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Designing, synthesis and antibacterial evaluation of novel oxazolidinone derivatives nitrogen-containing fused heterocyclic moiety

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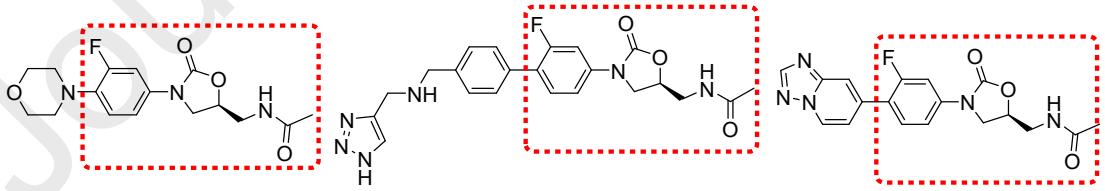
Abstract:

A series of novel oxazolidinone derivatives with nitrogen-containing fused heterocyclic moiety were designed and synthesized in this article. Their antibacterial activities were measured against *S.aureus*, MRSA and MSSA by MIC assay. Most of them exhibited potent activity against Gram-positive pathogens comparable to Linezolid and Radezolid. Compound **3b**, which exhibited significant antibacterial activity with MIC values ranging 0.5-1.0 μg/mL, might be a promising drug candidate for further investigation.

Keywords: Oxazolidinone derivatives; Nitrogen-containing fused heterocyclic moiety; Gram-positive bacteria; Antibacterial activities

Introduction

In recent years, with the widespread use and even abuse of antibacterial drugs, antibiotic-resistant infections have gradually become one of the most common diseases and threatened human health[1-5]. Among them, the resistance problem of Gram-positive bacteria (G^+) is particularly serious, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus Aureus* (MSSA), *etc* [6-7]. Thus, finding effective ways to reduce antibiotic-resistant crisis is an urgent task[8].



Linezolid Radezolid I

Fig. 1. Chemical structures of oxazolidinones

Oxazolidinones are a class of antibacterial agents against a variety of G^+, including MRSA, MSSA, and vancomycin-resistant Enterosphere (VRE), which are binded to 50S ribosomal subunit

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to inhibit bacterial protein biosynthesis[9-11]. Linezolid (**Fig.1**), the first clinically useful oxazolidinone antibacterial agent, has greatly alleviated the severe situation of G^+ infection[12]. Unfortunately, Linezolid-resistant Staphylococcus aureus and Enterococcus spp have emerged due to the overuse of Linezolid[13-14]. Thus, it is imperative to develop new oxazolidinone-type drug candidates against drug-resistant pathogens. The cocrystal structure of Linezolid shows that the oxazolidinone moiety occupied the domain and was engaged in a hydrogen bond with G 2540 (**Fig. 2**). But the morpholino moiety did not occupied the cavity completely. And we find there was no obvious interaction between the Linezolid terminal morpholine ring and the 50S ribosomal subunit [15]. Therefore, there has been considerable interests in the modification of the morpholine part of Linezolid.

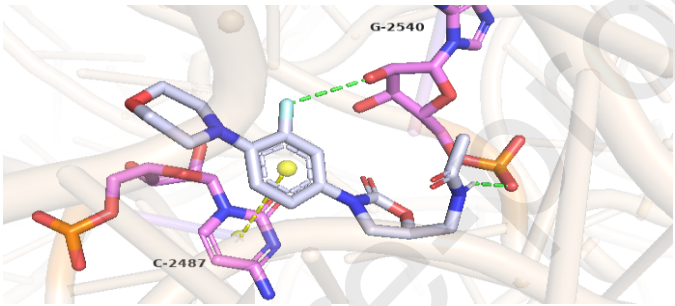


Fig. 2. The Co-crystal structure analysis of Linezolid

Radezolid (**Fig. 1**), which has a triazole ring as the "tail", was developed by Rib-X Laboratories. It is one of the most active and broad-spectrum antimicrobials in clinical development. The drug showed excellent antibacterial activity against G^+ such as enterococcus, staphylococcus and streptococcus (MIC range 0.125-1 μg/mL) [16]. As reported, oxazolidinone derivatives that contains biaryl scaffold showed increased antibacterial potency against G^+, especially antibiotic-resistant G^+ by forming π-π stacking interactions with the 50S subunit compared with Linezolid[17-18].

