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Synthesis and antibacterial activity of oxazolidinones containing pyridine substituted with heteroaromatic ring

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Abstract—A series of oxazolidinone derivatives, which morpholino group of linezolid was replaced with heteroaromatic ring substituted pyridine moiety, were newly synthesized, and their substituted effects on in vitro and in vivo antibacterial activities were evaluated against four problematic gram-positive strains including drug resistant strains and two gram-negative strains. Most compounds exhibited the enhanced in vitro activities with 4–16-fold and three compounds exerted more than 2-fold increased in vivo efficacies than linezolid.

1. Introduction

The rapidly increasing incidence of multiple drug-resistant gram-positive bacteria requires an urgent discovery of novel active agents against these pathogens.[1,2] The oxazolidinones, a new class of synthetic antimicrobial agent, are active against a variety of clinically important susceptible and resistant gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP).[3–5] These compounds have a unique mechanism of inhibiting bacterial protein synthesis at an early phase of translation by binding selectively to the central loop of domain of 23S rRNA of 50S ribosomal subunit and show no cross resistance with other classes of protein synthesis inhibitor[6–8] (Fig. 1).

Linezolid was approved for the treatment of multi-drug resistance gram-positive infections such as nosocomial and community-acquired pneumonia and skin infections,[9,10] since Dup-721 was introduced as the first candidate agent of oxazolidinones.[11] Recently, linezolid-resistant isolates have been reported,[12,13] and the development of resistance urgently required to explore a new oxazolidinone series with greater potency and broader spectra of activity by further modification of the basic oxazolidinone skeleton. Many groups have reported the synthesis and the activity of novel oxazolidinones,[14–20] particularly some analogues of nitrogen–carbon-linked oxazolidinone showed expanded spectrum of activity and improved activity against *H. influenzae*.[21] On this line, we designed some derivatives by introducing heteroaromatic rings on the concept that the electron affluence in the flat heteroaromatic ring structures could induce maximum interaction with the binding site. In the course of our efforts to improve spectrum and activity, we recently found that several oxazolidinone derivatives containing pyridine substituted with heteroaromatic ring exhibited the enhanced antibacterial activities against many antibiotic resistant strains compared to linezolid. We herein report the synthesis, in vitro biological activities, and preliminary in vivo efficacies.

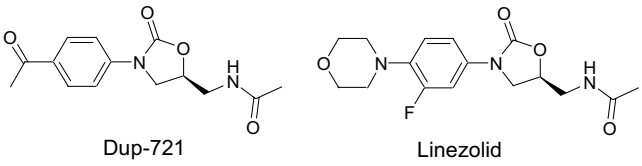

Figure 1. Structures of oxazolidinone antibacterial agents.

Keywords: Oxazolidinone; Heteroaromatic ring substitution; Pyridine; Antibacterial activity; In vivo efficacy.
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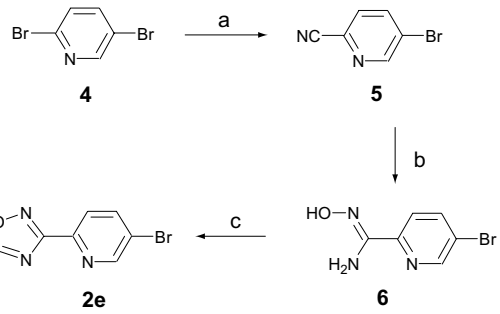
2. Chemistry

