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Synthesis and antibacterial evaluation of novel oxazolidinone derivatives containing a piperidinyl moiety

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Abstract：

In this article, a series of novel oxazolidinone derivatives containing a piperidinyl moiety was designed and synthesized. Their antibacterial activities were measured against *S.aureus*, MRSA, MSSA, LREF and VRE by MIC assay. Most of them exhibited potent activity against Gram-positive pathogens comparable to linezolid. Among them, compound **9h** exhibited comparable activity with linezolid against human MAO-A for safety evaluation and showed moderate metabolism in human liver microsome. The most promising compound **9h**, which showed remarkable antibacterial activity against *S.aureus*, MRSA, MSSA, LREF and VRE pathogens with MIC value of 0.25-1 μg/mL, was an interesting candidate for further investigation.

Keywords: Oxazolidinone derivatives; Piperidinyl moiety; Antibacterial activities; Linezolid

The resistance of bacteria developed rapidly and spreads widely throughout the world due to the long-term, inappropriate use and even abuse of antibiotics[1-3]. Among them, the resistance problem of Gram-positive bacteria (G^+) is particularly serious, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *vancomycin-resistant Enterosphere* (VRE), *etc*. The emergence of drug-resistant bacteria has led to a significant prolongation of treatment time and a mortality increase [4, 5]. Thus, it is imperative to develop new agents with great potency against drug-resistant pathogens[6].

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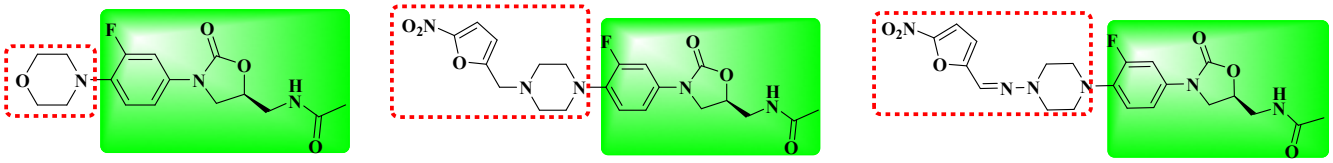


Fig. 1. Chemical structures of linezolid and other oxazolidinones.

Oxazolidinones are a new class of chemically synthesized antibacterial agents against a variety of Gram-positive bacteria, including MRSA, methicillin resistant *Staphylococcus Aureus* (MSSA), VRE, *etc*. The unique antibacterial mechanism of oxazolidinones, which was inhibiting the synthesis of bacterial proteins by binding to the bacterial 50S ribosomal subunit, had attracted much attention[7-10]. The first oxazolidinone antibacterial agent, linezolid (**Fig. 1**), was approved for the treatment of infections caused by multi-drug resistant G^+ bacteria in the United States in April 2000[11]. However, linezolid-resistant *Staphylococcus aureus* and *Enterococcus spp* had appeared since linezolid was released. Linezolid was also an inhibitor of monoamine oxidases (MAOs), which may result in drug−drug interactions with adrenergic and serotonergic agents[12-14]. Thus, there has been considerable interests in new oxazolidinone-type drug candidates[15]. The cocrystal structure of linezolid revealed that the oxazolidinone moiety occupied the active domain and was engaged in a hydrogen bond with G 2540[16]. And the morpholine "tail" was not occupied the cavity, completely (**Fig. 2**). Accordingly, the efficient incorporation of a group into the "tail" has attracted considerable interest among medicinal chemists.

