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Synthesis and antibacterial activities of novel oxazolidinones having spiro[2,4]heptane moieties

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ABSTRACT

The synthesis of a new series of oxazolidinones having spiro[2,4]heptane moieties is described. Their in vitro antibacterial activities against both Gram-positive and Gram-negative bacteria were tested and the effect of substituents on the oxazolidinone ring was investigated. A particular compound **Ih** having fluoro group showed the most potent antibacterial activity.

The emergence of bacterial resistance to the antibiotics poses a serious concern for medical professionals during the last decade.[1] In particular, multi-drug-resistant Gram-positive bacteria[2] including methicillin-resistant *Staphylococcus aureus* (MRSA)[3] and *Staphylococcus epidermidis* (MRSE), and vancomycin resistant enterococci (VRE) are of major concern.[4]

Oxazolidinones, a new class of synthetic antibacterial agents, exhibit activity against a large number of Gram-positive organisms. Linezolid is the first oxazolidinone approved for the treatment of Gram-positive bacterial infections in humans.[5] Since Linezolid, the many attractive traits of oxazolidinone series have encouraged further work in this area, and also the literature reveals extensive chemical programs exist.[6,7] At present, most efforts are focused on substituted phenyl oxazolidinones. Eperezolid and AZD2563 have been extensively used as the structural precursors for modification.[8]

In this Letter, we describe the synthesis and structure–activity relationship of oxazolidinones having spiro[2,4]heptane moieties instead of morpholine (Fig. 1). In addition, our approach for the improvement of antibacterial activity of oxazolidinones is also discussed.

It is revealed[9,10] that a spiro[2,4]heptane substituent could enhance largely the activity of quinolone antibiotics especially against both Gram-positive and Gram-negative bacteria. Based on the facts, a positive effect of a spiro[2,4]heptane moiety on the activity of oxazolidinone was anticipated.

7-Oxo-5-azaspiro[2,4]heptane **8**[11] was prepared via seven steps from diketene and benzylamine as shown in Scheme 1. Aceto compound **2** was obtained from diketene and benzylamine, which was then cyclized to compound **3** using 1,2-dibromoethane. Carbonyl group of **3** was protected by ethylene glycol and subsequently brominated to give **5**, which was converted to spiro[2,4]heptane **6** using sodium hydride. Cyclized compound **6** was reduced with lithium aluminum hydride to give **7**. The key compound **8** was obtained by hydrogenation of **7** in the presence of palladium carbon.

The syntheses of derivatives **Ia–i** are outlined in Schemes 2 and 3. 7-Oxo-5-azaspiro[2,4]heptane as a starting material on condensation with 3,4-difluoronitrobenzene in acetonitrile gave compound **9**.

Hydrogenation of compound **9** with 10% Pd–C/H_2 followed by condensation with benzyl chloroformate afforded the protected

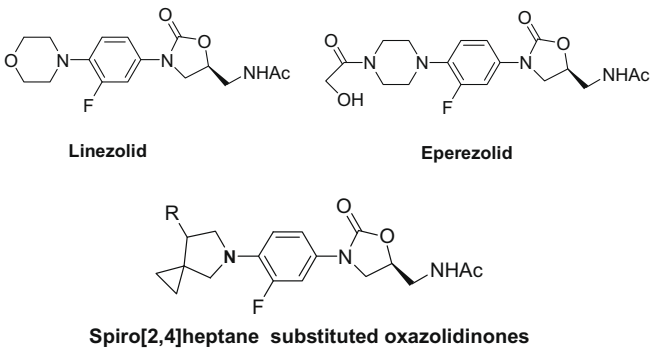

Figure 1. Structures of Linezolid, Eperezolid, and target molecules.

