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ARTICLE

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Structure−Activity Relationships of Antitubercular Nitroimidazoles. 3. Exploration of the Linker and Lipophilic Tail of ((*S*)-2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3] oxazin-6-yl)-(4-trifluoromethoxybenzyl)amine (6-Amino PA-824).

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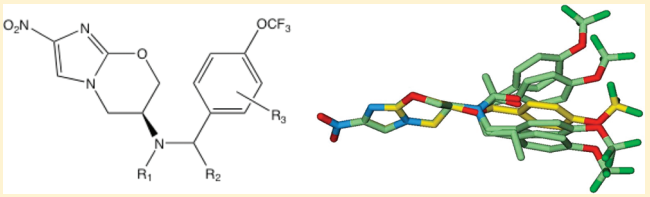
ABSTRACT: The (*S*)-2-nitro-6-(4-(trifluoromethoxy)benzyloxy)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine named PA-824 (**1**) has demonstrated antitubercular activity in vitro and in animal models and is currently in clinical trials. We synthesized derivatives at three positions of the 4-(trifluoromethoxy)-benzylamino tail, and these were tested for whole-cell activity against both replicating and nonreplicating *Mycobacterium tuberculosis* (Mtb). In addition, we determined their kinetic parameters as substrates of the deazaflavin-dependent nitroreductase (Ddn) from Mtb that reductively activates these pro-drugs. These studies yielded multiple compounds with 40 nM aerobic whole cell activity and 1.6 μM anaerobic whole cell activity: 10-fold improvements over both characteristics from the parent molecule. Some of these compounds exhibited enhanced solubility with acceptable stability to microsomal and in vivo metabolism. Analysis of the conformational preferences of these analogues using quantum chemistry suggests a preference for a pseudoequatorial orientation of the linker and lipophilic tail.



INTRODUCTION

The nitroimidazo-oxazines, including PA-824 (**1**),[1] and nitroimidazo-oxazoles, including OPC-67683[2,3] (Figure 1), are candidates for the treatment of tuberculosis (TB) that are currently in phase 2 clinical trials.[4] These compounds have shown good activity in animal models of disease both as single agents and in combination studies.[5−8] This class has attracted attention because of its ability to not only kill rapidly dividing bacteria but also to kill quiescent bacteria maintained under hypoxic conditions.[1] It is hoped that this activity against nonreplicating organisms may allow the shortening of the 6−8 month duration of TB chemotherapy required to achieve a durable cure.[9]

These nitroimidazoles are pro-drugs and require bioreductive activation to exert their bactericidal effect.[10] In *Mycobacterium tuberculosis* (*Mtb*) this reduction is mediated by a deazaflavin-dependent nitroreductase, Ddn,[11] and reduction occurs on the imidazole ring, resulting in the elimination of bactericidal reduced nitrogen species such as nitric oxide.[12] The anaerobic activity of compounds in this series correlates with the extent of production of reduced nitrogen species, and both aerobic and anaerobic activities correlate roughly with the efficiency of enzymatic reduction by Ddn.[13]

Recently, a significant amount of information on structure−activity relations (SAR) within this series of molecules has begun to emerge both in terms of whole cell activity and Ddn substrate preferences. The key determinants of these molecules as substrates include: an *S* configuration at C-6 of the oxazine ring, the nitro group, an 8-oxy substituted bicyclic nitroimidazole, and a lipophilic trifluoromethoxybenzyl tail.[14] The lipophilic tail has been the subject of considerable optimization chemistry since the series was first reported in the patent literature.[15,16] A wide variety of substituted aromatics have been employed, in general, with the most significant improvements in activity occurring with *para* substituents. The coincidental finding that *p*-trifluoromethoxy substitution (which appears in both candidate drugs) with linkers that vary in length by 4−5 Å consistently provided the most active derivatives prompted us to explore that more systematically with a homologous series of linkers increasing in carbon length. This study showed an optimal spacing of four carbons between the ether oxygen and the aromatic ring.[14] These findings and the extended lipophilic tail present in some

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analogues inspired further exploration of various substituted biphenyl analogues of this tail.[17] This study reported a correlation between lipophilicity of the side chain and identified analogues with significantly improved in vivo activity in a mouse model.

We have previously derived a 3D-quantitative structure−activity relationship (QSAR) pharmacophore model that was reasonably predictive for aerobic MIC among a series of 21 training and 22 test nitroimidazoles.[14] Although the best model suggested only one hydrophobic feature, other models suggested the possibility of two distinct hydrophobes in the tail region of **1** and raised the prospect that additional analogues could be synthesized, which would simultaneously engage both features. In this paper, we describe our attempts to explore this hypothesis with three series of analogues synthesized primarily from the more soluble 6-*S*-amino series of compounds. The first (R_1) employs the amine as an attachment site, the second (R_2) focuses on the benzylic carbon, and the third (R_3) explores additional diversity on the aromatic nucleus of the trifluoromethoxybenzyl moiety of the parent compound.

Chemistry. *R_1 Modifications.* **2** was synthesized as previously reported[14] and utilized as a starting point for R_1 (Scheme 1). Amide derivatives **4a**−**f** were synthesized by reacting **2** with the corresponding acyl chlorides in presence of NaH in DMF. Formylation of **2** with formic acid in the presence of acetic anhydride in THF yielded the *N*-formamide derivative (**3**) in 55% yield. The tertiary amine derivatives (**5a**−**c**) were synthesized by reductive amination of formaldehyde, propionaldehyde, or acetone with amine **2** in moderate yield. Reaction of **2** with triphosgene followed by ethylamine hydrochloride in the presence of triethylamine afforded the urea derivative **6** in 66% yield.

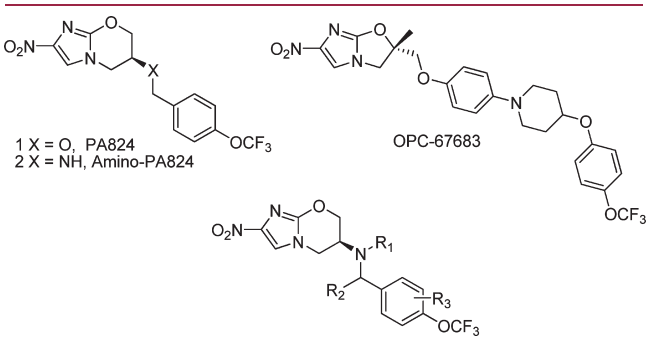
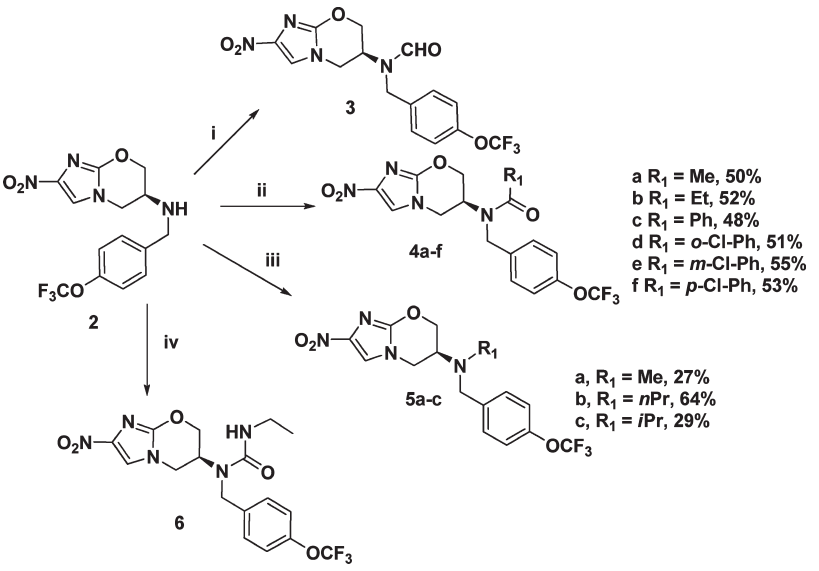


Figure 1

R_2 Modifications. Oxazol-2-yl(4-(trifluoromethoxy)phenyl)-methanone (**9a**) was prepared by copper mediated acylation[18] of oxazol-2-yl zinc chloride using 4-trifluoromethoxybenzoyl chloride and then subjected to reductive amination with amine (**7**) in the presence of $Ti(iPrO)_4$/AcOH/THF/$NaCNBH_3$ to provide **17a** as diastereomeric mixture (Scheme 2). Synthesis of the R_2 methyl analogue **17b** was accomplished by reductive amination of the commercially available 4-trifluoromethoxyacetophenone **9b**. Imidizolium ylide generated from *N*-benzylimidazole using diisopropylcarbamyl chloride, and diisopropylethylamine was treated with trifluoromethoxybenzaldehyde in refluxing CH_3CN to produce the carbamate, which upon hydrolysis afforded the benzylic alcohol (**11**). *N*-Debenzylation using Pd/C followed by mesylation of the benzylic alcohol and subsequent reaction with amine **7** in THF in the presence of NaH at room temperature produced the final product (**17c**) as a mixture of diastereomers. The three alkyl derivatives (**17d**−**f**) were all prepared in moderate yield by alkylation of amine **7** using the corresponding bromides **15a**−**c**, as illustrated in Scheme 2. Addition of ethyl and *n*-propyl Grignard reagents to 4-trifluoromethoxybenzaldehyde (**13**) afforded the corresponding benzylic alcohols (**14a**−**b**), while 1-(4-(trifluoromethoxy)phenyl)butan-1-ol (**14c**) was prepared by the addition of *n*-BuLi to trifluoromethoxybenzaldehyde. Alcohols **14a**−**c** were converted to their corresponding bromides (**15a**−**c**) using PBr_3 in ether and treated with amine (**7**) in DMF in the presence of K_2CO_3/KI at 90 °C to provide the required compounds **17d**−**f** as a mixture of diastereomers. Synthesis of carboxamide derivative **17g** was achieved by nucleophilic addition of TMSCN to the imine formed between the

Scheme 1[a]



[a] Reaction conditions: (i) HCO_2H, Ac_2O, THF, 0 °C, 1 h, 55%; (ii) R_1COCl, NaH, DMF rt to 70 °C; (iii) R_1CHO, $NaBH(OAc)_3$, MeOH/AcOH; (iv) triphosgene, $EtNH_2 \cdot HCl$, Et_3N, THF, 0 °C to rt, 66%.

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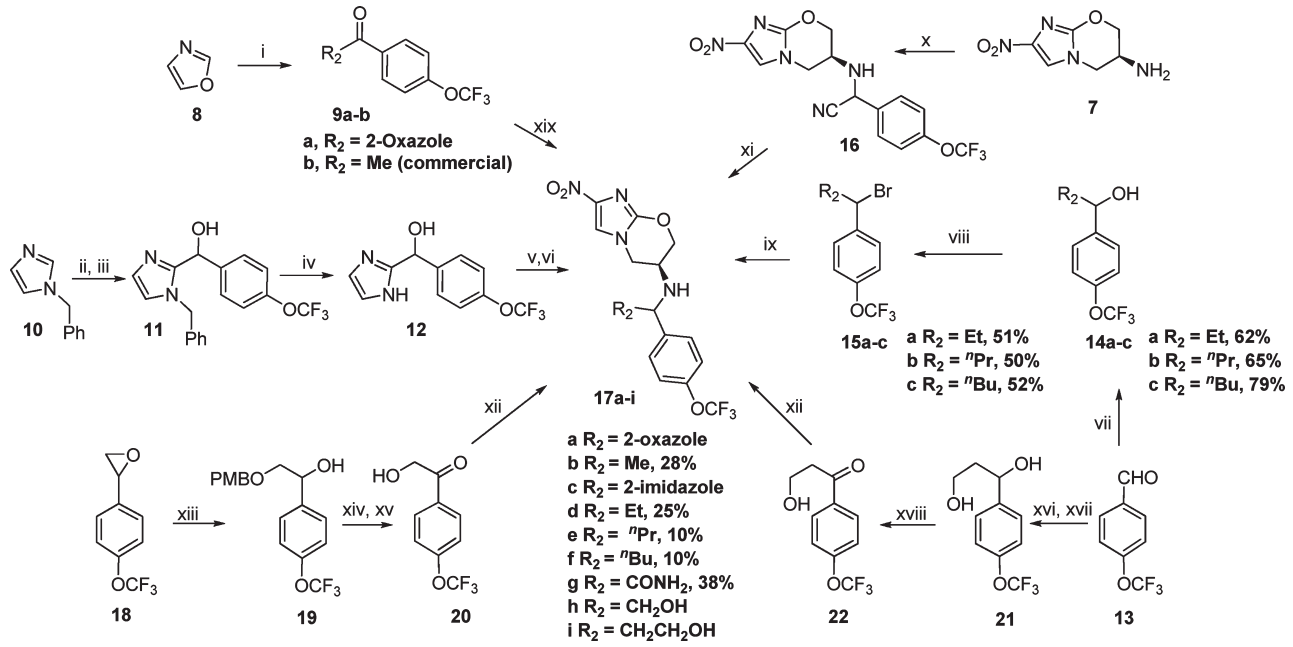
Scheme 2[a]



[a] Reaction conditions: (i) *n*-BuLi, $ZnCl_2$, CuI, THF, −78 °C to rt, 1.5 h, then 4-trifluoromethoxybenzoyl-chloride, rt, 1 h, 40%; (ii) diisopropylcarbamyl chloride, DIPEA, 4-trifluoromethoxybenzaldehyde, CH_3CN, reflux, 19 h, 73%; (iii) 50% TFA in water, THF, reflux, 15 h, 82%; (iv) H_2, Pd/C, MeOH, 1 atm, 81%; (v) MsCl, Et_3N, CH_2Cl_2, rt, 1 h; (vi) **7**, NaH, THF, rt, 40 h, 15%; (vii) RMgBr, THF, 0 °C to rt when R = Et and *n*-Pr; *n*-BuLi, THF, −78 °C to rt when R = *n*-Bu; (viii) PBr_3, ether, 0 °C to rt; (ix) **7**, K_2CO_3, DMF, KI, 90 °C; (x) 4-trifluoromethoxybenzaldehyde, neat, 100 °C, 5 min then TMSCN, 100 °C, 30 min, 50%; (xi) EtOH/HCl, −10 °C, 38%; (xii) **7**, $NaCNBH_3$, AcOH, EtOH, 5%; (xiii) 4-methoxybenzylalcohol, KO^{t}Bu, 60 °C, 2 h, 34%; (xiv) PDC, CH_2Cl_2, rt, 24 h, 62%; (xv) TBDMSOTf, CH_2Cl_2, rt, 5 min, 86%; (xvi) ethylbromoacetate, Zn, CH_2Cl_2, rt, 3 h; (xvii) $LiAlH_4$, THF, 0 °C to rt, 2 h, 25% over two steps; (xviii) MnO_2, CH_2Cl_2, rt, 6 h, 50%; (xix) **7**, Ti(*i*OPr)$_4$, AcOH, $NaBH_3CN$, 9%.

amine **7** and 4-trifluoromethoxybenzaldehyde (**13**) at 100 °C and subsequent hydrolysis in ethanolic HCl. Reaction of 4-((trifluoromethoxy)phenyl)oxirane **18** with 4-trifluoromethoxybenzyl alcohol in presence of KO*t*Bu at 60 °C afforded alcohol **19**. Further oxidation of the ring-opened product, followed by removal of the *p*-methoxybenzyl group using TBDMSOTf, afforded 2-hydroxy-1-(4-(trifluoromethoxy)phenyl)ethanone (**20**). 3-Hydroxy-1-(4-(trifluoromethoxy)-phenyl)propan-1-one (**22**) was synthesized from **13** in three steps involving a Reformatsky reaction with ethylbromoacetate in presence of Zn, $LiAlH_4$ mediated reduction of the ester group to yield **21**, and finally oxidation of the benzylic alcohol using MnO_2 to provide the hydroxy ketone **22**. Reductive amination of **20** and **22** with amine **7** afforded nitroimidazooxazines **17h**−**i**, respectively.

R_3 Modifications. R_3 modified nitroimidazooxazines were synthesized by the reductive amination of substituted trifluoromethoxybenzaldehydes with amine (**7**). Most of these aldehydes were not available commercially and were synthesized as described in Schemes 3, 4, and 5. Thus 2-hydroxy-4-trifluoromethoxybenzoic acid (**23**)[19] was used as a key intermediate in the synthesis of 2-hydroxy bearing analogues and the corresponding ether derivatives (**32a**−**c**, Scheme 3). Esterification of (**23**) with ethyl alcohol followed by alkylation with BnBr and cyclopropylmethylbromide gave the respective ether derivatives (**27b**−**c**) in excellent yields. Protection of the phenolic −OH group of **24** as its *O*-methoxymethyl ether was carried out using MOMCl/DIPEA to afford **27a**. $LiAlH_4$ mediated reduction of the ester group of **27a**−**c** and subsequent oxidation using PCC in CH_2Cl_2 afforded the required aldehydes **28a**−**c** in good yield.

2-Cyclopropyloxy-4-trifluoromethoxy benzaldehyde (**26**) was synthesized in five steps[20] from ethyl-2-hydroxy-4-trifluoromethoxybenzoate (**24**). Alkylation of **24** with 1-bromo-2-chloroethane using K_2CO_3 in DMF followed by saponification with KO*t*Bu in THF at 20 °C resulted in the 4-(trifluoromethoxy)-2-(vinyloxy)benzoic acid (**25**) in 63% yield. Cyclopropanation of **25** under Simmons−Smith conditions followed by reduction of the carboxylic acid group using BH_3·DMS and subsequent oxidation of the benzylic alcohol using PCC provided 2-*O*-cyclopropyl-4-trifluoromethoxybenzaldehyde (**26**) in 40% yield.

Reaction of ethyl-2-hydroxy-4-trifluoromethoxybenzoate (**24**) with 4-fluoronitrobenzene in the presence of NaH in DMF followed by Pd/C mediated reduction of the nitro group afforded the amine (**29**). Removal of the amino group by diazotization and subsequent reduction of the ester group using $LiAlH_4$ provided the benzylic alcohol derivative (**30**) with 48% yield. The required aldehyde **31** was then obtained by PCC mediated oxidation of (**30**). Reductive amination of these aldehydes **26**, **28a**−**d**, and **31** with amine **7** in presence of $NaBH(OAc)_3$ in DMF−AcOH provided the nitroimidazooxazines **32a**−**f**. Deprotection of the *O*-methoxymethyl ether using 6N HCl in THF provided **32g** in 67% yield.

