

Synthesis and antibacterial activity of arylpiperazinyl oxazolidinones with diversification of the *N*-substituents

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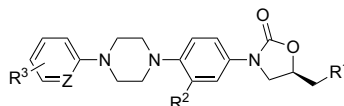
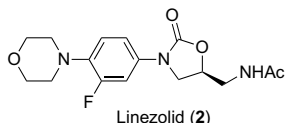
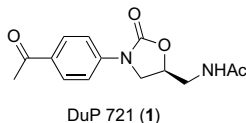
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Abstract—A series of 4-aryl-piperazin-1-yl-3-phenyloxazolidin-2-one derivatives with diversification of the *N*-substituents such as methylene *O*-linked heterocycles, thioamide, dithiocarbamate, thiourea, and thiocarbamate were synthesized and evaluated as antibacterial agents. Their in vitro activities (MIC) were evaluated against MRSA and VRE resistant Gram-positive strains such as *Staphylococcus* and *Enterococcus*. Most of the compounds were more potent in vitro but less active in vivo than linezolid.
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Many strains of multidrug resistance of Gram-positive pathogens cause clinical problems.¹ These include methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococcus faecium* (VREF). Since the first discovery of a new class of purely synthetic antibiotics DuP 721 (**1**)² was made, linezolid (Zyvox®) (**2**)³ was introduced in the market for the treatment of multidrug resistant Gram-positive infections such as nosocomial and community-acquired pneumonia and skin infections. It has novel action mechanism to inhibit protein synthesis by binding to the 50S ribosomal subunit with interference of fMet-tRNA binding to the P-site of the ribosomal peptidyltransferase center.⁴

After a few years of introducing linezolid already reported were linezolid-resistance *Enterococci*⁵ and linezolid-resistance *S. aureus*.⁶ Therefore, continuous efforts are necessary to develop more effective antibacterial agent as a new chemical entity. Many different approaches to alter the chemical structure of oxazolidinone antibiotic represented with linezolid and eperizolid can be classified into three different strategies: (1) modifying the core structure of oxazolin-2-one ring as its surrogates, (2) changing the pendent structure at C-4 of the oxazolin-2-one ring, and (3) diversifying the structure of *N*-acetylaminomethyl group. After the observation of the antibacterial activity with the compound bearing 4-(4-aryl)piperazinylphenyl group on the nitrogen of the oxazolidin-2-one⁷ we tried to alter the



3 R¹ = O-2-Pyridine, R² = F, R³ = 2-Cl, Z = H

4 R¹ = O-2-Pyrazine, R² = F, R³ = 2-Cl, Z = H

5 R¹ = O-2-Isoxazole, R² = F, R³ = 2-Cl, Z = H

6 R¹ = N(C=S)CH₃, R² = F, R³ = various substituents, Z = H or N

7 R¹ = N(C=S)SCH₃, R² = H or F, R³ = various substituents, Z = H or N

8 R¹ = N(C=S)NH₂, R² = H or F, R³ = various substituents, Z = H or N

9 R¹ = N(C=S)OCH₃, R² = F, R³ = various substituents, Z = H or N

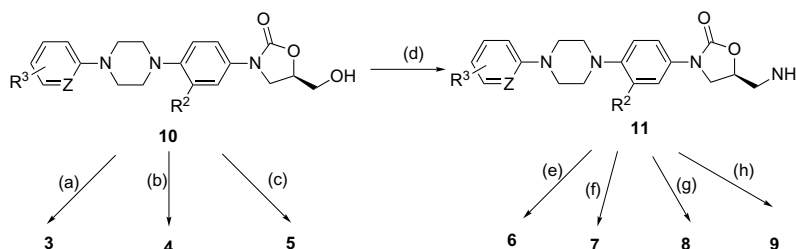
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structure of acetamide part with diversification of the *N*-substituents such as methylene *O*-linked heterocycles,⁸ thioamide, dithiocarbamate, thiourea, and thiocarbamate.⁹ In this report is described the synthesis and antibacterial activity of these compounds (**3–9**).

At first the parent molecule 4-(4-aryl)piperazinylphenyl-5-hydroxymethyloxazolidin-2-one **10** was synthesized by the reported method.⁷ The substitution of 5-hydroxymethyl to C-5 methyleneoxoheterocyclic compounds (**3**, **4**, and **5**) were achieved by the reaction with 2-hy-

droxypyridine, 2-chloropyrazine, and 3-hydroxyisoxazole, respectively. Azide displacement of the hydroxyl group on compound **10** and the subsequent hydrogenation yielded amine **11** that was further reacted with acetyl chloride to give acetamide. This was treated with Lawesson's reagents to give the corresponding thioamide **6** in 65–89% yields. *S*-Methyldithiocarbamate **7** was obtained from the corresponding amine by the reaction with CS₂ and iodomethane in good yield. Treatment of the amines with 1.1 mol equivalent of 1,1'-thiocarbonyldi-2(1*H*)-pyridone yielded thiocyanate that was



Scheme 1. Reagents and conditions: (a) 2-hydroxypyridine, PPh₃, DEAD, DMF; (b) 2-chloropyrazine, NaH, DMF; (c) 3-hydroxyisoxazole, PPh₃, DEAD, DMF; (d) (i) MsCl, TEA, CH₂Cl₂, 0 °C; (ii) NaN₃, DMF, 80 °C; (iii) Pd/C, H₂, EtOAc/EtOH; (e) (i) acetyl chloride, TEA, CH₂Cl₂, 0 °C; (ii) Lawesson's reagent, dioxane, 110 °C; (f) CS₂, TEA, EtOH, MeI; (g) (i) 1,1'-thiocarbonyldi-2(1*H*)-pyridone, CH₂Cl₂; (ii) 2.0 M NH₃ in MeOH, TEA, 0 °C; (h) (i) 1,1'-thiocarbonyldi-2(1*H*)-pyridone, CH₂Cl₂; (ii) MeOH, reflux.

