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# Solubility-Driven Optimization of (Pyridin-3-yl) Benzoxazinyl-oxazolidinones Leading to a Promising Antibacterial Agent

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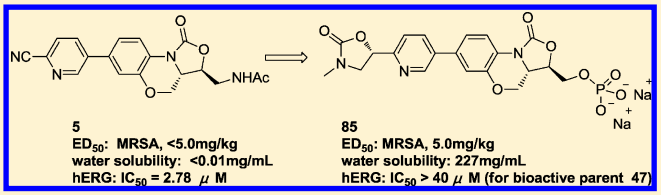
Supporting Information

**ABSTRACT:** The solubility-driven structural modification of (pyridin-3-yl) benzoxazinyl-oxazolidinones is described, which resulted in the development of a new series of benzoxazinyl-oxazolidinone analogues with high antibacterial activity against Gram-positive pathogens, including that against linezolid-resistant strains and low hERG inhibition. With regard to structure−activity relationship (SAR) trends among the various substituents on the pyridyl ring, relatively small and nonbasic substituents were preferable to sterically demanding or basic substituents. Oxazolidinone ring substitution on the pyridyl ring generated analogues with antibacterial activity superior to imidazolidinone ring. Solubility was enhanced by the incorporation of polar groups, especially when compounds were converted to their prodrugs. Among the prodrugs, compound **85** exhibited excellent solubility and a good pharmacokinetic profile. In a MRSA systemic infection model, compound **85** displayed an $ED_{50}$ = 5.00 mg/kg, a potency that is 2-fold better than that of linezolid.




## INTRODUCTION

The emergence and worldwide spread of bacterial pathogens resistant to existing antimicrobials has become a serious problem in hospitals and in the community.[1−3] In particular, multidrug-resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE), vancomycin-resistant *Enterococci* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) are the major concern. Oxazolidinones, a new class of synthetic antibacterial agents, have shown good activity against a broad range of Gram-positive bacteria such as MRSA, MRSE, and VRE. Linezolid[4] (**1**, Figure 1) was the first and only member of this class to achieve FDA approval and has resulted in important and new treatment options for infections caused by Gram-positive pathogens. Unfortunately, a short time after its launch, linezolid-resistant *Staphylococcus aureus* and *Enterococcus faecium* began to emerge.[5−7] The success of linezolid in treating bacterial infections and the emergence of linezolid-resistant strains have prompted significant interest in the development of new oxazolidinone drugs with a broad antimicrobial spectrum and activity especially against linezolid-resistant strains. Numerous reviews have been published by different groups on oxazolidinone antibacterial research.[8−12] Significant efforts have been made which have led to the clinical advancement of more than a dozen oxazolidinones. However, most of these oxazolidinones showed poor solubility which resulted in difficulties in developing an intravenous formulation.[13,14] In addition, poorly soluble compounds face other challenges, for example, high concentrations of poorly soluble drugs in organisms may result in crystallization and acute toxicity, as in the case of uric acid and gout. Currently, there are only two oxazolidinone compounds undergoing clinical evaluation: tedizolid phosphate (**2**), which is the phosphate prodrug of bioactive parent tedizolid (**3**),[15] and radezolid (**4**).[16]

In our previous efforts in the development of oxazolidinones,[17] we first reported a highly potent benzoxazinyl-oxazolidinone series as typified by the candidate compound **5**. Compared to linezolid, compound **5** showed a 3- to 4-fold increase in efficacy in vivo and an excellent pharmacokinetic (PK) profile. However, the poor solubility of compound **5** limited the development of its intravenous formulation and led to difficulty in purification. This prompted us to undertake SAR studies to maintain the antimicrobial activity, including that against linezolid-resistant strains, and good PK profile of compound **5**, while significantly increasing its aqueous solubility. In our tricyclic [6,6,5] fused benzoxazinyl-oxazolidinones, the pyridyl ring was preferable for the C ring. Therefore, we focused on modifications around the different substituent groups and their position on the pyridyl ring and the 3-position side chain of the tricyclic core structure. Our strategy to improve solubility included: (1) the incorporation of polar groups; (2) disruption of molecular planarity; (3) the

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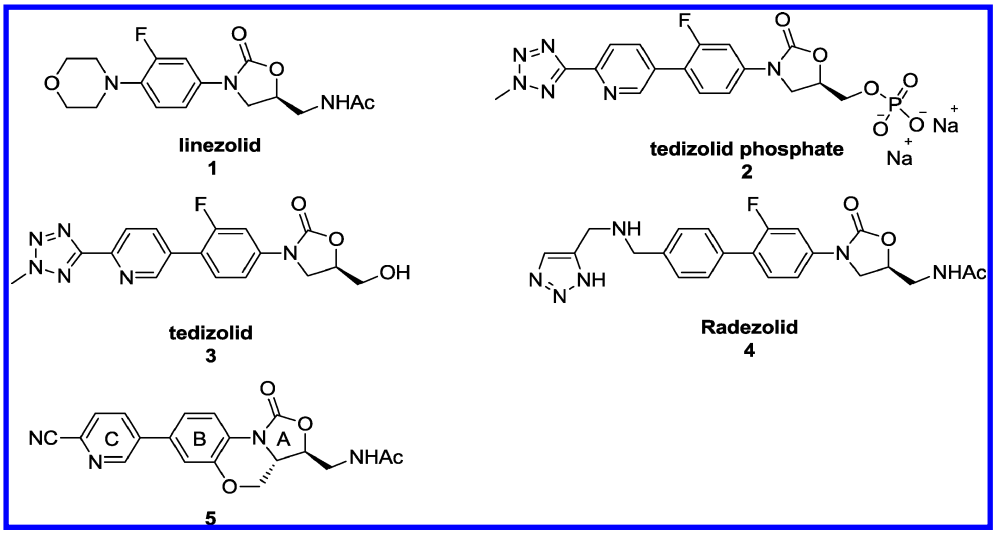
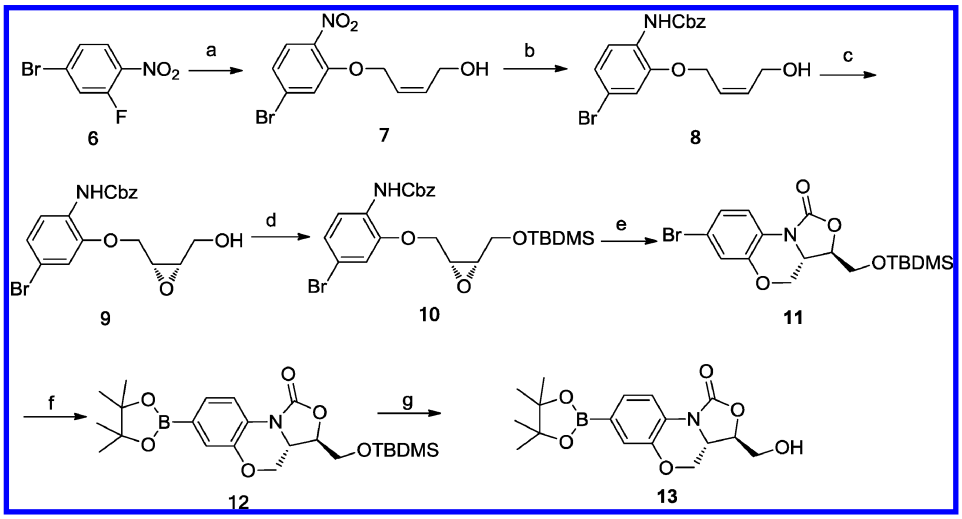