2-Fluoro and 2-chloro-4-trifluoromethoxybenzaldehyde (**34a**−**b**) were readily synthesized from commercially available 2-chloro and 2-fluoro substituted 4-trifluoromethoxyiodobenzene (**33a**−**b**) by lithiation using *n*-BuLi at −78 °C followed by quenching with DMF in 72% and 57% yield, respectively (Scheme 4). 2-Bromo-4-trifluoromethoxybenzaldehyde **37** was synthesized by oxidative cleavage of the product **36** of Pd(0)

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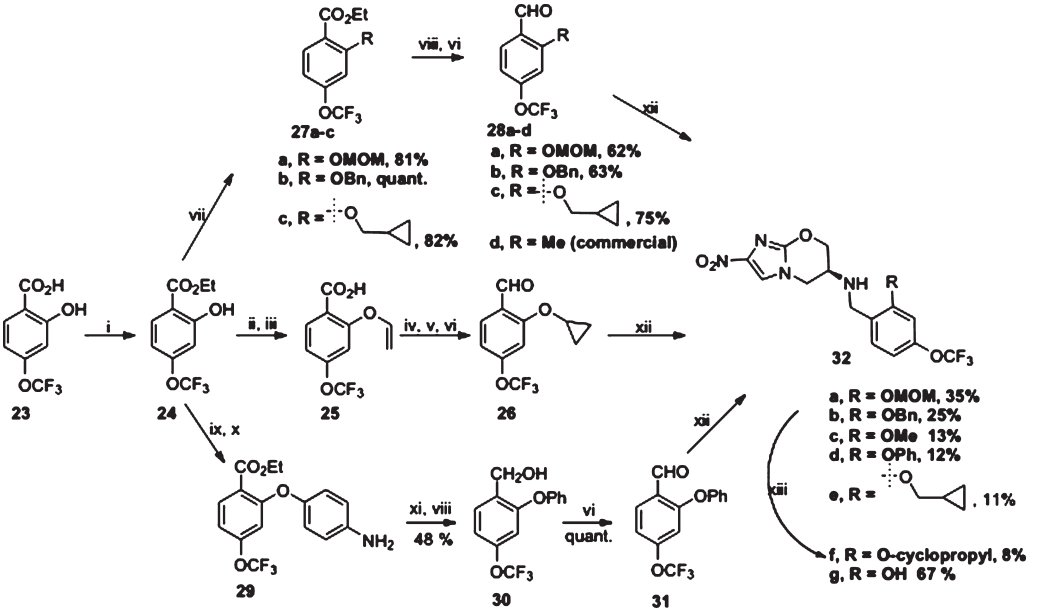
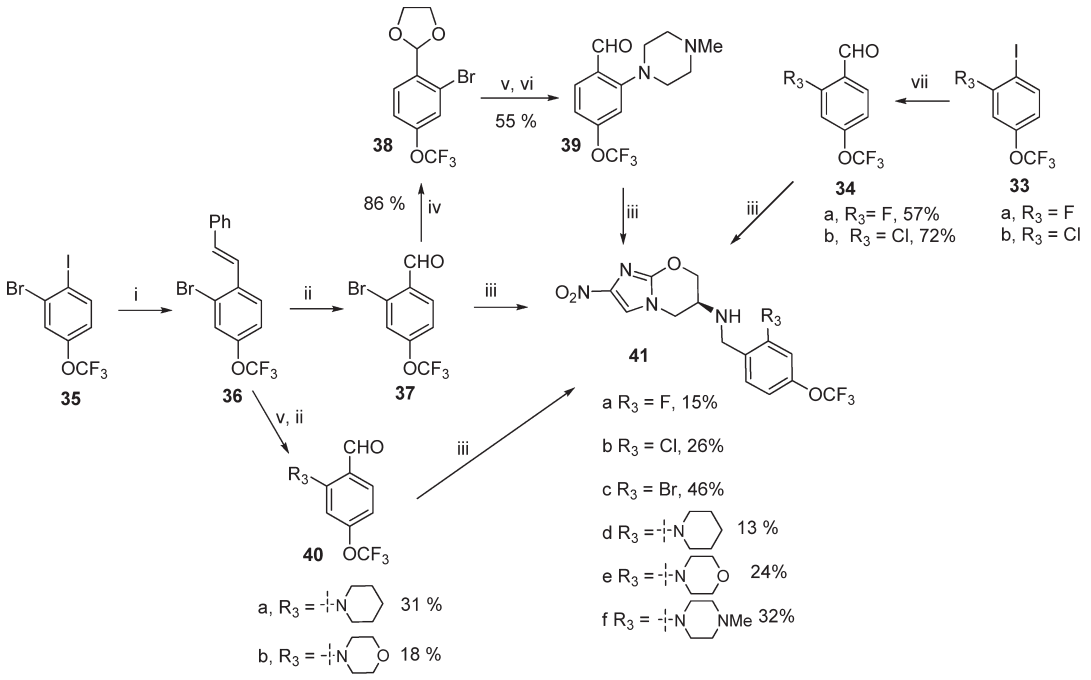
Scheme 3[a]



[a] Reaction conditions: (i) EtOH/H^+, 80 °C, 80%; (ii) 1-bromo-2-chloroethane, K_2CO_3, DMF, rt, 15 h, 80%; (iii) KO^{t}Bu, THF, 1 h, 20 °C, 78% over two steps; (iv) Et_2Zn, CH_2I_2, $ClCH_2CH_2Cl$, toluene, rt, 17 h, 51%; (v) BH_3-DMS, THF, reflux, 1.5 h; (vi) PCC, CH_2Cl_2, rt, 0.5 h, 80% over two steps; (vii) RX, K_2CO_3, DMF, 70 °C, 30 min to 2 h, when R = Bn, cyclopropylmethyl; MOMCl, DIPEA, CH_2Cl_2, rt, 3 h; (viii) $LiAlH_4$, THF, 0 °C to rt, 1 h; (ix) 4-fluoronitrobenzene, NaH, DMF, 100 °C, 2 h, 67%; (x) H_2, Pd/C, EtOAc, rt, 1.5 h, 87%; (xi) $NaNO_2$, H_3PO_2, 6N HCl, 50 °C, 1 h; (xii) $NaBH(OAc)_3$, AcOH, DMF, 20 h; (xiii) 6N HCl, THF, 1 h, rt.%.

Scheme 4[a]



[a] Reaction conditions: (i) styrene, $Pd(OAc)_2$, Et_3N, 95 °C, 16 h, 64%; (ii) OsO_4, $NaIO_4$, acetone−water, rt, 16 h, 16%; (iii) **7**, $NaCNBH_3$, AcOH, DMF, 20 h; (iv) ethylene glycol, *p*-TSA, benzene, 80 °C, 8 h, 86%; (v) $Pd(OAc)_2$, Cs_2CO_3, Xantphos, dioxane, amine, 90 °C, 8 h; (vi) THF, 6 N HCl, 30 min, room temperature; (vii) *n*-BuLi, THF, −78 °C, DMF, 15 min.

mediated Heck coupling between 2-bromo-4-trifluoromethoxy-iodobenzene (**35**) and styrene. Buchwald coupling of **36** with morpholine and piperidine followed by oxidative cleavage of the olefin afforded aldehydes **40a**−**b** in moderate yields.

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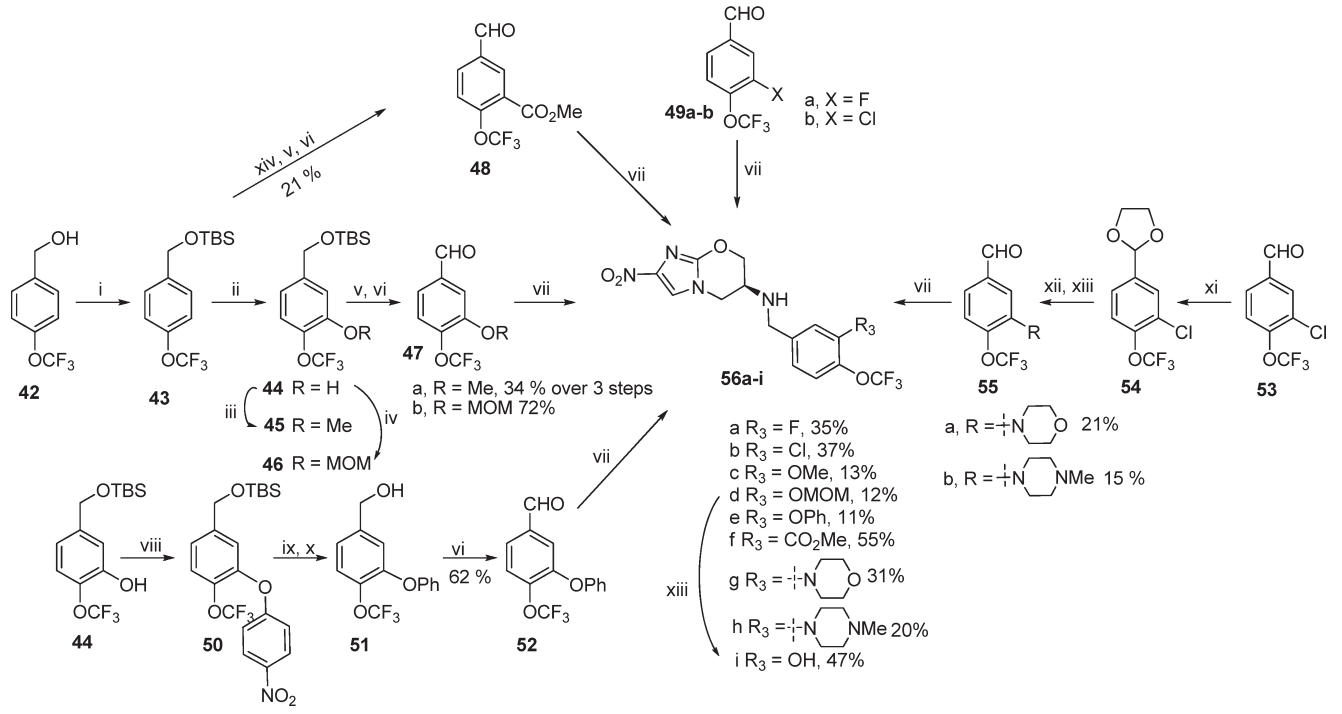
Scheme 5[a]



[a] Reaction conditions: (i) TBSCl, imidazole, CH_2Cl_2, rt, 60%; (ii) *s*-BuLi, TMEDA, THF, −78 °C, 1 h, then $FB(OMe)_2$, −78 °C, 30 min followed by alk, H_2O_2, 30 min, 28%; (iii) K_2CO_3, MeI, DMF, 70 °C; (iv) MOMCl, DIPEA, DMF, rt, 16 h, 82%; (v) TBAF, THF, rt, 1.5 h; (vi) PCC, CH_2Cl_2, rt, 1 h; (vii) **7**, $NaCNBH_3$, AcOH, DMF; (viii) 4-fluoronitrobenzene, NaH, DMF, 100 °C, 2 h, 30%; (ix) Fe/NH_4Cl, EtOAc−water, reflux, 1.5 h; (x) $NaNO_2$, H_3PO_2, 6N HCl, 50 °C, 1 h, 52% over two steps; (xi) ethylene glycol, *p*-TSA, benzene, 80 °C, 8 h, 72%; (xii) $Pd(OAc)_2$, Cs_2CO_3, Xantphos, dioxane, amine, 90 °C, 8 h; (xiii) THF, 6 N HCl, 30 min, rt; (xiv) *sec*-BuLi, MeOCOCl, THF, −78 °C to rt, 3 h.

2-(4-Methylpiperazin-1-yl)-4-(trifluoromethoxy)benzaldehyde **39** was synthesized by Buchwald coupling of *N*-methylpiperazine with 2-[2-bromo-4-(trifluoromethoxy)phenyl]-1,3-dioxolane (**38**) followed by deprotection of the acetal. The reductive amination of aldehydes **34a**−**b**, **37**, **39**, and **40a**−**b** with amine **7** afforded nitroimidazooxazines **41a**−**f**.

4-Trifluoromethoxybenzyl alcohol (**42**) was protected as a TBS ether (**43**) in 80% yield (Scheme 5), which was then reacted with *s*-BuLi at −78 °C in the presence of TMEDA and subsequently treated with $FB(OMe)_2$ followed by alkaline H_2O_2 hydrolysis, affording the required phenol (**44**) in 30% yield. Conversion of **44** to the corresponding *O*-methyl derivative (**45**) and *O*-methoxymethyl ether (**46**) was achieved by using MeI/K_2CO_3 and MOMCl/Et_3N, respectively. Cleavage of the TBS ether using TBAF followed by PCC-mediated oxidation produced the corresponding aldehydes **47a**−**b**. Synthesis of 3-phenoxy-4-trifluoromethoxybenzaldehyde **52** followed a similar protocol used for the synthesis of 2-phenoxy derivative **31**. Thus, 5-((*tert*-butyl(dimethyl)silyl)oxymethyl)-2-(trifluoromethoxy)-phenol (**44**) was converted to the 3-(4-nitrophenoxy) derivative **50**. Reduction to the corresponding aniline derivative followed by diazotization gave 3-phenoxy-4-trifluoromethoxybenzylic alcohol **51** in 52% yield. Oxidation of **51** with PCC in CH_2Cl_2 produced the required aldehyde **52**.

3-Morpholino- and 3-(1-pipiridyl)-substituted 4-trifluoromethoxybenzaldehydes **55a**−**b** were synthesized following a similar sequence of reaction used in the synthesis of **40a**−**b**. Thus, Buchwald coupling of the morpholine and piperidine with 2-(3-chloro-4-(trifluoromethoxy)phenyl)-[1,3]-dioxolane **54** and subsequent deprotection of the acetal afforded the required aldehydes **55a**−**b** in moderate yields. Methyl-5-formyl-2-(trifluoromethoxy)benzoate **48** was synthesized from **42** in three steps. Lithiation of **42** with *sec*-BuLi at −78 °C followed by the addition of methylchloroformate, subsequent deprotection of the TBS ether with TBAF, and PCC mediated oxidation of the benzylic alcohol afforded **48**.

Reductive amination of these aldehydes (**47a**−**b**, **48**, **49a**−**b**, **52**, **55a**−**b**) with amine **7** was carried out in presence of $NaCNBH_3$ in DMF containing AcOH to provide the nitroimidazole derivatives **56a**−**h** in 15−55% yields. Deprotection of the *O*-methoxymethyl ether **56d** was carried out as mentioned previously to provide **56i**. Syntheses of **1** derivatives **59a**−**c** with R_3 modification having 3-F, 3-OMe, and 3-OMOM substitutions was achieved by alkylation of alcohol **57** with the corresponding benzylic bromides **58a**−**b** (Scheme 6). Deprotection of the *O*-methoxymethyl ether in **59a** was carried out using 6N HCl in THF to afford **59d**.

RESULTS AND DISCUSSION

R_1 Modifications: SAR of Amides, Ureas, and Tertiary Amines. Both benzyl ether and amine analogues of nitroimidazooxazines have been shown to be equipotent against *M. tuberculosis*. The amenability of the benzylic amine in **2** allowed us to explore further modifications in the series shown in Table 1. *N*-Formylation (**3**) reduced both cellular activity and efficiency as a substrate for Ddn by 2-fold compared to the parent compound **2**, whereas *N*-acetylation of the amino group was

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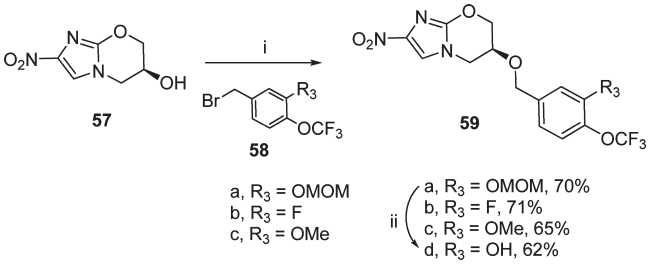
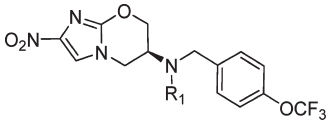
Scheme 6[a]



[a] Reaction conditions: (i) NaH, DMF, −78 °C to rt; (ii) 6N HCl, THF, rt, 4 h.

Table 1. Minimum Inhibitory Concentration (MIC) and Minimum Anaerobicidal Concentration (MAC) of R_1 Modifications



No	R_1	Mtb MIC_{99} μM (±SD)	Mtb MAC μM (±SD)	Ddn k_{cat}/K_M
1	--	0.63±0.22	8.8±3.4	0.15
2	-H	0.36±0.05	14±2	0.17
3	*N*-Formyl	0.79±0.01	12.5±8.8	0.07
4a	*N*-Acetyl	50±0.0	>50	0.02
4b	*N*-propionyl	Insoluble	ND	ND
4c	Benzoyl	8.8±3.4	>50	0.16
4d	*o*-Chlorobenzoyl	5.6±1.4	>50	0.13
4e	*m*-Chlorobenzoyl	7.5±2.8	21±7	0.25
4f	*p*- Chlorobenzoyl	1.3±0.5	7.8±3.1	0.37
5a	Me	0.15±0.05	2.5±0.9	0.25
5b	*n*Pr	0.11±0.06	3.8±1.4	0.32
5c	*i*Pr	0.34±0.05	11±3	0.04
6		17±7	69±38	0

detrimental to both MIC and MAC and reduced the catalytic efficiency of this as a substrate for Ddn by nearly 10-fold. The *N*-propionyl derivative **4b** was insoluble and could not be evaluated. *N*-Aroyl amide derivatives behaved in a similar fashion with both benzoyl (**4c**) and chlorobenzoyl (*ortho*, *meta*, and *para*) derivatives (**4d**−**f**) resulting in compounds that were significantly less potent against replicating and nonreplicating Mtb. We also explored one urea derivative (**6**) but did not elaborate on the series after the observation that reducing the basicity of nitrogen resulted in less potent compounds.

The catalytic activity of the simple amide substituted molecules as substrates for the Ddn enzyme, measured as k_{cat}/K_M for reoxidation of reduced F420, was generally lower than that of **1**, however, within this group there was in general only a weak correlation between enzymatic activity and MIC. The same was not true of the *N*-aroyl derivatives, some of which seem to be significantly better substrates for the enzyme. However, this improvement in substrate efficiency did not translate into improvements in MIC. One potential explanation for this phenomenon could be that the aroyl substituent, with its similarity to the *p*-trifluoromethoxybenzyl amine substituent, competes with this group and alters the binding mode of the substrate, altering product formation and hence efficacy. Alternatively, these could be transported into the cell less efficiently reducing the effective intracellular concentration.

The tertiary amine analogues (**5a**−**c**) showed significantly improved potency in comparison with the amide derivatives. Higher alkyl derivatives such as *N*-*n*-Pr derivative **5b** showed a 6-fold increase in the potency compared to **1** and was similar in potency to the *N*-Me derivative (**5a**). Both **5a** and **5b** were 2-fold more potent against anaerobic Mtb. However, the *N*-*i*-Pr derivative **5c** was less active against both replicating and nonreplicating Mtb when compared to *N*-*n*-Pr **5b**, which indicates that branched chains may not be suitable at this position. For **5a** and **5b**, improvements in MIC were paralleled by increases in k_{cat}/K_M, suggesting that the binding mode of the substrate was maintained to optimize aerobic activity. While these tertiary amines showed improvements in the overall activity profile, the intrinsic clearance of these compounds was unfortunately very high in mouse liver microsomes and we discontinued exploration of this series (Table 4).

R_2 Modifications: Flat SAR and a Potential Site for Metabolic Tailoring. Our efforts to systematically optimize the benzylic position by incorporating lower alkyl groups (**17b**, **17d**−**f**) resulted in compounds with either comparable or very slightly enhanced activity compared to **1** against both replicating and nonreplicating Mtb (Table 2). Elongation of the alkyl chain from methyl to *n*-butyl resulted in very slight enhancements in potency (2-fold), suggesting the presence of a hydrophobic pocket but the SAR here was notably flat, and the introduction of a hydroxymethyl group at this position resulted in compound (**17h**), which had activity profile similar to **2**. The derivatives with hydroxyethyl (**17i**) and carboxamide (**17g**) were 5- and 1.5-fold more potent respectively against replicating Mtb although their potency against anaerobic bacilli was compromised. Introduction of heterocycles such as oxazole (**17a**) or imidazole (**17c**) led to compounds with similar potency against both replicating and nonreplicating Mtb. In general, the presence of more polar groups resulted in the reduction of activity against nonreplicating Mtb, whereas the potency against replicating Mtb remained in the range of 0.15−0.4 μM. Although the stereochemistry of the C-6 substituent is critical for **1** activity (the *S* isomer being 100 fold more potent than the *R* isomer), the introduction of another chiral center at the benzylic position does not seem to affect the activity as testing the individual diastereoisomers showed equivalent activity in all assays. This flatness of the SAR is mirrored in the kinetics of F420 reoxidation when they were tested as substrates for Ddn. The k_{cat}/K_M was comparable to the parent compound except for the compounds (**17e**, **17f**) whose k_{cat}/K_M was 0.31 and 0.4, respectively (Table 2). This 2-fold increase in substrate efficiency translated into a slight improvement in MIC only for compound **17f**.

Benzylic positions in general are susceptible to metabolism (Metasite predictions, for example, highlight the benzylic carbon of **1** as the primary site of metabolic liability). Blocking of

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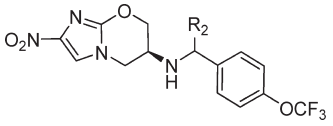
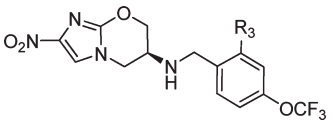
benzylic position would make this position metabolically more stable. The flat SAR observed at this position suggests that this does not form an important contact site for Ddn and therefore raises the possibility of further manipulation of this center to alter metabolic stability or other metabolic properties without compromising activity.

