Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 3069–3072
Bioorganic & Medicinal Chemistry Letters

The synthesis and antimicrobial evaluation of a new series of isoxazolinyl oxazolidinones

Michele A. Weidner-Wells,* Harvey M. Werblood, Raul Goldschmidt, Karen Bush, Barbara D. Foleno, Jamese J. Hilliard, John Melton, Ellyn Wira and Mark J. Macielag

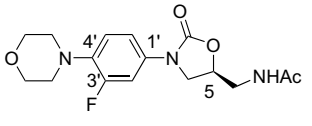
Antimicrobial Agents Research Team, Drug Discovery, Johnson & Johnson Pharmaceutical Research and Development, L. L. C., 1000 Route 202, Raritan, NJ 08869, USA

Received 12 February 2004; accepted 12 April 2004

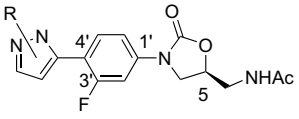
Abstract—A series of oxazolidinone antibacterial agents containing a 5-substituted isoxazol-3-yl moiety were synthesized via a nitrile oxide [3+2] dipolar cycloaddition reaction. These compounds were screened against a panel of susceptible and resistant Gram-positive organisms. Several analogs from this series were comparable to or more potent than linezolid in vitro.

The oxazolidinones, a new class of synthetic antibacterial agents, possess useful activity against a variety of susceptible and resistant Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP).[1,2] This class inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit and prevents the formation of a functional 70S initiation complex. Without this complex, protein synthesis does not commence.[3] Due to this unique mechanism of action, there is no cross-resistance between the oxazolidinones and other families of antibacterial agents. Linezolid (Zyvox®, **1**) is the only oxazolidinone to have been approved for the treatment of hospital- and community-acquired pneumonia as well as skin infections.

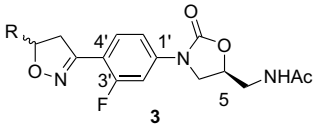
Previous literature on the structure–activity relationships for the oxazolidinones has demonstrated that the *S*-configuration at the C-5 position of the oxazolidinone ring is essential and the acetamide is usually optimal for activity.[4] In addition, there is a high tolerance for a wide variety of substituents at the 4′-position of the phenyl ring.[5] For example, Pharmacia has reported that compounds **2** containing a C-linked heteroaromatic ring attached to the phenyl ring are active against susceptible and resistant Gram-positive organisms.[6] Several patents from Pharmacia have generically disclosed (though not exemplified) isoxazole analogs where the pyrazole ring of **2** is replaced with an isoxazole (the point of attachment was not specified).[7,8] To our knowledge, the isoxazolines have not been investigated.



linezolid (**1**)



