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From Serendipity to Rational Antituberculosis Drug Discovery of Mefloquine-Isoxazole Carboxylic Acid Esters

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Both *in vitro* and *in vivo* metabolism studies suggested that 5-(2,8-bis(trifluoromethyl)quinolin-4-yloxymethyl)isoxazole-3-carboxylic acid ethyl ester (compound **3**) with previously reported antituberculosis activity is rapidly converted to two metabolites **3a** and **3b**. In order to improve the metabolic stability of this series, chemistry efforts were focused on the modification of the oxymethylene linker of compound **3** in the present study. Compound **9d** with an alkene linker was found to be both more metabolically stable and more potent than compound **3**, with a minimum inhibitory concentration (MIC) of 0.2 μM and 2.6 μM against replicating and nonreplicating *Mycobaterium tuberculosis*, respectively. These attributes make **9d** an interesting lead compound. A number of modifications were made to the structure of **9d**, and a series of active compounds were discovered. Although some neurotoxicity was observed at a high dosage, this new series was endowed with both improved *in vitro* anti-TB activity and metabolic stability in comparison to compound **3**.

Introduction

Tuberculosis (TB[a]) is presently regarded as one of the most dangerous infectious diseases worldwide and one of the major AIDS-associated infections. It has been estimated that more than 9.2 million people worldwide develop active TB, and 1.7 million die every year.[1] Despite these figures, no new anti-TB drugs have been introduced over the past 40 years.[2] Moreover, the current six-month treatment regimen required to cure the disease is accompanied by significant toxicity, making patient compliance difficult, and leading to the development of drug resistance. Successful treatment of multidrug resistant (MDR)-TB and extensively drug resistant (XDR)-TB is even more challenging and requires even longer term treatment.[3] Therefore, new drugs are urgently needed for the treatment of TB. 5-(2,8-Bis(trifluoromethyl)quinolin-4-yloxymethyl)isoxazole-3-carboxylic acid ethyl ester (compound **3**, structure shown in Figure 1), reported earlier by us as a promising antituberculosis lead, exhibited excellent activity against both replicating and nonreplicating *Mycobacterium tuberculosis (Mtb)*, with minimum inhibitory concentrations (MIC) of 0.9 μM and 12.2 μM, respectively.[4] Its activity appeared to be specific for organisms of the *Mtb* complex, and it effected significant reductions in colony forming units (CFU) in infected macrophages with an EC_{90} of 4.1 μM.[5] In this work, rational modifications of compound **3**, based on *in vitro* and *in vivo* assessment of its metabolic stability, are reported.

Metabolism of Compound 3 in Mouse and Human Liver Microsomes. After 30 min incubation of compound **3** with mouse and human liver microsomes in the presence of NADPH, two metabolite peaks were observed eluting at 16.1 and 24.7 min (**3a** and **3b**, named according to their retention times; Figure 1). Both were characterized by LC-MS-MS. The protonated form of **3a** was observed at m/z 282. On the basis of the nitrogen rule, **3a** was suspected to have only one nitrogen atom. A 20 mass unit loss was observed several times in the tandem mass spectrum of **3a** (Figure 2, 282 to 262, 214 to 194, 140 to 120), which is a characteristic loss of HF. This suggested that the structure of **3a** was related to the quinoline. Thus, the structure of **3a** was proposed to be 2,8-bis(trifluoromethyl)quinolin-4-ol (Figure 2). The protonated molecule of **3b** was observed at m/z 407, which is 28 mass units less than compound **3**, suggesting the loss of an ethyl group. The only possible site to lose an ethyl group in the structure is from the ester portion. The structure of **3b** was thus proposed to be the corresponding acid (Figure 2). After comparing the retention times and fragment patterns with those of the authentic compounds, the structures were confirmed as proposed. That these two metabolites were generated during the incubation of compound **3** in both mouse and human liver microsomes in the absence of NADPH (Figure 1) suggests the involvement of NADPH-independent enzymes. It was also noted that the acid **3b** can

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[a] Abbreviations: CMC, carboxymethyl cellulose; INH, isoniazid; LORA, low oxygen recovery assay; MABA, microplate Alamar Blue assay; MIC, minimum inhibitory concentration; MDR-TB, multidrug-resistant tuberculosis; *Mtb*, *Mycobacterium tuberculosis*; RMP, rifampin; TB, tuberculosis; XDRTB, extensively drug-resistant tuberculosis.

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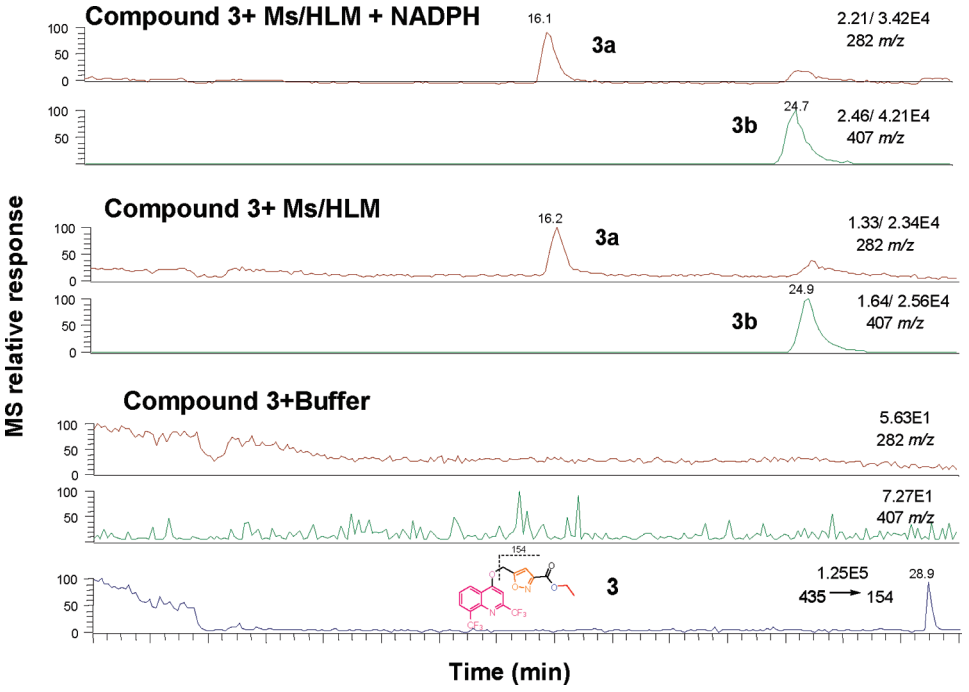


Figure 1. Metabolic profile of compound **3** in mouse and human liver microsomes.

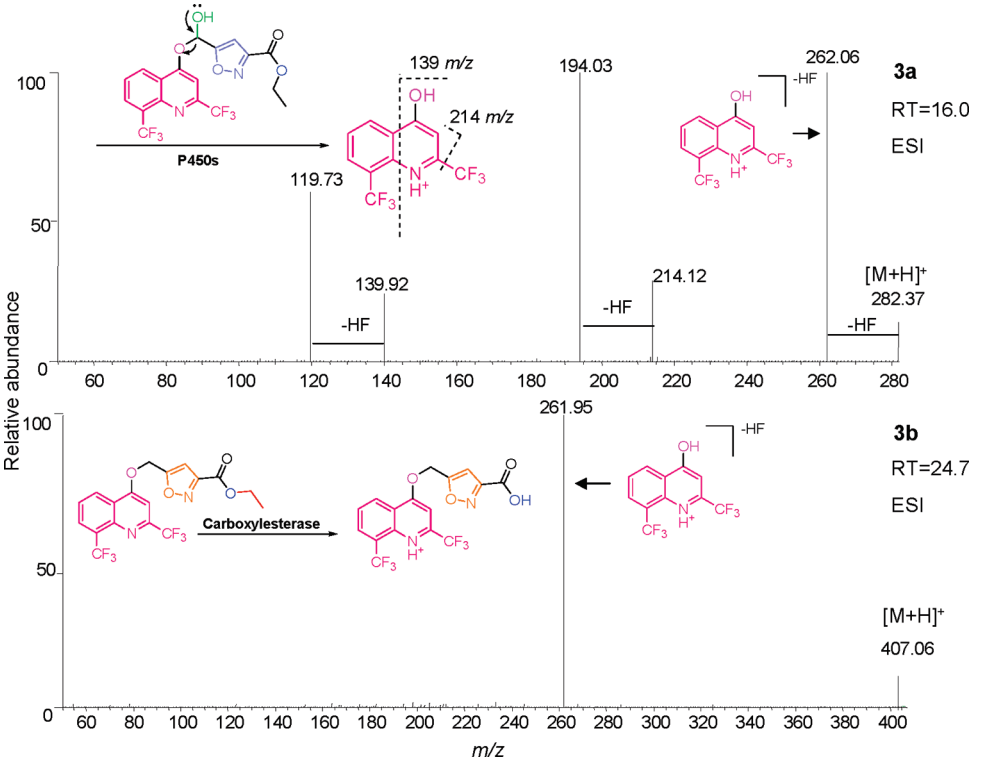


Figure 2. CID product ion mass spectra of metabolites **3a** and **3b**.

be metabolized into **3a** in both human and mouse liver microsomes. Interestingly, both metabolites were also observed after 30 min incubation of compound **3** in human serum (data not shown).

Plasma Concentrations of Compound 3 and Metabolites. BALB/c mice were dosed orally with a suspension of compound **3** at 400 mg/kg in carboxymethyl cellulose (CMC), and the plasma concentration of **3** and its metabolites were analyzed by LC-MS-MS. Consistent with the *in vitro* liver microsomal data, **3a** was the major metabolite detected in the mouse plasma. The C_{max} and T_{max} of **3a** were 2.71 μg/mL and 30 min, respectively. The acid **3b** could be detected at 5 min postdose. Unlike **3a**, the plasma concentration of **3b** (about 0.1 μg/mL) did not change appreciably over the 3 h time course, attaining a peak concentration of 0.40 μg/mL at 30 min. The highest plasma concentration of the parent compound **3** (0.026 μg/mL) occurred at 90 min postdose (Figure 3).