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Anaerobicidal Concentration (MAC) of R_2 Modifications



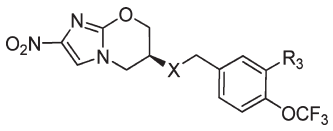
compd no.	R_2	Mtb MIC_{99} μM (±SD)	Mtb MAC μM (±SD)	Ddn k_{cat}/K_M
1	−H	0.63 ± 0.22	8.8 ± 3.4	0.15
2	−H	0.36 ± 0.05	14 ± 2	0.17
17a	2-oxazole	0.40 ± 0.18	7.8 ± 3.1	0.20
17b	methyl	0.44 ± 0.16	5.6 ± 1.4	0.09
17c	2-Imidazole	0.52 ± 0.23	17 ± 7	0.17
17d	Et	0.36 ± 0.18	3.1 ± 2.2	0.14
17e	*n*-Pr	0.27 ± 0.07	3.8 ± 1.4	0.31
17f	*n*-Bu	0.15 ± 0.00	4.4 ± 1.7	0.40
17g	$CONH_2$	0.41 ± 0.35	30 ± 11	0.12
17h	$-CH_2OH$	0.30 ± 0.22	11 ± 3	0.15
17i	$-CH_2CH_2OH$	0.13 ± 0.04	23 ± 6	0.21

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Anaerobicidal Concentration (MAC) of R_3 Modifications



No	R_3	Mtb MIC_{99} µM (±SD)	Mtb MAC µM (±SD)	Ddn k_{cat}/K_M
1	-H	0.63±0.22	8.8±3.4	0.15
2	-H	0.36±0.05	14±2	0.17
32b	-OBn	0.24±0.19	7.5±2.8	0.31
32c	-OMe	0.23±0.07	5.2±1.8	0.15
32d	-OPh	0.06±0.02	4.4±1.7	0.42
32e		0.10±0.04	4.2±1.8	0.37
32f		0.06±0.02	2.0±0.8	0.31
32g	-OH	0.09±0.01	4.4±1.7	0.18
41a	-F	0.12±0.05	2.5±0.9	0.20
41b	-Cl	0.13±0.06	2.1±0.9	0.27
41c	-Br	0.09±0.01	2.0±0.8	0.25
41d		0.21±0.13	11±3	0.42
41e		0.15±0.05	10±4	0.19
41f		2.2±0.5	33±14	0.17

Table 3. Continued



No	R_3	X	Mtb MIC_{99} µM (±SD)	Mtb MAC µM (±SD)	Ddn k_{cat}/K_M
1	-H	O	0.63±0.22	8.8±3.4	0.15
2	-H	NH	0.36±0.05	14±2	0.17
56a	-F	NH	0.12±0.07	4.2±1.8	0.21
59b	-F	O	0.17±0.03	7.5±2.8	0.33
56b	-Cl	NH	0.12±0.07	4.2±1.8	0.31
56c	-OMe	NH	0.07±0.02	2.1±0.9	0.19
59c	-OMe	O	0.13±0.04	4.2±1.8	0.24
56i	-OH	NH	0.16±0.06	7.5±2.8	0.13
59d	-OH	O	0.14±0.03	8.3±3.6	0.14
56e	-OPh	NH	0.14±0.06	3.9±1.6	0.13
56f	-COOMe	NH	0.14±0.03	11±3	0.17
56g		NH	0.15±0.05	7.0±3.9	0.10
56h		NH	0.17±0.02	7.8±3.1	0.16

R_3 Modifications: Hydrophobic Groups Preferred. Significant improvements in potency were realized upon substituting at the 2-position of the aromatic ring of the benzyl ether Table 3 (top). Substitution using halogens (**41a**−**c**) as well as hydroxyl (**32g**) groups improved MIC to lower than 100 nM. Phenoxy (**32d**) and cyclopropyloxy (**32f**) produced the most potent compounds in this series, showing MIC values down to 60 nM. The corresponding benzyloxy derivative (**32b**) was less potent against both aerobic and anaerobic Mtb but still three times as potent as **1**. Similarly, cyclopropylmethoxy (**32e**) group was tolerated less well in both cases, suggesting an optimal spacing between a cyclic hydrophobe and the ether oxygen that may play a critical role in binding to Ddn. Consistent with this hypothesis, both cyclopropylmethoxy and benzyloxy substituents are poorer substrates for Ddn by comparison with their correspondingly shorter analogues. In fact, phenoxy substituted **32d** was the best substrate for Ddn seen in the present study with a k_{cat}/K_M of 0.42. Compounds **41d**−**e** bearing a piperidine and morpholine group also showed improved MIC, while *N*-methylpiperazine analogue **41f** resulted in the least aerobically potent compound among these amine-bearing substitutions. All three compounds in this series resulted in disproportionate loss of anaerobic activity and an inconsistent correlation with their efficiency as substrates for Ddn.

The 3-position of the trifluoromethoxyphenyl ring likewise tolerated most of the substituents tested, resulting in compounds

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Table 4. Stability in Mouse Liver Microsomes and Solubility

				mouse liver microsomes		
compd no.	structure	ClogP	solubility pH 6.8 μg/mL	CL μL.min^{-1}mg^{-1}	$t_{1/2}$ min	predicted hepatic extraction ratio[21] (%)
5a	*N*-Me	3.2	10	124.9	11	85
5b	*N*-*n*Pr	4.2	<2	407	3.4	95
5c	*N*-*i*Pr	4.1	<2	433	3.2	95
32d	2-*O*Ph	4.7	3	216.6	6.4	90
32e	2-*O*-cyclopropylmethyl	3.7	6	866.3	1.6	97
32f	2-*O*-cyclopropyl	3.3	11	150.7	9.2	87
32g	2-*O*H	2.1	25	96.9	14.3	81
41a	2-F	2.9	72	95.6	14.5	81
41b	2-Cl	3.4	30	69.7	19.9	75
56a	3-F	2.7	43	58.7	23.6	72
56b	3-Cl	3.2	77	103.4	13.4	82
56i	3-*O*H	1.9	56	61.9	22.4	73
56e	3-*O*Ph	4.3	<2	149	9.3	87

with superior potency to **1**. Halogen substitutions (**56a**−**b**, **59b**) at the 3-position of the phenyl ring exhibited improved potency similar to that seen in the case of the corresponding 2-halo substituted analogues Table 3 (bottom). Compounds **56i** and **59d** both bear 3-OH moieties and differ in being the analogous benzyl ether and benzyl amines and behave identically in all assays, showing comparable aerobic and anaerobic potency to **1**. Compounds **56c** and **59c** are the corresponding methyl ethers and unremarkable except in the distinction that the amino analogue was slightly superior to the ether analogue. This compound **56c**, however, does not show a significant increase in k_{cat}/K_M, suggesting that the effect may be complex. Surprisingly, the 3-phenoxy substituted compound **56e** showed relatively similar activities to the hydroxy and methoxy substituents. The methyl carboxylate (**56f**), morpholine (**56g**), and piperazine (**56h**) all showed comparable cellular activity as well as enzymatic activity, resulting in a very flat picture of the SAR at the 3-position.

In general, *ortho* and *meta* positions of the trifluoromethoxyphenyl ring exhibited similar activity profile for the groups tested, except for *O*Me and *N*-methylpiperazine groups, for which a preference for *meta* position was observed. The loss of potency against nonreplicating Mtb was more significant when amine substituents were used in the R_3 modification. The activity of these analogues was not dependent on the nature of the substituents tested. Although benzylic ether analogues and their amine derivatives exhibited similar profile in their activities against both replicating and nonreplicating Mtb, the amino series exhibited a better solubility profile and a 2-fold improvement in potency.

Solubility, Microsomal Stability and in Vivo Clearance Rates in Mice. Solubility and stability in mouse liver microsomes were determined for selected potent compounds (Table 4). Hydroxyl group and halogen substitution on the aryl ring resulted in compounds with reasonable solubility, while *N*-alkyl derivatives such as **5a**−**c** and ether derivatives such as **32d**−**f** and **56e** had significantly reduced solubility. This is probably due to the higher lipophilicity of these derivatives as reflected in their ClogP values. Metabolic stability studies in mouse liver microsomes revealed high clearance rates for the more lipophilic compounds. Although the 3-Cl derivative **56b** and 2-F derivative **41a** were the most soluble compounds, their intrinsic clearance was comparatively higher than that of **56a** and **56i** (3-F and 3-OH derivatives respectively) that exhibited the most attractive metabolic stability. We used these intrinsic clearance rates to calculate a predicted hepatic extraction ratio.[21] On the basis of these values, we attempted to verify whether low in vitro clearance would be translated in vivo. We therefore performed in vivo pharmacokinetic studies with compounds **56a**, **56b**, and **56i**. Following intravenous injection at 5 mg/kg, compounds **56a** and **56b** showed low to moderate clearance with good systemic exposure and elimination half-lives of 1.3 and 4.3 h, respectively. Compound **56i**, on the other hand, was cleared more rapidly, with a half-life of 0.4 h resulting in low systemic exposure. This relative disconnect between in vitro and in vivo clearance for **56i** is suspected to be due to glucuronidation, which is not captured in microsomal stability studies that only measure phase I metabolism.

Ddn May Prefer Substrates in a Pseudoequatorial Conformation. We have previously shown that in this bicyclic system the lipophilic tail can adopt a pseudoaxial or pseudoequatorial conformation at C-6 and that the preferred form in crystalline **1** was pseudoaxial. In addition, we found that 7*R*-methylated **1** crystallized in a pseudoequatorial conformation.[22] To investigate the energetics of **1** in solution, we have calculated the Gibbs free energy of both conformers of **1** using density functional theory at the level of B3LYP/6-31G*[23] in a solvent reaction field of cyclohexane. These calculations reveal that the pseudoaxial form is 0.9 kcal/mol more stable than the pseudoequatorial form, indicating that only about 18% of **1** would be in the pseudoequatorial form in solution. Furthermore, the calculated energy barrier between the two conformers is less than 6 kcal/mol, suggesting that both conformers exist in solution at room temperature with rapid interconversion on the nanosecond time scale. Interestingly, the *ortho* substituted R_3 derivatives yielded the largest improvements in potency, with two candidates in the 60 nM range with substantial improvements in their activity as substrates for Ddn. Figure 2A (showing the pseudoequatorial conformer) and Figure 2B (showing the pseudoaxial conformer) show an overlay of the geometry optimized *ortho* substituted analogues including two promising molecules (2-phenoxy- (**32d**) and cyclopropyloxy- (**32f**)) as well as the methoxy- (**32c**), chloro- (**41b**), and *N*-methylpiperazino- (**41f**) analogues.

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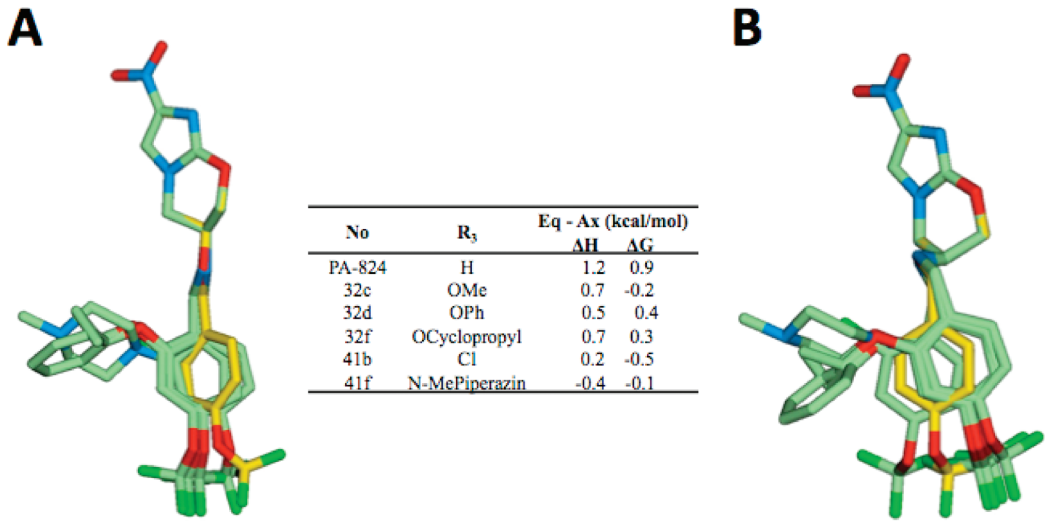

No	R_3	Eq - Ax (kcal/mol) ΔH	ΔG
PA-824	H	1.2	0.9
32c	OMe	0.7	-0.2
32d	OPh	0.5	0.4
32f	OCyclopropyl	0.7	0.3
41b	Cl	0.2	-0.5
41f	N-MePiperazin	-0.4	-0.1

Figure 2. Superpositions of PA-824 (**1**) and some R_3 derivatives that were geometry optimized at the level of B3LYP/6-31G*. In each, the head, tail, and linker region of **1** is shown with yellow carbons, in (A), the tail is shown in the pseudoequatorial conformation, while in (B), the tail is shown in the pseudoaxial conformation. (A) and (B) show R_3 analogues **32c**, **32d**, **32f**, **41b**, and **41f**. The inset table shows the difference in enthalpy (ΔH) and Gibbs free energy (ΔG) at 298.15 K calculated for the pseudoequatorial conformer compared with the pseudoaxial conformer for each compound with a solvent reaction field of cyclohexane. Atoms represented by colors are as follows: green, carbon; dark green, fluorine or chlorine; blue, nitrogen; red, oxygen. Hydrogen atoms are not shown.

Parts A and B of Figure 2 depict an overlay of the head portion of each *ortho* analogue with **1** to illustrate the conformational deviation of the tail portion of each derivative. In general, the tail group of the pseudoequatorial conformers better overlap with **1** than the tail groups of the pseudoaxial conformers. In terms of energetics, relative to **1**, each *ortho* substitution stabilizes the equatorial form from 0.5 kcal/mol (**32c**) to 1.6 kcal/mol (41f) as seen in the values of ΔH. Additional stabilization of the equatorial conformation from 0.1 kcal/mol (**32d**) to 0.9 kcal/mol (**32C**) arises from the larger vibrational entropy as seen in the calculated ΔG values; the equatorial conformation, which is more extended in molecular shape than the axial, is more flexible and thus tends to have larger vibrational entropy. This stabilization energy directly translates into an increase in the concentration of the pseudoequatorial conformer at equilibrium; for example, about 70% of compound **41b** would be in the pseudoequatorial form in cyclohexane. Accordingly, it can provide a rationale at a qualitative level for the 10- to 20-fold enhanced potency of the *ortho*-substituted compounds listed in Table 3A provided that Ddn preferentially recognizes the pseudoequatorial conformation. With larger and more polar substituents such as **41f** there might be other factors (e.g., steric repulsion) at the binding site of Ddn contributing to its relative inactivity. Besides the increase in the concentration of the pseudoequatorial conformer, the improved catalytic efficiency of several of these compounds, including 2-phenoxy (**32d**), cyclopropoxy (**32f**), Cl (**41b**), and Br (**41c**), suggests that these groups might well increase either the polarizability of the tail group by donating π-electron density (e.g., cyclopropoxy) and/or the separation of the electrostatic charge on the tail group by an electron withdrawing group (e.g., Cl). This increased polarizability and/or charge separation might be responsible for enhancing potential π-stacking interactions[24,25] with an aromatic residue at the binding site, resulting in improved catalytic efficiency of **32d**, **32f**, **41b**, and **41c**.

CONCLUSIONS

We have explored three positions of diversity in the 4-(trifluoromethoxy)benzylamino tail region of **1** using our previously described 3D-QSAR pharmacophore model for predicting MICs of compounds in this series. All of these analogues were predicted by our previously described QSAR model to be highly active (MIC <1 μM), and in general this was the case. In the case of the *N*-acylated and *N*-alkylated derivative in Table 1, however, there were some notable exceptions. Two of the three positions (R_1 and R_3) provided substantial SAR and improved lead compounds in terms of both potency and solubility, while one site provided little SAR (R_2). This is nonetheless a useful observation because this position could be potentially manipulated to modulate the pharmacological properties of additional derivatives. A recent report on a series of biphenyl analogues of these compounds highlighted the importance of microsomal stability in determining in vivo activity of compounds in this series.[17] In this study, we have confirmed good in vitro metabolic stability with in vivo pharmacokinetic studies. On the basis of our results, a subset of these compounds are both stable and soluble enough to merit further testing in animals. In general, the QSAR model was successful in predicting active compounds with the exception of compounds substituted at the benzylic nitrogen that are likely to affect the conformation of the oxazine ring. The present study provides an additional insight into the preferential binding mode of **1** to Ddn based on the conformational energetics, and this should facilitate further optimization of this exciting class of molecules for the treatment of TB.

EXPERIMENTAL SECTION

General Methods. Reagents and solvents were purchased from Aldrich, Acros, or other commercial sources and used without further purification. Thin layer chromatography (TLC) was carried out on

precoated Silica Gel 60 F_{254} plates from Merck. Compounds were visualized under UV light or phosphomolybdic acid (PMA) stain. NMR spectra were obtained on a Varian 400 MHz Oxford NMR. Preparative HPLC separation was performed using Agilent reverse phase HPLC with Atlantis dC18 column, 19 mm × 250 mm, 10 μm. Unless otherwise noted, purity of compounds was established to be >95% by LC/MS (Aquity UPLC with PDA detector and ELSD using Waters Quattro Micro-API Micromass with multimode ionization as detector and Acquity BEH C18, 50 mm × 2.1 mm, 1.7 μm column) and Waters Aquity UPLC with PDA detector, using Acquity BEH C18, 100 mm × 2.1 mm, 1.7 μm column. Column chromatography was carried out on silica gel (100−200 mesh). MIC, MAC, and enzyme kinetic assays were carried out as previously described.[14] MIC, MAC, and enzyme assays were carried out multiple times in two independent laboratories, and discrepancies were repeated until concordant results were obtained. Values reported are averages of multiple measurements, and reported errors are standard deviations.

(*S*)-*N*-(2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yl)-*N*-(4-trifluoromethoxybenzyl)-formamide (3). Acetic anhydride (26 μL, 0.28 mmol) was added to formic acid (0.11 mL, 2.78 mmol) at 0 °C. After 30 min at 0 °C, a solution of ((*S*)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yl)-(4-trifluoromethoxybenzyl)-amine **2** (50 mg, 0.14 mmol) in THF (1 mL) was added to the mixture and stirred for 1 h. The solvent was removed in vacuo and the residue partitioned between EtOAc and water. The organic layer was washed with water and brine and dried (anhyd Na_2SO_4) and concentrated under reduced pressure. Chromatographic purification of the residue on silica gel eluting with 1% MeOH in CH_2Cl_2 gave **3** (30 mg, 55%). ^{1}H NMR ($CDCl_3$): δ 4.03 (dd, 1H, *J* = 5.6, 12.0 Hz), 4.30−4.40 (m, 2H), 4.52−4.54 (m, 1H), 4.55 (s, 2H), 4.79 (dd, 1H, *J* = 6.8, 12.0 Hz), 7.17 (s, 1H), 7.23−7.24 (s, 4H), 8.37 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 44.8, 46.2, 50.9, 67.3, 114.8, 121.7, 121.9, 128.6, 129.1, 134.4, 147.0, 149.7, 164.0. HRMS calcd for $C_{15}H_{13}F_3N_4O_5$ [M + H^+] 387.0916, found 387.0926.

(*S*)-*N*-(2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yl)-*N*-(4-trifluoromethoxybenzyl)-acetamide (4a). To a solution of **2** (50 mg, 0.14 mmol) in dry DMF (0.5 mL) was added NaH (60% dispersion in mineral oil, 18 mg, 0.42 mmol) at 0 °C. After 0.5 h, acetyl chloride (29 μL, 0.42 mmol) was added, and stirring continued at room temperature for 2 h. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with 1N aqueous HCl (2 × 5 mL), water (10 mL), and brine (10 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure. Chromatographic purification of the residue on silica gel eluting with 1% MeOH in CH_2Cl_2 gave **4a** (28 mg, 50%). ^{1}H NMR ($CDCl_3$): δ 2.24 (s, 3H), 4.08 (dd, 1H, *J* = 5.6, 12.0 Hz), 4.30−4.40 (m, 2H), 4.52−4.54 (m, 1H), 4.55 (s, 2H), 4.79 (dd, 1H, *J* = 6.8, 12.0 Hz), 7.19 (d, 2H, *J* = 8.0 Hz), 7.23 (d, 2H, *J* = 8.0 Hz), 7.24 (s, 1H).

(*S*)-*N*-(2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yl)-*N*-(4-trifluoromethoxybenzyl)-propionamide (4b). To a solution of **2** (50 mg, 0.14 mmol) in dry THF (1 mL) was added NaH (18 mg, 0.42 mmol) at 0 °C. After 10 min, propionyl chloride (36 μL, 0.42 mmol) was added and the reaction mixture heated at 70 °C for 3 h. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure. Chromatographic purification of the residue on silica gel eluting with 1% MeOH in CH_2Cl_2 gave **4b** (30 mg, 52%). ^{1}HNMR ($CDCl_3$): δ 1.19 (t, 3H, *J* = 7.2 Hz), 2.45 (q, 2H, *J* = 7.2, 14.4 Hz), 4.08 (dd, 1H, *J* = 6.0, 12.5 Hz), 4.40 (dd, 2H, *J* = 7.2, 12.0 Hz), 4.61 (m, 3H), 4.77 (m, 1H), 7.17 (d, 2H, *J* = 8.0 Hz), 7.24−7.27 (m, 3H).

Using the same procedure, the following compounds were synthesized:

(*S*)-*N*-(2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yl)-*N*-(4-trifluoromethoxybenzyl)benzamide (4c). Yield 48%. ^{1}H NMR ($CDCl_3$): δ 4.08 (dd, 1H, *J* = 4.8, 12.0 Hz), 4.43−4.46 (m, 2H), 4.60−4.75 (m, 3H), 4.95 (m, 1H), 7.21 (m, 5H), 7.45 (m, 5H). HRMS calcd for $C_{21}H_{17}F_3N_4O_5$ [M + H^+] 463.1229, found 463.1232.

