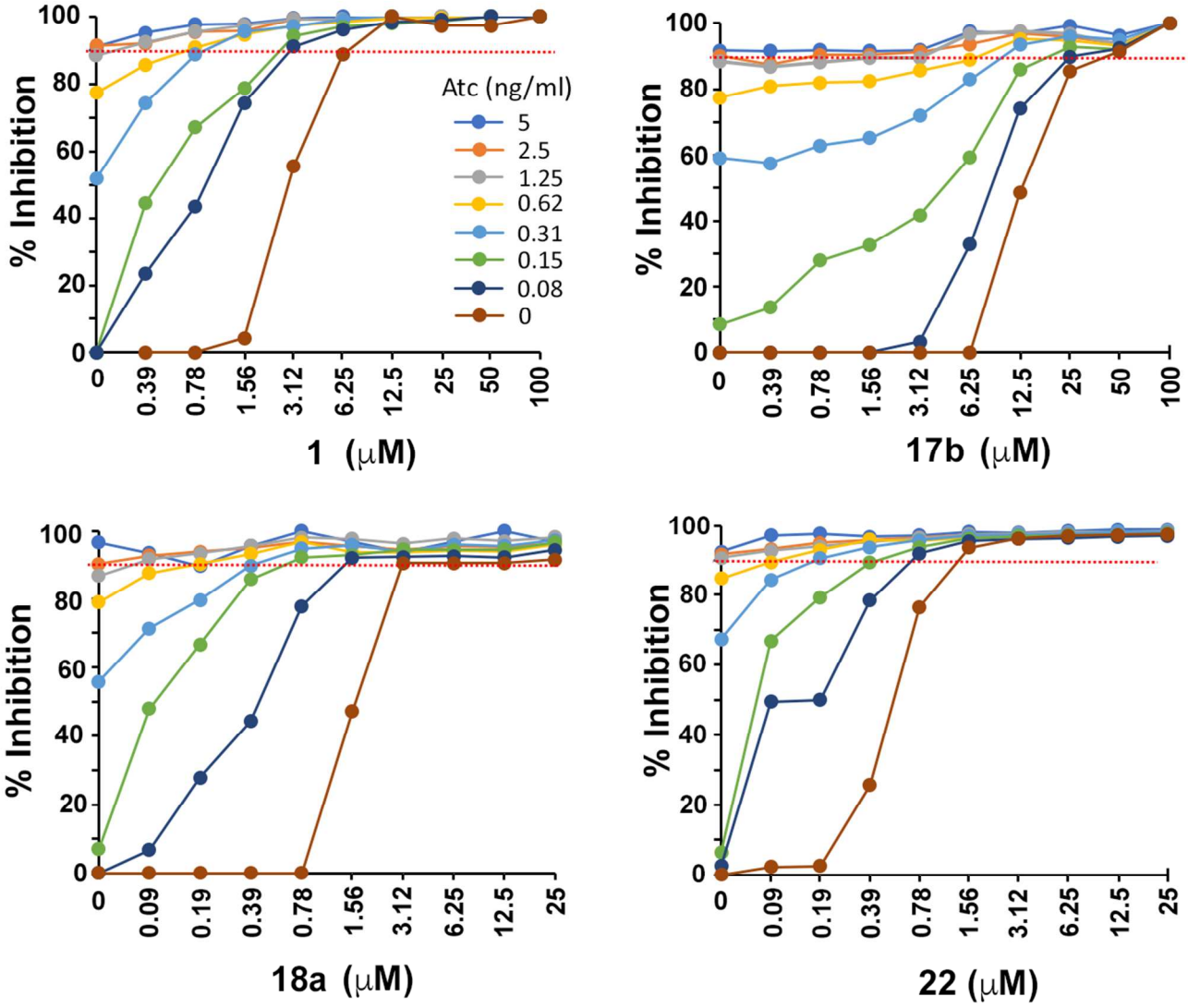


Figure 4. Knockdown of *Mtb*IMPDH2 hypersensitizes *Mtb* to **Q** compounds. Regulated expression of *guaB2* is achieved in a TET-OFF system, as previously described [11]. Addition of anhydrotetracycline (ATc) represses expression of *guaB2*, decreasing the level of *Mtb*IMPDH2 within the bacteria. ATc concentrations (ng/mL) are 0 (dark orange), 0.08 (dark blue), 0.15 (green), 0.31 (sky blue), 0.62 (yellow), 1.25 (gray), 2.5 (orange) and 5 (royal blue).

Table 5. Antibacterial activity against SRMV2.6. H37Rv is the wild-type strain. b. SRMV2.6 contains *Mtb*IMPDH2/Y487C, which confers resistance to the isoquinoline sulfonamide inhibitor VCC234718 [11]. a. Inhibition of purified wild-type *Mtb*IMPDH2. b. Inhibition of purified *Mtb*IMPDH2/Y487C c. Bacteria were cultured in 7H9/Glycerol/OADC/Tween and growth was measured with AlamarBlue as previously described [11], +ATc, 100 ng/mL. d. 20% inhibition at 50 μM. e. 5-10% inhibition at 15 μM. f. 80% inhibition at 50 μM.

Compound	WT[a] $K_{i,app}$ (nM)	Y487C[b] $K_{i,app}$ (μM)	Ratio $K_{i,app}$ (Y487C) /$K_{i,app}$ (WT)	MIC (μM)[c]		
				H37Rv	H37Rv (+ATc)	SRMV2.6
1	150 ± 50	>50[d]	>300	12.5	12.5	>100
17b	18 ± 3	1.1 ± 0.1	60	3.1	3.1	>100
18a	6.6 ± 0.4	~13[f]	~2000	3.1	3.1	3.1
22	360 ± 140	>15[e]	>40	25	25	25

were similarly increased in *Mtb*IMPDH2/Y487C, by at least 2000-fold and approximately 40-fold, respectively. These observations suggest that strain SRMV2.6 should also be resistant to **1** and **18a**, yet it remained sensitive to these compounds. Perhaps **1** and **18a** are concentrated or metabolized in the bacteria, or perhaps these compounds interfere with a moonlighting function. Alternatively, these compounds may engage an additional target(s). Irrespective of the ambiguous mechanism of action of **1** and **18a**, **17b** demonstrates that on-target inhibition of *Mtb*IMPDH2 can be impervious to guanine rescue, suggesting that it is a vulnerable target.

Evaluation of Human Cytotoxicity Activity. Compounds displaying potent antibacterial activity (**8l**, **8p**, **8u**, **17b, 18a** and **20**) were evaluated for cytotoxicity against HepG2 cells using a LDH release assay. Only compounds **8p**, **8u** and **17b** displayed measurable cytotoxicity (9-13% lactate dehydrogenase release) at 25 μM (LD_{50} > 25 μM, Figure 5). Since **18a** and **20** are esters of **19a**, we also examined the cytotoxicity of this compound, and again no cytotoxicity was observed. The cytotoxicity of **17b** (25 μM) was also less than 10% in Hela, HEK293T and MCF7 cells. These experiments demonstrate that all of the compounds display a greater than 10-fold selectivity for antibacterial activity versus cytotoxicity (the recommended criteria for *Mtb* [50]), with the selectivity of some exceeding 100-fold.

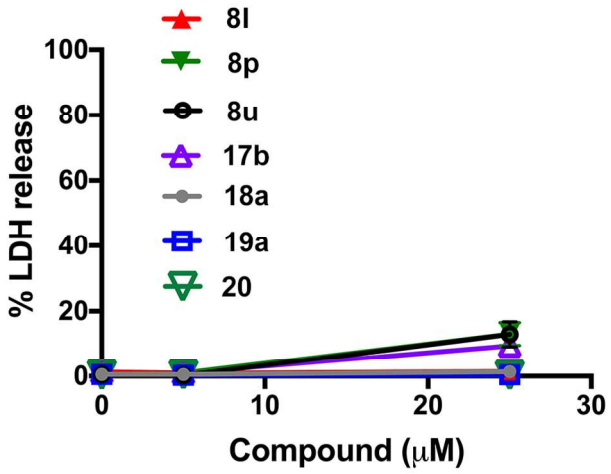


Figure 5. Cytotoxicity of select **Q** compounds in HepG2 cells after 24 h.

Preliminary pharmacokinetic evaluation of 17b. The above observations prompted us to consider evaluating the pharmacokinetics of **17b** and **18a** as a prelude to possible testing in a mouse model of tuberculosis. We first evaluated the stability of **17b** and **18a** in mouse liver microsomes. **18a** was rapidly metabolized ($t_{1/2}$ = 1.3 min) in an NADPH-independent process. Compound **19a**, the ester of **18a** was also metabolized in an NADPH-independent process, although with a much longer half-life ($t_{1/2}$ = 23 min). These observations suggest that both the ester and amide bonds of **18a** may be liabilities. Compound **17b** was metabolized in an NADPH-dependent process with $t_{1/2}$ = 26 min. No decomposition of **17b** was observed when it was incubated in mouse plasma at 37 ^{o}C for 2 h. Based on these results **17b** was selected for further analysis. This compound displayed promising pharmacokinetics in mice, with a single 20 mg/kg oral dose [formulated using Tween 80 (1%) and 0.5% (w/v) methylcellulose in water (99%)] producing a maximum plasma concentration level comparable to MIC (C_{max} = 3 μM) in 0.5 h (T_{max}) with a plasma elimination half-life of 5 h. However, **17b** also displays high serum protein

binding (>99%), which suggests that the free drug concentration is insufficient to achieve in vivo efficacy. Further optimization of **17b** to increase antibacterial activity and decrease plasma protein binding is ongoing.

CONCLUSIONS

The SAR of the benzoxazole-based IMPDH inhibitors (**Q**-series) has been expanded by replacing the 4-pyridyl with phenyl or benzyl groups containing a variety of hydrogen bonding and ionic-dipole interacting substituents. Modifications to the central amide and benoxazole also reinforced the importance of interactions between the inhibitors and Glu458, and the spatial orientation provided by the central heterocycle, respectively. Two *Mtb*IMPDH2 inhibitors displayed antibacterial activity with MIC values ≤ 1 μM, and another eight compounds displayed MICs ≤ 5 μM. The antibacterial activity of the best candidate, **17b**, derives from inhibition of *Mtb*IMPDH2, yet is not affected by the presence of guanine. This observation alleviates the concern that guanine salvage can rescue bacteria from *Mtb*IMPDH2 inhibition. We note that previously reported *Mtb*IMPDH2 inhibitors were not as potent as the current compounds, and suggest that the ability of guanine to protect bacteria derived from incomplete inhibition of *Mtb*IMDPH2. The values of $K_{i,app}$ for the best inhibitors are 25-fold more potent than the indazole sulfonamide inhibitor that called into question the vulnerability of *Mtb*IMPDH2,[10] suggesting that guanine salvage cannot overcome potent inhibition. **17b** was also non-toxic at 25 μM in four human cell lines. Overall, this study provides further evidence for the vulnerability of *Mtb*IMPDH2.

Experimental Section

Synthetic Chemistry. Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used without further purification. All reactions were performed under a nitrogen atmosphere in dried glassware unless otherwise noted. All NMR spectra were obtained using a 400 MHz spectrometer. For ^{1}H NMR, all chemical shifts are reported in δ units ppm and are referenced to tetramethylsilane (TMS). All chemical shift values are also reported with multiplicity, coupling constants, and proton count. Coupling constants (J) are reported in hertz. Column chromatography was carried out on SILICYCLE SiliaFlash silica gel F60 (40−63 μm, mesh 230−400). High-resolution mass spectra were obtained using a Q-tof UE521 mass spectrometer (University of Illinois, SCS, and Mass Spectrometry Laboratory). HPLC conditions: All final compounds have a chemical purity of >98% as determined by analysis using a Varian Prostar (380-LC) HPLC instrument equipped with a quaternary pump and a Varian Microsorb MV-100-5 C-8 column (250 mm × 4.6 mm). UV absorption was monitored at 332 nm. All samples were dissolved in THF (1-2 mg/mL) and the injection volume was 20 μL. HPLC gradient was 30% acetonitrile and 70% water (both solvents contain 0.05% formic acid) with a total run time of 20 min and a flow rate of 1.0 mL/min.

General Procedure for the Synthesis of 5-nitro-2-phenylbenzo[d]oxazoles (4): To a stirred solution of 2-amino-4-nitrophenol (1 mmol) and aromatic aldehydes (1 mmol) in anhydrous xylene DarcoKB (300 mg) was added. The solution was stirred under O_2 atmosphere at 140 ºC for 6-8 h. After completion of the reaction as observed from TLC, reaction mixture was filtered with the aid of Celite, which was washed with hot ethyl acetate (3×20 mL). The filtrate was concentrated, and the products were either used directly or recrystallized using ethyl acetate and hexane.

General Procedure for the Synthesis of 5: 5-Nitro-2-arylbenzo[d]oxazole **4** (1 mmol) was dissolved in 10 mL EtOAc:MeOH (1:1) and 10% Pd/C (catalytic) was added and stirred well under a H_2 atmosphere for 3 h. After the successful completion, the reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The crude amines were used directly or recrystallized using ethyl acetate and hexane.

General Procedure for the Synthesis of Phenyl Ethers 6: Substituted phenol (1 mmol) was added to the solution of methyl (+)-methyl D-lactate (1.38 mmol) in anhydrous THF (6 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C, followed by PPh_3 (1.20 mmol) was added portion wise to the stirred solution. DEAD (1.50 mmol) was added dropwise to the above solution and stirred well at room temperature for 4 h. After completion, solvent was removed under reduced pressure and the crude residue was purified by column chromatography on silica gel using ethyl acetate/n-hexane (10:90) to yield corresponding phenyl ether as a colorless liquid (85-90% Yield).

