Journal of Medicinal Chemistry Article

J. Med. Chem. **2009,** *52,* 6287–6296 **6287**
DOI: 10.1021/jm900513a

Synthesis, Biological Evaluation, and Structure−Activity Relationships for 5-[(*E*)-2-Arylethenyl]-3-isoxazolecarboxylic Acid Alkyl Ester Derivatives as Valuable Antitubercular Chemotypes

Marco Pieroni,[†] Annamaria Lilienkampf,[†] Baojie Wan,[‡] Yuehong Wang,[‡] Scott G. Franzblau,[‡] and Alan P. Kozikowski*[,†]

[†]*Drug Discovery Program, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, and* [‡]*Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612*

Received April 22, 2009

Tuberculosis (TB), mostly caused by *Mycobacterium tuberculosis* (*Mtb*), is one of the leading causes of death from infectious disease worldwide. Its coinfection with HIV and the emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains have further worsened the TB pandemic. Despite its global impact, TB is considered a neglected disease and no new anti-TB therapeutics have been introduced over the last four decades. The nonreplicating persistent form of TB (NRP-TB) is responsible for the length of the treatment and is the putative cause of treatment failure. Therefore, new anti-TB agents, which are active against both the replicating form of *Mtb* (R-TB) and NRP-TB, are urgently needed. Herein, we report the synthesis and structure−activity relationships (SAR) of a series of 5-[(*E*)-2-arylethenyl]-3-isoxazolecarboxylic acid alkyl esters as potent anti-TB agents. Several compounds had submicromolar minimum inhibitory concentrations (MIC) against R-TB and were active against NRP-TB in the low micromolar range, thus representing attractive lead compounds for the possible development of new anti-TB agents.

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

Introduction

Among the infectious diseases, tuberculosis (TB[a]), a lung infection caused mainly by *Mycobacterium tuberculosis* (*Mtb*), is considered one of the most threatening for public health. This concern is justified by recent reports from the World Health Organization (WHO), according to which there were 9.2 million new cases of TB and 1.7 million deaths from TB in 2006. Moreover, one-third of the world's population is estimated to be latently infected by *Mtb*.[1] The new cases, as well as the deaths, occur mostly in developing countries and the number of HIV-positive patients coinfected with *Mtb* is constantly rising.[2] In spite of the research efforts being made, no new drugs have actually been brought to the market for decades.[3−5] In fact, among the first-line anti-TB drugs, namely isoniazide (INH), pyrazinamide (PZA), ethambutol (EMB), and rifampin (RMP), the latter represents the most recent one, although discovered in 1966.[6] The current recommended therapeutic strategy Directly Observed Therapy, Short-course (DOTS)[7] is based on the coadministration of the four above-mentioned drugs for the first two months, followed by a prolonged treatment with INH and RMP for an additional 4−7 months.[7,8] The cause of this long therapy is the peculiar ability of *Mtb* to survive in a nonreplicating persistent form (NRP-TB) while withstanding chemotherapy.[9] Although the DOTS strategy has significantly improved the treatment outcome, the duration of the 6−12 month treatment, the high cost, and the undesired side effects often lead to a poor patient compliance, which, in turn, contributes to the emergence of multidrug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB).[10,11] MDR-TB is usually resistant to at least two of the first-line drugs, principally INH and RMP, whereas XDR-TB additionally fails to respond to any fluoroquinolone and at least one of three injectable second line drugs (amikacin, capreomycin, or kanamycin),[11] thereby being extremely difficult to cure. The treatment of resistant strains requires a prolongation of the therapy, employs more toxic drugs, and increases the financial burden, thus making TB a vicious cycle.

The foregoing facts highlight the crucial need for novel anti-TB agents, which are synthetically feasible, lack side effects, and have physicochemical properties allowing oral administration. Moreover, to shorten the duration of the treatment, they should target NRP-TB and have a novel mechanism of action.[12−15] As part of our efforts in developing new chemotherapeutics for TB,[16,17] we report herein the synthesis and an in-depth SAR study of new 5-[(*E*)-2-arylethenyl]-3-isoxazolecarboxylic acid alkyl ester derivatives (Chart 1) with excellent activity against both replicating *Mtb* (R-TB) and NRP-TB and devoid of apparent toxicity to Vero cells. We have previously reported the design and study of anti-TB mefloquine−isoxazole hybrid compounds, like compound **1**,[16b] followed by their optimization (compounds **2** and **3**)[18] in order to improve their potency and metabolic profile (Figure 1). The good activity shown by **2** and **3** encouraged us to search for a simplified structure that could be efficiently

*To whom correspondence should be addressed. Phone: +1-312-996-7577. Fax: +1-312-996-7107. E-mail: kozikowa@uic.edu.

[a] Abbreviations: EMB, ethambutol ; INH, isoniazid; LORA, low oxygen recovery assay; MABA, microplate Alamar Blue assay; MIC, minimum inhibitory concentration; MDR-TB, multidrug-resistant tuberculosis; MOX, moxifloxacin; *Mtb*, *Mycobacterium tuberculosis*; NRP-TB, nonreplicating persistent tuberculosis; PZA, pyrazinamide, R-TB, replicating tuberculosis; RMP, rifampin; TB, tuberculosis; XDR-TB, extensively drug-resistant tuberculosis.

© 2009 American Chemical Society

Published on Web 09/16/2009

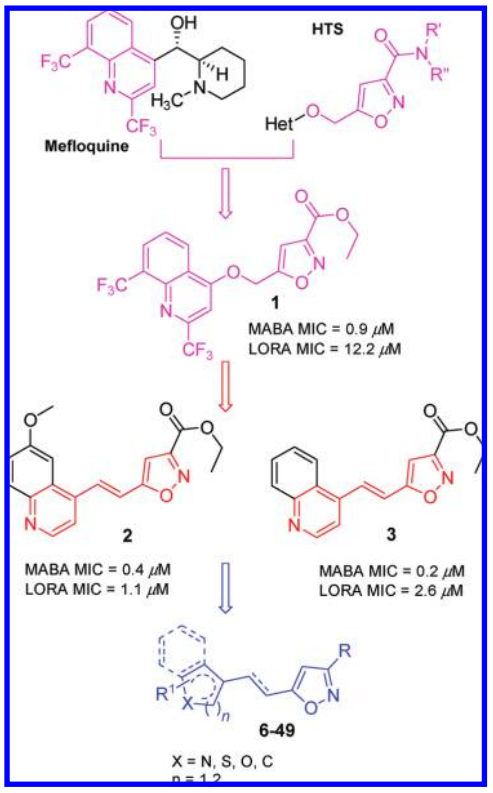


Figure 1. Examples of mefloquine−isoxazole hybrids and their simplified derivatives. For R and R^1, see Chart 1.

modified in order to investigate the SAR and to thereby elucidate the anti-TB potential of these isoxazole-based compounds. Hence, keeping intact the 5-ethenyl-3-isoxazolecarboxylic acid ester core, which is suggested to act as the pharmacophore,[16−18] we first substituted the quinoline ring with a naphthalene group (compound **6**) in order to assess whether the quinoline ring nitrogen was essential for the activity. Next, we investigated whether an appropriately substituted single aromatic ring, such as benzene or pyridine, could lead to good anti-TB activity. In our earlier studies,[16−18] halogenated and trifluoromethyl substituted compounds showed the best activity and thus we chose to further investigate the effect of these substituents in the present compound class. Substituents such as amino, hydroxyl, and methoxy were chosen to lower the ClogP in order to improve the drug-likeness of these compounds. More hindered groups were chosen as substituents to probe the presence and the size of any lipophilic pockets in the binding site. The majority of the new molecules synthesized herein were found to show excellent activity against *Mtb* while not exhibiting toxicity to Vero cells. For several compounds, the MICs were found to be in the submicromolar range against R-TB and in the low micromolar against NRP-TB. To some extent, the activity appeared to be influenced by the degree of lipophilicity, and thus for selected derivatives the ethyl ester was replaced with other more hindered esters, resulting in a slight improvement in their anti-TB activity as described below.

Chemistry

The target compounds **6**−**26**, **28**−**37**, and **47** were synthesized by employing straightforward and efficient Wittig protocols (Scheme 1). (3-Ethoxycarbonyl-5-isoxazolylmethyl)triphenylphosphonium bromide[19] (**5**) and (5-isoxazolylmethyl)-triphenylphosphonium bromide[20] (**51**) were prepared as described previously. A dipolar cycloaddition reaction of the nitrile oxide derived from ethyl 2-chloro-2-(hydroxyimino)-acetate with propargyl bromide gave the 5-bromomethylisoxazole derivative **4**,[21] which was subsequently reacted with triphenylphosphine in THF to give the salt **5**. Compound **51** was synthesized starting from 5-methylisoxazole, which was first brominated with NBS to give **50** and then reacted with triphenylphosphine to produce the desired intermediate. The Wittig reaction of **5** and **51** with an appropriate aldehyde in anhydrous THF using sodium bistrimethylsilylamide as a base produced the target compounds **6**−**10**, **12**−**26**, **28**−**36**, and **47**. For compounds **11** and **37**, NaH was employed as the base in anhydrous CH_2Cl_2 because the above conditions failed to afford the desired products. In most of the reactions carried out, the alkenes obtained largely possessed trans stereochemistry with yields ranging from 15% to 73%. In cases where some cis-isomer was formed (5−10% according to ^{1}H NMR), the pure trans-isomer was isolated either by additional column chromatography or by crystallization from EtOH. In the few cases where an approximately one to one mixture of both isomers was formed, as found in the case of **19a**, **19b**, **24a**, **24b**, **34a**, and **34b**, the isomers were separated by preparative HPLC. Ester hydrolysis of **6** and **9** using LiOH produced the corresponding acids **38** and **39** in excellent yields. Treatment of alkenes **9** and **22** with H_2/Pd in MeOH gave the alkyl derivatives **48** and **49** in satisfactory yields. The selective reduction of nitro derivative **24b** to the aniline **27** was accomplished by using $SnCl_2$ in EtOH. Finally, esters **40**−**46** were obtained in good yields by a $Ti(EtO)_4$ catalyzed transesterification between the corresponding ethyl ester and an excess of the appropriate alcohol.