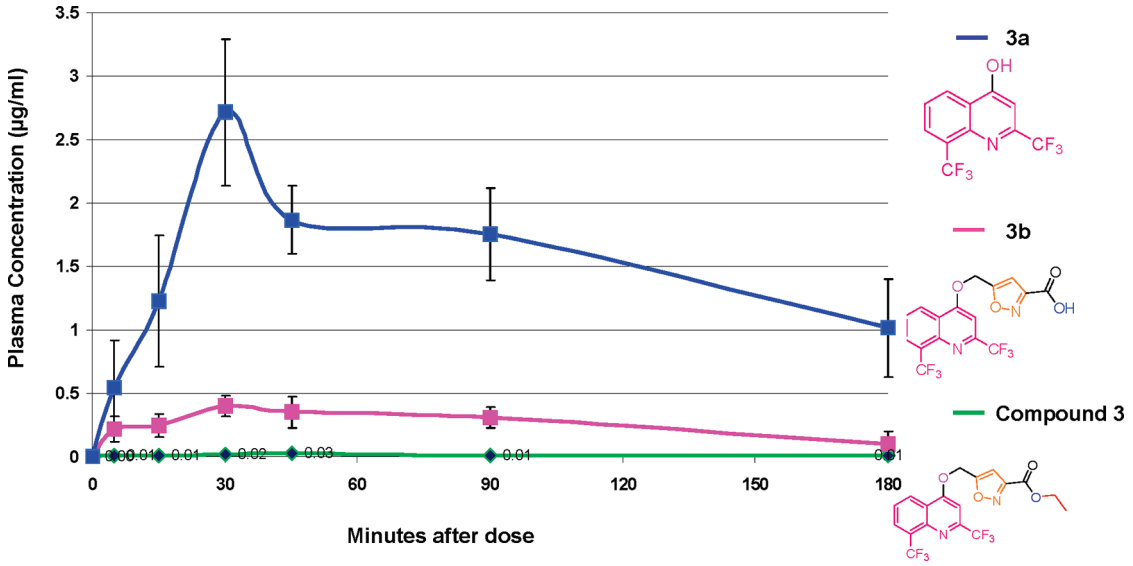
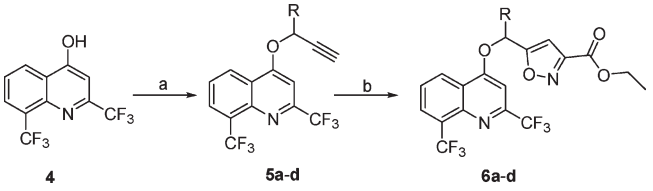


Figure 3. Compound **3** and its metabolites in plasma after oral dosing of mice.

Scheme 1. Synthesis of Analogues in Table 1



a. subsitituted propargyl bromides, K_2CO_3, acetone, reflux, overnight. b. ethyl chlorooximidaocetate, Et_2O, Et_3N, 6 h through syringe pump.

Neither of these metabolites exhibited antituberculosis activity in microplate Alamar Blue assay (MABA)[6] at pH 6.8. Therefore, in order to obtain *in vivo* efficacy in this series, chemistry efforts were focused on preventing the formation of **3a** and **3b**.

In order to avoid the biotransformation of **3b**, the ethyl ester was replaced by various ester bioisosteres, including the isomer ester, amides, and the oxadiazole analogues. None of them showed any antituberculosis activity (unpublished data).[5] These observations suggested that the ester may play a role as a prodrug, and the ethyl ester moiety was retained in the following modifications.

Metabolite **3a** was suspected to form through an oxidation step (Figure 2). Presumably, after the oxidation at the α-position of the isoxazole, **3a** is formed by breakdown of the hemiacetal. Therefore, several modifications on the oxymethylene linker of compound **3** were made with the intention of (1) increasing the steric hindrance at the soft spot and (2) replacing the oxymethylene linker with surrogate moieties.

Increasing Steric Hindrance at the Soft Spot. Various alkynes (**5a**−**d**) were prepared from 2,8-bis(trifluoromethyl)-quinolin-4-ol by reaction with substituted propargyl bromides in the presence of K_2CO_3 in anhydrous acetone. Next, nitrile oxide cycloaddition chemistry (NOC)[7] was performed by reacting the dipolarophiles **5a**−**d** with ethyl chlorooximidoacetate in the presence of triethylamine to afford compounds **6a**−**d** (Scheme 1). Remarkably, these modifications precluded the formation of **3a** when compounds **6a**−**d** were incubated in mouse liver microsomes for 30 min (Figure 4). However, the MICs (Table 1) evaluated with the MABA and the low oxygen recovery assay (LORA)[8] were poorly affected by the introduction of a racemic methyl group (compound **6a**) and a dimethyl group (compound **6b**). Insertion of more bulky groups such as a butyl group (compound **6c**) and a benzyl group (compound **6d**) led to a remarkable loss of activity.

Replacement of the Oxymethylene Linker. Removal of the oxymethylene linker between the quinoline and the isoxazole ring suggested the synthesis of compound **9a**. The intermediate **8** was prepared from 4-bromo-2,8-bis(trifluoromethyl)-quinoline (**7**) by reaction with trimethylsilylacetylene in the presence of $Pd(PPh_3)_4$ and CuI as catalysts, followed by treatment with KOH in methanol. The NOC reaction afforded 5-(2,8-bis(trifluoromethyl)quinolin-4-yl)isoxazole-3-carboxylic acid ethyl ester (**9a**) (Scheme 2). Although compound **9a** was stable as its corresponding acid form in mouse liver microsomes (Figure 5), it exhibited a 17-fold decrease in its anti-TB potency (MABA MIC = 16.5 μM, Table 2).

Next, the oxymethylene linker was replaced by an amide linker. 5-Hydroxymethylisoxazole-3-carboxylic acid ethyl ester was oxidized to its corresponding acid form (compound **11**) by use of Jone's reagent. The carbonyl chloride (compound **12**) was formed from compound **11** by treatment with oxalyl chloride. Compound **9b** was then generated by condensation of compound **12** and 2,8-bis(trifluoromethyl)-quinolin-4-ylamine (Scheme 2). Unfortunately, compound **9b** was not metabolically stable, as 2,8-bis(trifluoromethyl)-quinolin-4-ylamine was observed as the major metabolite after 30 min incubation in mouse liver microsomes (Figure 5). In addition, it showed less potency with a MABA MIC of 29.5 μM (Table 2).

A further modification involved replacement of the oxymethylene linker by an all carbon linker. Initially, the quinoline ring without any additional substitution was used to quickly compare the SAR with our previous SAR of the oxymethylene linker series.[5] The triphenylphosphonium ylide (**14**) was prepared from triphenylphosphine by reaction with 5-bromomethylisoxazole-3-carboxylic acid ethyl ester (**13**), a bromination product of 5-hydroxymethylisoxazole-3-carboxylic acid ethyl ester. Compounds **9c** and **9d** were then generated through the Wittig reaction. Compound **9d**

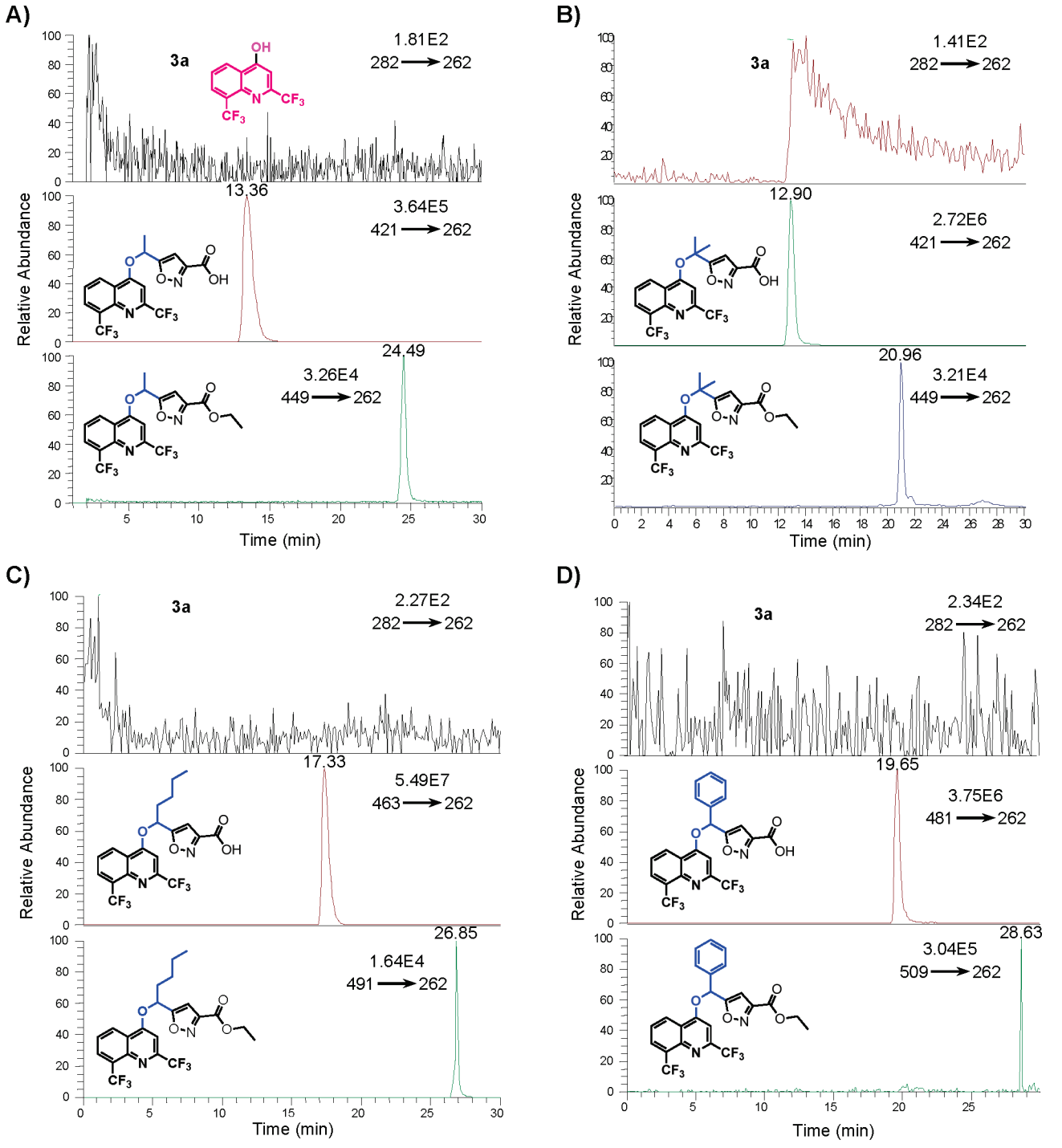


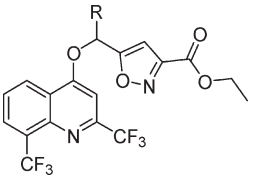
Figure 4. Metabolic profile of compounds **6a**−**d** in mouse liver microsomes.

was catalytically hydrogenated to afford compound **9e** (Scheme 2).