Hideyuki Suzuki and his coworkers replaced the morpholine ring of Linezolid with pyrazole [1,5-a] pyridine ring[19-21] and synthesized a series of new oxazolidone compounds, in which, compound I (**1**, **Fig. 3**)) exhibited potent antibacterial activiey with MIC values 0.25 and 0.5 μg/ mL against Staphylococcus aureus and Enterococcus faecalis, respectively. [21-22]

Hence, to overcome antibacterial resistance and discover a new oxazolidinone with the better activity, the modification of compound I and Radezolid provide new analogues with moderate to potent antibacterial activity. Based on our previous work in the laboratory[24-26] and the structure-activity relationship of oxazolidone, we utilized the biaryl structure of Radezolid to keep the π-π stacking with the 50S subunit (**Fig. 4**). At the same time, we introduced heterocycle moiety of compound **I** into the target compounds with the aim of trying to find new possible binding mode and occupy the cavity as far as possible. Compound **3a** was synthesized firstly in our work, while the antibacterial activity was weaker than Linezolid. Hence, there was an assumption that if introduction of nitrogen atom could enhance the activity in the "tail" of compound or not. Then, a

series of oxazolidinone derivatives containing nitrogen-containing fused heterocycle moieties (**3b-3h**) were synthesized to occupy the cavity as complete as possible. Of them, compound **3b** inhibited S.aureu, MIRSA and MASS with MIC values of 0.5 μg/mL, 0.5 μg/mL and 0.5 μg/mL respectively, which was more active than Linezolid.

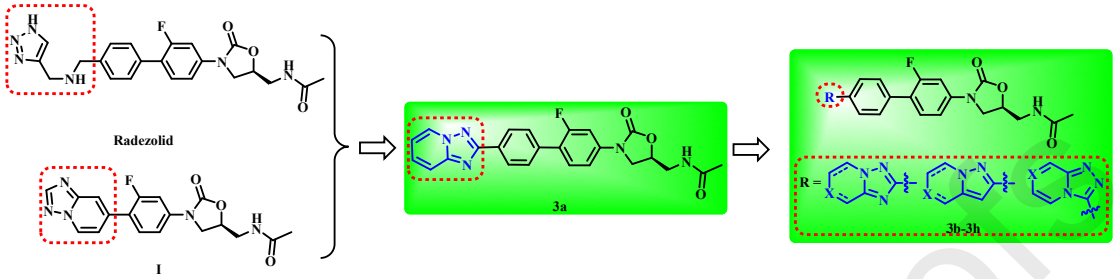
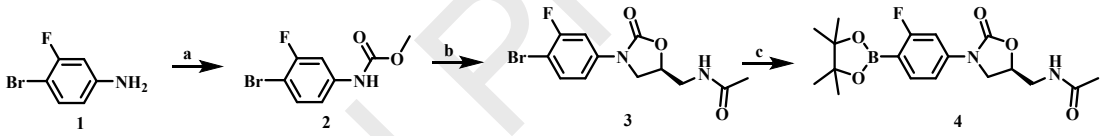


