Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1126–1129

Conformational constraint in oxazolidinone antibacterials. Part 2: Synthesis and structure–activity studies of oxa-, aza-, and thiabicyclo[3.1.0]hexylphenyl oxazolidinones

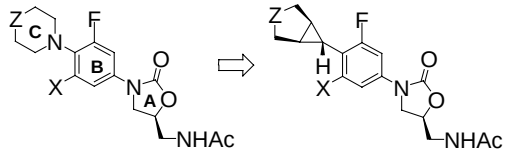
Adam R. Renslo,[a,*] Hongwu Gao,[a] Priyadarshini Jaishankar,[a] Revathy Venkatachalam,[a] Marcela Gómez,[a] Johanne Blais,[a] Michael Huband,[b] J. V. N. Vara Prasad[b] and Mikhail F. Gordeev[a]

[a]*Pfizer Global Research and Development, 34790 Ardentech Ct., Fremont, CA 94555, USA*
[b]*Pfizer Global Research and Development, 2800 Plymouth Rd., Ann Arbor, MI 48105, USA*

Received 8 November 2005; revised 23 November 2005; accepted 28 November 2005
Available online 4 January 2006

Abstract—A new class of oxazolidinone antibacterials incorporating oxygen-, nitrogen-, or sulfur-containing heterobicyclic C-rings is described. The in vitro potency and in vivo efficacy of these conformationally constrained oxazolidinone analogs are discussed.

The oxazolidinones are a promising new class of totally synthetic antibacterial protein synthesis inhibitors.[1] While they share with other antimicrobials a ribosomal target, the oxazolidinones bind in a distinct region of 23S rRNA near the peptidyl transferase center[2] and do not exhibit significant cross-resistance with the existing classes of antibacterials. Linezolid (**1**), the first oxazolidinone to receive regulatory approval, has become an important clinical option in the treatment of serious Gram-positive bacterial infections, including those caused by multi-drug resistant pathogens such as MRSA and VRE.



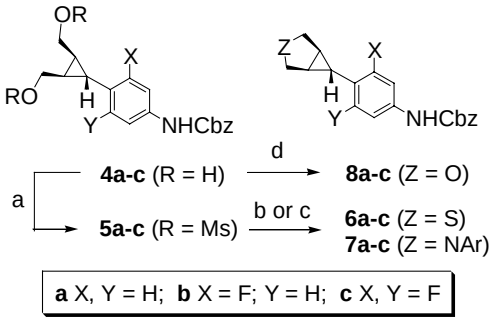
1 Z = O; X = H linezolid
2 Z = N-C(O)CH_2OH; X = H eperezolid
3 Z = SO_2; X = F **PNU-288034**

Other oxazolidinones that have advanced into clinical trials include the piperazine analog eperezolid (**2**) and the thiomorpholine analog **PNU-288034** (**3**). One focus of our early work in this field included the evaluation of conformationally constrained analogs of **1**–**3** in which the aliphatic C-ring is replaced by a rigid bicyclo[3.1.0]hexane ring system. Our initial efforts in this direction included the design and synthesis of (azabicyclo[3.1.0]hexyl)phenyloxazolidinones in which the bicyclic ring is connected to the aromatic B-ring via the pyrrolidine nitrogen atom. Many of these analogs exhibited enhanced antibacterial potency and spectrum, including activity against clinically relevant fastidious Gram-negative pathogens.[3] This initial success led us to consider a second generation of bicyclic analogs in which the cyclopropyl moiety is joined to the aromatic B-ring. Significantly, this structural modification would allow the preparation of thia-, oxa-, and azabicyclic analogs (see above, Z = S, N–R, and O) that closely mimic the steric nature of the unconstrained C-rings in the progenitor oxazolidinones **1**–**3**. In this letter, we describe the synthesis and antibacterial activities (in vitro and in vivo) of this new class of oxazolidinones.

