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Searching for New Cures for Tuberculosis: Design, Synthesis, and Biological Evaluation of 2-Methylbenzothiazoles

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The actual development and clinical use of new therapeutics for tuberculosis (TB) have remained stagnant for years because of the complexity of the disease process, the treatment of which at present requires the administration of drug combinations over a 6 month period. There is thus an urgent need for the discovery and development of novel, more active, and less toxic anti-TB agents. In this study, we report on the chemistry and biology of a series of potent 5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxamide derivatives, which proved to be active against replicating *Mycobacterium tuberculosis* (Mtb) $H_{37}Rv$. The most potent compounds **7j** and **7s** were found to inhibit Mtb growth at micromolar concentrations, with MIC values of 1.4 and 1.9 μM, respectively. Impressively, all active compounds were nontoxic toward Vero cells (IC_{50} > 128 μM). Moreover, the best of these compounds were also tested against protozoan parasites, and some of these compounds were found to show activity, especially against *Plasmodium falciparum*. These studies thus suggest that certain 2-methylbenzothiazole based compounds may serve as promising lead scaffolds for further elaboration as anti-TB drugs and as possible antimalaria drugs.

Introduction

Tuberculosis (TB[a]) represents a highly contagious, airborne disease that is caused by infection with *Mycobacterium tuberculosis* (Mtb), and it currently represents one of the most threatening health problems globally. According to the World Health Organization (WHO) data (2009),[1] Mtb has infected one-third of the world's population and results in the death of approximately 1.3−1.75 million patients in 2007. Furthermore, the emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) strains continues.[2,3] Although TB is curable, all of the aforementioned factors, along with the failure of some patients to complete the prescribed treatment, result in the burden of treating TB. Thus, the discovery and development of novel, safe, and more effective anti-TB agents is now being given more attention than ever before, in part because of the attention brought to the field by the Bill and Melinda Gates Foundation.[4]

To identify new chemical entities that could be used as a starting point in the development of new TB drugs, a 50 000 compound library (NOVACore Chembridge) was screened for activity against replicating Mtb using the microplate Alamar blue Assay (MABA).[5] Among the series of compounds identified having encouraging anti-TB activities were the two hit compounds **1** and **2** possessing a 5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxamide scaffold (Figure 1).

Herein, we describe the synthesis, biological evaluation, and analysis of the structure−activity relationships (SAR) of a series of 2-methylbenzothiazole derivatives as anti-TB agents. In order to identify agents with better potency, lack of toxicity, and reasonable physicochemical parameters, systematic structural modifications were made to the benzothiazole core, the oxymethylene linker, and the isoxazolecarboxamide group (Figure 1).

Chemistry

Modifications on the Isoxazolecarboxamide Group. Compounds **7a**−**w** possessing various amides were prepared using the methods outlined in Scheme 1. Commercially available benzothiazole **3** was reacted with 5-bromomethylisoxazole-3-carboxylic acid ethyl ester (**4**)[6] in the presence of K_2CO_3 and a catalytic amount of TBAI to afford **5** in an almost quantitative yield. After basic hydrolysis of **5** in MeOH, carboxylic acid **6** was obtained in a quantitative yield, and this intermediate was subsequently coupled with a set of amines to generate the desired ligands **1**, **2**, **7a**−**k**, **7n**−**q**, **7s**, **7t**, and **7v** according to one of four methods: compounds **7c**, **7g**, **7n**−**q**, **7t**, and **7v**−**w** were synthesized in the presence of EDC/HOBt and a catalytic amount of DMAP (method A) in yields of 14.1−93.9%; compound **7a** was obtained from the acyl chloride (method B) in 94.2% yield; compounds **1**, **2**, **7b**, **7d**, **7h**−**k**, and **7s** were synthesized in the presence of PyBOP and DIPEA (method C) in yields of

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[a] Abbreviations: INH, isoniazid; MABA, microplate Alamar blue assay; MIC, minimum inhibitory concentration; MDR-TB, multidrug-resistant tuberculosis; Mtb, *Mycobacterium tuberculosis*; RMP, rifampin; SAR, structure−activity relationships; TB, tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

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45.1−96.6%; compounds **7e** and **7f** were produced with CDI (method D) in 77.2−98.7% yield. Hydroxamate derivatives **7l** and **7m** were obtained by a two-step procedure consisting of generating the potassium salt of hydroxylamine followed by the addition to the esters **5** and **7j** in alcohol, respectively. Methylation of compound **7o** was carried out by reaction with MeI in the presence of K_2CO_3/DMF to yield compound **7r** in 88.2%. The ester **7j** was hydrolyzed by treatment with LiOH in MeOH/H_2O, and the resulting acid 7u was converted to amide **7w** following method C discussed for the synthesis of **7j** from **6**.

Modifications on the Oxymethylene Linker. Compounds **12a−c** were synthesized from the appropriate methoxy-substituted anilines as shown in Scheme 2. Amide bond formation was achieved using basic acylating conditions to afford **8a−c**, which were converted in turn to the corresponding thioamides **9a−c** by reaction with Lawesson's reagent in refluxing chlorobenzene. Subsequent cyclization to the corresponding methoxy-substituted 2-methylbenzothiazoles **10a−c** was accomplished using a modified procedure as described in the literature.[7,8] Whereas the *o*- and *p*-methoxythioacetanilides **9a** and **9b** gave only the single benzothiazoles **10a** and **10b**, respectively, in the case of the *m*-methoxythioacetanilide **9c**, a mixture of both the 5- and 7-substituted benzothiazoles was obtained. This mixture of isomers could be separated by silica gel chromatography either at this stage or after the following set of reactions. Subsequent demethylation with BBr_3 afforded the hydroxyl substituted 2-benzothiazoles **11a−c**. Lastly, compounds **12a−c** were obtained from compounds **11a−c**, respectively, following the same methodology as employed for the preparation of **7j** from **3**.

Modifications on the Benzothiazole Core. The coupling reaction of *m*-anisidine (**13**) and 3-(trifluoromethyl)benzoic acid (**14**) was carried out in the presence of EDC/HOBt to yield intermediate **15**, which was subsequently converted to the corresponding benzothiazole **16** by employing the same methodology as that used for the conversion of **8** to **11a** (Scheme 3). Target compounds **17**, **19a**, **19b** and **23** were synthesized from **16**, **18a**, and **18b**, respectively, using the same methodology as that used for the synthesis of **7j** from **3**. Target compound **21** was synthesized from **20** via method C from above.

Modifications on the Isoxazole Ring. For the preparation of **32**, ethyl thiooxamate (**24**) was condensed with 1,3-dichloroacetone (**25**) to provide 2,4-disubstituted

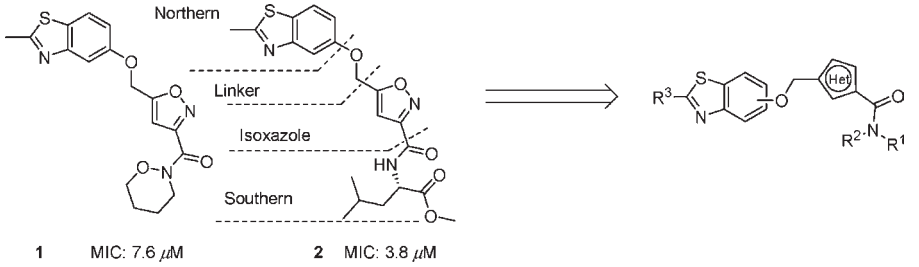
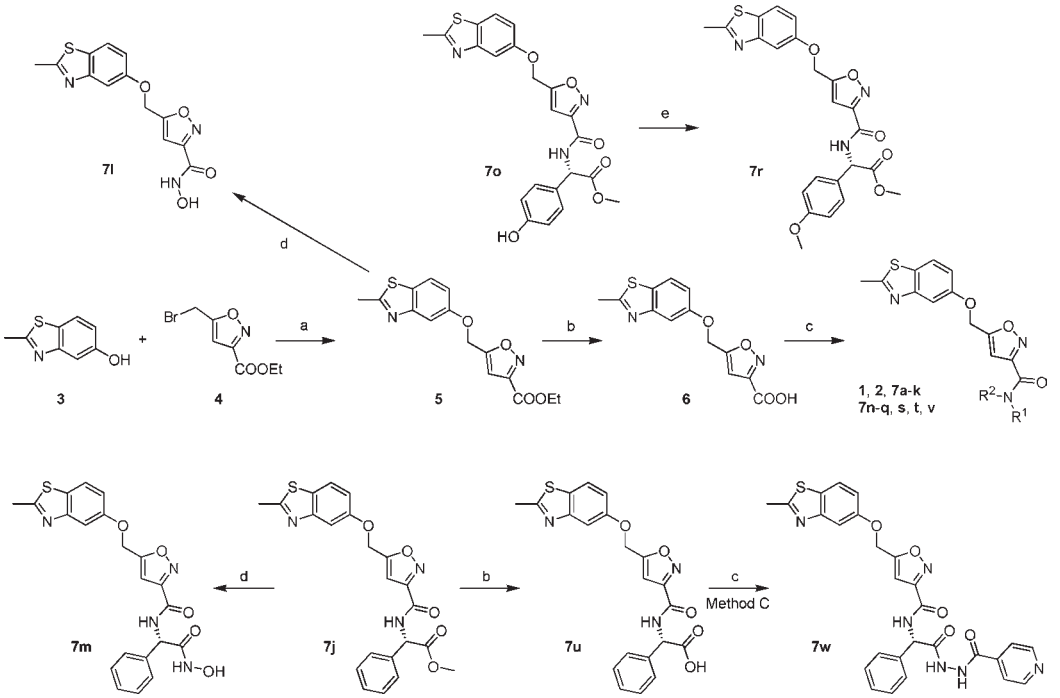


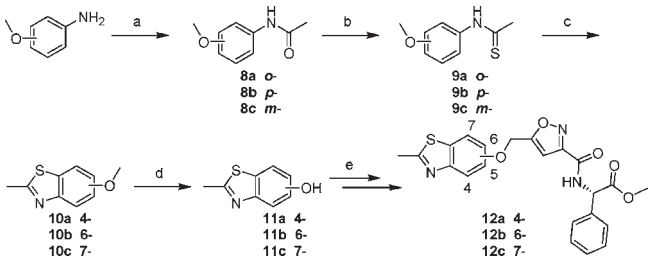
Figure 1. Systematic modifications to hits **1** and **2**.

