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SYNTHESIS AND *IN VITRO* ACTIVITY OF NEW OXAZOLIDINONE ANTIBACTERIAL AGENTS HAVING SUBSTITUTED ISOXAZOLES

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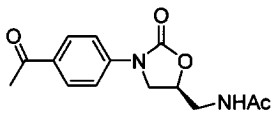
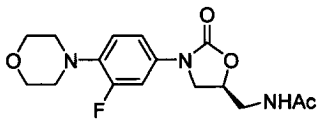
Abstract: Two series of oxazolidinone derivatives having substituted isoxazoles were synthesized and tested for antibacterial activities against several Gram-positive strains including the resistant strains of *Staphylococcus* and *Enterococcus*, such as MRSA, CRSA, MSSA and VRE. Some of them showed *in vitro* activities (MIC) comparable or superior to the reference compound vancomycin.

Introduction: A dramatic increase in antibiotic resistance among Gram-positive bacteria triggered a search for new antibiotics exerting novel modes of action for the treatment of infections caused by multidrug-resistant pathogens such as strains of *Staphylococcus*, *Streptococcus* and *Enterococcus* species.[1] One of such efforts resulted in the discovery of oxazolidinone **1** (Dup-721) by DuPont, a new class of synthetic antibacterial agent having activity against multidrug-resistant Gram-positive bacterial strains.[2] After a while, scientists at Pharmacia & Upjohn reported the development of linezolid (**2**) and eperezolid (**3**) which are currently under clinical trials.[3-4] And now the optimization of the activity of oxazolidinones became a hot topic in the antibiotics research area, and various heterocycles are being introduced.[5-7]

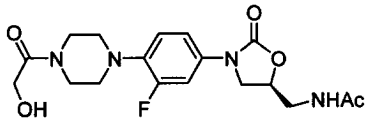
Recent findings in our laboratory revealed that an isoxazolyl substituent could enhance largely the activity of cephalosporins especially against Gram-positive bacteria.[8a-c] Based on the findings, a positive effect of an



Dup-721 (**1**)



Linezolid (**2**)



Eperezolid (**3**)

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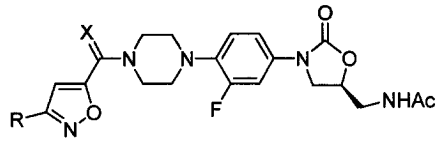
isoxazole group on the activity of oxazolidinone was anticipated. In the present report two series of oxazolidinones (**6** and **8**) having an isoxazole as a rigid bioisostere of hydroxymethyl group of eperezolid (**3**) were synthesized, and the effect of introducing the isoxazole on the activity was investigated. The isoxazole groups were introduced to the nitrogen of piperazine *via* a methylene or a carbonyl linkage.