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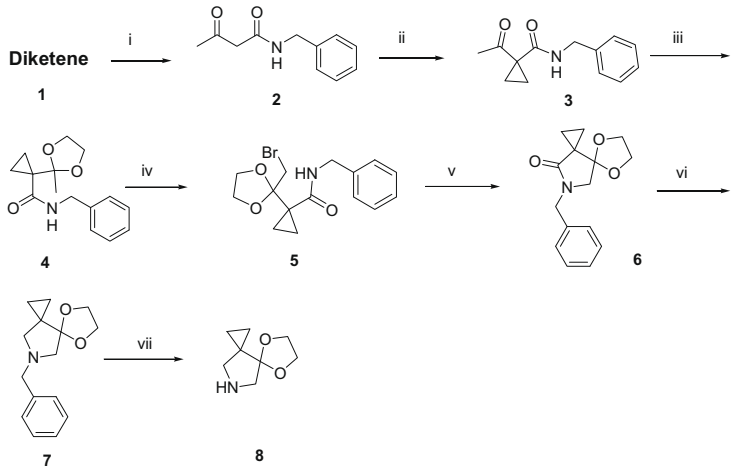
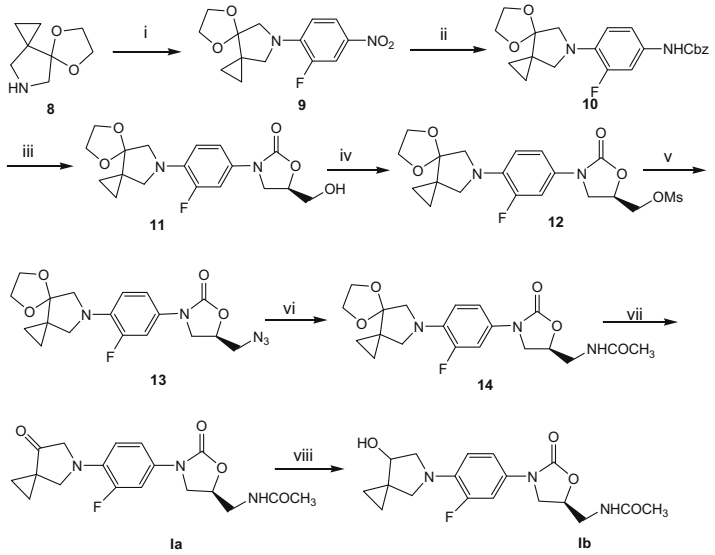
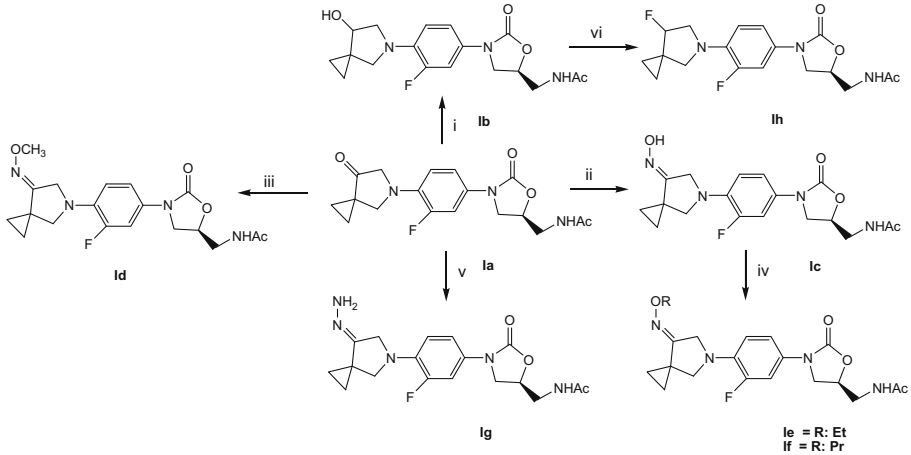


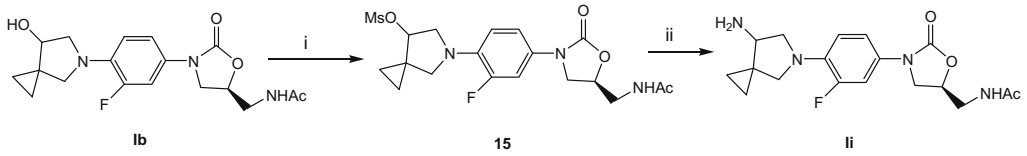
Scheme 1. Reagents and conditions: (i) benzylamine, CH_2Cl_2; (ii) 1,2-dibromoethane, K_2CO_3, DMF; (iii) ethylene glycol, *p*-toluensulfonic acid, benzene; (iv) Br_2, dioxane, ether; (v) NaH, DMF; (vi) $LiAlH_4$, THF; (vii) Pd/C, H_2, EtOH.