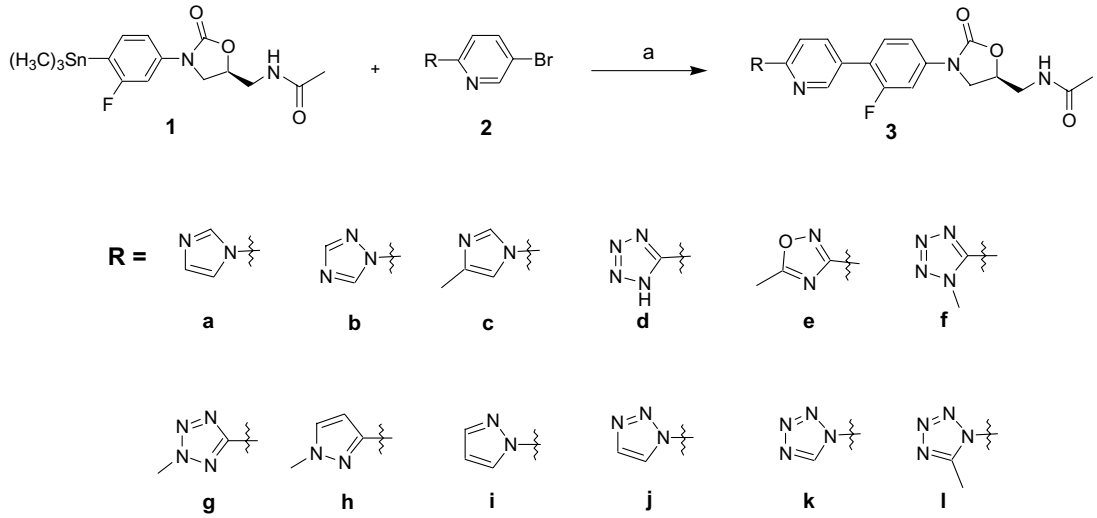
Synthesis of oxazolidinone derivatives is shown in Scheme 1. The Stille coupling reactions[22] of **1**[23] with the appropriate heteroaromatic ring containing pyridine bromides (**2a**–**l**) were carried out in solvent *N*-methyl-2-pyrrolidone with lithium chloride and palladium catalyst, and the products were afforded to 20–50% yield after chromatographic purification on silica gel (chloroform/methanol). Compounds **2a**–**c** and **2i**–**l** were prepared from 2,5-dibromopyridine and necessary reagents. The compounds **2d**,**f**, and **2g** were synthesized with introduction of tetrazole moiety followed by methylation as shown in Scheme 2. The compound **2e** was obtained by reaction of compound **5** first with hydroxylamine and subsequently with acetic anhydride as shown in Scheme 3. The compound **2h** was prepared as described in Scheme 4. Briefly, the compound **5** was treated with methylmagnesium bromide to produce the compound **7** and that was further reacted with DMF dimethylacetal and hydrazine to give compound **9**. Subsequent methylation of **9** afforded the compound **2h** (Scheme 4).



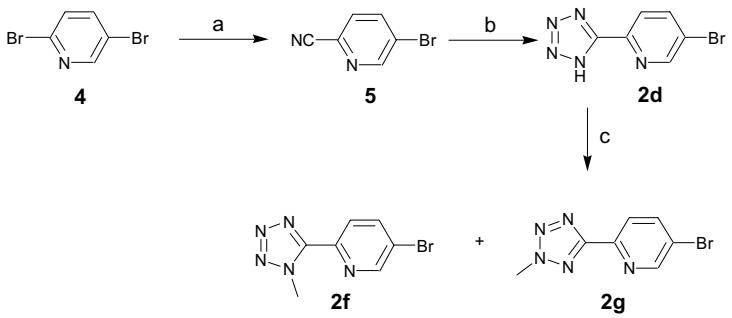
Scheme 3. Reagents and conditions: (a) NaCN, CuCN, DMF; (b) NH_2OH, $NaHCO_3$, EtOH, reflux, 2 h, 93%; (c) Ac_2O, reflux, 2 h, 29%.

3. Results and discussion

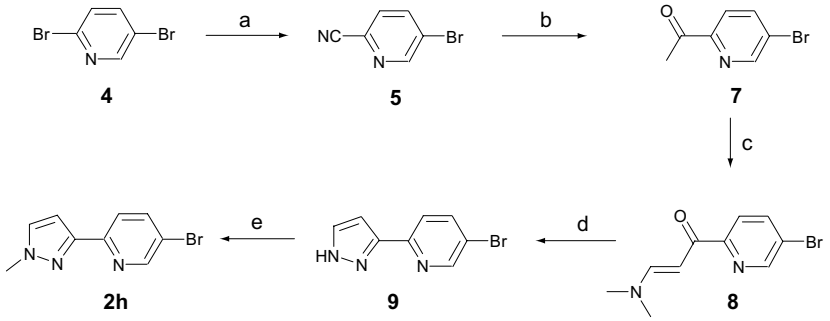
Twelve new oxazolidinone derivatives, bearing heteroaromatic ring substituted pyridine, were synthesized and evaluated for in vitro antibacterial activity against several clinically isolated strains and in vivo efficacy using standard lethal-systematic infection model in



Scheme 1. Synthesis of oxazolidinone derivatives. Reagents and conditions: (a) LiCl, $Pd(PPh_3)_2Cl_2$, NMP, 120 °C, 2 h, 50%.



