Journal of Medicinal Chemistry

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Identification of Novel Tricyclic Benzo[1,3]oxazinyloxazolidinones as Potent Antibacterial Agents with Excellent Pharmacokinetic Profiles against Drug-Resistant Pathogens

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Cite This: *J. Med. Chem.* 2021, 64, 3234−3248 Read Online

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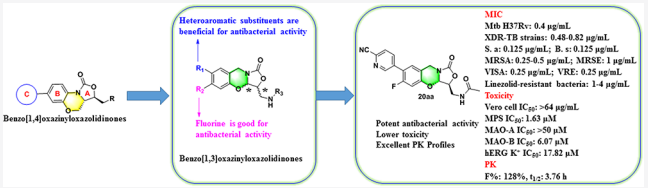
ABSTRACT: A series of conformationally constrained novel benzo[1,3]oxazinyloxazolidinones were designed, synthesized, and evaluated on their activities against *Mycobacterium tuberculosis*, Gram-positive bacteria, and Gram-negative bacteria. The studies identified a new compound **20aa** that displayed good to excellent antibacterial and antitubercular profiles against drug-resistant TB strains (MIC = 0.48−0.82 μg/mL), MRSA (MIC = 0.25−0.5 μg/mL), MRSE (MIC = 1 μg/mL), VISA (MIC = 0.25 μg/mL), and VRE (MIC = 0.25 μg/mL) and some linezolid-resistant strains (MIC 1−2 μg/mL). Compound **20aa** was demonstrated as a promising candidate through ADME/T evaluation including microsomal stability, cytotoxicity, and inhibition of hERG and monoamine oxidase. Notably, **20aa** showed excellent mouse PK profile with high plasma exposure ($AUC_{0-\infty}$ = 78 669 h·ng/mL), high peak plasma concentration (C_{max} = 10 253 ng/mL), appropriate half-life of 3.76 h, and superior oral bioavailability (128%). The present study not only successfully provides a novel benzo[1,3]oxazinyloxazolidinone scaffold with superior druggability but also lays a good foundation for new antibacterial drug development.



INTRODUCTION

Spanning decades, the use and misuse of antibiotics have increased the occurrence of drug resistant bacterial strains. This occurrence and prevalence of multidrug resistance strains pose one of the greatest risks to human health[1] and will inevitably increase in the future. In 2017, the World Health Organization released a list of 12 bacteria or bacterial families that pose the greatest threat to human health and for which new antibiotics are desperately needed. The list included a range of bacteria. Among them a quarter of the bacteria were Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and penicillin-resistant *Streptococcus pneumoniae* (PRSP). *Mycobacterium tuberculosis* (*M. tuberculosis*) was not added to the list due to the fact that it is a well-established priority for new drug discovery.[2] In 2019 alone, there were estimated 0.5 million new cases that displayed resistance to rifampicin, the most effective first-line drug, of which 78% were multidrug-resistant TB.[3] There is therefore a clear and urgent need to develop antibacterial agents bearing novel pharmacophores or new modes of action to combat these multidrug resistant bacteria.

Oxazolidinones were developed as a new class of synthetic antibacterial agents and exhibited their antibacterial activity through a unique mode of action. The compounds bind to 23S rRNA of the bacterial 50S ribosomal subunit and inhibit the bacterial protein synthesis at the initiation step.[4] As shown in Figure 1, linezolid (**1**)[5] was the first approved (2000) oxazolidinone antibacterial drug for the treatment of Gram-positive bacterial infections and has been off-label used in the treatment of complicated MDR-TB and XDR-TB. As one component in the Nix-TB regimen, consisting of bedaquiline, pretomanid, and linezolid, for the treatment of XDR-TB or treatment-intolerant/nonresponsive MDR-TB,[6] linezolid plays a key role in the efficacy of the treatment. As such, much effort has been devoted to improving the activity and ADME/T properties of linezolid. Extensive structural modifications along with the structure−activity relationship (SAR) studies were focused on the C-ring and C5 side chain (Figure 1) of the parent scaffold.[7−12] Tedizolid phosphate (**2**) was the second oxazolidinone drug approved by FDA for the treatment of MRSA skin infections in 2014.[13] Contezolid (**3**), developed by MicuRx Pharmaceuticals, phase III clinical trials were recently finished and the New Drug Application was submitted to regulators.[14] Furthermore, three oxazolidinones have been evaluated on their clinical effects with varying success such as

Received: December 14, 2020
Published: March 11, 2021

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
J. Med. Chem. 2021, 64, 3234−3248

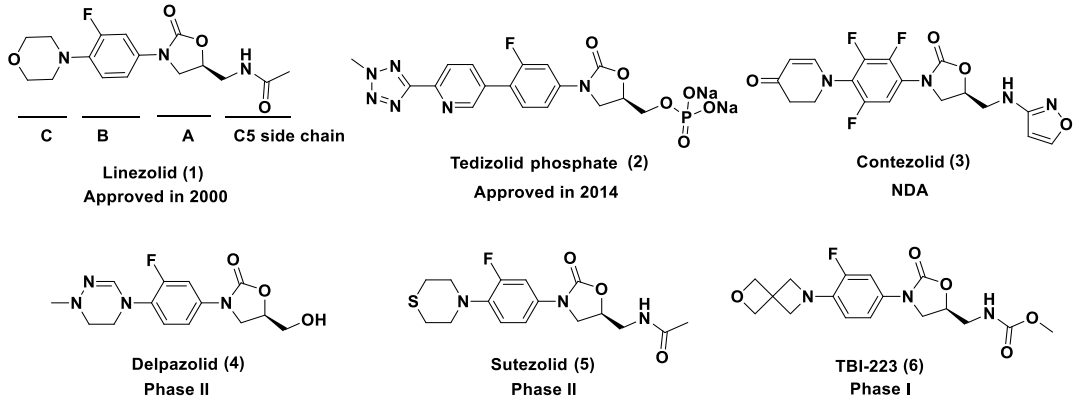


Figure 1. Representative structure modifications of linezolid focused on the C-ring and C5 side chain.

delpazolid (**4**) (for Gram-positive bacteria and TB),[15,16] sutezolid (**5**) (for TB),[17,18] and TBI-223 (**6**) (for TB).[19] Oxazolidinones can therefore be described as the privileged scaffold in antibacterial drug discovery, and further diversification of this important pharmacophore shows the potential to lead to the discovery of new antibacterial agents against both Gram-positive bacteria and *M. tuberculosis*.

Recently, the conformationally unique benzoxazinyloxazolidinone tricyclic scaffold has undergone intensive investigation. Yang and co-workers reported a series of benzo[1,4]oxazinyloxazolidinones[20,21] as antibacterial agents (Figure 2). The SAR showed that the compound with aryl

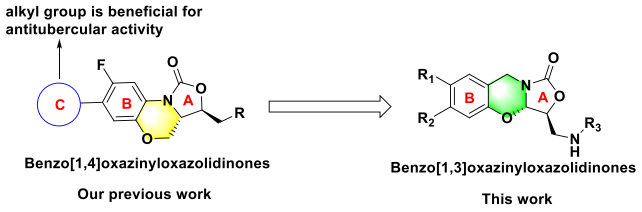


Figure 2. Design of novel conformational constraint oxazolidinones.

group as the C ring was more active against MRSA and MRSE than that with the corresponding alkyl group, while the derivatives with fluorine substitution on the B ring generated reduced activity. Interestingly, our previous work identified that the promising candidates exhibiting highly potent antitubercular activity contained an aliphatic heterocyclic group as the C ring of this interesting motif. The SAR expressed that F substitution on the B ring was beneficial for improving the antitubercular activity and significantly reducing overall cytotoxicity.[22,23] Although there are some differences in the SAR described between Yang's work and our reported work, this conformationally constrained tricyclic scaffold exhibited good to excellent antibacterial activities, with notably superior PK properties. These prominent characteristics displayed by this aforementioned benzo[1,4]oxazinyloxazolidinones led us to conclude that the exploration of chemical space between the phenyl (B ring) and the oxazolidinone (A ring) motif would pay dividends in relation to the discovery of potentially novel antibacterial agents bearing novel scaffolds.

Herein, we disclose our design and synthesis of novel oxazolidinones containing unique benzo[*e*]oxazolo[4,3-*b*]-[1,3]oxazin-1-one scaffold (Figure 2), and their subsequent evaluation for antibacterial and antitubercular properties. We aim to explore the effect of aromatic or nonaromatic groups and F substitution on the B ring, as well as the key methylene linkage between the B ring (phenyl ring) and A ring (oxazolidinone ring) on the antibacterial activity, cytotoxicity, and PK properties of this new type of molecule. Interestingly, the newly designed benzo[1,3]oxazinyloxazolidinone scaffold displayed differential SAR compared to that established by the benzo[1,4]oxazinyloxazolidinones and the traditional phenyloxazolidinones such as linezolid. In particular, this novel scaffold exhibited good to excellent activity against both Gram-positive bacteria and *M. tuberculosis*, particularly against drug-resistant bacteria including MRSA, MRSE, VISA, VRE, and TB strains. The present study demonstrated systematically that compound **20aa** exhibited not only highly potent antitubercular and antibacterial activities including moderate activity against linezolid-resistant isolated clinical strains but also superior PK profiles with oral bioavailability up to 128% in mice.

CHEMISTRY

The synthetic route of the critical tricyclic fused benzo[1,3]-oxazinyloxazolidinone intermediates (±)-**18a** and (±)-**18b** is depicted in Scheme 1. Compound **8a** was prepared from 4-fluoro-2-hydroxybenzoic acid (**7a**) according to the literature.[24] After esterification, the amination of **8a** gave the desired amide **9a** in 83% yield. The amide group of **9a** was subsequently reduced with BH_3 and followed by the protection with benzyl chloroformate (CbzCl) to afford compound **10a** at room temperature. The key oxazolidinone fragment 5-(hydroxymethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**13**) was synthesized from 4-methoxybenzylamine (**11**) and 2-(chloromethyl)oxirane (**12**) in the presence of K_2CO_3 as base.[25] The key intermediate **14** was produced from **13** via a Swern oxidation.[26] This was followed by a tandem condensation/cyclization strategy to construct the 1,3-oxazinane ring from **10a** and **14**.[27] As compound **14** has a chiral center, the cyclization will produce four distinct stereoisomers: two racemic diastereomers, (±)-**15a** (*trans*) and (±)-**15b** (*cis*), which were isolated by silica gel column chromatography directly. After cleavage of the 4-methoxybenzyl (PMB) group, the nitrogen atom in the intermediates (±)-**16a** and (±)-**16b** was Boc-protected to give (±)-**17a** and (±)-**17b**, respectively.

The benzo[*e*]oxazolo[4,3-*b*][1,3]oxazin-1-one scaffold was constructed by an ingenious tandem reaction. The oxazolidi-

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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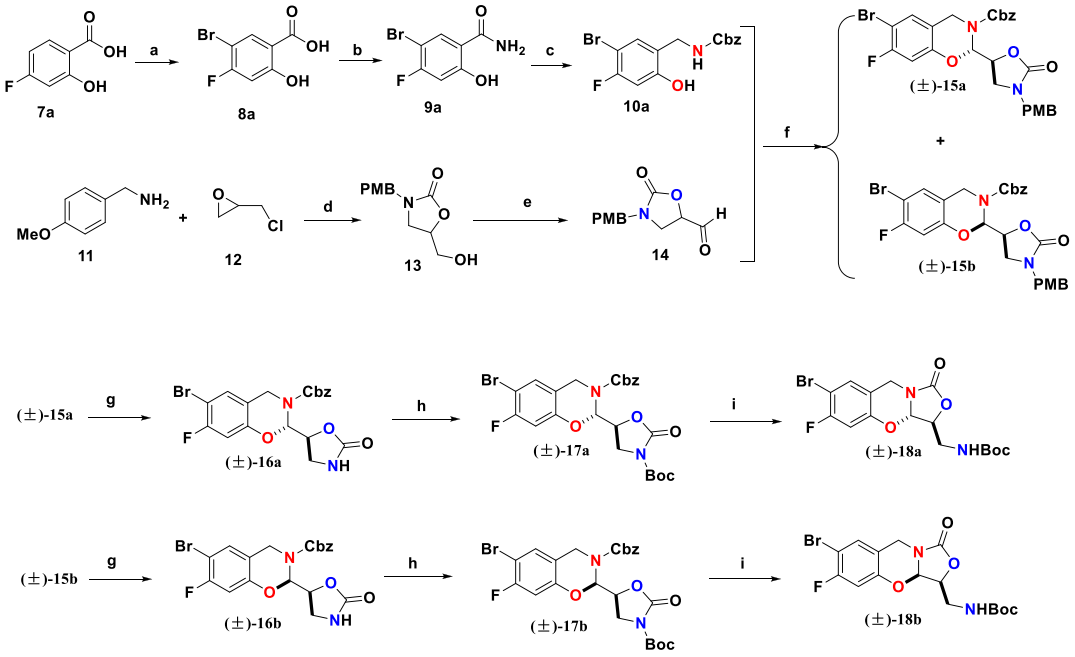
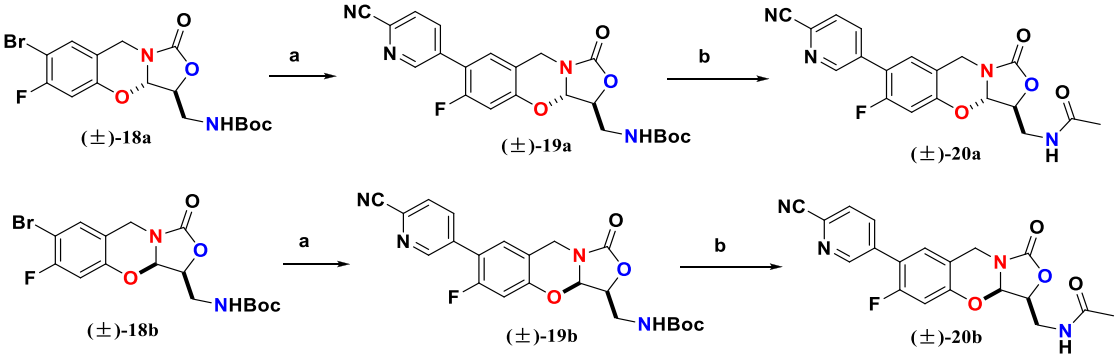
Scheme 1. Synthesis of Benzo[1,3]oxazinyloxazolidinone Intermediates (±)-18a and (±)-18b[a]