Scheme 1[a]



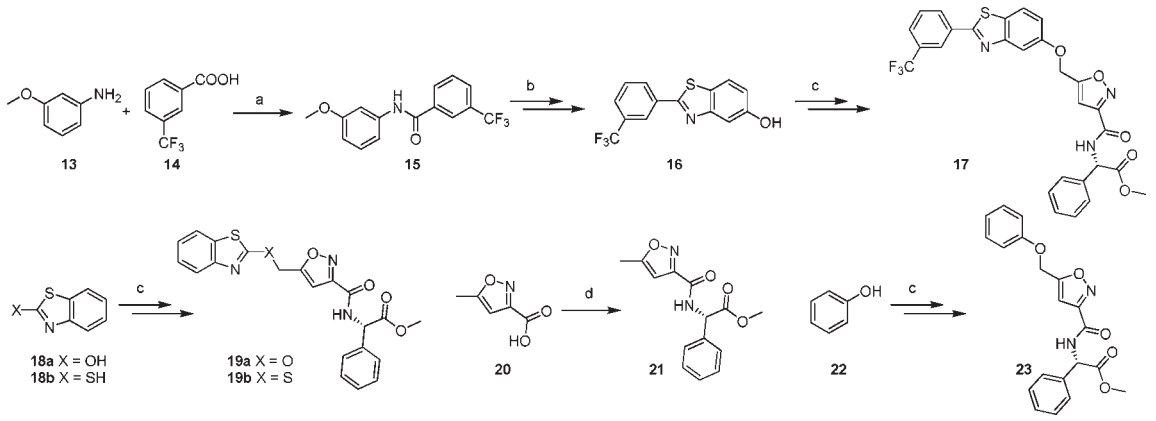
[a] Reagents and conditions: (a) K_2CO_3, TBAI, DMF, 50 °C; (b) LiOH·H_2O, MeOH-H_2O. (c) Method A: R_1R_2NH, EDC, HOBt, DIPEA, DMAP, 4 Å molecular sieves, DMF. Method B: (1) $(COCl)_2$, CH_2Cl_2; (2) R_1R_2NH, pyridine. Method C: R_1R_2NH, PyBOP, DIPEA, DMF. Method D: R_1R_2NH, CDI, DMF. (d) 1.76 M NH_2OK, MeOH; (e) MeI, K_2CO_3, TBAI, DMF. For complete structures see Table 1.

Scheme 2[a]



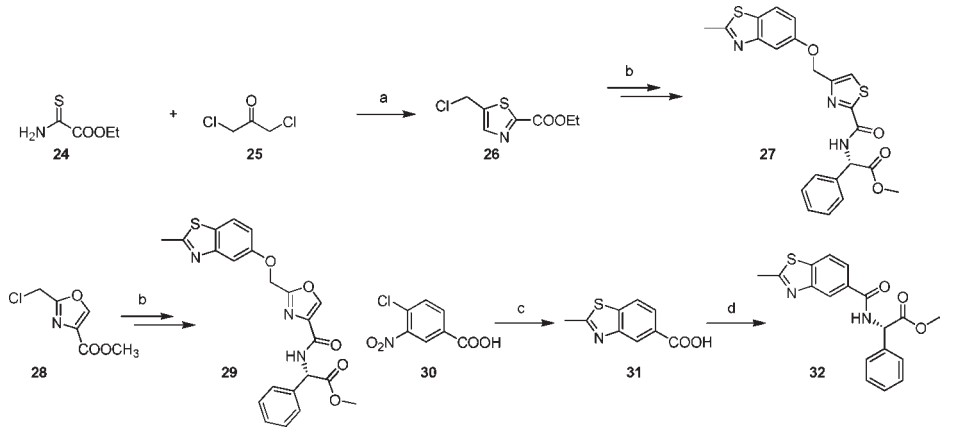
[a] Reagents and conditions: (a) Ac_2O, pyridine; (b) Lawesson's reagent, chlorobenzene, 135 °C, 3 h; (c) $K_3Fe(CN)_6$, KOH, H_2O; (d) BBr_3, CH_2Cl_2; (e) (1) **4**, K_2CO_3, TBAI, DMF, 50 °C; (2) LiOH·H_2O, MeOH−H_2O; (3) phenyl-Gly(OMe)·HCl, PyBOP, DIPEA, 4 Å molecular sieves, DMF.

Scheme 3[a]



[a] Reagents and conditions: (a) EDC, HOBt, DIPEA, DMAP, CH_2Cl_2; (b) (1) Lawesson's reagent, chlorobenzene, 135 °C, 3 h; (2) $K_3Fe(CN)_6$, KOH, H_2O; (3) BBr_3, CH_2Cl_2; (c) (1) **4**, K_2CO_3, TBAI, DMF, 50 °C; (2) LiOH·H_2O, MeOH−H_2O; (3) phenyl-Gly(OMe)·HCl, PyBOP, DIPEA, 4 Å molecular sieves, DMF; (d) phenyl-Gly(OMe)·HCl, PyBOP, DIPEA, 4 Å molecular sieves, DMF.

Scheme 4[a]



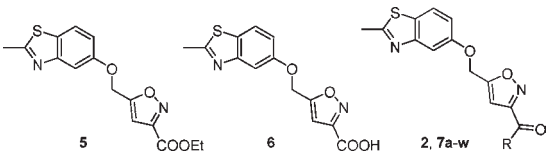
[a] Reagents and conditions: (a) toluene, reflux 2 h; (b) (1) **3**, K_2CO_3, TBAI, DMF, 50 °C; (2) LiOH·H_2O, MeOH−H_2O; (3) phenyl-Gly(OMe)·HCl, PyBOP, DIPEA, 4 Å molecular sieves, DMF; (c) (1) $Na_2S·9H_2O$, H_2O, 120 °C, 30 min; (2) Ac_2O, HOAc, reflux 2 h; (d) phenyl-Gly(OMe)·HCl, PyBOP, DIPEA, 4 Å molecular sieves, DMF.

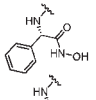
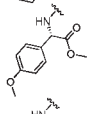
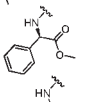
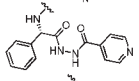
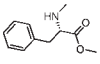
thiazole **26** in excellent yield (Scheme 4).[9] The target compounds **27** and **29** were synthesized from **26** and **28**, respectively, following the same methodology as that used in the preparation of **7j** from **4**. 2-Methylbenzothiazole-5-carboxylic acid (**31**) was obtained from 4-chloro-3-nitrobenzoic acid (**30**) in a one-pot reaction according to a modified procedure reported in the literature,[10,11] and this intermediate was then further converted to the final product **32** using method C.

Results and Discussion

2-Methylbenzothiazole derivatives were prepared and evaluated for the ability to inhibit the growth of Mtb strain $H_{37}Rv$ by using the microplate Alamar blue assay (MABA).[5] All the active compounds were further evaluated for their toxicity to Vero cells. Initially, we introduced variously substituted amides to the southern portion of the lead structure while keeping intact the 5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole scaffold (Table 1). Replacement of the amide by an ester **5**,

Table 1. In Vitro Anti-TB Activity of Analogues Modified at the Isoxazolecarboxamide Group



compd	R	MABA[a] MIC (μM)	Vero cells IC_{50} (μM)	compd	R	MABA[a] MIC (μM)	Vero cells IC_{50} (μM)
5	-	>128	ND[b]	**7l**		>128	ND
6	-	>128	ND	**7m**		>128	ND
7a		>128	ND	**7n**		>128	ND
7b		>128	ND	**7o**		15.4	>128
7c		>128	ND	**7p**		30.2	>128
7d		119.6	ND	**7q**		15.5	>128
7e		>128	ND	**7r**		12.2	>128
7f		>128	ND	**7s**		1.9	>128
7g		>128	ND	**7t**		>128	ND
7h		3.7	>128	**7u**		>128	ND
7i		>128	ND	**7v**		4.4	>128
7j		1.4	>128	**7w**		47.5	ND
7k		14.4	>128	**2**		3.8	>128
RMP[c]		0.1	127	**INH**[d]		0.5	>128

[a] Mtb $H_{37}Rv$. [b] ND: not determined. [c] RMP, rifampin. [d] INH, isoniazid.

an acid **6**, and a hydroxamate acid **7l** led to a loss of anti-TB activity. Compounds **7a**−**w** displayed a wide array of anti-TB activity with MICs ranging from 1.4 to >128 μM. Since the hit compound **1** showed good activity (MIC = 7.6 μM), the first round of substitutions at the southern amide contained small aliphatic and cycloaliphatic moieties (**7a**−**f**); unfortunately, these were found to be inactive. Different amino acids were then introduced to obtain compounds **7g**−**j**. Surprisingly, this slight modification of the alkyl appendage in the hit compound **2** (**7g** and **7i**) led to a complete loss of activity. Upon replacing the L-leucine methyl ester by an L-methionine methyl ester (**7h**), we found that the inhibitory activity was retained (MIC = 3.7 μM). The introduction of a hydrophobic phenyl group as an amide substituent (**7j**) enhanced activity to some extent (MIC = 1.4 μM), making compound **7j** an interesting starting point for further optimization.