Scheme 2. Reagents and conditions: (a) NaCN, CuCN, DMF, 150 °C, 3 h, 65%; (b) NaN_3, NH_4Cl, DMF, 120 °C, 4 h; (c) MeI, KOH, DMF, rt, 24 h.



Scheme 4. Reagents and conditions: (a) NaCN, CuCN, DMF; (b) MeMgBr, THF, −20 °C, 2 h; (c) DMF dimethylacetal, reflux, 15 h; (d) hydrazine hydrate, EtOH, reflux, 1 h, 71%; (e) MeI, KOH, DMF, 0 °C, 1 h, 72%.

mice. The in vitro activity was assessed by minimum inhibitory concentration (MIC_{50}) values expressed in μg/mL utilizing standard agar dilution method and the in vivo efficacy was evaluated by determination of effective dose (ED_{50}) expressed in mg/kg.

In order to procure highly potent oxazolidinone derivatives with a long half-life and elucidate the SAR based on the mode of action, we substituted oxazolidinone at the 4-position of phenyl ring of its mother nucleus. In a series of substitutions with hetero rings such as pyridine, pyrimidine, and pyridazine, the pyridine derivatives substituted at the 4-position of phenyl ring showed potent antibacterial activities than the pyrimidine and pyridazine derivatives (data not shown). Further substitutions using heteroaromatic ring were added to pyridine ring and their in vitro and in vivo activities were compared to those of linezolid. The results of the MIC_{50} and ED_{50} are presented in Table 1. These substituted compounds with heteroaromatic ring at pyridine (**3a**–**l**) exhibited the enhanced activity with 4–16-fold against six strains compared to linezolid. Especially, the compounds substituted with triazole (**3b**), oxadiazole (**3e**), and tetrazole (**3f**) showed high in vitro and in vivo activities. Among these derivatives, the tetrazole substituted oxazolidinone compound **3f** was found to be the most active compound with MICs of 0.39, 0.78, 0.39, 0.2, 1.56, and 3.13 μg/mL against MSSA, MRSA, VRE, PRSP, *M. catarrhalis*, and *H. influenzae*, respectively, and ED_{50} of 3.4 mg/kg. These superior antibacterial activities might be interpreted that the increased hydrophobic interaction of these heteroaromatic ring moieties in the oxazolidinone structure with 23S rRNA binding pocket might endow a stronger intrinsic binding attraction for the site of action as suggested by Gandhi et al.[24] although their activities vary with the structures and position or numbers of heteroaromatic ring.

In comparison with linezolid, especially, compound **3f** showed potential activity against *M. catarrhlis* and *H. influenzae* (MIC: 0.78 and 3.13 μg/mL, respectively) where linezolid was practically inactive (MIC: 6.25 and 12.5 μg/mL, respectively). However, the compounds substituted with diazole (**3c**,**h**, and **3j**) exhibited low

Table 1. In vitro and in vivo activities of oxazolidinone derivatives

Compound	MIC_{50} (μg/mL)						ED_{50}[g] (mg/kg)
	MSSA[a] (30)	MRSA[b] (20)	VRE[c] (21)	PRSP[d] (32)	*M. cat*[e] (35)	*H. inf*[f] (35)	
3a	0.39	0.39	0.2	0.2	1.56	6.25	12.0
3b	0.39	0.39	0.2	0.2	1.56	6.25	3.9
3c	1.56	1.56	0.39	—	—	>25	—
3d	—	>25	25	—	—	—	—
3e	0.39	0.78	0.39	0.2	1.56	6.25	3.9
3f	0.39	0.78	0.2	0.2	1.56	3.13	3.4
3g	0.78	0.78	0.2	—	—	6.25	6.7
3h	—	0.78	0.39	—	—	50	11.1
3i	0.78	0.78	0.39	—	1.56	12.5	>80
3j	0.39	0.39	0.2	0.1	1.56	3.13	14.9
3k	0.39	0.39	0.2	0.1	1.56	6.25	6.1
3l	—	0.78	0.39	—	—	3.13	8.1
Linezolid	3.13	3.13	1.56	1.56	6.25	12.5	8.0