**Figure 1.** Structures of oxazolidinone compounds.

**Scheme 1. General Synthesis of Key Intermediate 13**[α]




[α]Reagent and conditions: (a) *cis*-2-butene-1,4-diol, NaH, THF, 0 °C to room temp, 70%; (b) (i) Zn powder, $NH_4Cl$, THF, room temp, (ii) CbzCl, $NaHCO_3$, THF:$H_2O$ = 2:1, 0 °C to room temp, 74% for two steps; (c) L-(+)DET, TBHP, $Ti(O^iPr)_4$, 4 Å molecular sieves, $CH_2Cl_2$, −40 °C, 72%; (d) TBDMSCl, DMAP, imidazole, DMF, 0 °C, 78%; (e) $^n$BuLi, THF, −78 °C to room temp, 71%; (f) bis(pinacolato)diboron, Pd(dppf)$Cl_2$·$CH_2Cl_2$, KOAc, DMSO, 80 °C, 32.4%; (g) $^n Bu_4NF$, THF, 0 °C to room temp, 85%.

prodrug strategy. Herein, we report the discovery of a promising antibacterial agent (**85**), the prodrug of compound **47**, which has greater in vitro and in vivo activity than linezolid, significantly improved solubility, a good PK profile, and reduced hERG channel inhibition.

## CHEMISTRY

The general synthesis of key intermediate **13** is depicted in Scheme 1. The preparation involved a nucleophilic aromatic substitution reaction between *cis*-2-butene-1,4-diol and 4-bromo-2-fluoro-1-nitrobenzene (**6**) to give compound **7**. Reduction of the nitro group and Cbz protection of the resulting aniline gave intermediate **8**. Then, Sharpless asymmetric epoxidation of **8** afforded compound **9**. The hydroxyl group of **9** was then protected using *tert*-butyldimethylsilyl chloride (TBDMSCl) to give compound **10**. The core tricyclic ring scaffold **11** was constructed through a tandem cyclization of **10** with $^n$BuLi as the base at −78 °C.[18] The Miyaura coupling reaction between compound **11** and bis(pinacolato)diboron, catalyzed with Pd(dppf)$Cl_2$·$CH_2Cl_2$, yielded compound **12** and the partly deprotected product **13**. Compound **12** was converted to **13** by further deprotection.

The synthesis of the biaryl benzoxazinyl-oxazolidinones is outlined in Scheme 2. They were assembled by the Suzuki coupling reaction of key intermediate boric acid ester **13** with a series of bromide-substituted heteroaromatic fragments. Compounds **21** and **23** were generated from compounds **20** and **22** after reduction with sodium borohydride or $PPh_3$. Compounds **25** and **54** were obtained from **24** and **53** via cleavage of the N-Boc groups after Suzuki coupling. Compound **31** was synthesized from **30** with ethoxyamine hydrochloride using $K_2CO_3$ as the base at 40 °C.

