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Rational Design of 5-Phenyl-3-isoxazolecarboxylic Acid Ethyl Esters as Growth Inhibitors of *Mycobacterium tuberculosis*. A Potent and Selective Series for Further Drug Development

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New antituberculosis (anti-TB) drugs are urgently needed to shorten the 6−12 month treatment regimen and especially to battle drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strains. In this study, we have continued our efforts to develop isoxazole-based anti-TB compounds by applying rational drug design approach. The biological activity and the structure−activity relationships (SAR) for a designed series of 5-phenyl-3-isoxazolecarboxylic acid ethyl ester derived anti-TB compounds were investigated. Several compounds were found to exhibit nanomolar activity against the replicating bacteria (R-TB) and low micromolar activity against the nonreplicating bacteria (NRP-TB). The series showed excellent selectivity toward *Mtb*, and in general, no cytotoxicity was observed in Vero cells ($IC_{50} > 128$ μM). Notably, selected compounds also retained their activity against isoniazid (INH), rifampin (RMP), and streptomycin (SM) resistant *Mtb* strains. Hence, benzyloxy, benzylamino, and phenoxy derivatives of 5-phenyl-3-isoxazolecarboxylic acid ethyl esters represent a highly potent, selective, and versatile series of anti-TB compounds and as such present attractive lead compounds for further TB drug development.

Introduction

Mycobacterium tuberculosis (*Mtb*[a]) is the predominant cause of tuberculosis (TB), a life-threatening chronic infection primarily affecting the lungs. The World Health Organization (WHO) has estimated that one-third of the world's population is infected with *Mtb*, resulting in 1.7 million deaths from TB in 2006.[1] As a frequent coinfection, TB is aggravated by the spread of HIV and is a major cause of death among HIV/AIDS patients. Current TB treatment regimen DOTS (directly observed therapy short-course) requires patients to take a combination of three or four drugs, namely, isoniazid (INH), rifampin (RMP), pyrazinamide (PZA), and ethambutol (EMB) (or alternatively streptomycin (SM)), throughout a 6−12 month period. The long treatment regimen, which is necessary due to the presence of a nonreplicating persistent *Mtb* phenotype (NRP-TB),[2] results in poor patient compliance, causes undesired side effects, and makes a significant contribution to the emergence of drug resistant *Mtb* strains.[3] Multidrug-resistant TB (MDR-TB) is resistant to the most common first-line drugs, i.e., INH and RMP, whereas extensively drug-resistant TB (XDR-TB) is also resistant to the fluoroquinolones and at least one of the intravenous second-line drugs, i.e., kanamycin, capreomycin, or amikacin. According to the WHO, over 400 000 people develop MDR-TB each year, and cases have been reported across all regions of the world. Failed MDR-TB treatment regimen may result in XDR-TB, which in turn can be spread from one individual to another. Thus, in 2006, XDR-TB was named as a global threat to public health.[4] All the foregoing facts emphasize the urgent demand for new anti-TB drugs with novel mechanisms of action. However, TB is a neglected disease and new anti-TB drugs have not been introduced for the past 4 decades.[5] While more research has been placed on early stage drug discovery as a consequence of funding from the Bill and Melinda Gates Foundation and others, only a few compounds have been advanced to the clinic. In this connection we note that compounds such as arylquinoline (α*S*,β*R*)-6-bromo-α-[2-(dimethylamino)ethyl]-2-methoxy-α-1-naphthalenyl-β-phenyl-3-quinolineethanol (**1**) (TMC207)[6] and nitroimidazoles (6*S*)-6,7-dihydro-2-nitro-6-[[4-(trifluoromethoxy)phenyl]methoxy]-5*H*-imidazo[2,1-*b*][1,3]oxazine (**2**) (PA-824)[7] and (2*R*)-2,3-dihydro-2-methyl-6-nitro-2-[[4-[4-[4-(trifluoromethoxy)phenoxy]-1-piperidinyl]phenoxy]methyl]-imidazo[2,1-*b*]oxazole (**3**) (OPC-67683)[8] have entered clinical trials as new anti-TB agents.[9] **1** targets the c subunit of ATP synthase,[10] whereas **2** and **3** have been suggested to disrupt the synthesis of vital cell wall mycolic acids.[7,8] In addition, **2** kills NRP-TB by intracellular nitric oxide (NO) release.[11]

We have previously reported certain isoxazole-based compounds, created by combining phenotypic screening and rational drug design, to exhibit good activity against *Mtb*. When a 3-isoxazolecarboxylic acid ethyl ester moiety was linked to the quinoline core of the antimalarial drug mefloquine (*Mtb* MIC = 13 μM),[12] the original lead compound **4**

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[a] Abbreviations: DOTS, directly observed therapy short-course; INH, isoniazid; LORA, low oxygen recovery assay; MABA, microplate Alamar blue assay; MIC, minimum inhibitory concentration; MDR-TB, multidrug-resistant tuberculosis; *Mtb*, *Mycobacterium tuberculosis*; NRP-TB, nonreplicating persistent tuberculosis; R-TB, replicating tuberculosis; RMP, rifampin; SDR-TB, single drug resistant tuberculosis; SM, streptomycin; TB, tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

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(MIC = 0.9 μM) was obtained (Figure 1).[13] Equal activity was observed with compound **5** (MIC = 0.95 μM) in which the oxymethylene linker was replaced with a phenoxy linker.[14] In further optimization studies, the activity, as well as metabolic stability, was improved when the oxymethylene linker was replaced with a *trans*-ethenyl linker[15] and the quinoline core was replaced with a phenyl ring to obtain the structurally simplified derivative **6** (MIC = 0.73 μM).[16] Our previous work has also shown that the isoxazole[14] and the C3 ester moiety[13,14] are crucial for the anti-TB activity. However, the ester may act as a prodrug for the corresponding carboxylic acid that is formed within the bacteria possibly by esterase activity. The carboxylic acid derivatives are inactive in vitro which may be due to insufficient penetration through the *Mtb* cell wall.[13,16] In a related study, Lee and co-workers have recently reported certain derivatives of 5-phenyl-3-isoxazolinecarboxylic acid esters to have low micromolar activity against R-TB.[17]

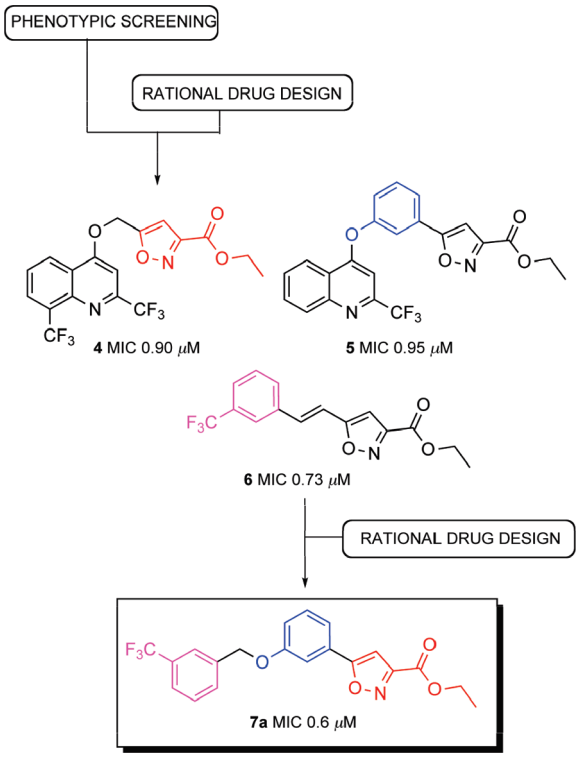


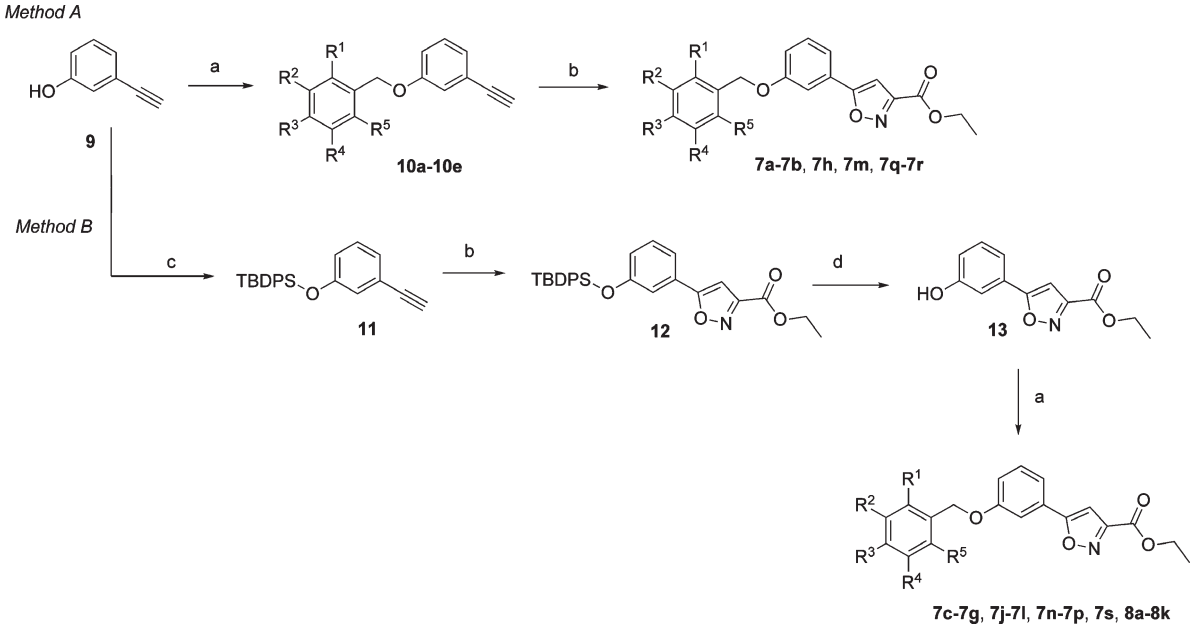
Figure 1. Evolution of isoxazole-based anti-TB agents.