[a] Methicillin-susceptible *S. aureus*.
[b] Methicillin-resistant *S. aureus*.
[c] Vancomycin-resistant enterococci.
[d] Penicillin-resistant *Streptococcus pneumoniae*.
[e] *Moraxella catarrhalis*.
[f] *Haemophilus influenzae*.
[g] Mouse was infected intraperitoneally with mucin suspension of *S. aureus* Smith and test compounds were administered orally 1 h after infection. ED_{50} was the amount of antibiotic in milligrams per kilogram of body weight required to cure 50% of the infected mice.

activity against *H. influenzae* with MICs of >12.5 μg/mL and low in vivo activity even though the antibacterial activity against gram-positive bacteria was nearly the same as that of **3f**. In case of tetrazole substitution, the effects of substituted forms of tetrazole ring and the position of methyl group in tetrazole ring on their antibacterial activity were briefly investigated. Antibacterial activities of the different-types of tetrazole ring structures substituted with methyl group like **3f**, **3g**, and **3k**, **3l** maintained the biological activity. However, the replacement of the methyl moiety with free acidic portion as found in **3d** resulted in complete loss of antibacterial activity against gram-positive bacteria. Thus, the presence of methyl group and its orientation can affect activity in these structures. Furthermore, compound **3f** showed a long in vivo half-life of 12.4 h in rats compared to 0.9 h of linezolid, and it could increase administration intervals in clinical use. This might be correlated with the higher in vivo activity than linezolid.

In conclusion, a series of heteroaromatic ring substituted pyridinyl oxazolidinone antibacterial agents were discovered with in vitro activity against clinically relevant resistant gram-positive organisms, *M. catarrhlis* and *H. influenzae* and with a long half-life. Diverse substituted forms of heteroaromatic ring are tolerated on the pyridine and the presence or orientation of methyl group in the heteroaromatic ring affected the antibacterial activity. Three of the most potent compounds, **3b**,**e**, and **3f**, were active against a variety of clinically relevant resistant gram-positive organisms and *M. catarrhlis* and *H. influenzae*, and also showed the higher in vivo efficacy with longer half-lives than linezolid.

4. Experimental

MICs were determined by the twofold serial agar dilution method with Mueller–Hinton agar.[25] In vivo experiments were also conducted according to the previously reported method.[26] The melting points were determined on Mettler Toledo FP62 apparatus. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrophotometer by $CDCl_3$ or DMSO-d_6 and TMS as an internal standard (chemical shift in δ ppm). IR spectra were measured on Nicolet Magna 550 Series II IR spectrophotometer. The mass spectra were recorded on a LC/GC–MS/MS, High Resolution Tandem Mass Spectrometer. The results were within ±0.4% of the theoretical values.

4.1. Synthesis

General procedure for the synthesis of oxazolidinone derivatives, (**3a**–**l**): To the solution of intermediate **1** in *N*-methyl-2-pyrrolidone at room temperature was added side chain (**2a**–**l**), lithium chloride and dichlorobistriphenylphosphine palladium (II). The reaction mixture was kept at 120 °C for 4 h with stirring and water was added thereto. The organic layer obtained after extraction with ethyl acetate was dehydrated, filtered, and concentrated and the residue was subjected to column chromatography to give **3a**–**l**.