Surprisingly, compounds **9c** and **9d** (Table 2), endowed with an alkene linker between the 4-position of the quinoline ring and the 5-position of the isoxazole, had markedly improved antituberculosis activity compared with compound **3**, and the *trans* isomer **9d** (MABA MIC = 0.2 μM) was found to have a MIC only 2-fold higher than rifampin, the most active anti-TB drug used today. Furthermore, **9d** showed better activity than the corresponding *cis* isomer **9c** (MABA MIC = 0.6 μM), while the hydrogenation of the alkene (compound **9e**) resulted in a sharp decrease in potency with an MIC of 2.7 μM. A metabolic stability assay was performed for **9d**. Although the parent compound was hydrolyzed to the acid within 5 min during the incubation with mouse liver microsomes, 80% of the acid remained after 30 min incubation (Figure 6). In contrast to the lack of antituberculosis activity of compound **3** acid, compound **9d** acid was active against *M. tuberculosis* $H_{37}Rv$ in MABA at pH = 6.8 (MIC = 12.5 μM) (Table 2).

In order to evaluate the pharmacokinetic behavior of the corresponding acid of compound **9d**, the parent compound was administered using the same formulation and oral dosage as compound **3** (Table 3). Although the C_{max} of **9d** (0.03 μg/mL) was similar to that found for compound **3**, the C_{max} of the corresponding acid of compound **9d** (16.4 μg/mL, 61.7 μM,

Table 1. *In Vitro* Activity of Mefloquine-Based Analogues Against *M. tuberculosis* $H_{37}Rv$



Compd	Structure R =	MIC (μM) MABA	MIC (μM) LORA	IC_{50} (μM) Vero	SI MABA	SI LORA	ClogP
1	Mefloquine	13	7	11	0.9	1.6	3.6
RMP	Rifampin	0.1	2	122	1220	61	-
3	H	0.9	12.2	>128	>144	>10.5	4.4
6a	CH_3	4.2	20.7	>128	>30.5	>6.2	4.8
6b	$(CH_3)_2$	6.8	22.1	23.0	0.6	1.0	5.3
6c	$(CH_2)_3CH_3$	115.8	11.9	42.7	0.3	3.6	6.3
6d	C_6H_5	>128	>128	>128	-	-	6.0

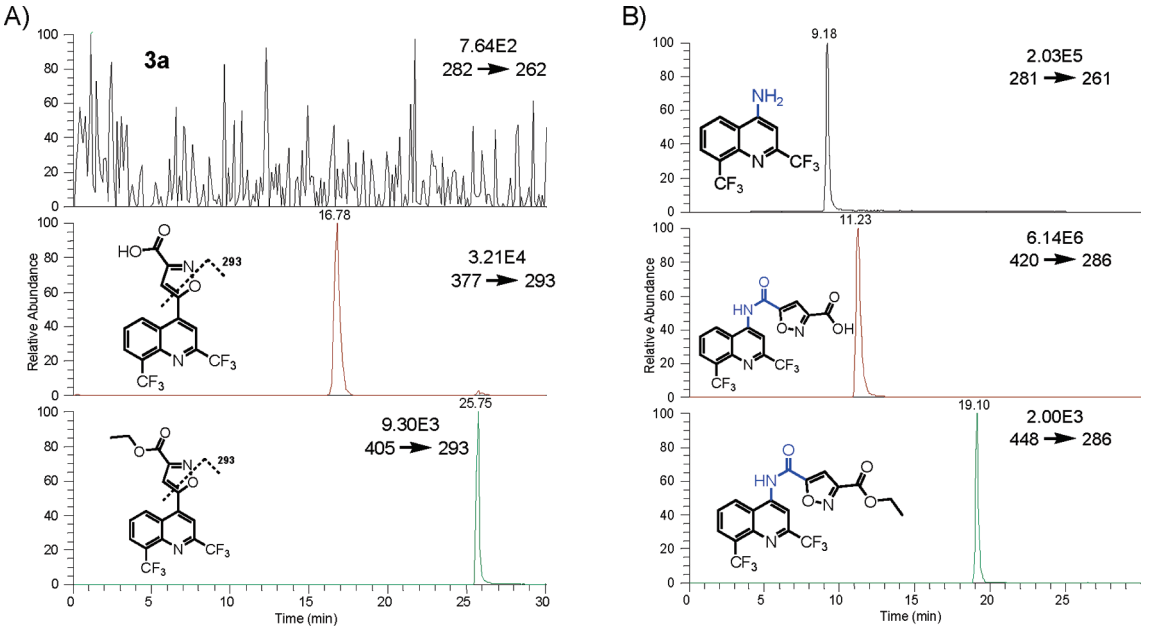


Figure 5. Metabolic profile of compounds **9a**,**b** in mouse liver microsomes.

almost 4-fold higher than its MIC at pH 6.8) was 60-fold higher than that of the corresponding acid of compound **3** (Table 3). The acid of compound **9d** also had a slightly longer half-life compared to that of compound **3** (1.9 h vs 1.6 h). Overall, the area under the curve (AUC) of the acid derived from **9d** was 70-fold higher than that of the acid derived from **3**.

These results indicate that, in addition to its improved *in vitro* anti-TB activity, compound **9d** is more metabolically stable than compound **3**, thus achieving our initial goals in modifying the linker of the latter.

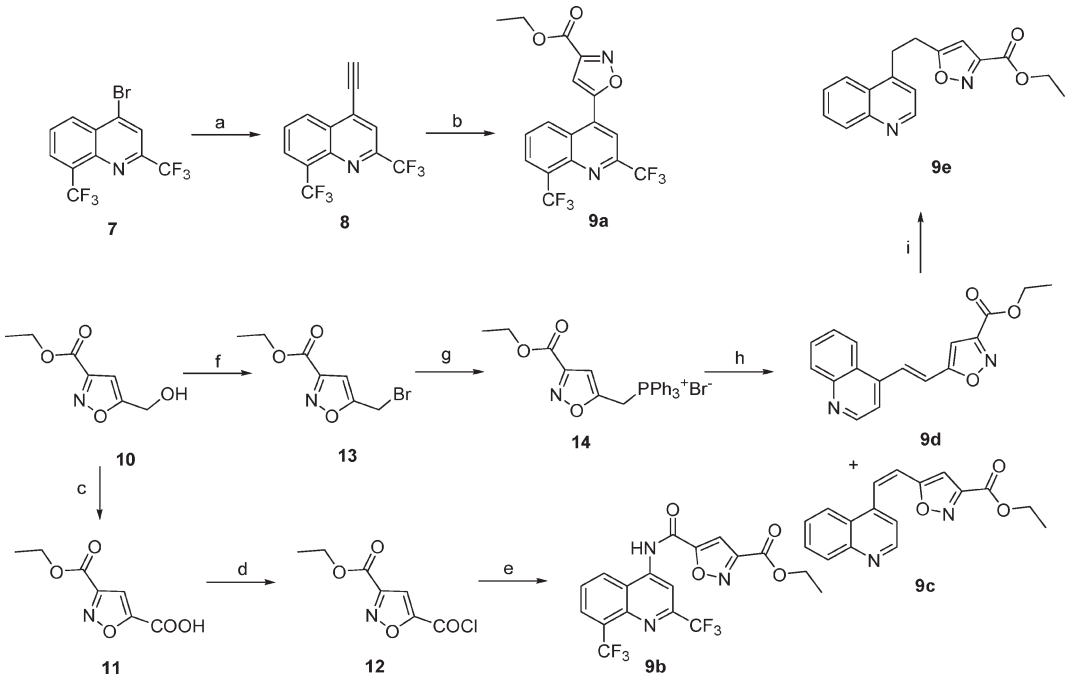
New Hybrids. Since the new alkene linker demonstrated both reasonable metabolic stability and an improved *in vitro* antituberculosis activity, we expected to design even more potent anti-TB ligands than compound **9d** in this alkene linker series by applying insights gained from the previous SARs studies derived from the oxymethylene linker series.[5]

Various alkene linker analogues **9f**−**h** and **9j**−**l** were generated through application of the Wittig reaction. Since some of required aldehydes were not commercially available, they were synthesized by two different routes with relatively low yields. Quinoline-6-carbaldehyde (**16**) was prepared from 6-methylquinoline by the Riley reaction with selenium dioxide at a high temperature, while aldehydes **17**, **20** and **21** were prepared by treatment of their corresponding bromides with *n*-butyllithium and then DMF (Scheme 3).

Based on the previously observed SAR with the oxymethylene linker,[5] the alkene linker was moved from the 4-position to the 6-position. The resulting *cis* isomer **9f** (MABA MIC = 1.3 μM) was slightly less active than the *trans* isomer **9g** (MABA MIC = 0.9 μM); however, both were about 4−6-fold less active than their corresponding 4-substituted compounds **9c** (MABA MIC = 0.6 μM) and **9d** (MABA MIC = 0.2 μM), respectively. These results suggested that the SAR gained from the oxymethylene linker might not be fully translatable to the new alkene linker series.

In order to confirm this, with the linker attached to the 4-position of the quinoline ring, the corresponding bis-(trifluoromethyl) analogue **9h** was prepared. According to our previous SAR, addition of two trifluoromethyl groups at

Scheme 2. Synthesis of Analogues in Table 2



a. (i) Me_3Si-CCH, Pd $(PPh_3)_4$, CuI, i-Pr_2NH, (ii) KOH/MeOH, 70%. b. ethyl chlorooximidaocetate, Et_2O, TEA, 6 h through syringe pump, 50%. c. Jone's reagent, acctone, 55 °C, 2 h. d. oxalyl chloride, CH_2Cl_2, rt, 3 h. e. 2,8-bis(trifluoromethyl)quinolin-4-amine, DIEA, CH_2Cl_2, 0 °C to rt, overnight. f. CBr_4/PPh_3, CH_2Cl_2, −15 °C, 2 h. g. PPh_3, acetonitrile, reflux, 2 h. h. NaHDMS, quinoline-4-carbaldehyde, THF, −78 °C to rt, 4 h. i. H_2, Pd/C, EtOAc, rt, 8 h.

the 2- and 8-positions of the quinoline ring boosted the antituberculosis activity more than 20-fold in the oxymethylene linker series.[5] In distinct contrast to the previous results, compound **9h** (MABA MIC = 2.7 μM) was 13-fold less active than the unsubstituted compound **9d** (MABA MIC = 0.2 μM). This trend was further observed in the saturated linker series as compound **9i** with 2,8-bis(trifloromethyl) groups (MABA MIC = 5.7 μM) was less active than the unsubstituted compound **9e** (MABA MIC = 2.7 μM).