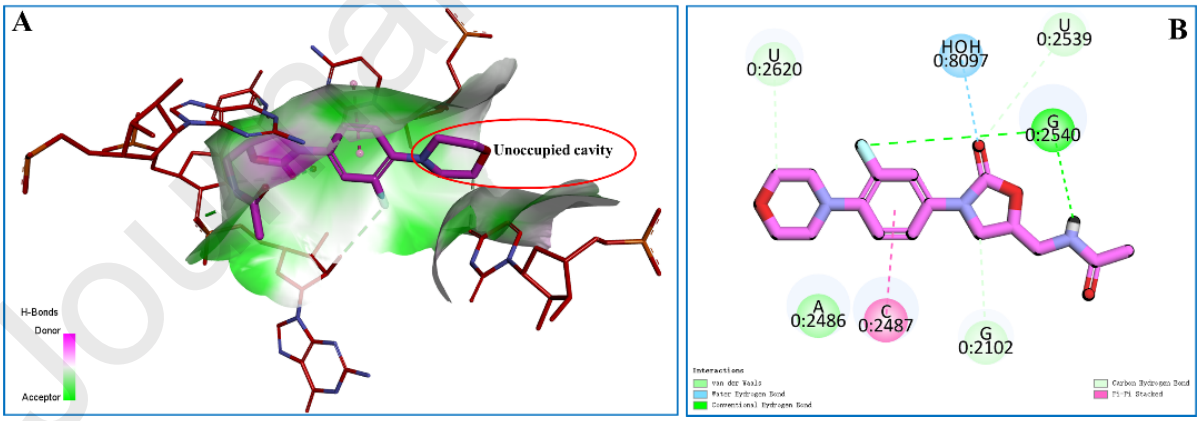


Fig. 2. The Co-crystal structure analysis of linezolid (PDB code: 3CPW)

Ranbezolid (**Fig. 1**), which had a furan ring as the terminal unit, was developed by Ranbaxy Laboratories, and its phase I clinical trial was performed in 2003[17-18]. Docking studies showed that the piperazine ring attached with a 2-methyl-5-nitrofuryl moiety had a higher total score than linezolid. Moreover, the nitrofuryl ring from the oxazolidinone extends to C2507, G2583 and U2584, and the nitro group forms a hydrogen bond with the base of G2583, which makes stronger interaction with the target pathogen[19]. In our previous work, a series of oxazolidinone

derivatives containing nitroheteroaromatic moieties was reported [20]. Of them, compound **I** (**Fig. 1**) inhibited *S.aureu*, MRSA and VRE with MIC values of 0.5 μg/mL, 1 μg/mL and 1 μg/mL, respectively, which was more active than Ranbezolid. However, it was contemplated that other antimicrobial compounds containing nitrofuryl group can be catalyzed in *vivo* by related enzymes into the nitro group through an active nitroso radical intermediate, which in turn produces cytotoxicity[21, 22]. At the same time, the presence of the hydrazone bond rendered the compound rigid and insoluble, and easily hydrolyzed, which was detrimental to metabolic stability.

Delamanid was initially approved by the European Medicines Agency (EMA) in 2014 for the treatment of adult pulmonary multi-drug resistant (MDR)-TB when an effective treatment regimen cannot otherwise be composed for reasons of resistance or tolerability. It has been approved by several other countries/regions.[23] The phenoxypiperidinyl "tail" of delamanid was necessary for the maintaining the antibacterial activities. In a continuing effort to develop new drug candidates, we herein described the design, synthesis, and antibacterial activities of a series of novel oxazolidinone derivatives. In these derivatives, the nitrohetero aromatic ring of compound **I** was replaced with various substituted phenyl group or benzyl group to avoid toxic side effects, and the piperazinyl group was replaced with a piperidinyl moiety by the bioisosteric principle caused by the hydrazone. At the same time, a NH acted as a hydrogen bond donor was introduced as a linker between the phenyl ring and piperidinyl group to increase the antibacterial activity (**Fig. 3**).

