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Discovery of isoxazolinone antibacterial agents. Nitrogen as a replacement for the stereogenic center found in oxazolidinone antibacterials

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Abstract—A series of potential antimicrobial derivatives possessing bioisosteric replacements for the central oxazolidinone ring found in oxazolidinone antibacterials have been prepared. The design concept involved replacement of the requisite sp^3-hybridized stereogenic center found at the 5-position of the oxazolidinone with a nitrogen atom. The synthesis and antibacterial activity of three such ring systems, the benzisoxazolinones, pyrroles, and isoxazolinones is described.

1. Introduction

The major impetus for much of antibacterial drug discovery in recent years has been the emergence of drug resistant strains of pathogenic microoganisms. Of particular importance are methicillin resistant *Staphylococcus aureus* (MRSA), penicillin resistant *Streptococcus pneumoniae* (PRSP), and vancomycin resistant *Enterococcus* (VRE) species.[1] In addition, there have been recent reports of vancomycin resistant *Staphylococcus aureus* isolated from patients in the United States.[2]

Oxazolidinone antibacterials were first identified by researchers at E.I. du Pont de Nemours and were shown to be potent antimicrobial agents.[3] Scientists at UpJohn were able to optimize this important new chemotype over the course of a decade culminating in the discovery of linezolid (Zyvox™).[4] Although many other research organizations have pursued the oxazolidinone class of antibacterials, and at least two have produced clinical candidates,[5] linezolid is the first and only oxazolidinone to make it to market, having been approved in April 2000 by the FDA for the treatment of bacterial infections due to Gram positive organisms. Linezolid has good activity against Gram positive organisms including those resistant to currently available antimicrobials, it is 100% orally bioavailable and since it is not derived from a natural product, resistance has been slow to emerge.[6] Herein, we detail our initial efforts to identify a new and potentially improved antibacterial agent using the oxazolidinone silhouette as a starting point.[7] One such approach involved searching for a bioisostere to replace the central oxazolidinone heterocycle (Fig. 1).

Prior to embarking on a search for a new oxazolidinone bioisostere with improved properties, we consulted the

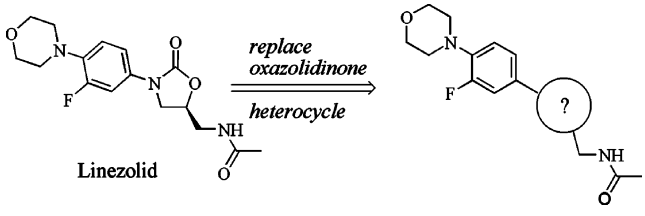

Figure 1. Strategy for discovering new linezolid analogs.

Keywords: Oxazolidinone; Isoxazolinone; Bioisostere; Antibacterial.
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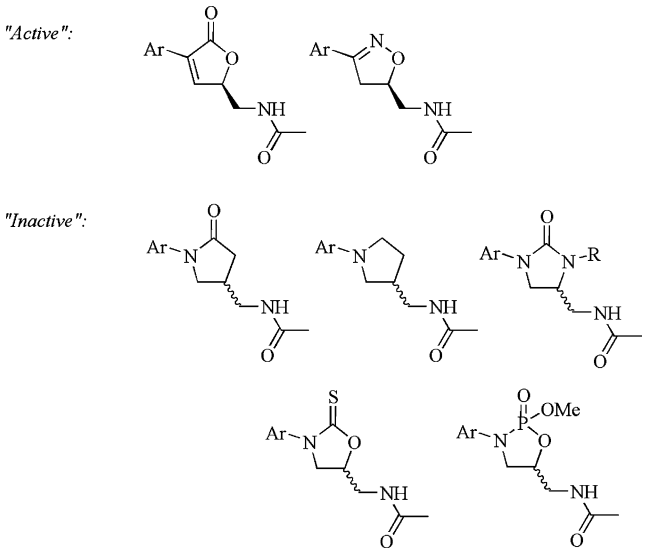


Figure 2. Other potential oxazolidinone bioisosteres reported in the literature.

