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Synthesis and In Vitro Activity of New Methylenepiperidinyl and Methylenepyrrolidinyl Oxazolidinone Antibacterial Agents

Hye Yeon Kim, Jae Seok Lee, Joo Hwan Cha, Ae Nim Pae, Yong Seo Cho, Moon Ho Chang and Hun Yeong Koh*

Biochemicals Research Center, Korea Institute of Science and Technology, Cheongryang, Seoul 130-650, South Korea

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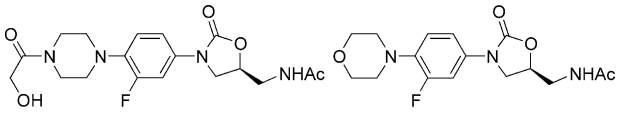
Abstract—We have prepared and evaluated the antibacterial activities of a series of substituted methylenepiperidinyl and methylenepyrrolidinyl oxazolidinones against several gram-positive strains including the resistant strains of *Staphyloccus* and *Enterococcus*, such as MRSA, CRSA, MSSA and VRE. Some of them showed comparable or superior in vitro activities (MIC) to vancomycin.

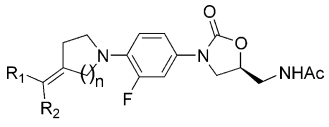
Introduction

The oxazolidinones are an exciting new development in the effective treatment of Gram-positive bacterial infections, including those infections caused by strains resistant to other antibiotics. The rising prevalence of multidrug resistant Gram-positive bacteria requires the discovery of novel active agents against these pathogens. New classes of antibacterial agents with novel mechanisms of action are urgently needed to combat the increase in multidrug resistant infections. The oxazolidinones, a new class of totally synthetic antibacterial agents, are active against a variety of clinically important susceptible and resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP). Scientists at DuPont originally discovered this class of agents in the late 1980's.[1] However, development of DuP-721, the drug candidate that emerged from these initial studies, was discontinued following Phase I clinical trials. Subsequently, researchers at Pharmacia and Upjohn identified two clinical candidates, eperezolid (**1**) and linezolid (**2**).[2] Linezolid is currently marketed for the treatment of multidrug resistant Gram-positive infections such as nosocomial and community-acquired pneumonia and skin infections.



eperezolid (**1**) linezolid (**2**)

Recently, our laboratory revealed that two series of oxazolidinones having an isoxazole as a rigid bioisostere of hydroxymethyl group of eperezolid were synthesized and the effect of introducing the isoxazole moiety on the activity was investigated.[3] To improve biological activity, in the present work we describe the synthesis and antibacterial activity of a related family of compounds **5** and **6** in which the substituted methylenepiperidinyl and methylenepyrrolidinyl ring were introduced. Several potent compounds were studied in vivo activities and pharmakokinetic profile.



5. n = 1
6. n = 2

Chemistry

The synthesis of the oxazolidinone derivatives is outlined in Scheme 1. To prepare the oxazolidinone derivatives shown in Table 1, we could use the Knoevenagel condensation reaction as a key step.[4] The ketone intermediates **3** and **4**, which were prepared by known

*Corresponding author. Tel.: +822-958-5146; fax: +822-958-5189; e-mail: hykoh@kist.re.kr