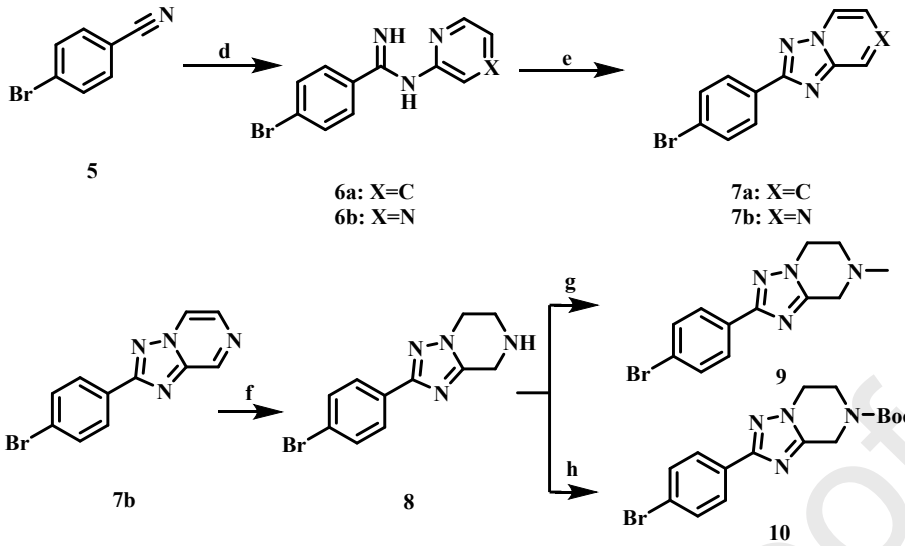
Fig. 3. Design strategies for the novel oxazolidinone analogues containing a nitrogen-containing fused heterocycle

The synthetic route of key intermediate **4** was depicted in **Scheme 1**. Intermediate **2** was prepared from commercially available 4-bromo-3-fluoroaniline **1** by acylation with chloroformate in dichloromethane. Subsequently, intermediate **2** was reacted with (S)-1-((acetylamino)methyl)-2-chloroethyl acetate in the presence of *t*-BuOLi to afford intermediate **3**. And then Miyaura coupling occurred in intermediate **3** and bis(pinacolato)diboron to yield the key intermediate **4**.



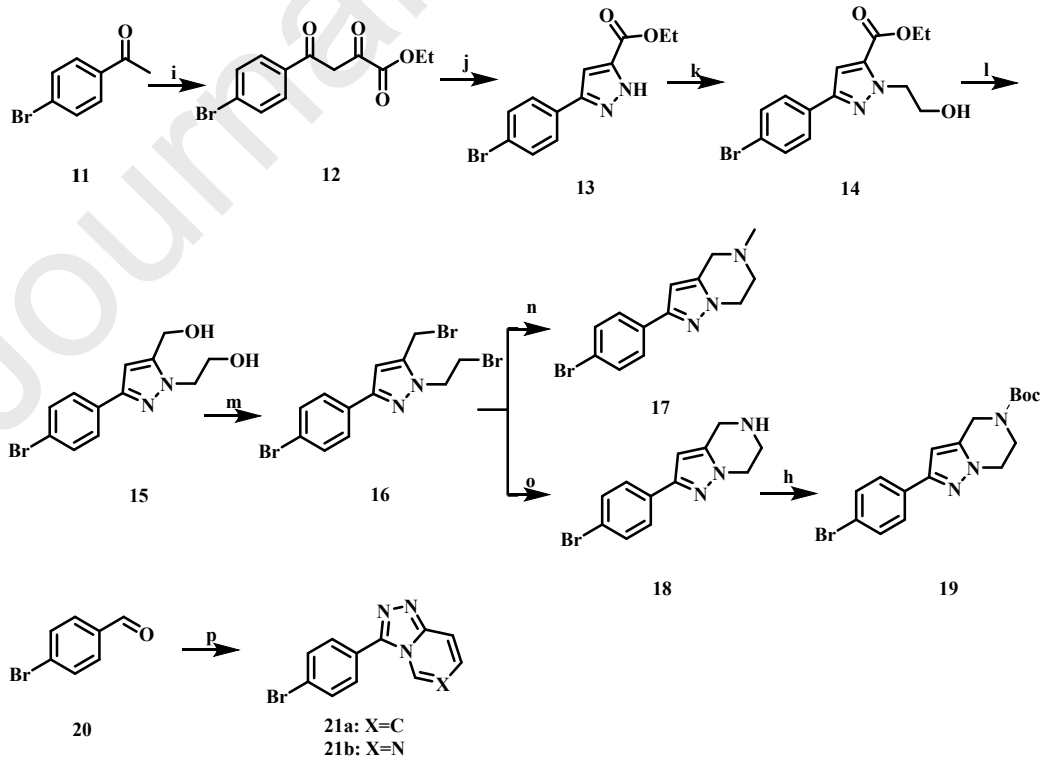
Scheme 1. Synthetic route of key intermediate 4. Reagents and conditions: (a) methyl chloroformate, pyridine, DCM, 25 °C, 2 h; (b) (S)-1-((acetylamino)methyl)-2-chloroethyl acetate, *t*-BuOLi, DMF, CH_3OH, r.t, 20 h; (c) bis(pinacolato)diboron, $Pd(PPh_3)_2Cl_2$, potassium acetate, 1,4-dioxane, 90 °C, 20 h.