literature and found many examples of oxazolidinone isosteres with varying levels of activity. Figure 2 illustrates some of the 'active' (butenolide[8,9] and isoxazoline[10]) as well as 'inactive' isosteres.[9,11] As there has yet to appear a crystal structure of an oxazolidinone bound to its prokaryotic target (the 50S ribosomal subunit),[4] one is left to infer the critical interactions, which differentiate active five membered ring heterocycles from those that are inactive. Although one of linezolids critical structural elements resulting in good antimicrobial activity has been shown empirically to be the 5-(*S*)-configured stereocenter, we became intrigued by the idea of replacing this stereogenic carbon with a nitrogen atom. With this design element in mind, three potential oxazolidinone bioisosteres wherein a nitrogen atom would replace the sp^3-hybridized stereogenic center found in the oxazolidinone were surveyed. Our work on the benzisoxazolinones, pyrroles, and isoxazolinones is described below.[12]

2. Benzisoxazolinones

At an early stage of the program we became aware of a disclosure by Wierenga et al. describing the antimicrobial properties of a select series of benzisoxazolinones.[13] As these derivatives possessed primarily Gram negative activity, we decided to investigate the possibility of designing a hybrid structure, which might incorporate the Gram negative activity of the benzisoxazolinones with the Gram positive activity of the oxazolidinones.

As shown in Figure 3, the proposed benzisoxazolinone (**A**) overlays reasonably well with the oxazolidinone (**B**). A series of benzisoxazolinone analogs were synthesized as shown in Scheme 1 starting from commercially available nitrobenzoates. Partial reduction of the nitro group led to the intermediate hydroxylamines, which were cyclized in alkaline solution to the parent benzisoxazolinone. Hydrazine and Raney nickel were used in

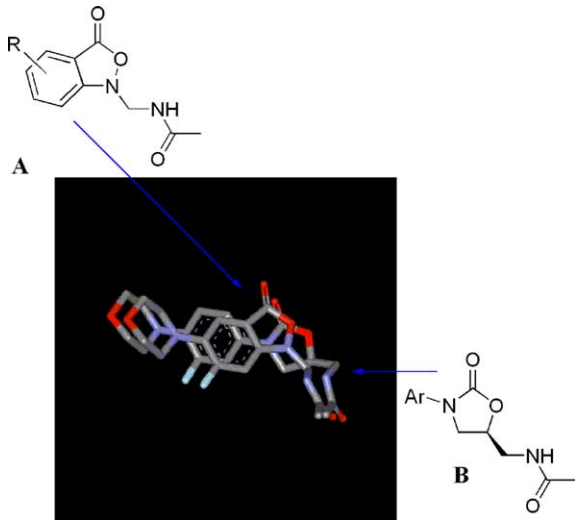
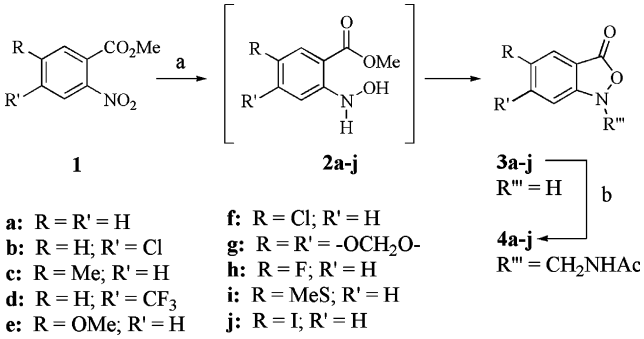


Figure 3. Overlay of benzisoxazolinone with linezolid.