These results indicate that the new series of analogues with carbon linkers exhibit a considerably different SAR compared with what we obtained from the previous oxymethylene linked analogues. To further explore the SAR of this new series, compounds **9j**−**k** with an electron-donating methoxyl substitution and compound **9l** with 2,6-bis(trifluoromethyl) substitutes were prepared and tested. The MABA MIC values of compound **9k** and **9l** (both at 0.4 μM) were on the same order as derivative **9d**, which were 7-fold more potent than the 2,8-bis(trifluoromethyl) compound **9h** (MABA MIC = 2.7 μM). In addition, compound **9k** was shown to be very potent in the LORA assay as well (LORA MIC = 1.1 μM).

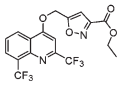
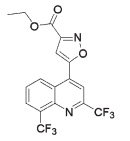
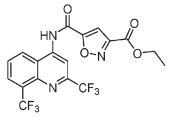
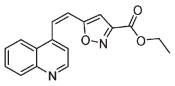
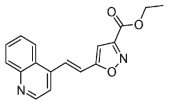
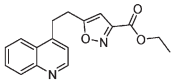
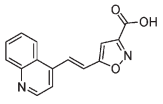
On the basis of the SAR obtained from **9c**−**l**, it is suggested that an alkene linker is important for activity, and a *trans*-alkene is more favored. The position(s) of the substitution(s) on the quinoline ring may also affect the activity to some extent, while the electron effect of the substitution(s) is less important. A pharmacophore model with the target protein structure may help to provide a more explicit SAR. On the basis of a literature search, peptide deformylase (PDF) and ATPase were considered as promising targets for the series. However, our preliminary studies suggested that neither of these are the target for the series, and there is no evidence to suggest an alternative target at this time. In addition, transposon mutants with resistance to compound **3** all mapped to genes with no known function (S. Warit, personal communication). Once the target has been determined, a systematic exploration of this carbon linker series will be carried out with the guidance of a pharmacophore model.

The most promising compound **9d** was chosen for an *in vivo* efficacy test. On the basis of previous work with other compounds in this series, maximum tolerance of compound **9d** was set to 400 mg/kg in CMC. At this dosage, neurotoxicity was observed in BALB/c mice. This suggested that *in vivo* toxicity assessment should be addressed earlier for later investigation in this series.

Summary

Compound **3**, previously reported as a promising antituberculosis lead, was found to be converted to metabolites **3a** and **3b** both *in vitro* and *in vivo*. Upon the basis of data gleaned from both metabolism and pharmacokinetic studies in concert with rational drug design principles, metabolically more stable derivatives endowed with potent anti-TB activity were synthesized. Thus, after various modifications of the oxymethylene linker, compound **9d** with an alkene linker was found to be both metabolically more stable and more potent than compound **3**, with a MABA MIC of 0.2 μM, only 2-fold higher than that of rifampin. Moreover, compound **9d** acid turned out to be active against *M. tuberculosis* $H_{37}Rv$ in MABA at pH 6.8 (MIC = 12.5 μM). The AUC of the acid derived from compound **9d** is 70-fold higher than the acid derived from **3** (**3b**) when dosed orally at 400 mg/kg in CMC, and the C_{max} of compound **9d** acid is almost 4-fold higher than its MIC. The fact that pyrazinamide (the first-line antituberculosis drug) and its active form pyrazinoic acid (POA) are only active *in vitro* at pH 5.6 but not at pH 6.8 suggests that a localized acidic

Table 2. *In Vitro* Activity of Analogues with Different Linkers Against *M. tuberculosis* $H_{37}Rv$[a]

Compd.	Structure R =	MIC (μM) MABA	MIC (μM) LORA	IC_{50} (μM) Vero	SI MABA	SI LORA	ClogP
1	Mefloquine	13	7	11	0.9	1.6	3.6
RMP	Rifampin	0.1	2	122	1220	61	-
3		0.9	12.2	> 128	> 144	> 10.5	4.4
9a		16.5	48.1	> 128	> 7.8	> 2.7	4.4
9b		29.5	> 128	> 128	> 4.3	-	3.6
9c		0.6	3.0	> 128	> 213	> 42.7	3.2
9d		0.2	2.6	> 128	> 640	> 49.2	3.2
9e		2.7	22.5	> 128	> 47.5	> 5.7	3.4
9d acid		12.5	NA	> 128	>10.3	NA	2.9

[a] NA: not available.

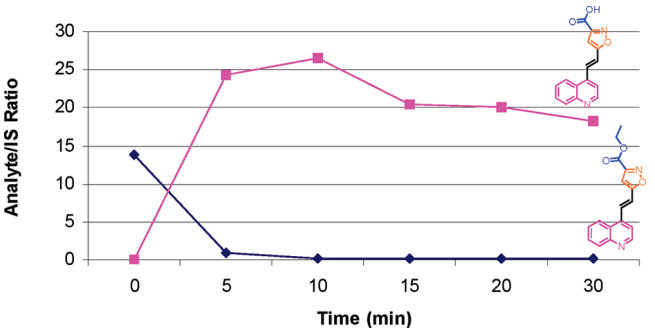


Figure 6. Mouse liver microsomal stability study of compound **9d**.

pH environment, which facilitates the penetration of POA, is likely produced in the lungs of TB patients as a consequence of inflammation.[9,10] As it is easier for acids to penetrate the cell wall under acidic conditions, the antituberculosis activity of compound **9d** acid may be superior *in vivo*. We have an ongoing effort to develop a robust low pH MIC assay to further test this hypothesis.

Table 3. Comparison of PK Profile of Compound **9d** and **3**

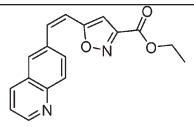
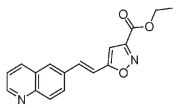
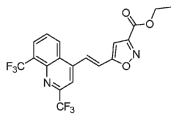
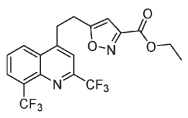
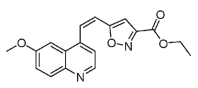
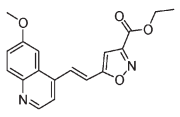
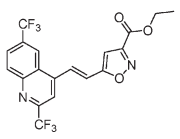
acid of compound	$T_{1/2}$ (h)	C_{max} (μM)	oral AUC (μM h)
9d	1.9	61.7	156.4
3	1.6	1.0	2.2

On the basis of these results, compound **9d** was identified as a new lead. A new series with an alkene linker was found to contain several potent compounds and SAR that differed from that observed with the oxymethylene linker. The new SAR suggested that, for this alkene series, the position(s) of substitution(s) plays a role in the antituberculosis activity. The target protein for these compounds is under investigation, and a systematic exploration of the alkene linker series will be carried out with early *in vivo* toxicity assessment.

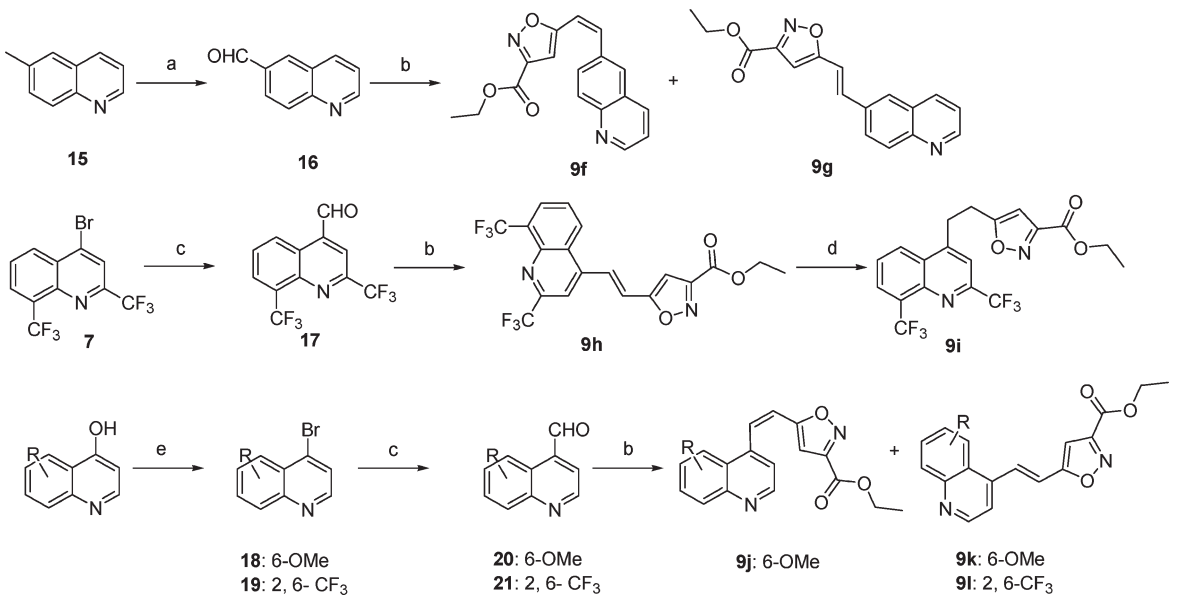
Experimental Section

1. Drug Metabolism and Pharmacokinetics. General Information. Reference compounds were synthesized in-house. Pooled mouse and human liver microsomes (protein concentration: 20 mg/mL) were purchased from XenoTech (Lenexa, KS).

Table 4. *In Vitro* Activity of Mefloquine Analogues with Carbon Linkers Against *M. tuberculosis* $H_{37}Rv$

Compd.	Structure	MIC (μM)		IC_{50} (μM)	SI		ClogP
		MABA	LORA	Vero	MABA	LORA	
9f		1.3	12.4	> 128	> 98.4	> 10.3	3.2
9g		0.9	9.3	> 128	> 142	> 13.8	3.2
9h		2.7	38.9	> 128	> 47.5	> 3.3	5.2
9i		5.7	12.6	> 128	> 22.5	> 10.2	5.4
9j		1.6	2.0	> 128	> 80	> 64	3.3
9k		0.4	1.1	> 128	> 320	> 116	3.3
9l		0.4	8.4	> 128	> 320	> 15.2	5.2

Scheme 3. Synthesis of Analogues in Table 4



a. SeO_2, 160 °C, 12 h, 33%. b. NaHDMS, 14, THF, −78 °C to rt, 4 h. c. (i) *n*-BuLi, Et_2O, −78 °C, 30 min; (ii) DMF, Et_2O, −78 °C, 3 h. d. H_2, Pd/C, EtOAc, rt, 8 h. e. PBr_3, DMF, rt, 2 h.