Table 1. Prepared compounds **3–9** and their in vitro antibacterial activity against four different bacterial strains (MIC, µg/mL)^a

No.	Compound				Microorganism ^b			
	R ¹	R ²	R ³	Z	S.a. ¹	S.p.	S.a. ²	E.f.
3	O-2-Pyridine	F	2-Cl	H	>64	>64	>64	>64
4	O-2-Piperazine	F	2-Cl	H	>64	>64	>64	>64
5	O-2-Isioxazole	F	2-Cl	H	>64	>64	>64	>64
6a	N(C=S)CH ₃	F	H	H	0.12	0.06	0.12	0.25
6b	N(C=S)CH ₃	F	H	N	0.06	0.03	0.12	0.25
6c	N(C=S)CH ₃	F	2-Cl	H	0.12	0.12	0.12	>64
6d	N(C=S)CH ₃	F	3-Cl	H	0.03	0.03	0.03	0.06
6d	N(C=S)CH ₃	F	4-Cl	H	0.12	0.15	0.25	0.25
6e	N(C=S)CH ₃	F	2-Me	H	0.12	0.12	0.12	0.05
6f	N(C=S)CH ₃	F	3-F	H	0.12	0.12	0.12	0.03
7a	N(C=S)SCH ₃	F	H	N	0.06	0.06	0.25	0.12
7b	N(C=S)SCH ₃	F	3-Cl	H	0.12	0.50	1.00	0.50
7c	N(C=S)SCH ₃	H	3-OMe	H	0.12	0.12	0.25	0.12
7d	N(C=S)SCH ₃	F	2-Me	H	0.12	0.12	0.12	0.12
8a	N(C=S)NH ₂	H	H	H	0.12	0.06	0.25	0.12
8b	N(C=S)NH ₂	F	H	H	0.06	0.06	0.06	0.12
8c	N(C=S)NH ₂	F	2-OMe	H	0.25	0.12	0.25	0.25
8d	N(C=S)NH ₂	F	H	N	0.12	0.06	0.12	0.25
8e	N(C=S)NH ₂	H	2-Cl	H	0.03	0.008	0.06	0.06
8f	N(C=S)NH ₂	F	2-Cl	H	0.12	0.12	0.12	0.25
8g	N(C=S)NH ₂	F	3-Cl	H	0.02	0.004	0.008	0.06
8h	N(C=S)NH ₂	F	3-OMe	H	0.03	0.06	0.06	0.06
8i	N(C=S)NH ₂	F	4-Cl	H	0.12	0.12	0.12	0.50
8j	N(C=S)NH ₂	F	2-Me	H	0.12	0.06	0.12	0.25
8k	N(C=S)NH ₂	F	3-CF ₃	H	0.25	0.12	0.12	0.25
9a	N(C=S)OCH ₃	F	H	H	0.25	0.12	0.12	0.25
9b	N(C=S)OCH ₃	F	H	N	0.12	0.06	0.06	0.12
9c	N(C=S)OCH ₃	F	3-Cl	H	0.12	0.06	0.12	0.12
9d	N(C=S)OCH ₃	F	3-OMe	H	0.12	0.06	0.12	0.12
Linezolid					0.25	0.50	0.50	1.00

^a Agar dilution method, Mueller–Hinton agar, 104 CFU/spot.

^b S.a.¹ = *Staphylococcus aureus* ATCC 25923, S.p. = *Staphylococcus pyogenes* ATCC 8668, S.a.² = *Staphylococcus agalactiae* ATCC 13813, E.f. = *Enterococcus faecalis* ATCC 29212.

served as the precursor for the synthesis of thiourea and methylthiocarbamate. When this was treated with ammonia in methanol or methanol only, thio-analogs of thioureas **8** and methylthiocarbamates **9** were yielded, respectively, in more than 85% yield in most cases (Scheme 1).

Antibacterial activity of compounds **4–9** were tested in vitro against Gram-positive pathogenic bacteria with linezolid as the reference compound. Minimum inhibitory concentration (MIC) values were determined using agar dilution or broth microdilution methodology. Compounds were incorporated into MH (Mueller–Hinton) agar medium at concentration of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03 mg/L. The test organism was grown in MH broth medium at 35 °C for 16–18 h, the broths were adjusted to the turbidity of 0.5 McFarland standards, and then the bacterial suspensions were inoculated onto the drug-supplemented MH agar plates at 35 °C for 18–20 h. The MIC was defined as the lowest concentration of drug that completely inhibited growth of the organism and the results are shown in Table 1.

Most analogs exhibited excellent antibacterial activity against the Gram-positive strains we tested except compounds **4**, **5**, and **6** with methylene *O*-linked heterocycles. Thiocarbonyl compounds such as thioamide, dithiocarbamate, thiourea, and thiocarbamate were more active in vivo than linezolid tested as the internal reference. Selected compounds **6d**, **7a**, **8g**, and **9b** representing each group of compounds were also evaluated for in vivo efficacy against *S. aureus* with lethally infected mice. However, the activities of all three compounds were less potent as >16 mg/kg than linezolid 4 mg/kg in terms of ED₅₀s.

In summary, a series of 4-arylpiperazin-1-yl-3-phenyl-oxazolidin-2-one derivatives with diversification of the *N*-substituents such as methylene *O*-linked heterocycles, thioamide, dithiocarbamate, thiourea, and thiocarbamate were synthesized efficiently and evaluated as antibacterial agents in vitro. Most of the compounds were more potent in vitro but less active in vivo than linezolid.

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