Scheme 2. Reagents and condition: (i) 3,4-difluoronitrobenzene, CH_3CN, reflux, 67%; (ii) (1) H_2, Pd/C, THF (2) benzylchloroformate, $NaHCO_3$, acetone–H_2O; (iii) *n*-BuLi, (*R*)-glycidyl butyrate, −78 °C, 65%; (iv) MsCl, TEA, CH_2Cl_2, 5 °C, 79%; (v) NaN_3, DMF, 75 °C, 78%; (vi) (1) H_2, Pd/C, EtOAc (2) Ac_2O, pyridine, 69%; (vii) *p*-toluenesulfonic acid monohydrate, acetone–H_2O; (viii) $NaBH_4$, THF, −5 °C, 75%.



Scheme 3. Reagents and conditions: (i) $NaBH_4$, THF, −5 °C, 75%; (ii) $NH_2OH{\cdot}HCl$, TEA, EtOH, 60 °C, 70%; (iii) $NH_2OCH_3{\cdot}HCl$, TEA, MeOH, rt, 65%; (iv) **Ie**: C_2H_5Br, KOH, DMF, 40 °C, 71%; **If**: C_3H_7Br, KOH, DMF, 40 °C, 73%; (v) N_2H_4, TEA, EtOH, 60 °C, 55%; (vi) DAST, CH_2Cl_2, −75° C to rt, 53%.



Scheme 4. Reagents and conditions: (i) MsCl, TEA, CH_2Cl_2, 5 °C, 76%; (ii) (1) NaN_3, DMF, 75 °C, 78% (2) H_2, Pd/C, EtOH, 69%.

Table 1
In vitro antibacterial activity(MIC, μg/ml) of oxazolidinone derivatives against standard strains

Compound	S. a.[a]	C. s.[b]	E. f.[c]	E. f.[d]	S. p.[e]	S. p.[f]	S. a.[g]	H. i.[h]
Ia	3.12	3.12	6.25	1.56	0.78	3.12	1.56	1.56
Ib	12.5	12.5	12.5	6.25	3.12	6.25	6.25	6.25
Ic	12.5	25.0	25.0	12.5	12.5	6.25	6.25	12.5
Id	6.25	12.5	12.5	6.25	3.12	6.25	6.25	6.25
Ie	12.5	12.5	25.0	12.5	12.5	12.5	12.5	12.5
If	25.0	12.5	25.0	12.5	12.5	12.5	12.5	25.0
Ig	25.0	12.5	25.0	25.0	25.0	12.5	25.0	25.0
Ih	3.12	3.12	1.56	0.78	1.56	1.56	0.78	0.39
Ii	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
Linezolid	3.12	3.12	1.56	1.56	0.78	1.56	1.56	1.56

[a] S. a. = *Staphylococcus aureus* C463.
[b] C. s. = *Coagulase negative staphylococci.*
[c] E. f. = *Enterococcus faecalis* C474.
[d] E. f. = *Enterococccus faecium* C803.
[e] S. p. = *Streptococcus pneumoniae* C402.
[f] S. p. = *Streptococcus pyogenes* ATCC8736.
[g] S. a. = *Streptococcus agalactiae* ATCC2901.
[h] H. i. = *Haemophilus influenza.*

compound **10**. Conversion of compound **10** to oxazolidinone **11** was accomplished by use of *n*-butyllithium and (*R*)-glycidyl butyrate in dry THF at −78 °C. Compound **11** was reacted with methanesulfonyl chloride and subsequent treatment with sodium azide to yield azide **13**. Reduction and acylation of compound **13** and subsequent deprotection using *p*-toluenesulfonic acid provided gave the title compound **Ia**.

The title compounds **Ib**–**Ih** were prepared from **Ia** as shown in Scheme 3. Reduction of **Ia** with sodium borohydride in THF gave the hydroxyl compound **Ib**. Preparation of oxime **Ic** and methoxyimino compound **Id** was accomplished by treatment of the carbonyl group with hydroxyl and methoxyl amine. The oxime **Ic** was converted to the ethyloxyimino **Ie** and propyloxyimino **If** using ethyl bromide and propyl bromide, respectively, in the presence of potassium hydroxide.

Preparation of the hydrazone **Ig** was accomplished by treatment of the carbonyl group with hydrazine.

Introduction of fluoro group of compound **Ih** was carried out by treatment of **Ib** with DAST in CH_2Cl_2.

Compound **Ib** was reacted with methane sulfonyl chloride and subsequent treatment with sodium azide to yield azide compound. The title compound **Ii** was obtained by hydrogenation of azide in the presence of palladium carbon (Scheme 4).