Thus, further modifications to compound **7j** were carried out. Introduction of various substituents on the phenyl ring of the phenyl-Gly(OMe) group (**7o**, **7q**, and **7r**) were well tolerated, although not leading to any improvement in potency compared to **7j**. Introducing an alkyl spacer between the phenyl ring and the amide group (**7k**) was also well tolerated. In contrast, removal of a methylene group between the phenyl ring and the methyl ester (**7p**) had a detrimental effect on inhibitory activity (MIC = 30.2 μM). Substitution of the methyl ester with a hydroxamic acid (**7m**), hydrolysis to acid (**7u**), reduction to alcohol (**7n**), as well as its removal (**7t**) led to a complete loss of anti-TB potency in every case. Likely, these negative results are a consequence of the poor permeability of these compounds, indicating that the ester group may well play an important role in penetration of the cell wall of the TB bacteria.

As isoniazid (INH) is a first-line anti-TB medication,[12] we imagined that it might be valuable to incorporate this molecule into our benzothiazoles, and thus the derivatives **7v** and **7w** were prepared. Compound **7v** (MIC = 4.4 μM) retained

good anti-TB potency, while the potency decreased considerably for compound **7w** (MIC = 47.5 μM), possibly because of its steric bulkiness. It is worth mentioning that the enantiomer **7s** of compound **7j** was found to have an excellent anti-TB activity with an MIC of 1.9 μM.

To investigate the role of the linker position on activity, four regioisomeric benzothiazoles (**7j**, **12a**−**c**) varying in points of attachment to the phenyl ring of the benzothiazole were prepared as displayed in Table 2. Shifting the linker to the 4-position (**12a**) or to the 6-position (**12b**) resulted in about a 2- to 5-fold decrease in anti-TB activity when compared to the 5-position (**7j**). Attachment to the 7-position (**12c**) completely abolished activity (MIC > 128 μM), showing that linkage to the 5-position is preferable.

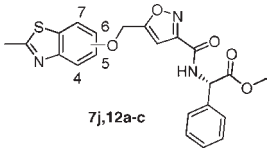
With the above SAR data in hand, we combined the most efficacious amide and the best regioisomer to generate compounds **17**, **19a**, **19b**, **21**, and **23** in which modifications to the northern portion of the starting scaffold were now explored (Table 3). Attaching a phenyl group to the C-2 position of the benzothiazole ring (**17**) led to a dramatic decrease in the anti-TB activity (MIC = 59.5 μM). Relocation of the linker to the thiazole ring of the benzothiazole, using either an oxygen atom (**19a**) or a sulfur atom (**19b**) in the linker, also resulted in compounds having higher MIC values of 15.2 and 13.0 μM, respectively. On the other hand, removal of the benzothiazole ring (**21**) resulted in a 20-fold decrease in potency compared to **7j**. In contrast, the deletion of the thiazole ring alone (**23**) maintained activity compared to hit compound **2**, but this analogue was still 2-fold less potent than **7j**, suggesting that the benzothiazole ring might be the best choice at the northern part.

Futhermore, replacement of the isoxazole ring by a thiazole ring (**27**), an oxazole ring (**29**), or deletion of the isoxazole ring (**32**) resulted in a loss of potency, suggesting a preference for the isoxazole ring in maximizing anti-TB activity (Table 4).

Finally, Vero cells were used for the in vitro toxicity evaluation of the active compounds. In general, the present compounds failed to show any toxicity (IC_{50} > 128 μM), thus suggesting that their anti-TB activity was not due to some general cytotoxicity. Several of the present compounds were also evaluated for their potency against four protozoan parasites as shown in Table 5.[13] In this preliminary screening, some of our compounds were found to show inhibitory activity toward *P. falciparum*. Interestingly, hydroxamate **7m** showed on top of its excellent activity against *T.b.rhodesiense* a good activity against the other three parasites, a result that is not particularly surprising, as the hydroxamate group of **7m** could interact with any of a number of relevant metalloproteases. Additionally, these data are suggestive of both the selectivity of this compound series and the possible commonality of their biological target between TB and malaria.

From the above in vitro results, it may be concluded that 5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxamide represents a reasonable starting scaffold in generating new chemical entities possessing anti-TB properties. In an attempt to understand the possible biological target for our

Table 2. In Vitro Anti-TB Activity of Analogues Modified by Point of Attachment of Linker to the Benzothiazole Ring

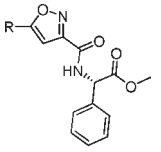


7j,12a-c

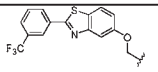
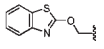
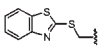
compd	linker position	MABA[a] MIC (μM)	Vero cells IC_{50} (μM)
12a	4	7.4	> 128
7j	5	1.4	> 128
12b	6	3.4	> 128
12c	7	> 128	> 128
RMP[b]		0.1	127
INH[c]		0.5	> 128

[a] Mtb $H_{37}Rv$. [b] RMP, rifampin. [c] INH, isoniazid.

Table 3. In Vitro Anti-TB Activity of Analogues Modified at the Benzothiazole Ring

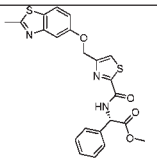
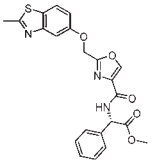
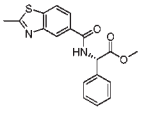


17, 19a, 19b, 21, 23

compd	R	MABA[a] MIC (μM)	Vero cells IC_{50} (μM)
17		59.5	ND[b]
19a		15.2	>128
19b		13.0	>128
21	CH_3	37.0	>128
23		3.5	>128
RMP[c]		0.1	127
INH[d]		0.5	>128

[a] Mtb $H_{37}Rv$. [b] ND: not determined. [c] RMP, rifampin. [d] INH, isoniazid.

Table 4. In Vitro Anti-TB Activity of Analogues Modified at the Isoxazole Ring

compd	structure	MABA[a] MIC (μM)	Vero cells IC_{50} (μM)
27		62.5	ND[b]
29		55.4	>128
32		13.0	>128
RMP[c]		0.1	127
INH[d]		0.5	>128

[a] Mtb $H_{37}Rv$. [b] ND: not determined. [c] RMP, rifampin. [d] INH, isoniazid.

Table 5. In Vitro Antiparasite Activity of Several Active Anti-TB Agents

	IC_{50} (μM)			
compd	*T.b. rhodesiense*	*T. cruzi*	*L. donovani*	*P. falciparum*
2	18.7	12.3	2.79	4.57
7j	74.2	>90	27.5	3.97
7l	1.85	15.9	6.06	2.76
7m	0.415	6.2	3.25	1.82
7n	31.2	>90	>90	2.56
7o	88.9	26.4	4.48	2.5
7s	89.5	37.5	22.3	3.33
7t	86.9	>90	8.8	3.43
7u	89.5	>90	32.3	>5
7v	38	>90	>90	>5
12a	>90	18.8	4.12	>5
12b	>90	25.4	4.27	3.4
12c	66.8	14.7	4.89	4.89
19a	88.6	26.8	4.99	>5
19b	88.9	16.9	6.03	>5
21	>90	>90	56.3	>5
23	>90	45.9	13.5	>5
27	37.5	14.5	4.44	4.86
29	73.8	15.2	5.01	4.65
melarsoprol	0.004			
benznidazole		0.521		
miltefosine			0.142	
chloroquine				0.053

compounds, we made a search of the literature for structurally related compounds. We found that several similar compounds containing a nitrobenzothiazole fragment inhibit HisG,[14] which is an ATP-phosphoribosyl transferase (ATP-PRTase) that catalyzes the first step in the biosynthesis of histidine and that represents a potential drug target for TB. Although our compounds share a benzothiazole group, more careful analysis reveals common pharmacophoric elements including the presence of an aryl group at either end of the molecule and a hydrophobic aryl group in the middle. We thus hypothesized that they may also share the same target in Mtb. On the basis of these previously published results,[14] we then utilized the Protein Data Bank structure 1NH8, for which the active site is known to be centered about Tyr116, to investigate the fit of our compounds to this putative target. An automated docking analysis of 2-methylbenzothiazoles reported in Tables 1−4 was performed into the crystallographic structure of HisG, using the Surflex-Dock suite (Sybyl 8.0, Tripos, Inc.). These molecules were found to fit reasonably well into the active site, with one primary binding mode being observed in which the ligands are located within the ATP binding site. The predicted binding modes for two compounds (**7j** and **7s**) are shown in Figure 2. The benzothiazole ring of both molecules was found to interact with the hydrophobic pocket next to Leu12, Leu71, and Pro50. For compound **7j** (Figure 2a), a hydrogen bond formed between the nitrogen atom present in the benzothiazole ring and the hydrogen atom of the amino group of Lys51, while a second hydrogen bond formed between the carboxylate oxygen and the hydrogen of the hydroxyl group in Ser90. In contrast, the terminal phenyl ring in the southern portion of **7s** was predicted to be close to the P-loop (Gly157-Ser158, Gly159)[14] (Figure 2b); one hydrogen bond formed between the carboxylate oxygen and the phenolic hydroxyl group in Try116, and a second formed between the oxygen in the linker and the hydrogen atom of the amino group of Ala11. Figure 2c shows two previously identified HisG inhibitors in complex with the HisG enzyme as taken from ref 14. As is apparent, our compounds share similar binding sites within this target protein with these reference compounds. While these modeling studies are very preliminary, these studies along with literature reports support the possibility that the target of our 2-methylbenzothiazole series might be Mtb HisG.

