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Article

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The Discovery of a Conformationally Constrained Oxazolidinone with Improved Safety and Efficacy Profiles for the Treatment of Multidrug-Resistant Tuberculosis

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KEYWORDS. oxazolidinone, multidrug-resistant tuberculosis, mitochondrial protein synthesis, myelosuppression, antitubercular agent

ABSTRACT. Tuberculosis (TB) remains a serious public health challenge, and the research and development of new antituberculosis drugs is an essential component of the global strategy to eradicate TB. In this work, we discovered a conformationally constrained oxazolidinone **19c** with improved antituberculosis activity and safety profile through a focused lead optimization effort. Compound **19c** displayed superior *in vivo* efficacy in a mouse TB infection model compared to linezolid and sutezolid. The druggability of compound **19c** was demonstrated in a panel of assays including microsomal stability, cytotoxicity, CYP450 enzyme inhibition and pharmacokinetics in animals. Compound **19c** demonstrated an excellent safety profile in a battery of safety assays, including mitochondrial protein synthesis, hERG K^+, hCav1.2 and Nav1.5 channel, monoamine oxidase, and genotoxicity. In a 4-week repeated dose toxicology study in rats, **19c** appeared to have less bone marrow suppression than linezolid, which has been a major liability of the oxazolidinone class.

INTRODUCTION

Tuberculosis (TB) is a major public health challenge, leading to about 1.2 million deaths in 2018. Multidrug-resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) have emerged rapidly and make the treatment and prevention of tuberculosis extremely difficult. Three countries with the largest share of the global MDR-TB burden were India (27%), China (14%) and the Russian Federation (9%).[1] There is an urgent unmet medical need to develop new therapies with a novel mechanism of action for the treatment of MDR- and XDR-TB.

Oxazolidinones is a relatively new class of antibacterial agents originally developed for the treatment of Gram-positive bacterial infections. This class of compounds inhibits bacterial protein synthesis by binding competitively to the 23S rRNA in the catalytic site of the bacterial 50S ribosomes.[2,3] Certain members of the oxazolidinone class have shown activity against *M. tuberculosis* and are currently under clinical development for the treatment of MDR- and XDR-TB (Figure 1).[4] Linezolid (**1**) is the first oxazolidinone approved for commercialization (2000) and this compound has exhibited bacteriostatic activity against *M. tuberculosis* with a MIC of about 1 μg/mL.[5,6] Linezolid is a key component of the Nix-TB regimen (bedaquiline, pretomanid and linezolid) approved by the Food and Drug Administration (FDA) in 2019 for the treatment of XDR-TB or treatment-intolerant/non-responsive MDR-TB.[7] Sutezolid (**2**), originally discovered by Upjohn company,[8] is in Phase II studies for TB. Sutezolid differs from linezolid by having a thiomorpholine substituent and has displayed a better antituberculosis activity and improved safety profile compared to linezolid.[9,10] In addition, delpazolid (**3**)[11,12] and TBI-223 (**4**)[13] are also under clinical development for the treatment of TB. The development of AZD5847 (**5**) by AstraZeneca for the treatment of Gram-positive infections and TB has been discontinued.[14-16] The long duration required for the treatment of TB with linezolid has been associated with severe side effects such

as anemia, thrombocytopenia, optic and peripheral neuropathy. The toxicity of the oxazolidinone class is thought to be mediated by the inhibition of mitochondrial protein synthesis (MPS) and monoamine oxidase (MAO).[17-19] Therefore discovery of new oxazolidinones with an improved safety and efficacy profile is extremely important for the treatment of TB.[20]

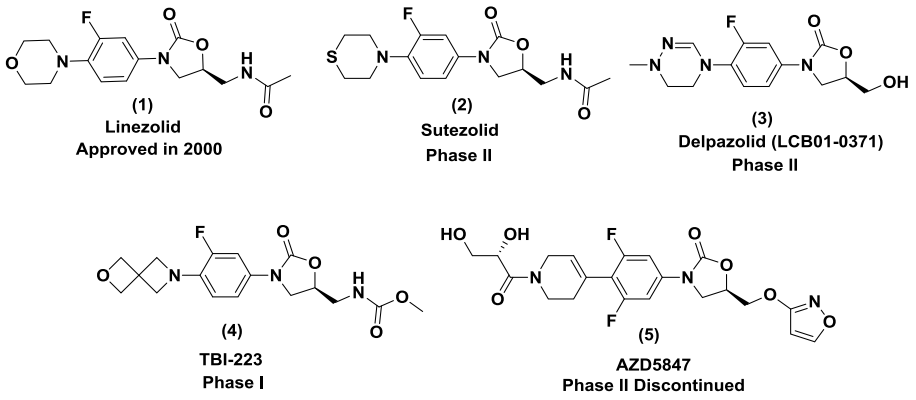


Figure 1. Chemical structure of oxazolidinones as anti-TB agents

A series of conformationally constrained tricyclic benzoxazinyl-oxazolidinones have been reported to have excellent pharmacokinetics profile and potent antibacterial activity against Gram-positive bacteria.[21,22] These reports promoted us to take this novel series as lead to optimize for antituberculosis activity. In our previous work, we have identified a novel series of conformationally constrained oxazolidinones, as exemplified by compound **6**, for the treatment of MDR-TB.[23] These compounds contain a common 3a,4-dihydro-1*H*,3*H*-benzo[*b*]oxazolo[3,4-*d*][1,4]oxazin-1-one core and exhibited an improved pharmacokinetic profile compared to linezolid. However, the *in vitro* anti-TB activity and MPS inhibitory activity of **6** were similar to linezolid (Figure 2). In order to identify an oxazolidinone with improved anti-TB activity, reduced toxicity and improved drug-like properties, we undertook a focused lead optimization effort based on the 3a,4-dihydro-1*H*,3*H*-benzo[*b*]oxazolo[3,4-*d*][1,4]oxazin-1-one scaffold (Figure 2). The stereochemistry of the core structure was first investigated, which indicated that the 3*S*,3a*S*

configuration was optimal for antituberculosis activity. Further optimization was centered around the (3*S*,3a*S*)-3a,4-dihydro-1*H*,3*H*-benzo[*b*]oxazolo[3,4-*d*][1,4]oxazin-1-one core and focused on the variation of the R^1 group on the phenyl ring and the R^2 group on the oxazolidinone ring. Morpholine, 2-oxa-6-azaspiro[3.3]heptane, 4,4-difluoro-piperidine and thiomorpholine were introduced to R^1 position. In addition, the known sulfoxide and sulfone metabolites of the thiomorpholine were also prepared. To increase the metabolic stability of the thiomorpholine group, a bridge-thiomorpholine was also prepared with the goal to increase steric hindrance. At the R^2 position, various substituents, including amides, heterocycles, carbamates and sulfonamides were introduced. Compound **19c** was identified through this effort as a promising development candidate for the treatment of MDR-/XDR-TB.

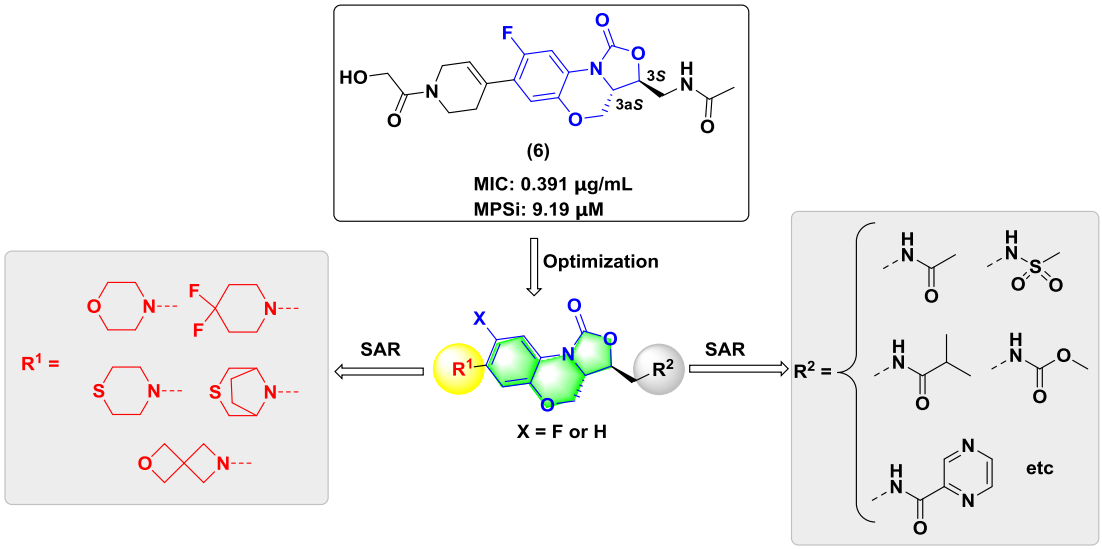


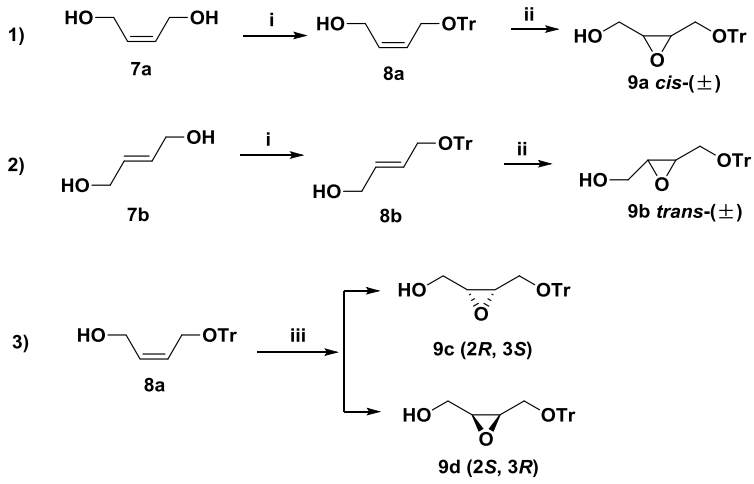
Figure 2. Lead optimization strategy of the 3a,4-dihydro-1*H*,3*H*-benzo[*b*]oxazolo[3,4-*d*][1,4]oxazin-1-one

CHEMISTRY

As shown in Scheme 1, the key epoxides **9a–d** were synthesized from but-2-ene-1,4-diol (**7a** and **7b**). **7a** was reacted with trityl chloride (TrCl) in the presence of triethylamine and 4-

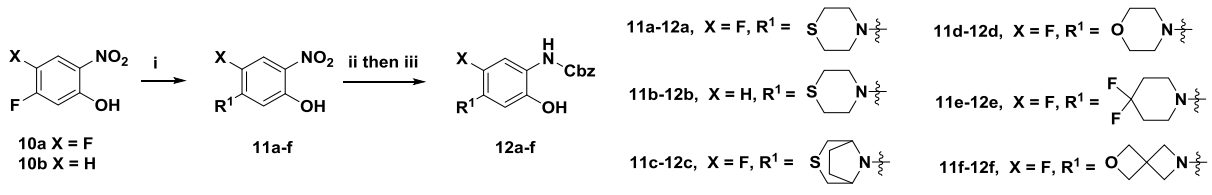
dimethylaminopyridine (DMAP) to afford compound **8a**. (*E*)-but-2-ene-1,4-diol (**7b**) reacted with TrCl in the presence of 2,4,6-collidine and tetrabutylammonium iodide (TBAI) to yield compound **8b**.[24] Compounds **8a** and **8b** were converted to the racemic epoxides **9a** and **9b** via oxidation reaction using 3-chloroperoxybenzoic acid (*m*-CPBA). A Sharpless asymmetric epoxidation reaction was carried out to convert **8a** and **8b** into epoxides **9c** and **9d** respectively.[25] Nucleophilic substitution of **10a** and **10b** with different amines afforded compounds **11a−f**, followed by reduction of the nitro group using catalytic hydrogenation or Zinc/acetic acid, and then the aniline product was protected with benzyl chloroformate (CbzCl) to afford compounds **12a−f** (Scheme 2).

Scheme 1. Synthesis of Compounds 9a−d[a]



[a]Reagents and conditions: (i) TrCl, Et_3N, DMAP, DCM, 0°C-rt for **8a**; TrCl, 2,4,6-Collidine, TBAI, DCM, 0°C-rt, for **8b**; (ii) *m*-CPBA, DCM, 30°C; (iii) TBHP, $Ti(OiPr)_4$, 4Å MS, DCM, -40°C to -20°C, *D*-(-)-DET for **9c**, *L*-(+)-DET for **9d**.

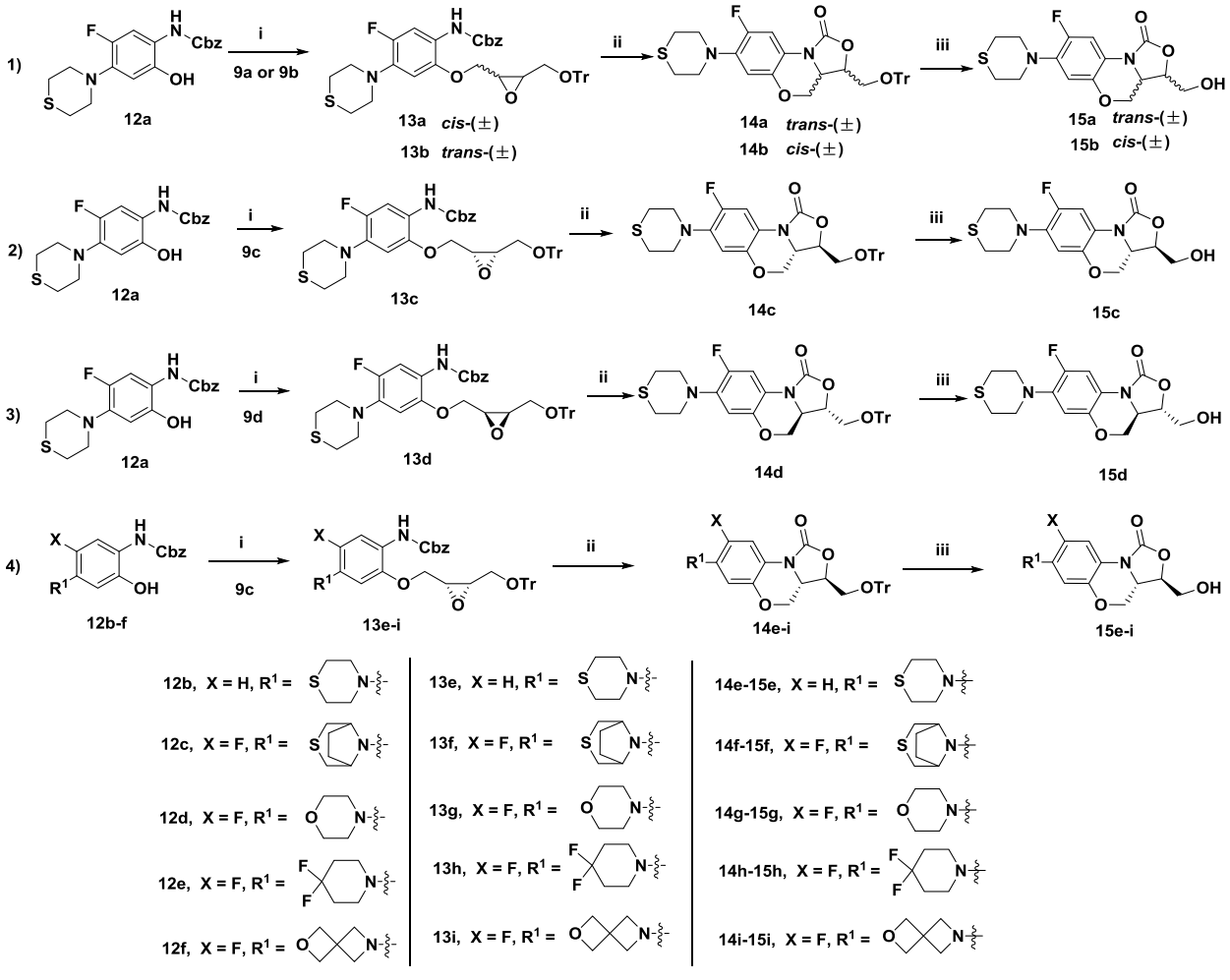
Scheme 2. Synthesis of Compounds 12a−f[a]



[a]Reagents and conditions: (i) amine, *N*-methylmorpholine (NMM), MeCN, 80°C; (ii) H_2, Raney Ni, THF, 20-50 psi, rt for **12a**, **12c−e**; Zn, AcOH, THF, 30-40°C for **12b** and **12f**; (iii) CbzCl, $NaHCO_3$, THF/H_2O, 0°C.

The synthesis of intermediates **15a−i** was depicted in Scheme 3. Compound **12a** reacted with epoxides **9a−d** via Mitsunobu reaction to furnish compounds **13a−d** respectively. Subsequently, treatment of compounds **13a−d** with *t*-BuOLi or *n*-BuLi afforded the key tricyclic intermediates **14a−d**. Cleavage of trityl protective group yielded products **15a−d**. Compounds **12b−f** reacted with epoxide **9c** via Mitsunobu reaction to furnish compounds **13e−i**, subsequently, compounds **15e−i** were obtained via cyclization and deprotection.

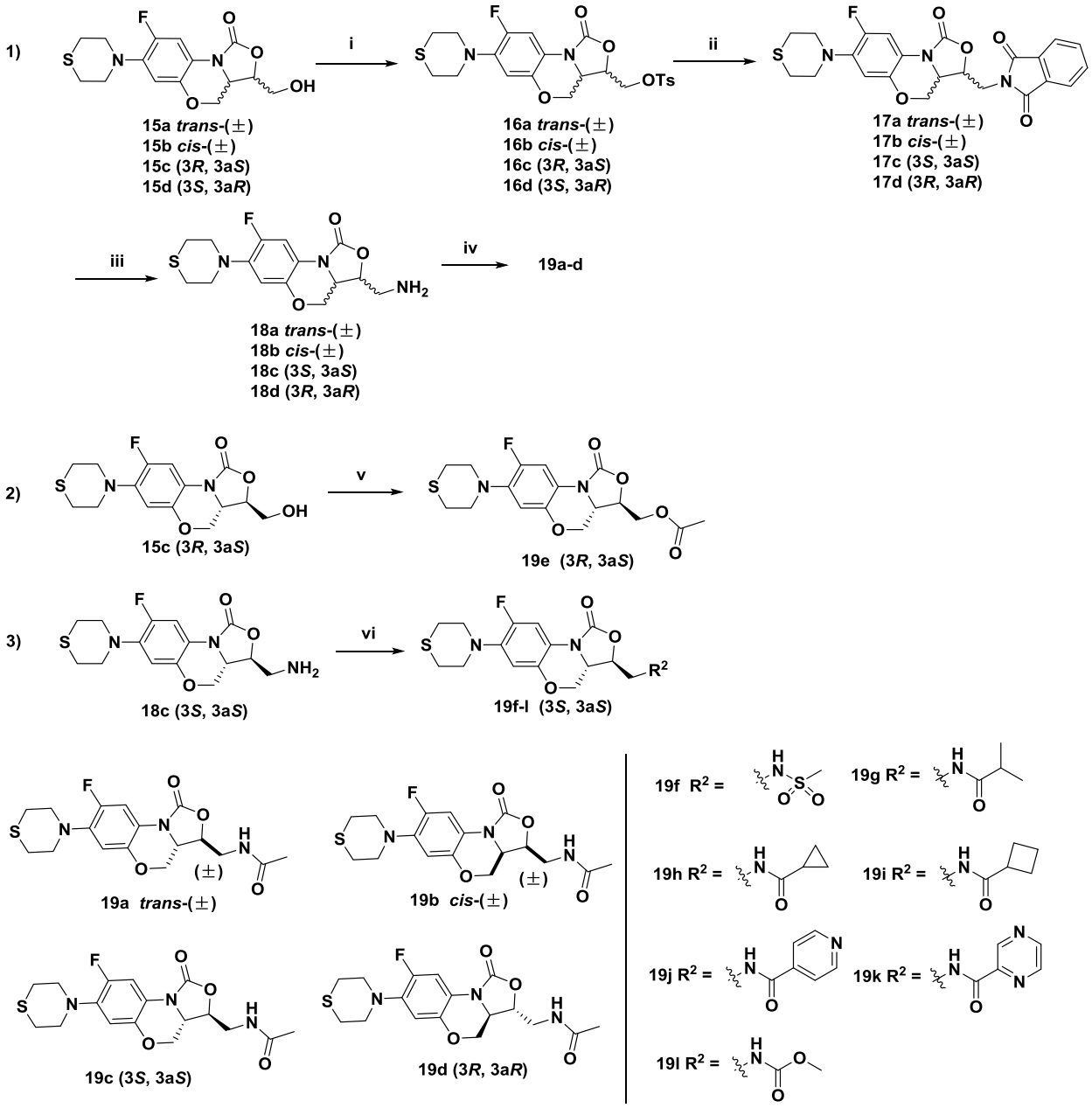
Scheme 3. Synthesis of Compounds 15a−i[a]



[a]Reagents and conditions: (i) ADDP, PPh_3, THF or DCM, 0°C-rt; (ii) *t*-BuOLi, THF, rt for **14a**, **14b** and **14d**; *n*-BuLi, THF, -78°C-rt for **14c** and **14e−i**; (iii) TFA, DCM, rt for **15a−f** and **15h**; TsOH·H_2O, DCM, MeOH, rt for **15g** and **15i**.

The synthetic route of the target compounds **19a−l** was illustrated in Scheme 4. Compounds **15a−d** reacted with 4-methylbenzenesulfonyl chloride to obtain compounds **16a−d**. Nucleophilic substitution of **16a−d** with potassium phthalimide afforded compounds **17a−d,** which reacted with methylamine in MeOH to yield **18a−d**. Finally, the target compounds **19a−d** were prepared from **18a−d** by acetylation. Compound **19e** was prepared by reaction of intermediate **15c** with acetyl chloride and Et_3N in DCM. The target compounds **19f−i** were synthesized by amidation of **18c** with different acyl chlorides. Compound **18c** was reacted with isonicotinic acid, pyrazine-2-carboxylic acid and MeOH in the presence of EDCI/HOBt and CDI to afford the final products **19j−l** respectively.

Scheme 4. Synthesis of Compounds 19a−l[a]



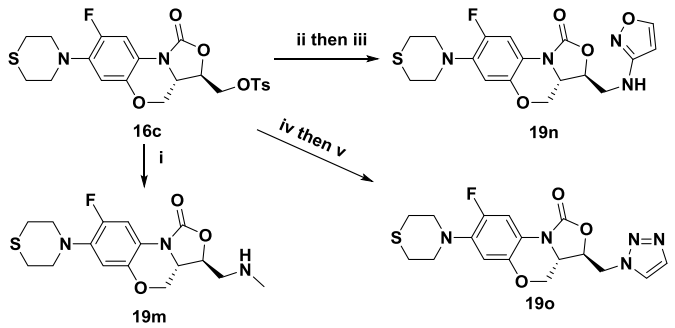
[a]Reagents and conditions: (i) TsCl, Et_3N, DMAP, DCM, 0°C-rt; (ii) potassium phthalimide, DMF, 80°C; (iii) $MeNH_2$, MeOH, reflux; (iv) acetic anhydride, pyridine, DCM, 0°C-rt; (v) acetyl chloride, Et_3N, DCM, 0°C-rt; (vi) methylsulfonyl chloride, Et_3N, DCM, 0°C for **19f**; acyl chloride, Et_3N, DCM, 0°C for **19g–i**; isonicotinic acid, EDCI, HOBt, Et_3N, DMF, rt for **19j**; pyrazine-2-carboxylic acid, EDCI, HOBt, Et_3N, DMF, rt for **19k**; CDI, THF, rt, then MeOH, THF, rt for **19l**.

Scheme 5 displayed the synthesis of the target compounds **19m–o**. Intermediate **16c** was reacted with $MeNH_2$ in a sealed tube to furnish compound **19m**. Compound **19n** was obtained from the reaction of **16c** with *tert*-butyl isoxazol-3-yl-carbamate[26] in the presence of NaH, followed by

removing the amino-protective group using HCl in EtOAc. Nucleophilic substitution of **16c** with sodium azide at 80°C in DMF, followed by cycloaddition reaction with bicyclo[2.2.1]hepta-2,5-diene,[27] gave compound **19o**.