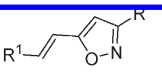
Results and Discussion

All of the target compounds were tested for their ability to inhibit the growth of R-TB strain H_{37}Rv in a microplate Alamar Blue assay (MABA)[22] and subsequently tested for their activity against NRP-TB in a low oxygen recovery assay (LORA).[23]

The majority of the compounds were found to be highly active in inhibiting R-TB, some of them being active at submicromolar concentrations (Table 1). The naphthyl derivative **6** retained good activity against R-TB (MIC 1.6 μM) although less active than the corresponding quinoline derivative **3** (MIC 0.2 μM). When the structure was simplified by replacing the naphthyl core with simple aromatic rings, i.e., pyridine and benzene (compound **7** MIC 15.5 μM and compound **8** MIC 11.7 μM), the activity decreased significantly as compared to **6**. The introduction of various substituents to the pyridine and benzene rings led to a variable range of activities and allowed us to construct a plausible SAR as will be described below. The MICs were, in almost all the cases, lower than those of the unsubstituted species. Introduction of a lipophilic trifluoromethyl group in the meta position of the aromatic ring led to a sharp increase in potency, with compound **9** being very active against R-TB with an MIC of 0.73 μM. The meta position was found to be favored because moving the trifluoromethyl group around the benzene ring, as with derivatives **10** (MIC 3.9 μM) and **11** (MIC 3.9 μM), led to a decrease in anti-TB potency. The replacement of the trifluoromethyl group with a chlorine atom (compound **18**) led to a slight decrease in activity (MIC 2.3 μM), with **18** being 3-fold less active than **9**. The appendage of an additional chlorine atom in the benzene ring led to derivatives **21** and **35** (MIC 5.4

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

Chart 1. Structures of the Anti-TB Derivatives Synthesized in This Study



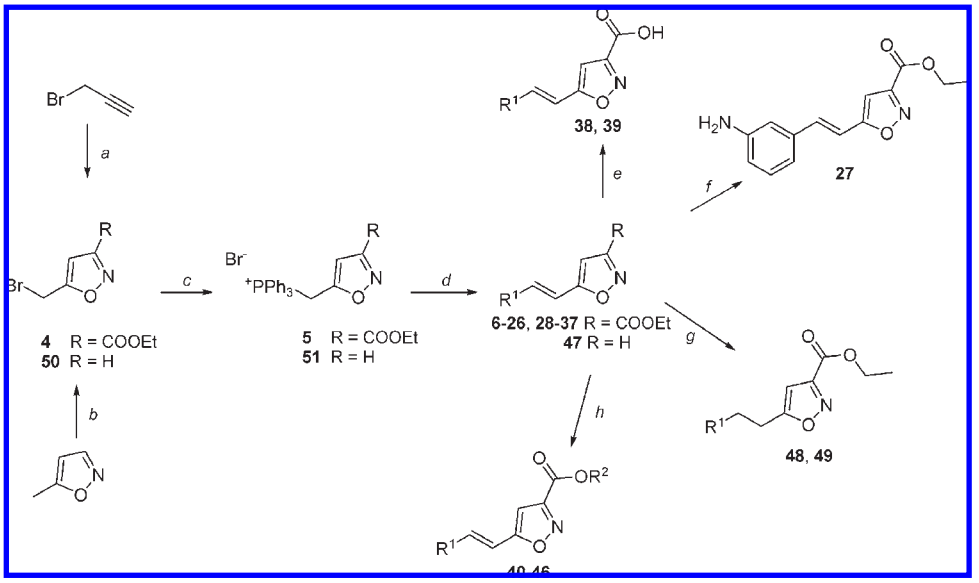
Compd	R^1	R	Compd	R^1	R	Compd	R^1	R
6			**22**			**38**		
7			**23**			**39**		
8[24]			**24a**[a]			**40**		
			24b					
9			**25**			**41**		
10			**26**			**42**		
11			**27**			**43**		
12			**28**			**44**		
13			**29**			**45**		
14			**30**			**46**		
15			**31**			**47**		**H**
16			**32**[26]			**48**		
17			**33**			**49**		
18[25]			**34a**[a]					
			34b					
19a[a]			**35**					
19b								
20			**36**					
21			**37**					

[a] cis-isomer.

and 2.5 μM, respectively), both of which showed comparable activity to that of **18**. The dichloro substitution in the ortho position of the pyridine ring produced the most potent derivative in the series against R-TB (compound **30**, MIC 0.59 μM). The replacement of the chlorine atoms with fluorine at the benzene ring led to derivatives with the same range of activities as the dichloro substituted analogues, compounds **12** and **19b** having an MIC of 5.3 and 3.7 μM, respectively. Introduction of small alkyl groups to the benzene ring was generally well tolerated (**14** and **15**), with the ortho-ethyl group producing the best activity (**14**, MIC 0.98 μM). Conversely, the meta-substituted toluene derivative **17** (MIC 6.5 μM) was found to be one of the least potent compounds in the series. Derivative **13**, bearing a chlorine, a fluorine, and a methyl group at the benzene ring, had an MIC of 3.5 μM, with a difficult assessment of its collocation within the SAR. With methoxy substitution, in both the benzene and in the pyridine ring, a good activity was still maintained. The 6-methoxypyridine derivative **29** (MIC 1.4 μM) was 3-fold more potent than the corresponding regioisomer **34b** (MIC 3.8 μM). Monomethoxy substitution at the meta position of the benzene ring was well tolerated (**26**, MIC 3.7 μM), and the introduction of an additional methoxy group at the other meta position of the benzene ring (**16**, MIC 2.0 μM) did not significantly alter the potency. Derivative **20** (MIC 0.96 μM), bearing an ortho-methylthio group, also showed good potency. Next, bulkier substituents were introduced in order to investigate the presence of lipophilic pockets in the binding site. When a second aromatic ring was attached to the phenyl group, either directly (compound **33**, MIC 2.0 μM) or via an oxymethylene linker (compound **25**, MIC 1.7 μM), the activity was retained. However, the dibenzyloxy substituted derivative **22** (MIC 6.3 μM) was found to be 4-fold less active than the corresponding monosubstituted compound. In addition to the pyridine and benzene rings, five-membered heterocycles were investigated. The thiophene derivative **31** and the furan derivative **32** both showed good activity. In particular, **31** (MIC 1.6 μM) was found to be only 3-fold less active than the most potent compound **30**. As expected, more polar compounds, such as the amino-derivative **27**, the phenol **28**, and much more markedly, the N-methylpiperazine derivative **37**, showed lower activities with MICs of 7.5, 7.8, and 15.2 μM, respectively.

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

Scheme 1[a,b]



[a] Reagents and conditions: (a) Ethyl 2-chloro-2-(hydroxyimino)acetate, triethylamine, anhydrous Et_2O, 10 h through syringe pump, rt (58%); (b) NBS, benzoylperoxide (cat.), anhydrous CH_2Cl_2, 3 h, 80 °C (68%); (c) Ph_3P, MeCN, reflux, 8 h (95%); (d) R^1CHO, $Na[(CH_3)_3Si]_2N$, anhydrous THF, −78 °C to rt, overnight (15−73%) or R^1CHO, NaH, anhydrous CH_2Cl_2, 0 °C to rt, overnight, (49−61%); (e) LiOH, THF−MeOH−H_2O 3:1:1, rt, 1 h; (f) $SnCl_2$, EtOH, reflux, 3 h (51%); (g) Pd/C 5%, H_2, EtOH, rt, 1 h (76%); (h) $Ti(EtO)_4$ (cat), R^2OH, neat, 80 °C, 3 h (59−89%).
[b] For complete structures, see Chart 1.