Conclusions

In this study a new class of 2-methylbenzothiazole analogues showing good anti-TB properties was identified, with several compounds found to possess MICs in the low micromolar

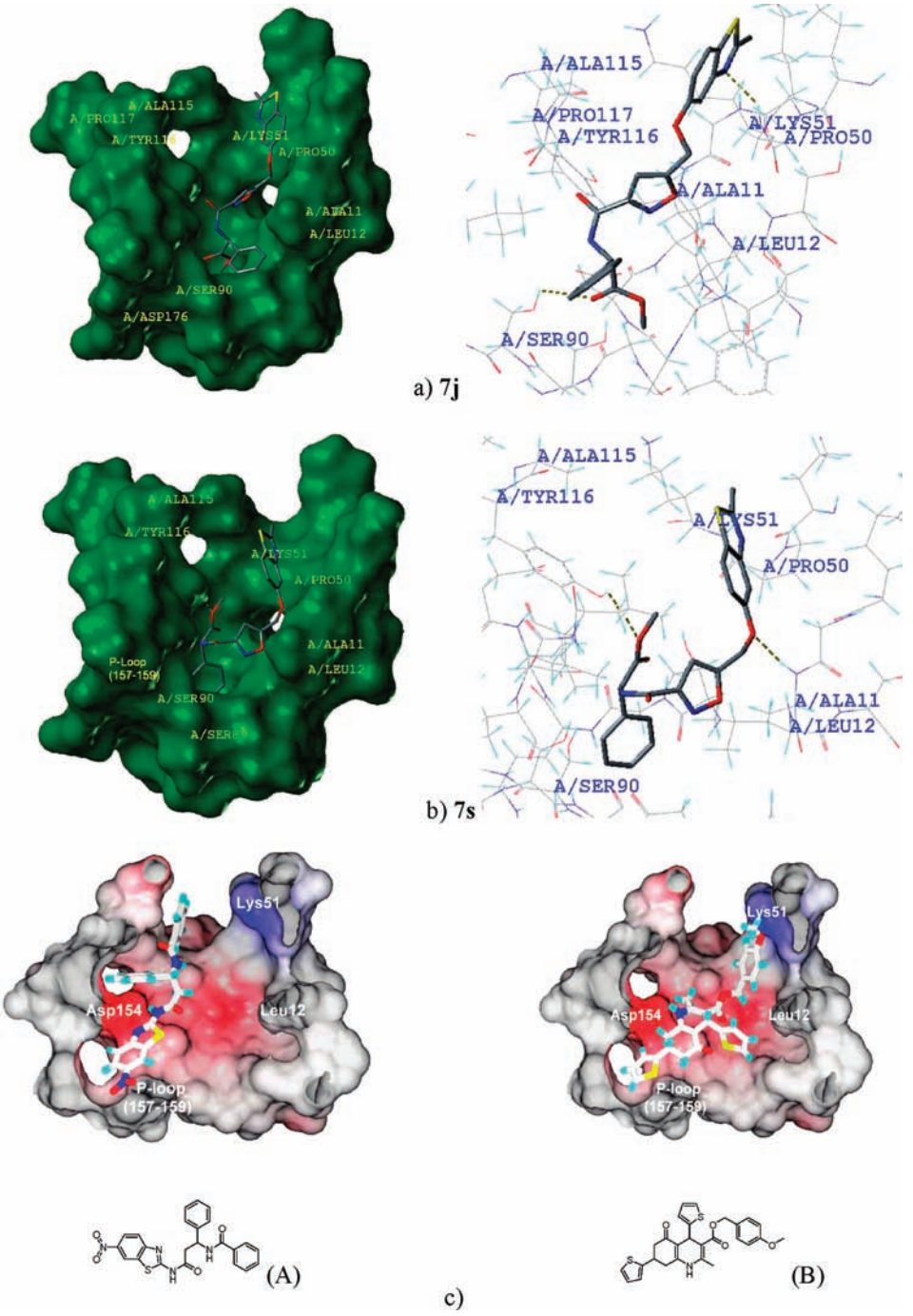


Figure 2. Docked structures of **7j** (a) and **7s** (b) with HisG as determined using the Surflex-Dock suite (Sybyl 8.0, Tripos, Inc.). (c) Docked structures of two HisG inhibitors taken from ref 14.

range against replicating TB in the MABA. Variations in the substitution of the southern amide site were well tolerated, with groups ranging in size from the small dimethylamino group to substituted amino acid esters providing compounds with good anti-TB activity. The most potent compounds **7j** and **7s** exhibited MICs of 1.4 and 1.9 μM, respectively, which amount to a 5-fold increase in potency compared to hit compound **1** and a 2-fold increase in potency compared to hit compound **2**. Upon migrating the linker around the phenyl ring of the benzothiazole, we found that substitution at the 5-position was preferred. Attempts to replace either the benzothiazole or the isoxazole led to a substantially reduced or complete loss in activity. As such, the 2-methylbenzothiazole−isoxazole core appears essential to retaining activity. In general, the active compounds failed to show any toxicity to the Vero cells at 128 μM. Moreover, several of the present compounds showed moderate inhibitory activity toward *P. falciparum*. Preliminary molecular modeling results along with other literature reports suggest that the target of this 2-methylbenzothiazole series might be associated with Mtb HisG. The overall results thus provide a useful starting point in the quest for chemically distinctive classes of anti-TB agents and as possible antiparasitic agents.

Experimental Section

Biology. MABA and Cytotoxicity Assays. These were carried out according to the published protocols.[15]

Antiprotozoal Assays. The in vitro activities against the protozoan parasites *T.b. rhodesiense*, *T. cruzi*, *L. donovani*, and *P. falciparum* were determined as described earlier.[16] The following strains and parasite forms were used: *T.b. rhodesiense*, STIB900, trypomastigote forms; *T. cruzi*, Tulahuen C2C4, amastigote forms in L-6 rat myoblasts; *L. donovani*, MHOM/ET/67/L82, axenic amastigote forms; *P. falciparum*, K1 (chloroquine and pyrimethamine resistant), erythrocytic stages.

Chemistry. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer at 400 and 100 MHz, respectively, with TMS as an internal standard. Mass spectra were measured in the ESI mode at an ionization potential of 70 eV with LCMS MSD (Hewlett Packard); TLC was performed with Merck 60 F_{254} silica gel plates. Column chromatography was performed using Merck silica gel (40−60 mesh). Purity of compounds (>95%) was established by HPLC, which was carried out with two methods: (1) on an ACE AQ column (100 mm × 4.6 mm and 250 mm × 10 mm), with detection at 254 and 280 nm on a Shimadzu SPD-10A VP detector, flow rate = 2.0−3.6 mL/min, from 10% acetonitrile in water to 100% acetonitrile with 0.05% TFA; (2) on an Agilent 1100 HPLC system with a Synergi 4 μm Hydro-RP 80A column, with detection at 254 on a variable wavelength detector G1314A, flow rate = 1.4 mL/min, gradient elution over 20−29 min, from 30% CH_3CN-H_2O to 100% CH_3CN with 0.05% TFA.

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid Ethyl Ester (5). A mixture of commercial 2-methylbenzothiazol-5-ol **3** (165 mg, 1 mmol), 5-bromomethylisoxazole-3-carboxylic acid ethyl ester **4** (304 mg, 1.3 mmol), anhydrous K_2CO_3 (691 mg, 5 mmol), and TBAI (36.9 mg, 0.1 mmol) in anhydrous DMF (2 mL) was heated overnight at 50 °C. After cooling to room temperature, the mixture was diluted with EtOAc (50 mL), washed with H_2O (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using hexane−EtOAc (2:1) as the eluent to give **5** as a white solid (318 mg, 99.9%). ^{1}H NMR ($CDCl_3$) δ 7.73 (d, J = 12.0 Hz, 1H), 7.50 (s, 1H), 7.06 (d, J = 12.0 Hz, 1H), 6.80 (s, 1H), 5.29 (s, 2H), 4.46 (q, J = 8.0 Hz, 2H), 2.78 (s, 3H), 1.43 (t, J = 8.0 Hz, 3H).