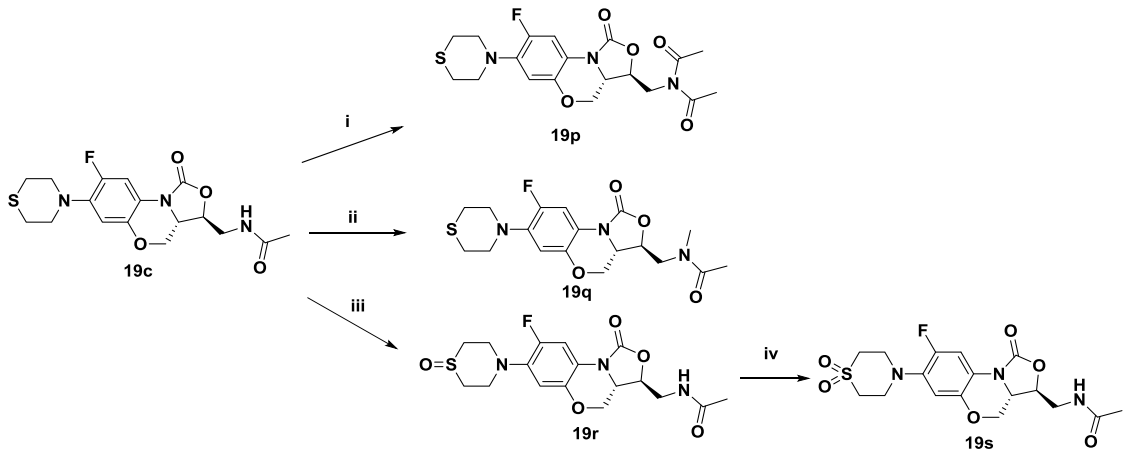
The synthesis of compounds **19p−s** was outlined in Scheme 6. **19c** could be readily acetylated and methylated to afford compounds **19p** and **19q**, employing acetic anhydride/DMAP and MeI/NaH, respectively. The corresponding sulfoxide **19r** was prepared by treating **19c** with $NaIO_4$, and the sulfone **19s** was prepared by treating **19r** with $NaIO_4$ at higher temperature.

Scheme 5. Synthesis of Compounds 19m−o[a]



[a]Reagents and conditions: (i) $MeNH_2$, MeOH, THF, sealed tube, 100°C; (ii) *N*-Boc-3-aminoisoxazole, NaH, DMF, 0-70°C; (iii) HCl, EtOAc rt then H_2O, $NaHCO_3$, rt; (iv) NaN_3, DMF, 80°C; (v) bicyclo[2.2.1]hepta-2,5-diene, dioxane, reflux.

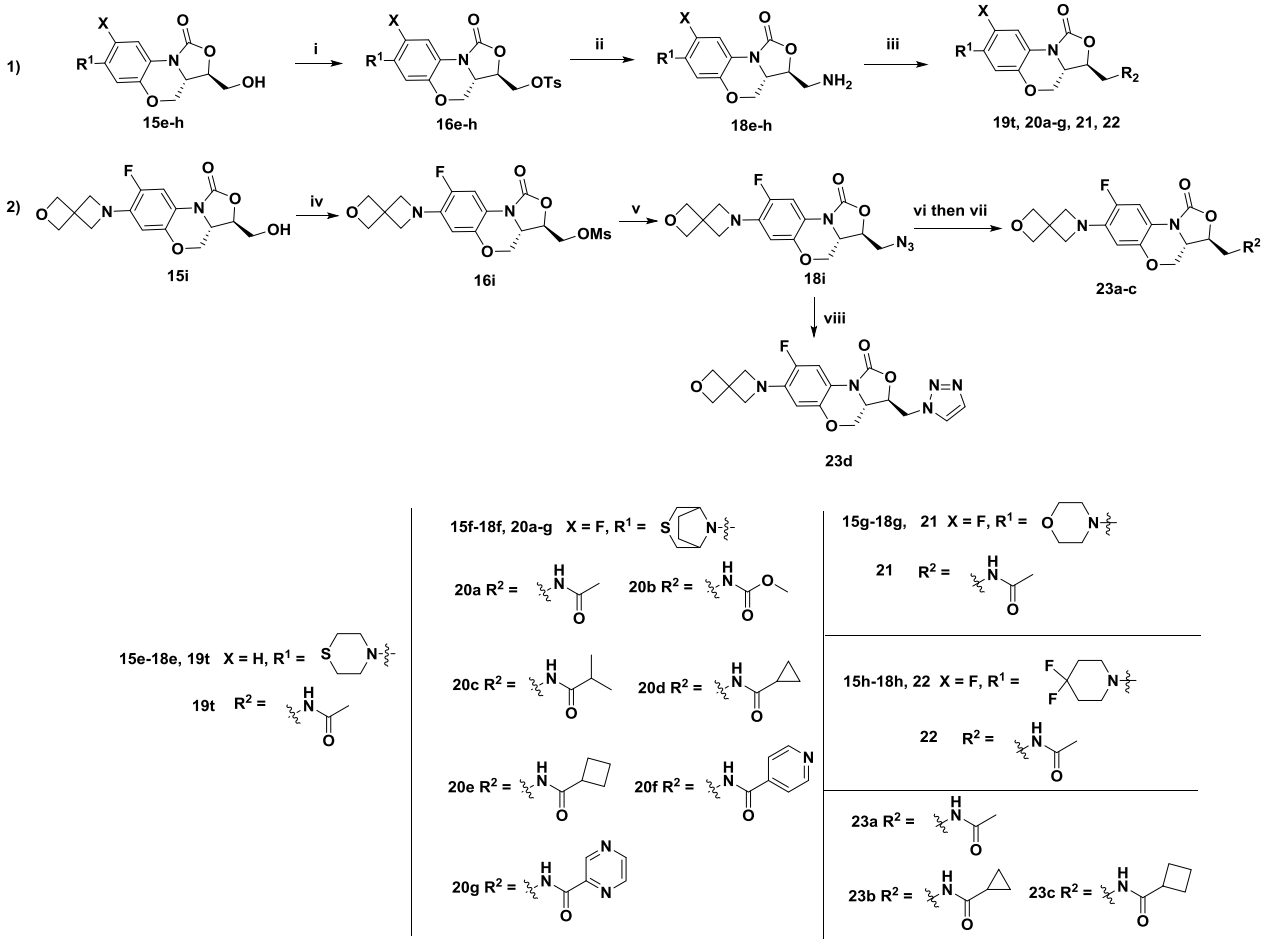
Scheme 6. Synthesis of Compounds 19p−s[a]



[a]Reagents and conditions: (i) acetic anhydride, DMAP, 100°C; (ii) MeI, NaH, THF, reflux; (iii) $NaIO_4$, MeOH, H_2O, rt; (iv) $NaIO_4$, MeOH, H_2O, 60 °C.

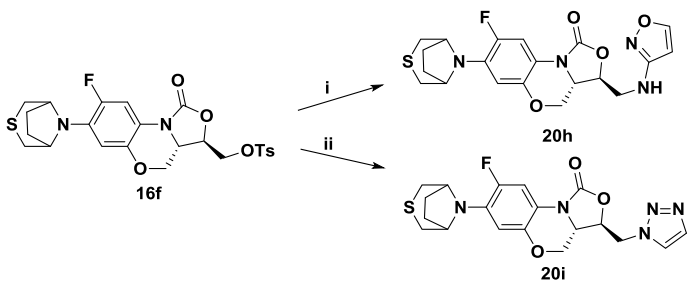
The synthetic procedure of compounds **19t**, **20a−g** (containing bridge-thiomorpholine[28]), **21**, **22** and **23a−d** were shown in Scheme 7. Compounds **15e−h** were reacted with 4-methylbenzenesulfonyl chloride to obtain compounds **16e−h**. Nucleophilic substitution of **16e−h** with ammonium hydroxide afforded compounds **18e−h**. Compounds **19t**, **20a**, **21** and **22** were prepared from **18e−h** by the acetylation, respectively. The desired products **20b−g** were synthesized by the same sequence of steps as compounds **19g−l**. The hydroxyl of **15i** was reacted with methylsulfonyl chloride to obtain compound **16i**. Next, substitution of the sulphonate group in the presence of sodium azide afforded compound **18i**, followed by hydrogenation to afford amine intermediate, and then compounds **23a−c** were synthesized via condensation. Compound **18i** underwent cycloaddition to give compound **23d**. The target compounds **20h** and **20i** were synthesized utilizing the synthetic route shown in Scheme 8.

Scheme 7. Synthesis of Compounds 19t, 20a−g, 21, 22 and 23a−d [a]



[a]Reagents and conditions: (i) TsCl, Et_3N, DMAP, DCM, rt; (ii) ammonium hydroxide, THF, sealed tube, 100°C; (iii) acetic anhydride, pyridine, DCM, 0°C-rt for **19t**, **20a**, **21** and **22**; CDI, THF, rt, then MeOH, THF, rt for **20b**; acyl chloride, Et_3N, DCM, 0°C for **20c−e**; isonicotinic acid, EDCI, HOBt, Et_3N, DMF, rt for **20f**; pyrazine-2-carboxylic acid, EDCI, HOBt, Et_3N, DMF, rt for **20g**; (iv) MsCl, NMM, DCM, rt; (v) NaN_3, DMF, 70°C; (vi) H_2, Pd/C, THF; (vii) acetic anhydride, pyridine, THF, 0°C-rt for **23a**; acyl chloride, Et_3N, THF, 0°C for **23b−c**; (viii) bicyclo[2.2.1]hepta-2,5-diene, dioxane, reflux.

Scheme 8. Synthesis of Compounds 20h and 20i[a]



[a]Reagents and conditions: (i) *N*-Boc-3-aminoisoxazole, NaH, DMF, 0-70°C then HCl, EtOAc rt then H_2O, $NaHCO_3$, rt; (ii) NaN_3, DMF, 80°C then bicyclo[2.2.1]hepta-2,5-diene, dioxane, reflux.

The absolute configuration of **19c** was confirmed by X-ray crystallography (Figure 3). The lattice parameters were a = 12.150, b = 26.738, c = 23.805 Å and the lattice volume was 7713.78 $Å^3$. There were 8 molecules in one lattice. The configurations of chiral centers are (3*S*,3a*S*).

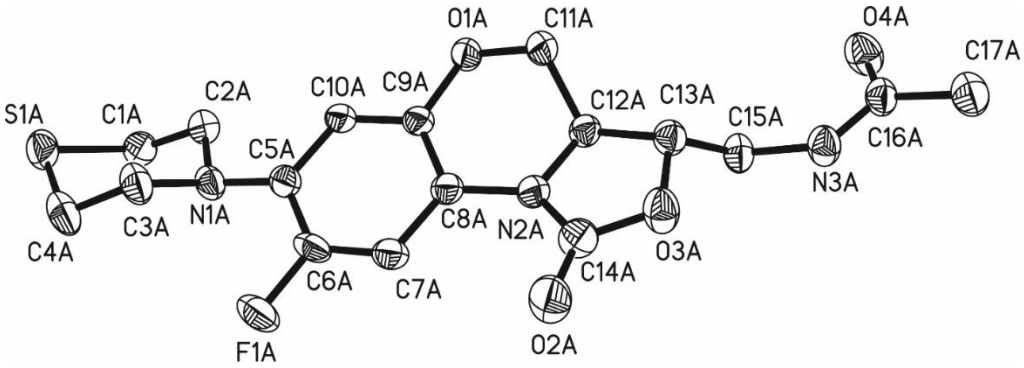


Figure 3. X-ray crystallographic structure of compound **19c**

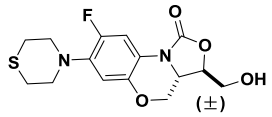
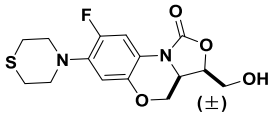
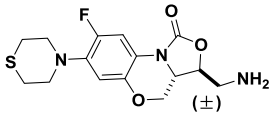
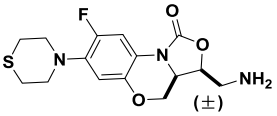
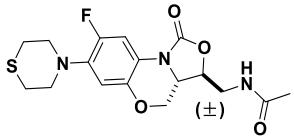
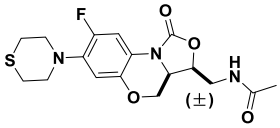
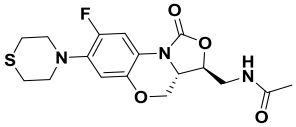
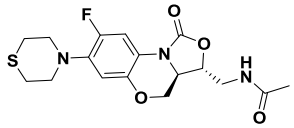
RESULTS AND DISCUSSION

All final compounds were evaluated for their inhibitory activity against *M. tuberculosis* H37Rv in microplate alamar blue assay (MABA) and cytotoxicity against Vero cell line. Selected compounds were also tested for their MPS inhibitory activity.

Firstly, the impact of the stereochemistry of the 3a,4-dihydro-1*H*,3*H*-benzo[*b*]oxazolo[3,4-*d*][1,4]oxazin-1-one core on the activity was investigated. Linezolid and other oxazolidinone currently under clinical development possess only one chiral center and the 5-*S* configuration is known to be important for antibacterial activity.[5] Due to two chiral centers existed in this novel benzoxazinyl-oxazolidinone scaffold, it is essential to explore the relationship between the configurations and anti-TB activity. As shown in Table 1, compounds containing the racemate trans configuration (**15a**, **18a** and **19a**) appeared significantly more potent than those with the racemate cis configuration (**15b**, **18b** and **19b**) against *M. tuberculosis* H37Rv. It is anticipated

that the (3*S*,3a*S*) isomer **19c** with trans configuration appeared much more potent than its isomer **19d** with (3*R*,3a*R*) configuration. This result is consistent with our previous finding that the (3*S*,3a*S*) configuration is essential for activity.[23]

Table 1. *In Vitro* Antitubercular Activity of 3a,4-Dihydro-1*H*,3*H*-benzo[*b*]oxazolo[3,4-*d*][1,4]oxazin-1-ones Containing Thiomorpholine

Compds	Structure	H37Rv MIC (μg/mL)
15a		3.79
15b		>32
18a		0.10
18b		>32
19a		0.16
19b		>32
19c		0.04
19d		>32
Linezolid		0.88
Sutezolid		0.12

Subsequently, further optimization was centered around the (3*S*,3a*S*)-3a,4-dihydro-1*H*,3*H*-benzo[*b*]oxazolo[3,4-*d*][1,4]oxazin-1-one core. As shown in Table 2, analogues with various R^1

and R^2 substitutions were prepared and evaluated for their antituberculosis activity, cytotoxicity and MPS activity.

Among various R^1 substitutions explored, the thiomorpholine substituted compound **19c** appeared to display the best antituberculosis activity with a MIC 0.03 μg/mL against the *M. tuberculosis* H37Rv strain. Deletion of the fluorine on the phenyl ring of **19c** to give compound **19t** resulted in a 25-fold loss of activity, indicating the importance of fluorine substitution on the activity. Compounds with other substitutions **21**, **22** and **23a** appeared less active with MICs 0.89, 0.91 and 3.92 μg/mL respectively. The 3-thia-8-azabicyclo[3.2.1]octane substituted analogues **20a–i** were designed with the intention to minimize the metabolism of thiomorpholine group. However, these compounds were much less active against *M. tuberculosis* H37Rv strain with MIC ranging from 0.92 to >32 μg/mL.

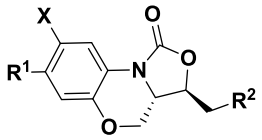
S-Oxidation of sulfur containing therapeutics is a well-known metabolic transformation catalyzed by cytochrome P450s or by flavin monooxygenases.[29] Pharmacokinetics and metabolism study of sutezolid had shown that it is well absorbed orally but with significant first pass metabolism to sulfoxide and to a lesser extent of sulfone metabolite.[8] Therefore, the potential metabolites of **19c** were prepared and evaluated for antituberculosis activity against the H37Rv strain. We found that the sulfoxide **19r** and sulfone **19s** of **19c** maintained good antituberculosis activity with MIC values 0.44 and 0.93 μg/mL, respectively, but less potent than **19c**.

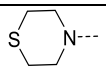
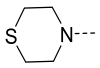
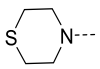
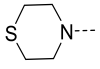
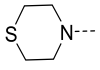
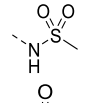
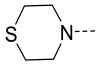
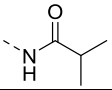
Further optimization of the R^2 group around the thiomorpholine-substituted compound **19c** provided a series derivatives **18c** and **19e–19q** with a variety of substitutions at the R^2 position. When R^2 was amino, compound **18c** showed potent activity and low MPS inhibition consistent with **19c**. Several compounds, including the cyclopropanecarboxamido **19h**, cyclobutanecarboxamido **19i**, methyl carbamate **19l** and isoxazolamino **19n**, all exhibited potent

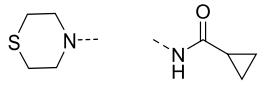
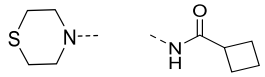
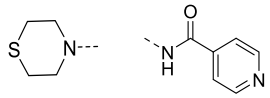
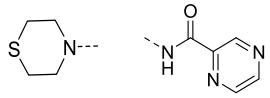
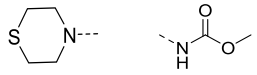
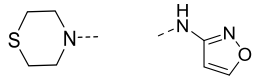
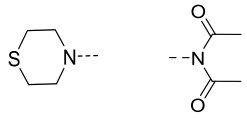
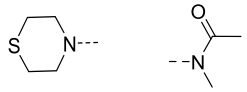
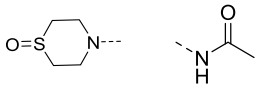
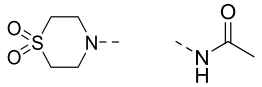
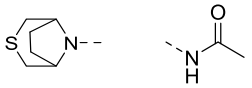
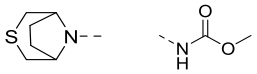
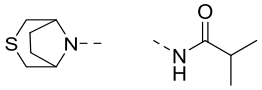
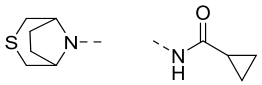
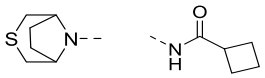
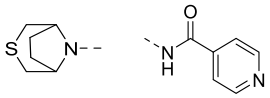
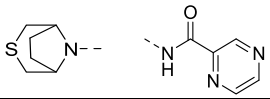
antituberculosis activity with MICs similar to **19c** and better than sutezolid. However, compound **19p** with diacetylamine group at R^2 had 10-fold decrease in antibacterial activity than **19c**, and when R^2 was methylacetylamine or ester group, the related compounds **19q** and **19e** had almost no activity.

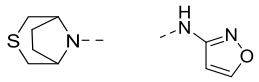
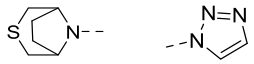
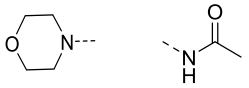
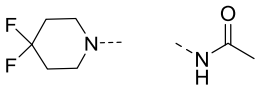
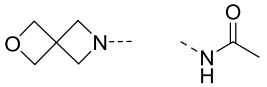
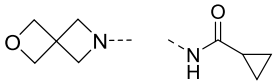
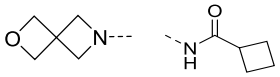
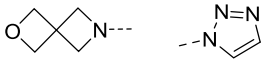
The majority compounds with a variety of R^1 and R^2 groups exhibited low cytotoxicity against the Vero cell line (Table 2). The 50% inhibitory concentrations (IC_{50}) for most compounds were >64 μg/mL. Compounds in the (3*S*,3a*S*)-3a,4-dihydro-1*H*,3*H*-benzo[*b*]oxazolo[3,4-*d*][1,4]oxazin-1-one series appeared to have less mitochondrial protein synthesis (MPS) inhibition compared to both linezolid and sutezolid. The MPS IC_{50} for compound **19c** and many its analogues were >100 μg/mL, compared to 7.98 μg/mL for linezolid and 8.17 μg/mL for sutezolid. This series compounds appeared to have a major advantage in minimizing toxicity caused by inhibition of mitochondrial protein synthesis.

Table 2. Antituberculosis Activity, Cytotoxicity and MPS Inhibition of Target Compounds



Compds	R^1	R^2	X	MIC[a] (μg/mL)	Cytotoxicity[b] IC_{50} (μg/mL)	MPSi[c] IC_{50} (μM)
18c			F	0.03	36.05	>100
19c			F	0.03	>64	>100
19t			H	0.79	>64	
19e			F	>1		
19f			F	3.90	>64	
19g			F	0.10	>64	>100

19h			F	0.03	>64	>100
19i			F	0.03	>64	53.69
19j			F	0.16	>64	>100
`19k			F	0.12	40.69	>100
19l			F	0.03	>64	>100
19m			F	>32	46.54	
19n			F	0.03	>64	>100
19o			F	0.23	>64	>100
19p			F	0.34	>64	
19q			F	>1		
19r			F	0.44	>64	60.57
19s			F	0.93	>64	25.97
20a			F	0.92	58.5	
20b			F	1.68	>64	
20c			F	1.48	>64	
20d			F	0.98	>64	
20e			F	0.97	34.67	
20f			F	2.83	>64	
20g			F	>32	9.53	

20h			F	2.52	>64	
20i			F	>32	>64	
21			F	0.89	>64	51.2
22			F	0.91	>64	10.12
23a			F	3.92	>64	
23b			F	3.09	>64	
23c			F	4.20	>64	
23d			F	>32	>64	
Linezolid				0.45	>64	7.98
Sutezolid				0.10	>64	8.17
INH				0.03		
RFP				0.06		

[a]*M. tuberculosis* H37Rv; [b]Vero cell lines; [c]Mitochondrial protein synthesis inhibition.

Table 3. Microsome Stability of Selected Compounds

Compds	MLM[a]	
	Remaining%[b]	Stability[c]
19c	48.7	stable
18c	15.8	stable
19g	6.02	stable
19h	15.0	stable
19i	4.0	stable
19j	20.8	stable
19k	6.85	stable
19l	3.59	stable
19n	0	stable
19o	21.7	stable
19r	100	stable
Linezolid	110.9	stable
Sutezolid	54.8	stable

[a]Mouse Liver Microsome; [b]Substrate concentrations were determined in incubations with NADPH after 30 min and normalized to concentrations at time zero. [c]Stability was determined without NADPH cofactor.

Selective compounds with potent antituberculosis activities were further evaluated for *in vitro* metabolic stability (Table 3). Compound **19c** showed similar metabolic stability against mouse liver microsomes compared with sutezolid. Compound **18c** with an amino group at the R^2 position was less stable. Compounds **19g−l**, and **19n−o,** with a different R^2 substituent such as aliphatic amide, aryl amide or heterocycles, all displayed lower stability than **19c**. While compound **19r**, the sulfoxide metabolite of **19c** exhibited excellent metabolic stability.

Based on its potent activity against *M. tuberculosis* H37Rv, low MPS inhibition and good microsome stability, compound **19c** and its sulfoxide metabolite **19r** were further evaluated against drug-resistant TB strains (Table 4) using linezolid and sutezolid as comparators. Compound **19c** displayed a better potency than linezolid and a similar potency to sutezolid against MDR-TB clinical isolated 12525 strain. Compound **19c** was more potent than compound **19r**, linezolid, and sutezolid against the linezolid-resistant (L-R) strain, with MIC 0.24 μg/mL. It is worth noting that there is about a 4- to10-fold increase in MIC value for linezolid-resistant (L-R) strain compared with that for H37Rv for all four compounds **19c**, **19r**, linezolid and sutezolid, indicating that **19c** is likely have the same binding site as other oxazolidinones. Based on these results, compound **19c** was therefore identified as a potential development candidate for further investigation.

Table 4. *In Vitro* Activities of 19c and 19r against Drug-resistant TB

Compds	MIC (μg/mL)		
	H37Rv	12525[a]	L-R[b]
19c	0.06	0.03	0.24
19r	0.67	0.46	6.68
Linezolid	0.32	0.23	3.92
Sutezolid	0.12	0.06	0.96

INH	0.03	>10	0.04
RFP	0.04	>10	0.03

[a]Resistance to isoniazid (INH) and rifampicin (RFP); [b]Resistance to linezolid.

To better understand the druggability of compound **19c**, a battery of *in vitro* ADME assays were performed. As shown in Table 5, compound **19c** showed good membrane permeability and excellent metabolic stability against human liver microsomes. Compound **19c** also showed excellent metabolic stability in hepatocytes from different species. Compound **19c** was also tested against several isoforms of the cytochrome P450 (CYP450) enzymes, CYP1A2, CYP2D6, CYP2C9, CYP2C19, and CYP3A4. The IC_{50} values against all these CYP450 isoforms were all > 45 μM, indicating their low potential for drug-drug interactions.

