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Article

Benzoheterocyclic Oxime Carbamates Active against *Mycobacterium tuberculosis*: Synthesis, Structure−Activity Relationship, Metabolism, and Biology Triaging

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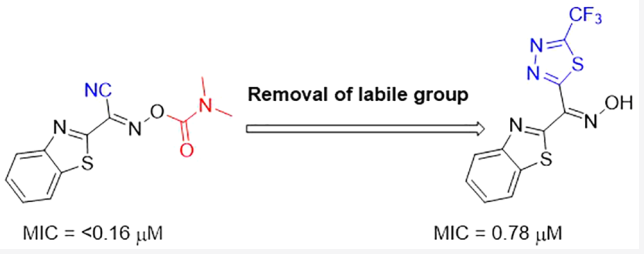
ABSTRACT: Screening of a library of small polar molecules against *Mycobacterium tuberculosis* (*Mtb*) led to the identification of a potent benzoheterocyclic oxime carbamate hit series. This series was subjected to medicinal chemistry progression underpinned by structure−activity relationship studies toward identifying a compound for proof-of-concept studies and defining a lead optimization strategy. Carbamate and free oxime frontrunner compounds with good stability in liver microsomes and no hERG channel inhibition liability were identified and evaluated *in vivo* for pharmacokinetic properties. *Mtb*-mediated permeation and metabolism studies revealed that the carbamates were acting as prodrugs. Toward mechanism of action elucidation, selected compounds were tested in biology triage assays to assess their activity against known promiscuous targets. Taken together, these data suggest a novel yet unknown mode of action for these antitubercular hits.



INTRODUCTION

Tuberculosis (TB), caused by a single infectious pathogen *Mycobacterium tuberculosis* (*Mtb*), represents a serious global public health concern. The current treatment for drug-sensitive TB, also called "first-line" TB treatment, was developed over 40 years ago and requires multiple drugs to be taken, often daily, for 6 to 9 months.[1] This drug treatment can cure active drug-sensitive TB if treatment is completed properly with no interruptions. However, the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and so-called totally drug-resistant (TDR) *Mtb* strains complicates TB treatment severely.[2−4] New drugs, novel targets, and treatment strategies are critical to control the TB epidemic.

Among the existing clinically used TB drugs, which occupy a broad chemical space, are small polar molecules exemplified by isoniazid (INH) and pyrazinamide (PZA).[5] These hydrophilic molecules occupy a unique chemical space with respect to molecular weight (<250 Da) and lipophilicity (clog P < 2.5) and are able to cross the otherwise highly lipophilic mycobacterial cell-wall to reach their site of action.[6,7] This permeation is apparently facilitated by the presence of hydrophilic channels that allow access to polar nutrients.[8] These observations encouraged researchers at the Novartis Institute for Tropical Diseases (NITD) to conduct a high-throughput phenotypic screen of an ∼6000-member library of small polar molecules (M_w 150−350 Da and clog P −1 to 3.5) against *Mtb*. One of the hit series identified from this effort was a novel pyrrolo[3,4-*c*]pyridine-1,3(2*H*)-dione series, which we previously reported.[9]

In continuation of our efforts to exploit chemical matter arising from the screen, we now report the exploration of another hit series containing oxime carbamates (Figure 1). Gratifyingly, these compounds were also potently active against a panel of five drug-susceptible clinical *Mtb* isolates.[9] The hits were seemingly selective toward mycobacteria as they were inactive (MIC > 125 μM) against a panel of 6 ESKAPE bacterial pathogens (Table S1). These compounds exhibited good selectivity *versus* a mammalian cell line as they were not cytotoxic to Chinese hamster ovary (CHO) cells at concentrations of 50 μM. This selective antimycobacterial activity encouraged further progression of these compounds. In this paper, we report the strategies that were adopted to explore the *in vitro* anti-*Mtb* structure−activity relationship

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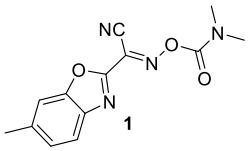
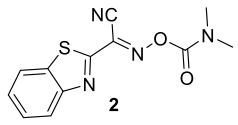


1

H37RvMa MIC = <0.156 μM
Clinical isolate MIC = <0.08 – 0.31 μM
ESKAPE bacteria MIC = 31.2-125 μM
CHO IC_{50} = >50 μM
cLogP = 2.04
TPSA = 92.7
MW = 272



2

H37RvMa MIC = <0.156 μM
Clinical isolate MIC = <0.08 μM
ESKAPE bacteria MIC = >125 μM
CHO IC_{50} = >50 μM
cLogP = 2.16
TPSA = 107
MW = 274

Figure 1. HTS hit compounds **1** and **2** from screening a focused library. cLog *P* and TPSA calculated by the ACD Percepta module. ESKAPE bacterial strains: *Enterobacter cloacae* (ATCC 700323), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC BAA-1705), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923).

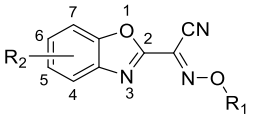
(SAR), cytotoxicity, solubility, and ADME/pharmacokinetic (PK) profiles of the series toward identifying a compound for proof-of-concept studies.

RESULTS AND DISCUSSIONS

Chemistry. We attempted to synthesize the 2-benzoxazolyl acetonitrile intermediates by reacting 2-aminophenol with malononitrile, as reported by Das and colleagues.[10] However, the yields obtained were in the region of 20%. After several attempts with different reagents and conditions, a different protocol was adapted, which increased the yield of 2-benzoxazolyl acetonitrile to about 69%.[11] The starting point was the activation of malononitrile with absolute ethanol in the presence of chlorotrimethylsilane (TMSCl) to give a more reactive 2-cyanoacetimidic acid ethyl ester hydrochloride intermediate **3** in high yields (Scheme 1). Appropriately substituted 2-aminophenols were then reacted with **3** under reflux in dichloromethane (DCM) for about 20 h to give the corresponding 2-benzoxazolyl acetonitrile-based intermediates in moderate to high yields. Oxime formation at the methylene carbon was achieved *via* a nitrosation reaction, following a literature protocol.[12] In this case, a frozen solution of the appropriate 2-benzoxazolyl acetonitrile-based intermediate in glacial acetic acid (AcOH) was slowly treated with sodium nitrite. The 6-nitro intermediate was converted to the aromatic amine by reduction under a hydrogen atmosphere followed by acylation with acetyl chloride under basic conditions to give **4**. Finally, the oxime carbamate was introduced in the presence of NEt_3 by reaction of oximes with dimethylcarbamoyl chloride to give a single geometric isomer, as revealed by ^{1}H NMR. The sulfamoyl analogue **8** could be prepared under similar conditions from **5** and *N*,*N*-dimethylsulfamoyl chloride.

We initially set out to explore the SAR of this series (Table 1). The oxime carbamate moiety was required for anti-*Mtb*

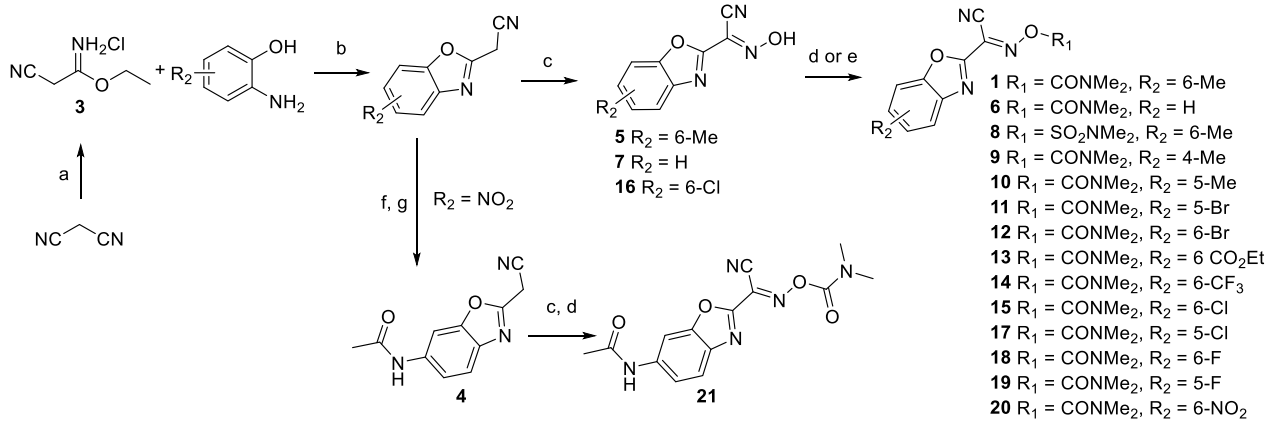
Table 1. Optimization of the Benzoxazole Core



compound	R_1	R_2	MIC (μM)[a]	CHO IC_{50} (μM)[b]
1	$CONMe_2$	6-Me	<0.16	>100
5	H	6-Me	>160	>100
6	$CONMe_2$	H	0.30	>100
7	H	H	>160	n.d.
8	SO_2NMe_2	6-Me	5.0	n.d.
9	$CONMe_2$	4-Me	<0.24	>100
10	$CONMe_2$	5-Me	<0.24	>100
11	$CONMe_2$	5-Br	<0.24	n.d.
12	$CONMe_2$	6-Br	<0.24	>100
13	$CONMe_2$	6-CO_2Me	<0.24	7.05
14	$CONMe_2$	6-CF_3	<0.24	46.3
15	$CONMe_2$	6-Cl	<0.24	>100
17	$CONMe_2$	5-Cl	<0.24	>100
18	$CONMe_2$	6-F	<0.24	>100
19	$CONMe_2$	5-F	<0.24	n.d.
20	$CONMe_2$	6-NO_2	<0.24	46.8
21	$CONMe_2$	6-NHCOMe	5.20	>100
Rifampicin			0.009	n.d.
INH			0.14	n.d.

[a]14 day Alamar Blue readout against *Mtb* H37RvMa in GAST/Fe medium. [b]Cytotoxicity against CHO cells. n.d., not determined.

Scheme 1. Synthesis of Benzoxazole analogues[a]



[a]Reagents and conditions: (a) EtOH, TMSCl, 0 °C, 17 h; (b) DCM, appropriately substituted 2-aminophenol, 25 °C, 10 min then reflux, 20 h; (c) AcOH, $NaNO_2$, 0–25 °C, 12 h; (d) dimethylcarbamoyl chloride or *N*,*N*-dimethylsulfamoyl chloride, triethanolamine, DCM, reflux, 15 h; (e) dimethylcarbamoyl chloride, MeCN, K_2CO_3, reflux, 1 h; (f) H_2, Pd/C, 15 h; and (g) MeCN, K_2CO_3, acetyl chloride, 40 °C, 0.5 h.