Here, we describe the design, synthesis, and biological evaluation of a series of anti-TB agents based on a 5-phenyl-3-isoxazolecarboxylic acid ethyl ester core. Compounds **4**, **5**, and **6** were used as lead compounds, and a rational approach was employed to design compound **7a** that showed excellent activity against *Mtb* with an MIC of 0.6 μM (Figure 1). Encouraged by this finding, we engaged in a more in-depth study of the structure−activity relationships (SAR) for this isoxazole-based anti-TB compound series. The modifications were focused on the substitution pattern of the side-chain aryl moiety, on the oxymethylene linker, and in one example on the isoxazole ring. The target compounds were evaluated for their activity against R-TB and NRP-TB. The 5-phenyl-3-isoxazolecarboxylic acid ethyl ester derivatives were found, in several cases, to exhibit nanomolar activity against R-TB and also to have low micromolar activity against NRP-TB. These 5-phenyl-3-isoxazolecarboxylic acid ethyl esters were also found to have activity against single drug-resistant *Mtb* strains (SDR-TB), as described herein.

Chemistry

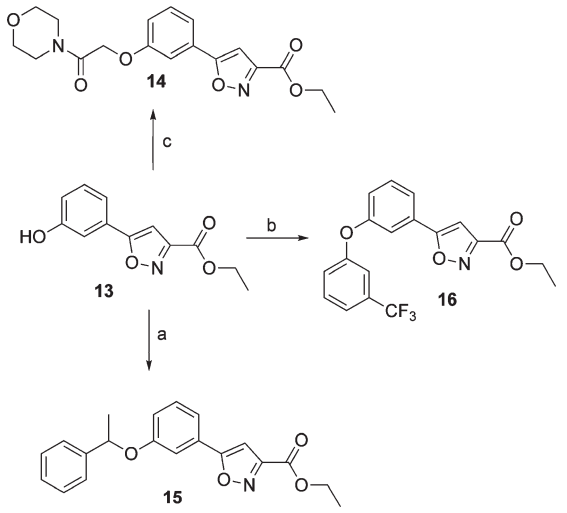
Two different strategies were employed in the synthesis of target compounds **7a**−**s** and **8a**−**k**. Compounds **7a**, **7b**, **7h**, **7m**, and **7q**−**r** were synthesized in two steps starting from 3-hydroxyphenylacetylene (**9**) (Scheme 1, method A). Alkylation of **9** with a suitably substituted benzyl bromide produced the acetylene intermediates **10a**−**e** in excellent yields (81−91%). Next, a dipolar cycloaddition of the nitrile oxide

Scheme 1[a]



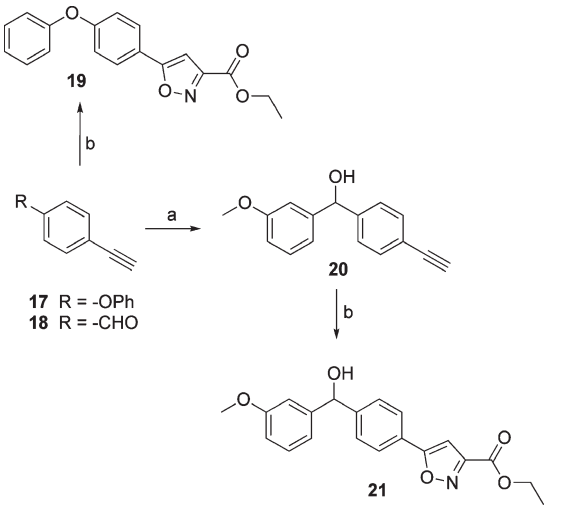
[a] Reagents and conditions: (a) $ArCH_2Br$ or $ArCH_2Cl$, K_2CO_3, KI, acetone, reflux; (b) ethyl 2-chloro-2-(hydroxyimino)acetate, Et_3N, ether or THF, room temp; (c) TBDPSCl, imidazole, CH_2Cl_2; (d) TBAF, THF, room temp.

Scheme 2[a]



[a] Reagents and conditions: (a) (1-bromoethyl)benzene, K_2CO_3, KI, acetone, reflux; (b) 3-(trifluoromethyl)phenylboronic acid, $Cu(OAc)_2$, Et_3N, CH_2Cl_2, room temp; (c) 4-(chloroacetyl)morpholine, K_2CO_3, KI, acetone, reflux.

Scheme 3[a]



[a] Reagents and conditions: (a) 3-methoxyphenylmagnesium bromide, THF, 0 °C to room temp; (b) ethyl 2-chloro-2-(hydroxyimino)acetate, Et_3N, THF, room temp.

derived from ethyl 2-chloro-2-(hydroxyimino)acetate with the acetylene intermediates furnished the desired 5-phenyl-3-isoxazolecarboxylic acid ethyl esters **7a**, **7b**, **7h**, **7m**, and **7q**−**r** in 43−77% yield. In an alternative route, we chose to synthesize 5-(3-hydroxyphenyl)-3-isoxazolecarboxylic acid ethyl ester (**13**) as an intermediate, which could efficiently be employed in the focused library synthesis of the monosubstituted derivatives **7c**−**g**, **7j**−**l**, **7n**−**p**, and **7s** and the di- and trisubstituted compounds **8a**−**k** (Scheme 1, method B). The protection of **9** with TBDPSCl produced the intermediate **11**, which in turn gave the isoxazole intermediate **12** via the dipolar cycloaddition reaction. Deprotection of **12** with TBAF in THF gave the key intermediate **13** in 67% yield. The final compounds **7c**−**g**, **7j**−**l**, **7n**−**p**, **7s**, and **8a**−**k** were synthesized from **13** in 63−99% yields by straightforward Williamson reaction with various benzyl halides, as described above. All the benzyl halides employed were commercially available with the exception of [3-(chloromethyl)phenyl]-4-morpholinylmethanone, which was synthesized from morpholine and 3-(chloromethyl)benzoylchloride. The 3-amino derivative **7i** was synthesized by reducing the corresponding 3-nitro derivative **7h** with $SnCl_2$. The acetylmorpholine derivative **14** was synthesized from phenol **13** and 4-(chloroacetyl)morpholine (Scheme 2).