Table 5. ADME Profiles of Compound 19c

Species	Metabolic stability of hepatocytes		Caco-2 Papp×10^{-6}cm/s	HLM[a] %[b]	CYP Inhibition[c] (IC_{50}, μM)
	$T_{1/2}$ (min)	Clint (mL/min/million cell)			
Human	> 500	< 1.39			1A2 (>50)
Monkey	315	2.20			2C9 (>50)
Dog	68.0	10.2	67.58	81.5	2C19 (48.2)
Rat	100	6.90			2D6 (>50)
Mouse	76.2	9.10			3A4 (>50)

[a]Human Liver Microsome, Positive control: Dextromethorphan ($T_{1/2}$ 26.7 min), Midazolam ($T_{1/2}$ 3.65 min), Phenacetin ($T_{1/2}$ 53.3 min), Diclofenac ($T_{1/2}$ 6.1 min), Omeprazole ($T_{1/2}$ 47.8 min); [b]Substrate concentrations were determined in incubations with NADPH after 30 min and normalized to concentrations at time zero. [c]Positive control, for 1A2, Naphthoflavone (IC_{50}): 0.018 μM; for 2C9, Sulfaphenazole (IC_{50}): 0.77 μM; for 2C19, Tranylcypromine (IC_{50}): 5.86 μM; for 2D6, Quinidine (IC_{50}): 0.044 μM; for 3A4, Ketoconazole(IC_{50}): 0.069 μM

Subsequently, compound **19c** was further progressed into *in vivo* pharmacokinetic study (Table 6). Compound **19c** was quickly absorbed after oral administration at 25 mg/kg dose in mice, with T_{max} value of 0.25 h, C_{max} of 3 μg/mL and half-life ($t_{1/2}$) of 14.7 h. The exposure of compound **19c** after oral administration was high with area under the curve (AUC) value of > 6000 ng·h/mL. As anticipated, sulfoxide **19r** and sulfone **19s** were identified as the metabolites of **19c** with oral

exposure in the order of **19r**>**19c**>**19s**. After intravenous administration, compound **19c** and its metabolite **19r** were detected in the whole blood samples, with **19c**>**19r** in AUC. The concentration of metabolite **19s** was extremely low (<10 ng/mL). The oral bioavailability of compound **19c** reached 55.9%, supporting the further evaluation of its *in vivo* efficacy via oral administration.

Table 6. Mouse PK Properties of Compound 19c

Parameters		po[a] (25 mg/kg)			iv[b] (2.5 mg/kg)	
		19c	**19r**	**19s**	**19c**	**19r**
$t_{1/2\beta}$	h	14.7	6.28	7.64	0.923	3.81
T_{max}	h	0.25	0.50	1.00		0.70
C_{max}	ng/mL	3000	1518	118	4202	115
$AUC_{(0-t)}$	ng/mL *h	6478	8752	1254	1159	443
$AUC_{(0-\infty)}$	ng/mL *h	7621	9021	1475	1194	592
$MRT_{(0-t)}$	h	7.59	9.83	10.3	0.540	3.52
$MRT_{(0-\infty)}$	h	15.2	10.8	14.3	0.691	6.22
CL	mL/h/kg				2189	
V	mL/kg				2722	
F%		55.9%				

[a] Five mice for po. [b] Five mice for iv.

The *in vivo* efficacy of compound **19c** was assessed in BALB/c mice in an acute TB infection model (Table 7). Compound **19c** was orally administered at 100 mg/kg/day, whereas the positive controls linezolid and isoniazid (INH) were given at 100 mg/kg/day and 25 mg/kg/day, respectively. After three weeks of treatment, compound **19c** showed better efficacy than linezolid at the same dose level (**19c**, $\log_{10}$CFU/lung = 1.954 vs linezolid, $\log_{10}$CFU/lung = 4.136). Subsequently, a dose-response study was conducted in an acute TB model in BALB/c mice (Figure 4). In this study, sutezolid was added as the positive control besides INH and linezolid, the efficacy of linezolid dosed at 50 and 100 mg/kg/day, sutezolid at 25 and 50 mg/kg/day and **19c** at 25, 50 and 100 mg/kg/day was evaluated. CFUs were enumerated in lungs following 3 weeks of treatment

by oral gavage dosing 5 days of one week. We were pleased to find that **19c** exhibited better antitubercular activity than linezolid at all doses. Compound **19c** also showed better efficacy than sutezolid at the same dose levels.

Table 7. In Vivo Efficacy of Compound 19c

Group	Dosage (mg/kg)	Weight (g)	log_{10}CFU/lung
CMC	-	21.29±0.84	7.102±0.152
19c	100	21.25±0.88	1.954±0.159
Linezolid	100	20.83±0.43	4.136±0.498
INH	25	21.30±0.66	1.929±0.213

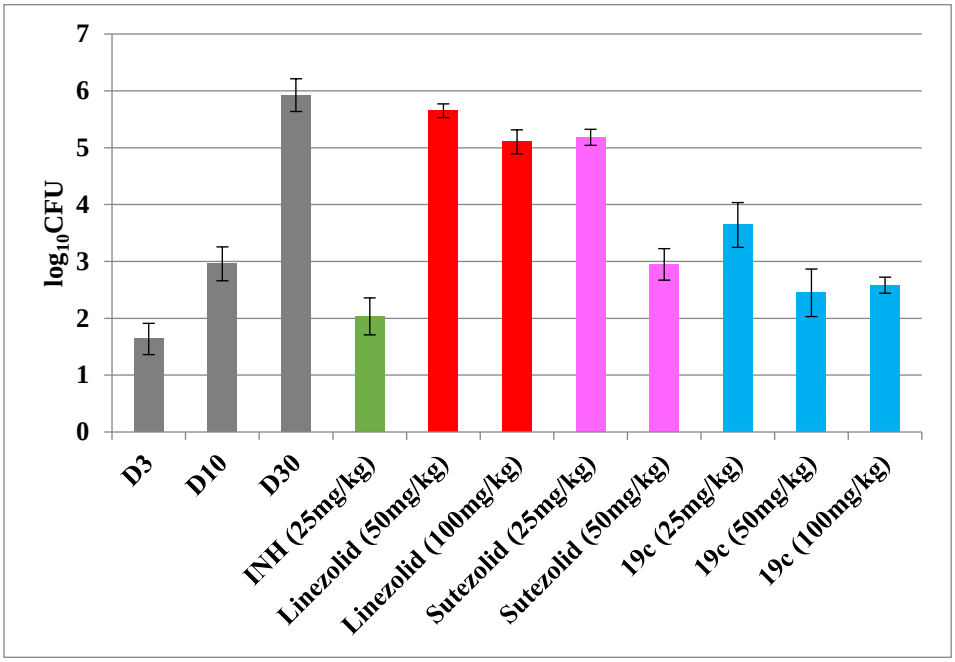


Figure 4. Efficacy in the Mouse Model of Acute *M. tuberculosis* Infection

As a potential candidate, the preliminary toxicity of compound **19c** and its main metabolite **19r** were further evaluated and the results were summarized in Table 8. Both compounds were not cytotoxic with high IC_{50} values >64 μg/mL against HepG2 cell line. Both compounds showed no inhibition of the hERG K^+, hCav1.2 and Nav1.5 channels at the highest concentration tested (>30

μM), indicating that **19c** and **19r** had low potential for cardiotoxicity. As the main adverse effect of peripheral neuropathy related to inhibition of MAOs during the long-term use of oxazolidinone, the *in vitro* MAO-A and MAO-B inhibition activity was conducted to assess the risk of **19c** for peripheral neuropathy toxicity. The inhibitory activity of compound **19c** against MAOs was significantly lower, with a 4-fold improvement over sutezolid, while the metabolite **19r** had an IC_{50} > 100 μM. These results suggested that **19c** might have an improved safety profile in terms of the risk for peripheral neuropathy. In addition, genotoxicity studies of compound **19c** and its metabolite **19r** were all negative.

Table 8. Preliminary Toxicity Properties of Compound 19c and 19r

Compds	Cytotoxicity HepG2	Mice Bone Marrow Micronucleus[a]	Chromosome Aberration[b]	Mini-Ames[c]	hERG K^+	hCav1.2	Nav1.5	MAO-A	MAO-B
	IC_{50}, μg/mL				IC_{50}, μM				
19c	> 64	Negative			> 30	> 30	> 30	44.8	3.2
19r	> 64	Negative			> 30	> 30	> 30	> 100	> 100
Sutezolid	> 64							13	0.7

[a]ICR mice, at dose levels of 100, 200, 500, 1000 and 2000 mg/kg. [b]Compounds **19c** and **19r** were tested at concentrations from 20 to 150 μg/mL with or without metabolic activation (+/- S9) using CHO-WBL cell. [c]Compounds **19c** and **19r** were tested at concentrations from 0.005 to 1000 μg/well with or without metabolic activation (+/- S9) using the two tester strains TA98 and TA100.

To further assess the risk of compound **19c** for myelosuppression, a repeated dose toxicity study in SD rat (12 female and 12 male/group) was performed. Compound **19c** was administered orally once daily at doses of 50, 150, and 450 mg/kg/day for 4 weeks, followed by a two-week recovery period. No mortality and significant body weight loss was observed in any of the compound **19c** groups. Compound **19c** had no significant effect on food consumption, eye examination, urine examination and neuropsychic symptoms of SD rats. The histopathology revealed no changes in main organs after 28-day administration and 14-day recovery period in any groups. More importantly, there were no bone marrow histopathological changes found in the highest 450 mg/kg/day dosing group. Furthermore, a hematologic analysis further confirmed that **19c** had less

myelosuppressive effect (Table 9). No significant reductions in white blood cell (WBC) and red blood cells (RBC) counts were observed in any of the compound **19c** dosing groups compared to the vehicle control. Although the platelet (PLT) count in the 150 mg/kg/day and 450 mg/kg/day female rat groups didn't show statistically significant differences ($P \geq 0.05$) compared to the control group, the drop in the mean value was observed. The decline of PLT count should be evaluated in GLP toxicity study. According to the reported data,[30] there was a pronounced drop in WBC, RBC and PLT counts in 100 mg/kg/day rat group of linezolid in a repeated dose toxicity study. The results of MPS assay and repeated dose toxicity in rat demonstrated that compound **19c** displayed lower potential risk of myelosuppressive toxicity compared to linezolid.

Table 9. Repeated Dose Toxicity of 19c: Hematology Parameters in Rat[a]

Parameter		Group[b]			
		50 mg/kg/day	150 mg/kg/day	450 mg/kg/day	vehicle
WBC(10^9/L)	F[c]	4.2±1.44	3.04±1.16	2.82±1.40	3.61±0.75
	M[d]	6.22±2.17	6.11±0.88	5.06±0.86	6.12±1.10
RBC(10^{12}/L)	F	6.85±0.58	6.61±1.43	6.53±1.20	7.32±0.20
	M	7.29±0.54	7.62±0.44	7.46±0.52	7.86±0.32
PLT(10^9/L)	F	1094±91	787±411	735±418	1051±122
	M	955±300	998±57	1038±45	1077±117
MPV(fL)	F	7.1±0.4	7.4±1.1	7.5±1.2	7.1±0.5
	M	7.0±0.6	7.0±0.4	6.9±0.6	7.0±0.4
MCV (fL)	F	56.5±1.3	56.6±0.6	56.5±1.3	56.1±0.8
	M	59.7±1.5	58.5±1.3	58.7±1.0	57.5±2.0
MCH(pg)	F	18.5±0.5	18.8±0.5	18.7±0.5	18.5±0.6
	M	18.7±0.6	18.5±0.5	18.7±0.4	18.3±0.4
HGB(g/L)	F	126±10	124±27	122±22	136±7
	M	136±10	141±11	139±9	144±5
HCT(%)	F	38.7±3.1	37.4±8.0	36.9±6.5	41.1±1.4
	M	43.5±3.5	44.5±2.9	43.8±2.7	45.2±1.7
%Retic(%)	F	3.25±0.72	2.89±0.53	2.93±0.73	2.85±0.53
	M	2.97±0.49	3.15±0.49*	2.99±0.40	2.48±0.34
%Neut(%)	F	14.8±4.5	12.2±4.8	11.8±3.6	18.5±5.3
	M	13.0±2.6	17.0±6.3	13.7±3.5	15.9±3.4
%Lymph(%)	F	80.7±5.2	83.0±6.0	83.7±4.0*	76.9±4.6
	M	82.0±2.9	77.3±6.4	80.7±5.3	77.4±3.7

[a]WBC, white blood cells; RBC, red blood cells; PLT, platelets; MPV, mean platelet volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; HGB, hemoglobin; HCT, hematocrit; %Neut, neutrophils percentage; %Lymph, lymphocytes percentage; %Retic, reticulocytes percentage. [b]Results are expressed as the mean ± SD (n = 12 for each group). [c]F, Female rat. [d]M, Male rat. *P<0.05 vs vehicle.

CONCLUSION

A novel conformationally constrained oxazolidinone **19c** has been identified through a focused lead optimization effort. Compound **19c** displayed potent activity against susceptible *M. tuberculosis* H37Rv and drug-resistant MDR-TB isolated and linezolid-resistant strain. Further evaluation of compound **19c** revealed that this compound possesses favorable mouse and human microsomal stability, low cytotoxicity, low liability for drug-drug interactions and an excellent PK profile. Compound **19c** exhibited better *in vivo* efficacy in a mouse TB model than linezolid and sutezolid. Compound **19c** exhibited an excellent safety profile in a panel of safety assessments, including inhibition of MPS, inhibition of hERG K^+, hCav1.2 and Nav1.5 channels, inhibition of MAO-A and MAO-B enzymes, and genotoxicity assays. Compound **19c** showed reduced potential for myelosuppression in the 4-week repeated dose toxicity study. Compound **19c** was therefore selected as an antituberculosis drug candidate and currently under preclinical development.

EXPERIMENTAL SECTION

Chemistry. *General.* All solvents used were of analytical grade. Inert atmosphere operations were conducted under argon in flame-dried glassware. All melting points were measured with a micro melting point apparatus (MP-J3, Yanaco) and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 NMR or 500 NMR or 600 NMR spectrometer using $CDCl_3$ or DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard. Chemical shift (δ) are reported in parts per million (ppm) and coupling constants (*J*) are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs; broad singlet. HR-MS spectra were obtained on a ThermoFisher Exactive Plus mass spectrometer (ThermoFisher Scientific, Bremen, Germany). Optical rotations were measured with a Rudolph Research Analytical (Autopol IV-T). Reactions were monitored by TLC (silica gel GF254). Column

chromatography was carried out using silica gel (200-300 mesh), and the solvent proportions were expressed on a volume: volume basis. The purity of all final compounds (⩾95%) was established by HPLC, which was carried out on a ThermoFisher Accela HPLC system (ThermoFisher Scientific, Bremen, Germany) with an agilent Zorbax SB-C18 column (5 μm, 2.1 × 50 mm), column temperature 40°C; detection wavelength at 254 nm; flow rate = 0.3 mL/min; and gradient of 5−95% MeCN in water (both containing 0.1 vol % of HCOOH) in 10 min.

Synthesis of compounds 9a-d

(Z)-4-(Trityloxy)but-2-en-1-ol (8a). To a mixture of (*Z*)-1,4-butenediol **7a** (30 g, 340 mmol), Et_3N (24 ml, 170 mmol) and DMAP (1 g) in DCM (250 mL) cooled with ice-water bath was slowly added a solution of triphenylchloromethane (31 g, 113.6 mmol) in DCM (100 mL). The reaction mixture was stirred overnight at room temperature. The mixture was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and then the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (10%~25% EtOAc in PE) to give compound **8a** (27 g, 73.0%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49−7.41 (m, 6 H), 7.36−7.18 (m, 9 H), 5.87−5.64 (m, 2 H), 4.03 (d, *J* = 6.0 Hz, 2 H), 3.71 (d, *J* = 5.6 Hz, 2 H).

(E)-4-(Trityloxy)but-2-en-1-ol (8b). To a mixture of (*E*)-1,4-butenediol **7b** (0.88 g, 10 mmol), 2,4,6-Collidine (0.4 mL, 3 mmol) and TBAI (50 mg) in DCM (10 mL) cooled with ice-water bath was added triphenylchloromethane (0.56 g, 2 mmol). The reaction mixture was stirred overnight at room temperature. The mixture was washed with 0.5 N HCl, water and brine, dried over anhydrous Na_2SO_4, filtered and then the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (15% EtOAc in PE) to give compound **8b** (0.32 g, 48.3%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49−7.44 (m, 6 H), 7.32−7.27 (m, 6 H),

7.25−7.19 (m, 3 H), 6.10−5.69 (m, 2 H), 4.17 (d, *J* = 4.8 Hz, 2 H), 3.71−3.56 (m, 2 H), 1.34 (brs, 1 H).

cis-(3-((Trityloxy)methyl)oxiran-2-yl)methanol (9a). To a solution of **8a** (10 g, 30.4 mmol) in DCM (80 mL) was added *m*-CPBA (6.4 g, 31.8 mmol). The reaction mixture was stirred overnight. After filtration, the filtrate was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. To the residue was added diethyl ether and stirred for 3 h. After filtration, the filter cake was washed with diethyl ether and dried to afford **9a** (7.3 g, 69.2%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47−7.41 (m, 6 H), 7.38−7.17 (m, 9 H), 3.65−3.47 (m, 3 H), 3.30−3.16 (m, 2 H), 3.07 (dd, *J* = 10.4, 5.6 Hz, 1 H) 1.87 (brs, 1 H). HR-MS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{23}H_{22}NaO_3$: 369.1461, found: 369.1445.

trans-(3-((Trityloxy)methyl)oxiran-2-yl)methanol (9b). To a solution of **8b** (2.2 g, 6.7 mmol) in DCM (25 mL) was added *m*-CPBA (75%, 1.7 g, 7 mmol) in portionwise. The reaction mixture was stirred overnight. After filtration, the filtrate was washed with saturated sodium bicarbonate solution and brine, dried over anhydrous Na_2SO_4, filtered and concentrated. To the residue was added EtOAc/PE (1/5, 10 mL) and stirred for 2.5 h. After filtration, the filter cake was washed with the mixture solution and dried to afford **9b** (1.1 g, 47.4%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48−7.42 (m, 6 H), 7.34−7.28 (m, 6 H), 7.26−7.21 (m, 3 H), 3.94 (dd, *J* = 12.8, 2.4 Hz, 1 H), 3.64 (dd, *J* = 12.4, 4.0 Hz, 1 H), 3.38 (dd, *J* = 10.4, 2.4 Hz, 1 H), 3.25−3.15 (m, 2 H), 3.12−3.11 (m, 1 H). HR-MS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{23}H_{22}NaO_3$: 369.1461, found: 369.1455.

(2R,3S)-(3-((Trityloxy)methyl)oxiran-2-yl)methanol (9c). To a four-necked flask were added 4Å molecular sieve (12 g) and anhydrous DCM (330 mL). The mixture was cooled to -40^{o}C under argon. *D*-(-)-diethyl tartrate (13.6 mL, 79.2 mmol) was added, followed by titanium

tetraisopropanolate (18.8 mL, 63.4 mmol), and the reaction mixture turned to yellow. After stirring for 0.5 h, a solution of **8a** (26.1 g, 79.2 mmol) in DCM (120 mL) was added to the mixture and stirred for 0.5 h. A solution of *tert*-butyl hydroperoxide in toluene (3.8 M, 50 mL, 190 mmol) was added and stirred overnight at -20°C. After the reaction completed monitored by TLC, tartaric acid solution (10%, 200 mL) containing $FeSO_4 \cdot 7H_2O$ (30 g) was added and stirred at 0°C for 1 h before layering. The organic layer was separated, and the water layer was extracted with DCM for twice. The organic phase was combined and washed with brine for twice, filtered, and evaporated to give a solid, which was stirred in *n*-hexane and filtered, followed by recrystallizing with petroleum ether/ethyl acetate to afford **9c** (15 g, 57.5%) as an off-white solid. The corresponding alcohol was analyzed by Chiral HPLC (OD-H, 5 μm, 4.6 mm×250mmL; eluent: hexane/ethanol, 90/10; flow rate: 1 mL/min; λ = 215 nm; T = 20°C): t_R = 6.46 min (96.49%), t_R = 12.58 min (3.51%). The enantiomeric purity of **9c** was determined to be 92.98% *ee*. $[\alpha]_D^{24}$ = +31.9 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.48−7.41 (m, 6 H), 7.35−7.19 (m, 9 H), 3.64−3.50 (m, 3 H), 3.30−3.15 (m, 2 H), 3.07 (dd, *J* = 10.4, 5.6 Hz, 1 H), 1.90 (brs, 1 H). HR-MS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{23}H_{22}NaO_3$: 369.1461, found: 369.1427.

(2S,3R)-(3-((Trityloxy)methyl)oxiran-2-yl)methanol (9d). Compound **9d** (6 g, 51.3%) was prepared from **8a** in the same manner as described for **9c** by replacing *D*-(-)-diethyl tartrate with *L*-(+)-diethyl tartrate. The enantiomeric purity of **9d** was determined to be 96.84% *ee*. $[\alpha]_D^{24}$ = -31.1 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49−7.20 (m, 15 H) 3.65−3.50 (m, 3 H), 3.28−3.17 (m, 2 H), 3.06 (dd, *J* = 10.4, 5.6 Hz, 1 H), 1.85 (t, *J* = 6.4 Hz, 1 H). HR-MS (ESI): *m/z* $[M+Na]^+$ calcd for $C_{23}H_{22}NaO_3$: 369.1461, found: 369.1448.

Synthesis of compounds 12a-f

4-Fluoro-2-nitro-5-thiomorpholinophenol (11a). To a solution of 4,5-difluoro-2-nitrophenol **10a** (1.75 g, 10 mmol) in MeCN (20 mL) was added *N*-methylmorpholine (1.5 mL), followed by thiomorpholine (11 mL, 11 mmol). The reaction mixture was heated at 80°C for 3 h. After cooling, water (20 mL) was added to form precipitate which was filtered and washed with water, dried to afford 2.53 g of **11a** as an orange solid (98.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.86 (s, 1 H), 7.73 (d, *J* = 13.2 Hz, 1 H), 6.42 (d, *J* = 7.6 Hz, 1 H), 3.70−3.61 (m, 4 H), 2.81−2.74 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{10}H_{12}FN_2O_3S$: 259.0547, found: 259.0543.

Benzyl (5-fluoro-2-hydroxy-4-thiomorpholinophenyl)carbamate (12a). To a suspension of **11a** (4 g, 15.5 mmol) in THF (40 mL) was added Raney nickel (1 g). The mixture was hydrogenated at 20-50 psi for 2 h. The reaction mixture was filtered into a flask containing sodium bicarbonate (2.6 g, 31 mmol) and water (10 mL) in an ice bath under argon. Benzyl chloroformate (2.0 mL, 14.4 mmol) was added in dropwise, and the mixture was stirred for 20 minutes. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give a red solid. The crude product was purified by column chromatography eluted with petroleum ether/ethyl acetate = 7/3 to give **12a** (4.5 g, 80.4%) as a pink solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.51 (s, 1 H), 8.45 (s, 1 H), 7.37−7.28 (m, 6 H), 6.48 (d, *J* = 8.8 Hz, 1 H), 5.07 (s, 2 H), 3.10 (t, *J* = 4.8 Hz, 4 H), 2.68 (t, *J* = 4.8 Hz, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{20}FN_2O_3S$: 363.1173, found: 363.1170.

2-Nitro-5-thiomorpholinophenol (11b). Compound **11b** (13.1 g, 94.9%) was prepared from 5-fluoro-2-nitrophenol **10b** (9 g, 57.3 mmol) in the same manner as described for **11a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.24 (s, 1 H), 7.96 (d, *J* = 10.0 Hz, 1 H), 6.38 (dd, *J* = 10.0, 2.8 Hz, 1 H), 6.27 (d, *J* = 2.8 Hz, 1 H), 4.00−3.72 (m, 4 H), 2.82−2.56 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{10}H_{13}N_2O_3S$: 241.0641, found: 241.0638.

Benzyl (2-hydroxy-4-thiomorpholinophenyl)carbamate (12b). To a suspension of **11b** (3.7 g, 15.5 mmol) in THF (80 mL) was added Zn dust (4 g, 62 mmol). AcOH (4.4 mL, 77.5 mmol) was added to the reaction mixture under argon and kept the inner temperature between 30-40°C. After reaction completed, the mixture was filtered into a flask containing sodium bicarbonate (3.9 g, 46.5 mmol) and water (40 mL) in an ice bath under argon. Benzyl chloroformate (2.1 mL, 15.5 mmol) was added dropwise, and the mixture was stirred for 30 minutes. After removing the solvent, the residue was partitioned between ethyl ether and H_2O, and then extracted with ethyl ether. The organic solution was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography eluted with DCM/MeOH = 99/1 to give **12b** (3.8 g, 74.6%) as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86 (s, 1 H), 7.46−7.32 (m, 5 H), 6.90 (d, *J* = 8.8 Hz, 1 H), 6.63 (brs, 1 H), 6.53 (d, *J* = 2.8 Hz, 1 H), 6.41 (dd, *J* = 8.4, 2.4 Hz, 1 H), 5.22 (s, 2 H), 3.64−3.39 (m, 4 H), 2.81−2.64 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{21}N_2O_3S$: 345.1267, found: 345.1259.

