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Synthesis and antibacterial evaluation of isoxazolinyl oxazolidinones: Search for potent antibacterial ☆

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ABSTRACT

A series of (5S) *N*-(3-{3-fluoro-4-[4-(3-aryl-4,5-dihydro-isoxazole-5-carbonyl)-piperazin-1-yl]-phenyl}-2-oxo-oxazolidin-5-ylmethyl)-acetamide(**6a–o**) were synthesized and their in vitro antibacterial activity against various resistant Gram-positive and Gram-negative bacteria were evaluated. Most of the synthesized compounds showed 2 to 10 fold lower MIC values compared to linezolid against *Staphylococcus aureus* **ATCC 25923, ATCC 70069, ATCC 29213,** *Bacillus cereus* **MTCC 430,** *Enterococcus faecalis* **MTCC439,** *Klebsiella pneumoniae* **ATCC 27736,** and *Streptococcus pyogens*.

In spite of available potent antimicrobials for management of infections, development of bacterial resistance to existing drugs is a major concern in antibacterial therapy and necessitates continuing research for new classes of antibacterial. Of particular concern are severe infections caused by multi drug-resistant Gram-positive pathogens, which result in high mortality rates especially in the hospital settings. The individual organisms responsible include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE) and penicillin-resistant *Streptococcus pneumoniae*. There are several antibiotics effective against these organisms but these organisms easily acquire resistance against them. Resistant development is inherent in the mode of action of all antibiotics and hence posses challenges in the development of new antimicrobial agents to overcome it. Some bacterial strains have been found to be resistant to all currently available antibacterial agents.[1]

Among the antibacterial, oxazolidinone covers the important Gram-positive pathogens and show unique mechanism of action. Linezolid[2] (Fig. 1), the first commercially available oxazolidinone shows activity against Gram-positive bacteria[3] including multi-drug-resistant bacteria. Linezolid is a bacteriostatic antibiotic that exerts its action by binding to 50S subunit of bacterial ribosome, blocking synthesis of bacterial proteins.[4] Despite of its unique mode of action resistance against linezolid was reported in early 2001.[5]

As a result, new antimicrobial agents are being developed and old ones are reevaluated as potential alternatives for control of infections due to multiple resistant Gram-positive microorganism. The hybrid approach has been one of the alternate approaches followed by several research groups[6] to treat resistant bacterial strains. Among oxazolidinone family, Eperazolid (Fig. 1) is another lead molecule but it was not taken for further commercial development due to its shorter half life[7] and bone marrow toxicity.[8] However it has been modified by various groups[9] to enhance activity and to reduce its toxicity level. Johnson[10] and other research groups[9a] reported synthesis and activity of isoxazolinyl oxazolidinone (2, Fig. 2). Barbachyn et al.[11] replaced oxazolidinone ring by isoxazoline ring (3, Fig. 2), these compounds are showing good activity against resistant microorganisms.

These literature findings prompted us to synthesize hybrid analogues of oxazolidinones and isoxazoline **6a–o** (Fig. 3). We herein report the synthesis of novel hybrid analogues of Eperazolid and isoxazoline and their in vitro activity against Gram-positive and Gram-negative bacteria resistant to various drugs in this Letter.

Key intermediate **1** was synthesized from 3,4-difluoro nitrobenzene by reported protocol[12] (Scheme 1).

The isoxazoline components were synthesized by following the literature precedence. Conversion of aldehydes **2a–o** to the oxime **3a–o** by hydroxylamine hydrochloride followed by chlorination with N-chlorosuccinimide produced the nitrile oxide precursor.[13] Dehydrohalogenation of chloroxime with base in the presence of dipolarophile ethylacrylate afforded exclusively the 5-substituted cycloadduct (**4a–o**) in good yield with none of the regioisomeric 4-substituted product being observed by NMR.[14] A doublet of dou-

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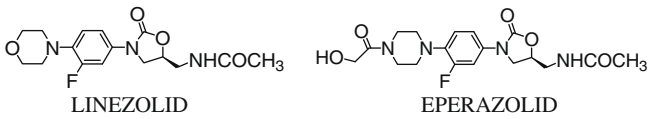


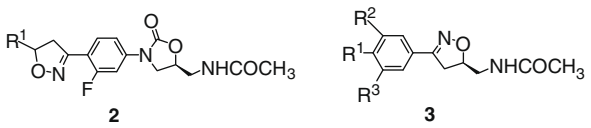


Figure 2.