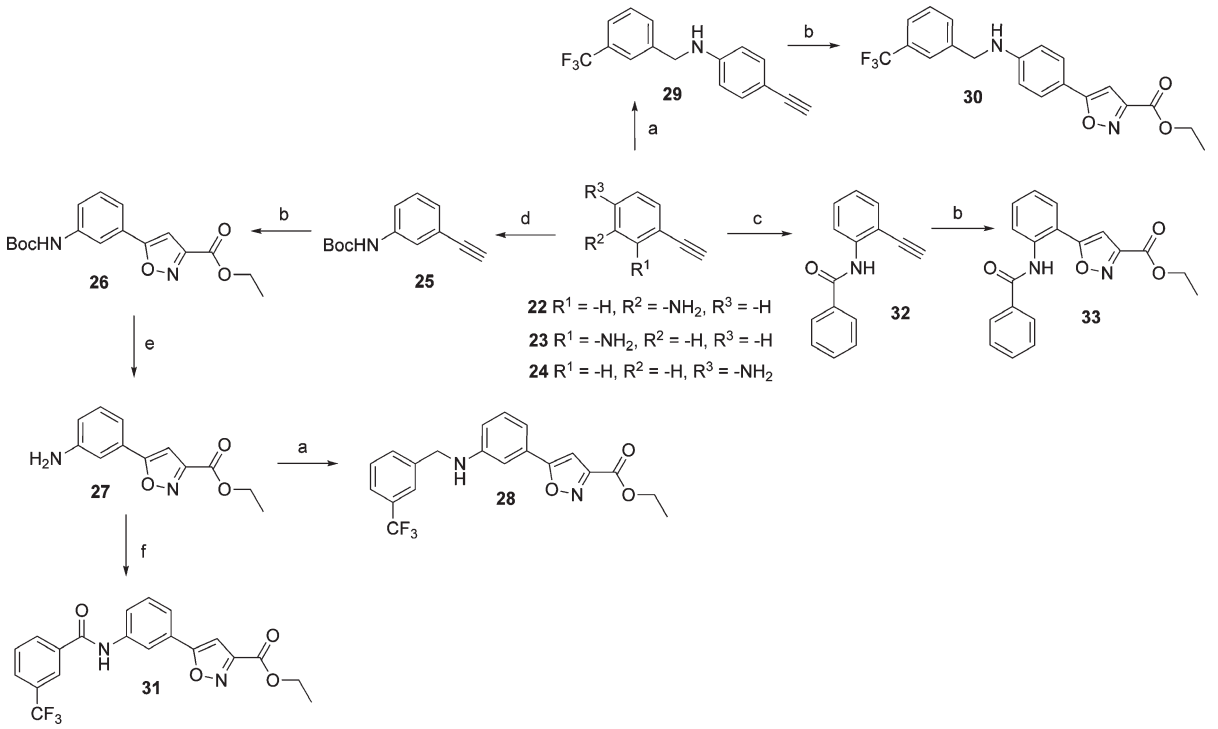
Next, we focused our efforts on the modifications of the oxymethylene linker moiety. The α-methyl derivative **15** was synthesized from the phenol **13** and (1-bromoethyl)benzene by employing the same strategy as described above (Scheme 2). The diphenyl ether derivative **16**, bearing the phenoxy moiety at the meta position, was obtained in 67% yield by $Cu(OAc)_2$-catalyzed coupling of **13** with 3-(trifluoromethyl)phenylboronic acid (Scheme 2), whereas the para substituted derivative **19** was synthesized from the commercially available 1-ethynyl-4-phenoxybenzene (**17**) as outlined above (Scheme 3). Grignard reaction of 3-methoxyphenylmagnesium bromide with 4-ethynylbenzaldehyde (**18**) gave the acetylene intermediate **20** which in turn gave **21**, bearing a hydroxymethylene linker at the para position.

Keeping the linker in the original meta position, $NaB(OAc)_3H$ mediated reductive amination of 3-(trifluoromethyl)-benzaldehyde with 5-(3-aminophenyl)-3-isoxazolecarboxylic acid ethyl ester (**27**) gave **28** in 65% yield (Scheme 4). The aniline intermediate **27** was in turn synthesized from 3-ethynylaniline (**22**) via Boc-protected intermediate **26** according to a published procedure.[18] Similarly compound **30**, bearing an aminomethylene linker at the para position, was synthesized in two steps via reductive amination and the dipolar cycloaddition. The meta amide linked derivative **31** was also synthesized from **27** in 79% yield by an amide coupling reaction with 3-(trifluoromethyl)benzoic acid. The ortho amide **33** was obtained via the acetylene intermediate **32**. The pyrazole derivative **34** was synthesized from the corresponding acetylene intermediate **10f** and ethyl diazoacetate under microwave irradiation (Scheme 5). According to ^1H NMR, **34** is a mixture of 1-*H* and 2-*H* tautomers in DMSO-d_6.

Results and Discussion

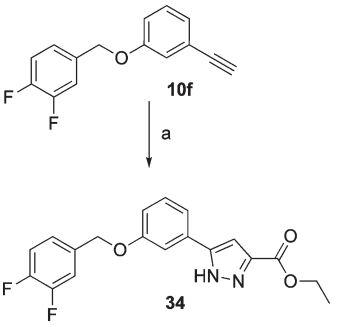
A total of 42 compounds were synthesized and evaluated first for their activity against the *Mtb* strain $H_{37}Rv$ in a microplate Alamar blue assay (MABA).[19] In addition, the compounds were further evaluated for their potency in a low oxygen recovery assay (LORA),[20] which is a luminescence-based high-throughput assay suggested for assessment of activity against NRP-TB in oxygen-deprived conditions. Several compounds were found to effectively inhibit the growth of R-TB in MABA with nanomolar MICs. In addition, several compounds exhibited low micromolar activity. First, we investigated the effect of the aromatic substitution pattern of the benzyloxy moiety on the anti-TB activity (Table 1), which was shown to affect compound potency and from which some whole cell SAR could be derived. In all modifications, the oxymethylene linker was kept at the meta position in relation to the isoxazole moiety. The designed lead compound **7a**, which had an excellent MIC of 0.6 μM, carries a $-CF_3$ group at the meta position (R^2) of the benzene ring. Removal of this substituent (**7b**, MIC = 1.2 μM) led to 2-fold reduced activity relative to **7a**, indicating that substitution of the ring plays some role in the anti-TB activity. At the meta position, various substituents were investigated. All the tested halogen substituents (−F, −Cl, and −Br), as well as $-OCF_3$, −CN, and $-NO_2$ (compounds **7c**−**h**), yielded activity comparable to that of the lead **7a** with MICs ranging between 0.6 and 1.0 μM.

Scheme 4[a]



[a] Reagents and conditions: (a) 3-(trifluoromethyl)benzaldehyde, $NaB(OAc)_3H$, AcOH, 1,2-dichloroethane, room temp; (b) ethyl 2-chloro-2-(hydroxyimino)acetate, Et_3N, ether or THF, room temp; (c) benzoylchloride, Et_3N, DMAP, CH_2Cl_2, room temp; (d) Boc_2O, THF, reflux, 16 h; (e) TFA, CH_2Cl_2, room temp; (f) 3-(trifluoromethyl)benzoic acid, DMAP, EDCI, CH_2Cl_2, 0 °C to room temp.

Scheme 5[a]

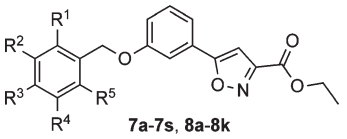


[a] Reagents and conditions: (a) N_2CHCO_2Et, benzene, microwave, 140 °C.

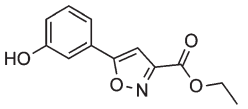
Although still active, $-CH_3$, $-OCH_3$, and $-NH_2$ substitution led to slightly decreased potency (compounds **7i**–**k**, MIC = 1.2–2.1 μM). However, the activity was completely lost with **7l**, bearing a 4-morpholinecarbonyl moiety, probably because of increased steric hindrance at the target site. The electronic character of the substituent apparently contributes to the anti-TB activity as can be seen when comparing $-CH_3$ and $-CF_3$ (**7k**, MIC = 1.2 μM vs **7a**, MIC = 0.6 μM) or $-OCH_3$ and $-OCF_3$ (**7j**, MIC = 2.1 μM vs **7f**, MIC = 0.7 μM). Introduction of $-CF_3$ group, as well $-NO_2$, to the ortho position (R^1) decreased the potency 2- to 3-fold, although **7m** (MIC = 1.5 μM) and **7p** (MIC = 1.5 μM) still exhibited good anti-TB activity. With halogens as ortho substituents, i.e., $-Cl$ and $-F$, the activity was retained as compared to the corresponding meta substituted compounds **7n** and **7o** having an MIC of 0.9 and 1.1 μM, respectively. The introduction of a $-CF_3$ group to the para position (R^3) of the benzene ring (compound **7q**) led to a slight improvement in the activity relative to the meta substituted lead **7a** (MIC of 0.4 vs 0.6 μM). Comparable activity was obtained with **7r** (MIC = 0.4 μM), bearing a $-CO_2Et$ moiety at the para position, while $-F$ (**7s**, MIC = 1.8 μM) substitution was somewhat detrimental. In general, the preferred monosubstitution position seemed to be para ≥ meta > ortho. A range of substituents were tolerated at the meta position where the electronic character of the substitution seemed to influence the activity. However, at the ortho position, small substituents seemed to be favored, whereas at the para position larger groups yielded the best activity.

Next, various di- and trisubstitution patterns were explored with halogens and the $-CF_3$ group (**8a**–**k**, Table 1). Fluorine was chosen as the preferred substituent because of its small size and previous success (good overall potency) in the monosubstituted series. Several difluorosubstitution patterns were investigated. With the exception of **8e** (R^2,R^4 = F, MIC = 4.4 μM), all the difluorinated compounds (**8a**, **8c**, and **8f**–**h**, MIC = 0.6–1.6 μM) exhibited very good activity against *Mtb*, in particular **8c** (R^2,R^3 = F) with an MIC of 0.6 μM. However, the corresponding dichloro substitution pattern (R^2,R^3 = Cl) led to decreased activity (**8d**, MIC = 2.7 μM). Compound **8b**, bearing both an R^1 = F and an R^2 = CF_3 group in the ring, also exhibited good activity, as did **8i** (R^1 = Cl, R^4 = F), with MICs of 0.6 and 0.9 μM, respectively. Trifluorosubstitution patterns, namely, R^1, R^2, and R^3 (**8j**, MIC = 0.9 μM) or R^1, R^4, and R^5 (**8k**, MIC = 1.0 μM), were also well tolerated. In addition, the benzyloxy side chain in question was shown to be as important as the intermediate 5-(3-hydroxyphenyl)-3-isoxazolecarboxylic acid ethyl ester (**13**, MIC = 7.3 μM), as well as the acetylmorpholine derivative **14** (MIC = 13.8 μM), exhibited reduced anti-TB activity.

