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Analogs of *N*′-hydroxy-*N*-(4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide inhibit *Mycobacterium tuberculosis* methionine aminopeptidases

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ABSTRACT

Our previous target validation studies established that inhibition of methionine aminopeptidases (MtMetAP, type 1a and 1c) from *Mycobacterium tuberculosis* (Mtb) is an effective approach to suppress Mtb growth in culture. A novel class of MtMetAP1c inhibitors comprising of *N*′-hydroxy-*N*-(4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide (**4c**) was uncovered through a high-throughput screen (HTS). A systematic structure–activity relationship study (SAR) yielded variants of the hit, **4b**, **4h**, and **4k**, bearing modified A- and B-rings as potent inhibitors of both MtMetAPs. Except methanimidamide **4h** that showed a moderate Mtb inhibition, a desirable minimum inhibitory concentration (MIC) was not obtained with the current set of MtMetAP inhibitors. However, the SAR data generated thus far may prove valuable for further tuning of this class of inhibitors as effective anti-tuberculosis agents.

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1. Introduction

It is estimated that as much as one-third of the world's population is infected with *Mycobacterium tuberculosis* (Mtb). Globally mortality due to Mtb infection is second only to HIV/AIDS,[1] and an expanding epidemic of multi-drug resistant, extensively drug-resistant, and totally drug-resistant[2] tuberculosis (TB) coupled with Mtb (including latent) and HIV co-infection is a serious health threat requiring urgent attention.[3] Since the approval of rifampin as a treatment for TB by the US Food and Drug Administration (FDA) in 1971, there has been a long hiatus with no new and significant anti-TB drugs brought to the clinic. Recently there has been an awakening to the urgency of the situation, and now there are several candidate anti-TB drugs in various stages of development.[4] Continued emphasis on drug discovery is needed as new drugs with novel modes of action are required to catch up with the rapidly evolving resistant strains of Mtb. To this end we have been actively pursuing inhibitors of Mtb methionine aminopeptidases (MetAPs) as novel targets.

MetAPs are metalloproteases that perform the vital function of removing the initiator methionine from the nascent polypeptides emanating from ribosomes, and hence are expressed and present in every life form. In prokaryotes, and in organelles like mitochondria and plastids the start codon is translated by *N*-formylmethionine, and a prerequisite step of deformylation by peptide deformylase (PDF) is required.[5] The importance of these two enzymes is underscored by the study by Solbiati et al. showing that null mutations in PDF or MetAP gene are lethal to *Escherichia coli*.[6] The genome of the H37Rv strain of Mtb contains two MetAP genes, mapA and mapB encoding for Mtb methionine aminopeptidase 1a (MtMetAP1a) and MtMetAP1c, respectively. We have previously characterized these two isozymes both biochemically and by X-ray crystallography.[7] The crystal structure revealed the presence of an SH3 domain at the N-terminal extension (40 amino acids) suggesting a mode of association with the ribosome. MtMetAP1a isozyme is a minimal protein exhibiting optimal enzyme activity at 55 °C whereas MtMetAP1c is fully active at 37 °C. The two isozymes also differ in their specificity towards substrates and metal ion cofactors. Further, Zhang et al.[8] determined that the mRNA levels for the two isoforms were vastly different in different growth phases—*mapA* gene was expressed more in log phase, while *mapB* transcript levels were higher in stationary phase—suggesting that the two MetAPs are important for Mtb growth in different phases of its unique life cycle. Using a combination of chemical and genetic approaches, we had validated MtMetAPs to be viable targets. MtMetAP1a had emerged as a more sensitive target in the culture setting of Mtb.[9] In an effort to identify novel inhibitors of MtMetAPs, we conducted a high-throughput screen (HTS) of a 175,000 compound library (from ASDI, Inc., Newark, DE) against MtMetAP1c. One of the most potent hits against MtMetAP1c was *N*′-hydroxy-*N*-(7-meth-

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oxy-4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide (**4c**, see Table 1). This article is an account of our medicinal chemistry effort at optimizing this hit for potent inhibition of both MtMetAPs.

2. Chemistry

Compounds **3n** through **3r** were from the ASDI compound library and they were verified by ESI-MS analysis to confirm the presence of expected molecular ions. The *N*′-hydroxy-*N*-(thiazol-2-yl)methanimidamides were prepared using a three-step protocol starting from ketones **1** (Scheme 1). The ketones unavailable from commercial sources, namely **1f**,[10] **1g**,[11] **1h**,[12] **1j**,[13] and **1k**[14] were prepared following literature procedures. Aminothiazoles **2a**–**2m** were prepared using a variation of Hantzsch thiazole synthesis,[15] where α-halo ketones generated in situ (from **1a**–**1m**) were reacted with thiourea. Amide **3s** was synthesized by coupling a silyl-protected (*S*)-lactic acid with aminothiazole **2c** and by subsequent removal of the silyl protection revealing the free hydroxy group. The Schiff bases **3t** and **3v** were made from **2c** by treating them with furfural and salicylaldehyde, respectively, following a reported procedure.[16] The Schiff base **3t** was reduced using sodium borohydride to form the alkylated thiazole **3u**. The aminothiazoles (**2a**–**2m**) were further converted to the intermediates *N*′,*N*′-dimethylamidines **3a**–**3m** by refluxing with DMF–dimethylacetal. And finally, the formamidine intermediates (**3a**–**3m**) were transformed into *N*′-hydroxy-*N*-(thiazol-2-yl)methanimidamides **4a**–**4m** by treating with hydroxylamine, as per the protocol of Sasaki et al.[17] The overall yields of **4a**–**4m** starting from the ketones (**1a**–**1m**) ranged from 37% to 75%.