Table 5. SRMs and Retention Times of Mefloquine-Based Analogues Detected by LC-MS-MS

compound		SRM ion pairs (m/z)	CE (eV)	tube lens	RT (min)
6a	Parent	449 to 262	25	87	24.49
	Acid	421 to 262	25	87	13.36
	3a	282 to 262	21	96	-
6b	Parent	463 to 262	53	113	20.96
	Acid	435 to 262	27	92	12.90
	3a	282 to 262	21	96	-
6c	Parent	491 to 262	35	100	26.85
	Acid	463 to 262	35	100	17.33
	3a	282 to 262	21	96	-
6d	Parent	509 to 262	40	94	28.63
	Acid	481 to 262	40	94	19.65
	3a	282 to 262	21	96	-
9a	Parent	405 to 293	32	96	25.75
	Acid	377 to 293	37	92	16.78
	3a	282 to 262	21	96	-
9b	Parent	448 to 286	47	70	19.10
	Acid	420 to 286	47	70	11.23
	2,8-Bis(trifluoromethyl)-quinolin-4-amine	281 to 261	33	96	9.18

NADPH was purchased from Sigma Aldrich (St. Louis, MO). All solvents were HPLC grade and purchased from Fisher Scientific (Hanover Park, IL).

Microsomal Incubation. Compound stock solutions were prepared in acetonitrile. The final concentration of acetonitrile in the incubation media was 0.2% (v/v). The 100 μL incubation mixture contained a test compound (10 μM), 1 mM NADPH, and 2.0 mg/mL mouse or human liver microsomes in 50 mM potassium phosphate buffer (pH 7.4). Controls consisted of the same reaction mixtures without either NADPH or microsomes. The reaction mixture was prewarmed at 37 °C for 5 min before adding NADPH (1 mM). Incubations were carried out in a 37 °C water bath for 30 min, and the reaction was stopped by adding 300 μL acetonitrile. The samples were centrifuged at 4000*g* for 10 min before analysis using liquid chromatography−tandem mass spectrometry (LC-MS-MS).

Microsomal Stability Assay. Compound stock solutions were prepared in acetonitrile. The final concentration of acetonitrile in the incubation media was 0.2% (v/v). The stability of compound **9d** in microsomes was determined in triplicate after its incubation at 1 μM with mouse liver microsomes (0.5 mg/mL) in 50 mM potassium phosphate buffer (pH 7.4) at 37 °C. The total incubation volume was 0.8 mL. The reaction mixture was prewarmed at 37 °C for 5 min before adding NADPH (1 mM). Aliquots (100 μL) of the reaction mixture at 0, 5, 10, 15, 20, and 30 min were added to acetonitrile (300 μL) containing the internal standard. 5-(2-Quinolin-4-yl-ethyl)isoxazole-3-carboxylic acid ethyl ester at 0.01 μM. The samples were centrifuged at 4000*g* for 10 min before liquid chromatography/tandem mass spectrometry (LC-MS-MS) analysis of the parent compound. For control experiments, NADPH was omitted from these incubations.

LC-MS-MS Assay for Metabolite Identification. Qualitative assessments of compound metabolism were conducted using a Finnigan TSQ quantum triple quadrapole mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Reversed-phase HPLC separations during LC-MS-MS were carried out using an XTerra MS C_{18} column (2.1 × 150 mm, 2.5 μm, Waters) connected to a Waters 2695 HPLC system.

Method A. A binary gradient consisted of a mixture of a 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.2 mL/min. The LC gradient was programmed as follows: 15% to 70% B over 30 min, followed by an isocratic hold at 95% B over the next 5 min. The column was re-equilibrated for 7 min between injections. The column temperature was 30 °C, and the autosampler was maintained at 4 °C. Ionization was conducted in the positive ionization mode. The electronspray voltage was 3 kV, and the capillary temperature was 320 °C. Under these conditions, compound **3** and its most abundant metabolites eluted at 28.9, 24.9, and 16.1 min, respectively. Initial Q1 scans were performed between m/z 150 and 490. Tandem mass spectra were acquired at a collision energy of 30 eV using nitrogen as the collision gas at a pressure of 1.5 mTorr.

Method B. A binary gradient consisted of a mixture of a 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.2 mL/min. The LC gradient was programmed as follows: 50% to 65% B over 25 min, then followed by an isocratic hold at 95% B over the next 5 min. The column was re-equilibrated for 7 min between injections. The column temperature was 30 °C, and the autosampler was maintained at 4 °C. Ionization was conducted in the positive ionization mode. The electronspray voltage was 3 kV, and the capillary temperature is 320 °C. Initial Q1 scans were performed between m/z 150 and 550. The limit of detection (LOD) of 2,8-bis(trifluoromethyl)-quinolin-4-ol and quinolin-4-ol was 120 pM and 160 pM, respectively, and the retention times for these two compounds were 8.92 and 4.35 min, respectively. The ion transitions with the collision energy, tube lens, and retention times during selected reaction monitoring (SRM) for compounds **6a**−**d** and **9a**,**b** are listed in Table 5. Three SRMs were set up for each compound, including the parent, the corresponding acid, and the corresponding quinoline metabolite.

Dosing and *in Vivo* Sample Collection. Groups of twelve female BALB/c mice (20−22 g) were administered a single oral 400 mg/kg dose of compound **3** or **9d**. The dose was formulated in 0.5% carboxymethyl cellulose (CMC) at the target concentration of 20 mg/mL. Whole blood samples (250 μL) were collected aseptically from the retro orbital sinus at 0, 15, 30, 45, 90, and 120 min postdose. Plasma was prepared from the whole blood by centrifugation at 2000*g* for 30 min. Three volumes of acetonitrile containing the internal standard (IS1: 4-amino-2,8-bis(trifluoromethyl)quinoline 0.01 μM for compound **3**; IS2: 5-(2-quinolin-4-yl-ethyl)isoxazole-3-carboxylic acid ethyl ester 0.01 μM for compound **9d**) were added to each sample, and the samples were centrifuged at 4000*g* for 10 min before LC-MS-MS analysis.

LC-MS-MS Quantitation. Quantitation of compounds and their metabolites was carried out using LC-MS-MS with positive ion electrospray with selected reaction monitoring on a Finnigan TSQ quantum triple quadrupole mass spectrometer coupled with a Waters 2695 HPLC system. Separations were carried out using a Phenomenex (Torrance, CA) Luna C_{18} column 2.0 mm × 30 mm, 4 μm particle size. A binary gradient consisted of a mixture of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of

Table 6. SRMs, Retention Times, and Standard Curves of Compound **3** and Metabolites Detected by LC-MS-MS

compd.	SRM ion pairs (m/z)	CE (eV)	tub lens	RT (min)	standard curve
3	435 to 154	27	96	3.2	$Y = 0.7515x + 0.0007$ ($R = 0.9953$)
3a	282 to 262	21	96	2.4	$Y = 0.6067x + 0.0576$ ($R = 0.9980$)
3b	407 to 262	27	92	1.8	$Y = 0.1715x - 0.0029$ ($R = 0.9946$)
IS1	281 to 261	33	96	1.5	
9d	295 to 154	25	90	2.8	$Y = 0.6438x + 0.005$ ($R = 0.9938$)
9d acid	267 to 154	27	92	2.0	$Y = 0.0571x + 0.1702$ ($R = 0.9988$)
IS2	297 to 156	25	90	2.6	

0.4 mL/min. The HPLC gradient was programmed as follows: 40% to 65% B over 2.5 min, followed by an isocratic hold at 95% B over the next 1 min. The column was re-equilibrated for 1.5 min between injections. The column temperature was 30 °C, and the autosampler was maintained at 4 °C. The collision energy, tube lens, and retention time during selected reaction monitoring for compound **3**, **3a**, **3b**, IS1, compound **9d**, the acid of compound **9d**, and IS2 are included in Table 6. The ratio of peak area responses of analytes to IS was used to construct a standard curve.

PK Parameter Calculations. The half-life $T_{1/2}$ was calculated by the equation $T_{1/2} = 0.693/k$, and the areas of all trapezoids were calculated and summed to give AUC from $t = 0$ to $t = \infty$.

2. Chemistry. General Information. All starting materials were purchased from Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer at 400 or 300 MHz and 100 or 75 MHz, respectively, with TMS as an internal standard. Positive or negative ion mass spectra were measured using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) at an ionization potential of 70 eV; high resolution mass spectrometry (HRMS) was carried out using Micromass (Manchester, UK) hybrid quadrupole time-of-flight mass spectrometer (Q-TOF-2TM). Thin-layer chromatography (TLC) was performed with Merck 250 mm $60F_{254}$ silica gel plates. Column chromatography was performed using Merck silica gel (40−60 mesh). The purity of the target compounds was determined to be >98% by analytical HPLC.

4-(1-Methylprop-2-ynyloxy)-2,8-bis(trifluoromethyl)quinoline (5a, general procedure for 5a-d). To a solution of the 2,8-bis-(trifluoromethyl)quinolin-4-ol (1.000 g, 3.6 mmol) in dry acetone (25 mL) was added excess anhydrous K_2CO_3 (5 g) and the mixture was refluxed for 0.5 h. To the mixture, 3-bromobut-1-yne (0.47 g, 3.6 mmol) was added dropwise. The resulting mixture was refluxed for an additional 14 h and then cooled and filtered, and the filtrate was evaporated and then dried *in vacuo* to obtain a white solid **5a** (1.1 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, $J = 7.6$ Hz, 1H), 8.14 (d, $J = 7.2$ Hz, 1H), 7.64 (t, $J = 7.6$ Hz, 1H), 7.09 (s, 1H), 5.21 (q, $J = 6.4$ Hz, 1H), 2.64 (s, 1H), 1.94 (d, $J = 6.4$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.9, 149.2, 144.3, 128.9, 128.8, 125.9, 125.7, 124.6, 122.1, 119.4, 98.8, 79.8, 75.8, 64.6, 21.4.

4-(1,1-Dimethylprop-2-ynyloxy)-2,8-bis(trifluoromethyl)quinoline (5b). The synthesis is similar to compound **5a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.51 (d, $J = 8.0$ Hz, 1H), 8.16 (d, $J = 7.2$ Hz, 1H), 7.90 (s, 1H), 7.76 (t, $J = 7.2$ Hz, 1H), 2.82 (s, 1H), 1.93 (s, 1H).

