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Synthesis and In Vitro Activity of Novel Isoxazolyl Tetrahydropyridinyl Oxazolidinone Antibacterial Agents

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Abstract—A series of isoxazolyl tetrahydropyridinyl oxazolidinones with various substituents at the 3-position of the isoxazole ring have been synthesized and their in vitro antibacterial activities (MIC) were evaluated against several Gram-positive strains including the resistant strains of *Staphyloccus* and *Enterococcus*, such as MRSA and VRE. One of the most potent compounds synthesized, **4f**, showed comparable or better activity against selected bacterial strains than those of linezolid and vancomycin.

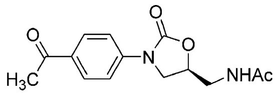
Introduction

The oxazolidinones represent a new class of antibacterial agents which showed activity against a wide spectrum of Gram-positive bacterial infections, including those infections caused by strains resistant to other antibiotics. The oxazolidinones 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).

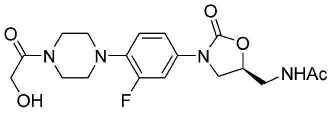
The mechanism of action of the oxazolidinones has been revealed that they inhibit bacterial protein synthesis at an early event in the initiation step of protein synthesis.[1]

The oxazolidinone, exemplified by DuP 721 (**1**), is a relatively new class of orally active, totally synthetic-antibacterial agent discovered by scientists at DuPont.[2] However, DuP 721 was discontinued following Phase I clinical trials because it was shown that DuP 721 exhibited lethal toxicity in rats in drug safety studies conducted at the Upjohn Co. Subsequent studies at Pharmacia and Upjohn Co. have resulted in two potent oxazolidinone antibacterial agents, eperezolid (**2**) and linezolid (**3**).[3]

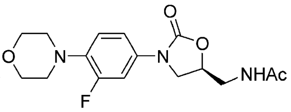
Linezolid (Zyvox®) is the first member of this class recently licensed in the USA and Europe for the treatment of multidrug resistant Gram-positive infections such as nosocomial and community-acquired pneumonia and skin infections.



DuP 721 (**1**)



eperezolid (**2**)



linezolid (**3**)

In our previous study, we have described the synthesis of two series of oxazolidinones having an isoxazole as a rigid bioisostere of hydroxymethyl group of eperezolid and the investigation of the effect of introducing the isoxazole moiety on the activity.[4]

Recently, we have reported on another oxazolidinone analogues having methylenepiperidinyl and methylene-

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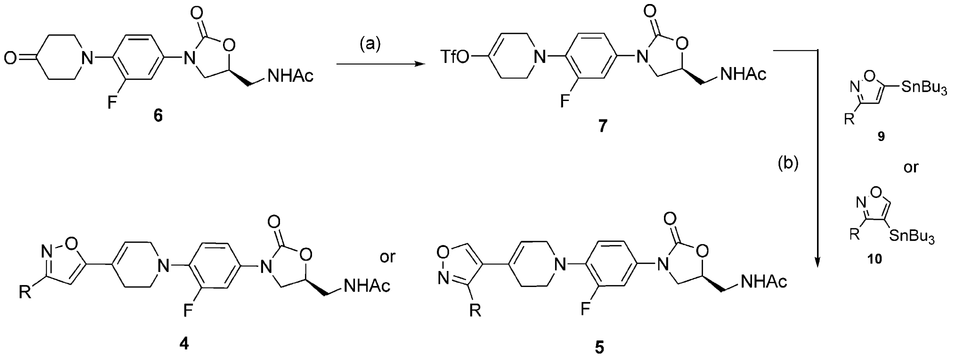
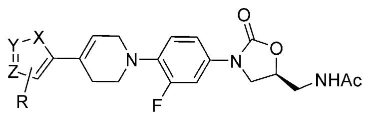


Scheme 1. (a) LDA, $PhNTf_2$, HMPA/THF, −50 °C; (b) $Pd_2(dba)_3$ $CHCl_3$, PPh_3, **9** (or **10**), $ZnCl_2$, NMP, THF, −78 °C to rt.

pyrrolidinyl groups and their evaluation of their antibacterial activities.[5] From the continuing study on anti-infectives, we have explored the possibility of synthesis of novel antibacterials based on the conformationally constrained analogues of the oxazolidinones. Herein, we describe the synthesis and antibacterial activity of a related family of compound **4** and **5** in which the substituted isoxazolyl tetrahydropyridinyl groups were introduced.