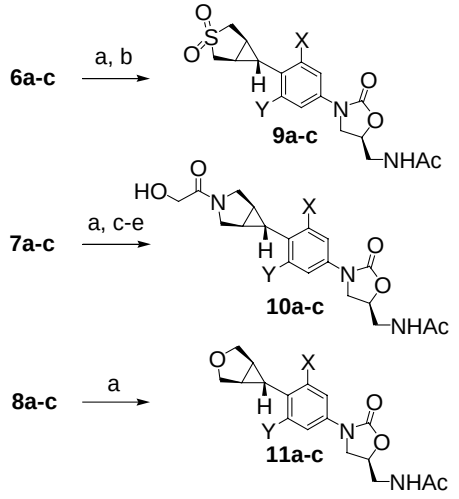
The bicyclic analogs described herein were synthesized as shown in Schemes 1–5. All three types of bicyclic C-rings (e.g., **6**–**8**) were prepared from the key diol intermediates **4a**–**c** (Scheme 1). We recently described a novel synthesis of diols **4a**–**c** (and **6**–**8**) employing as a key step

Keywords: Oxazolidinone; Antibacterials.
* Corresponding author. Tel.: +1 510 290 6171; fax: +1 510 653 8009; e-mail: renslo@mindspring.com

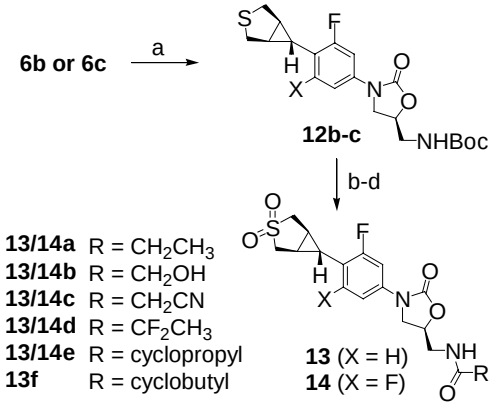
0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.11.093



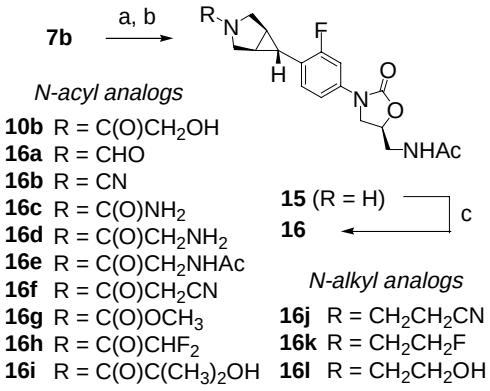
Scheme 1. Reagents and conditions: (a) Ms_2O, Et_3N, CH_2Cl_2; (b) Na_2S, DMSO, 79–88%; (c) NH_2Ar (Ar = 4-$MeOC_6H_4CH_2$–), rt, 63–92%; (d) 2 equiv *n*-BuLi, THF, 1.2 equiv MsCl, −78 °C; then 1.2 equiv *n*-BuLi, 37–47%.



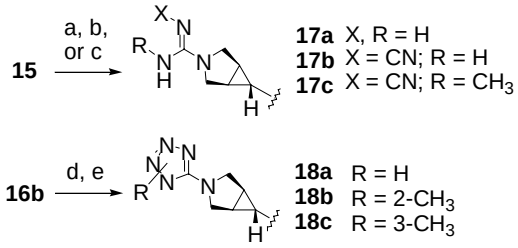
Scheme 2. Reagents: (a) 3.0 equiv LiO-*t*-Bu, 2.0 equiv (*S*)-$ClCH_2CH(OAc)CH_2NHAc$, 2 equiv MeOH, DMF, 41–71%; (b) AcOOH, THF, 82–92%; (c) for **10b**,**c**: H_2, $Pd(OH)_2$/C, MeOH, EtOAc, for **10a**: $ClCO_2CH(Cl)CH_3$, MeOH; (d) $BnOCH_2COCl$, Et_3N, CH_2Cl_2, 74% for two steps; (e) H_2, Pd/C, MeOH, CH_2Cl_2, 95%.