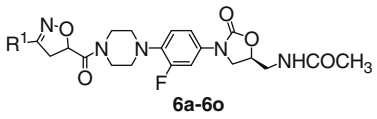
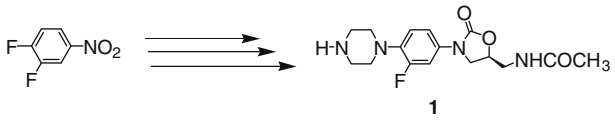


Figure 3. R^1 = Substituted aryl/substituted heteroaryl.



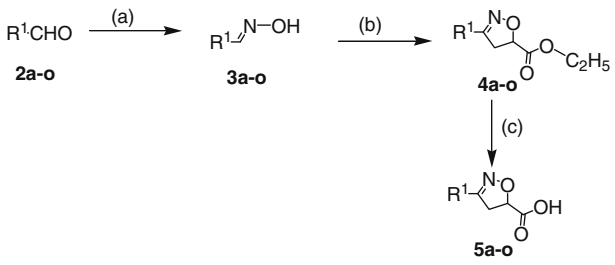
Scheme 1.

blet for C-5 proton of isoxazoline appeared at δ 5.10 (J = 10.6, 7.6 Hz) and signal of C-4 H_{cis} appeared at δ 3.66 showed characteristic geminal and cis coupling (J = 16.9, 7.6 Hz).The C-4H_{trans} appearing at δ 3.58 showed geminal and trans coupling constant 16.8 and 10.6 Hz, respectively. Isoxazoline ester **4a–o** were hydrolyzed in the presence of LiOH to furnish compounds **5a–o** (Scheme 2).

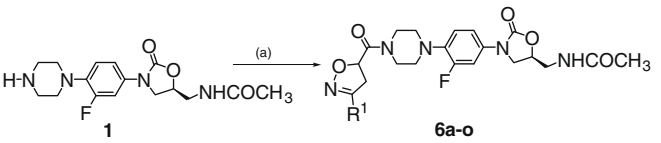
Compounds **5a–o** were coupled with intermediate **1** mediated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) as coupling reagent at 0 °C in the presence of triethylamine to afford novel isoxazolinyl oxazolidinone analogues **6a–o** in good yields (Scheme 3).

This series of oxazolidinone antimicrobial agents was screened against panel of susceptible and resistant bacteria. Compounds were tested against both Gram-negative and Gram-positive strains where linezolid and vancomycin were used as standard drug.

The bacterial strains were grown on nutrient agar at 37 °C. After 24 h of incubation, bacterial cells were suspended in normal saline containing Tween 20 at 0.05% at a concentration of approximately



Scheme 2. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, NaOH, H_2O; (b) NCS, ethylacrylate, Et_3N, 0 °C; (c) LiOH, THF:MeOH:H_2O, (3:3:1).



Scheme 3. Reagents and conditions: (a) compounds **5a–o**, EDC·HCl, HOBt, Et_3N, 0 °C, overnight.

1.0–2.0 × 10^7 cells/mL by matching with 0.5 McFarland standards. The activity of compounds was determined as per NCCLS protocol using Mueller Hinton broth (Becton Dickinson, USA) in 96-well tissue culture plates. Proper growth control, drug control and the negative control were adjusted onto the plate. Compounds were dissolved in DMSO at a concentration of 1 mg/mL and 20 μL of this was added to each well of 96-well tissue culture plate having 180 μL Mueller Hinton broth. From here the solution was serially diluted resulting in twofold dilution of the test compounds in subsequent wells. 100 μL of Mc Farland matched bacterial suspension was diluted in 10 ml of media and then 100 μL of it was added in each well and kept for incubation. The maximum concentration of compounds tested was 50 μg/mL. The micro-titer plates were incubated at 35 °C in a moist, dark chamber and MICs were recorded spectrophotometrically after 24 h using SOFT max Pro 4.3 Software (Molecular Devices, Sunnyvale, USA).