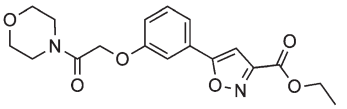
Table 1. The effect of the benzyl side chain substitution on the anti-TB activity



7a-7s, **8a-8k**



13



14

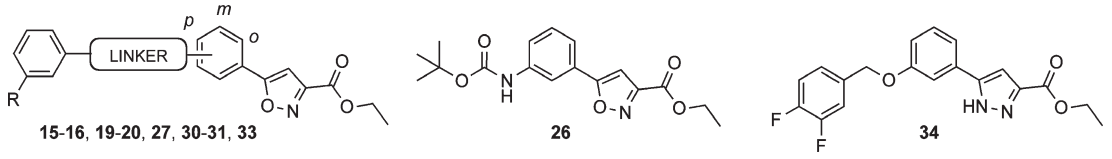
	R^1	R^2	R^3	R^4	R^5	MABA[a] MIC (μM)	LORA[a] MIC (μM)	Vero cells IC_{50} (μM)
7a	H	$-CF_3$	H	H	H	0.6	8.0	> 128
7b	H	H	H	H	H	1.2	41.5	> 128
7c	H	-F	H	H	H	0.8	18.9	> 128
7d	H	-Cl	H	H	H	1.0	10.8	> 128
7e	H	-Br	H	H	H	0.8	12.7	> 128
7f	H	$-OCF_3$	H	H	H	0.7	13.1	101
7g	H	$-C\equiv N$	H	H	H	0.6	26.5	> 128
7h	H	$-NO_2$	H	H	H	0.6	8.7	> 128
7i	H	$-NH_2$	H	H	H	1.5	25.8	> 128
7j	H	$-OCH_3$	H	H	H	2.1	26.7	> 128
7k	H	$-CH_3$	H	H	H	1.2	14.9	> 128
7l	H	$-CON(CH_2CH_2)_2O$	H	H	H	> 128	> 128	> 128
7m	$-CF_3$	H	H	H	H	1.5	34.8	> 128
7n	-Cl	H	H	H	H	0.9	10.1	> 128
7o	-F	H	H	H	H	1.1	19.1	nd[b]
7p	$-NO_2$	H	H	H	H	1.5	25.8	> 128
7q	H	H	$-CF_3$	H	H	0.4	11.8	> 128
7r	H	H	$-CO_2Et$	H	H	0.4	32.7	> 128
7s	H	H	-F	H	H	1.8	24.0	nd
8a	-F	-F	H	H	H	1.6	26.8	> 128
8b	-F	$-CF_3$	H	H	H	0.6	7.0	> 128
8c	H	-F	-F	H	H	0.6	19.2	> 128
8d	H	-Cl	-Cl	-H	H	2.7	10.6	nd
8e	H	-F	H	-F	H	4.4	16.6	> 128
8f	-F	H	H	-F	H	0.9	23.7	> 128
8g	-F	H	H	H	-F	1.2	12.8	> 128
8h	-F	H	-F	H	H	1.0	15.7	> 128
8i	-Cl	H	H	-F	H	0.9	15.1	nd
8j	-F	-F	-F	H	H	0.9	12.6	> 128
8k	-F	H	H	-F	-F	1.0	10.9	nd
13						7.3	113.9	nd
14						13.8	48.9	nd
RMP						0.1	1.9	127−144
INH						0.5	> 128	> 128
2						0.3	3.8	nd

[a] *Mtb* strain $H_{37}Rv$; [b] not determined.

Finally, we investigated the effect of the oxymethylene linker moiety on the anti-TB activity (Table 2). The introduction of an α-methyl group to the linker (compound **15**) led to complete loss of the activity. Elimination of the methylene moiety, to give the phenoxy derivative **16** (MIC = 1.0 μM), did not significantly alter the potency. Insertion of a nonsubstituted phenoxy moiety to the para position resulted in similar activity (compound **19**, MIC = 0.9 μM). A hydroxymethylene linker at the para position was also tolerated (compound **21**, MIC = 2.8 μM), which is interesting because the related compound **15** was found to be inactive. This suggests that hydrophilic, but not hydrophobic, branching at the linker may be acceptable. The original oxymethylene linker could also be successfully replaced with an aminomethylene linker, as in compounds **28** (MIC = 1.1 μM) and **30** (MIC = 0.6 μM). In fact, **30**, bearing the aminomethylene linker at the para position, was 2-fold more active than the corresponding meta derivative **28**, which in turn was 2-fold less active than the corresponding oxymethylene derivative **7a**. The planar amide linker at the meta position (**31**, MIC = 0.9 μM) yielded activity comparable to that of the corresponding aminomethylene linker derivative, **28**, and slightly decreased activity as compared to the lead **7a**. However, an amide linker at the ortho position (compound **33**), which significantly alters the 3D geometry of the molecule, was not tolerated and the activity was lost. Surprisingly, the Boc-protected intermediate **26**, with a carbamate linker and a bulky *tert*-butyl moiety, also exhibited good activity against *Mtb*. Although modifications of the isoxazole moiety in the past have been unsuccessful, we decided to synthesize the pyrazole derivative **34**. The pyrazole ring was chosen, since our earlier SAR studies on the isoxazole moiety had suggested that the position of the ring nitrogen at this moiety may play an important role in the anti-TB activity.[14] However, the pyrazole derivative **34** had significantly reduced activity with an MIC of 32.9 μM, thus further confirming the crucial role of the isoxazole moiety for the anti-TB activity.

The compounds were also tested for potency in LORA, a plausible model for NRP-TB. Notably, these compounds also exhibited micromolar activity in LORA (Tables 1 and 2). In general, the compounds that were the most active in MABA also showed good activity in LORA. In particular, **8b** and **30**

Table 2. The effect of the linker moiety and its position on the anti-TB activity



	Linker position	Linker	R	MABA MIC (μM)	LORA MIC (μM)	Vero cells IC_{50} (μM)
15	*meta*	-CH(CH_3)O-	H	> 128	> 128	nd
16	*meta*	-O-	-CF_3	1.0	10.5	> 128
19	*para*	-O-	H	0.9	13.8	> 128
21	*para*	-CH(OH)-	-OCH_3	2.8	23.9	nd
28	*meta*	-CH_2NH-	-CF_3	1.1	15.3	> 128
30	*para*	-CH_2NH-	-CF_3	0.6	6.3	> 128
31	*meta*	-CONH-	-CF_3	1.4	28.3	> 128
33	*ortho*	-CONH-	H	> 128	> 128	nd
26				1.1	12.6	> 128
34[c]				32.0	> 128	nd
RMP				0.1	1.9	127−144

[a] *Mtb* strain H_{37}Rv; [b] not determined; [c] A mixture of 1-*H* and 2-*H* tautomers.

retained their activity relatively well against the oxygen starved bacteria with a LORA MIC of 7.0 and 6.3 μM, respectively. In addition, several other compounds, namely, **7a**, **7d**−**f**, **7h**, **7n**, **7q**, **8d**, **8g**, **8j**−**k**, **16**, and **26**, had LORA MIC values of $\leq$13 μM. This can be considered as an encouraging finding, since RMP has a LORA MIC of ~2 μM and is thus 20-fold less active under low oxygen conditions. Among the current TB drugs, only RMP, SM, and PZA have been reported to show good activity against this phenotype and it has been suggested that the key to shortening the long TB treatment is to target NRP-TB.

To eliminate the possibility that the anti-TB activity arises from general toxicity, Vero cells were used for an in vitro cytotoxicity evaluation. These compounds are highly selective toward *Mtb*, and with the exception of **7f** (IC_{50} = 101 μM, selectivity index of 144), all the compounds had IC_{50} > 128 μM. In addition, these compounds are not broad-spectrum antibiotics as was shown by the fact that **7a** did not exhibit activity against *M. smegmatis*, *E. coli*, *S. aureus*, or fungus *C. albicans* (MIC > 100 μM). In addition, no activity was found against parasite *T. gondii*. Finally, selected compounds were evaluated against *Mtb* strains that are resistant to three first-line TB drugs (Table 3). All the tested compounds, namely, **7a**, **7e**, **7n**, **8a**, **8b**, **8f**, and **8g**, retain their activity against RMP, INH, and SM resistant strains, as do **4** and **5**,[14] suggesting a different mode of action and indicating that this compound class also holds promise as lead structures for drug-resistant TB.