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid (6). The ester **5** (318 mg, 1 mmol) was dissolved into MeOH−H_2O (4:1, 10 mL) and cooled to 0 °C. LiOH·H_2O (126 mg, 3.0 mmol) was added, and the reaction mixture was stirred at room temperature for 5 h. After completion the reaction was quenched with H_2O (50 mL) and the mixture was acidified with 6 M HCl (pH ~2), extracted with EtOAc (2 × 30 mL), washed with brine (20 mL), and dried with Na_2SO_4. After filtration the solvent was evaporated to give **6** in 100% yield as a white powder. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 7.75 (d, J = 12.0 Hz, 1H), 7.50 (s, 1H), 7.10 (d, J = 12.0 Hz, 1H), 6.83 (s, 1H), 5.32 (s, 2H), 2.84 (s, 3H).

General Procedures for the Synthesis of Compounds 1, 2, 7a−k, 7n−q,s,t,v. Method A. To a solution of acid **6** (24 mg, 0.083 mmol) in DMF (1 mL) was added 4 Å molecular sieves (24 mg), HOBt (34 mg, 0.25 mmol), and DMAP (cat.). After the mixture was stirred at room temperature for 30 min, morpholine (0.02 mL, 0.25 mmol) and EDC (48 mg, 0.25 mmol) were added. The mixture was stirred overnight, diluted with EtOAc (40 mL), washed with 1 N HCl (10 mL), saturated $NaHCO_3$ (20 mL), H_2O (20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using $CHCl_3$−MeOH (20:1) as the eluent to give **7c** as a yellow solid (4.2 mg, 14.1%).

Method B. To a solution of **6** (10 mg, 0.034 mmol) in anhydrous CH_2Cl_2 (1 mL) was added $(COCl)_2$ (2 M in CH_2Cl_2, 0.05 mL, 0.102 mmol) and 1 drop of DMF at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred for 2 h. After evaporation of the solvent, the residue was dissolved in pyridine (1 mL) and then dimethylamine hydrochloride (28 mg, 0.34 mmol) was added at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred for 15 h and then diluted with EtOAc (40 mL), washed with 1 N HCl (10 mL), saturated $NaHCO_3$ (20 mL), H_2O (20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using $CHCl_3$−MeOH (20:1) as the eluent to give **7a** as a white solid (10.3 mg, 94.2%).

Method C. To a solution of acid **6** (10 mg, 0.034 mmol) in DMF (1 mL) were added 4 Å molecular sieves (10 mg), PyBOP (54 mg, 0.10 mmol), and DIPEA (22 mg, 0.17 mmol). After the mixture was stirred at room temperature for 30 min, diethylamine (4 mg, 0.051 mmol) was added. The mixture was stirred overnight and diluted with EtOAc (40 mL), washed with 1 N HCl (10 mL), saturated $NaHCO_3$ (20 mL), H_2O (20 mL), brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using $CHCl_3$−MeOH (20:1) as the eluent to give **7b** as a yellow solid (11.5 mg, 96.6%).

Method D. To a solution of acid **6** (15 mg, 0.052 mmol) in DMF (1 mL) was added CDI (13 mg, 0.078 mmol). After the mixture was stirred at room temperature for 1 h, piperazine (9 mg, 0.10 mmol) was added. The mixture was stirred overnight and diluted with EtOAc (40 mL), washed with saturated $NaHCO_3$ (20 mL), H_2O (20 mL), and brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using $CHCl_3$−MeOH (20:1) as the eluent to give **7e** as a yellow solid (14.3 mg, 77.2%).

[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazol-3-yl][1,2]oxazinan-2-yl-methanone (1). Method C was used. Yield: 70.8% (white solid). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 7.72 (d, J = 12.0 Hz, 1H), 7.50 (d, J = 2.0 Hz, 1H), 7.07 (dd, J = 12.0, 2.0 Hz, 1H), 6.71 (s, 1H), 5.28 (s, 2H), 4.05 (m, 2H), 3.94 (m, 2H), 2.83 (s, 3H), 1.89 (m, 4H); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 169.5, 168.2, 158.2, 157.0, 154.1, 128.6, 122.2, 115.1, 106.4, 104.5, 74.7, 61.5, 43.6, 24.1, 22.4, 20.0; HPLC purity 98.0%; MS (ESI) m/z 360 $(M + H)^+$.

4-Methyl-2-{[5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}pentanoic Acid Methyl Ester (2). Method C was used. Yield: 55.6% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.8 Hz, 1H), 7.49 (d, J = 2.0 Hz, 1H), 7.14 (d, J = 8.4 Hz, 1H), 7.05 (dd, J = 8.8, 2.4 Hz, 1H), 6.81 (s, 1H), 5.27 (s, 2H), 4.79 (m, 1H), 3.77 (s, 3H), 2.83 (s, 3H), 1.71 (m, 3H), 0.97 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.1, 169.0, 168.4, 157.9, 157.8, 156.2, 154.0, 128.5, 121.5, 114.5, 106.1, 103.1, 61.0, 52.1, 50.4, 41.1, 24.5, 22.4, 21.4, 19.8; HPLC purity 99.4%; MS (ESI) m/z 456 $(M + K)^+$.

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid Dimethylamide (7a). Method B was used. Yield: 94.2% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 12.0 Hz, 1H), 7.52 (s, 1H), 7.07 (d, J = 12.0 Hz, 1H), 6.69 (s, 1H), 5.27 (s, 2H), 3.30 (s, 3H), 3.14 (s, 3H), 2.84 (s, 3H); HPLC purity 96.1%; MS (ESI) m/z 318 $(M + H)^+$.

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid Diethylamide (7b). Method C was used. Yield: 96.6% (yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 12.0 Hz, 1H), 7.52 (s, 1H), 7.06 (d, J = 12.0 Hz, 1H), 6.68 (s, 1H), 5.26 (s, 2H), 3.56 (m, 4H), 2.84 (s, 3H), 1.25 (m, 6H); HPLC purity 97.0%; MS (ESI) m/z 346 $(M + H)^+$.

[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazol-3-yl]-morpholin-4-ylmethanone (7c). Method A was used. Yield: 14.1% (yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 12.0 Hz, 1H), 7.52 (s, 1H), 7.06 (d, J = 12.0 Hz, 1H), 6.70 (s, 1H), 5.25 (s, 2H), 3.94 (m, 2H), 3.80 (m, 4H), 3.74 (m, 2H), 2.84 (s, 3H); HPLC purity 99.0%; MS (ESI) m/z 360 $(M + H)^+$.

[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazol-3-yl]piperidin-1-ylmethanone (7d). Method C was used. Yield: 73.6% (yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 12.0 Hz, 1H), 7.75 (s, 1H), 7.17 (d, J = 12.0 Hz, 1H), 6.70 (s, 1H), 5.30 (s, 2H), 3.74 (m, 4H), 2.96 (s, 3H), 1.68 (m, 6H); HPLC purity 99.7%; MS (ESI) m/z 358 $(M + H)^+$.

[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazol-3-yl]piperazin-1-ylmethanone (7e). Method D was used. Yield: 77.2% (yellow

solid). ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J = 8.0 Hz, 1H), 7.66 (s, 1H), 7.14 (d, J = 8.0 Hz, 1H), 6.80 (s, 1H), 5.32 (s, 2H), 4.37 (m, 2H), 4.13 (m, 2H), 3.43 (br, 1H), 4.34 (m, 4H), 2.92 (s, 3H); HPLC purity 99.4%; MS (ESI) m/z 359 $(M + H)^+$.

[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazol-3-yl]-(4-methylpiperazin-1-yl)methanone (7f). Method D was used. Yield: 98.7% (yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.06 (dd, J = 8.0, 2.0 Hz, 1H), 6.69 (s, 1H), 5.26 (s, 2H), 3.91 (m, 4H), 2.82 (s, 3H), 2.55 (m, 4H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 168.5, 167.6, 158.6, 158.1, 156.4, 154.0, 128.4, 121.6, 114.5, 106.0, 104.6, 61.0, 54.7, 53.9, 46.2, 45.2, 41.8, 19.8; HPLC purity 95.4%; MS (ESI) m/z 373 $(M + H)^+$.

(3*S*)-Methyl-2-{[5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}pentanoic Acid Methyl Ester (7g). Method A was used. Yield: 30.6% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 12.0 Hz, 1H), 7.50 (s, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 12.0 Hz, 1H), 6.81 (s, 1H), 5.28 (s, 2H), 4.77 (dd, J = 8.0, 5.2 Hz, 1H), 3.78 (s, 3H), 2.84 (s, 3H), 2.02 (m, 1H), 1.53 (m, 1H), 1.28 (m, 1H), 0.97 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 171.7, 169.6, 169.0, 158.5, 156.9, 154.7, 129.1, 122.3, 115.2, 106.7, 103.7, 61.6, 56.8, 52.5, 38.2, 25.4, 20.4, 15.8, 11.7; HPLC purity 99.0%; MS (ESI) m/z 418 $(M + H)^+$.

2-{[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}-4-methylsulfanylbutyric Acid Methyl Ester (7h). Method C was used. Yield: 51.7% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.8 Hz, 1H), 7.49 (d, J = 2.4 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.05 (dd, J = 8.8, 2.4 Hz, 1H), 6.81 (s, 1H), 5.28 (s, 2H), 4.90 (m, 1H), 3.80 (s, 3H), 2.83 (s, 3H), 2.58 (t, J = 7.2 Hz, 2H), 2.28 (m, 1H), 2.11 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 171.1, 169.1, 168.4, 157.9, 157.7, 156.2, 154.0, 128.5, 121.7, 114.5, 106.1, 103.0, 61.0, 52.3, 51.1, 31.2, 29.5, 19.8, 15.1; HPLC purity 98.6%; MS (ESI) m/z 436 $(M + H)^+$.