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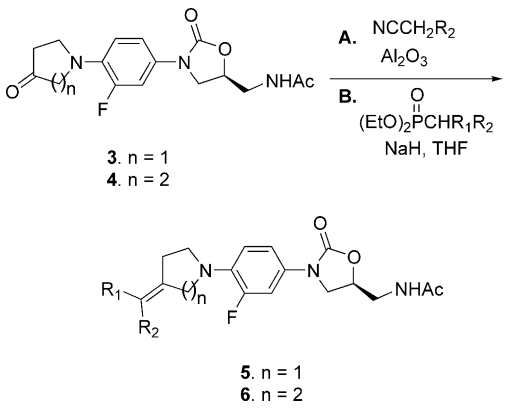
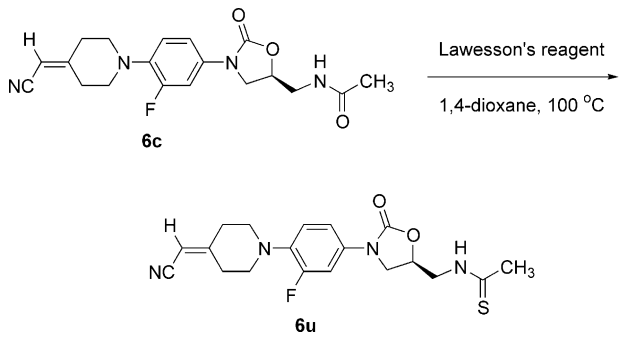
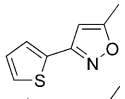
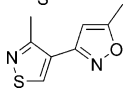


Scheme 1.



Scheme 2.

Table 1. Synthesized oxazolidinones of **5** and **6**

Compd	Method[a]	R_1	R_2	Yield (%)[b]
5a	A	CN	CN	51
5b	A	CN	CO_2Et	50
5c	B	H	CN	98
5d	B	CH_3	CN	98
6a	A	CN	CN	69
6b	A	CN	CO_2Et	46
6c	B	H	CN	64
6d	B	H	CO_2Et	64
6e	B	H	$COCH_3$	91
6f	B	CH_3	CO_2Et	54
6g	B	H	COONa	13
6h	B	Cl	CO_2Et	35
6i	B	CN	CH_3	32
6j	B	H	CHO	8
6k	B	H	CH(NOH)	71
6l	B	H	$CH(NOCH_3)$	57
6m	B	H	$C(NOH)CH_3$	43
6n	B	H	$C(NOCH_3)CH_3$	74
6o	B	H	$CH(OH)CH_3$	77
6p	B	H	$CH(OAc)CH_3$	57
6q	B	H	$C(OCOCH_2Cl)_2CH_3$	70
6r	B	H	$C(OCOCHCl_2)CH_3$	69
6s	B	H		20
6t	B	H		15

[a]Method A; Knoevenagel condensation, Method B; Wadsworth–Horner–Emmons reaction.
[b]Isolated yield.

method,[5,6] with the malononitrile and cyanoacetic ester using aluminum oxide gave the product **5** and **6** in 46–69% yield after chromatographic purification on silica gel (5% methanol-ethyl acetate).[7]

In another way, the new oxazolidinone derivatives also can be prepared via Wadsworth–Horner–Emmons reaction.[8] As shown in Scheme 1, a variety of phosphonate anions have been prepared via reaction of phosphonates with sodium hydride or *n*-butyllithium in freshly distilled tetrahydrofuran for 1 h. Then compounds **3** or **4** were added to the preformed phosphonate anions, and the reaction mixture was stirred for a few hours, for some time, relatively rigorous reaction condition such as reflux was required to confirm the reactions completed in 15–98%.[9]

As reported by Upjohn,[10] thioacetamide of 5-position of oxazolidinone showed 2-fold good activity compared to the acetamide form. Thus, the most potent compound **6c** synthesized was treated with Lawesson's reagent to transform into thioacetamide **6u** (Scheme 2) and it was also evaluated in vitro activity.

In Vitro and In Vivo Activity

In vitro antibacterial activities of all the compounds prepared and references were determined by the Mueller–Hinton agar dilution method.[11] The activities of compounds synthesized were compared with linezolid, and vancomycin as references. Data for selected Gram-positive organisms are reported as a minimum inhibitory concentration (MIC) expressed in υμg/mL (Tables 2 and 3). Most of the compounds synthesized exhibited good antibacterial activities against Gram-positive strains and the resistant strains including MRSA (methicillin-resistant *Staphylococcus aureus*), CRSA (ciprofloxacin-resistant *Staphylococcus aureus*), MSSA (methicillin-susceptible *Staphylococcus aureus*) and VRE (vancomycin-resistant *Enterococcus faecium*).