The synthetic route of intermediates **7a**, **7b**, **9** and **10** was depicted in **Scheme 2**. The intermediates **7a** and **7b** were prepared from 4-bromobenzonitrile by condensation and cyclization. Intermediate **8** was prepared from intermediate **7b** by reduction of pyrazine, which then underwent *N*-methylation in the presence of iodomethane to form intermediate **9**. Intermediate **8** reacted with di-*tert*-butyl pyrocarbonate to form intermediate **10**.



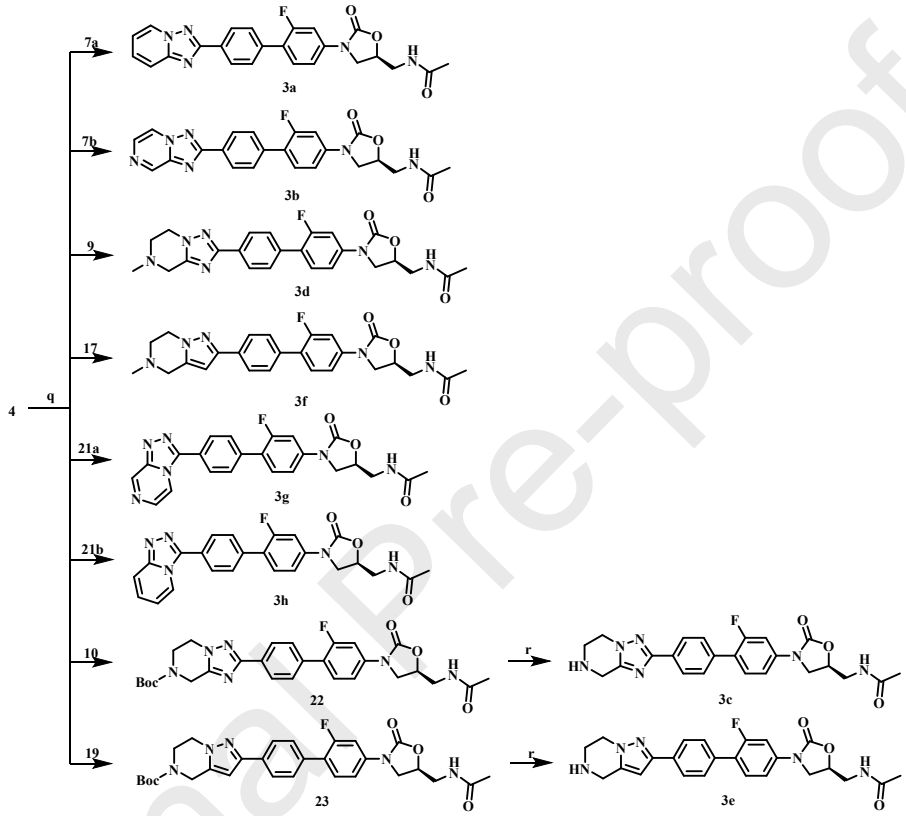
Scheme 2. Synthetic route of key intermediates 7a, 7b, 9 and 10. Reagents and conditions: (d) 2-aminopyridine or 2-aminopyrazine, NaH, DMF, 25 °C, 10 h; (e) I_2, KI, K_2CO_3, DMSO, 120 °C, 8 h; (f) sodium borohydride, $Pd(PPh_3)_2Cl_2$, DCM/EtOH, 25 °C, 24 h; (g) iodomethane, K_2CO_3, DMF, 25 °C, 10 h; (h) di-tert-butyl pyrocarbonate, DIPEA, DMAP, DMF, 25 °C, 5 h.