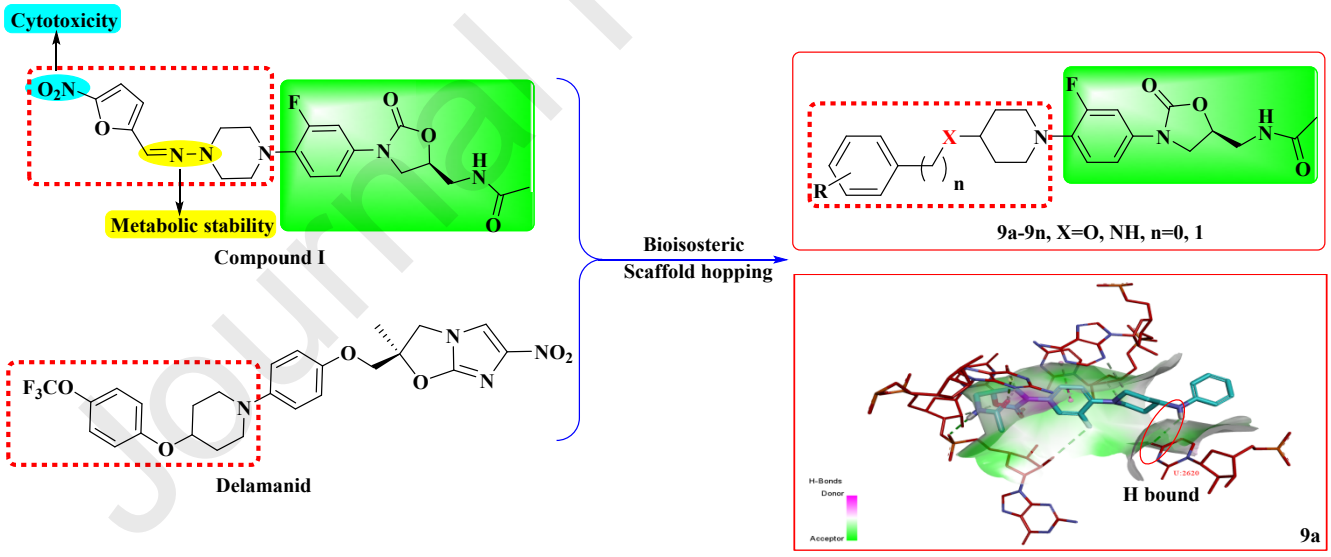
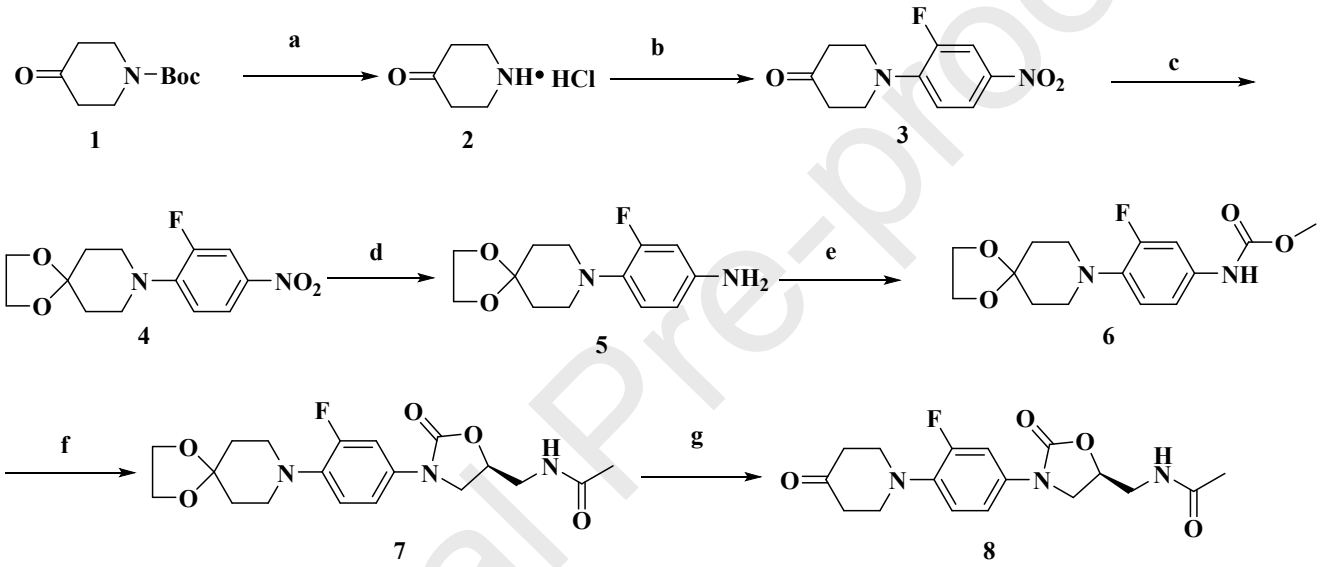


Fig. 3. Design strategies for the novel oxazolidinone analogues containing a piperidinyl moiety.