General Procedure for Ester Hydrolysis for Preparation of 7: LiOH (1.5 mmol) was added portion wise to the stirred solution of ester **6** (1 mmol) in 5 mL THF:MeOH (2:3) at 0 ºC. The reaction was brought to room temperature and stirred well for 4 h. The solvents were removed under reduced pressure, 1N HCl was added to a pH of 4 and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried using anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude acids were used in the next step without purification.

(*S*)-2-(2,3-Dichlorophenoxy)-N-(2-(pyridin-4-yl)benzo[d]oxazol-5-yl)propanamide (8a): Prepared and characterized previously.[39]

General Procedure for Synthesis of 8b-8v: EDC·HCl (1.5 mmol) was added to the stirred solution of acid **7** (1 mmol) and amine **5** (1 mmol) in dry DMF at 0 ºC under nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. After completion of the reaction, excess water was added and extracted with ethyl acetate. The organic layer was washed with brine and dried using anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the crude residue was purified through column chromatography using ethyl acetate/n-hexane.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(2-methoxypyridin-4-yl)benzo[d]oxazol-5-yl)propanamide (8b): White solid (306 mg, 66%), ^{1}H NMR (DMSO-d_6) 10.34 (s, 1H, NH), 8.93 (d, J = 2.00 Hz, 1H, CH), 8.39-8.36 (m, 1H, CH), 8.06 (d, J = 2.00 Hz, 1H, CH), 7.69 (d, J = 8.80 Hz, 1H, CH), 7.52 (dd, J = 8.90 Hz, J = 2.00 Hz, 1H, CH), 7.30-7.26 (m, 1H, CH), 7.22 (t, J = 1.20 Hz, 1H, CH), 7.02-7.00 (m, 2H, 2×CH), 4.98 (q, J = 6.80 Hz, 1H, CH), 3.92 (s, 3H, -OCH_3), 1.59 (d, J = 6.80 Hz, 3H, CH_3). Purity 98% (t_R = 15.13). m.p. 165-166 C.

(*S*)-2-(2,3-dichlorophenoxy)-*N*-(2-(6-methoxypyridin-3-yl)benzo[d]oxazol-5-yl)propanamide (8c): White solid (315 mg, 69%), ^{1}H NMR (DMSO-d_6) 10.34 (s, 1H, NH), 8.92 (m, 1H, CH), 8.39-8.37 (m, 1H, CH), 8.06-8.05 (m, 1H, CH), 7.69-7.67 (m, 1H, CH), 7.53- 7.51 (m, 1H, CH), 7.27-7.20 (m, 2H, 2×CH), 7.01-6.98 (m, 2H, 2×CH), 5.01 (q, J = 6.80 Hz, 1H, CH), 3.91 (s, 3H, -OCH_3), 1.58 (d, J = 6.80 Hz, 3H, CH_3). Purity 97% (t_R = 14.85). m.p. 156-157 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-methoxyphenyl)benzo[d]oxazol-5-yl)propanamide (8d): White solid (351 mg, 77%), ^{1}H NMR (DMSO-d_6) 10.32 (s, 1H, NH), 8.08 (d, J = 8.80 Hz, 2H, 2×CH), 8.03 (d, J = 2.00 Hz, 1H, CH), 7.65 (d, J = 8.80 Hz , 1H, CH), 7.50-7.48 (m, 1H, CH), 7.27 (t, J = 8.20 Hz, 1H, CH), 7.21-7.19 (m, 1H, CH), 7.10 (d, J = 8.80 Hz, 2H, 2×CH),

7.01-6.99 (m, 1H, CH), 4.98 (q, *J* = 6.80 Hz, 1H, CH), 3.82 (s, 3H, -OCH_3), 1.59 (d, *J* = 6.80 Hz, 3H, CH_3). Purity 99% (t_R = 15.30). m.p. 230 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-(trifluoromethoxy)phenyl)benzo[d]oxazol-5-yl)propanamide (8e): White solid (357 mg, 70%), ^{1}H NMR (DMSO-d_6) 10.41 (s, 1H, NH), 8.31 (d, *J* = 8.80 Hz, 2H, 2×CH), 8.16 (m, 1H, CH), 7.77 (d, *J* = 9.00 Hz, 1H, CH), 7.61 (d, *J* = 8.00 Hz, 2H, 2×CH), 7.59 (m, 1H), 7.32 (t, *J* = 8.20 Hz, 1H, CH), 7.26-7.24 (m, 1H, CH), 7.05 (d, *J* = 6.40 Hz, 1H, CH), 5.03 (q, *J* = 6.20 Hz, 1H, CH), 1.64 (d, *J* = 6.40 Hz, 3H, CH_3). Purity 99% (t_R = 17.15). m.p. 140-141 C.

(*S*)-*N*-(2-(4-Cyanophenyl)benzo[d]oxazol-5-yl)-2-(2,3-dichlorophenoxy)propanamide (8f): Yellow solid (311 mg, 69%), ^{1}H NMR (DMSO-d_6) 10.38 (s, 1H, NH), 8.28 (dd, *J* = 8.20 Hz, *J* = 1.20, 2H, 2×CH), 8.14 (m, 1H, CH), 8.02 (dd, *J* = 8.20 Hz, *J* = 1.20 Hz, 2H, 2×CH), 7.75-7.73 (m, 1H, CH), 7.58-7.56 (m, 1H, CH), 7.26 (t, *J* = 1.60 Hz, 1H, CH), 7.20 (m, 1H, CH), 7.00 (d, *J* = 8.40 Hz m, 1H, CH), 5.01 (q, 1H, *J* = 6.80 Hz, CH), 1.58 (d, 3H, *J* = 6.80 Hz, CH_3). Purity 99% (t_R = 14.85). m.p. 183-184 C.

(*S*)-2-(2,3-Dichlorophenoxy)-N-(2-(4-fluorophenyl)benzo[d]oxazol-5-yl)propanamide (8g): White solid (351 mg, 79%), ^{1}H NMR (DMSO-d_6) 10.42 (s, 1H, NH), 8.26-8.23 (m, 2H, 2×CH), 8.13 (m, 1H, CH), 7.76-7.74 (m, 1H, CH), 7.59-7.44 (m, 3H, 3×CH), 7.32-7.26 (m, 2H, 2×CH), 7.06-7.04 (m, 1H, CH), 5.04 (q, *J* = 6.00 Hz, 1H, CH), 1.64 (d, *J* = 6.00 Hz, 3H, CH_3). Purity 98% (t_R = 15.73). m.p. 213-214 C.

Methyl (*S*)-4-(5-(2-(2,3-dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)benzoate (8h): White solid (329 mg, 68%), ^{1}H NMR (DMSO-d_6) 10.37 (s, 1H, NH), 8.29-8.26 (m, 1H, CH), 8.11 (d, *J* = 8.80 Hz, 2H, 2×CH), 7.86 (m, 1H, CH), 7.73 (dd, *J* = 8.80 Hz, *J* = 3.20 Hz, 1H, CH), 7.56 (d, *J* = 8.20 Hz, 1H, CH), 7.40 (m, 1H, CH), 7.27-7.20 (m, 2H, 2×CH), 7.00 (dd, *J* =

8.80 Hz, *J* = 2.00 Hz, 1H, CH), 5.02 (q, 1H, *J* = 6.80 Hz,, 1H, CH), 3.85 (s, 3H, -$COOCH_3$), 1.59 (d, 3H, *J* = 6.00 Hz, CH_3). Purity 99% (t_R = 15.60). m.p. 197-198 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-hydroxyphenyl)benzo[d]oxazol-5-yl)propanamide (8i): White solid (313 mg, 71%), ^{1}H NMR (DMSO-d_6) 10.30 (s, 1H, NH), 8.00-7.99 (m, 1H, CH), 7.97 (d, *J* = 8.40 Hz, 2H, 2×CH), 7.62 (d, *J* = 8.80 Hz, 1H, CH), 7.48-7.46 (m, 1H, CH), 7.27 (t, *J* = 8.00 Hz, 1H, CH), 7.00 (d, *J* = 8.40 Hz, 1H, CH), 6.91 (d, *J* = 8.80 Hz, 2H, 2×CH), 4.47 (q, *J* = 6.40 Hz, 1H, CH), 1.58 (d, *J* = 6.80 Hz, 3H, CH_3). Purity 99% (t_R = 13.11). m.p. 213.5-214.5 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(3-hydroxyphenyl)benzo[d]oxazol-5-yl)propanamide (8j): White solid (300 mg, 68%), ^{1}H NMR (DMSO-d_6) 10.38 (s, 1H, NH), 9.75 (s, 1H, OH), 8.12 (s, 1H, CH), 7.74 (d, *J* = 9.20Hz, 1H, CH), 7.61-7.55 (m, 3H, 3×CH), 7.40 (t, *J* = 8.00 Hz, 1H, CH), 7.32 (t, *J* = 8.20 Hz, 1H, CH), 7.26 (m, 1H), 7.06-7.01 (m, 2H, 2×CH), 5.03 (q, *J* = 6.80 Hz, 1H, CH), 1.64 (d, *J* = 6.40 Hz, 3H, CH_3). Purity 98% (t_R = 13.36). m.p. 259 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(2-hydroxyphenyl)benzo[d]oxazol-5-yl)propanamide (8k): White solid (287 mg, 65%), ^{1}H NMR (DMSO-d_6) 11.10 (s, 1H, OH), 10.41 (s, 1H, NH), 8.13 (d, *J* = 2.00 Hz, 1H, CH), 7.97 (dd, *J* = 7.60 Hz , *J* = 1.20 Hz, 1H, CH), 7.76 (d, *J* = 8.40 Hz, 1H, CH), 7.55 (dd, *J* = 8.40 Hz, *J* = 2.00 Hz, 1H, CH), 7.54-7.48 (m, 1H, CH), 7.26 (t, *J* = 8.00 Hz, 1H, CH), 7.19 (m, 1H, CH), 7.09-7.00 (m, 3H, 3×CH), 5.00 (q, *J* = 6.80 Hz, 1H, CH), 1.59 (d, *J* = 6.60 Hz, 3H, CH_3). Purity 99% (t_R = 16.99). m.p. 209-210 C.

(*S*)-2-(2,3-Difluorophenoxy)-*N*-(2-(pyridin-4-yl)benzo[d]oxazol-5-yl)propanamide (8l): White solid (268 mg, 68%), ^{1}H NMR (DMSO-d_6) 10.40 (s, 1H, NH), 8.79 (dd, *J* = 4.20 Hz, *J* = 1.20 Hz, 2H, CH), 8.16 (m, 1H, CH), 8.04 (t, *J* = 2.00 Hz, 2H, 2×CH), 7.77 (d, *J* = 8.80 Hz, 1H,

CH), 7.62 (m, 1H, CH), 7.62-7.60 (m, 1H, CH), 4.96 (q, J = 6.80 Hz, 1H, CH), 1.58 (d, J = 6.80 Hz, 3H, CH_3). Purity 99% (t_R = 14.45). m.p. 189-190 C.

(*S*)-2-(2,3-Difluorophenoxy)-*N*-(2-(4-methoxyphenyl)benzo[d]oxazol-5-yl)propanamide (8m): White solid (322 mg, 76%), ^{1}H NMR (DMSO-d_6) 10.32 (s, 1H, NH), 8.08 (d, J = 9.20 Hz, 2H, 2×CH), 8.02 (d, J = 1.60 Hz, 1H, CH), 7.64 (d, J = 8.40 Hz, 1H, CH), 7.50 (d, J = 6.80 Hz, 1H, CH), 7.10 (d, J = 8.80 Hz, 3H, 3×CH), 6.99 (m, 1H, CH), 6.89 (t, J = 2.00 Hz, 1H, CH), 4.95 (q, J = 6.80 Hz, 1H, CH), 1.57 (d, J = 6.40 Hz, 3H, CH_3). Purity 97% (t_R = 13.95). m.p. 179-180 C.

(*S*)-2-(2,3-Difluorophenoxy)-*N*-(2-(4-hydroxyphenyl)benzo[d]oxazol-5-yl)propanamide (8n): White solid (291 mg, 71%), ^{1}H NMR (DMSO-d_6) 10.36 (s, 1H, OH), 10.33 (s, 1H, NH), 8.04 (d, J = 2.00 Hz, 2H, 2×CH), 8.01 (m, 1H, CH), 7.67 (d, J = 8.80 Hz, 1H, CH), 7.52 (d, J = 8.20 Hz,, 1H, CH), 7.15-6.97 (m, 3H, 3×CH), 6.96 (d, J = 8.40 Hz, 2H, 2×CH), 4.99 (q, 1H, J = 6.80 Hz, CH), 1.62 (d, 3H, J = 6.40 Hz, CH_3). Purity 98% (t_R = 11.36). m.p. 182-183 C.

(*S*)-2-(2,3-Difluorophenoxy)-*N*-(2-(6-methoxypyridin-3-yl)benzo[d]oxazol-5-yl)propanamide (8o): White solid (311 mg, 69%), ^{1}H NMR (DMSO-d_6) 10.49 (s, 1H, NH), 8.97 (s,, 1H, CH), 8.40 (dd, J = 8.40 Hz, J = 2.00 Hz, 1H, CH), 8.11 (m, 1H, CH), 7.73 (d, J = 8.00 Hz, 1H, CH), 7.59-7.56 (m, 1H, CH), 7.19-6.98 (m, 3H, 3×CH), 6.94 (t, J = 2.40 Hz, 1H, CH), 5.00 (q, J = 6.40 Hz, 1H, CH), 3.17 (s, 3H, -OCH_3), 1.63 (d, J = 6.40 Hz, 3H, CH_3). Purity 99% (t_R = 13.25). m.p. 177-178 C.

