Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4537–4542
Bioorganic & Medicinal Chemistry Letters

Antibacterial activity of pyrrolopyridine-substituted oxazolidinones: synthesis and in vitro SAR of various C-5 acetamide replacements

Steven D. Paget, Christine M. Boggs,† Barbara D. Foleno, Raul M. Goldschmidt, Dennis J. Hlasta,† Michele A. Weidner-Wells, Harvey M. Werblood, Karen Bush and Mark J. Macielag*

Johnson & Johnson Pharmaceutical Research and Development, L.L.C., Route 202, PO Box 300, Raritan, NJ 08869, USA

Received 6 April 2006; revised 7 June 2006; accepted 8 June 2006
Available online 27 June 2006

Abstract—A series of pyrrolopyridine-substituted oxazolidinones containing various C-5 acetamide isosteres was synthesized and the structure–antibacterial activity relationships determined against a representative panel of susceptible and resistant Gram-positive bacteria.

Introduction of the oxazolidinone class of antimicrobial agents has provided a means for the healthcare community to combat the increasing incidence of drug-resistant Gram-positive bacteria found in both the hospital and community settings. Linezolid, the first and only FDA-approved drug in the class, is indicated for both hospital and community-acquired pneumonia, skin infections including cases due to methicillin-resistant *Staphylococcus aureus* (MRSA), and infections associated with vancomycin-resistant *Enterococcus faecium* (VREF), including cases of bloodstream infection.[1] However, if the healthcare community wishes to stay ahead of bacterial innovation, such as the development of resistance to vancomycin[2,3] and β-lactam antibiotics,[4] the discovery of novel members of this class will be crucial.

In previous publications from this group, various pyrroloaryl-substituted oxazolidinones were detailed,[5–10] several of which had superior in vitro antimicrobial activity to linezolid. Following the identification of the pyrrolo[3,4-*b*]pyridinyl oxazolidinone core structure (**1**, Fig. 1) as the optimal template for antibacterial activity, we next shifted our focus to the modification of the C-5 acetamide moiety as a means of increasing potency and enhancing efficacy. A handful of strategies have been employed to replace the acetamide group of oxazolidinone antibacterial agents with varying success;[11–15] some of these same approaches were utilized in the present study. The effect of novel isosteric replacements on antibacterial activity was also investigated.[16]

The synthesis of the key intermediates, **8** and **9**, in the preparation of acetamide isosteres is depicted in Scheme 1. To begin the sequence an inverse electron demand Diels–Alder reaction of acyclic precursor **2** with extrusion of hydrogen cyanide provided the regioisomeric pyrrolopyridines **3** and **4**.[17] Separation of the desired regioisomer by column chromatography followed by acid hydrolysis of the carbamate protecting group of **3** provided the pyrrolopyridine salt **5**. Following the work of the Upjohn group[18] an S_NAr reaction of 3,4-difluoronitrobenzene with **5**, followed by transfer hydrogenation of the nitro group with palladium on carbon and ammonium formate and benzyloxycarbonylation of the resulting amine furnished the Cbz-protected aniline **6**. Nucleophilic attack of the lithium anion of **6** on (*R*)-glycidyl butyrate provided oxazolidinone carbinol derivative **7**. Mesylation of **7** provided the first key intermediate **8**. Intermediate **9** was accessed by displacement with potassium phthalimide and subsequent hydrazinolysis.

Keywords: Pyrrolopyridine-substituted oxazolidinones; Antibacterial agents; C-5 acetamide isosteres; Methicillin-resistant *Staphylococcus aureus*; Vancomycin-resistant *Enterococcus faecium*.
* Corresponding author. Tel.: +1 609 655 6950; fax: +1 609 655 6930; e-mail: mmaciela@prdus.jnj.com
† Present address: Johnson & Johnson Pharmaceutical Research and Development, L.L.C., Welsh and McKean Roads, Spring House, PA 19477, USA.