(*S*)-2-Chloro-*N*-(2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]-oxazin-6-yl)-*N*-(4-trifluoromethoxybenzyl)-benzamide (4d). Yield 51%. ^{1}H NMR (DMSO-d_6): δ 4.10−4.20 (m, 1H), 4.20−4.70 (m, 4H), 4.70−5.05 (m, 2H), 7.00−7.70 (m, 8H), 7.91 (s, 1H). HRMS calcd for $C_{21}H_{16}ClF_3N_4O_5$ [M + H^+] 497.0840, found 497.0847. HPLC purity: 88%.

(*S*)-3-Chloro-*N*-(2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]-oxazin-6-yl)-*N*-(4-trifluoromethoxybenzyl)-benzamide (4e). Yield 55% yield. ^{1}H NMR ($CDCl_3$): δ 4.09 (dd, 1H, *J* = 4.8, 12.0 Hz), 4.43−4.61 (m, 2H), 4.65−4.73 (m, 3H), 4.93 (m, 1H), 7.18−7.26 (m, 6H), 7.32 (d, 1H, *J* = 7.2 Hz), 7.83 (t, 1H, *J* = 7.6 Hz), 7.45 (br s, 1H). HRMS calcd for $C_{21}H_{16}ClF_3N_4O_5$ [M + H^+] 497.0840, found 497.0829.

(*S*)-4-Chloro-*N*-(2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]-oxazin-6-yl)-*N*-(4-trifluoromethoxybenzyl)-benzamide (4f). Yield 53%. ^{1}H NMR ($CDCl_3$): δ 4.11 (m, 1H), 4.42−4.73 (m, 5H), 4.94 (m, 1H), 7.21−7.41 (m, 9H). ^{13}C NMR ($CDCl_3$): δ 44.8, 49.5, 67.6, 115.1, 122.0, 124.9, 127.3, 128.3, 130.7, 131.2, 134.9, 136.7. HRMS calcd for $C_{21}H_{16}ClF_3N_4O_5$ [M + H^+] 497.0840, found 497.0827. HPLC purity: 91.3%.

(*S*)-*N*-Methyl-2-nitro-*N*-4-trifluoromethoxybenzyl-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (5a). To solution of **2** (70 mg. 0.196 mmol) in a mixture of methanol (1 mL) and AcOH (0.2 mL) was added aqueous formaldehyde (5.9 μL, 0.196 mmol) followed by $NaBH(OAc)_3$ (24.6 mg, 0.392 mmol). After stirring at room temperature for 3 h, the solvent was removed under reduced pressure and the residue partitioned between water and ethyl acetate. The organic layer was washed with water and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 10−20% gradient mixture of ethyl acetate/chloroform as eluent to give **5a** (20 mg, 27%). ^{1}H NMR ($CDCl_3$): δ 2.35 (s, 3H), 3.34 (m, 1H), 3.74 (ABq, 2H, *J* = 14.0, 19.0 Hz), 4.15 (m, 2H), 4.53 (m, 2H), 7.18 (d, 2H, *J* = 8.0 Hz), 7.29 (d, 2H, *J* = 8.0 Hz), 7.40 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 38.6, 45.3, 53.5, 58.4, 68.0, 115.0, 121.4, 129.7, 136.8, 147.8, 148.8. HRMS calcd for $C_{15}H_{15}F_3N_4O_4$ [M + H^+] 373.1124, found 373.1110. HPLC purity: 93.5%.

(*S*)-2-Nitro-*N*-propyl-*N*-(4-trifluoromethoxybenzyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (5b). To a stirred solution of **2** (75 mg, 0.3 mmol) in THF (2 mL) containing AcOH (0.5 mL) was added propionaldehyde (53 μL, 0.73 mmol)) and $Ti(i\text{-}OPr)_4$ (0.27 mL, 0.9 mmol) at room temperature. After 30 min, $NaBH(OAc)_3$ (190 mg, 0.9 mmol) was added and the reaction was stirred for 15 h. The reaction mixture was neutralized with satd aq $NaHCO_3$ and extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using gradient mixture of 0.5−1% MeOH in $CHCl_3$ to afford 75 mg of **5b** (64%). ^{1}H NMR (DMSO-d_6): 0.77 (t, 3H, *J* = 7.2 Hz), 1.38 (m, 2H), 2.50 (m, 2H), 3.44 (m, 1H), 3.77 (ABq, 2H, *J* = 14.8, 20.4 Hz), 4.13 (m, 2H), 4.52 (d, 2H, *J* = 6.0 Hz), 7.29 (d, 2H, *J* = 8.0 Hz), 7.39 (d, 2H, *J* = 8.0 Hz), 7.98 (s, 1H). HRMS calcd for $C_{17}H_{19}F_3N_4O_4$ [M + H^+] 401.1437, found 401.1434.

(*S*)-*N*-Isopropyl-2-nitro-*N*-(4-trifluoromethoxybenzyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (5c). To a stirred solution of **2** (100 mg, 0.279 mmol) and acetone (0.2 mL, 1.39 mmol) in methanol (1.5 mL) containing AcOH (1.5 mL) was added titanium isopropoxide (0.5 mL, 1.67 mmol) at room temperature. After stirring for 2 h, $NaBH(OAc)_3$ (235 mg, 1.1 mmol) was added and the reaction was stirred for 3 days. Saturated aq $NaHCO_3$ was added, and the

reaction mixture extracted with ethyl acetate (2 × 75 mL). The organic layer was washed with water and brine, dried (anhyd Na_2SO_4), and then concentrated under reduced pressure. The residue was purified over silica gel column chromatography using 1% MeOH in $CHCl_3$ as eluent to give **5c** (30 mg, 29%). ^{1}H NMR ($CDCl_3$): δ 1.13, 1.14 (2 d, 6H, J = 6.8 Hz), 3.15 (m, 1H), 3.55 (m, 1H), 3.73 (d, 1H, J = 15.2, Hz), 3.86 (d, 1H, J = 15.2 Hz), 3.99 (m, 2H), 4.31 (dd, 1H, J = 8.8, 11.2 Hz), 4.47 (dd, 1H, J = 2.4, 11.2 Hz), 7.16 (d, 2H, J = 8.0 Hz), 7.25 (s, 1H), 7.29 (d, 2H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ 19.3, 20.8, 46.2, 49.1, 49.2, 68.7, 115.0, 119.0, 121.0, 128.6, 138.7, 143.6, 147.5, 148.3. HRMS calcd for $C_{17}H_{19}F_3N_4O_4$ [M + H$^+$] 401.1437, found 401.1451.

(*S*)-3-Ethyl-1-(2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]-oxazin-6-yl)-1-(4-trifluoromethoxybenzyl)-urea (6). To a stirred solution of ethylamine hydrochloride (42 mg, 0.52 mmol) and triphosgene (312 mg, 1.05 mmol) in dry THF (4 mL) at 0 °C was added Et_3N (0.5 mL, 2.1 mmol). After 15 min, a solution of **2** (75 mg, 0.2 mmol) in THF (2 mL) was added and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried (anhyd Na_2SO_4), and then concentrated under reduced pressure. The residue thus obtained (50 mg) was redissolved in DMF (3 mL) and treated with Et_3N (83 μL, 0.6 mmol), followed by ethylamine hydrochloride (49 mg, 0.6 mmol). After stirring for 3 h at room temperature, the reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine (10 mL), dried (anhyd Na_2SO_4), and then concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using 1−2% MeOH in $CHCl_3$ as eluent to afford 35 mg (66%) of **6**. ^{1}H NMR (DMSO-d_6): 0.99 (t, 3H, J = 7.2 Hz), 3.08 (m, 2H), 4.15 (d, 2H, J = 7.2 Hz), 4.36 (dd, 1H, J = 3.2, 10.4 Hz), 4.46−4.68 (m, 4H), 6.78 (t, 1H, J = 4.8 Hz), 7.26 (d, 2H, J = 8.8 Hz), 7.30 (d, 2H, J = 8.8 Hz), 7.98 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 15.4, 36.1, 45.8, 48.4, 49.1, 68.5, 115.2, 122.1, 127.3, 135.2, 143.9, 157.6.

Oxazol-2-yl-(4-trifluoromethoxyphenyl)methanone (9a). To a solution of oxazole (600 mg, 8.69 mmol) in dry THF (10 mL) at −78 °C was added *n*-BuLi (2.9 M in hexane, 3.3 mL, 9.57 mmol). After 30 min at −78 °C, $ZnCl_2$ (0.5 M in THF, 13.7 mL, 17.39 mmol) was added and the mixture warmed to room temperature. After 45 min, CuI (1.65 g, 8.69 mmol) was added and stirring was continued for 10 min. A solution of 4-trifluoromethoxybenzoylchloride (obtained by refluxing 4-trifluoromethoxybenzoic acid (2.14 g, 9.57 mmol) in dry THF (15 mL) containing oxalyl chloride (4.16 mL) and cat. pyridine for 3 h followed by evaporation) in THF (15 mL) was added at room temperature, and the mixture was stirred for 1 h. The reaction mixture was diluted with water (5 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a gradient of 1−2% MeOH in $CHCl_3$ to afford **9a** (900 mg, 40%). ^{1}H NMR ($CDCl_3$): δ 7.35 (d, 2H, J = 8.0 Hz), 7.44 (s, 1H), 7.93 (s, 1H), 8.60 (d, 2H, J = 8.0 Hz). ESI MS: m/z 258.0 (M + H).

(6*S*)-2-Nitro-*N*-(oxazol-2-yl(4-trifluoromethoxyphenyl)-methyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (17a). To a stirred solution of oxazol-2-yl-(4-(trifluoromethoxy)-phenyl)methanone (280 mg, 1.09 mmol) and amine **7** (200 mg, 1.09 mmol) in dry THF (4 mL) containing AcOH (2 mL) was added Ti(*i*-OPr)$_4$ (0.98 mL, 3.27 mmol) and stirred at room temperature. After 15 h, $NaBH_3CN$ (203 mg, 3.27 mmol) was added and stirring continued for 2 h. The reaction mixture was quenched with water, neutralized to pH 7 (satd aq $NaHCO_3$), and extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL) dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified using preparative HPLC to afford **17a** (40 mg, 9%) as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6): δ 3.20 (m, 1H), 3.51−3.57 (m, 1H), 4.06−4.14 (m, 2H), 4.37−4.46 (m, 2H), 5.39 (d, 1H, J = 9.0 Hz), 7.19 (s, 1H), 7.34−7.37 (m, 2H), 7.55−7.587 (m, 2H), 7.97 and 8.00 (2s, 1H), 8.07 (s, 1H). HRMS calcd for $C_{17}H_{14}F_3N_5O_5$ [M + H$^+$] 426.1025, found 426.1025.

(6*S*)-2-Nitro-*N*-(1-(4-trifluoromethoxyphenyl)ethyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (17b). Compound **17b** was prepared in a similar fashion to **17a** as a mixture of diastereomers from **9b** and amine **7** in 28% yield. ^{1}H NMR (DMSO-d_6): δ 1.26 (d, 3H, J = 6.4 Hz), 2.77 (m, 1H), 3.01 (br s, 1H), 3.78 (m, 1H), 3.92−4.07 (m, 2H), 4.19−4.35 (m, 2H), 7.31 (t, 2H, J = 8.0 Hz), 7.49 (t, 2H, J = 8.0 Hz), 7.94 and 8.01 (2s, 1H). FT-ICR HRMS calcd for $C_{15}H_{15}F_3N_4O_4$ [M + H$^+$] 373.1118, found 373.1120.

(1-Benzyl-1*H*-imidazol-2-yl)(4-trifluoromethoxyphenyl)-methanol (11). To a stirred solution of *N*-benzylimidazole (1.0 g, 6.32 mmol) in acetonitrile (15 mL) was added diisopropylcarbamoyl chloride (1.24 g, 7.59 mmol), 4-trifluoromethoxybenzaldehyde (1.4 mL, 9.49 mmol), and DIPEA (3.4 mL, 19.61 mmol) at 0 °C under nitrogen atmosphere. The reaction mixture was heated at reflux for 19 h, quenched with water (30 mL), and extracted with EtOAc (20 mL × 2). The combined organic layer was washed with water (20 mL) and brine (10 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a gradient mixture of 0−15% of EtOAc in hexane as eluent to afford 2.2 g (73%) of (1-benzyl-1*H*-imidazol-2-yl)(4-(trifluoromethoxy)phenyl)-methyldiisopropylcarbamate. ^{1}H NMR (DMSO-d_6): δ 1.00−1.15 (m, 12H), 3.81 (br s, 2H), 5.24−5.40 (m, 2H), 6.92 (d, 2H, J = 8.0 Hz), 7.06 (d, 2H, J = 8.0 Hz), 7.21 (s, 1H), 7.22−7.35 (m, 5H), 7.41 (d, 2H, J = 8.0 Hz). ESI MS: m/z 476.1 (M + H). To a stirred solution of the above product (2.2 g, 4.6 mmol) in THF (20 mL) was added TFA (2 mL) and water (2 mL). After heating at reflux for 15 h, the mixture was quenched with aqueous $NaHCO_3$ (30 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with water (20 mL) and brine solution (10 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure to afford 1.32 g (82%) of **11**. ^{1}H NMR (DMSO-d_6): δ 5.21 (s, 2H), 5.92 (d, 1H, J = 4.0 Hz), 6.38 (d, 1H, J = 4.0 Hz), 6.83 (br s, 1H), 6.95−7.10 (m, 3H), 7.23−7.25 (m, 5H), 7.39 (d, 2H, J = 8.0 Hz). ESI MS: m/z 349.1 (M + H).

(1*H*-Imidazol-2-yl)(4-trifluoromethoxyphenyl)methanol (12). To a stirred solution of **11** (500 mg, 1.43 mmol) in MeOH (10 mL) was added 5% Pd/C (100 mg) and two drops of AcOH and stirred under hydrogen (balloon) atmosphere at room temperature for 2.5 h. The reaction mixture was filtered through a Celite pad and washed with EtOAc (50 mL). The filtrate was washed with aqueous $NaHCO_3$ solution (20 mL), water (10 mL), and brine solution (10 mL). The organic phase was dried (anhyd Na_2SO_4) and concentrated under reduced pressure to afford **12** (300 mg, 81%). ^{1}H NMR (DMSO-d_6): δ 5.76 (s, 1H), 6.26 (s, 1H), 6.89 (s, 2H), 7.30 (d, 2H, J = 8.4 Hz), 7.51 (d, 2H, J = 8.4 Hz), 11.94 (br s, 1H). ESI MS: m/z 259.0 (M + H).

(6*S*)-*N*-((1*H*-Imidazol-2-yl)(4-trifluoromethoxyphenyl)-methyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (17c). To a stirred solution of **12** (300 mg, 1.20 mmol) in CH_2Cl_2(10 mL) was added methanesulfonyl chloride (0.11 mL, 1.45 mmol) and triethylamine (0.25 mL, 1.81 mmol) at 0 °C under nitrogen atmosphere and stirred at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (10 mL) and brine solution (10 mL). The organic phase was dried (anhyd Na_2SO_4) and concentrated under reduced pressure to afford 400 mg of mesylate, which was redissolved in dry THF (3 mL) and added to a previously stirred solution of (2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yl)-(4-trifluoromethoxybenzyl)amine (0.19 mg, 1.03 mmol) in THF (10 mL) containing NaH (85 mg, 60% dispersion in mineral oil, 2.06 mmol) at room temperature under the nitrogen

atmosphere. After 40 h, the reaction was quenched with aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (20 mL × 2). The combined organic layer was washed with water (20 mL) and brine (10 mL) and dried (anhyd Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a gradient mixture of 0−10% of MeOH in EtOAc as eluent to afford **17c** (65 mg, 15%) as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6): δ 3.10−3.30 (m, 2H), 3.90−4.10 (m, 1H), 4.13 (dd, 1H, J = 4.0, 12.0 Hz), 4.30−4.45 (m, 2H), 5.17 (d, 1H, J = 7.6 Hz), 6.60−7.10 (br s, 2H), 7.25−7.35 (m, 2H), 7.51 (dd, 2H, J = 2.4, 8.8 Hz), 7.98 (d, 1H, J = 2.4 Hz), 11.8 (br s, 1H). HRMS calcd for $C_{17}H_{15}F_3N_6O_4$ [M + H$^+$] 425.1185, found 425.1187.

1-(4-Trifluoromethoxyphenyl)propan-1-ol (14a). To a stirred solution of 4-trifluoromethoxybenzaldehyde (5 g, 26.31 mmol) in THF was added ethylmagnesium bromide (2 M solution, 32.8 mL, 65.7 mmol) at −15 °C and warmed to room temperature over 30 min. After 3 h, the reaction mixture was quenched with aqueous NH_4Cl and extracted with ethyl acetate. The organic layer was washed with water (2 × 200 mL and brine (200 mL), dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a solvent gradient mixture of 0−5% EtOAc in hexane as eluent to afford **14a** (3.5 g, 62%). ^{1}H NMR (DMSO-d_6): δ 0.81 (t, 3H, J = 7.6 Hz), 1.50−1.70 (m, 2H), 4.48 (m, 1H), 5.24 (d, 1H, J = 4.4 Hz), 7.29 (d, 2H, J = 8.4 Hz), 7.42 (d, 2H, J = 8.4 Hz).

In a similar fashion, the following compounds were synthesized:

1-(4-Trifluoromethoxyphenyl)butan-1-ol (14b). Yield 65%. ^{1}H NMR (DMSO-d_6): δ 0.88 (t, 3H, J = 7.2 Hz), 1.22−1.36 (m, 2H), 1.34−1.61 (m, 2H), 4.53−4.58 (m, 1H), 5.20 (d, 1H, J = 4.0 Hz), 7.29 (d, 2H, J = 8.0 Hz), 7.43 (d, 2H, J = 8.0 Hz).

1-(4-Trifluoromethoxyphenyl)pentan-1-ol (14c). To solution of 4-trifluoromethoxy benzaldehyde (3 g, 15.78 mmol) in THF at −78 °C was added *n*-BuLi (1.6 M in hexane, 11 mL, 17.36 mmol) and warmed to room temperature over 30 min. After 2 h, the reaction mixture was quenched with ice and extracted with diethyl ether (2 × 60 mL). The combined organic layer was washed with water and brine solution, dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a solvent gradient mixture of 0−2% EtOAc in hexane as eluent to afford **14c** (3.1 g, 79%). ^{1}H NMR (DMSO-d_6): δ 0.82−0.89 (m, 3H), 1.15−1.41 (m, 4H), 1.50−1.63 (m, 2H), 4.51−4.57 (m, 1H), 5.21−5.24 (m, 1H), 7.26−7.32 (m, 2H), 7.40−7.45 (m, 2H).

1-(1-Bromopropyl)-4-trifluoromethoxybenzene (15a). To a stirred solution of **14a** (3.5 g, 15.98 mmol, 1 equiv) in ether was added PBr_3 (0.5 g, 0.75 mL, 7.99 mmol) at 0 °C. After stirring at 0 °C for 1 h, the reaction mixture was diluted with cold water and extracted into ether (800 mL). The organic layer was washed with water (150 mL) and brine (150 mL), dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (100−200mesh) using a 5−10% gradient mixture of EtOAc in hexane as eluent to afford **15a** (2.5 g, 51%). ^{1}H NMR ($CDCl_3$): δ 1.00 (t, 3H, J = 6.8 Hz), 2.10−2.30 (m, 2H), 4.80−4.96 (m, 1H), 7.18 (d, 2H, J = 8.4 Hz), 7.41 (d, 2H, J = 8.4 Hz).

In a similar fashion, the following compounds were synthesized:

1-(1-Bromobutyl)-4-trifluoromethoxybenzene (15b). Yield 50%. ^{1}H NMR ($CDCl_3$): δ 0.86 (t, 3H, J = 7.6 Hz), 1.26−1.57 (m, 2H), 2.03−2.12 (m, 1H), 2.18−2.29 (m, 1H), 4.94 (t, 1H, J = 7.6 Hz), 7.18 (d, 2H, J = 8.0 Hz), 7.42 (d, 2H, J = 8.0 Hz).

1-(1-Bromopentyl)-4-trifluoromethoxybenzene (15c). Yield 52%. ^{1}H NMR ($CDCl_3$): δ 0.87−0.98 (m, 3H), 1.25−1.49 (m, 4H), 2.00−2.40 (m, 2H), 4.90−5.00 (m, 1H), 7.16−7.20 (m, 2H), 7.39−7.44 (m, 2H).

