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Novel 4-N-substituted aryl but-3-ene-1,2-dione derivatives of piperazinyloxazolidinones as antibacterial agents ☆

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ABSTRACT

Novel (5*S*)-*N*-[3-(3-fluoro-4-{4-[2-oxo-4-(substituted aryl)-but-3-enoyl]-piperazin-1-yl}-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-acetamide **3a–j** analogues were synthesized and their in vitro antibacterial activity was evaluated. Most of the compounds of series showed superior in vitro activity against Gram-positive resistant strains than linezolid. Compound **3f** is the most potent compound in the series with 0.04–0.39 μg/mL MIC.

Drug development in the area of the infectious diseases has been ever challenging due to the continuous development of resistance to the antibiotics few years after their introduction. The situation is getting particularly critical in hospital settings where Gram-positive pathogens like Staphylococci are becoming resistant to β-lactam,[1] vancomycin[2] and other antibiotics.

Oxazolidinones, a new class of antimicrobial agents were first introduced by chemists[3] at E. I. DuPont Nemours and Co. (DUP-721, Fig. 1) and finds no congeners amongst the natural products. Linezolid (Zyvox®,[4] Fig. 1), discovered by Pharmacia and Upjohn, is the first member of oxazolidinone family approved by FDA in 2000 which shows anti-bacterial activity against a wide spectrum of Gram-positive bacteria including multi drug resistant strains.[5] It was presumed that oxazolidinones were not prone to resistance development due to their unique mode of action.[6,7] However resistance to all antimicrobial agents is predictable and hence resistance to linezolid has also been observed in clinical isolates of *Staphylococcus aureus* and *Enterococcus* sp.[8] The frequency of emergence of resistant strains against linezolid is increasing rapidly. Thus there is urgent need to initiate program in development of new member of this class to be ready, if resistance becomes more of an issue.

Eperezolid (Fig. 1), another lead molecule of oxazolidinone family is not approved for treating bacterial infection due to its side effects. However, several research groups[9] have attempted modification of eperezolid to get superior analogs devoid of side effects.

Derivatives of 4-piperazinoaryl oxazolidinones have higher stability and low toxicity hence several libraries containing this motif have been synthesized and their antibacterial activities were evaluated in the past.[10] Lohray[9] et al. have been attempted to synergize the antibacterial activities by incorporating unsaturated carbonyl scaffold on the piperazinyl aryl oxazolidinone derivatives **1** (Fig. 2). They also have investigated the antimicrobial activity of aryl-pent-2-ene-1,4-dione derivative **2** (Fig. 2). Similar glyoxamide analog[11a] of eperazolid and alkylated derivatives[11b] of **4** have recently been reported. We herein report the synthesis of *N*-[3-(3-fluoro-4-{4-[2-oxo-4-(substituted aryl)-but-3-enoyl]-piperazin-1-yl}-phenyl)-2-oxo-oxazolidin-5-ylmethyl]-acetamide **3a–j** (Fig. 2) and their in vitro antibacterial activity against Gram-positive and Gram-negative resistant strains.

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

Compounds **5a–j**, the potassium salts of 2-oxo-4-aryl-but-3-enoic acid were prepared by Knoevenagel reaction of pyruvic acid

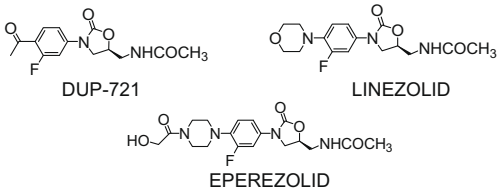

Figure 1.

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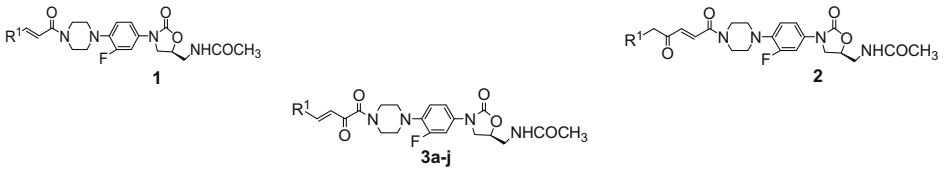
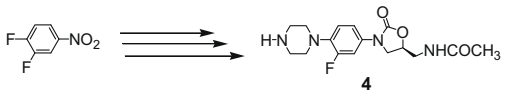
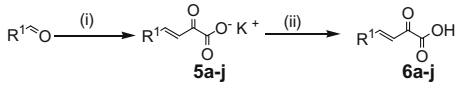


Figure 2.



Scheme 1.



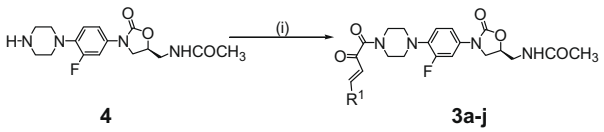
Scheme 2. Reagents and conditions: (i) pyruvic acid, methanolic KOH, 0 °C; (iii) 5 % aqueous HCl.

with different substituted aromatic aldehydes in presence of methanolic KOH.[12b] Treatment of **5a–j** with aqueous HCl afforded 2-oxo-4-aryl-3-butenoic acid **6a–j** (Scheme 2).

Compounds **6a–j** were coupled with intermediate **5** using EDC and HOBt in presence of triethylamine at 0 °C afforded novel oxazolidinone analogues **3a–j** in good yields (Scheme 3).