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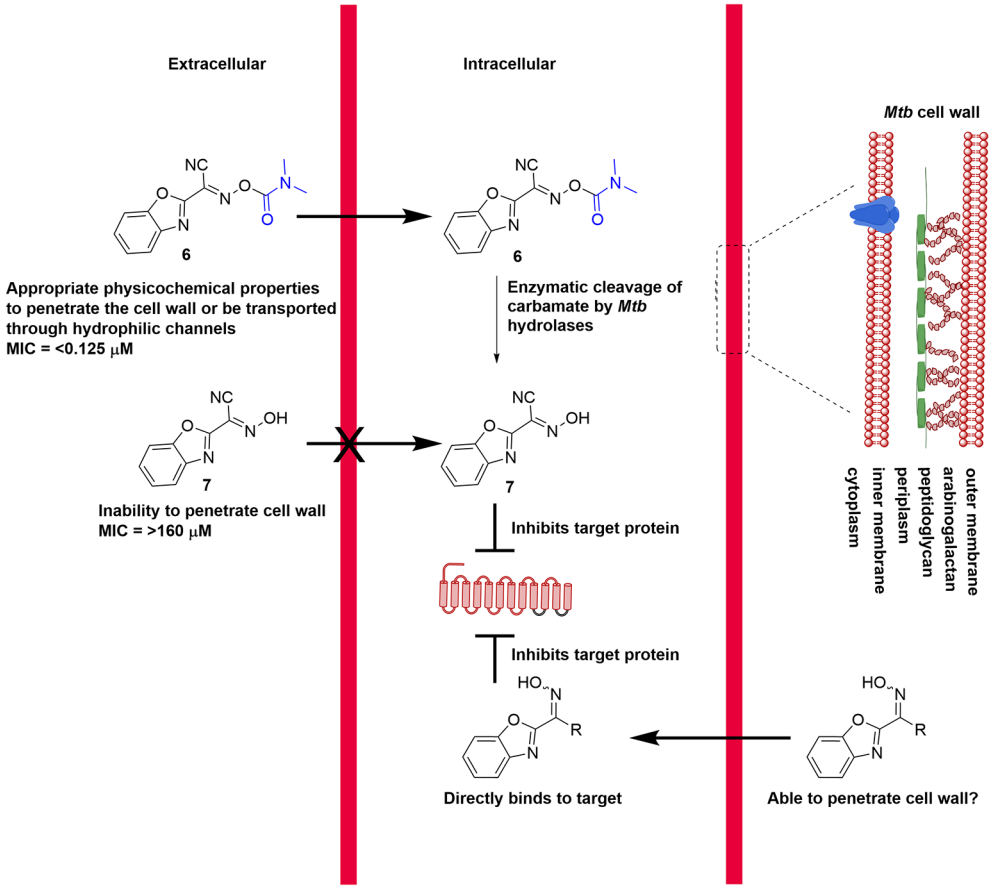

Figure 2. Proposed prodrug-based activity of carbamate-functionalized oxime compounds.

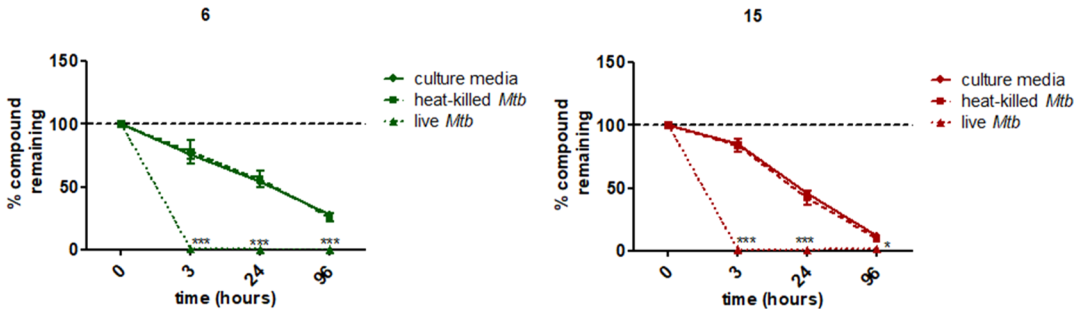

Figure 3. *Mtb*-mediated *in vitro* metabolism of benzoxazole-based carbamates **6** and **15**. Compounds were incubated in 7H9/OADC (culture medium) and added to log-phase *Mtb* H37RvMa culture (live or heat-killed). PARs were calculated by dividing the peak area of the analyte by the peak area of the internal standard, and all values were calculated as the percentage of the average PAR at time-point zero. Statistical significance was assessed using one-way ANOVA with Dunnett's multiple comparison test, comparing against the culture media incubation control at each time point. *P < 0.05, **P < 0.01, and ***P < 0.001.

activity as the corresponding free oxime **5** resulted in complete loss of activity. The carbonyl of the carbamate group could be replaced with a sulfone in compound **8**, resulting in a drop in potency (MIC = 5 μM). The 6-Me group could be removed, while activity was retained (compound **6** MIC of 0.3 μM). Compounds incorporating small electron-withdrawing groups were equipotent to **1**. The 4-, 5-, and 6-positions around the benzoxazole core were tolerant of various electron-withdrawing groups (F, Cl, Br, ester, and nitro). However, the presence of an ester group in the 6-position for **13** increased cytotoxicity (IC_{50} = 7.05 μM) compared to **1**. The single example of an electron-donating group, such as an amide group **21**, caused a loss in potency.

***Mtb*-Mediated Permeation and Metabolism.** *Mtb* is known to possess enzymes that can mediate biochemical transformations on specific chemical groups of drug molecules such as ester hydrolysis, addition of alkyl groups, and reduction−oxidation reactions to name a few, which can activate prodrugs or be detrimental if the drug molecules are deactivated.[13] Small polar anti-TB drugs, such as INH and PZA, are prodrugs that are specifically activated within the mycobacterial cell.[14−16] In this regard, we hypothesized that the carbamate group in our compounds masks the oxime to improve its *Mtb* cell-membrane permeability in an analogous fashion, as described in Figure 2. Once the carbamate crosses the *Mtb* cell membrane and is within the bacilli, it can then be

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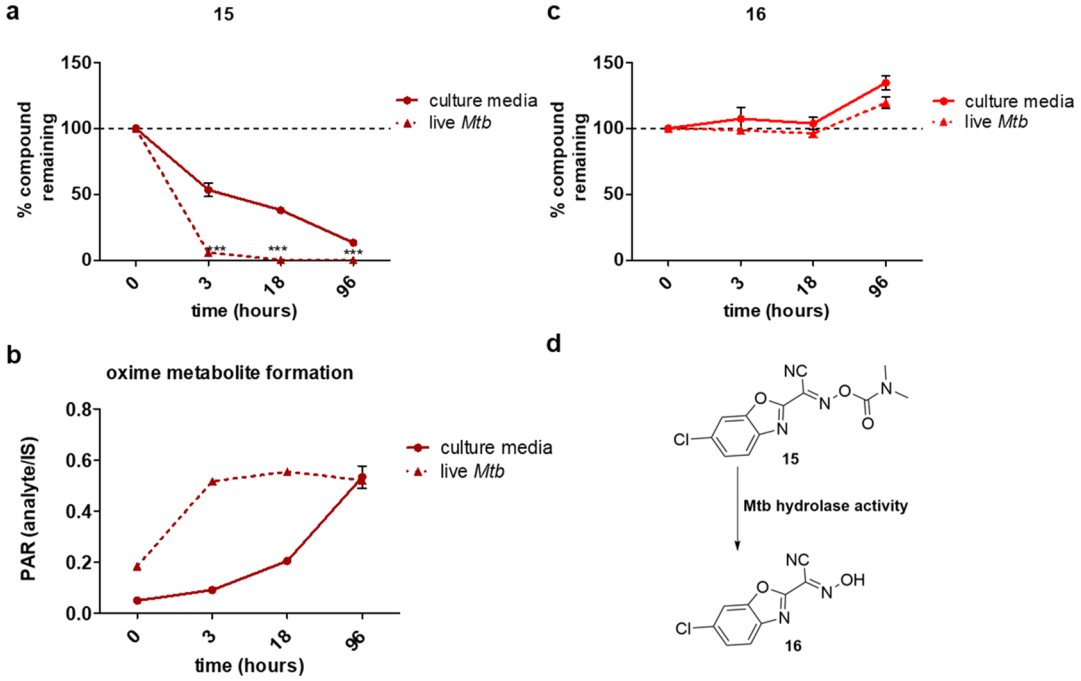

Figure 4. Carbamate metabolism by *Mtb* yields the free oxime, while the oxime remains stable. (a) Compound **15** undergoes rapid *Mtb*-mediated metabolism, resulting in the accumulation of the free oxime metabolite **16**, (b). (c) **16** does not undergo metabolism or spontaneous degradation. (d) *Mtb* hydrolase activity hydrolyzes the carbamate to form the oxime. Compounds were incubated in 7H9/OADC growth medium (culture media) and added to log-phase *Mtb* H37RvMa culture (live). PAR was calculated by dividing the peak area of the analyte by the peak area of the internal standard and values calculated as the percentage of the average PAR at time-point zero.

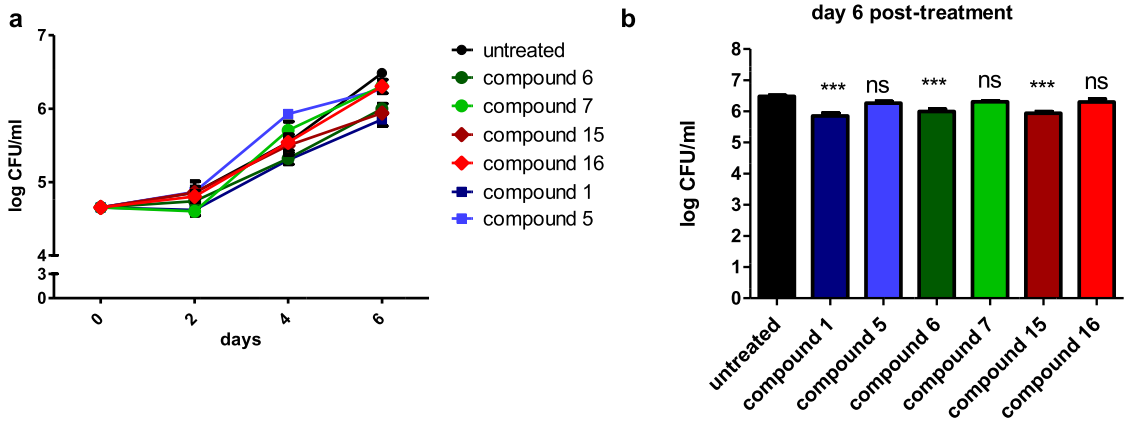

Figure 5. Effect of carbamates and their corresponding free oximes on intracellular *Mtb* growth. (a) *In vitro* infected THP-1 macrophages treated with selected compounds at 10 μM (showing how both oximes and the carbamates fail to control *Mtb* growth at high concentrations). (b) Carbamates reduce intracellular growth in comparison to the untreated control. Carbamates (**6**, **15**, and **1**) are shown in dark green, maroon, and dark blue, respectively; corresponding oximes (**7**, **16**, and **5**) are shown in light green, red, and light blue, respectively. Statistical significance was assessed using one-way ANOVA with Dunnett's multiple comparison test, comparing against the untreated control day 6 post treatment. ns not significant; *P < 0.05, **P < 0.01, and ***P < 0.001.

enzymatically cleaved through esterase activity to form the free oxime which then in turn binds to the target protein. This hypothesis is in part supported by the observation that the free oxime **7** is completely inactive at the highest concentration tested, 160 μM.