4-(1-Ethynylpentyloxy)-2,8-bis(trifluoromethyl)quinoline (5c). The synthesis of **5c** is similar to compound **5a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.47 (d, $J = 7.6$ Hz, 1H), 8.15 (d, $J = 7.2$ Hz, 1H), 7.68 (t, $J = 7.6$ Hz, 1H), 7.28 (s, 1H), 5.07 (t, $J = 5.6$ Hz, 1H), 2.64 (s, 1H), 2.15 (m, 2H), 1.69 (m, 2H), 1.45 (m, 2H), 1.01 (t, $J = 7.2$ Hz, 3H).

4-(1-Phenylprop-2-ynyloxy)-2,8-bis(trifluoromethyl)quinoline (5d). The synthesis of **5d** is similar to compound **5a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, $J = 7.6$ Hz, 1H), 8.14 (d, $J = 7.2$ Hz, 1H), 7.64 (t, $J = 7.6$ Hz, 1H), 7.36 (m, 5H), 7.09 (s, 1H), 5.21 (s, 1H), 2.65 (s, 1H).

5-[1-(2,8-Bis(trifluoromethyl)quinolin-4-yloxy)ethyl]isoxazole-3-carboxylic acid Ethyl Ester (6a). To a vigorously stirred solution of ethyl chlorooximidoacetate (1.57 g, 10.34 mmol) and 4-(1-methylprop-2-ynyloxy)-2,8-bis(trifluoromethyl)quinoline (1.14 g, 3.4 mmol) in Et_2O (15 mL) was added triethylamine (1.45 mL, 10.34 mmol) in Et_2O (12 mL) via syringe pump over a 6 h period. The mixture was diluted with Et_2O (20 mL), the organic layer separated, washed with H_2O (10 mL), dried ($MgSO_4$), and concentrated *in vacuo* to afford an oil, which was purified by column chromatography (EtOAc/hexane = 1: 4) to give the product as a white solid with a yield of 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (d, $J = 7.6$ Hz, 1H), 8.19 (d, $J = 7.2$ Hz, 1H), 7.72 (t, $J = 7.6$ Hz, 1H), 7.17 (s, 1H), 6.74 (s, 1H), 5.96 (q, $J = 6.4$ Hz, 1H), 4.45 (q, $J = 7.2$ Hz, 2H), 1.98 (d, $J = 6.4$ Hz, 3H), 1.43 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 160.9, 159.3, 156.6, 149.2, 144.3, 129.7, 128.9, 126.6, 126.1, 124.8, 122.2, 119.4, 102.7, 98.3, 69.2, 62.5, 19.8, 14.1. Electrospray MS: m/z 449.0 ($[M + H]^+$, 100). Electrospray HRMS calcd for $[C_{19}H_{14}F_6N_2O_4 + H]^+$: 449.0936; found: 449.0914.

5-[1-(2,8-Bis(trifluoromethyl)quinolin-4-yloxy)-1-methylethyl]-isoxazole-3-carboxylic Acid Ethyl Ester (6b). The synthesis of **6b** is similar to that of **6a**. Yield: 54%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.49 (d, $J = 8.0$ Hz, 1H), 8.16 (d, $J = 7.2$ Hz, 1H), 7.90 (s, 1H), 7.76 (t, $J = 7.2$ Hz, 1H), 6.78 (s, 1H), 4.46 (q, $J = 7.2$ Hz, 2H), 2.06 (s, 6H), 1.43 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.0, 159.1, 158.9, 156.3, 148.6, 144.6, 129.1, 129.0, 128.1, 126.1, 124.5, 123.3, 121.7, 101.4, 62.2, 26.6, 13.7. Electrospray MS: m/z 461 ($[M - H]^-$, 100). Electrospray HRMS calcd for $[C_{20}H_{16}F_6N_2O_4 - H]^-$:461.0936; found: 461.0935.

5-[1-(2,8-Bis(trifluoromethyl)quinolin-4-yloxy)pentyl]isoxazole-3-carboxylic Acid Ethyl Ester (6c). The synthesis of **6c** is similar to that of **6a**. Yield: 52%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, $J = 7.6$ Hz, 1H), 8.20 (d, $J = 7.2$ Hz, 1H), 7.73 (t, $J = 7.6$ Hz, 1H), 7.10 (s, 1H), 6.98 (s, 1H), 5.78 (t, $J = 5.6$ Hz, 1H), 4.45 (q, $J = 7.2$ Hz, 2H), 2.18 (m, 2H), 1.67 (m, 2H), 1. 49 (m, 2H), 1.41 (t, $J = 7.2$ Hz, 3H), 1.01 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 160.9, 158.9, 156.2, 148.6, 144.5, 129.2, 128.6, 128.1, 126.3, 125.6, 125.6, 121.9, 102.7, 97.9, 72.8, 62.1, 33.6, 29.3, 26.5, 21.8, 13.7. Electrospray MS: m/z 491.0 ($[M + H]^+$, 100). Electrospray HRMS calcd for $[C_{22}H_{20}F_6N_2O_4 + H]^+$: 491.1405; found: 491.1400.

5-[(2,8-Bis(trifluoromethyl)quinolin-4-yloxy)phenylmethyl]isoxazole-3-carboxylic Acid Ethyl Ester (6d). The synthesis of **6d** is similar to that of **6a**. Yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (d, $J = 7.6$ Hz, 1H), 8.15 (d, $J = 7.2$ Hz, 1H), 7.66 (t, $J = 7.6$ Hz, 1H), 7.39 (m, 5H), 7.09(s, 1H), 6.54 (s, 1H), 5.56 (s, 1H), 4.40 (q, $J = 7.2$ Hz, 2H), 1.41 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167. 6, 159.1, 158.9, 156.3, 148.6, 144.6, 129.1, 129.0, 128.6, 128.5, 128.1, 126.5, 125.9, 125.6, 124.5, 123.3, 121.7, 102.4, 99.6, 73.0, 61.9, 13.8. Electrospray MS: m/z 509.0 ($[M - H]^-$, 100). Electrospray HRMS calcd for $[C_{24}H_{16}F_6N_2O_4 - H]^-$: 509.0936; found: 509.0955.

4-Ethynyl-2,8-bis(trifluoromethyl)quinoline (8). A mixture of 4-bromo-2,8-bis(trifluoromethyl)quinoline (0.52 g, 1.5 mmol), (trimethylsilyl) acetylene (0.45 g, 4.5 mmol), CuI (17.1 mg, 0.09 mmol), and $Pd(PPh_3)_4$ (102.9 mg, 0.09 mmol) in 15 mL of toluene−*i*-Pr_2NH (7:3) mixture was stirred at 80 °C in a sealed flask for 36 h. After allowing to cool to room temperature, the reaction mixture was diluted with chloroform and passed

through a short silica gel column eluting with chloroform. After concentrating *in vacuo*, the oily residue was purified by column chromatography on silica gel (EtOAc/hexanes 1:4) to afford a crude product as a dark-red crystalline material. It was dissolved in 40 mL of THF and added dropwise into a stirred solution of KOH (0.25 g, 4.5 mmol) in 100 mL of methanol. The resulting mixture was stirred at room temperature for 40 min, poured into water, and extracted with ether. The organic fraction was washed successively with water, conc. NH_4Cl solution, water, and brine, and dried over Na_2SO_4. Concentration *in vacuo* afforded a solid material, which was purified by column chromatography on silica gel (EtOAc/hexanes 1:10) to afford product with 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ, 8.23 (d, J = 7.2 Hz, 1H), 8.58 (d, J = 8.0 Hz, 1H), 7.82 (t, J = 7.6 Hz, 1H), 7.98 (s, 1H), 3.85 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.2, 132.7, 130.3, 129.0, 128.2, 127.1, 121.3, 108.2, 98.3, 88.0, 85.9.

5-(2,8-Bis(trifluoromethyl)quinolin-4-yl)isoxazole-3-carboxylic Acid Ethyl Ester (9a). The synthesis of **9a** is similar to that of **6a**. Yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.62 (d, J = 8.0 Hz, 1H), 8.31 (d, J = 7.2 Hz, 1H), 8.14 (s, 1H), 7.90 (t, J = 7.6 Hz, 1H), 7.11 (s, 1H), 4.46 (q, J = 7.2 Hz, 2H), 1.42 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.7, 163.5, 162.8, 157.0, 148.7, 144.6, 129.9, 128.6, 127.8, 125.8, 124.3, 123.4, 118.0, 99.8, 97.6, 62.4, 14.2. Electrospray MS: m/z 405.2 ([M + H]$^+$, 100). Electrospray HRMS calcd for $[C_{17}H_{10}F_6N_2O_3 + H]^+$: 405.0674; found: 405.0689.

3,5-Isoxazoledicarboxylic Acid, 3-Ethyl Ester (11). Potassium dichromate (0.95 g, 3.2 mmol) was added dropwise to concentrated sulfuric acid (5 mL), and the resulting mixture was diluted with water (5 mL). The diluted solution was slowly added to a solution of ethyl 5-(hydroxymethyl)-3-isoxazolecarboxylate (361 mg, 90%, 1.9 mmol) in acetone (40 mL). The reaction was stirred at 55 °C for 2 h, and the resulting mixture was extracted with ethyl acetate (40 mL × 2). Evaporation of the solvents provided the crude product (480 mg), which was dissolved in 0.5 M NaOH solution (20 mL) and washed with ethyl acetate (20 mL × 2). The aqueous phase was acidified with concentrated HCl to pH 2 and was extracted with ethyl acetate (20 mL × 2). The combined organic phase was dried over Na_2SO_4. Evaporation of the solvents provided **11** as an oil (310 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.71 (br s, 1H), 7.42 (s, 1H), 4.50 (q, 2H, J = 6.7 Hz), 1.45 (t, 3H, J = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.4, 159.4, 159.1, 157.3, 111.1, 63.1, 14.3.

5-Chlorocarbonyl-isoxazole-3-carboxylic Acid Ethyl Ester (12). To a solution of **11** (37 mg, 0.2 mmol) in dichloromethane (0.5 mL) was added a solution of oxalyl chloride in dichloromethane (2.0 M, 0.5 mL, 1.0 mmol). A solution of DMF in dichloromethane was added (1 drop of DMF was dissolved in 1.5 mL of dichloromethane, and 0.5 mL of the solution was used). The reaction was stirred at room temperature for 3 h. Evaporation of the solvents provided the crude **12** as an oil (40 mg, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.54 (s, 1H), 4.50 (q, 2H, J = 6.7 Hz), 1.45 (t, 3H, J = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.6, 158.4, 157.5, 154.8, 113.4, 63.2, 14.2.