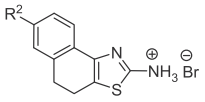
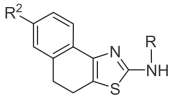
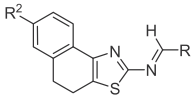
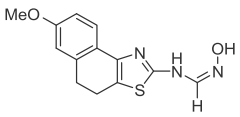
N′-Hydroxy-*N*-(thiazol-2-yl)methanimidamide can exist as two distinct tautomers as shown in Scheme 2. In the case of methanimidamide **4c**, we were able to discern by 2D-NOESY experiment that the prevailing tautomer in acetone is **4c–2**. This observation is in complete agreement with the literature reports where *N*′-hydroxy-*N*-arylmethanimidamides were seen in this tautomeric form in the X-ray structures.[18,19]

3. Results and discussion

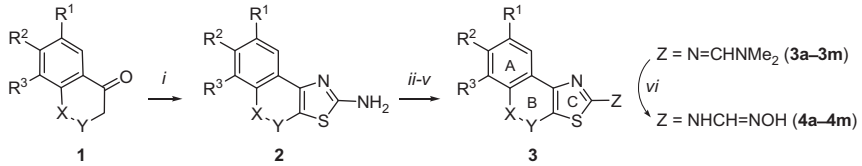
A target-based HTS of a 175,000 compound library from ASDI, Inc., Newark, DE, was performed to identify novel inhibitors of MtMetAP1c using a coupled enzyme assay[20] with Met-Pro-pNA as the substrate, Co^{2+} as the cofactor, and proline aminopeptidase from *Bacillus coagulans* (BcProAP) as the coupling enzyme.[8] The HTS furnished us a hit bearing a novel *N*′-hydroxy-*N*-(7-methoxy-4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide structure, **4c** with an IC_{50} of 1.33 μM. We then undertook a systematic structure–activity relationship (SAR) study to fine–tune the structure for inhibition against MtMetAPs. As mentioned previously, each of the isoforms, MtMetAP1a and MtMetAP1c, appears to be important for TB during different growth phases, and hence, we assayed all the compounds against both isoforms. Also, it is still unclear what the physiologically relevant metal cofactors are for these two isoforms. In the in vitro assay setting, Wang's group demonstrated that highest catalytic activity of MtMetAP1a could be achieved with Co^{2+} as the cofactor followed by Mg^{2+} and Mn^{2+} with nearly 2- and 3.5-fold less efficiency, respectively when compared to Co^{2+} activation.[8] On the other hand, Ye's group reported that the most efficient activators of MtMetAP1a were Ni^{2+} and Co^{2+} ions (in that order).[21] When it comes to MtMetAP1c, Co^{2+}, Mn^{2+}, Zn^{2+}, and Mg^{2+} (in that order) were shown to be the effective activators by Wang's group;[2] whereas Ye's group established k_{cat}/k_M (in M^{-1}, S^{-1}) to be 659, 232, and 107 for Ni^{2+}, Co^{2+}, and Mn^{2+}, respectively.[22] Under our assay conditions, Co^{2+} and Mn^{2+} (in that order) were the most effective metal ion cofactors for the MtMetAPs and hence we chose to carry out all the in vitro enzyme inhibition assays using these two metal ions. A likelihood of the coupling enzyme (BcProAP) inhibition in the MtMetAP assay was ruled out by performing an additional control experiment.

First we wanted to interrogate the importance of the *N*′-hydroxymethanimidamide functionality of the hit **4c**, and thus we browsed through the ASDI library and tested structurally related analogs. Nei-

Table 1
4*H*,5*H*-Naphtho[1,2-*d*]thiazole derivatives—MtMetAP assays

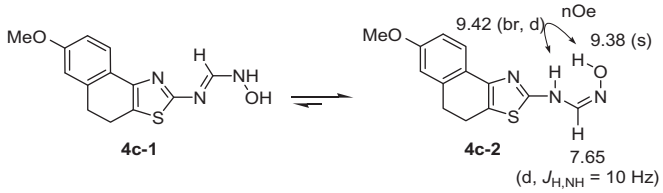
Entry	Structure	Substituents (when not specified, R# = H)	IC_{50} (μM), MtMetAP			
			1a, Co^{2+}	**1a**, Mn^{2+}	**1c**, Co^{2+}	**1c**, Mn^{2+}
3n		—	>50	>50	>50	>50
3o		R^2 = OMe				
3p		R^2 = OMe; R = COMe				
3q		R = $CO(CH_2)_7Me$				
3r		R^2 = OMe; R = $CO(CH_2)_7Me$				
3s		R^2 = OMe; R = (*S*)-COCH(OH)Me				
3u		R^2 = OMe; R = 2-furanyl				
3a		$R = NMe_2$	28.9 ± 5.7	27.7 ± 13.9	36.3 ± 9.2	>50
3t		R^2 = OMe; R = 2-furanyl	>50	>50	>50	>50
3v		R^2 = OMe; R = Ph(2-OH)				
4c		R^2 = OMe	5.35 ± 1.5	>50	1.33 ± 0.24	>50

Note: IC_{50} values are the average of at least three independent experiments, each consisting of triplicates and ±standard deviation is given.