Table 1. Anti-TB Activity of Compounds **6**−**49** against *M. tuberculosis* Strain $H_{37}Rv$

compd	MABA MIC (μM)	LORA MIC (μM)	Vero cells IC_{50} (μM)	ClogP[a]	compd	MABA MIC (μM)	LORA MIC (μM)	Vero cells IC_{50} (μM)	ClogP[a]
6	1.6	4.0	> 128	4.8	**30**	0.59	5.1	> 128	3.1
7	15.5	52.4	> 128	2.1	**31**	1.6	26.9	> 128	3.2
8	11.7	18.0	nd[b]	3.6	**32**	7.9	50.2	> 128	2.8
9	0.73	21.1	> 128	4.5	**33**	2.0	12.3	113	4.2
10	3.9	6.8	nd	4.5	**34a**	27.5	45.9	nd	2.9
11	3.9	12.1	nd	4.5	**34b**	3.8	16.0	nd	2.9
12	5.3	13.8	> 128	3.9	**35**	2.5	3.5	nd	5.0
13	3.5	7.0	> 128	4.9	**36**	3.8	10.7	nd	3.8
14	0.98	2.0	> 128	4.6	**37**	15.2	37.4	nd	2.7
15	3.1	8.3	> 128	5.0	**38**	> 128	> 128	nd	4.2
16	2.0	3.6	> 128	3.6	**39**	> 128	> 128	nd	3.9
17	6.5	7.4	> 128	4.1	**40**	1.8	7.6	nd	4.6
18	2.3	6.7	> 128	4.3	**41**	2.0	6.7	nd	4.0
19a	29.2	12.3	> 128	3.9	**42**	7.9	8.9	> 128	5.0
19b	3.7	8.9	> 128	3.9	**43**	10.9	9.2	> 128	6.8
20	0.96	7.8	> 128	4.1	**44**	8.3	24.2	> 128	6.6
21	5.4	5.9	> 128	5.0	**45**	7.3	9.3	nd	6.3
22	6.3	4.7	> 128	7.1	**46**	1.1	1.8	> 128	4.8
23	17.3	3.6	> 128	5.3	**47**	> 128	> 128	nd	2.8
24a	5.4	10.9	> 128	3.3	**48**	14.2	15.6	nd	4.0
24b	1.2	5.1	> 128	3.3	**49**	> 128	> 128	nd	1.8
25	1.7	9.9	> 128	5.3	**RMP**	0.1	1.9	127	
26	3.7	8.8	> 128	3.5	**INH**	0.84	> 128	128	
27	7.5	20.7	> 128	2.4	**MOX**	0.46	> 128		
28	7.8	28.7	> 128	2.3					
29	1.4	86.6	> 128	2.9					

[a] Calculated with ChemDraw Ultra 7.0, CambridgeSoft. [b] Not determined

The above results suggest that the overall lipophilicity, more than the position of the substituents at the ring, may be the main variable responsible for the achievement of satisfactory anti-TB activity. The cis-isomers were found to be less potent than the corresponding trans-isomers, the difference in activity ranging from 4-fold (**24a** vs **24b**) to 8-fold (**19a** vs **19b**). The reduction of the planar ethenyl moiety to an ethylene had a detrimental effect on the potency. In fact, compound **48** (MIC 7.5 μM) was more than 20-fold less active that the corresponding unsaturated derivative **9**. The dihydroxy derivative **49** did not show any anti-TB activity, however, the loss of activity may be due to its more polar character that may prevent penetration through the thick and greasy *Mtb* cell wall. Finally, more hindered esters, which could exhibit improved cellular penetration due to increased lipophilicity, were investigated. The *n*-butyl ester **40** (MIC 1.8 μM) was found to be

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

slightly more active than the parent compound **26**, whereas **41** (MIC 2.0 μM) had a potency comparable to the corresponding ethyl ester **29**. Compound **46**, derived from the ethyl ester **2**, was less active than the parent compound. Further elongation of the ester alkyl chain, as for compounds **42**−**45**, led to a pronounced decrease in potency along with an unfavorable increase in ClogP. The acid derivatives **38** and **39**, and the derivative **47**, which lack the carboxylic acid moiety on the isoxazole ring, showed no anti-TB activity. It is possible that the acid, resulting from the hydrolysis of the ester, may be the species responsible for the anti-TB activity;[24] however, the marked polar character of the acid may prevent its penetration through the cell wall. On the other hand, the lack of activity of **47** may be due to the absence of a carboxylic acid precursor, demonstrating that this moeity may represent a crucial element for anti-TB activity.

With the exceptions of derivatives **23** and **43**, the MICs in LORA were found to be higher than those in MABA, although in the low micromolar range (< 10 μM) for almost half of the compounds. With few exceptions, the overall SAR outlined for MABA activity may, to a certain extent, be applied to activity against NRP-TB. In fact, the most active compounds in LORA, namely **46** (LORA MIC 1.8 μM), **14** (LORA MIC 2.0 μM), **35** (LORA MIC 3.5 μM), and **16** (LORA MIC 3.6 μM), were also found to be among the most potent compounds in MABA. The dichloro-substituted pyridine **30**, which in the MABA assay had the best MIC, also maintained satisfactory activity in LORA. Compounds **9** and **29**, which showed good activity in MABA, failed to maintain good activity in LORA with MICs that were 27-fold and 62-fold higher than those exhibited against R-TB, respectively. In contrast to the general trend, some of the more hindered esters, namely **42**, **43**, and **45**, exhibited very similar activities both in MABA and in LORA. Surprisingly, **23** was 5-fold more potent against the NRP-TB than the R-TB.

Next, the selectivity of the two most promising compounds against *Mtb* was evaluated by testing their activity against three other organisms. Compounds **9** and **30** failed to inhibit the growth of a Gram positive microorganism (*Staphylococcus aureus*), a Gram negative microorganism (*Escherichia coli*), a fungus (*Candida albicans*), and another mycobacterium (*Mycobacterium smegmatis*), thus indicating selectivity toward *Mtb* (Table 2, Supporting Information). Compound **30** was also evaluated for its ability to induce mutations in *Mtb* at a concentration 4-fold higher than its MIC. The frequency of mutation to resistance was found to be 1.6×10^{-7}, similar to that shown by RMP and superior to that of INH. With the exception of **33** (IC_{50} 113 μM), the compounds synthesized in this study did not show toxicity to Vero cells up to 128 μM test concentration (Table 1).

Our previous studies have indicated that the 3-isoxazolecarboxylic moiety is crucial for the anti-TB activity. Inhibition of the *Mtb* virulence factor protein tyrosine phosphatase (MptpB) by isoxazole-3-carboxylic acid derivatives has been reported.[25] Thus, if indeed the ester moiety acts a prodrug for the corresponding acid, our compounds may be ligands for MptpB. However, MptpB is not essential for the survival of *Mtb* in vitro. Hence, the mechanism by which these the 5-[(*E*)-2-arylethenyl]-3-isoxazolecarboxylic acid alkyl esters exhibit their activity against R-TB and NRP-TB remains unknown.

Conclusions

Utilizing simple and flexible synthetic chemistry protocols, we have built a small library of 5-[(*E*)-2-arylethenyl]-3-isoxazolecarboxylic acid alkyl esters as anti-TB compounds active against both R-TB and NRP-TB. Most of the synthesized derivatives exhibited good activity, with several compounds being active in the submicromolar range against R-TB and in the low micromolar range against NRP-TB. 5-[(*E*)-2-(3,5-Dichloro-4-pyridinyl)ethenyl]-3-isoxazolecarboxylic acid ethyl ester (**30**) was the most active compound in the series against R-TB (MABA MIC 0.59 μM), whereas 5-[(*E*)-2-(6-methoxy-4-quinolinyl)ethenyl]-3-isoxazolecarboxylic acid butyl ester (**46**) showed the best activity against NRP-TB (LORA MIC 1.8 μM). These MIC values are slightly higher than those of RMP, a first line anti-TB drug, and comparable or better than those of other anti-TB agents currently marketed or in clinical trials. Moreover, **30** and **46**, as almost all of the derivatives synthesized in this study, are devoid of toxicity to Vero cells and possess overall satisfactory drug-like properties. The SAR from the series provided interesting insights and, in general, all the compounds endowed with a higher ClogP value, a feature likely to promote *Mtb* cell permeability, were found to show good anti-TB activity. Therefore halogens, small alkyl appendages, bulkier aromatic and heteroaromatic rings, and methoxy groups were all found to be suitable substituents in order to obtain good anti-TB properties. The more hydrophilic substituents, i.e., hydroxyl, amino, and *N*-methylpiperazine, had a detrimental effect on the activity. The stereochemistry of the alkene linker played an important role, with a 4−8-fold difference in potency between the more active trans- and the less active cis-isomers. Moreover, the reduction of the double bond led to a marked decrease of potency. The *n*-butyl ester derivatives were found to have good anti-TB potency, especially against NRP-TB, while bulkier alkyl esters had an unfavorable impact on the activity. Finally, hydrolysis of the ester or the removal of the carboxylic function led to a loss of activity. These findings provide significant information about the 5-[(*E*)-2-arylethenyl]-3-isoxazolecarboxylic acid alkyl esters as a valuable lead scaffold for the development of new anti-TB drugs active against both R-TB and NRP-TB. Further studies on this promising anti-TB phenotype are currently underway in our laboratory.

Experimental Section

Biology. The MICs were determined using *Mtb* H_{37}Rv ATCC 27294 in MABA[21] and LORA[22] assays according to published procedures. Reported MICs are an average of three individual measurements. For a description of the biological, assays see Supporting Information.

Chemistry. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 400 and 100 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. Standard abbreviation indicating multiplicity was used as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quadruplet, m = multiplet, and br = broad. HRMS experiments were performed on Q-TOF-2TM (Micromass). TLC was performed with Merck 60 F_{254} silica gel plates. Column chromatography was performed using Combi-Flash Rf system with RediSep columns or alternatively using Merck silica gel (40−60 mesh). Preparative HPLC was carried out on a Shimadzu SCL-10A VP instrument with an ACE 5-AQ (21.2 mm × 150 mm) column. Analytical HPLC was carried out on Agilent 1100 HPLC system with a Synergi 4 μ Hydro-RP 80A column, on a variable wavelength detector G1314A. Method 1: flow rate = 1.4 mL/min; gradient eluation over 20 min, from 30% CH_3CN-H_2O to 100% CH_3CN with 0.05% TFA. Method 2: flow rate = 1.4 mL/min; gradient eluation over 20 min, from 10% CH_3CN-H_2O to 100% CH_3CN with 0.05% TFA.

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

Method 3: flow rate = 1.4 mL/min; gradient eluation over 20 min, from 50% CH_3CN-H_2O to 70% CH_3CN with 0.05% TFA. The purity of the target compounds was determined to be >95% by analytical HPLC.