The preparation of chiral fragments for compounds **47** and **48** is illustrated in Scheme 3. The route chosen for the chiral pyridyl fragments was based on previous literature.[19] The reaction of 2,5-dibromopyridine with Weinreb amide **61** generated chloroacetylpyridine (compound **62**). After the Noyori asymmetry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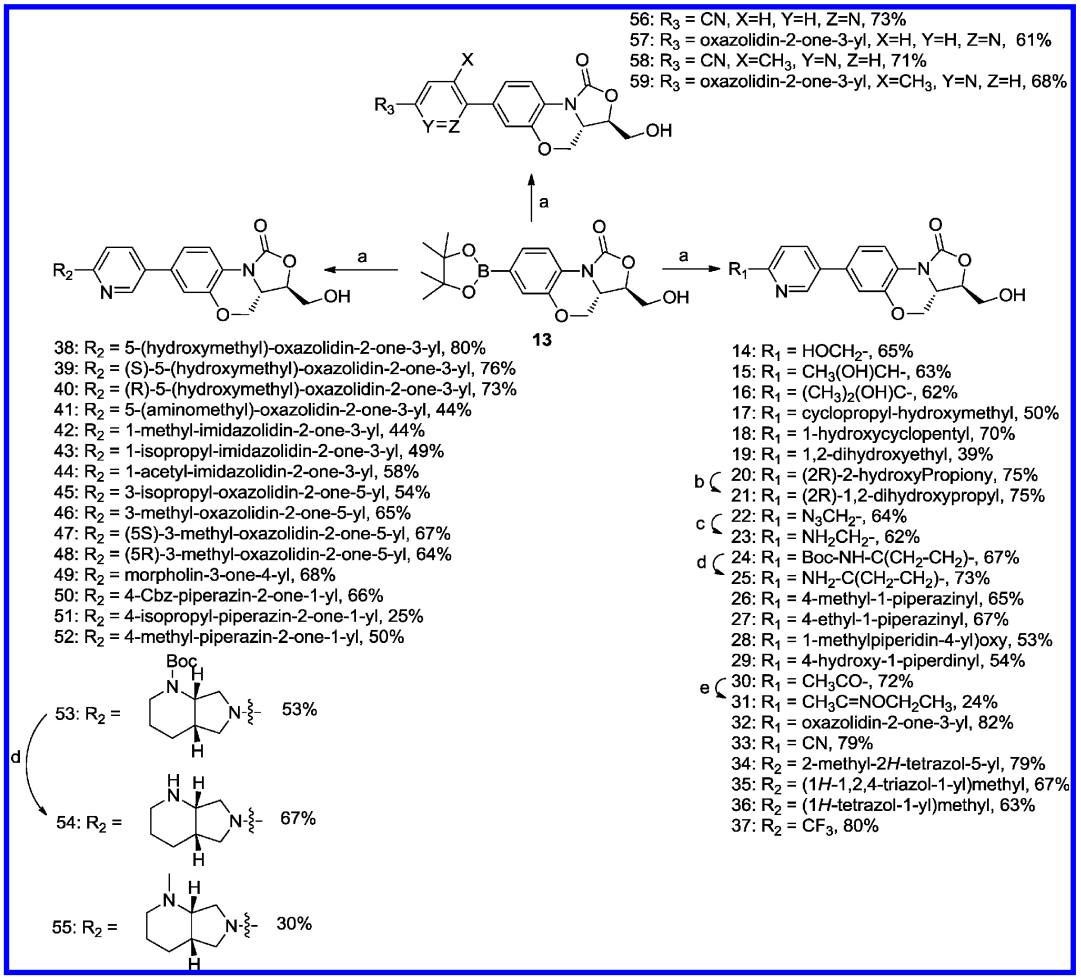
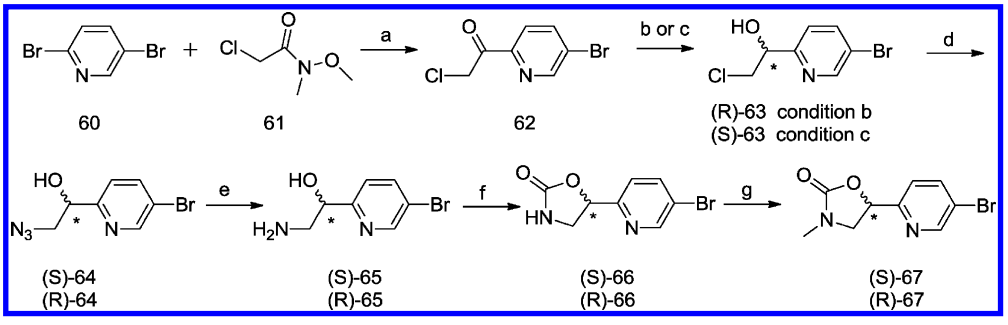
**Scheme 2. Synthesis of the Biaryl Benzoxazinyl-oxazolidinones**[α]




[α]Reagent and conditions: (a) ArBr, $Pd(PPh_3)_4$, $Cs_2CO_3$, dioxane/$H_2O$, 80 °C; (b) $NaBH_4$, $CH_2Cl_2$/MeOH, room temp; (c) $PPh_3$, THF/$H_2O$, 45 °C to room temp; (d) $CF_3COOH$, $CH_2Cl_2$/MeOH, room temp; (e) ethoxyamine hydrochloride, $K_2CO_3$, $CH_2Cl_2$/MeOH, 40 °C.

**Scheme 3. Asymmetry Synthesis of Chiral Fragments (*R*)- and (*S*)-67**[α]




[α]Reagent and conditions: (a) $^n$BuLi, toluene, −78 °C, 73%; (b)HCOOH, $Et_3N$, dichloro(*p*-cymene)ruthenium(II)dimer, (1*S*,2*S*)-(+)-*N*-(4-toluenesulphonyl)-1,2- ethane diamine, MTBE, room temp, 89%; (c) HCOOH, $Et_3N$, dichloro(*p*-cymene) ruthenium(II)dimer, (1*R*,2*R*)-(+)-*N*-(4-toluenesulphonyl)-1,2-ethane diamine, MTBE, room temp, 74%; (d) $NaN_3$, DMF, 90 °C, 83% for (*S*)-**64** and 82% for (*R*)-**64**; (e) $PPh_3$, THF/$H_2O$, 45 °C to room temp, 81% for (*S*)-**65** and 78% for (*R*)-**65**; (f) CDI, DMAP, DMF, room temp, 87% for (*S*)-**66** and 81% for (*R*)-**66**; (g) MeI, NaH, THF, 0 °C to room temp, 91% for (*S*)-**67** and 92% for (*R*)-**67**.

reduction of **62**, chiral chlorohydrins (*R*)-**63** and (*S*)-**63** were obtained, respectively, and the ee value was raised to 99.5% through recrystallization in ethyl acetate/hexane. The chlorine of compound **63** was substituted by azide to give **64**. Then reduction of compound **64** with $PPh_3$ yielded the desired compound **65**. Oxazolidinone ring compound **66** was prepared by an intramolecular condensation reaction using the *N*,*N*′-carbonyldiimidazole (CDI) as the agent in DMF. Finally, a methyl group was introduced into compound **66** to obtain the target fragments (*S*)-**67** and (*R*)-**67**.