1, 2, 3, and 4: Substituents (when not specified, R#, X–Y = H)			
Code	R^1, R^2, R^3	X–Y	Z
a	–	CH_2CH_2	**3a** – **3m**, Z = N=CHNMe$_2$
b	R^1 = OMe		**4a** – **4m**, Z = NHCH=NOH
c	R^2 = OMe		Other thiazole **3** derivatives: R^1, R^2, R^3, and X–Y defined by **a** or **b** but differing in Z
d	R^3 = OMe		
e	R^1, R^2 = OMe		**3n** = **a**, while Z = $NH_3^+Br^-$
f	R^1–R^2 = OCH_2O		**3o** = **b**, while Z = $NH_3^+Br^-$
g	R^2 = OMe	OCH_2	**3p** = **b**, while Z = NHCOCH$_3$
h	R^1–R^2 = OCH_2O		**3q** = **a**, while Z = NHCO(CH$_2$)$_7$CH$_3$
i	R^2 = OMe	(*RS*)-OCH(Ph)	**3r** = **b**, while Z = NHCO(CH$_2$)$_7$CH$_3$
j	R^1–R^2 = OCH_2O	$(CH_2)_3$	**3s** = **b**, while Z = (*S*)-NHCOCH(OH)CH$_3$
k		$O(CH_2)_2$	**3t** = **b**, while Z = NH=CH(2-furanyl)
l	R^2 = OMe	CH_2	**3u** = **b**, while Z = NCH$_2$(2-furanyl)
m	R^1, R^2 = OMe	H (no ring)	**3v** = **b**, while Z = N=CHPh(2-OH)

Scheme 1. Synthesis of thiazole derivatives. Reagents and conditions: (i) I_2/$(NH_2)_2C$=S, EtOH, 100 °C, 2–3 h; then free-base with $NaHCO_3$; (ii) furfural or salicylaldehyde, anhydrous $MgSO_4$, 1:1 CH_2Cl_2–MeOH, rt, 6 h; (iii) $(OMe)_2CHNMe_2$/toluene, reflux, 4–6 h; (iv) (*S*)-(*O*)-TBDPS-lactic acid, HBTU/*i*-Pr_2NEt, THF, 16 h, then *n*-Bu_4NF/THF; (v) **3**, $NaBH_4$/MeOH, rt, 16 h; (vi) NH_2OH·HCl, MeOH, rt, 18 h.



Scheme 2. Tautomers of *N*′-hydroxymethanimidamides, example **4c–1** and **4c–2**. Evidence form NOESY suggesting a preponderence of **4c–2**.

ther of the simple naphthothiazolyl amine salts **3n** and **3o** nor their amide derivatives **3p**, **3q**, and **3r** inhibited MtMetAPs (Table 1). At this point we reasoned that the presence of an oxygen atom in α or β position with respect to the nitrogen atom of aminothiazole might be necessary for the inhibitory activity, and we prepared and tested lactamide **3s**, 2-furyl derivative **3u**, and Schiff bases **3t** and **3v**. All the analogs listed above failed to inhibit either MtMetAPs. Only methanimidamide **3a** showed a weak inhibition of both MtMetAPs. Thus we concluded *N*′-hydroxy-*N*-(thiazol-2-yl)methanimidamide to be the optimal pharmacophore and focused our attention on varying substitutions on the A-ring (see **3** in Scheme 1 for ring designation).

With examples **4a** through **4f** (see Table 2), we systematically probed the effect of methoxy substitution on the A ring (**4b**, **4c**, and **4d**), where compound **4b** stood out for its good potency against the two metalloforms of MtMetAPs. Dimethoxy derivative **4e** was less potent against MtMetAPs and the methylenedioxy substitution (**4f**) led to even poorer inhibition. We then altered the B-ring by replacing the benzylic methylene with an oxygen atom (examples **4g**, **4h**, and **4i**). A single methoxy group substitution on the A-ring combined with or without an additional phenyl group substitution on the B-ring (**4g** or **4i**), produced compounds exhibiting moderate inhibition of both the metalloforms of MtMetAP1a, but they did not inhibit MtMetAP1c. Contrary to the case of **4f**, methylenedioxy substituted compound **4h** turned out to be potent at inhibiting the cobalt-form of MtMetAP1c (730 nM) with good inhibition of MtMetAP1a as well (1.43 μM, Co^{2+}-MtMetAP1a). We then synthesized the B-ring expanded versions **4j** and **4k**, and gratifyingly, **4k** became the most potent MtMetAP inhibitor. Unlike the benzosuberan congener **4j**, benzoxepine derivative **4k** inhibited both the metalloforms of MtMetAPs while having an IC_{50} of 1.26 μM and 380 nM for Co^{2+}-MtMetAP1a and Co^{2+}-MtMetAP1c, respectively.

To explore the SAR further about the B-ring, we tested a ring-contracted version, indan derivative **4l**, and a ring-excised version **4m**. Of the two, **4l** was not an inhibitor of MtMetAPs, but **4m** did inhibit MtMetAP1c with moderate potency, emphasizing the relevance of B-ring, important perhaps in fixing the orientation of the *N*′-hydroxymethanimidamide pharmacophore.