General Procedure for the Synthesis of 6−26, 28−37, and 47. To a stirred mixture of (3-ethoxycarbonyl-5-isoxazolylmethyl)triphenylphosphonium bromide[19] (**5**) (1 equiv) or (5-bromomethylisoxazole)triphenylphosphonium bromide[20] (**51**) (1 equiv) in anhydrous THF at −78 °C, sodiumbistrimethylsilylamide (1.05 equiv) was added dropwise. After 0.5 h, the appropriate aldehyde (1 equiv) in anhydrous THF was added dropwise and the reaction mixture was stirred at rt until consumption of the starting material according to TLC. The mixture was cooled to 0 °C and sat. aq NH_4Cl (10 mL) was added, followed by removal of the solvent under reduced pressure. H_2O (20 mL) was added and the mixture was extracted with chloroform (3 × 20 mL), and then the organic layers were separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography to obtain the product. Analytical data for compounds **8**,[26] **18**,[27] and **32**[28] matched the data previously published.

5-[(*E*)-2-(1-Naphthyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (6). Purified by column chromatography (EtOAc−hexane 1:4). Yield 51% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.47 (t, J = 7.2 Hz, 3H), 4.95 (q, J = 7.2 Hz, 2H), 6.76 (s, 1H), 7.07 (d, J = 16.4 Hz, 1H), 7.50−7.65 (m, 3H), 7.99 (d, J = 7.2 Hz, 1H), 7.90 (d, J = 8.0 Hz, 2H), 8.20−8.27 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 101.5, 114.5, 122.9, 123.8, 125.1, 125.9, 126.4, 128.4, 129.5, 130.9, 132.2, 132.7, 133.3, 156.4, 159.6, 169.9. HRMS (ESI) calculated for $C_{18}H_{15}NO_3$ $[M + Na]^+$ 316.0944, found 316.0955.

5-[(*E*)-2-(4-Pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (7). Purified by column chromatography (EtOAc−hexane 3:7). Yield 49% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.77 (s, 1H), 7.16 (d, J = 16.4 Hz, 1H), 7.30−7.40 (m, 3H), 8.66 (d, J = 6.0 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.9, 102.9, 116.0, 120.8, 132.9, 141.8, 150.2, 156.5, 159.3, 168.5. HRMS (ESI) calculated for $C_{13}H_{12}N_2O_3$ $[M + H]^+$ 245.0920, found 245.0922.

5-[(*E*)-2-Phenylethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester[26] (8). Purified by column chromatography (EtOAc−hexane 1:4). Yield 50% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.69 (s, 1H), 6.99 (d, J = 16.4 Hz, 1H), 7.33−7.45 (m, 4H), 7.55 (d, J = 7.2 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 101.2, 111.9, 126.9, 128.6, 129.2, 134.7, 135.7, 156.3, 159.6, 169.9. HRMS (ESI) calculated for $C_{14}H_{14}NO_3$ $[M + H]^+$ 244.0968, found 244.0974.

5-[(*E*)-2-(3-Trifluoromethylphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (9). Purified by column chromatography (EtOAc−hexane 1:4). Yield 46% (white crystals). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.73 (s, 1H), 7.06 (d, J = 16.8 Hz, 1H), 7.44 (d, J = 16.8 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.79 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.9, 102.0, 113.6, 123.4 (q, J = 4 Hz), 123.5 (q, J = 271 Hz), 125.5 (q, J = 4 Hz), 129.1, 129.9, 131.1 (q, J = 32 Hz), 133.9, 135.5, 156.4, 159.5, 169.1. HRMS (ESI) calculated for $C_{15}H_{12}F_3NO_3$ $[M + H]^+$ 312.0842, found 312.0854.

5-[(*E*)-2-(4-Trifluoromethylphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (10). Purified by column chromatography (EtOAc−hexane 1:4). Yield 39% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 6.8 Hz, 3H), 4.48 (q, J = 6.8 Hz, 2H), 6.75 (s, 1H), 7.06 (d, J = 16.8, 1H), 7.44 (d, J = 16.8 Hz, 1H), 7.65−7.69 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.9, 102.2, 114.2, 123,5 (q, J = 270 Hz), 125.6 (q, J = 4 Hz), 127.0, 130.8 (q, J = 40 Hz), 133.9, 138.1, 156.4, 159.5, 169.0. HRMS (ESI) calculated for $C_{15}H_{12}F_3NO_3$ $[M + H]^+$ 312.0842, found 312.0853.

5-[(*E*)-2-(2,4-Difluorophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (12). Purified by column chromatography (EtOAc−hexane 1:4) and recrystallized by EtOH. Yield 39% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.71 (s, 1H), 6.85−6.98 (m, 2H), 7.04 (d, J = 16.8 Hz, 1H), 7.45 (d, J = 16.8 Hz, 1H), 7.50−7.62 (m, 1H). ^{19}F NMR ($CDCl_3$) δ −111.9 (d, J = 8.0 Hz), −108.1 (d, J = 8.0 Hz). HRMS (ESI) calculated for $C_{14}H_{11}F_2NO_3$ $[M + H]^+$ 280.0779, found 280.0793.

5-[(*E*)-2-(6-Chloro-2-fluoro-3-methylphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (13). Purified by column chromatography (EtOAc−hexane 1:4) followed by recrystallization with EtOH. Yield 47% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 2.28 (s, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.74 (s, 1H), 7.05−7.18 (m, 2H), 7.31 (d, J = 16.8 Hz, 1H), 7.58 (d, J = 16.8 Hz, 1H). ^{19}F NMR ($CDCl_3$) δ −113.4. HRMS (ESI) calculated for $C_{15}H_{13}ClFNO_3$ $[M + H]^+$ 310.0640, found 310.0653.

5-[(*E*)-2-(2-Ethylphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (14). Purified by column chromatography (EtOAc−hexane 1:9). Yield 46% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.6 Hz, 3H), 1.45 (t, J = 7.2 Hz, 3H), 2.82 (q, J = 7.6 Hz, 2H), 4.81 (q, J = 7.2 Hz, 2H), 6.68 (s, 1H), 6.91 (d, J = 16.2 Hz, 1H), 7.22−7.36 (m, 3H), 7.59 (d, J = 7.2 Hz, 1H), 7.65 (d, J = 16.2 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 15.3, 26.0, 61.8, 101.1, 113.1, 125.4, 126.0, 128.9, 129.2, 133.0, 133.2, 142.8, 156.4, 159.7, 170.1. HRMS (ESI) calculated for $C_{16}H_{17}NO_3$ $[M + H]^+$ 272.1281, found 272.1285.

5-[(*E*)-2-[3-(1-Methylethyl)phenyl]ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (15). Purified by column chromatography (EtOAc−hexane 1:9). Yield 39% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.29 (d, J = 6.8 Hz, 6H), 1.45 (t, J = 7.2 Hz, 3H), 2.88−2.99 (m, 1H), 4.47 (q, J = 7.2 Hz, 2H), 6.68 (s, 1H), 6.99 (d, J = 16.4 Hz, 1H), 7.22−7.28 (m, 1H), 7.30−7.45 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 13.8, 23.5, 33.7, 61.8, 101.0, 111.7, 124.4, 125.1, 127.5, 128.5, 134.7, 136.1, 149.2, 156.3, 159.7, 170.1. HRMS (ESI) calculated for $C_{17}H_{19}NO_3$ $[M + H]^+$ 286.1437, found 286.1442.

5-[(*E*)-2-(3,5-Dimethoxyphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (16). Purified by column chromatography (EtOAc−hexane 1:4) followed by recrystallization with EtOH. Yield 33% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 3.85 (s, 6H), 4.47 (q, J = 7.2 Hz, 2H), 6.48−6.50 (m, 1H), 6.69 (bds, 3H), 6.90 (d, J = 16.4 Hz, 1H), 7.34 (d, J = 16.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 55.0, 61.8, 101.3, 101.4, 104.9, 112.4, 135.7, 136.7, 156.3, 159.6, 160.4, 171.4. HRMS (ESI) calculated for $C_{16}H_{17}NO_5$ $[M + H]^+$ 304.1179, found 304.1188.

5-[(*E*)-2-(3-Methylphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (17). Purified by column chromatography (EtOAc−hexane 1:4). Yield 45% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 2.40 (s, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.67 (s, 1H), 6.97 (d, J = 16.4 Hz, 1H), 7.19 (d, J = 7.2 Hz, 1H), 7.27−7.41 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 13.8, 21.0, 61.8, 101.0, 111.7, 124.1, 127.6, 128.4, 130.0, 134.6, 135.9, 138.2, 156.3, 159.6, 170.0. HRMS (ESI) calculated for $C_{15}H_{15}NO_3$ $[M + H]^+$ 258.1124, found 258.1135.

5-[(*E*)-2-(3-Chlorophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (18).[27] Purified by column chromatography (EtOAc−hexane 1:4) followed by recrystallization with EtOH. Yield 63% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.70 (s, 1H), 6.99 (d, J = 16.4 Hz, 1H), 7.30−7.42 (m, 4H), 7.54 (bds, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.9, 101.8, 113.2, 125.1, 126.6, 129.0, 129.8, 134.1, 134.6, 136.5, 156.4, 159.5, 169.2. HRMS (ESI) calculated for $C_{14}H_{12}ClNO_3$ $[M + H]^+$ 278.0578, found 278.0588.

5-[(*Z*)-2-(3,5-Difluorophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (19a). Purified by preparative HPLC. Yield 21% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.41 (t, J = 7.2 Hz, 3H), 4.43 (q, J = 7.2 Hz, 2H), 6.48 (s, 1H), 6.59 (d, J = 12.4 Hz, 1H), 6.78−6.93 (m, 4H). ^{19}F NMR ($CDCl_3$) δ −109.9. HRMS (ESI) calculated for $C_{14}H_{11}F_2NO_3$ $[M + H]^+$ 280.0779, found 280.0794.