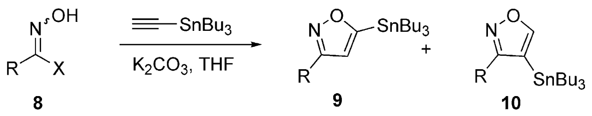
4; X = O, Y = N, Z = C
5; X = C, Y = O, Z = N

Chemistry

The synthesis of the novel oxazolidinone derivatives having isoxazolyl tetrahydropyridinyl groups is shown in Scheme 1. The key intermediate **6** was prepared according to the known method.[6] To synthesize the oxazolidinone derivatives shown in Table 2, we surveyed reaction conditions suitable for Pd^0-catalysed Stille coupling reaction with triflate **7** and various tributylstannanyl isoxazoles.

Lithium enolate of **6** was prepared with LDA in THF/HMP at −50 °C, and then $PhNTf_2$ was added and the mixture was kept at −50 °C for 1.5 h. This sequence provided the triflate **7** which is an intermediate of the coupling reactions.[7]

As shown in Scheme 2, a variety of tributylstannanyl isoxazoles has been prepared via [2 + 3]cycloaddition with various substituted chloro oximes in the presence of tributylethynyltin and potassium carbonate in CH_2Cl_2.



Scheme 2.

Table 1. Prepared tributylstannanyl isoxazoles of **9** and **10**

Substrates	Method[a]	R	X	Products (yield, %)[b]	
				9	**10**
8a	A	CH_3	H	47	—
8b	B	CN	Cl	46	48
8c	B	Cl	Cl	51	27
8d	B	CF_3	Cl	50	—
8e[8]	B	CO_2Et	Cl	82	—

[a] Method A: NCS, tributylethynyltin, Et_3N in THF at 65 °C; method B: K_2CO_3, tributylethynyltin in CH_2Cl_2 at room temperature.
[b] Isolated yield.

As shown in Table 1, most of oximes gave 3-substituted 5-tributylstannanyl isoxazoles **9**. In the cases of cyclization reaction of **8b** and **8c**, however, two regioisomers, **9** and **10**, were obtained.

To prepare 3-methyl-5-tributylstannanyl isoxazole (**9a**), we used different method because chlorination of methyl oxime with NCS was needed for the isoxazole ring cyclization. Chlorinated acetaldoxime with *N*-chlorosuccinimide in THF at 65 °C was formed in situ. Subsequent addition of tributyletynyltin and triethylamine gave the product successfully (47%).

The Stille-coupling reaction (method C) of triflate **7** and various tributylstannanyl isoxazoles with $[Pd_2(dba)_2 \cdot CH_3Cl]$, PPh_3 and $ZnCl_2$ in THF/NMP at room temperature for 3 days afforded the corresponding products.

Table 2. Synthesized oxazolidinones of **4** and **5**

Compd	Method[a]	R	Yield (%)[b]
4a	C	CH_3	86
4b	C	CN	58
4c	C	Cl	52
4d	C	CF_3	52
4e[9]	C	CO_2Et	61
4f[10]	D	$CONH_2$	80
4g	D	$CON(CH_3)_2$	63
5a	C	Cl	93
5b	C	CN	91

[a] Method C: Stille-coupling reaction; method D: amidation reaction of **4e**.
[b] Isolated yield.