The preparation of C-3-substituted amine compound **70** from **33** is illustrated in Scheme 4. First, the hydroxyl group in **33** was converted into the corresponding mesylate group with mesyl chloride. Then the resulting compound **68** was reacted with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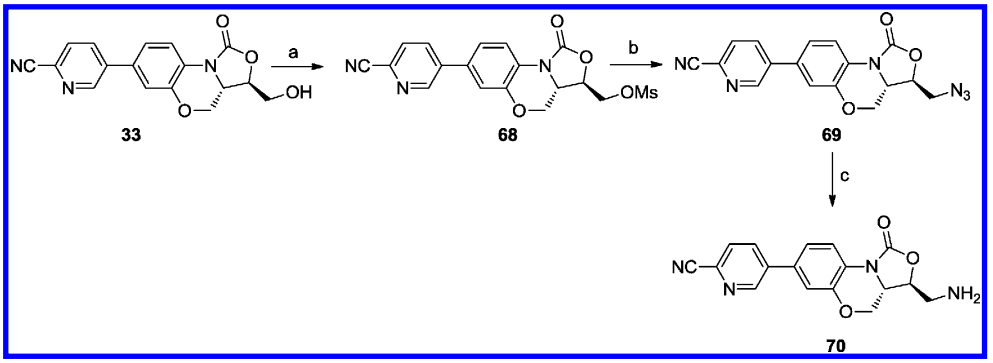
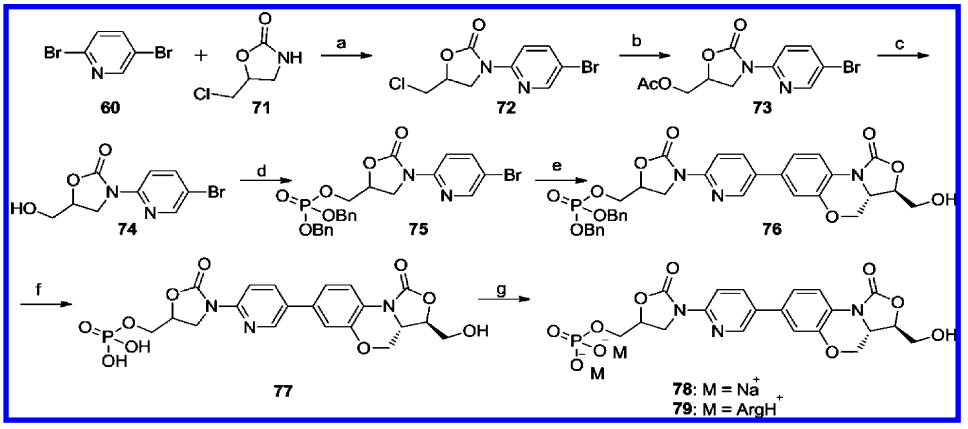
**Scheme 4. Synthesis of Compound 70**[α]



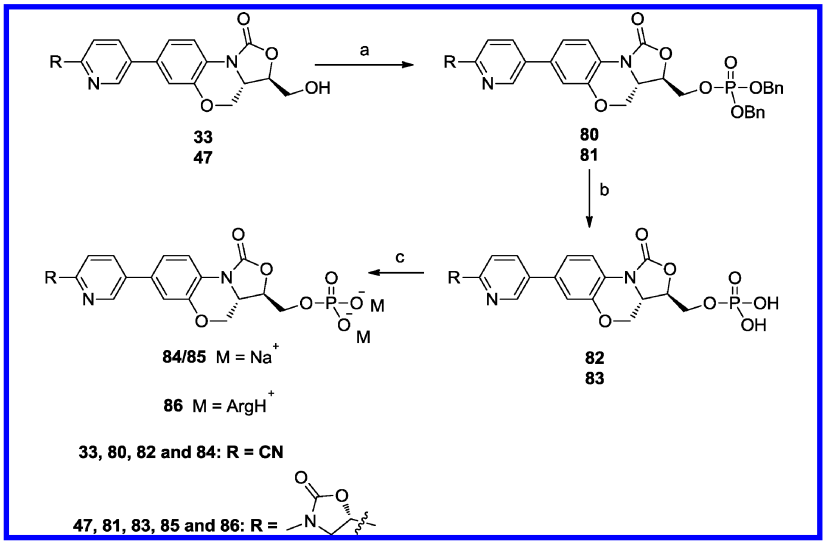

[α]Reagent and conditions: (a) MsCl, $Et_3N$, $CH_2Cl_2$, 0 °C to room temp, 77%; (b) $NaN_3$, DMF, 90 °C, 90%; (c) $PPh_3$, THF/$H_2O$, 45 °C to room temp, 54%.

**Scheme 5. Synthesis of Prodrugs of Compound 38**[α]




[α]Reagent and conditions: (a) $Pd_2(dba)_3$, xantphos, $Cs_2CO_3$, toluene, 100 °C, 88%; (b) KOAc, DMF, 80 °C, 92%; (c) $K_2CO_3$, MeOH, room temp, 70%; (d) (i) $(BnO)_2PN(^iPr)_2$, 4,5-dicyanoiazole, $CH_2Cl_2$, room temp, (ii) 3-chloroperbenzoic acid, $CH_2Cl_2$, 0 °C, 92%, for two steps; (e) intermediate **13**, $Pd(PPh_3)_4$, $Cs_2CO_3$, dioxane/$H_2O$, 80 °C, 34%; (f) 10% Pd/C, $H_2$, room temp, 86%; (g) sodium 2-ethylhexanoate or arginine, acetone/$H_2O$, room temp, 62% for **78** or 65% for **79**.

**Scheme 6. Synthesis of Prodrugs of Compounds 33 and 47**[α]




[α]Reagent and conditions: (a) (i) $(BnO)_2PN(^iPr)_2$, 4,5-dicyanoiazole, $CH_2Cl_2$, room temp, (ii) 3-chloroperbenzoic acid, $CH_2Cl_2$, 0 °C, 75% for **80** or 72% for **81**; (b) 10% Pd/C, $H_2$, room temp, 65% for **82** or 80% for **83**; (c) sodium 2-ethylhexanoate or arginine, acetone/$H_2O$, room temp.

sodium azide in DMF to afford compound **69**. Finally, reduction of **69** with $PPh_3$ provided compound **70**.

Because of the good in vitro antibacterial activity of compounds **33**, **38**, and **47**, their various prodrugs were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synthesized in order to improve water solubility for intravenous administration.