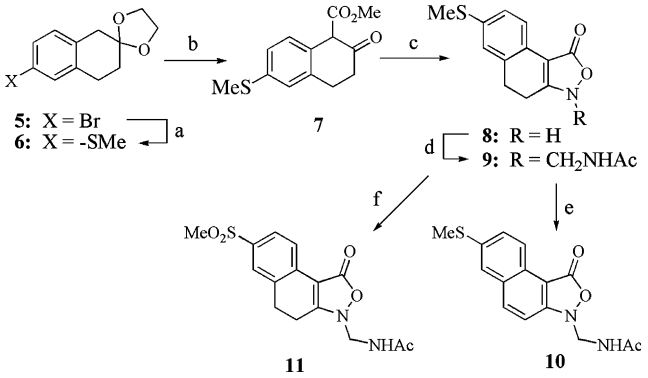


Scheme 1. Reagents: (a) (1) Zn, NH_4Cl (for **3h**, Hydrazine/RaNi), (2) NaOH; 31% for **3a**, 66% for **3b**, 21% for **3c**, 66% for **3d**, 3% for **3e**, 67% for **3f**, 41% for **3g**, 53% for **3h**, 69% for **3i**, 59% for **3j**; (b) K_2CO_3, $AcOCH_2NHAc$, DMF; 21% for **4a**, 57% for **4b**, 68% for **4c**, 72% for **4d**, 0% for **4e**, 53% for **4f**, 17% for **4g**, 47% for **4h**, 26% for **4i**, 66% for **4j**.

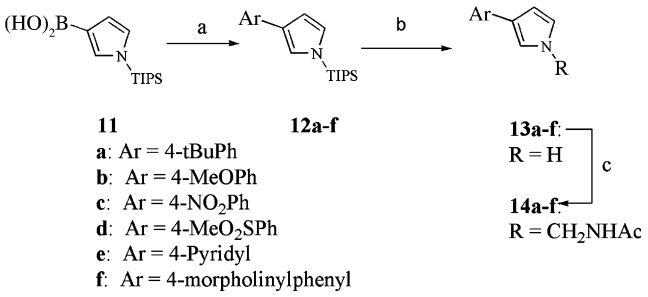
order to avoid over-reduction when R = 4-F (**3h**). The requisite acetamidomethyl side chain was appended by making use of an acetamidomethyl acetate electrophile described by Barnes et al.[14] Unfortunately benzisoxazolinones **4a**–**j** were found to be devoid of antibacterial activity. It was thought that tricyclic derivatives **9**–**11** might more closely mimic the spatial arrangement of linezolid. The synthesis of these analogs is outlined in Scheme 2. Disappointingly, **9**–**11** also showed a lack of antibacterial activity.

3. Pyrroles

Concurrent with our work exploring the feasibility of benzisoxazolinones as antibacterial agents, we began to explore pyrroles as a potential oxazolidinone bioisostere wherein the pyrrole nitrogen atom would replace the sp^3-hybridized stereogenic center found in the oxazolidinone ring. Commercially available N-TIPS pyrrole was selectively iodinated at the C-3 position and converted to the boronic acid using known conditions[15]



Scheme 2. Reagents: (a) BuLi, $(MeS)_2$; 48%, (b) (1) $AcOH/H_2O$; (2) NaH, Me_2CO_3; 63%; (c) NH_2OH; 40%; (d) K_2CO_3, $AcOCH_2NHAc$, DMF; 60%; (e) DDQ; 50%; (f) Peracetic acid; 99%.



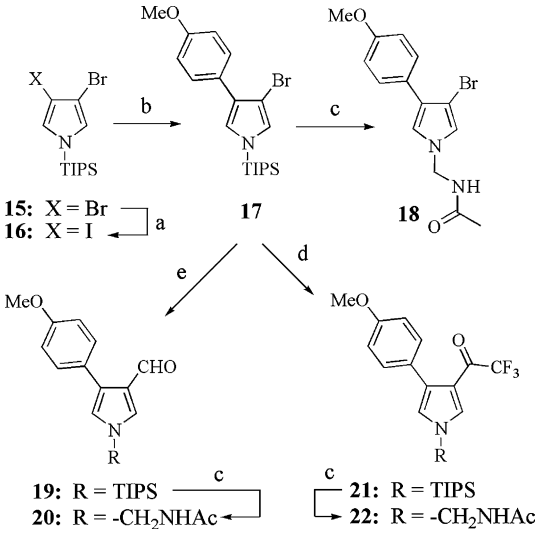
Scheme 3. Reagents: (a) ArX, $Pd(Ph_3P)_4$, Na_2CO_3, MeOH, reflux; 90% for **6a**, 86% for **6b**, 34% for **6c**, 70% for **6d**, 61% for **6e**, 40% for **6f**; (b) TBAF; (c) K_2CO_3, $AcOCH_2NHAc$, DMF; Yield for steps b and c combined: 12% for **8a**, 19% for **8b**, 24% for **8c**, 64% for **8d**, 4% for **8e**, 20% for **8f**.

