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Design, Synthesis, and Structure−Activity Relationship Studies of Highly Potent Novel Benzoxazinyl-Oxazolidinone Antibacterial Agents

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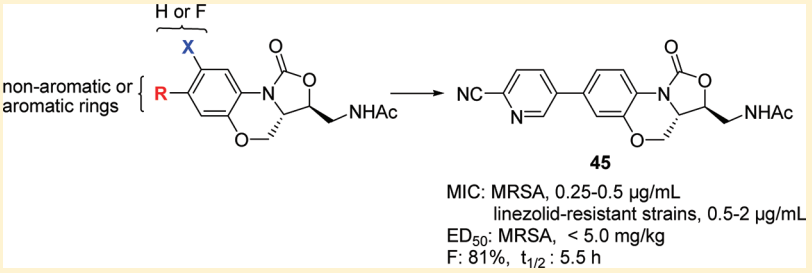
Supporting Information

ABSTRACT:



A series of novel benzoxazinyl-oxazolidinones bearing nonaromatic heterocycle or aryl groups were designed and synthesized. Their in vitro and in vivo antibacterial activities were investigated. Most of the (3*S*, 3a*S*) biaryl benzoxazinyl-oxazolidinones exhibited potent activity against Gram-positive pathogens. SAR trends were observed; a pyridyl C ring was preferable to other 5- or 6-member aryl C rings, while fluorine substitution on the B ring generated derivatives with reduced activity. Various substituent group positions on the pyridyl ring were also evaluated. The resulting compounds displayed excellent activity against linezolid-resistant strains. Compound **45** exhibited excellent in vitro activity, with a MIC value of 0.25−0.5 μg/mL against MRSA and an activity against linezolid-resistant strains of 8−16-fold higher potency than linezolid. In a MRSA systemic infection model, compound **45** displayed an ED_{50} < 5.0 mg/kg, a potency that is nearly 3-fold better than that of linezolid. This compound also showed excellent pharmacokinetic profiles, with a half-life of more than 5 h as well as an oral bioavailability of 81% in rats.

INTRODUCTION

Antibiotic resistance is an emerging threat to global health.[1−3] Infections caused by resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococcus faecalis* (VRE) are the most serious problems. Linezolid **1**[4,5] (Scheme 1), the first and only oxazolidinone drug, was approved in 2000 by the Food and Drug Administration (FDA) for the treatment of multidrug resistant Gram-positive bacterial infections, including the above-mentioned strains. However, after its commercial release, linezolid-resistant strains of *Staphylococcus aureus*[6] and *Enterococcus faecium*[7−9] began to appear in the clinic, underscoring the increasingly urgent need for improved antibiotics with potent antimicrobial activity and with activity toward linezolid-resistant strains. Therefore, many research groups have attempted to improve the potency and the antibacterial spectrum of this new drug.[10−13]

Previous reports have described the design and synthesis of a series of [6,5,5] tricyclic fused oxazolidinones, exemplified by **2**,[14,15] which display very weak antibacterial activity. During the course of our initial efforts to develop novel oxazolidinones, we chose to explore the novel [6,6,5] tricyclic fused benzoxazinyl-oxazolidinone **3**.[16] Although **3** also exhibited only weak antibacterial activity, it was a novel lead suitable for further structural modifications. Initially, [6,6,5] tricyclic fused benzoxazinyl-oxazolidinones of the general structure **4** with heterocycles attached and the (3*S*, 3a*S*) configuration were investigated, however, all showed much lower potency compared with linezolid.

Meanwhile, a number of studies suggested that an aryl C ring of the oxazolidinone subtype would significantly improve potency.[17−20] We therefore focused on structure−activity relationship studies of biaryl [6,6,5] tricyclic fused benzoxazinyl-oxazolidinone derivatives **4** (5- or 6-member aryl C rings). As anticipated, a pyridyl ring[17,19] was preferable to other C ring subtypes. Next, the effects of changes to the substituent group position on the pyridyl ring was evaluated. Herein, we

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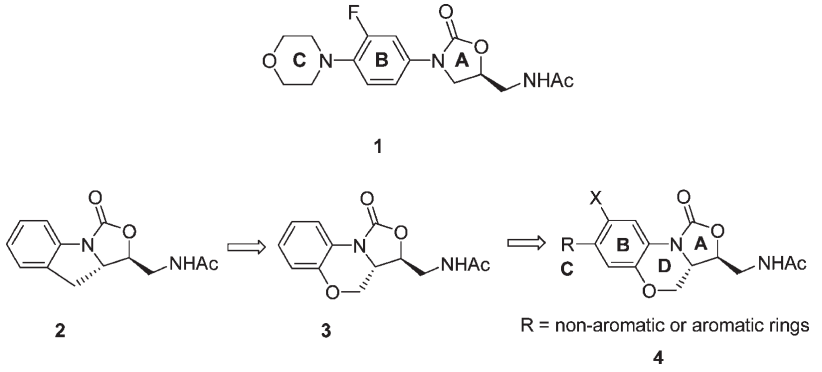
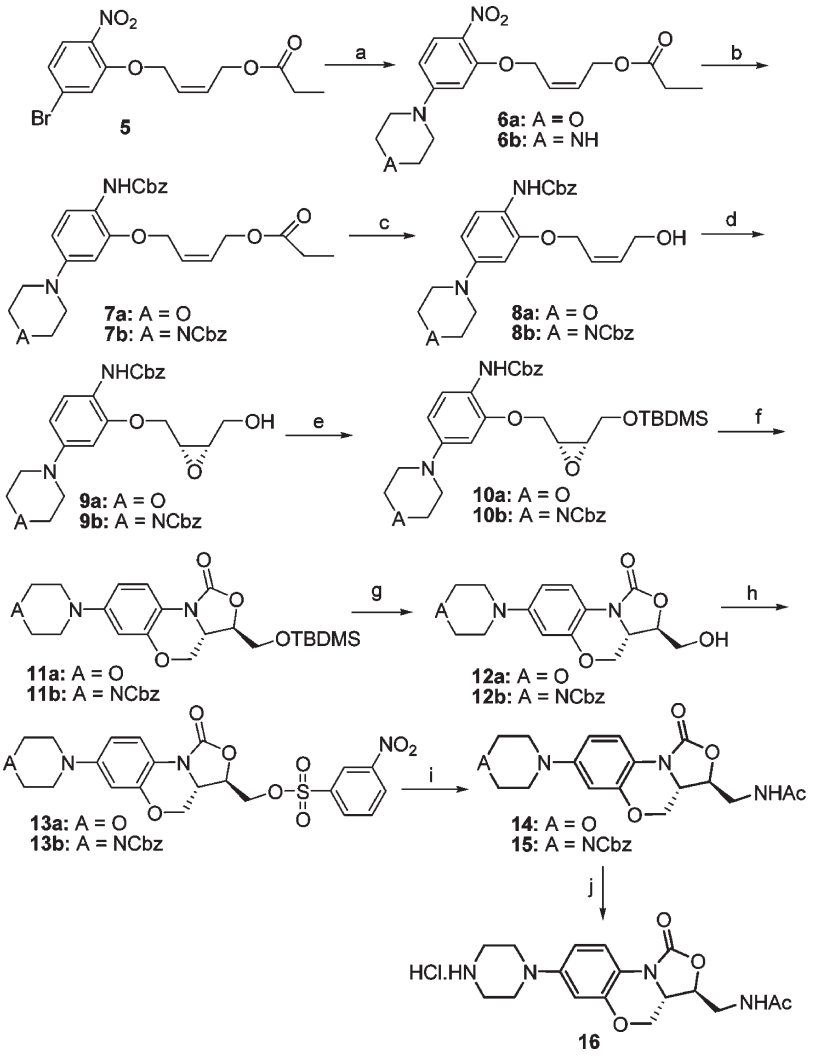
Scheme 1