As results of screening of compounds **6a–t**, compound **6c** and **6u** showed the most potent activities in this series relative to linezolid and vancomycin against Gram-positive organisms (Table 2). Especially, **6u** showed 8-fold and 4-fold good activity than linezolid against *Staphylococcus pyogenes* C6003 (S.p.2) and VRE, respectively. The cyano group might be playing crucial role for the activity as a substituent.

In our previous study,[3] 5-isoxazolyl moiety was effective to the activity as a rigid bioisostere of the hydroxyacetamide functionality of eperezolid (**1**). According to this result, heterocyclyl methylene **6s–t** were synthesized and evaluated. The replacement of cyanomethylene with heterocyclyl methylene led to less potent compounds. By investigation of the effect of substituents R_1 and R_2, the introduction of polar, simple functional group to the oxzolidinone derivatives were effective for the activity. The replacement by thioacetamide achieved 2-fold increase of activities against most of strains tested and especially 8-fold enhancement against VRE. For the

Table 2. In vitro antibacterial activity of oxazolidinone derivatives against 11 bacterial strains (MIC, μg/mL)[a]

Compd	Microorganism[b]										
	S.a.	MRSA	CRSA	MSSA	*S.e.* 1	*S.e.* 2	*E.f.* 1	*E.f.* 2	*S.p.* 1	*S.p.* 2	VRE
6a	2	2	4	4	1	2	4	2	1	4	4
6b	4	4	4	4	1	2	4	2	1	4	2
6c	2	1	2	4	0.5	1	2	2	0.5	2	4
6d	16	8	8	16	4	8	8	8	2	8	8
6e	8	16	16	16	1	8	16	8	0.25	16	8
6f	16	8	8	16	4	8	8	8	4	16	8
6g	8	4	8	8	1	1	8	4	2	8	8
6h	32	16	16	8	2	4	4	2	2	8	4
6i	2	2	2	2	0.5	2	2	2	0.5	2	1
6j	8	4	4	8	1	2	8	4	2	8	8
6k	8	4	8	8	1	4	4	4	2	4	4
6l	8	4	4	8	1	2	4	4	1	2	4
6m	8	4	8	8	1	4	4	4	1	4	4
6n	16	8	16	16	8	8	8	8	2	8	4
6o	8	4	8	16	2	4	8	4	2	8	4
6p	16	8	16	16	2	8	8	8	0.25	8	4
6q	16	8	16	16	2	8	16	8	2	8	4
6r	16	8	16	16	2	8	8	8	2	8	4
6s	32	16	16	16	4	8	16	16	2	16	16
6t	16	8	8	16	2	4	8	8	2	8	4
6u	2	2	2	2	0.5	0.5	1	1	0.5	0.25	0.5
LZ[c]	4	2	2	4	0.5	2	2	2	0.5	2	2
VCM[d]	1	1	2	1	1	2	2	1	0.12	4	>32

[a]Agar dilution method, Mueller–Hinton agar, 10^4 CFU/spot.
[b]*S.a.* = *Staphylococcus aureus* ATCC 29213, MRSA = methicillin-resistant *Staphylococcus aureus* C6068, CRSA = ciprofloxacin-resistant *Staphylococcus aureus* C6043, MSSA = methicillin-susceptible *Staphylococcus aureus* C2214, *S.e.* 1 = *Staphylococcus epidermis* ATCC 1228, *S.e.* 2 = *Staphylococcus epidermis* C2235, *E.f.* 1 = *Enterococcus faecalis* C6291, *E.f.* 2 = *Enterococcus faecalis* C6301, *S.p.* 1 = *Staphylococcus pyogenes* ATTCC8668, *S.p.* 2 = *Staphylococcus pyogenes* C6003, VRE = *vancomycin-resistant Enterococcus faecium*, C6487.
[c]LZ = linezolid.
[d]VCM = vancomycin.