4.2. (*S*)-[N-3-(4-(2-(1-methyl-5-tetrazolyl)-pyridin-5-yl)-3-fluorophenyl)-2-oxo-5-oxazolidinyl]methyl acetamide (3f)

This was obtained from the 2-(1-methyl-5-tetrazolyl)-5-bromopyridine (**2f**) (510 mg, 2.12 mmol) and intermediate **1** (1.17 g, 2.82 mmol); yield 530 mg (1.28 mmol, 45%), mp 217–219 °C. ^{1}H NMR ($CDCl_3$): δ 8.89 (s, 1H, pyridine), 8.29 (d, 1H), 8.00 (m, 1H), 7.61 (dd, 1H, ArH), 7.46 (t, 1H), 7.26 (dd, 1H), 6.12 (t, 1H, –NH–), 4.80 (m, 1H, –CH–), 4.45 (s, 3H, –NCH_3), 4.07 (t, 1H), 3.83 (dd, 1H), 3.67 (m, 2H), 2.02 (s, 3H, –$COCH_3$). IR (KBr, cm^{-1}): 3430, 3036, 2987, 2953, 1743, 1677, 1226. HRMS $C_{19}H_{18}FN_7O_3$, calcd (M): 411.1455; meas.: 411.1460 (Table 2).

4.3. 2-(1,2,3,4-Tetrazolyl)-5-bromopyridine (2k)

To the solution of 2,5-dibromopyridine (1.0 g, 4.22 mmol) in *N*-methyl-2-pyrrolidone (10 mL), 1,2,3,4-tetrazole (0.5 g, 7.14 mmol) along with 1.75 g of potassium carbonate was added. The reaction mixture was heated at 100 °C for 3 h with stirring. After completion of the reaction, the reaction mixture was added to water and extracted with ethyl acetate. The organic layer thus obtained was dehydrated, filtered, and concentrated and the concentrate was subjected to column chromatography to give **2k** (0.8 g, 3.54 mmol, 84%). TLC (ethyl acetate/hexane: 1:4, R_f: 0.4), ^{1}H NMR (DMSO-d_6): δ 10.17 (s, 1H), 8.80 (d, 1H), 8.40 (dd, 1H), 8.00 (d, 1H).

4.4. 2-Cyano-5-bromopyridine (5)

2,5-Dibromopyridine (10.0 g, 42.21 mmol) was dissolved in dimethylformamide (100 mL) and copper cyanide (3.0 g, 33.50 mmol) and sodium cyanide (1.7 g, 34.69 mmol) were added to this solution. The reaction mixture was heated to reflux for 3 h. After completion of the reaction, the reaction mixture was added to the water and extracted with ethyl acetate. The organic layer obtained was dehydrated, filtered, and concentrated. The crude product was triturated with *n*-hexane to afford **5** (5 g, 27.32 mmol, 65%) after filtration. TLC (ethyl acetate/hexane: 1:8, R_f: 0.5), ^{1}H NMR ($CDCl_3$): δ 8.76 (s, 1H), 7.96 (dd, 1H), 7.57 (dd, 1H).

4.5. 2-(5-1,2,3,4-Tetrazolyl)-5-bromopyridine (2d)

To the solution of **5** (4.5 g, 24.59 mmol) in dimethylformamide (50 mL) at room temperature were added ammonium chloride (4.2 g, 78.52 mmol) and sodium azide (2.4 g, 36.92 mmol). After stirring at 120 °C for 4 h, the reaction mixture was added to ice water and controlled to pH 2 with a 6 N HCl solution. After additional stirring for 1 h at room temperature, the reaction mixture was extracted with ethyl acetate. The organic layer thus collected was dehydrated, filtered, and concentrated to give crude **2d**, which was used for next step without further purification. TLC ($CHCl_3$/MeOH: 5:1, R_f: 0.2), ^{1}H