Scheme 2[a]



[a] Reagents and conditions: (a) K_2CO_3, 4 Å molecular sieves, DMF; 120 °C; (b) (i) $SnCl_2 \cdot 2H_2O$, EtOH, reflux, (ii) CbzCl, pyridine, CH_2Cl_2; (c) KOH, THF; (d) L-(+)-DET, TBHP, $Ti(O^iPr)_4$, 4 Å molecular sieves, CH_2Cl_2, −40 °C ; (e) TBDMSCl, imidazole, DMAP, DMF, 0 °C; (f) ^{n}BuLi, THF, −78 °C to room temp; (g) $^n Bu_4NF$, THF, 0 °C; (h) *meta*-nitro benzene sulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C to room temp; (i) (i) 25% $NH_3 \cdot H_2O$, acetonitrile/2-propanol, 55 °C, (ii) CH_3COCl, Et_3N, CH_2Cl_2, 0 °C; (j) 10% Pd-C/H_2, CH_2Cl_2/MeOH.

disclose our efforts toward the design and synthesis of a novel class of benzoxazinyl-oxazolidinones, which exhibit excellent activity against Gram-positive bacteria, including linezolid-resistant strains, resulting in the discovery of a promising antibacterial drug candidate **45**.

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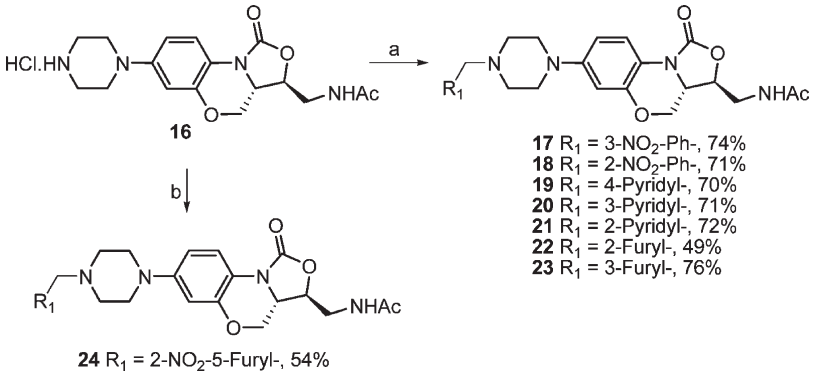
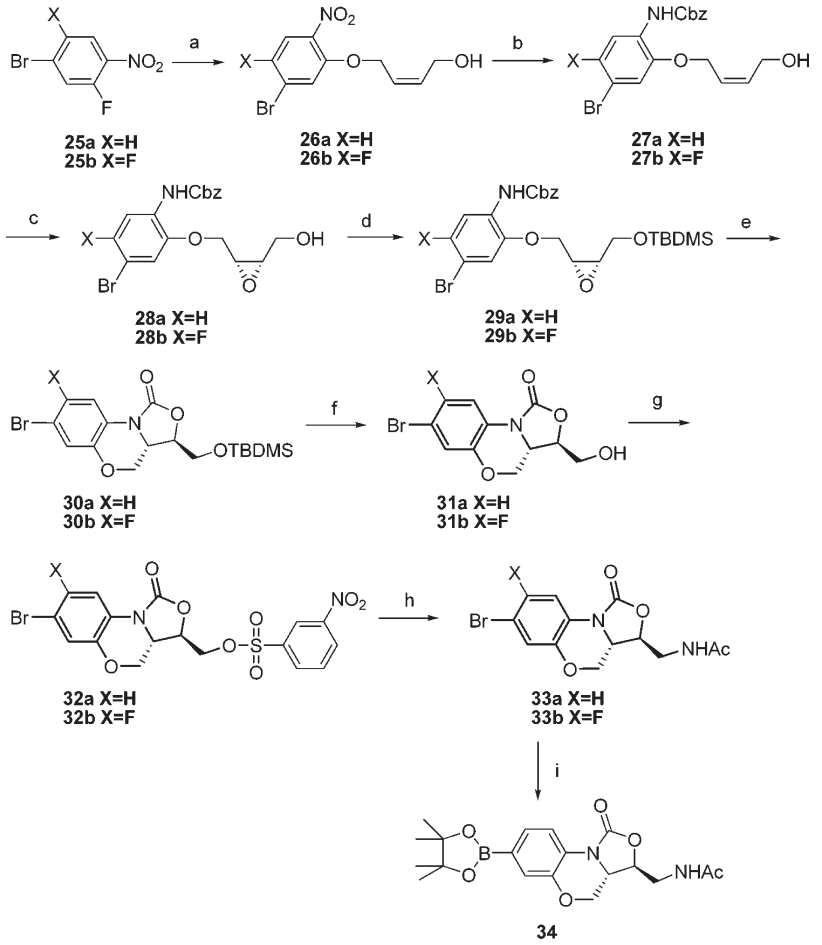
Scheme 3[a]



[a] Reagents and conditions: (a) ArCHO, HCO_2H, DMF, 100 °C, (b) 2-chloromethyl-5-nitrofuran, DIEA, acetonitrile, 50 °C.