(Scheme 3). Cross-coupling followed by deprotection and appendage of the acetamidomethyl side chain resulted in analogs **14a**–**f**. As in the case of the benzisoxazolinones, no antibacterial activity was detected. Since pyrroles **14a**–**f** lack a carbonyl or a ring oxygen found in the oxazolidinones, synthesis of pyrrole derivatives **20** and **22**, which posses potential hydrogen bond acceptors in the form of a formyl and trifluoromethyl group were prepared as outlined in Scheme 4. Again, a lack of antibacterial activity was observed for these analogs.

Possibilities for the lack of activity of the pyrroles and benzisoxazolinones include decreased binding to the ribosomal target, decreased permeability or increased efflux relative to the oxazolidinones.[16] Since the oxazolidinone literature suggests a ring oxygen as well as a carbonyl group are essential elements for antibacterial activity, it was thought that an isoxazolinone might more closely meet these requirements.

4. Isoxazolinones

The isoxazolinone ring system has found limited use in medicinal chemistry presumably due to inherent instabilities associated with the labile N–O bond,[17] potential Michael accepting properties, and hydrolytically labile



Scheme 4. Reagents and conditions: (a) (1) *n*-BuLi, THF, −78 °C; (2) I_2; 99%; (b) 4-MeOPh$(BOH)_2$, $Pd(Ph_3P)_4$, $CsCO_3$, Tol/MeOH; 78%; (c) (1) TBAF, (2) K_2CO_3, $AcOCH_2NHAc$, DMF; 50% for **18**, 67% for **20**, 60% for **22**; (d) (1) *t*-BuLi, THF, −78 °C, (2) $MeN(OMe)COCF_3$; 47%; (e) (1) *t*-BuLi, THF, −78 °C, (2) DMF; 64%.

ring carbonyl[18] all of which have been amply documented.[19] Examination of the overlay between the two heterocycles (Fig. 4) however, was compelling and taken together with the relative simplicity of the chemistry required to test our hypothesis, the preparation of representative examples was undertaken.

In the event, commercially available phenylacetic acids were converted to their corresponding esters, formylated by treatment with sodium hydride in ethyl formate and cyclodehydrated with hydroxylamine[20] (Scheme 5). The acetamidomethyl side chain was appended to provide the final isoxazolinone derivatives **25** required for microbiological testing. Since there was concern about the stability of the acetamidomethyl side chain, retro-amide derivatives **26** were also prepared via alkylation with iodoacetamide. It is noteworthy that the synthesis of these analogs compares favorably with the corresponding oxazolidinones, starting with readily available esters and providing the final isoxazolinone analogs in only three steps.[21,22]

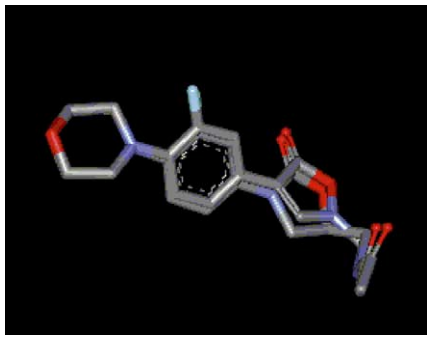
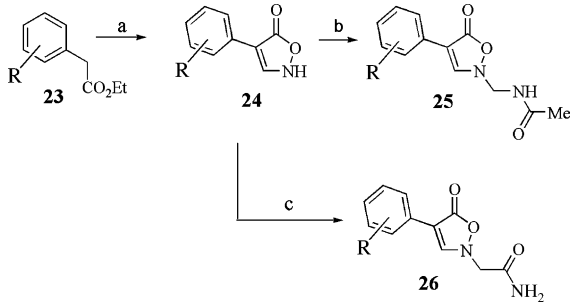


Figure 4. Overlay of isoxazolinone (top) and linezolid (bottom).



Scheme 5. Reagents: (a) (1) NaH, ethyl formate (2) hydroxylamine; 35–49% over two steps; (b) K_2CO_3, $AcOCH_2NHAc$, DMF; 14–65%; (c) K_2CO_3, iodoacetamide; 45–50%.