5-[(*E*)-2-(3,5-Difluorophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (19b). Purified by preparative HPLC. Yield 29% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J =

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

7.2 Hz, 2H), 6.73 (s, 1H), 6.78−6.84 (m, 1H), 6.98 (d, J = 16.4 Hz, 1H), 7.03−7.08 (m, 2H), 7.32 (d, J = 16.4 Hz, 1H). ^{19}F NMR ($CDCl_3$) δ −110.0. HRMS (ESI) calculated for $C_{14}H_{11}F_2NO_3$ $[M + H]^+$ 280.0779, found 280.0793.

5-[(*E*)-2-(2-Methylthiophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (20). Purified by column chromatography (EtOAc−hexane 1:4). Yield 45% (pale-yellow powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 2.50 (s, 3H), 4.46 (q, J = 7.2 Hz, 2H), 6.73 (s, 1H), 6.93 (d, J = 15.6 Hz, 1H), 7.16−7.34 (m, 3H), 7.57 (d, J = 1.8 Hz, 1H), 7.87 (d, J = 15.6 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 16.3, 61.8, 101.2, 113.6, 125.4, 125.9, 127.2, 129.3, 132.8, 134.0, 138.2, 156.3, 159.6, 170.1. HRMS (ESI) calculated for $C_{15}H_{15}NO_3S$ $[M + H]^+$ 290.0845, found 290.0859.

5-[(*E*)-2-(3,5-Dichlorophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (21). Purified by column chromatography (EtOAc−hexane 1:4). Yield 73% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.48 (q, J = 7.2 Hz, 2H), 6.72 (s, 1H), 6.99 (d, J = 16.0 Hz, 1H), 7.24−7.42 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.9, 102.4, 114.4, 125.1, 128.7, 132.7, 135.2, 137.7, 156.5, 159.4, 168.7. HRMS (ESI) calculated for $C_{14}H_{11}NO_3Cl_2$ $[M + H]^+$ 312.0188, found 312.0197.

5-[(*E*)-2-[3,5-Bis(phenylmethoxy)phenyl]ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (22). Purified by column chromatography (EtOAc−hexane 1:4). Yield 29% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 5.00 (s, 4H), 6.64−6.70 (m, 2H), 6.78 (d, J = 1.8 Hz, 1H), 6.93 (d, J = 16.0 Hz, 1H), 7.28−7.48 (m, 11H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 69.8, 101.4, 103.0, 106.1, 112.5, 127.1, 127.7, 128.3, 135.6, 136.2, 136.7, 156.5, 159.6, 159.8, 169.7. HRMS (ESI) calculated for $C_{28}H_{25}NO_3$ $[M + H]^+$ 456.1805, found 456.1810.

5-[(*E*)-2-(4-Methyl-1-naphthyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (23). Purified by column chromatography (EtOAc−hexane 1:4). Yield 37% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.47 (t, J = 7.2 Hz, 3H), 2.75 (s, 3H), 4.49 (q, J = 7.2 Hz, 2H), 6.73 (s, 1H), 7.03 (d, J = 16.0 Hz, 1H), 7.37 (d, J = 7.2 Hz, H) 7.50−7.74 (m, 3H), 8.01−8.08 (m, 1H), 8.21−8.25 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 19.2, 61.6, 102.4, 116.3, 124.4, 125.4, 125.7, 125.8, 125.9, 126.0, 130.5, 130.9, 132.4, 135.4, 135.8, 155.8, 159.6, 169.0. HRMS (ESI) calculated for $C_{19}H_{17}NO_3$ $[M + H]^+$ 308.1281, found 308.1296.

5-[(*Z*)-2-(3-Nitrophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (24a). Purified by preparative HPLC. Yield 15% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.41 (t, J = 7.2 Hz, 3H), 4.46 (q, J = 7.2 Hz, 2H), 6.48 (s, 1H), 6.67 (d, J = 12.4 Hz, 1H), 6.97 (d, J = 12.4 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.73 (d, J = 7.6 Hz, 1H), 8.22−8.24 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 13.7, 61.9, 99.6, 103.9, 116.2, 123.1, 123.2, 129.2, 133.7, 133.9, 146.2, 156.0, 159.8, 168.8. HRMS (ESI) calculated for $C_{14}H_{12}N_2O_5$ $[M + H]^+$ 289.0819, found 289.0824.

5-[(*E*)-2-(3-Nitrophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (24b). Purified by preparative HPLC. Yield 29% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.46 (t, J = 7.2 Hz, 3H), 4.48 (q, J = 7.2 Hz, 2H), 6.77 (s, 1H), 7.13 (d, J = 16.4 Hz, 1H), 7.47 (d, J = 16.4 Hz, 1H), 7.61 (t, J = 8.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 8.21−8.23 (m, 1H), 8.42 (brs, 1H). ^{13}C NMR ($CDCl_3$) δ 14.4, 62.4, 103.0, 115.1, 121.5, 123.8, 130.0, 133.0, 133.2, 136.9, 148.0, 156.9, 159.8, 169.0. HRMS (ESI) calculated for $C_{14}H_{12}N_2O_5$ $[M + H]^+$ 289.0819, found 289.0829.

5-[(*E*)-2-[(3-Phenylmethoxy)phenyl]ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (25). Purified by column chromatography (EtOAc−hexane 1:4) followed by recrystallization with EtOH. Yield 41% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 5.12 (s, 2H), 6.68 (s, 1H), 6.92−7.01 (m, 2H), 7.14−7.16 (m, 2H), 7.28−7.48 (m, 7H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 69.7, 101.2, 112.3, 113.0, 115.7, 119.8, 127.1, 127.7, 128.3, 129.6, 135.6, 136.1, 136.3, 156.3, 158.8, 159.6, 169.8. HRMS (ESI) calculated for $C_{21}H_{19}NO_4$ $[M + H]^+$ 350.1386, found 350.1390.

5-[(*E*)-2-(3-Methoxyphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (26). Purified by column chromatography (EtOAc−hexane 1:4) followed by recrystallization with EtOH. Yield 51% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 3.87 (s, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.69 (s, 1H), 6.93 (dd, J_1 = 2 Hz, J_2 = 8.0 Hz, 1H), 6.97 (d, J = 16.4 Hz, 1H), 7.06 (bds, 1H), 7.14 (d, J = 7.2 Hz, 1H), 7.30−7.41 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 101.6, 104.3, 111.8, 114.0, 119.2, 127.5, 129.0, 156.4, 159.4, 159.6, 161.8, 164.1, 169.8. HRMS (ESI) calculated for $C_{15}H_{15}NO_4$ $[M + H]^+$ 274.1073, found 274.1084.

5-[(*E*)-2-(3-Hydroxyphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (28). Purified by column chromatography (EtOAc−hexane 1:4). Yield 39% (white powder). ^{1}H NMR (CD_3OD) δ 1.41 (t, J = 7.2 Hz, 3H), 4.43 (q, J = 7.2 Hz, 2H), 6.77−6.87 (m, 2H), 7.00−7.17 (m, 3H), 7.22 (t, J = 8.0 Hz, 1H), 7.39 (d, J = 16.4 Hz, 1H). ^{13}C NMR (CD_3OD) δ 12.6, 61.4, 100.6, 111.5, 112.8, 116.0, 118.2, 129.2, 135.8, 136.2, 156.3, 157.3, 159.4, 170.4. HRMS (ESI) calculated for $C_{14}H_{13}NO_4$ $[M + H]^+$ 260.0917, found 260.0929.

5-[(*E*)-2-(6-Methoxy-3-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid] Ethyl Ester (29). Purified by column chromatography (EtOAc−hexane 1:4) followed by recrystallization with EtOH. Yield 46% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.44 (t, J = 7.2 Hz, 3H), 3.99 (s, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.66 (s, 1H), 6.80 (d, J = 8.8 Hz, 1H), 6.87 (d, J = 16.4 Hz, 1H), 7.35 (d, J = 16.4 Hz, 1H), 7.80 (dd, J_1 = 2.4 Hz, J_2 = 8.4 Hz, 1H) 8.28 (d, J = 2.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 53.4, 61.8, 101.0, 111.0, 111.2, 124.1, 132.0, 135.2, 147.0, 156.4, 159.6, 164.4, 169.7. HRMS (ESI) calculated for $C_{14}H_{14}N_2O_4$ $[M + H]^+$ 275.1026, found 275.1037.

5-[(*E*)-2-(3,5-Dichloro-4-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (30). Purified by column chromatography (EtOAc−hexane 1:4). Yield 50% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.48 (q, J = 7.2 Hz, 2H), 6.85 (s, 1H), 7.40 (d, J = 17.2 Hz, 1H), 7.47 (d, J = 17.2 Hz, 1H), 8.56 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 62.0, 103.8, 122.6, 126.4, 130.8, 138.5, 148.0, 156.6, 159.2, 168.0. HRMS (ESI) calculated for $C_{13}H_{10}Cl_2N_2O_3$ $[M + H]^+$ 313.0141, found 313.0152.

5-[(*E*)-2-(3-Thiophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (31). Purified by column chromatography (EtOAc−hexane 1:4) followed by recrystallization with EtOH. Yield 59% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.44 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.64 (s, 1H), 6.81 (d, J = 16.4 Hz, 1H) 7.33−7.46 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 100.9, 111.8, 124.2, 125.8, 126.6, 129.6, 137.8, 156.3, 159.6, 170.0. HRMS (ESI) calculated for $C_{14}H_{14}N_2O_4$ $[M + H]^+$ 275.1026, found 275.1037.