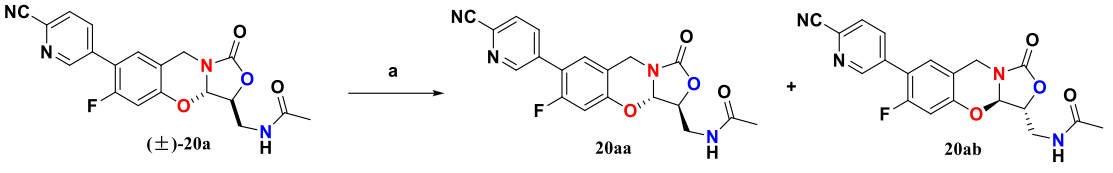
[a]Reagents and conditions: (a) NBS, conc H_2SO_4, MeCN, rt, 1 h, 84%; (b) (i) conc H_2SO_4, MeOH, reflux, 32 h; (ii) NH_4OH, MeOH, 55 °C, 6 h, 83% of two steps; (c) (i) BH_3, THF, reflux, 5 h; (ii) CbzCl, $NaHCO_3$, THF/H_2O = 5:1, rt, 3 h, 72% of two steps; (d) K_2CO_3, TEA, MeOH, reflux, 12 h, 60%; (e) $(COCl)_2$, DMSO, TEA, THF, −78 °C to rt, 90%; (f) TsOH·H_2O, toluene, reflux, 6 h, 49% for **(±)-15a**, 25% for **(±)-15b**; (g) ceric ammonium nitrate (CAN), MeCN/H_2O = 9:1, rt, 2 h, 78% for **(±)-16a**, 76% for **(±)-16b**; (h) $(Boc)_2O$, TEA, DMAP, DCM, rt, 2 h, 78% for **(±)-17a**, 82% for **(±)-17b**; (i) Cs_2CO_3, MeOH, rt, 3h, 89% for **(±)-18a**, 91% for **(±)-18b**.

Scheme 2. Synthesis of Compounds (±)-20a and (±)-20b[a]



[a]Reagents and conditions: (a) 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinonitrile, $Pd(PPh_3)_4$, Na_2CO_3, DMSO/H_2O = 6:1, 100 °C, 5 h, 84% for **(±)-19a**, 81% for **(±)-19b**; (b) (i) TFA, DCM, rt, 2 h; (ii) acetyl chloride, TEA, DCM, 0 °C, 2 h, 87% for **(±)-20a**, 80% for **(±)-20b**.

Scheme 3. Separation of Compound (±)-20a[a]



[a]Reagents and conditions: (a) CHIRALPAK IA column, MeOH/EtOAc = 80:20, 1.0 mL/min, λ = 254 nm, 25 °C.

none ring of **(±)-17a** and **(±)-17b** was opened in the presence of Cs_2CO_3 to afford the required oxazolidinone ring immediately and gave the key intermediates **(±)-18a** and **(±)-18b** with the novel tricyclic scaffold, respectively.

The coupling of **(±)-18a** or **(±)-18b** with boronic acid ester afforded compounds **(±)-19a** or **(±)-19b**. Finally, **(±)-20a** and **(±)-20b** were obtained via cleavage of the protective carbamate group, followed by acetylation with acetyl chloride (Scheme 2). Compound **(±)-20a** was separated as

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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the pure enantiomers **20aa** and **20ab** with chiral HPLC (Scheme 3). The absolute configurations of the above prepared compounds **20aa** and **20ab** were determined by the comparison of the experimental ECD spectrum and the corresponding calculated ECD spectra of compounds **20aa** and **20ab** (Figure 3). The ECD spectrum recorded for **20aa**

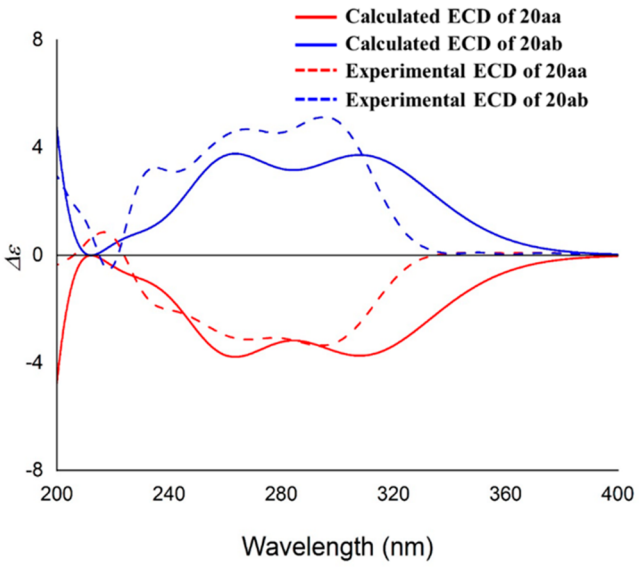


Figure 3. ECD spectra of compounds **20aa** and **20ab**.

matches the calculated ECD curve of **20aa** but is opposite to that of **20ab**. Therefore, compounds **20aa** and **20ab** were deduced to have the (3*S*,3a*S*) and (3*R*,3a*R*) absolute configuration, respectively.

The synthetic route of the fluoride-free compound (±)-**24** is outlined in Scheme 4. Compound (±)-**24** was prepared from 5-bromo-2-hydroxybenzamide (**9b**) in a similar procedure as shown in Scheme 1. The synthesis of the chiral pure target compounds **20aa**, **30**−**38** are outlined in Scheme 5. First, the pure enantiomers **18a** and **24** were obtained by chiral HPLC separation and then reacted with arylboronate ester or morpholine to afford intermediates **25**−**29** followed by subsequent deprotection of the *N*-Boc group to deliver amine compounds **30**−**34**. The desired products, **20aa** and **35**−**38**, were obtained from compounds **30**−**34** via the acetylation of the NH_2 group with acetyl chloride. The absolute configuration of the synthetic target compound was determined by chiral HPLC compared with the optically pure **20aa** obtained by chiral chromatography separation (Figure S4).

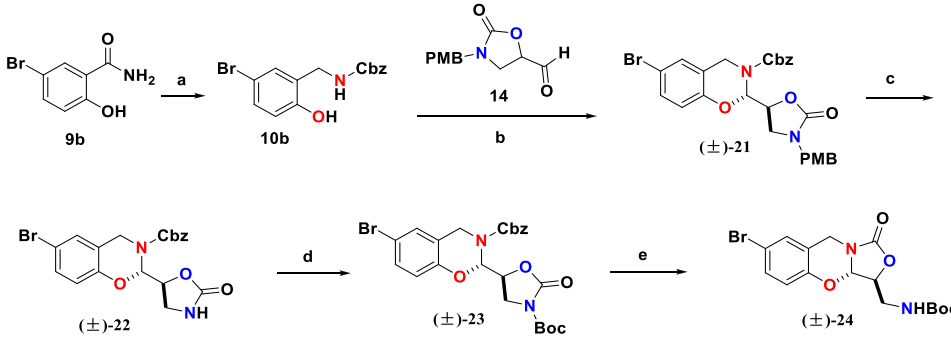
Our next goal was to produce compounds (±)-**45**−**48**, with the fluorine on the phenyl ring at the *para* position relative to the oxygen of benzo[*e*]oxazolo[4,3-*b*][1,3]oxazin-1-one scaffold. As illustrated in Scheme 6, 4-bromo-5-fluoro-2-hydroxybenzamide (**9c**) was used as the starting material and the target compounds (±)-**45**−**48** were prepared in a similar procedure as shown in Scheme 1. Compound **9c** was synthesized from 4-bromo-5-fluoro-2-hydroxybenzoic acid via esterification, amination nucleophilic substitution, and deprotection sequentially (Supporting Information, Scheme S1).

RESULTS AND DISCUSSION

As mentioned, the benzo[1,3]oxazinyloxazolidinone scaffold was first designed to elucidate its antibacterial activity, with the aim to characterize the intrinsic ability of this novel tricyclic fused oxazolidinone as an antibacterial agent. Due to the *N*,*O*-acetal fragment within the benzo[1,3]oxazinyloxazolidinone scaffold, we initially explored its microsome stability, as this would determine the subsequent success of this scaffold. We subjected compound **49** (Supporting Information, Scheme S2) with the key pharmacophore in the scaffold to two stability tests. As shown in Table 1, compound **49** exhibited excellent stability not only in mouse liver microsomes but also in human liver microsomes compared to the known compound benzo[1,4]oxazinyloxazolidinone **50**.

Supported by the positive results of the above microsomal stability assay, the target compounds with the benzo[*e*]oxazolo[4,3-*b*][1,3]oxazin-1-one scaffold were synthesized and screened for their *in vitro* activities against Mtb H37Rv to confirm our molecular design strategy (Table 2). Compound *trans* (±)-**20a** displayed good MIC (1.58 μg/mL) against Mtb H37Rv, while compound *cis* (±)-**20b** showed low activity. These results clearly indicated that the *trans* configuration was optimal for anti-TB activity. Subsequently, compound *trans* (±)-**20a** was separated into optical isomers **20aa** and **20ab** through chiral resolution. To our delight, compound **20aa** (3*S*,3a*S*) showed more potent *in vitro* anti-TB activity than linezolid. By contrast, compound **20ab** (3*R*,3a*R*) was found to be virtually inactive (MIC > 32 μg/mL). The results disclosed

Scheme 4. Synthesis of Compound (±)-24[a]



[a]Reagents and conditions: (a) (i) BH_3, THF, reflux, 5 h; (ii) CbzCl, $NaHCO_3$, THF/H_2O = 5:1, rt, 3 h, 72% of two steps; (b) $TsOH{\cdot}H_2O$, toluene, reflux, 6 h, 50%; (c) CAN, MeCN/H_2O = 9:1, rt, 2 h, 76%; (d) $(Boc)_2O$, TEA, DMAP, DCM, rt, 2 h, 90%; (e) Cs_2CO_3, MeOH, rt, 3 h, 84%.

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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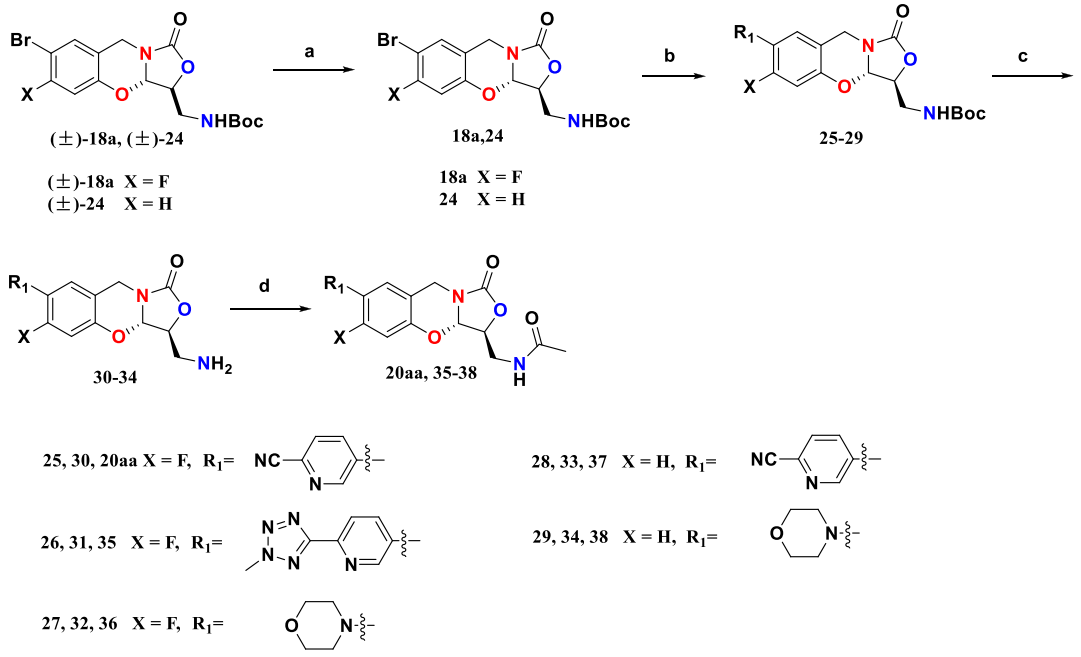
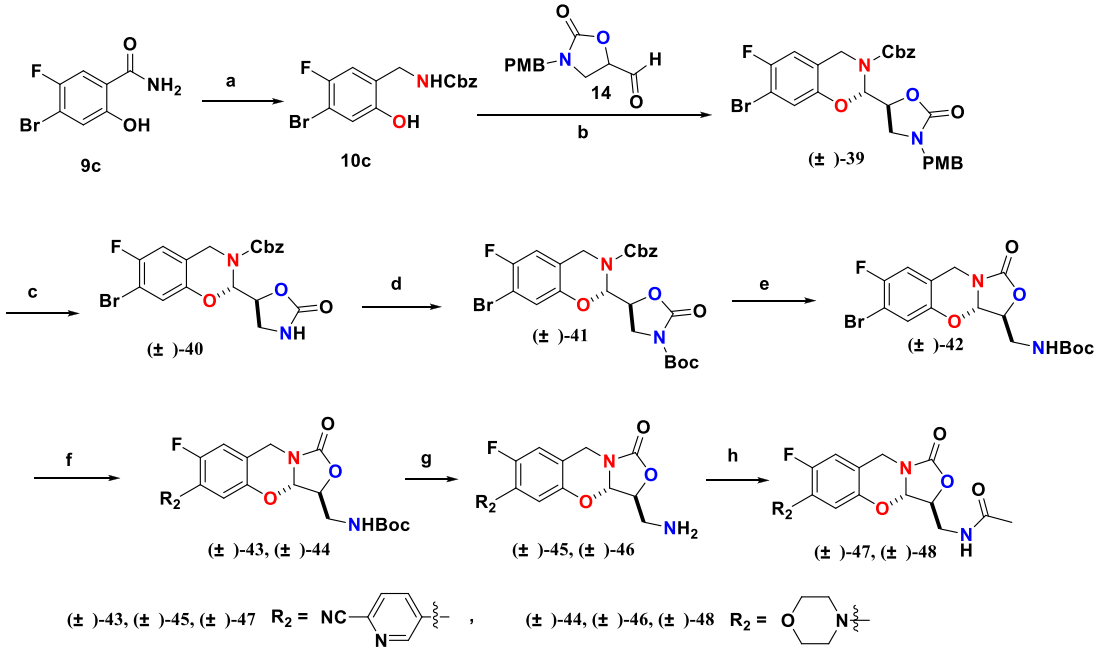
Scheme 5. Synthesis of Target Compounds 20aa and 30−38[a]