2

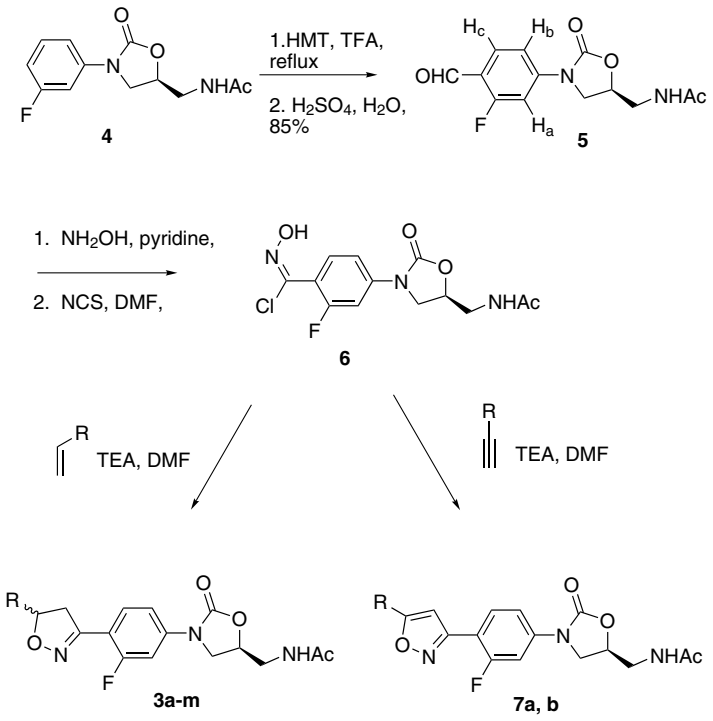


3

This paper describes the synthesis and microbiological evaluation of a series of 3-isoxazolinyl oxazolidinones **3**. The key step is a late stage nitrile oxide dipolar [3+2] cycloaddition reaction with an appropriately substituted olefinic dipolarophile, which enables access to a diverse array of analogs.

Keywords: Antibacterial; Oxazolidinone; Resistant bacteria.
* Corresponding author. Tel.: +1-908-704-5933; fax: +1-908-203-8109; e-mail: mwells@prdus.jnj.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.04.037



Scheme 1. Synthesis of 3-isoxazolinyl and 3-isoxazolyloxazolidinones.

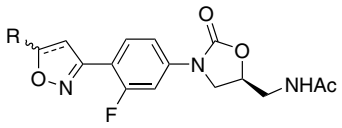
Compounds **3a**–**m** were readily synthesized in several steps from the known intermediate **4** (Scheme 1).[9] Formylation of **4**, utilizing modified Duff conditions, occurs regioselectively to afford aldehyde **5** in excellent yield.[10] Examination of the splitting pattern of the protons in the aromatic region indicated that formylation occurred exclusively *para* to the oxazolidinone ring. Proton H_c is a triplet at δ 7.88 ($J = 8.5$ Hz) indicating *meta* fluorine and *ortho* hydrogen coupling. Proton H_a is a doublet of doublets at δ 7.63 ($J = 13.6$, 1.9 Hz) indicating *ortho* fluorine and *meta* hydrogen coupling. Lastly, H_b is a doublet of doublets at δ 7.28 ($J = 8.5$, 1.9 Hz) indicating *ortho* and *meta* proton couplings. This is the typical coupling pattern seen in other series of oxazolidinones. Additional evidence for this regiochemical assignment is the appearance of the aldehyde proton as a singlet indicating that the fluorine is an even number of bonds removed since 5-bond fluorine coupling to aldehydic protons has been observed in other systems.[10]

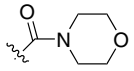
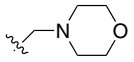
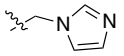
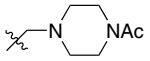
Conversion to the oxime followed by chlorination with *N*-chlorosuccinimide produced the nitrile oxide precursor **6**.[11] Dehydrohalogenation of chlorooxime **6** with base in the presence of a dipolarophile afforded the 5-substituted cycloadducts **3** in good yields with none of the regioisomeric 4-substituted cycloadduct being observed by NMR or LC/MS. Isoxazolines **3** were presumably formed as a diastereomeric mixture although diastereomers were not detected by proton NMR, LC/MS (several solvent systems), or TLC. HPLC analysis of the crude reaction mixture utilizing a variety of solvent systems as well as chiral stationary phases suggested that both diastereomers were present in an approximate 1:1 ratio. However, we were unable to develop a practical analytical method to determine a more accurate ratio or to separate the diastereomers. Isoxazole cycloadducts **7** were produced when an alkynyl dipolarophile was utilized in the cycloaddition reaction.

Oxazolidinones **3** and **7** were characterized in vitro against a representative methicillin-susceptible *S. aureus* strain in broth, as well as in the presence of 50% mouse serum, using a twofold dilution series of concentrations for testing.[12] The minimum inhibitory concentrations (MIC) for these compounds are reported in Table 1. A fourfold or greater 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 blood to inhibit the growth of bacteria effectively in vivo.