To determine the potential metabolism of carbamates by *Mtb*, we prepared three samples for representative compounds **6** and **15** as follows: (1) *Mtb* H37RvMa cultures incubated with the compound of interest at sub-MIC concentration (0.1 μM), over 96 h, (2) similarly cultured, heat-killed *Mtb* control, and (3) an equal volume of sterile growth media as a control incubated with the same compound's final concentration. Control incubations were performed using heat-killed cells to distinguish between enzymatic metabolism and aqueous degradation. Samples were analyzed using liquid chromatography coupled with mass spectrometry (LC−MS/MS), and the results are represented as the percentage remaining by comparing the analyte/internal standard peak area ratios (PARs) at each time point with those at the beginning of the incubation.

We found that, over time, the relative levels of carbamates reduced gradually following incubation in culture media, suggesting that the compounds were susceptible to spontaneous aqueous degradation (Figure 3). However, the relative

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levels reduced much more rapidly in incubations with *Mtb* live cultures, with complete loss of compounds by 3 h (the earliest time-point sampled). In contrast, no significant differences were detected between culture media and heat-killed cultures.

To further test our prodrug hypothesis, we sought to determine if the free oxime formed from the metabolism of carbamates. We incubated compound **15** and its corresponding oxime **16** in culture media alone and in the presence of live *Mtb* (Figure 4). Carbamate hydrolysis was accompanied by the formation of **16** as the only metabolite, with the same MS transitions and retention time as a solvent-spiked oxime compound. The free oxime compound **16** remained stable when incubated with *Mtb*. These data confirm our hypothesis that the carbamates permeated the *Mtb* cell membrane; moreover, it seemed that hydrolysis occurs rapidly to form the oxime which is then presumably the compound species that reaches the molecular target.

Activity against Intracellular *Mtb*. We next assessed the uptake of compounds **1**, **6**, and **15** and their oximes in *Mtb*-infected THP-1 macrophages using a method reported by Prideaux *et al.* 2015 and the associated impact on intracellular bacillary survival.[17] Owing to natural compound degradation in growth media, macrophages were exposed to drug media, replenished every 24 h to increase the chances of drug cellular uptake. Analysis of macrophage cell cultures exposed to compounds at high concentration, greater than 10× MIC for all carbamates (10 μM), revealed significantly lower analyte signals compared to media spiked with similar amounts of the drug (Figure S2). To confirm whether or not this macrophage uptake translated into effective intracellular killing, we treated infected macrophages at the same concentration over a 6 day period. We found that both the carbamates and their free oxime derivatives failed to control intracellular bacterial growth, presumably due to poor host cell penetration for the highly active carbamates (Figure 5a). Even though bacteria continued to grow, the carbamates did reduce the intracellular bacterial counts in comparison to the untreated control (Figure 5b).

An increase in compound concentrations for the selected representative compound **15** and its free oxime revealed that the carbamates could penetrate the host macrophage cells and reduce intracellular bacterial burden in a dose-dependent manner with approximately a 2-log reduction at 80 μM in comparison to untreated cells (Figure 6).

Microsomal Metabolic Stability and Metabolite Identification. The unusual oxime carbamate moiety presented us with a significant optimization challenge as it was anticipated that it would be labile under biological conditions. Indeed, it was found that the hit compounds displayed low metabolic stability in microsomal and S9 fractions. Metabolite identification studies showed that **2** was metabolized extensively in liver microsomes and plasma by hydrolysis of the carbamate to yield the corresponding oxime **22**. This was confirmed using a sample of oxime **22** which had the same retention time and fragmentation pattern as the identified metabolite (Figure S1). No other metabolites were detected. This metabolic reaction occurs even in the absence of NADPH, indicating contribution by non-CYP450 enzymes. The metabolic stability of **8** could not be determined due to low solubility, while a carbonate (R_1 = CO_2Me) was found to be chemically unstable.

The poor microsomal stability of the oxime carbamate prompted a two-fold strategy to replace the carbamate moiety

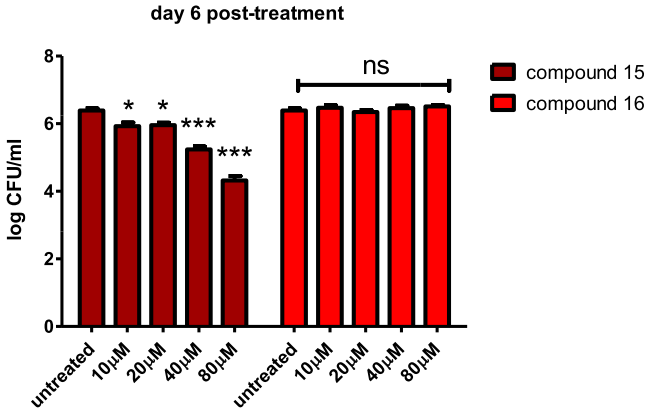
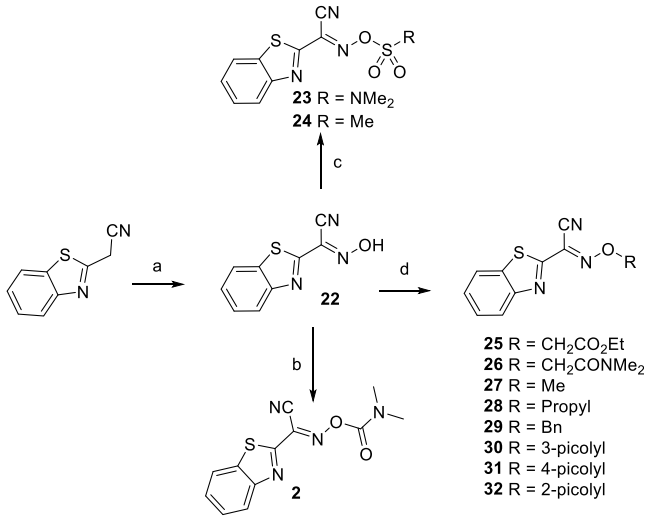


Figure 6. Effect of increasing concentration on intracellular *Mtb* growth. Increasing extracellular compound concentration up to 80 μM reduced intracellular bacterial growth by approximately 2-log in comparison to the untreated control. Statistical significance was assessed using one-way ANOVA with Dunnett's multiple comparison test, comparing against the untreated control day 6 post treatment. ns not significant; *P < 0.05, **P < 0.01, and ***P < 0.001.

with a metabolically more stable group and/or modify the physicochemical properties of the free oxime to the extent that it may cross the cell membrane (Figure 2). We endeavored to find a stable replacement for the oxime group. The oxime was introduced into the benzothiazole core by reacting with sodium nitrite using the same procedure as for the benzoxazole (Scheme 2). Oxime carbamates and sulfamoyl compounds

Scheme 2. Synthesis of Benzothiazole analogues[a]



[a]Reagents and conditions: (a) $NaNO_2$, AcOH, 0−25 °C, 12 h; (b) dimethylcarbamoyl chloride, NEt_3, DCM, reflux, 15 h (c) MsCl or *N*,*N*-dimethylsulfamoyl chloride, NEt_3, DCM, reflux, 15 h; and (d) alkyl halide, NEt_3, DCM, reflux, rt.

were prepared using the same protocol to give hit compound **2** and sulfamoyl **23**. The oxime **22** was reacted with mesyl chloride to give **24** under the same conditions. Alkyl ethers of the oxime (**25**−**32**) were synthesized by alkylation of the oxime with various alkyl halides.

Similar to the benzoxazole core, removal of the carbamate group resulted in complete loss of activity for compound **22** (Table 2). Acyl sulfonamide and sulfone oximes maintained

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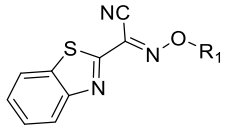
Table 2. Optimization of Acyl Oxime Functionalities



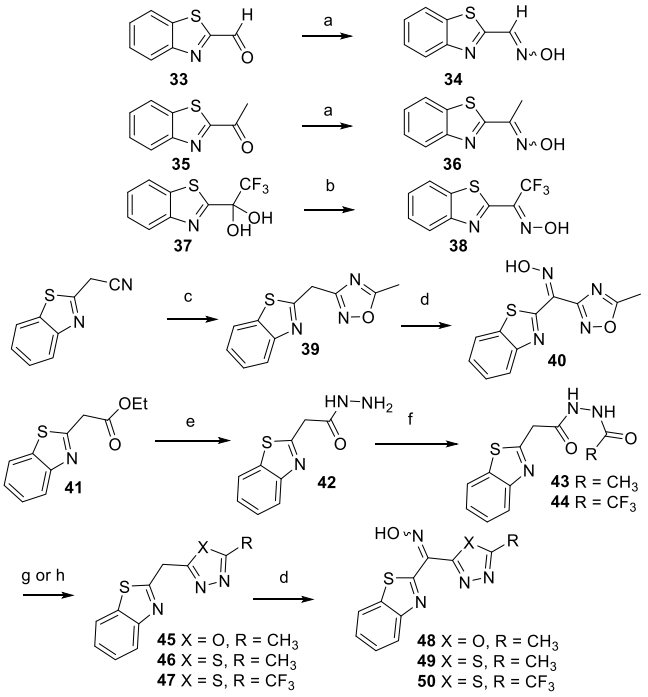
Compound	R_1	MIC[a] (μM)	CHO IC_{50}[b] (μM)
2	$CONMe_2$	<0.16	>100
22	H	>160	>100
23	SO_2NMe_2	0.30	91.3
24	SO_2Me	5.0	n.d.
25	CH_2CO_2Et	>160	n.d.
26	CH_2CONMe_2	>160	n.d.
27	Me	160	n.d.
28	Propyl	>160	n.d.
29	Bn	20	n.d.
30		>160	n.d.
31		20	n.d.
32		>160	n.d.
Rifampicin		0.009	n.d.
INH		0.14	n.d.

[a]14 day Alamar Blue readout against H37RvMa in GAST/Fe medium. [b]Cytotoxicity against CHO cells. n.d., not determined.

some activity, indicating that other potentially labile groups are also tolerated albeit would likely suffer the same fate as the oxime carbamates. Alkylation of the oxime with a variety of alkyl groups led to loss in potency in most cases. Benzyl **29** and 4-picolyl **31** ethers maintained some activity but were significantly less active compared to **2**.