Although *N*′-hydroxymethanimidamide moiety appears to be intuitively acid labile, it has proved to be quite useful in improving the oral bioavailability of methanimidamide containing drug candidates, in particular as a prodrug approach[23] (examples: antithrombotic agent sibrafiban[18] and a direct thrombin inhibitor called ximelagatran[24]). Another salient example of an *N*′-hydroxymethanimidamide is an inhibitor of 20-hydroxyeicosatetraenoic acid, called TS-011 which is being evaluated for the treatment of cerebral ischaemia.[25] Considering these successes, the MtMetAP inhibitors we have described here are not likely to defy the druggability criteria.

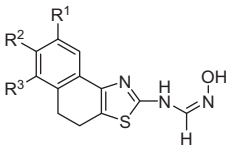
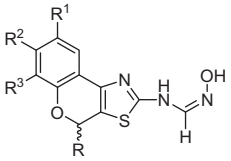
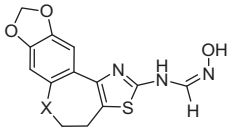
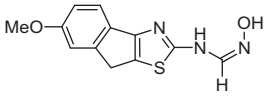
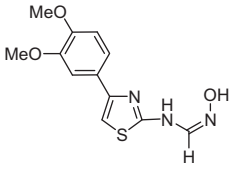
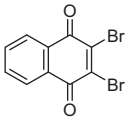
3.1. Metal complexation studies

We recorded UV–vis spectra of **4g**, **4h**, and **4k**, in the MetAP assay buffer (at 10 μM) with or without 10 μM cobalt or manganese chlorides, which closely emulated the enzyme assay conditions. All three compounds form metal complexes and yet despite such a close structural relationship **4g** did not inhibit either metalloforms of MtMetAP1c. It should be noted that salicylaldimine like **3v** is a well-established metal ion chelator, but it did not inhibit MtMetAPs. Thus, it appears that akin to our earlier experience with the human MetAP inhibitors,[26] ability to complex metal ions is not a sufficient or necessary criterion for rationalizing MtMetAP inhibition.

3.2. Anti-tuberculosis activity

We determined the minimum inhibitory concentrations (MICs) of methanimidamides **4a**–**4m** in the culture of Mtb (H37Rv strain).

Table 2
Analogs of *N*′-Hydroxy-*N*-(4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide—MtMetAP assays and MICs (Mtb, H37Rv)

Entry	Structure	Substituents (when not specified, R# = H)	IC_{50} (μM), MtMetAP				MIC (μg/mL)
			1a, Co^{2+}	**1a**, Mn^{2+}	**1c**, Co^{2+}	**1c**, Mn^{2+}	
4a		—	11.53 ± 3.5	>50	1.05 ± 0.39	>50	100
4b		R^1 = OMe	1.90 ± 0.5	>50	0.64 ± 0.08	2.33 ± 0.39	200
4c		R^2 = OMe	5.35 ± 1.5	>50	1.33 ± 0.24	>50	>200
4d		R^3 = OMe	11.26 ± 4.1	11.67 ± 3.2	1.89 ± 0.3	10.30 ± 3.9	>200
4e		R^1,R^2 = OMe	14.56 ± 3.3	14.6 ± 3.6	3.77 ± 0.4	>50	>200
4f		R^1–R^2 = OCH_2O	15.44 ± 4.6	>20	>20	12.5 ± 3.1	>200
4g		R^2 = OMe	4.9 ± 1.9	4.7 ± 1.2	>50	>50	>200
4h		R^1–R^2 = OCH_2O	1.43 ± 0.38	>10	0.73 ± 0.09	4.4 ± 0.9	100
4i		R^2 = OMe; R = Ph	6.7 ± 1.9	9.7 ± 2.3	>50	>50	>200
4j		X = CH_2	15.4 ± 4.1	>50	1.4 ± 0.25	14.5 ± 3.6	>200
4k		X = O	1.26 ± 0.2	24.5 ± 2.2	0.38 ± 0.05	3.13 ± 0.9	>200
4l		—	>50	>50	>50	>50	100
4m		—	>20	>20	6.6 ± 1.8	7.0 ± 1.7	200
NQ[a]		—	1.14	ND	0.71	ND	25

Note: IC_{50} values are the average of at least three independent experiments, each consisting of triplicates and ±standard deviation is given.
[a] Presented in our earlier work[8] and is given here for comparison.

Except **4a**, **4h**, and **4l,** which exhibited MICs of a meager 100 μg/mL, the remaining compounds did not affect the growth of Mtb. The positive control on the other hand, 2,3-dibromonaphthoquinone (**NQ**, IC_{50}s, 1.14 [Co^{2+}-MtMetAP1a], and 0.71 [Co^{2+}-MtMetAP1c) had an MIC of 25 μg/mL matching our previous results.[8] On the basis of enzyme inhibition data, we were expecting **4b**, **4h**, and **4k** to manifest superior MICs, but counter to our expectations only **4h** had a weak MIC of 100 μg/mL. Although methanimidamide **4l** did not display any MtMetAP inhibitory activity, it nevertheless registered an MIC of 100 μg/mL. This result is quite puzzling, because MICs did not correlate with the enzyme inhibition. In the MtMetAP target validation study we conducted previously, **NQ** was effective in blocking the Mtb growth. A plausible interpretation of this puzzling result of MICs is that the methanimidamides **4a**–**4m** either lack the ability to permeate the bacterial cell or they succumb to an intrinsic efflux mechanism.[27,28] A control experiment was conducted to allay suspicion of instability of *N*′-hydroxymethanimidamide functionality and unsurprisingly compounds **4a–c** and **4k** were quite stable to the Mtb culturing conditions (see Supplementary data). Curiously, an MtMetAP inhibitor bengamide analog was reported to exhibit a reasonable inhibition of replicating Mtb (IC_{50}s, μM: 6.9 [Mn^{2+}-MtMetAP1a], 0.54 [Mn^{2+}-MtMetAP1c], MIC: 18.5 μg/mL).[29]