Scheme 3. Reagents: (a) 2.5 equiv LiO-*t*-Bu, 1.3 equiv (*S*)-$ClCH_2CH(OH)CH_2NHBoc$, DMF, 73–81%; (b), TFA, CH_2Cl_2; (c) RCOCl, DIEA, DMF or RCOOH, EDCI, HOBT, DMF; for **13/14b**: $AcOCH_2COCl$, DIEA DMF, then aq LiOH, THF, 49–84% for two steps; (d) AcOOH, THF, 82–96%.



Scheme 4. Reagents: (a) 3.0 equiv LiO-*t*-Bu, 2.0 equiv (*S*)-$ClCH_2CH(OAc)CH_2NHAc$, 2 equiv MeOH, DMF, 64%; (b) H_2, $Pd(OH)_2$/C, MeOH, EtOAc, 95%; (c) for **16d**–**i**, RCOCl, DIEA, DMF or RCOOH, EDCI, HOBT, DMF, 41–74%; for **16j**–**l**, RCH_2CH_2I or CH_2CHCN, 16–56%; for **16b** CNBr, 41%; for **16c** TMSNCO, 40%; for **16a**, HCOOH, Ac_2O, 40%.



Scheme 5. Reagents and conditions: (a) for **17a**, CbzNHC(=NCbz)SMe, AgOTf, Et_3N, then H_2, Pd/C, 95%; (b) for **17b**, $NaN(CN)_2$, HCl, *n*-BuOH, reflux, 36%; (c) for **17c**, MeNHC(=N–CN)SMe, AgOTf, Et_3N, 33%; (d) NaN_3, NH_4Cl, DMF, 100 °C, 54%; (e) MeI, DIEA, DMF (for **18b**,**c**), 30%.

the intramolecular cyclopropanation reaction of diazoacetates.[4] The conversion of diols **4a**–**c** to bicyclic intermediates **6**–**8** is summarized in Scheme 1. Thia- and azabicyclic compounds **6a**–**c** and **7a**–**c** were prepared in two steps from **4a**–**c** via initial formation of the bis-mesylates **5a**–**c** followed by reaction with sodium sulfide in DMSO (for **6a**–**c**) or with neat 4-methoxybenzylamine (for **7a**–**c**). The oxabicyclic intermediates **8a**–**c** were prepared from **4a**–**c** using a one-pot mesylation–cyclization reaction.

Scheme 2 illustrates the synthesis of oxazolidinone analogs with the privileged acetamidomethyl C-5 side chain. The reaction of thia-, aza-, and oxabicyclic aniline intermediates **6**–**8** with (1*S*)-2-(acetylamino)-1-(chloromethyl)ethyl acetate and lithium *tert*-butoxide in DMF furnished the desired oxazolidinones (e.g., **9a**–**c** to **11a**–**c**) in a single synthetic step.[5] For the thiabicyclic analogs, a subsequent sulfur oxidation step provided the sulfone analogs **9a**–**c**. For the azabicyclic analogs, the 4-methoxybenzyl group was removed via hydrogenolysis and the hydroxyacetamide side chain installed in two steps (see Scheme 2) to provide the desired analogs **10a**–**c**.

The synthesis of thiabicyclic analogs with atypical (i.e., non-acetamidomethyl) C-5 side chains is illustrated in

Scheme 3. Reaction of **6b** (mono-fluoro B-ring) or **6c** (bis-fluoro B-ring) with *tert*-butyl (2*S*)-3-chloro-2-hydroxypropylcarbamate[5] and lithium *tert*-butoxide provided the Boc-protected aminomethyl oxazolidinones **12b**,**c**, respectively. Next, the Boc group was removed and the resulting amine coupled to carboxylic acid or acid chloride building blocks to provide the desired amides. A final sulfur oxidation step then completed the synthesis of oxazolidinone analogs **13a**–**f** and **14a**–**e**.