The phosphate prodrugs of **38** were synthesized from **60** as illustrated in Scheme 5. First, the Buchwald−Hartwig coupling reaction of **60** with **71** resulted in **72**, and then nucleophilic substitution of **72** by potassium acetate afforded compound **73**. The ester **73** was hydrolyzed with $K_2CO_3$ as the base in methanol to give compound **74**. Then the hydroxyl group in **74** was converted to a dibenzyl phosphate ester **75**. The Suzuki coupling reaction of **75** with key intermediate **13** gave **76**, and then benzyl group was cleaved in 10% Pd/C under $H_2$ to give monophosphate ester **77**. Finally, compound **77** was reacted with sodium 2-ethylhexanoate or arginine to obtain phosphate salts **78** or **79**.

As shown in Scheme 6, to synthesize the phosphate prodrug of **33**, the hydroxyl group in **33** was first converted to a dibenzyl phosphate ester **80**, and then benzyl group was cleaved in 10% Pd/C under $H_2$ to give monophosphate ester **82**. Finally, **82** was reacted with sodium 2-ethylhexanoate to obtain disodium phosphate salt **84**. In addition, various amino acid-conjugated ester salt compounds of **33** were synthesized. Unfortunately, these compounds decomposed either under reaction conditions or during purification by column chromatography (data not shown). Using the same synthetic procedures as those in Scheme 6, the phosphate prodrugs of **47** were prepared

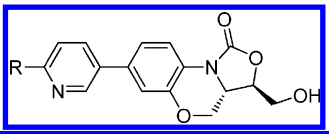
## RESULTS AND DISCUSSION

**In Vitro Antibacterial Activity.** 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 1−4.

First, a series of analogues with hydroxyl or amino groups were designed and synthesized. The introduction of these polar groups have two purposes: (1) they can, to an extent, directly improve the water solubility of compounds, (2) they supply potent sites for further modification, such as prodrug design or formation of salts. As shown in Table 1, for alcohols, compounds bearing a relatively small substituent (compounds **14**, **15**, and **17**) displayed good in vitro antibacterial activity and were similar to that of linezolid. Substituents that were more sterically demanding yielded reduced activity (**16** and **18**). Dialcohols were more potent than the corresponding monoalcohols (**19** vs **15**). Oxidation of alcohols to ketones lowered the antibacterial activity, and if ketones were converted further into oximes, the activity was lost (**30** and **31**). However, it is worth noting that $\alpha$-hydroxy ketone compound **20** exhibited activity several times more potent than that of linezolid and was the most potent of this series, with MIC values of 0.25, 0.125−0.5, 0.125−0.5, 0.25−0.5, 0.125−0.5, and 1 $\mu$g/mL against MSSA, MRSA, MSSE, MRSE, PRSP, and *Enterococcus faecalis*, respectively. Most of analogues containing substituents with basic groups, no matter the basic groups were simple alkyl (**23** and **25**), or nonaromatic rings (**26**−**28**, **51**−**52**, and **54**−**55**), had activity that was lower than that of linezolid. The best compound within the basic series was compound **25** with MIC values of only 1−4 $\mu$g/mL against all the tested strains, which was equipotent to linezolid.

For further modification, nonaromatic ring substituents were introduced into the 3-pyridyl core structure, as shown in Table 2. Molecule **32** showed excellent antibacterial activity, which was 2- to 4-fold greater than that of linezolid. To improve water solubility, further polar groups were introduced into the 5-position of the oxazolidinone ring of compound **32** which resulted in analogues **38**−**41**. As shown in Table 2, analogues substituted at the 5-position of the oxazolidinone ring with hydroxymethyl (**38**−**40**) showed similar activity to the unsubstituted parent **32**. Moreover, the same potency of compound **39** and **40** showed that the absolute configuration of this chiral center hardly had an impact on the antibacterial activity. The replacement of the hydroxymethyl of **38** with aminomethyl (**41**) resulted in reduced activity compared with

**Table 1. In Vitro Antibacterial Activity of Substituted Pyridyl Benzoxazinyl-oxazolidinones**



| compd | R | MIC ($\mu$g/mL)[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | MSSA | MRSA | MSSE | MRSE | PRSP | E.f |
| **14** | $HOCH_2$ | 1-2 | 1-2 | 0.5 | 0.5-1 | 2-4 | 4 |
| **15** | $CH_3CHOH$ | 1-2 | 1-2 | 1 | 1-2 | 2-4 | 2-4 |
| **16** |  | 1-4 | 1-4 | 1-4 | 2 | 2-4 | 4 |
| **17** |  | 1 | 1-2 | 0.5-1 | 1 | 1-2 | 2 |
| **18** |  | 2 | 1-2 | 1-2 | 1-2 | 2 | 2-4 |
| **19** |  | 0.5-1 | 0.5-1 | 0.5 | 0.5 | 0.5 | 1-2 |
| **20** |  | 0.25 | 0.125-0.5 | 0.125-0.5 | 0.125-0.5 | 0.25-0.5 | 1 |
| **21** |  | 2 | 2 | 1-2 | 1-2 | 2 | 4-8 |
| **29** |  | 2-4 | 2-4 | 4 | 2-4 | 2-8 | >32 |
| **30** |  | 2-4 | 2-4 | 4 | 4-8 | 8-16 | 2 |
| **31** |  | >32 | >32 | >32 | >32 | >32 | >32 |
| **23** | $NH_2CH_2$ | 2-4 | 2-4 | 1-2 | 0.5-4 | 4 | 4-8 |
| **25** |  | 2-4 | 1 | 1 | 1-2 | 1-2 | 2 |
| **26** |  | 2-4 | 4-8 | 2-4 | 4 | 4 | 2-4 |
| **27** |  | 4 | 4-8 | 2-4 | 4-8 | 8 | 4-8 |
| **28** |  | 4-8 | 4-8 | 4 | 8 | 16 | >32 |
| **54** |  | 4-16 | 8 | 4-8 | 4-8 | 8-16 | 16 |
| **55** |  | 8 | 8 | 4-8 | 8-16 | >32 | >32 |
| **51** |  | 2-4 | 2-4 | 2-4 | 2-4 | 4 | 16 |
| **52** |  | 2-4 | 2-4 | 2-4 | 2-4 | 4-8 | 16 |
| **49** |  | 2-4 | 1-2 | 2-4 | 2-4 | 2-4 | 2-4 |
| **Linezolid** | | 1-2 | 1-2 | 1-2 | 1-2 | 2 | 2-4 |

[a]Abbreviations: MIC, minimum inhibitory concentration; MSSA, methicillin-sensitive *Staphylococcus aureus*, 5 strains; MRSA, methicillin-resistant *Staphylococcus aureus*, 6 strains; MSSE, methicillin-sensitive *Staphylococcus epidermidis*, 5 strains; MRSE, methicillin-resistant *Staphylococcus epidermidis*, 5 strains; PRSP, penicillin-resistant *Streptococcus pneumoniae*, 4 strains; E.f., *Enterococcus faecalis*, 6 strains.