Table 3. In vitro antibacterial activity of oxazolidinone derivatives against 11 bacterial strains (MIC, μg/mL)[a]

Compd	Microorganism[b]										
	S.a. 1.	*S.a.* 2	MRSA 1	MRSA 2	*S.e.* 1	*S.e.* 2	*S.e.* 3	*E.f.* 1	*E.f.* 2	VRE 1	VRE 2
5a	8	16	8	16	4	4	8	16	16	16	16
5b	16	32	16	16	8	8	16	32	32	16	16
5c	0.5	1	0.5	0.5	0.5	0.5	0.5	1	1	1	1
5d	1	2	1	1	1	1	1	4	4	2	2
6c	0.5	0.5	0.5	0.5	0.25	0.5	0.5	1	1	1	1
LZ[c]	0.5	1	1	0.5	0.5	0.5	1	2	1	1	1
VCM[d]	1	1	2	1	1	1	1	4	64	64	64

[a]Agar dilution method, Mueller–Hinton agar, 10^4 CFU/spot.
[b]*S.a.* 1 = *Staphylococcus aureus* 77, *S.a.* 2 = *Staphylococcus aureus* SA011, MRSA 1 = methicillin-resistant *Staphylococcus aureus* 241, MRSA 2 = methicillin-resistant *Staphylococcus aureus* K283, *S.e.* 1 = *Staphylococcus epidermiidis* Q004, *S.e.* 1 = *Staphylococcus epidermiidis* Q033, *S. e.* 1 = *Staphylococcus epidermiidis* R005, *E. f.* 1 = *Enterococcus faecalis* 29212A, *E. f.* 2 = *Enterococcus faecalis* 2009, VRE 1 = vancomycin-resistant *Enterococcus faecium* 2006, VRE 1 = vancomycin-resistant *Enterococcus faecium* 2153.
[c]LZ: linezolid.
[d]VCM = vancomycin.

investigation of the effectiveness of ring size, a series of the five membered pyrrolidinyl derivatives **5** were synthesized and evaluated (Table 3). Cyanomethylenyl analogue **5c** showed also the best activity but 2-fold less active than **6c**. The pharmacokinetic profile of some active compounds were studied, only **6c** showed meaningful result by comparing with Linezolid (Table 4). In vivo activity of **6c** (PD_{50} = 4.60 mg/kg) was comparable to that of linezolid (PD_{50} = 4.07 mg/kg) against *S. aureus* Smith (Table 5). Further optimization of the structure of oxazolidinone analogues for in vitro and in vivo activities are in progress.

Table 4. Single dose pharmacokinetic parameters for oxazolidinone antibiotics in male rates

Compd	AUC (μg.h/mL)	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$(h)
6c	6.807	0.5	1.321	8.139
Linezolid	4.239	0.333	1.389	3.093

Each compound was dissolved in 50% PEG solution and orally administered to rats at a dose of 15 mg/Kg.

Table 5. In vivo activity of selected compounds

Microorganism	Challenge dose (cfu/mouse)	Route	Compd	MIC (υγμg/mL)	PD_{50}[a] (mg/kg)
S. aureus Smith	1.8×107	po	**6c**	2	4.60
			6i	2	>8.0
			6k	8	>8.0
			Linezolid	2	4.07

[a]Each test compound was suspended in 0.5% carboxymethyl cellulose and administered orally 1 h following intraperitoneally bacterial inoculation in mice.

Acknowledgements

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7. Representative procedure: **6a** To a solution of *N*-[3-{3-fluoro-4-(4-oxo-piperidin-1-yl)-phenyl}-2-oxo-oxazolidin-5-ylmethyl]-acetamide (20 mg, 0.06 mmol), aluminium oxide (Basic, I, Aldrich, 17.2 mg) and malononitrile (3.8 mg, 0.06 mmol) in dichloromethane (0.5 mL) was stirred at 40 °C for 15 h. The reaction mixture was poured into a mixture of dichloromethane and water. The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 mL×3). The collected organic layer was washed with brine, dried over anhydrous $MgSO_4$, evaporated and purified by column chromatography (5% methanol–ethyl acetate) to give the product **6a** (14.8 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 1.2, 1.2 Hz, 1H), 7.09 (dd, *J* = 1.1, 6.6 Hz, 1H),