3-Methyl-2-{[5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}butyric Acid Methyl Ester (7i). Method C was used. Yield: 45.1% (yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 8.0 Hz, 1H), 7.48 (s, 1H), 7.23 (d, J = 8.0 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 6.80 (s, 1H), 5.26 (s, 2H), 4.70 (m, 1H), 3.76 (s, 3H), 2.82 (s, 3H), 2.25 (m, 1H), 0.98 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 171.2, 169.0, 168.4, 158.0, 157.9, 156.3, 154.0, 128.5, 121.6, 114.5, 106.1, 103.1, 61.0, 56.8, 51.9, 31.0, 19.8, 18.6, 17.4; HPLC purity 97.6%; MS (ESI) m/z 404 $(M + H)^+$.

{[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}phenylacetic Acid Methyl Ester (7j). Method C was used. Yield: 54.6% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 7.2 Hz, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.49 (d, J = 2.4 Hz, 1H), 7.37 (m, 5H), 7.04 (dd, J = 8.4, 2.0 Hz, 1H), 6.79 (s, 1H), 5.72 (d, J = 7.2 Hz, 1H), 5.26 (s, 2H), 3.77 (s, 3H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.1, 168.4, 157.7, 157.5, 156.2, 154.0, 135.3, 128.7, 128.5, 127.0, 121.6, 114.5, 106.1, 103.1, 61.0, 56.1, 52.7, 19.8; HPLC purity 99.4%; MS (ESI) m/z 460 $(M + Na)^+$.

2-{[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}-3-phenylpropionic Acid Methyl Ester (7k). Method C was used. Yield 54.7% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 8.4 Hz, 1H), 7.49 (s, 1H), 7.29 (M, 3H), 7.16 (d, J = 6.8 Hz, 2H), 7.04 (d, J = 8.8 Hz, 1H), 6.79 (s, 1H), 5.26 (s, 2H), 5.07 (m, 1H), 3.75 (s, 3H), 3.21 (m, 2H), 2.83 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.7, 169.0, 168.5, 157.7, 156.3, 154.0, 135.3, 128.8, 128.5, 128.3, 126.9, 121.7, 114.5, 106.0, 103.0, 61.0, 52.9, 52.1, 37.6, 19.8; HPLC purity 97.3%; MS (ESI) m/z 474 $(M + Na)^+$.

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid (2-Hydroxy-1-phenylethyl)-(*S*)-amide (7n). Method A was used. Yield: 90.3% (white solid). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 7.73 (d, J = 8.0 Hz, 1H), 7.48 (s, 1H), 7.33 (m, 5H), 7.07 (d, J = 8.0 Hz, 1H), 6.82 (s, 1H), 5.30 (s, 2H), 5.18 (m, 1H), 3.89 (m, 2H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.7, 172.4, 162.0, 161.5, 159.7, 156.7, 141.7, 131.5, 131.4, 130.6, 129.6, 125.1, 118.0, 109.2, 106.3, 67.8, 64.2, 58.7, 22.6; HPLC purity 98.9%; MS (ESI) m/z 410 $(M + H)^+$.

(4-Hydroxyphenyl){[5-(2-methylbenzothiazol-5-yloxymethyl-isoxazole-3-carbonyl]-(*S*)-amino}acetic Acid Methyl Ester (7o). Method A was used. Yield: 93.9% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.87 (br, 1H), 7.82 (d, J = 6.8 Hz, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.45 (d, J = 2.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.03 (dd, J = 8.8, 2.8 Hz, 1H), 6.80 (d, J = 8.4 Hz, 2H), 6.76 (s, 1H), 5.61 (d, J = 7.2 Hz, 1H), 5.20 (s, 2H), 3.74 (s, 3H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.4, 169.0, 157.7, 157.6, 156.7, 156.3, 153.5, 128.4, 128.2, 126.5, 121.5, 115.7, 114.8, 105.7, 103.1, 60.9, 55.7, 52.6, 19.6; HPLC purity 96.8%; MS (ESI) m/z 454 $(M + H)^+$.

2-{[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]amino}benzoic Acid Methyl Ester (7p). Method A was used. Yield: 14.9% (white solid). ^{1}H NMR ($CDCl_3$) δ 12.4 (s, 1H), 8.82 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.52 (s, 1H), 7.18 (t, J = 8.0 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 6.81 (s, 1H), 5.31 (s, 2H), 4.00 (s, 3H), 2.83 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.3, 168.0, 159.1, 156.7, 156.3, 140.0, 134.3, 130.7, 123.0, 121.6, 120.3, 115.5, 114.5, 106.2, 103.1, 61.1, 52.3, 19.8; HPLC purity 98.5%; MS (ESI) m/z 424 $(M + H)^+$.

(2-Chlorophenyl){[5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}acetic Acid Methyl Ester (7q). Method A was used. Yield: 59.4% (yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.86 (d, J = 7.2 Hz, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.58 (s, 1H), 7.44 (m, 2H), 7.30 (m, 2H), 7.07 (d, J = 8.8 Hz, 1H), 6.80 (s, 1H), 6.10 (d, J = 7.2 Hz, 1H), 5.27 (s, 2H), 3.79 (s, 3H), 2.87 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.0, 169.4, 168.8, 158.0, 157.9, 156.6, 154.4, 134.0, 133.7, 130.2, 130.1, 130.0, 127.4, 122.0, 114.9, 106.5, 103.5, 61.3, 54.7, 53.2, 20.2; HPLC purity 97.3%; MS (ESI) m/z 510 $(M + K)^+$.

{[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*R*)-amino}phenylacetic Acid Methyl Ester (7s). Method C was used. Yield: 61.9% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 7.2 Hz, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.39 (m, 5H), 7.04 (dd, J = 8.8, 2.4 Hz, 1H), 6.79 (s, 1H), 5.72 (d, J = 7.6 Hz, 1H), 5.26 (s, 2H), 3.77 (s, 3H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.1, 168.4, 157.7, 157.5, 156.2, 154.0, 135.3, 128.7, 128.5, 128.4, 127.0, 121.6, 114.5, 106.1, 103.1, 61.0, 56.1, 52.6, 19.8; HPLC purity 97.7%; MS (ESI) m/z 438 $(M + H)^+$.

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid Benzylamide (7t). Method A was used. Yield: 70.6% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 8.8 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.32 (m, 6H), 7.04 (dd, J = 8.8, 2.4 Hz, 1H), 6.83 (s, 1H), 5.25 (s, 2H), 4.61 (d, J = 5.6 Hz, 2H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.3, 168.8, 158.6, 158.4, 156.4, 154.4, 137.3, 128.8, 128.6, 127.8, 122.0, 114.9, 106.4, 103.5, 61.4, 43.5, 20.2; HPLC purity 99.4%; MS (ESI) m/z 380 $(M + H)^+$.

Isonicotinic acid *N*′-[5-(2-methylbenzothiazol-5-yloxymethyl)-isoxazole-3-carbonyl]hydrazide (7v). Method A was used. Yield: 74.4% (white solid). ^{1}H NMR ($CDCl_3$+CD_3OD) δ 8.63 (d, J = 4.0 Hz, 2H), 7.76 (d, J = 5.6 Hz, 2H), 7.67 (d, J = 8.8 Hz, 1H), 7.41 (d, J = 1.5 Hz, 1H), 7.02 (dd, J = 8.8, 2.0 Hz, 1H), 6.82 (s, 1H), 5.26 (s, 2H), 2.74 (s, 3H); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 169.8, 169.5, 165.1, 158.4, 157.1, 156.9, 153.6, 149.7, 140.1, 128.4, 122.1, 121.9, 115.0, 106.0, 103.4, 61.1, 19.3; HPLC purity 99.1%; MS (ESI) m/z 432 $(M + Na)^+$.

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid Hydroxyamide (7l). A suspended solution of hydroxylamine hydrochloride (2.35 g, 0.034 mol) in MeOH (12 mL) was refluxed under argon until a clear solution was obtained. To this hot and clear solution was added a solution of KOH (3.35 g, 0.05 mol) in MeOH (7 mL). After refluxing 30 min, the mixture was cooled to room temperature and the upper clean solution (about 1.76 M NH_2OK in methanol[17]) was used directly. To a suspension of ester **5** (100 mg, 0.31 mmol) in MeOH (2 mL) was added the above NH_2OK solution (1.8 mL, 3.14 mmol). The mixture was stirred at room temperature overnight and then acidified to pH 5−6 with 1 N HCl at 0 °C. The resulting mixture was diluted with EtOAc (40 mL), washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by short

column chromatography on silica gel using EtOAc as the eluent to give **7l** as a white solid (49.2 mg, 51.3%). ^{1}H NMR (DMSO) δ 11.6 (s, 1H), 9.42 (s, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.63 (s, 1H), 7.13 (d, J = 8.0 Hz, 1H), 6.94 (s, 1H), 5.44 (s, 2H), 2.77 (s, 3H); HPLC purity 97.5%; MS (ESI) m/z 306 $(M + H)^+$.