4. Conclusions

Starting from an *N*′-hydroxy-*N*-(naphthothiazolyl)methanimidamide **4c** as a hit in an HTS, we established *N*′-hydroxymethanimidamide as the required pharmacophore for the inhibition of the two Mtb MetAP isoforms. Three inhibitors **4b**, **4h**, and **4k** possessing potent activity against both MtMetAP1a and MtMetAP1c were uncovered through an SAR campaign where benzoxepine derivative **4k** was identified as the best inhibitor. Unfortunately the MtMetAP inhibitory potency of these methanimidamides did not transpire into good MICs in Mtb culture. Undoubtedly, the *N*′-hydroxymethanimidamide inhibitors need to be tuned further with more SAR to find analogs that are effective in Mtb culture, and the SAR described herein is an important step towards achieving that goal.

5. Experimental

5.1. Chemistry: general methods

Unless stated otherwise, all non-aqueous reactions were carried out under ambient atmosphere in oven-dried glassware. Indicated reaction temperatures refer to those of the reaction bath, while

room temperature (rt) is noted as 25 °C. All the solvents were of reagent grade purchased from Fisher Scientific or VWR and used as received. Commercially available starting materials and reagents were purchased from Acros, Aldrich, or TCI America and were used as received, unless stated otherwise.

Analytical thin layer chromatography (TLC) was performed using Analtech Uniplates (silica gel HLF, W/UV254, 250 μm). Visualization was achieved using 254 nm UV light, or additionally by staining with iodine, or ceric ammonium molybdate stain. The retention factor is reported as follows: R_f (eluent system). Crude products were purified by air-flashed column chromatography over silica gel (0.06–0.2 mm, 60 Å, from Acros) using indicated eluents. Melting points were recorded on a Mel-Temp II apparatus and are uncorrected. NMR data were collected on a Varian Unity-400 (400 MHz ^{1}H, 100.6 MHz ^{13}C) or Bruker-Spectrospin 400 MHz spectrometers. Chemical shifts are reported in ppm (δ) with the solvent resonance or 0.1% tetramethylsilane contained in the deuterated solvent as the internal reference. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, m = multiplet, br = broad, app = apparent, exch = exchangeable), coupling constants (*J*, reported in Hertz, Hz), and number of protons. Low resolution mass spectra were acquired on a Thermo-Finnigan MAT, LCQ Classic ESI-mass spectrometer or Voyager DE-STR, MALDI-TOF (Applied Biosystems) instruments. The MALDI-samples were prepared by mixing the droplets of the sample solutions in chloroform or methanol and 2,5-dihydroxybenzoic acid solution in acetone, where the latter served as the matrix. Data are reported in the form *m/z* (% abundance, ion).

Samples were analyzed for purity on a JASCO HPLC equipped with a Rainin Microsorb-MV C18 column (5 μm, 4.6 × 100 mm). The mobile phase set at a flow rate of 1 mL/min was programmed for a gradient elution starting from a 75:20:5–10:75:15 mixture of H_2O–MeCN–MeOH over 10 min, and the mixture was held steady at that ratio for six more minutes, and during the next minute it was reverted back to the original ratio of 75:20:5, making each run a total of 17 min long. Purity of final compounds was determined to be >95%, using a 10 μL injection (approximately 1 mM in acetonitrile) with quantitation by area under the curve (AUC) at 254, 275, and 296 nm (JASCO Diode Array Detector). The retention time (t_R) is reported in minutes with the AUC given in parentheses.

5.1.1. (*S*)-2-Hydroxy-*N*-(7-methoxy-4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)propanamide (3s)

7-Methoxy-4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-amine (85 mg, 365 μmol) was coupled with (*S*)-*O*-(*tert*-butyldiphenylsilyl)lactic acid[30] using HBTU (152 mg, 1.1 equiv) and *i*-Pr2NEt (230 μL, 3.5 equiv) in THF (10 mL). After stirring the reaction mixture overnight, water (10 mL) was added and the product was extracted into EtOAc (2× 15 mL). The pooled organic phase was evaporated and the product was diluted with THF (10 mL) and treated with *n*-Bu_4NF (1.2 mL, 1 M in THF) at room temperature for 4 h. The reaction mixture was concentrated and the residue was subjected to flash column chromatography over silica gel (eluent, 1:5 EtOAc/hexanes) to yield an off-white solid. Yield: 59 mg, 53% (over two steps); R_f (3:7 EtOAc/hexanes): 0.21 (UV active); t_R = 9.080 min (98%); ^{1}H NMR (400 MHz, $CDCl_3$): δ, 7.55 (d, *J* = 9.1, 1H, H-9), 7.37 (br s, 1H, NH), 6.79 (m, 2H, H-6,7), 4.54 (m, 1H, H-1′), 3.83 (s, 3H, OMe), 3.07–2.86 (m, 4H, H-4,5), 1.28 (d, *J* = 6.7 Hz, 3H, H-3′); MALDI-TOF: *m/z*, 305 (30%, MH^+), 327 (80%, $M+Na^+$).