The minimal inhibitory concentrations (MICs) were determined by the agar dilution method using test agar. An overnight culture of bacteria in tryptosoy broth was diluted to about 10^6 cells/mL with the same broth and inoculated with an inoculating device onto agar containing serial twofold dilutions of the tested compounds. Organisms were incubated at 37 °C for 18–20 h. The MICs of a compound were defined as the lowest concentration that visibly inhibited growth.

In particular, compound **Ih** having fluoro substituted spiro[2,4]heptane moiety was 2–3-fold superior active against most of the targeted both Gram-positive and vancomycin-resistant strains to Linezolid. As to the substituents on the oxazolidinone ring, it was found that compounds **Ib** and **Ii** having hydroxy and amino groups, respectively didn't show good activity compared to other compounds. With increasing bulkiness from methyl in compound **Id** to ethyl or propyl in compounds **Ie** and **If**, respectively, the activity was found to be decreased.

The in vitro antibacterial activities of the new oxazolidinones (**Ia**–**i**) prepared above against both Gram-positive and Gram-negative organism such as *Haemophilus influenzae* are listed in Tables 1 and 2. For comparison, the MIC values of Linezolid are also listed. Among the tested compounds, **Ia** and **Ih**[12] displayed superior or similar antibacterial activities to Linezolid against Gram-positive, methicillin- and vancomycin-resistant strain sequence.

In case of **Ie** and **If**, any bulky substituents also led to significant loss in antibacterial activity. This suggests that bulky substituents are not favorable.

In summary, the introduction of 7-fluoro-5-azaspiro[2,4]heptane moiety to oxazolidinone afforded potent compound with in vitro antibacterial activity comparable or superior to Linezolid against Gram-positive, methicillin- and vancomycin-resistant strains.

Table 2
In vitro antibacterial activity (MIC, μg/mL) of oxazolidinone derivatives against MRSA and VRE

Compound	MRSA	VRE 1	VRE 2	VRE 3	VRE 4	VRE 5	VRE 6	VRE 7	VRE 8
Ia	3.12	1.56	1.56	3.12	1.56	1.56	3.12	3.12	1.56
Ib	6.25	6.25	6.25	3.12	6.25	6.25	6.25	6.25	3.12
Ic	12.5	12.5	12.5	12.5	6.25	6.25	6.25	6.25	12.5
Id	6.25	6.25	6.25	6.25	3.12	6.25	6.25	6.25	6.25
Ie	12.5	12.5	12.5	12.5	6.25	6.25	12.5	6.25	6.25
If	12.5	25	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Ig	25	50	25	12.5	25	12.5	25	12.5	25
Ih	0.78	1.56	1.56	1.56	1.56	3.12	0.78	0.78	1.56
Ii	6.25	12.5	6.25	12.5	6.25	6.25	6.25	6.25	12.5
Linezolid	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56

MRSA: methicillin-resistant *Staphylococcus aureus*, VRE 1: vancomycin-resistant *Enterococcus faecalis*, VRE 2: vancomycin-resistant *Enterococccus faecium*, VRE 3: vancomycin resistant enterococci 1, VRE 4: vancomycin resistant enterococci 2, VRE 5: vancomycin resistant enterococci 3, VRE 6: vancomycin resistant enterococci 4, VRE 7: vancomycin resistant enterococci 5, VRE 8: vancomycin resistant enterococci 6.

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12. Compound **Ih**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (q, 2H, J = 3.6 Hz), 1.44 (q, 2H, J = 3.5 Hz), 2.02 (s, 3H), 3.4 (s, 1H), 3.67 (t, 2H, J = 3.0 Hz), 3.75 (t, 2H, J = 7.8 Hz), 3.96 (d, 2H, J = 1.6 Hz), 4.02 (t, 2H, J = 8.9 Hz), 4.77 (m, 1H), 6.69 (t, 1H, J = 9.3 Hz), 7.08 (dd, 1H, J = 1.8, 1.9 Hz), 7.42 (dd, 1H, J = 2.5 Hz, J = 2.5 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ 18.1, 23.2, 41.9, 47.7, 55.3, 58.3, 71.8, 108.1, 114.4, 115.9, 130.5, 133.1, 151.0, 154.4, 171.2. HRMS(FAB) Calcd for $C_{18}H_{21}F_2N_3O_3$ 365.1551, Found 365.1554.