Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1829–1832

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Novel Piperidinyloxy Oxazolidinone Antimicrobial Agents

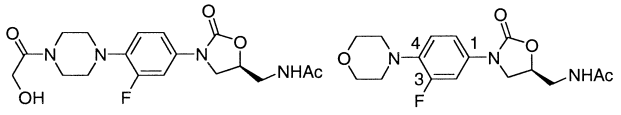
Michele A. Weidner-Wells,* Christine M. Boggs, Barbara D. Foleno, Ellyn Wira, Karen Bush, Raul M. Goldschmidt and Dennis J. Hlasta

Drug Discovery, The R. W. Johnson Pharmaceutical Research Institute, 1000 Route 202, Raritan, NJ 08869, USA

Received 29 March 2001; accepted 26 April 2001

Abstract—Oxazolidinone antibacterial agents, where the *N*-substituted piperazinyl group of eperezolid was replaced with a *N*-substituted piperidinyloxy moiety, were synthesized and shown to be active against a variety of resistant and susceptible Gram-positive organisms. The effect of ring size, positional isomerism, and fluorine substitution on antibacterial activity was examined.

The rising prevalence of multidrug resistant Gram-positive bacteria requires the discovery of novel agents active against these pathogens. Recent reports indicate that in 1998, at least 21% of all nosocomial enterococcal infections in US hospitals were due to vancomycin-resistant enterococci (VRE).[1] The oxazolidinones, a new class of synthetic antibacterial agents, are active against a variety of clinically important susceptible and resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), VRE, and penicillin-resistant *Streptococcus pneumoniae* (PRSP). Scientists from Pharmacia identified two clinical candidates from this class, eperezolid (**1**) and linezolid (**2**).[2] Linezolid (Zyvox^{R}) is currently marketed for the treatment of multidrug resistant Gram-positive infections such as nosocomial and community-acquired pneumonia and skin infections.

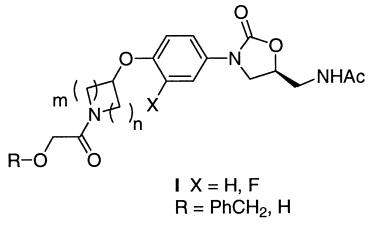


eperezolid (**1**) linezolid (**2**)

The oxazolidinone class of antibacterial agents selectively binds to the 23S RNA component of the 50S ribosomal subunit, inhibiting protein synthesis at an early phase of translation.[3] Due to its unique mechanism of action, it is believed that there will be a lack of cross-resistance with other classes of protein synthesis inhibitors.[4]

*Corresponding author. Fax: +1-908-203-8109; e-mail: mwells@prius.jnj.com

Previous investigations of the SAR of eperezolid and linezolid have demonstrated a high tolerance for substitution at the 4-position of the phenyl ring, while the oxazolidinone ring as well as the *S*-configuration at C-5 of this ring are essential for activity.[5] Several moieties (such as a methyl carbamate and small heterocycles) can replace the acetamide without a significant loss of activity, however, the acetamide functionality is usually optimal.[5,6] Based on these considerations, we have developed a novel series of oxazolidinone antibacterial agents **I** in which the piperazinyl group of eperezolid is replaced with a 4-piperidinyloxy moiety ($m=n=2$).[7,8]