Scheme 4[a]



[a] Reagents and conditions: (a) *cis*-2-butene-1,4-diol, NaH, THF, 0 °C to room temp; (b) (i) Zn powder, NH_4Cl, THF (**9a**) or CH_3OH (**9b**), room temp, (ii) CbzCl, $NaHCO_3$, THF:H_2O = 2:1 (**9a**) or acetone:H_2O = 2:1 (**9b**), 0 °C to room temp; (c) L-(+)-DET, TBHP, $Ti(O^iPr)_4$, 4 Å molecular sieves, CH_2Cl_2, −40 °C; (d) TBDMSCl, DMAP, imidazole, DMF, 0 °C; (e) ^{n}BuLi, dry THF, −78 °C to room temp; (f) $^n Bu_4NF$, THF, 0 °C to room temp; (g) *meta*-nitro benzene sulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C to room temp; (h) (i) 25% $NH_3 \cdot H_2O$, acetonitrile/2-propanol, 55 °C, (ii) CH_3COCl, Et_3N, CH_2Cl_2; 0 °C; (i) **33a** to **34**: bis(pinacolato)diboron, $PdCl_2(dppf) \cdot CH_2Cl_2$, KOAc, DMSO, 80 °C.

CHEMISTRY

Compound **14** and the key intermediate **16** were prepared using the same synthetic route, as described in Scheme 2. Nucleophilic substitution of bromobenzene **5**[16] afforded compound **6**, before reduction of the nitro functional group using $SnCl_2$. The aniline produced was protected with benzyl chloroformate (CbzCl) to

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afford compound **7**. After cleavage of the propionic ester, Sharpless asymmetric epoxidation of **8** at −40 °C, gave the desired compound **9** (**2*R*,3*S***). The hydroxyl group of **9** was then protected using *tert*-butyldimethylsilyl chloride (TBDMSCl). Next, a tandem cyclization was used to construct the core [6,6,5] tricyclic ring system,[14] to give **11** (**3*R*, 3a*S***) from **10**, using ^{n}BuLi as the base, at −78 °C. *meta*-Nitrobenzene sulfonyl chloride was used to form an aryl sulfonate in **13**, to provide a good leaving group, after cleavage of the protecting TBDMS group. Amination of **13** with aqueous ammonia, followed by acetylation with acetyl chloride, gave compounds **14** and **15**. The key intermediate **16** was produced from **15** by further deprotection.

The synthesis of benzoxazinyl-oxazolidinones bearing non-aromatic heterocycles is outlined in Scheme 3. Compounds **17**−**23** were obtained from key intermediate **16** by reaction with aromatic aldehydes. A nucleophilic substitution between key intermediate **16** and 2-(chloromethyl)-5-nitrofuran yielded compound **24**.

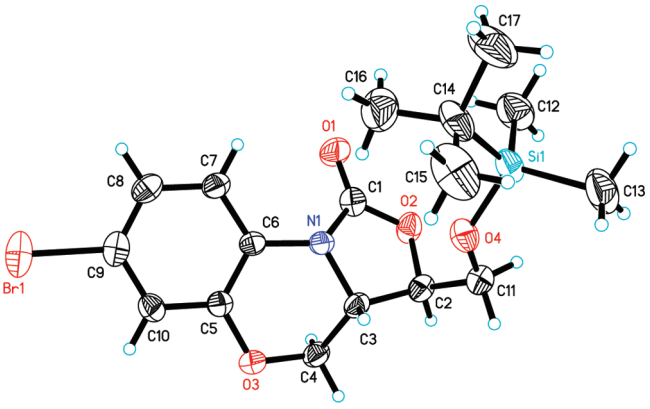
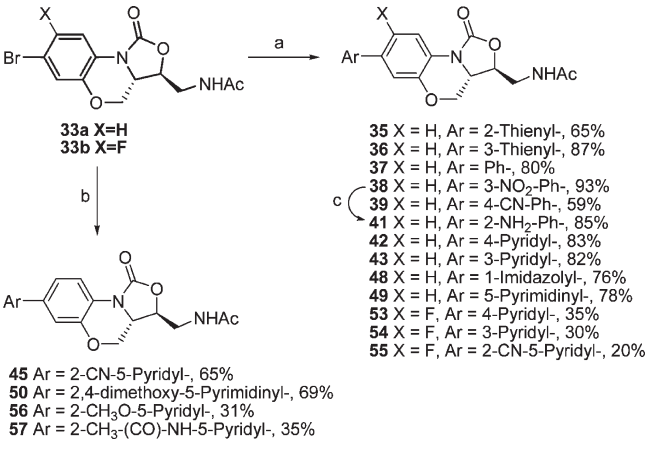


Figure 1. X-ray structure of compound **30a**.