5-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-4-fluoro-2-nitrophenol (11c). To a solution of 4,5-difluoro-2-nitrophenol **10a** (3.55 g, 20 mmol) in acetonitrile (40 mL) was added *N*-methylmorpholine (6.7 mL, 60 mmol) and 3-thio-8-azodicyclo[3.2.1]octane hydriodate (5.14 g, 20 mmol). The reaction mixture was stirred at 80°C for 8 h before cooling. After adding water (30 mL), the solid was precipitated, which was filtered, washed with water, and dried to give **11c** (4.3 g, 75.7%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.05 (s, 1 H), 7.74 (d, *J* = 14.8 Hz, 1 H), 6.29 (d, *J* = 7.6 Hz, 1 H), 4.72 (s, 2 H), 3.28 (dd, *J* = 13.6, 2.0 Hz, 2 H), 2.31−2.10 (m, 6 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{12}H_{14}FN_2O_3S$: 285.0704, found: 285.0698.

Benzyl-(4-(3-thia-8-azabicyclo[3.2.1]octan-8-yl)-5-fluoro-2-hydroxyphenyl)carbamate (12c). Compound **12c** (4.2 g, 81.6%) was prepared from **11c** (4 g, 14.1 mmol) in the same manner as

described for **12a**. Pink solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.34 (s, 1 H), 8.44 (s, 1 H), 7.57−7.08 (m, 6 H), 6.44 (d, J = 8.8 Hz, 1 H), 5.11 (s, 2 H), 4.26 (s, 2 H), 3.13 (d, J = 12.8 Hz, 2 H), 2.17−1.97 (m, 6 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{22}FN_2O_3S$: 389.1330, found: 389.1327.

4-Fluoro-5-morpholino-2-nitrophenol (11d). Compound **11d** (2.3 g, 56.1%) was prepared from 4,5-difluoro-2-nitrophenol **10a** (3 g, 17.1 mmol) in the same manner as described for **11a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.86 (s, 1 H), 7.74 (d, J = 13.2 Hz, 1 H), 6.43 (d, J = 7.6 Hz, 1 H), 4.00−3.70 (m, 4 H), 3.49−3.21 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{10}H_{12}FN_2O_4$: 243.0776; found: 243.0773.

Benzyl (5-fluoro-2-hydroxy-4-morpholinophenyl)carbamate (12d). Compound **12d** (1.8 g, 62.7%) was prepared from **11d** (2 g, 8.3 mmol) in the same manner as described for **12a**. Red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.52 (s, 1 H), 8.49 (s, 1 H), 7.62−7.16 (m, 6 H), 6.49 (d, J = 8.4 Hz, 1 H), 5.12 (s, 2 H), 3.72 (t, J = 4.4 Hz, 4 H), 2.90 (t, J = 4.4 Hz, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{20}FN_2O_4$: 347.1402, found: 347.1392.

5-(4,4-Difluoropiperidin-1-yl)-4-fluoro-2-nitrophenol (11e). To a solution of 4,5-difluoro-2-nitrophenol **10a** (2.8 g, 16 mmol) in acetonitrile (15 mL) was added *N*-methylmorpholine (4 mL) and 4,4-difluoropiperidine hydrochloride (3.5 g, 22 mmol). The reaction mixture was heated at 80^{o}C for 4 h. After cooling, water was added (15 mL) and stayed overnight. The precipitation was filtered and the filter cake was purified by column chromatography (petroleum ether/DCM = 80/20) to give **11e** (3 g, 68.2%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.83 (s, 1 H), 7.75 (d, J = 13.2 Hz, 1 H), 6.47 (d, J = 7.6 Hz, 1 H), 3.47 (t, J = 6.0 Hz, 4 H), 2.22−2.07 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{12}F_3N_2O_3$: 277.0795, found: 277.0791.

Benzyl-(4-(4,4-difluoropiperidin-1-yl)-5-fluoro-2-hydroxyphenyl)carbamate (12e). Compound **12e** (4.7 g, 77.0%) was prepared from **11e** (3 g, 10.87 mmol) in the same manner as described for **12a**. Light pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49−7.30 (m, 5 H), 7.01 (brs, 1 H), 6.87−6.55 (m, 2 H), 5.22 (s, 2 H), 3.18 (brs, 4 H), 2.17 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{20}F_3N_2O_3$: 381.1421, found: 381.1408.

4-Fluoro-2-nitro-5-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenol (11f). To a solution of 4,5-difluoro-2-nitrophenol **10a** (4.0 g, 22.9 mmol) in acetonitrile (30 mL) was added *N*-methylmorpholine (4 mL, 45.8 mmol) and 2-oxa-6-azaspiro[3.3]heptane hemioxalate (4 g, 13.7 mmol). The reaction mixture was stirred at 80°C for 5 h before cooling. After adding water (30 mL), the solid was precipitated, which was filtered, washed with water, and dried to give **11f** (5.0 g, 86.2%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.19 (s, 1 H), 7.65 (d, *J* = 12.8 Hz, 1 H), 5.83 (d, *J* = 7.6 Hz, 1 H), 4.86 (s, 4 H), 4.37 (d, *J* = 2.0 Hz, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{11}H_{12}FN_2O_4$: 255.07761; found: 255.0765.

Benzyl(5-fluoro-2-hydroxy-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)phenyl)carbamate (12f). Compound **12f** (1.24 g, 46.3%) was prepared from **11f** (1.9 g, 7.5 mmol) in the same manner as described for **12b**. Red solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.36 (s, 1 H), 8.37 (s, 1 H), 7.55−7.25 (m, 5 H), 7.16 (s, 1 H), 6.00 (d, *J* = 8.8 Hz, 1 H), 5.09 (s, 2 H), 4.69 (s, 4 H), 3.97 (d, *J* = 2.0 Hz, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{20}FN_2O_4$: 359.1402, found: 359.1386.

Synthesis of compounds 15a-i

cis-Benzyl (5-fluoro-4-thiomorpholino-2-((3-((trityloxy)methyl)oxiran-2-yl)methoxy)phenyl) carbamate (13a). To a 100 mL three-necked round bottom flask were added **12a** (1 g, 2.8 mmol), **9a** (1.1 g, 3.3 mmol), PPh_3 (1.47 g, 5.6 mmol) and anhydrous THF (20 mL), and then ADDP (1.4 g, 5.6 mmol) was added in four batches at 0°C. Then, the reaction was allowed to warm to room

temperature and monitored by TLC. After reaction completed, *n*-hexane was added for dilution, the mixture was filtered, and then the filtrate was concentrated. The crude product was purified by column chromatography eluted with petroleum ether/ethyl acetate = 80/20 to give **13a** (1.3 g, 67.0%) as a pink foam solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 12.4 Hz, 1 H), 7.47−7.21 (m, 20 H), 7.13 (s, 1 H), 6.44 (brs, 1 H), 5.18 (s, 2 H), 4.15 (dd, *J* = 11.2, 2.4 Hz, 1 H), 3.81 (dd, *J* = 11.2, 6.4 Hz, 1 H), 3.45 (dd, *J* = 10.4, 5.2 Hz, 1 H), 3.38−3.28 (m, 2 H), 3.27−3.11 (m, 5 H), 2.79 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{41}H_{40}FN_2O_5S$: 691.2636, found: 691.2605.

trans-8-Fluoro-7-thiomorpholino-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (14a). To a solution of **13a** (1 g, 1.45 mmol) in anhydrous THF (20 mL) was added *t*-BuOLi (0.17 g, 2.2 mmol). After reaction completed, saturated NH_4Cl aqueous (2 mL) was added to the reaction mixture and then evaporated. MeOH was added to the residue and stirred for 1 h. It was filtered and the filter cake was washed with water and MeOH, and dried to give **14a** (837 mg, 99.2%) as a pink solid. ^{1}H NMR (400 M, $CDCl_3$) δ: 7.75 (d, *J* = 12.8 Hz, 1 H), 7.44−7.25 (m, 15 H), 6.55 (d, *J* = 7.6 Hz, 1 H), 4.37 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.29−4.18 (m, 1 H), 3.99−3.91 (m, 1 H), 3.79 (t, *J* = 10.4 Hz, 1 H), 3.53−3.45 (m, 2 H), 3.34−3.19 (m, 4 H), 2.85−2.74 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{34}H_{32}FN_2O_4S$: 583.2061, found: 583.2039.

trans-8-Fluoro-3-(hydroxymethyl)-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (15a). To a solution of **14a** (800 mg, 1.37 mmol) in DCM (18 mL) was added trifluoroacetic acid (1.8 mL, 23.3 mmol) under ice bath, and stirred overnight at room temperature. The reaction mixture was adjusted to weak alkalinity with a solution of saturated $NaHCO_3$, and extracted with DCM. The combined organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by column

chromatography (DCM/MeOH=98/2) to afford **15a** (360 mg, 77.3%) as a pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, *J* =13.2 Hz, 1 H), 6.56 (d, *J* = 7.6 Hz, 1 H), 4.46 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.40−4.31(m, 1 H), 4.19−4.09 (m, 1 H), 4.07−3.98 (m, 1 H), 3.94−3.80 (m, 2 H), 3.36−3.20 (m, 4 H), 2.88−2.76 (m, 4 H), 2.05 (t, *J* = 6.0 Hz, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 153.6, 149.1 (d, *J* = 236.0 Hz), 140.5 (d, *J* = 2.0 Hz), 136.9 (d, *J* = 10.0 Hz), 117.5 (d, *J* = 11.0 Hz), 108.5 (d, *J* = 3.0 Hz), 106.0 (d, *J* = 28.0 Hz), 76.5, 65.9, 60.7, 52.94, 52.92, 51.6, 27.2. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}FN_2O_4S$: 341.0966; found: 341.0953.

trans-Benzyl(5-fluoro-4-thiomorpholino-2-((3-((trityloxy)methyl)oxiran-2-yl)methoxy) phenyl)carbamate (13b). Compound **13b** (304 mg, 45.9%) was prepared from **12a** (349 mg, 0.96 mmol) and **9b** (505 mg, 1.45 mmol) in the same manner as described for **13a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.94 (s, 1 H), 7.58−7.16 (m, 21 H), 6.63 (brs, 1 H), 5.20 (s, 2 H), 4.29 (d, *J* = 11.0 Hz, 1 H), 3.90 (dd, *J* = 11.5, 6.0 Hz, 1 H), 3.39−3.15 (m, 8 H), 2.81 (s, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{41}H_{40}O_5N_2FS$: 691.2636, found: 691.2610.

cis-8-Fluoro-7-thiomorpholino-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo [3,4-d][1,4]oxazin-1-one (14b). Compound **14b** (81 mg, 46.3%) was prepared from **13b** (204 mg, 0.3 mmol) in the same manner as described for **14a**. Pale-pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.93 (d, *J* = 13.2 Hz, 1 H), 7.43−7.20 (m, 15 H), 6.69 (s, 1 H), 4.87−4.74 (m, 1 H), 4.35−4.24 (m, 2 H), 3.91 (t, *J* = 10.4 Hz, 1 H), 3.44 (dd, *J* = 10.4, 3.2 Hz, 1 H), 3.32 (brs, 4 H), 3.17 (dd, *J* = 10.4, 3.2 Hz, 1 H), 2.86 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{34}H_{32}FN_2O_4S$: 583.2061, found: 583.2059.

cis-8-Fluoro-3-(hydroxymethyl)-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo [3,4-d][1,4]oxazin-1-one (15b). Compound **15b** (684 mg, 58.5%) was prepared from **14b** (2 g, 3.44 mmol) in the same manner as described for **15a**. Pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ:

7.81 (d, *J* = 13.2 Hz, 1 H), 6.73 (s, 1 H), 4.84−4.80 (m, 1 H), 4.52 (dd, *J* = 10.8, 2.8 Hz, 1 H), 4.34−4.26 (m, 1 H), 4.19 (t, *J* = 10.4 Hz, 1 H), 3.96 (d, *J* = 13.6 Hz, 1 H), 3.81 (d, *J* = 12.8 Hz, 1 H), 3.39−3.26 (m, 4 H), 2.86 (brs, 4 H), 2.04 (s, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 153.3, 150.3 (d, *J* = 236.0 Hz), 140.3 (d, *J* = 2.0 Hz), 136.7 (d, *J* = 11.0 Hz), 117.9 (d, *J* = 11.0 Hz), 108.5 (d, *J* = 3.0 Hz), 105.3 (d, *J* = 28.0 Hz), 75.2, 63.5, 59.0, 53.0, 52.0, 27.2. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}FN_2O_4S$: 341.0958; found: 341.0966.

Benzyl (5-fluoro-4-thiomorpholino-2-(((2R,3S)-3-((trityloxy)methyl)oxiran-2-yl)methoxy) phenyl)carbamate (13c). To a 25 mL three-necked round bottom flask were added **12a** (3.0 g, 8.28 mmol), **9c** (3.7 g, 10.76 mmol), triphenylphosphine (4.3 g, 16.56 mmol) and anhydrous DCM (60 mL), and then ADDP (4.2 g, 16.56 mmol) was added in portions at 0°C. Then, the reaction was allowed to warm to room temperature and monitored by TLC. After reaction completed, *n*-hexane was added for dilution, the mixture was filtered, and then the filtrate was concentrated. The crude product was purified by column chromatography eluted with petroleum ether/DCM = 60/40 to give **13c** (4.2 g, 73.7%) as a red foam solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 12.4 Hz, 1 H), 7.47−7.21 (m, 20 H), 7.13 (s, 1 H), 6.47 (brs, 1 H), 5.18 (s, 2 H), 4.16 (dd, *J* = 10.4, 2.8 Hz, 1 H), 3.81 (dd, *J* = 11.2, 6.8 Hz, 1 H), 3.45 (dd, *J* = 10.4, 5.2 Hz, 1 H), 3.38−3.28 (m, 2 H), 3.27−3.11 (m, 5 H), 2.79 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{41}H_{40}O_5N_2FS$: 691.2636, found: 691.2606.

(3R,3aS)-8-Fluoro-7-thiomorpholino-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (14c). To a solution of **13c** (690 mg, 1 mmol) in anhydrous THF (11 mL) under argon at -78°C was added *n*-BuLi (1.6 M in *n*-hexane, 0.69 mL, 1.1 mmol) dropwise. After addition, the resulting mixture was stirred for 1 h, then warmed to room temperature and stirred overnight. Saturated ammonium chloride was added to quench the reaction. The solvent

was concentrated under reduced pressure, followed by adding ethyl acetate and water. The organic phase was separated, and water phase was extracted with ethyl acetate again. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by column chromatography eluted with petroleum ether/ethyl acetate = 75/25 to give **14c** (450 mg, 77.3%) as a pink solid. The product was analyzed by Chiral HPLC (OZ-H, 5 μm, 4.6 mm×250mmL; eluent: hexane/isopropanol, 70/30; flow rate: 1 mL/min; λ = 240 nm; T = 20°C): t_R = 11.12 min (2.43%), t_R = 18.28 min (97.57%). The enantiomeric purity of **14c** was determined to be 95.14% *ee*. $[\alpha]_D^{24}$ = -15.4 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.75 (d, *J* = 13.2 Hz, 1 H), 7.46−7.39 (m, 6 H), 7.37−7.23 (m, 9 H), 6.57 (d, *J* = 8.0 Hz, 1 H), 4.37 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.27−4.20 (m, 1 H), 4.00−3.92 (m, 1 H), 3.79 (t, *J* = 10.4 Hz, 1 H), 3.54−3.43 (m, 2 H), 3.34−3.19 (m, 4 H), 2.85−2.75 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{34}H_{32}FN_2O_4S$: 583.2061, found: 583.2053.

(3*R*,3a*S*)-*8-Fluoro-3-(hydroxymethyl)-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (15c).* Compound **15c** (1.2 g, 98.4%) was prepared from **14c** (2.1 g, 3.6 mmol) in the same manner as described for **15a**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.73 (d, *J* = 12.8 Hz, 1 H), 6.61 (d, *J* = 6.4 Hz, 1 H), 4.45 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.37−4.31 (m, 1 H), 4.17−4.08 (m, 1 H), 4.02 (dd, *J* = 12.4, 3.6 Hz, 1 H), 3.92−3.81 (m, 2 H), 3.33−3.22 (m, 4 H), 2.85−2.76 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}FN_2O_4S$: 341.0966; found: 341.0964.

Benzyl (5-fluoro-4-thiomorpholino-2-(((2S,3R)-3-((trityloxy)methyl)oxiran-2-yl)methoxy)phenyl)carbamate (13d). Compound **13d** (5.9 g, 85.2%) was prepared from **12a** (3.1 g, 8.56 mmol) and **9d** (4 g, 11.56 mmol) in the same manner as described for **13c**. Red foam solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 (d, *J* = 12.4 Hz, 1 H), 7.47−7.21 (m, 20 H), 7.13 (brs, 1 H), 6.45 (brs, 1 H),

5.18 (s, 2 H), 4.16 (dd, *J* = 11.2, 2.8 Hz, 1 H), 3.81 (dd, *J* = 11.6, 6.8 Hz, 1 H), 3.45 (dd, *J* = 10.4, 5.2 Hz, 1 H), 3.38−3.28 (m, 2 H), 3.27−3.11 (m, 5 H), 2.79 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{41}H_{40}FN_2O_5S$: 691.2636, found: 691.2628.

(3S,3aR)-8-Fluoro-7-thiomorpholino-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo [b]oxazolo[3,4-d][1,4]oxazin-1-one (14d). Compound **14d** (3.9 g, 93.1%) was prepared from **13d** (5.9 g, 8.55 mmol) in the same manner as described for **14a**. Pale pink solid. The enantiomeric purity of **14d** was determined to be 99.20% *ee*. $[\alpha]_D^{24}$ = +15.4 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.75 (d, *J* = 12.8 Hz, 1 H), 7.56−7.18 (m, 15 H), 6.57 (d, *J* = 7.6 Hz, 1 H), 4.37 (dd, *J* = 10.8, 3.2 Hz, 1 H), 4.27−4.19 (m, 1 H), 4.00−3.92 (m, 1 H), 3.79 (t, *J* = 10.4 Hz, 1 H), 3.54−3.43 (m, 2 H), 3.34−3.19 (m, 4 H), 2.85−2.75 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{34}H_{32}FN_2O_4S$: 583.2061, found: 583.2048.

(3S,3aR)-8-Fluoro-3-(hydroxymethyl)-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxaz olo[3,4-d][1,4]oxazin-1-one (15d). Compound **15d** (2.1 g, 92.1%) was prepared from **14d** (3.9 g, 6.7 mmol) in the same manner as described for **15a**. Pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 12.8 Hz, 1 H), 6.58 (d, *J* = 7.8 Hz, 1 H), 4.46 (dd, *J* = 10.8, 3.2 Hz, 1 H), 4.38−4.32 (m, 1 H), 4.17−4.09 (m, 1 H), 4.07−3.98 (m, 1 H), 3.92−3.81 (m, 2 H), 3.34−3.20 (m, 4 H), 2.85−2.75 (m, 4 H), 2.11 (t, *J* = 5.6 Hz, 1 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}FN_2O_4S$: 341.0966; found: 341.0956.

Benzyl (4-thiomorpholino-2-(((2R,3S)-3-((trityloxy)methyl)oxiran-2-yl)methoxy)phenyl) carbamate (13e). Compound **13e** (3.2 g, 71.0%) was prepared from **12b** (1.5 g, 4.36 mmol) and **9c** (2.26 g, 6.54 mmol) in the same manner as described for **13c**. Red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.95 (s, 1 H), 7.59−7.18 (m, 20 H), 7.17−6.89 (m, 1 H), 6.67−6.19 (m, 2 H), 5.17 (s, 2

H), 4.13 (brs, 1 H), 3.95−3.72 (m, 1 H), 3.55−3.25 (m, 7 H), 3.18 (brs, 1 H), 2.72 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{41}H_{41}N_2O_5S$: 673.2731, found: 673.2712.

(3R,3aS)-7-Thiomorpholino-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (14e). Compound **14e** (2.97 g, 77.7%) was prepared from **13e** (2.7 g, 4 mmol) in the same manner as described for **14c**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.87 (d, *J* = 8.4 Hz, 1 H), 7.53−7.14 (m, 15 H), 6.81−6.36 (m, 2 H), 4.38 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.28−4.18 (m, 1 H), 4.03−3.93 (m, 1 H), 3.84 (t, *J* = 10.4 Hz, 1 H), 3.60−3.42 (m, 6 H), 2.72 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{34}H_{33}N_2O_4S$: 565.2156, found: 565.2137.

(3R,3aS)-3-(Hydroxymethyl)-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (15e). Compound **15e** (626 mg, 68.5%) was prepared from **14e** (1.6 g, 2.84 mmol) in the same manner as described for **15a**. Pink solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.65 (d, *J* = 8.8 Hz, 1 H), 6.61 (dd, *J* = 9.2, 2.8 Hz, 1 H), 6.52 (d, *J* = 2.8 Hz, 1 H), 4.54−4.44 (m, 1 H), 4.43−4.36 (m, 1 H), 4.08−3.86 (m, 2 H), 3.78−3.61 (m, 2 H), 3.49−3.41 (m, 4 H), 2.69−2.61 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{19}N_2O_4S$: 323.1060; found: 323.1050.

Benzyl (4-(3-thia-8-azabicyclo[3.2.1]octan-8-yl)-5-fluoro-2-(((2R,3S)-3-((trityloxy)methyl) oxiran-2-yl)methoxy)phenyl)carbamate (13f). Compound **13f** (2.3 g, 33.3%) was prepared from **12c** (3.62 g, 10 mmol) and **9c** (4.5 g, 13 mmol) in the same manner as described for **13c**. Off-white foam solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (d, *J* = 13.6 Hz, 1 H), 7.47−7.19 (m, 20 H), 7.03 (s, 1 H), 6.34 (d, *J* = 7.6 Hz, 1H), 5.18 (s, 2 H), 4.27 (brs, 2 H), 4.18 (d, *J* = 11.6 Hz, 1 H), 3.83−3.74 (m, 1 H), 3.43 (dd, *J* = 10.8, 5.6 Hz, 1 H), 3.36−3.23 (m, 4 H), 3.14 (dd, *J* = 10.4, 4.8 Hz, 1 H), 2.17−1.93 (m, 6 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{43}H_{42}FN_2O_5S$:717.2793, found: 717.2767.

(3R,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (14f). Compound **14f** (1.7 g, 94.4%) was prepared from **13f** (2.1 g, 3 mmol) in the same manner as described for **14c**. Light purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (d, *J* = 14.4 Hz, 1 H), 7.47−7.41 (m, 6 H), 7.36−7.23 (m, 9 H), 6.40 (d, *J* = 8.0 Hz, 1 H), 4.38−4.31 (m, 3 H), 4.27−4.20 (m, 1 H), 4.01−3.93 (m, 1 H), 3.81 (t, *J* = 10.4 Hz, 1 H), 3.54−3.43 (m, 2 H), 3.38−3.28 (m, 2 H), 2.21−1.99 (m, 6 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{36}H_{34}FN_2O_4S$: 609.2218, found: 609.2198.

(3R,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-3-(hydroxymethyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (15f). Compound **15f** (0.83 g, 84.0%) was prepared from **14f** (1.64 g, 2.7 mmol) in the same manner as described for **15a**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 14.4 Hz, 1 H), 6.43 (d, *J* = 8.0 Hz, 1 H), 4.44 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.41−4.31 (m, 3 H), 4.18−4.10 (m, 1 H), 4.02 (dd, *J* = 12.4, 4.0 Hz, 1 H), 3.93−3.82 (m, 2 H), 3.41−3.31 (m, 2 H), 2.22−2.02 (m, 7 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}FN_2O_4S$: 367.1122; found: 367.1118.

Benzyl(5-fluoro-4-morpholino-2-(((2R,3S)-3-((trityloxy)methyl)oxiran-2-yl)methoxy) phenyl)carbamate (13g). Compound **13g** (2.3 g, 74.2%) was prepared from **12d** (1.7 g, 4.59 mmol) and **9c** (2.3 g, 6.63 mmol) in the same manner as described for **13c**. Off-white foam solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 13.2 Hz, 1 H), 7.51−7.19 (m, 20 H), 7.12 (s, 1 H), 6.43 (d, *J* = 7.2 Hz, 1 H), 5.18 (s, 2 H), 4.17 (dd, *J* = 11.2, 2.8 Hz, 1 H), 3.95−3.68 (m, 5 H), 3.45 (dd, *J* = 10.4, 5.2 Hz, 1 H), 3.39−3.23 (m, 2 H), 3.16 (dd, *J* = 10.4, 4.8 Hz, 1 H), 3.04−2.87 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{41}H_{40}FN_2O_6$: 675.2865, found: 675.2858.