(*S*)-*N*-(2-(4-Cyanophenyl)benzo[d]oxazol-5-yl)-2-(2,3-difluorophenoxy)propanamide (8p): White solid (289 mg, 69%), ^{1}H NMR (DMSO-d_6) 10.44 (s, 1H, NH), 8.34 (d, J = 8.40 Hz, 2H, 2×CH), 8.19 (d, J = 1.60 Hz, 1H, CH), 8.07 (d, J = 8.8 Hz, 2H, 2×CH), 7.79 (d, J = 8.80 Hz, 1H, CH), 7.65-7.62 (m, 1H, CH), 7.17-7.12 (m, 1H, CH), 7.06-7.02 (m, 1H, CH), 6.95 (t, J =

2.00 Hz, 1H, CH), 5.01 (q,, J = 6.40 Hz, 1H, CH), 1.63 (d, J = 6.8 Hz, 3H, CH_3). Purity 98% (t_R = 13.24). m.p. 176-177 C.

(*S*)-2-(2,3-Difluorophenoxy)-*N*-(2-(4-fluorophenyl)benzo[d]oxazol-5-yl)propanamide (8q): White solid (317 mg, 77%), ^{1}H NMR (DMSO-d_6) 10.40 (s, 1H, NH), 8.24 (dd, J = 8.60 Hz, J = 5.40 Hz, 2H, 2×CH), 8.13 (d, J = 1.60 Hz,1H, CH), 7.74 (d, J = 9.20 Hz,, 1H, CH), 7.58 (dd, J = 8.80 Hz, J = 2.00 Hz, 1H, CH), 7.46 (td, J = 8.80, J = 1.00 Hz, 2H, 2×CH), 7.17-7.12 (m, 1H, CH), 7.06-7.02 (m, 1H, CH), 6.96-6.92 (m, 1H, CH), 5.00 (q, J = 6.40 Hz, 1H, CH), 1.63 (d, J = 6.80 Hz, 3H, CH_3). Purity 97% (t_R = 14.29). m.p. 194-195 C.

(*S*)-*N*-(2-(4-Cyanophenyl)benzo[d]oxazol-5-yl)-2-(2,3,4-trifluorophenoxy)propanamide (8r): White solid (270 mg, 62%), ^{1}H NMR (DMSO-d_6) 10.42 (s, 1H, NH), 8.34 (d, J = 6.80 Hz, 2H, 2×CH), 8.19 (s, 1H, CH), 8.08 (d, J = 7.20 Hz , 2H, 2×CH), 7.80 (d, J = 8.00 Hz, 1H, CH), 7.63 (d, J = 8.40 Hz, 1H, CH), 7.27 (d, J = 10.00 Hz 1H, CH), 6.98 (m, 1H, CH), 4.98 (q, J = 6.40 Hz, 1H, CH), 1.61 (d, J = 6.80 Hz, 3H, CH_3). Purity 99% (t_R = 13.60). m.p. 184-185 C.

(*S*)-2-(2-Cyanophenoxy)-*N*-(2-(pyridin-4-yl)benzo[d]oxazol-5-yl)propanamide (8s): White solid (261 mg, 68%), ^{1}H NMR (DMSO-d_6) 10.49 (s, 1H, NH), 8.84 (dd, J = 4.00 Hz, J = 1.60 Hz, 2H, 2×CH), 8.21 (d, J = 2.00 Hz, 1H, CH), 8.09 (dd, J = 4.40 Hz, J = 1.60 Hz, 2H, 2×CH), 7.83-7.77 (m, 2H, 2×CH), 7.68-7.63 (m, 2H, 2×CH), 7.14-7.11 (m, 2H, 2×CH), 5.11 (q, J = 6.80 Hz, 1H, CH), 1.66 (d, J = 6.80 Hz, 3H, CH_3). Purity 98% (t_R = 12.67). m.p. 229-230 C.

(*S*)-2-(2-Cyano-3-fluorophenoxy)-*N*-(2-(pyridin-4-yl)benzo[d]oxazol-5-yl)propanamide (8t): White solid (285 mg, 71%), ^{1}H NMR (DMSO-d_6) 10.51 (s, 1H, NH), 8.84 (dd, J = 6.00 Hz, J = 1.00 Hz, 2H, 2×CH), 8.20 (s, 1H, CH), 8.09 (dd, J = 6.00 Hz, J = 1.00 Hz, 2H, 2×CH), 7.84-7.81 (m, 1H, CH), 7.73-7.63 (m, 2H, 2×CH), 7.10 (t, J = 8.80 Hz, 1H, CH), 6.99-6.97 (d, J =

8.80 Hz, 1H, CH), 5.16 (q, *J* = 6.40 Hz, 1H, CH), 1.67 (d, *J* = 6.80 Hz, 3H, CH_3). Purity 98% (t_R = 13.44). m.p. 189-dec C.

(*S*)-2-(2-Cyanophenoxy)-*N*-(2-(4-methoxyphenyl)benzo[d]oxazol-5-yl)propanamide (8u): White solid (318 mg, 77%), ^{1}H NMR (DMSO-d_6) 10.41 (s, 1H, NH), 8.13 (d, *J* = 8.80 Hz, 2H, 2×CH), 8.07 (m, 1H, CH), 7.78 (d, *J* = 8.80 Hz, 1H, CH), 7.72 (m, 2H, 3×CH), 7.54 (d, *J* = 8.00 Hz, 1H, CH), 7.21-7.04 (m, 4H, 4×CH), 5.09 (q, *J* = 6.00 Hz, 1H, CH), 3.82 (s, 3H, OCH_3), 1.65 (d, *J* = 6.40 Hz, 3H, CH_3). Purity 99% (t_R = 12.99). m.p. 162-163 C.

(*S*)-2-(2-(Benzyloxy)phenoxy)-*N*-(2-(4-cyanophenyl)benzo[d]oxazol-5-yl)propanamide (8v): White solid (337 mg, 69%), ^{1}H NMR (DMSO-d_6) 10.23 (s, 1H, NH), 8.34 (d, *J* = 8.00 Hz, 2H, 2×CH), 8.21 (m, 1H, CH), 8.08 (d, *J* = 8.00 Hz, 2H, 2×CH), 7.76 (d, *J* = 8.80 Hz, 1H, CH), 7.61-7.58 (m, 1H, CH), 7.50 (d, *J* = 7.60 Hz, 2H, 2×CH), 7.38-7.29 (m, 3H, 3×CH), 7.08 (m, 2H, 2×CH), 6.93(m, 2H, 2×CH), 5.17 (s, 2H, -OCH_2-), 4.82 (q, *J* = 6.40 Hz, 1H, CH), 1.57 (d, *J* = 6.80 Hz, 3H, CH_3). Purity 99% (t_R = 15.31). m.p. 144-145 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-methoxyphenyl)benzo[d]oxazol-5-yl)propanethioamide (9): A mixture of amide **8** (Ar = 4-OMePh, R = 2,3-diCl, 1 mmol) and Lawesson's reagent (1 mmol) was dissolved in dry dioxane (5 mL) and refluxed well for 2h. After cooling to room temperature, dioxane was removed under reduced pressure and the crude product was purified through column chromatography. White solid (335 mg, 71%), ^{1}H NMR (DMSO-d_6) 11.72 (s, 1H, NH), 8.10-8.08 (m, 3H, 3×CH), 7.76-7.70 (m, 1H, CH), 7.51-7.50 (m, 1H, CH), 7.32-7.29 (m, 1H, CH), 7.22-7.20 (m, 1H, CH), 7.14-7.02 (m, 2H, 2×CH), 7.02-7.00 (m, 1H, CH), 5.20 (q, *J* = 6.80 Hz, 1H, CH), 1.70 (d, *J* = 6.80 Hz, 3H, CH_3). Purity 98% (t_R = 17.09). m.p. 132-139 C.

(*S*)-2-(2,3-Dichlorophenoxy)propanal (10): DIBAL-H (1.0 M, in cyclohexane, 2 mmol) was added dropwise to the stirred solution of ester **6a** (1 mmol) in dry CH_2Cl_2 (4 mL) under a nitrogen atmosphere at -78 ºC. After completion of the addition, stirring was continued at the same temperature for 1 h. Aq. HCl (1 N, 3 mL) was added carefully. The bath was removed and the mixture was extracted with Et_2O (3 ×30 mL). The combined organic layers were washed with water (10 mL) and brine (10 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the crude reaction mixture was purified through column chromatography. Colorless oil (193 mg, 89%), ^{1}H NMR ($CDCl_3$) 9.72 (s, 1H, CH), 7.10 (m, 2H, 2×CH), 6.74-6.71 (m, 1H, CH), 4.62-4.60 (m, 1H, CH), 1.51 (d, J = 7.20 Hz, 3H, CH_3).

(*S*)-*N*-(2-(2,3-Dichlorophenoxy)propyl)-2-(4-methoxyphenyl)benzo[d]oxazol-5-amine (11): Aldehyde **10** (1 mmol) was dissolved in DCE (25 mL) at 0 °C. Benzoxazole amine **5** (Ar = 4-OMePh, 1 mmol) and sodium triacetoxyborohydride (2 mmol) were added to the above solution and stirred well. The reaction mixture was stirred for 1.5 h and quenched with satd. $NaHCO_3$ solution (20 mL), and the product was extracted with CH_2Cl_2 (2 × 20 mL). The organic layer was washed with brine and dried using anhydrous $MgSO_4$. The solvent was removed under reduced pressure and crude mixture was purified through column chromatography. Colorless oil (351 mg, 81%), ^{1}H NMR ($CDCl_3$) 8.11 (dd, J = 9.20 Hz, J = 2.00 Hz, 2H, 2×CH), 7.29 (dd, J = 8.80 Hz, J = 2.40 Hz, 1H, CH), 7.05-7.02 (m, 2H, 2×CH), 6.96 (dd, J = 9.00 Hz, J = 2.60 Hz, 2H, 2×CH), 6.92-6.91 (m, 1H, CH), 6.81-6.78 (m, 1H, CH), 6.59 (dd, J = 8.60 Hz, J = 2.20 Hz, 1H, CH), 4.64 (m, 1H, CH), 4.21 (bs, 1H, NH), 3.84 (s, 3H, -OCH_3), 3.47-3.39 (m, 2H, -CH_2-), 1.39 (d, J = 6.20 Hz, 3H, CH_3). Purity 98% (t_R = 17.11).

2-(4-Methoxyphenyl)-6-nitroimidazo[1,2-a]pyridine (12): 5-Nitropyridin-2-amine (1 mmol) and $NaHCO_3$ (2 mmol) was added to a stirred solution of 2-bromo-4′-methoxyacetophenone (1

mmol) in ethanol at room temperature and the reaction mixture was refluxed for overnight. After completion, the solvent was removed under reduced pressure and the crude material was dissolved in water and extracted with ethyl acetate. The organic layer was washed with brine and dried using anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The product was recrystallized using ethyl acetate and hexane. Brown solid (237 mg, 88%), ^{1}H NMR (DMSO-d_6) 9.76 (m, 1H, CH), 8.47 (s, 1H, CH), 7.90-7.88 (m, 3H, 3×CH), 7.67-7.65 (m, 1H, CH), 7.61 (d, 2H, J = 8.80 Hz, 2×CH, 3.78 (s, 3H, -OCH_3).

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-methoxyphenyl)imidazo[1,2-a]pyridin-6-yl)propanamide (13): To the stirred solution of 2-(4-methoxyphenyl)-6-nitroimidazo[1,2-a]pyridine **12** (1 mmol) in ethanol and ethyl acetate, $SnCl_2$ (2 mmol) was added and refluxed for 2h. Solvent was removed under reduced pressure and the reaction was quenched by adding a saturated solution of $NaHCO_3$ solution and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude amine was used in the next step without any purification. EDC·HCl (1.5 mmol) was added to the stirred solution of acid **7** (R = 2,3-diCl, 1 mmol) and amine (1 mmol) in dry DMF at 0 ºC under a nitrogen atmosphere. The reaction mixture was stirred overnight at room temperature. After completion of the reaction, water was added and extracted with ethyl acetate. The organic layer was washed with brine and dried using anhydrous $MgSO_4$. The solvent was removed under reduced pressure. The crude residue was purified through column chromatography to give the desired product. Brown solid (273 mg, 60%), ^{1}H NMR (DMSO-d_6) 10.35 (s, 1H, NH), 9.13 (s, 1H, CH), 8.33 (s, 1H, CH), 7.84 (d, J = 8.40 Hz, 2H, 2×CH), 7.53 (d, J = 8.00 Hz, 1H, CH), 7.24-6.92 (m, 6H, 6×CH), 5.02 (q, J = 6.40 Hz, 1H, CH), 3.79 (s, 3H, -OCH_3), 1.62 (d, J = 6.40 Hz, 3H, CH_3). Purity 97% (t_R = 12.00). m.p. 146-147 C.

2-(4-Methoxybenzyl)-5-nitrobenzo[d]oxazole (14): DABCO (2 mmol) was added to the stirred solution of 1-(2,2-dibromovinyl)-4-methoxybenzene (1 mmol) and 2-amino-4-nitrophenol (1 mmol) in NMP (5 mL) under nitrogen atmosphere. The solution was stirred well for 24 h at 100 ºC. After completion, reaction was quenched by the addition of water and extracted with ethyl acetate. The organic layer was washed with brine and dried using $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified using column chromatography. Yellow solid (171 mg, 60%), ^{1}H NMR ($CDCl_3$) 8.57-8.56 (m, 1H, CH), 8.28-8.25 (m, 1H, CH), 7.57-7.55 (m, 1H, CH), 7.31-7.29 (m, 2H, 2×CH), 6.91-6.89 (m, 2H, 2×CH), 4.66 (s, 2H, -CH_2-), 3.80 (s, 3H, -OCH_3).