5. Biological activity of isoxazolinones

Table 1 illustrates in vitro antibacterial activity of the initial set of isoxazolinones. The initial unsubstituted isoxazolinone **25a** showed promising antibacterial activity. Substitution of the phenyl ring at the C-3 position by a variety of functional groups (**25–f**) resulted in analogs with marginal Gram positive activity and no detectable Gram negative potency. Substitution of the 4-position however, provided isoxazolinones with much improved Gram positive and measurable Gram negative antibacterial activity as illustrated by examples **25–m**. Disubstitution, in general, produced analogs with drastically reduced antibacterial activity (**25o–s**; **25n** appeared to be a lone exception to this empirical observation). As a result of this initial data, the majority of analogs prepared henceforth were 4-substituted. The serum effect for selected analogs was in some cases significant (e.g., **25h**). In other cases, however, incubation with serum resulted in a more modest 2–4-fold increase in MIC (e.g., **25k** and **25n**). To address concerns about the stability of the acetamidomethyl side chain appended to the isoxazolinone ring nitrogen, the antibacterial activity of the corresponding N–H isoxazolinones **24j**, **24k**, **24l**, and **24n** was investigated. As these unalkylated isoxazolinones were devoid of antibacterial activity while their respective fully elaborated derivatives **25i**, **25k**, **25l**, and **25n** possessed good potency it can be surmised that the final products **25a–s** have good stability[23] under the assay conditions.[24] The retro-amide present in **26a** and **26b** results in inactive isoxazolinones. Lastly, a series of related isoxazolinones not described in this disclosure were evaluated against a human carcinoma HEP2 cell line. In general, a 200-fold spread between MIC and CC_{50} was found suggesting that antibacterial activity is not due to cytotoxicity.

In conclusion, we have outlined the discovery of a new class of antibacterial agents based on the oxazolidinone antibacterial chemotype. The design concept involved replacement of an sp^3-hybridized asymmetric carbon atom with a nitrogen atom, thereby rendering the resultant analogs achiral and greatly simplifying the synthetic sequence. Initial efforts to prepare benzisoxazolinones and pyrroles provided compounds devoid of antibacte-

Table 1. Antibacterial activity of selected isoxazolinones

Compound	R	MIC without and (with 50% calf serum) (μg/mL)			
		S. aureus[a]	*MRSA*[b]	*E. faecalis*[c]	*H. influenzae*[d]
24j	4-MeS	>128 (>64)	>128 (>64)	>128 (>64)	>64
24l	4-Ph	>128 (>64)	>128 (>64)	>128 (>64)	>64
24k	4-MeO	>128 (>64)	>128 (>64)	>128 (>64)	>64
24n	3,4-Methylenedioxy	>128 (>64)	>128 (>64)	>128 (>64)	>64
25a	H	8 (16)	4 (8)	16 (16)	8
25b	3-F	8 (ND)	4 (ND)	16 (ND)	>64
25c	3-MeO	32 (64)	16 (>64)	32 (>64)	>64
25d	3-CF_3	32 (64)	16 (64)	64 (>64)	>64
25e	3-Cl	8 (>64)	4 (16)	16 (>64)	32
25f	3-Me	8 (ND)	4 (ND)	32 (ND)	>64
25g	4-F	>128 (>64)	>128 (>64)	>128 (>64)	32
25h	4-Br	4 (>64)	2 (>64)	8 (>64)	8
25i	4-Cl	8 (ND)	4 (ND)	8 (ND)	32
25j	4-MeS	2 (16)	1 (8)	1 (16)	8
25k	4-MeO	2 (4)	1 (4)	2 (8)	16
25l	4-Ph	4 (>64)	1 (64)	4 (64)	16
25m	4-iPr	1 (4)		2 (8)	
25n	3,4-Methylenedioxy	2 (4)	1 (4)	1 (8)	16
25o	3,4-Di-MeO	128 (>64)	64 (>64)	>128 (>64)	>64
25p	3,4-Di-F	128 (>64)	>128 (>64)	>128 (>64)	>64
25q	3,5-Di-MeO	>128	>128	>128	64
25r	3,5-Di-F	8 (ND)	8 (ND)	32 (ND)	>64
25s	3,5-Bis-CF_3	>128	>128	>128	>64
26a	H	>128	>128	>128	>64
26b	4-Br	>128	>128	>128	>64
Linezolid		1 (1)	1 (1)	0.5 (0.5)	8