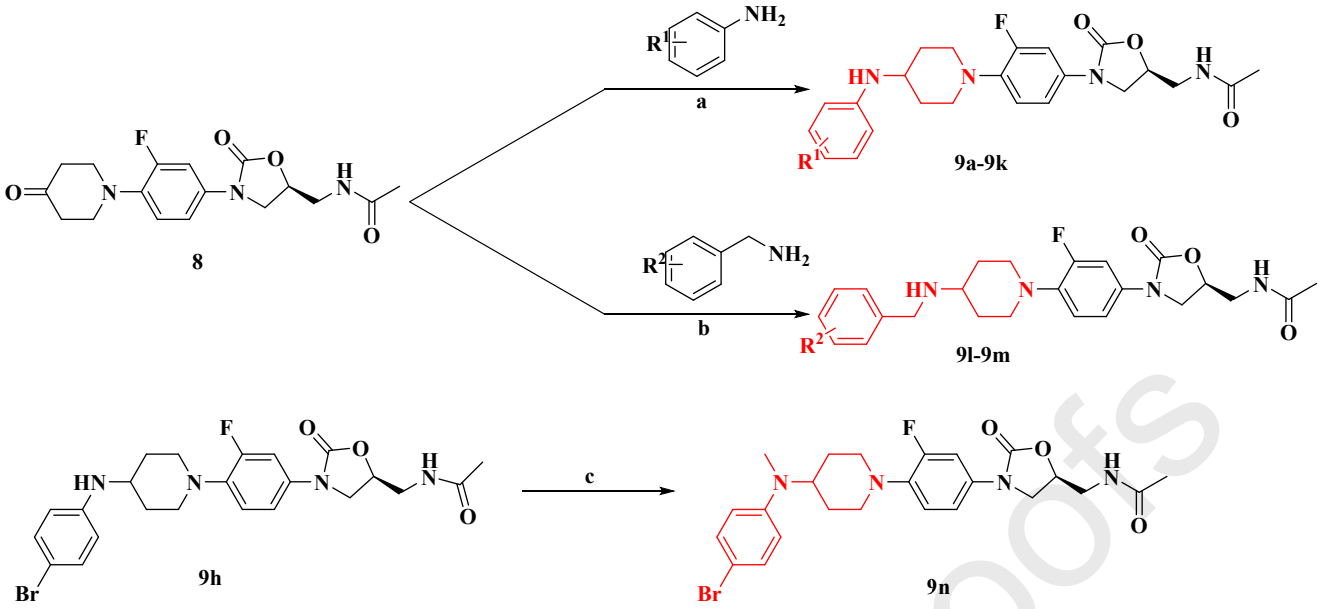
The routes used to synthesize key intermediate **8** and compounds **9a-9n** were depicted in **Scheme 1** and **Scheme 2,** respectively. Intermediate **2** was prepared from commercially available 1-(*tert*-butyloxycarbonyl)piperidin-4-one **1** by deprotection with HCl in 1,4-dioxane. Subsequently, intermediate **2** was reacted with 3,4-difluoronitrobenzene in

the presence of acetonitrile to afford **3**. Intermediate **3** was protected with ethylene glycol to yield the precursor **4**, which was reduced in the presence of 5% palladium on carbon in methanol and then reacted with methyl chloroformate to yield intermediate **6**. The oxazolidinone ring was constructed to the intermediate **7** by reaction of **6** with (*S*)-1-acetamido-3-chloropropan-2-yl acetate in a facile one-step process[24-27]. Removal of the carbonyl protecting group of intermediate **7** with *p*-toluenesulfonic acid afforded key intermediate **8**.

Compounds **9a−9m** were prepared through reductive amination of various substituted anilines/benzylamines with key intermediate **8** in the presence of sodium triacetoxyborohydride in DCM. Compound **9h** was methylated with iodomethane to generate compound **9n**. The structures of all target compounds were confirmed by ^{1}H NMR and MS.