[a]Reagents and conditions: (a) CHIRALPAK AD-H column, MeOH = 100%, 1.0 mL/min, λ = 214 nm, 35 °C, for **18a**; CHIRALPAK IA column, hexane/EtOH = 50:50, 1.0 mL/min, λ = 254 nm, 25 °C, for **24**; (b) (i) arylboronic acid ester, $Pd(PPh_3)_4$, Na_2CO_3, DMSO/H_2O = 6:1, 100 °C, 5 h, 57−89%, for **25**, **26**, and **28**; (ii) morpholine, X-PHOS, $Pd(OAc)_2$, Cs_2CO_3, toluene, reflux, 100 °C, 6 h, 44−66%, for **27** and **29**; (c) TFA, DCM, rt, 3 h, 63−94%; (d) acetyl chloride, TEA, DCM, 0 °C, 2 h, 78−99%.

Scheme 6. Synthesis of Target Compounds (±)-45−48[a]



[a]Reagents and conditions: (a) (i) BH_3, THF, reflux, 5 h; (ii) CbzCl, $NaHCO_3$, THF/H_2O = 5:1, rt, 3 h, 71% of two steps; (b) TsOH·H_2O, toluene, reflux, 6 h, 51%; (c) CAN, MeCN/H_2O = 9:1, rt, 2 h, 67%; (d) $(Boc)_2O$, TEA, DMAP, DCM, rt, 2 h, 74%; (e) Cs_2CO_3, MeOH, rt, 3h, 82%; (f) (i) 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinonitrile, $Pd(PPh_3)_4$, Na_2CO_3, DMSO/H_2O = 6:1, 100 °C, 5 h, 84%, for (±)-**43**; (ii) morpholine, X-PHOS, $Pd(OAc)_2$, Cs_2CO_3, toluene, reflux, 100 °C, 6 h, 38%, for (±)-**44**; (g) TFA, DCM, rt, 3 h, 78−86%; (h) acetyl chloride, TEA, DCM, 0 °C, 2 h, 76−86%.

that the absolute configuration (3*S*,3a*S*) for this novel tricyclic fused oxazolidinone was essential for potency in accordance with that previously observed for the benzo[1,4]oxazinyl-oxazolidinone scaffold.[23] Compounds **20aa** and **20ab** were also evaluated for their antibacterial activities against Gram-positive bacteria *S. aureus* ATCC 29213. As anticipated, compound

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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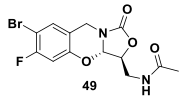
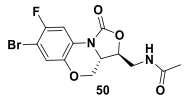
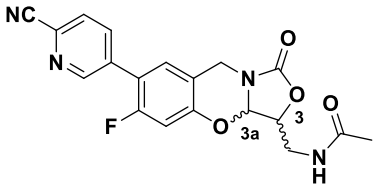
Table 1. Microsome Stability of Compounds 49 and 50

Compd[e]	MLM[a]		HLM[b]	
	Substrate remaining (%)[c]	Stability[d]	Substrate remaining (%)[c]	Stability[d]
 49	105	102	111	93.8
 50	73.4	125	71.4	110

[a]Mouse liver microsome. [b]Human liver microsome. [c]Substrate concentrations were determined in incubations with NADPH after 30 min and normalized to concentrations at time zero. [d]Stability was determined without the NADPH cofactor. [e]Compound **50** was prepared according to the literature.[23,28]

Table 2. Antibacterial Activities of Different Isomers of 20a

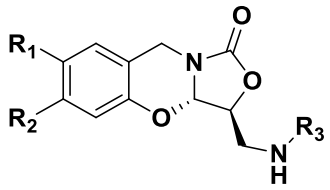


compd	configuration (3,3a)	chiral purity	MIC (μg/mL), Mtb H37Rv	MIC (μg/mL), *S. aureus* 29213
(±)-**20a**	*trans*	racemic	1.58	
(±)-**20b**	*cis*	racemic	15.51	
20aa	(3*S*,3a*S*)	99.97%	0.40	0.125
20ab	(3*R*,3a*R*)	99.94%	>32	>64
linezolid			0.88	0.125

20aa displayed potent antibacterial activity (MIC = 0.125 μg/mL), while its enantiomer, **20ab**, showed no activity (MIC > 64 μg/mL). This result further demonstrated that the 3*S*,3a*S*-configuration is essential for maintaining potency.

After confirming **20aa**, the 3*S*, 3a*S*-isomer, as the most potent compound, we conducted extensive SAR studies on the phenyl ring and C5 side chain. The target compounds were evaluated for their activities against a series of bacteria including Mtb, Gram-positive and Gram-negative strains (Table 3). Keeping fluoro substitution at R_2, compounds with R_1 as aryl group showed more potent *in vitro* anti-TB activity (**20aa**, **30**, **31**, and **35**), whether R_3 was H or an acetyl group. In contrast, when R_1 was morpholinyl, compounds **32** and **36** had no activity. For Gram-positive bacteria, compounds **20aa** and **35** containing an aryl group at R_1 displayed equal or more potent activity (MIC = 0.125 μg/mL) compared to the reference linezolid (MIC = 0.125−0.25 μg/mL). Compound **36** bearing a morpholine motif showed moderate antibacterial activity but lower than compound **20aa**. Fluorine on the phenyl ring has been illustrated to have a beneficial effect on the antibacterial activity for linezolid analogues;[5] thus we assessed the activities of the fluoride-free compounds (**33**, **37** and **34**, **38**). As illustrated in Table 3, compounds **33** and **37** containing a pyridine group displayed significantly reduced anti-TB activity, while compounds **34** and **38** bearing a morpholine group totally lost anti-TB activity. Although compound **37** showed good activity against Gram-positive pathogens (*S. aureus* and *B. subtilis*), its activity was lower

Table 3. Antibacterial Activities of Target Compounds



Compd	R_1	R_2	R_3	MIC (μg/mL)			
				Mtb H37Rv	S. a[a]	B. s[b]	E. coli[c]
30		F	H	0.26	-	-	-
20aa		F	Ac	0.48	0.125	0.125	>64
31		F	H	0.47	32	>64	>64
35		F	Ac	0.54	0.125	0.125	>64
32		F	H	>32	>64	>64	>64
36		F	Ac	31.70	2	8	>64
33		H	H	2.94	>64	>64	>64
37		H	Ac	12.23	0.5	1	>64
34		H	H	>32	-	-	-
38		H	Ac	>32	-	-	-
(±)-45	F		H	>32	>64	>64	>64
(±)-47	F		Ac	>32	>64	>64	>64
(±)-46	F		H	>32	-	-	-
(±)-48	F		Ac	>32	-	-	-
linezolid				0.88	0.125	0.25	>64
vancomycin					1	0.25	64
ampicillin					0.25	0.25	2

[a]*Staphylococcus aureus* 29213. [b]*Bacillus subtilis* 168. [c]*Escherichia coli* 25922.

compared to **20aa**. These results implied that fluorine on the phenyl ring of this novel tricyclic fused oxazolidinone was not only essential for the anti-TB activity but also critical for its antibacterial activity. The effect of fluorination on anti-TB activity was consistent with benzo[1,4]oxazinyl-oxazolidinones;[22,23] however, for antibacterial activity, the effect was contrary to that previously reported.[20] Subsequently, we investigated the effect on the activity by switching the F and pyridine (or morpholine) positions. Obviously, compounds (±)-**45**−**48** with F at the R_1 position resulted in a complete loss of antitubercular activity (MIC > 32 μg/mL); (±)-**45** and (±)-**47** even with a pyridine group at R_2 displayed no antibacterial activity (MIC > 64 μg/mL), indicating that the positions of the substituents on the B ring also have an

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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Table 4. Activities against Drug-Resistant Bacteria of Compounds 20aa and 35

	MIC (μg/mL)					
compd	Mtb 13946[a]	Mtb 14862[b]	MRSA[c]	MRSE[d]	VISA[e]	VRE[f]
20aa	0.48	0.82	0.25−0.5	1	0.25	0.25
35	0.39	0.85	0.5−4	1	0.5	0.25
INH	2.38	>10				
RIF	>10	>10				
ampicillin			>32	>32		
vancomycin			0.5−1	<2	>32	>32

[a]Resistance to isoniazid (INH), streptomycin (SM), rifampicin (RFP), ethambutol (EMB), rifabutin (RBT), *p*-aminosalicylate (PAS), and ofloxacin (OLFX). [b]Resistance to INH, SM, RFP, EMB, PAS, prothionamide (1321), and capreomycin (CPM). [c]Methicillin resistant *Staphylococcus aureus*, four strains. [d]Methicillin resistant *Staphylococcus epidermidis*. [e]Vancomycin intermediate *Staphylococcus aureus*. [f]Vancomycin resistant *Enterococcus*.

important effect on activity. Unsurprisingly, all these compounds were inactive against Gram-negative bacteria *E. coli* similar to the reference linezolid.

Prompted by two promising compounds **20aa** and **35** which displayed significant potency against Mtb H37Rv and Gram-positive bacteria, we subsequently concentrated on evaluating these two compounds for their activities against drug-resistant TB strains and drug-resistant Gram-positive strains (Table 4). We were pleased to find that compounds **20aa** and **35** displayed equivalent potency against two clinically isolated strains 13946 and 14862 (extensively drug-resistant TB) compared with the drug-sensitive strain. Meanwhile, as displayed in Table 4, **20aa** and **35** exhibited excellent antibacterial activity against all tested Gram-positive drug-resistant bacteria, with MIC values of 0.25−0.5 μg/mL (**20aa**) and 0.5−4 μg/mL (**35**) against MRSA, 1 μg/mL (**20aa** and **35**) against MRSE, 0.25 μg/mL (**20aa**) and 0.5 μg/mL (**35**) against VISA, and 0.25 μg/mL (**20aa** and **35**) against VRE. Furthermore, we investigated the activities of compounds **20aa** and **35** against linezolid-resistant bacteria (Table 5). Although **20aa** (MIC = 3.63 μg/mL) and **35** (MIC = 2.32 μg/mL) exhibited some activity against linezolid-resistant TB strain, for drug-sensitive strain Mtb H37Rv, these two compounds displayed reduced activity as about 3- to 7-fold increase in MIC values (**20aa** 0.48 μg/mL and **35** 0.54 μg/mL). In addition, we evaluated the activities of **20aa** and **35** against linezolid-resistant *Enterococcus* and *Staphylococcus* strains. The results showed that **20aa** and **35** exhibited superior activities against linezolid-resistant *Enterococcus* (MIC = 1−2 μg/mL) and linezolid-resistant *Staphylococcus* (MIC = 2−4 μg/mL). However, the antibacterial activity of **20aa** and **35** against linezolid-resistant Gram-positive bacteria was substantially inferior compared to that against the standard strains. The results were similar to antituberculosis activities of compounds **20aa** and **35** against linezolid-resistant TB strain, indicating that this novel benzo[1,3]oxazinyloxazolidinone scaffold might have a similar mode of action as linezolid.

Table 5. Activities Against Linezolid-Resistant Bacteria of Compounds 20aa and 35

	MIC (μg/mL)		
	linezolid	**20aa**	**35**
Mtb	14.54	3.63	2.32
E. f[a]	2	0.5	0.5
E. f[b]	4−8	1−2	1−2
E. f[c]	2	1	1
E. f[d]	8	2	2
S. c[e]	16 to >16	2−4	2−4

[a]*E. faecalis* ATCC 29212. [b]Linezolid-resistant *E. faecalis*, six strains. [c]*E. faecium* ATCC 49624. [d]Linezolid-resistant *E. faecium* 13549. [e]Linezolid-resistant *S. capitis*, five strains.