5-[(*E*)-2-(2-Furanyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester[28] (32). Purified by column chromatography (EtOAc−hexane 1:4) followed by recrystallization with EtOH. Yield 49% (pale-yellow powder). ^{1}H NMR ($CDCl_3$) δ 1.44 (t, J = 6.8 Hz, 3H), 4.46 (q, J = 6.8 Hz, 2H), 6.49 (dd, J_1 = 2 Hz, J_2 = 3.2 Hz, 1H), 6.63 (s, 1H), 6.87 (d, J = 16.0 Hz, 1H), 7.17 (d, J = 16.0 Hz, 1H), 7.49 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 101.3, 109.8, 111.9, 112.6, 122.4, 143.6, 150.9, 156.4, 159.6, 169.6. HRMS (ESI) calculated for $C_{12}H_{11}NO_4$ $[M + H]^+$ 234.0760, found 234.0769.

5-[(*E*)-2-[4-(2-Pyridinyl)phenyl]ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (33). Purified by column chromatography (EtOAc−hexane 1:4). Yield 48% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.70 (s, 1H), 7.05 (d, J = 16.4 Hz, 1H), 7.20−7.30 (m, 1H), 7.45 (d, J = 16.4 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.76−7.80 (m, 2H), 8.06 (d, J = 8.4 Hz, 2H), 8.72 (d, J = 4.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 101.3, 112.3, 120.1, 122.1, 127.0, 127.3, 135.1, 135.2, 136.4, 139.9, 149.4, 156.0, 156.4, 159.6, 169.8. HRMS (ESI) calculated for $C_{19}H_{16}N_2O_3$ $[M + H]^+$ 321.1233, found 321.1242.

5-[(*Z*)-2-(6-Methoxy-2-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (34a). Purified by preparative HPLC. Yield 19%

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

(white powder). ^{1}H NMR ($CDCl_3$) δ 1.42 (t, J = 7.2 Hz, 3H), 3.91 (s, 3H), 4.42 (q, J = 7.2 Hz, 2H), 6.59 (d, J = 12.8 Hz, 1H), 6.71−6.78 (m, 2H), 6.97 (d, J = 7.2 Hz, 1H), 7.37 (s, 1H), 7.55−7.62 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 13.7, 53.5, 61.7, 105.4, 111.2, 115.1, 118.6, 134.3, 138.4, 151.0, 155.9, 159.7, 163.3, 169.0. HRMS (ESI) calculated for $C_{14}H_{14}N_2O_4$ $[M + H]^+$ 275.1026, found 275.1039.

5-[(*E*)-2-(6-Methoxy-2-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (34b). Purified by preparative HPLC. Yield 29% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.02 (s, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.70−6.75 (m, 2H), 6.94 (d, J = 6.8 Hz, 1H), 7.33 (d, J = 16.4 Hz, 1H), 7.55−7.62 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 52.9, 61.8, 102.3, 111.6, 115.0, 117.3, 134.1, 138.7, 150.2, 156.4, 159.6, 163.3, 169.7. HRMS (ESI) calculated for $C_{14}H_{14}N_2O_4$ $[M + H]^+$ 275.1026, found 275.1039.

5-[(*E*)-2-(2,6-Dichlorophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (35). Purified by column chromatography (EtOAc−hexane 1:4). Yield 41% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.77 (s, 1H), 7.16−7.22 (m, 2H), 7.39 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 16.8 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.9, 102.3, 120.3, 128.5, 129.1, 129.3, 131.9, 134.4, 156.4, 159.5, 169.0. HRMS (ESI) calculated for $C_{14}H_{11}Cl_2NO_3$ $[M + H]^+$ 312.0188, found 312.0199.

5-[(*E*)-2-(2-Fluoro-4-methoxyphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (36). Purified by column chromatography (EtOAc−hexane 0.5:4.5). Yield 25% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.44 (t, J = 7.2 Hz, 3H), 3.84 (s, 3H), 4.47 (q, J = 7.2 Hz, 2H), 6.66−6.70 (m, 2H), 6.75 (dd, J_1 = 2 Hz, J_2 = 8.4 Hz, 1H) 6.97 (d, J = 16.8 Hz, 1H), 7.42−7.49 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 55.3, 61.8, 100.8, 101.4, 110.4, 112.0, 115.3, 128.4, 128.7, 156.3, 159.9, 161.5, 162.8, 170.2. HRMS (ESI) calculated for $C_{15}H_{14}FNO_4$ $[M + H]^+$ 292.0979, found 292.0985.

5-[(*E*)-2-(3,5-Dichloro-4-pyridinyl)ethenyl]-isoxazole (47). Purified by column chromatography (EtOAc−hexane 3:7). Yield 38% (orange powder). ^{1}H NMR ($CDCl_3$) 6.53 (s, 1H), 7.39−7.47 (m, 2H), 8.31 (s, 1H), 8.55 (bds, 2H). ^{13}C NMR ($CDCl_3$) δ 103.1, 123.0, 125.3, 130.8, 139.0, 147.8, 150.4, 165.6. HRMS (ESI) calculated for $C_{10}H_6Cl_2N_2O$ $[M + H]^+$ 240.9929, found 240.9936.

5-[(*E*)-2-[4-(4-Methyl-1-piperazinyl)phenyl]ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (37). To a stirred solution of 3-ethyl-isoxazolecarboxylate-5-methyltriphenylphosphonium bromide **5** (267 mg, 0.54 mmol) in anhydrous CH_2Cl_2 (12 mL), NaH (82 mg, 3.43 mmol) was added at 0 °C. After 20 min, 4-(4-methylpiperazin-1-yl)benzaldehyde (100 mg, 0.49 mmol) in CH_2Cl_2 (2 mL) was added dropwise and the reaction mixture was stirred at rt overnight. The mixture was quenched with H_2O, and the organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by flash chromatography (CH_2Cl_2−MeOH 9:1) gave the title compound as a yellow powder. Yield 61%. ^{1}H NMR ($CDCl_3$) δ 1.44 (t, J = 7.2 Hz, 3H), 2.38 (s, 3H), 2.58−2.60 (t, 4H), 3.29−3.33 (t, 4H), 4.46 (q, J = 7.2 Hz, 2H), 6.60 (s, 1H), 6.81 (d, J = 16.4 Hz, 1H), 6.91 (d, J = 8.4 Hz, 2H), 7.32 (d, J = 16.4 Hz, 1H), 7.47 (d, J = 8.4 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 13.8, 45.8, 47.6, 54.5, 61.7, 99.6, 99.5, 108.6, 114.8, 125.4, 128.2, 135.6, 151.5, 159.8, 170.7. HRMS (ESI) calculated for $C_{19}H_{23}N_3O_3$ $[M + H]^+$ 342.1812, found 342.1811.

5-[(*E*)-2-(2-Trifluoromethylphenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (11). Synthesized as **37**, except 2-(trifluoromethyl)-benzaldehyde was used as a starting material. Purified by preparative HPLC. Yield 49% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J = 7.2 Hz, 3H), 4.48 (q, J = 7.2 Hz, 2H), 6.8 (s, 1H), 6.98 (d, J = 16.0 Hz, 1H), 7.47 (t, J = 7.6 Hz, 1H), 7.60 (t, J = 7.6 Hz, 1H), 7.70−7.80 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.9, 102.0, 115.9, 123.3 (q, J = 270 Hz), 125.8 (q, J = 6 Hz), 126.8, 128.2, 128.6, 130.4 (q, J = 32 Hz), 131.7, 135.5, 156.4, 159.5, 169.1. HRMS (ESI) calculated for $C_{15}H_{12}F_3NO_3$ $[M + H]^+$ 312.0842, found 312.0846.

5-[(*E*)-2-(1-Naphthyl)ethenyl]-3-isoxazolecarboxylic Acid (38). To a stirred solution of **6** (20 mg, 0.31 mmol) in a mixture of THF-H_2O-MeOH 3:1:1 (4 mL), $LiOH \cdot H_2O$ (8.9 mg, 1.23 mmol) was added portionwise. After 30 min at rt, the mixture was concentrated under reduced pressure to evaporate the organic layer and then 0.1N HCl was cautiously added dropwise until a precipitate appeared. The precipitate was collected to give the title compound **38** (Yield 93%) as a white solid without further purification. ^{1}H NMR (CD_3OD) δ 6.95 (s, 1H), 7.25 (d, J = 16.2 Hz, 1H), 7.51−7.63 (m, 3H), 7.85−7.93 (m, 3H), 8.25−8.34 (m, 2H). ^{13}C NMR (CD_3OD) δ 102.2, 116.2, 123.5, 124.7, 125.2, 125.6, 125.8, 127.9, 128.2, 130.3, 133.0, 133.4, 135.4, 156.3, 160.6, 169.0. HRMS (ESI) calculated for $C_{16}H_{11}NO_3$ $[M + H]^+$ 266.0811, found 266.0820.

5-[(*E*)-2-(3-Trifluoromethylphenyl)ethenyl]-3-isoxazolecarboxylic Acid (39). Synthesized as **38**, except **9** was used as a starting material. Yield 86% (white solid). ^{1}H NMR (CD_3OD) δ 6.89 (s, 1H), 7.34 (d, J = 16.4 Hz, 1H), 7.53 (d, J = 16.8 Hz, 1H), 7.59−7.66 (m, 2H), 7.88−7.94 (m, 2H). ^{13}C NMR (CD_3OD) δ 102.2, 116.2, 123.5, 124.7, 125.2, 125.6, 125.8, 127.9, 128.2, 130.3, 133.0, 133.4, 135.4, 156.3, 160.6, 169.0. HRMS (ESI) calculated for $C_{13}H_8F_3NO_3$ $[M + H]^+$ 284.0529, found 284.0525.