Scheme 1. Synthetic route of key intermediate 8. Reagents and conditions: (a). hydrochloric acid, methanol, 25 °C, 5h; (b). 3,4-difluoronitrobenzene, *N*-ethyldiisopropylamine, acetonitrile, 80 °C, 8h; (c). Ethylene glycol, *p*-toluenesulfonic acid, toluene, 110 °C,7h; (d). 5% palladium on carbon, methanol, 25 °C, 1h； (e). methyl carbonochloridate, pyridine, dichloromethane, 25 °C, 4h; (f). (*S*)-1-acetamido-3-chloropropan-2-yl acetate, *t*-BuOLi, methanol, dimethyl formamide, 25 °C, 23h; (g). *p*-toluenesulfonic acid, H_2O, acetone, 70 °C, 5h.



Scheme 2. Synthetic route of target compounds 9a-9n. Reagents and conditions: (a). Sodium triacetoxyborohydride, acetic acid, dichloromethane, 25 °C, 24h; (b). Sodium triacetoxyborohydride, acetic acid, dichloromethane, 25 °C, 24h; (c). Iodomethane, K_2CO_3, dimethyl formamide, 35 °C, 6h.

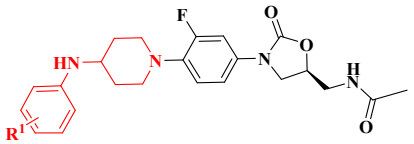
The antibacterial activities of the target compounds **9a-9n** were evaluated *in vitro* against five bacterial strains including S.aureus, MRSA, MSSA, LREF and VRE by MIC assay. The results were summarized in **Table 1**.

According to the data in **Table 1**, most of the tested compounds displayed potent antibacterial activity against Gram-positive organisms and selected antibiotic-susceptible and antibiotic-resistant isolates comparable to linezolid, and the activity ranges from 2 to 0.25 μg/mL. The preliminary SARs (structure-activity relationships) of the oxazolidinone derivatives were studied. Compound **9h** showed 64-fold more potent against LREF than that of linezolid, which was the most active compounds having a MIC value of 0.25 μg/mL. Compound **9a** with hydrogen group and **9c** with 4-OCF_3 group exhibited similar levels of *in vitro* antibacterial activity, while compound **9d** with 3-OCF_3 group and **9e** with 2-OCF_3 group showed nearly 2-fold weaker *in vitro* antibacterial activity compared to linezolid. This result indicated that the substituent on the *para*-position of phenyl maybe superior to *ortho*-position or *meta*-position. This observation can be obtained on the compound **9h**, **9i** and **9j**. By observing the antibacterial activity of compounds **9h-9j**, the potency order of antibacterial activity was *para*-position > *ortho*-position = *meta*-position. In order to determine whether the extension of linker had an influence on activity, compounds **9a**, **9f** and **9l**, **9m** were synthesized. Compounds **9l**, **9m** exhibited weaker activity than **9a**, **9f** against LREF and VRE, respectively. These results implied that extension of linker may be not conducive to the improvement of activity.

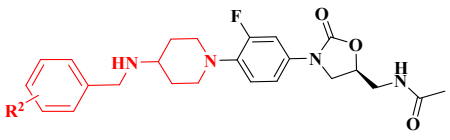
Methylation of compound **9h** on the nitrogen atom of the linker reduced the activity (**9n**, MIC=2 μg/mL, 2 μg/mL, 2 μg/mL, 1 μg/mL and 1 μg/mL), which deduced that the secondary amine as linker may be essential for the activity. Thus, we speculated that the N- piperidinyl moiety may form extra hydrogen bonds and interact with the

binding site of other antibiotic. And this phenomenon could be explained by the result of molecular docking models. Overall, most compounds exhibited moderate-to-significant antibacterial activity against Grampositive organisms and selected antibiotic-susceptible and antibiotic-resistant isolates as compared with linezolid. Then, **9a** and **9h** were selected for further clinical isolates investigation.