As we desired to assess the druggability of the most potent compounds, their safety profile should be conducted as the first pass assessment. Compound **20aa** was selected for evaluation on *in vitro* toxicity, due to its highly potent antibacterial activity, particularly against MRSA. As shown in Table 6, compound **20aa** showed no cytotoxicity against Vero cell lines (IC_{50} > 64 μg/mL). Its low hERG K^+ channel inhibition precluded its low QT prolongation risk. Compound **20aa** showed comparable mitochondrial protein synthesis (MPS) inhibition compared to linezolid (IC_{50} = 9.28 μM).[29] This suggests that compound **20aa** has a similar safety profile as linezolid in terms of myelosuppression risk. Monoamine oxidase (MAO) inhibition is one of the adverse effects of oxazolidinone during long-term dosing; therefore, the MAO-A and MAO-B inhibition profiles of compound **20aa** were evaluated to assess the potential risk of toxicity. Compound **20aa** showed no activity against MAO-A and moderate activity against MAO-B, which implies that compound **20aa** could have an improved safety property. Bearing good safety profile, compound **20aa** was subsequently tested for its metabolic

Table 6. Representative Properties of Compound 20aa

	MLM[a]		HLM[b]			IC_{50} (μM)			
compd	substrate remaining (%)[c]	stability[d]	substrate remaining (%)[c]	stability[d]	Vero cytotoxicity, IC_{50} (μg/mL)	hERG K^+ inhibition	MPS[e] inhibition	MAO-A[f]	MAO-B[f]
20aa	95.5	108	118	106	>64	17.82	1.63	>50	6.07

[a]Mouse liver microsome. [b]Human liver microsome. [c]Substrate concentrations were determined in incubations with NADPH after 30 min and normalized to concentrations at time zero. [d]Stability was determined without the NADPH cofactor. [e]Mitochondrial protein synthesis. [f]Positive control, leflunomide (IC_{50}): 9.40 μM for MAO-A, 5.58 μM for MAO-B.

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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stability. It was noted that this compound showed excellent stability against mouse and human liver microsomes. This further demonstrated that this novel tricyclic fused benzo[1,3]oxazinyloxazolidinone scaffold may have an acceptable PK profile for further development.

The aforementioned results encouraged us to further investigate the pharmacokinetic properties of compound **20aa** in BALB/c mice by following a single oral administration (dose, 10 mg/kg) and an intravenous injection (dose, 1 mg/kg). As illustrated in Table 7, compound **20aa** exhibited

Table 7. Mouse PK Properties of Compound 20aa

parameter	unit	iv (1 mg/kg)	po (10 mg/kg)
$t_{1/2}$[a]	h	4.08	3.76
T_{max}	h		1.33
C_0/C_{max}	ng/mL	1791	10253
$AUC_{(0-t)}$[b]	h·ng/mL	6080	77649
$AUC_{(0-\infty)}$	h·ng/mL	6162	78669
$MRT_{(0-\infty)}$[c]	h	4.71	5.86
V[d]	L/kg	0.762	
CL[e]	(mL/min)/kg	2.71	
F[f]	%		128

[a]Plasma elimination half-life. [b]Plasma exposure. [c]Mean residence time. [d]Apparent volume of distribution. [e]Clearance rate. [f]Oral bioavailability.

excellent PK profiles with high plasma exposure ($AUC_{0-\infty}$ = 78 669 h·ng/mL), high maximal plasma concentration (C_{max} = 10 253 ng/mL), appropriate half-life ($t_{1/2}$ = 3.76 h), and excellent oral bioavailability (128%). Furthermore, compound **20aa** was rapidly absorbed after oral administration, with 8290 ng/mL plasma concentration after 15 min, and remained at 1730 ng/mL after 12 h (Figure 4, Supporting Information

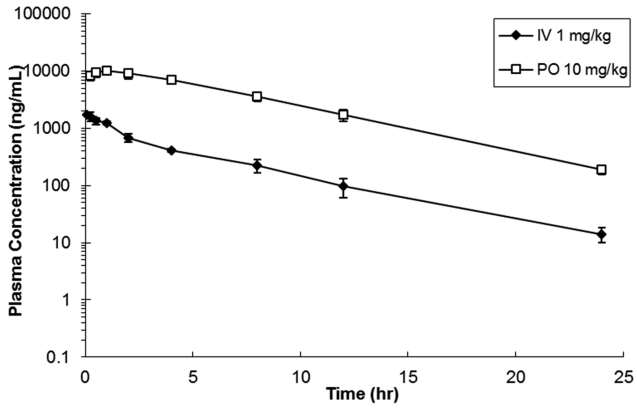

Figure 4. Mean blood concentration−time profiles of compound **20aa** in mouse after oral administration (10 mg/kg) and intravenous injection (1 mg/kg) of compound **20aa** (n = 3). The data are presented as the means ± SD (Table S1).

Table S1). The high plasma concentration and quick absorption indicated that compound **20aa** could be an excellent antibacterial agent in a clinical setting.

CONCLUSIONS

In summary, we have developed a series of novel oxazolidinones containing the benzo[*e*]oxazolo[4,3-*b*][1,3]-oxazin-1-one scaffold and assessed them as highly potent antibacterial agents against both Gram-positive strains and *M. tuberculosis*. It was found that a (3S,3aS) absolute configuration for the novel tricyclic fused oxazolidinone is required for potency. The systematic SAR evaluation exhibited that these new oxazolidinones differ from the previously described benzo[1,4]oxazinyloxazolidinones. Fluorine on the phenyl ring was not only essential for anti-TB activity but also significant for antibacterial activity. Compounds with heteroaromatic substituents at the para-position relative to the oxygen of benzo[1,3]oxazinyl framework exhibited relatively high potency against *M. tuberculosis* and Gram-positive strains; however, the compounds bearing a morpholine group lost antituberculosis activity and displayed moderate activity against *S. aureus* and *B. subtilis*. Two compounds, **20aa** and **35**, displayed good to excellent *in vitro* antibacterial activity including against drug-resistant TB strains, MRSA, MRSE, VISA, and VRE, as well as certain activities to some linezolid-resistant strains. The most superior compound **20aa** exhibited good druggability exemplified by potent antibacterial activity, excellent microsomal stability, no cytotoxicity against Vero cells, low hERG K^+ channel inhibition, comparable MPS inhibition, and acceptable inhibitory activity of MAO. Moreover, compound **20aa** exhibited an excellent mouse PK profile with high plasma exposure, high maximal plasma concentration, appropriate half-life, and excellent oral bioavailability after oral administration. Thus, we believe that **20aa** is a promising lead compound for further investigation and subsequent development. This novel conformationally constrained benzo[1,3]oxazinyloxazolidinone scaffold could provide new insight into the identification of new generation oxazolidinone for the treatment of serious infection caused by drug-resistant bacteria, which is still the unmet medical needs worldwide.

EXPERIMENTAL SECTION

Chemistry. General. All solvents and reagents were obtained commercially and used without further purification unless otherwise stated. ^{1}H NMR or ^{13}C NMR spectra were recorded on Varian 400 or 500 MHz spectrometer using $CDCl_3$, DMSO-d_6, or acetone-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) were referenced to the residual solvent peak and reported in ppm, and all coupling constantd (J) were given in Hz. The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, and (brs) broad. High-resolution mass spectra were recorded using a Thermo Exactive Orbitrap plus mass spectrometer (ESI). TLC was performed on silica gel plates (GF254) with visualization of components by UV light (254 nm). Silica gel 300−400 mesh was used for all flash column chromatography experiments. Melting points were determined on Yanaco MP-J3 microscope melting point apparatus. The purity of all final compounds (≥95%) was established by high-performance liquid chromatography (HPLC), which was carried out on a Thermo Fisher Accela HPLC system with an Agilent Zorbax SB-C18 column (5 μm, 2.1 mm × 50 mm), a column temperature of 40 °C, detection wavelength at 254 nm, flow rate = 0.3 mL/min, and a gradient of 5−95% MeCN in water (both containing 0.1 vol% of HCOOH) in 10 min.

Synthetic Procedure for Compounds (±)-20a and (±)-20b. *Benzyl 6-Bromo-7-fluoro-2-(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)-2H-benzo[e][1,3]oxazine-3(4H)-carboxylate ((±)-15a) and ((±)-15b).* A mixture of compound **14** (9.2 g, 39.1 mmol), **10a** (5.5 g, 15.5 mmol), and *p*-toluenesulfonic acid monohydrate (0.3 g, 1.6 mmol) in toluene (80 mL) was refluxed under continuous removal of water using Dean−Stark apparatus for 6 h. After cooling down to room temperature, the mixture was washed with water and dried over sodium sulfate. The products of (±)-**15a** and (±)-**15b** were obtained by chromatography eluting with petroleum/ethyl acetate. (±)-**15a**

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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(4.4 g, yield: 49%), white solid. Mp: 150−152 °C. LC−MS (ESI) m/z $[M + H]^+$: 571.0879 and 573.0859. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.52 (d, J = 7.6 Hz, 1 H), 7.45−7.34 (m, 5 H), 7.21 (d, J = 8.0 Hz, 2 H), 6.89 (d, J = 8.4 Hz, 2 H), 6.78 (d, J = 9.6 Hz, 1 H), 6.05 (d, J = 8.4 Hz, 1 H), 5.23 (d, J = 12.4 Hz, 1 H), 5.19 (d, J = 12.4 Hz, 1 H), 5.07 (d, J = 17.6 Hz, 1 H), 4.86 (td, J = 5.2, 8.4 Hz, 1 H), 4.46 (d, J = 17.2 Hz, 1 H), 4.38 (d, J = 14.4 Hz, 1 H), 4.32 (d, J = 14.8 Hz, 1 H), 3.78 (s, 3 H), 3.62 (t, J = 9.2 Hz, 1 H), 3.49 (dd, J = 5.2, 9.2 Hz, 1 H). **(±)-15b** (2.2 g, yield: 24.7%), colorless oil. LC−MS (ESI) m/z $[M + H]^+$: 571.0879 and 573.0859. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.47 (d, J = 7.6 Hz, 1 H), 7.41−7.34 (m, 5 H), 7.23 (d, J = 8.4 Hz, 2 H), 6.93 (d, J = 8.4 Hz, 2 H), 6.80 (d, J = 9.6 Hz, 1 H), 6.08 (d, J = 6.8 Hz, 1 H), 5.22 (d, J = 12.4 Hz, 1 H), 5.17 (d, J = 12.4 Hz, 1 H), 5.05 (d, J = 17.2 Hz, 1 H), 4.95−4.89 (m, 1 H), 4.45 (d, J = 17.2 Hz, 1 H), 4.33 (d, J = 14.8 Hz, 1 H), 4.28 (d, J = 15.2 Hz, 1 H), 3.79 (s, 3 H), 3.66 (t, J = 8.8 Hz, 1 H), 3.36 (dd, J = 6.0, 9.2 Hz, 1 H).

Benzyl 6-Bromo-7-fluoro-2-(-2-oxooxazolidin-5-yl)-2H-benzo[e]-[1,3]oxazine-3(4H)-carboxylate ((±)-16a). To a mixed solvent ($MeCN/H_2O$ = 85.0 mL:9.5 mL) were added **(±)-15a** (3.4 g, 5.9 mmol) and CAN (12.9 g, 23.6 mmol). The mixture was stirred for 2 h at room temperature and then poured into water (200 mL). The resulting solution was extracted with DCM (100 mL × 3). The combined organic layer was washed with water, brine and dried over sodium sulfate. The crude product was purified by chromatography (DCM/MeOH = 100:1) to afford **(±)-16a** as a white solid (2.1 g, 78%). Mp: 85−87 °C. LC−MS (ESI) m/z $[M + H]^+$: 451.0318 and 453.0296. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.55 (d, J = 7.6 Hz, 1 H), 7.45−7.42 (m, 2 H), 7.39−7.33 (m, 3 H), 6.83 (d, J = 10.0 Hz, 1 H), 6.69 (s, 1 H), 6.18 (d, J = 8.4 Hz, 1H), 5.25 (d, J = 12.4 Hz, 1 H), 5.21 (d, J = 12.8 Hz, 1 H), 5.10 (d, J = 17.2 Hz, 1 H), 4.95 (td, J = 5.6, 8.8 Hz, 1 H), 4.49 (d, J = 17.2 Hz, 1 H), 3.77 (td, J = 0.8, 9.2 Hz, 1 H), 3.70−3.66 (m, 1 H).

Benzyl 6-Bromo-7-fluoro-2-(-2-oxooxazolidin-5-yl)-2H-benzo[e]-[1,3]oxazine-3(4H)-carboxylate ((±)-16b). Compound **(±)-16b** (1.6 g, 76%) was prepared from **(±)-15b** (2.5 g, 4.3 mmol) according to the procedure described for **(±)-16a**. Mp: 80−82 °C. LC−MS (ESI) m/z $[M + H]^+$: 451.0280 and 453.0260. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.49 (d, J = 7.6 Hz, 1 H), 7.45−7.34 (m, 5H), 6.88 (d, J = 10.0 Hz, 1 H), 6.61 (s, 1 H), 6.12 (d, J = 7.2 Hz, 1 H), 5.24 (s, 2 H), 5.09 (d, J = 17.2 Hz, 1 H), 5.04−4.98 (m, 1 H), 4.48 (d, J = 17.2 Hz, 1 H), 3.80 (t, J = 9.2 Hz, 1 H), 3.47 (dd, J = 6.4, 9.2 Hz,1 H).

Benzyl 6-Bromo-2-(-3-(tert-butoxycarbonyl)-2-oxooxazolidin-5-yl)-7-fluoro-2H-benzo[e][1,3]oxazine-3(4H)-carboxylate ((±)-17a). A mixture of **(±)-16a** (2.2 g, 4.8 mmol), TEA (1.0 mL, 7.3 mmol), $(Boc)_2O$ (1.2 g, 5.7 mmol), DMAP (49.0 mg, 0.1 mmol) in DCM (20.0 mL) was stirred at room temperature for 2 h. The solution was washed with water and brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by chromatography (PE/DCM = 6:4) to give **(±)-17a** as a white solid (2.1 g, 78%). LC−MS (ESI) m/z $[M + Na]^+$: 573.0637 and 575.0617. Mp: 161−163 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.56 (d, J = 7.6 Hz, 1 H), 7.44−7.41 (m, 2 H), 7.40−7.32 (m, 3 H), 6.88 (d, J = 9.6 Hz, 1 H), 6.28 (d, J = 8.8 Hz, 1 H), 5.25 (d, J = 12.4 Hz, 1 H), 5.21 (d, J = 12.8 Hz, 1 H), 5.11 (d, J = 17.2 Hz, 1 H), 4.98−4.92 (m, 1 H), 4.50 (d, J = 16.8 Hz, 1 H), 4.16−4.08 (m, 2 H), 1.50 (s, 9 H).