With the knowledge that activity could not be maintained when replacing the acyl group with more metabolically stable groups, we next investigated whether permeation of the free oxime could be improved by modification of the nitrile functionality. Free oximes with the nitrile replaced by H and Me were prepared by reaction of the commercially available aldehyde **33** or ketone **35** with hydroxylamine under basic conditions (Scheme 3) to give a mixture of *E*/*Z* isomers. Similarly, the hydrate **37**[18] could be converted to the oxime **38** to afford a CF_3 replacement of the nitrile as a single isomer. 1,2,4-Oxadiazole **40** was prepared by reaction of 2-benzothiazoleacetonitrile with hydroxylamine followed by cyclization with acetic anhydride. Intermediates **45-47** were synthesized by reacting ester **41** with hydrazine. The hydrazide was then acylated and converted to oxadiazole and thiadiazoles by reaction with $POCl_3$ and Lawesson's reagent, respectively. These benzothiazole methylene compounds were then converted to oximes using $NaNO_2$ in acetic acid, which yielded a mixture of *E*/*Z* isomers in most cases. Benzothiazole was transformed to the required ketones by reacting with acid chlorides or Weinreb amides (Scheme 4). Benzothiazole ketone dimer **53** was formed by the reaction of triphosgene with benzothiazole in the presence of triethylamine and

Scheme 3. Synthesis of Benzothiazole analogues[a]



[a]Reagents and conditions: (a) NH_2OH, $NaHCO_3$, IPA, 65 °C; (b) NH_2OH, Na_2CO_3, water, EtOH, 75 °C;(c) (1) NH_2OH, EtOH, reflux, (2) Ac_2O, PhMe, 120 °C; (d) $NaNO_2$, AcOH, water, rt; (e) hydrazine hydrate, EtOH, rt; (f) Ac_2O or TFA_2O Pyr, PhMe, 100 °C; (g) $POCl_3$, 80 °C; and (h) Lawesson's reagent, dioxane, 100 °C.

DMAP.[19] The formed ketones were converted to oximes **52** and **54-60** by reaction with hydroxylamine or O-methylhydroxylamine.

Replacement of the nitrile group with H and Me substituents resulted in no improvement in activity when compared to free oxime **22** (Table 3). However, the CF_3 replacement did contribute to the observed weak activity. Replacement of the nitrile group with five-membered heterocyclic isosteres was next investigated. While oxazole **52** and methyloxadiazoles **40** and **48** were inactive, thiadiazole **49** showed moderate activity. This activity was maintained with methylthiazole **51**. Activity of **49** could be improved to submicromolar levels by replacement of the methyl with a CF_3 group in compound **50**. Interestingly, a benzothiazole dimer **54** also displayed good activity, while the methyl ether **55** lost all activity, which was consistent with what was observed with the oxime carbamates. A six-membered heterocyclic replacement **56** in the form of a pyrazine also maintained some potency, but unfortunately cytotoxicity had eroded. Six-membered carbocyclic **58** and cyclic alkyl groups **59-60** resulted in complete loss of activity.

Alternative cores were synthesized similar to earlier compounds by preparing the oxime from commercially available nitriles followed by installation of the carbamate (Scheme 5). 2-Benzothiazoleacetonitrile was transformed to 4-amino-1,2,5-oxadiazole **67** in a one-pot two-step procedure, as described in Scheme 5. Furoxane **68** was formed *via* cycloaddition of the corresponding nitrile-oxide after chlorination of oxime **34**.

Replacing benzoxazole or benzothiazole cores of the hit compounds with a benzimidazole resulted in loss of

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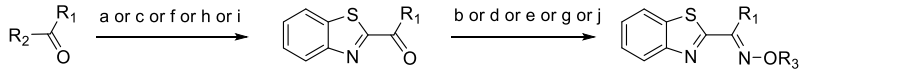
Scheme 4. Synthesis of Benzothiazole analogues[a]



R_2 = OH, MeO(Me)NH or Cl

53 R_1 = Benzothiazol-2-yl

52 R_1 = oxazol-5-yl, R_3 = H
54 R_1 = benzothiazol-2-yl, R_3 = H
55 R_1 = benzothiazol-2-yl, R_3 = CH_3
56 R_1 = pyrazine, R_3 =H
57 R_1 = benzothiazol-6-yl, R_3 = H
58 R_1 = Ph, R_3 = H
59 R_1 = cyclopentyl, R_3 = H
60 R_1 = cyclohexyl, R_3 = H

[a]Reagents and conditions: (a) (1) $SOCl_2$, $CHCl_3$, cat. DMF, (2) benzothiazole, DMAP, NEt_3, MeCN, 80 °C; (b) NH_2OH, K_2CO_3, EtOH, water, 50 °C; (c) benzothiazole, triphosgene, DMAP, MeCN, NEt_3, 80 °C; (d) NH_2OH·HCl, $NaHCO_3$, EtOH, aniline, THF, 50 °C; (e) *O*-methylhydroxylamine, cat. aniline, $NaHCO_3$, MeOH, THF, 50 °C; (f) (1) benzothiazole, *n*-BuLi, −78 °C, THF, (2) Weinreb amide, THF, rt; (g) NH_2OH·HCl, $NaHCO_3$, cat. aniline, MeOH, THF, 60 °C; (h) benzothiazole, DMAP, NEt_3, MeCN, 80 °C; (i) (1) benzothiazole, isoprpylmagnesium chloride, −15 °C, THF, (2) Weinreb amide, THF, rt; and (j) NH_2OH·HCl, NaOAc, MeOH, reflux.

antitubercular activity, suggesting that hydrogen-bond donors were less favored compared to hydrogen-bond acceptors or that the additional benzimidazole NH impacts permeability (Table 4). Replacement of the core with a phenyl and 2-pyridyl led to the same reduction in potency, while removal of the carbamate resulted in the loss of the marginal activity. Replacement of the oxime with a trifluoromethyl ketone hydrate **37** was not tolerated. Cyclization of the nitrile and oxime in the form of an oxadiazole **67** or furoxanes **68** was not tolerated.

We further profiled frontrunners from the oxime carbamate and free oxime series with the aim of identifying metabolically stable compounds that could potentially be evaluated *in vivo*. Compound **6** retained the potency of the hit compounds and displayed improved microsomal metabolic stability despite the presence of the oxime carbamate (Table 5). Solubility was low, human S9 fraction stability was moderate, and no human ether-a-go-go-related gene (hERG) liability was found.

Dosing of **6** in mice showed clearance higher than mouse liver blood flow notwithstanding the moderate to good *in vitro* stability in microsomes, and the compound was not detected in blood following oral administration at 20 mg/kg (Table 6). The free oxime compound **50** had moderate potency but improved solubility and microsomal metabolic stability compared to the hit compounds. With the labile carbamate group now removed and a better balance of activity, solubility, and microsomal stability properties observed, the compound was assessed in a mouse PK study (Table 6). The compound was rapidly distributed into tissue, with a high volume of distribution and rapidly cleared following IV administration in the mouse, resulting in a short elimination half-life. Oral PK showed that **50** was only 3% orally bioavailable. Taken together, the PK exposure of **6** and **50** were not sufficient for evaluation of *in vivo* efficacy in a mouse model of tuberculosis.

BIOLOGY TRIAGING TOWARD MECHANISM OF ACTION DETERMINATION

To explore the mechanism of action, selected compounds were tested in biology triage assays to assess their activity against promiscuous targets. The tested compounds **1**, **2**, **6**, **8**, **49**, and **50** retained activity against a QcrB mutant (A396T) and did not show MIC modulation against a cytochrome-*bd* oxidase knockout mutant strain (*cyd*KO), thereby eliminating the respiratory system of *Mtb* as a potential mechanism of action.[20] The compounds did not show a positive signal in two standard bioluminescence reporter assays: PiniB-LUX which detects modulation in *iniB* expression if the test compound damages the *Mtb* cell-wall and PrecA-LUX which detects modulation in *recA* expression—an indicator of compounds inducing DNA damage. Several attempts of generating spontaneous resistant mutants failed where a range of inoculum (10^{-8} to 10^{-10} CFU) was used. Nonetheless, based on this initial triage process, the compounds were identified as novel hits involving a potential novel mode of action in *Mtb*.

CONCLUSIONS

Phenotypic whole-cell screening of a hydrophilic small molecule library yielded NCEs with excellent antimycobacterial activity against both laboratory and clinical *Mtb* strains. While the parent carbamates were highly potent, the corresponding free oximes were inactive, due to their inability to permeate the *Mtb* cell-wall. Attempts at replacing the carbamate moiety with more metabolically stable groups remained a challenge. On the other hand, modifying the physicochemical properties of the free oxime proved successful in delivering potent free oxime compounds exemplified by **50**. These active free oxime compounds can potentially benefit from further optimization of *in vivo* intrinsic clearance. This work also demonstrated that these compounds do not hit promiscuous cell-wall biosynthesis and respiratory targets that are so often confirmed as targets of whole-cell phenotypic screening hits. Coupled with the potential of these compounds to act *via* a novel mode of action in *Mtb*, this further justifies continued future work to optimize this chemical series and/or develop formulation strategies to improve permeation across the *Mtb* cell-wall and identification of the target.

EXPERIMENTAL SECTION

General Methods. Purification of compounds were carried out by column chromatography on silica gel 60 (Fluka), particle size 0.063−0.2 mm (70−230 mesh), as the stationary phase. All target compounds and intermediates were characterized by ^{1}H NMR and LC−MS. NMR spectra were recorded on either a Varian Mercury-300 (^{1}H 300.1 MHz, ^{13}C 75.5 MHz) or Bruker-400 (^{1}H 400.2 MHz, ^{13}C 100.6 MHz) instrument using $CDCl_3$, CD_3OD, and DMSO-d_6 as solvents. LC−MS analysis was performed using an Agilent 1260 Infinity Binary Pump, Agilent 1260 Infinity Diode Array Detector (DAD), Agilent 1290 Infinity Column Compartment, Agilent 1260 Infinity Standard Autosampler, and a Agilent 6120 Quadrupole (Single) mass spectrometer, equipped with APCI and ESI multimode ionization sources. Purities were determined by Agilent LC−MS using a Kinetex Core C18 2.6 μm column (50 × 3 mm); Mobile Phase B: 0.4% acetic acid and 10 mM ammonium acetate in a 9:1 ratio of

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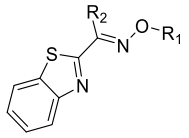
Table 3. Optimization of Nitrile Functionality



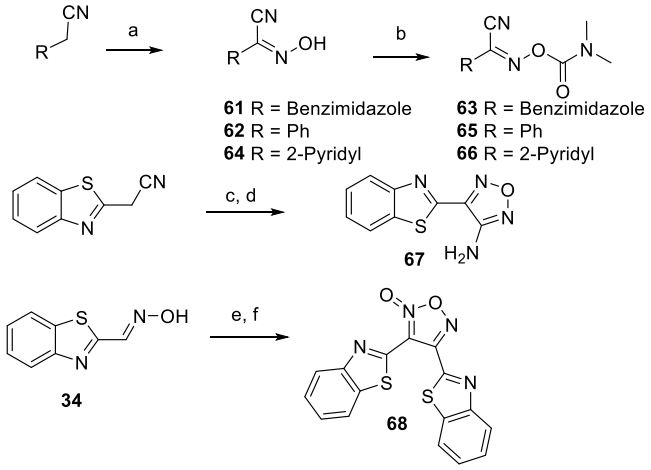
Compound	R_1	R_2	MIC[a] (μM)	CHO IC_{50}[b] (μM)
2	$CONMe_2$	CN	<0.16	>100
22	H	CN	>160	>100
34	H	H	>160	n.d.
36	H	Me	>125	>100[c]
38	H	CF_3	37	n.d.
40	H		>160	n.d.
48	H		160	n.d.
49	H		2.5	38
50	H		0.78	15.6
51	H		3.13	29[c]
52	H		50	>100[c]
54	H		0.80	>100[c]
55	Me		>125	>50[c]
56	H		3.6	11.7[c]
57	H		>125	>50[c]
58	H		>50	>100[c]
59	H		>125	83.5[c]
60	H		>125	51.6[c]
Rifampicin			0.009	n.d.
INH			0.14	n.d.