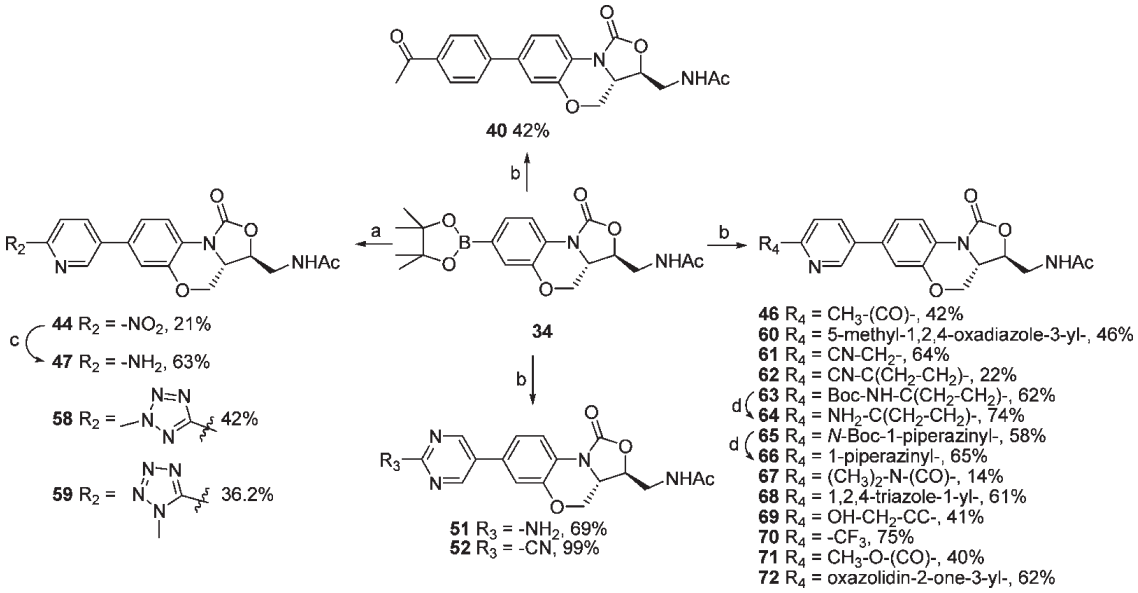
The synthesis of **33a** and its fluorine substituted analogue **33b** was initiated from nitrofluorobenzene **25** (Scheme 4). Nucleophilic substitution of **25** with *cis*-2-butene-1,4-diol gave compound **26**. Because the reduction of the nitro group of **26** using $SnCl_2$[16] resulted in a low yield, zinc powder was instead chosen as the reductant. The yield obtained over two steps (nitro reduction and protection of the aniline with Cbz) was increased to 73% (**27a**) and 56% (**27b**), respectively, by adopting this alternative reducing agent. The synthetic procedures used subsequently were similar to those used in the synthesis of compound **14** (Scheme 2). The absolute configuration of **30a**, which is a good representative of this novel [6,6,5] tricyclic ring system, was confirmed by X-ray crystallography (Figure 1).

Scheme 5[a]



[a] Reagents and conditions: (a) $ArB(OH)_2$, $Pd(PPh_3)_4$, 2 N Cs_2CO_3, THF, reflux; (b) ArB(pin), $Pd(PPh_3)_4$, 2 N KF, dioxane, 80 °C; (c) 10% Pd-C, H_2, room temp.

Scheme 6[a]



[a] Reagents and conditions: (a) ArBr, $PdCl_2(dppf)\cdot CH_2Cl_2$, K_2CO_3, dioxane/EtOH/H_2O, reflux; (b) ArBr, $Pd(PPh_3)_4$, 2 N Cs_2CO_3, dioxane, 80 °C; (c) 10% Pd-C, H_2, room temp; (d) CF_3COOH, CH_2Cl_2, room temp.

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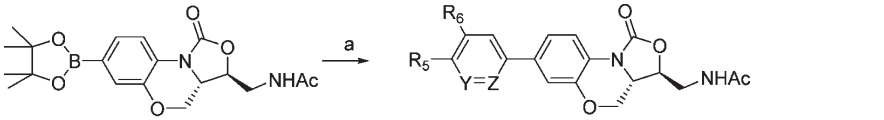
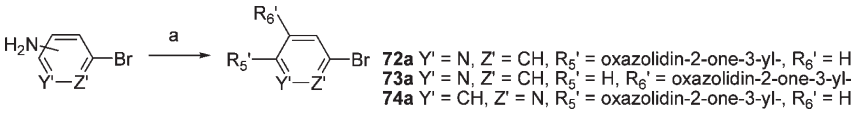
Scheme 7[a]



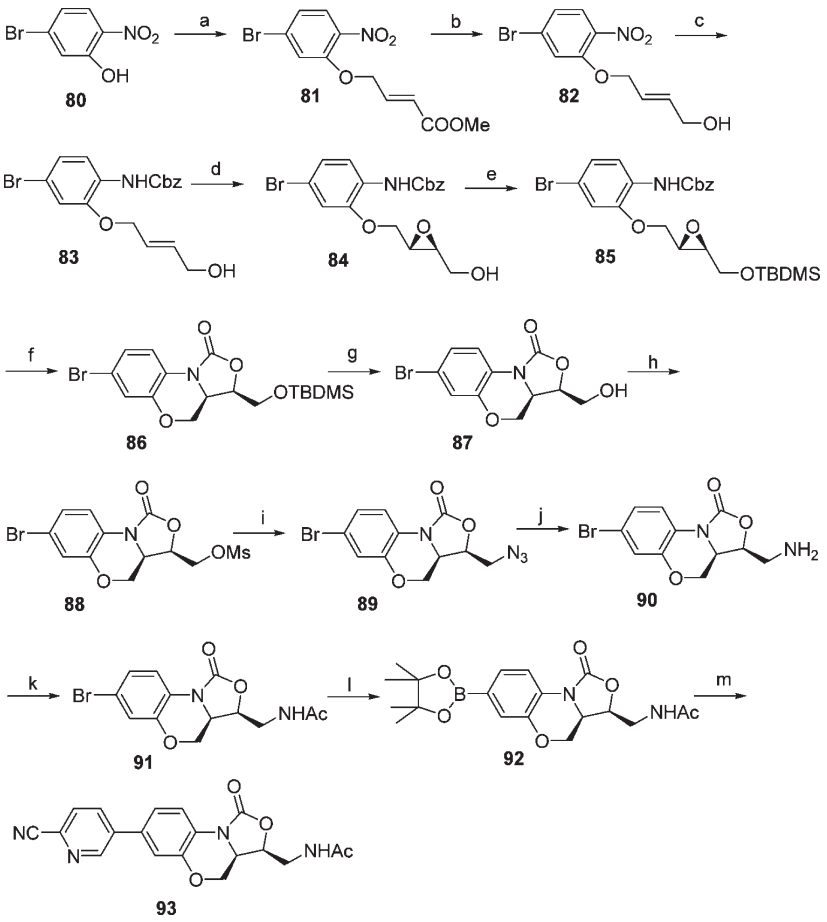
[a] Reagents and conditions: (a) ArBr, $Pd(PPh_3)_4$, 2 N Cs_2CO_3, dioxane, 80 °C.