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.06.023

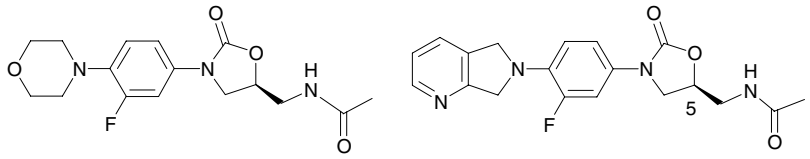
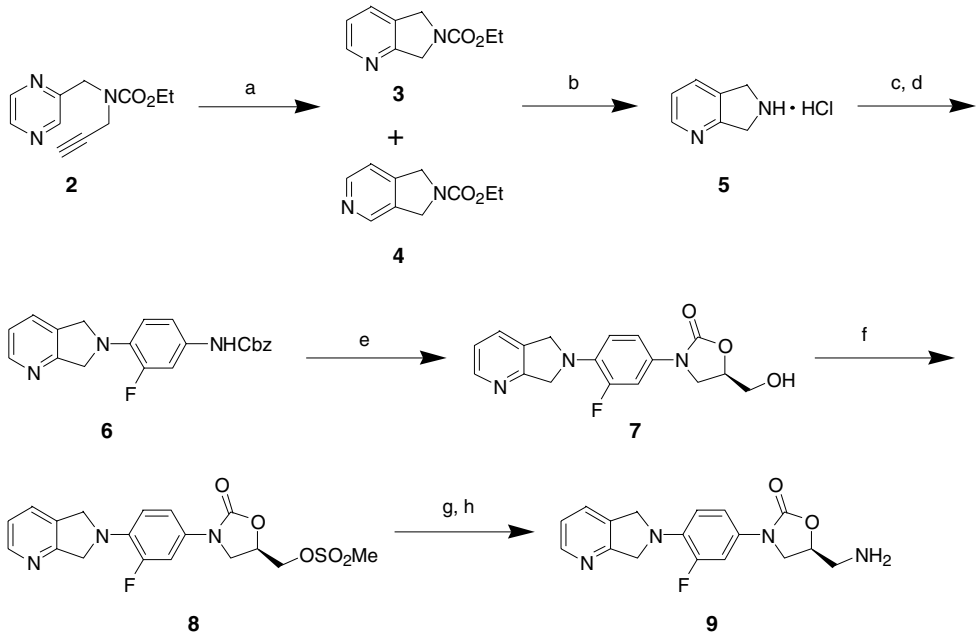


Figure 1. Linezolid and pyrrolo[3,4-*b*]pyridinyl oxazolidinone (**1**) structures.



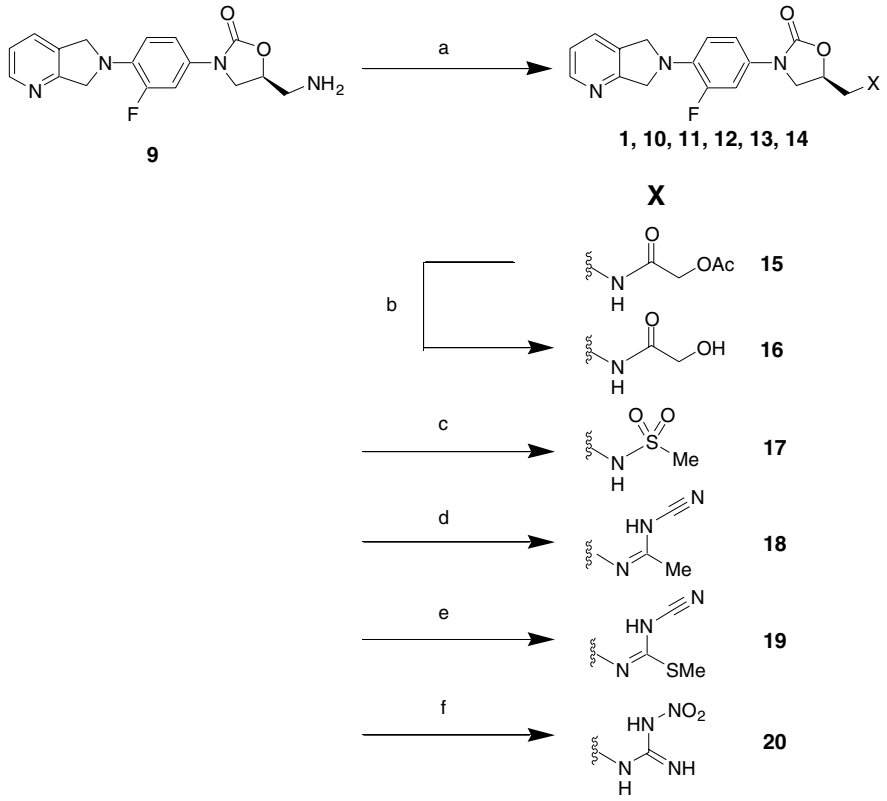
Scheme 1. Reagents and conditions: (a) undecane, 180 °C; separate isomers (**3**, 60%; **4**, 23%); (b) concd aq HCl, reflux (97%); (c) 3,4-difluoronitrobenzene, DIPEA, DMF, 60 °C (94%); (d) 10% Pd/C, $HCOONH_4$, THF, MeOH; CbzCl, $NaHCO_3$, H_2O, acetone (97%); (e) *n*-BuLi, THF then (*R*)-glycidyl butyrate, −78 °C to rt (92%); (f) MsCl, Et_3N, DMF (95%); (g) potassium phthalimide, DMF, 50 °C; (h) H_2NNH_2, MeOH, reflux.