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid (Hydroxycarbamoylphenylmethyl)-(*S*)-amide (7m). The synthesis involved starting with **7j** and following the same methodology as employed for the preparation of **7l** from **5**. Yield: 90.7% (white solid). ^{1}H NMR (CD_3OD) δ 7.78 (d, J = 8.0 Hz, 1H), 7.48 (m, 3H), 7.36 (m, 3H), 7.11 (d, J = 8.0 Hz, 1H), 6.85 (s, 1H), 5.57 (s, 1H), 5.35 (s, 2H), 2.78 (s, 3H); HPLC purity 96.3%; MS (ESI) m/z 439 $(M + H)^+$.

(4-Methoxyphenyl)-{[5-(2-methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}acetic Acid Methyl Ester (7r). To a solution of **7o** (22 mg, 0.05 mmol) in DMF (1 mL) was added K_2CO_3 (20 mg, 0.15 mmol), n-Bu_4NI (5 mg) and MeI (0.02 mL, 0.24 mmol). The mixture was stirred at room temperature for 2 h and then diluted with EtOAc (40 mL), washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using hexane−EtOAc (1:2) as the eluent to give **7r** as a yellow solid (20 mg, 88.2%). ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J = 8.8 Hz, 2H), 7.55 (s, 1H), 7.35 (d, J = 8.0 Hz, 2H), 7.06 (d, J = 8.0 Hz, 1H), 6.91 (d, J = 8.4 Hz, 2H), 6.80 (s, 1H), 5.65 (d, J = 7.2 Hz, 1H), 5.27 (s, 2H), 3.81 (s, 3H), 3.78 (s, 3H), 2.86 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.4, 169.0, 168.4, 159.5, 157.7, 157.4, 156.2, 154.0, 128.5, 128.3, 127.4, 121.6, 114.5, 114.1, 106.1, 103.1, 61.0, 56.2, 55.6, 52.5, 19.8; HPLC purity 99.1%; MS (ESI) m/z 468 $(M + K)^+$.

{[5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}phenylacetic Acid (7u). The synthesis involved starting with **7j** and following the same methodology as employed for the preparation of **6** from **5**. Yield: 54.4% (white solid). ^{1}H NMR (CD_3OD) δ 7.87 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 2.4 Hz, 1H), 7.48 (m, 2H), 7.35 (m, 3H), 7.19 (dd, J = 8.8, 2.4 Hz, 1H), 6.88 (s, 1H), 5.65 (s, 1H), 5.40 (s, 2H), 2.88 (s, 3H); HPLC purity 98.0%; MS (ESI) m/z 446 $(M + Na)^+$.

5-(2-Methylbenzothiazol-5-yloxymethyl)isoxazole-3-carboxylic Acid {2-Oxo-1-phenyl-2-[*N*′-(pyridine-4-carbonyl)hydrazino]ethyl}-(*S*)-amide (7w). The synthesis involved starting with **7u** and following the method C. Yield: 29.9% (white solid). ^{1}H NMR ($CDCl_3$+CD_3OD) δ 8.76 (m, 2H), 7.87 (m, 2H), 7.67 (d, J = 8.8 Hz, 1H), 7.50 (d, J = 7.2 Hz, 2H), 7.45 (s, 1H), 7.36 (m, 3H), 7.01 (d, J = 8.8 Hz, 1H), 6.78 (s, 1H), 5.78 (s, 1H), 5.24 (s, 2H), 2.78 (s, 3H); HPLC purity 96.6%; MS (ESI) m/z 543 $(M + H)^+$.

{[5-(2-Methylbenzothiazol-4-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}phenylacetic Acid Methyl Ester (12a). The synthesis involved starting with **11a** and following the same methodology as employed for the preparation of **7j** from **3**. Yield: 63.0% for three steps (white solid). ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 7.2 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.41 (m, 5H), 7.26 (m, 1H), 6.90 (d, J = 8.0 Hz, 1H), 6.79 (s, 1H), 5.70 (d, J = 7.2 Hz, 1H), 5.55 (s, 2H), 3.76 (s, 3H), 2.85 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.4, 165.8, 157.7, 157.5, 150.1, 143.3, 137.5, 135.3, 128.7, 128.4, 126.9, 125.0, 114.8, 108.9, 103.3, 61.6, 56.1, 52.6, 19.8; HPLC purity 97.7%; MS (ESI) m/z 476 $(M + K)^+$.

{[5-(2-Methylbenzothiazol-6-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}phenylacetic Acid Methyl Ester (12b). The synthesis involved starting with **11b** and following the same methodology as employed for the preparation of **7j** from **3**. Yield: 79.0% for three steps (yellow solid). ^{1}H NMR ($CDCl_3$) δ 7.84 (d, J = 8.8 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.38 (m, 6H), 7.08 (dd, J = 8.8, 2.4 Hz, 1H), 6.78 (s, 1H), 5.72 (d, J = 7.2 Hz, 1H), 5.26 (s, 2H), 3.77 (s, 3H), 2.79 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.0, 165.0, 157.7, 157.4, 154.8, 148.4, 136.5, 135.3, 128.7, 128.5, 127.0, 122.7, 114.9, 105.6, 103.0, 61.3, 56.1, 52.6, 19.6; HPLC purity 96.2%; MS (ESI) m/z 438 $(M + H)^+$.

{[5-(2-Methylbenzothiazol-7-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}phenylacetic Acid Methyl Ester (12c). The synthesis involved starting with **11c** and following the same methodology as employed for the preparation of **7j** from **3**. Yield: 71.1% for three steps (white solid). ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 7.2 Hz, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.40 (m, 6H), 6.83 (d, J = 8.4 Hz, 1H), 6.81 (s, 1H), 5.72 (d, J = 7.2 Hz, 1H), 5.35 (s, 2H), 3.77 (s, 3H), 2.83 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1, 168.7, 167.5, 157.7, 157.4, 155.0, 151.5, 135.3, 128.7, 128.5, 127.0, 126.3, 124.4, 116.1, 105.8, 103.1, 61.0, 56.1, 52.6, 19.7; HPLC purity 97.7%; MS (ESI) m/z 460 $(M + Na)^+$.

Phenyl({5-[2-(3-trifluoromethylphenyl)benzothiazol-5-yloxymethyl]isoxazole-3-carbonyl}-(*S*)-amino)acetic Acid Methyl Ester (17). The synthesis involved starting with **16** and following the same methodology as employed for the preparation of **7j** from **3**. Yield: 24.4% for three steps (white solid). ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.79 (m, 3H), 7.63 (m, 2H), 7.42 (m, 5H), 7.13 (d, J = 8.8 Hz, 1H), 6.84 (s, 1H), 5.74 (d, J = 7.2 Hz, 1H), 5.32 (s, 2H), 3.79 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.5, 169.3, 167.8, 158.1, 157.8, 157.1, 155.0, 135.7, 134.3, 131.8(q, J = 32.6 Hz), 130.5, 129.6, 129.1, 128.8, 128.4, 127.4, 127.3, 124.2, 124.1, 122.4, 116.4, 106.9, 103.5, 61.3, 56.5, 53.0; HPLC purity 96.3%; MS (ESI) m/z 606 $(M + K)^+$.

{[5-(Benzothiazol-2-yloxymethyl)isoxazole-3-carbonyl]-(*S*)-amino}phenylacetic Acid Methyl Ester (19a). The synthesis involved starting with **18a** and following the same methodology as employed for the preparation of **7j** from **3**. Yield: 49.8% for three steps (white solid). ^{1}H NMR ($CDCl_3$) δ 7.73 (d, J = 7.2 Hz, 1H), 7.45 (d, J = 7.6 Hz, 1H), 7.36 (m, 6H), 7.21 (m, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.63 (s, 1H), 5.69 (d, J = 7.2 Hz, 1H), 5.27 (s, 2H), 3.75 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.3, 167.5, 157.9, 157.2, 135.4, 135.3, 128.7, 128.5, 126.9, 126.4, 123.6, 122.6, 122.0, 110.1, 102.8, 56.1, 52.6, 37.2; HPLC purity 95.8%; MS (ESI) m/z 446 $(M + Na)^+$.

{[5-(Benzothiazol-2-ylsulfanylmethyl)isoxazole-3-carbonyl]-(*S*)-amino}phenylacetic Acid Methyl Ester (19b). The synthesis involved starting with **18b** and following the same methodology as employed for the preparation of **7j** from **3**. Yield: 53.8% for three steps (white solid). ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 8.0 Hz, 2H), 7.39 (m, 7H), 6.75 (s, 1H), 5.69 (d, J = 7.2 Hz, 1H), 4.72 (s, 2H), 3.76 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.1, 169.8, 163.1, 157.8, 157.6, 152.3, 135.4, 135.1, 128.7, 128.4, 127.0, 125.9, 124.3, 121.5, 120.8, 103.0, 56.1, 52.6, 26.6; HPLC purity 95.8%; MS (ESI) m/z 478 $(M + K)^+$.

[(5-Methylisoxazole-3-carbonyl)-(*S*)-amino]phenylacetic Acid Methyl Ester (21). The synthesis involved starting with **20** and following method C. Yield: 84.7% (white solid). ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 6.8 Hz, 1H), 7.41 (d, J = 6.8 Hz, 2H), 7.34 (m, 3H), 6.38 (s, 1H), 5.70 (d, J = 7.2 Hz, 1H), 3.72 (s, 3H), 2.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.9, 170.2, 158.1, 157.7, 135.5, 128.6, 128.3, 127.0, 101.0, 56.0, 52.5, 11.8; HPLC purity 96.7%; MS (ESI) m/z 297 $(M + Na)^+$.