The discovery of 5-phenyl-3-isoxazolecarboxylic acid esters as potent anti-TB compounds, although driven by rational design, originates from phenotypic screening, thus proving the power of the approach of bringing lead discovery to the level of the organism. Phenotypic screening is well justified for *Mtb*, since one of the major challenges in TB drug discovery is the compound penetration through the thick and waxy *Mycobacterium* cell wall. However, the major disadvantage of phenotypic screening is that often the molecular target of a compound remains unknown and lead optimization depends solely on ligand-based design and the chemists' intuition. Although SAR can be derived for this anti-TB compound class, it should be noted that the whole cell SAR not only includes the binding affinity to a plausible molecular target but also is affected by *Mtb* cell permeability and intracellular

Table 3. Activity against drug-resistant strains of *Mtb*

	MABA MIC (μM)		
	r-RMP[a]	r-INH[b]	r-SM[c]
7a	1.0	0.9	0.9
7e	1.0	1.0	1.0
7n	1.0	1.0	1.0
8a	1.0	1.0	1.0
8b	1.0	0.9	1.0
8f	1.0	1.0	1.2
8g	1.0	1.1	1.1
RMP	> 32	0.1	0.1
INH	0.4	> 128	0.5
SM	0.2	0.4	> 32

[a] RMP resistant strain. [b] INH resistant strain. [c] SM resistant strain.

metabolism. The SAR obtained from this series, including the particular structural requirements for the activity, i.e., the isoxazole moiety and the carboxy functionality at its C3 position, suggests that a specific, yet to be identified, molecular target may exist for these anti-TB compounds. Although not much can be said regarding the target at this point, the additional activity against NRP-TB indicates that the target is not likely to lie somewhere along the *Mtb* cell wall synthesis pathway. The fact that a variety of substituents and substitution patterns on the side chain benzene ring, as well as various linker moieties, are tolerated suggests that while the planar 5-phenyl-3-isoxazolecarboxylic moiety may reside in a specific binding cavity, the flexible benzylic side chain of the molecule may, at least partly, extend to the surface area. Alternatively, the side chain may occupy a relatively large and flexible hydrophobic cavity. The lack of activity of ortho amide **33** can be explained by the difference in the spatial orientation of side chain.

Conclusions

Benzyloxy, benzylamino, and phenoxy derivatives of 5-phenyl-3-isoxazolecarboxylic acid ethyl esters are highly potent anti-TB agents with several compounds exhibiting nanomolar activity against R-TB phenotype and thus comparable activity to the current first-line anti-TB drugs. In addition, although being somewhat weaker, these anti-TB compounds show activity against the NRP-TB phenotype in low oxygen conditions. Various mono-, di-, and trisubstitution patterns

on the benzyloxy moiety were explored, for which a range of substituents yield good anti-TB activity. The preferred monosubstitution position seems to be para ≥ meta > ortho, with a variety of substituents being tolerated at the meta position. At the ortho position, small substituents are preferred, while at the para position larger groups furnish the best activity. Also, di- and trisubstituted derivatives, mostly fluorinated compounds, yield good anti-TB activity. The nature and the position of the linker moiety affect the anti-TB activity. The original oxymethylene linker at the meta position can be replaced with an oxy, aminomethylene, or amide linker, of which the oxy linker yields comparable activity, with respect to the original system. Insertion of the aminomethylene linker to the para position is beneficial for the activity.

Tested compounds, namely, certain benzyloxy derivatives, retain their activity against *Mtb* strains that are resistant to first-line TB drugs INH and RMP or to second-line drug SM. The compound class shows high selectivity toward *Mtb* and, in general, does not exhibit cytotoxity toward Vero cells up to 128 μM. Overall, the high selectivity and potency against drug susceptible and drug-resistant R-TB, as well as the excellent activity against NRP-TB, establish these 5-phenyl-3-isoxazolecarboxylic acid ethyl esters derivatives as a promising anti-TB chemotype.

Experimental Section

Chemistry. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer at 400 and 100 MHz or 300 and 75 MHz, respectively, with TMS as an internal standard. ^{19}F NMR spectra were recorded on Bruker spectrometer at 376 MHz with TFA as an external standard. HRMS experiments were performed on Q-TOF-2TM (Micromass). TLC was performed with Merck 60 F_{254} silica gel plates and column chromatography by using CombiFlash Rf system with RediSep columns. Preparative HPLC was carried out on a Shimadzu SCL-10A VP instrument with an ACE 5-AQ (21.2 mm × 150 mm) column. The purity of the target compounds was determined to be ≥95% by analytical HPLC using Agilent 1100 HPLC system with a Synergi 4 μm Hydro-RP 80A column, with detection at 254 nm on a variable wavelength detector G1314A; flow rate = 1.4 mL/min; gradient elution over 20 min, from 30% MeOH−H_2O to 100% MeOH with 0.05% TFA or alternatively from 10% MeOH−H_2O to 100% MeOH with 0.05% TFA.

General Procedure A for the Synthesis of 7a, 7b, 7h, 7m, 7q, 7r, 12, 19, 21, 30, and 33. The appropriate acetylene intermediate (1 equiv) and Et_3N (3 equiv) were dissolved into anhydrous THF or ether (15 mL/mmol). Subsequently, ethyl 2-chloro-2-(hydroxyimino)acetate (3 equiv) in anhydrous THF or ether (2 mL/mmol) was added to the solution via syringe pump over 8 h, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was filtered and washed with THF, and the filtrate was evaporated in vacuo. The crude material was purified by flash chromatography using gradient elution from hexane to 30−40% EtOAc−hexane to give the product. The reactions were typically performed in 100−300 mg quantities.

General Procedure B for the Synthesis of 7c−g, 7j−l, 7n−p, 7s, 8a−k, 14, and 15. Anhydrous K_2CO_3 (6 equiv) and **13** (1 equiv) in acetone (12 mL/mmol, HPLC grade) were refluxed for 15 min. Subsequently, KI (0.5 equiv) and an appropriate benzyl halide or alkyl halide (1.05 equiv) were added and the reaction mixture was refluxed for 0.5−3 h until disappearance of the starting material on TLC (1:4 EtOAc−hexane as an eluent). The reaction mixture was cooled and filtered, and the filtrate was evaporated in vacuo. The crude product was purified by flash chromatography using gradient elution from hexane to 30−90% EtOAc−hexane. The reactions were typically performed in 50−100 mg quantities.

5-[3-[[(3-Trifluoromethyl)phenyl]methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7a). Procedure A (in ether) was used. Yield 64% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.18 (2H, s), 6.92 (1H, s), 7.09 (1H, m), 7.42 (3H, m), 7.53 (1H, m), 7.63 (2H, m), 7.74 (1H, br s). ^{13}C NMR ($CDCl_3$) δ 14.3, 62.4, 69.5, 100.4, 112.2, 117.5, 119.2, 124.2 (q, J = 272 Hz), 124.3 (q, J = 4 Hz), 125.1 (q, J = 4 Hz), 128.1, 129.3, 130.6, 130.8, 131.2 (q, J = 32 Hz), 137.6, 157.2, 159.0, 160.1, 171.5. HRMS (ESI) calculated for $C_{20}H_{16}F_3NO_4$ $[M + H]^+$ 392.1104, found 392.1111.

5-[3-[(Phenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7b). Procedure A (in ether) was used. Purification was by preparative HPLC. Yield 68% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.14 (2H, s), 6.91 (1H, s), 7.08 (1H, m), 7.33−7.47 (8H, m). HRMS (ESI) calculated for $C_{19}H_{17}NO_4$ $[M + H]^+$ 324.1230, found 324.1247.

5-[3-[(3-Fluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7c). Procedure B was used. Yield 97% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.13 (2H, s), 6.91 (1H, s), 7.01−7.09 (2H, m), 7.17−7.23 (2H, m), 7.34−7.42 (4H, m). HRMS (ESI) calculated for $C_{19}H_{16}FNO_4$ $[M + H]^+$ 342.1136, found 342.1151.

5-[3-[(3-Chlorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7d). Procedure B was used. Yield 93% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.44 (3H, t, J = 7.1 Hz), 4.47 (2H, q, J = 7.1 Hz), 5.10 (2H, s), 6.91 (1H, s), 7.07 (1H, m), 7.32 (3H, m), 7.38−7.41 (3H, m), 7.46 (1H, br s). HRMS (ESI) calculated for $C_{19}H_{16}ClNO_4$ $[M + H]^+$ 358.0841, found 358.0853.

5-[3-[(3-Bromophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7e). Procedure B was used. Yield 99% (white powder). ^{1}H NMR ($CDCl_3$) 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.10 (2H, s), 6.92 (1H, s), 7.07 (1H, m), 7.28 (1H, m), 7.37−7.42 (4H, m), 7.48 (1H, m), 7.46 (1H, br s). HRMS (ESI) calculated for $C_{19}H_{16}BrNO_4$ $[M + H]^+$ 402.0336 found 402.0355.