[a] *Staphylococcus aureus* A15090.
[b] Methicillin resistant *Staphylococcus aureus* A27223.
[c] *Enterococcus faecalis* A20688.
[d] *Haemophilus influenzae* A20191.

rial activity. The isoxazolinone ring system however, has proven itself to be an effective bioisostere of the oxazolidinone heterocycle found in oxazolidinone antibacterials. The pharmacological profiles of the isoxazolinone versions of linezolid and eperezolid[4] as well as details of our efforts to design an improved version linezolid will be the subject of future reports.

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16. Compounds **10**, **18**, **20**, and **22** exhibited no antibacterial activity against either AcrA:KANA *E. coli* (A2891) or AcrB:KANA *H. influenzae* (A2935) efflux pump mutants. It is therefore likely that the lack of antibacterial activity observed with the pyrrole and benzisoxazolinones is not due to increased efflux.
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21. Experimental procedure for compound **25a**: To a stirred solution of ethyl phenylacetate (30 mL; 0.19 mol) in ethyl formate (600 mL) was added sodium hydride (60% in oil; 30 g; 0.75 mol) portionwise over 45 min under a constant stream of nitrogen. After 15 additional minutes LC indicated consumption of starting material. The reaction was poured into a mixture of 1 N HCl/ice, extracted with ether, washed with brine, and dried over $MgSO_4$ providing 49 g of an amber liquid, which was used without purification. The amber liquid was dissolved in methanol/water (600 mL/60 mL), hydroxylamine hydrochloride (25 g) was added and the solution was heated to reflux for 1.5 h. The solution was concentrated to near dryness, and residual water was azeotroped further from methanol (3×). The solid was transferred to a Buchner funnel, washed with water (200 mL) and ether (250 mL) and dried in vacuo to provide **24a** (18.4 g; 59% over two steps) as a colorless solid.[18] To a stirred solution of $AcOCH_2NHAc$[13] (8.1; 62.1 mmol) in dichloromethane (120 mL) was added **24a** (2.0 g; 12.4 mmol). After 18 h, 1 N HCl was added until the reaction mixture was acidic, the aqueous was extracted with chloroform, and the organics were dried over $MgSO_4$ providing a pale liquid. Sonication with ether provided 1.9 g (67%) **25a** as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 8.95 (s, 1H), 8.93 (t, J = 6.1 Hz, 1H), 7.76 (d, J = 7.7 Hz, 2H), 7.38 (t, J = 9.4 Hz, 2H), 7.25 (t, J = 8.3 Hz, 1H), 5.03 (d, J = 6.2 Hz, 2H), 1.84 (s, 3H); ^{13}C NMR (DMSO-d_6): δ 170.6, 168.7, 150.4, 129.4, 128.6, 126.8, 124.6, 102.3, 55.4, 22.3; IR (KBr pellet) 3288, 3057, 1722, 1667 cm^{-1}; LC purity >99%; HRMS (FAB) calcd for $C_{12}H_{12}N_2O_3$: 233.093005, found: 233.09300.
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23. Additional stability tests in acidic and basic media were done. Acidic stability (TFA/CH_2Cl_2; or 1 N HCl/THF) was excellent. Storage in citrate buffer for 18 h (pH 4.5) resulted in <1% decomposition. Storage in basic media (pH 8.5) showed loss of the acetamidomethyl side chain in a time and temperature dependent fashion (a maximum of 40% loss over the course of 18 h was observed with related isoxazolinones). Solid state stability was excellent with no degradation evident after storage at ambient temperature for >3 years.
24. The minimum inhibitory concentration (MIC) of the compound was obtained using a microbroth dilution susceptibility test in accordance to the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Mueller-Hinton broth was used to test *S. aureus* and *E. faecalis*. Haemophilus test medium broth was used to test *H. influenzae*. The final bacterial inoculum contained approximately 5×10^5 CFU/well in a microtiter plate. The volume of each well was 100 μL and the plates were incubated at 35 °C for 18 h in ambient air. The MIC was defined as the lowest drug concentration that prevented visible growth of the bacterium.