The routes to synthesize intermediates **17**, **19**, **21a** and **21 b** were depicted in **Scheme 3**. The 4-bromino acetophenone **11** reacted with diethyl oxalate to form intermediate **12**. The pyrazole ring was constructed to intermediate **13** by reaction of **12** with hydrazine hydrate. Treatment of intermediate **13** with 2-bromoethanol gave intermediate **14**, which was reduced in the presence of lithium aluminum hydride in tetrahydrofuran to give 15. Intermediate 15 reacted with PBr_3 to yield key intermediate **16**. Subsequently, construction of the piperazine ring afforded the intermediate **17** and **18**. Intermediate **18** was protected with di-*tert*-butyl pyrocarbonate to yield intermediate **19**. Intermediates **21a** and **21b** were constructed respectively through cyclization.



Scheme 3. Synthetic route of intermediates 17, 19, 21a and 21 b. Reagents and conditions: (i) Na, EtOH,diethyl oxalate, 25 °C, 10 h; (j) hydrazine hydrate, 78 °C, 10 h; (k) 2-bromoethanol, K_2CO_3, DMF, 25 °C, 12 h; (l) $LiAlH_4$, THF, 25 °C, 2 h; (m) PBr_3, DCM, 25 °C, 10 h; (n) methylamine hydrochloride, K_2CO_3, 25 °C, 6 h; (o) NH_3, K_2CO_3, 25 °C, 2 h; (h) di-tert-butyl pyrocarbonate, DIPEA, DMAP, DMF, 25 °C, 5 h; (p) 2-hydrazinylpyridine or 2-hydrazinylpyrazine, I_2, TBHP, K_2CO_3, 1,4-dioxane, 60 °C, 1 h.

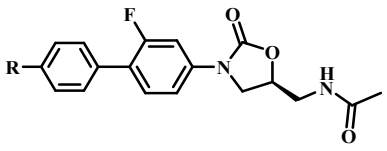
The synthetic route of the target compounds **3a-3h was** indicated in **Scheme 4**. Suzuki coupling reaction of compound **4** with the above intermediates gave the target compounds **3a**-**3b** and **3f-3h**. Compounds **3c** and **3e** were obtained by removing the amino protecting group of intermediate **22** and **23,** respectively.

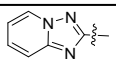
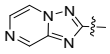
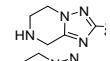
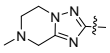
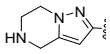
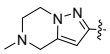


Scheme 3. Synthetic route of target compounds 3a-3h. Reagents and conditions: (q) $Pd(PPh_3)_2Cl_2$, K_2CO_3, DMF/ H_2O, 75 °C, 8-12 h; (r) TFA, DCM, 25 °C, 5h.

The antibacterial activities of the target compounds were evaluated in *vitro* against five bacterial strains including *S.aureus*, MRSA, MSSA, LREF and VRE by MIC assay. The results were shown in **Table 1**.

Table 1 Structures and in vitro antibacterial activities of compound 3a-3h.