5.1.2. *N*-(Furan-2-ylmethyl)-7-methoxy-4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-amine (3u)

7-Methoxy-4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-amine (325 mg, 1.4 mmol) and freshly distilled furfural (162 μL, 1.7 mmol) were added to a 1:1 CH_2Cl_2–MeOH mixture containing anhydrous $MgSO_4$ (1 g) and the reaction mixture was stirred under argon for 6 h (the solution turned yellow after 30 min). The mixture was filtered and the filtrate was concentrated. The previously known product (**3t**) was purified by column chromatography over silica gel (eluent: 1:9 EtOAc/hexanes). Yield: 404 mg, 94%; R_f (3:7 EtOAc/hexanes): 0.75 (UV active); ^{1}H NMR (400 MHz, acetone-d_6): δ, 7.97 (s, 1H, HC=N), 7.79 (d, *J* = 9.2 Hz, 1H, H-9), 7.35 (d, *J* = 2.1 Hz,1H, H-5′), 6.85 (m, 3H, H-6,8; H-3′), 6.77 (m, 1H, H-4′), 3.82 (s, 3H, OMe), 2.86–2.82 (m, 4H, H-4,5); MALDI-TOF: *m/z*, 311 (100%, MH^+), 350 (15%, $M+K^+$).

The Schiff base **3t** (20 mg, 64.5 μmol) was dissolved in MeOH (8 mL) and $NaBH_4$ (15 mg, 395 μmol) was added and the mixture was stirred overnight. After concentrating, the reaction mixture was purified by flash column chromatography over silica gel (1.5:8.5 EtOAc/hexanes). Yield: 29 mg, 96%; R_f (3:7 EtOAc/hexanes): 0.73 (UV active); t_R = 12.400 min (98%); ^{1}H NMR (400 MHz, acetone-d_6): δ, 7.81 (d, *J* = 9.2 Hz, 1H, H-9), 7.34 (d, *J* = 2.1 Hz,1H, H-5′), 6.89 (m, 3H, H-6,8; H-3′), 6.78 (m, 1H, H-4′), 4.66 (br s, 1H, NH), 4.32 (s, 2H, CH_2-furyl), 3.85 (s, 3H, OMe), 2.86–2.82 (m, 4H, H-4,5); δ, MALDI-TOF: *m/z*, 313 (100%, MH^+), 335 (20%, $M+Na^+$).

5.1.3. Analogs of *N*′-hydroxy-*N*-(4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide

These derivatives were prepared using a three-step protocol starting from an appropriate ketone. As a representative example, synthesis of methanimidamide **4b** is described below.

5.1.3.1. Synthesis of 8-methoxy-4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-amine (2b). Naphthothiazole **2b** was prepared using a known procedure.[15] Briefly, thiourea (3.88 g, 51 mmol) and iodine (4.75 g, 18.7 mmol) were added to a solution of tetralone **1b** (3 g, 17 mmol) in absolute ethanol. The reaction mixture was heated at 100 °C in an open vessel for 3 h, and in the end all the solvent was allowed to evaporate. The residue was washed with ether (3× 15 mL), dissolved in water (50 mL) and heated for 30 min and cooled. The white solid was filtered, dried, and recrystallized from 9:1 EtOH–H_2O and dried under vacuum to afford the hydroidide salt of **2b**. Upon a quick free-basing by washing with 5% NaOH and evaporating the dichloromethane layer, the sample was characterized by ^{1}H NMR and MALDI-TOF and it was carried over to the next step.

5.1.3.2. Synthesis of *N*′-hydroxy-*N*-(8-methoxy-4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide (4b). A two step sequence described in the literature[17] was applied to making methanimidamide **4b**. Aminothiazole **2b** (1.4 mmol) from the previous step was heated at 100 °C with *N*,*N*-dimethyl-formamide dimethyl acetal (220 μL, 1.64 mmol) in toluene (15 mL) for 5 h and the solvent was evaporated. The residue was recrystallized from cyclohexane and the resulting solid (**4b**) was stirred with hydroxylamine hydrochloride (575 mg, 8.3 mmol) in methanol (20 mL) at room temperature for 16 h. The reaction mixture was concentrated and it was neutralized by adding 10% Na_2CO_3 solution dropwise (pH 9). A brown precipitate was formed and it was filtered and washed with water and recrystallized further from 1,4-dioxane to afford pure methanimidamide **4b**.

5.1.3.3. Methanimidamide 4b. Yield: 52% over three steps; R_f (3:7 EtOAc/hexanes): 0.42 (UV active); t_R = 8.013 min (98%); ^{1}H NMR (400 MHz, acetone-d_6): δ, 9.38 (br s, 2H, NH, OH), 9.26 (s, 1H, CH=N), 7.52 (d, *J* = 8.6 Hz, 1H, H-6), 7.37 (d, *J* = 1.9 Hz,1H, H-9), 6.85 (dd, *J* = 8.6, 1.9 Hz, 1H, H-7), 3.75 (s, 3H, OMe), 3.11–2.92 (m, 4H, H-4,5); MALDI-TOF: *m/z*, 276 (35%, MH^+), 298 (20%, $M+Na^+$).