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

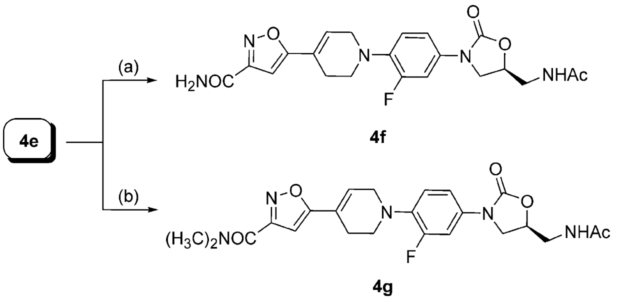
Compd	Microorganism[b]										
	*S. a.*1	*S. a.*2	*S. a.*3	MRSA	*S. e.* 1	*S. e.* ^{MR}	*E. f.*1	*E. f.*2	VRE.1	VRE.2	VRE.3
4a	2	1	4	2	1	2	4	4	2	2	2
4b	2	1	4	2	1	4	4	4	4	4	4
4c	8	8	32	4	8	16	64	64	32	32	32
4d	> 64	> 64	> 64	> 64	> 64	> 64	> 64	> 64	> 64	> 64	> 64
4e	8	4	4	4	4	16	16	8	16	8	8
4f	0.5	0.5	0.5	0.5	0.5	1	1	1	1	1	1
4g	4	8	4	8	2	8	8	8	8	8	8
5a	2	2	8	2	2	8	8	8	8	8	8
5b	1	1	2	1	0.5	4	2	2	2	2	2
LZ[c]	0.5	0.5	2	0.5	0.5	1	2	2	2	2	2
VCM[d]	0.5	1	1	2	1	2	4	4	64	> 64	> 64

[a] Agar dilution method, Mueller-Hinton agar, 10^4 CFU/spot.

[b] *S. a.*1, *Staphylococcus aureus* 6538p; *S. a.*2, *Satphylococcus aureus* 77; *S. a.*3, *Staphylococcus aureus* 29213A; MRSA, methicillin-resistant *Staphylococcus aureus* 241; *S. e.* 1, *Staphylococcus epidermidis* Q004; *S. e.* ^{MR}, *Staphylococcus epidermidis*^{MR} 887E; *E. f.* 1, *Enterococcus faecalis* 29212A; *E. f.* 2, *Enterococcus faecalis* EFS008; VRE.1, vancomycin-resistant *Enterococcus faecium* or2; VRE.2, vancomycin-resistant *Enterococcus faecium* 2006; VRE.3, vancomycin-resistant *Enterococcus faecium*2164.

[c] LZ, linezolid.

[d] VCM, vancomycin.



Scheme 3. (a) aq NH_4OH, NH_4Cl, MeOH, rt; (b) $(CH_3)_2NH{\cdot}HCl$, $InBr_3$, K_2CO_3, MeOH, rt.

In another way, the new oxazolidinone derivatives, amides **4f** and **4g**, could be synthesized via amidation reaction (method D) of isoxazolyl ethylester **4e** with NH_4OH or dimethyl amine in methanol at room temperature as shown in Scheme 3. In the case of **4g**, however, $InBr_3$ was required as a Lewis acid to activate the ester group of **4e**.

In vitro activity

In vitro antibacterial activities of all the compounds prepared and references were determined by the Mueller-Hinton agar dilution method[11] against Gram-positive strains and the resistant strains including MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant *Enterococcus faecium*). The activities of the 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 μg/mL. The results are presented in Table 3. Most of the compounds synthesized exhibited good antibacterial activities.

The carbamoyl isoxazole **4f** demonstrated MIC values comparable or 2–4-fold better than those of linezolid and vacomycin against all of tested organisms including MRSA and VRE (Table 3). The amide group of isoxazole moiety of **4f** might be playing crucial role for the activity as a substituent. Although **4b**, **4c** and **4d** have strong electronegative group such as –CN, –Cl and $–CF_3$ at the same position as amide group of **4f**, they showed lower activity than **4f**. Therefore we suggest that the hydrophilic group of 3-position of isoxazole in **4f** might have important function for antibacterial activity.