Reaction of amine **9** with various acyl chloride or chloroformate reagents, or alternatively with methanesulfonyl chloride in the case of sulfonamide **17**, provided a range of simple amide (**10**–**12** and **15**) or carbamate (**13** and **14**) derivatives (Scheme 2). Acetyloxyacetamide **15** was saponified with K_2CO_3 in methanol to supply hydroxyacetamide **16**. More exotic isosteres such as *N*-cyano-ethanimidamide **18**,[19] methyl *N*-cyano-carbimidothioate **19**,[20] and *N*-nitro-guanidine **20**[21] were prepared by the literature procedures.

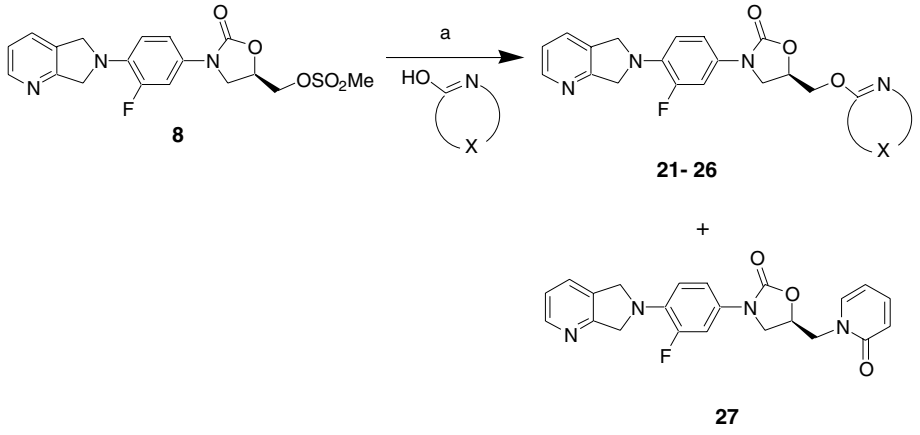
A series of alkoxyheterocycles was constructed by reaction of mesylate **8** with the sodium salt of the hydroxyheterocycle of interest. In general the displacement proceeded smoothly to provide analogs **21**–**26** but at times gave the alternate N-linked regioisomer, exemplified by compound **27**, in which the amide is constrained to a '*transoid*' conformation (Scheme 3). This side product, while unintended, gave an additional isosteric structure for investigation.

Minimum inhibitory concentrations (MIC) were measured against a panel of susceptible and resistant strains of Gram-positive bacteria with linezolid as a standard. A set of four strains was used as the primary screening panel: *S. aureus* OC 4172 (Smith strain) is methicillin-susceptible (MSSA); *S. aureus* OC 2878 is methicillin-resistant (MRSA); *Enterococcus faecalis* ATCC 29212 and *E. faecium* OC 3312 are susceptible and resistant to vancomycin, respectively. Compounds were also tested against *S. aureus* OC 4172 in the presence of 50% mouse serum to gauge the extent of protein binding or inactivation due to serum. Broth microdilution MIC (lowest concentration of compound inhibiting visible growth) determinations were performed according to National Committee for Clinical Laboratory Standards methods.[22]

Within this series of C-5 acetamide analogs (Table 1)[23] the lead compound, **1**, was the most active, with MIC values fourfold lower than linezolid against the four organisms tested. Although **1** was slightly less active when tested in the presence of mouse serum, it was still twofold more potent than linezolid. Among simple amide replacements, propionamide **10** and cyclopropylcarboxamide **11** were about equally active as linezolid but, like acetamide **1**, they were slightly less potent in the presence of mouse serum. Further increase in the size and lipophilicity of the C-5 amide substituent, as in cyclobutylcarboxamide analog **12**, had a detrimental effect on antibacterial activity. Similarly, introduction of polar functionality reduced antibacterial activity. In particular



Scheme 2. Reagents and conditions: (a) R_1COCl, Et_3N, DMF (25–87%); (b) K_2CO_3, MeOH; (c) MsCl, pyridine, CH_2Cl_2 (38%); (d) (EtO)(Me)C=N–CN, Et_3N, CH_3CN; (e) $(MeS)_2C$=N–CN, toluene, reflux (20%); (f) $(MeS)(NH_2)C$=N–NO_2, MeOH, reflux (24%).