Benzyl 6-Bromo-2-(-3-(tert-butoxycarbonyl)-2-oxooxazolidin-5-yl)-7-fluoro-2H-benzo[e][1,3]oxazine-3(4H)-carboxylate ((±)-17b). In a similar manner to **(±)-17a**, compound **(±)-17b** (1.4 g, 82%) was prepared from **(±)-16b** (1.4 g, 3.1 mmol). Mp: 92−94 °C. LC−MS (ESI) m/z $[M + Na]^+$: 573.0620 and 575.0604. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.51 (d, J = 7.6 Hz, 1 H), 7.45−7.43 (m, 2 H), 7.40−7.34 (m, 3 H), 6.88(d, J = 10.0 Hz, 1 H), 6.24 (d, J = 7.2 Hz, 1 H), 5.25 (s, 2 H), 5.10 (d, J = 16.8 Hz, 1 H), 5.04−4.98 (m, 1 H), 4.52 (d, J = 17.2 Hz, 1 H), 4.19 (dd, J = 8.8, 10.4 Hz, 1 H), 3.86 (dd, J = 6.4, 10.4 Hz, 1 H), 1.49 (s, 9 H).

tert-Butyl ((7-Bromo-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo-[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate ((±)-18a). To a solution of MeOH (20 mL) containing **(±)-17a** (2.1 g, 3.8 mmol) was added Cs_2CO_3(4.5 g, 7.6 mmol). The mixture was stirred for 3 h at room temperature, then poured into water (100 mL). The precipitate was filtered and washed with water and dried to afford **(±)-18a** as a white solid (1.4 g, 89%). Mp: 182−184 °C. LC−MS (ESI) m/z $[M + Na]^+$: 439.0265 and 441.0246. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26 (d, J = 7.2 Hz, 1 H), 6.70 (d, J = 8.8 Hz, 1 H), 5.45 (s, 1 H), 4.89 (brs, 1 H), 4.77 (d, J = 16.8 Hz, 1 H), 4.63 (t, J = 4.8 Hz, 1 H), 4.37 (d, J = 16.8 Hz, 1 H), 3.62−3.48 (m, 2 H), 1.44 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.3 (d, J_{C-F} = 247.0 Hz), 156.3, 156.2, 152.4 (d, J_{C-F} = 11.0 Hz), 130.8 (d, J_{C-F} = 1.0 Hz), 116.2 (d, J_{C-F} = 4.0 Hz), 106.6 (d, J_{C-F} = 26.0 Hz), 102.1 (d, J_{C-F} = 21.0 Hz), 83.6, 80.5, 78.9, 41.4, 39.8, 28.3.

tert-Butyl ((7-Bromo-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo-[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate ((±)-18b). Compound **(±)-18b** (1.1 g, 91%) was prepared from **(±)-17b** (1.6 g, 2.9 mmol) according to the procedure described for **(±)-18a**. Mp: 88−90 °C. LC−MS (ESI) m/z $[M + Na]^+$: 439.0258 and 441.0236. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28 (d, J = 7.2 Hz, 1 H), 6.72 (d, J = 9.2 Hz, 1 H), 5.43 (d, J = 5.2 Hz,1 H), 4.91 (brs, 1 H), 4.83−4.77 (m, 2 H), 4.41 (d, J = 16.4 Hz, 1 H), 3.85−3.78 (m, 1 H), 3.62−3.55 (m, 1 H),1.46 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.3 (d, J_{C-F} = 247.0 Hz), 156.3, 155.8, 152.1 (d, J_{C-F} = 11.0 Hz), 130.9 (d, J_{C-F} = 2.0 Hz), 116.2 (d, J_{C-F} = 4.0 Hz), 106.7 (d, J_{C-F} = 25.0 Hz), 102.4 (d, J_{C-F} = 22.0 Hz), 82.0, 80.3, 75.8, 40.0, 39.0, 28.4.

tert-Butyl ((7-(6-Cyanopyridin-3-yl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate ((±)-19a). To a three-necked bottle containing **(±)-18a** (270 mg, 0.65 mmol), 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-picolinonitrile (194 mg, 0.84 mmol), $Pd(PPh_3)_4$(74.8 mg, 0.06 mmol), Na_2CO_3(137.3 mg, 1.30 mmol) was added solvent (DMSO/H_2O = 9.0 mL: 1.5 mL) under an atmosphere of argon. The reaction was stirred for 5 h at 100 °C and then cooled to room temperature. The resulting mixture was poured into water (30 mL) and extracted with DCM (15 mL × 3). The combined organic layer was washed with water and brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by chromatography (DCM/MeOH = 100:1) to afford **(±)-19a** as an off-white solid (240 mg, yield: 84%). Mp: 167−169 °C. LC−MS (ESI) m/z $[M + H]^+$: 441.1558. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.83 (s, 1 H), 7.97−7.94 (m, 1 H), 7.77 (dd, J = 0.8, 8.0 Hz, 1 H), 7.17 (d, J = 8.0 Hz, 1 H), 6.79 (d, J = 11.2 Hz, 1 H), 5.56 (s, 1 H), 4.90 (brs, 1 H), 4.86 (d, J = 16.8 Hz, 1 H), 4.67 (t, J = 4.8 Hz, 1 H), 4.47 (d, J = 16.4 Hz, 1 H), 3.65−3.50 (m, 2 H), 1.45 (s, 9 H).

tert-Butyl ((7-(6-Cyanopyridin-3-yl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate ((±)-19b). Compound **(±)-19b** (155 mg, 81%) was prepared from **(±)-18b** (200 mg, 0.48 mmol) in the same manner as described for **(±)-19a**. Mp: 98−100 °C. LC−MS (ESI) m/z $[M + H]^+$: 441.1562. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.83 (s, 1 H), 7.97−7.94 (m, 1 H), 7.77 (dd, J = 0.8, 8.0 Hz, 1 H), 7.19 (d, J = 8.4 Hz, 1 H), 6.81 (d, J = 11.2 Hz, 1 H), 5.52 (d, J = 4.8 Hz, 1 H), 4.94−4.88 (m, 2 H), 4.84−4.80 (m, 1 H), 4.51 (d, J = 16.8 Hz, 1 H), 3.90−3.82 (m, 1 H), 3.65−3.59 (m, 1 H), 1.47 (s, 9 H).

N-((7-(6-Cyanopyridin-3-yl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide ((±)-20a). Trifluoroacetic acid (7.0 mL) was added to a solution of compound **(±)-19a** (404.4 mg, 0.91 mmol) in DCM (20 mL). The reaction mixture was stirred for 2 h at room temperature and then neutralized with saturated sodium bicarbonate aqueous solution. The resulting mixture was extracted with DCM (10 mL × 3). The combined organic layer was wash with water, brine, dried over sodium sulfate, and filtered. To this filtered solution were added TEA (190.0 μL, 1.4 mmol) and acetyl chloride (84.0 μL, 1.2 mmol) sequentially. The mixture was stirred for 2 h at 0 °C. Then the solution was washed with water and brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by chromatography (DCM/MeOH = 100:1) to give **(±)-20a** as an off-white solid (305 mg, 87%). Mp: 210−212 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{19}H_{16}FN_4O_4$ 383.1156, found 383.1144. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.82 (s, 1 H), 7.95 (dt, J = 1.6, 8.0 Hz, 1 H), 7.76 (dd, J = 0.4, 8.0 Hz, 1 H), 7.16 (d, J = 8.0 Hz, 1 H), 6.79 (d, J = 11.2 Hz, 1 H), 6.05 (t, J = 6.4 Hz,1 H), 5.51 (d, J = 1.2 Hz, 1 H), 4.84 (d, J =

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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16.4 Hz, 1 H), 4.70 (t, J = 4.8 Hz, 1 H), 4.49 (d, J = 16.4 Hz, 1 H), 3.71 (t, J = 5.6 Hz, 2 H), 2.04 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.1, 159.1 (d, J_{C-F} = 250.0 Hz), 156.2, 154.2 (d, J_{C-F} = 12.0 Hz), 150.6 (d, J_{C-F} = 3.0 Hz),136.7 (d, J_{C-F} = 4.0 Hz), 134.2 (d, J_{C-F} = 2.0 Hz), 132.5, 128.2, 128.1 (d, J_{C-F} = 4.0 Hz), 118.7 (d, J_{C-F} = 15.0 Hz), 117.2, 115.8 (d, J_{C-F} = 4.0 Hz), 106.5 (d, J_{C-F} = 25.0 Hz), 83.8, 78.9, 40.2, 40.1, 23.1.

*N-((7-(6-Cyanopyridin-3-yl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide ((±)-**20b**).* Compound (±)-**20b** (154 mg, 80%) was prepared from (±)-**19b** (222 mg, 0.50 mmol) in the same manner as described for (±)-**20a**. Mp: 204−206 °C. HRMS (ESI) m/z [M + H]$^+$: calcd for $C_{19}H_{16}FN_4O_4$ 383.1156, found 383.1141. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.83 (s, 1 H), 7.97−7.94 (m, 1 H), 7.77 (dd, J = 0.8, 8.0 Hz, 1H), 7.20 (d, J = 8.4 Hz, 1 H), 6.81 (d, J = 10.8 Hz, 1 H), 5.94 (t, J = 6.8 Hz, 1 H), 5.53 (d, J = 4.8 Hz, 1 H), 4.90 (d, J = 16.4 Hz, 1 H), 4.86−4.81 (m, 1 H), 4.52 (d, J = 16.8 Hz, 1 H), 4.20−4.14 (m, 1 H), 3.57−3.50 (m, 1 H), 2.06 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.7, 159.2 (d, J_{C-F} = 250.0 Hz), 156.2, 153.9 (d, J_{C-F} = 12.0 Hz), 150.6 (d, J_{C-F} = 3.0 Hz), 136.7 (d, J_{C-F} = 4.0 Hz), 134.1, 132.6, 128.2, 128.1, 119.0 (d, J_{C-F} = 14.0 Hz), 117.1, 115.8 (d, J_{C-F} = 4.0 Hz), 106.6 (d, J_{C-F} = 26.0 Hz), 82.1, 75.8, 40.2, 38.1, 23.2.

*N-(((3S,3aS)-7-(6-Cyanopyridin-3-yl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide (**20aa**) and N-(((3R,3aR)-7-(6-Cyanopyridin-3-yl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide (**20ab**).* Chiral resolution of racemic (±)-**20a** afforded **20aa** and **20ab**. HPLC separation condition: CHIRALPAK IA column, MeOH/EtOAc = 80:20, 1 mL/min, λ = 254 nm, 25 °C. Rt (**20aa**) = 2.95 min, Rt (**20ab**) = 3.52 min. $[\alpha]_D^{29}$ (**20aa**) = −298.8 (c 0.332, $CHCl_3$), $[\alpha]_D^{29}$ (**20ab**) = +268.9 (c 0.238, $CHCl_3$).

Synthetic Procedure for Compound (±)-24. *Benzyl 6-Bromo-2-(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)-2H-benzo[e][1,3]-oxazine-3(4H)-carboxylate ((±)-**21**).* Compound (±)-**21** (3.7 g, 50%) was prepared from **10b** and **14** in the same manner as described for (±)-**15a**. Mp: 134−136 °C. LC−MS (ESI) m/z [M + Na]$^+$: 575.0792 and 577.0781. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.42−7.25 (m, 7 H), 7.19 (d, J = 8.0 Hz, 2 H), 6.85 (d, J = 8.0 Hz, 2 H), 6.74 (d, J = 8.8 Hz, 1 H), 5.98 (d, J = 8.8 Hz, 1 H), 5.21 (d, J = 12.4 Hz, 1 H), 5.16 (d, J = 12.4 Hz, 1 H), 5.03 (d, J = 17.2 Hz, 1 H), 4.83−4.77 (m, 1 H), 4.46 (d, J = 16.8 Hz, 1 H), 4.35 (d, J = 14.8 Hz, 1 H), 4.28 (d, J = 15.2 Hz, 1 H), 3.75 (s, 3 H), 3.57 (t, J = 9.2 Hz, 1 H), 3.49−3.43 (m, 1 H).

*Benzyl 6-Bromo-2-(2-oxooxazolidin-5-yl)-2H-benzo[e][1,3]-oxazine-3(4H)-carboxylate ((±)-**22**).* Compound (±)-**22** (1.9 g, 76%) was prepared from (±)-**21** (3.2 g, 5.7 mmol) in the same manner as described for (±)-**16a**. Mp: 72−74 °C. LC−MS (ESI) m/z [M + H]$^+$: 433.0388 and 435.0368. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.45−7.30 (m, 6 H), 6.83 (d, J = 8.8 Hz, 1 H), 6.67 (s, 1 H), 6.14 (d, J = 8.4 Hz, 1 H), 5.25 (d, J = 12.4 Hz, 1 H), 5.21 (d, J = 12.4 Hz, 1 H), 5.08 (d, J = 17.2 Hz, 1H), 4.95−4.89 (m, 1 H), 4.52 (d, J = 17.6 Hz, 1 H), 3.77−3.65 (m, 2 H).