5.1.3.4. Methanimidamide 4d. Methanimidamide **4d** was prepared from the commercially available 5-mehtoxy-1-tetralone. Final compound was purified by flash chromatography over silica gel (eluent: 2% MeOH–DCM). Yield: 61% over 3 steps; R_f (3:7 EtOAc/hexanes): 0.33 (UV active); t_R = 9.547 min (98%); ^{1}H NMR (400 MHz, acetone-d_6): δ, 9.18 (br s, 2H, NH, OH), 9.12 (s, 1H, CH=N), 8.05 (d, J = 8.7 Hz, 1H, H-9), 7.38 (d, J = 8.7 Hz, 1H, H-8), 6.85 (d, J = 8.6 Hz, 1H, H-7), 3.79 (s, 3H, OMe), 3.21–2.97 (m, 4H, H-4,5); MALDI-TOF: m/z, 276 (60%, MH^+), 298 (40%, $M+Na^+$).

5.1.3.5. Methanimidamide 4e. This was prepared from the commercially available 6,7-dimehtoxy-1-tetralone (**1e**). Yield: 48% over three steps; R_f (5% MeOH/DCM): 0.28 (UV active); t_R = 8.187 min (96%); ^{1}H NMR (400 MHz, acetone-d_6): δ, 9.61 (br s, 2H, NH, OH), 7.81 (s, 1H, CH=N), 7.39 (s, 1H, H-9), 6.83 (s, 1H, H-6), 3.83 and 3.81 (2× s, 6H, OMe), 3.22–2.85 (m, 4H, H-4,5); MALDI-TOF: m/z, 306 (20%, MH^+), 328 ($M+Na^+$), 290 (100%, M^+-O).

5.1.3.6. Methanimidamide 4f. This was prepared from 6,7-(methylenedioxy)-1-tetralone (**1f**).[10] Yield: 67% over three steps; R_f (3:7 EtOAc/hexanes): 0.39 (UV active); t_R = 9.507 min (97%); ^{1}H NMR (400 MHz, acetone-d_6): δ, 9.55 (br s, 2H, NH, OH), 7.89 (s, 1H, CH=N), 7.25 (s, 1H, H-9), 6.85 (s, 1H, H-6), 6.02 (d, J = 10.3 Hz, 2H, OCH_2O), 3.21–2.95 (m, 4H, H-4,5); MALDI-TOF: m/z, 290 (90%, MH^+), 312 (30%, $M+Na^+$).

5.1.3.7. Methanimidamide 4g. This was prepared from 7-methoxy-2*H*,3*H*,4*H*-chromen-4-one.[11] Yield: 69% over three steps; R_f (3:7 EtOAc/hexanes): 0.43 (UV active); t_R = 8.943 min (97%); ^{1}H NMR (400 MHz, acetone-d_6): δ 9.51 and 9.48 (2× br s, 2H, NH and OH), 9.36 (s, 1H, CH=N), 7.65 (d, J = 9.2 Hz, 1H, H-9), 7.57 (d, J = 9.2, Hz,1H, H-8), 6.70 (d, J = 6 Hz, 1H, H-6), 5.35 (d, J = 9.6 Hz, 2H, H-4); MALDI-TOF: m/z, 278 (76%, MH^+), 300 (55%, $M+Na^+$).

5.1.3.8. Methanimidamide 4h. Methanimidamide **4h** was prepared from 6,7-dihydro-8*H*-1,3-dioxolo[4,5-*g*]benzopyran-8-one.[12] Yield: 55% over three steps; R_f (3:7 EtOAc/hexanes): 0.36 (UV active); t_R = 9.320 min (96%); ^{1}H NMR (400 MHz, acetone-d_6): δ 9.64 and 9.55 (2× br s, 2H, NH and OH), 7.79 (s, 1H, CH=N), 7.17 (s, 1H, H-9), 6.51 (s, 1H, H-6), 6.02 (d, J = 10.6 Hz, 2H, OCH_2O), 5.33 (d, J = 9.8 Hz, 2H, H-4); MALDI-TOF: m/z, 292 (80%, MH^+), 314 (45%, $M+Na^+$).

5.1.3.9. Methanimidamide 4i. This was prepared from the commercially available 7-methoxyflavanone. Yield: 61% over three steps; R_f (3:7 EtOAc/hexanes): 0.54 (UV active); t_R = 10.403 min (97%); ^{1}H NMR (400 MHz, methanol-d_4): δ, 8.31 (br s, 2H, NH and OH, partially exchanged), 7.75 (dd, J = 8.8, 1.4 Hz, 1H, H-9), 7.52–7.33 (m, 6H, CH=N, phenyl), 6.55 (dd, J = 8.8 and 2.4 Hz, 1H, H-8), 6.49 (dd, J = 2.4 and 1.4 Hz, 1H, H-6), 5.08 (s, 1H, H-4), 3.78 (s, 3H, OMe); MALDI-TOF: m/z, 354 (65%, MH^+), 376 (40%, $M+Na^+$).

5.1.3.10. Methanimidamide 4j. This compound was prepared from 6,7,8,9-tetrahydro-5*H*-cyclohepta[*f*]-1,3-benzodioxol-5-one.[13] Yield: 37% over three steps; R_f (3:7 EtOAc/hexanes): 0.45 (UV active); t_R = 9.987 min (98%); ^{1}H NMR (400 MHz, acetone-d_6): δ, 9.57 (br s, 2H, NH, OH), 7.90 (s, 1H, CH=N), 7.27 (s, 1H, H-10), 6.88 (s, 1H, H-7), 6.05 (d, J = 10.3 Hz, 2H, OCH_2O), 3.11–2.85 (m, 4H, H-4,6), 2.28 (m, 2H, H-5); MALDI-TOF: m/z, 304 (70%, MH^+), 326 (40%, $M+Na^+$).