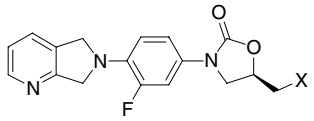
Scheme 3. Reagents: (a) NaH, DMF (**22**–**26**, 15–93%; **21**, 5%; **27**, 30%).

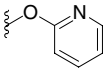
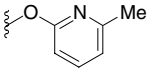
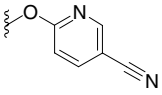
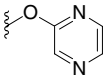
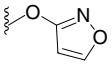
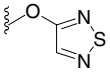
the MIC values of acetoxyacetamide **15** and hydroxyacetamide **16** were 32- to 512-fold higher than **1**.

Other acetamide isosteres (**13**, **14**, and **17**–**20**) were also investigated but of these only methyl carbamate **13**, *N*-cyano-ethanimidamide **18**, and methyl *N*-cyano-carbimidothioate **19** had MIC values of 4 μg/mL or less against all bacterial strains tested in the absence of serum. With the exception of *N*-nitro-guanidine analog **20**, the antibacterial activity of these isosteric replacements decreased 4- to 8-fold in the presence of mouse serum, likely due to protein binding. We have found that oxazolidinones with MIC values $\geqslant$8 μg/mL in 50% mouse serum have limited efficacy in a *S. aureus* Smith murine systemic lethal infection model, and therefore these compounds were not pursued beyond susceptibility testing.

In exploring C-5 substituents with proven success, the acetamide was replaced with alkoxyheterocycles, a strategy employed by researchers at AstraZeneca in the 4-tetrahydropyridinyl and 4-dihydropyranyloxazolidinone series.[13,14] This approach was ineffective in the current pyrrolopyridine oxazolidinone series; alkoxyheterocycle compounds (**21**–**26**) were generally poorly active with the notable exception of thiadiazolyloxy analog **26**,

Table 1. Antibacterial activity (MIC[a] values in μg/mL) for pyrrolo[3,4-*b*]pyridine-substituted oxazolidinones



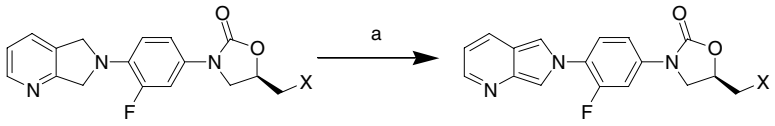
Compound	X	*E. faecalis*	*E. faecium*	MRSA	MSSA in broth	MSSA + 50% mouse serum
Linezolid		2	2	1	2	2
1	NHCOMe	0.5	0.5	0.25	0.5	1
10	NHCOEt	2	2	1	2	4
11	NHCOcyclopropyl	2	2	2	2	4
12	NHCOcyclobutyl	8	8	4	8	32
13	$NHCO_2Me$	4	2	2	4	16
14	$NHCO_2Et$	8	8	4	8	64
15	$NHCOCH_2OAc$	16	16	128	32	32
16	$NHCOCH_2OH$	32	16	16	32	32
17	$NHSO_2Me$	8	4	2	4	16
18	N=C(Me)–NH–CN	4	4	2	2	8
19	N=C(SMe)–NH–CN	4	2	2	2	8
20	NHC=NH($NH–NO_2$)	16	8	8	8	16
21		>64	>64	32	>128	>128
22		>32	>64	>128	>128	>128
23		>16	>64	>64	>64	>64
24		>32	>32	>32	>32	>32
25		>32	16	8	>32	>64
26		8	2	1	2	8
27		>32	>32	16	>32	64

[a] The variance in the determination of MIC values is twofold such that an MIC difference of at least fourfold is significant.

which had an MIC range of 1–8 μg/mL. The reasons for the generally poor antibacterial activity of the O-linked heterocycles in this series are not entirely clear. The calculated log *P* values (Advanced Chemistry Development Software V8.19 for Solaris) of AstraZeneca's lead oxazolidinone, AZD-2563,[24] and compound **25**, both of which contain a 3-isoxazolyloxy acetamide replacement, are 1.60 and 2.32, respectively. Thus, it is unlikely that differences in physicochemical properties are responsible. Instead, the structure–activity relationships of the C5-substituent would appear to be dependent on the structure of the heterocyclic moiety appended to position 4 of the phenyl ring. Interestingly, a similar trend has recently been reported for a series of arylpiperazinyl oxazolidinone antibacterial agents.[25] The N-linked pyridone analog **27** was also found to be poorly active, possibly due to increased steric demand, the need for an H-bond donor, or conformational factors.