(6*S*)-2-Nitro-*N*-(1-(4-trifluoromethoxyphenyl)propyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (17d). To a stirred solution of amine **7** (200 mg, 1.086 mmol) and 1-(1-bromopropyl)-4-(trifluoromethoxy)benzene (500 mg, 1.63 mmol) in acetonitrile was added K_2CO_3 (449 mg, 3.26 mmol) and KI (16 mg, 0.108 mmol). After heating at reflux for 48 h, the solvent was evaporated and the residue partitioned between ethyl acetate and water. The organic layer was washed with brine (150 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (100−200 mesh) using a solvent gradient mixture of 0−10% MeOH in $CHCl_3$ to afford **17d** (100 mg, 25%) as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6): δ 0.67, 0.72 (2 t, 3H, J = 7.6 Hz), 1.40−1.70 (m, 2H), 2.70−2.80 (m, 1H), 2.90−3.00 (m, 1H), 3.60−4.10 (m, 3H), 4.10−4.46 (m, 2H), 7.33 (d, 2H, J = 8.0 Hz), 7.47 (d, 2H, J = 8.0 Hz), 7.94 and 8.01 (2s, 1H). HRMS calcd for $C_{16}H_{17}F_3N_4O_4$ [M + H$^+$] 387.1280, found 387.1267.

In a similar manner, the following compounds were synthesized:

(6*S*)-2-Nitro-*N*-(1-(4-trifluoromethoxyphenyl)butyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (17e). Yield 10%. ^{1}H NMR (DMSO-d_6): δ 0.70−0.85 (m, 3H), 1.00−1.25 (m, 2H), 1.35−1.70 (m, 2H), 2.72−2.79 (m, 1H), 2.95 (s, 1H), 3.74−3.83 (m, 1H), 4.01−4.05 (m, 2H), 4.15−4.36 (m, 2H), 7.32 (m, 2H), 7.46−7.49 (m, 2H), 7.96 and 8.02 (2 s, 1H). HRMS calcd for $C_{17}H_{19}F_3N_4O_4$ [M + H$^+$] 401.1437, found 401.1431.

(6*S*)-2-Nitro-*N*-(1-(4-trifluoromethoxyphenyl)pentyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (17f). Yield 10%. ^{1}H NMR (DMSO-d_6): δ 0.75−0.79 (2 t, 3H, J = 5.2 Hz), 1.00−1.23 (m, 4H), 1.42−1.63 (m, 2H), 2.71−2.76 (m, 1H), 2.95 (br s, 1H), 3.67−3.80 (m, 2H), 4.01−4.05 (m, 1H), 4.16−4.34 (m, 2H), 7.30−7.43 (m, 2H), 7.46, 7.48 (2 dd, 2H, J = 8.0 Hz), 7.95 and 8.01 (2s, 1H). HRMS calcd for $C_{18}H_{21}F_3N_4O_4$ [M + H$^+$] 415.1593, found 415.1600.

2-((*S*)-2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-ylamino)-2-(4-trifluoromethoxyphenyl)-acetamide (17g). A mixture of amine **7** (100 mg, 0.543 mmol) and trifluoromethoxybenzaldehyde (206 mg, 1.086 mmol) were heated at 100 °C for 5 min. The reaction mixture was brought to room temperature and added TMSCN (216 mg, 2.172 mmol) to the reaction mixture. After heating for 30 min, the reaction mixture was cooled to room temperature and diluted with ethyl acetate (50 mL). Organic layer was washed with water (2 × 10 mL) and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (100−200 mesh) using a solvent gradient of 10−60% EtOAc−hexane as eluent to afford 105 mg (50%) of 2-((*S*)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-ylamino)-2-(4-trifluoromethoxyphenyl)acetonitrile (**16**). ^{1}H NMR (DMSO-d_6): δ 3.46−3.52 (m, 1H), 3.80−3.95 (m, 1H), 4.00−4.10 (m, 1H), 4.17−4.20 (m, 1H), 4.42−4.59 (m, 2H), 5.33−5.42 (m, 1H), 7.42−7.47 (m, 2H), 7.55−7.61 (m, 2H), 8.00 and 8.08 (2s, 1H). ESI MS: m/z 384.0 (M + H). A solution of **16** (100 mg, 0.257 mmol) in ethanol (5 mL) was purged with HCl gas for 1 h at −10 °C. After 15 min, reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted with ethyl acetate (2 × 30 mL). The organic layer was washed with water and brine, dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a solvent gradient of 15% MeOH in $CHCl_3$ as eluent to afford 40 mg (38%) of **17g** as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6): δ 3.11−3.16 (m, 2H), 3.95−4.20 (m, 2H), 4.38−4.46 (m, 3H), 7.19 (s, 1H), 7.31−7.35 (m, 2H), 7.52−7.56 (m, 3H), 7.97 and 8.05 (2s, 1H). FT-ICR MS calcd for $C_{15}H_{14}F_3N_5O_5$ [M + H$^+$] 402.1019, found 402.1023. HPLC purity 95.6%.

2-(4-Methoxybenzyloxy)-1-(4-trifluoromethoxyphenyl)-ethanol (19). To a mixture of 2-(4-trifluoromethoxyphenyl)oxirane (2 g, 9.80 mmol) and *p*-methoxybenzyl alcohol (2.19 g, 19.60 mmol) was added KO*t*Bu (2.19 g, 19.6 mmol) and heated at 60 °C for 2 h. The reaction mixture was diluted with water (100 mL) and extracted with

ethyl acetate (150 mL). The organic layer was washed with water (2 × 50 mL) and brine (1 × 50 mL), dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (100−200 mesh) using a solvent gradient of 0−5% EtOAc in hexane as eluent to afford **19** (1.3 g, 34%). ^{1}H NMR (DMSO-d_6): δ 3.35−3.55 (m, 2H), 3.73 (s, 3H), 4.42 (s, 2H), 4.74−4.77 (m, 1H), 5.52 (d, 1H, J = 4.8 Hz), 6.87 (d, 2H, J = 8.8 Hz), 7.19 (d, 2H, J = 8.0 Hz), 7.30 (d, 2H, J = 8.0 Hz), 7.47 (d, 2H, J = 8.8 Hz).

2-Hydroxy-1-(4-trifluoromethoxyphenyl)ethanone (20). To a solution of **19** (1.3 g, 3.82 mmol) in CH_2Cl_2 (20 mL) was added pyridinium dichromate (2.6 g, 68.8 mmol) and 4 Å molecular sieves powder and stirred at room temperature for 24 h. The reaction mixture was filtered through Celite and filtrate concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a solvent gradient of 0−30% EtOAc in hexane as eluent to afford 800 mg (62%) of 2-(4-methoxybenzyloxy)-1-(4-(trifluoromethoxy)phenyl)ethanone. This compound (800 mg, 2.36 mmol) was dissolved in CH_2Cl_2 (10 mL) and added TBDMSOTf (1.08 mL, 4.73 mmol) at 0 °C. After stirring for 15 min at room temperature, the reaction mixture was quenched with satd $NaHCO_3$ solution and extracted with ethyl acetate (75 mL). The organic layer was washed with water (2 × 50 mL) and brine (50 mL) and dried (over anhyd Na_2SO_4) and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a solvent gradient of 0−30% EtOAc in hexane as eluent to afford **20** (450 mg, 86%). ^{1}H NMR (DMSO-d_6): δ 4.80 (d, 2H, J = 5.6 Hz), 5.19 (t, 1H, J = 6.0 Hz), 7.51 (d, 2H, J = 8.0 Hz), 8.06 (d, 2H, J = 8.0 Hz).

2-((*S*)-2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-ylamino)-2-(4-trifluoromethoxyphenyl)-ethanol (17h). A mixture of amine **7** (300 mg, 1.63 mmol) and 2-hydroxy-1-(4-trifluoromethoxyphenyl)ethanone (430 mg, 1.95 mmol) in ethanol (2 mL) was heated at 70 °C for 1 h. After cooling, the reaction mixture to room temperature, AcOH (1 drop) and $NaCNBH_3$ (101.08 mg, 1.63 mmol) were added, and stirring continued for 16 h. The solvent was removed and the residue partitioned between ethyl acetate and water. The organic layer was washed with satd aq $NaHCO_3$ solution (2 × 50 mL), water (50 mL), and brine (1 × 50 mL), dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatogrpahy eluting with 2% MeOH in $CHCl_3$ to afford 30 mg (5%) of **17h** as a mixture of diasteromers. ^{1}H NMR (DMSO-d_6): δ 2.69 (br s, 1H), 3.09 (s, 1H), 3.30−3.43 (m, 2H), 3.70−4.60 (m, 5H), 4.80−5.00 (m, 1H), 7.20−7.60 (m, 4H), 7.91 and 8.04 (2s, 1H). HRMS calcd for $C_{15}H_{15}F_3N_4O_5$ [M + H$^+$] 389.1073, found 389.1079.

1-(4-Trifluoromethoxyphenyl)propane-1,3-diol (21). To a stirred solution of 4-trifluoromethoxybenzaldehyde (6.0 g, 31.57 mmol) in THF (40 mL) and methyl bromoacetate (11.5 mL, 126.31 mmol), Zn powder (16.5 g, 252.6 mmol) was added portionwise at room temperature and sonicated for 3 h. The reaction mixture was filtered through Celite and washed with CH_2Cl_2 (500 mL). The filtrate was concentrated under reduced pressure and the crude residue purified by column chromatography over silica gel using a solvent gradient of 0−5% EtOAc in hexane as eluent to afford 3 g of ethyl 3-hydroxy-3-(4-trifluoromethoxyphenyl)propanoate. This compound (3 g, 10.8 mmol) was dissolved in anhydrous THF (30 mL) and treated with $LiAlH_4$ (514 mg, 15.15 mmol) at 0 °C. After 2 h, the reaction mixture was quenched at 0 °C with satd aq Na_2SO_4 solution and filtered. The filtrate was washed with water (2 × 75 mL) and brine (75 mL), dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a solvent gradient of 0−40% EtOAc in hexane as eluent to afford **21** (1.8 g, 25%). ^{1}H NMR ($CDCl_3$): δ 1.80−2.10 (m, 2H), 2.31 (s, 1H), 3.31 (s, 1H), 3.90 (s, 2H), 4.80−5.05 (m, 1H), 7.10−7.30 (m, 2H), 7.35−7.45 (m, 2H). ESI MS: m/z 237.0 (M + H).

3-Hydroxy-1-(4-trifluoromethoxyphenyl)propan-1-one (22). A solution of compound **21** (1.2 g, 5.08 mmol) in THF (20 mL) was treated with MnO_2 (4.2 g, 50.84 mmol) at room temperature. After 6 h, the reaction mixture was filtered through Celite, washed with excess CH_2Cl_2 (100 mL), and concentrated. The residue was purified by column chromatography over silica gel using a solvent gradient of 0−30% EtOAc in hexane as eluent to afford **22** (450 mg, 50%). ^{1}H NMR (DMSO-d_6): δ 3.14−3.18 (t, 2H, J = 6.0 Hz), 3.78 (m, 2H), 4.650 (t, 1H, J = 4.8 Hz), 7.51(d, 2H, J = 8.2 Hz), 8.10 (d, 2H, J = 8.2 Hz).

2-((*S*)-2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-ylamino)-2-(4-trifluoromethoxyphenyl)-ethanol (17i). A mixture of amine **7** (300 mg, 1.630 mmol) and **22** (457 mg, 1.456 mmol) in ethanol (5 mL) was heated at 70 °C for 36 h. After cooling the reaction mixture to room temperature, AcOH (1 drop) and $NaCNBH_3$ (202 mg, 3.26 mmol) were added and stirring continued for 4 h. The solvent was removed and the residue partitioned between ethyl acetate and water. The organic layer was washed with satd aq $NaHCO_3$ solution (2 × 50 mL), water (50 mL), and brine (1 × 50 mL), dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography eluting with 2% MeOH in $CHCl_3$ to afford 30 mg (5%) of **17i** as a mixture of diastereomers. ^{1}H NMR (DMSO-d_6): δ 1.69−1.70 (br s, 2H), 2.23 (s, 1H), 2.64 (br s, 2H), 3.24 (s, 1H), 3.92 (d, 1H, J = 12.0 Hz), 4.15 (d, 1H, J = 12.0 Hz), 4.29−4.33 (m, 1H), 4.39−4.45 (m, 1H), 4.66 (s, 1H), 5.39 (m, 1H), 7.28−7.31 (m, 2H), 7.40−7.44 (m, 2H), 8.06 (s, 1H). HRMS calcd for $C_{16}H_{17}F_3N_4O_5$ [M + H$^+$] 403.1229, found 403.1218.

Ethyl-2-benzyloxy-4-trifluoromethoxybenzoate (27b). To a stirred solution of ethyl-2-hydroxy-4-trifluoromethoxybenzoate **24** (300 mg, 1.20 mmol) in DMF (4 mL) was added benzyl bromide (0.17 mL, 1.44 mmol) and K_2CO_3 (331 mg, 2.4 mmol) and heated at 70 °C for 30 min. It was diluted with EtOAc (50 mL) and washed with water (30 mL × 2) and brine solution (20 mL). The organic phase was separated, dried (anhyd Na_2SO_4), and concentrated under reduced pressure to afford 400 mg (quant) of **27b**. ^{1}H NMR ($CDCl_3$): δ 1.37 (t, 3H, J = 7.2 Hz), 4.36 (q, 2H, J = 3.2, 14.4 Hz), 5.15 (s, 2H), 6.84 (m, 2H), 6.29−7.49 (m, 5H), 7.87 (d, 1H, J = 8.4 Hz). ESI MS: m/z 341.1 (M + H).

Ethyl 2-Methoxymethoxy-4-trifluoromethoxybenzoate (27a). To a solution of ethyl-2-hydroxy-4-trifluoromethoxybenzoate **24** (500 mg, 2.00 mmol) in CH_2Cl_2 (5 mL) was added diisopropylethylamine (1.09 mL, 6.72 mmol) followed by methoxymethylchloride (0.53 mL, 6.72 mmol) at room temperature. After 3 h, the reaction mixture was poured into water and extracted with CH_2Cl_2 (10 mL). The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure to give crude product, which upon purification by silica gel column chromatography using 2% ethyl acetate in hexane as eluent gave 480 mg (81%) **27a**. ^{1}H NMR ($CDCl_3$): δ 1.38 (t, 3H, J = 7.2 Hz), 3.52 (s, 3H), 4.36 (q, 2H, J = 3.2, 14.4 Hz), 5.25 (s, 2H), 6.89 (d, 1H, J = 8.4 Hz), 7.05 (s, 1H), 7.82 (d, 1H, J = 8.4 Hz). ESI MS: m/z 295.0 (M + H).

Ethyl (2-Cyclopropylmethoxy)-(4-trifluoromethoxy)benzoate (27c). To a solution of **24** (300 mg, 1.20 mmol) in DMF (4 mL) was added bromomethylcyclopropane (0.17 mL, 1.44 mmol) and K_2CO_3 (331 mg, 2.4 mmol). After heating at 100 °C for 3 h, the reaction mixture was poured into water and extracted with EtOAc (50 mL). The organic layer was washed with water (30 mL × 2) and brine solution (20 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a gradient mixture of 0−3% of EtOAc−hexane as eluent to afford **27c** (300 mg, 82%). ^{1}H NMR ($CDCl_3$): δ 0.39−0.43 (m, 2H), 0.63−0.67 (m, 2H), 1.22−1.29 (m, 1H), 1.37 (t, 3H, J = 7.2 Hz), 3.89 (d, 2H, J = 6.8 Hz), 4.37 (q, 2H, J = 3.2, 14.4 Hz), 6.75 (s, 1H), 6.82 (d, 1H, J = 8.4 Hz), 7.82 (d, 1H, J = 8.4 Hz). ESI MS: m/z 305.0 (M + H).

2-Benzyloxy-4-trifluoromethoxybenzaldehyde (28b). To a stirred solution of **27b** (400 mg, 1.17 mmol) in THF (10 mL) was added $LiAlH_4$ (66 mg, 1.76 mmol) at 0 °C under nitrogen atmosphere. After stirring 0 °C for 1 h, the reaction mixture was quenched with water (30 mL) and then extracted with EtOAc (20 mL × 2). The organic layer was washed with water (20 mL) and brine solution (10 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure to afford 320 mg of (2-benzyloxy-4-trifluoromethoxyphenyl)methanol which was dissolved in CH_2Cl_2 (10 mL) and treated with PCC (300 mg) at room temperature under nitrogen atmosphere. After 1 h, the reaction mixture was filtered through Celite pad and the filtrate concentrated under reduced pressure. The residue was passed through a short pad of silica gel eluting with EtOAc−hexane (1:5) to afford 220 mg (63%) of **28b**. ^{1}H NMR (DMSO-d_6): δ 5.34 (s, 2H), 7.08 (d, 1H, J = 8.4 Hz), 7.34 (s, 1H), 7.35 −7.44 (m, 4H), 7.51 (d, 1H, J = 7.2 Hz), 7.84 (d, 1H, J = 8.4 Hz), 10.35 (s, 1H). ESI MS: m/z 297.0 (M + H).

2-(Methoxymethoxy)-4-(trifluoromethoxy)benzaldehyde (28a). To a stirred solution of **27a** (480 mg, 1.63 mmol) in THF (5 mL) at (0 °C) was added $LiAlH_4$ (74.2 mg, 1.96 mmol) and stirred at room temperature for 30 min. The reaction mixture was quenched with moist ethyl acetate. The organic layer was washed with water and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude product was filtered through short silica plug using 10% ethyl acetate in hexane as eluent to get 380 mg of (2-methoxymethoxy-4-trifluoromethoxyphenyl)-methanol, which was dissolved (380 mg, 1.29 mmol) in CH_2Cl_2 (5 mL) and treated with pyridinium chlorochromate (760 mg) at room temperature for 1 h. The reaction mixture was filtered through Celite and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using gradient mixture of 0−2% ethyl acetate in hexanes as eluent to give **28a** (350 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 3.54 (s, 3H), 5.31 (s, 2H), 6.94 (d, 1H, J = 8.4 Hz), 7.08 (s, 1H), 7.89 (d, 1H, J = 8.4 Hz), 10.43 (s, 1H).

2-Cyclopropylmethoxy-4-trifluoromethoxybenzaldehyde (28c). To a stirred solution of **27c** (280 mg, 0.92 mmol) in THF (10 mL) was added $LiAlH_4$ (52 mg, 1.38 mmol) at 0 °C under nitrogen atmosphere. After stirring 0 °C for 1 h, the reaction mixture was quenched with water (30 mL) and extracted with EtOAc (20 mL × 2). The organic layer was washed with water (20 mL) and brine solution (10 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure to afford 190 mg of (2-cyclopropylmethoxy-4-trifluoromethoxyphenyl)methanol. This compound was dissolved in CH_2Cl_2 (10 mL) and added PCC (400 mg) at room temperature under nitrogen atmosphere. After 2 h, the reaction mixture was filtered through Celite pad and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a gradient mixture of 0−2% EtOAc in hexane as eluent to afford **28c** (180 mg, 75%). ^{1}H NMR ($CDCl_3$): δ 0.38−0.42 (m, 2H), 0.67−0.72 (m, 2H), 1.25−1.33 (m, 1H), 3.93 (d, 2H, J = 6.8 Hz), 6.75 (s, 1H), 6.86 (d, 1H, J = 8.4 Hz), 7.88 (d, 1H, J = 8.4 Hz), 10.48 (s, 1H). ESI MS: m/z 261.0 (M + H).

4-(Trifluoromethoxy)-2-(vinyloxy)benzoic Acid (25). To a stirred solution of **24** (800 mg, 3.20 mmol) in DMF (5 mL) was added K_2CO_3 (1.32 g, 9.60 mmol) and 1-bromo-2-chloroethane (1.32 mL, 16.00 mmol) under nitrogen atmosphere and stirred at room temperature for 15 h. It was diluted with cold water (20 mL) and extracted with EtOAc (15 mL × 2). The combined organic layer was washed with water (10 mL) and brine. The organic phase was dried (anhyd Na_2SO_4) and then concentrated under reduced pressure to give crude compound. The residue was purified by column chromatography over silica gel using a gradient mixture of 0−5% EtOAc−hexane as eluent to afford 800 mg (80%) of ethyl-[2-(2-chloro-ethoxy)-4-trifluoromethoxy]benzoate. ^{1}H NMR ($CDCl_3$): δ 1.39 (t, 3H, J = 6.8 Hz), 3.87 (t, 2H, J = 6.0 Hz), 4.28 (t, 2H, J = 6.0 Hz), 4.36 (q, 2H, J = 6.8 Hz), 6.77 (s, 1H), 6.89 (d, 1H, J = 8.4 Hz), 7.86 (d, 1H, J = 8.4 Hz). ESI MS: m/z 313.0 (M + H). To a stirred solution of ethyl-[2-(2-chloro-ethoxy)-4-trifluoromethoxy]-benzoate (800 mg, 2.56 mmol) in THF (10 mL) was added KO-t-Bu (575 mg, 5.12 mmol) at 0 °C under nitrogen atmosphere. After stirring at 20 °C for 1 h, saturated NH_4Cl solution (20 mL) was added and the reaction mixture extracted with EtOAc (15 mL × 2). The combined organic layer was washed with water (10 mL) and brine. The organic phase was dried (anhyd Na_2SO_4) and concentrated under reduced pressure to afford **25** (500 mg, 78%). ^{1}H NMR ($CDCl_3$): δ 4.86 (m, 1H), 5.09 (dd, 1H, J = 2.0, 13.2 Hz), 6.60 (dd, 1H, J = 5.6, 13.2 Hz), 6.94 (s, 1H), 7.07 (d, 1H, J = 8.0 Hz), 8.19 (d, 1H, J = 8.0 Hz). ESI MS: m/z 249.0 (M + H).