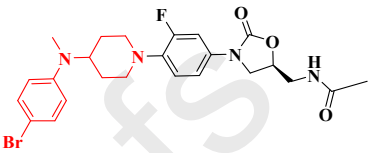
Table 1 The antibacterial activity of target compounds



9a-9k



9l-9m



9n

Compound	R^1/R^2	MIC (μg/mL)				
		S.aureus[a]	MRSA[b]	MSSA[c]	LREF[d]	VRE[e]
9a	**H**	**1**	**1**	**1**	**0.5**	**1**
9b	4-isopropyl	2	1	2	0.5	0.5
9c	4-OCF_3	1	1	1	0.5	0.5
9d	3-OCF_3	2	2	2	2	2
9e	2-OCF_3	2	2	2	1	2
9f	4-F	1	0.5	1	1	1
9g	4-Cl	2	2	2	2	2
9h	**4-Br**	**1**	**0.5**	**1**	**0.25**	**0.25**
9i	3-Br	2	2	2	2	2
9j	2-Br	2	2	2	2	2
9k	2-F, 4-Br	2	2	2	1	2
9l	H	2	1	1	2	2
9m	4-F	1	1	1	2	2
9n	-	2	2	2	1	1
Linezolid	-	1	1	1	＞16	2
Ranbezolid	-	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*.

To ascertain their spectrum of activity against multiple strains of MDR-S. aureus, compounds **9a** and **9h** were evaluated against several antibiotic-susceptible and antibiotic-resistant clinical isolates, and the results were summarized in **Table 2**. Clinical strains of gram-positive bacteria 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 **9a** displayed moderate activity against the tested clinical isolates with MIC values of 0.125-1 μg/mL. Significantly, compound **9h** showed high activity against the tested clinical isolates with

MIC values in the range of 0.0625–1 μg/mL, which was 64-fold more potent against clinical isolates of LREF than that of linezolid

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

Isolates	Compound	MICs (μg/mL)
MRSA[a] (4)[b]	**9a**	0.5-1
	9h	0.5-1
	linezolid	1-4
MSSA[c] (4)	**9a**	0.5-1
	9h	0.5-1
	linezolid	1-2
VRE[d](4)	**9a**	0.25-0.5
	9h	0.125-0.25
	linezolid	1-4
LREF[e](4)	**9a**	0.125-0.25
	9h	0.0625-0.25
	linezolid	>16

[a] Clinical strains of methicillin-resistant *Staphylococcus aureus*.

[b] Number of bacterial strains tested are given in parentheses.

[c] Clinical strains of methicillin-sensitive *Staphylococcus aureus*.

[d] Clinical strains of vancomycin-resistant *Enterococcus faecium*.

[e] Clinical strains of linezolid-resistant *Enterococcus faecalis*.

Linezolid and other oxazolidinone antibacterial agents were reversible, nonselective inhibitors of human MAO due to their structural similarity with toloxatone, a known MAO inhibitor. Clinical use of the oxazolidinone antibacterial agents has shown that these agents had a side effect of hypertension because of MAOI [28]. To determine the safety profile of compounds synthesized, we evaluated the MAO-A inhibitory activities of several representative compounds (**9a, 9h**) and the results were summarized in **Fig. 4**. Compound **9h** exhibited weaker activity than linezolid against human MAO-A, which may be a drug candidate from a safety viewpoint.

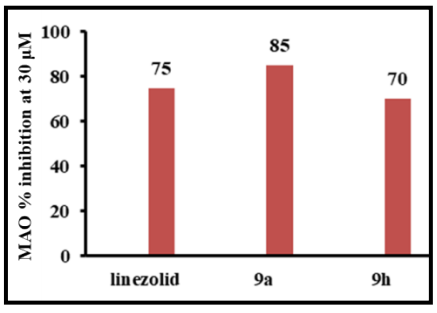


Fig. 4. MAO-A inhibition of potent compounds

Based on the in *vitro* antibacterial activity and inhibition of MAO-A, **9h** was selected for *in vitro* cytotoxicity and metabolic stability, and the results were outlined in **Table 4**. Compound **9h** and linezolid exhibited IC_{50} values > 64 μM against HepG2 cell, which indicated they were safety to hepatic cells to some extent. The human liver

microsome studies showed that **9h** had a long half-life (501.99 min) and low liver microsomal clearance (3.46 mL/min/kg) in human liver microsome，which indicated that **9h** was stable in human liver microsome.