Acknowledgements

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7. Experimental Procedure: **7**; To a solution of diisopropylamine (92 μL, 0.66 mmol) in THF (2 mL) was added dropwise *n*-BuLi (1.45 M in hexane, 452 μL, 0.66 mmol) at 0 °C under N_2. After stirring for 30 min, **6** (100 mg, 0.30 mmol) in THF (7 mL)/HMPA (2.7 mL) was added dropwise for 20 min at −50 °C. After stirring for 3 h, *N*-phenyltrifluoromethanesulfonimide (117 mg, 0.33 mmol) in THF (2 mL) was added dropwise. After stirring for 1.5 h at −50 °C, The reaction mixture was poured into a mixture of ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (20 mL×3). The collected organic layer was washed with brine, dried over anhydrous $MgSO_4$,

evaporated and purified by column chromatography with neutralized SiO_2 by Et_3N (5% methanol-ehtyl acetate) to give the product **7** (54 mg, 37%).

8. Representative procedure: **8e**: To a solution of ethyl chloro oximidoacetate (100 mg, 0.66 mmol) in CH_2Cl_2 (2 mL) was added tributyl ethynyl tin (191 μL, 0.66 mmol) followed by K_2CO_3 (100 mg, 0.73 mmol). After stirring 5 h at room temperature, the reaction mixture was poured into a mixture of CH_2Cl_2 and water. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (20 mL×3). The collected organic layer was washed with brine, dried over anhydrous $MgSO_4$, evaporated and purified by column chromatography (hexane–ethyl acetate = 40:1) to give the product **8e** (232 mg, 82%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.81 (s, 1H), 4.45 (q, J = 13.8, 2H), 1.57 (m, 6H), 1.44 (t, J = 6.6, 3H), 1.33 (m, 6H), 1.23 (t, J = 8.29, 6H), 0.92 (t, J = 7.05, 9H).

9. Representative procedure: **4e**; To a solution of **7** (53 mg, 0.11 mmol) in THF (1 mL) was added $[Pd_2(dba)_3]\cdot CHCl_3$ (2.3 mg, 8 mol%) at $-78\,^\circ$C, followed by PPh_3 (2.3 mg, 2 mol%) and ethyl 5-tributylstannanyl-3-isoxazole carboxylate (**8e**) (48 mg, 0.11 mmol) in THF (1 mL). Finally, a solution of $ZnCl_2$ in hexane (1 M, 110 μL, 0.11 mmol) was added. The cooling bath was removed and the reaction temperature was rapidly raised to ambient temperature using a water bath. An intense red color developed on warming. After stirring 3 days at ambient temperature, the reaction mixture was poured into a mixture of ethyl acetate and water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (20 mL×3). The collected organic layer was washed with brine, dried over anhydrous $MgSO_4$, evaporated and purified by column chromatography (2% methanol–ethyl acetate) to give the product **4e** (34 mg, 61%).

^{1}H NMR ($CDCl_3$, 300MHz) δ 7.48 (dd, J = 2.40, 2.40, 1H), 7.11 (d, J = 9.60, 1H), 6.98 (t, J = 8.70, 1H), 6.73 (s, 1H), 6.59 (s, 1H), 6.20 (m, 1H), 4.80 (m, 1H), 4.45 (q, J = 7.20 1H), 4.06 (t, J = 8.70, 1H), 3.86 (s, 1H), 3.73 (m, 2H), 3.38 (m, 2H), 2.66 (s, 2H), 2.05 (s, 3H), 1.45 (t, J = 7.2, 3H). IR (KBr, cm^{-1}) 1736, 1652, 1522, 1422, 1226.