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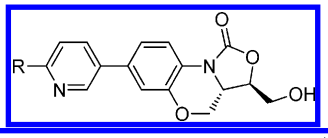
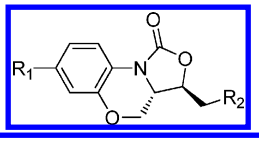
**Table 2. In Vitro Antibacterial Activity of Substituted Pyridyl Benzoxazinyl-oxazolidinones**



| compd | R | MIC (μg/mL)[α] | | | | | |
|---|---|---|---|---|---|---|---|
| | | MSSA | MRSA | MSSE | MRSE | PRSP | E.f |
| **32** |  | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 1 |
| **38** |  | 0.5-1 | 0.5-1 | 0.25-0.5 | 0.5-1 | 0.5-1 | 0.5-1 |
| **39** |  | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 |
| **40** |  | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 |
| **41** |  | 1-2 | 1-2 | 0.5-1 | 2 | 2 | 1-2 |
| **42** |  | 2-4 | 2-4 | 2-4 | 4 | 4 | 8 |
| **43** |  | 4-8 | 2-4 | 2-4 | 4-8 | 4-8 | 8 |
| **44** |  | 8-16 | 4-8 | 4-8 | 8-16 | 8-16 | 4-8 |
| **45** |  | 1-2 | 1-2 | 1-2 | 2-4 | 2 | 1-2 |
| **46** |  | 0.5-1 | 0.5-1 | 0.25-0.5 | 0.5-1 | 1-2 | 1 |
| **47** |  | 0.5-1 | 0.5 | 0.25-0.5 | 0.25-0.5 | 0.5-1 | 1 |
| **48** |  | 1-2 | 1-2 | 0.5-1 | 1-2 | 2-4 | 2 |
| **Linezolid** | | 1-2 | 1-2 | 1-2 | 1-2 | 2 | 2-4 |

[α]Abbreviations: MIC, minimum inhibitory concentration; MSSA, methicillin-sensitive *Staphylococcus aureus*, 5 strains; MRSA, methicillin-resistant *Staphylococcus aureus*, 6 strains; MSSE, methicillin-sensitive *Staphylococcus epidermidis*, 5 strains; MRSE, methicillin-resistant *Staphylococcus epidermidis*, 5 strains; PRSP, penicillin-resistant *Streptococcus pneumoniae*, 4 strains. E.f., *Enterococcus faecalis*, 6 strains.

**Table 3. In Vitro Antibacterial Activity of Substituted Pyridyl Benzoxazinyl-oxazolidinones**



| compd | $R_1$ | $R_2$ | MIC (μg/mL)[α] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | MSSA | MRSA | MSSE | MRSE | PRSP | E.f |
| **33** |  | OH | 0.25-0.5 | 0.125-0.25 | 0.25 | 0.125-0.25 | 0.25-0.5 | 1 |
| **58** |  | OH | 0.25-0.5 | 0.5-1 | 0.25-0.5 | 0.25-0.5 | 1-2 | 4-8 |
| **70** |  | $NH_2$ | >16 | >16 | >16 | >16 | >16 | >16 |
| **32** |  | OH | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 1 |
| **59** |  | OH | 0.5-1 | 0.5-1 | 0.5-1 | 1 | 2 | 4-16 |
| **34** |  | OH | 1-4 | 1-2 | 1-2 | 1-2 | 1-2 | 4 |
| **35** |  | OH | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-1 | 0.5-2 | 1-2 |
| **36** |  | OH | 2-4 | 2-4 | 1-2 | 1-2 | 4 | 4 |
| **37** |  | OH | >16 | >16 | >16 | >16 | >16 | >16 |
| **LZ** | | | 1-2 | 1-2 | 1-2 | 2 | 2 | 2-4 |

[α]Abbreviations: MIC, minimum inhibitory concentration; MSSA, methicillin-sensitive *Staphylococcus aureus*, 5 strains; MRSA, methicillin-resistant *Staphylococcus aureus*, 6 strains; MSSE, methicillin-sensitive *Staphylococcus epidermidis*, 5 strains; MRSE, methicillin-resistant *Staphylococcus epidermidis*, 5 strains; PRSP, penicillin-resistant *Streptococcus pneumoniae*, 4 strains; E.f., *Enterococcus faecalis*, 6 strains; LZ, linezolid.

**32**. On the basis of the bioisosterism approach, some imidazolidinone ring substituted analogues (**42**−**44**) of compound **32** were synthesized and their in vitro antibacterial activity was evaluated. Unfortunately, most compounds only displayed moderate activity and were less potent than linezolid. In addition, the link position between the oxazolidinone ring and the pyridyl ring was changed from 3-substituted to 5-substituted (**45**−**48**). The antibacterial activity results demonstrated that, for the 5-substituted oxazolidinone series, compounds bearing a smaller N-substituent such as methyl exhibited more potent activity (**45** vs **46**). The obtained lead compound **46** was further fine-tuned by structure−activity relationship (SAR) studies on the absolute configuration of the oxazolidinone substituent. This led to the discovery of compound **47** with greater activity than compound **48**, which indicates that the *S*-configuration is preferable.

Disruption of molecular planarity can improve water solubility,[20] therefore, a methyl group was introduced at the para-position of the *meta*-pyridyl ring (Table 3). Unfortunately, although the resulting compounds **58** and **59** retained the excellent antibacterial activity of the unsubstituted parent compounds **33** and **32** against *Staphylococcus aureus* and *Staphylococcus epidermidis*, their activity against *Enterococcus faecalis* was significantly reduced. Analogues substituted on the pyridyl ring with −CN and $-CF_3$ electron-withdrawing moieties (**33** and **37**) exhibited quite different activity. Compound **33** displayed excellent activity against all the tested strains, while **37** almost lost its antibacterial activity. It is worth noting that replacement of the hydroxymethyl at the 3-position of the [6,6,5] tricyclic core structure with aminomethyl resulted in loss of antibacterial activity (**33** vs **70**).