Scheme 8[a]



[a] Reagents and conditions: (a) (i) 2-chloroethyl carbonochloridate, K_2CO_3, CH_3CN, 0 °C to room temp, (ii) reflux.

Scheme 9[a]



[a] Reagents and conditions: (a) methyl 4-bromocrotonate, K_2CO_3, KI, acetone, reflux; (b) DIBAL-H, toluene, 0 °C; (c) (i) Zn powder, NH_4Cl, THF, room temp, (ii) CbzCl, $NaHCO_3$, THF:H_2O = 2:1, 0 °C to room temp; (d) L-(+)-DET, TBHP, $Ti(O^iPr)_4$, 4 Å molecular sieves, CH_2Cl_2, −40 °C; (e) TBDMSCl, DMAP, imidazole, DMF, 0 °C; (f) ^{n}BuLi, dry THF, −78 °C to room temp; (g) $^n Bu_4NF$, THF, 0 °C to room temp; (h) MsCl, Et_3N, CH_2Cl_2, Et_3N, CH_2Cl_2, 0 °C to room temp; (i) NaN_3, DMF, 80 °C; (j) PPh_3, H_2O, THF, 45 °C to room temp; (k) CH_3COCl, Et_3N, CH_2Cl_2; 0 °C; (l) bis(pinacolato)diboron, $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, DMSO, 80 °C; (m) 5-bromo-2-pyridinecarbonitrile, $Pd(PPh_3)_4$, Cs_2CO_3, dioxane/H_2O, 80 °C, 82%.

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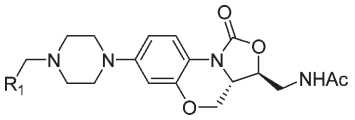
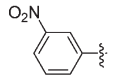
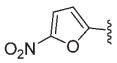
Table 1. In Vitro Antibacterial Activity of Benzoxazinyl-oxazolidinones Bearing Nonaromatic Heterocycles



compd	R_1	MIC (μg/mL)[a]				
		S.a	MRSA	MRSE	PRSP	E.f
14		4	4-8	4-8	8-16	8-16
17		>16	>16	>16	>16	>16
18		8	8-16	8-16	16->16	16->16
19		16	16->16	16->16	>16	>16
20		16	16->16	>16	>16	>16
21		16	16->16	>16	>16	>16
22		>16	16	16->16	16->16	>16
23		16	8->16	8-16	16->16	>16
24		4	4-16	2-16	8->16	>16
1		1	1-2	1-2	1-2	1-4

[a] Abbreviations: S.a., *Staphylococcus aureus*. ATCC 29213. MRSA, methicillin-resistant *Staphylococcus aureus*, 6 strains. MRSE, methicillin-resistant *Staphylococcus epidermidis*, 5 strains. PRSP, penicillin-resistant *Streptococcus pneumoniae*, 4 strains. E.f., *Enterococcus faecalis*, 6 strains.

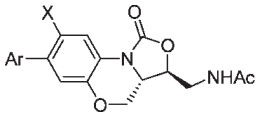
The boric acid ester **34** was synthesized by reacting **33a** and bis(pinacolato)diboron, mediated with $PdCl_2(dppf)\cdot CH_2Cl_2$, resulting in a high yield (87.9%).[19] However, the fluorine substituted analogue of boric acid ester **34** could not be obtained in the same manner. The use of other catalytic systems ($Pd(OAc)_2$/biphenyl-2-yl di-*tert*-butylphosphine, $Pd(dba)_2$/biphenyl-2-yl di-*tert*-butylphosphine, and $PdCl_2(dppf)\cdot CH_2Cl_2$/CuI), other bases (^{t}BuONa, ^{t}BuOK and Et_3N), or longer reaction times (24−48 h) did not result in any reaction.

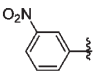
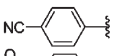
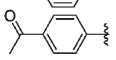
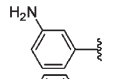
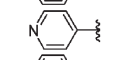
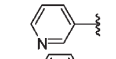
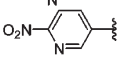
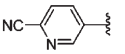
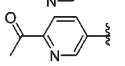
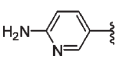
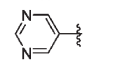
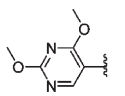
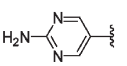
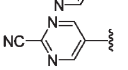
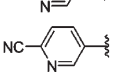
The synthesis of the biaryl benzoxazinyl-oxazolidinones is outlined in Schemes 5, 6, and 7. They were assembled by Suzuki coupling of benzoxazinyl-oxazolidinones **33** with a series of arylboronic acids or boric acid esters (Scheme 5) or key intermediate boric acid ester **34** with bromide-substituted heteroaromatic fragments (Schemes 6 and 7).

Compounds **41** and **47** were generated from compounds **38** and **44** by catalytic hydrogenation of the nitro moieties to amines (Schemes 6 and 7). Compounds **64** and **66** were obtained from compounds **63** and **65** via cleavage of the N-Boc groups after Suzuki coupling (Scheme 7).

The novel fragments **72a**, **73a**, and **74a** were generated from aminopyridine bromides by one-pot acylation and nucleophilic substitution with commercially available 2-chloroethyl carbonochloridate[21] (Scheme 8).