6.92 (t, $J=9.1$ Hz, 1H), 6.31 (m, 1H), 4.76 (m, 1H), 4.02 (t, $J=8.8$ Hz, 1H), 3.76 (t, $J=8.7$ Hz, 1H), 3.62 (m, 2H), 3.26 (m, 4H), 2.92 (m, 4H), 1.92 (s, 3H), IR (KBr, cm^{-1}) 2232, 1750, 1660, 1518, 1418, 1382, 1216, 1012, 752, ^{13}C NMR($CDCl_3$, 300 MHz) $CDCl_3$, 300 MHz), δ 180.21, 171.83, 157.38, 154.68, 135.65, 134.25, 134.36, 134.22, 120.44, 114.25, 111.53, 108.46, 107.79, 84.52, 73.36, 51.39, 47.28, 42.31, 34.72, 23.51, HRMS (FAB, M + H) Calcd. for $C_{20}H21N_5O_3$ 398.1628, found 398.1579.

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9. Representative procedure: **5c**; To a solution of *tert*-butoxide (0.84 g, 1.49 mmol) in THF (30 mL) was added dropwise diethyl cyanomethyl phosphonate (1.35 g, 7.6 mmol) in THF (30 mL). After stirring for 1 h, *N*-[3-{3-fluoro-4-(3-oxopyrrolidin-1-yl)-phenyl}-2-oxo-oxazolidin-5-ylmethyl]-acetamide (0.50 g, 0.24 mmol) in THF (90 mL) was dropwise for 1 h at −78 °C. After stirring for 7 h at the same temperature, The reaction mixture was poured into a mixture of dichloromethane and water. The organic layer was separated and the aqueous layer was extracted with dichloromethane (20 mL×3). The collected organic layer was washed with brine, dried over anhydrous $MgSO_4$, evaporated and purified by column chromatography (5% methanol–ethyl acetate) to give the product **5c** (525.7 mg, 98%). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40 (dd, 1H, $J=2.5$, 2.5 Hz), 6.70 (m, 2H), 5.38 (d, 1H, $J=2.4$ Hz), 4.77 (m, 1H), 4.13 (s, 1H), 4.01 (t, 1H, $J=8.9$ Hz), 3.76 (t, 1H, $J=7.9$ Hz), 3.66 (d, 2H, $J=2.7$ Hz), 3.48 (m, 2H), 2.92 (m, 2H), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.69, 166.61, 154.95, 154.84, 151.40, 133.31, 133.20, 133.17, 131.38, 131.25, 117.10, 117.03, 116.37, 116.40, 114.80, 114.76, 108.41, 108.06, 91.91, 91.58, 77.89, 77.46, 77.05, 72.40, 56.02, 55.93, 50.04, 49.98, 49.66, 49.60, 48.13, 42.31, 32.96, 32.12, 23.40. HRMS (FAB, M + H) Calcd. for $C_{18}H_{20}FN_4O_3$ 359.1519, found 359.1471.

6c; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (dd, $J=2.70$, 2.40 Hz, 1H), 7.08 (dd, $J=2.49$, 2.46 Hz, 1H), 6.93 (t, $J=9.00$ Hz, 1H), 6.05 (m, 1H), 5.20 (s, 1H) 4.78 (m, 1H), 4.03 (t, $J=9.3$, 1H), 3.78–3.59 (m, 3H), 3.16 (m, 4H), 2.78 (t, $J=4.80$ Hz, 2H), 2.55 (t, $J=5.10$ Hz, 2H), 2.03 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.92, 164.45, 157.01, 155.10, 136.80, 133.98, 120.56, 114.65, 108.33, 108.15, 94.57, 72.65, 52.49, 52.05, 48.34, 42.62, 35.98, 33.50, 23.70. HRMS (FAB, M + H) cacld. for $C_{19}H_{22}FN_4O_3$ 373.1676, found 373.1676.

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