*Benzyl 6-Bromo-2-(3-(tert-butoxycarbonyl)-2-oxooxazolidin-5-yl)-2H-benzo[e][1,3]oxazine-3(4H)-carboxylate ((±)-**23**).* Compound (±)-**23** (2.0 g, 90%) was prepared from (±)-**22** (1.8 g, 4.1 mmol) in the same manner as described for (±)-**17a**. Mp: 135−137 °C. LC−MS (ESI) m/z [M + H]$^+$: 555.0742 and 557.0724. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.44−7.33 (m, 7 H), 6.87 (d, J = 8.4 Hz, 1 H), 6.23 (d, J = 8.8 Hz, 1 H), 5.25 (d, J = 12.4 Hz, 1 H), 5.22 (d, J = 12.4 Hz, 1 H), 5.10 (d, J = 17.2 Hz, 1 H), 5.00−4.85 (m, 1 H), 4.53 (d, J = 17.2 Hz, 1 H), 4.10 (d, J = 7.2 Hz, 2 H), 1.50 (s, 9 H).

*tert-Butyl ((7-Bromo-1-oxo-3,3a-dihydro-1H,9H-benzo[e]-oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate ((±)-**24**).* Compound (±)-**24** (1.2 g, 84%) was prepared from (±)-**23** (1.9 g, 3.5 mmol) in the same manner as described for (±)-**18a**. Mp: 165−167 °C. LC−MS (ESI) m/z [M + Na]$^+$: 421.0364 and 423.0343. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28 (dd, J = 2.4, 8.8 Hz, 1 H), 7.21 (d, J = 2.0 Hz, 1 H), 6.78 (d, J = 8.8 Hz, 1 H), 5.40 (s, 1 H), 4.91 (brs, 1 H), 4.77 (d, J = 16.8 Hz, 1 H), 4.63 (t, J = 4.8 Hz, 1 H), 4.39 (d, J = 16.8 Hz, 1 H), 3.61−3.49 (m, 2 H), 1.44 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.4, 156.1, 151.3, 131.5, 129.4, 120.8, 119.8, 114.9, 83.4, 80.4, 79.0, 41.5, 40.2, 28.3.

Synthetic Procedure for Compounds 20aa and 35−38. *tert-Butyl (((3S,3aS)-7-bromo-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e] oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate (**18a**).* Chiral resolution of (±)-**18a** afforded **18a**. HPLC condition: CHIRALPAK AD-H column, MeOH = 100%, 1.0 mL/min, λ = 214 nm, 35 °C. Rt: 5.5 min. Mp: 130−132 °C. $[\alpha]_D^{29}$ = −204.9 (c 0.287, $CHCl_3$).

*tert-Butyl (((3S,3aS)-7-Bromo-1-oxo-3,3a-dihydro-1H,9H-benzo-[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate (**24**).* Chiral resolution of (±)-**24** afforded **24**. HPLC condition: CHIRALPAK IA column, hexane/EtOH = 50:50, 1.0 mL/min, λ = 254 nm, 25 °C. Rt: 9.3 min. Mp: 114−115 °C. $[\alpha]_D^{29}$ = −200.7 (c 0.300, $CHCl_3$).

*tert-Butyl (((3S,3aS)-7-(6-Cyanopyridin-3-yl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)-methyl)carbamate (**25**).* Compound **25** (185 mg, 88%) was prepared from **18a** (200 mg, 0.48 mmol) according to the procedure described for (±)-**19a**. Mp: 148−150 °C. LC−MS (ESI) m/z [M + H]$^+$: 441.1573.

*tert-Butyl (((3S,3aS)-6-Fluoro-7-(6-(2-methyl-2H-tetrazol-5-yl)-pyridin-3-yl)-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b]-[1,3]oxazin-3-yl)methyl)carbamate (**26**).* Compound **26** (150 mg, 57%) was prepared from **18a** (220 mg, 0.53 mmol) and 2-(2-methyl-2*H*-tetrazol-5-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-pyridine (181.6 mg, 0.63 mmol) according to the procedure described for (±)-**19a**. Mp: 95−97 °C. LC−MS (ESI) m/z [M + H]$^+$: 498.1878. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.87 (s, 1 H), 8.30 (d, J = 8.0 Hz,1 H), 7.98 (dt, J = 8.0, 1.6 Hz, 1 H), 7.20 (d, J = 8.0 Hz, 1 H), 6.79 (d, J = 11.2 Hz, 1 H), 5.54 (s, 1 H), 4.92−4.85 (m, 2 H), 4.67 (td, J = 1.2, 4.8 Hz, 1 H), 4.48 (d, J = 16.4 Hz, 1 H), 4.47 (s, 3 H), 3.64−3.51 (m, 2 H), 1.45 (s, 9 H).

*tert-Butyl (((3S,3aS)-6-Fluoro-7-morpholino-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate (**27**).* To a three necked-bottle containing **18a** (300 mg, 0.72 mmol), morpholine (125.0 mg, 1.44 mmol), X-PHOS (103.0 mg, 0.22 mmol), $Pd(OAc)_2$ (24.2 g, 0.11 mmol), and Cs_2CO_3(351.6 mg, 1.08 mmol) was added toluene (15.0 mL) under an atmosphere of argon. The mixture was stirred at 100 °C for 6 h and then filtered. The filtered solution was concentrated and purified by chromatography (PE/EtOAc = 3:1) to give **27** as an off-white solid (134 mg, 44%). Mp: 89−91 °C. LC−MS (ESI) m/z [M + H]$^+$: 424.1880. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.67−6.62 (m, 2 H), 5.38 (s, 1 H), 4.87 (s, 1 H), 4.73 (d, J = 16.4 Hz, 1 H), 4.61 (t, J = 4.8 Hz, 1 H), 4.37 (d, J = 16.4 Hz, 1 H), 3.88−3.86 (m, 4 H), 3.56−3.49 (m, 2 H), 3.02−3.0 (m, 4 H), 1.44 (s, 9 H).

*tert-Butyl (((3S,3aS)-7-(6-Cyanopyridin-3-yl)-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)-carbamate (**28**).* Compound **28** (187 mg, 89%) was prepared from **24** (200 mg, 0.5 mmol) according to the procedure described for **25**. Mp: 151−153 °C. LC−MS (ESI) m/z [M + H]$^+$: 423.1658. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.88 (dd, J = 0.8, 2.4 Hz, 1 H), 7.94 (dd, J = 2.4, 8.0 Hz, 1 H), 7.75 (dd, J = 0.8, 8.4 Hz, 1 H), 7.43 (dd, J = 2.0, 8.4 Hz, 1 H), 7.32 (d, J = 2.4 Hz,1 H), 7.05 (d, J = 8.4 Hz, 1 H), 5.52 (s, 1 H), 4.91−4.87 (m, 2 H), 4.68 (td, J = 4.8, 1.2 Hz, 1 H), 4.52 (d, J = 17.2 Hz, 1 H), 3.65−3.51 (m, 2 H), 1.45 (s, 9 H).

*tert-Butyl (((3S,3aS)-7-Morpholino-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate (**29**).* Compound **29** (283 mg, 66%) was prepared from **24** (400 mg, 1 mmol) according to the procedure described for **27**. Mp: 85−87 °C. LC−MS (ESI) m/z [M + H]$^+$: 406.1983. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.84 (d, J = 8.8 Hz, 1 H), 6.79 (dd, J = 2.8, 8.8 Hz, 1 H), 6.59 (d, J = 2.8 Hz, 1 H), 5.33 (s, 1 H), 4.87 (brs, 1 H), 4.76 (d, J = 16.4 Hz, 1 H), 4.61 (td, J = 1.2, 4.8 Hz, 1 H), 4.40 (d, J = 16.4 Hz, 1 H), 3.86−3.84 (m, 4 H), 3.55−3.52 (m, 2 H), 3.07−3.05 (m, 4 H), 1.44 (s, 9 H).

*5-((3S,3aS)-3-(Aminomethyl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-7-yl)picolinonitrile (**30**).* Trifluoroacetic acid (4.0 mL) was added to a solution of compound **25** (240 mg, 0.54 mmol) in DCM (15 mL). The reaction mixture was stirred for 2 h at room temperature and then neutralized with

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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saturated sodium bicarbonate aqueous solution. The resulting mixture was extracted with DCM (15 mL × 3). The combined organic layer was washed with water, brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by chromatography (DCM/MeOH/NH_4OH = 100:2:0.5) to afford **30** as a white solid (175 mg, 94%). Mp: 157−159 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{17}H_{14}FN_4O_3$ 341.1050, found 341.1039. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.84 (s, 1 H), 7.96 (dt, J = 2.0, 8.0 Hz, 1 H), 7.76 (dd, J = 0.8, 8.4 Hz, 1 H), 7.19 (d, J = 8.0 Hz, 1 H), 6.80 (d, J = 11.2 Hz, 1 H), 5.41 (d, J = 1.2 Hz,1 H), 4.86 (d, J = 16.4 Hz, 1 H), 4.61 (td, J = 1.2, 5.2 Hz, 1 H), 4.52 (d, J = 16.4 Hz, 1 H), 3.18−3.06 (m, 2 H), 1.29 (brs, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 159.1 (d, J_{C-F} = 250 Hz), 156.2, 154.4 (d, J_{C-F} = 12.5 Hz), 150.6, 136.7, 134.3, 132.5, 128.2, 118.6 (d, J_{C-F} = 13.8 Hz), 117.2, 116.0, 106.4 (d, J_{C-F} = 25 Hz), 84.0, 81.0, 42.7, 39.9.

*(3S,3aS)-3-(Aminomethyl)-6-fluoro-7-(6-(2-methyl-2H-tetrazol-5-yl)pyridin-3-yl)-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]-oxazin-1-one (**31**).* Compound **31** (85 mg, 63%) was prepared from **26** (170 mg, 0.34 mmol) according to the procedure described for **30**. Mp: 125−127 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{18}H_{17}FN_7O_3$ 398.1377, found 398.1353. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.88 (s, 1 H), 8.30 (dd, J = 0.8, 8.0 Hz, 1 H), 7.99 (dt, J = 8.0, 1.6 Hz, 1 H), 7.22 (d, J = 8.0 Hz, 1 H), 6.79 (d, J = 10.8 Hz, 1 H), 5.40 (d, J = 1.6 Hz, 1 H), 4.87 (d, J = 16.4 Hz, 1 H), 4.61 (td, J = 1.2, 5.6 Hz, 1 H), 4.53 (d, J = 16.4 Hz, 1 H), 4.47 (s, 3 H), 3.17−3.05 (m, 2 H), 1.34 (brs, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 164.8, 159.2 (d, J_{C-F} = 248.0 Hz), 156.4, 153.6 (d, J_{C-F} = 12.0 Hz), 149.9 (d, J_{C-F} = 4.0 Hz), 145.7, 137.1, 132.1, 128.2 (d, J_{C-F} = 4.0 Hz), 122.1, 119.9 (d, J_{C-F} = 15.0 Hz), 115.7, 106.3 (d, J_{C-F} = 25.0 Hz), 84.0, 81.1, 42.9, 40.1, 39.8.

*(3S,3aS)-3-(Aminomethyl)-6-fluoro-7-morpholino-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-1-one (**32**).* Compound **32** (75 mg, 70%) was prepared from **27** (140 mg, 0.33 mmol) according to the procedure described for **30**. Mp: 152−157 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{15}H_{19}FN_3O_4$ 324.1360, found 324.1347. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.66−6.62 (m, 2 H), 5.25 (d, J = 1.6 Hz, 1 H), 4.74 (d, J = 16.4 Hz, 1 H), 4.55 (td, J = 1.2, 4.8 Hz, 1 H), 4.41 (d, J = 16.4 Hz,1 H), 3.87−3.85 (m, 4 H), 3.14−2.98 (m, 6 H), 1.37 (brs, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.5, 155.0 (d, J_{C-F} = 247.0 Hz), 147.5 (d, J_{C-F} = 12.0 Hz), 135.7 (d, J_{C-F} = 10.0 Hz), 116.3 (d, J_{C-F} = 4.0 Hz), 114.4 (d, J_{C-F} = 4.0 Hz), 106.4 (d, J_{C-F} = 24.0 Hz), 83.6, 81.0, 67.0, 51.4, 51.3, 42.9, 40.2.

*5-((3S,3aS)-3-(Aminomethyl)-1-oxo-3,3a-dihydro-1H,9H-benzo-[e]oxazolo[4,3-b][1,3]oxazin-7-yl)picolinonitrile (**33**).* Compound **33** (98 mg, 69%) was prepared from **28** (187 mg, 0.44 mmol) according to the procedure described for **30**. Mp: 154−156 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{17}H_{15}N_4O_3$ 323.1144, found 323.1130. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.89 (s, 1 H), 7.94 (d, J = 8.0 Hz, 1 H), 7.75 (d, J = 8.0 Hz, 1 H), 7.44 (d, J = 7.6 Hz, 1 H), 7.34 (s, 1 H), 7.06 (d, J = 8.8 Hz, 1 H), 5.39 (s, 1 H), 4.90 (d, J = 16.8 Hz, 1 H), 4.64−4.55 (m, 2 H), 3.18−3.06 (m, 2 H), 1.32 (brs, 2 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 156.4, 153.6, 149.3, 138.8, 134.5, 132.1, 130.2, 128.5, 127.4, 125.8, 120.1, 119.3, 117.4, 83.9, 81.1, 42.9, 40.4.

*(3S,3aS)-3-(Aminomethyl)-7-morpholino-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-1-one (**34**).* Compound **34** (154 mg, 77%) was prepared from **29** (267 mg, 0.66 mmol) according to the procedure described for **30**. Mp: 173−175 °C. LC−MS (ESI) m/z $[M + H]^+$: 306.1148. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.84 (d, J = 8.8 Hz, 1 H), 6.79 (dd, J = 2.8, 8.8 Hz, 1 H), 6.60 (d, J = 2.8 Hz, 1 H), 5.23 (d, J = 1.2 Hz, 1 H), 4.75 (d, J = 16.4 Hz, 1 H), 4.59−4.56 (td, J = 1.6, 4.8 Hz, 1 H), 4.44 (d, J = 16.8 Hz, 1 H), 3.86−3.84 (m, 4 H), 3.15−3.02 (m, 6 H), 1.57 (brs, 2 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 156.6, 147.0, 146.1, 119.5, 118.6, 117.0, 113.7, 83.6, 81.1, 66.9, 50.3, 43.0, 40.8.