Table 3. *In vitro* cytotoxicity and ADME results

Compounds	clogD (pH=7.4)[a]	HepG2 cytotoxicity IC_{50} (μM)	Human liver microsome (HLM)			
			$T_{1/2}$ (min)	Cl_{int} (mL/min/kg)	Substrate remaining(%)[b]	Stability
9h	**2.91**	**>64**	**501.99**	**3.46**	**97**	**stable**
linezolid	**0.64**	**>64**	∞	**0**	**104.18**	**stable**

[a]Calculated using instant JChem.

[b]Substrate remaining were determined in incubations with NADPH after 45 min

To understand the possible mechanism of action of compound **9h**, molecular superposition of **9h** and linezolid were performed based on the crystal structure of 50S ribosome unit of *E. coli* with linezolid (PDB code: 3CPW [16]). As shown in **Fig. 5**, **9h** was found to form two hydrogen bonds *via* the NH of acetamide and "tail" aniline moiety with G 2540 and U 2620, respectively. Compound **9h** and linezolid have a high degree of overlap (**Fig. 5D**). Furthermore, the *N*-piperidinyl part of compound **9h** extended beyond the morpholine ring of linezolid. As shown in **Table 1**, compound **9n** exhibited weaker activity than **9h** against the different bacteria. On the other hand, the docking results of compound **9n** suggested that the methylated N atom could not form hydrogen bond with U 2620. Meanwhile, the introduction of methyl group resulted the forming of unfavorable bump. Therefore, the NH of played an important role. This result was also consisted with the *in vitro* antibacterial activity, which the methylated product **9n** displayed lower potency than compound **9h**.