Following the clinical use of linezolid, linezolid-resistant strains of *Staphylococcus aureus* and *Enterococcus faecium* began to emerge. Therefore, one of the hallmarks in the next generation of oxazolidinones is the demonstration of significant improvements in activity against linezolid-resistant strains. The antibacterial activity of some highly active pyridyl benzoxazinyl-oxazolidinones was further evaluated against linezolid-resistant strains. As shown in Table 4, all compounds, especially compounds **33**, **38**, and **47**, were more potent than linezolid against common linezolid-resistant pathogens. Compound **47** exhibited a 4- to 8-fold increase in activity compared with linezolid against all the linezolid-resistant strains tested. It is worth noting that the antibacterial activity of compound **47** against linezolid-resistant strains was greater than that of tedizolid, which is the only oxazolidinone at clinical phase III, especially against linezolid-resistant *Staphylococcus aureus*.

**hERG $K^+$ Channel Inhibition.** In most cases, compounds with high affinity for the hERG ion channel can induce QT interval prolongation frequently associated with potentially lethal arrhythmias.[21] Consequently, the in vitro hERG channel activity in mammalian cell lines can be tested to predict QT prolongation risk. Compound **5**, the highly potent oxazolidinone we previously reported, also had an unsuitable inhibition activity


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**Table 4. In Vitro Activity of Selected Pyridyl Benzoxazinyl-oxazolidinones against Linezolid-Resistant Bacteria**

| | MIC ($\mu$g/mL)[α] | | | | |
|---|---|---|---|---|---|
| compd | S.a. | LRSA | LRSE | LREFL | LREFA |
| **19** | 0.5 | 16 | 8 | 4–8 | 4–8 |
| **20** | 0.25 | 8 | 4 | 8 | 4–8 |
| **32** | 0.5 | 16 | 2 | 4–8 | 4–8 |
| **33** | 0.25 | 4 | 4 | 4 | 2–4 |
| **35** | 1 | 16 | 8 | 8 | 8 |
| **38** | 0.5 | 8 | 4 | 4 | 4 |
| **47** | 0.5 | 4 | 2 | 2 | 2 |
| tedizolid | 0.25 | 8–16 | 2 | 2–4 | 2–4 |
| linezolid | 1 | >16 | 8 | 16 | 16 |

[α]Abbreviations: MIC, minimum inhibitory concentration; S.a., *Staphylococcus aureus* ATCC 29213; LRSA, linezolid-resistant *Staphylococcus aureus*, 3 strains; LRSE, linezolid-resistant *Staphylococcus epidermidis*, 1 strain; LREFL, linezolid-resistant *Enterococcus faecalis*, 3 strains; LREFA, linezolid-resistant *Enterococcus faecium*, 3 strains.

for the hERG channel, with $IC_{50}$ = 2.78 $\mu$M. Therefore, to estimate the risk, potent compounds were selected to evaluate their affinity for the hERG channel. As shown in Table 5, all tested compounds displayed significantly less hERG inhibition than compound **5**.

**Table 5. hERG $K^+$ Channel Inhibition of Selected Compounds**

| compd | hERG $K^+$ channel inhibition |
|---|---|
| **20** | 18.4% @ 30 $\mu$M |
| **32** | 13.3% @ 30 $\mu$M |
| **33** | $IC_{50}$ = 18.35 $\mu$M |
| **38** | $IC_{50}$ > 40 $\mu$M |
| **47** | $IC_{50}$ > 40 $\mu$M |
| **5** | $IC_{50}$ = 2.78 $\mu$M |

**Water Solubility of Prodrugs.** As compounds **33**, **38**, and **47** exhibited excellent in vitro antibacterial activities, including that against linezolid-resistant strains, their prodrugs were synthesized in order to dramatically improve their solubility for intravenous administration. Because of the instability of the amino acid-conjugated ester prodrugs, these prodrugs were not included (data not shown). The phosphate salts were synthesized and evaluated for water solubility. The solubilities of **78**–**79** and **84**–**86** are shown in Table 6. All compounds showed high water solubility. Therefore, all compounds were subjected to pharmacokinetic studies.

**Table 6. Solubility of Prodrugs in Water**

| compd | solubility (mg/mL) |
|---|---|
| **78** | 534 |
| **79** | 210 |
| **84** | 15 |
| **85** | 227 |
| **86** | 196 |

**Pharmacokinetic Properties.** The pharmacokinetic properties of prodrugs **78**–**79**, **84**–**86** and their parent compounds **38**, **33**, and **47** were measured in rats following oral administration and intravenous injection. The study results are summarized in Table 7.

As shown in Table 7, different phosphate salts had an important influence on the pharmacokinetic properties of prodrugs. The pharmacokinetic profiles of **84** were not as good as parent compound **33**, which indicated that prodrug **84** may be partly metabolized before it is converted into **33**. Compared to **38**, its prodrugs **78** and **79** did not show notable improvements in pharmacokinetic properties.

For compound **47**, its prodrugs **85** and **86** were rapidly converted into **47** after intravenous injection or oral administration. After oral administration, the $T_{max}$ was shorter and was 0.44 and 0.38 h for **85** and **86**, respectively. The AUC of **85** was significantly improved by 57% compared with **47** following oral administration, while the AUC of **85** was about 1.34-fold greater than that of **47** after intravenous injection. This may explain why the oral bioavailability of **85** only improved from 44.8% to 52.5%. In addition, the $T_{1/2}$ of compound **85** was longer following intravenous or oral administration.