General Procedure for the Synthesis of Esters 40−46. Under nitrogen atmosphere, the ethyl ester derivative (1 equiv), the appropriate alcohol (10 equiv) and $Ti(EtO)_4$ (10% mol) were stirred at 80 °C until consumption of the limiting reagent. After the addition of H_2O, the mixture was extracted with $CHCl_3$ and then the organic layer was separated, washed with brine, dried over Na_2SO_4 anhydrous, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc−hexane 1:4) to give the product.

5-[(*E*)-2-(3-Methoxyphenyl)ethenyl]-3-isoxazolecarboxylic Acid Butyl Ester (40). Synthesized from **26**. Yield 83% (white powder). ^{1}H NMR ($CDCl_3$) δ 0.99 (t, J = 7.2 Hz, 3H), 1.43−1.54 (m, 2H), 1.76−1.83 (m, 2H), 3.86 (s, 3H), 4.44 (t, J = 7.2 Hz, 2H), 6.68 (s, 1H), 6.93 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 1H), 6.97 (d, J = 16.4 Hz, 1H), 7.06 (br s, 1H), 7.14 (d, J = 7.6 Hz, 1H), 7.33 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 16.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) 13.4, 18.7, 30.1, 54.9, 65.6, 101.2, 112.0, 112.2, 114.9, 119.5, 129.6, 135.6, 136.1, 156.3, 159.6, 159.7, 169.8. HRMS (ESI) calculated for $C_{17}H_{19}NO_4$ $[M + H]^+$ 302.1386, found 302.1402.

5-[(*E*)-2-(6-Methoxy-3-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid Butyl Ester (41). Synthesized from **29**. Yield 89% (white powder). ^{1}H NMR ($CDCl_3$) δ 0.95−1.00 (m, 3H), 1.43−1.52 (m, 2H), 1.75−1.82, (m, 2H), 3.98 (s, 3H), 4.42 (t, J = 6.8 Hz, 2H), 6.65 (s, 1H), 6.80 (d, J = 4.4 Hz, 1H), 6.87 (d, J = 16.4 Hz, 1H), 7.34 (d, J = 16.4 Hz, 1H), 7.78−7.81 (m, 1H), 8.28 (br s, 1H). ^{13}C NMR ($CDCl_3$) 13.3, 18.7, 30.1, 53.4, 65.6, 101.0, 111.0, 111.2, 124.1, 132.0, 135.2, 147.0, 156.3, 159.7, 164.4, 169.7. HRMS (ESI) calculated for $C_{16}H_{18}N_2O_4$ $[M + H]^+$ 303.1339, found 303.1351.

5-[(*E*)-2-(3,5-Dichloro-4-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid 3-Pentyl Ester (42). Synthesized from **30**. Yield 59% (white powder). ^{1}H NMR ($CDCl_3$) δ 0.98 (t, J = 7.2 Hz 6H), 1.74−1.79 (m, 4H), 5.09−5.12 (m, 1H), 6.84 (s, 1H), 7.39 (d, J = 16.8 Hz, 1H), 7.47 (d, J = 16.8 Hz, 1H), 8.56 (s, 2H). ^{13}C NMR ($CDCl_3$) 9.24, 25.9, 37.6, 44.0, 79.2, 103.0, 122.6, 126.4, 130.7, 138.0, 147.2, 157.3, 159.2, 168.0. HRMS (ESI) calculated for $C_{16}H_{16}Cl_2N_2O_3$ $[M + H]^+$ 355.0610, found 355.0620.

5-[(*E*)-2-(3,5-Dichloro-4-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid Octyl Ester (43). Synthesized from **30**. Yield 81% (white powder). ^{1}H NMR ($CDCl_3$) δ 0.89−0.91 (m, 3H), 1.28−1.50 (m, 10H), 1.78−1.83, (m, 2H), 4.42 (t, J = 6.8 Hz, 2H,), 6.84 (s, 1H), 7.39 (d, J = 16.8 Hz, 1H), 7.47 (d, J = 16.8 Hz, 1H), 8.56 (s, 2H). ^{13}C NMR ($CDCl_3$) 13.7, 22.2, 25.4, 28.1, 28.8, 28.9, 31.4, 66.1, 103.8, 122.6, 126.4, 130.8, 138.5, 148.0, 156.2, 159.3, 167.9. HRMS (ESI) calculated for $C_{19}H_{22}Cl_2N_2O_3$ $[M + H]^+$ 397.1080, found 397.1086.

5-[(*E*)-2-(3,5-Dichloro-4-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid 2-Octyl Ester (44). Synthesized from **30**. Yield 64% (white powder). ^{1}H NMR ($CDCl_3$) δ 0.86−0.92 (m, 3H), 1.28−1.42 (m,

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a

11H), 1.63−1.82, (m, 2H), 5.24−5.28 (m, 1H), 6.84 (s, 1H), 7.39 (d, J = 16.8 Hz, 1H), 7.47 (d, J = 16.8 Hz, 1H), 8.56 (s, 2H). ^{13}C NMR ($CDCl_3$) 13.7, 19.5, 22.2, 25.0, 28.6, 31.3, 35.4, 73.5, 103.8, 122.6, 126.3, 130.8, 138.6, 148.0, 156.8, 158.9, 167.8. HRMS (ESI) calculated for $C_{19}H_{22}Cl_2N_2O_3$ $[M + H]^+$ 397.1080, found 397.1087.

5-[(*E*)-2-(3,5-Dichloro-4-pyridinyl)ethenyl]-3-isoxazolecarboxylic Acid 3-Cyclopentanepropyl Ester (45). Synthesized from **30**. Yield 69% (white powder). ^{1}H NMR ($CDCl_3$) δ 1.02−1.12 (m, 2H), 1.41−1.89 (m, 9H), 4.41 (d, J = 6.8 Hz, 2H), 6.84 (s, 1H), 7.40 (d, J = 16.8 Hz, 1H), 7.47 (d, J = 16.8 Hz, 1H), 8.56 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 24.8, 27.4, 31.7, 32.2, 39.3, 66.3, 103.8, 122.6, 126.4, 130.8, 138.5, 148.0, 156.6, 159.3, 167.9. HRMS (ESI) calculated for $C_{19}H_{20}Cl_2N_2O_3$ $[M + H]^+$ 395.0923, found 395.0930.

5-[(*E*)-2-(6-Methoxy-4-quinolinyl)ethenyl]-3-isoxazolecarboxylic Acid Butyl Ester (46). Synthesized from **2**. Yield 69% (yellow powder). ^{1}H NMR ($CDCl_3$) δ 1.00 (t, J = 7.2 Hz, 3H), 1.43−1.54 (m, 2H), 1.78−1.85 (m, 2H), 4.01 (s, 3H), 4.44 (t, J = 6.8 Hz, 2H), 6.81 (s, 1H), 7.19 (d, J = 16.4 Hz, 1H), 7.35 (d, J = 2.8 Hz, 1H), 7.44 (dd, J_1 = 2.8 Hz, J_2 = 9.2 Hz, 1H), 7.55 (d, J = 4.4 Hz, 1H), 8.05−8.09 (m, 2H), 8.80 (d, J = 4.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.3, 18.7, 30.1, 55.4, 65.8, 99.6, 100.7, 103.0, 117.3, 122.0, 126.7, 130.2, 131.4, 138.7, 144.6, 147.1, 156.6, 158.0, 159.5, 168.7. HRMS (ESI) calculated for $C_{20}H_{20}N_2O_4$ $[M + H]^+$ 353.1495, found 353.1503.

5-[2-(3-Trifluoromethylphenyl)ethyl]-3-isoxazolecarboxylic Acid Ethyl Ester (48). 5% Pd/C (100 mg) was added to a solution of **9** (76 mg, 0.24 mmol) in anhydrous EtOH (10 mL) and the flask was saturated with H_2. After 10 min, Pd/C was filtered over celite and the filtrate evaporated under reduced pressure. The crude material was purified by flash chromatography (EtOAc−hexane 1:4) to give the title compound as a white solid (yield 75%). ^{1}H NMR ($CDCl_3$) δ 1.41 (t, J = 7.2 Hz, 3H), 3.09−3.18 (m, 4H), 4.44 (q, J = 7.2 Hz, 2H), 6.38 (s, 1H), 7.37−7.51 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 13.7, 27.8, 32.8, 61.7, 101.7, 123.2 (q, J = 4 Hz), 123.6 (q, J = 271 Hz), 124.6 (q, J = 4 Hz), 128.7, 130.6 (q, J = 32 Hz), 131.3, 140.0, 156.0, 159.6, 173.2. HRMS (ESI) calculated for $C_{15}H_{14}F_3NO_3$ $[M + H]^+$ 314.0998, found 314.1006.

5-[2-(3,5-Dihydroxyphenyl)ethyl]-3-isoxazolecarboxylic Acid Ethyl Ester (49). Synthesized as **48** by using **22** as a starting material. Purified by column chromatography (EtOAc−hexane 3:7). Yield 87% (white powder). ^{1}H NMR (CD_3OD) δ 1.38 (t, J = 7.2 Hz, 3H), 2.88 (t, J = 7.2 Hz, 3H), 3.11 (t, J = 7.2 Hz, 3H), 4.39 (q, J = 7.2 Hz, 2H), 6.10−6.16 (m, 3H), 6.47 (s, 1H). ^{13}C NMR (CD_3OD) δ 12.5, 27.3, 32.6, 61.3, 99.9, 101.0, 105.9, 141.6, 155.8, 157.8, 159.5, 174.8. HRMS (ESI) calculated for $C_{14}H_{15}NO_5$ $[M + H]^+$ 278.1023, found 278.1035.