[a]14 day Alamar Blue readout against H37RvMa in GAST/Fe medium. [b]Cytotoxicity against CHO cells. [c]Cytotoxicity against kidney epithelial cells extracted from an African green monkey (VERO). n.d., not determined.

HPLC-grade methanol and type 1 water, Mobile Phase A: 0.4% acetic acid in 10 mM ammonium acetate in HPLC-grade (type 1) water, with flow rate = 0.9 mL/min; detector: diode array (DAD) and all compounds were confirmed to have ≥95% purity. Compound **51** was a generous gift from Pfizer. 2-Benzothiazoleacetonitrile was acquired form Sigma-Aldrich.

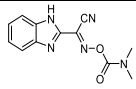
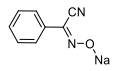
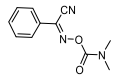
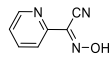
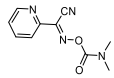
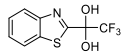
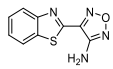
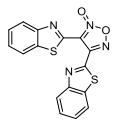
*General Procedure for Synthesis of Benzoxazole Oxime Carbamates **1**, **6**, and **9**–**20**.* Triethylamine (5.0 equiv) and dimethylcarbamoyl chloride (3.0 equiv) were successively added to a suspension of the appropriate oxime intermediate (1.0 equiv) in anhydrous DCM at room temperature. This solution was purged with nitrogen gas for 10 min, heated to reflux for 15 h, and then cooled to room temperature, and water (20 mL) was added. The mixture was extracted with DCM (3 × 20 mL), and the combined organic phases were rinsed with brine (2 × 50 mL), dried over anhydrous $MgSO_4$,

Scheme 5. Synthesis of Alternative cores[a]



[a]Reagents and conditions: (a) $NaNO_2$, AcOH, 0–25 °C, 12 h; (b) dimethylcarbamoyl chloride or *N*,*N*-dimethylsulfamoyl chloride, triethanolamine, DCM, reflux, 15 h; (c) $NaNO_2$, aq. HCl, MeOH, rt; (d) aq. NaOH, NH_2OH, reflux; (e) NCS, HCl gas, DMF, 18 h; and (f) NEt_3, EtOAc, 2 days.

Table 4. SAR of Alternative Core Structures

Compound	Structure	MIC[a] (μM)	Solubility[b] (μM)
63		9.4	29
62		>50	156
65		9.4	202
64		>50	202
66		9.4	175
37		>50	184
67		>50	5
68		>50	<5
Rifampicin		0.009	n.d.
INH		0.14	n.d.

[a]14 day Alamar Blue readout against H37RvMa in GAST/Fe medium. [b]Kinetic solubility assay at pH 6.5.

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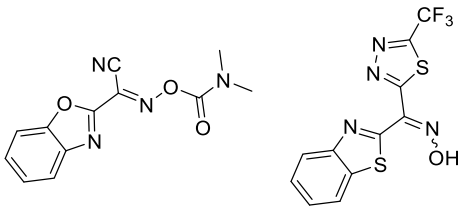
Table 5. Further Profiling of Leading Compounds



compound	6	50
Mtb MIC (μM) GAST/Fe[a]	0.30	0.78
Mtb MIC (μM) 7H9[b]	0.60	1.2
ESKAPE bacteria[c]	>125	>125
solubility (μM)[d]	18	67
H/R/MLM % remaining[e]	>99/98/>99	>99/86/71
human S9 % remaining[f]	60	n.d.
CHO IC_{50} (μM)[g]	>100	15.6
hERG IC_{50} (μM)[h]	>33	n.d.
PPB (fu)	n.d.	0.002
clog *P*[i]	1.53	2.64
TPSA[i]	91.7	128

[a]14 day Alamar Blue readout against H37RvMa in GAST/Fe medium. [b]14 day Alamar Blue readout against H37RvMa in Middlebrook 7H9 medium. [c]ESKAPE bacteria strains: *Enterobacter cloacae* (ATCC 700323), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC BAA-1705), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923). [d]Kinetic solubility assay at pH 6.5. [e]Percentage remaining at 30 min assessed in human, rat, and mouse liver microsomes. [f]Percentage remaining at 40 min assessed in the human S9 fraction. [g]Cytotoxicity against CHO cells. [h]Human ether-a-go-go related gene (hERG) K^+ channel inhibition determined with electrophysiology-based hERG assay using IonWorks HT. [i]Calculated using the ACD Percepta module. PPB = plasma protein binding; n.d., not determined.

Table 6. PK Parameters of Compound 6 and 50 in C57/BL6 Mice Following Intravenous and Oral Administration[a]

	6		50	
parameters	IV	PO	IV	PO
nominal dose (mg/kg)	5.0	20	5.0	20.0
C_{max} (μM)	3.9	n.d.	5.4	0.13
T_{max} (h)				0.17
$t_{1/2}$ terminal (h)	0.13	n.d.	2.6	1.8
CL_b (mL/min/kg)	165		157	
V_d (L/kg)	1.82		34.7	
$AUC_{0-\infty}$ (min. μmol/L)	118	n.d.	98	12
F (%)		<1		3

[a]Mean values from three animals. n.d., not determined due to low oral exposure. Dash indicates that the value was not measured or was not relevant.

and concentrated to give a residue, which was column-chromatographed (EtOAc/hexane) to give the desired product.

*N-((Dimethylcarbamoyl)oxy)-6-methylbenzo[d]oxazole-2-carbimidoyl Cyanide (**1**).* Yield (0.312 g, 77%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.3 Hz, 1H), 7.44 (d, *J* = 0.7 Hz, 1H), 7.29 (dd, *J* = 8.3, 1.0 Hz, 1H), 3.18 (s, 3H), 3.13 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 153.55, 152.89, 151.38, 139.61, 139.04, 127.47, 127.00, 121.01, 111.51, 107.07, 37.51, 36.19, 22.06. LC−MS (ESI): *m*/*z* 318.1 $[M + 46]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)benzo[d]oxazole-2-carbimidoyl Cyanide (**6**).* Yield (0.252 g, 79%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.89 (d, *J* = 7.9 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 1H), 7.52 (t, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 1H), 3.17 (s, 3H), 3.11 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 153.49, 151.38, 151.13, 141.16, 128.56, 127.00, 126.10, 121.83, 111.76, 107.14, 37.67, 36.33. LC−MS (ESI): *m*/*z* 304.1 $[M + 46]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)-4-methylbenzo[d]oxazole-2-carbimidoyl Cyanide (**9**).* Yield (0.233 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, *J* = 7.7 Hz, 1H), 7.44−7.38 (m, 1H), 7.26 (dt, *J* = 7.2, 1.0 Hz, 1H), 3.19 (s, 3H), 3.14 (s, 3H), 2.70 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 152.51, 151.34, 150.86, 140.64, 132.68, 128.12, 127.05, 126.23, 108.72, 107.08, 37.51, 36.19, 16.40. LC−MS (ESI): *m*/*z* 318.1 $[M + 46]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)-5-methylbenzo[d]oxazole-2-carbimidoyl Cyanide (**10**).* Yield (0.288 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (dt, *J* = 1.6, 0.8 Hz, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.35 (ddd, *J* = 8.5, 1.7, 0.6 Hz, 1H), 3.19 (s, 3H), 3.14 (s, 3H), 2.53 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 153.35, 151.29, 149.32, 141.29, 136.10, 129.77, 126.97, 121.30, 110.94, 107.05, 37.52, 36.19, 21.48. LC−MS (ESI): *m*/*z* 318.1 $[M + 46]^+$. HPLC purity >99%.

*5-Bromo-N-((dimethylcarbamoyl)oxy)benzo[d]oxazole-2-carbimidoyl Cyanide (**11**).* Yield (0.105 g, 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, *J* = 2.0 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.67 (dd, *J* = 8.5, 2.1 Hz, 1H), 3.13 (s, 3H), 3.09 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.21, 152.77, 141.98, 130.12, 126.53, 122.86, 122.71, 117.74, 112.87, 107.65, 37.41, 36.28. LC−MS (ESI): *m*/*z* 382.0, 384.0 (1:1) $[M + 46]^+$. HPLC purity >99%.

*6-Bromo-N-((dimethylcarbamoyl)oxy)benzo[d]oxazole-2-carbimidoyl Cyanide (**12**).* Yield (0.187 g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (d, *J* = 1.9 Hz, 1H), 7.77 (d, *J* = 8.5, 1H), 7.63 (dd, *J* = 8.5, 1.9 Hz, 1H), 3.19 (s, 3H), 3.14 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 153.75, 151.33, 151.13, 140.15, 129.73, 126.54, 122.58, 121.89, 115.15, 106.82, 37.57, 36.21. LC−MS (ESI): *m*/*z* 382.0, 384.0 (1:1) $[M + 46]^+$. HPLC purity >99%.

*Methyl 2-(cyano(((dimethylcarbamoyl)oxy)imino)methyl)benzo[d]oxazole-6-carboxylate (**13**).* Yield (0.282 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (d, *J* = 1.1 Hz, 1H), 8.20 (dd, *J* = 8.5, 1.4 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 4.00 (s, 3H), 3.20 (s, 3H), 3.15 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.83, 155.52, 151.05, 150.65, 144.32, 130.28, 127.39, 126.56, 121.38, 113.24, 106.79, 52.66, 37.60, 36.23. LC−MS (ESI): *m*/*z* 362.1 $[M + 46]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)-6-(trifluoromethyl)benzo[d]oxazole-2-carbimidoyl Cyanide (**14**).* Yield (0.105 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (d, *J* = 8.5, 1H), 7.95 (d, *J* = 2.0, 1H), 7.79 (dd, *J* = 8.5, 2.0 Hz, 1H), 3.20 (s, 3H), 3.15 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.48, 150.99, 150.36, 143.38, 130.39 (q), 126.37, *124*.89, 123.19, 122.31, 109.49, 106.72, 37.61, 36.22. LC−MS (ESI): *m*/*z* 372.1 $[M + 46]^+$. HPLC purity >99%.