Table 2. Characterization data of target compounds

3a	Yield: 35%; mp: 228 °C; ^{1}H NMR (DMSO-d_6): δ 8.70 (s, 1H), 8.58 (s, 1H), 8.21 (m, 2H), 7.99 (m, 2H), 7.69 (m, 2H), 7.48 (dd, 1H), 7.14 (s, 1H), 4.77 (m, 1H), 4.19 (t, 1H), 3.78 (dd, 1H), 3.43 (t, 2H), 1.83 (s, 3H); HRMS $C_{20}H_{18}FN_5O_3$, calcd (M): 395.1394; meas.: 395.1363
3b	Yield: 32%; mp: 224–225 °C; ^{1}H NMR (DMSO-d_6): δ 9.41 (s, 1H, triazole), 8.90 (d, 1H, pyridine), 8.72 (s, 1H), 8.33 (s, 1H, triazole), 8.29–8.25 (m, 2H, –NH–, pyridine), 7.99 (d, 1H), 7.76–7.62 (m, 2H, ArH), 7.46 (dd, 1H), 4.76 (m, 1H, –CH–), 4.17 (t, 1H), 3.79 (dd, 1H), 3.43 (t, 2H), 1.83 (s, 3H, –OCH_3); IR (KBr, cm^{-1}): 3347, 3123, 2988, 2928 1739, 1417, 987; HRMS $C_{19}H_{17}FN_6O_3$, calcd (M): 396.1346; meas.: 396.1350
3c	Yield: 40%; ^{1}H NMR (DMSO-d_6): δ 8.74 (s, 1H), 8.21 (m, 2H), 7.74 (m, 2H), 7.68 (m, 2H), 7.48 (dd, 1H), 6.94 (dd, 1H), 4.76 (m, 1H), 4.18 (t, 1H), 3.80 (dd, 1H), 3.43 (t, 2H), 2.25 (s, 3H), 1.87 (s, 3H); HRMS $C_{21}H_{20}FN_5O_3$, calcd (M): 409.1550; meas.: 409.1512
3d	Yield: 35%; mp: 236 °C; ^{1}H NMR (DMSO-d_6): δ 8.97 (s, 1H, pyridine), 8.29 (m, 3H), 7.72 (t, 1H, ArH), 7.65 (dd, 1H), 7.47 (dd, 1H), 4.78 (m, 1H, –CH–), 4.18 (t, 1H), 3.81 (dd, 1H), 3.44 (dd, 2H), 1.83 (s, 3H, –OCH_3); IR (KBr, cm^{-1}): 3333, 2973, 2887, 2727, 1754, 1624, 1395, 842; HRMS $C_{18}H_{16}FN_7O_3$, calcd (M): 397.1299; meas.: 397.1304
3e	Yield: 36%; mp: 215 °C; ^{1}H NMR ($CDCl_3$): δ 8.85 (s, 1H, pyridine), 8.09 (d, 1H), 7.97 (m, 1H), 7.58 (dd, 1H, ArH), 7.45 (t, 1H), 7.26 (dd, 1H), 6.50 (t, 1H, –NH–), 4.81 (m, 1H, –CH–), 4.10 (t, 1H), 3.84 (dd, 1H), 3.68 (m, 2H), 2.67 (s, 3H, oxadiazole), 2.01 (s, 3H, –OCH_3); IR (KBr, cm^{-1}): 3306, 3096, 1774, 1761, 1407, 1221; HRMS $C_{20}H_{18}FN_5O_4$, calcd (M): 411.1343; meas.: 411.1340
3f	Yield: 50%; mp: 217–219 °C; ^{1}H NMR ($CDCl_3$): δ 8.89 (s, 1H, pyridine), 8.29 (d, 1H), 8.00 (m, 1H), 7.61 (dd, 1H, ArH), 7.46 (t, 1H), 7.26 (dd, 1H), 6.12 (t, 1H, –NH–), 4.80 (m, 1H, –CH–), 4.45(s, 3H, –NCH_3), 4.07 (t, 1H), 3.83 (dd, 1H), 3.67 (m, 2H), 2.02 (s, 3H, –$COCH_3$); IR (KBr, cm^{-1}): 3430, 3036, 2987, 2953, 1743, 1677, 1226, HRMS $C_{19}H_{18}FN_7O_3$, calcd (M): 411.1455; meas.: 411.1460
3g	Yield: 28%; ^{1}H NMR (DMSO-d_6): δ 8.92 (s, 1H), 8.35 (m, 3H), 7.79 (m, 1H), 7.70 (dd, 1H), 7.46 (dd, 1H), 4.78 (m, 1H), 4.44 (s, 3H), 4.14 (t, 1H), 3.84 (dd, 1H), 3.43 (t, 2H), 1.83 (s, 3H); HRMS $C_{19}H_{18}FN_7O_8$, calcd (M): 411.1455; meas.: 411.1486
3j	Yield: 35%; ^{1}H NMR (DMSO-d_6): δ 8.88 (s, 1H), 8.76 (s, 1H), 8.28 (d, 1H), 8.21 (d, 1H), 8.01 (s, 1H), 7.70 (m, 2H), 7.51 (d, 1H), 4.76 (m, 1H), 4.17 (t, 1H), 3.79 (m, 1H), 3.42 (t, 2H), 1.83 (s, 3H); HRMS $C_{19}H_{17}FN_6O_3$, calcd (M): 396.1346; meas.: 396.1332
3k	Yield: 20%; mp: 210 °C; ^{1}H NMR (DMSO-d_6): 10.22 (s, 1H, tetrazole), 8.83 (s, 1H, pyridine), 8.39 (dd, 1H, pyridine), 8.30 (t, 1H, –NH–), 8.15 (d, 1H, pyridine), 7.76–7.64 (m, 2H, ArH), 7.47 (dd, 1H), 4.78 (m, 1H, –CH–), 4.16 (t, 1H), 3.80 (dd, 1H), 3.43 (t, 2H),1.83 (s, 3H, –OCH_3); IR (KBr, cm^{-1}): 3408, 3113, 2927, 1740, 1667, 1225; HRMS: $C_{18}H_{16}FN_7O_3$, calcd (M): 397.1299; meas.: 397.1307
3l	Yield: 37%; ^{1}H NMR (DMSO-d_6): δ 8.83 (s, 1H), 8.34 (d, 1H), 8.24 (t, 1H, NH), 8.07 (d, 1H), 7.77 (t, 1H), 7.65 (dd, 1H), 7.48 (dd, 1H), 4.77 (m, 1H), 4.18 (t, 1H), 3.80 (dd, 1H), 3.43 (t, 2H), 2.82 (s, 3H), 1.83 (s, 3H); HRMS $C_{19}H_{18}FN_7O_3$, calcd (M): 411.1455; meas.: 411.1442