5-[(*E*)-2-(3-Aminophenyl)ethenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (27). To a stirred solution of **24b** (35 mg, 0.12 mmol) in anhydrous EtOH (5 mL), $SnCl_2 \cdot H_2O$ (270 mg, 1.20 mmol) was added portionwise and the mixture was refluxed until consumption of the starting material on TLC. The solvent was evaporated under reduced pressure, and an aqueous solution of 1N NaOH (1 mL) was added. The mixture was extracted several times with EtOAc and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative TLC (EtOAc−hexane 3:7), to give the title compound as a pale-yellow solid (yield 51%). ^{1}H NMR ($CDCl_3$) δ 1.44 (t, J = 7.2 Hz, 3H), 3.76 (s, 2H), 4.47 (q, J = 7.2 Hz, 2H), 6.66 (s, 1H), 6.70 (dd, J_1 = 2.0 Hz, J_2 = 8.0 Hz, 1H), 6.85 (br s, 1H), 6.90−6.99 (m, 2H), 7.16−7.22 (m, 1H), 7.32 (d, J = 16.4 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 13.8, 61.8, 101.4, 112.1, 113.4, 116.4, 117.9, 129.8, 135.4, 136.4, 146.8, 156.4, 159.7, 170.1. HRMS (ESI) calculated for $C_{14}H_{14}N_2O_3$ $[M + H]^+$ 259.1077, found 259.1082.

Acknowledgment. TB Alliance is acknowledged for financial support. A. Lilienkampf thanks The Academy of Finland (grant 120441) and The Finnish Cultural Foundation for fellowships. Dr. S. C. Cho and Dr. K. Sidell are acknowledged for the selectivity and the frequency of mutations assays.

Supporting Information Available: HPLC purity determinations for the target compounds **6**−**49** and a brief description of the biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) World Health Organization. *Global Tuberculosis Control—Surveillance, Planning, Financing*; World Health Organization: Geneva, **2008**; http://www.who.int/tb/publications/global_report/en/index.html (accessed February **2009**).
(2) Brooks, J. T.; Kaplan, J. E.; Holmes, K. K.; Benson, C.; Pau, A.; Masur, H. HIV-associated opportunistic infections—going, going, but not gone: the continued need for prevention and treatment guidelines. *Clin. Infect. Dis.* **2009**, *48*, 609–611.
(3) Zhang, Y.; Post-Martens, K.; Denkin, S. New drug candidates and therapeutic targets for tuberculosis therapy. *Drug Discovery Today* **2006**, *11*, 21–27.
(4) Rivers, E. C.; Mancera, R. L. New anti-tuberculosis drugs in clinical trials with novel mechanism of action. *Drug Discovery Today* **2008**, *13*, 23–24.
(5) Chaisson, R. E. Tuberculosis chemotherapy. Still a double-edged sword. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 1461–1466.
(6) Maggi, N.; Pasqualucci, C. R.; Ballotta, R.; Sensi, P. Rifampicin, a new orally active rifamycin. *Farmaco Sci.* **1966**, *21*, 68–75.
(7) *The Five Elements of DOTS*; World Health Organization: Geneva, **2009**; http://www.who.int/tb/dots/whatisdots/en/index.html (accessed February **2009**).
(8) Blumberg, H. M.; Burman, W. J.; Chaisson, R. E.; Daley, C. L.; Etkind, S. C.; Friedman, L. N.; Fujiwara, P.; Grzemska, M.; Hopewell, P. C.; Iseman, M. D.; Jasmer, R. M.; Koppaka, V.; Menzies, R. I.; O'Brien, R. J.; Reves, R. R.; Reichman, L. B.; Simone, P. M.; Starke, J. R.; Vernon, A. A. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: Treatment of Tuberculosis. *Am. J. Respir. Crit. Care Med.* **2003**, *15*, 603–662.
(9) (a) McKinney, J. D.; Jacobs, W. R. J.; Bloom, B. R. Persisting problems in Tuberculosis. In *Emerging Infections*; Krause, R. M., Ed.; Academic Press: New York, 1998; pp 51−146. (b) Honer zu Bentrup, K.; Russell, D. G. Mycobacterial persistence: adaptation to a changing environment. *Trends Microbiol.* **2001**, *9*, 597–605. (c) Boshoff, H. I. M.; Barry, C. E. Tuberculosis—metabolism and respiration in the absence of growth. *Nat. Rev. Microbiol.* **2005**, *3*, 70–80.
(10) Johnson, R.; Streicher, E. M.; Louw, G. E.; Warren, R. M.; Van Helden, P. D.; Victor, T. C. Drug resistance *Mycobacterium tuberculosis*. *Curr. Issues Mol. Biol.* **2006**, *8*, 97–112.
(11) (a) Dorman, S. E.; Chaisson, R. E. From magic bullets back to the magic mountain: the rise of extensively drug-resistant tuberculosis. *Nat. Med* **2007**, *3*, 295–298. (b) CNC. Extensively drug-resistant tuberculosis—United States, 1993−2006. MMWR. *Morb. Mortal. Wkly Rep.* **2007**, *56*, 250–253.
(12) Global alliance for tuberculosis drug development. Executive summary of the scientific blueprint for TB drug development. *Tuberculosis* **2001**, *81*, (Suppl.), 1−52.
(13) O'Brien, R. J.; Nunn, P. P. The need for new drugs against tuberculosis. Obstacles, opportunities, and next steps. *Am. J. Respir. Crit. Care Med.* **2001**, *163*, 1055–1058.
(14) Dunkan, K.; Sacchettini, J. D. Approaches to tuberculosis drug development. In: Hatfull, J. F., Jacobs, W. R. J., Eds.; *Molecular Genetics of Mycobacteria*; ASM Press: Washington, DC; 2000, 297−307.
(15) Zhang, Y. The magic bullets and tuberculosis drug targets. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 529–564.
(16) (a) Mao, J.; Wan, B.; Wang, Y.; Franzblau, S. G.; Kozikowski, A. P. HTS chemical hybridization, and drug design identify a chemically unique antituberculosis agents coupling serendipity and rational approaches to drug discovery. *ChemMedChem* **2007**, *2*, 811–813. (b) Mao, J.; Wang, Y.; Wan, B.; Kozikowski, A. P.; Franzblau, S. G. Design, synthesis, and pharmacological evaluation of mefloquine-based ligands as novel antituberculosis agents. *ChemMedChem* **2007**, *2*, 1624–1630.
(17) 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.
(18) Mao, J.; Yuan, H.; Wang, Y.; Pieroni, M.; Kim, K. H.; Huang, Q.; Wan, B.; van Breemen, R. B.; Kozikowski, A. P.; Franzblau, S. G. Unpublished results.

(19) Bravo, P.; Ricca, A.; Ticozzi, C.; Vajna de Pava, O. New α-oxo-γ-(enaminoalkyl)triphenylphosphonium salts and ylides from isoxazole derivatives: synthesis of α,γ-dioxox olefins. *Gazz. Chim. Ital*. **1976**, *106*, 743–759.
(20) DeShong, P.; Cipollina, J. A.; Lowmaster, N. K. A general method for the synthesis of tetramic acid derivatives. *J. Org. Chem*. **1988**, *53*, 1356–1364.
(21) 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-, 5-alkylthiomethyl-, and 5-dialkylaminomethylisoxazoles. *Heterocycles* **1985**, *23*, 571–583.
(22) Franzblau, S. G.; Witzig, R. S.; Mclaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. *J. Clin. Microbiol*. **1998**, *36*, 362–366.
(23) Cho, S. H.; Warit, S.; Wan, B.; Hwang, C. H.; Pauli, G. F.; Franzblau, S. G. Low-Oxygen-Recovery Assay for high-throughput screening of compounds against nonreplicating *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother*. **2007**, *51*, 1380–1385.
(24) Mao, J.; Yuan, H.; Wang, Y.; Wan, B.; Pak, D.; Lilienkampf, A.; Pieroni, M.; Franzblau, S. G.; Kozikowski, A. P. Unpublished results.
(25) Soellner, M. B.; Rawls, K. A.; Grundner, C.; Alber, T.; Ellman, J. A. Fragment-Based Substrate Activity Screening Method for the Identification of Potent Inhibitors of the *Mycobacterium tuberculosis* Phosphatase PtpB. *J. Am. Chem. Soc*. **2007**, *129*, 9613–9615.
(26) Omar, F. A.; Abdel-Hafez, A. A.; Ahmed, M. S. Design and synthesis of some p-substituted styrylisoxazole carboxylic acid derivatives as anti-inflammatory agents. *Bull. Pharm. Sci. Assiut Univ*. **2004**, *27*, 171–186.
(27) Mokhtar, H. M. Synthesis of nitrogenous compounds. Part II. *Pak. J. Sci. Ind. Res*. **1990**, *33*, 30–36.
(28) Soliman, G.; Rateb, L. Synthesis of heterocyclic compounds from δ-unsaturated 1,3-dioxo esters. *J. Chem. Soc*. **1956**, 3663–3668.

Downloaded by TATA INST OF FUNDAMENTAL RES on November 5, 2009 | http://pubs.acs.org
Publication Date (Web): September 16, 2009 | doi: 10.1021/jm900513a