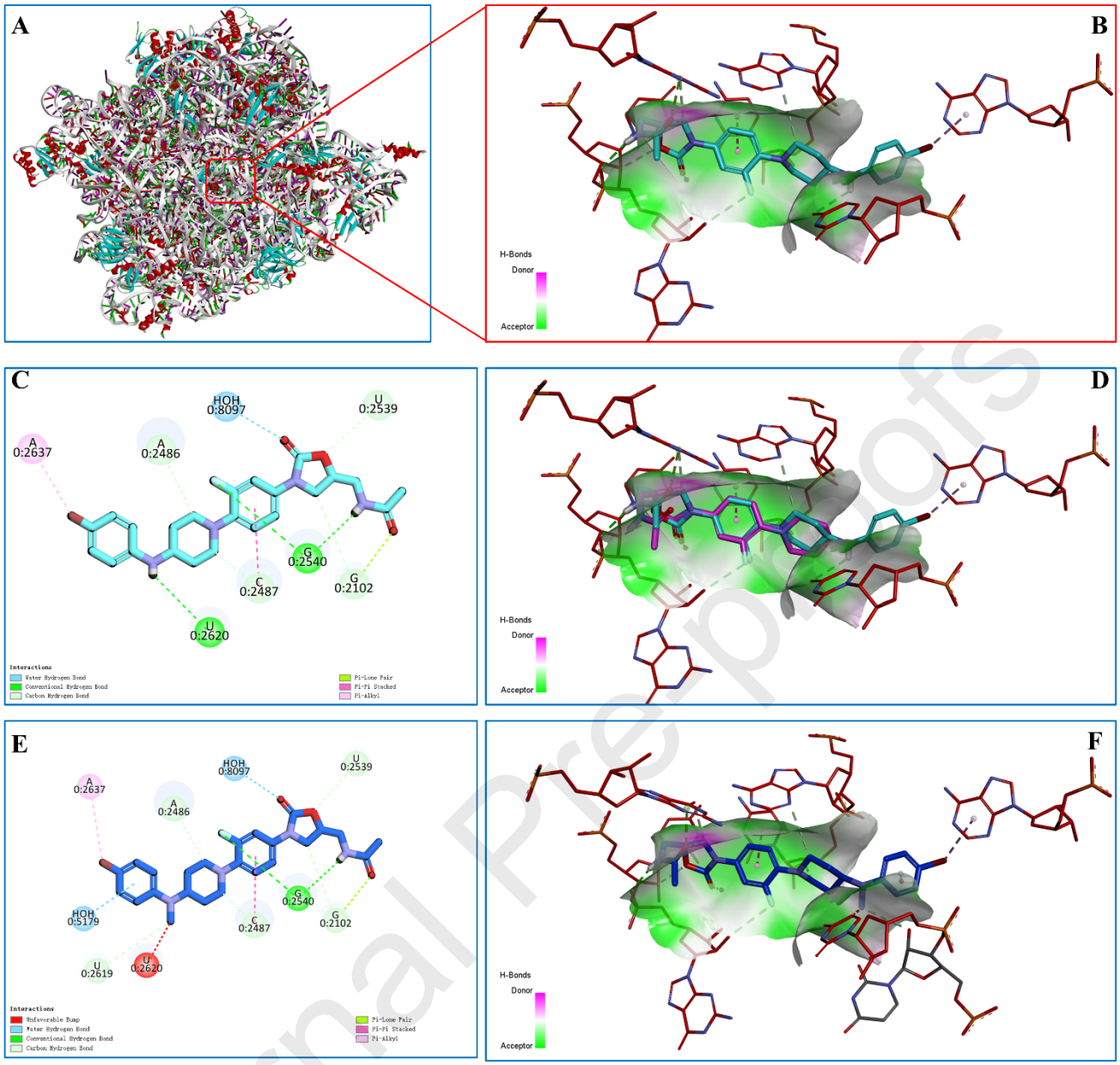


Fig. 5. Superposition of compound **9h** and **9n**. (A) and (B): Predicted binding conformation for **9h** (light blue sticks) in the binding site cavity, (C): 2D diagram of the interaction between **9h** and the binding site cavity, (D) **9h** overlapping with linezolid (pink sticks), (E): 2D diagram of the interaction between **9n** and the binding site cavity, (F) predicted binding conformation for **9n** (blue sticks) in the binding site cavity.

In summary, a series of novel oxazolidinone derivatives containing a piperidinyl moiety were synthesized as potential antibacterial agents and their structures were confirmed by ^{1}H NMR, and MS spectra. Most compounds exhibited potent antibacterial activity against *S.aureus,* MRSA, MSSA, LREF and VRE pathogens as compared with linezolid and radezolid. Compounds **9a** and **9h** were more potent against the tested clinical isolates of MRSA, MSSA, VRE and LREF than linezolid. The exploration of preliminary structure-activity relationships showed that the introduction of *N*-piperidinyl moiety can improve the potency against linezolid-susceptible and linezolid-resistant Gram-positive bacteria. Compound **9h** showed the most potent antibacterial activity with MIC values of 0.25-1 μg/mL against five Gram-positive bacteria, and its potency against LREF was 64-fold higher than that of linezolid.

Moreover, compound **9h** was non-cytotoxic with an IC_{50} value of > 64 μM against HepG2 cell, and **9h** exhibited comparable inhibitory activity with linezolid against human MAO-A. This study provided the researchers more insights about the antibacterial agents.

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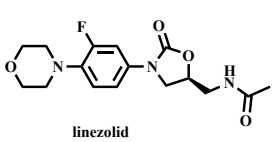
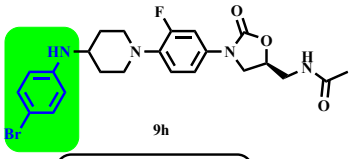


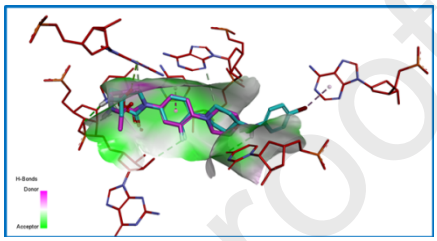
linezolid

S.aureus	MIC= 1 ug/mL
MRSA	MIC= 1 ug/mL
MSSA	MIC= 1 ug/mL
LREF	MIC >16 ug/mL
VRE	MIC= 2 ug/mL



9h

S.aureus	MIC= 1 ug/mL
MRSA	MIC= 0.5 ug/mL
MSSA	MIC= 1 ug/mL
LREF	MIC= 0.25 ug/mL
VRE	MIC= 0.25 ug/mL



Superposition of compound 9h and linezolid

[29]

Declaration of interests

☑The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

[30]