The analogues **6a–o** of this series exhibited better activity than linezolid against Gram-positive resistant strain but did not show any activity against *Escherichia coli* (ATCC 9637) and *Pseudomonas aeruginosa* (ATCC-BAA 427). Surprisingly, these compounds showed excellent activity against a Gram-negative bacteria *Klebsiella pneumoniae* (ATCC 27736) resistant to various drugs. Minimum inhibitory concentration MIC (μM) of these novel compounds are reported in Table 1.

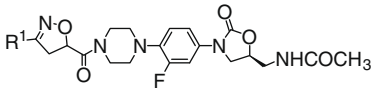
Antibacterial activity evaluation largely depended on various substitutions in aryl ring present at 3-position of isoxazoline ring. Attempts were made to correlate antibacterial activity of these compounds with changing substitution at aryl ring. Compounds (**6a–o**) demonstrated a range of MIC 0.0866–24.5580 μM against resistant bacteria tested in this study. All compounds except **6a, 6m** and **6n** were more potent than linezolid. From the results in Table 1 it is clear that unsubstituted phenyl ring at 3-position of isoxazoline (**6a**) showed higher MIC values (lower activity) against all bacterial strains tested. Substitution of chloro group either at 4 position or at 3,4 position of phenyl ring increased antibacterial activity of compounds (**6c** and **6e**) These compounds **6c** and **6e** showed MIC of 0.1559–0.3499 μM against all strains except *S. aureus* ATCC 29213 with MIC of 2.879 μM and 2.7036 μM, respectively. Compounds **6b** and **6d** with fluoro substitution at 3 and at 3,4 position respectively were less potent than compounds **6c** and **6e** with chloro substitution suggesting electronegative substituent was not suitable for antibacterial activity.

Compound **6h** having CF_3 group at meta position was proved to be better than compound **6g** having CF_3 at para position against all resistant strains evaluated. This indicated that substitution at meta position enhanced the antibacterial activity.

Compound **6i** was the most potent compound of series (**6a–o**) showing MIC of 0.0866 μM against *E. faecalis* MTCC 439 suggesting electron withdrawing group at meta position enhanced the activity.

Among the *S. aureus* resistant strains ATCC 25293, ATCC 70069, ATCC 29213 compounds **6c** (p-chloro), **6k** (3,4-methylenedioxy) were the most potent compound showing MIC 0.1657 and 0.1627 μM, respectively. Compound **6o** (pyridyl) was the most active compound among heterocyclic analogues with MIC of 0.3725–1.5294 μM. Thiophenyl analogues **6m** and **6n** were inferior to fura-