^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.33, 160.31, 157.44, 156.88, 154.56, 154.18, 136.08, 133.33, 133.19, 128.03, 123.86, 119.80, 114.20, 108.02, 107.67, 100.22, 99.82, 72.15, 62.48, 49.74, 47.91, 47.60, 42.23, 29.96, 26.18, 23.40, 14.42. HRMS (FAB, M + H) calcd for $C_{23}H_{26}FN_4O_6$ 473.1836, found 473.1836.

4c: ^{1}H NMR (DMSO, 300 MHz) δ 8.26 (m, 1H), 7.53 (dd, J = 1.80, 2.40, 1H), 7.17 (m, 2H), 6.95 (s, 1H), 6.75 (s, 1H), 4.73 (m, 1H), 4.11 (t, J = 9.3, 1H), 3.75 (m, 3H), 3.34–3.27 (m, 2H), 1.85 (s, 3H). IR (KBr, cm^{-1}) 1740, 1654, 1567, 1520, 1424, 1226, 1192. ^{13}C NMR (DMSO, 75 MHz) δ 172.01, 170.76, 156.93, 154.80, 153.67, 135.57, 135.45, 134.22, 134.09, 129.61, 123.69, 120.64, 114.83, 107.56, 107.21, 101.13, 72.28, 49.86, 49.21, 48.03, 47.55, 42.13, 25.76, 23.15, 17.93. HRMS (FAB, M + H) calcd for $C_{20}H_{21}ClFN_4O_4$ 435.1235, found 435.1235.

10. Representative procedure: **4f**: To a solution of **4e** (18 mg, 0.04 mmol) in methanol (3 mL) was added NH_4OH (28% in water, 500 μL, excess) at room temperature, followed by NH_4Cl (50 mg, excess) and stirred for 21 h. After removing methanol, a mixture of CH_2Cl_2 and water was poured into the reaction pot. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (20 mL×3). The collected organic layer was washed with brine, dried over anhydrous $MgSO_4$, evaporated and purified by column chromatography (2% methanol–dichloromethane) to give the product **4f** (13 mg, 80%).

^{1}H NMR (DMSO, 300 MHz) δ 8.09 (s, 1H), 7.80 (s, 1H), 7.49 (dd, J = 1.5, 1.8, 1H), 7.16 (m, 2H), 6.82 (s, 1H), 6.71 (s, 1H), 4.69 (m, 1H), 4.07 (t, J = 8.70, 1H), 3.76 (s, 2H), 3.69 (m, 1H), 2.55 (s, 2H), 2.05 (s, 3H). IR (KBr, cm^{-1}) 11738, 1684, 1659, 1520, 1232. ^{13}C NMR (DMSO, 75 MHz) δ 171.14, 170.87, 161.05, 160.10, 154.92, 135.77, 134.16, 128.64, 123.98, 120.69, 114.92, 107.69, 107.34, 100.01, 72.40, 49.98, 48.14, 47.77, 42.26, 26.19, 23.30. HRMS (FAB, M + H) calcd for $C_{21}H_{23}FN_5O_5$ 444.1683, found 444.1683.

4g: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46 (dd, J = 2.43, 2.37, 1H), 7.10 (d, J = 8.79, 1H), 6.96 (t, J = 9.03, 1H), 6.68 (s, 1H), 6.47 (s, 1H), 5.99 (m, 1H), 4.77 (m, 1H), 4.03 (t, J = 8.91, 1H), 3.84 (s, 1H), 3.76 (m, 2H), 3.36 (t, J = 5.64, 2H), 3.31 (s, 3H), 2.63 (s, 2H), 2.03 (s, 3H). IR (KBr, cm^{-1}) 1732, 1672, 1628, 1518, 1230. ^{13}C NMR (DMSO, 75 MHz) δ 171.14, 170.87, 161.05, 160.10, 154.92, 135.77, 134.16, 128.64, 123.98, 120.69, 114.92, 107.69, 107.34, 100.01, 72.40, 49.98, 48.14, 47.77, 42.26, 26.19, 23.30. HRMS (FAB, M + H) calcd for $C_{23}H_{27}FN_5O_5$ 472.1996, found 472.1996.

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