Ethyl 5-[[[2,8-bis(trifluoromethyl)-4-quinolinyl]amino]carbonyl]-3-isoxazolecarboxylate (9b). To a solution of 2,8-bis-(trifluoromethyl)-4-quinolinamine (56 mg, 0.2 mmol) and diisopropylethylamine (78 μL, 0.4 mmol) in dichloromethane (1 mL) at 0 °C was slowly added a solution of crude **12** (40 mg, ~0.2 mmol) in dichloromethane (2 mL). The reaction was allowed to warm to room temperature and was stirred overnight. The resulting mixture was diluted with dichloromethane (20 mL), washed with 10% aqueous $KHSO_4$ (10 mL), saturated $NaHCO_3$ (10 mL), and brine (10 mL). The organic phase was dried over Na_2SO_4 and concentrated, and the residue was purified by column chromatography on silica gel (EtOAc/hexanes 1:6) to afford **9b** as a pale solid (50 mg, 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.71 (br s, 1H), 8.82 (d, 1H, J = 8.0 Hz), 8.68 (d, 1H, J = 4.0 Hz), 8.39 (d, 1H, J = 8.0 Hz), 7.99 (t, 1H, J = 8.0 Hz), 4.48 (q, 2H, J = 6.7 Hz), 1.42 (t, 3H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.0, 159.6, 158.3, 155.5, 149.1 (q, J = 34.7 Hz), 145.4, 144.4, 130.9 (q, J = 5.5 Hz), 129.0 (q, J = 29.9 Hz), 128.4, 128.3, 124.7 (q, J = 253 Hz), 126.1, 122.5 (q, J = 273 Hz), 111.4, 109.1, 63.1, 14.3. Electrospray HRMS: calcd for 446.0581 $[C_{18}H_{11}F_6N_3O_4 - H]^-$, found 446.0590.

Ethyl 5-Bromomethyl-3-isoxazolecarboxylate (13). To a solution of ethyl 5-(hydroxymethyl)-3-isoxazolecarboxylate **10** (1.54 g, 90%, 8.12 mmol) in dichloromethane (20 mL) at −15 °C was added triphenylphosphine (2.44 g, 9.35 mmol) and tetrabromomethane (3.03 g, 9.15 mmol). The reaction was stirred at the same temperature for 2 h. The resulting mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (EtOAc/hexanes 1:12) to give **13** as a colorless oil (1.56 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.74 (s, 1H), 4.51 (s, 2H), 4.45 (q, 2H, J = 7.2 Hz), 1.42 (t, 3H, J = 7.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 159.7, 156.9, 104.6, 62.6, 18.0, 14.3.

[[3-(Ethoxycarbonyl)-5-isoxazolyl]methyl]triphenylphosphonium Bromide (14). To a solution of **13** (1.56 g, 6.7 mmol) in acetonitrile (20 mL) was added triphenylphosphine (1.91 g, 7.25 mmol). The solution was refluxed for 2 h. The resulting mixture was allowed to cool to room temperature and was further cooled to 0 °C using an ice bath. The formed white solid was filtered and washed with ice-cool acetonitrile (1 mL × 2) to give **14** (2.76 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.8−8.0 (m, 9H), 7.6−7.8 (m, 6H), 7.12 (s, 1H), 6.20 (d, 2H, J = 12.0 Hz), 4.45 (d, 2H, J = 4.0 Hz), 1.42 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8 (J = 9.8 Hz), 159.2, 157.1, 135.7 (J = 2.7 Hz), 134.3 (J = 10.4 Hz), 130.6 (J = 13.0 Hz), 117.6 (J = 86.7 Hz), 108.4 (J = 7.1 Hz), 62.5, 24.4 (J = 51.8 Hz), 14.2.

***Z*-Ethyl 5-[2-(4-quinolinyl)ethenyl]-3-isoxazolecarboxylate (9c) and *E*-Ethyl 5-[2-(4-quinolinyl)ethenyl]-3-isoxazolecarboxylate (9d).** To a solution of **14** (39 mg, 0.079 mmol) in THF (1 mL) at −78 °C was added a solution of sodium hexamethyldisilazide in THF (1 M, 83 μL, 0.083 mmol). The mixture was stirred for 1 h at −30 °C and was cooled to −78 °C. A solution of 4-quinolinecarboxaldehyde (12.7 mg, 0.079 mmol) in THF (1 mL) was added dropwise. The resulting mixture was allowed to warm to room temperature and was stirred for 3 h. Saturated ammonium chloride solution was added at 0 °C to quench the reaction. Solvents were removed under reduced pressure, and the residue was repartitioned in dichloromethane (20 mL) and water (20 mL). The organic phase was separated, dried over Na_2SO_4, and concentrated. The residue was separated by column chromatography on silica gel (EtOAc/hexanes 1:1). Evaporation of the faster eluting fractions gave **9c** as a yellow solid (6 mg, 26%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.94 (d, 1H, J = 4.0 Hz), 8.19 (d, 1H, J = 8.0 Hz), 7.90 (d, 1H, J = 8.0 Hz), 7.78 (t, 1H, J = 8.0 Hz), 7.57 (t, 1H, J = 8.0 Hz), 7.36 (d, 1H, J = 4.0 Hz), 7.32 (d, 1H, J = 12.0 Hz), 6.96 (d, 1H, J = 12.0 Hz), 6.11 (s, 1H), 4.34 (q, 2H, J = 8.0 Hz), 1.33 (t, 3H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 159.8, 156.6, 150.2, 148.3, 142.6, 132.5, 130.4, 130.3, 127.6, 126.0, 124.5, 120.5, 118.9, 104.2, 62.4, 14.3. Electrospray HRMS: calcd 295.1077 for $[C_{17}H_{14}N_2O_3 + H]^+$, found 295.1088. Evaporation of the slower eluting fractions gave **9d** as a light yellow solid (12 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (d, 1H, J = 4.4 Hz), 8.1−8.2 (m, 3H), 7.78 (t, 1H, J = 9.2 Hz), 7.65 (t, 1H, J = 7.4 Hz), 7.59 (d, 1H, J = 4.8 Hz), 7.21 (d, 1H, J = 16.0 Hz), 6.83 (s, 1H), 4.49 (q, 2H, J = 8.0 Hz), 1.46 (t, 3H, J = 6.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.2, 160.0, 157.2, 150.3, 149.0, 140.7, 130.6, 130.5, 130.0, 127.5, 126.2, 123.4, 118.3, 117.6, 103.7, 62.6, 14.4. Electrospray HRMS: calcd 295.1077 for $[C_{17}H_{14}N_2O_3 + H]^+$, found 295.1088.

***E*-Ethyl 5-[2-(4-Quinolinyl)ethenyl]-3-isoxazolecarboxylic Acid (9d Acid).** To a solution of compound **9d** (0.58 g, 2 mmol)

in THF/H2O/MeOH (3: 1: 1, 15 mL) was added lithium hydroxide monohydrate (0.6 g, 13.6 mmol). The solution was allowed to stir for 4 h at ambient temperature and the solvent was removed in vacuo. The residue was dissolved in water and extracted with EtOAc (3 × 15 mL). The aqueous layer was acidified to pH 2 with 6 M HCl and extrated with EtOAc (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo to give a colorless product (0.5 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.96 (d, 1H, J = 4.4 Hz), 8.1−8.2 (m, 3H), 7.78 (t, 1H, J = 9.2 Hz), 7.63 (t, 1H, J = 7.4 Hz), 7.61 (d, 1H, J = 4.8 Hz), 7.21 (d, 1H, J = 16.0 Hz), 6.85 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7, 160.0, 157.2, 150.3, 149.5, 140.7, 130.6, 130.5, 130.0, 128.5, 126.2, 123.4, 118.3, 117.6, 103.7. Electrospray HRMS: calcd 266.0727 for $[C_{15}H_{10}N_2O_3 + H]^+$, found 266.0720.

Ethyl 5-[2-(4-Quinolinyl)ethyl]-3-isoxazolecarboxylate (9e). To a solution of **9d** (7 mg) in ethyl acetate (3 mL) was added 5% palladium on activated carbon (2 mg). The mixture was stirred at room temperature under a H_2 atmosphere for 8 h. The mixture was extracted by ethyl acetate, and the palladium on carbon was removed by filtration through a silica gel pad. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel (EtOAc/hexanes 1:1) to give **9e** as a light yellow solid (7 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.83 (d, 1H, J = 4.4 Hz), 8.16 (d, 1H, J = 4.4 Hz), 8.03 (d, 1H, J = 4.4 Hz), 7.75 (t, 1H, J = 7.2 Hz), 7.62 (t, 1H, J = 7.2 Hz), 7.21 (d, 1H, J = 4.4 Hz), 6.41 (s, 1H), 4.43 (q, 2H, J = 9.3 Hz), 3.53 (t, 2H, J = 8.0 Hz), 3.30 (t, 2H, J = 8.0 Hz), 1.41 (t, 3H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.6, 160.2, 156.7, 150.4, 148.6, 145.4, 130.7, 129.7, 127.2, 123.0, 120.9, 102.5, 62.4, 29.9, 27.1, 14.4. Electrospray HRMS: calcd 297.1077 for $[C_{17}H_{14}N_2O_3 + H]^+$, found 297.1074.

6-Quinolinecarboxaldehyde (16). 6-Methylquinoline (2.0 g, 13.8 mmol) was heated to 160 °C and selenium dioxide (1 g, 9.2 mmol) was added. The mixture was stirred for 12 h, cooled to room temperature, and diluted with ethyl acetate (10 mL). The solution was decanted, and the residue was extracted with ethyl acetate (10 mL × 2). The combined organic phase was concentrated, and the residue was purified by column chromatography on silica gel (EtOAc/hexanes 1:2) to give **16** as a light gray solid (715 mg, 33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.18 (s, 1H), 9.04 (dd, 1H, J_1 = 4.0 Hz, J_2 = 4.0 Hz), 8.3 (m, 2H,), 8.2 (m, 2H), 7.50 (dd, 1H, J_1 = 8.0 Hz, J_2 = 4.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 191.4, 153.1, 150.8, 137.4, 134.3, 133.7, 130.8, 127.7, 126.6, 122.2.

2,8-Bis(trifluoromethyl)-4-quinolinecarboxaldehyde (17). To a solution of *n*-BuLi (2 M in hexanes, 0.49 mL, 0.49 mmol) in anhydrous diethyl ether (10 mL) at −78 °C was slowly added a solution of 4-bromo-2,8-bis(trifluoromethyl)quinoline (334 mg, 0.97 mmol) in diethyl ether (4 mL). The resulting mixture was stirred at −78 °C for 30 min. A solution of anhydrous DMF (87 μL, 1.2 mmol) was added dropwise. The reaction was stirred at −78 °C for 3 h and quenched by water. Diethyl ether (50 mL) was added, and the organic phase was washed with saturated NH_4Cl (25 mL), saturated $Na_2S_2O_3$ (25 mL) and brine (25 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexanes 1:8) to give **17** as a light yellow solid (105 mg, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.56 (br s 1H), 9.31 (d, 1H, J = 8.0 Hz), 8.29 (d, 1H, J = 8.0 Hz), 8.23 (s, 1H), 7.94 (t, 1H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 191.4, 149.2 (q, J = 36.3 Hz), 146.0, 138.9, 130.4, 130.0 (q, J = 5.3 Hz), 129.4 (q, J = 30.5 Hz), 129.0, 125.2, 123.5 (q, J = 272 Hz), 122.4, 121.0 (q, J = 274 Hz).