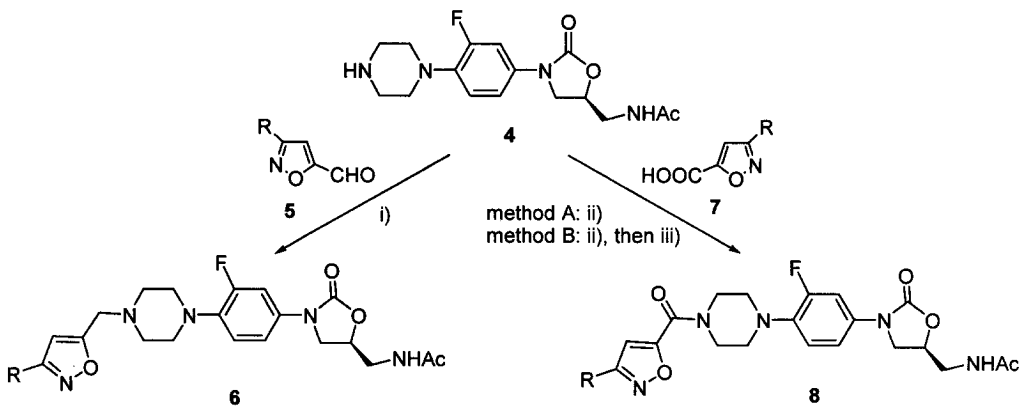


6 : X = H_2
8 : X = O

R = H, alkyl, alkoxy, aryl, heterocyclyl, halogen

Chemistry: The synthesis of oxazolidinone derivatives **6** having an isoxazolylmethyl group is shown in Scheme 1. Reductive alkylation[9] of the amine intermediate **4**, which was prepared by a known procedure,[4] with the isoxazole aldehydes **5** using sodium triacetoxyborohydride gave the products **6** in 50-80% yield after chromatographic purification on silica gel (5% methanol-ethyl acetate).[10] The other series of compounds **8** were prepared by simply acylating the amine **4** with isoxazolecarboxylic acids **7** by the action of phosphorus pentachloride and triethylamine in 40-80% yield.[11] The products were purified by column chromatography using 5% methanol-ethyl acetate as eluent. Compounds **6** and **8** thus prepared are summarized in Table 1.

Scheme 1. Synthesis of oxazolidinones **6** and **8**



i) $NaBH(OAc)_3$, CH_2Cl_2, rt, MS 4A(50-80%), ii) PCl_5, Et_3N, CH_2Cl_2, rt (40-80%), iii) $Pd(PPh_3)_4$, THF, rt (when R = allyloxycarbonyl), or *m*-cresol, 50 °C (when R = *p*-methoxybenzyloxy)

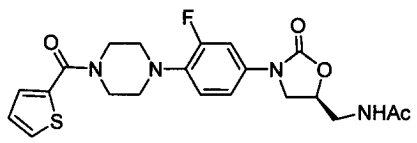
Table 1. Synthesized oxazolidones **6** and **8**

No	R	mp (°C)	Yield[a]	No	R	mp (°C)	Yield
6a	4-Fluorophenyl	181-184	61 %	**8d**	OCH_3	184-185	28 %
6b	4-Chlorophenyl	196-198	50 %	**8e**	CF_3	192-196	60 %
6c	4-Cyanophenyl	164(d)[b]	50 %	**8f**	CN	146-148	45 %
6d	4-Tolyl	192-194	55 %	**8g**	$C(O)NH_2$	198(d)	57 %
6e	3-Methoxyphenyl	144	73 %	**8h**	$CH_2OC(O)NH_2$	180(d)	45 %
6f	3,4-Dimethoxyphenyl	180(d)	78 %	**8i**	$C(O)N(CH_3)_2$	186-187	73 %
6g	4-Phenoxyphenyl	161	69 %	**8j**	$CH_2OC(O)N(CH_3)_2$	142-143	63 %
6h	*trans*-Styryl	181	73 %	**8k**	Br	185(d)	47 %
6i	2-Pyridyl	150-153	52 %	**8l**	Cl	201(d)	50 %
6j	2-Thiophenyl	172-173(d)	78 %	**8m**	2-Thiophenyl	185	66 %
6k	CH_3	132-135	37 %	**8n**	2-Thiazolyl	174-176	30 %
6l	OCH_3	150	90 %	**8o**	COOH	200(d)	73 %[c]
8a	H	172(d)	53 %	**8p**	OH	190(d)	72 %[c]
8b	CH_3	189(d)	36 %	**8q**	1,2,3-Thiadiazol-4-yl	163	80 %
8c	OCH_2CH_2F	159	13 %	**8r**	1,2,5-Thiadiazol-3-yl	156	54 %

[a]The yield of reductive alkylation or acylation. [b]d; decompose. [c]Overall yield of two steps, acylation and deprotection (method B).

On the other hand, the compounds **9** and **10**, which lack the isoxazole, were also prepared for comparison. Otherwise specified, all the products were subjected to biological evaluation as HCl salts (from ethereal HCl).

Biological Study: *In vitro* antibacterial activities (MIC) of all the compounds prepared and references were



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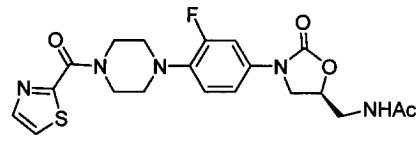

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determined by the Mueller-Hinton agar dilution method.[12] The activities of compounds synthesized were compared with linezolid, eperezolid and vancomycin as references. The results for the selected compounds are summarized in Table 2. Most of the 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*). Regarding the linkage between the isoxazole and the