*6-Chloro-N-((dimethylcarbamoyl)oxy)benzo[d]oxazole-2-carbimidoyl Cyanide (**15**).* Yield (0.160 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (dd, *J* = 8.6, 0.5 Hz, 1H), 7.67 (dd, *J* = 1.9, 0.5 Hz, 1H), 7.47 (dd, *J* = 8.6, 1.9 Hz, 1H), 3.19 (s, 3H), 3.14 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 153.92, 151.11 (2C), 139.74, 134.43, 126.98, 126.51, 122.20, 112.17, 106.81, 37.56, 36.20. LC−MS (ESI): *m*/*z* 338.1 $[M + 46]^+$. HPLC purity >99%.

*5-Chloro-N-((dimethylcarbamoyl)oxy)benzo[d]oxazole-2-carbimidoyl Cyanide (**17**).* Yield (0.207 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (dd, *J* = 2.1, 0.5 Hz, 1H), 7.60 (dd, *J* = 8.8, 0.5 Hz, 1H), 7.52 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.19 (s, 3H), 3.14 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 154.49, 151.08, 149.52, 141.95, 131.71, 128.82, 126.51, 121.49, 112.41, 106.79, 37.57, 36.21. LC−MS (ESI): *m*/*z* 338.1 $[M + 46]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)-6-fluorobenzo[d]oxazole-2-carbimidoyl Cyanide (**18**).* Yield (0.225 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (dd, *J* = 8.8, 5.0 Hz, 1H), 7.83 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.35 (ddd, *J* = 9.8, 8.8, 2.3 Hz, 1H), 3.19 (s, 3H), 3.14 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 163.77, 161.28, 153.97, 151.09, 137.47, 126.50, 122.44, 114.77, 106.85, 99.70, 37.55, 36.19. LC−MS (ESI): *m*/*z* 322.1 $[M + 46]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)-5-fluorobenzo[d]oxazole-2-carbimidoyl Cyanide (**19**).* Yield (0.167 g, 62%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.83 (dd, *J* = 8.9, 4.0 Hz, 1H), 7.78 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.40 (ddd, *J* = 9.6, 8.9, 2.7 Hz, 1H), 3.20 (s, 3H), 3.16 (s, 3H).

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^{13}C NMR (101 MHz, DMSO-d_6): δ 164.08, 160.23, 155.98, 148.31, 142.20, 126.57, 122.91, 114.57, 112.81, 107.49, 37.53, 36.21. LC−MS (ESI): m/z 322.1 $[M + 46]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)-6-nitrobenzo[d]oxazole-2-carbimidoyl Cyanide (**20**).* Yield (0.114 g, 55%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.57 (d, J = 2.0 Hz, 1H), 8.45 (dd, J = 8.8, 2.0 Hz, 1H), 8.05 (d, J = 8.8 Hz, 1H), 3.21 (s, 3H), 3.16 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 157.18, 150.82, 150.13, 147.29, 145.41, 126.05, 121.96, 121.74, 108.31, 106.54, 37.66, 36.25. LC−MS (ESI): m/z 349.1 $[M + 46]^+$. HPLC purity 96%.

*6-Acetamido-N-((dimethylcarbamoyl)oxy)benzo[d]oxazole-2-carbimidoyl Cyanide (**21**).* Yield (0.071 g, 47%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.41 (br s, 1H), 8.27 (d, J = 2.1 Hz, 1H), 7.86 (d, J = 8.9 Hz, 1H), 7.55 (dd, J = 8.9, 2.1 Hz, 1H), 3.09 (s, 3H), 3.05 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 169.13, 153.97, 151.59, 151.42, 140.03, 136.04, 127.62, 121.59, 118.41, 108.41, 101.59, 37.62, 36.03, 24.76. LC−MS (ESI): m/z 361.1 $[M + 46]^+$. HPLC purity >99%.

*(E)-N-((Dimethylcarbamoyl)oxy)benzo[d]thiazole-2-carbimidoyl Cyanide (**2**).* Triethylamine (0.700 mL, 5.00 mmol) and dimethylcarbamoyl chloride (0.276 mL, 3.00 mmol) were successively added to a suspension of oxime 22 (0.200 g, 0.984 mmol) in anhydrous DCM (10 mL) at 0 °C. This solution was stirred at 0 °C for 30 min and left at rt o/n. Water (10 mL) was added, the mixture was extracted with DCM (3 × 20 mL), and the combined organic phases were rinsed with brine (2 × 50 mL), dried over anhydrous $MgSO_4$, and concentrated to give a residue, which was purified by flash column chromatography with EtOAc/hexane (3/7) to give **2** (108 mg, 40%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23−8.18 (m, 1H), 7.97−7.90 (m, 1H), 7.62−7.49 (m, 2H), 3.17 (s, 3H), 3.10 (s, 3H). LC−MS (ESI): m/z 275.0 $[M + 1]^+$. HPLC purity 94%.

*(E)-N-Hydroxybenzo[d]thiazole-2-carbimidoyl Cyanide (**22**).* The synthesis of compound **22** was performed according to the literature procedure of Ilkun *et al.*[12]

*N-((N,N-Dimethylsulfamoyl)oxy)benzo[d]thiazole-2-carbimidoyl Cyanide (**23**).* Triethylamine (0.700 mL, 5.00 mmol) and *N,N*-dimethylsulfamoyl chloride (0.322 mL, 3.00 mmol) were successively added to a suspension of oxime 22 (0.200 g, 0.984 mmol) in anhydrous DCM (10 mL) at 0 °C. This solution was stirred at 0 °C for 30 min and left at rt o/n. Water (10 mL) was added, the mixture was extracted with DCM (3 × 20 mL), and the combined organic phases were rinsed with brine (2 × 50 mL), dried over anhydrous $MgSO_4$, and concentrated to give a residue, which was purified by flash column chromatography with EtOAc/hexane (10/90 to 20/80) to give **23** (55 mg, 18%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20−8.11 (m, 1H), 7.86 (ddd, J = 7.1, 2.0, 0.7 Hz, 1H), 7.62−7.44 (m, 2H), 3.08 (s, 6H). LC−MS (ESI): m/z 311.0 $[M + 1]^+$. HPLC purity 99%.

*N-((Methylsulfonyl)oxy)benzo[d]thiazole-2-carbimidoyl Cyanide (**24**).* Triethylamine (0.700 mL, 5.00 mmol) and MsCl (0.232 mL, 3.00 mmol) were successively added to a suspension of oxime **22** (0.200 g, 0.984 mmol) in anhydrous DCM (10 mL) at 0 °C. This solution was stirred at 0 °C for 30 min and left at rt o/n. Water (10 mL) was added, the mixture was extracted with DCM (3 × 20 mL), and the combined organic phases were rinsed with brine (2 × 50 mL), dried over anhydrous $MgSO_4$, and concentrated to give a residue, which was purified by flash column chromatography with EtOAc/hexane (20/80 to 50/50) to give **24** (71 mg, 26%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30−8.21 (m, 1H), 8.00−7.92 (m, 1H), 7.68−7.55 (m, 2H), 3.37 (s, 3H). LC−MS (ESI): m/z 282.0 $[M + 1]^+$. HPLC purity 95%.

*General Procedure for Synthesis of **25**, **26**, **28-32**.* To a solution of **22** (1 equiv) in DCM (0.1−0.2 M) was added triethylamine (5 equiv) followed by the appropriate bromide or chloride (3 equiv) under a nitrogen atmosphere. The mixture was stirred o/n at rt or until the starting material was consumed, diluted with DCM, and washed with water. After drying over $MgSO_4$, filtration, and concentration, the crude product was purified by flash column chromatography using EtOAc/hexane to yield the desired product.

*Ethyl (E)-2-(((Benzo[d]thiazol-2-yl(cyano)methylene)amino)-oxy)acetate (**25**).* Yield (55 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (ddd, J = 8.2, 1.3, 0.7 Hz, 1H), 7.89 (ddd, J = 7.8, 1.4, 0.7 Hz, 1H), 7.65−7.36 (m, 2H), 5.00 (s, 2H), 4.30 (q, J = 7.1 Hz, 2H), 1.32 (t, J = 7.1 Hz, 3H). LC−MS (ESI): m/z 290.0 $[M + 1]^+$. HPLC purity 94%.

*(E)-N-(2-(Dimethylamino)-2-Oxoethoxy)benzo[d]thiazole-2-carbimidoyl Cyanide (**26**).* Yield (135 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (ddd, J = 8.1, 1.4, 0.7 Hz, 1H), 7.96−7.79 (m, 1H), 7.64−7.43 (m, 2H), 5.13 (s, 2H), 3.07 (s, 3H), 3.01 (s, 3H). LC−MS (ESI): m/z 289.1$[M + 1]^+$. HPLC purity 99%.

*(E)-N-Propoxybenzo[d]thiazole-2-carbimidoyl Cyanide (**28**).* Yield (51 mg, 21%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (ddd, J = 8.2, 1.3, 0.7 Hz, 1H), 7.82 (ddd, J = 7.8, 1.4, 0.7 Hz, 1H), 7.50−7.37 (m, 2H), 4.41 (t, J = 6.7 Hz, 2H), 1.81 (h, J = 7.4 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H). LC−MS (ESI): m/z 246.1 $[M + 1]^+$. HPLC purity >99%.

*(E)-N-(Benzyloxy)benzo[d]thiazole-2-carbimidoyl Cyanide (**29**).* Yield (130 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (ddd, J = 8.1, 1.4, 0.7 Hz, 1H), 7.80 (ddd, J = 7.8, 1.4, 0.6 Hz, 1H), 7.55−7.26 (m, 7H), 5.43 (s, 2H). LC−MS (ESI): m/z 294.0 $[M + 1]^+$. HPLC purity >99%.

*(E)-N-(Pyridin-3-ylmethoxy)benzo[d]thiazole-2-carbimidoyl Cyanide (**30**).* Yield (100 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (s, 1H), 8.71 (d, J = 4.5 Hz, 1H), 8.22−8.11 (m, 1H), 8.03 (d, J = 8.1 Hz, 1H), 7.94−7.79 (m, 1H), 7.63−7.44 (m, 3H), 5.57 (s, 2H). LC−MS (ESI): m/z 295.1 $[M + 1]^+$. HPLC purity 97%.