5.1.3.11. Methanimidamide 4k. This was prepared from 7*H*,8*H*-1,3-dioxolo[4,5-*h*]benzoxepin-9(6*H*)-one.[14] Yield: 51% over three steps; R_f (3:7 EtOAc/hexanes): 0.32 (UV active); t_R = 8.640 min (96%); ^{1}H NMR (400 MHz, methanol-d_4): δ, 7.69 (s, 1H, CH=N), 6.49 (s, 1H, H-10), 5.92 (s, 1H, H-7), 5.91 (d, J = 6 Hz, 2H, OCH_2O), 4.28 (t, J = 5.6 Hz, 2H, H-5) 3.18 (t, J = 5.6 Hz, H-4); MALDI-TOF: m/z, 306 (85%, MH^+), 328 (50%, $M+Na^+$).

5.1.3.12. Methanimidamide 4l. This prepared from the commercially available 5-mehtoxy-1-indanone. Yield: 71% over three steps; R_f (3:7 EtOAc/hexanes): 0.34 (UV active); t_R = 8.040 min (97%); ^{1}H NMR (400 MHz, DMSO-d_6): δ, 10.51 (br s, 1H, NH or OH), 10.33 (s, 1H, CH=N), 7.63 (s, 1H, NH or OH), 7.40 (d, J = 8.8 Hz, 1H, H-8), 7.18 (d, J = 1.4 Hz, 1H, H-5), 6.88 (d, J = 8.8 Hz, 1H, H-6), 3.79 (s, 3H, OMe), 3.76 (d, J = 11.2 Hz, 2H, H-4); MALDI-TOF: m/z, 262 (66%, MH^+), 284 (20%, $M+Na^+$).

5.1.3.13. Methanimidamide 4m. This was prepared from the commercially available 3,4-methylenedioxyacetophenone. Yield: 75% over three steps; R_f (5% MeOH/DCM): 0.42 (UV active); t_R = 7.187 min (97%); ^{1}H NMR (400 MHz, acetone-d_6): δ, 8.67 (br s, 2H, NH and OH), 7.18 (s, 1H, CH=N), 6.91 (d, J = 8.8 Hz, 1H, H-6), 6.62 (d, J = 2.4 Hz,1H, H-10), 6.41 (s, 1H, H-5), 6.03 (d, J = 8.8 Hz, 1H, H-7), 2.94 and 2.91 (2× s, 6H, OMe); MALDI-TOF: m/z, 280 (70%, MH^+), 263 (MH^+-OH).

5.2. Biological assays

5.2.1. MetAP enzyme assays

The protein expression and purification of C-terminal 6×His-tagged MtMetAP1a and N-terminal 6×His-tagged MtMetAP1c were carried out as described before. HTS was also conducted as was described in our earlier work.[9] However the MtMetAP assays in the present study were performed with slight modification as explained underneath.

Eleven different concentrations of naphthothiazole derivatives (50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, 0.098, 0.049 μM) were pipetted into a 96-well plate and they were incubated for 20 min at room temperature with an assay buffer (40 mM HEPES [pH 7.5], 100 mM NaCl and 10 μM $CoCl_2$ or $MnCl_2$). The assay buffer also contained either 200 nM MtMetAP1a or 100 nM MtMetAP1c, and 1.5 U/mL proline aminopeptidase (from *Bacillus coagulans*, BcProAP) as the coupling enzyme.[31] After 20 min preincubation with the compounds, the enzyme reaction was initiated by adding methionylprolyl-*p*-nitroanilide as the substrate (20 μL, 3 mM; such that a final concentration of 600 μM was attained in a 100 μL of total reaction volume in each well) and the change in absorbance at 405 nm was measured using a microplate reader (BMG Labtech, Offenburg, Germany) for a period of 20 min. Reaction progress was computed against the data from the wells containing DMSO alone taken for uninhibited reactions. In the control experiments to rule out the possibility of the inhibition of BcProAP itself, the assay protocol was modified where prolyl-*p*-nitroanilide was used as the substrate (obviously MtMetAP was eliminated). EC_{50} values were determined by plotting the data using nonlinear regression analysis in the GraphPad Prism 4.0 (La Jolla, CA) and the data from at least three independent experiments (each consisting of a triplicate) and IC_{50}s reported are the average while the error indicated is the standard deviation.

5.2.2. Determination of MICs

Compound concentrations prepared directly in the medium were 0, 0.8, 1.6, 3.2, 6.3, 12.5, 25, 50, 100, 200 and 400 μg/mL. A 100 μL volume of Middlebrook 7H9 broth (Difco, USA) with compound in different concentrations was dispensed into each well of a 96-well cell culture plate (BD). A standard log-phase growth bacterial suspension (*Mycobacterium tuberculosis*, H37Rv strain) was prepared and diluted to $\approx 10^6$ CFU/mL in 7H9 broth and a 100 μL inoculum was used to inoculate each well of the plate. A sterile control without inoculum was also included. Plates were

sealed and incubated at 37 °C for two weeks. The MIC was read as the minimum compound concentration at which the well was clear indicating that no bacterial growth had occurred.

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Supplementary data

Supplementary data (these data include representative MtMetAP inhibition curves, representative UV–vis spectra recorded as part of the metal complexation studies, and HPLC chromatograms attesting to the stability of *N*′-hydroxymethanimidamides (**4a**, **4b**, **4c**, and **4k**) under Mtb culturing conditions) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.05.022.

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