2-Cyclopropoxy-4-trifluoromethoxybenzaldehyde (26). To a solution of Et_2Zn (4 mL, 4.01 mmol) in dichloroethane (5 mL) at −20 °C was added CH_2I_2 (0.32 mL, 4.01 mmol) under nitrogen atmosphere and stirred for 10 min at −20 °C. A cold (0 °C) solution of 4-trifluoromethoxy-2-vinyloxybenzoic acid (500 mg, 2.01 mmol, solution in 30% toluene−dichloroethane) was added at −20 °C, and the resulting mixture stirred at room temperature for 17 h. It was quenched with 2N aqueous HCl (20 mL) and then extracted with EtOAc (10 mL × 2). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a gradient mixture of 0−20% EtOAc in hexane as eluent to afford 270 mg (51%) of 2-cyclopropoxy-4-trifluoromethoxybenzoic acid. ^{1}H NMR (DMSO-d_6): δ 0.69 (m, 2H), 0.83 (m, 2H), 3.98 (m, 1H), 7.00 (d, 1H, J = 8.0 Hz), 7.35 (s, 1H), 7.76 (d, 1H, J = 8.0 Hz), 12.84 (s, 1H). ESI MS: m/z 261.0 (M-H). To a cold stirred solution of 2-cyclopropoxy-4-trifluoromethoxybenzoic acid (270 mg, 1.03 mmol) in THF (10 mL) at 0 °C was added BH_3-DMS (0.19 mL, 2.06 mmol) under nitrogen atmosphere. After refluxing for 1.5 h at room temperature, the reaction mixture was cooled to 0 °C and quenched with saturated aqueous NH_4Cl solution (20 mL). The crude product was extracted with EtOAc (10 mL × 2), organic layer washed with water (10 mL) and brine, dried (anhyd Na_2SO_4), and evaporated to afford 230 mg of (2-cyclopropoxy-4-trifluoromethoxyphenyl)methanol as colorless oil. This compound (230 mg, 0.927 mmol) was dissolved in CH_2Cl_2 (10 mL) and treated with PCC (500 mg) under nitrogen atmosphere at room temperature. After 0.5 h, the mixture was filtered through Celite pad and the filtrate concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a gradient mixture of 0−5% EtOAc in hexane as eluent to afford **26** (200 mg, 80%). ^{1}H NMR ($CDCl_3$): δ 0.89 (m, 4H), 3.84 (m, 1H), 6.89 (d, 1H, J = 8.0 Hz), 7.18 (s, 1H), 7.86 (d, 1H, J = 8.0 Hz), 10.32 (s, 1H). ESI MS: m/z 247.0 (M + H).

Ethyl 2-(4-Aminophenoxy)-4-(trifluoromethoxy)benzoate (29). To a stirred solution of **24** (250 mg, 1.0 mmol) in DMF (5 mL) was added NaH (45 mg, 1.20 mmol) at 0 °C under nitrogen atmosphere and stirred at 0 °C for 10 min. 1-Fluoro-4-nitrobenzene (0.3 mL, 3.0 mmol) was added and the mixture heated at 100 °C. After 2 h, the reaction mixture was cooled to 0 °C, diluted with water (50 mL), and then extracted with EtOAc (20 mL × 2). The combined organic layer was washed with water (50 mL) and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a gradient mixture of 0−4% EtOAc in hexane as eluent to afford 250 mg (67%) of ethyl-2-(4-nitrophenoxy)-4-trifluoromethoxybenzoate. ^{1}H NMR ($CDCl_3$): δ 1.81 (t, 3H, J = 7.2 Hz), 4.21 (q, 2H, J = 7.2, 14.0 Hz), 6.94−6.99 (m, 3H), 7.22 (d, 1H, J = 8.0 Hz), 8.10 (d, 1H, J = 8.0 Hz), 8.21−8.24 (m, 2H). ESI MS: m/z 370.0 (M − H). To a stirred solution of ethyl-2-(4-nitrophenoxy-4-trifluoromethoxybenzoate (250 mg, 0.65 mmol) in EtOAc (15 mL) was added 5% Pd/C (20 mg) and stirred under the hydrogen atmosphere. After 1.5 h, the reaction mixture was filtered through Celite pad and the filtrate concentrated under reduced pressure to afford **29** (200 mg, 87%). ^{1}H NMR (DMSO-d_6): δ 1.23 (t, 3H, J = 7.2

Hz), 4.25 (q, 2H, J = 7.2, 14.0 Hz), 5.10 (br s, 2H), 6.59−6.63 (m, 3H), 6.78 (d, 2H, J = 8.2 Hz), 7.10 (d, 1H, J = 8.2 Hz), 7.86 (d, 1H, J = 8.2 Hz). ESI MS: m/z 342.0 (M + H).

(2-Phenoxy-4-(trifluoromethoxy)phenyl)methanol (30). To a solution of **29** (190 mg, 0.55 mmol) in aqueous 6N HCl (5 mL) was added aqueous $NaNO_2$ solution (50 mg, 0.72 mmol in 1 mL of water) at 0 °C. After stirring at 0 °C for 30 min, a cold solution of 50% H_3PO_2 (0.3 mL, 5.57 mol) was added and stirred at 50 °C for 1 h. The reaction mixture was diluted with water (15 mL) and extracted with EtOAc (10 mL × 2). The combined organic layer was washed with water (10 mL) and brine (10 mL), dried (anhyd Na_2SO_4), and concentrated to afford 130 mg of ethyl-2-phenoxy-4-trifluoromethoxybenzoate (0.39 mmol), which was dissolved in THF (10 mL) and treated with $LiAlH_4$ (22 mg, 0.59 mmol) at 0 °C under nitrogen atmosphere. After stirring at room temperature for 30 min, the reaction mixture was quenched with ice and filtered. The filtrate was diluted with EtOAc (20 mL), washed with water (20 mL) and brine solution (10 mL), and dried (anhyd Na_2SO_4). Evaporation under reduced pressure and purification of the residue by column chromatography over silica gel using a gradient mixture of 0−10% EtOAc in hexane as eluent afforded **30** (55 mg, 48%). ^{1}H NMR ($CDCl_3$): δ 4.77 (d, 2H, J = 5.6 Hz), 6.67 (s, 1H), 6.97−6.99 (m, 2H), 7.03 (d, 1H, J = 8.0 Hz), 7.18 (t, 1H, J = 7.2 Hz), 7.38 (t, 2H, J = 7.2 Hz), 7.48 (d, 1H, J = 8.0 Hz). ESI MS: m/z 283.1 (M − H).

2-Phenoxy-4-trifluoromethoxybenzaldehyde (31). To a stirred solution of **30** (55 mg, 0.19 mmol) in CH_2Cl_2 (10 mL) was added PCC (120 mg) at room temperature under nitrogen atmosphere. After 30 min, the reaction mixture was filtered through Celite and the filtrate concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluting with 10% EtOAc in hexane to afford **31** (50 mg, quant). ^{1}H NMR ($CDCl_3$): δ 6.65 (s, 1H), 7.00 (d, 1H, J = 8.0 Hz), 7.11 (d, 2H, J = 8.2 Hz), 7.24−7.28 (m, 1H), 7.45 (t, 2H, J = 8.0 Hz), 7.98 (d, 1H, J = 8.2 Hz), 10.45 (s, 1H). ESI MS: m/z 283 (M + H).

(*S*)-*N*-(2-Benzyloxy-4-trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (32b). To a stirred solution of **28b** (100 mg, 0.33 mmol) in DMF−AcOH (5 mL, 1:1) was amine **7** (68 mg, 0.37 mmol) at 0 °C under the nitrogen atmosphere. After stirring at room temperature for 1 h, $NaBH(OAc)_3$ (214 mg, 1.01 mmol) was added and stirring continued for 24 h. The reaction mixture was diluted with EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ solution (20 mL) and water (20 mL), and brine solution. The organic layer was dried (anhyd Na_2SO_4), evaporated under reduced pressure, and the residue purified by column chromatography over silica gel using a gradient mixture of 0−1% MeOH in $CHCl_3$ as eluent to afford **32b** (35 mg, 24%). ^{1}H NMR (DMSO-d_6): 3.78 (br s, 2H), 3.94−3.97 (m, 1H), 4.11−4.13 (m, 1H), 4.34−4.40 (m, 3H), 5.15 (s, 2H), 6.88 (d, 1H, J = 8.0 Hz), 7.04 (s, 1H), 7.30−7.40 (m, 5H), 7.44 (d, 1H, J = 7.8 Hz), 7.94 (s, 1H), ^{13}C NMR (acetone-d_6): δ 45.7, 48.3, 49.0, 70.3, 71.2, 106.6, 113.4, 117.4, 128.6, 129.1, 129.6, 131.2, 137.7, 149.8, 158.5. HRMS calcd for $C_{21}H_{19}F_3N_4O_5$ [M + H$^+$] 465.1386, found 465.1387.

The following compounds were synthesized in a similar manner:

(*S*)-*N*-(2-Methoxy-4-trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (32c). Yield 23%. ^{1}H NMR (DMSO-d_6): δ 3.20−3.30 (m, 1H), 3.75 (d, 3H, J = 9.2 Hz), 3.81 (s, 3H), 3.90−4.10 (dd, 1H, J = 5.6, 16.0 Hz), 4.16 (dd, 1H, J = 5.6, 16.0 Hz), 4.30−4.50 (m, 2H), 6.89 (d, 1H, J = 10.2 Hz), 6.95 (s, 1H), 7.39 (d, 1H, J = 10.2 Hz), 8.01 (s, 1H). HRMS calcd for $C_{15}H_{15}F_3N_4O_5$ [M + H$^+$] 389.1059, found 389.1061. HPLC purity: 94%.

(*S*)-*N*-(2-Methoxymethoxy-4-trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (32a). Yield 35%. ^{1}H NMR ($CDCl_3$): δ 3.37−3.39 (m, 1H), 3.46 (s, 3H), 3.90 (s, 2H), 3.94 (dd, 1H, J = 4, 12.4 Hz), 4.15 (dd, 1H, J = 4, 12.4 Hz), 4.32−4.44 (m, 2H), 5.20 (s, 2H), 6.86 (d, 1H, J = 8.4 Hz), 7.00 (s, 1H), 7.27 (m, 1H), 7.38 (s, 1H). ESI MS: m/z 419.2 (M + H).

(*S*)-*N*-(2-Cyclopropylmethoxy-4-trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (32e). Yield 11%. ^{1}H NMR (DMSO-d_6): δ 0.32−0.35 (m, 2H), 0.55−0.59 (m, 2H), 1.19−1.25 (m, 1H), 3.27 (m, 1H), 3.78 (d, 2H, J = 6.8 Hz), 3.87 (d, 2H, J = 6.8 Hz), 4.02 (dd, 1H, J = 3.2, 13.0 Hz), 4.17 (dd, 1H, J = 4.0, 13.0 Hz), 4.41 (m, 2H), 6.87 (d, 1H, J = 8.4 Hz), 6.91 (s, 1H), 7.39 (d, 1H, J = 8.4 Hz), 8.02 (s, 1H). HRMS calcd for $C_{18}H_{19}F_3N_4O_5$ [M + H$^+$] 429.1386, found 429.1391.

(*S*)-*N*-(2-Cyclopropoxy-4-trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (32f). Yield 8%. ^{1}H NMR (DMSO-d_6): δ 0.69 (m, 2H), 0.81 (m, 2H), 3.21 (br s, 1H), 3.68 (d, 2H, J = 6.8 Hz), 3.92−4.00 (m, 2H), 4.14 (dd, 1H, J = 4.0, 12.0 Hz), 4.37−4.43 (m, 2H), 6.92 (d, 1H, J = 8.0 Hz), 7.19 (s, 1H), 7.39 (d, 1H, J = 8.0 Hz), 8.02 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 6.3, 45.5, 47.5, 47.7, 51.4, 69.4, 106.2, 112.8, 115.2, 125.4, 130.1, 143.5, 147.3, 149.3, 157.6. HRMS calcd for $C_{17}H_{17}F_3N_4O_5$ [M + H$^+$] 415.1229, found 415.1231. HPLC purity: 93.88%.

(*S*)-2-Nitro-*N*-(2-phenoxy-4-trifluoromethoxybenzyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (32d). Yield 12%. ^{1}H NMR (DMSO-d_6): δ 3.28 (br s, 1H), 3.83 (d, 2H, J = 6.0 Hz), 4.00 (br d, 1H, J = 12.0 Hz), 4.14 (dd, 1H, J = 3.2, 12.0 Hz), 4.35−4.43 (m, 2H), 6.73 (s, 1H), 7.02 (d, 2H, J = 8.0 Hz), 7.14−7.20 (m, 2H), 7.41 (t, 2H, J = 7.2 Hz), 7.60 (d, 2H, J = 8.0 Hz), 8.00 (s, 1H). HRMS calcd for $C_{20}H_{17}F_3N_4O_5$ [M + H$^+$] 451.1270, found 451.1251.

(*S*)-2-((2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-ylamino)methyl)-5-trifluoromethoxyphenol (32g). To a solution of **32a** (200 mg, 0.47 mmol) in THF (2 mL) was added 6N aqueous HCl (5 mL) and stirred at room temperature for 4 h. The reaction mixture was neutralized using aq $NaHCO_3$ solution and extracted with ethyl acetate (2 × 50 mL). The organic layer was washed with water and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 0−5% MeOH in $CHCl_3$ as eluent to give **32g** (120 mg, 67%). ^{1}H NMR (DMSO-d_6): δ 3.25 (m, 1H), 3.76 (s, 2H), 4.01 (d, 1H, J = 12.0 Hz), 4.15 (d, 1H, J = 12.0 Hz), 4.41 (br s, 2H), 6.69−6.71 (m, 2H), 7.26 (d, 1H, J = 7.6 Hz), 8.01 (s, 1H), 10.21 (br s, 1H). ^{13}C NMR (CD_3OD): δ 47.3, 48.0, 48.7, 70.0, 109.1, 112.5, 117.8,123.1, 125.0, 131.6, 143.9, 149.2, 150.6, 158.8. HRMS calcd for $C_{14}H_{13}F_3N_4O_5$ [M + H$^+$] 375.0916, found 375.0916.

2-Chloro-4-trifluoromethoxybenzaldehyde (34b). To a solution of 2-chloro-4-triflouromethoxyiodobenzene (100 mg, 0.310 mmol) in THF (4 mL) at 78 °C was added *n*-butyl lithium (1 M in hexane, 0.62 mL, 0.021 mmol) and stirred at −78 °C for 30 min. The pale-yellow suspension was treated with a solution of DMF (0.048 mL, 0.621 mmol) in diethyl ether (1 mL) at −78 °C. The reaction mixture was stirred for 15 min and quenched with dilute aqueous H_2SO_4. The organic layer was separated. The aqueous layer was extracted with diethylether, and the combined organic layer washed with water and brine, dried (anhyd Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography over silica gel by using mixture of 0−3% ethyl acetate in hexane to get **34b** (50 mg, 72%). ^{1}H NMR ($CDCl_3$): δ 7.28−7.33(m, 1H), 7.45 (d, 1H, J = 8.0 Hz), 7.53 (t, 1H, J = 8.0 Hz), 10.45 (s, 1H).

2-Fluoro-4-trifluoromethoxybenzaldehyde (34a). To a solution of 2-fluoro-4-triflouromethoxyiodobenzene (1.0 g, 3.86 mmol) in THF (20 mL) at −78 °C was added *n*-butyl lithium (1 M solution, 7.7 mL, 7.72 mmol) and stirred at −78 °C for 30 min. The pale-yellow suspension was treated with a solution of DMF (0.59 mL, 7.72 mmol) in diethyl ether (5 mL) at −78 °C. The reaction mixture was stirred for 15 min and quenched with dilute aqueous H_2SO_4. The organic layer was separated. The aqueous layer was extracted with diethyl ether, and the combined organic layer was washed with water and brine, dried

(Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography over silica gel by using mixture of 0−3% ethyl acetate in hexane to get 460 mg (57%) of **34a**. ^{1}H NMR ($CDCl_3$): δ 7.06 (d, 1H, J = 10.0 Hz), 7.13 (d, 1H, J = 8.0 Hz), 7.95 (t, 1H, J = 8.0 Hz), 10.32 (s, 1H).

(*E*)-2-Bromo-1-styryl-4-(trifluoromethoxy)benzene (36). To a solution of 2-bromo-1-iodo-4-trifluoromethoxybenzene (200 mg, 0.544 mmol) in acetonitrile (5 mL) was added styrene (0.08 mL, 0.708 mmol) and triethylamine (0.1 mL, 0.71 mmol). After purging with argon for 30 min, $Pd(OAc)_2$ (10 mg, 0.04 mmol) was added and the reaction mixture was stirred at 95 °C. After 16 h, the reaction mixture was cooled to room temperature and poured into 10% aq HCl (10 mL). The product was extracted using diethyl ether. The combined organic layer was washed with water and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel by using a solvent gradient mixture of 0−1% EtOAc in hexane to afford **36** (120 mg, 64%). ^{1}H NMR ($CDCl_3$): δ 7.02 (d, 1H, J = 16.0 Hz), 7.20 (m, 1H), 7.29−7.32 (m, 1H), 7.37−7.42 (m, 3H), 7.47 (m, 1H), 7.54 (d, 2H, J = 8.0 Hz), 7.68 (d, 1H, J = 8.0 Hz).

2-Bromo-4-(trifluoromethoxy)benzaldehyde (37). To a solution of **36** (1.0 g, 2.91 mmol) in acetone (20 mL) and water (5 mL) at 0 °C was added OsO_4 (0.4 M in *t*-BuOH, 0.072 mL, 0.029 mmol) and $NaIO_4$ (931 mg, 4.37 mmol). After 16 h at room temperature, the reaction mixture was diluted with aq sodium metabisulphite solution and extracted with EtOAc (2 × 50 mL). The combined organic layer was washed with water and brine solution, dried (Na_2SO_4), and concentrated under reduced pressure. The residue upon purification by column chromatography over silica gel using a gradient mixture of 0−2% EtOAc in hexane afforded **37** (130 mg, 16%). ^{1}H NMR ($CDCl_3$): δ 7.27−7.30 (m, 1H), 7.52 (dd, 1H, J = 1.2, 2.4 Hz), 7.98 (d, 1H, J = 8.5 Hz), 10.32 (s, 1H).

2-(Piperidin-1-yl)-4-(trifluoromethoxy)benzaldehyde (40a). To a stirred solution of **36** (1.0 g, 2.91 mmol), piperidine (0.34 mL, 3.49 mmol), and Cs_2CO_3 (2.83 g, 8.73 mmol) in dioxane (15 mL) was added Xantphos (804 mg, 0.873 mmol) under argon. After degassing, the reaction mixture with argon for 30 min, $Pd(OAc)_2$ (65 mg, 0.291 mmol) was added and continued the argon purging for another 10 min. The resulting reaction mixture was stirred at 80 °C for 16 h. After cooling to room temperature, the reaction mixture was diluted with water (20 mL) and extracted with diethyl ether. The organic layer was washed with water and brine, dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel by using a solvent gradient of 0−10% EtOAc in hexane as eluent to afford 800 mg of (*E*)-1-(2-styryl-5-(trifluoromethoxyphenyl)piperidine. This compound (0.8 g, 2.30 mmol) was dissolved in acetone (30 mL), water (6 mL) was added OsO_4 (1 M solution in *t*-BuOH, 72 μL, 0.028 mmol), and $NaIO_4$ (920 mg, 4.32 mmol) was added at 0 °C and stirred at room temperature for 16 h. The reaction mixture was quenched with aq metabisulphite solution and extracted with ethyl acetate. The combined ethyl acetate layer was washed with aq $Na_2S_2O_3$, water and brine and dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel (100−200 mesh) by using a solvent gradient mixture of 0−15% EtOAc in hexane as eluent to afford **40a** (250 mg, 31%). ^{1}H NMR ($CDCl_3$): δ 1.58−1.77 (m, 6H), 3.10 (t, 4H, J = 5.2 Hz), 6.86−6.89 (m, 2H), 7.82 (d, 1H, J = 8.4 Hz), 10.18 (s, 1H).