(*S*)-2-(2,3-dichlorophenoxy)-*N*-(2-(4-methoxybenzyl)benzo[d]oxazol-5-yl)prop-anamide (15): 5-Nitro-2-arylbenzo[d]oxazoles (1 mmol) was dissolved in 10 mL EtOAc:MeOH (1:1) and 10% Pd/C (catalytic) was added and stirred well under a H_2 atmosphere for 2-3 h. After the successful completion, reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The crude amine was recrystallized using ethyl acetate and hexane. The amine was proceeded to next step without any chromatographic purification. EDC·HCl (1.5 mmol) was added to the stirred solution of acid **7** (R = 2,3-diCl, 1 mmol), amine (1 mmol) in dry DMF at 0 ºC under nitrogen atmosphere. Reaction mixture was stirred well for overnight at room temperature. After completion of the reaction, excess water was added and extracted with ethyl acetate. The organic layer was washed with brine and dried using $MgSO_4$. The solvent was removed under reduced pressure and crude residue was purified through column chromatography using ethyl acetate/n-hexane. White solid (315 mg, 67%), ^{1}H NMR ($CDCl_3$) 8.71 (s, 1H, NH), 7.97 (bs, 1H, CH), 7.46 (d, J = 8.00 Hz, 1H, CH), 7.39 (d, J = 8.20 Hz, 1H, CH), 7.28 (d, J = 8.00 Hz, 2H, 2×CH), 7.18-7.16 (m, 2H, 2×CH), 6.91-6.86 (m, 3H, 3×CH), 4.86

(q, J = 6.80 Hz, 1H, CH), 4.19 (s, 2H, -CH_2-), 3.78 (s, 3H, -OCH_3), 1.73 (d, J = 7.20 Hz, 3H, CH_3). Purity 97% (t_R = 14.78). m.p. 143-147 C.

Synthesis of 17: A mixture of L-alanine (163 mg, 1.82 mmol), Cs_2CO_3 (1.19 g, 3.65 mmol), and CuI (69.7 mg, 0.36 mmol) was added to the stirred solution of 1,2-dichloro-3-iodobenzene or 1,2-difluoro-3-iodobenzene (1.83 mmol) in dry DMF (3 mL) under a nitrogen atmosphere. The reaction mixture was heated at 90 °C for 24 h. After completion, the reaction mixture was allowed to cool and water was added. The pH was adjusted to 3−5 by the addition of 2N HCl and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude acid **16** was used in the next step without further purification. Acid **16** (1 mmol) was added to the stirred solution of amine **5** (Ar = 4-CNPh, 1 mmol) in dry DMF at 0 ºC followed by HATU (1.2 mmol) and stirred well for overnight at room temperature. After completion, excess water was added to the reaction and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography using ethyl acetate/n-hexane.

(*S*)-*N*-(2-(4-Cyanophenyl)benzo[d]oxazol-5-yl)-2-((2,3-dichlorophenyl)amino)propanamide (17a): Light brown powder (202 mg, 45%), ^{1}H NMR (DMSO-d_6) 10.39 (s, 1H, NH), 8.34 (d, J = 8.00 Hz, 2H, 2×CH), 8.20 (s, 1H, CH), 8.07 (d, J = 8.40 Hz, 2H, 2×CH), 7.81 (d, J = 8.40 Hz, 1H, CH), 7.60-7.58 (m, 1H, CH), 7.18 (t, J = 8.20 Hz, 1H, CH), 6.88 (d, J = 7.60 Hz, 1H, CH), 6.65 (d, J = 8.40 Hz, 1H, CH), 5.61 (d,J = 8.00 Hz, 1H, NH), 4.26 (m, 1H, CH), 1.54 (d, J = 6.8Hz, 3H, CH_3). Purity 99% (t_R = 14.95). m.p. 201-202 C.

(*S*)-*N*-(2-(4-Cyanophenyl)benzo[d]oxazol-5-yl)-2-((2,3-difluorophenyl)amino)propanamide (17b): Yellow powder (225 mg, 54%) ^{1}H NMR (DMSO-

d_6) 10.29 (s, 1H, NH), 8.33 (d, J = 8.40 Hz, 2H, 2×CH), 8.20 (m, 1H, CH), 8.07 (d, J = 8.40 Hz, 1H, CH), 7.77 (d, J = 8.80 Hz, 1H, CH), 7.60 (d, J = 8.80 Hz, 1H, CH), 6.96 (q, J = 7.60 Hz, 1H, CH), 6.59 (m, 1H, CH), 6.51 (t, J = 7.60 Hz, 1H, CH), 5.92 (d,J = 7.60 Hz, 1H, NH), 4.18 (q, J = 6.80 Hz, 1H, CH), 1.51 (d, J = 6.40 Hz, 3H, CH_3). Purity 99% (t_R = 12.95). m.p. 197-198 C.

Synthesis of 18: Arylbenzoxazole **8i** or **8j** (1 mmol) was dissolved in dry DMF (5 mL) in an oven dried round bottom flask and cooled to 0 ºC. K_2CO_3 (2 mmol) was added to the cold solution and stirred well for ten minutes, then ethyl bromoacetate (1.5 mmol) was added and stirred well at room temperature for 6 h. After completion, the reaction was quenched with water and extracted with ethyl acetate (20 mL) and the organic layer was washed with brine. The organic layer was dried using anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified through column chromatography using hexane: ethyl acetate.

Ethyl (*S*)-2-(4-(5-(2-(2,3-dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)phenoxy)acetate (18a): White powder (438 mg, 83%), ^{1}H NMR (DMSO-d_6) 10.31 (s, 1H, NH), 8.08-8.03 (m, 3H, 3×CH), 7.66-7.64 (m, 1H, CH), 7.50-7.48 (m, 1H, CH), 7.29-6.98 (m, 5H, 5×CH), 4.97 (q, J = 6.40 Hz, 1H, CH), 4.87 (s, 2H, -OCH_2-), 4.13 (q, J = 7.20 Hz, 2H, -OCH_2-), 1.58 (d, J = 6.80 Hz, 3H, CH_3), 1.17 (t, J = 7.20 Hz, 3H, CH_3). Purity 99% (t_R = 15.02). m.p. 144-145 C.

Ethyl(*S*)-2-(3-(5-(2-(2,3-dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)phenoxy)acetate (18b): White powder (422 mg, 80%), ^{1}H NMR (DMSO-d_6) 10.35 (s, 1H, NH), 8.09 (m, 1H, CH), 7.76 (d, J = 8.00 Hz, 1H, CH), 7.70 (d, J = 8.00 Hz, 1H, CH), 7.62-7.61 (m, 1H, CH), 7.55-7.47 (m, 2H, 2×CH), 7.27 (t, J = 6.20 Hz, 1H, CH), 7.21-7.18 (m, 2H, 2×CH), 7.02-7.00 (m, 1H, CH), 4.98 (q, J = 6.80 Hz, 1H, CH), 4.89 (s, 2H, -OCH_2-), 4.15 (q, J = 7.20

Hz, 2H, -OCH_2-), 1.59 (d, J = 6.40 Hz, 3H, CH_3), 1.18 (t, J = 6.80 Hz, 3H, CH_3). Purity 99% (t_R = 13.28). m.p. 175-176 C.

Synthesis of 19: The ester **18** was subjected to ester hydrolysis using the general procedure described for **7**.

(*S*)-2-(4-(5-(2-(2,3-Dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)phenoxy)acetic acid (19a): White powder (440 mg, 88%), ^{1}H NMR (DMSO-d_6) 10.36 (s, 1H, NH), 8.12 (d, J = 8.80 Hz, 2H, 2×CH), 8.08 (m, 1H, CH), 7.70 (d, J = 8.80 Hz, 1H, CH), 7.50 (m, 1H, CH), 7.32 (t, J = 8.40 Hz, 1H, CH), 7.25 (m, 1H), 7.13 (d, J = 8.80 Hz, 2H, 2×CH), 7.05 (d, J = 8.00 Hz, 1H, CH), 5.02 (q, J = 6.40 Hz, 1H, CH), 4.82 (s, 2H, -OCH_2-), 1.63 (d, J = 6.40 Hz, 3H, CH_3). Purity 98% (t_R = 12.64). m.p. 238-239 C.

(*S*)-2-(3-(5-(2-(2,3-Dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)phenoxy)acetic acid (19b): White powder (430 mg, 86%), ^{1}H NMR (DMSO-d_6) 13.07 (bs, 1H, COOH), 10.35 (s, 1H, NH), 8.09 (m, 1H, CH), 7.76-7.70 (m, 2H, 2×CH), 7.59-7.46 (m, 3H, 3×CH), 7.26 (t, J = 8.00 Hz, 1H, CH), 7.21-7.14 (m, 2H, 2×CH), 7.01 (d, J = 8.00 Hz, 1H, CH), 4.98 (q, J = 6.40 Hz, 1H, CH), 4.79 (s, 2H, -OCH_2-), 1.59 (d, J = 6.40 Hz, 3H, CH_3). Purity 97% (t_R = 12.77). m.p. 191-dec C.

Prop-2-yn-1-yl (*S*)-2-(4-(5-(2-(2,3-dichlorophenoxy)propanamido)benzo[d]oxa-zol-2-yl)phenoxy)acetate (20): Arylbenzoxazole **19a** (1 mmol) was dissolved in dry DMF (5 mL) in an oven dried round bottom flask and cooled to 0 ºC. K_2CO_3 (2 mmol) was added to the cold solution and stirred well for ten minutes, followed by propargyl bromide 80% solution in toluene (1.5 mmol) was added and stirred well at room temperature for 6 h. After completion, reaction was quenched with water and extracted with ethyl acetate (20 mL) and organic layer was washed with brine. The organic layer was dried using $MgSO_4$, filtered and concentrated under reduced

pressure. The crude product was purified through column chromatography. White solid (435 mg, 81%), ^{1}H NMR (DMSO-d_6) 10.37 (s, 1H, NH), 8.12 (d, J = 8.80 Hz, CH, 2×CH), 8.08 (m, 1H, CH), 7.72-7.70 (d, J = 8.80 Hz, 1H, CH), 7.55 (d, J = 8.00 Hz, 1H, CH), 7.34-7.26 (m, 2H, 2×CH), 7.17 (d, J = 8.40 Hz, 2H, 2×CH), 7.05 (d, J = 8.00 Hz, 1H, CH), 5.03-5.00 (m and s, 3H, CH, -OCH_2-), 4.83 (s, 2H, -OCH_2-), 3.64 (bs, 1H, CH), 1.63 (d, J = 6.00 Hz, 3H, CH_3). Purity 98% (t_R = 14.69). m.p. 132-133 C.

(*S*)-4-(5-(2-(2,3-Dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)-*N*-hydroxybenzamide (21): The ester **8h** (1 mmol) was dissolved in methanol and hydroxylamine hydrochloride (2 mmol) was added at 0 ºC and stirred well. KOH (3 mmol) was added to the cold solution and stirred overnight at room temperature. After completion, the solvent was removed under reduced pressure and the residue was dissolved in water and extracted with ethyl acetate. The organic layer was washed with brine and dried using anhydrous $MgSO_4$. Solvent was removed under reduced pressure and the product was purified using column chromatography. White powder (310 mg, 65%), ^{1}H NMR (DMSO-d_6) 13.29 (bs, 1H, -OH), 10.37 (s, 1H, NH), 8.26 (d, J = 8.00 Hz, 2H, 2×CH), 8.11 (t, J = 8.40 Hz, 3H, 3×CH), 7.74(d, J = 9.20 Hz, 1H, CH), 7.56 (d, J = 8.80 Hz, 1H, CH), 7.26 (t, J = 8.00 Hz, 1H, CH), 7.20 (m, 1H, CH), 7.00 (d, J = 8.00 Hz, 1H, CH), 4.99 (q, J = 6.80 Hz, 1H, CH), 1.59 (d, J = 6.80 Hz, 3H, CH_3). Purity 98% (t_R = 13.37). m.p. 253-254 C.

(*S*)-4-(5-(2-(2,3-Dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)benzoic acid (22): Ester **8h** (1 mmol) was dissolved in THF:MeOH:H_2O (3:1:1) at room temperature and NaOH (1 mmol) was added and stirred well for 2 h. After completion, the solvents were removed under reduced pressure. The crude residue was dissolved in water and pH was adjusted to 3−5 by the addition of 2N HCl and extracted with ethyl acetate. The organic layer was washed with brine

and dried using anhydrous $MgSO_4$. The crude acid was recrystallized using ethyl acetate and hexane. White powder (423 mg, 90%), ^{1}H NMR (DMSO-d_6) 10.43 (s, 1H, NH), 8.30 (d, J = 8.40 Hz, 2H, 2×CH), 8.15 (t, J = 9.00, 3H, 3×CH), 7.79 (d, J = 8.80 Hz, 1H, CH), 7.61 (d, J = 8.80 Hz, 1H, CH), 7.32 (t, J = 8.20 Hz, 1H, 1×CH), 7.26-7.24 (m, 1H, CH), 7.05 (d, J = 8.00 Hz, 1H, CH), 5.04 (q, J = 6.80 Hz, 1H, CH), 1.64 (d, J = 6.40 Hz, 3H, CH_3). Purity 98% (t_R = 13.33). m.p. 244 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-(hydrazinecarbonyl)phenyl)benzo[d]oxazol-5-yl)propanamide (23): Hydrazine hydrate (3 mmol) was added to the stirred solution of **8h** (1 mmol) in ethanol (10 mL) at room temperature and refluxed for 5 h. After completion, the solvent was removed under reduced pressure, water was added and extracted with ethyl acetate. The organic layer was washed with brine and dried using anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the crude mixture was purified through column chromatography. White powder (324 mg, 67%), ^{1}H NMR (DMSO-d_6) 10.41 (s, 1H, NH), 9.97 (s, 1H, NH), 8.25 (d, J = 6.80 Hz, 2H, 2×CH), 8.16 (m, 1H, CH), 8.03 (d, J = 6.80 Hz, 2H, 2×CH), 7.77 (d, J = 9.2 Hz, 1H, CH), 7.60 (d, J = 8.40 Hz, 1H, CH), 7.32 (t, J = 7.60, 1H, CH), 7.25 (m, 1H), 7.05 (d, J = 8.40 Hz, 1H, CH), 5.03 (q, J = 6.80 Hz, 1H, CH), 4.60 (bs, 2H, NH_2), 1.58 (d, J = 6.80 Hz, 3H, CH_3). Purity 97% (t_R = 10.37). m.p. 247-248 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-(5-oxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)phenyl)benzo[d]oxazol-5-yl)propanamide (24): To the stirred solution of **23** (1 mmol) in anhydrous DMF (2 mL), carbonyldiimidazole (1.2 mmol) and diisopropylethylamine (1.2 mmol) was added. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with water and extracted with ethyl acetate. The combined extract was washed with brine, dried using anhydrous $MgSO_4$, filtered and concentrated. The residue was purified through

colum chromatography (2−8% MeOH gradient in CH_2Cl_2) to give the title compound. White solid (336 mg, 66%), ^{1}H NMR (DMSO-d_6) 12.78 (s, 1H, NH), 10.44 (s, 1H, NH), 8.33 (d, J = 7.60 Hz, 2H, 2×CH), 8.17(m, 1H, CH), 8.01 (d, J = 8.00 Hz, 2H, 2×CH), 7.78 (d, J = 5.20 Hz, 1H, CH), 7.61 (d, J = 8.80 Hz, 1H, CH), 7.32 (t, J = 8.40 Hz, 1H, CH), 7.25 (m, 1H, CH), 7.06 (d, J = 8.00 Hz, 1H, CH),, 5.04 (q, J = 6.80 Hz, 1H, CH), 1.64 (d, J = 6.00 Hz, 3H, CH_3). Purity 99% (t_R = 13.05). m.p. 288-dec C.