5-[3-[[(3-Trifluoromethoxy)phenyl]methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7f). Procedure B was used. Yield 71% (colorless oil). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.15 (2H, s), 6.92 (1H, s), 7.08 (1H, m), 7.24 (1H, m), 7.33 (1H, br s), 7.38−7.44 (5H, m). HRMS (ESI) calculated for $C_{20}H_{16}F_3NO_5$ $[M + H]^+$ 408.1053, found 408.1036.

5-[3-[(3-Cyanophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7g). Procedure B was used. Yield 95% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.17 (2H, s), 6.93 (1H, s), 7.08 (1H, m), 7.43 (3H, m), 7.53 (1H, m), 7.67 (2H, m), 7.78 (1H, br s). HRMS (ESI) calculated for $C_{20}H_{16}N_2O_4$ $[M + H]^+$ 349.1183, found 349.1200.

5-[3-[(3-Nitrophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7h). Procedure A (in ether) was used. Yield 77% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.49 (2H, q, J = 7.1), 5.24 (2H, s), 6.94 (1H, s), 7.11 (1H, m), 7.45 (3H, m), 7.61 (1H, apparent t, J = 7.9 Hz), 7.81 (1H, d, J = 7.5), 7.23 (1H, d, J = 7.9), 8.37 (1H, s). HRMS (ESI) calculated for $C_{19}H_{16}N_2O_6$ $[M + H]^+$ 369.1081, found 369.1097.

5-[3-[(3-Methoxyphenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7j). Procedure B was used. Yield 96% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.46 (3H, t, J = 7.1 Hz), 3.84 (3H, s), 4.49 (2H, q, J = 7.1 Hz), 5.12 (2H, s), 6.90 (2H, m), 7.02−7.11 (3H, m), 7.33 (1H, apparent t, J = 7.8 Hz), 7.42 (3H, m). HRMS (ESI) calculated for $C_{20}H_{19}NO_5$ $[M + H]^+$ 354.1336, found 354.1354.

5-[3-[(3-Methylphenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7k). Procedure B was used. Yield 97% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.44 (3H, t, J = 7.1 Hz), 2.38 (3H, s), 4.48 (2H, q, J = 7.1 Hz), 5.09 (2H, s), 6.90 (1H, s), 7.08 (1H, m), 7.16 (1H, m), 7.24−7.31 (3H, m), 7.39−7.43 (3H, m). HRMS

(ESI) calculated for $C_{20}H_{19}NO_4$ $[M + H]^+$ 338.1387 found 388.1399.

5-[3-[[3-(4-Morpholinylcarbonyl)phenyl]methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7l). Procedure B was used. Yield 92% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 3.35−3.90 (8H, m), 4.48 (2H, q, J = 7.1 Hz), 5.17 (2H, s), 6.92 (1H, s), 7.08 (1H, m), 7.37−7.53 (7H, m). HRMS (ESI) calculated for $C_{24}H_{24}N_2O_6$ $[M + H]^+$ 437.1707, found 437.1728.

5-[3-[[(2-Trifluoromethyl)phenyl]methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7m). Procedure A (in ether) was used. Purification was by preparative HPLC. Yield 43% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.33 (2H, s), 6.91 (1H, s), 7.07 (1H, m), 7.43 (4H, m), 7.59 (1H, m), 7.74 (2H, m). HRMS (ESI) calculated for $C_{20}H_{16}F_3NO_4$ $[M + H]^+$ 392.1104, found 392.1114.

5-[3-[(2-Chlorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7n). Procedure B was used. Yield 88% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.44 (3H, t, J = 7.1 Hz), 4.47 (2H, q, J = 7.1 Hz), 5.22 (2H, s), 6.92 (1H, s), 7.09 (1H, m), 7.29 (2H, m), 7.42 (4H, m), 7.57 (1H, m). HRMS (ESI) calculated for $C_{19}H_{16}ClNO_4$ $[M + H]^+$ 358.0841, found 358.0857.

5-[3-[(2-Fluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7o). Procedure B was used. Yield 93% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.20 (2H, s), 6.92 (1H, s), 7.08−7.21 (3H, m), 7.32−7.44 (4H, m), 7.53 (1H, m). HRMS (ESI) calculated for $C_{19}H_{16}FNO_4$ $[M + H]^+$ 342.1136, found 342.1149.

5-[3-[(2-Nitrorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7p). Procedure B was used. Yield 63% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.56 (2H, s), 6.94 (1H, s), 7.10 (1H, m), 7.42 (3H, m), 7.53 (1H, apparent t, J = 7.7 Hz), 7.72 (1H, apparent t, J = 7.6 Hz), 7.91 (1H, d, J = 7.9 Hz), 8.20 (1H, d, J = 7.9 Hz). HRMS (ESI) calculated for $C_{19}H_{16}N_2O_6$ $[M + H]^+$ 369.1081, found 369.1101.

5-[3-[[(4-Trifluoromethyl)phenyl]methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7q). Procedure A (in ether) was used. Purification was by preparative HPLC. Yield 57% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.46 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.20 (2H, s), 6.92 (1H, s), 7.07 (1H, m), 7.42 (3H, m), 7.58 (2H, d, J = 8.2 Hz), 7.67 (2H, d, J = 8.2 Hz). HRMS (ESI) calculated for $C_{20}H_{16}F_3NO_4$ $[M + H]^+$ 392.1104, found 392.1101.

5-[3-[(4-Ethoxycarbonylphenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7r). Procedure A (in ether) was used. Yield 77% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, J = 7.1 Hz), 1.45 (3H, t, J = 7.1 Hz), 4.39 (2H, q, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.20 (2H, s), 6.91 (1H, s), 7.07 (1H, m), 7.41 (3H, m), 7.53 (2H, d, J = 8.2 Hz), 8.08 (2H, d, J = 8.3 Hz). HRMS (ESI) calculated for $C_{22}H_{21}NO_6$ $[M + H]^+$ 396.1442, found 396.1460.

5-[3-[(4-Fluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7s). Procedure B was used. Yield 99% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.10 (2H, s), 6.91 (1H, s), 7.08 (3H, m), 7.43 (5H, m). HRMS (ESI) calculated for $C_{19}H_{16}FNO_4$ $[M + H]^+$ 342.1136, found 342.1138.

5-[3-[(2,3-Difluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8a). Procedure B was used. Yield 99% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.22 (2H, s), 6.93 (1H, s), 7.08−7.20 (3H, m), 7.29 (1H, m), 7.42 (3H, m). HRMS (ESI) calculated for $C_{19}H_{15}F_2NO_4$ $[M + H]^+$ 360.1042, found 360.1056.

5-[3-[[2-Fluoro-(3-trifluoromethyl)phenyl]methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8b). Procedure B was used. Yield 91% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.24 (2H, s), 6.93 (1H, m), 7.10 (1H, m), 7.30 (1H, m), 7.44 (3H, m), 7.62 (1H, m), 7.77 (1H, m). HRMS (ESI) calculated for $C_{20}H_{15}F_4NO_4$ $[M + H]^+$ 410.1010, found 410.1028.

5-[3-[(3,4-Difluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8c). Procedure B was used. Yield 99% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.08 (2H, s), 6.92 (1H, s), 7.07 (1H, m), 7.17−7.32 (3H, m), 7.42 (3H, m). HRMS (ESI) calculated for $C_{19}H_{15}F_2NO_4$ $[M + H]^+$ 360.1042, found 360.1061.

5-[3-[(3,4-Dichlorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8d). Procedure B was used. Yield 99% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.09 (2H, s), 6.92 (1H, s), 7.06 (1H, m), 7.29 (1H, dd, J = 1.7 Hz, J = 8.3 Hz), 7.42 (3H, m), 7.48 (1H, d, J = 8.2 Hz), 7.57 (1H, d, J =1.6 Hz). HRMS (ESI) calculated for $C_{19}H_{15}Cl_2NO_4$ $[M + H]^+$ 392.0451, found 392.0462.

5-[3-[(3,5-Difluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8e). Procedure B was used. Yield 89% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.12 (2H, s), 6.78 (1H, m), 6.92 (1H, s), 6.99 (2H, m), 7.05 (1H, m), 7.41−7.43 (3H, m). HRMS (ESI) calculated for $C_{19}H_{15}F_2NO_4$ $[M + H]^+$ 360.1042, found 360.1060.

5-[3-[(2,5-Difluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8f). Procedure B was used. Yield 91% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.18 (2H, s), 6.93 (1H, s), 6.98−7.11 (3H, m), 7.25 (1H, m), 7.42 (3H, m). HRMS (ESI) calculated for $C_{19}H_{15}F_2NO_4$ $[M + H]^+$ 360.1042, found 360.1061.

5-[3-[(2,6-Difluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8g). Procedure B was used. Yield 90% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.44 (3H, t, J = 7.1 Hz), 4.47 (2H, q, J = 7.1 Hz), 5.20 (2H, s), 6.95 (3H, m), 7.11 (1H, m), 7.32−7.45 (4H, m). HRMS (ESI) calculated for $C_{19}H_{15}F_2NO_4$ $[M + H]^+$ 360.1042, found 360.1053.