To further explore C-ring SAR, the azabicyclic C-ring intermediate **15** was functionalized with various acyl, alkyl, guanidino, or heterocyclic substituents (Schemes 4 and 5). The formyl and cyano analogs **16a**,**b** were prepared from **15** by reaction with HCOOH/Ac_2O or cyanogen bromide, respectively. The *N*-acyl analogs **16d**–**i** were prepared by reaction of **15** with acid chlorides or via coupling to carboxylic acids. The *N*-alkyl analogs **16j**–**l** were prepared from **15** via alkylation or conjugate addition reactions.

Scheme 5 illustrates the synthesis of azabicyclic analogs bearing guanidino or tetrazole functionality. Guanidine and cyanoguanidine analogs **17a**–**c** were prepared using established procedures, for example by reaction of **15** with imidothiocarbamate reagents.[6] Tetrazole analog **18a** was prepared from the cyanamide **16b** by reaction with sodium azide in DMF.[7] Methylation of **18a** then provided a separable mixture of 2-methyl and 3-methyl tetrazole analogs **18b**,**c**.

The new bicyclic oxazolidinone analogs were tested against a panel of Gram-positive and fastidious Gram-negative bacteria (Tables 1–4). Minimum inhibitory concentration (MIC) values were determined using standard broth microdilution methods.[8] Inspection of Table 1 reveals interesting SAR trends relating to both B-ring and C-ring types. All three bicyclic C-ring subtypes are viable, although thia- and azabicyclic analogs **9a**–**c** and **10a**–**c** were generally more potent than the oxabicyclic analogs **11a**–**c**. For the thiabicyclic analogs **9a**–**c**, MIC values improve with increasing degrees of B-ring fluorination, **9c** showing the best potency. In contrast, a mono-fluorinated B-ring is preferred in the case of aza- and oxabicyclic analogs (cf. **10/11b** and **10/11a**,**c**). These B-ring SAR trends hold for both the Gram-positive and Gram-negative strains examined. The bis-fluoro B-ring thiabicyclic analog **9c** exhibited the best overall spectrum and potency among these initial acetamide analogs. Notably, five analogs in Table 1 (**9b**,**c**, **10a**,**b**, and **11b**) had improved in vitro activity against *Hae-*

Table 1. Minimum inhibitory concentration (MIC) values for linezolid (**1**) and thia-, aza-, and oxabicyclic analogs **9**–**11** against Gram-positive and fastidious Gram-negative bacteria[a]

Compound	MIC (μg/mL)				
	S.a.	*S.p.*	*E.f.*	*H.i.*	*M.c.*
Linezolid **1**	4	2	4	16	8
9a	4	4	8	16	8
9b	4	2	4	8	8
9c	2	1	2	8	4
10a	4	4	4	8	4
10b	2	2	2	4	8
10c	4	2	4	16	8
11a	8	8	16	32	8
11b	4	2	4	8	4
11c	8	4	8	32	8

[a] Strains: *S.a.*: *Staphylococcus aureus* UC-76 SA-1; *S.p.*: *Streptococcus pneumoniae* SVI SP-3; *E.f.*: *Enterococcus faecalis* MGH-2 EF1-1; *H.i.*: *Haemophilus influenzae* HI-3542; *M.c.*: *Moraxella catarrhalis* BC-3531.

Table 2. Minimum inhibitory concentration (MIC) values for thiabicyclic analogs bearing various C-5 amide side chains

Compound	MIC (μg/mL)				
	S.a.	*S.p.*	*E.f.*	*H.i.*	*M.c.*
9b	4	2	4	8	8
13a	4	1	2	8	8
13b	8	4	8	8	8
13c	8	4	8	8	8
13d	8	2	8	16	8
13e	4	2	4	4	4
13f	8	2	8	32	32
9c	2	1	2	8	4
14a	2	1	2	4	4
14b	4	2	4	8–16	8–16
14c	4	2	4	4–8	8–16
14d	4	1	2	16	8
14e	2	1	2	8	4

Strains: see Table 1.