(*S*,*Z*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-(*N*'-hydroxycarbamimidoyl)phenyl)benzo[d]oxazol-5-yl)propanamide (25): (*S*)-*N*-(2-(4-cyanophenyl)benzo[d]oxazol-5-yl)-2-(2,3-dichlorophenox-y)propanamide **8f** (1 mmol) was dissolved in anhydrous ethanol (5 mL) followed by hydroxylamine hydrochloride (3 mmol) and triethylamine (6 mmol) was added. The reaction mixture was refluxed for 4 h and the solvent was removed under reduced pressure. The residue was suspended in water and extracted with DCM (20 mL). Organic layer was washed with brine and dried using anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified through column chromatography (2−8% MeOH gradient in CH_2Cl_2) to give the title compound. White solid (396 mg, 82%), ^{1}H NMR (DMSO-d_6) 10.34 (s, 1H, NH), 9.85 (s, 1H, OH), 8.13-8.08 (m, 2H, 2×CH), 7.86-7.84 (m, 2H, 2×CH), 7.71-7.68 (m, 1H, CH), 7.53-7.51 (m, 1H, CH), 7.28-7.18 (m, 3H, 3×CH), 7.00-6.98 (m, 1H, CH), 5.90 (s, 2H, NH_2), 4.97 (m, 1H, CH), 1.57 (m, 3H, CH_3). Purity 97% (t_R = 14.97). m.p. 196-197 C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(4-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)phenyl)benzo-[d]oxazol-5-yl)propanamide (26): To the stirred solution of **25** (1 mmol) in anhydrous DMF (2 mL), carbonyldiimidazole (1.2 mmol) and diisopropylethylamine (1.2 mmol) was added. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture

was diluted with water and extracted with ethyl acetate. The combined extract was washed with brine, dried using anhydrous $MgSO_4$, filtered and concentrated. The residue was purified through colum chromatography (2−8% MeOH gradient in CH_2Cl_2) to give the title compound. White solid (321 mg, 61%), ^{1}H NMR (DMSO-d_6) 10.49 (s, 1H, NH), 9.60 (bs, 1H, NH), 8.32 (d, J = 8.40 Hz, 2H, 2×CH), 8.15 (m, 1H, CH), 7.99 (d, J = 8.40 Hz, 2H, 2×CH), 7.72 (m, 1H, CH), 7.58 (m, 1H, CH), 7.27 (t, J = 7.60 Hz, 1H, CH), 7.21 (m, 1H, CH), 7.02 (d, J = 8.40 Hz, 1H, CH), 5.03 (q, J = 6.80 Hz, 1H, CH), 1.59 (d, J = 6.80 Hz, 3H, CH_3). Purity 98% (t_R = 13.04). m.p. 260 C.

(*S*)-*N*-(2-(4-(1H-Tetrazol-5-yl)phenyl)benzo[d]oxazol-5-yl)-2-(2,3-dichlorophenoxy)propanamide (27): A mixture of (*S*)-*N*-(2-(4-cyanophenyl)benzo[d]oxazol-5-yl)-2-(2,3-dichlorophenoxy)propanamide **8f** (1 mmol), NaN_3 (2 mmol), and NH_4Cl (2 mmol) in DMF (1.5 mL) was heated at 100 °C for 6 h. Solvent was removed under reduced pressure, water was added and extracted with ethyl acetate. The organic layer was washed with brine, dried using anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was purified through column chromatography. White solid (306 mg, 62%), ^{1}H NMR (DMSO-d_6) 11.11 (s, 1H, NH), 10.38 (s, 1H, NH), 8.36-8.34 (m, 1H, CH), 8.22 (d, J = 5.80 Hz, 2H, 2×CH), 8.13 (m, 1H, CH), 7.90 (m, 1H, CH), 7.76-7.70 (m, 1H, CH), 7.59-7.50 (m, 1H, CH), 7.30-7.21 (m, 2H, 2×CH), 7.00 (m, 1H, CH), 5.01 (m, 1H, CH), 1.59 (m, 3H, CH_3). Purity 99% (t_R = 12.25). m.p. 257 C.

(*S*)-4-(5-(2-(2,3-Dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)benzamide (28): To the stirred solution of (*S*)-*N*-(2-(4-cyanophenyl)benzo[d]oxazol-5-yl)-2-(2,3-dichlorophenoxy)prop-anamide **8f** (1 mmol) in dry tert-butyl alcohol (4 mL/mmol), KOtBu (3 mmol), was added. The reaction mixture was stirred at room temperature for 12 h under a

nitrogen atmosphere, and progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with brine, dried using anhydrous $MgSO_4$ and solvent was removed under reduced pressure. The crude mixture was purified through column chromatography. White solid (253 mg, 54%), ^{1}H NMR (DMSO-d_6) 10.36 (s, 1H, NH), 8.21 (d, J = 8.00 Hz, 2H, 2×CH), 8.12 (s, 2H, NH_2), 8.03 (d, J = 8.00 Hz, 2H, 2×CH), 7.73 (d, J = 8.40 Hz, 1H, CH), 7.55 (d, J = 8.40 Hz, 1H, CH), 7.51 (m, 1H, CH), 7.26 (t, J = 8.00 Hz, 1H, CH), 7.20 (m, 1H), 7.00 (d, J = 8.40 Hz, 1H, CH) 4.98 (q, J = 6.80 Hz, 1H, CH), 1.59 (d, J = 6.80 Hz, 3H, CH_3). Purity 98% (t_R = 11.56). m.p. 232 C.

(*S*)-*N*-(2-(4-(Aminomethyl)phenyl)benzo[d]oxazol-5-yl)-2-(2,3-dichlorophenoxy)propanamide (29): To a stirred solution of (*S*)-*N*-(2-(4-cyanophenyl)benzo[d]oxazol-5-yl)-2-(2,3-dichloroph-enoxy)propanamide **8f** (2.0 mmol) in THF: MeOH (15 mL), Boc_2O (873 mg, 4.0 mmol) and $NiCl_2{\cdot}6H_2O$ (48 mg, 0.2 mmol) were added at 0 ºC and stirred for five minutes. After five minutes, $NaBH_4$ (530 mg, 14.0 mmol) was added in small portions over 30 min followed by stirring at room temperature for 2h. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The crude residue was poured into 1N HCl and extracted with ethyl acetate. The organic layer was washed with brine and dried using anhydrous $MgSO_4$. Solvent was removed under reduced pressure and crude reaction mixture was purified through column chromatography. Trifluoroacetic acid (1 mL) was added to the stirred solution of Boc protected amine (1 mmol) in dry DCM (5 mL) at 0 ºC. After 4 h, the reaction was quenched with a saturated $NaHCO_3$ solution and extracted with DCM. The organic layer was washed with brine and dried using anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the

product was recrystallized using ethyl acetate and hexane. White solid (305 mg, 67%), ^{1}H NMR (DMSO-d_6) 10.40 (s, 1H, NH), 8.14-8.10 (m, 3H, 3×CH), 7.73 (d, J = 9.20 Hz , 1H, CH), 7.58-7.56 (m, 3H, 3×CH), 7.32 (t, J = 8.20 Hz, 1H, CH), 7.26-7.24 (m, 1H, CH), 7.05 (d, J = 8.00 Hz, 1H, CH), 5.03 (q, J = 6.80 Hz, 1H, CH), 3.82 (s, 3H, -OCH_3), 1.64-1.63 (d, J = 6.00 Hz, 3H, CH_3). Purity 97% (t_R = 14.63). m.p. 189-dec C.

(*S*)-2-(2,3-Dichlorophenoxy)-*N*-(2-(2-oxo-1,2-dihydropyridin-4-yl)benzo[d]oxazol-5-yl)propanamide (30): To the stirred solution of **8b** (1 mmol) in DMF, LiCl (5 mmol) and p-TSA (5 mmol) was added and stirred well for 2 h at 120 ºC. After completion, reaction was quenched with saturated $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with brine and dried using $MgSO_4$. Solvent was removed under reduced pressure and crude residue was purified through column chromatography. White solid (341 mg, 77%), ^{1}H NMR (DMSO-d_6) 12.20 (bs, 1H, NH), 10.30 (s, 1H, NH), 8.19 (s, 1H, CH), 8.02 (dd, J = 9.80 Hz, J = 2.20 Hz, 1H, CH), 7.98 (m, 1H, CH), 7.61 (d, J = 9.20 Hz, 1H, CH), 7.46 (m, 1H, CH), 7.27 (t, J = 8.00 Hz, 1H, CH), 7.19 (m, 1H), 6.99 (d, J = 8.80 Hz, 1H, CH), 6.47 (d, J = 9.60 Hz, 1H, CH), 4.97 (q, J = 6.40 Hz, 1H, CH), 1.58 (d, J = 6.80 Hz, 3H, CH_3). Purity 97% (t_R = 10.25). m.p. 240-241 C.

(*S*)-4-(5-(2-(2,3-Dichlorophenoxy)propanamido)benzo[d]oxazol-2-yl)pyridine1-oxide (31): *m*-Chloroperoxybenzoic acid (2 mmol) was added to the stirred solution of **8a** (1 mmol) in dry DCM at rt and stirred well for overnight. After completion, reaction was quenched with saturated $NaHCO_3$ solution and extracted with DCM. The organic layer was washed with brine, dried using $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified through column chromatography. White solid (327 mg, 74%), ^{1}H NMR (DMSO-d_6) 10.43 (s, 1H, NH), 8.38 (d, J = 6.80 Hz, 2H, 2×CH), 8.17 (m, 1H, CH), 8.09 (d, J = 6.80 Hz, 2H, 2×CH),

7.78-7.76 (m, 1H, CH), 7.63-7.60 (m, 1H, CH), 7.16-7.12 (m, 1H, CH), 7.06-7.02 (m, 1H, CH), 6.95-6.92 (m, 1H, CH), 5.00 (q, *J* = 6.80 Hz, 1H, CH), 1.62 (d, *J* = 6.80 Hz, 3H, CH_3). Purity 99% (t_R = 10.80). m.p. 125-dec C.

(*S*)-2-(2-(Aminomethyl)phenoxy)-*N*-(2-(4-methoxyphenyl)benzo[d]oxazol-5-yl)propanamide (32): The reaction was proceeded with **8u** using the procedure, which used for the synthesis of **29**. White solid (283 mg, 68%), ^{1}H NMR (DMSO-d_6) 11.10 (bs, 1H, NH), 8.13-8.11 (d, *J* = 9.2 Hz, 2H, 2×CH), 8.02-8.01 (m, 1H, CH), 7.67 (d, *J* = 8.2 Hz, 1H, CH), 7.54-7.51 (m, 1H, CH), 7.29-7.14 (m, 3H, 3×CH), 7.04-7.02 (m, 1H, CH), 6.93-6.91 (m, 1H, CH), 5.06 (q, *J* = 6.80 Hz, 1H, CH), 3.71 (s, 3H, -OCH_3), 1.62 (d, *J* = 6.80 Hz, 3H, CH_3). Purity 97% (t_R = 14.31). m.p. 160-161 C.

(*S*)-*N*-(2-(4-Cyanophenyl)benzo[d]oxazol-5-yl)-2-(2-hydroxyphenoxy)propanamide (33): Compound **8v** (1 mmol) was dissolved in 10 mL EtOAc: MeOH (1:1) and 10% Pd/C (catalytic) was added and stirred well under a H_2 atmosphere for 3 h. After the successful completion, reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The crude product was purified through column chromatography. White solid (359 mg, 90%), ^{1}H NMR (DMSO-d_6) 10.26 (s, 1H, NH), 9.29 (s, 1H, OH), 8.34 (d, *J* = 8.4 Hz, 2H, 2×CH), 8.22-8.21 (m, 1H, CH), 8.08 (d, *J* = 8.80 Hz, 2H, 2×CH), 7.81 (d, *J* = 8.80 Hz, 1H, CH), 7.68-7.66 (m, 1H, CH), 7.05-7.03 (d, *J* = 7.6 Hz, 1H, CH), 6.88-6.83 (m, 2H, 2×CH), 6.71-6.73 (m, 1H, CH), 4.87 (q, *J* = 6.80 Hz, 1H, CH), 1.57 (d, *J* = 6.80 Hz, 3H, CH_3). Purity 99% (t_R = 11.10). m.p. 185-186 C.