5-[3-[(2,4-Difluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8h). Procedure B was used. Yield 77% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.46 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.14 (2H, s), 6.84−6.94 (3H, m), 7.08 (1H, m), 7.41 (3H, m), 7.49 (1H, m). HRMS (ESI) calculated for $C_{19}H_{15}F_2NO_4$ $[M + H]^+$ 360.1042, found 360.1055.

5-[3-[(2-Chloro-5-fluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8i). Procedure B was used. Yield 95% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.20 (2H, s), 6.93 (1H, s), 7.00 (1H, dt, J = 2.9, Hz, J = 8.2 Hz), 7.09 (1H, m), 7.34 (1H, dd, J = 2.9 Hz, J = 9.1 Hz), 7.38 (1H, m), 7.44 (3H, m). HRMS (ESI) calculated for $C_{19}H_{15}ClFNO_4$ $[M + H]^+$ 376.0746, found 376.0758.

5-[3-[(2,3,4-Trifluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8j). Procedure B was used. Yield 88% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.49 (2H, q, J = 7.1 Hz), 5.16 (2H, s), 6.93 (1H, s), 7.01−7.09 (2H, m), 7.24 (1H, m), 7.43 (3H, m). HRMS (ESI) calculated for $C_{19}H_{14}F_3NO_4$ $[M + H]^+$ 378.0948, found 378.0957.

5-[3-[(2,3,6-Trifluorophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (8k). Procedure B was used. Yield 75% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 5.21 (2H, s), 6.91 (2H, m), 7.11 (1H, m), 7.19 (1H, m), 7.43 (3H, m). HRMS (ESI) calculated for $C_{19}H_{14}F_3NO_4$ $[M + H]^+$ 378.0948, found 378.0960.

5-[3-[(3-Aminophenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7i). $SnCl_2 \cdot 2H_2O$ (1.2 g, 6.2 mmol) was added portionwise to compound **7h** (1.23 g, 0.62 mmol) in 50% EtOH−EtOAc (50 mL), and the reaction mixture was stirred overnight at room temperature. Approximately $^3/_4$ of the solvent was evaporated under reduced pressure, and 1 M NaOH (15 mL) was added to the residue. The mixture was extracted with EtOAc (3 × 25 mL), and combined organic layers were washed with brine (20 mL) and dried with Na_2SO_4. After filtration, the solvent was evaporated and the residue was purified by flash chromatography using gradient elution from 10% EtOAc−hexane to 50% EtOAc−hexane to give **7i** as a pale-yellow powder in 61% yield. ^{1}H NMR ($CDCl_3$) δ 1.45 (3H,

t, J = 7.1 Hz), 3.71 (2H, br s), 4.48 (2H, q, J = 7.1 Hz), 5.05 (2H, s), 6.66 (1H, m), 6.78 (1H, m), 6.82 (1H, d, J = 7.6 Hz), 6.90 (1H, s), 7.07 (1H, m), 7.18 (1H, t, J = 7.7 Hz), 7.39 (2H, m), 7.42 (1H, m). HRMS (ESI) calculated for $C_{19}H_{18}N_2O_4$ $[M + H]^+$ 339.1339, found 339.1351.

3-[(*tert*-Butyldiphenyl)silanyloxy]phenylacetylene (11). To 3-hydroxyphenylacetylene (0.85 g, 7.2 mmol) in anhydrous CH_2Cl_2 (20 mL), imidazole (0.65 g, 9.4 mmol) was added followed by *tert*-butyldiphenylchlorosilane (2.4 mL, 9.4 mmol), and the reaction mixture was stirred for 1 h at room temperature. Upon completion, the reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was purified by flash chromatography using gradient elution from hexane to 10% EtOAc−hexane to give **11** as a pale-yellow oil in 90% yield. ^{1}H NMR ($CDCl_3$) δ 1.09 (9H, s), 2.97 (1H, s), 6.67 (1H, m), 6.99 (3H, m), 7.35−7.43 (6H, m), 7.70 (4H, m). ^{13}C NMR ($CDCl_3$) δ 19.5, 26.5, 76.8, 83.4, 120.6, 122.9, 123.4, 125.1, 127.8, 129.1, 130.0, 132.5, 135.5, 155.3.

5-[[(*tert*-Butyldiphenyl)silanyloxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (12). 12 was synthesized as described above in general method A (in THF) and purified by flash chromatography from hexane to 30% EtOAc−hexane. Yield 83% (colorless oil). ^{1}H NMR ($CDCl_3$) δ 1.13 (9H, s), 1.43 (3H, t, J = 7.1 Hz), 4.45 (2H, q, J = 7.1 Hz), 6.8 (1H, s), 6.80 (1H, m), 7.17 (2H, m), 7.30 (1H, m), 7.36−7.45 (6H, m), 7.70 (4H, m). ^{13}C NMR ($CDCl_3$) δ 14.2, 19.5, 26.5, 62.2, 100.0, 117.2, 118.7, 122.2, 127.6, 127.9, 130.0, 130.2, 132.4, 135.5, 156.1, 156.8, 160.0, 171.5.

5-(3-Hydroxyphenyl)-3-isoxazolecarboxylic Acid Ethyl Ester (13). To compound **12** (1.8 g, 3.9 mmol) in anhydrous THF (20 mL), 1 M TBAF in THF (5.8 mL, 5.8 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 3 h. The solvent was evaporated in vacuo, saturated NH_4Cl (aq, 50 mL) was added to the residue, and the mixture was stirred for 30 min followed by extraction with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried with Na_2SO_4, and evaporated. The residue was purified by flash chromatography using gradient elution from 25% EtOAc−hexane to 100% EtOAc to give **13** as a white powder in 67% yield. ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, t, J = 7.1 Hz), 4.39 (2H, q, J = 7.1 Hz), 6.94 (1H, s), 7.29−7.41 (4H, m), 9.90 (1H, s). ^{13}C NMR (DMSO-d_6) δ 14.0, 61.9, 100.7, 112.2, 116.7, 118.1, 127.1, 130.6, 156.8, 157.9, 159.4, 171.2. HRMS (ESI) calculated for $C_{12}H_{11}NO_4$ $[M + H]^+$ 234.0761, found 234.0771.

5-[3-[(1-Methyl-1-phenyl)methoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (15). 15 was synthesized as described in the general method B by using (1-bromoethyl)benzene as a benzyl halide. Yield 71% (white solid). ^{1}H NMR ($CDCl_3$) δ 1.44 (3H, t, J = 7.1 Hz), 1.67 (3H, d, J = 6.4 Hz), 4.46 (2H, q, J = 7.1 Hz), 5.38 (1H, q, J = 6.4 Hz), 6.83 (1H, s), 6.95 (1H, m), 7.24−7.40 (8H, m). HRMS (ESI) calculated for $C_{20}H_{19}NO_4$ $[M + H]^+$ 338.1387, found 338.1394.

5-[2-(4-Morpholinyl)-2-oxoethoxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (15). 15 was synthesized as described in the general method B by using 4-(chloroacetyl)morpholine as an alkyl halide. Yield 88% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 3.61−3.69 (8H, m), 4.48 (2H, q, J = 7.1 Hz), 4.78 (2H, s), 6.93 (1H, s), 7.09 (1H, m), 7.38−7.44 (3H, m). HRMS (ESI) calculated for $C_{18}H_{20}N_2O_6$ $[M + H]^+$ 361.1394, found 361.1404.

5-[(3-(3-Trifluoromethyl)phenoxy)phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (16). The phenol **13** (55 mg, 0.24 mmol), 3-(trifluoromethyl)phenylboronic acid (90 mg, 0.47 mmol), Et_3N (0.3 mL, 2.1 mmol), and powdered 4 Å molecular sieves (500 mg) were mixed in anhydrous CH_2Cl_2 (10 mL). $Cu(OAc)_2$ (64 mg, 0.35 mmol) was added, and the reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with CH_2Cl_2 (10 mL) and filtered, and the filtrate was evaporated in vacuo. The crude product was purified by flash chromatography using gradient elution from hexane to 50% EtOAc−hexane to give the product as a white solid in 67% yield. ^{1}H NMR δ 1.46 (3H, t, J = 7.1 Hz), 4.49 (2H, q, J = 7.1 Hz), 6.94 (1H, s), 7.15 (1H, m), 7.22−7.31 (2H, m), 7.42−7.54 (4H, m), 7.63 (1H, m). ^{13}C NMR ($CDCl_3$) δ 14.4, 62.5, 100.8, 116.0 (q, J = 4 Hz), 116.7, 120.7, (q, J = 4 Hz), 121.6, 121.7, 122.2, 123.8 (q, J = 273 Hz), 128.7, 130.8, 131.2, 132.8 (q, J = 33 Hz), 157.25, 157.26, 157.30, 160.1, 170.9. HRMS (ESI) calculated for $C_{19}H_{14}F_3NO_4$ $[M + H]^+$ 378.0948, found 378.0950.