2-Morpholino-4-(trifluoromethoxy)benzaldehyde (40b). To a stirred solution of **36** (1.5 g, 4.37 mmol) in 1,4-dioxane (20 mL) was added morpholine (0.42 mL, 5.24 mmol) and Cs_2CO_3 (4.2 g, 13.11 mmol). After degassing the reaction mixture with argon, $Pd(OAc)_2$ (98 mg, 0.43 mmol) was added and stirred at 100 °C. After 18 h, the reaction mixture was filtered through Celite. The filtrate was diluted with water (40 mL) and extracted with EtOAc (2 × 30 mL). The organic layer was washed with water (20 mL) and brine, dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a gradient of 0−7% EtOAc in hexane as eluent to afford 900 mg (59%) of (*E*)-4-(2-styryl-5-(trifluoromethoxy)phenyl)morpholine. ^{1}H NMR ($CDCl_3$): δ 2.99 (t, 4H, J = 4.8 Hz)), 3.89 (t, 4H, J = 4.8 Hz), 6.85 (br s, 1H), 6.94 (d, 1H, J = 8.8 Hz), 7.03 (d, 1H, J = 16.0 Hz), 7.29 (d, 1H, J = 7.2 Hz), 7.35 (d, 1H, J = 8.4 Hz), 7.39 (t, 2H, J = 7.2 Hz), 7.52 (d, 2H, J = 7.2 Hz), 7.58 (d, 1H, J = 8.8 Hz). ESI MS: m/z 350.1 (M + H). To a solution of (*E*)-4-(2-styryl-5-(trifluoromethoxy)phenyl)morpholine (900 mg, 2.57 mmol) in acetone (15 mL), water (30 mL) at 0 °C was added $NaIO_4$ (823 mg, 3.86 mmol) followed by OsO_4 (1 M solution in *t*-BuOH, 0.05 mL, 0.02 mmol). After stirring at room temperature for 19 h, the reaction mixture was quenched with aq sodium metabisulfite solution (20 mL) and extracted with EtOAc (2 × 15 mL). The combined organic layer was washed with water (10 mL) and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using a gradient of 0−5% EtOAc in hexane as eluent to afford **40b** (350 mg, 49%). ^{1}H NMR ($CDCl_3$): δ 3.10 (t, 4H, J = 4.8 Hz), 3.91 (t, 4H, J = 4.8 Hz), 6.88 (s, 1H), 6.97 (d, 1H, J = 8.4 Hz), 7.85 (d, 1H, J = 8.4 Hz), 10.23 (s, 1H).

2-(2-Bromo-4-(trifluoromethoxy)phenyl)-1,3-dioxolane (38). To a stirred solution of 2-bromo-4-trifluoromethoxybenzaldehyde (1.5 g, 5.57 mmol) in benzene (30 mL) was added *p*-TSA (15 mg) and ethylene glycol (0.94 mL, 16.72 mmol). After heating at reflux using Dean−Stark apparatus for 8 h, the reaction mixture was cooled to room temperature. The organic layer was diluted with ethyl acetate and washed with satd aq $NaHCO_3$ (50 mL), water, and brine. The organic layer was dried (anhyd Na_2SO_4), evaporated under reduced pressure, and the residue purified by column chromatography over neutral alumina using a solvent gradient of 0−1% EtOAc in hexane as eluent to afford **38** (1.5 g, 86%). ^{1}H NMR ($CDCl_3$): δ 4.06−4.17 (m, 4H), 6.06 (s, 1H), 7.21 (d, 1H, J = 8.0 Hz), 7.45 (br s, 1H), 7.64 (d, 1H, J = 8.0 Hz).

In a similar manner, 2-(3-chloro-4-(trifluoromethoxy)phenyl)-1,3-dioxolane (**54**) was prepared in 72% yield from 3-chloro-4-trifluoromethoxybenzaldehyde. ^{1}H NMR ($CDCl_3$): δ 4.00−4.15 (m, 4H), 5.80 (s, 1H), 7.32 (d, 1H, J = 8.4 Hz), 7.39−7.42 (m, 1H), 7.61 (d, 1H, J = 1.6 Hz).

2-(4-Methylpiperazin-1-yl)-4-(trifluoromethoxy)benzaldehyde (39). To a stirred solution of **38** (200 mg, 0.63 mmol), *N*-methylpiperzine (0.085 mL, 0.766 mmol) and Cs_2CO_3 (622 mg, 1.914 mmol) in dioxane (3 mL) was added Xantphos (110 mg, 0.191 mmol) and degassed with argon for 20 min. $Pd(OAc)_2$ (14 mg, 0.063 mmol) was added and continued the argon purging for another 10 min. After stirring at 90 °C for 8 h, the reaction mixture was cooled to room temperature, diluted with ethyl acetate (10 mL), and washed with water and brine and dried (anhyd Na_2SO_4). Evaporation of the organic solvent under reduced pressure and purification of the residue by column chromatography over neutral alumina using a solvent gradient of 0−1% MeOH in $CHCl_3$ as eluent to afford 1-(2-(1,3-dioxolan-2-yl)-5-(trifluoromethoxyphenyl)-4-methylpiperazine (120 mg). ^{1}H NMR (DMSO-d_6): δ 2.22 (s, 3H), 2.48 (m, 4H), 2.96 (m, 4H), 3.95−4.11 (m, 4H), 5.95 (s, 1H), 7.01 (br s, 1H), 7.06 (d, 1H, J = 8.0 Hz), 7.56 (d, 1H, J = 6.4 Hz). ESI MS: 333.0 (M + H). This compound (120 mg, 0.361 mmol) was dissolved in THF (5 mL) and treated with 6 N HCl (10 mL) at room temperature for 30 min. The reaction mixture was neutralized with solid $NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with water and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure to afford **39** (100 mg, 55%). ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H), 2.52 (m, 4H), 3.10 (m, 4H), 7.08 (m, 2H), 7.81 (d, 1H, J = 9.6 Hz), 10.09 (s, 1H). ESI MS: 289.0 (M + H).

Adopting a similar procedure, the following compounds were synthesized:

3-Morpholino-4-(trifluoromethoxy)benzaldehyde (55a). Yield 21%. ^{1}H NMR ($CDCl_3$): δ 3.12 (t, 4H, J = 5.2 Hz), 3.87 (t, 4H,

J = 5.2 Hz), 7.38 (dd, 1H, J = 2.0, 8.0 Hz), 7.50−7.60 (m, 2H), 9.96 (s, 1H).

3-(4-Methylpiperazin-1-yl)-4-(trifluoromethoxy)benzaldehyde (55b). Yield 15%. ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 3H), 2.59 (t, 4H, J = 4.4 Hz), 3.16 (t, 4H, J = 4.4 Hz), 7.35 (dd, 1H, J = 1.2, 8.0 Hz), 7.50 (dd, 1H, J = 2.0, 8.0 Hz), 7.53 (d, 1H, J = 1.6 Hz), 9.95 (s, 1H).

***tert*-Butyldimethyl-(4-(trifluoromethoxy)benzyloxy)silane (43).** To a solution of (4-(trifluoromethoxy)phenyl)methanol (1.49 g, 7.76 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added imidazole (686 mg, 10.08 mmol) followed by TBDMSCl (1.40 g, 9.31 mmol). After stirring for 16 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, washed with water and brine and dried (anhyd Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 10% ethyl acetate in hexane as eluent to give **43** (1.70 g, 60%). ^{1}H NMR ($CDCl_3$): δ 0.10 (s, 6H), 0.94 (s, 9H), 4.73 (s, 2H), 7.17 (d, 2H, J = 8.0 Hz), 7.34 (d, 2H, J = 8.0 Hz).

5-((*tert*-Butyldimethylsilyloxy)methyl)-2-(trifluoromethoxy)-phenol (44). To a solution of **43** (500 mg, 1.63 mmol) in THF (10 mL) at −78 °C was added TMEDA (0.245 mL, 1.63 mmol), followed by *sec*-BuLi (1.4 M in cyclohexane, 1.16 mL, 1.63 mmol). After stirring at −78 °C for 1 h, fluorodimethoxyborane−dimethyl ether complex (prepared by adding $BF_3 \cdot OEt_2$ (0.17 mL, 1.40 mmol) and trimethylborate (0.311 mL, 2.81 mmol) in a separate flask followed by diethyl ether (0.27 mL, 2.81 mmol) was added at −78 °C. After warming the reaction mixture to room temperature over 30 min, alkaline H_2O_2 was added and stirred for 20 min. The reaction mixture was poured into 1N HCl and extracted with ether. The organic layer was washed with water and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using 10% ethyl acetate in hexane as eluent to give **44** (150 mg, 28%). ^{1}H NMR (DMSO-d_6): δ 0.08 (s, 6H), 0.91 (s, 9H), 4.63 (s, 2H), 6.76 (d, 1H, J = 8.0 Hz), 6.99 (s, 1H), 7.18 (d, 1H, J = 8.0 Hz), 10.07 (s, 1H).

3-Methoxy-4-(trifluoromethoxy)benzaldehyde (47a). To the solution of **44** (3 g, 9.31 mmol) in dry DMF (10 mL) was added K_2CO_3 (2.57 g, 18.63 mmol) followed by MeI (1.16 mL, 18.63 mmol) and stirred for 3 h at room temperature. Reaction mixture was diluted with ether (50 mL) and washed with water (2 × 10 mL) and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure to afford 3 g (8.928 mmol) of product which was dissolved in dry THF (12 mL) and treated with TBAF·3H_2O (2.812 g, 8.928 mmol) at room temperature. After stirring the reaction mixture for 1.5 h, it was diluted with ether (30 mL). The organic layer was washed with water (2 × 5 mL) and brine solution, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue upon purification by column chromatography over silica gel (100−200 mesh) using a solvent gradient of 20−23% EtOAc in hexane as eluent afforded 3-methoxy-4-(trifluoromethoxy)-benzyl alcohol (1.0 g, 52%). ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 3H), 4.50 (d, 2H, J = 5.6 Hz), 5.28 (t, 1H, J = 5.6 Hz), 6.95 (d, 1H, J = 8.4 Hz), 7.17 (s, 1H), 7.27 (d, 1H, J = 8.4 Hz). A solution of the above benzylic alcohol (900 mg, 4.484 mmol) in CH_2Cl_2 (10 mL) was added PCC (966 mg, 4.484 mmol) at room temperature. After stirring the reaction mixture for 1 h, it was diluted with CH_2Cl_2 (25 mL), washed with water (2 × 5 mL) and brine, and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a solvent gradient of 10−15% EtOAc−petroleum ether as eluent to afford **47a** (700 mg, 91%) of 3-methoxy-4-(trifluoromethoxy)benzaldehyde. ^{1}H NMR (DMSO-d_6): δ 3.95 (s, 3H), 7.62 (s, 2H), 7.71 (s, 1H), 10.01 (s, 1H).

***tert*-Butyl-(3-(methoxymethoxy)-4-(trifluoromethoxybenzyloxy))dimethylsilane (46).** To a solution of **44** (150 mg, 0.46 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added diisopropylethylamine (0.09 mL, 0.56 mmol) followed by methoxymethylchloride (0.04 mL, 0.56 mmol). After stirring at room temperature for 16 h, the reaction mixture was diluted with CH_2Cl_2, washed with water and brine, dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The residue upon purification by column chromatography over silica gel using a solvent gradient of 10% EtOAc in hexane as eluent afforded **46** (140 mg, 82%). ^{1}H NMR (DMSO-d_6): δ 0.00 (s, 6H), 0.82 (s, 9H), 3.30 (s, 3H), 4.63 (s, 2H), 5.17 (s, 2H), 6.92 (d, 1H, J = 8.00 Hz), 7.19 (s, 1H), 7.25 (d, 1H, J = 8.0 Hz).

3-(Methoxymethoxy)-4-(trifluoromethoxy)benzaldehyde (47b). To a solution of **46** (280 mg, 0.82 mmol) in THF (2 mL) was added tetra-*n*-butyl ammonium fluoride trihydrate (250 mg, 0.92 mmol). After stirring at room temperature for 30 min, it was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried (anhyd Na_2SO_4), and concentrated to give (3-(methoxymethoxy)-4-(trifluoromethoxyphenyl)methanol (160 mg, 0.65 mol), which was dissolved in CH_2Cl_2 (4 mL) and treated with pyridinium chlorochromate (240 mg, 1.1 mmol). After 1 h at room temperature, the reaction mixture was diluted with ethyl acetate. The organic layer was washed with water and brine, dried (anhyd Na_2SO_4), and evaporated under reduced pressure. The residue was purified over silica gel eluting with 10% EtOAc in hexane to give **47b** (140 mg, 73%). ^{1}H NMR ($CDCl_3$): δ 3.51 (s, 3H), 5.30 (s, 2H), 7.41 (d, 1H, J = 8.0 Hz), 7.55 (dd, 1H, J = 1.2, 8.0 Hz), 7.75 (d, 1H, J = 1.2 Hz), 9.96 (s, 1H).

Methyl 5-Formyl-2-(trifluoromethoxy)benzoate (48). To a solution of **43** (3.0 g, 9.8 mmol) in dry THF (45 mL) at −78 °C was added *sec*-BuLi (1.4 M in cyclohexane, 14.7 mL, 20.56 mmol). After 1 h at −78 °C, a solution of methyl chloroformate (1.13 mL, 14.75 mmol) in dry THF (5 mL) was added and the reaction mixture allowed to warm to room temperature over 1 h. After 3 h at room temperature, the reaction was quenched with satd aq NH_4Cl solution and extracted with diethyl ether (2 × 30 mL). The organic layer was washed with brine (50 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure to give 4 g of crude product, which was dissolved in dry THF (40 mL) and treated with TBAF·3H_2O (4.16 g, 13.1 mmol) for 1 h. The reaction mixture was diluted with water (50 mL), extracted with diethyl ether (2 × 50 mL), washed with water (3 × 50 mL) and brine (50 mL), dried (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude product (3 g, 12.0 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and treated with PCC (3.0 g, 13.9 mmol). After 30 min, the reaction mixture was diluted with diethyl ether (50 mL), filtered through Celite, and concentrated. The residue was purified by column chromatography over silica gel by using a solvent gradient mixture of 2−3% EtOAc in hexane as eluent to afford **48** (500 mg, 21%). ^{1}H NMR ($CDCl_3$): δ 3.98 (s, 3H), 7.51 (d, 1H, J = 8.4 Hz), 8.10 (dd, 1H, J = 1.6, 8.4 Hz), 8.47 (d, 1H, J = 1.6 Hz), 10.06 (s, 1H).

***tert*-Butyldimethyl(3-(4-nitrophenoxy)-4-(trifluoromethoxy)-benzyloxy)silane (50).** To a stirred solution of **44** (250 mg, 0.776 mmol) in DMF (3 mL) at 0 °C was added NaH (60% in oil, 40 mg, 0.931 mmol) and followed by 4-nitro-1-fluorobenzene (0.25 mL, 2.33 mmol) and stirred at 100 °C for 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate (2 × 20 mL). The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel (100−200 mesh) by using gradient mixture of 0−10% ethyl acetate in hexane as eluent to afford **50** (100 mg, 30%). ^{1}H NMR ($CDCl_3$): δ 0.09 (s, 6H), 0.91 (s, 9H), 4.73 (s, 2H), 7.02 (d, 2H, J = 9.0 Hz), 7.15 (br s, 1H), 7.22 (dd, 1H, J = 1.2, 8.8 Hz), 7.35 (d, 1H, J = 8.4 Hz), 8.22 (d, 2H, J = 8.8 Hz).

(3-(4-Phenoxy)-4-(trifluoromethoxy)phenyl)methanol (51). To stirred solution of **50** (300 mg, 0.677 mmol) in ethyl acetate (5 mL) was added iron powder (380 mg, 6.77 mmol), NH_4Cl (109 mg, 2.031 mmol), and H_2O (1.5 mL) at room temperature. The reaction mixture was heated at reflux for 16 h. Reaction mixture was filtered through Celite and the filtrate diluted with ethyl acetate. The organic layer was

washed with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure to afford 200 mg (0.48 mmol) of 4-(5-((*tert*-butyldimethylsilyloxy)methyl)-2-(trifluoromethoxy)phenoxy)aniline, which was dissolved in 6N aqueous HCl (3 mL) and treated with aqueous $NaNO_2$ (43 mg, 0.62 mmol). After stirring at 0 °C for 30 min, a cold solution of 50% H_3PO_2 (50%, 0.290 mL, 2.672 mmol) was added and stirred at 50 °C for 1 h. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (2 × 10 mL). The combined organic layer was washed with aq $NaHCO_3$, water and brine, and dried (Na_2SO_4) and concentrated under reduced pressure to afford **51** (50 mg, 52%). ^{1}H NMR ($CDCl_3$): δ 4.63 (s, 2H), 7.00 (m, 3H), 7.10−7.14 (m, 2H), 7.31−7.37 (m, 3H).

3-Phenoxy-4-(trifluoromethoxy)benzaldehyde (52). To a stirred solution of **51** (25 mg, 0.088 mmol) in CH_2Cl_2 (2 mL) was added PCC (19 mg, 0.088 mmol). After stirring for 1 h at room temperature, the reaction mixture was filtered through Celite. The filtrate was evaporated under reduced pressure and the residue purified by column chromatography over silica gel using a gradient mixture of 0−5% EtOAc in hexane as eluent to afford **52** (15 mg, 62%). ^{1}H NMR ($CDCl_3$): δ 7.04 (d, 2H, J = 8.4 Hz), 7.21 (t, 1H, J = 7.2 Hz), 7.42 (t, 2H, J = 7.2 Hz), 7.45 (s, 1H), 7.50 (d, 1H, J = 8.4 Hz), 7.63 (d, 1H, J = 8.4 Hz), 9.89 (s, 1H).

(*S*)-*N*-(2-Chloro-4-trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (41b). A mixture of **34b** (483 mg, 2.16 mmol) and amine **7** (200 mg 1.08, mmol) in DMF (5 mL) containing AcOH (1 mL) was stirred at room temperature for 1 h. $NaBH_3CN$ (165 mg, 2.52 mmol) was added to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic layer was washed with water and brine solution, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography over silica gel using a gradient mixture of 0−5% MeOH-$CHCl_3$ to afford **41b** (110 mg, 26%). ^{1}H NMR (DMSO-d_6): δ 2.81−2.83 (m, 1H), 3.88 (d, 2H, J = 6.8 Hz), 4.01−4.04 (br d, 1H, J = 12.0 Hz), 4.118 (dd, 1H, J = 4.0, 12.0 Hz), 4.39−4.48 (m, 2H), 7.36 (d, 1H, J = 8.0 Hz), 7.52−7.53 (m, 1H), 7.61 (d, 1H, J = 8.0 Hz), 8.03 (s, 1H). HRMS calcd for $C_{14}H_{12}ClF_3N_4O_4$ [M + H$^+$] 393.0577, found 393.0572.

Adopting similar procedure, the following compounds were synthesized:

(*S*)-*N*-(2-Fluoro-4-(trifluoromethoxy)benzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (41a). Yield 15%. ^{1}H NMR ($CDCl_3$): δ 3.34 (br s, 1H), 3.97 (m, 3H), 4.19 (dd, 1H, J = 4.0, 12.0 Hz), 4.39−4.43 (m, 2H), 6.97−6.98 (m, 1H), 7.02 (s, 1H, J = 8.0 Hz), 7.38 (d, 1H, J = 8.0 Hz), 7.40 (s, 1H). HRMS calcd for $C_{14}H_{12}F_4N_4O_4$ [M + H$^+$] 377.0873, found 377.0878. HPLC purity: 95.86%.

(*S*)-*N*-(2-Bromo-4-(trifluoromethoxy)benzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (41c). Yield 46%. ^{1}H NMR (DMSO-d_6): δ 2.81−2.85 (m, 1H), 3.32−3.34 (m, 1H), 3.85 (d, 2H, J = 7.0 Hz), 4.03 (dd, 1H, J = 2.4, 120 Hz), 4.20 (dd, 1H, J = 4.0, 12.0 Hz), 4.38−4.48 (m, 2H), 7.40 (dd, 1H, J = 1.2, 7.5 Hz), 7.0 (d, 1H, J = 7.5 Hz), 7.66 (d, 1H, J = 1.6 Hz), 8.04 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 47.5, 47.9, 49.9, 69.4, 115.6, 120.1, 121.5, 123.5, 125.3, 130.7, 136.9, 143.3, 147.2, 148.4. HRMS calcd for $C_{14}H_{12}BrF_3N_4O_4$ [M + H$^+$] 437.0072, found 437.0064. HPLC purity: 94.5%.

(*S*)-2-Nitro-*N*-(2-(piperidin-1-yl)-4-(trifluoromethoxy)benzyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (41d). Yield 13%. ^{1}H NMR (DMSO-d_6): δ 1.50−1.65 (m, 6H), 2.69−2.84 (m, 5H), 3.24 (m, 1H), 3.77 (d, 2H, J = 7.2 Hz), 4.00 (dd, 1H, J = 3.2, 13.0 Hz), 4.17 (dd, 1H, J = 3.2, 13.0 Hz), 4.42−4.45 (m, 2H), 6.93 (br s, 1H), 6.98 (d, 1H, J = 8.4 Hz), 7.48 (d, 1H, J = 8.4 Hz), 8.03 (s, 1H). FT-ICR MS calcd for $C_{19}H_{22}F_3N_5O_4$ [M + H$^+$] 442.1696, found 442.1698.