Methyl (*S*)-2-(2-bromo-3-formylphenoxy)propanoate (34): 2-Bromo-3-hydroxybenzaldehyde (1 mmol) was added to the solution of (+)-methyl-D-lactate (1.38 mmol) in anhydrous THF (6 mL) under a nitrogen atmosphere and the solution was cooled to 0 °C. PPh_3

(1.20 mmol) was added portion wise and stirred for 10 min followed by the dropwise addition of DEAD (1.50 mmol) over 20 min. The reaction mixture was stirred for 2 h at room temperature. After completion, the solvent was removed under reduced pressure and the crude mixture was purified through column chromatography using ethyl acetate/n-hexane (10:90) to yield methyl ((S)-2-(2-bromo-3-formylphenoxy)propanoate (236 mg, 83%) as a white solid.

Methyl (*S*)-2-(2-bromo-3-((methoxymethoxy)methyl)phenoxy)propanoate (35): Under a nitrogen atmosphere, $NaBH_4$ (1.5 mmol) was added to the stirred solution of ((S)-2-(2-bromo-3-formylphenoxy)propanoate **34** (1 mmol) in ethanol at 0 ºC. After 1 h at room temperature, the reaction was quenched with a saturated $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with brine and dried using anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was dissolved in dry DCM and cooled to 0 ºC. DIPEA (5 mmol) was added, followed by dropwise addition of MOMCl (3 mmol) and stirred for overnight at room temperature. After completion, the reaction was quenched with saturated NH_4Cl and extracted with DCM. The organic layer was dried using anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The crude residue was purified through column chromatography. White solid (239 mg, 72%), ^{1}H NMR (DMSO-d_6) 7.32-7.28 (m, 1H, CH), 7.12-7.09 (m, 1H, CH), 6.90-6.88 (m, 1H, CH), 5.06 (m, 1H, CH), 4.70 (bs, 3H, -OCH_3), 4.61-4.51 (m, 4H, 2×-OCH_2-), 3.68 (s, 3H, -$COOCH_3$), 1.55 (m, 3H, CH_3).

Methyl (*S*)-2-(3-((methoxymethoxy)methyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxa-borolan-2-yl)phenoxy)propanoate (36): To a solution of methyl (S)-2-(2-bromo-3-((methoxymethoxy)methyl)phenoxy)propanoate **35** (400 mg, 1.145 mmol) in 1,4-dioxane (6 mL) was added KOAc (483 mg, 4.923 mmol), Pin_2B_2 (349 mg, 1.37 mmol) and $Pd(Ph_3P)_2Cl_2$ (80 mg, 0.114 mmol) under an argon atmosphere. The reaction flask was placed under vacuum and then

backfilled with argon (two times). Then the reaction was stirred at 95 ºC for overnight. The solvent was removed under reduced pressure and the residue was suspended in water and extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was purified through column chromatography using hexane/ethyl acetate. White solid (243 mg, 64%), ^{1}H NMR (DMSO-d_6) 7.28-7.24 (m, 1H, CH), 6.93-6.88 (m, 1H, CH), 6.68-6.66 (m, 1H, CH), 4.90-4.86 (m, 1H, CH), 4.56 (bs, 4H, 2×-OCH_2-), 4.47 (bs, 3H, -OCH_3), 3.67 (s, 3H, -$COOCH_3$), 1.45 (m, 3H, CH_3), 1.29 (bs, 12H, 4×CH_3).

(*S*)-*N*-(2-(4-Cyanophenyl)benzo[d]oxazol-5-yl)-2-((1-hydroxy-1,3-dihydrobenzo-[c][1,2]oxaborol-7-yl)oxy)propanamide (37): Ester **36** (1 mmol) was dissolved in 5 mL THF: MeOH (2:3) and stirred at 0 ºC. LiOH (1.5 mmol) was added portion wise to the solution and stirred well at room temperature for 4 h. The solvents were removed under reduced pressure, crude residue was dissolved in ethyl acetate and 1N HCl was added until a pH of 4 was reached. The product was extracted with ethyl acetate and the organic extracts were combined, washed with brine, dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and crude acid was used in the next step without chromatographic purification. EDC·HCl (1.5 mmol) was added to the stirred solution of acid (1 mmol), amine **5** (Ar = 4-CNPh, 1 mmol) in dry DMF at 0 ºC under nitrogen atmosphere. Reaction mixture was stirred well for overnight at room temperature. After completion of the reaction, excess water was added and extracted with ethyl acetate. The organic layer was washed with brine and dried using $MgSO_4$. The solvent was removed under reduced pressure. The crude residue was purified through column chromatography give desired coupled product. To a solution of this MOM protected material (140 mg, 0.363 mmol) in THF (0.9 mL) 4 N HCl (0.43 mL, 18.1 mmol) was added. The reaction

was stirred at room temperature for 4 h, upon completion ethyl acetate was added and extracted. The organic phase was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified through column chromatography using hexane/ethyl acetate. Brown solid (122 mg, 28%).^{1}H NMR (DMSO-d_6) 10.13 (s, 1H, NH), 9.20 (s, 1H, OH), 8.34 (d, J = 8.4 Hz, 2H, 2×CH), 8.22 (m, 1H, CH), 8.08 (d, J = 8.40 Hz, 2H, 2×CH), 7.80 (d, J = 9.2 Hz, 1H, CH), 7.70 (m, 1H, CH), 7.45 (m, 1H, CH), 7.04 (d, J = 7.60 Hz, 1H, CH), 6.93 (d, J = 7.60 Hz, 1H, CH), 5.03 (q, J = 6.80 Hz, 1H, CH), 4.98 (s, 2H, -OCH_2-), 1.62 (d, J = 6.8 Hz, 3H, CH_3). Purity 97% (t_R = 11.27). m.p. hygroscopic.

Inhibition of *Mtb*IMPDH. The $K_{i,app}$ values were determined by measuring the initial velocities at varying concentrations of the inhibitors (1-10,000 nM) with fixed concentrations of IMP (0.5 mM) and NAD^+ (1.5 mM) and *Mtb*IMPDH2 (20-50 nM). Inhibition of human IMPDH2 was assayed IMP using (0.25 mM) and NAD^+ (0.060 mM) and *h*IMPDH2 (250 nM). Inhibition of human GMPR2 was assayed using GMP (0.050 mM), NADPH (0.045 mM) and enzyme (100 nM). The assay buffer contained 50 mM TrisCl, pH 8.0, 100 mM KCl and 1 mM dithiothreitol.

The values of $K_{i,app}$ were obtained using the equations (3) and (4)

$$v_i = v_0 / (1 + [I]/ IC_{50}) \quad (3)$$

$$K_{i,app} = IC_{50} - [E]/2 \quad (4)$$

where v_i is the initial velocity in the presence of inhibitor and v_0 is the initial velocity in the absence of the inhibitor. If the IC_{50} value is comparable to the enzyme concentration, the Morrison tight binding equation was used to determine $K_{i,app}$ (5)

$v_i/v_0 = 1-(([E] + [I] + K_{i,app}) - (([E] + [I] + K_{i,app})^2 - 4[E][I])^{0.5})/(2[E])$ (5)

where [E] is the concentration of the enzyme. All the initial velocity measurements were performed in triplicates. The $K_{i,app}$ values reported are the average of at least two independent experiments unless otherwise noted.

MIC determinations. MICs were determined as previously described.[9] MIC values were determined in at least triplicate according to the broth microdilution methods using compounds from DMSO stock solutions. Isoniazid was used as a positive control and DMSO was utilized as a negative control. Isolated *Mtb* cells (ATCC 27294) were cultured to an OD 0.2–0.3 in the required medium, then diluted to deliver approximately 1 x 10^4 bacteria per well of a 96 well clear round-bottom plate. Plates were read after 1 week with an inverted enlarging mirror plate reader and graded as either growth or no growth. GAST/Fe medium (per liter) consisted of 0.3 g of Bacto Casitone (Difco), 4.0 g of dibasic potassium phosphate, 2.0 g of citric acid, 1.0 g of L-alanine, 1.2 g of magnesium chloride hexahydrate, 0.6 g of potassium sulfate, 2.0 g of ammonium chloride, 1.80 ml of 10 N sodium hydroxide, and 10.0 ml of glycerol, 0.05% Tween 80 and 0.05 g of ferric ammonium citrate adjusted to pH 6.6. 7H9/glycerol/glucose/BSA/Tween medium consisted of Middlebrook 7H9 broth base supplemented per liter with 0.2% glucose, 0.2% glycerol, 0.5% BSA fraction V, 0.08% NaCl and 0.05% Tween 80. Cultures were supplemented with 200 μM guanine as noted.

***Mtb*IMPDH2 downregulation and susceptibility of SRMV2.6.** *Mtb* strains *guaB2* cKD (*guaB2-B3* Tet-OFF *attB*::*guaB3*) and SRMV2.6, which carries *guaB2*^{Y487C}, were cultured in

Middlebrook 7H9 media (Difco) supplemented with 0.2% glycerol, Middlebrook oleic acid-albumin-dextrose-catalase (OADC) enrichment (Difco) and 0.05% Tween 80 (7H9/Glycerol/OADC/Tween). Hygromycin (Hyg), kanamycin (Km) and gentamycin (Gm) were used in *guaB2* cKD culture at final concentrations of 50, 25 and 2.5 μg/mL, respectively. ATc (Sigma) was used at concentrations up to 100 ng/mL. For pairwise combination (checkerboard) assays, a two-dimensional array of serial dilutions of test compound and ATc was prepared in 96-well plates, as previously described.[11] MIC testing was carried out by broth microdilution using the AlamarBlue (AB, Invitrogen) assay [51, 52].

Mammalian Cell Culture. Hep G2 cells (ATCC, purchased February 2017) were cultivated in EMEM supplemented with 10% heat inactivated FBS and 1X penicillin/streptomycin under standard conditions (37 ^{o}C in a 5% CO_2 humidified atmosphere). HEK293T, MCF7 and HELA cells were cultured in DMEM with 10% heat inactivated FBS and 1X penicillin/streptomycin. Active cell cultures routinely tested for presence of Mycoplasma (MycoAlert^{TM} detection kit, Lonza) and confirmed to be Mycoplasma free.

LDH Cytotoxicity Assay. All compounds were dissolved in DMSO and further diluted with culture medium before use in tissue culture assays (final DMSO concentrations were ≤ 0.1%). To determine cytotoxicity, LDH release was measured with the LDH Cytotoxicity Assay Kit (Pierce) according to manufacturer's protocol. Briefly, 96 well plates were seeded with 13,000 Hep G2 cells (all other cell lines seeded at 6,000 to 8,000 cells per well) and the cells were cultured for 24 h prior to drug treatment. The cells were incubated in 110 μL of EMEM containing compound or DMSO (vehicle only, control) for 24 h at 37^{o}C. At least four wells from each plate were used as either 'spontaneous' LDH controls or as 'maximum' LDH controls per manufacturer's instructions. Cytotoxicity was determined by measuring absorbance on a

microplate reader. Data represent two independent experiments each performed in quadruplicate (n =8).

ADMET studies were performed by GVK Biosciences, (Hyderbad India).

Ancillary Information

Supporting Information: CSV file with molecular formula strings and the associated biochemical and biological data.

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Abbreviations Used: BSA: bovine serum albumin, CBS: cystathione β-synthetase, CDI: carbonyl diimidazole, DCM: dichloromethane, DEAD: diethylazodicarboxylate, DIPEA:

diisopropylethylamine, EDC•HCl: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, EDG: electron donating group, EWG: electron withdrawing group, Gua: guanine, HATU: (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate), IMP: inosine 5'-monophosphate, IMPDH2: inosine 5'-monophosphate dehydrogenase 2, LDH: lactate dehydrogenase, *m*-CPBA: *meta*-chloroperoxybenzoic acid, MIC: minimum inhibitory concentration, *Mtb*, *Mycobacterium tuberculosis*; NAD: nicotinamide adenine dinucleotide, NMP: N-methyl-2-pyrrolidone, *p*-TSA: *para*-toluene sulfonic acid, SAR: structure-activity relationship, TEA: triethylamine, TFA: trifluoroacetic acid, XMP: xanthosine 5'-monophosphate.