Isoxazolinyl ester **3a** was fourfold less potent than linezolid and suffered a dramatic increase in MIC in the presence of mouse serum, such that it no longer inhibited bacterial growth at concentrations as high as 128 μg/mL. The esterases in serum may have hydrolyzed the ester to the corresponding acid, which may no longer be able to enter the bacterial cell. In contrast, the corresponding primary amide **3b** and the nitrile analog **3c** were equipotent to linezolid in broth and twofold more potent in the presence of serum. In order to increase the aqueous solubility of amide **3b**, secondary amide **3d**, in which an alkyl ether is appended to the amide nitrogen was prepared. This compound was less potent than both **3b** and the secondary amide analog **3e**. Tertiary amide **3f** was also significantly less active than **3b**, suggesting limited steric bulk tolerance in this region of the molecule.

Table 1. MIC values (μg/mL) for oxazolidinones **3a**–**m** and **7a**,**b**[a]



Compds	R	Bond[b]	*S. aureus* OC 4172	
			Without serum	With serum
3a	CO_2Et	s	8	>128
3b	$C(O)NH_2$	s	2	1
3c	CN	s	2	1
3d	$C(O)NHCH_2CH_2OMe$	s	8	8
3e	C(O)NHMe	s	4	4
3f		s	8	8
3g		s	16	16
3h		s	>32	>32
3i	CH_2NMe_2	s	8	2
3j	4-Pyridyl	s	4	4
3k	2-Pyridyl	s	2	4
3l	CH_2NHAc	s	8	8
3m		s	8	8
7a	CO_2Me	d	8	>128
7b	2-Pyridyl	d	4	8
1	—	—	2	2

[a] The variance in the determination of MIC values is twofold such that an MIC difference of more than fourfold is significant.
[b] s indicates a single bond (isoxazoline); d indicates a double bond (isoxazole).

Morpholine analog **3g** and imidazole analog **3h** were significantly less potent than linezolid, thus indicating that a sterically demanding basic functionality is not tolerated. Although, the less bulky dimethylamino analog **3i** was slightly more potent than either **3g** or **3h**, the MIC value of **3i** was fourfold higher than that of linezolid. Incorporation of a pyridyl group onto the isoxazoline ring enhanced the in vitro potency of the series. Regioisomeric analogs **3j** and **3k** were two- to fourfold more potent than dimethylamino analog **3i** with the 2-pyridyl isomer **3k** being optimal.

In light of the above structure–activity relationships, the antimicrobial activity of several acylated amines was investigated. Compounds **3l** and **3m** were equipotent to dimethylamino analog **3i** against the *S. aureus* strain, but were fourfold less potent than linezolid.

From the limited number of examples, there appears to be little difference in the in vitro activity between the aromatic and the dihydro species (**3a** vs **7a** and **3k** vs **7b**).

This series of oxazolidinone antimicrobial agents was screened against an expanded panel of susceptible and resistant Gram-positive and Gram-negative organisms (Table 2). As is evident from the data, analogs **3b**, **3c**, **3j**, **3k**, and **7b** exhibit microbiological activity comparable to linezolid against MRSA, *E. faecalis*, and VRE. Like linezolid, this series did not have an effect on an *E. coli* wild type strain (OC 2605), but did exhibit marginal activity against a mutant *E. coli* strain (OC 2530) that is hypersensitive to antibacterial agents due to a defective outer membrane. The lack of activity for this class against Gram-negative organisms may be due in part to active efflux, effectively lowering the intracellular concentration of test compound.[13]

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

Compd	MRSA OC2878	*E. faecalis* ATCC 29212	*E. faecium* OC 3312 (VRE)	*E. coli* OC 2605	*E. coli* OC 2530 (HS)[a]
3a	4	16	8	>128	128
3b	2	4	4	>64	32
3c	1	2	2	128	16
3d	16	16	16	>64	64
3e	4	4	4	>128	32
3f	8	8	8	>128	32
3g	8	16	32	64	32
3h	>32	>32	>32	>32	>32
3i	8	8	8	128	64
3j	1	4	4	>64	32
3k	2	2	2	>64	8
3l	16	16	16	>64	64
3m	8	8	16	>32	16
7a	4	16	8	>128	64
7b	2	4	4	>128	64
1	2	4	4	>32	16

[a] HS: hypersensitive.