(3R,3aS)-8-Fluoro-7-morpholino-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo

[3,4-d][1,4]oxazin-1-one (14g). Compound **14g** (1.78 g, 92.7%) was prepared from **13g** (2.3 g, 3.4 mmol) in the same manner as described for **14c**. Pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 13.2 Hz, 1 H), 7.57−7.14 (m, 15 H), 6.53 (d, *J* = 7.6 Hz, 1 H), 4.38 (dd, *J* = 10.4, 2.4 Hz, 1 H), 4.28−4.18 (m, 1 H), 4.06−3.92 (m, 1 H), 3.92−3.72 (m, 5 H), 3.56−3.43 (m, 2 H), 3.15−2.89 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{34}H_{32}FN_2O_5$: 567.2290, found: 567.2284.

(3R,3aS)-8-Fluoro-3-(hydroxymethyl)-7-morpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo [3,4-d][1,4]oxazin-1-one (15g). To a mixture of **14g** (1.7 g, 3 mmol), MeOH (1.3 mL) and DCM (9 mL) was added $TsOH·H_2O$ (1.14 g, 6 mmol). The resulting mixture was stirred overnight. Saturated $NaHCO_3$ aqueous was added to adjust to weak alkalinity, and extracted with DCM. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. To the residue was added a mixture of DCM (20 mL) and petroleum ether (20 mL) and stirred for 1 h. The mixture was filtered and the filter cake was dried to give **15g** (865 mg, 89.0%) as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.75 (d, *J* = 13.2 Hz, 1 H), 6.55 (d, *J* = 8.0 Hz, 1 H), 4.46 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.38−4.31 (m, 1 H), 4.18−4.10 (m, 1 H), 4.07−3.97 (m, 1 H), 3.96−3.77 (m, 6 H), 3.13−2.92 (m, 4 H), 2.23 (brs, 1 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{18}FN_2O_5$: 325.1194, found: 325.1192.

Benzyl(4-(4,4-difluoropiperidin-1-yl)-5-fluoro-2-(((2R,3S)-3-((trityloxy)methyl)oxiran-2-yl)methoxy)phenyl)carbamate (13h). Compound **13h** (2.2 g, 76.3%) was prepared from **12e** (1.6 g, 4.2 mmol) and **9c** (1.87 g, 5.4 mmol) in the same manner as described for **13c**. Pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.92 (d, *J* = 13.2 Hz, 1 H), 7.51−7.19 (m, 20 H), 7.13 (s, 1 H), 6.44 (d, *J* = 7.6 Hz, 1 H), 5.18 (s, 2 H), 4.17 (dd, *J* = 11.2, 2.0 Hz, 1 H), 3.81 (dd, *J* = 11.2, 6.4 Hz, 1 H), 3.46 (dd, *J* = 10.4, 4.8 Hz, 1 H), 3.37−3.29 (m, 2 H), 3.16 (dd, *J* = 10.4, 4.8 Hz, 1 H), 3.05 (t,

J = 5.6 Hz, 4 H), 2.22−2.01 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{42}H_{40}F_3N_2O_5$: 709.2884, found: 709.2866.

(3R,3aS)-7-(4,4-Difluoropiperidin-1-yl)-8-fluoro-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (14h). Compound **14h** (2.97 g, 77.7%) was prepared from **13h** (4.5 g, 6.36 mmol) in the same manner as described for **14c**. Light pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 13.2 Hz, 1 H), 7.54−7.18 (m, 15 H), 6.55 (d, *J* = 7.6 Hz, 1 H), 4.37 (dd, *J* = 10.4, 2.4 Hz, 1 H), 4.29−4.17 (m, 1 H), 4.02−3.90 (m, 1 H), 3.78 (t, *J* = 10.4 Hz, 1 H), 3.59−3.41 (m, 2 H), 3.23−3.03 (m, 4 H), 2.28−2.00 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{35}H_{32}F_3N_2O_4$: 601.2309, found: 601.2305.

(3R,3aS)-7-(4,4-Difluoropiperidin-1-yl)-8-fluoro-3-(hydroxymethyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (15h). Compound **15h** (2.97 g, 77.7%) was prepared from **14h** (2.9 g, 4.83 mmol) in the same manner as described for **15a**. Light pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.78 (d, *J* = 13.2 Hz, 1 H), 6.75 (d, *J* = 7.6 Hz, 1 H), 4.47 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.39−4.32 (m, 1 H), 4.18−4.11 (m, 1 H), 4.03 (dd, *J* = 12.4, 4.0 Hz, 1 H), 3.92−3.83 (m, 2 H), 3.28−3.13 (m, 4 H), 2.28−2.14 (m, 5 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{18}F_3N_2O_4$: 359.1213, found: 359.1208.

Benzyl(5-fluoro-4-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-2-(((2R,3S)-3-((trityloxy)methyl) oxiran-2-yl)methoxy)phenyl)carbamate (13i). Compound **13i** (488 mg, 71.1%) was prepared from **12f** (358 mg, 1 mmol) and **9c** (467 mg, 1.35 mmol) in the same manner as described for **13c**. Yellow semisolid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, *J* = 12.0 Hz, 1 H), 7.49−7.18 (m, 20 H), 7.00 (s, 1 H), 5.93 (d, *J* = 8.0 Hz, 1 H), 5.17 (s, 2 H), 4.81 (s, 4 H), 4.13 (dd, *J* = 11.2, 2.8 Hz, 1 H), 4.05−3.93 (m, 4 H), 3.81 (dd, *J* = 11.6, 6.8 Hz, 1 H), 3.44 (dd, *J* = 10.8, 5.6 Hz, 1 H), 3.36−3.25

(m, 2 H), 3.17 (dd, *J* = 10.4, 4.8 Hz, 1 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{42}H_{40}FN_2O_6$: 687.2865, found: 687.2842.

(3R,3aS)-8-Fluoro-7-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-3-((trityloxy)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (14i). Compound **14i** (425 mg, 56.6%) was prepared from **13i** (0.9 g, 1.3 mmol) in the same manner as described for **14c**. White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 12.8 Hz, 1 H), 7.48−7.18 (m, 15 H), 6.03 (d, *J* = 8.0 Hz, 1 H), 4.82 (s, 4 H), 4.35 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.26−4.17 (m, 1 H), 4.05 (d, *J* = 2.0 Hz, 4 H), 3.98−3.89 (m, 1 H), 3.78 (t, *J* = 10.0 Hz, 1 H), 3.53−3.41 (m, 2 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{35}H_{32}FN_2O_5$: 579.2290, found: 579.2297.

(3R,3aS)-8-Fluoro-3-(hydroxymethyl)-7-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (15i). Compound **15i** (60 mg, 95.2%) was prepared from **14i** (107 mg, 0.19 mmol) in the same manner as described for **15g**. Semisolid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.47 (d, *J* = 13.6 Hz, 1 H), 6.15 (d, *J* = 8.8 Hz, 1 H), 4.69 (s, 4 H), 4.57−4.31 (m, 2 H), 4.08−3.84 (m, 6 H), 3.78−3.58 (m, 2 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{18}FN_2O_5$: 337.1194, found: 337.1189.

Synthesis of the target compounds 19a−t

trans-(8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl 4-methylbenzenesulfonate (16a). To a mixture of **15a** (172 mg, 0.51 mmol), triethylamine (0.11 mL, 0.77 mmol) and DMAP (5 mg) in DCM (7 mL) was added *p*-methylbenzenesulfonyl chloride (116 mg, 0.61 mmol) in portionwise at 0°C. The reaction mixture was stirred for 1.5 h and diluted with DCM. The organic phase was washed sequentially with water, 10% citric acid solution and brine, dried over anhydrous sodium sulfate, filtered and concentrated to give **16a** (197 mg, 67.1%) as a white solid. ^{1}H NMR (500 MHz，$CDCl_3$) δ: 7.81 (d, *J* = 7.5 Hz,

2 H), 7.67 (d, *J* = 13.0 Hz, 1 H), 7.39 (d, *J* = 8.0 Hz, 2 H), 6.62 (brs, 1 H), 4.51−4.40 (m, 2 H), 4.37−4.21 (m, 2 H), 4.08−3.98 (m, 1 H), 3.82 (t, *J* = 10.5 Hz, 1 H), 3.37−3.20 (m, 4 H), 2.82 (brs, 4 H), 2.47 (s, 3 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}FN_2O_6S_2$: 495.1054; found: 495.1032.

trans-2-((8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)isoindoline-1,3-dione (17a). To a solution of **16a** (572 mg, 1.16 mmol) in DMF (6 mL) was added potassium phthalimide (429 mg, 2.32 mmol). The reaction mixture was stirred at 80ºC for 2 h and cooled to room temperature. Water (8 mL) was added. The precipitation was formed and filtered. The filter cake was washed with water and dried to afford **17a** (471 mg, 86.6%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.90 (dd, *J* = 5.5, 3.0 Hz, 2 H), 7.78 (dd, *J* = 5.0, 3.0 Hz, 2 H), 7.71 (d, *J* = 12.5 Hz, 1 H), 6.64 (brs, 1 H), 4.65−4.61 (m, 1 H), 4.42 (dd, *J* = 10.5, 3.0 Hz, 1 H), 4.22 (dd, *J* = 14.0, 6.5 Hz, 1 H), 4.10−3.98 (m, 2 H), 3.83 (t, *J* = 10.0 Hz, 1 H), 3.37−3.22 (m, 4 H), 2.83 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{21}FN_3O_5S$: 470.1180; found: 470.1182.

trans-3-(Aminomethyl)-8-fluoro-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18a). To a suspension of **17a** (441 mg, 0.94 mmol) in MeOH (6 mL) was added a solution of methylamine in methanol (33%, 3 mL). The reaction mixture was refluxed for 2.5 h and cooled to room temperature. After cooling, water was added for dilution, the mixture was extracted for 3 times with EtOAc. The combined organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (DCM/MeOH/ammonium hydroxide =100/2/1) to afford **18a** (281 mg, 88.1%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.75 (d, *J* = 13.0 Hz, 1 H), 6.55 (d, *J* = 7.5 Hz, 1 H), 4.45 (dd, *J* = 10.5, 3.0 Hz, 1 H), 4.30−4.22

(m, 1 H), 4.08−3.98 (m, 1 H), 3.86 (t, *J* = 10.0 Hz, 1 H), 3.34−3.20 (m, 4 H), 3.15 (dd, *J* = 13.5, 4.5 Hz, 1 H), 3.08 (dd, *J* = 14.0, 5.5 Hz, 1 H), 2.80 (t, *J* = 4.5 Hz, 4 H), 1.30 (brs, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.5, 150.3 (d, *J* = 239.0 Hz), 140.4 (d, *J* = 2.0 Hz), 137.8 (d, *J* = 11.0 Hz), 117.2 (d, *J* = 11.0 Hz), 108.2 (d, *J* = 4.0 Hz), 107.2 (d, *J* = 24.0 Hz), 77.1, 66.9, 53.4, 53.30, 53.28, 44.1, 27.9. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{19}O_3N_3FS$: 340.1126; found: 340.1116.

trans-N-((-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (19a). To a mixture of **18a** (123 mg, 0.36 mmol) and pyridine (0.044 mL, 0.54 mmol) in DCM (5 mL) in an ice bath was added acetic anhydride (0.044 mL, 0.47 mmol). The reaction was stirred for 1.5 h and diluted with DCM. The organic phase was washed sequentially with water, diluted hydrochloric acid and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by column chromatography (DCM/MeOH=99/1) to give **19a** (112 mg, 81.8%) as an off-white solid. Mp: 220–222°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 13.0 Hz, 1 H), 6.58 (d, *J* = 7.5 Hz, 1 H), 6.06 (t, *J* = 5.5 Hz, 1 H), 4.50 (dd, *J* = 10.5, 2.5 Hz, 1 H), 4.43−4.34 (m, 1 H), 3.96−3.87 (m, 1 H), 3.83 (t, *J* = 10.0 Hz, 1 H), 3.78−3.63 (m, 2 H), 3.34−3.20 (m, 4 H), 2.81 (t, *J* = 4.5 Hz, 4 H), 2.05 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.9, 153.1, 150.3 (d, *J* = 239.0 Hz), 140.4 (d, *J* = 3.0 Hz), 138.0 (d, *J* = 10.0 Hz), 116.8 (d, *J* = 11.0 Hz), 108.3 (d, *J* = 3.0 Hz), 107.0 (d, *J* = 28.0 Hz), 75.2, 66.4, 53.28, 53.25, 53.0, 41.2, 27.9, 23.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_3O_4S$: 382.1231; found: 382.1220.

cis-(8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl 4-methylbenzenesulfonate (16b). Compound **16b** (887 mg, 72.4%) was prepared from **15b** (842 mg, 2.48 mmol) in the same manner as described for **16a**. Pale-orange foam solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.85−7.74 (m, 3 H), 7.39 (d, *J* = 6.0 Hz, 2 H), 6.76 (s,

1 H), 5.00−4.89 (m, 1 H), 4.53 (dd, *J* = 8.8, 1.6 Hz, 1 H), 4.31 (t, *J* = 6.8 Hz, 1 H), 4.18−4.12 (m, 2 H), 3.94 (t, *J* = 8.4 Hz, 1 H), 3.41−3.25 (m, 4 H), 2.86 (brs, 4 H), 2.47 (s, 3 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}FN_2O_6S_2$: 495.1040; found: 495.1054.

cis-2-((8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)isoindoline-1,3-dione (17b). Compound **17b** (675 mg, 85.7%) was prepared from **16b** (830 mg, 1.68 mmol) in the same manner as described for **17a**. White solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.95−7.82 (m, 3 H), 7.82−7.74 (m, 2 H), 7.05 (s, 1 H), 5.30−5.17 (m, 1 H), 4.59 (d, *J* = 9.5 Hz, 1 H), 4.33 (t, *J* = 8.5 Hz, 1 H), 4.15−4.00 (m, 2 H), 3.75 (dd, *J* = 14.0, 3.5 Hz, 1 H), 3.42 (s, 4 H), 2.96 (s, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{21}FN_3O_5S$: 470.1170; found: 470.1180.

cis-3-(Aminomethyl)-8-fluoro-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18b). Compound **18b** (330 mg, 76.0%) was prepared from **17b** (600 mg, 1.28 mmol) in the same manner as described for **18a**. White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 12.8 Hz, 1 H), 6.55 (d, *J* = 8.0 Hz, 1 H), 4.82−4.71 (m, 1 H), 4.50 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.30−4.17 (m, 1 H), 4.07 (t, *J* = 10.4 Hz, 1 H), 3.35−3.18 (m, 4 H), 3.08−2.91 (m, 2 H), 2.87−2.74 (m, 4 H), 1.25 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.5, 150.3 (d, *J* = 238.0 Hz), 140.4 (d, *J* = 2.0 Hz), 137.7 (d, *J* = 10.0 Hz), 117.6 (d, *J* = 12.0 Hz), 108.2 (d, *J* = 3.0 Hz), 107.2 (d, *J* = 28.0 Hz), 76.2, 63.8, 53.30, 53.27, 52.9, 41.4, 27.9. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{19}O_3N_3FS$: 340.1118; found: 340.1126.

cis-N-((-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (19b). Compound **19b** (82 mg, 74.2%) was prepared from **18b** (100 mg, 0.29 mmol) in the same manner as described for **19a**. White solid. Mp: 253–255°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.79 (d, *J* = 13.0 Hz, 1 H), 6.91 (s, 1 H), 6.03 (s, 1 H), 4.90 (t, *J* =

8.5 Hz, 1 H), 4.52 (d, *J* = 10.5 Hz, 1 H), 4.27 (t, *J* = 9.0 Hz, 1 H), 3.90 (t, *J* = 10.5 Hz, 2 H), 3.38 (s, 4 H), 3.23−3.07 (m, 1 H), 2.92 (s, 4 H), 2.04 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.4, 152.9, 150.3 (d, *J* = 240.0 Hz), 140.3 (d, *J* = 2.0 Hz), 138.0, 117.2 (d, *J* = 9.0 Hz), 108.4 (d, *J* = 2.0 Hz), 107.1 (d, *J* = 28.0 Hz), 74.0, 63.3, 53.30, 53.27, 52.5, 39.0, 27.9, 23.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}O_4N_3FS$: 382.1221; found: 382.1231.

((3R,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl 4-methylbenzenesulfonate (16c). Compound **16c** (1.59 g, 85.0%) was prepared from **15c** (1.2 g, 3.53 mmol) in the same manner as described for ***16a***. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88−7.73 (m, 2 H), 7.67 (d, *J* = 12.8 Hz, 1 H), 7.46−7.33 (m, 2 H), 6.62 (d, *J* = 7.0 Hz, 1 H), 4.50−4.40 (m, 2 H), 4.33 (dd, *J* = 11.2, 4.4 Hz, 1 H), 4.26 (dd, *J* = 11.2, 6.0 Hz, 1 H), 4.07−3.99 (m, 1 H), 3.82 (t, *J* = 10.4 Hz, 1 H), 3.33−3.23 (m, 4 H), 2.86−2.78 (m, 4 H), 2.47 (s, 3 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}FN_2O_6S_2$: 495.1054; found: 495.1056.

2-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)isoindoline-1,3-dione (17c). Compound **17c** (243 mg, 88.0%) was prepared from **16c** (0.29 g, 0.59 mmol) in the same manner as described for **17a**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94−7.87 (m, 2 H), 7.83−7.72 (m, 3 H), 6.91 (brs, 1 H), 4.67−4.60 (m, 1 H), 4.44 (dd, *J* = 10.8, 3.2 Hz, 1 H), 4.22 (dd, *J* = 14.4, 6.8 Hz, 1 H), 4.10−4.01 (m, 2 H), 3.82 (t, *J* = 10.4 Hz, 1 H), 3.38 (brs, 4 H), 2.92 (brs, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{21}O_5N_3FS$: 470.1180; found: 470.1181.

(3S,3aS)-3-(Aminomethyl)-8-fluoro-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18c). Compound **18c** (130 mg, 89.2%) was prepared from **17c** (200 mg, 0.43 mmol) in the same manner as described for **18a**. Off-white solid. ^{1}H NMR

(400 MHz, $CDCl_3$) δ: 7.75 (d, *J* = 12.8 Hz, 1 H), 6.55 (d, *J* = 7.6 Hz, 1 H), 4.45 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.29−4.23 (m, 1 H), 4.07−4.00 (m, 1 H), 3.86 (t, *J* = 10.4 Hz, 1 H), 3.32−3.20 (m, 4 H), 3.19−3.04 (m, 2 H), 2.83−2.76 (m, 4 H), 1.41 (brs, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.5, 150.3 (d, *J* = 238.0 Hz), 140.4 (d, *J* = 3.0 Hz), 137.8 (d, *J* = 11.0 Hz), 117.2 (d, *J* = 11.0 Hz), 108.2 (d, *J* = 3.0 Hz), 107.2 (d, *J* = 28.0 Hz), 77.1, 66.9, 53.4, 53.33, 53.31, 44.2, 28.0. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{19}FN_3O_3S$: 340.1126; found: 340.1111.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (19c). Compound **19c** (128 mg, 63.4%) was prepared from **18c** (0.18 g, 0.53 mmol) in the same manner as described for **19a**. Off-white solid. Mp: 190–192°C. The corresponding product was analyzed by Chiral HPLC (IC, 5 μm, 4.6 mm×250mmL; eluent: hexane/ethanol, 65/35; flow rate: 1 mL/min; λ = 254 nm; T = 20°C): t_R = 17.40 min (0.09%), t_R = 24.44 min (99.91%). The enantiomeric purity of 1**9c** was determined to be 99.82% *ee*. $[\alpha]_D^{24}$ = -18.3 (c = 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.69 (d, *J* = 12.5 Hz, 1 H), 6.59 (d, *J* = 6.0 Hz, 1 H), 6.28 (t, *J* = 5.5 Hz, 1 H), 4.51 (dd, *J* = 10.0, 2.5 Hz, 1 H), 4.43−4.37 (m, 1 H), 3.95−3.88 (m, 1 H), 3.85 (t, *J* = 10.5 Hz, 1 H), 3.79−3.63 (m, 2 H), 3.35−3.20 (m, 4 H), 2.81 (brs, 4 H), 2.05 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.1, 153.2, 150.2 (d, *J* = 238.8 Hz), 140.4, 137.9 (d, *J* = 10.0 Hz), 116.8 (d, *J* = 11.2 Hz), 108.4, 106.9 (d, *J* = 27.5 Hz), 75.3, 66.4, 53.2, 53.0, 41.1, 27.8, 23.0. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_3O_4S$: 382.1231; found: 382.1222.

((3S,3aR)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl 4-methylbenzenesulfonate (16d). Compound **16d** (2.9 g, 100.0%) was prepared from **15d** (2 g, 5.88 mmol) in the same manner as described for **16a**. Pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, *J* = 8.0 Hz, 2 H), 7.66 (d, *J* = 12.0 Hz, 1 H), 7.39 (d, *J* = 8.0 Hz, 2 H), 6.67 (d, *J* = 7.6 Hz, 1 H), 4.50−4.40 (m, 2 H), 4.37−4.22 (m, 2 H), 4.07−3.99 (m, 1 H), 3.82 (t,

J = 10.4 Hz, 1 H), 3.35−3.20 (m, 4 H), 2.86−2.78 (m, 4 H), 2.47 (s, 3 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}FN_2O_6S_2$: 495.1054; found: 495.1041.

2-(((3R,3aR)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)isoindoline-1,3-dione (17d). Compound **17d** (2.55 g, 92.7%) was prepared from **16d** (2.9 g, 5.86 mmol) in the same manner as described for **17a**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.94−7.87 (m, 2 H), 7.83−7.72 (m, 2 H), 6.69 (d, *J* = 12.8 Hz, 1 H), 6.64 (d, *J* = 8.0 Hz, 1 H), 4.67−4.60 (m, 1 H), 4.42 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.22 (dd, *J* = 14.0, 6.8 Hz, 1 H), 4.10−4.01 (m, 2 H), 3.83 (t, *J* = 10.4 Hz, 1 H), 3.33−3.18 (m, 4 H), 2.87−2.72 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{21}O_5N_3FS$: 470.1180; found: 470.1174.

(3R,3aR)-3-(Aminomethyl)-8-fluoro-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18d). Compound **18d** (1.64 g, 90.6%) was prepared from **17d** (2.5 g, 5.33 mmol) in the same manner as described for **18a**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.75 (d, *J* = 13.0 Hz, 1 H), 6.55 (d, *J* = 7.8 Hz, 1 H), 4.45 (dd, *J* = 10.4, 3.0 Hz, 1 H), 4.29−4.23 (m, 1 H), 4.07−4.00 (m, 1 H), 3.86 (t, *J* = 10.2 Hz, 1 H), 3.32−3.20 (m, 4 H), 3.19−3.04 (m, 2 H), 2.83−2.76 (m, 4 H), 1.41 (brs, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.5, 150.3 (d, *J* = 238.0 Hz), 140.4 (d, *J* = 3.0 Hz), 137.8 (d, *J* = 11.0 Hz), 117.2 (d, *J* = 11.0 Hz), 108.2 (d, *J* = 3.0 Hz), 107.2 (d, *J* = 28.0 Hz), 77.1, 66.9, 53.4, 53.31, 53.28, 44.1, 28.0. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{19}FN_3O_3S$: 340.1126; found: 340.1115.

N*-(((*3R,3aR*)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (19d). Compound **19d** (1.44 g, 80.1%) was prepared from **18d** (1.6 g, 4.72 mmol) in the same manner as described for **19a**. Off-white solid. Mp: 210–212°C. The enantiomeric purity of 1**9d** was determined to be 100% *ee*. $[\alpha]_D^{24}$ = +15.1 (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68 (d, *J* = 13.2 Hz, 1 H), 6.55 (d, *J* = 8.0 Hz, 1 H), 6.38 (t, *J* = 6.0

Hz, 1 H), 4.50 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.45−4.37 (m, 1 H), 3.97−3.88 (m, 1 H), 3.83 (t, *J* = 10.0 Hz, 1 H), 3.71 (t, *J* = 4.0 Hz, 2 H), 3.32−3.19 (m, 4 H), 2.86−2.72 (m, 4 H), 2.05 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.1, 153.2, 150.2 (d, *J* = 239.0 Hz), 140.4 (d, *J* = 3.0 Hz), 138.0 (d, *J* = 12.0 Hz), 116.8 (d, *J* = 11.0 Hz), 108.3 (d, *J* = 5.0 Hz), 106.9 (d, *J* = 28.0 Hz), 75.3, 66.4, 53.3, 53.2, 53.0, 41.2, 27.9, 23.0. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_3O_4S$: 382.1231; found: 382.1230.