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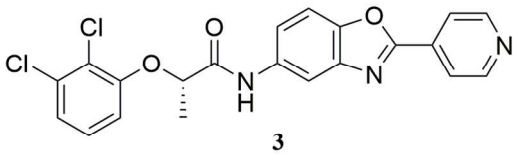
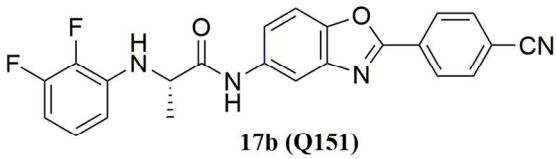
Table of Content graphic



3

*Mtb*IMPDH2 IC_{50} = 14 nM

Mtb MIC = 12 μM



17b (Q151)

*Mtb*IMPDH2 IC_{50} = 18 nM

Mtb MIC = 1.5 μM

HepG2 cell LD_{50} > 25μM

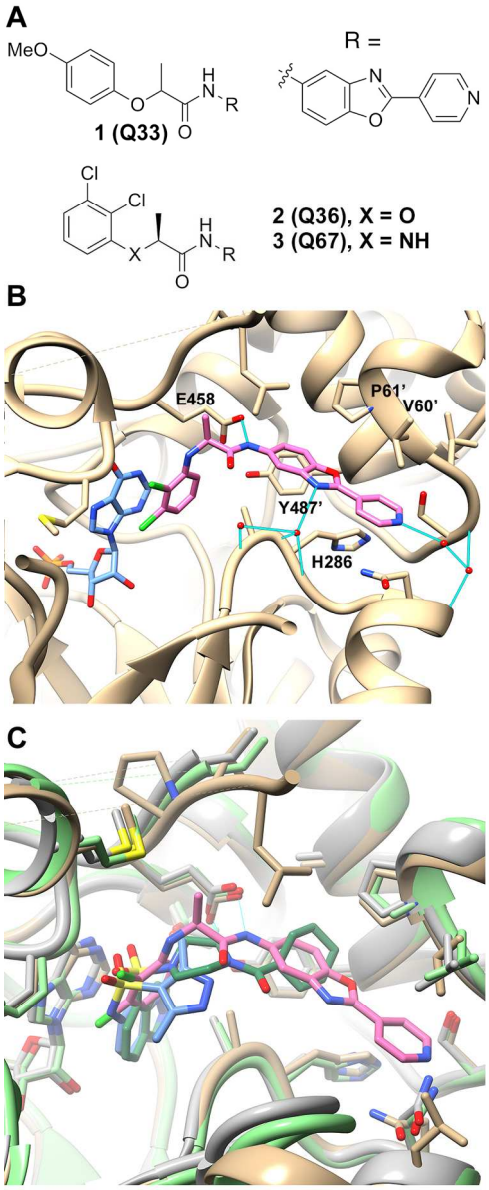


Figure 1. Inhibitors of MtbIMPDH2. A) Structures of 1, 2 and 3. B) Crystal structure of MtbIMPDH2ΔCBS •IMP•3 (PDB 4ZQO)9. 3 is pink, IMP is blue, hydrogen bonds are shown in cyan, residues from the adjacent subunit are marked with '. C) Crystal structures of M. thermoresistible IMPDH2 in complex with IMP and cyclohexyl[4-(5-isoquinolinylsulfonyl)-1-piperazinyl]methanone (VCC234718, PDB 5J5R, protein and IMP are spring green, inhibitor is forest green) 11, and N-1H-indazol-6-yl-3,5-dimethyl-1H-pyrazole-4-sulfonamide (6Q9, PDB 5K4X, protein and IMP are gray, inhibitor is blue) 10. The structure of MtbIMPDH2ΔCBS •IMP•3 (PDB 4ZQO, protein and IMP are tan, 3 is pink)9 is also included.

81x203mm (300 x 300 DPI)

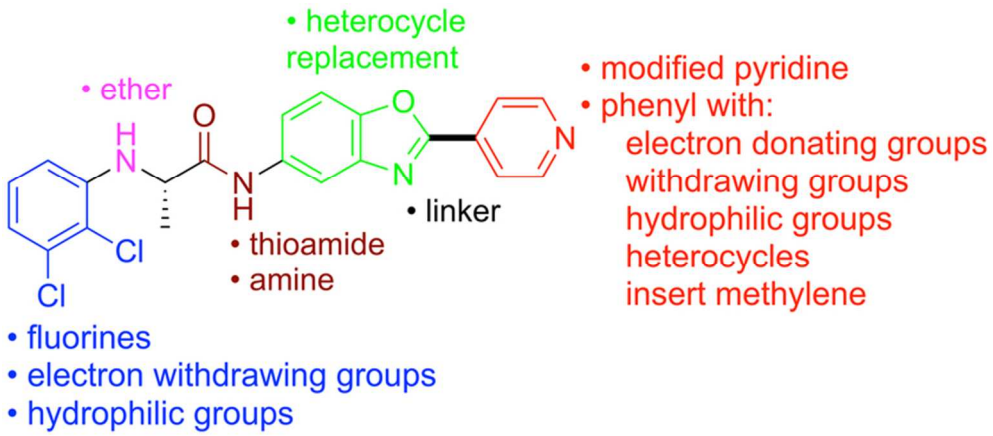


Figure 2. An overview of the SAR explored in this study.

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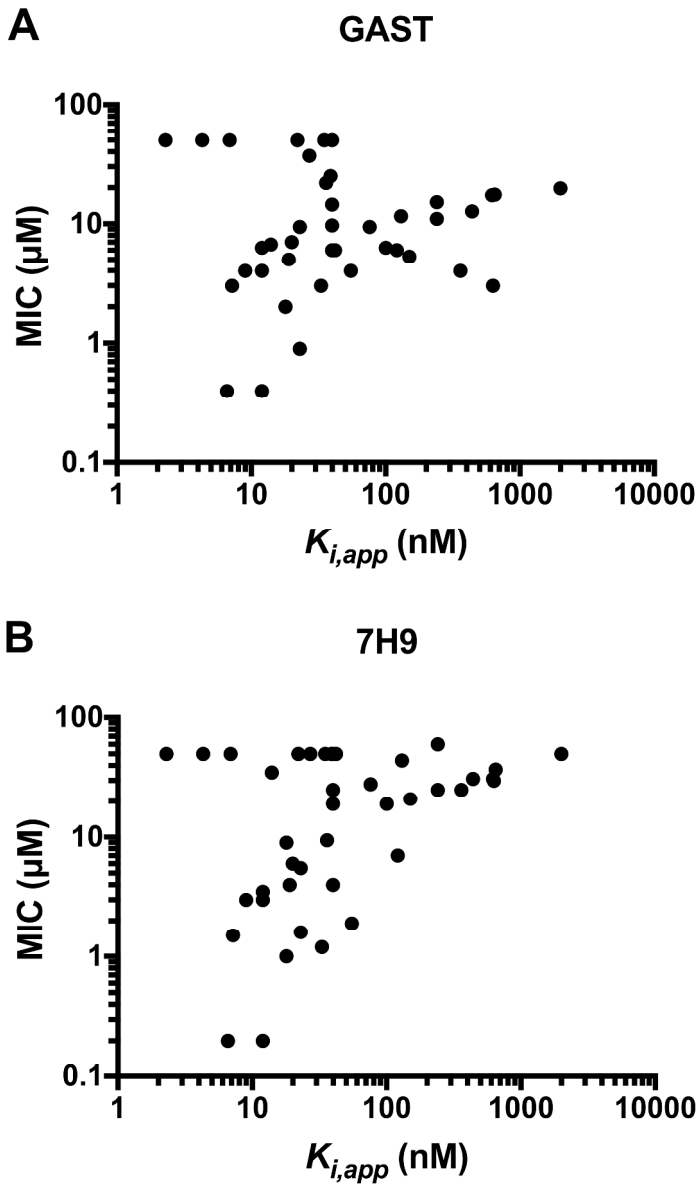

Figure 3. Correlation between antibacterial activity and enzyme inhibition for Q series compounds. Values from Table 4 and Makowska-Grzyska et al 9. Mtb H37Rv cultured in A) GAST medium and B) 7H9 medium.

136x223mm (600 x 600 DPI)

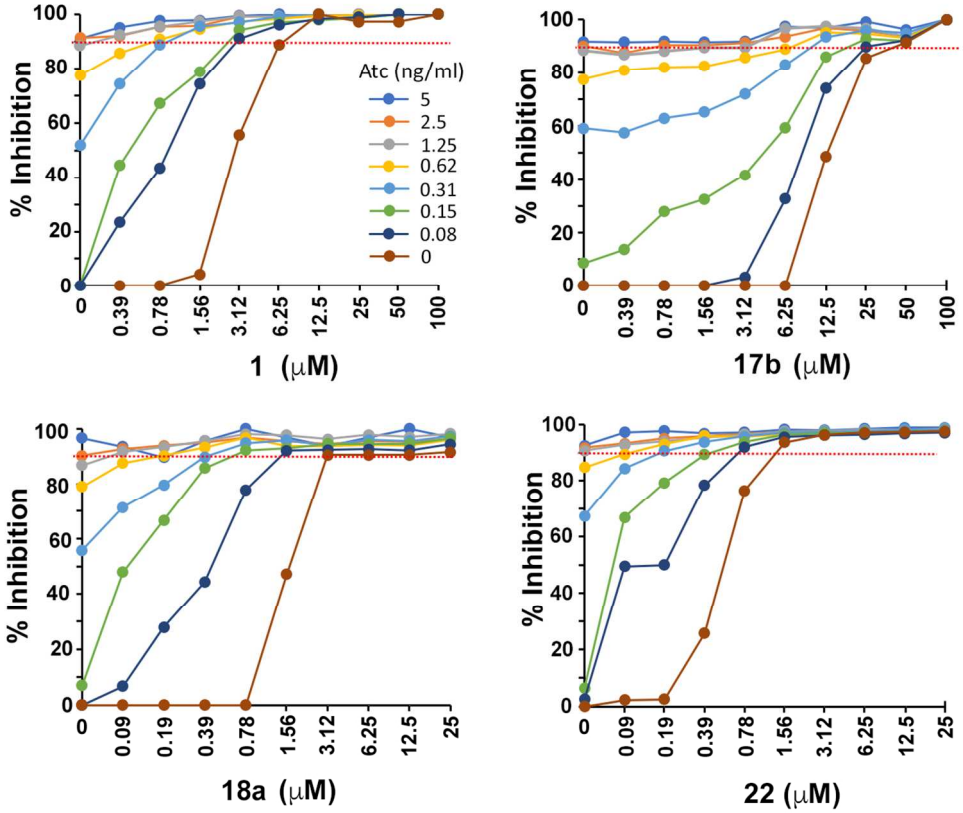

Figure 4. Knockdown of MtbIMPDH2 hypersensitizes Mtb to Q compounds. Regulated expression of guaB2 is achieved in a TET-OFF system, as previously described 11. Addition of anhydrotetracycline (ATc) represses expression of guaB2, decreasing the level of MtbIMPDH2 within the bacteria. ATc concentrations (ng/mL) are 0 (dark orange), 0.08 (dark blue), 0.15 (green), 0.31 (sky blue), 0.62 (yellow), 1.25 (gray), 2.5 (orange) and 5 (royal blue).

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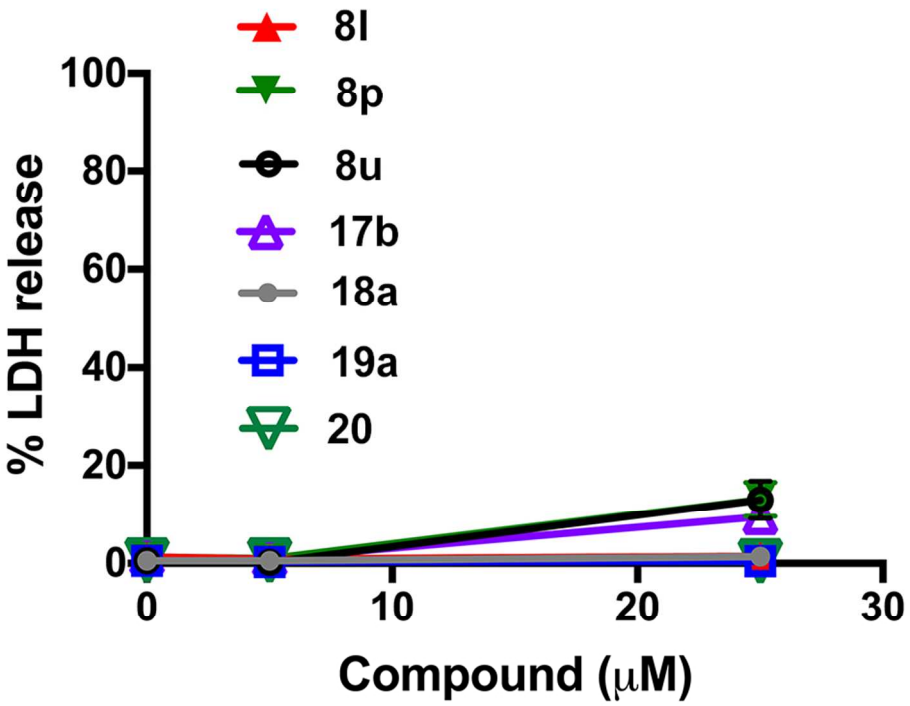

Figure 5. Cytotoxicity of select Q compounds in HepG2 cells after 24 h.