Compounds	R	MICs, μg/mL				
		S.aureus[a]	MRSA[b]	MSSA[c]	LREF[d]	VRE[e]
3a		2.0	1.0	2.0	1.0	0.5
3b		0.5	0.5	0.5	0.5	0.5
3c		2.0	2.0	1.0	1.0	1.0
3d		1.0	1.0	1.0	2.0	1.0
3e		2.0	2.0	2.0	2.0	0.5
3f		1.0	1.0	1.0	1.0	0.5
3g		1.0	1.0	1.0	1.0	1.0
3h		2.0	2.0	1.0	1.0	2.0
Linezolid		1.0	1.0	1.0	＞16	2.0
Radezolid		0.5	0.5	0.5	0.5	0.5

[a] Standard *Staphylococcus aureus* (29213).
[b] Methicillin-resistant *Staphylococcus aureus*.
[c] Methicillin-sensitive *Staphylococcus aureus*.
[d] Linezolid-resistant *Enterococcus faecalis*.
[e] Vancomycin-resistant *Enterococcus faecium*.

According to the data in **Table 1**, compounds **3d**, **3f** and **3g** appeared to exhibit similar antibacterial activity in comparison with Linezolid. Of them, compound **3b,** with the MIC value 0.5 μg/mL, was comparable to Linezolid. Compound **3a** and 3h with pyridinyl moiety exhibited weaker in vitro anti-bacterial activity than other compounds. The SARs (structure-activity relationships) of the oxazolidinone derivatives were studied. It was speculated that piperazinyl or pyrazinyl moiety may conducive to the keep of activity. Methylation of compound **3f** on the nitrogen atom of piperazinyl moiety showed 2 fold potency advantages compared to compound **3e**, which deduced that the tertiary amine maybe conducive to the keep of activity when piperazinyl was used. In addition, compound **3e** with pyrazolyl moiety and **3c** with triazolo moiety exhibited similar levels of in *vitro* antibacterial activity. In conclusion, most of compounds exhibited good to moderate antibacterial activity against G^+ and selected antibiotic-susceptible and Linezolid-resistant isolates. Compound **3b** was selected for further clinical isolate investigation in the next experiment.

To elucidate the activity against multiple strains of MDR-*S.aureus*, compounds **3b** was evaluated against several antibiotic-susceptible and antibiotic-resistant clinical isolates, and the results were summarized in **Table 2**. Clinical strains of G^+ included *S.aureus* (4 isolates of MRSA and 4 isolates of MSSA), *E.faecium* (4 isolates of VRE) and *E. faecalis* (4 isolates of LREF), and all the isolates were collected in PLA 309 hospital. Compound **3b** with the R-substituent group replaced by [1,2,4]triazolo[1,5-a]pyrazine displayed increased activity against the tested clinical isolates with MIC values of 0.5-1 μg/mL, which was 32-fold more potent against clinical iso-lates of LREF than that of Linezolid.

Table 2. **In vitro antibacterial activity against clinical isolates.**

Compounds	MICs, μg/mL			
	MRSA[a](4)[b]	MSSA[c](4)	LREF[d](4)	VRE[e](4)
3b	0.5-1	0.5-1	0.5	0.5
Linezolid	1-4	1-4	＞16	1-4

[a]. Methicillin-resistant *Staphylococcus aureus*.
[b] Number of bacterial strains tested are given in parentheses.
[c] Methicillin-sensitive *Staphylococcus aureus*.
[d] Vancomycin-resistant *Enterococcus faecium*.
[e] Linezolid-resistant *Enterococcus faecalis*.

With the interest of the binding modes of compound **3b**, the docking analysis of compound **3b** and Linezolid were performed depended on the crystal structure of 50S ribosome unit of *E. coli* with linezolid (PDB code: 3CPW [15]). As shown in **Fig. 4**, compound **3b** has the similar binding pattern with Linezolid (**Fig. 4,** B). The condensed heteroaromatic moiety of **3b** took over more cavity than the morpholinyl moiety in Linezolid, and had π-π stacking interactions with U 2619 and U 2620 (**Fig. 4,** A), which could result in high antibacterial activity.