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

Compds.	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	8	4	8	8	2	4	4	4	2	2	4
6b	>32	16	>32	32	16	32	>32	32	4	4	32
6c	16	8	16	16	4	8	16	16	2	2	8
6d	32	8	32	32	4	8	16	8	4	4	8
6e	4	4	2	4	4	4	8	4	4	1	1
6f	4	2	4	4	2	4	4	4	2	1	2
6g	8	4	8	8	2	4	4	4	1	1	4
6h	>32	32	>32	>32	2	16	16	8	2	2	8
6i	2	2	2	4	1	1	2	2	0.5	0.5	2
6j	4	4	2	4	4	1	2	2	1	1	2
6k	16	16	16	16	16	16	16	16	16	16	16
6l	8	4	8	8	2	4	8	4	1	1	4
8a	2	1	2	2	1	1	2	1	0.5	0.5	2
8b	4	2	4	8	0.5	1	4	2	0.5	0.5	2
8c	4	2	2	2	1	2	2	1	0.5	0.5	1
8d	4	2	4	4	0.5	0.5	2	2	0.5	0.5	2
8e	4	2	4	4	2	2	4	4	1	1	4
8f	2	1	2	2	0.5	0.5	2	1	0.25	0.25	1
8g	2	1	2	2	0.5	0.5	1	1	0.25	0.25	1
8h	2	1	2	2	0.5	0.5	1	1	0.5	0.5	1
8i	8	2	8	8	2	2	4	4	1	1	4
8j	4	2	4	4	1	2	2	2	0.5	0.5	2
8k	4	1	2	2	1	2	2	4	1	1	2
8l	4	1	2	4	1	1	2	2	1	1	2
8m	1	1	1	1	0.25	1	1	1	0.5	0.5	1
8n	2	0.5	0.5	1	0.5	0.5	1	1	0.25	0.5	0.5
8o	>32	>32	>32	>32	>32	>32	>32	>32	16	16	>32
8p	>32	>32	>32	>32	>32	>32	>32	>32	16	16	>32
8q	2	1	2	2	0.5	1	1	1	0.5	0.5	1
8r	4	1	2	4	1	1	2	2	0.5	0.5	1
9	2	1	2	2	0.5	1	2	2	1	1	2
10	4	2	4	4	1	2	4	4	1	1	4
EZ	2	1	2	2	0.5	1	2	1	0.25	0.5	1
LZ	2	1	2	2	1	1	2	1	0.5	0.5	2
VCM	0.5	1	1	0.5	0.5	1	1	0.5	0.25	0.25	>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 faecium* C6301, *S. p.* 1= *Staphylococcus pyogenes* ATTCC8668, *S. p.* 2= *Staphylococcus pyogenes* C6003, VRE= vancomycin-resistant *Enterococcus faecium, C6487,* [c]N.D= No Determination, EZ= eperezolid, LZ= linezolid, VCM= vancomycin

piperazine ring, carbonyl group was more effective than methylene group. The series of oxazolidinone derivatives **8** having an isoxazolylcarbonyl group were about 2-32 fold more potent than **6** having an isoxazolylmethyl group by comparing the activities of **6k** with **8b**, **6j** with **8m**, and **6l** with **8d** (Table 2). According to our 3D QSAR study, it might be due to the difference in their lipophilicities.[13] The tendency of increasing activity with decreasing lipophilicity was observed in the study.

Among the compounds **6** containing methylene linkage, the compounds **6i** and **6j** having a pyridyl and a thiophenyl substituent, respectively, showed the best activities comparable to those of linezolid. The compounds **6b**, **6g**, and **6h** didn't show good activities possibly because of their poor solubility in water. Among the compounds **8** containing carbonyl linkage, **8m** and **8n** were more potent than any of the reference compounds (Table 2). The activity of **8n** was noteworthy. The compound **8n** was 2-4 fold more potent than linezolid and comparable or superior to those of vancomycin, especially, against VRE. By comparing the activities of **8m** with **9**, and **8n** with **10**, it could be found that the isoxazole moiety contributed significantly to the activities. Introduction of the isoxazole moiety enhanced the activity of **9** and **10** by factors of 2 and 4, respectively (Table 2). Further optimization of the structure of these compounds and *in vivo* studies are now in progress.