(3R,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl acetate (19e). To a solution of **15c** (340 mg, 1 mmol) in DCM (6 mL) was added Et_3N (0.21 mL, 1.5 mmol) in an ice bath. Acetyl chloride (0.086 mL, 1.2 mmol) was added and stirred for 0.5 h at room temperature. The reaction mixture was washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was stirred in a solution of EtOAc (4 mL) and PE (2 mL) to give **19e** (308 mg, 80.6%) as a white solid. Mp: 195–197°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 12.5 Hz, 1 H), 6.58 (d, *J* = 7.0 Hz, 1 H), 4.52−4.32 (m, 4 H), 4.05−3.95 (m, 1 H), 3.87 (t, *J* = 10.0 Hz, 1 H), 3.36−3.20 (m, 4 H), 2.81 (brs, 4 H), 2.14 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.3, 152.9, 150.3 (d, *J* = 239.0 Hz), 140.3 (d, *J* = 2.0 Hz), 138.0 (d, *J* = 10.0 Hz), 116.9 (d, *J* = 11.0 Hz), 108.3 (d, *J* = 3.0 Hz), 107.3 (d, *J* = 28.0 Hz), 73.2, 66.5, 63.2, 53.28, 53.25, 52.8, 27.9, 20.6. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}FN_2O_5S$: 383.1071; found: 383.1058.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)methanesulfonamide (19f). Compound **19f** (43 mg, 51.8%) was prepared from **18c** (70 mg, 0.175 mmol) and methylsulfonyl chloride (0.016 mL, 0.21 mmol) in the same manner as described for **19e**. Off-white solid. Mp: 234–235°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.61−7.51 (m, 2 H), 6.68 (d, *J* = 8.4 Hz, 1 H), 4.55−4.47 (m, 2 H), 4.04−3.94 (m, 2

H), 3.50−3.35 (m, 2 H), 3.24−3.12 (m, 4 H), 2.97 (s, 3 H), 2.77−2.69 (m, 4 H). ^{13}C NMR (150 MHz, DMSO-d_6) δ: 153.1, 149.1 (J = 237.0 Hz), 140.5 (J = 1.5 Hz), 137.0 (J = 10.5 Hz), 117.3 (J = 10.5 Hz), 108.5 (J = 3.0 Hz), 105.9 (J = 27.0 Hz), 74.6, 65.7, 52.9, 52.5, 44.1, 40.0, 27.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{21}FN_3O_5S_2$: 418.0901; found: 418.0894.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)isobutyramide (19g). Compound **19g** (38 mg, 58.5%) was prepared from **18c** (55 mg, 0.16 mmol) and isobutyryl chloride (0.020 mL, 0.18 mmol) in the same manner as described for **19e**. Off-white solid. Mp: 193–195ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, J = 13.2 Hz, 1 H), 7.01 (brs, 1 H), 6.06 (t, J = 5.6 Hz, 1 H), 4.51 (dd, J = 10.0, 2.4 Hz, 1 H), 4.45−4.36 (m, 1 H), 4.00−3.87 (m, 1 H), 3.86−3.62 (m, 3 H), 3.50−3.33 (m, 4 H), 2.94 (brs, 4 H), 2.49−2.36 (m, 1 H), 1.17 (d, J = 4.0 Hz, 3 H), 1.16 (d, J = 4.0 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 177.9, 153.2, 150.3 (d, J = 239.0 Hz), 140.5 (d, J = 2.0 Hz), 137.9, 117.0, 108.4 (d, J = 1.0 Hz), 107.2 (d, J = 28.0 Hz), 75.3, 66.4, 53.3, 53.1, 41.3, 35.5, 27.9, 19.6, 19.4. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{25}FN_3O_4S$: 410.1544; found: 410.1528.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)cyclopropanecarboxamide (19h). Compound **19h** (86 mg, 70.5%) was prepared from **18c** (0.10 g, 0.30 mmol) and cyclopropanecarbonyl chloride (0.035 mL, 0.39 mmol) in the same manner as described for **19e**. Off-white solid. Mp. 209–211ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, J = 13.2 Hz, 1 H), 6.60 (d, J = 7.6 Hz, 1 H), 6.16 (t, J = 6.0 Hz, 1 H), 4.48 (dd, J = 10.4, 2.8 Hz, 1 H), 4.43−4.36 (m, 1 H), 3.95−3.88 (m, 1 H), 3.82 (t, J = 10.0 Hz, 1 H), 3.79–3.65 (m, 2 H), 3.32–3.23 (m, 4 H), 2.86–2.76 (m, 4 H), 1.45–1.36 (m, 1 H), 1.01–0.94 (m, 2 H), 0.85−0.77 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.8, 153.3, 150.3 (d, J = 240.0 Hz), 140.5 (d, J = 3.0 Hz), 137.8 (d, J = 12.0 Hz), 117.1 (d, J = 9.0 Hz), 108.5 (d, J = 2.0 Hz), 107.0 (d, J =

28.0 Hz), 75.5, 66.5, 53.4, 53.3, 53.1, 41.3, 27.9, 14.6, 7.8. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{23}FN_3O_4S$: 408.1388; found: 408.1379.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)cyclobutanecarboxamide (19i). Compound **19i** (30 mg, 24.8%) was prepared from **18c** (98 mg, 0.29 mmol) and cyclobutanecarbonyl chloride (0.037 mL, 0.37 mmol) in the same manner as described for **19e**. Off-white solid. Mp. 176–178°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 12.8 Hz, 1 H), 6.63 (d, *J* = 7.2 Hz, 1 H), 5.83 (t, *J* = 6.4 Hz, 1 H), 4.50 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.42–4.36 (m, 1 H), 3.94–3.86 (m, 1 H), 3.82 (t, *J* = 10.4 Hz, 1 H), 3.78–3.71 (m, 1 H), 3.71–3.62 (m, 1 H), 3.36–3.22 (m, 4 H), 3.11–2.97 (m, 1 H), 2.89–2.76 (m, 4 H), 2.34–2.11 (m, 4 H), 2.07–1.81 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.9, 153.2, 150.3 (d, *J* = 239.0 Hz), 140.4 (d, *J* = 3.0 Hz), 138.0 (d, *J* = 10.0 Hz), 116.8 (d, *J* = 11.0 Hz), 108.3 (d, *J* = 3.0 Hz), 107.1 (d, *J* = 28.0 Hz), 75.3, 66.4, 53.29, 53.26, 53.1, 41.2, 39.6, 27.9, 25.3, 18.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{25}FN_3O_4S$: 422.1544; found: 422.1534.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)isonicotinamide (19j). **18c** (60 mg, 0.18 mmol), isonicotinic acid (26 mg, 0.21 mmol), EDCI (40 mg, 0.21 mmol), HOBt (28 mg, 0.21 mmol) and triethylamine (0.050 mL, 0.35 mmol) were added to a 5 mL flask. DMF (2 mL) was added and stirred overnight at room temperature. The solid can be precipitated by adding ice water and filtered. The obtained solid was purified by column chromatography (DCM/EtOAc/MeOH=50/50/1) to afford **19j** (46 mg, 59.0%) as a white solid. Mp. 135–137°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.77 (d, *J* = 4.4 Hz, 2 H), 7.69 (d, *J* = 5.6 Hz, 2 H), 7.65 (d, *J* = 12.8 Hz, 1 H), 7.35 (t, *J* = 6.0 Hz, 1 H), 6.55 (d, *J* = 8.0 Hz, 1 H), 4.61–4.49 (m, 2 H), 4.03–3.93 (m, 2 H), 3.92–3.80 (m, 2 H), 3.33–3.18 (m, 4 H), 2.86–2.75 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.4, 153.2, 150.6, 150.2 (d, *J* = 240.0 Hz), 140.5 (d, *J* = 3.0

Hz), 138.2 (d, *J* = 11.0 Hz), 121.1, 116.6 (d, *J* = 9.0 Hz), 108.4 (d, *J* = 3.0 Hz), 107.0 (d, *J* = 28.0 Hz), 75.2, 66.4, 53.3, 53.2, 42.0, 27.9. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{22}FN_4O_4S$: 445.1340; found: 445.1324.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)pyrazine-2-carboxamide (19k). Compound **19k** (41 mg, 51.9%) was prepared from **18c** (60 mg, 0.18 mmol) and pyrazine-2-carboxylic acid (25 mg, 0.21 mmol) in the same manner as described for **19j**. Pale-yellow solid. Mp. 212–214ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.38 (d, *J* = 1.6 Hz, 1 H), 8.80 (d, *J* = 2.0 Hz, 1 H), 8.57 (dd, *J* = 2.4, 1.6 Hz, 1 H), 8.27 (t, *J* = 6.0 Hz, 1 H), 7.73 (d, *J* = 12.8 Hz, 1 H), 6.68 (brs, 1 H), 4.60–4.46 (m, 2 H), 4.08–3.81 (m, 4 H), 3.37–3.22 (m, 4 H), 2.83 (brs, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.0, 153.0, 150.3 (d, *J* = 239.0 Hz), 147.9, 144.4, 143.4, 142.8, 140.4 (d, *J* = 2.0 Hz), 137.8 (d, *J* = 10.0 Hz), 116.9 (d, *J* = 11.0 Hz), 108.4 (d, *J* = 3.0 Hz), 107.1 (d, *J* = 28.0 Hz), 75.0, 66.4, 53.29, 53.26, 53.2, 41.2, 27.9. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{21}FN_5O_4S$: 446.1298; found: 446.1276.

Methyl (((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)carbamate (19l). To a solution of **18c** (0.10 g, 0.29 mmol) in THF (9 mL) was added CDI (0.49 g, 3 mmol) and stirred at room temperature for 50 mins. Anhydrous methanol (3 mL) was added and stirred overnight at room temperature. The solvent was evaporated, and then the residue was diluted with DCM. The resulting mixture was washed with saturated ammonium chloride solution, brine, dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The residue was purified by column chromatography (petroleum ether/EtOAc = 60/40) to afford **19l** (77 mg, 64.7%) as a white solid. Mp. 155–156ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 13.2 Hz, 1 H), 6.66 (d, *J* = 5.6 Hz, 1 H), 5.16 (brs, 1 H), 4.49 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.41–4.35 (m, 1 H), 3.99–3.91 (m, 1 H), 3.84 (t, *J* = 10.0 Hz,

1 H), 3.70 (s, 3 H), 3.67–3.58 (m, 2 H), 3.36–3.24 (m, 4 H), 2.83 (t, J = 4.8 Hz, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.4, 153.1, 150.3 (d, J = 239.0 Hz), 140.4 (d, J = 2.0 Hz), 137.8 (d, J = 8.0 Hz), 117.1 (d, J = 8.0 Hz), 108.5 (d, J = 2.0 Hz), 107.2 (d, J = 28.0 Hz), 75.2, 66.5, 53.39, 53.37, 52.9, 52.7, 43.0, 27.9. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_3O_5S$: 398.1180; found: 398.1172.

(3S,3aS)-8-Fluoro-3-((methylamino)methyl)-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (19m). A solution of **16c** (0.090 g, 0.17 mmol) and methylamine methanol solution (3 mL) in THF (3 mL) was heated at 100ºC for 1 h in a sealed tube. The reaction solution was concentrated and purified by column chromatography (DCM/MeOH/ammonium hydroxide = 100/2/1) to afford **19m** (30 mg, 50.0%) as an off-white solid. Mp: 155–156ºC. ^{1}H NM*R* (400 MHz, $CDCl_3$) δ: 7.75 (d, J = 12.8 Hz, 1 H), 6.54 (d, J = 8.0 Hz, 1 H), 4.44 (dd, J = 10.4, 3.2 Hz, 1 H), 4.39–4.31 (m, 1 H), 4.08–3.98 (m, 1 H), 3.84 (t, J = 10.4 Hz, 1 H), 3.33–3.20 (m, 4 H), 3.04–2.91 (m, 2 H), 2.83–2.75 (m, 4 H), 2.51 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.4, 150.3 (d, J = 239.0 Hz), 140.4 (d, J = 2.0 Hz), 137.8 (d, J = 11.0 Hz), 117.2 (d, J = 11.0 Hz), 108.2 (d, J = 3.0 Hz), 107.2 (d, J = 28.0 Hz), 75.5, 66.9, 53.9, 53.8, 53.33, 53.31, 36.7, 28.0. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{21}FN_3O_3S$: 354.1282; found: 354.1275.

(3S,3aS)-8-Fluoro-3-((isoxazol-3-ylamino)methyl)-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (19n). To a solution of *N*-Boc-3-aminoisoxazole (0.074 g, 0.4 mmol) in anhydrous DMF (2 mL) cooled with in an ice-water bath was added NaH (60%, 17 mg, 0.44 mmol). After stirred for 5 minutes, **16c** (197 mg, 0.4 mmol) was added. The reaction mixture was heated at 70ºC for 2 h and then water was added, extracted with DCM, the organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by column chromatography (petroleum ether/EtOAc = 85/15) to give 181 mg

oil with a yield of 89.4%. To a solution of the above product (141 mg, 0.28 mmol) in EtOAc (2 mL) was added a solution of HCl in methanol (5 N, 4 mL), the resulting solution was stirred at room temperature for 0.5 h. Solvent was evaporated, and water (3 mL) was added to the residue. Saturated sodium bicarbonate was used to adjust the pH to alkalinity. The precipitation was filtered. The filter cake was washed with water, dried to afford **19n** (94 mg, 82.7%) as a white solid. Mp. 148–150ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (d, *J* = 2.0 Hz, 1 H), 7.73 (d, *J* = 13.2 Hz, 1 H), 6.55 (d, *J* = 8.0 Hz, 1 H), 5.88 (d, *J* = 1.6 Hz, 1 H), 4.61–4.54 (m, 1 H), 4.51 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.35 (t, *J* = 6.4 Hz, 1 H), 4.05–3.98 (m, 1 H), 3.86 (t, *J* = 10.0 Hz, 1 H), 3.82–3.74 (m, 1 H), 3.74–3.65 (m, 1 H), 3.31–3.20 (m, 4 H), 2.79 (t, *J* = 5.2 Hz, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.3, 158.6, 153.2, 150.3 (d, *J* = 239.0 Hz), 140.4 (d, *J* = 2.0 Hz), 139.0 (d, *J* = 10.0 Hz), 116.9 (d, *J* = 11.0 Hz), 108.3 (d, *J* = 3.0 Hz), 107.1 (d, *J* = 28.0 Hz), 96.3, 74.6, 66.6, 53.27, 53.25, 45.9, 27.9. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{20}FN_4O_4S$: 407.1184; found: 407.1174.

(3S,3aS)-3-((1H-1,2,3-Triazol-1-yl)methyl)-8-fluoro-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (19o). To a solution of **16c** (494 mg, 1 mmol) in DMF (10 mL) was added sodium azide (130 mg, 2 mmol). The reaction mixture was heated at 80ºC for 2.5 h and cooled to room temperature. Ice water (10 mL) was added. The precipitation was filtered and washed with water, and dried to give 343 mg of azide product as an off-white solid with a yield of 87.7%. A solution of azide product (0.11 g, 0.3 mmol) and bicyclo[2.2.1]hepta-2,5-diene (0.31 mL, 3 mmol) in 1,4-dioxane (3 mL) was refluxed for 5 h. Then, the reaction was cooled to room temperature and concentrated under reduced pressure. The crude residue was purified by column chromatography (DCM/MeOH = 99/1) to give **19o** (83 mg, 70.9%) as an off-white solid. Mp. 223–225ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80–7.77 (m, 2 H), 7.64 (d, *J* = 12.8 Hz, 1 H), 6.64 (d, *J* = 6.8 Hz, 1 H), 4.84 (d, *J* = 4.8 Hz, 2 H), 4.72–4.66 (m, 1 H), 4.46 (dd, *J* = 10.4, 3.2 Hz,

1 H), 4.08–4.00 (m, 1 H), 3.84 (t, J = 10.4 Hz, 1 H), 3.35–3.23 (m, 4 H), 2.83 (t, J = 4.4 Hz, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.2, 150.3 (d, J = 239.0 Hz), 140.4 (d, J = 2.0 Hz), 137.4 (J = 2.0 Hz), 134.7, 125.1, 117.2, 108.9, 107.4 (d, J = 28.0 Hz), 73.5, 66.2, 53.52, 53.50, 53.1, 51.3, 27.7. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{19}FN_5O_3S$: 392.1187; found: 392.1178.

N-Acetyl-N-(((3S,3aS)-8-fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (19p). To a solution of **19c** (381 mg, 1 mmol) in acetic anhydride (3 mL) was added DMAP (122 mg, 1 mmol). The mixture was heated at 100ºC for 17 h. Saturated $NaHCO_3$ aqueous was added to adjust the pH to alkalinity. The mixture was extracted with EtOAc for twice. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by PTLC (DCM/EtOAc = 80/20) to give **19p** (93 mg, 22.0%) as a pale yellow solid. Mp. 176–178ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, J = 12.8 Hz, 1 H), 6.58 (d, J = 8.0 Hz, 1 H), 4.59 (dd, J = 10.4, 2.8 Hz, 1 H), 4.47–4.39 (m, 1 H), 4.23 (dd, J = 15.2, 2.0 Hz, 1 H), 4.03–3.87 (m, 2 H), 3.82 (t, J = 10.4 Hz, 1 H), 3.37–3.15 (m, 4 H), 2.86–2.72 (m, 4 H), 2.50 (s, 6 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 173.3, 152.4, 150.3 (J = 240.0 Hz), 140.3 (J = 1.5 Hz), 138.0 (J = 10.5 Hz), 116.7 (J = 10.5 Hz), 108.4 (J = 1.5 Hz), 106.9 (J = 28.5 Hz), 75.5, 66.4, 54.1, 53.27, 53.25, 47.9, 27.9, 26.4. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{23}FN_3O_5S$: 424.1337; found: 424.1322.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)-N-methylacetamide (19q). To a solution of **19c** (381 mg, 1 mmol) in anhydrous THF (6 mL) cooled with in an ice-water bath was added NaH (60%, 148 mg, 1.2 mmol). After stirred for 5 minutes, MeI (0.075 mL, 1.2 mmol) was added. The reaction mixture was refluxed for 1.5 h, and the solvent was evaporated. The resulting residue was purified by column chromatography (DCM/MeOH = 99/2) to give an oil which was purified by recrystallization

(MeOH) to give **19q** (220 mg, 55.7%) as a white solid. Mp. 197–199ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 12.8 Hz, 1 H), 6.58 (d, *J* = 8.0 Hz, 1 H), 4.53 (dd, *J* = 10.0, 2.8 Hz, 1 H), 4.50–4.44 (m, 1 H), 4.00–3.86 (m, 2 H), 3.81 (t, *J* = 10.0 Hz, 1 H), 3.58 (dd, *J* = 14.4, 6.4 Hz, 1 H), 3.33–3.23 (m, 4 H), 3.20 (s, 3 H), 2.84–2.74 (m, 4 H), 2.14 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.8, 153.0, 150.2 (*J* = 239.0 Hz), 140.4 (*J* = 2.0 Hz), 137.8 (*J* = 11.0 Hz), 117.0 (*J* = 11.0 Hz), 108.3 (*J* = 4.0 Hz), 106.8 (*J* = 26.0 Hz), 76.0, 66.4, 53.5, 53.3, 53.2, 50.2, 38.5, 27.9, 21.6. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{23}FN_3O_4S$: 396.1388; found: 396.1383.

N-(((3S,3aS)-8-Fluoro-7-(1-oxidothiomorpholino)-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (19r). To a mixture of **19c** (0.1 g, 0.26 mmol) and sodium periodate (0.11 g, 0.52 mmol) were added methanol (4 mL) and water (1.5 mL), the reaction mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure, and methanol was added to the residue. The mixture was filtered to remove solid and the filtrate was purified by column chromatography (EtOAc/MeOH=96/4) to afford **19r** (74 mg, 71.8%) as a white solid. Mp. 214-216ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 12.8 Hz, 1 H), 6.68 (d, *J* = 8.0 Hz, 1 H), 6.04 (t, *J* = 6.0 Hz, 1 H), 4.52 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.43–4.37 (m, 1 H), 3.96–3.89 (m, 1 H), 3.83 (t, *J* = 10.4 Hz, 1 H), 3.79–3.63 (m, 4 H), 3.31–3.20 (m, 2 H), 3.06–2.93 (m, 4 H), 2.05 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.0, 153.1, 150.2 (d, *J* = 239.0 Hz), 140.6 (d, *J* = 2.0 Hz), 135.8 (d, *J* = 11.0 Hz), 118.1 (d, *J* = 11.0 Hz), 108.7 (d, *J* = 3.0 Hz), 107.3 (d, *J* = 27.0 Hz), 75.3, 66.4, 53.0, 46.0, 42.4, 42.3, 41.3, 23.2. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_3O_5S$: 398.1180; found: 398.1162.

N-(((3S,3aS)-7-(1,1-Dioxidothiomorpholino)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (19s). To a mixture of **19r** (1 g, 2.5 mmol) and sodium periodate (1.35 g, 6.3 mmol) was added methanol (9 mL) and water (5 mL).

The mixture was stirred at 60ºC for 10 h. After cooling, water (20 mL) was added to form a solid precipitate which was filtered and washed with water, dried to afford **19s** (535 mg, 51.9%) as a pink solid. Mp. 226–228ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 12.8 Hz, 1 H), 6.59 (d, *J* = 8.0 Hz, 1 H), 6.07 (t, *J* = 6.4 Hz, 1 H), 4.52 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.44–4.37 (m, 1 H), 3.96–3.89 (m, 1 H), 3.83 (t, *J* = 10.0 Hz, 1 H), 3.79–3.63 (m, 2 H), 3.59–3.52 (m, 4 H), 3.23–3.16 (m, 4 H), 2.05 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.0, 153.1, 150.0 (d, *J* = 239.0 Hz), 140.5 (d, *J* = 3.0 Hz), 135.2 (d, *J* = 12.0 Hz), 118.1 (d, *J* = 11.0 Hz), 109.0 (d, *J* = 2.0 Hz), 107.4 (d, *J* = 27.0 Hz), 75.3, 66.4, 53.0, 51.9, 49.54, 49.51, 41.2, 23.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_3O_6S$: 414.1130; found: 414.1126.

((3R,3aS)-1-Oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl 4-methylbenzenesulfonate (16e). Compound **16e** (0.81 g, 89.6%) was prepared from **15e** (0.61 g, 1.9 mmol) in the same manner as described for **16a**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90−7.73 (m, 3 H), 7.48−7.34 (m, 2 H), 6.62 (brs, 2 H), 4.52−4.40 (m, 2 H), 4.36−4.22 (m, 2 H), 4.07−3.99 (m, 1 H), 3.87 (t, *J* = 10.4 Hz, 1 H), 3.54 (brs, 4 H), 2.74 (brs, 4 H), 2.48 (s, 3 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{25}N_2O_6S_2$: 477.1149; found: 477.1133.

(3S,3aS)-3-(Aminomethyl)-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18e). To a suspension of **16e** (0.66 g, 1.39 mmol) in tetrahydrofuran (10 mL) in a sealed tube was added ammonium hydroxide (5 mL). The reaction mixture was heated at 100ºC for 7 h. After cooling, THF was evaporated, and the aqueous phase was extracted with EtOAc for three times. The combined organic phase was washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by column chromatography (DCM/MeOH/ammonium hydroxide = 98/2/1) to give **18e** (240 mg, 53.8%) as a light red solid.^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (d, *J* = 8.4 Hz, 1 H), 6.56 (dd, *J* = 8.8, 2.8 Hz, 1 H), 6.46 (d, *J* =

2.4 Hz, 1 H), 4.43 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.24 (dd, *J* = 11.6, 5.2 Hz, 1 H), 4.04−3.96 (m, 1 H), 3.90 (t, *J* = 10.0 Hz, 1 H), 3.55−3.42 (m, 4 H), 3.18−3.01 (m, 2 H), 2.81−2.65 (m, 4 H), 1.32 (s, 2 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{20}N_3O_3S$: 322.1220; found: 322.1210.

N-(((3S,3aS)-1-Oxo-7-thiomorpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (19t). Compound **19t** (30 mg, 33.0%) was prepared from **18e** (80 mg, 0.25 mmol) in the same manner as described for **19a**. White solid. Mp. 152-154ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, *J* = 8.8 Hz, 1 H), 6.57 (d, *J* = 6.8 Hz, 1 H), 6.48 (s, 1 H), 6.10 (brs, 1 H), 4.49 (d, *J* = 8.0 Hz, 1 H), 4.42–4.32 (m, 1 H), 3.97–3.82 (m, 2 H), 3.80–3.60 (m, 2 H), 3.50 (t, *J* = 4.8 Hz, 4 H), 2.73 (brs, 4 H), 2.04 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ: 170.8, 153.4, 145.1, 120.1, 111.2, 105.2, 74.9, 66.7, 53.2, 52.2, 41.5, 26.6, 23.2. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{22}N_3O_4S$: 364.1326; found: 364.1337.

Synthesis of the target compounds 20a−i

((3R,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl 4-methylbenzenesulfonate (16f). Compound **16f** (1.07 g, 95.5%) was prepared from **15f** (0.79 g, 2.16 mmol) in the same manner as described for **16a**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (dd, *J* = 6.8, 1.6 Hz, 2 H), 7.62 (d, *J* = 14.4 Hz, 1 H), 7.40 (dd, *J* = 8.0, 0.4 Hz, 2 H), 6.41 (d, *J* = 8.0 Hz, 1 H), 4.48−4.30 (m, 5 H), 4.26 (dd, *J* = 10.8, 5.6 Hz, 1 H), 4.06−4.00 (m, 1 H), 3.84 (t, *J* = 10.4 Hz, 1 H), 3.34 (t, *J* = 11.6 Hz, 2 H), 2.48 (s, 3 H), 2.21-1.99 (m, 6 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{26}FN_2O_6S_2$: 521.1211; found: 521.1204.