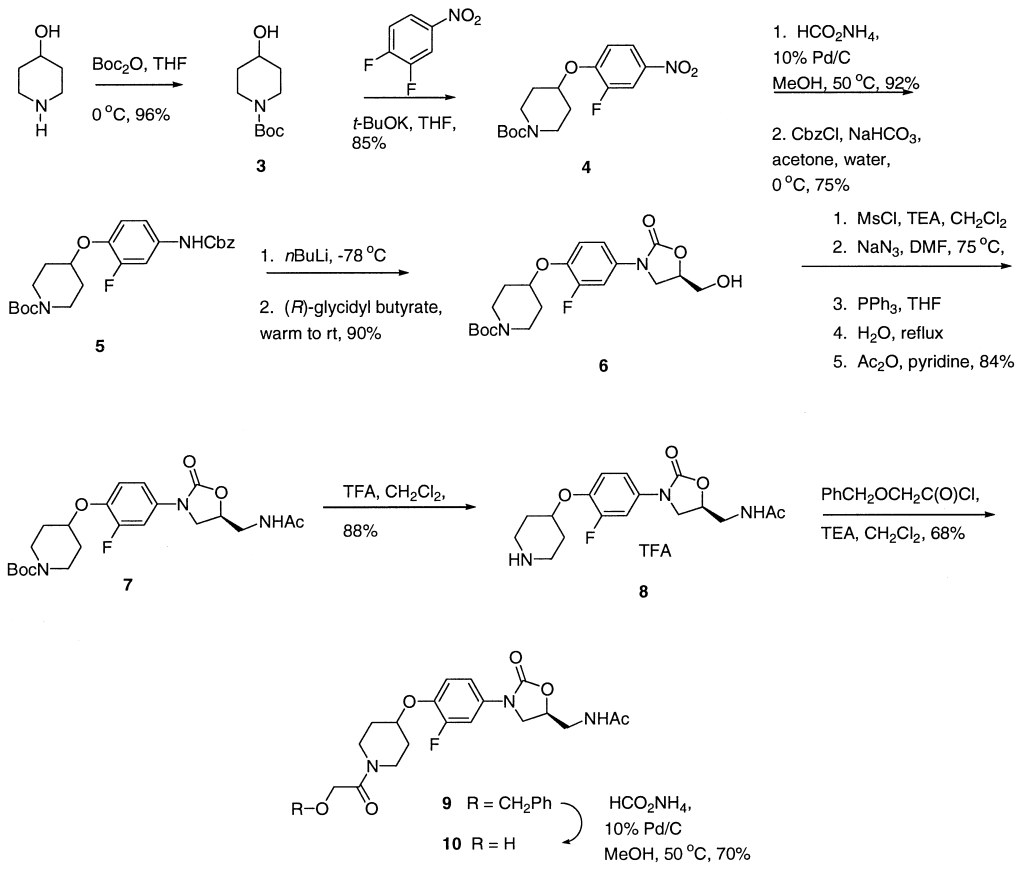


I X = H, F
R = $PhCH_2$, H

In addition, the regioisomeric 3-piperidinyloxy analogues were synthesized. The pyrrolidinyloxy and azetidinyloxy analogues were prepared in order to investigate the importance of ring size on antibacterial activity. The role of the aromatic fluorine substituent was examined in the 4-piperidinyloxy series. The in vitro antibacterial activity of these compounds is reported in this paper.

The target 4-piperidinyloxy oxazolidinones **9** and **10** were synthesized as shown in Scheme 1. *N*-*t*-Butoxycarbonylpiperidin-4-ol (**3**), prepared from 4-hydroxypiperidinol, was reacted with potassium *t*-butoxide and 3,4-difluoronitrobenzene to afford nitro compound **4**.

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00305-5



Scheme 1. Synthesis of 4-piperidinyloxy oxazolidinones **9** and **10**.

Reduction to the aniline followed by protection as the Cbz derivative gave intermediate **5**. The oxazolidinone ring was formed by reaction of the anion of Cbz derivative **5** with (*R*)-glycidyl butyrate to afford alcohol **6**. Functional group manipulation of alcohol **6** yielded acetamide **7** in several steps in good overall yield. The Boc moiety was readily removed by treatment with trifluoroacetic acid in methylene chloride. The TFA salt of amine **8** was acylated with α-benzyloxyacetyl chloride. Removal of the benzyl protecting group from **9** under hydrogen transfer conditions led to desired α-hydroxyacetamide **10**.

The 3-piperidinyloxy analogues **11** and **12** were prepared in a similar manner starting with *N*-*t*-butoxycarbonylpiperidin-3-ol.

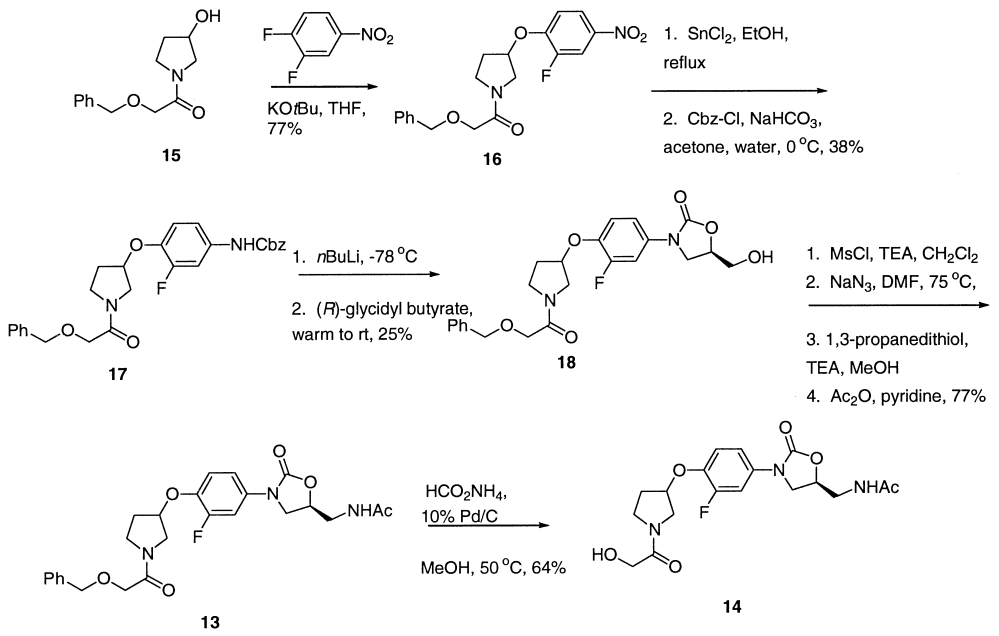
Pyrrolidinyloxy analogues **13** and **14** were prepared in a similar fashion utilizing *N*-(α-benzyloxyacetyl)pyrrolidin-3-ol in the nucleophilic aromatic substitution reaction (Scheme 2). However, in this series, the azide intermediate was reduced to the amine with 1,3-propanedithiol and triethylamine. The target compounds were submitted for biological testing as a mixture of diastereomers.