Compounds **3b**, **3c**, **3k**, and **7b** were tested in a lethal murine systemic infection model with *S. aureus* Smith (OC 4172) (Table 3). The isoxazolinyl compounds **3b**, **3c**, and **3k** protected the mice from infection following oral or subcutaneous administration, however, they were several fold less potent than linezolid **1** despite comparable in vitro activity against this organism. This difference in in vivo potency may be due to poor solubility in the vehicle or suboptimal pharmacokinetics. Isoxazole **7b** was inactive when tested subcutaneously.

In conclusion, we have identified a series of isoxazolinyl oxazolidinones with in vitro microbiological activity against resistant and susceptible Gram-positive organisms comparable to linezolid. Several analogs from this series also exhibited in vivo activity in a lethal murine infection model when administered either subcutaneously or orally, but were less effective than linezolid.

Table 3. In vivo efficacy in a murine lethal infection model

Compd	ED_{50} (sc) (mg/kg/day)	ED_{50} (po) (mg/kg/day)
3b	11.8	40
3c	22.4	22.1
3k	22.8	31.6
7b	>40	Not tested
1	5.4	6.8

Minimum inhibitory concentration (MIC) determinations: Antibacterial susceptibility testing was performed according to the broth microdilution method of the National Committee for Clinical Laboratory Standards.[12]

Mouse protection assay: In vivo efficacy was assessed in a murine septicemia model of infection caused by *S. aureus* Smith. Female Swiss-Webster mice were infected ip with approximately 6×10^5 CFU/mL of the challenging strain. Protecting compounds were administered subcutaneously in 40% hydroxypropyl-β-cyclodextrin or orally in 0.5% Methocel at 1 and 3 h after infection. The dose allowing survival of 50% of the animals (ED_{50}) was calculated using the logistic routine of the SAS suite of statistical programs.

References and notes

1. Diekema, D. J.; Jones, R. N. *Drugs* **2000**, *59*, 7–16.
2. Kaatz, G. W.; Rybak, M. J. *Emerging Drugs* **2001**, *6*, 43–55.
3. Barbachyn, M. R.; Ford, C. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 2010–2023.
4. Genin, M. J. *Exp. Opin. Ther. Patents* **2000**, *10*, 1405–1414.
5. Hutchinson, D. K. *Curr. Top. Med. Chem.* **2003**, *3*, 1021–1042.
6. Lee, C. S.; Allwine, D. A.; Barbachyn, M. R.; Grega, K. C.; Dolak, L. A.; Ford, C. W.; Jensen, R. M.; Seest, E. P.; Hamel, J. C.; Schaadt, R. D.; Stapert, D.; Yagi, B. H.; Zurenko, G. E.; Genin, M. J. *Bioorg. Med. Chem.* **2001**, *9*, 3243–3253.
7. Hester, J. B., Jr.; Nidy, E. G.; Perricone, S. C.; Poel, T.-J. U.S. 6,255,304 (Pharmacia), 2001.
8. Barbachyn, M. R.; Brickner, S. J. U.S. 5,801,246 (Pharmacia), 1998.
9. Barbachyn, M. R.; Brickner, S. J. WO 93/09103 (Upjohn), 1993.
10. Weidner-Wells, M. A.; Fraga-Spano, S. A. *Synth. Commun.* **1996**, *14*, 3775–3783.
11. Liu, K.-C.; Shelton, B. R.; Howe, R. K. *J. Org. Chem.* **1980**, *45*, 3916–3918.
12. 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.
13. 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), C-42, 1996, New Orleans, LA.