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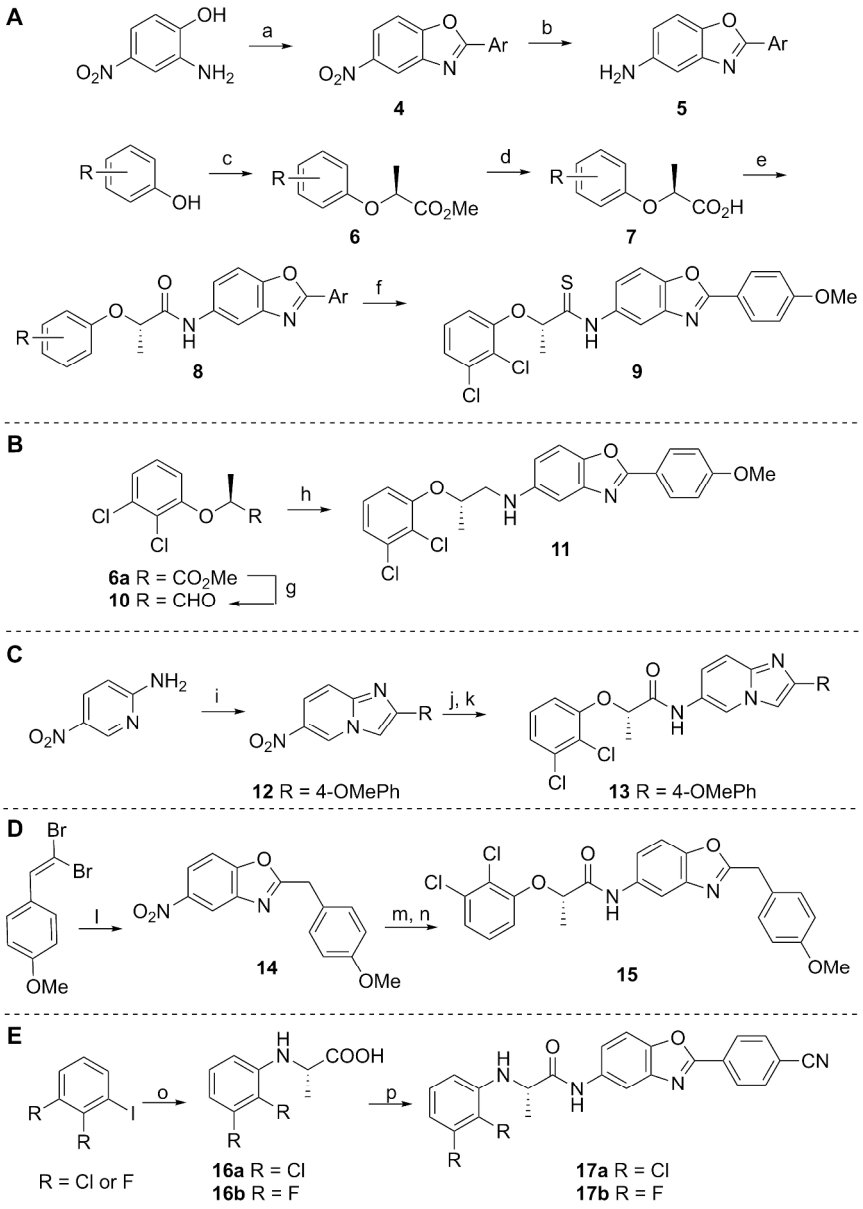
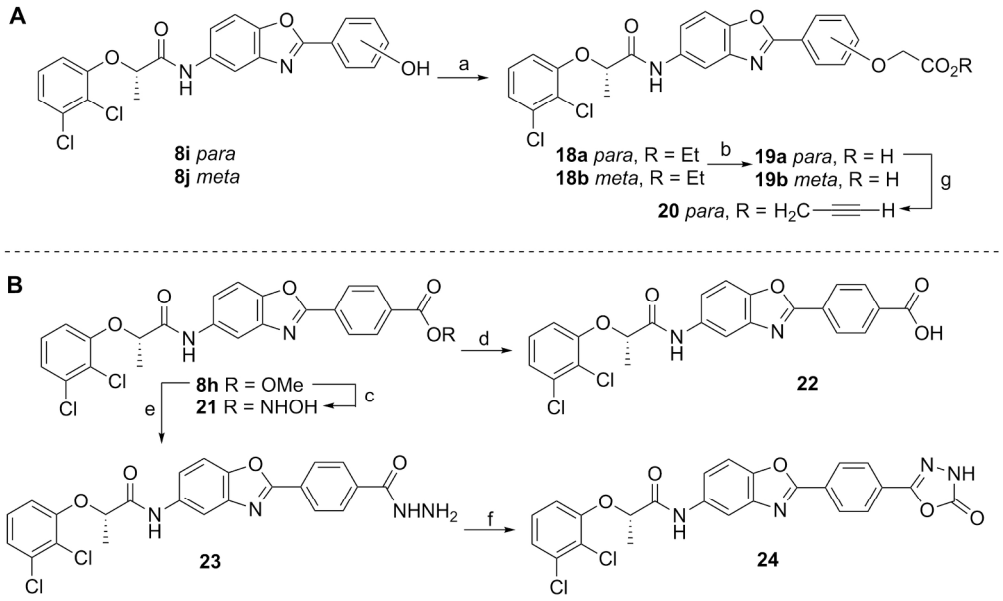


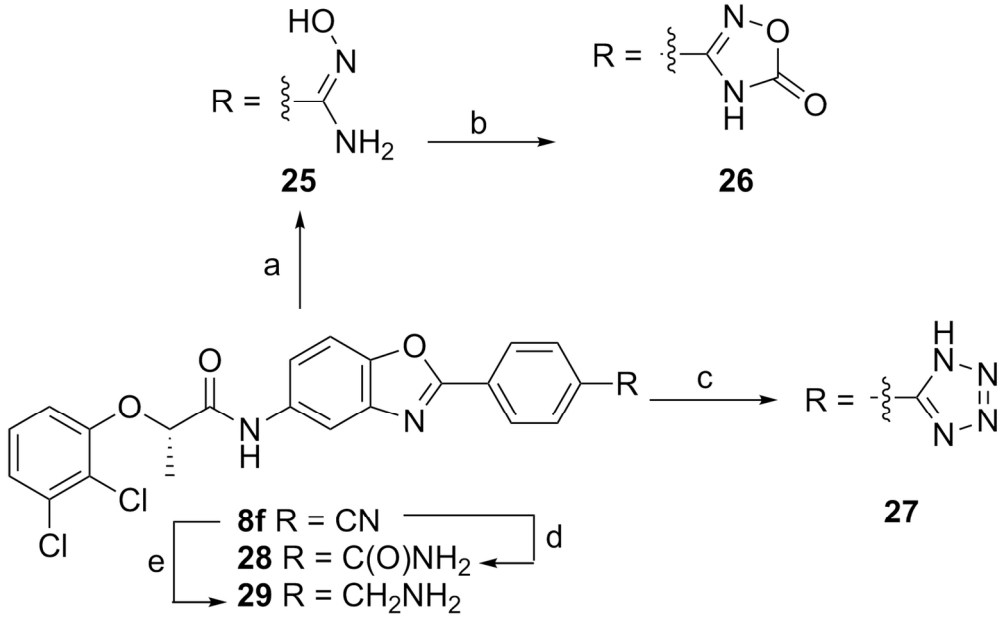
Scheme 1. Synthesis of various aryl benzoxazoles 8, thioamide 9, amine 11, imidazo[1,2-a]pyridine 13, methylene linked derivative 15 and N-arylated benzoxazoles 17a-b.

147x204mm (600 x 600 DPI)



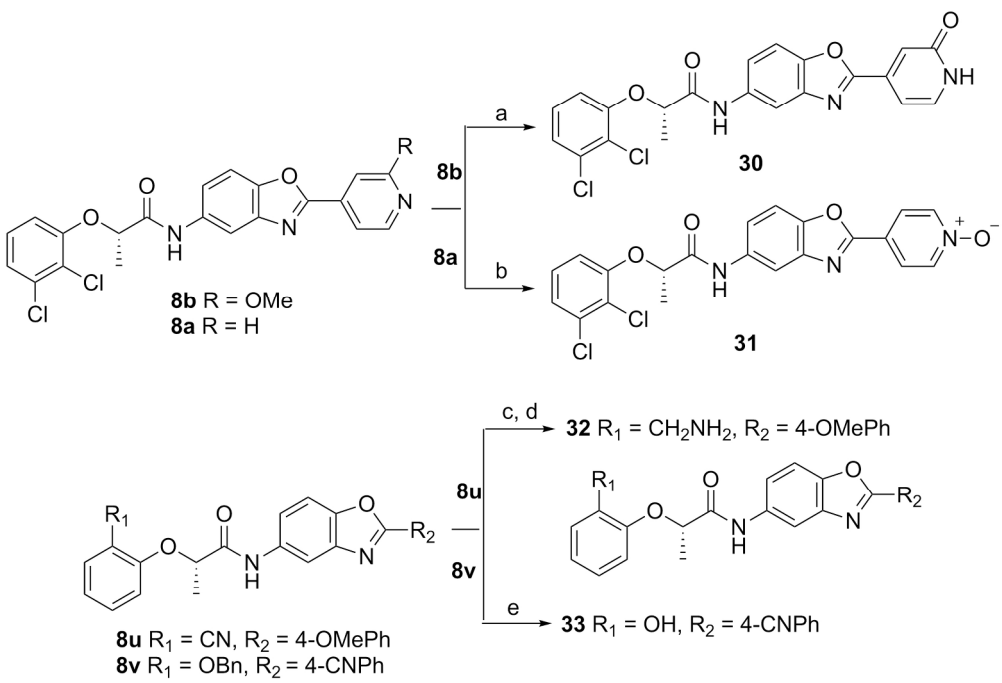
Scheme 2. Synthesis of derivatives 18a-b, 19a-b and 20-24.

100x59mm (600 x 600 DPI)



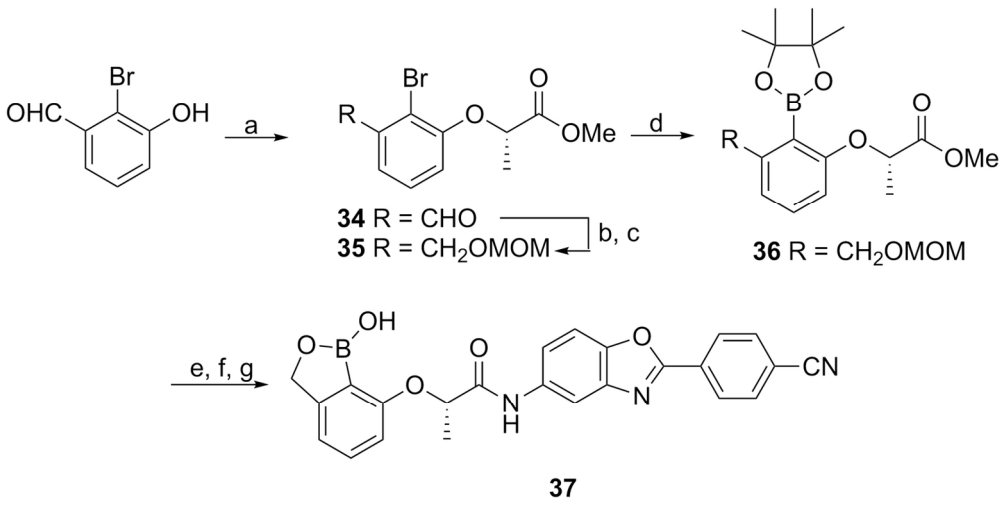
Scheme 3. Synthesis of derivatives 25-29 from 8f.

68x42mm (600 x 600 DPI)



Scheme 4. Synthesis of derivatives 30-33.

106x71mm (600 x 600 DPI)



Scheme 5. Synthesis of benzoxaborole 37.

68x34mm (600 x 600 DPI)

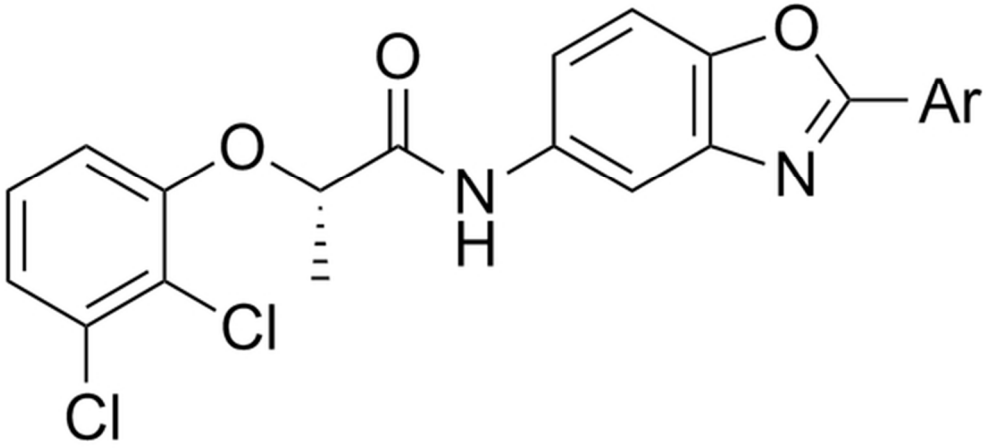


Table 1. reference structure

25x11mm (600 x 600 DPI)

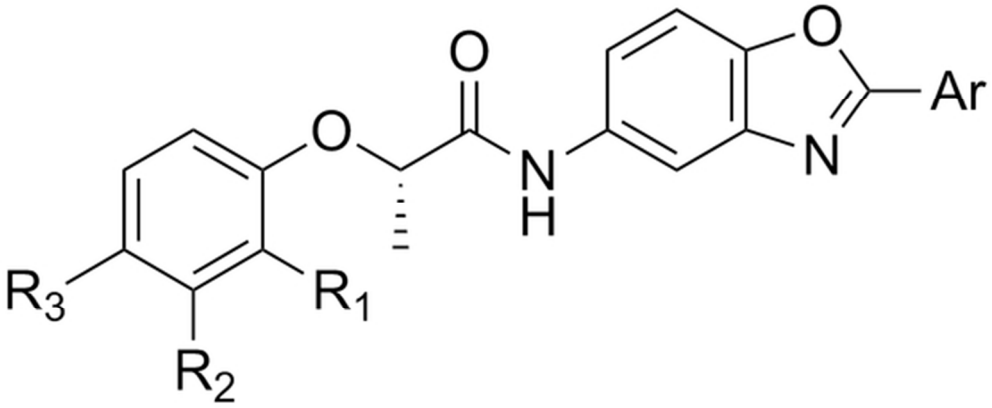
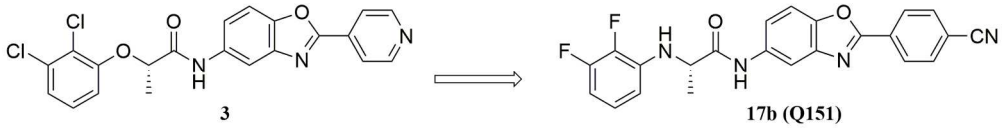


Table 2 reference structure

26x11mm (600 x 600 DPI)



TOC

175x38mm (300 x 300 DPI)