Table 1
MIC values (μM) for oxazolidinones **6a–o**



6a-6o

Compounds	R^1	S.a.[a]	S.a[b]	S.a.[c]	B.c.[d]	E.f.[e]	K.pn.[f]	S.p.[g]
6a	Phenyl	24.5580	24.5580	24.5580	24.5580	24.5580	24.5580	24.5580
6b	4-Fluorophenyl	0.7400	0.7400	0.7400	0.7400	0.7400	1.4800	0.7400
6c	4-Chlorophenyl	0.1657	0.3499	2.8729	0.3499	0.3499	0.1657	0.3490
6d	3,4-Difluorophenyl	0.3486	0.3486	0.7155	0.3486	0.3486	0.7155	0.3486
6e	3,4-Dichlorophenyl	0.3292	0.3292	2.7036	0.1559	0.3292	0.3292	0.3292
6f	4-Tolyl	0.3632	1.4913	2.9827	0.3632	0.7456	0.3633	0.7456
6g	3-Trifluoromethyl phenyl	1.3518	1.3518	2.7036	1.3518	1.3518	2.7036	1.3518
6h	4-Trifluoromethyl phenyl	0.6759	0.6759	2.7036	0.6759	0.3292	0.6759	0.6759
6i	3-Nitrophenyl	0.3429	0.7039	0.7039	0.1624	0.0866	0.7039	0.7039
6j	3-Hydroxy-4-methoxyphenyl	0.3423	0.7027	0.7027	0.3423	0.3423	0.3423	0.7027
6k	3,4-Methylene dioxyphenyl	0.1627	0.3435	2.8209	0.3435	0.3435	0.1627	0.3435
6l	2-Furanyl	0.7815	1.5631	6.2525	1.5631	0.7815	1.5631	0.7815
6m	2-Thiophenyl	6.0582	24.2718	24.2718	6.0582	24.2718	6.0583	12.1359
6n	3-Thiophenyl	12.1359	6.0583	24.2718	12.1359	12.1359	6.0583	12.1359
6o	2-Pyridyl	0.7647	1.5294	1.5294	0.7647	0.3725	0.7647	0.3725
Linezolid		4.6290	4.6290	9.2581	2.3145	9.2581	4.6290	4.6290
Vancomycin		0.2624	4.2066	1.0499	0.1278	0.5249	0.2624	0.2624

[a] *Staphylococcus aureus* **ATCC 25923** (floxacin and methicillin-resistant).
[b] *Staphylococcus aureus* **ATCC 70069** (methicillin-resistant).
[c] *Staphylococcus aureus* **ATCC 29213** (methicillin and vancomycin-resistant).
[d] *Bacillus cereus* **MTCC 430**.
[e] *Enterococcus faecalis* **MTCC 439**.
[f] *Klebsiella pneumoniae* **ATCC 27736**.
[g] *Streptococcus pyogens*.

nyl analogue **6l** against all strains evaluated. All Compounds except **6a, 6m, and 6n** of this series were more potent than linezolid against *Bacillus cereus* MTCC 430. Compounds **6e** and **6i** were as par with vancomycin against *B. cereus* MTCC 430 with MIC 0.1559 μM and 0.1624 μM. Rest of the compounds was either equipotent or inferior to vancomycin against *B. cereus*. Among *Staphylococcus* strains, all compounds except **6i, 6j, 6b** and **6c** were more potent than vancomycin against *S. aureus* ATCC 29213 (resistant to vancomycin) in similar comparison of the respective MIC values expressed in μg/mL. All compounds except **6a**, **6m** and **6n** were found to be more active than vancomycin against methicillin-resistant *S. aureus* ATCC 70069.

It is interesting to note that these compounds were also active against Gram-negative bacterial strains. Generally oxazolidinones are devoid of activity against Gram-negative bacteria but to our great surprise these compounds showed promising activity against K. pneumoniae ATCC 27736, a Gram-negative bacteria, resistant to various drugs with MIC of 0.1627–6.05825 μM. Compounds **6c** and **6k** showed excellent activity against this Gram-negative bacteria with MIC 0.1657 μM and 0.1627 μM, respectively. Compounds **6c**, **6e, 6i** and **6k** were the potent compounds of the series showed MIC of 0.0862–2.8729 μM against different resistant bacterial strains.

In conclusion, novel hybrid analogues of Eperazolid and isoxazoline **6a–o** were synthesized and their antibacterial activities against various resistant Gram-positive and Gram-negative bacteria were evaluated. All compounds except **6a**, **6m** and **6n** are more potent than linezolid. This is remarkable in view of the fact that these compounds showed promising activity against resistant Gram-negative bacteria *K. pneumoniae*. As racemic isoxazolinyl oxazolidinones exhibited superior in vitro activity against several resistant bacterial strains, It is of high interest to evaluate the antibacterial activities of enantiomerically pure isoxazolinyl oxazolidinones. Further work in this direction and lead optimization of the series are in progress.

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Supplementary data

Supplementary data (the physical and spectral data of all synthesized compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.133.

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