5-[(4-Phenoxy)phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (19). 19 was synthesized as described in general method A (in ether) by using 1-ethynyl-4-phenoxybenzene (**17**) as a starting material and purified by preparative HPLC. Yield 63% (white powder). ^{1}H NMR δ 1.45 (3H, t, J = 7.1 Hz), 4.48 (2H, q, J = 7.1 Hz), 6.85 (1H, s), 7.08 (4H, d, J = 8.1), 7.20 (1H, m), 7.40 (2H, m), 7.77 (2H, d, J = 8.5). ^{13}C NMR ($CDCl_3$) δ 14.4, 62.4, 99.3, 118.7, 120.1, 121.5, 124.6, 127.9, 130.2, 156.0, 157.2, 160.1, 160.3, 171.5. HRMS (ESI) calculated for $C_{18}H_{15}NO_4$ $[M + H]^+$ 310.1074, found 310.1088.

5-[4-[(3-Methoxyphenyl)hydroxymethyl]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (21). 21 was synthesized as described in general method A (in ether) by using intermediate **20** as a starting material. Yield 50% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.44 (3H, t, J = 7.1 Hz), 2.35 (1H, br s), 3.79 (3H, s), 4.47 (2H, m, J = 7.1 Hz), 5.86 (1H, s), 6.83 (1H, m), 6.90 (1H, s), 6.95 (2H, m), 7.27 (1H, m), 7.52 (2H, d, J = 8.2 Hz), 7.77 (2H, d, J = 8.2 Hz). ^{13}C NMR ($CDCl_3$) δ 14.2, 55.3, 62.4, 75.8, 99.9, 112.2, 113.3, 118.9, 125.8, 126.1, 127.1, 129.8, 144.9, 146.4, 157.0, 159.9, 160.0, 171.5. HRMS (ESI) calculated for $C_{20}H_{19}NO_5$ $[M + H]^+$ 354.1336, found 354.1338.

5-[3-[[[(3-Trifluoromethyl)phenyl]methyl]amino]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (28). To a solution of 3-(trifluomethyl)benzaldehyde (75 mg, 0.43 mmol) in 1,2-dichloroethane (8 mL) were added aniline **27** (100 mg, 0.43 mmol), $NaB(OAc)_3H$ (0.11 g, 0.52 mmol), and AcOH (0.03 mg, 0.027 mL, 0.47 mmol). The mixture was stirred at room temperature for 3 h. The reaction mixture was poured into water (30 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with saturated $NaHCO_3$ (20 mL) and brine (20 mL) and dried with $MgSO_4$. After filtration, the solvent was evaporated and the crude product was purified first by flash chromatography (elution from hexane to 40% EtOAc−hexane) followed by purification by preparative HPLC. Yield 65% (beige powder). ^{1}H NMR ($CDCl_3$) δ 1.44 (3H, t, J = 7.1 Hz), 4.47 (2H, m, J = 7.1 Hz), 6.71 (2H, d, J = 8.8 Hz), 6.85 (1H, s), 7.08 (1H, br s), 7.16 (1H, m), 7.28 (2H, m), 7.49 (1H, m), 7.58 (2H, m), 7.66 (1H, br s). ^{13}C NMR ($CDCl_3$) δ 14.4, 48.0, 62.4, 100.1, 110.0, 115.4, 115.9, 124.25 (q, J = 273 Hz), 124.33 (q, J = 4 Hz), 124.6 (q, J = 4 Hz), 127.8, 129.5, 130.4, 130.9, 131.4 (q, J = 33 Hz), 140.1, 148.4, 157.1, 160.3, 172.3. HRMS (ESI) calculated for $C_{20}H_{17}F_3N_2O_3$ $[M + H]^+$ 391.1264, found 391.1274.

5-[4-[[[(3-Trifluoromethyl)phenyl]methyl]amino]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (30). 30 was synthesized as described in general method A (in THF) by using intermediate **29** as a starting material. Yield 54% (pale-yellow powder). ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, t, J = 7.1 Hz), 4.36 (2H, q, J = 7.1 Hz), 4.46 (2H, d, J = 6.0 Hz), 6.69 (2H, d, J = 8.8 Hz), 7.07 (2H, m), 7.57−7.71 (6H, m). ^{13}C NMR (DMSO-d_6) δ 14.0, 45.3, 61.8, 97.1, 112.3, 113.8, 123.6 (two over lapping quartets), 124.3 (q, J = 273 Hz), 127.3, 129.2 (q, J = 31 Hz), 129.5, 131.3, 141.3, 150.6, 156.6, 159.7, 172.2. HRMS (ESI) calculated for $C_{20}H_{17}F_3N_2O_3$ $[M + H]^+$ 391.1264, found 391.1273.

5-[3-[[3-(Trifluoromethyl)benzoyl]amino]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (31). A mixture of the aniline **27** (80 mg, 0.34 mmol), DMAP (34 mg, 0.28 mmol), and EDCI (80 mg, 0.52 mmol) in CH_2Cl_2 (20 mL) was cooled to 0 °C. (3-Trifluoromethyl)benzoic acid (65 mg, 0.34 mmol) was added, and the reaction mixture was stirred at 0 °C for 4 h followed by overnight at room temperature. Upon completion, CH_2Cl_2

(40 mL) was added and the mixture was washed with 5% HCl (2 × 20 mL), 1 M NaOH (2 × 20 mL), and brine (20 mL). The organic layer was dried with Na_2SO_4, filtered, and evaporated. The crude product was purified by flash chromatography using gradient elution from hexane to 70% EtOAc−hexane. Yield 79% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.43 (3H, t, J = 7.1 Hz), 4.46 (2H, q, J = 7.1 Hz), 6.98 (1H, s), 7.51 (1H, m), 7.64 (2H, m), 7.82 (2H, m), 8.14 (4H, m). ^{13}C NMR ($CDCl_3$) δ 14.3, 62.5, 100.6, 118.0, 122.4, 123.1, 123.8 (q, J = 273 Hz), 124.4 (q, J = 4 Hz), 127.5, 128.8 (q, J = 4 Hz), 129.6, 130.2, 130.8, 131.5 (q, J = 33 Hz), 135.5, 138.7, 157.2, 160.1, 165.0, 171.4. HRMS (ESI) calculated for $C_{20}H_{15}F_3N_2O_4$ $[M + H]^+$ 405.1057, found 405.1073.

5-[2-(Benzoylamino)phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (33). 33 was synthesized as described in general method A (in THF) by using intermediate **32** as a starting material except that the product was further purified by three consecutive recrystallizations from EtOAc. Yield 41% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7.1 Hz), 4.49 (2H, q, J = 7.1 Hz), 6.96 (1H, s), 7.27 (1H, m), 7.51−7.60 (4H, m), 7.66 (1H, dd, J = 1.4 Hz, J = 7.8), 7.96 (2H, m), 8.58 (1H, d, J = 8.3), 9.28 (1H, br s). ^{13}C NMR ($CDCl_3$) δ 14.4, 62.8, 102.9, 117.0, 123.6, 125.0, 127.3, 129.2, 129.3, 132.3, 132.5, 134.5, 136.1, 157.2, 159.8, 165.8, 171.8. HRMS (ESI) calculated for $C_{19}H_{16}N_2O_4$ $[M + H]^+$ 337.1182, found 337.1197.

5-[3-[(3,4-Difluorophenyl)methoxy]phenyl]-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (34). Ethyl diazoacetate (0.53 g, 3.9 mmol, 0.48 mL) was added to acetylene intermediate **10f** (0.80 g, 3.3 mmol) in benzene (3 mL). The reaction mixture was heated to 140 °C for 1.5 h in a sealed vessel under microwave irradiation (Biotage Initiator). After the mixture was cooled, the solvent was evaporated and the residue was purified by preparative HPLC to give the title compound as a light-yellow powder in 80% yield. According to ^{1}H NMR, the product is a mixture of 1-*H* and 2-*H* tautomers in DMSO-d_6. ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, m), 4.31 (2H, m), 7.00 (1H, m), 7.25−7.59 (7H, m), 13.92 (N−H), 14.05 (N−H). HRMS (ESI) calculated for $C_{19}H_{16}F_2N_2O_3$ $[M + H]^+$ 359.1202, found 359.1194.

Biology. The MICs were determined using *Mtb* H_{37}Rv ATCC 27294 in MABA[19] and LORA[20] assays according to published procedures. The reported MICs are average values from two to three individual experiments. Similarly, cytotoxicities were determined on Vero cells according to a published procedure.[19b] For a brief description of the biological assays see the Supporting Information.

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Supporting Information Available: Synthesis of acetylene intermediates **10a−f**, **20**, **29**, and **32**, synthesis of 3-[-(chloromethyl)phenyl]-4-morpholinylmethanone, and a brief description of the biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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