Table 3. Minimum inhibitory concentration (MIC) values for azabicyclic analogs with acyl or alkyl C-ring substituents

Compound	MIC (μg/mL)				
	S.a.	*S.p.*	*E.f.*	*H.i.*	*M.c.*
15	8	2	16	32	8
10b	2	2	2	4	8
16a	2	1	2	8	8
16b	2	1	1	8	4
16c	2	2	2	8	8
16d	16	1	4	16	16
16e	4	4	4	32	32
16f	1	1	1	8	4
16g	4	2	4	32	8
16h	4	1	2	16	8
16i	8	2	4	32	16
16j	2	1	2	16	8
16k	4	1	4	16	8
16l	4	0.5	8	8	8

Strains: see Table 1.

Table 4. Minimum inhibitory concentration (MIC) values for azabicyclic analogs with guanidino or heterocyclic C-ring substituents

Compound	MIC (μg/mL)				
	S.a.	*S.p.*	*E.f.*	*H.i.*	*M.c.*
17a	64	4	64	64	16
17b	1	1	2	8	8
17c	4	2	4	16	32
18a	64	64	32	64	64
18b	2	1	2	16	8
18c	2	1	2	32	8

Strains: see Table 1.

mophilus influenzae as compared to linezolid, and the most potent of these—azabicyclic analog **10b**—was fourfold more potent (*H. influenzae* MIC = 4 μg/mL).

SAR of the oxazolidinone ring C-5 side chain was evaluated in the context of the thiabicyclic C-ring series (analogs **13a–f** and **14a–e**, Scheme 3 and Table 2). The majority of the C-5 groups examined were well tolerated. Propionamide analogs **13a** and **14a** equaled or bettered the activity of the corresponding acetamides **9b**,**c**. Difluoropropionamide, hydroxyacetamide, and cyanoacetamide analogs were somewhat less potent than the propionamides, particularly against the Gram-negative pathogens (cf. **14a** vs. **14b–d**). The cyclopropyl amides **13e** and **14e** exhibited a combination of Gram-positive and Gram-negative activities at least as good as the corresponding acetamides **9b**,**c**. However, the slightly larger cyclobutyl amide **13f** was notably less active against the Gram-negative strains. The extent of B-ring fluorination was again important. Hence, bis-fluoro B-ring analogs **14a–e** were typically 2- to 4-fold more potent than mono-fluoro analogs **13a–e**. In total, nine analogs in Table 2 had improved in vitro activity against *H. influenzae* as compared to linezolid.

The impact of C-ring substitution was examined via the introduction of acyl, alkyl, or heterocyclic groups in a series of azabicyclic analogs (Tables 3 and 4). The analog **10b** represents a benchmark compound for this series in that it contains the privileged hydroxyacetamide side chain of eperezolid. Indeed, **10b** displayed improved potency and spectrum as compared to linezolid. Among the various side chains examined, those containing a nitrile substituent often produced analogs with significantly improved activity against the Gram-positive strains (e.g., **16b**,**f**, and **j**). In contrast, analogs possessing a primary or secondary amine were much less active (e.g., **15** and **16d**). A tertiary amine however is surprisingly well tolerated (e.g., *N*-alkyl analogs **16j–l**). Analogs incorporating more lipophilic side chains (e.g., **16g–i**) generally had reduced activity against *H. influenzae*.

Table 4 presents MIC data for azabicyclic analogs with guanidine and tetrazole substituents. Strongly basic or acidic functionality is clearly not tolerated; guanidine **17a** and tetrazole **18a** were essentially inactive. Antimicrobial activity could be restored however by attenuating the basicity as in cyanoguanidines **17b**,**c** or by alkylation of the acidic tetrazole ring (*N*-Me analogs **18b**,**c**). No significant difference in activity was observed for regioisomeric tetrazoles **18b**,**c**.