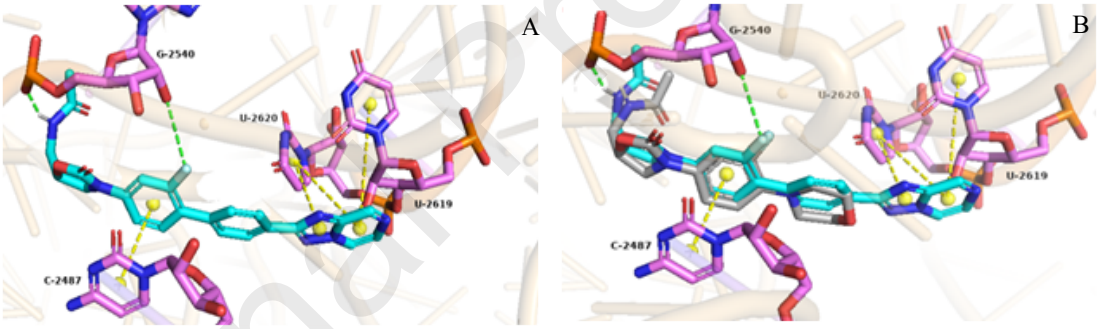


Fig. 4. The Co-crystal structure analysis of **3b**. (A) Predicted binding conformation for **3b** (light blue sticks) and the interaction between **3b** and the binding site cavity, (B) **3b** overlapping with Linezolid (gray sticks).

Then we used the free SwissADME web tool (http://www. swissadme.ch/) to predict physicochemical, ADME parameters and drug-likeness properties of above compounds. As we can see from the Table 3, all the compounds fitted well with Lipinski's rule, which means all these compounds are hopeful to develop into oral drugs. And all the compounds showed no BBB permeability and high GI values, which indicated that these compounds were unlikely to cause CNS adverse effects.

Table 3. In silico Molecular properties prediction at SwissADME.

compounds	MWa	logP[b]	Hbond donors[c]	Hbond acceptors[d]	GI[e]	TPSA[f]	lipinski[g]	logS[h]	BBB[i]
3h	445.45	3.23	1	6	High	88.83	0	-4.87	No

3g	446.43	2.53	1	7	High	101.72	0	-4.20	No
3f	463.50	2.63	1	6	High	79.70	0	-4.02	No
3e	449.48	2.31	2	6	High	88.49	0	-3.65	No
3d	464.49	2.40	1	7	High	92.59	0	-3.88	No
3a	445.45	3.32	1	6	High	88.83	0	-4.60	No
3b	446.43	2.62	1	7	High	101.72	0	-3.93	No
3c	450.47	2.12	2	7	High	101.38	0	-3.51	No
Linezolid	337.35	1.20	1	5	High	71.11	0	-2.22	No
Radezolid	438.45	2.01	3	7	High	112.24	0	-3.12	No

a Molecular weight.
b Logarithm of compound partition coefficient between n-octanol and water (<5).
c Number of hydrogen bond donor (<5).
d Number of hydrogen bond acceptors (<10).
e Human gastrointestinal absorption.
f Topological polar surface area (140).
g Lipinski's rule of five..
h Predicted aqueous solubility.
i Predicted brain/blood partition coefficient.

In summary, a series of novel oxazolidinone derivatives with nitrogen-containing fused heterocyclic moiety was synthesized as potential antibacterial agents and their structures were confirmed by ^{1}H NMR and MS spectra. Their anti-bactierial activities were evaluated against *S. aureus*, MRSA, MSSA, LREF and VRE pathogens and most of them exhibited potent in *vitro* antibacterial activity as compared with Linezolid. Notably, compound **3b** showed the most potent antibacterial activity with MIC values of 0.5-1 μg/mL against five G^{+}, which was comparable to Linezolid. This study provided the researchers more insights about the antibacterial agents.

Acknowledgements

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