Table 2. In Vitro Antibacterial Activity of Biaryl Benzoxazinyl-oxazolidinones



compd	X	Ar	MIC (μg/mL)[a]				
			S.a	MRSA	MRSE	PRSP	E.f
35	H		1	1-4	1-4	1-8	4-8
36	H		2	2-4	2-4	2-8	4-8
37	H		0.5	0.5-2	1-2	0.5-2	2
38	H		0.5	0.5-2	0.5-2	0.5-2	2
39	H		0.25	0.5-1	0.5-1	0.5-1	1
40	H		1	1-2	0.5-2	2	1-2
41	H		0.5	1-2	1-2	0.5-2	1-2
42	H		0.5	0.5-1	1	1	1
43	H		0.5	0.5-1	0.5-1	0.5-2	1-2
44	H		0.125	0.25-0.5	0.25-1	0.5	0.5-1
45	H		0.125	0.25-0.5	0.25-0.5	0.25-0.5	0.5
46	H		0.5	0.25-0.5	0.25-0.5	0.5	0.5
47	H		0.5	0.5	0.5	0.5	0.5-1
49	H		0.25	0.5-1	0.5	1	1
50	H		1	1	1	1	1
51	H		1	1-2	1	1-2	2
52	H		1	1-2	0.5-1	2	2
53	F		2	4	2-4	8	8
54	F		2	1-4	1-2	2-4	2-4
55	F		2	1-2	1-2	2-4	2-4
1			1	1-2	1-2	2	2-4

[a] Abbreviations: S.a., *Staphylococcus aureus*. ATCC 29213. MRSA, methicillin-resistant *Staphylococcus aureus*, 6 strains. MRSE, methicillin-resistant *Staphylococcus epidermidis*, 5 strains. PRSP, penicillin-resistant *Streptococcus pneumoniae*, 4 strains. E.f., *Enterococcus faecalis*, 6 strains.

Compound **93**, a diastereomer of **45**, was synthesized as outlined in Scheme 9. 5-Bromo-2-nitrophenol (**80**) was reacted with the *E*-isomer of alkene methyl 4-bromocrotonate in a basic solution, and the product was then reduced with diisobutylaluminium hydride

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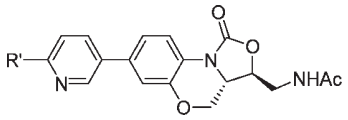
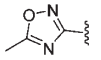
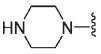
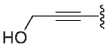
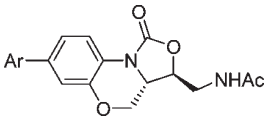
Table 3. In Vitro Antibacterial Activity of Substituted Pyridyl Benzoxazinyl-oxazolidin-ones

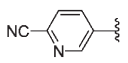
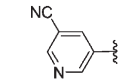
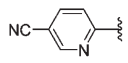
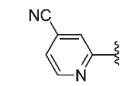
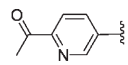
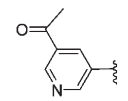
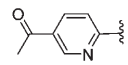
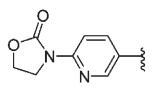
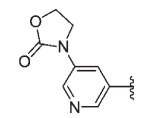
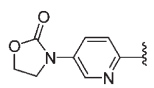


compd	R'	MIC (μg/mL)[a]				
		S.a	MRSA	MRSE	PRSP	E.f
48		0.5	0.5-1	0.5	0.5-1	1
56	CH_3O	0.25	0.5	0.5	0.5-1	0.5-1
57		0.5	0.5-1	1	1	1
58		0.5	0.5-1	0.5-1	0.5-1	1
59		0.25	0.25-0.5	0.25	0.25-0.5	0.5
60		0.25	0.5-1	0.5-1	1	1
61		0.25	0.25-0.5	0.25-0.5	0.5	0.5-1
62		0.5	0.5	0.5-1	0.5-1	0.5-1
64		0.25	0.25-1	0.25-1	0.5-1	0.5-1
66		0.5	1	0.5-2	1-2	0.5-1
67		2	1-2	1-2	2	2
68		0.5	1-2	1-2	2	2
69		0.5	0.25-0.5	0.25-0.5	0.5	0.5
70	CF_3	2	1-2	1-2	2-4	2-4
71		0.5	0.5-1	0.5-1	1	1
72		0.25	0.125-0.5	0.125-0.25	0.25-0.5	0.5
1		1	1-2	1-2	2	2-4

[a] Abbreviations: S.a., *Staphylococcus aureus*. ATCC 29213. MRSA, methicillin-resistant *Staphylococcus aureus*, 6 strains. MRSE, methicillin-resistant *Staphylococcus epidermidis*, 5 strains. PRSP, penicillin-resistant *Streptococcus pneumoniae*, 4 strains. E.f., *Enterococcus faecalis*, 6 strains.

Table 4. In Vitro Antibacterial Activity of Substituted Pyridyl Benzoxazinyl-oxazolidinones



compd	Ar	MIC (μg/mL)[a]				
		S.a	MRSA	MRSE	PRSP	E.f
45		0.125	0.25-0.5	0.25-0.5	0.25-0.5	0.5
75		0.5	0.5-1	0.5-1	0.5-1	1
76		2	1-2	1-2	2-4	2
77		0.5	0.5-1	0.25-0.5	0.25-0.5	1
46		0.5	0.25-0.5	0.25-0.5	0.5	0.5
78		1	1	0.5-1	1-2	1-2
79		2	2-4	2	2-4	4
72		0.25	0.125-0.5	0.125-0.25	0.25-0.5	0.5
73		1	0.5-1	0.5-2	1-2	0.5-1
74		1	1-2	1	1-2	2
93		>16	>16	>16	>16	>16
1		1	1-2	1-2	2	2-4

[a] Abbreviations: S.a., *Staphylococcus aureus*. ATCC 29213. MRSA, methicillin-resistant *Staphylococcus aureus*, 6 strains. MRSE, methicillin-resistant *Staphylococcus epidermidis*, 5 strains. PRSP, penicillin-resistant *Streptococcus pneumoniae*, 4 strains. E.f., *Enterococcus faecalis*, 6 strains.

(DIBAL-H) to yield (*E*)-4-(5-bromo-2-nitrophenoxy)but-2-en-1-ol (**82**). After a similar reaction scheme to that depicted in Schemes 2 and 4, the desired product **93** was obtained.