The in vivo efficacy of selected thia- and azabicyclic analogs was evaluated in a murine septicemia infection model (Table 5). Thiabicyclic analogs **9a–c** demonstrated oral efficacy similar to that of linezolid. Azabicyclic analog and eperezolid isostere **10b** was the most efficacious analog examined (ED_{50} = 2.2 mg/kg), while the corresponding urea derivative **16c** was notably less active in vivo (ED_{50} = 15.3 mg/kg).

Table 5. In vivo efficacy of selected analogs in a systemic mouse infection model

Compound	Administration route	*S. aureus* UC 9213 ED_{50}[a] (mg/kg)
9a	po	7.2 (L 4.6)
9b	po	4.2 (L 2.5)
9c	po	3.1 (L 2.8)
10b	po	2.2 (L 2.8)
16c	po	15.3 (L 4.4)

[a] ED_{50} is the amount of drug required to cure 50% of infected mice. Value for linezolid control is given in parentheses (L = linezolid).

In summary, conformationally constrained thia-, oxa-, and azabicyclo[3.1.0]hexane heterocycles are valid bioisosteres of thiomorpholine, morpholine, and piperazine ring systems as applied to the oxazolidinone class of antibacterials. Analogs bearing these novel heterocycles possess in vitro and in vivo activities comparable and in some cases superior to those of the progenitor unconstrained analogs, including linezolid.

References and notes

1. For recent reviews, see: (a) Brickner, S. J. *Curr. Pharm. Des.* **1996**, *2*, 175; (b) Barbachyn, M. R.; Ford, C. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 2010; (c) Hutchinson, D. K. *Curr. Top. Med. Chem.* **2003**, *3*, 1021; (d) Nilus, A. M. *Curr. Opin. Invest. Drugs* **2003**, *4*, 149.
2. (a) Shinabarger, D. *Expert Opin. Invest. Drugs* **1999**, *8*, 1195; (b) Colca, J. R.; McDonald, W. G.; Waldon, D. J.; Thomasco, L. M.; Gadwood, R. C.; Lund, E. T.; Cavey, G. S.; Mathews, W. R.; Adams, L. D.; Cecil, E. T.; Pearson, J. D.; Bock, J. H.; Mott, J. E.; Shinabarger, D. L.; Xiong, L.; Mankin, A. S. *J. Biol. Chem.* **2003**, *278*, 21972.
3. Renslo, A. R.; Jaishankar, P.; Venkatachalam, R.; Hackbarth, C.; Lopez, S.; Patel, D. V.; Gordeev, M. F. *J. Med. Chem.* **2005**, *48*, 5009.
4. Renslo, A. R.; Gao, H.; Jaishankar, P.; Venkatachalam, R.; Hackbarth, C.; Lopez, S.; Gordeev, M. F. *Org. Lett.* **2005**, *7*, 2627.
5. (a) Perrault, W. R.; Pearlman, B. A.; Godrej, D. B. U.S. Patent 6,887,995, 2005; (b) Perrault, W. R.; Pearlman, B. A.; Godrej, D. B.; Jeganathan, A.; Yamagata, K.; Chen, J. J.; Lu, C. V.; Herrinton, P. M.; Gadwood, R. C.; Chan, L.; Lyster, M. A.; Maloney, M. T.; Moeslein, J. A.; Greene, M. L.; Barbachyn, M. R. *Org. Process Res. Dev.* **2003**, *7*, 533.
6. (a) Gadekar, S. M.; Nibi, S.; Cohen, E. *J. Med. Chem.* **1968**, *11*, 811; (b) DeMong, D. E.; Williams, R. M. *J. Am. Chem. Soc.* **2003**, *125*, 8561.
7. Garbrecht, W. L.; Herbst, R. M. *J. Org. Chem.* **1953**, *18*, 1003.
8. NCCLS (National Committee for Clinical Laboratory Standards). 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically—5th ed.; Approved Standard. NCCLS Document M7-A5, Vol. 20, No 2.