4-Bromo-6-methoxyquinoline (18). To a solution of 6-methoxy-4-quinolinol (876 mg, 5 mmol) in anhydrous DMF (8 mL) under a N_2 atmosphere was added PBr_3 (484 μL, 5.15 mmol) dropwise. The reaction was stirred at room temperature for 2 h and quenched with water. The pH of the resulting mixture was adjusted to 10 by adding $NaHCO_3$. The formed precipitate was separated by filtration, washed with water, and dried under reduced pressure to give **18** as a white solid (0.83 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.53 (d, 1H, J = 4.8 Hz), 8.00 (d, 1H, J = 8.4 Hz), 7.66 (d, 1H, J = 4.8 Hz), 7.40 (m, 2H), 3.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.2, 147.4, 145.3, 132.7, 131.7, 129.3, 125.5, 123.5, 104.6, 55.9.

4-Bromo-2,6-bis(trifluoromethyl)quinoline (19). The synthesis of **19** is similar to that of **18**. Yield: 77%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H), 8.38 (d, 1H J = 8.8), 8.14 (s, 1H), 8.06 (dd, 1H, J_1 = 1.6, J_2 = 8.8). ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.2, 148.2, 136.6, 131.2, 127.5, 127.0, 124.5, 121.7.

6-Methoxy-4-quinolinecarboxaldehyde (20). The synthesis of **20** is similar to that of **17**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.39 (s, 1H), 9.02 (d, 1H, J = 4.0 Hz), 8.43 (s, 1H), 8.07 (d, 1H, J = 8.0 Hz), 7.72 (d, 1H, J = 4.0 Hz), 7.43 (d, 1H, J = 8.0 Hz), 3.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 193.7, 160.6, 147.6, 145.9, 135.3, 131.4, 127.4, 125.3, 123.3, 102.5, 55.9.

2,6-Bis(trifluoromethyl)quinoline-4-carbaldehyde (21). The synthesis of **21** is similar to that of **17**. The product was used for the next step without purification.

***Z*-Ethyl 5-[2-(6-quinolinyl)ethenyl]-3-isoxazolecarboxylate (9f) and *E*-Ethyl 5-[2-(6-quinolinyl)ethenyl]-3-isoxazolecarboxylate (9g).** The synthesis of **9f**,**g** is similar to that of **9c**,**d**. **9f**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.95 (d, 1H, J = 2.4 Hz), 8.12 (dd, 2H, J_1 = 13.6 Hz, J_2 = 8.4 Hz), 7.86 (s, 1H), 7.58 (d, 1H, J = 16.8 Hz), 7.43 (dd, 1H, J_1 = 8.4 Hz, J_2 = 4.0 Hz), 7.10 (d, 1H, J = 12.8 Hz), 6.64 (d, 1H, J = 12.4 Hz), 6.46 (s, 1H), 4.39 (q, 2H, J = 6.7 Hz), 1.37 (t, 3H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 160.0, 156.6, 151.4, 148.3, 136.46, 136.41, 133.9, 130.1, 129.8, 128.3, 128.1, 121.9, 115.6, 103.8, 62.4, 14.3. Electrospray HRMS: calcd 295.1077 for $[C_{17}H_{14}N_2O_3 + H]^+$, found 295.1074. **9g**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.93 (d, 1H, J = 2.4 Hz), 8.15 (dd, 2H, J_1 = 22.0 Hz, J_2 = 8.0 Hz), 7.9−8.0 (m, 2H), 7.58 (d, 1H, J = 16.4 Hz), 7.44 (dd, 1H, J_1 = 8.4 Hz, J_2 = 4.4 Hz), 7.12 (d, 1H, J = 16.4 Hz), 6.74 (s, 1H), 4.48 (q, 2H, J = 7.1 Hz), 1.45 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 160.2, 157.0, 151.3, 149.0, 136.5, 135.4, 133.5, 130.5, 128.6, 128.3, 126.9, 122.1, 113.8, 102.3, 62.5, 14.4. Electrospray HRMS: calcd 295.1077 for $[C_{17}H_{14}N_2O_3 + H]^+$, found 295.1075.

***E*-Ethyl 5-[2-[2,8-bis(trifluoromethyl)-4-quinolinyl]ethenyl]-3-isoxazolecarboxylate (9h).** The synthesis of **9h** is similar to that of **9c**,**d**. Only the *E*-isomer was obtained. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (d, 1H, J = 4.4 Hz), 8.24 (d, 1H, J = 6.8 Hz), 8.16 (d, 1H, J = 16.4 Hz), 7.99 (s, 1H), 7.83 (t, 1H, J = 8.0 Hz), 7.31 (d, 1H, J = 16.0 Hz), 6.88 (s, 1H), 4.49 (q, 2H, J = 6.7 Hz), 1.46 (t, 3H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 159.7, 157.3, 148.5 (q, J = 35.1 Hz), 144.5, 143.6, 129.7 (q, J = 5.3 Hz), 129.6 (q, J = 30.3 Hz), 128.7, 128.0, 127.7, 127.1, 123.6 (q, J = 272 Hz), 121.3 (q, J = 234.2 Hz), 120.3, 114.2, 104.9, 62.7, 14.3. Electrospray HRMS: calcd 431.0825 for $[C_{19}H_{12}F_6N_2O_3 + H]^+$, found 431.0812.

Ethyl 5-[2-[2,8-Bis(trifluoromethyl)-4-quinolinyl]ethyl]-3-isoxazolecarboxylate (9i). The synthesis of **9i** is similar to that of **9e**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (d, 1H, J = 8.4 Hz), 8.19 (d, 1H, J = 7.2 Hz), 7.79 (t, 1H, J = 7.8 Hz), 7.66 (s, 1H), 6.46 (s, 1H), 4.44 (q, 2H, J = 7.2 Hz), 3.63 (t, 2H, J = 8.0 Hz), 3.33 (t, 2H, J = 7.8 Hz), 1.41 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6, 159.9, 156.8, 148.5, 148.4 (q, J = 35.2 Hz), 144.0, 129.8 (q, J = 31.0 Hz), 129.3 (q, J = 5.3 Hz), 128.2, 127.8, 127.3, 123.7 (q, J = 272 Hz), 121.2 (q, J = 273.7 Hz), 117.4, 102.7, 62.5, 30.3, 27.1, 14.3. Electrospray HRMS: calcd 433.0981 for $[C_{19}H_{14}F_6N_2O_3 + H]^+$, found 431.0975.

***Z*-Ethyl 5-[2-(6-Methoxy-4-quinolinyl)ethenyl]-3-isoxazolecarboxylate (9j) and *E*-Ethyl 5-[2-(6-methoxy-4-quinolinyl)ethenyl]-3-isoxazolecarboxylate (9k).** The synthesis of **9j**,**k** is similar to that of **9c**,**d**. **9j**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (d, 1H, J = 4.4 Hz), 8.07 (d, 1H, J = 9.2 Hz), 7.41 (dd, 2H, J_1 = 8.4 Hz, J_2 = 2.4 Hz), 7.32 (s, 1H), 7.26 (d, 1H, J = 13.2 Hz), 7.07 (d, 1H, J = 2.4 Hz), 6.94 (d, 1H, J = 12.4 Hz), 6.13 (s, 1H), 4.34

(q, 2H, J = 6.7 Hz), 3.88 (s, 3H), 1.33 (t, 3H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4, 159.7, 158.4, 156.5, 147.6, 144.8, 140.6, 132.8, 131.9, 126.9, 122.8, 120.6, 118.6, 103.9, 102.0, 62.4, 55.7, 14.2. Electrospray HRMS: calcd 325.1183 for $[C_{18}H_{16}N_2O_4 + H]^+$, found 325.1194. **9k**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.78 (d, 1H, J = 4.4 Hz), 8.05 (s, 1H), 8.02 (d, 1H, J = 12.8 Hz), 7.51 (d, 1H, J = 4.4 Hz), 7.41 (dd, 1H, J_1 = 9.2 Hz, J_2 = 2.0 Hz), 7.32 (s, 1H), 7.17 (d, 1H, J = 16.4 Hz), 6.80 (s, 1H), 4.48 (q, 2H, J = 6.7 Hz), 3.99 (s, 3H), 1.45 (t, 3H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 159.9, 158.5, 157.1, 147.6, 145.1, 139.2, 131.9, 130.7, 127.2, 122.5, 117.9, 117.7, 103.5, 101.2, 62.5, 55.9, 14.3. Electrospray HRMS: calcd 325.1183 for $[C_{18}H_{16}N_2O_4 + H]^+$, found 325.1192.

***Z*-Ethyl-5-[2-(2,6-bis(trifluoromethyl)quinolin-4-yl)vinyl]isoxazole-3-carboxylate (9l).** The synthesis of **9l** is similar to that of **9d**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.84 (s, 1H), 8.42 (d, J = 9.0, 1H), 8.15 (d, J = 16.2, 1H), 8.07 (d, J = 9.0, 1H), 8.01 (s, 1H), 7.36 (d, J = 16.2, 1H), 6.93 (s, 1H), 4.51 (q, J = 7.2, 2H), 1.48 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.0, 159.5, 157.1, 150.1, 149.7, 148.8, 144.3, 132.3, 130.7, 128.0, 126.7, 125.7, 121.3, 121.2, 120.5, 114.3, 104.8, 65.5, 14.1. Electrospray MS: m/z 431.0 ([M+ H]$^+$, 100). Electrospray HRMS calcd for $[C_{19}H_{12}F_6N_2O_3 + H]^+$: 431.0825; found 431.0816.

3. Biology. General Information. Minimum inhibitory concentrations against replicating and nonreplicating cultures of *Mycobacterium tuberculosis* H37Rv were performed using the microplate Alamar Blue assay (MABA)[6] and low oxygen recovery assay (LORA)[8] as previously described. Cytotoxicity for green monkey kidney (VERO) cells was determined following 72 h exposure as previously described.[11]

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Supporting Information Available: HPLC purity determinations of the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org

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