[(5-Phenoxymethylisoxazole-3-carbonyl)-(*S*)-amino]phenylacetic Acid Methyl Ester (23). The synthesis involved starting with **22** and following the same methodology as employed for the preparation of **7j** from **3**. Yield: 59.1% for three steps (white solid). ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J = 7.2 Hz, 1H), 7.46 (m, 2H), 7.39 (m, 3H), 7.32 (m, 2H), 7.03 (m, 1H), 6.96 (d, J = 8.8 Hz, 2H), 6.78 (s, 1H), 5.74 (d, J = 7.2 Hz, 1H), 5.19 (s, 2H), 3.78 (s, 3H), 2.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.5, 169.8, 158.1, 158.0, 157.5, 135.8, 129.7, 129.1, 128.8, 127.4, 122.0, 114.8, 103.3, 60.9, 56.5, 53.0; HPLC purity 98.4%; MS (ESI) m/z 389 $(M + Na)^+$.

{[4-(2-Methylbenzothiazol-5-yloxymethyl)thiazole-2-carbonyl]-(*S*)-amino}phenylacetic Acid Methyl Ester (27). The synthesis involved starting with **26** and following the same methodology as employed for the preparation of **7j** from **4**. Yield: 56.9% for three steps (white solid). ^{1}H NMR ($CDCl_3$) δ 8.17 (d, J = 7.2 Hz, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.60 (s, 1H), 7.53 (d, J = 2.0 Hz, 1H), 7.47 (m, 2H), 7.36 (m, 3H), 7.08 (dd, J = 8.8, 2.0 Hz, 1H), 5.76 (d, J = 7.2 Hz, 1H), 5.28 (s, 2H), 3.79 (s, 3H), 2.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.3, 168.2, 162.4, 158.3, 156.9, 154.1, 153.1, 135.5, 128.7, 128.4, 127.1, 121.9, 121.5, 114.6, 106.0, 65.9, 56.2, 52.6, 19.8; HPLC purity 97.4%; MS (ESI) m/z 476 $(M + Na)^+$.

{**[2-(2-Methylbenzothiazol-5-yloxymethyl)oxazole-4-carbonyl]-(*S*)-amino}phenylacetic Acid Methyl Ester (29).** The synthesis involved starting with **28** and following the same methodology as employed for the preparation of **7j** from **4**. Yield: 34.6% for three steps (white solid). ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 1H), 7.85 (d, J = 7.2 Hz, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.54 (d, J = 2.0 Hz, 1H), 7.44 (m, 2H), 7.34 (m, 3H), 7.06 (dd, J = 8.8, 2.0 Hz, 1H), 5.73 (d, J = 7.2 Hz, 1H), 5.19 (s, 2H), 3.76 (s, 3H), 2.80 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.8, 168.7, 159.5, 159.3, 156.8, 154.4, 142.4, 136.0, 129.0, 128.8, 128.7, 127.4, 122.0, 114.9, 106.5, 62.4, 56.2, 52.9, 20.1; HPLC purity 100%; MS (ESI) m/z 438 $(M + H)^+$.

[(2-Methylbenzothiazole-5-carbonyl)-(*S*)-amino]phenylacetic Acid Methyl Ester (32). The synthesis involved starting with **31** and following method C. Yield: 50.9% (white solid). ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1H), 7.79 (s, 2H), 7.80 (d, J = 6.8 Hz, 1H), 7.44 (d, J = 7.2 Hz, 2H), 7.34 (m, 3H), 5.80 (d, J = 6.8 Hz, 1H), 3.74 (s, 3H), 2.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.1, 168.2, 166.0, 152.7, 138.8, 136.1, 131.4, 128.6, 128.2, 127.0, 123.2, 121.1, 120.7, 56.6, 52.5, 19.8; HPLC purity 99.5%; MS (ESI) m/z 341 $(M + H)^+$.

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Supporting Information Available: Details for the synthesis of compounds **4**, **11a**−**c**, **15**, **16**, **26**, and **31**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *Global Tuberculosis Control 2009: Epidemiology, Strategy, Financing*; WHO Report 2009. WHO Library Cataloguing-in-Publication Data; World Health Organization: Geneva, Switzerland, 2009.
(2) Faustini, A.; Hall, A. J.; Perucci, C. A. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. *Thorax* **2006**, *61*, 158–163.
(3) Fraser, A.; Paul, M.; Attamna, A.; Leibovici, L. Treatment of latent tuberculosis in persons at risk for multidrug-resistant tuberculosis: systematic review. *Int. J. Tuberculosis Lung Dis.* **2006**, *10*, 19–23.
(4) Wright, A.; Bai, G.; Barrera, L.; Boulahbal, F.; Martin-Casabona, N.; Gilpin, C.; Drobniewsji, F.; Havelkova, M.; Lepe, R.; Lumb, R.; Metchock, B.; Portaels, F.; Rodrigues, M.; Rusch-Gerdes, S.; Van Deun, A.; Vincent, V.; Leimane, V.; Riekstina, V.; Skenders, G.; Holtz, T.; Pratt, R.; Laserson, K.; Wells, C.; Cegielski, P.; Shah, N. S. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs—Worldwide, 2000−2004. *JAMA, J. Am. Med. Assoc.*. **2006**, *295*, 2349–2351.
(5) Falzari, K.; Zhu, Z. H.; Pan, D. H.; Liu, H. W.; Hongmanee, P.; Franzblau, S. G. In vitro and in vivo activities of macrolide derivatives against *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother*. **2005**, *49*, 1447–1454.
(6) Micetich, R. G.; Shaw, C. C.; Hall, T. W.; Spevak, P.; Fortier, R. A.; Wolfert, P.; Foster, B. C.; Bains, B. K. The 5-alkoxymethyl-isoxazoles, 5-alkylthiomethyl-isoxazoles and 5-dialkylamino-methyl-isoxazoles. *Heterocycles* **1985**, *23*, 571–583.
(7) Kashiyama, E.; Hutchinson, I.; Chua, M. S.; Stinson, S. F.; Phillips, L. R.; Kaur, G.; Sausville, E. A.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. Antitumor benzothiazoles. 8. Synthesis, metabolic formation, and biological properties of the C- and N-oxidation products of antitumor 2-(4-aminophenyl)benzothiazoles. *J. Med. Chem.* **1999**, *42*, 4172–4184.
(8) Mathis, C. A.; Wang, Y. M.; Holt, D. P.; Huang, G. F.; Debnath, M. L.; Klunk, W. E. Synthesis and evaluation of C-11-labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents. *J. Med. Chem.* **2003**, *46*, 2740–2754.
(9) Lee, C. B.; Wu, Z. C.; Zhang, F.; Chappell, M. D.; Stachel, S. J.; Chou, T. C.; Guan, Y. B.; Danishefsky, S. J. Insights into long-range structural effects on the stereochemistry of aldol condensations: a practical total synthesis of desoxyepothilone F. *J. Am. Chem. Soc.* **2001**, *123*, 5249–5259.
(10) Klar, U.; Buchmann, B.; Schwede, W.; Skuballa, W.; Hoffinann, J.; Lichtner, R. B. Total synthesis and antitumor activity of ZK-EPO: the first fully synthetic epothilone in clinical development. *Angew. Chem., Int. Ed.* **2006**, *45*, 7942–7948.
(11) Lynch, D. C.; Miller, J. R.; Spawn, T. D. A convenient synthesis of 2-methyl-5-(methylthio)benzothiazole. *Synth. Commun.* **1997**, *27*, 897–905.
(12) Timmins, G. S.; Deretic, V. Mechanisms of action of isoniazid. *Mol. Microbiol.* **2006**, *62*, 1220–1227.
(13) Arai, M.; Alavi, Y. I. H.; Mendoza, J.; Billker, O.; Sinden, R. E. Isonicotinic acid hydrazide: an anti-tuberculosis drug inhibits malarial transmission in the mosquito gut. *Exp. Parasitol.* **2004**, *106*, 30–36.
(14) Cho, Y.; Ioerger, T. R.; Sacchettini, J. C. Discovery of novel nitrobenzothiazole inhibitors for *Mycobacterium tuberculosis* ATP phosphoribosyl transferase (HisG) through virtual screening. *J. Med. Chem.* **2008**, *51*, 5984–5992.
(15) Lilienkampf, A.; Mao, J.; Wan, B.; Wang, Y.; Franzblau, S. G.; Kozikowski, A. P. Structure−activity relationships for a series of quinoline-based compounds active against replicating and nonreplicating *Mycobacterium tuberculosis*. *J. Med. Chem.* **2009**, *52*, 2109–2118.
(16) Ganapaty, S; Steve, T. P; Karagianis, G; Waterman, P. G.; Brun, R. Antiprotozoal and cytotoxic naphthalene derivatives from *Diospyros assimilis*. *Phytochemistry* **2006**, *67*, 1950–1956.
(17) Fieser, M. *Fieser and Fieser's Reagents for Organic Synthesis*; Wiley Interscience: New York, 1967; Vol. 1, p 478.