*(E)-N-(Pyridin-4-ylmethoxy)benzo[d]thiazole-2-carbimidoyl Cyanide (**31**).* Yield (46 mg, 31%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.70−8.54 (m, 2H), 8.26−8.08 (m, 2H), 7.70−7.52 (m, 2H), 7.51−7.40 (m, 2H), 5.66 (s, 2H). LC−MS (ESI): m/z 295.0 $[M + 1]^+$. HPLC purity 99%.

*(E)-N-(Pyridin-2-ylmethoxy)benzo[d]thiazole-2-carbimidoyl Cyanide (**32**).* Yield (116 mg, 39%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (dd, J = 4.6, 2.2 Hz, 1H), 8.21−8.11 (m, 1H), 7.92−7.71 (m, 2H), 7.63−7.43 (m, 3H), 7.38−7.28 (m, 1H), 5.68 (s, 2H). LC−MS (ESI): m/z 295.0 $[M + 1]^+$. HPLC purity 98%.

*(E)-N-Methoxybenzo[d]thiazole-2-carbimidoyl Cyanide (**27**).* To a solution of **22** (200 mg, 0.984 mmol) in DCM (10 mL) was added triethylamine (0.411 mL, 2.95 mmol) followed by methyl iodide (0.123 mL, 1.968 mmol) under a nitrogen atmosphere. The mixture was stirred for 3 h at rt, diluted with DCM, and washed with water. After drying over $MgSO_4$, filtration, and concentration, the crude product was purified by flash column chromatography using EtOAc/hexane (10/90 to 30/70) to yield **27** (44 mg, 21%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21−8.09 (m, 1H), 7.92−7.84 (m, 1H), 7.58−7.44 (m, 2H), 4.33 (s, 3H). LC−MS (ESI): m/z 218.0 $[M + 1]^+$. HPLC purity 95%.

*N-[(1,3-Benzothiazol-2-yl)methylidene]oxime (**34**).* Benzothiazole-2-carboxaldehyde (200 mg, 1.22 mmol) was dissolved in methanol (3 mL) and tetrahydrofuran (THF) (3 mL) and placed in a pressure tube. To this mixture was added a solution of hydroxylamine hydrochloride (500 mg, 7.20 mmol) in saturated $NaHCO_3$ (1 mL) solution, and the mixture was stirred at 60 °C for 18 h. The cooled mixture was diluted with 60 mL of water and then extracted with EtOAc (2 × 80 mL). The organic phase was dried over Na_2SO_4 and evaporated to give **34** (197 mg, 90%) as a light-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ [isomer ratio 2:1] 8.52 & 8.15 (s & s 1H), 8.21−8.07 (m, 1H), 8.04−7.88 (m, 2H), 7.63−7.44 (m, 2H). LC−MS (ESI): m/z 179.0 $[M + 1]^+$. HPLC purity 98%.

*N-[-1-(1,3-Benzothiazol-2-yl)ethylidene]hydroxylamine (**36**).* 2-Acetylbenzothiazole (80 mg, 0.45 mmol) was dissolved in isopropanol (2 mL); then, a solution of hydroxylamine hydrochloride (500 mg, 7.20 mmol) in $NaHCO_3$ (2 mL) solution was added, and the mixture was heated to 65 °C overnight. The mixture was diluted with 50 mL of $NaHCO_3$ solution and extracted with EtOAc (2 × 50 mL). The organic phase was dried over Na_2SO_4 and evaporated. Flash chromatography with hexane/DCM/EtOAc (70/20/10 to 50/30/20) and crystallization from EtOAc yielded **36** (62 mg, 71%) in the form of white crystals. ^{1}H NMR (600 MHz, MeOD): δ [isomer ratio 1:1] 8.20−8.13 & 8.13−8.06 (m & m, 2H), 7.67−7.61 & 7.61−7.55

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(m & m, 2H), 2.81 & 2.78 (s & s, 3H). LC−MS (ESI): m/z 193.1 $[M + 1]^+$. HPLC purity >99%.

*N-[1-(1,3-Benzothiazol-2-yl)-2,2,2-trifluoroethylidene]-hydroxylamine (**38**).* **37** (700 mg, 2.80 mmol) was dissolved in water (3 mL) and ethanol (5 mL). Hydroxylamine hydrochloride (800 mL, 11.5 mmol) and Na_2CO_3 (1.0 g, 9.43 mmol) were added, and the mixture was heated to 75 °C for 24 h. The reaction mixture was evaporated, and the remaining material was diluted with a $NaHCO_3$ (50 mL) solution and extracted with EtOAc (2 × 70 mL). The organic phase was dried over Na_2SO_4 and evaporated. Flash chromatography with hexane/EtOAc (90/10 to 70/30) yielded **38** (250 mg, 36%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 14.25 (br s, 1H), 8.12−8.08 (m, 1H), 7.98−7.94 (m, 1H), 7.62−7.49 (m, 2H). LC−MS (ESI): m/z 247.0 $[M + 1]^+$. HPLC purity 99%.

*General Procedure for Synthesis of **40**, **48**, **49** and **50**.* To a solution of either **39**, **45**, **46**, or **47** (1 equiv) in acetic acid (7.5 mL) and water (0.75 mL) was added sodium nitrite (1 equiv). After stirring for 2 h at rt, the reaction mixture was diluted with water (40 mL) and the desired product was isolated by filtration of the precipitate that had formed.

*Benzo[d]thiazol-2-yl(5-methyl-1,2,4-oxadiazol-3-yl)methanone Oxime (**40**).* Yield (109 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.64 (s, 1H), 8.29 (dddd, J = 11.9, 8.1, 1.4, 0.7 Hz, 2H), 7.80−7.55 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 169.18, 153.13, 152.71, 149.43, 144.98, 134.74, 127.76, 124.45, 123.26, 23.74. LC−MS (ESI): m/z 261.0 $[M + 1]^+$. HPLC purity >99%.

*Benzo[d]thiazol-2-yl(5-methyl-1,3,4-oxadiazol-2-yl)methanone Oxime (**48**).* ^{1}H NMR (300 MHz, DMSO-d_6): δ [isomer ratio 5:1] 8.29−8.22 & 8.18−8.14 (m & m, 1H), 8.13−8.09 & 8.03−8.00 (m & m, 1H), 7.65−7.58 & 7.56−7.52 (m & m, 2H), 2.65 & 2.63 (s & s, 3H). LC−MS (ESI): m/z 261.0 $[M + 1]^+$. HPLC purity 99%.

*Benzo[d]thiazol-2-yl(5-methyl-1,3,4-thiadiazol-2-yl)methanone Oxime (**49**).* ^{1}H NMR (300 MHz, $CDCl_3$): δ [isomer ratio 1:1] 8.23−8.07 & 8.07−8.03 (m & m, 1H), 7.71−7.40 (m, 2H), 2.93 (s, 1H), 2.85 (s, 1H). LC−MS (ESI): m/z 277.0 $[M + 1]^+$. HPLC purity 96%.

*Benzo[d]thiazol-2-yl(5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)-methanone Oxime (**50**).* Yield (200 mg, 46%). ^{1}H NMR (600 MHz, DMSO-d_6): δ [isomer ratio 2:3] 8.26 & 8.23 (d, J = 8.0 Hz & d, J = 8.1 Hz, 1H), 8.18 & 8.10 (d, J = 7.8 Hz, & d, J = 8.0 Hz, 1H), 7.65 & 7.63−7.58 (d, J = 7.7 Hz & m, 1H), 7.58−7.51 (m, 1H). LC−MS (ESI): m/z 331.2 $[M + 1]^+$. HPLC purity 97%.

*(1,3-Benzothiazol-2-yl)(1,3-oxazol-5-yl)methanone Oxime (**52**).* To a solution of (1,3-benzothiazol-2-yl)(1,3-oxazol-5-yl)methanone (100 mg, 0.43 mmol) in EtOH (10 mL) was added a solution of hydroxylamine hydrochloride (650 mg, 9.34 mmol) in water (5 mL) and $NaHCO_3$ (2 mL) solution and K_2CO_3 (250 mg). This mixture was heated to 50 °C for 3 days. The organics were evaporated, the remaining mixture was extracted with EtOAc (2 × 50 mL), and the organic layer was dried over Na_2SO_4 and evaporated. Flash chromatography with EtOAc/hexane (1/3) yielded **52** (51 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.79 (s, 1H), 8.21 (s, 1H), 8.12−8.02 (m, 2H), 7.63−7.50 (m, 2H). LC−MS (ESI): m/z 246.1$[M + 1]^+$. HPLC purity 95%.

*Bis(benzo[d]thiazol-2-yl)methanone Oxime (**54**).* 53 (200 mg, 0.67 mmol) was dissolved in ethanol (20 mL) and THF (20 mL). A solution of hydroxylamine hydrochloride (800 mg, 11.5 mmol) in sat. $NaHCO_3$ (2 mL) solution was added, and the mixture was heated with aniline (0.2 mL, 2.15 mmol) to 50 °C for 16 h. The cooled mixture was diluted with a 50 mL $NaHCO_3$ solution and extracted with EtOAc (2 × 50 mL). The organic phase was dried over Na_2SO_4 and evaporated. Flash chromatography with hexane/EtOAc/$CHCl_3$ (50/15/35 to 40/25/35 to 0/70/30) and crystallization from EtOAc yielded **54** (58 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23−8.09 (m, 3H), 7.99−7.96 (m, 1H), 7.69−7.48 (m, 4H). LC−MS (ESI): m/z 312.0 $[M + 1]^+$. HPLC purity >99%.

*Bis(benzothiazol-2-yl)methanone O-methyl Oxime (**55**).* A mixture of 53 (70 mg, 0.236 mmol), O-methylhydroxylamine (500 mg, 10.63 mmol, dissolved in 1 mL $NaHCO_3$ solution), and 10 drops of aniline in THF (2 mL) and MeOH (2 mL) was heated in a sealed pressure tube to 50 °C for 5 h. The mixture was cooled and evaporated onto silica gel. Flash chromatography with hexane/EtOAc/DCM (90/10/0 to 40/10/50) yielded **55** (35 mg, 46%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30−8.19 (m, 1H), 8.11−7.91 (m, 1H), 7.67−7.50 (m, 2H), 4.41 (s, 3H). LC−MS (ESI): m/z 326.0 $[M + 1]^+$. HPLC purity >99%.

*N-[(1,3-Benzothiazol-2-yl) (pyrazin-2-yl)methylidene]-hydroxylamine (**56**).* Benzothiazol-2-yl(pyrazin-2-yl)methanone (100 mg, 0.414 mmol) was dissolved in THF (3 mL) and MeOH (3 mL). A solution of hydroxylamine hydrochloride (500 mg, 7.19 mmol) in $NaHCO_3$ (1 mL) solution was added, and the mixture was heated in a pressure tube with 1 drop of aniline to 60 °C for 18 h. The cooled mixture was treated with $NaHCO_3$ (50 mL) solution and extracted with EtOAc (50 mL). The organic phase was dried over $NaSO_4$ and evaporated. Flash chromatography with EtOAc/DCM (40/60) yielded **56** (80 mg, 76%) as a white solid after crystallization from EtOAc. ^{1}H NMR (300 MHz, $CDCl_3$): δ (isomer ratio 4:1) 9.95 & 9.51 (s & s, 1H), 8.85−8.68 (m, 2H), 8.26−7.94 (m, 2H), 7.69−7.52 (m, 2H). HPLC purity 97%.