(*S*)-*N*-(2-Morpholino-4-(trifluoromethoxy)benzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (41e). Yield 24%. ^{1}H NMR (DMSO-d_6):.δ 2.67 (br s, 1H), 2.80−2.95 (m, 4H), 3.26−3.37 (m, 1H), 3.70−3.82 (m, 6H), 4.01 (dd, 1H, J = 3.6, 12.4 Hz), 4.18 (dd, 1H, J = 3.6, 12.4 Hz), 4.44 (d, 2H, J = 2.4 Hz), 6.97 (s, 1H), 7.03 (d, 1H, J = 8.0 Hz), 7.49 (d, 1H, J = 8.0 Hz), 8.05 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 46.17, 47.9, 52.9, 67.1, 69.1, 112.9, 115.3, 116.2, 130.7, 132.0, 143.4, 147.2, 149.1, 152.5. HRMS calcd for $C_{18}H_{20}F_3N_5O_5$ [M + H$^+$] 444.1495, found 444.1490. HPLC purity: 95.4%.

(*S*)-*N*-(2-(4-Methylpiperazin-1-yl)-4-(trifluoromethoxy)-benzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (41f). Yield 32%. ^{1}H NMR (DMSO-d_6): δ 2.24 (s, 3H), 2.40−2.60 (m, 4H), 2.67 (br s, 1H), 2.80−3.00 (m, 4H), 3.20−3.40 (br s, 1H), 3.77 (br s, 2H), 3.98−4.02 (m, 1H), 4.16−4.20 (m, 1H), 4.43 (br s, 2H), 6.94 (s, 1H), 7.00 (d, 1H, J = 8.4 Hz), 7.48 (d, 1H, J = 8.4 Hz), 8.04 (s, 1H). FT-ICR MS calcd for $C_{19}H_{23}F_3N_6O_4$ [M + H$^+$] 457.1805, found 457.1804.

(*S*)-*N*-(3-Morpholino-4-(trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (56g). Yield 31%. ^{1}H NMR (DMSO-d_6): δ 2.80−2.90 (m, 1H), 2.90−3.00 (m, 4H), 3.20−3.30 (m, 1H), 3.65−3.85 (m, 6H), 4.00 (dd, 1H, J = 2.8, 12.8 Hz), 4.15 (dd, 1H, J = 4.0, 12.8 Hz), 4.35−4.45 (m, 2H), 7.00−7.10 (m, 2H), 7.21 (d, 1H, J = 8.4 Hz), 8.01 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 38.6, 39.8, 41.5, 42.9 (2C), 58.6 (2C), 61.2, 108.3, 111.2, 113.2, 113.7, 132.0, 133.0, 134.4, 136.9, 139.9. HRMS calcd for $C_{18}H_{20}F_3N_5O_5$ [M + H$^+$] 444.1495, found 444.1506. HPLC purity: 95.3%.

(*S*)-*N*-(3-(4-Methylpiperazin-1-yl)-4-(trifluoromethoxy)-benzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (56h). Yield 20% yield. ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H), 2.60−2.70 (t, 4H, J = 4.0 Hz), 3.05−3.15 (t, 4H, J = 4.0 Hz), 3.35−3.45 (m, 1H), 3.88 (d, 2H, J = 10.0 Hz), 3.94 (dd, 1H, J = 4.4, 12.0 Hz), 4.16 (dd, 1H, J = 4.8, 12.0 Hz), 4.35−4.45 (m, 2H), 6.92 (dd, 1H, J = 2.0, 8.0 Hz), 6.96 (d, 1H, J = 2.0 Hz), 7.13 (dd, 1H, J = 1.2, 8.0 Hz), 7.38 (s, 1H). HRMS calcd for $C_{19}H_{23}F_3N_6O_4$ [M + H$^+$] 457.1811, found 457.1798. HPLC purity: 93.4%.

(*S*)-Methyl 5-((2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]-oxazin-6-ylamino)methyl)-2-(trifluoromethoxy)benzoate (56f). Yield 55%. ^{1}H NMR (DMSO-d_6): δ 2.93−2.94 (m, 1H), 3.26−3.29 (m, 1H), 3.84−3.87 (m, 5H), 3.97−4.00 (m, 1H), 4.15 (dd, 1H, J = 3.6, 12.4 Hz), 4.37−4.45 (m, 2H), 7.46 (d, 1H, J = 8.4 Hz), 7.67 (d, 1H, J = 8.4 Hz), 7.88 (s, 1H), 7.99 (s, 1H). ESI MS: m/z 417.1 (M + H). HRMS calcd for $C_{16}H_{15}F_3N_4O_6$ [M + H$^+$] 417.1022, found 417.1014. HPLC purity: 95.3%.

(*S*)-*N*-(3-Methoxy-4-(trifluoromethoxy)benzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (56c). Yield 13%. ^{1}H NMR (DMSO-d_6): δ 2.80−2.90 (m, 1H), 3.24 (br s, 1H), 3.65−3.88 (m, 5H), 3.90−4.00 (m, 1H), 4.10−4.20 (m, 1H), 4.38−4.45 (m, 2H), 6.95 (d, 1H, J = 11.0 Hz), 7.19 (s, 1H), 7.25 (d, 1H, J = 11.0 Hz), 8.00 (s, 1H). FT-ICR HRMS calcd for $C_{15}H_{14}F_3N_4O_5$ [M + H$^+$] 389.1067, found 389.1069.

(*S*)-*N*-(3-(Methoxymethoxy)-4-(trifluoromethoxy)benzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (56d). Yield 12%. ^{1}H NMR ($CDCl_3$): δ 3.41 (m, 1H), 3.45 (s, 3H), 3.90 (d, 2H, J = 10 Hz), 3.94 (m, 1H), 4.15 (dd, 1H, J = 4.0, 12.0 Hz), 4.35−4.45 (m, 2H), 5.21 (s, 2H), 6.95 (d, 1H, J = 8.4 Hz), 7.19−7.21 (m, 2H), 7.38 (s, 1H). ESI MS: m/z 419.2 (M + H).

(*S*)-*N*-(3-Fluoro-4-trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (56a). Yield 35%. ^{1}H NMR ($CDCl_3$): δ 3.40 (br s, 1H), 3.88−3.97 (m, 4H), 4.20 (dd, 1H, J = 4.0, 12.0 Hz), 4.38−4.46 (m, 2H), 7.11 (d, 1H, J = 8.0 Hz), 7.22 (dd, 1H, J = 1.6, 10.0 Hz), 7.28 (d, 1H, J = 8.0 Hz), 7.39 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 47.5, 47.8, 49.7, 69.3, 115.5, 116.4, 116.6, 119.1, 121.6, 123.7, 135.4, 140.3, 143.3, 147.3, 153.2, 155.7. HRMS calcd for $C_{14}H_{12}F_4N_4O_5$ [M + H$^+$] 378.0742, found 378.0729.

(*S*)-*N*-(3-Chloro-4-trifluoromethoxybenzyl)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (56b). Yield

37%. ^{1}H NMR ($CDCl_3$): δ 3.40 (br s, 1H), 3.87−3.97 (m, 4H), 4.19 (dd, 1H, J = 4.4, 12.0 Hz), 4.18−4.46 (m, 2H), 7.26−7.30 (m, 2H), 7.40 (s, 1H), 7.46 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 47.5, 47.9, 49.6, 69.3, 115.4, 119.1, 121.7, 122.6, 127.2, 127.5, 130.1, 139.6, 143.4, 144.2, 147.3. HRMS calcd for $C_{14}H_{12}ClF_3N_4O_4$ [M + H$^+$] 393.0577, found 393.0574.

(*S*)-2-Nitro-*N*-(3-phenoxy-4-(trifluoromethoxy)benzyl)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-amine (56e). Yield 11%. ^{1}H NMR ($CDCl_3$): δ 3.35 (br s, 1H), 3.80−3.90 (m, 3H), 4.14 (dd, 1H, J = 4.0, 12.0 Hz), 4.30−4.41 (m, 2H), 6.95 (d, 1H, J = 1.6 Hz), 6.98 (d, 2H, J = 8.0 Hz), 7.07 (dd, 1H, J = 1.6, 8.0 Hz), 7.16 (t, 1H, J = 8.0 Hz), 7.30 (d, 1H, J = 8.4 Hz), 7.34−7.38 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 47.6, 47.7, 50.0, 69.2, 115.2, 118.5, 119.5, 122.9, 123.5, 123.8, 129.8, 139.4, 143.4, 147.2, 149.5, 156.4. HRMS calcd for $C_{20}H_{17}F_3N_4O_5$ [M + H$^+$] 451.1229, found 451.1228.

(*S*)-5-((2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-ylamino)methyl)-2-(trifluoromethoxy)-phenol (56i). To a solution of **56d** (20 mg, 0.047 mmol) in THF (1 mL) was added 6N HCl (1 mL). After 1 h, the reaction mixture was neutralized with aq $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with water and brine and dried (anhyd Na_2SO_4) and concentrated under reduced pressure. The residue was triturated with ether/pentane to obtain **56i** (8.0 mg 47%). ^{1}H NMR ($CDCl_3$): δ 3.40 (m, 1H), 3.91 (d, 2H, J = 10 Hz), 3.95 (m, 1H), 4.16 (dd, 1H, J = 4.0, 12.0 Hz), 4.39−4.44 (m, 2H), 5.50 (br s, 1H), 6.86 (d, 1H, J = 8.4 Hz), 7.02 (br s, 1H), 7.19 (d, 1H, J = 8.4 Hz), 7.35 (s, 1H). HRMS calcd for $C_{14}H_{13}F_3N_4O_5$ [M + H$^+$] 375.0916, found 375.0929. HPLC purity: 95.7%.

4-(Bromomethyl)-2-(methoxymethoxy)-1-(trifluoromethoxy)-benzene (58a). To a solution of **46** (1.1 g, 3.0 mmol) in THF (10 mL) was added TBAF (1.0 g, 3.6 mmol). After stirring at room temperature for 1 h, the reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried (anhyd Na_2SO_4), and concentrated to give (3-(methoxymethoxy)-4-(trifluoromethoxyphenyl)methanol (600 mg, 1.039 mmol), which was dissolved in CH_2Cl_2 (20 mL) and treated with triphenylphosphine (644 mg, 2.46 mmol) and NBS (437 mg, 2.458 mmol) at 0 °C. After stirring for 30 min at room temperature, the reaction mixture was poured into water (5 mL) and extracted with diethyl ether (2 × 15 mL). The organic layer was washed with water, $NaHCO_3$ solution, and brine and dried (Na_2SO_4). The solvent was evaporated under reduced pressure and the residue passed through a short silica pad to afford **58a** (500 mg, 66%), which was contaminated with 15% triphenylphosphine oxide. This material was used as such for next reaction, without further purification. ^{1}H NMR (DMSO-d_6): δ 3.40 (s, 3H), 4.69 (s, 2H), 5.29 (s, 2H), 7.17 (dd, 1H, J = 2.0, 8.4 Hz), 7.37 (d, 1H, J = 8.4 Hz), 7.41 (d, 1H, J = 2.0 Hz).

In a similar manner, 4-(bromomethyl)-2-methoxy-1-(trifluoromethoxy)benzene (**58c**) was prepared from (3-methoxy-4-(trifluoromethoxy)phenyl)methanol in 46% yield. ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H), 4.46 (s, 2H), 6.96 (dd, 1H, J = 2.8, 10.8 Hz), 7.02 (d, 1H, J = 2.8 Hz), 7.18 (dd, 1H, J = 1.2, 10.8 Hz).

(*S*)-6-(3-(Methoxymethoxy)-4-(trifluoromethoxy)benzyloxy)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (59a). To a solution of 4-(bromomethyl)-2-(methoxymethoxy)-1-(trifluoromethoxy)benzene (0.3 g, 0.9 mmol) (*S*)-2-nitro-6,7-dihydro-5*H*-imidazo-[2,1-*b*][1,3]oxazin-6-ol (**57**, 0.15 g, 0.81 mmol) in DMF (10 mL) was added NaH (60% in mineral oil, 36 mg, 0.9 mmol) at −78 °C and warmed to room temperature over 1 h. Water (5 mL) was added and the reaction mixture extracted with EtOAc (2 × 30 mL). The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by column chromatography over silica gel using a solvent gradient of 0−50% EtOAc in petroleum ether to afford **59a** (115 mg, 34%). ^{1}H NMR (DMSO-d_6): δ 3.37 (s, 3H), 4.25 (m, 3H), 4.46 (d, 1H, J = 12.4 Hz), 4.60−4.70 (m, 3H), 5.26 (s, 2H), 7.02 (d, 1H, J = 8.4 Hz), 7.22 (s, 1H), 7.34 (d, 1H, J = 8.4 Hz), 8.03 (s, 1H). ESI MS: m/z 420.3 (M + H).

In a similar manner, (*S*)-6-(3-methoxy-4-(trifluoromethoxy)-benzyloxy)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (**59c**) was synthesized in 30% yield. ^{1}H NMR (DMSO-d_6): δ 3.80 (s, 3H), 4.20−4.30 (m, 3H), 4.48 (m, 1H), 4.60−4.70 (m, 3H), 6.96 (d, 1H, J = 8.4 Hz), 7.14 (s, 1H), 7.31 (d, 1H, J = 8.4 Hz), 8.03 (s, 1H). FT-ICR HRMS calcd for $C_{15}H_{14}F_3N_3O_6$ [M + H$^+$] 390.0907, found 390.0909.

(*S*)-6-(3-Fluoro-4-(trifluoromethoxy)benzyloxy)-2-nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine (59b). To a stirred solution of **49b** (7.0 g, 33.65 mmol) in methanol (50 mL) was added $NaBH_4$ (1.9 g, 50.48 mmol) at room temperature under the N_2 atmosphere. After 1 h, ice water (100 mL) was added and the reaction mixture extracted with EtOAc (3 × 60 mL). The combined organic layer was washed with water (50 mL) and brine, dried over (anhyd Na_2SO_4), and concentrated under reduced pressure to afford 3-fluoro-4-trifluoromethoxybenzyl alcohol (7 g, quant.). ^{1}H NMR (DMSO-d_6): δ 4.53 (d, 2H, J = 5.6 Hz), 5.44 (t, 1H, J = 5.6 Hz), 7.26 (d, 1H, J = 8.4 Hz), 7.40 (d, 1H, J = 11.2 Hz), 7.48−7.54 (t, 1H, J = 8.4 Hz). ESI MS: m/z 211.0 (M + H). To a solution of 3-fluoro-4-trifluoromethoxybenzyl alcohol (7 g, 33.33 mmol) in CH_2Cl_2 (70 mL) at 0 °C was added triphenylphosphene (13.0 g, 49.99 mmol), followed by NBS (8.9 g, 49.99 mmol) in portionwise at room temperature under the N_2 atmosphere. After 1 h, water (100 mL) was added and the reaction mixture extracted with EtOAc (3 × 40 mL). The combined organic layer was washed with water (30 mL) and brine, dried over (anhyd Na_2SO_4), and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica gel using a gradient of 0−5% EtOAc−hexane as eluent to afford 4-(bromomethyl)-2-fluoro-1-(trifluoromethoxy)benzene (**58b**, 6.0 g, 66%). ^{1}H NMR (DMSO-d_6): 4.73 (s, 2H), 7.42 (d, 1H, J = 8.4 Hz), 7.55−7.71 (m, 2H). Alkylation of **57** using **58b** was carried out as described previously during the synthesis of **59a** to afford **59b** (32% yield). ^{1}H NMR (DMSO-d_6): δ 4.20−4.35 (m, 3H), 4.47 (d, 1H, J = 11.6 Hz), 4.65−4.75 (m, 3H), 7.26 (d, 1H, J = 8.0 Hz), 7.44 (d, 1H, J = 10.8 Hz), 7.55 (t, 1H, J = 8.0 Hz), 8.04 (s, 1H). ^{13}C NMR (CD_3OD): δ 58.3, 68.6, 69.2, 70.1, 117.1, 117.3, 117.7, 120.6, 123.1, 124.8, 124.9, 136.8, 141.0, 143.9, 149.1, 154.4, 156.9. HRMS calcd for $C_{14}H_{11}F_4N_3O_5$ [M + H$^+$] 378.0742, found 378.0729.

(*S*)-5-((2-Nitro-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazin-6-yloxy)methyl)-2-(trifluoromethoxy) phenol (59d). To a solution of **59a** (150 mg, 0.357 mmol) in THF (5 mL) was added 6N HCl (10 mL) and stirred for 1 h at room temperature. The reaction mixture was neutralized with satd $NaHCO_3$ solution and extracted with EtOAc (2 × 20 mL). The organic layer was washed with water and brine solution, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was triturated with diethyl ether/pentane (1:1) mixture to afford **59d** (90 mg, 67%). ^{1}H NMR (DMSO-d_6): δ 4.21−4.27 (m, 3H), 4.47 (d, 1H, J = 12.0 Hz), 4.55−4.67 (m, 3H), 6.78 (dd, 1H, J = 1.6, 8.4 Hz), 6.94 (d, 1H, J = 1.2 Hz), 7.20 (d, 1H, J = 8.0 Hz), 8.03 (s, 1H), 10.16 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 46.7, 66.5, 67.8, 68.9, 116.3, 118.0, 119.0, 121.0, 122.8, 135.3, 138.3, 142.1, 147.0, 149.7. HRMS calcd for $C_{14}H_{12}F_3N_3O_6$ [M + H$^+$] 376.0756, found 376.0750.

In Vivo Pharmacokinetic Studies. The Novartis Institute for Tropical Disease's Animal Care and Use Committee approved all animal experimental protocols and animals use. The compounds were formulated in 10% w/v hydroxypropyl-β-cyclodextrin (Acros) and 10% w/v lecithin (Acros/Organics, New Jersey, USA). The formulation was centrifuged, and the supernatant was injected into animals via the intravenous route at 5 mg/kg. Blood samples were collected at various time points between 1 min and 24 h post dose. Plasma samples were extracted and analyzed using optimum LC/MS/MS conditions. Pharmacokinetic parameters were determined using WinNonLin Professional, version 5.0.1 (Pharsight, CA, USA), by noncompartmental analysis. The extraction ratio (ER) was calculated using the formula as previously described.[21]

Quantum Chemistry and Superposition Study. In our modeling studies, both pseudoequatorial and pseudoaxial forms of **1**

were used as reference structures.[22] The pseudoequatorial conformer of **1** was built by replacing the methyl of 7-(*R*)-methyl-**1** (X-ray structure) with a hydrogen atom while the X-ray structure of **1** was used for the pseudoaxial conformer without further modification. The geometries of these two conformers of **1** were then energy minimized with the density functional theory at the level of B3LYP/6-31G*.[23] The two resulting conformers were subsequently modified to build structures of the corresponding conformers of **32c**, **32d**, **32f**, **41b**, and **41f**. These compounds (each with two conformations) were then energy minimized at the level of B3LYP/6-31G* in cyclohexane utilizing the polarized continuum solvent model with the UAKS parameters set. The resulting structures were taken as the assumed geometries of these compounds. Alternative conformers were considered by varying the hydrogen position of the linker group (NH), which can H-bond with the corresponding *ortho*-substituent. This was done by first changing the dihedral angle of H−N−C6−C5, and then after another cycle of full geometry optimization, the respective low energy conformers were chosen for comparison (see Figure 2). For the superposition study, the optimized structures of **32c**, **32d**, **32f**, **41b**, and **41f** were overlaid onto the geometry optimized **1** with the rigid fit of Quanta 2008 (Accelrys) using the nine heavy atoms of the imidazo-oxazine as a common alignment site. The root-mean-square deviations of such fit for each compound were all less than 0.01 Å and 0.03 Å for the pseudoequatorial and the pseudoaxial conformers, respectively. The energy barrier for the conversion of pseudoaxial **1** to the pseudoequatorial form was calculated by varying the dihedral angle of O8−C7−C6−O and then further optimizing the geometry with the highest enegy on the potential energy surface with the keyword, opt=(ts,calcfs,noeigentest) as implemented in Gausssian 09 software.[23]

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ABBREVIATIONS USED

AcOH, acetic acid; DIPEA, *N*,*N*-diiospropylethylamine; DMF, dimethylformamide; ER extraction ratioMAC, minimum anaerobicidal concentration; MIC, minimum inhibitory concentration; MsCl, methanesulfonyl chloride; Mtb, *Mycobacterium tuberculosis*; Mtz, metronidazole; NBS, *N*-bromosuccinimide; SAR, structure−activity relationship; TB, tuberculosis; TBAF, tetra-*n*-butylammonium fluoride; TBS or TBDMS, *tert*-butyldimethylsilyl; Tf, triflate; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMEDA, tetramethlyethylenediamine; TMSCN, trimethylsilyl cyanide

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