NMR (DMSO-d_6): δ 8.91 (br s, 1H), 8.31 (br s, 1H), 8.13 (br, 1H).

4.6. 2-(1-Methyl-5-tetrazolyl)-5-bromopyridine (2f) and 2-(2-methyl-5-tetrazolyl)-5-bromopyridine (2g)

To the solution of crude **2d** (6.0 g, 26.55 mmol) in dimethylformamide (60 mL) were added iodomethane (15 g, 106.18 mmol) and potassium hydroxide (3.7 g, 66.37 mmol). After stirring for 24 h at room temperature, work-up as described before followed by column chromatography gave the polar desired product **2f** (1.0 g, 4.17 mmol) along with the less polar **2g** (2.2 g, 9.16 mmol) as a by-product.

Compound **2f**: TLC (ethyl acetate/hexane: 1:4, R_f: 0.3), ^{1}H NMR ($CDCl_3$): δ 8.80 (d, 1H), 8.11 (d, 1H), 7.96 (dd, 1H), 4.42 (s, 3H from CH_3 on tetrazole ring).

Compound **2g**: TLC (ethyl acetate/hexane: 1:4, R_f: 0.5), ^{1}H NMR ($CDCl_3$): δ 8.77 (d, 1H), 8.25 (d, 1H), 8.03 (dd, 1H), 4.48 (s, 3H, from CH_3 on tetrazole ring).

Compounds **2f** and **2g** were easily distinguished from each other by comparison of the chemical shifts of CH_3 on tetrazole ring and their polarities on TLC.[27,28]

4.7. 2-(2-Amino-1-oxime)-5-bromopyridine (6)

To the solution of **5** (2.0 g, 10.93 mmol) in EtOH (20 mL) were added hydroxylamine (3.8 g, 54.68 mmol) and sodium hydrogen carbonate (4.6 g, 54.76 mmol) and the reaction mixture was heated to reflux for 2 h. The ordinary work-up was conducted to give crude **6** (2.2 g, 10.18 mmol, 93%). TLC (ethyl acetate/hexane: 1:1, R_f: 0.2), ^{1}H NMR (DMSO-d_6): δ 10.02 (s, 1H), 8.65 (d, 1H), 8.01 (dd, 1H), 7.78 (dd, 1H).