*Benzothiazol-2-yl(benzothiazol-6-yl)methanone Oxime (**57**).* Benzothiazol-2-yl(benzothiazol-6-yl)methanone (100 mg, 0.337 mmol) and 3 drops of aniline were dissolved in THF (3 mL) and MeOH (3 mL). A solution of hydroxylamine hydrochloride (600 mg, 8.63 mmol) was added in $NaHCO_3$ (1 mL) solution. The mixture was heated in a sealed pressure tube to 50 °C for 16 h, and 50 mL of $NaHCO_3$ solution was added to the cooled mixture and extracted with EtOAc (2 × 50 mL), then dried over Na_2SO_4, and evaporated. Flash chromatography with hexane/EtOAc/DCM (50/30/20) and crystallization from the almost evaporated product fractions yielded a mixture of isomers **57** (86 mg, 82%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ [isomer ratio 1:1] 13.33 & 12.70 (bs & s, 1H), 9.48 & 9.45 (s & s, 1H), 8.44 & 8.35 (d & d, J = 3.0 & 3.0 Hz, 1H), 8.27−7.88 (m, 3H), 7.84 & 7.70 (dd & dd, J = 6.0 & 3.0 Hz & J = 6.0 & 3.0 Hz, 1H), 7.61−7.44 (m, 2H). LC−MS (ESI): m/z 312.0 $[M + 1]^+$. HPLC purity 97%.

*(1,3-Benzothiazol-2-yl)(phenyl)methanone Oxime (**58**).* A solution of (1,3-benzothiazol-2-yl)(phenyl)methanone (200 mg, 0.83 mmol) in THF (15 mL) and EtOH (20 mL) was treated with a 50% solution of NH_2OH (1.2 mL, 18.2 mmol) in water and stirred for 3 days at 50 °C. The organic solvents were evaporated, and the remaining mixture was extracted with EtOAc (2 × 50 mL), dried over Na_2SO_4, and evaporated. Purification by flash chromatography with hexane/EtOAc (90/10) yielded **58** (80 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11−7.95 (m, 1H), 7.90−7.80 (m, 1H), 7.70−7.30 (m, 7H), 7.20−7.14 (m, 1H). LC−MS (ESI): m/z 255.0 $[M + 1]^+$. HPLC purity 99%.

*Benzothiazol-2-yl(cyclopentyl)methanone Oxime (**59**).* A mixture of benzo[d]thiazol-2-yl(cyclopentyl)methanone (282 mg, 1.22 mmol), hydroxylamine hydrochloride (93 mg, 1.34 mmol), and sodium acetate (110 mg, 1.34 mmol) in methanol was heated to reflux for 2 h under a nitrogen atmosphere. The reaction mixture was cooled, and the methanol was removed under reduced pressure. The residue was diluted with EtOAc (30 mL) and extracted with 2 N NaOH (15 mL). The organic layer was washed with water (15 mL) and concentrated under reduced pressure. Flash chromatography with hexane/EtOAc (70/30) gave **59** (230 mg, 77%) as a mixture of two isomers as a green solid. ^{1}H NMR (300 MHz, DMSO-d_6) [isomer ratio 3:1]: δ 12.66 & 12.27 (s & s, 1H), 8.20−7.98 (m, 2H), 7.62−7.44 (m, 2H), 3.85−3.65 (m, 1H), 2.20−1.54 (m, 8H). LC−MS (ESI): m/z 247.1 $[M + 1]^+$. HPLC purity >99%.

*Benzo[d]thiazol-2-yl(cyclohexyl)methanone Oxime (**60**).* A mixture of benzo[d]thiazol-2-yl(cyclohexyl)methanone (100 mg, 0.408 mmol), hydroxylamine hydrochloride (31.2 mg, 0.449 mmol), and sodium acetate (36.8 mg, 0.449 mmol) in 5 mL of methanol was heated to reflux for 2 h under a nitrogen atmosphere. The reaction mixture was cooled, and the methanol was removed under reduced pressure. The residue was diluted with EtOAc (20 mL), and 2 N NaOH (7 mL) was added. The organic layer was washed with water (7 mL) and concentrated under reduced pressure. Flash chromatography with hexane/EtOAc (70/30) yielded **60** (81 mg, 76%) as a

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mixture of two isomers in the form of a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ [isomer ratio 7:3] 12.70 & 12.21 (s & s, 1H), 8.23−8.03 (m, 2H), 7.62−7.45 (m, 2H), 3.60−3.20 (m, 1H), 2.30−1.20 (m, 10H). LC−MS (ESI): m/z 261.1 $[M + 1]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)-1H-benzo[d]imidazole-2-carbimidoyl Cyanide (**63**).* Triethylamine (0.700 mL, 5.00 mmol) and dimethylcarbamoyl chloride (0.276 mL, 3.00 mmol) were successively added to a suspension of oxime **61** (0.200 g, 1.07 mmol) in anhydrous DCM (10 mL) at 0 °C. This solution was stirred at 0 °C for 30 min and left at rt o/n. Water (10 mL) was added, the mixture was extracted with DCM (3 × 20 mL), and the combined organic phases were rinsed with brine (2 × 50 mL), dried over anhydrous $MgSO_4$, and concentrated to give a residue, which was purified by flash column chromatography with EtOAc/hexane (5/5 to 7/3) to give **63** (157 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76−7.66 (m, 2H), 7.45−7.33 (m, 2H), 3.17 (s, 3H), 3.11 (s, 3H). LC−MS (ESI): m/z 258.1 $[M + 1]^+$. HPLC purity 99%.

*N-((Dimethylcarbamoyl)oxy)benzimidoyl Cyanide (**65**).* Triethylamine (0.953 mL, 6.84 mmol) and dimethylcarbamoyl chloride (0.378 mL, 4.11 mmol) were successively added to a suspension of oxime **62** (0.200 g, 1.37 mmol) in anhydrous DCM (10 mL) and dimethylformamide (DMF) (2 mL) at 0 °C. This solution was stirred at 0 °C for 30 min and left at rt o/n. Water (10 mL) was added, and the solid that formed was purified by recrystallization (DCM/hexane) to give **63** (163 mg, 55%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03−7.90 (m, 2H), 7.61−7.43 (m, 3H), 3.12 (s, 3H), 3.07 (s, 3H). LC−MS (ESI): m/z 218.0 $[M + 1]^+$. HPLC purity >99%.

*N-((Dimethylcarbamoyl)oxy)picolinimidoyl Cyanide (**66**).* Triethylamine (0.953 mL, 6.84 mmol) and dimethylcarbamoyl chloride (0.378 mL, 4.11 mmol) were successively added to a suspension of oxime **64** (0.200 g, 1.07 mmol) in anhydrous DCM (10 mL) at 0 °C. This solution was stirred at 0 °C for 30 min and left at rt o/n. Water (10 mL) was added, the mixture was extracted with DCM (20 mL x 3), and the combined organic phases were rinsed with brine (2 × 50 mL), dried over anhydrous $MgSO_4$, and concentrated to give a residue, which was purified by flash column chromatography with EtOAc/hexane (6/4) to give **66** (82 mg, 35%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.85−8.57 (m, 1H), 8.17 (dt, J = 8.0, 1.1 Hz, 1H), 7.81 (td, J = 7.8, 1.8 Hz, 1H), 7.45 (ddd, J = 7.5, 4.8, 1.1 Hz, 1H), 3.16 (s, 3H), 3.09 (s, 3H). LC−MS (ESI): m/z 219.1 $[M + 1]^+$. HPLC purity >99%.

*4-(Benzo[d]thiazol-2-yl)-1,2,5-oxadiazol-3-amine (**67**).* The oxime **22** (200 mg, 1.00 mmol) was dissolved in MeOH (3.33 mL), and the pH was adjusted to 12 with 50% aq. NaOH. 50% aqueous NH_2OH (2.73 mL) was added, and the mixture was stirred for 2 h at 90 °C. The solid that formed was isolated by filtration to give **67** (120 mg, 55%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.47−8.06 (m, 2H), 7.87−7.34 (m, 2H), 6.83 (s, 2H). LC−MS (ESI): m/z 219.0 $[M + 1]^+$. HPLC purity >99%.

*3,4-Bis(benzo[d]thiazol-2-yl)-1,2,5-oxadiazole 2-Oxide (**68**).* *N*-[(1,3-Benzothiazol-2-yl)methylidene]hydroxylamine (197 mg, 1.10 mmol) was dissolved in DMF (3 mL); then, NCS (195 mg, 1.46 mmol) was added, and ∼10 mL of HCl gas (use HCl gas over conc. HCl) was filled into the flask and then closed with a plastic stopper. The mixture was stirred at rt overnight. EtOAc (50 mL) was added, and the solution was washed with water (2 × 50 mL) and dried over Na_2SO_4. To this solution was added triethylamine (500 mg, 4.94 mmol), and the solution was stirred at rt for 2 days. This solution was washed with 50 mL of water/1 mL of HCl, dried over Na_2SO_4, and evaporated to ∼3 mL, and the product was allowed to crystallize to give **68** (42 mg, 22%) in the form of an orange solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30−8.03 (m, 4H), 7.67−7.52 (m, 4H). LC−MS (ESI): m/z 352.9 $[M + 1]^+$. HPLC purity 98%.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00707.

Synthetic procedures and procedures used for *in vitro Mtb* studies and PK and metabolism studies (PDF)

SMILES file with *Mtb* MIC values (CSV)

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Notes

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■ ABBREVIATIONS

AcOH, acetic acid; ADME, absorption, distribution, metabolism and excretion; CHO, Chinese hamster ovarian; DCM, dichloromethane; DMAP, 4-(dimethylamino)pyridine; hERG, human ether-a-go-go-related gene; HLM, human liver microsome; HTS, high-throughput screening; INH, isoniazid; LC−MS/MS, liquid chromatography coupled with mass spectrometry; MDR, multidrug-resistant; MIC, lowest concentration of the drug that inhibits growth of more than 90% of the bacterial population; MLM, mouse liver microsome; *Mtb*, *Mycobacterium tuberculosis*; NCE, new chemical entity; NITD, Novartis Institute for Tropical Diseases; NMR, nuclear magnetic resonance; PAR, peak area ratio; PK, pharmacokinetics; PPB, plasma protein binding; PZA, pyrazinamide; RLM, rat liver microsome; SAR, structure−activity relationship; TB, tuberculosis; TDR, totally drug-resistant; TMSCl, chlorotrimethylsilane; TPSA, total polar surface area; VERO, kidney epithelial cells extracted from an African green monkey; XDR, extensively drug-resistant

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