*N-(((3S,3aS)-7-(6-Cyanopyridin-3-yl)-6-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)-acetamide (**20aa**).* To a solution of **30** (75 mg, 0.22 mmol) in DCM (10.0 mL) were added TEA (45.9 μL, 0.33 mmol) and acetyl chloride (20.4 μL, 0.29 mmol) sequentially. The mixture was stirred for 2 h at 0 °C. Then the solution was washed with water and brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by chromatography (DCM/MeOH = 100:1) to give **20aa** as an off-white solid (66.0 mg, 78%). Mp: 159−161 °C. $[\alpha]_D^{19}$ = −294.8 (c 0.384, $CHCl_3$). HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{19}H_{16}FN_4O_4$ 383.1156, found 383.1146. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.83 (s, 1 H), 7.95 (dt, J = 1.6, 8.0 Hz, 1 H), 7.76 (d, J = 8.0 Hz, 1 H), 7.16 (d, J = 8.0 Hz, 1 H), 6.79 (d, J = 11.2 Hz, 1 H), 5.91 (t, J = 5.6 Hz, 1 H), 5.51 (s, 1 H), 4.85 (d, J = 16.4 Hz, 1 H), 4.70 (t, J = 4.8 Hz,1 H), 4.49 (d, J = 16.8 Hz, 1 H), 3.72 (t, J = 5.2 Hz, 2 H), 2.05 (s, 3 H).

*N-(((3S,3aS)-6-Fluoro-7-(6-(2-methyl-2H-tetrazol-5-yl)pyridin-3-yl)-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide (**35**).* Compound **35** (44 mg, 89%) was prepared from **31** (45 mg, 0.11 mmol) according to the procedure described for **20aa**. Mp: 108−110 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{20}H_{19}FN_7O_4$ 440.1483, found 440.1455. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.87 (s, 1 H), 8.30 (d, J = 8.4 Hz, 1 H), 7.98 (dt, J = 1.6, 8.0 Hz, 1 H), 7.20 (d, J = 8.0 Hz, 1 H), 6.77 (d, J = 10.8 Hz, 1 H), 6.05 (t, J = 6.4 Hz, 1 H), 5.48 (d, J = 1.2 Hz, 1 H), 4.84 (d, J = 16.4 Hz, 1 H), 4.70 (t, J = 4.4 Hz, 1 H), 4.50 (d, J = 16.4 Hz, 1 H), 4.47 (s, 3 H), 3.73−3.70 (m, 2 H), 2.05 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.1, 164.7, 159.2 (d, J_{C-F} = 249.0 Hz), 156.3, 153.4 (d, J_{C-F} = 12.0 Hz), 149.9, 145.7, 137.1 (d, J_{C-F} = 3.0 Hz), 132.0, 128.1 (d, J_{C-F} = 5.0 Hz), 122.0, 120.0 (d, J_{C-F} = 15.0 Hz), 115.5 (d, J_{C-F} = 4.0 Hz), 106.2 (d, J_{C-F} = 25.0 Hz), 83.7, 78.9, 40.3, 40.2, 39.8, 23.1.

*N-(((3S,3aS)-6-Fluoro-7-morpholino-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide (**36**).* Compound **36** (58.4 mg, 86%) was prepared from **32** (60 mg, 0.14 mmol) according to the procedure described for **20aa**. Mp: 118−120 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{17}H_{21}FN_3O_5$ 366.1465, found 366.1453. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.65−6.59 (m, 2 H), 6.32 (brs, 1 H), 5.32 (d, J = 1.2 Hz, 1 H), 4.70 (d, J = 16.4 Hz, 1 H), 4.63 (t, J = 5.2 Hz, 1 H), 4.38 (d, J = 16.4 Hz, 1 H), 3.86−3.84 (m, 4 H), 3.67 (t, J = 5.6 Hz, 2 H), 2.99−2.97 (m, 4 H), 2.02 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.2, 156.6, 155.1 (d, J_{C-F} = 246.0 Hz), 147.4 (d, J_{C-F} = 11.0 Hz), 135.9 (d, J_{C-F} = 9.0 Hz), 116.3 (d, J_{C-F} = 4.0 Hz), 114.3 (d, J_{C-F} = 3.0 Hz), 106.5 (d, J_{C-F} = 24.0 Hz), 83.5, 79.0, 67.0, 51.4, 51.3, 40.4, 40.3, 23.1.

*N-(((3S,3aS)-7-(6-Cyanopyridin-3-yl)-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide (**37**).* Compound **37** (81 mg, 93%) was prepared from **33** (77 mg, 0.24 mmol) according to the procedure described for **20aa**. Mp: 229−231 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{19}H_{17}N_4O_4$ 365.1250, found 365.1240. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.88 (d, J = 1.6 Hz, 1 H), 7.94 (dd, J = 2.4, 8.0 Hz, 1 H), 7.75 (d, J = 8.0 Hz, 1 H), 7.43 (dd, J = 2.4, 8.8 Hz, 1 H), 7.32 (d, J = 2.4 Hz, 1 H), 7.05 (d, J = 8.8 Hz, 1 H), 5.93 (t, J = 6.4 Hz, 1 H), 5.46 (d, J = 1.2 Hz, 1 H), 4.88 (d, J = 16.8 Hz, 1 H), 4.71 (t, J = 4.4 Hz, 1 H), 4.54 (d, J = 16.8 Hz, 1 H), 3.75−3.67 (m, 2 H), 2.05 (s, 3 H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 171.2, 156.3, 153.4, 149.3, 138. 8, 134.5, 132.2, 130.4, 128.5, 127.5, 125.7, 119.9, 119.3, 117.3, 83.6, 78.94, 40.6, 40.3, 23.2.

*N-(((3S,3aS)-7-Morpholino-1-oxo-3,3a-dihydro-1H,9H-benzo[e]-oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide (**38**).* Compound **38** (82 mg, 99%) was prepared from **34** (74 mg, 0.24 mmol) according to the procedure described for **20aa**. Mp: 160−162 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{17}H_{22}N_3O_5$ 348.1560, found 348.1554. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.85−6.79 (m, 2 H), 6.59 (s, 1 H), 5.94 (t, J = 6.0 Hz, 1 H), 5.27 (d, J = 1.6 Hz, 1 H), 4.75 (d, J = 16.4 Hz, 1 H), 4.65−4.62 (m, 1 H), 4.42 (d, J = 16.8 Hz, 1 H), 3.87−3.85 (m, 4 H), 3.76−3.70 (m, 1 H), 3.65−3.58 (m, 1 H), 3.07−3.05 (m, 4 H), 2.03 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.8, 156.5, 119.3, 118.7, 117.2, 113.70, 83.4, 78.9, 66.9, 50.3, 40.9, 40.4, 23.2.

Synthetic Procedure for Compounds (±)-47 and (±)-48. *Benzyl 7-Bromo-6-fluoro-2-(3-(4-methoxybenzyl)-2-oxooxazolidin-5-yl)-2H-benzo[e][1,3]oxazine-3(4H)-carboxylate ((±)-**39**).* Compound (±)-**39** (3.2 g, 51%) was prepared from **10c** (3.9 g, 11.0 mmol) according to the procedure described for (±)-**15a**. Mp: 110−112 °C. LC−MS (ESI) m/z $[M + H]^+$: 571.0892 and 573.0837. ^1H NMR (400 MHz, acetone-d_6) δ: 7.45−7.32 (m, 5 H), 7.22−7.19 (m,

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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3 H), 7.10 (d, J = 6.0 Hz, 1 H), 6.88 (d, J = 8.0 Hz, 2 H), 6.02 (d, J = 8.8 Hz, 1 H), 5.22 (d, J = 12.0 Hz, 1 H), 5.19 (d, J = 12.4 Hz, 1 H), 5.05 (d, J = 17.6 Hz, 1 H), 4.86−4.80 (m, 1 H), 4.45 (d, J = 17.6 Hz, 1 H), 4.37 (d, J = 14.8 Hz, 1 H), 4.31 (d, J = 14.8 Hz, 1 H), 3.78 (s, 3 H), 3.61 (t, J = 9.2 Hz, 1 H), 3.50−3.45 (m, 1 H).

Benzyl 7-Bromo-6-fluoro-2-(2-oxooxazolidin-5-yl)-2H-benzo[e][1,3]oxazine-3(4H)-carboxylate ((±)-40). Compound (±)-**40** (1.9 g, 67%) was prepared from (±)-**39** (3.6 g, 6.3 mmol) according to the procedure described for (±)-**16a**. Mp: 150−152 °C. LC−MS (ESI) m/z $[M + H]^+$: 451.0299 and 453.0280. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.45−7.42 (m, 2 H), 7.39−7.33 (m, 3 H), 7.22 (d, J = 8.8 Hz, 1 H), 7.16 (d, J = 6.0 Hz, 1 H), 6.67 (s, 1 H), 6.15 (d, J = 8.8 Hz, 1 H), 5.25 (d, J = 12.4 Hz, 1 H), 5.21 (d, J = 12.4 Hz, 1 H), 5.08 (d, J = 17.6 Hz, 1 H), 4.95−4.90 (m, 1 H), 4.48 (d, J = 17.2 Hz, 1 H), 3.76 (t, J = 8.8 Hz, 1 H), 3.69−3.65 (m, 1 H).

Benzyl 7-Bromo-2-(3-(tert-butoxycarbonyl)-2-oxooxazolidin-5-yl)-6-fluoro-2H-benzo[e][1,3]oxazine-3(4H)-carboxylate ((±)-41). Compound (±)-**41** (3.2 g, 74%) was prepared from (±)-**40** (3.4 g, 7.6 mmol) according to the procedure described for (±)-**17a**. Mp: 170−172 °C. LC−MS (ESI) m/z $[M + Na]^+$: 573.0613 and 575.0594. ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.44−7.32 (m, 5 H), 7.24−7.21 (m, 2 H), 6.24 (d, J = 8.8 Hz, 1 H), 5.25 (d, J = 12.4 Hz, 1 H), 5.21 (d, J = 12.4 Hz, 1 H), 5.08 (d, J = 17.6 Hz, 1 H), 4.96−4.90 (m, 1 H), 4.50 (d, J = 17.6 Hz, 1 H), 4.16−4.08 (m, 2 H), 1.50 (s, 9 H).

tert-Butyl ((6-Bromo-7-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate ((±)-42). Compound (±)-**42** (2.2 g, 82%) was prepared from (±)-**41** (3.7 g, 6.7 mmol) according to the procedure described for (±)-**18a**. Mp: 107−109 °C. LC−MS (ESI) m/z $[M + Na]^+$: 439.0256 and 441.0236. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.11 (d, J = 6.0 Hz,1 H), 6.86 (d, J = 8.0 Hz,1 H), 5.40 (s,1 H), 4.88 (brs, 1 H), 4.75 (d, J = 16.8 Hz, 1 H), 4.62 (td, J = 1.2, 5.2 Hz,1 H), 4.35 (d, J = 17.2 Hz, 1 H), 3.60−3.48 (m, 2 H), 1.44 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.3, 156.1, 154.5 (d, J_{C-F} = 242.0 Hz), 148.6 (d, J_{C-F} = 2.0 Hz), 122.7, 119.2 (d, J_{C-F} = 7.0 Hz), 113.6 (d, J_{C-F} = 24.0 Hz), 108.3 (d, J_{C-F} = 23.0 Hz), 83.6, 80.5, 79.0, 41.4, 40.4, 40.3, 28.3.

tert-Butyl ((6-(6-Cyanopyridin-3-yl)-7-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate ((±)-43). Compound (±)-**43** (160 mg, 84%) was prepared from (±)-**42** (180 mg, 0.43 mmol) according to the procedure described for (±)-**19a**. Mp: 60−62 °C. LC−MS (ESI) m/z $[M + H]^+$: 441.1574. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.86 (s,1 H), 8.00−7.97 (m, 1 H), 7.78 (dd, J = 0.8, 8.0 Hz, 1 H), 7.01−6.90 (m, 2 H), 5.49 (s, 1 H), 4.90 (s, 1 H), 4.86 (d, J = 17.2 Hz, 1 H), 4.66 (t, J = 4.8 Hz, 1 H), 4.47 (d, J = 17.6 Hz, 1 H), 3.64−3.52 (m, 2 H), 1.45 (s, 9 H).

tert-Butyl ((7-Fluoro-6-morpholino-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)carbamate ((±)-44). Compound (±)-**44** (35 mg, 38%) was prepared from (±)-**42** (90 mg, 0.22 mmol) according to the procedure described for **27**. Mp: 135−137 °C. LC−MS (ESI) m/z $[M + H]^+$: 424.1871. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.75 (d, J = 12.0 Hz, 1 H), 6.44 (d, J = 7.2 Hz, 1 H), 5.35 (s, 1 H), 4.92 (brs, 1 H), 4.70 (d, J = 16.4 Hz, 1 H), 4.60 (t, J = 4.8 Hz, 1 H), 4.3 (d, J = 16.4 Hz, 1 H), 3.85 (t, J = 4.4 Hz, 4 H), 3.55−3.51 (m, 2 H), 3.05−3.02 (m, 4 H), 1.43 (s, 9 H).