**In Vivo Efficacy of Compound 85.** Compound **85** was tested in comparison to linezolid in a staphylococcal systemic infection model using both MRSA and MSSA strains in mice. As shown in Table 8, the efficacy of compound **85** was not as good as that of linezolid in the MSSA infection model following intravenous administration. This may be because compound **85** had a relatively shorter MRT (mean retention time) of 1.25 h after intravenous administration. Nonetheless, when administered orally, lower dosages of **85** were required to cure both the MSSA and MRSA infections compared to linezolid. In the MSSA and MRSA infection models, the $ED_{50}$ values for **85** were 6.65 and 5.00 mg/kg for oral administration, compared to 8.28 and 9.87 mg/kg for linezolid, respectively.

## CONCLUSION

Starting with benzoxazinyl-oxazolidinones represented by compound **5**, an extensive plan of solubility-driven structural modifications was undertaken with the goal of maintaining the high antimicrobial activity including that against linezolid-resistant strains and good PK profile of compound **5** while significantly increasing aqueous solubility. The SAR study demonstrated that relatively small and nonbasic substituents were preferable to sterically demanding or basic substituents. On the basis of the lead compound **32**, a further SAR study was performed, and this resulted in some excellent compounds possessing potent antibacterial activity such as compounds **38**, **39**, **40**, **46**, and **47**. Analogues with a methyl group at the para-position of the *meta*-pyridyl ring retained excellent antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*; however, their activity against *Enterococcus faecalis* was significantly reduced. Compared to compound **5**, selected compounds exhibited reduced hERG ion channel inhibition. Several prodrugs of the highly active compounds **33**, **38**, and **47** exhibited promising results, especially compound **85**. This compound exhibited high in vivo activity for treating MSSA and MRSA infected mice. Moreover, it had high aqueous solubility and an excellent pharmacokinetic profile that displayed rapid conversion to the active antibacterial compound, a long half-life, and good oral bioavailability. These favorable drug properties led to the promotion of **85** as a promising drug candidate. Further preclinical evaluation of compound **85** is currently underway.

## EXPERIMENTAL SECTION

**Minimum Inhibitory Concentration Testing.** The minimum inhibitory concentrations of the novel compounds against Gram-


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Table 7. Comparison of the Pharmacokinetic Properties of Parent Compounds and Their Prodrugs in Rats[e]

| compd | route | dose (mg/kg) | $C_{max}$ (ng/mL) | $T_{max}$ (h) | $T_{1/2}$ (h) | AUC (ng·h/mL) | CLz (L/h/kg) | $F$ (%) |
|---|---|---|---|---|---|---|---|---|
| **33** | po | 50 | 3015 | 4.50 | 4.69 | 33419 | | NT[a] |
| **84** | po | 10[b] | 15.2 | 1.31 | 3.17 | 81.1 | | NT |
| **38** | po | 10 | 332 | 0.38 | 2.25 | 1491 | | 27.5 |
| | iv | 5.6 | | | 1.08 | 2805 | 2.00 | |
| **78** | po | 15[c] | 650 | 0.25 | 1.61 | 1227 | | 19.7 |
| | iv | 10[c] | | | 1.00 | 3999 | 2.03 | |
| **79** | po | 15[c] | 416 | 0.25 | 1.45 | 914 | | 32.6 |
| | iv | 10[c] | | | 0.26 | 1803 | 2.78 | |
| **47** | po | 15 | 809 | 1.00 | 1.93 | 2167 | | 44.8 |
| | iv | 5 | | | 2.51 | 1556 | 3.22 | |
| **85** | po | 10[d] | 524 | 0.44 | 4.61 | 1665 | | 52.5 |
| | iv | 10[d] | | | 4.88 | 3165 | 2.42 | |
| **86** | po | 10[d] | 170 | 0.38 | 3.88 | 523 | | 34.8 |
| | iv | 10[d] | | | 9.43 | 1445 | 3.37 | |

[a]NT: not tested. [b]A dose of 10 mg/kg of **84** is equivalent to 7.2 mg/kg of **33**. All values were measured based on the amount of the active antibacterial agent **33** detected. [c]A dose of 15 and 10 mg/kg of **78** is equivalent to 11.5 and 7.7 mg/kg of **38**, respectively. A dose of 15 and 10 mg/kg of **79** is equivalent to 7.4 and 4.9 mg/kg of **38**, respectively. All values were measured based on the amount of the active antibacterial agent **38** detected. [d]A dose of 10 mg/kg of **85** is equivalent to 7.6 mg/kg of **47**. A dose of 10 mg/kg of **86** is equivalent to 4.8 mg/kg of **47**. All values were measured based on the amount of the active antibacterial agent **47** detected. [e]Abbreviations: $C_{max}$, the peak plasma concentration of a drug after administration; $T_{max}$, time to reach $C_{max}$; $T_{1/2}$, elimination half-life; AUC, area under the concentration−time curve; Clz, clearance; $F$, bioavailability.

Table 8. In Vivo Efficacy ($ED_{50}$)[α] of 85 in the Mouse Systemic Infection Models

| $ED_{50}$ MSSA (mg/kg) (ATCC 19636) | | | | $ED_{50}$ MRSA (mg/kg) (ATCC 33591) | |
|---|---|---|---|---|---|
| **85** | | Linezolid | | **85** | Linezolid |
| po | iv | po | iv | po | po |
| 6.65 | 14.15 | 8.28 | 7.07 | 5.00 | 9.87 |

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

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.** Compound **85** and linezolid were studied in a mouse systemic infection model. Institute of Cancer Research mice (female, Sippr/BK Lab Animal Ltd.) 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 or via intravenous injection 1 h after infection at doses of 2.5, 5, 10, and 20 mg/kg for compounds **85**, 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).

**MRSA Infection Model.**[24] Female Institute of Cancer Research mice (B&K Universal Group Limited), weighing between 23 and 25 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 $10^6$ 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 **85** and linezolid was used as a control. The $ED_{50}$ was calculated 24 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).

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Experimental details and pharmacokinetic studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

MRSA, methicillin-resistant *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; VRE, vancomycin-resistant *Enterococci*; FDA, Food and Drug Administration; TBDMSCl, *tert*-butyldimethylsilyl chloride; CbzCl, benzyl chloroformate; SAR, structure−activity relationship; hERG, human ether-a-go-go related gene; QT, the length of time between the start of the Q wave and end of the T wave on an electrocardiogram

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