RESULTS AND DISCUSSION

In Vitro and in Vivo Activities. The target compounds were evaluated for their antibacterial activity against a panel of susceptible and resistant Gram-positive organisms, including linezolid-resistant strains. The results of these studies are summarized in Tables 1−6. Most target compounds displayed excellent in vitro antibacterial activity. Compound **45**, which was the most potent compound tested, exhibited activity several times more potent than linezolid, with minimum inhibitory concentration (MIC) values of 0.125−0.5 μg/mL against *S. aureus* ATCC 29213, 0.25−0.5 μg/mL against MRSA, MRSE, and PRSP and 0.5 μg/mL against *E. faecalis*.

As shown in Table 1, benzoxazinyl-oxazolidinones bearing nonaromatic heterocycles showed very weak in vitro activity. Compound **14**, an analogue of linezolid, was the most potent of these and exhibited MIC values of only 4−16 μg/mL against all strains. This compound is therefore less potent than linezolid.

Exchanging the substituted piperazine for aromatic rings produced biaryl benzoxazinyl-oxazolidinones. To our delight, a significant improvement in potency was observed. As shown in

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Table 2, most of the unsubstituted 6-membered aryl analogues (**37**, **42**, **43**, and **49**) exhibited activity superior to linezolid, while 2-thienyl **35** and 3-thienyl **36** displayed lower activity, similar to that of linezolid. In previous SAR studies[5] for linezolid and similar molecules, a *meta* fluorine on the phenyl B ring has often been found to improve antibacterial activity. By contrast, in our [6,6,5] study, the fluorinated benzoxazinyl-oxazolidinones **53**, **54**, and **55** were less active than their unsubstituted analogues **42**, **43**, and **45**.

Analogues substituted on the phenyl ring with $-NO_2$, $-CN$, CH_3-(CO)$-$, and $-NH_2$ moieties (**38**−**41**) exhibited similar activities to those observed for the unsubstituted parent **37**. The replacement of the pyridyl group of **43** with substituted pyridyls (**44**−**47**) resulted in more active compounds than **43**. Pyridyl rings substituted with both electron-withdrawing groups ($-NO_2$ and $-CN$, compounds **44**, **45** vs **43**) and electron-donating groups ($-NH_2$, **47**) led to compounds with significantly increased activity. However, compounds containing pyrimidyl rings substituted with either electron-donating groups (OMe, $-NH_2$, compounds **50** and **51**) or an electron-withdrawing group ($-CN$, compound **52**) had activity that was significantly reduced. Therefore, these SAR studies have established that the substituents follow the trend pyridyl > phenyl > pyrimidyl > thienyl with respect to in vitro activity.

Accordingly, the 3-pyridyl core structure was chosen as a model for further modifications, including substitution with heteroaromatic rings, simple alkyl groups, and nonaromatic rings. The results are summarized in Table 3. All the derivatives tested displayed similar activity to **43**, with the exception of **67**, **68**, and **70**. Compounds **59**, **61**, **64**, and **69** showed 2- to 4-fold lower MICs than that of linezolid. Compound **72** was equipotent with compound **45** and exhibited an activity that is 4- to 8-fold higher than that of linezolid.

Because of the very potent in vitro activity of compounds **45**, **46**, and **72**, a more in-depth survey of the effects of substituents on the various pyridines was investigated (Table 4). Compounds **73**, **75**, and **78**, which feature *meta*-substitution on the *meta*-pyridyl ring, exhibited reduced activity. Compounds **74**, **76**, and **79**, which have *meta*-substitution on the *ortho*-pyridyl ring, exhibited dramatically reduced activity. While compound **77**, which has *para*-substitution on the *ortho*-pyridyl ring, showed improved activity (**77** vs **75**, **76**), it remained less potent than **45**.

Inverting one chiral center of **45** gave the (**3*S***, **3a*R***) diastereomeric compound **93**, and this compound was found to be virtually inactive (Table 4). This result indicates that a (**3*S***, **3a*S***) absolute configuration for the [6,6,5] tricyclic ring (**45**) system is required for potency.

The in vitro activities of selected pyridyl benzoxazinyl-oxazolidinones against linezolid-resistant strains are shown in Table 5. All compounds tested exhibited more potent activity against common linezolid-resistant pathogens than linezolid. Compounds **45**, **46**, and **72** displayed 8−16-fold more potency than linezolid against all the linezolid-resistant strains tested.

Selected compounds were evaluated for in vivo efficacy in a mouse systemic infection model via oral dosing. As shown in Table 6, compound **45** displayed an efficacy about 4-fold (ED_{50}: 2.5 mg/kg) and 3-fold (ED_{50}: <5.0 mg/kg) higher than linezolid against *S. aureus* and MRSA, respectively. Compound **72** demonstrated oral efficacy comparable (ED_{50}: 9.5 mg/kg) with linezolid (ED_{50}: 14.1 mg/kg) in the MRSA infection model. It is interesting to note that compound **46** exhibited disappointingly low efficacy in this in vivo study (ED_{50} > 20.0 mg/kg) against *S. aureus* despite a good in vitro antibacterial profile.

Pharmacokinetic (PK) Profiles of the Selected Antibacterial Agents. Next, the highly potent compounds **45** and **72** were subjected to pharmacokinetic performance assessment in rats (Table 7). Both compounds exhibited favorable pharmacokinetic

Table 5. In Vitro Activity of Selected Pyridyl Benzoxazinyl-oxazolidinones against Linezolid-Resistant Bacteria

	MIC (μg/mL)[a]				
compd	S.a.	LRSA	LRSE	LREF	LREFA
44	0.125	2−4	2	1−2	1
45	0.125	2	1	1	0.5−1
46	0.5	2	1	1	1
47	0.5	16	4	2−4	2−4
56	0.25	4	2	2	1−2
59	0.25	4	2	1−2	1
62	0.5	8	1	1	1
64	0.25	16	4	4−8	4
69	0.5	4	1	1	1
72	0.25	2	1	1−4	0.5−1
1	1	>16	16	8−16	8−16

[a] Abbreviations: S.a., *Staphylococcus aureus*. ATCC 29213. LRSA, linezolid-resistant *Staphylococcus aureus*, 3 strains. LRSE, linezolid-resistant *Staphylococcus epidermidis*, 1 strain. LREF, linezolid-resistant *Enterococcus faecalis*, 3 strains. LRFEA, linezolid-resistant *Enterococcus faecium*, 3 strains.