5-(3-(Aminomethyl)-7-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-6-yl)picolinonitrile ((±)-45). Compound (±)-**45** (91 mg, 78%) was prepared from (±)-**43** (150 mg, 0.43 mmol) according to the procedure described for **30**. Mp: 84−86 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{17}H_{14}FN_4O_3$ 341.1050, found 341.1042. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.86 (s, 1 H), 8.01−7.98 (m, 1 H), 7.78 (dd, J = 0.8, 8.0 Hz, 1 H), 7.02−7.00 (m, 2 H), 5.35 (d, J = 1.2 Hz, 1 H), 4.87 (d, J = 17.6 Hz, 1 H), 4.60 (td, J = 1.6, 5.6 Hz, 1 H), 4.53 (d, J = 17; 2 Hz, 1 H), 3.17−3.05 (m, 2 H), 1.49 (brs, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.3, 154.7 (d, J_{C-F} = 244.0 Hz), 150.7 (d, J_{C-F} = 4.0 Hz), 148.9 (d, J_{C-F} = 2.0 Hz), 136.9 (d, J_{C-F} = 4.0 Hz), 134.1, 133.0, 128.2, 123.9, 121.8 (d, J_{C-F} = 8.0 Hz), 119.1 (d, J_{C-F} = 2.0 Hz), 117.1, 114..4 (d, J_{C-F} = 26.0 Hz), 83.9, 81.1, 42.8, 40.3.

3-(Aminomethyl)-7-fluoro-6-morpholino-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-1-one ((±)-46). Compound (±)-**46** (72 mg, 86%) was prepared from (±)-**44** (110 mg, 0.26 mmol) according to the procedure described for **30**. Mp: 64−66 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{15}H_{19}FN_3O_4$ 324.1360, found 324.1349. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.77 (d, J = 12.4 Hz, 1 H), 6.45 (d, J = 7.6 Hz, 1 H), 5.24 (d, J = 1.6 Hz, 1 H), 4.71 (d, J = 16.4 Hz, 1 H), 4.53 (td, J = 1.2, 5.6 Hz, 1 H), 4.38 (d, J = 16.4 Hz, 1 H), 3.85 (t, J = 4.8 Hz, 4 H), 3.12−2.99 (m, 6 H), 1.25 (brs, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.5, 151.1 (d, J_{C-F} = 241.0 Hz), 148.5 (d, J_{C-F} = 2.0 Hz), 140.1 (d, J_{C-F} = 10.0 Hz), 113.6 (d, J_{C-F} = 23.0 Hz), 111.9 (d, J_{C-F} = 7.0 Hz), 108.0 (d, J_{C-F} = 3.0 Hz), 83.7, 81.1, 66.9, 50.8, 50.7, 43.0, 40.0.

N-((6-(6-Cyanopyridin-3-yl)-7-fluoro-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide ((±)-47). Compound (±)-**47** (68 mg, 76%) was prepared from (±)-**45** (80 mg, 0.24 mmol) according to the procedure described for **20aa**. Mp: 115−117 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{19}H_{16}FN_4O_4$ 383.1156, found 383.1141. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.85 (s, 1 H), 7.98 (d, J = 8.0 Hz, 1 H), 7.78 (d, J = 8.0 Hz, 1 H), 7.00−6.97 (m, 2 H), 6.22 (brs, 1 H), 5.45 (s, 1 H), 4.84 (d, J = 17.2 Hz, 1 H), 4.69 (t, J = 4.8 Hz, 1 H), 4.49 (d, J = 17.2 Hz, 1 H), 3.72−3.69 (m, 2 H), 2.04 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.2, 156.4, 154.8 (d, J_{C-F} = 245.0 Hz), 150.7 (d, J_{C-F} = 4.0 Hz), 148.8 (d, J_{C-F} = 2.0 Hz), 137.0 (d, J_{C-F} = 4.0 Hz), 134.1, 132.9, 128.2, 124.0 (d, J_{C-F} = 15.0 Hz), 121.6 (d, J_{C-F} = 8.0 Hz), 119.2 (d, J_{C-F} = 3.0 Hz), 117.1, 114.4 (d, J_{C-F} = 25.0 Hz), 83.7, 79.1, 40.5, 40.2, 23.1.

N-((7-Fluoro-6-morpholino-1-oxo-3,3a-dihydro-1H,9H-benzo[e]oxazolo[4,3-b][1,3]oxazin-3-yl)methyl)acetamide ((±)-48). Compound (±)-**48** (44 mg, 86%) was prepared from (±)-**46** (45 mg, 0.14 mmol) according to the procedure described for **20aa**. Mp: 202−205 °C. HRMS (ESI) m/z $[M + H]^+$: calcd for $C_{17}H_{21}FN_3O_5$ 366.1465, found 366.1443. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.76 (d, J = 12.0 Hz, 1 H), 6.48 (d, J = 7.2 Hz, 1 H), 5.93 (brs, 1 H), 5.30 (d, J = 1.2 Hz, 1 H), 4.70 (d, J = 16.4 Hz, 1 H), 4.63 (t, J = 5.2 Hz, 1 H), 4.36 (d, J = 16.4 Hz, 1 H), 3.87 (t, J = 4.4 Hz, 4 H), 3.75−3.60 (m, 2 H), 3.07−3.04 (m, 4 H), 2.03 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.9, 156.4, 151.1 (d, J_{C-F} = 241.0 Hz), 150.8, 148.3 (d, J_{C-F} = 2.0 Hz), 113.5 (d, J_{C-F} = 24.0 Hz), 111.9 (d, J_{C-F} = 7.0 Hz), 108.2 (d, J_{C-F} = 3.0 Hz), 83.5, 78.9, 66.8, 50.8, 50.7, 40.3, 40.2, 23.2.

Minimum Inhibitory Concentration Testing (Antitubercular Activity). MICs of compounds against *M. tuberculosis* were determined by the microplate alamar blue assay (MABA). INH and RFP were included as positive controls. Compound stock solutions and the range of final testing concentrations were 32−0.5 μg/mL. For the active compounds, the stock concentration and final testing concentration range were lowered to 2−0.016 μg/mL. *M. tuberculosis* H37Rv or clinical isolates were grown to late log phase (70 to 100 Klett units) in Difco Middlebrook 7H9 broth supplemented with 0.2% (v/v) glycerol, 0.05% Tween 80, and 10% (v/v) albumin-dextrose-catalase (7H9-ADC-TG). Two-fold dilution of compounds was prepared in 7H9-ADC-TG in a volume of 100 μL in 96-well, black, clear-bottom microplates. *M. tuberculosis* (100 μL containing 2 × 10^5 CFU) was added, yielding a final testing volume of 200 μL. The plates were incubated at 37 °C, and on day 7 of incubation, 12.5 μL of 20% Tween 80 and 20 μL of alamar blue were added to all wells. After incubation at 37 °C for 16 h, the fluorescence was read at an excitation of 530 nm and an emission of 590 nm. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of ≥90% relative to controls.

Minimum Inhibitory Concentration Testing (Antibacterial Activity). Antimicrobial susceptibility tests were conducted in 96-well microplates using the broth microdilution procedure. Cation-adjusted Mueller−Hinton broth for all the *S. aureus* strains or brain heart infusion broth for VRE or Mueller−Hinton broth for the other strains was used in the assays. After incubation at 37 °C for 18 h, the absorbance at 600 nm was recorded using a microplate reader (Bio-Rad Laboratory Ltd., U.K.) and then the percentage of bacterial cell inhibition versus vehicles (1% DMSO) was calculated. The MIC was defined as the lowest compound concentration at which the growth of bacteria was inhibited by ≥90%. According to Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically

https://dx.doi.org/10.1021/acs.jmedchem.0c02153
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(29th edition, M100), for *Enterococcus*, linezolid resistant MIC ≥ 8 μg/mL, linezolid intermediate MIC = 4 μg/mL, linezolid susceptible MIC ≤ 2 μg/mL; for *Staphylococcus*, linezolid resistant MIC ≥ 8 μg/mL, linezolid susceptible MIC ≤ 4 μg/mL.

Cytotoxicity Assay. Vero cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS). The cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. Stocks of cells were cultured in 25 cm^2 tissue culture flasks and subcultured two to three times per week. Cytotoxicity testing was performed in a transparent 96-well microplate. Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. The cells were incubated at 37 °C under 5% CO_2 until confluent and then diluted with culture medium to 4×10^5 cells/mL. Three-fold serial dilutions of the stock solutions resulted in final concentrations of 64−0.26 μg/mL in a final volume of 100 μL. After incubation at 37 °C for 48 h, the medium was removed, and the monolayers were washed twice with 100 μL of warm Hanks' balanced salt solution (HBSS). Warm medium (100 μL) and 10 μL of freshly made methylthiazolyldiphenyltetrazolium bromide (MTT) were added to each well, and then the plates were incubated for 4 h. The absorbance was determined at 492 nm.

Liver Microsome Stability Assay. The assay was performed with liver microsomes from male CD-1 mouse and pooled human. The incubation was performed as follows: microsomes in 0.05 M phosphate buffer, pH 7.4 (1 mg/mL microsomal protein), tested compounds (final concentration 0.1 μM, cosolvent (DMSO)), and then NADPH (1 mM) at 37 °C for 30 min. The reaction can be started by the addition of NADPH or the same volume of buffer. Aliquots were sampled at 0 and 30 min incubation, and enzymatic reaction was quenched by addition of acetonitrile. After centrifugation, samples were then analyzed by LC−MS. The assay evaluated the metabolic stability of compounds by measuring the substrate remaining with or without NADPH cofactor.

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 were digitalized by Digidata1440A with sampling frequency at 10 kHz using Patchmaster or pClamp10. hERG current inhibition in the presence of five 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.

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 prewarmed PBS and detached using trypsin. After centrifugation (1000 rpm for 5 min at rt), the cells were resuspended with cell culture medium and seeded at 1500 cells/well into a 384-well plate. After 18 h, compounds **20aa** and **35**, with final concentrations of 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, were 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 performed for every compound. MPS inhibition was evaluated by MitoBiogenesis In-Cell ELISA. IC_{50} calculation was performed using GraphPad Prism 5 software.

MAO Inhibition. Test compound working solutions were made (4-fold dilution, six nonzero concentrations) from 10 mM test compound DMSO stock with DMSO. Control compound working solution was made (4-fold dilution, six nonzero concentrations) from 40 mM leflunomide DMSO stock with DMSO. 40 μM substrate working solution was made from 20 mM kynuramine DMSO stock with phosphate buffer. Thaw MAO-A and MAO-B recombinase in 37 °C water bath. 0.01 mg/mL MAO-A and 0.04 mg/mL MAO-B working solutions were made with phosphate buffer. Add 99 μL of substrate working solution into 1.1 mL incubation tubes and then add 1 μL of the test compound/control working solution or DMSO (vehicle control), mix well, and preincubate at 37 °C for 5 min. Add 100 μL of preincubated MAO-A or MAO-B working solution to incubation tubes to start the reaction, mix well, and incubate at 37 °C for 15 min. Following the incubation, immediately add 200 μL of quenching solution and vortex to mix. Centrifuge all samples at 4 °C for 15 min, then transfer 100 μL of supernatant with 100 μL of water for LC−MS/MS analysis. Corresponding metabolite 4-hydroxyquinolinol is detected. The inhibition potentials (% inhibition) are calculated using formation with test compound or control compound compared to that with the vehicle control. IC_{50} values are calculated using GraphPad Prism.

Pharmacokinetic Studies in Mouse. The animal protocol was approved by Institute Animal Care and Welfare Committee. Compound **20aa** was subjected to pharmacokinetic studies in BALB/c mouse (female) weighing 16−21 g with three mice in oral administration group and three mice in intravenous injection group. Compound **20aa** was formulated at a concentration of 5 mg/mL for a dose of 10 mg/kg given orally (po) and at 1 mg/mL for a dose of 1 mg/kg given intravenously (iv). Compound **20aa** was formulated with 0.5% carboxymethyl cellulose for po administration and with 10% DMSO/50% PEG400/40% water for iv administration. Blood samples were collected at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h after oral dosing and 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h after iv administration. Analyte quantification was performed by a LC/MS/MS Quantum Access mass spectrometer (AB Sciex 5500). 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 noncompartmental 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, $F = [(AUC_{po} \times dose_{iv})/(AUC_{iv} \times dose_{po})] \times 100\%$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c02153.

Synthesis of compounds **10a−c**, **14**, and **49**; NMR spectra of all compounds; chiral HPLC of compounds (±)-**18a**, **18a**, (±)-**20a**, **20aa**, **20ab**, (±)-**24**, and **24**; plasma exposure of compound **20aa** in mouse (PDF)

Molecular formula strings (CSV)

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https://dx.doi.org/10.1021/acs.jmedchem.0c02153
J. Med. Chem. 2021, 64, 3234−3248

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The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research is supported by the National Natural Science Foundation of China (Grant 81502917) and the National Natural Science Foundation of China (Grant 81703333). The linezolid resistant strains are gifts from Prof. Min Li of Renji Hospital of Shanghai Jiaotong University and Dr. Wenchang Yuan of Guangzhou Medical University.

■ ABBREVIATIONS USED

MRSA, methicillin-resistant *Staphylococcus aureus*; VISA, vancomycin intermediate *Staphylococcus aureus*; VRE, vancomycin resistant *Enterococcus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; TB, tuberculosis; MDR-TB, multidrug-resistant TB; XDR-TB, extensively drug resistant TB; CbzCl, benzyl chloroformate; PMB, 4-methoxybenzyl; Boc, *tert*-butoxycarbonyl; DMAP, 4-dimethylaminopyridine; DCM, dichloromethane; EtOAc, ethyl actate; THF, tetrahydrofuran; CAN, ceric ammonium nitrate; ECD, electrostatic circular dichroism; TFA, trifluoroacetic acid; MIC, minimum inhibitory concentration; MPS, mitochondrial protein synthesis; MAO, monoamine oxidase; PK, pharmacokinetic; NADPH, nicotinamide adenine dinucleotide phosphate

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