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10. Representative procedure: To a solution of (*S*)-*N*-[[3-[3-fluoro-4-(*N*-1-piperazinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]acetamide (**4**) (25.7 mg, 73.8 mmol) in 4 mL of methylene chloride was added 5-(2-thiophenyl)isoxazole aldehyde (**5**) (16.5 mg, 91.1 mmol) and sodium triacetoxyborohydride (19.5 mg, 92.0 mmol). After stirring the mixture at rt for 3 h, the reaction mixture was washed with brine, dried with anhydrous $MgSO_4$, concentrated and purified by column chromatography (5% methanol-ethyl acetate) to give the product **6j** (27.1 mg, 55.5 mmol, 77.7 %). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.50-7.40 (m, 3H), 7.13 (dd, 1H, J = 5.1 Hz, J = 3.7 Hz), 7.07 (dd, 1H, J = 12.8 Hz, J = 1.9 Hz), 6.93 (t, 1H, J = 9.1 Hz), 6.58 (s, 2H), 6.08 (t, 1H, J = 7.1 Hz), 4.77 (m, 1H), 4.02 (t, 1H, J = 9.1 Hz), 3.87 (s, 2H), 3.74 (dd, 1H, J = 9.1 Hz, J = 6.8 Hz), 3.68 (dd, 1H, J = 6.3 Hz, J = 3.5 Hz), 3.64 (t, 1H, J = 6.0 Hz), 3.08 (brs, 4H), 2.83 (brs, 4H), 2.01 (s, 3H); IR (KBr, cm^{-1}): 1730, 1652, 1520, 1434, 1234, 752; ^{13}C NMR (75 MHz, $CDCl_3$, δ): 171.18, 164.44, 157.76, 157.24, 156.64, 154.29, 153.97, 128.35, 127.63, 126.92, 119.77, 113.91, 107.69, 107.35, 106.63, 106.08, 71.94, 51.22, 50.51, 47.59, 46.63, 42.90, 41.93, 23.03; HRMS (M+H, FAB): calcd. for $C_{24}H_{27}FN_5O_4S$, 500.1768, found 500.1772.

11. Representative procedure: 5-[3-(2-thiazolyl)isoxazole]carboxylic acid (**7**) (53.9 mg, 0.275 mmol) and phosphorus pentachloride (68.7 mg, 0.450 mmol) were dissolved in 2 mL of THF and the mixture was stirred at rt for 30 min, then the amine **4** (77.0 mg, 0.229 mmol) and triethylamine (64 μL, 0.458 mmol) were added and stirred at rt for 2 h. After completion of the reaction, the reaction mixture was diluted with ethyl acetate, and washed with water, saturated aqueous sodium bicarbonate and brine. The organic layer was dried with abhydrous $MgSO_4$, concentrated and purified by column chromatography (5% methanol-ethyl acetate) to afford the desired product **8n** (35.8 mg, 0.07 mmol, 30.4%). ^{1}H-NMR (300 MHz, $CDCl_3$, δ): 7.97 (d, 1H, J = 3.2 Hz), 7.52 (d, 1H, J = 3.2 Hz,), 7.44 (dd, 1H), 7.30 (s, 1H J = 14.1 Hz, J = 2.5 Hz), 7.08 (dd, 1H, J = 1.8 Hz J = 8.6 Hz), 6.94 (t, 1H, J = 9.2 Hz), 6.58 (t, 1H), 4.77 (m, 1H, J = 6.1 Hz), 4.00 (t, 1H, J = 9.1 Hz), 3.92 (brs, 4H), 3.78 (t, 1H, J = 6.8 Hz), 3.70-3.57 (m, 2H), 3.10 (brs, 4H), 2.01 (s, 3H); IR (KBr, cm^{-1}): 1748, 1658, 1518, 1420, 1226, 982, 928, 750; ^{13}C NMR (75 MHz, $CDCl_3$, δ) 171.22, 164.64, 158.39, 156.45, 155.14, 154.30, 144.06, 135.40, 133.86, 121.52, 119.72, 113.88, 107.64, 107.29, 106.16, 71.95, 51.15, 50.40, 47.57, 46.71, 42.81, 41.87, 22.96; HRMS (M+H, FAB): calcd. for $C_{23}H_{24}FN_6O_5S$ 515.1513, found 515.1509

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