Table 6. Antibacterial Efficacies (ED_{50}) of Selected Compounds in Mice Systemic Infection Models

compd	strain	ED_{50} (mg/kg)[a]
45	*S. aureus* (ATCC 19636)	2.5
	MRSA(ATCC 33591)	<5.0
46	*S. aureus* (ATCC 19636)	>20.0
72	*S. aureus* (ATCC 19636)	11.3
	MRSA (ATCC 33591)	9.5
1	*S. aureus* (ATCC 19636)	9.5
	MRSA (ATCC 33591)	14.1

[a] ED_{50} is the dose at which 50% of infections had been successfully treated after oral administration.

Table 7. Pharmacokinetic Parameters of Compounds 45 and 72

compd	route	dose (mg/kg)	C_{max}[a] (μg/mL)	T_{max}(h)	$t_{1/2}$	$AUC_{0-\infty}$ (μ g·h/mL)	clearance[b] (mL/h/kg)	F (%)
45	po	15	5 ± 1	4.0 ± 1.4	5.5 ± 0.7	66 ± 10	229 ± 31	81
	iv	15	7	2.0	5.6 ± 0.2	82 ± 8	184 ± 18	
72	po	50	9 ± 1	6.0 ± 1.2	4.7 ± 0.7	123 ± 23	nc	18
	iv	10	na	na	3.7 ± 0.1	136 ± 33	77 ± 21	

[a] na = not applicable. [b] nc = not calculated.

profiles with excellent half-lives, areas under the concentration-time curve (AUCs). The oral bioavailability of compound 45 is 81% in rats.

CONCLUSIONS

In conclusion, we have identified a novel class of antibacterial agent, the benzoxazinyl-oxazolidinones. Nonaromatic heterocycles or aromatic rings attached to the [6,6,5] tricyclic fused core structure significantly improved the potency. The SAR profile of this new class is quite different from the profiles found in previous SAR studies on related compounds in that the fluorination of this core structure led to a reduction in activity. A remarkable diversity of functionality was tolerated at C-2 of the pyridyl ring. Most of the target compounds exhibited potent activity against Gram-positive pathogens, and particularly noteworthy was the excellent activity shown by compounds **45**, **46**, and **72** against linezolid-resistant strains. Compound **45** exhibited an excellent PK profile as well as activity that was 3- to 4-fold higher than linezolid in vivo. The favorable in vitro and in vivo activities of this compound, combined with its excellent PK profile, make it a very promising drug candidate. Evaluation of the safety of this drug candidate is currently under investigation.

EXPERIMENTAL SECTION

Minimum Inhibitory Concentration Testing. The minimum inhibitory concentrations of the novel compounds against Gram-positive bacteria were tested using linezolid as a positive control. Minimum inhibitory concentration (MIC) values were determined using an agar dilution method according to the methods of National Committee for Clinical Laboratory Standards (NCCLS).[22] Compounds were dissolved in 50% water in DMSO to prepare a stock solution that had a concentration of 320 μg/mL. Serial 2-fold dilutions were prepared from the stock solution with sterile water and then 10-fold diluted with Mueller−Hinton (MH) agar medium to provide concentration ranges of 16−0.03125 μg/mL. The tested organisms were grown in MH broth medium at 35 °C for 8 h and were adjusted to the turbidity of the 0.5 McFarland standard. The bacterial suspensions were inoculated onto the drug-supplemented MH agar plates with a multipoint inoculator and incubated at 35 °C for 16 h.

***S. aureus* Systemic Infection Model.** Compounds **45**, **46**, and **72** and linezolid were studied in a mouse systemic infection model. Institute of Cancer Research mice (female) weighing 20−22 g were used in the study, with six mice in each group. A lethal systemic *S. aureus* infection was given to the mice by the injection of 0.5 mL of an inoculum of *S. aureus* 10^7−10^8 CFU/mL via intraperitoneal injection. Compounds were administered orally (dissolved in 20% cyclodextrin) 1 h after infection. The ED_{50} was calculated 48 h after treatment by the method of Reed Muench[23] and using the Hill equation (GraphPad Prism 5.0; GraphPad Software, Inc., San Diego, CA, USA).

MRSA Infection Model[24]. Female NSA mice (Harlan, Indianapolis, IN, USA), weighing between 18 and 28 g, were rendered neutropenic by treatment with 150 mg/kg of cyclophosphamide intraperitoneally 4 days prior to infection, and a further 100 mg/kg was given 2 days prior to infection. Neutropenic animals were inoculated intraperitoneally with ~0.5 mL of an inoculum containing $(1-5) \times 10^6$ CFU/mL of bacteria. Mice were administered with the test compounds orally 1 h after infection at doses of 5, 10, 20, and 40 mg/kg for compounds **45** and **72**, and linezolid was used as a control. The ED_{50} was calculated 48 h after treatment by the method of Reed Muench[23] and using the Hill equation (GraphPad Prism 5.0; GraphPad Software, Inc., San Diego, CA, USA).

Pharmacokinetic Studies. Compounds **45** and **72** were tested in pharmacokinetic studies on Sprague−Dawley rats weighing 200−250 g, with four mice (male) in each group. The tested compounds were administered orally at a dose of 15 and 50 mg/kg or were administered intravenously at a dose of 15 mg/kg and 10 mg/kg. Serial specimens (0.3 mL) were collected via the retrobulbar vein, and quantification was performed by LC-MS. Pharmacokinetic parameters were calculated from the mean plasma concentration by noncompartmental analysis.

ASSOCIATED CONTENT

Supporting Information. Experimental details, elemental analysis, HPLC data, and X-ray structure of **30a** are available (PDF and CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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ABBREVIATIONS USED

MRSA, methicillin-resistant *Staphylococcus aureus*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; VRE, vancomycin-resistant *Enterococcus faecalis*; FDA, Food and Drug Administration; CbzCl, benzyl chloroformate; TBDMSCl, *tert*-butyldimethylsilyl chloride; DIBAL-H, diisobutylaluminium hydride; MIC, minimum inhibitory concentration; SAR, structure−activity relationship; AUCs, areas under the concentratione-time curve; NCCLS, National Committee for Clinical Laboratory Standards

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