(3S,3aS)-3-(Aminomethyl)-7-(3-thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18f). Compound **18f** (464 mg, 82.5%) was prepared from **16f** (0.8 g, 1.54 mmol) in the same manner as described for **18e**. Off-white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (d, *J* = 14.0 Hz, 1 H), 6.41 (d, *J* = 8.0 Hz, 1 H), 4.43 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.39−4.31 (m, 2 H), 4.29−4.22 (m, 1 H), 4.07−3.99 (m, 1 H), 3.87 (t, *J* = 10.4 Hz, 1 H), 3.38−3.28 (m, 2 H), 3.19−3.04 (m, 2 H), 2.23−2.00 (m, 6 H), 1.29 (brs, 2 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_3O_3S$: 366.1282; found: 366.1265.

N-(((3S,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (20a). Compound **20a** (43 mg, 62.3%) was prepared from **18f** (62 mg, 0.17 mmol) in the same manner as described for **19a**. White solid. Mp: 235–236°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67 (d, *J* = 14.4 Hz, 1 H), 6.42 (d, *J* = 8.4 Hz, 1 H), 6.14 (t, *J* = 6.0 Hz, 1 H), 4.48 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.43–4.31 (m, 3 H), 3.95–3.88 (m, 1 H), 3.84 (t, *J* = 10.0 Hz, 1 H), 3.78–3.63 (m, 2 H), 3.39–3.29 (m, 2 H), 2.21–1.99 (m, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.0, 153.2, 147.6 (d, *J* = 237.0 Hz), 140.8, 132.8 (d, *J* = 10.0 Hz), 113.8 (d, *J* = 11.0 Hz), 107.8 (d, *J* = 29.0 Hz), 105.9 (d, *J* = 5.0 Hz), 75.2, 66.6, 57.4, 57.2, 53.1, 41.3, 30.2, 30.0, 28.34, 28.31, 23.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{23}FN_3O_4S$: 408.1388; found: 408.1368.

Methyl-(((3S,3aS)-7-(3-thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)carbamate (20b). Compound **20b** (25 mg, 34.7%) was prepared from **18f** (62 mg, 0.17 mmol) in the same manner as described for **19l**. Off-white solid. Mp. 149–151°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (d, *J* = 14.0 Hz, 1 H), 6.44 (d, *J* = 7.6 Hz, 1 H), 5.19 (s, 1 H), 4.47 (dd, *J* = 10.4, 2.8 Hz, 1 H), 4.43–4.31 (m, 3 H), 3.95 (brs, 1 H), 3.85 (t, *J* = 10.4 Hz, 1 H), 3.71 (s, 3 H), 3.67–3.56 (m, 2 H), 3.33 (t, *J* = 11.2 Hz, 2 H), 2.22–2.00 (m, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.4, 153.1, 147.7 (d, *J* = 237.0 Hz), 140.8 (d, *J* = 1.0 Hz), 132.6 (d, *J* = 10.0 Hz), 114.0 (d, *J* = 11.0 Hz), 107.9 (d, *J* = 28.0 Hz), 105.9 (d, *J* = 5.0 Hz),

75.1, 66.6, 57.5, 57.3, 52.9, 52.7, 43.1, 30.2, 30.1, 28.33, 28.30. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{23}FN_3O_5S$: 424.1337; found: 424.1326.

N-(((3S,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)isobutyramide (20c). Compound **20c** (54 mg, 73.0%) was prepared from **18f** (62 mg, 0.17 mmol) and isobutyryl chloride (0.022 mL, 0.21 mmol) in the same manner as described for **19g**. White solid. Mp: 164–165°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.65 (d, *J* = 13.2 Hz, 1 H), 6.41 (brs, 1 H), 6.06 (t, *J* = 6.0 Hz, 1 H), 4.47 (dd, *J* = 10.0, 2.8 Hz, 1 H), 4.43–4.28 (m, 3 H), 3.97–3.79 (m, 2 H), 3.79–3.61 (m, 2 H), 3.34 (t, *J* = 11.6 Hz, 2 H), 2.49–2.36 (m, 1 H), 2.21–1.99 (m, 6 H), 1.17 (d, *J* = 2.8 Hz, 3 H), 1.16 (d, *J* = 2.8 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.0, 153.3, 147.6 (d, *J* = 236.0 Hz), 140.9, 132.8 (d, *J* = 10.0 Hz), 113.8 (d, *J* = 11.0 Hz), 107.9 (d, *J* = 29.0 Hz), 105.9 (d, *J* = 5.0 Hz), 75.2, 66.6, 57.4, 57.1, 53.1, 41.3, 35.5, 30.2, 30.0, 28.4, 28.3, 19.6, 19.4. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{27}FN_3O_4S$: 436.1701; found: 436.1680.

N-(((3S,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)cyclopropanecarboxamide (20d). Compound **20d** (57 mg, 77.0%) was prepared from **18f** (62 mg, 0.17 mmol) and cyclopropanecarbonyl chloride (0.019 mL, 0.21 mmol) in the same manner as described for **19h**. Off-white solid. Mp. 169–171°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (d, *J* = 14.4 Hz, 1 H), 6.41 (d, *J* = 8.0 Hz, 1 H), 6.23 (t, *J* = 6.0 Hz, 1 H), 4.46 (dd, *J* = 10.0, 2.8 Hz, 1 H), 4.43–4.28 (m, 3 H), 3.96–3.63 (m, 4 H), 3.39–3.25 (m, 2 H), 2.23–1.95 (m, 6 H), 1.47–1.35 (m, 1 H), 1.02–0.92 (m, 2 H), 0.84–0.74 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.6, 153.2, 147.8 (d, *J* = 236.0 Hz), 140.8 (d, *J* = 1.0 Hz), 132.8 (d, *J* = 10.0 Hz), 113.9 (d, *J* = 11.0 Hz), 107.7 (d, *J* = 29.0 Hz), 105.9 (d, *J* = 5.0 Hz), 75.4,

66.6, 57.4, 57.1, 53.1, 41.4, 30.2, 30.0, 28.4, 28.3, 14.6, 7.81, 7.80. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{25}FN_3O_4S$: 434.1544; found: 434.1525.

N-(((3S,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)cyclobutanecarboxamide (20e). Compound **20e** (65 mg, 85.5%) was prepared from **18f** (62 mg, 0.17 mmol) and cyclobutanecarbonyl chloride (0.020 mL, 0.21 mmol) in the same manner as described for **19i**. White solid. Mp. 195–196ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 14.0 Hz, 1 H), 6.41 (d, *J* = 7.6 Hz, 1 H), 5.87 (t, *J* = 6.0 Hz, 1 H), 4.47 (dd, *J* = 10.0, 2.4 Hz, 1 H), 4.43–4.28 (m, 3 H), 3.97–3.79 (m, 2 H), 3.79–3.61 (m, 2 H), 3.34 (t, *J* = 10.8 Hz, 2 H), 3.14–2.95 (m, 1 H), 2.35–1.80 (m, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.9, 153.2, 147.6 (d, *J* = 236.0 Hz), 140.9, 132.8 (d, *J* = 10.0 Hz), 113.8 (d, *J* = 11.0 Hz), 107.8 (d, *J* = 29.0 Hz), 105.9 (d, *J* = 5.0 Hz), 75.2, 66.6, 57.4, 57.2, 53.1, 41.3, 39.6, 30.2, 30.0, 28.4, 28.3, 25.4, 25.3, 18.2. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{27}FN_3O_4S$: 448.1701; found: 448.1683.

N-(((3S,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)isonicotinamide (20f). Compound **20f** (54 mg, 67.5%) was prepared from **18f** (62 mg, 0.17 mmol) and isonicotinic acid (25 mg, 0.2 mmol) in the same manner as described for **19j**. White solid. Mp. 149–150ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.78 (dd, *J* = 4.8, 1.6 Hz, 2 H), 7.68 (dd, *J* = 4.8, 1.6 Hz, 2 H), 7.63 (d, *J* = 14.4 Hz, 1 H), 7.17 (d, *J* = 6.0 Hz, 1 H), 6.41 (d, *J* = 8.0 Hz, 1 H), 4.58–4.48 (m, 2 H), 4.35 (brs, 2 H), 4.04–3.95 (m, 2 H), 3.94–3.82 (m, 2 H), 3.36–3.27 (m, 2 H), 2.21–2.00 (m, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.4, 153.2, 150.7, 147.5 (d, *J* = 236.0 Hz), 140.9 (d, *J* = 1.0 Hz), 140.4, 133.0 (d, *J* = 11.0 Hz), 121.0, 113.5 (d, *J* = 11.0 Hz), 107.7 (d, *J* = 29.0 Hz), 105.9 (d, *J* = 5.0 Hz), 75.1, 66.5, 57.2, 57.1,

53.3, 42.1, 30.1, 30.0, 28.4, 28.3. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{24}FN_4O_4S$: 471.1497; found: 471.1479.

N-(((3S,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)pyrazine-2-carboxamide (20g). Compound **20g** (64 mg, 80.0%) was prepared from **18f** (62 mg, 0.17 mmol) and pyrazine-2-carboxylic acid (25 mg, 0.2 mmol) in the same manner as described for **19k**. Pale yellow solid. Mp. >250°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.39 (s, 1 H), 8.80 (d, *J* = 2.4 Hz, 1 H), 8.58-8.55 (m, 1 H), 8.28 (t, *J* = 6.0 Hz, 1 H), 7.67 (d, *J* = 14.0 Hz, 1 H), 6.41 (d, *J* = 7.2 Hz, 1 H), 4.56–4.46 (m, 2 H), 4.34 (brs, 2 H), 4.05–3.83 (m, 4 H), 3.38–3.26 (m, 2 H), 2.20–1.99 (m, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.0, 153.0, 147.9, 147.6 (d, *J* = 236.0 Hz), 144.5, 143.5, 142.8, 140.8 (d, *J* = 1.0 Hz), 132.9 (d, *J* = 10.0 Hz), 113.7 (d, *J* = 11.0 Hz), 107.8 (d, *J* = 29.0 Hz), 105.8 (d, *J* = 5.0 Hz), 74.9, 66.6, 57.3, 57.1, 53.2, 41.3, 30.1, 30.0, 28.3. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{23}FN_5O_4S$: 472.1449; found: 472.1430.

(3S,3aS)-7-(3-Thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-3-((isoxazol-3-ylamino)methyl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (20h). Compound **20h** (65 mg, 68.4%) was prepared from **16f** (0.11 g, 0.22 mmol) and *N*-Boc-3-aminoisoxazole (0.041 g, 0.22 mmol) in the same manner as described for **19n**. Pale pink solid. Mp. 180–182°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (s, 1 H), 7.70 (d, *J* = 14.0 Hz, 1 H), 6.45 (d, *J* = 8.0 Hz, 1 H), 5.89 (s, 1 H), 4.58 (brs, 1 H), 4.53–4.46 (m, 1 H), 4.42–4.31 (m, 2 H), 4.06–3.98 (m, 1 H), 3.87 (t, *J* = 10.0 Hz, 1 H), 3.81–3.65 (m, 2 H), 3.44–3.34 (m, 2 H), 2.22–2.02 (m, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.3, 158.6, 153.2, 147.7 (d, *J* = 236.0 Hz), 140.8, 132.2 (d, *J* = 10.0 Hz), 114.5 (d, *J* = 12.0 Hz), 107.9 (d, *J* = 29.0 Hz), 106.1 (d, *J* = 3.0 Hz), 96.4, 74.5, 66.8, 57.8, 57.5, 53.3, 46.0, 30.3,

30.1, 28.29, 28.25. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{22}FN_4O_4S$: 433.1340; found: 433.1321.

(3S,3aS)-3-((1H-1,2,3-Triazol-1-yl)methyl)-7-(3-thia-8-azabicyclo[3.2.1]octan-8-yl)-8-fluoro-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (20i). Compound **20i** (70 mg, 65.4%) was prepared from **16f** (135 mg, 0.26 mmol) in the same manner as described for **19o**. Off-white solid. Mp. 225–227°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (d, *J* = 0.8 Hz, 1 H), 7.78 (d, *J* = 1.2 Hz, 1 H), 7.58 (d, *J* = 14.0 Hz, 1 H), 6.39 (d, *J* = 8.0 Hz, 1 H), 4.86–4.81 (m, 2 H), 4.72–4.66 (m, 1 H), 4.44 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.39–4.30 (m, 2 H), 4.07–3.99 (m, 1 H), 3.86 (t, *J* = 10.4 Hz, 1 H), 3.37–3.26 (m, 2 H), 2.20–2.00 (m, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.3, 147.6 (d, *J* = 236.0 Hz), 140.8 (d, *J* = 2.0 Hz), 134.7, 133.1 (d, *J* = 10.0 Hz), 125.1, 113.2 (d, *J* = 11.0 Hz), 107.9 (d, *J* = 29.0 Hz), 105.8 (d, *J* = 5.0 Hz), 73.5, 66.3, 57.3, 57.1, 53.2, 51.4, 30.1, 30.0, 28.34, 28.30. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{21}FN_5O_3S$: 418.1344; found: 418.1324.

Synthesis of the target compound 21

((3R,3aS)-8-Fluoro-7-morpholino-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl 4-methylbenzenesulfonate (16g). Compound **16g** (1.06 g, 88.3%) was prepared from **15g** (0.8 g, 2.5 mmol) in the same manner as described for **16a**. Pale purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, *J* = 8.4 Hz, 2 H), 7.67 (d, *J* = 13.2 Hz, 1 H), 7.39 (d, *J* = 8.0 Hz, 2 H), 6.55 (d, *J* = 7.6 Hz, 1 H), 4.51–4.40 (m, 2 H), 4.37–4.22 (m, 2 H), 4.07–4.00 (m, 1 H), 3.94–3.72 (m, 5 H), 3.10–2.91 (m, 4 H), 2.47 (s, 3 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}FN_2O_7S$: 479.1283, found: 479.1277.

(3S,3aS)-3-(Aminomethyl)-8-fluoro-7-morpholino-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18g). Compound **18g** (418 mg, 61.6%) was prepared

from **16g** (1.0 g, 2.09 mmol) in the same manner as described for **18e**. Off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 13.2 Hz, 1 H), 6.53 (d, *J* = 7.6 Hz, 1 H), 4.46 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.30–4.22 (m, 1 H), 4.09–3.99 (m, 1 H), 3.95–3.75 (m, 5 H), 3.28–2.87 (m, 6 H), 1.28 (s, 2 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{15}H_{19}FN_3O_4$: 324.1354, found: 324.1345.

N-(((3S,3aS)-8-Fluoro-7-morpholino-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (21). Compound **21** (81 mg, 72.1%) was prepared from **18g** (100 mg, 0.31 mmol) in the same manner as described for **19a**. Off-white solid. Mp. 179–181ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.73 (d, *J* = 13.2 Hz, 1 H), 6.57 (d, *J* = 8.0 Hz, 1 H), 6.10 (brs, 1 H), 4.51 (dd, *J* = 10.0, 1.6 Hz, 1H), 4.44–4.36 (m, 1 H), 3.99–3.80 (m, 6 H), 3.79–3.61 (m, 2 H), 3.19–2.93 (m, 4 H), 2.05 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.9, 153.1, 150.1 (d, *J* = 239 Hz), 140.5 (d, *J* = 2.0 Hz), 137.0 (d, *J* = 10.0 Hz), 116.6 (d, *J* = 11.0 Hz), 107.14 (d, *J* = 28.0 Hz), 107.10 (d, *J* = 4.0 Hz), 75.2, 66.9, 66.4, 53.0, 51.0, 50.9, 41.3, 23.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{21}FN_3O_5$: 366.1460; found: 366.1457.

Synthesis of the target compound 22

((3R,3aS)-7-(4,4-Difluoropiperidin-1-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl-4-methylbenzenesulfonate (16h). Compound **16h** (1.07 g, 95.5%) was prepared from **15h** (0.4 g, 1.12 mmol) in the same manner as described for **16a**. Off-white foam solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, *J* = 8.0 Hz, 2 H), 7.68 (d, *J* = 12.8 Hz, 1 H), 7.39 (d, *J* = 8.0 Hz, 2 H), 6.61 (d, *J* = 7.6 Hz, 1 H), 4.51–4.39 (m, 2 H), 4.33 (dd, *J* = 10.8, 4.0 Hz, 1 H), 4.26 (dd, *J* = 10.8, 5.6 Hz, 1 H), 4.08–4.00 (m, 1 H), 3.82 (t, *J* = 10.4 Hz, 1 H), 3.23–3.08 (m, 4 H), 2.47 (s, 3 H), 2.25–2.08 (m, 4 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{24}F_3N_2O_6S$: 513.1302, found: 513.1300.

(3S,3aS)-3-(Aminomethyl)-7-(4,4-difluoropiperidin-1-yl)-8-fluoro-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18h). Compound **18h** (330 mg, 86.4%) was prepared from **16h** (1.07 g, 2.09 mmol) in the same manner as described for **18e**. Off-white foam solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 13.2 Hz, 1 H), 6.55 (d, *J* = 8.0 Hz, 1 H), 4.45 (dd, *J* = 10.8, 3.2 Hz, 1 H), 4.31–4.23 (m, 1 H), 4.10–4.00 (m, 1 H), 3.86 (t, *J* = 10.4 Hz, 1 H), 3.26–2.97 (m, 6 H), 2.22–2.05 (m, 4 H), 1.34 (brs, 2 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{19}F_3N_3O_3$: 358.1373, found: 358.1368.

N-(((3S,3aS)-7-(4,4-Difluoropiperidin-1-yl)-8-fluoro-1-oxo-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (22). Compound **22** (95 mg, 76.6%) was prepared from **18h** (112 mg, 0.31 mmol) in the same manner as described for **19a**. Pale yellow solid. Mp. 230–232°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 13.2 Hz, 1 H), 6.56 (d, *J* = 8.0 Hz, 1 H), 6.25 (t, *J* = 6.0 Hz, 1 H), 4.51 (dd, *J* = 10.0, 2.8 Hz, 1 H), 4.45–4.35 (m, 1 H), 3.97–3.88 (m, 1 H), 3.83 (t, *J* = 10.0 Hz, 1 H), 3.78–3.62 (m, 2 H), 3.20–3.04 (m, 4 H), 2.22–2.08 (m, 4 H), 2.05 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.0, 153.2, 150.1 (d, *J* = 239.0 Hz), 140.4 (d, *J* = 2.0 Hz), 136.4 (d, *J* = 11.0 Hz), 121.4 (t, *J* = 240.0 Hz), 116.9 (d, *J* = 10.0 Hz), 108.0 (d, *J* = 3.0 Hz), 107.1 (d, *J* = 28.0 Hz), 75.3, 66.4, 53.0, 47.9 (q, *J* = 6.0 Hz), 41.2, 34.1 (q, *J* = 23.0 Hz), 23.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{21}F_3N_3O_4$: 400.1479; found: 400.1458.

Synthesis of the target compounds 23a−d

((3R,3aS)-8-Fluoro-1-oxo-7-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl methanesulfonate (16i). To a solution of **15i** (0.4 g, 1.2 mmol) in DCM (10 mL) cooled to 0°C with ice-water bath was added *N*-methylmorpholine (0.26 mL, 2.4 mmol), and then methanesulfonyl chloride (0.11 mL, 1.4 mmol) was added. The reaction mixture was stirred for 5.5 h at room temperature, and concentrated to give a solid. The

residue was stirred with water and filtered to give **16i** (0.49 g, 99.4%) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.47 (d, J = 13.2 Hz, 1 H), 6.16 (d, J = 8.4 Hz, 1 H), 4.78–4.66 (m, 4 H), 4.64–4.49 (m, 3 H), 4.06–3.89 (m, 6 H), 3.27 (s, 3 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{17}H_{20}FN_2O_7S$: 415.0970, found: 415.0961.

(3S,3aS)-3-(Azidomethyl)-8-fluoro-7-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (18i). To a solution of compound **16i** (384 mg, 0.93 mmol) in DMF (10 mL) was added sodium azide (120 mg, 1.86 mmol). The reaction mixture was heated at 70ºC for 3 h and cooled to room temperature. Water (10 mL) was added to the mixture, the resulting solid was filtered and washed with water, dried to give **18i** (285 mg, 84.8%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.65 (d, J = 12.8 Hz, 1 H), 6.06 (d, J = 8.4 Hz, 1 H), 4.82 (s, 4 H), 4.42 (dd, J = 10.4, 3.2 Hz, 1 H), 4.37–4.29 (m, 1 H), 4.07 (d, J = 2.0 Hz, 4 H), 4.02–3.94 (m, 1 H), 3.82 (t, J = 10.4 Hz, 1 H), 3.78–3.63 (m, 2 H). HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{16}H_{17}FN_5O_4$: 362.1259, found: 362.1243.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)acetamide (23a). To a solution of compound **18i** (70 mg, 0.19 mmol) in tetrahydrofuran (5 mL) was added Pd/C (10%, 10 mg). The resulting mixture was stirred in hydrogen atmosphere for 2 h and then filtered. To the filtrate was added pyridine (0.031 mL, 0.38 mmol) at 0ºC and then acetic anhydride (0.029 mL, 0.3 mmol) was added in dropwise. The mixture was warmed to room temperature and stirred overnight. The mixture was diluted with DCM. The organic phase was washed sequentially with 0.5 N hydrochloric acid and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by column chromatography (DCM/methanol=98/2) to give compound **23a** (42 mg, 58.3%) as an off-white solid. Mp. 105–107ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.63 (d, J = 12.8 Hz, 1 H), 6.05 (d, J

= 8.0 Hz, 1 H), 5.96 (t, *J* = 6.0 Hz, 1 H), 4.82 (s, 4 H), 4.50–4.43 (m, 1 H), 4.39–4.33 (m, 1 H), 4.06 (d, *J* = 2.0 Hz, 4 H), 3.92–3.77 (m, 2 H), 3.76–3.61 (m, 2 H), 2.04 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.9, 153.2, 147.0 (d, *J* = 236 Hz), 140.8 (d, *J* = 2.0 Hz), 136.4 (d, *J* = 12.0 Hz), 113.5 (d, *J* = 10.0 Hz), 107.0 (d, *J* = 26.0 Hz), 102.3 (d, *J* = 4.0 Hz), 81.0, 75.0, 66.5, 62.9, 62.8, 53.0, 41.3, 39.72, 39.69, 23.1. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{21}FN_3O_5$: 378.1460; found: 378.1449.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)cyclopropanecarboxamide (23b). To a solution of compound **18i** (70 mg, 0.19 mmol) in tetrahydrofuran (5 mL) was added Pd/C (10%, 10 mg). The resulting mixture was stirred in hydrogen atmosphere for 3 h and then filtered. To the filtrate was added triethylamine (0.054 mL, 0.38 mmol) at 0°C and then cyclopropanecarbonyl chloride (0.025 mL, 0.27 mmol) was added in dropwise. The mixture was warmed to room temperature and stirred for 1 h. The solvent was evaporated and the residue was purified by column chromatography (DCM/methanol=99/1) to give compound **23b** (45 mg, 58.4%) as an off-white solid. Mp. 181–183°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.65 (d, *J* = 12.8 Hz, 1 H), 6.10 (t, *J* = 6.0 Hz, 1 H), 6.04 (d, *J* = 8.4 Hz, 1 H), 4.82 (s, 4 H), 4.45 (dd, *J* = 10.0, 2.8 Hz, 1 H), 4.40–4.34 (m, 1 H), 4.05 (d, *J* = 2.0 Hz, 4 H), 3.93–3.72 (m, 3 H), 3.72–3.63 (m, 1 H), 1.45–1.35 (m, 1 H), 1.02–0.95 (m, 2 H), 0.84–0.76 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 153.2, 147.0 (d, *J* = 236.0 Hz), 140.8 (d, *J* = 2.0 Hz), 136.4 (d, *J* = 13.0 Hz), 113.6 (d, *J* = 10.0 Hz), 107.0 (d, *J* = 26.0 Hz), 102.4 (d, *J* = 5.0 Hz), 81.0, 75.3, 66.6, 62.87, 62.86, 53.0, 41.4, 39.72, 39.70, 14.6, 7.8. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{23}FN_3O_5$: 404.1616; found: 404.1608.