Azetidinyloxy analogues **19** and **20** were synthesized from commercially available *N*-(diphenylmethyl)-azetidin-3-ol. Removal of the benzhydryl protecting group was accomplished on the fully elaborated oxazolidinone acetamide utilizing standard conditions with α-chloroethylchloroformate.[9] The resulting HCl salt of the amine was functionalized as in Scheme 1.

Utilization of 4-fluoronitrobenzene in place of 3,4-difluoronitrobenzene in the displacement reaction followed by formation of the oxazolidinone ring afforded the des-fluoro 4-piperidinyloxy analogues **21** and **22**.

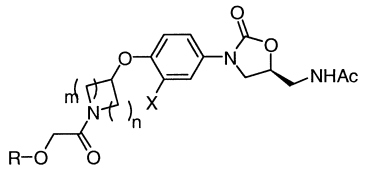
Oxazolidinones **9**–**14** and **19**–**22** were tested for in vitro antibacterial activity against a panel of important Gram-positive pathogens. The minimum inhibitory concentration (MIC) for these compounds against a representative methicillin-susceptible *S. aureus* strain is reported in Table 1. Compounds were tested in broth as well as in the presence of 50% mouse serum in order to give an indication of serum protein binding. At least a 4-fold increase in the MIC in the presence of serum suggests that the compound binds to serum proteins or is inactivated by components of the serum. As a result, there may no longer be a sufficient concentration of free drug in the serum to inhibit the growth of bacteria effectively in vivo.

Within the series of α-benzyloxyacetamides, the 4-substituted piperidinyloxy derivative **9** was the most potent and was only 2-fold less potent than linezolid. The regioisomeric 3-piperidinyloxy derivative **11** was less potent than **9**. In addition, contraction of the six-membered ring to a five-membered ring resulted in a decrease



Scheme 2. Synthesis of the pyrrolidinyloxy oxazolidinones **13** and **14**.

Table 1. MIC (μg/mL) for oxazolidinones **9**–**14** and **19**–**22**[a]



Compd	R	*m*	*n*	X	*S. aureus* OC 4172	
					Without serum	With serum
9	$PhCH_2$	2	2	F	4	16
11	$PhCH_2$	1	3	F	32	32
13	$PhCH_2$	1	2	F	16	16
19	$PhCH_2$	1	1	F	8	16
21	$PhCH_2$	2	2	H	16	64
10	H	2	2	F	4	4
12	H	1	3	F	8	8
14	H	1	2	F	4	4
20	H	1	1	F	8	8
22	H	2	2	H	16	32
Linezolid	—	—	—	—	2	2

[a]The variance in the determination of MIC values is 2-fold such that an MIC difference of at least 4-fold is significant.

in activity (**13** vs **9**). Interestingly, further contraction of the ring leading to the azetidinyloxy derivative **19** restores some of the in vitro activity. This may be due to the altered disposition of the nitrogen substituent in the symmetrical four- and six-membered ring systems (**9** and **19**) versus the unsymmetical five- and six-membered ring systems (**11** and **13**).

However, this trend was not observed in the α-hydroxyacetamide series. Except for the des-fluoro analogue **22**, the compounds were either equipotent or were 2-fold less potent than **9** in vitro.

In agreement with the literature SAR for the oxazolidinones, removal of the fluorine atom from the phenyl ring decreases in vitro potency several fold compared to the fluoro analogues.[2] The des-fluoro compounds **21** and **22** were 4-fold less potent than the parent compounds **9** and **10**. Linezolid was more potent than compounds **9**–**14** and **19**–**22** by at least 2-fold against this *S. aureus* strain.