4.8. 2-(5-Methyl-1,2,4-oxadiazol-3-yl)-5-bromopyridine (2e)

Compound **6** (8.6 g, 39.81 mmol) was dissolved in acetic anhydride (250 mL) and the solution was heated to reflux for 2 h. Excess acetic anhydride was removed under reduced pressure and the resulting residue was purified on column chromatography to yield **2e** (2.8 g, 11.66 mmol, 29%). TLC (ethyl acetate/hexane: 1:2, R_f: 0.7), ^{1}H NMR ($CDCl_3$): δ 8.80 (dd, 1H), 7.96 (dd, 2H), 2.67 (s, 3H).

4.9. 2-Acetyl-5-bromopyridine (7)

To the solution of **5** (1 g, 5.46 mmol) in THF (50 mL) at -20 °C was added drop by drop methyl magnesiumbromide (54 mL). And the reaction mixture was stirred for 2 h and warmed up to room temperature slowly. The ordinary work-up was conducted to give crude **7** (0.5 g, 2.50 mmol), which was used without further purification. TLC (ethyl acetate/hexane: 1:4, R_f: 0.6), ^{1}H NMR ($CDCl_3$): δ 8.70 (dd, 1H), 7.92 (dd, 2H), 2.67 (s, 3H).

4.10. 2-(3-Pyrazolyl)-5-bromopyridine (9)

2-Acetyl-5-bromopyridine (**7**) (2 g, 10.00 mmol) was refluxed in dimethylformamide dimethylacetal for 15 h. After evaporating excess solvent, the resulting residue (crude **8**) was dissolved in ethanol and hydrazine hydrate (5 mL, 99.88 mmol) was added thereto. The mixture was refluxed for 1 h. The crude product obtained from the ordinary work-up was further purified by column chromatography to give the desired product **9**. It was purified by column chromatography to produce the desired product **9** (1.6 g, 7.14 mmol, 71%). TLC (ethyl acetate/hexane: 1:4, R_f: 0.1), ^{1}H NMR ($CDCl_3$): δ 11.56 (s, 1H, NH), 8.68 (d, 1H), 7.83 (dd, 1H), 7.70 (d, 1H), 7.66 (s, 1H), 6.81 (d, 1H).

4.11. 2-(1-Methyl-3-pyrazolyl)-5-bromopyridine (2h)

To the solution of **9** (300 mg, 1.34 mmol) in dimethylformamide were added iodomethane (760 mg, 5.36 mmol) and potassium hydroxide (230 mg, 4.10 mmol). After stirring for 1 h at 0 °C, work-up as described before and purification by column chromatography gave the desired product **2h** (230 mg, 0.97 mmol, 72%). TLC (ethyl acetate/hexane: 1:1, R_f: 0.4), ^{1}H NMR ($CDCl_3$): δ 8.63 (t, 1H), 7.78 (m, 2H), 7.38 (d, 1H), 6.81 (d, 1H), 3.94 (s, 3H).

4.12. In vitro activity

The MICs were determined using an agar dilution method by NCCLS guidelines. An overnight culture was inoculated with an inoculating device onto Muller–Hinton agar containing serial twofold dilutions of the test compounds. Organisms were incubated at 37 °C for 18–24 h. The MIC of a compound was defined as the lowest concentration that visibly inhibited growth.

4.13. In vivo efficacy

Four-week-old male outbred ICR mice (18–20 g) were given an intraperitoneal injection of *S. aureus* Smith (2.9×10^7 CFU/mice) suspended in 5% mucin. Eight mice per group were used. Test compounds were orally administered 1 h after bacterial infection. Mice were observed for mortality over seven days. The total number of survivors at each dose was used to calculate the effective dose that protected 50% of the infected mice from death (ED_{50}). ED_{50} and 95% confidence limits determinations were performed by Probit analysis within each test.

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