N-(((3S,3aS)-8-Fluoro-1-oxo-7-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-3-yl)methyl)cyclobutanecarboxamide (23c). Compound **23c**

(44 mg, 55.7%) was prepared from **18i** (70 mg, 0.19 mmol) in the same manner as described for **23b**. Off-white solid. Mp. 118–120ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.60 (d, *J* = 12.8 Hz, 1 H), 6.03 (d, *J* = 8.4 Hz, 1 H), 5.82 (t, *J* = 6.0 Hz, 1 H), 4.81 (s, 4 H), 4.45 (dd, *J* = 10.0, 2.4 Hz, 1 H), 4.38–4.32 (m, 1 H), 4.05 (d, *J* = 2.0 Hz, 4 H), 3.91–3.68 (m, 3 H), 3.67–3.59 (m, 1 H), 3.08–2.95 (m, 1 H), 2.32–2.10 (m, 4 H), 2.05–1.78 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 175.9, 153.3, 147.0 (d, *J* = 235 Hz), 140.8 (d, *J* = 2.0 Hz), 136.4 (d, *J* = 13.0 Hz), 113.5 (d, *J* = 11.0 Hz), 107.1 (d, *J* = 25.0 Hz), 102.4 (d, *J* = 4.0 Hz), 81.0, 75.2, 66.5, 62.87, 62.85, 53.1, 41.3, 39.72, 39.70, 39.6, 25.33, 25.31, 18.2. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{21}H_{25}FN_3O_5$: 418.1773; found: 418.1762.

(3S,3aS)-3-((1H-1,2,3-Triazol-1-yl)methyl)-8-fluoro-7-(2-oxa-6-azaspiro[3.3]heptan-6-yl)-3a,4-dihydro-1H,3H-benzo[b]oxazolo[3,4-d][1,4]oxazin-1-one (23d). Compound **23d** (42 mg, 56.8%) was prepared from **18i** (70 mg, 0.19 mmol) in the same manner as described for **19o**. Off-white solid. Mp. 219–221ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (d, *J* = 0.8 Hz, 1 H), 7.78 (d, *J* = 0.8 Hz, 1 H), 7.53 (d, *J* = 12.8 Hz, 1 H), 6.03 (d, *J* = 8.4 Hz, 1 H), 4.85–4.80 (m, 6 H), 4.69–4.63 (m, 1 H), 4.42 (dd, *J* = 10.4, 3.2 Hz, 1 H), 4.05 (d, *J* = 2.0 Hz, 4 H), 4.04–3.97 (m, 1 H), 3.82 (t, *J* = 10.4 Hz, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.3, 147.0 (d, *J* = 235 Hz), 140.7 (d, *J* = 3.0 Hz), 136.6 (d, *J* = 2.0 Hz), 134.6, 125.0, 113.0 (d, *J* = 11.0 Hz), 107.2 (d, *J* = 26.0 Hz), 102.3 (d, *J* = 5.0 Hz), 81.0, 73.3, 66.3, 62.83, 62.81, 53.1, 51.4, 39.71, 39.68. HR-MS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{19}FN_5O_4$: 388.1416; found: 388.1405.

Minimum Inhibitory Concentration. MIC values of compounds against *M. tuberculosis* (H37Rv and DR-TB strains) were determined by the microplate alamar blue assay (MABA) according to the published protocol.[31]

Cytotoxicity. The potential cytotoxicity of the tested compounds on Vero cells or HepG2 cells was evaluated using MTT assay as previously reported.[31]

Mitochondrial protein synthesis inhibition. Mitochondrial protein synthesis was measured on Cardiac (H9C2) cell line that was obtained from National Platform of Experimental Cell Resources for Sci-Tech. The H9C2 cells were kept in DMEM (Gibico) with 10% FBS (Hyclone) and 1× Glutamine and NEAA at 37°C, 5% CO_2. The cell monolayer was washed with pre-warmed PBS and detached using trypsin. After centrifugation (1000 rpm for 5 min at rt), the cells were re-suspended with cell culture medium, and seeded at 1500 cells/well into a 384-well plate. After 18 h, the target compound**s** with the final concentrations 200 μM, 66.67 μM, 22.22 μM, 7.41 μM, 2.47 μM, 0.82 μM, 0.27 μM, 0.09 μM, 0.03 μM was added to the appropriate well, and then incubated at 37°C 5% CO_2 for 5 days. A negative control with 0.5% DMSO was for every compound. MPS inhibition was evaluated by MitoBiogenesis In-Cell ELISA. IC_{50} calculation was performed using Graph Pad Prim 5 software.

Caco-2 permeability assay. The Caco-2 cell line is derived from a human colon adenocarcinoma. The cells were seeded in Transwells (Millipore, 0.4 μm pore size) and formed a confluent monolayer after 21 days culture. On day 21, the target compound (50 μM) was added into the apical side of the membrane and the concentration of the compound across Caco-2 cell monolayer was quantified by UPLC after 1 hour incubation at 37 °C. Transepithelial electrical resistance (TEER) should be determined before as well as after transport experiments and should be over 500Ω×cm^2. The apparent permeability coefficient (Papp) for the compound is calculated from the following equation: Papp = $(dQ/dt)/(C_0 \times A)$, where dQ/dt is the rate of permeation of the drug across the cells, C_0 is the initial concentration and A is the area of the cell monolayer.

Liver microsome stability. The assay was performed with liver microsomes from male CD-1 mouse (Gibco) and pooled human (Xenotech). Compounds of interest were tested at 1 μM with a final concentration of microsomal protein of 1 mg/mL. The reaction was initiated by the addition of NADPH (1 mM), and samples were incubated for up to 60 min at 37 °C in a shaking incubator. The reaction was terminated at 0, 5, 15 and 30 min by the addition of ice-cold ACN/MeOH (50:50) spiked with internal standard. An aliquot of reaction mixture was removed at 0, 5, 15, 30, and 60 min, respectively, followed by addition of ice-cold ACN/MeOH (50:50) spiked with internal standard. Samples were centrifuged at 4000 rpm for 15 min and the supernatants were analyzed by LC-MS/MS. The assay evaluated the metabolic stability of compounds by measuring the amount of parent remaining to test compounds with or without NADPH cofactor.

Hepatocyte stability. The assay was performed with hepatocytes from male CD-1 mouse (BioreclamationIVT), male SD rat (BioreclamationIVT), male beagle dog (BioreclamationIVT), male cynomolgus monkey (RILD) and pooled human (BioreclamationIVT). Compounds of interest were tested at 1 μM with a final hepatocyte concentration of 1 million cells/mL. The reaction was initiated by addition of pre-warmed hepatocyte working solution (2 million cells/mL) to the compound working solution (2 μM). Reaction mixtures were incubated for up to 120 min at 37 °C in a CO_2 incubator. At the predetermined time points (0, 15, 30, 60, 90 and 120 min), 30 μL of the reaction mixtures was removed and reaction was terminated by addition of 200 mL of methanol/ACN (1:1, v/v) with 0.1% formic acid with internal standard. Samples were mixed well and then were centrifuged at 4000 rpm at 4 ^{o}C for 15 min. 100 μL supernatants were removed to a 96-well plate for LC-MS/MS analysis. The assay evaluated the metabolic stability of compounds in hepatocytes by measuring amount of parent remaining of the test compounds.

CYP inhibition. Compounds were incubated with human liver microsome (0.2 mg/mL) contained phosphate buffer for 20 min. The compound was tested at a range of concentrations (0.1-50 μM), alongside a relevant positive control. The positive controls, which are CYP isoform-specific (1A2, 2C9, 2C19, 2D6 and 3A4) substrates were also incubated with human liver microsomes at a range of the tested compound concentrations. At the end of the incubation, the amount of parent remaining to each substrate is monitored by LC-MS/MS at each of the tested compound concentrations. IC_{50} was calculated using Sigma-plot software.

Pharmacokinetic Studies. All animal protocols were approved by Institute Animal Care and Welfare Committee. The selected compound **19c** was subjected to pharmacokinetic studies in BALB/c mouse (female) weighing 20 to 25 g with five mice in the oral administration group and five mice in the intravenous injection group. The tested compound was given orally (p.o.) at dose of 25 mg/kg and intravenously (i.v.) at dose of 2.5 mg/kg. The tested compound was formulated with 0.5% carboxymethyl cellulose for p.o. administration and with 10% DMSO/physiological saline for i.v. administration. Blood samples were collected at 5, 15, 30 min, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72 h after oral dosing and 2, 5, 15, 30 min, 1, 2, 4, 6, 8, 12, 24, 36, 48 h after i.v. administration. Analyte quantitation was performed by a LC/MS/MS Quantum Access mass spectrometer (Applied Biosystems). Chromatographic separation performed on a Zobax SB-C18 column (50 mm×2.1 mm, 3.5 μm) with an isocratic mobile phase of methanol/water at 0.3 mL/min flow rate. Compound detection on the mass spectrometer was performed in electrospray positive ionization mode. The pharmacokinetic parameters were calculated using WinNonlin software version 6.3 based on non-compartmental analysis (Pharsight Corporation, Mountain View, USA). The oral bioavailability was calculated as the ratio between the area under the curve (AUC)

following intravenous administration corrected for dose ($F = (AUC_{p.o.} \times dose_{i.v.})/(AUC_{i.v.} \times dose_{p.o.}) \times 100\%$).

***In vivo* TB infection assay.** All animal protocols were approved by Institute Animal Care and Welfare Committee. SPF Balb/c mice (female) were used in this study. Each treated group was composed of 6 mice. Mice were infected via aerosol with a suspension of 5×10^6 CFU/mL *M. tuberculosis* (H37Rv) using a Glas-Col inhalation system, to deposit 50-100 bacilli into the lungs of each animal. The course of infection was followed by plating homogenates of harvested organs [n = 3] on 7H11 agar plates (7H11 plates containing 10% oleic acidalbumin-dextrose-catalase (OADC) enrichment and 50 mg/mL cycloheximide, 200 U/mL polymyxin B, 50 μg/mL carbenicillin, and 20 μg/mL trimethoprim) and determining CFU on days 3, 10, and 30 post infection. INH, linezolid, sutezolid and compound **19c** were suspended in 0.5% CMC and administered by oral gavage. The control group was received only 0.5% CMC. Mice were treated on day 10 post infection and 5 times per week. The treatment period was 3 weeks. Mice were sacrificed the day after the last day of treatment, lungs removed, homogenized, and serially diluted in 10-fold steps in HBSS. 100 μL were spread on 7H11 agar in duplicate. The plates were incubated at 37 ºC for 3 weeks. Data are expressed as the log10 (and as log10 reduction) provided by a given dose of the compound against the growth of the organism in the untreated control group. Mean log10 values were calculated from bacterial burden counts. Student's *t*-test was used to compare means between the test and control groups. A P value of ≤ 0.05 was considered significant.

Mice bone marrow micronucleus assay. An animal care and use application for the study was approved by Pharmaron Institutional Animal Care and Use Committee (IACUC). Compounds **19c** and **19r** were subjected to the assay in CD-1 (ICR) mouse (male, n = 42) weighing 28.3 to 33.3 g. The mice were randomly divided into 7 groups (solvent control, positive control, **19c** or **19r** at

dose levels of 100, 200, 500, 1000 and 2000 mg/kg) with 3 mice in per group. Animals were fed ad libitum with rodent diet and water were provided ad libitum via water bottles. Animals were dosed 2 consecutive days via oral gavage with the vehicle control and the test article. The second dose occurred 24 hours (± 30 minutes) after the first dose. At 18-24 hours post the last dose, animals were sacrificed for bone marrow harvest. The bone marrow from each animal were examined microscopically to determine micronucleus frequency and the proportion of PCE to total erythrocytes. Three concentrations of compounds **19c** and **19r** were analyzed for micronucleus formation, i.e., 500, 1000 and 2000 mg/kg. The top dose level analyzed was the limit dose recommended by ICH S2R1. The results indicated that compounds **19c** and **19r** did not induce statistically significant increases in bone marrow micronucleus formation at dose levels up to 2000 mg/kg.

Chromosome aberration test. Chinese hamster ovary (CHO) cells, clone WBL, were cultured in a humidified atmosphere of 5% CO_2 in air at 37°C in McCoy's 5A medium, supplemented with 10% fetal bovine serum, 2 mM GlutaMAX^{TM}, 100 units/mL penicillin and 100 µg/mL streptomycin. Cells were seeded 20-24 hours before treatment at 0.4×10^6/5 mL/25 cm^2 flask. Cultures treated with the metabolic activation system were re-fed with serum-free S-9 mix just before treatment. Compounds were dissolved in DMSO, added to cultures, and the cultures were incubated at 37°C. Treatments were for either 3 or 20 h, and aberrations were scored in cells harvested 20 hours from the beginning of treatment. At the termination of the 3-hour treatments, the cultures were washed twice with Hanks Balanced Salt Solution (HBSS) and re-fed with fresh complete medium. Cultures were incubated for a further 17 hours. Colcemid (0.1 µg/mL) was added 2 hours before monolayers were harvested by trypsinization. A cell sample was counted by Coulter counter to determine cell number as an indicator of cytotoxicity. The viable cells were

verified by checking samples for trypan blue dye exclusion. The cells were treated with hypotonic KCl (75 mM) for 3 minutes at room temperature, washed twice with fixative (methanol:glacial acetic acid, 3:1, v/v), dropped onto slides, air-dried, and stained with Giemsa stain. 300 metaphase cells from each concentration (150 per duplicate flask) were examined and scored for chromosome aberrations. Compound **19c** was tested for the chromosome analysis at the concentrations of 20, 60 and 150 μg/mL in group A and B, and 20, 60 and 135 μg/mL in group C, respectively. Compound **19r** was tested at the concentrations of 90, 180 and 397.1 μg/mL for the chromosome analysis. Statistical analysis of chromosome aberration data was performed using SAS 9.2 software.

Mini-Ames assay. Two strains of Salmonella typhimurium bacteria (TA98, TA100) were used in the study and were originally obtained from Molecular Toxicology (Boone, NC), USA. DMSO was used as the solvent/vehicle control and the highest concentration tested was 1000 μg/well, which was equal to the OECD limit concentration of 5000 μg/plate in standard Ames assay. Bacteria, test compound or vehicle/positive control formulation, and 10% S9 mixture or PBS buffer were added to molten agar at 45°C, mixed rapidly, and poured onto 6-well plate containing minimal agar media. After the agar was solidified, the plates were inverted and incubated at 37°C for 48-72 hours. Revertant colonies were counted manually and the background lawn was inspected for signs of cytotoxicity. The results were considered to be positive for mutagenic potential if the increase in mean revertants at the peak of the dose response was equal to or greater than 2-fold the mean solvent/vehicle control value and the increase should be dose related. The results indicated that compound **19c** and **19r** were not cytotoxic to the tester strains and was not mutagenic at concentrations up to 1000 μg/well.

Inhibition evaluation on hERG K^+ channel. HEK 293 cells were stably transfected with human Ether-à-go-go related gene (hERG) channel. The voltage-gated hERG potassium channel current was recorded at room temperature (25°C) from randomly selected transfected cells under whole-cell manual patch clamp systems equipped with EPC10 USB (HEKA) or Multiclamp 700B amplifier (Molecular Devices), while electrical data was digitalized by Digidata1440A with sampling frequency at 10 kHz using Patchmaster or pClamp10 respectively. hERG current inhibition in presence of 5 concentrations, including 30, 10, 3.0, 1.0 and 0.3 μM, was tested for IC_{50} determination. Dofetilide was also included as a positive control to ensure the accuracy and sensitivity of the test system. All experiments were performed in duplicate for IC_{50} determination. The compound with $IC_{50} > 30$ μM was generally considered to have a lower potential for hERG K^+ channel inhibition.

HEK-293 Cell-line/hCav1.2 assay. HEK-293 cells stably expressing the human Cav1.2 L-type Ca channel (α1c, α2/δ, and β2a subunits) along with the inward rectifier potassium channel Kir2.3 were grown in media contained 90%DMEM culture media supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin -streptomycin, 40 μg/mL zeocin, 100 μg/mL hygromycin B, and 100 μg/mL G418 (geneticin). Before the experiment, the cells were inoculated to 1.2×10^4 cells per well and incubated overnight in a 384 well cell culture plate, each well containing 10 μL of DMEM medium supplemented with 1% FBS and 100 U/mL penicillin-streptomycin. To each well of the cell plate, 10 μL of 2×fluorochrome containing 5 mM probenecid, 4 μM fluo-4 AM, and 1 mM brilliant black were added. Then, the cells were incubated at 37°C for 60 min in a 5% CO_2 atmosphere. Duplicate per dose were measured with test compounds at the concentration of 1.52, 4.57, 13.72, 41.15, 123.46, 370.37, 1111.11, 3333.33, 10000.00, 30000.00 nM on FLIPR^{Tetra}. The

fluorescence was read at an excitation of 470-495 nm and an emission of 515-575 nm. Calcium channel inhibition rate was calculated by the following equation:

Calcium channel inhibition rate (%) = (1-(U-Cn)/(Cp-Cn)) ×100%

U: fluorescence signal intensity of test compound; Cp: fluorescence signal intensity of positive control (EC80 of calcium channel activator KCl); Cn: fluorescence signal intensity of solvent control.

IC_{50} was performed using XLfit software.

CHO Cell-line/Nav1.5 assay. CHO cells expressing Nav1.5 channel were maintained in 90%F12K supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin -streptomycin, 20 mM HEPES, and 700 μg/mL G418 (geneticin) in a humidified incubator with an atmosphere of 5% CO_2 at 37°C. For electrophysiological recordings, the cells were superfused by extracellular saline containing: 132 mM NaCl, 4 mM KCl, 3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 11.1 mM Glucose, 10 mM HEPES (pH 7.35). Compounds for testing were dissolved in DMSO at the concentrations of 30 mM, 10 mM, 3 mM, 1 mM and 0.3 mM and then diluted in extracellular saline to 30 μM, 10 μM, 3.0 μM, 1.0 μM and 0.3 μM. The glass electrodes for whole-cell patch-clamp recording were filled with intracellular saline containing: 140 mM KCl, 2 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES and 5 mM MgATP (pH 7.35). Cells were clamped to a holding potential of -80 mV and then depolarized to -10 mV using a 20-ms long voltage followed by a repolarization to -80 mV. Current induced intervals were 15 s. The sampling frequency was 50 kHz, and filter frequency was 10 kHz. The instantaneous peak current under depolarization was indicated as Nav1.5 channel current. The inhibition rate was calculated by the following equation:

The percentage of inhibition (%) = (1-Peak current $_{compound}$/Peak current $_{blank\ control}$) × 100%

IC_{50} was performed using Graphpad Prism 6.0 software.

MAO inhibition. Stock solution of the test article was prepared in DMSO at 50 mM concentration and stored frozen at -20°C. Serial dilutions of the stock solution were prepared in acetonitrile/DMSO (8:2) for testing. The test article was incubated at seven increasing concentrations (0.1 μM-100 μM) in duplicate with human, recombinant MAO-A or MAO-B (50 μg/mL) in 100 mM potassium phosphate (pH 7.4) containing 5 mM magnesium chloride and a probe substrate, kynuramine (50 μM for MAO-A and 25 μM for MAO-B). Human Monoamine Oxidase A and Human Monoamine Oxidase B were purchased from Corning (catalog numbers 456283 and 456284, respectively).

The control MAO inhibitor (tranylcypromine) was screened alongside the test article as a positive control for each isoform. After 20 min incubation, the reactions were terminated by addition of methanol containing propranolol as an analytical internal standard for quantification. The quenched samples were incubated at 4°C for 10 minutes and then centrifuged at 4°C for 10 minutes. The supernatant was removed and the formed probe metabolite (4-hydroxyquinoline) was analyzed by LC-MS/MS. A decrease in the formation of the probe metabolite compared to vehicle control was used to calculate an IC_{50} value.

Repeated dose toxicity study. All animal protocols were approved by Institute Animal Care and Welfare Committee. 6-7 weeks SD rats were used in this study. 96 rats were divided into four dose groups: vehicle control group, low, medium and high dose group. Each group was composed of 24 rats (♀12 and ♂12). Compound **19c** was administered orally once daily at doses of 50, 150, and 450 mg/kg/day for 4 weeks, followed by a 2 weeks recovery period.

After the last administration, 8 male and 8 female animals were dissected in each group. After 14 days of recovery period, the remained 4 male and 4 female animals were dissected in each group. The clinical symptoms of the animals were observed before administration, after

administration, and in the afternoon during the experiment. Neurological symptoms were inspected at the day before the end of administration and the day before the end of recovery period. Weight of rats were weighed once a week and the dosage volume was adjusted according to the latest rat weight. The food consumption was weighed once a week. Ophthalmic examination was conducted during quarantine domestication, the end of drug administration and the end of recovery period within 7 days; Urine examination was conducted within 7 days before the end of administration and recovery period. Blood samples were collected at the end of administration for hematological and serum biochemical examination, weighing of organ, bone marrow smear, and pathological examination of tissue samples.

Bartlett test was used to test for variance homogeneity. If the result showed no significance ($P \geq 0.05$), one-way analysis of variance (ANOVA) was used. If ANOVA showed significance ($P < 0.05$), Dunnett's test (parameter method) was used for multiple comparisons to identify statistically significant differences between the control group and each test article group.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

NMR spectra of all compounds; chiral HPLC of compounds **9a**, **9c-d**, **14a**, **14c-d**, **19a** and **19c-d** (PDF)

X-ray crystal structure data of compound **19c** (CIF)

Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

TB, tuberculosis; MDR-TB, multidrug-resistant TB; XDR-TB, extensively drug resistant TB; MPS, mitochondrial protein synthesis; MAO, monoamine oxidase; PK, pharmacokinetic; TrCl, trityl chloride; DMAP, 4-dimethylaminopyridine; TBAI, tetrabutylammonium iodide; *m*-CPBA, 3-chloroperoxybenzoic acid; CbzCl, benzyl chloroformate; DCM, dichloromethane; EtOAc, ethyl

actate; DMF, *N*,*N*-dimethylforamide; EDCI, 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; CDI, 1,1'-carbonyldiimidazole; THF, tetrahydrofuran; TBHP, *tert*-butyl hydroperoxide; *D*-(-)-DET, *D*-(-)-diethyl tartrate; *L*-(+)-DET, *L*-(-)-diethyl tartrate; NMM, N-methylmorpholine; Boc, *tert*-butoxycarbonyl; NADPH, nicotinamide adenine dinucleotide phosphate; CYP450, cytochrome P450; Mini-Ames, bacterial reverse mutation.

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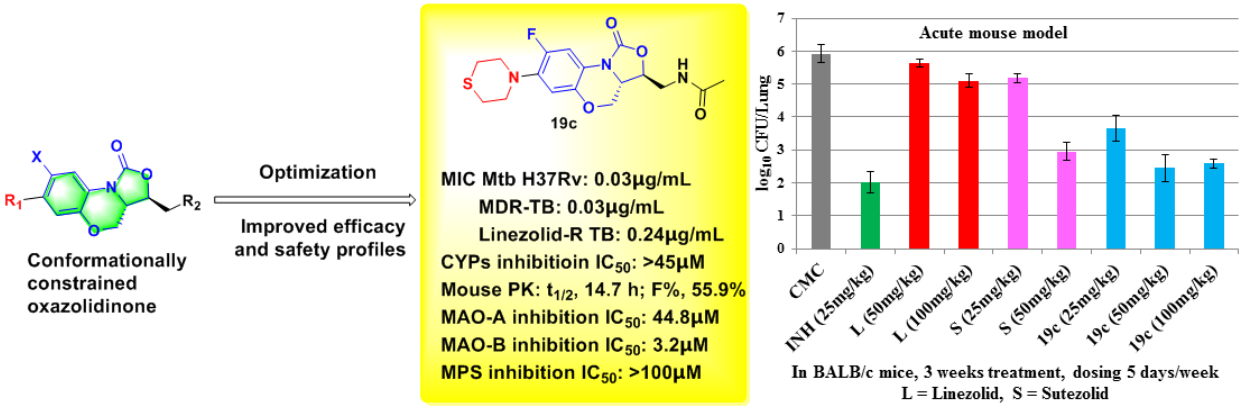

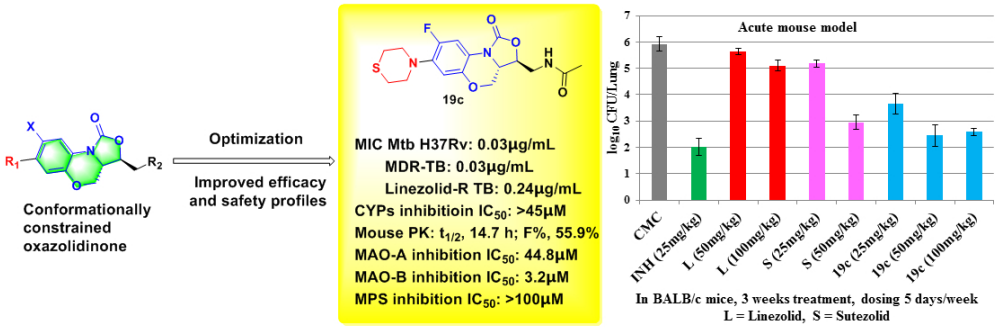

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