Several of the α-benzyloxyacetamides bound to or were inactivated by serum proteins, since these compounds tended to exhibit an increase in the MIC in the presence of mouse serum, whereas only one of the α-hydroxyacetamides did. This is most likely due to the increased lipophilicity of the α-benzyloxyacetamides relative to the α-hydroxyacetamides.

This series of oxazolidinone antimicrobial agents was active against a variety of susceptible as well as resistant Gram-positive organisms (Table 2). In contrast to linezolid, none of these compounds exhibited significant activity against Gram-negative organisms such as *Escherichia coli*. As is evident from the data in Table 2, none of the oxazolidinones **9**–**14** nor **19**–**22** was active against either an *E. coli* wild type strain (OC 2605) or against a mutant strain (OC 2530) that is hypersensitive to antimicrobial agents due to a defective outer membrane. Lack of Gram-negative activity may be attributed to several factors. Either the compounds did not penetrate the outer membrane of the bacteria or the compounds entered the organism but were actively effluxed as described for linezolid.[10] Alternatively, the compounds may have entered the organism, but were not active at the biological target.

In conclusion, we have discovered a novel series of oxazolidinone antibacterial agents. These compounds are active against a variety of susceptible, as well as

Table 2. Spectrum of activity against susceptible and resistant Gram-positive and Gram-negative organisms (MIC (μg/mL))

Compd	*S. aureus* OC 4172	MRSA OC2878	*E. faecalis* ATTC 29212	*E. faecium* OC 3312 (VRE)	*E. coli* OC 2605	*E. coli* OC 2530 (HS)
9	4	2	4	4	>128	>32
11	32	32	64	64	>128	>64
13	16	16	16	16	>128	128
19	8	8	16	16	>128	64
21	16	8	16	16	128	128
10	4	4	8	8	128	128
12	8	8	16	16	>128	128
14	4	8	16	16	128	128
20	8	8	16	16	>128	>128
22	16	16	32	32	>128	>128
Linezolid	2	2	2	2	32	8

E. faecalis: *Enterococcus faecalis*; *E. faecium*: *Enterococcus faecium*.

resistant, Gram-positive organisms. Modifications to improve the potency of this series by diversification of the piperidinyl nitrogen are currently being explored. These results will be reported in the future.

Minimum Inhibitory Concentration (MIC) Determinations

Antibacterial susceptibility testing was performed following the broth microdilution method of the National Committee for Clinical Laboratory Standards.[11]

Acknowledgements

The authors wish to thank Dr. Mark J. Macielag for his suggestions and critical reading of the manuscript.

References and Notes

1. Centers for Disease Control and Prevention. Summary of Notifiable Diseases—United States, 1998. *MMWR Morb. Mortal. Wkly. Rep.* **1999**, *47*, 1.
2. Brickner, S. J.; Hutchinson, D. K.; Barbachyn, M. R.; Manninen, P. R.; Ulanowicz, D. A.; Garmon, S. A.; Grega, K. C.; Hendges, S. K.; Toops, D. S.; Ford, C. W.; Zurenko, G. E. *J. Med. Chem.* **1996**, *39*, 673.
3. Swaney, S. M.; Aoki, H.; Ganoza, M. C. *Antimicrob. Agents Chemother.* **1998**, *42*, 3251.
4. Clemett, D.; Markham, A. *Drugs* **2000**, *59*, 815.
5. Genin, M. J. *Expert Opin. Ther. Patents* **2000**, *10*, 1405 and references cited within.
6. Gravestock, M. B. WO 00/21960, 2000.
7. Boggs, C. M.; Weidner-Wells, M. A.; Nelson, E. A.; Goldschmidt, R. M.; Foleno, B. D.; Bush, K.; Hlasta, D. J. 220th National Meeting of the American Chemical Society, Washington, DC, 2000; MEDI-098.
8. Weidner-Wells, M. A.; Boggs, C. M.; Nelson, E. A.; Foleno, B. D.; Goldschmidt, R. M.; Hilliard, J. J.; Melton, J.; Montenegro, D. A.; Wira, E.; Bush, K.; Hlasta, D. J. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Toronto, Canada, 2000; F-1828.
9. Bilotte, S. Sy*nlett* **1998**, 379.
10. Buysse, J. M.; Demyan, W. F.; Dunyak, D. S.; Stapert, D.; Hamel, J. C.; Ford, C. W. 36th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), New Orleans, LA, 1996; C-42.
11. National Committee for Clinical Laboratory Standards, 1997. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 4th ed. Approved standard. NCCLS Document M7-A4. National Committee for Clinical Laboratory Standards: Villanova, PA.