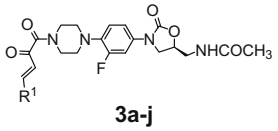
All compounds **3a–j** were well characterized[13] and were submitted for the assessment of in vitro antibacterial activity against a panel of susceptible and resistant Gram-positive and Gram-negative bacteria. None of these compounds exhibited any activity against Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The data for selected Gram-positive organisms have been reported as minimum inhibitory concentration (MIC) expressed in μg/mL (Table 1).

MICs were determined by agar dilution method using doubling dilutions in cation adjusted Mueller Hinton agar over a concentration range of 50–0.019 μg/mL and incubation in air at 35 °C for 24 h. 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 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 on to the plates. 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 SOFTmaxPro 4.3 Software (Molecular Devices, Sunnyvale, USA).



Scheme 3. Reagents and conditions :(i) compounds **6a–j**, EDC·HCl, HOBt, Et_3N, 0 °C.

Table 1
MIC values (μg/mL) for oxazolidinones **3a–j**



Compounds	R^1	*C* log *P*	S.a.[a]	S.a[b]	S.a.[c]	B.c.[d]	E.f.[e]	S.p.[f]
3a		0.925	0.78	0.39	0.78	0.39	0.78	0.39
3b		1.086	0.78	0.78	1.56	0.39	0.78	0.39
3c		1.638	0.78	0.78	0.78	0.39	0.78	0.39
3d		1.141	0.78	1.56	3.12	0.39	0.78	0.78
3e		2.824	>50	>50	>50	>50	>50	>50
3f		1.420	0.19	0.19	0.39	0.19	0.04	0.09
3g		1.808	0.78	0.78	1.56	1.56	0.19	0.19
3h		0.101	0.39	1.56	3.12	0.78	0.78	0.78
3i		0.571	0.39	0.78	1.56	0.39	0.78	0.39
3j		0.571	0.78	0.78	1.56	0.78	0.78	0.39
Linezolid			1.56	1.56	3.12	0.78	3.12	1.56
Vancomycin			0.39	6.25	1.56	0.19	0.78	0.39

[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] *Streptococcus pyogens*.

Most of the compounds of this series exhibited a range of MIC 0.04–1.56 μg/mL some of which are superior as compared to that of linezolid against Gram-positive bacterial strains. The most potent compound of the series was **3f** , displaying MIC range between 0.04 and 0.39 μg/mL which was lower than MIC value of linezolid (0.78–3.12 μg/mL) and vancomycin (0.19–6.25 μg/mL). The compound **3f** was found to have better activity against *Enterococcus faecalis* (MIC value: 0.04 μg /mL) than against *Streptococcus pyogenes* (MIC value 0.09 μg/mL).

Compound **3a** was found either equipotent or more potent than compound **3b** against all strains examined in this study. It is obvious from this data that electronegative substitution at para position decreased activity against *S. aureus* (ATCC 70069 and ATCC 29213). This observation was confirmed by the similar trend observed in the MIC values of compound **3f** and **3g**. Compound **3f** (R^1 = 4-CH_3Ph) exhibited MIC range 0.04–0.19 μg/mL which is lower than the MICs of compound **3g** (R^1 = 4-CF_3Ph) 0.19–1.56 μg/mL.

Compound **3d** (R^1 = 3,4-difluorophenyl) was either equipotent or less potent than compound **3b** (R^1 = 4-fluorophenyl). The observed data indicated that mono substituted phenyl ring was favored over disubstituted phenyl ring. Compound **3e** (R^1 = 2,3,4-trichlorophenyl) was found to be devoid of any activity. From this observation it may be concluded that incorporation of ortho substituent is detrimental for the anti-bacterial activity.

Among heterocyclic compounds, the thiophenyl analogues **3i** and **3j** with MIC range of 0.39–1.56 μg/mL were more potent than linezolid against all strains screened. Compound **3i** (2-thiophenyl) derivative was superior to its regioisomer **3j** (3-thiophenyl) against all bacterial strains screened except *S. aureus* ATCC 25923. Compound **3i** was found to be more potent than **3j** against this strain with MIC value at 0.39 μg/m. Compound **3h** (furanyl) exhibited MICs in same range 0.39–3.12 that of linezolid against *S. aureus* (ATCC 29213 and ATCC 70069) and was found to be more potent than linezolid against rest of the bacterial strains examined.

Compound **3f** was the only compound in the series which was more potent than vancomycin against all strains except *Bacillus cereus*. All compounds except **3g** were either equipotent or less potent than vancomycin against *E. faecalis* and *S. pyogenes*. All compounds except **3e** exhibited lower MIC than vancomycin against *S. aureus* ATCC 70069 strain. On the contrary, all compounds except **3b**, **3c** and **3f** showed higher MIC than vancomycin against *S. aureus* ATCC 29213 strain.

Earlier several groups attempted[14] to correlate biological activities with *C* log *P*. We also attempted for the same but failed to establish any significant correlation. The *C* log *P* value[15] of the compounds in this series was in the range 0.10–2.824 showing variable MIC values against strains evaluated. Instead of having same *C* log *P* value at 0.571, the compounds **3i** and **3j** exhibited different MIC values against bacterial strains screened. Thus no correlation of computed *C* log *P* of the compounds with their respective MIC values could be established. This clearly indicates that lipophilicity of compounds is not critical factor responsible for their antibacterial activity. Other factors like steric and electronic might be responsible for the variation in antibacterial activity.

In conclusion, a novel series of oxazolidinone analogues were synthesized exhibiting excellent in vitro activity against several Gram–positive resistant strains. The best compound of the series is **3f** more potent than linezolid as well as vancomycin against all bacterial strains evaluated. Further lead optimisation in the series is in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.106.

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