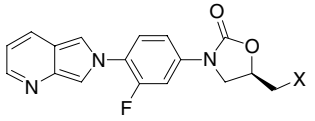
Oxidation of select pyrrolo[3,4-*b*]pyridinyl oxazolidinones with manganese dioxide[26] provided the corresponding fully aromatic pyrrolopyridinyl oxazolidinones **28**–**40** (Scheme 4). [23]

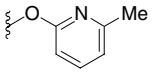
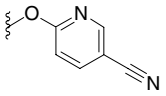
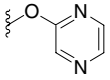
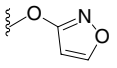
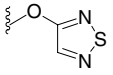
In general, oxidation of the heterocycle proved to be detrimental to antibacterial activity (Table 2). MIC values for analogs **28**–**34** were 2- to greater than 32-fold higher than for the corresponding analogs in the dihydro series. The effect on antibacterial activity was especially apparent for the C-5 sulfonamidomethyl derivative (**32**), in which MIC values increased by more than eightfold compared to the dihydro analog (**17**) against all bacteria in the testing panel. In select cases, antibacterial activity improved for alkoxyheterocycle analogs in the fully aromatic series compared to the parent series. In particular, the MIC value of pyrazine **37** against the MRSA strain was at least fourfold lower



Scheme 4. Reagents: (a) MnO_2, CH_2Cl_2 (4–64%).

Table 2. Antibacterial activity (MIC[a] values in μg/mL) for pyrrolopyridine-substituted oxazolidinones



Compound	X	*E. faecalis*	*E. faecium*	MRSA	MSSA in broth	MSSA + 50% mouse serum
Linezolid		2	2	1	2	2
28	NHCOMe	1	1	0.5	1	2
29	NHCOEt	16	16	16	32	32
30	NHCOcyclopropyl	16	8	8	8	16
31	$NHCO_2Me$	16	16	16	16	32
32	$NHSO_2Me$	>64	>64	>64	>64	>64
33	N=C(SMe)–NH–CN	8	8	8	8	64
34	NHC=NH(NH–NO_2)	32	32	>128	64	128
35		>64	>128	>64	>128	>128
36		>32	>64	>64	>128	>128
37		64	64	8	>32	>32
38		16	8	4	8	64
39		4	4	2	8	16
40		>128	>128	>128	>128	>128

[a] The variance in the determination of MIC values is twofold such that an MIC difference of at least fourfold is significant.

than the analogous compound **24**, and the antibacterial activity of isoxazole **38** was at least twofold better than **25** against all strains tested.

Despite the overall weaker antibacterial activity of the fully aromatic series (**28**–**40**) compared to the parent series, the SAR trends were generally quite similar but with greater sensitivity to structural alteration of the C-5 acetamide substituent. Once again, acetamide **28** was the most potent analog in this series, with MIC values twofold lower than linezolid. Increasing substituent size and lipophilicity produced an increase in the MIC value, as was evident from the 16- to 32-fold increase in MIC for propionamide **29** compared to acetamide **28**, for example. In the alkoxyheterocycle subseries the thiadiazole analog, **39**, showed modest antibacterial activity (MIC values of 2–16 μg/mL) with the remainder of the alkoxyheterocycles (**35**–**38**) and the pyridone analog (**40**) significantly less potent.

In summary, although diverse strategies were employed to identify a replacement for the C-5 acetamide functionality, no improvement was found over the lead compound (**1**) in the pyrrolo[3,4-*b*]pyridinyl oxazolidinone series of antibacterial agents. Compound **1** and the oxidized congener, **28**, exhibited the best antibacterial profile in our abbreviated testing panel with MIC ranges of 0.25–0.5 μg/mL and 0.5–1 μg/mL, respectively, against staphylococci, including MRSA, and 0.5 and 1 μg/mL, respectively, against enterococci, including VRE. Unfortunately, many analogs with larger amide or other isosteric replacements for the C-5 acetamide

were significantly less potent than the lead compound (**1**), although propionamide **10** and cyclopropylcarboxamide **11** were equally active as linezolid against the bacterial strains tested. In contrast to the AstraZeneca series of 4-tetrahydropyridinyl- and 4-dihydropyranyloxazolidinones,[13,14] pyrrolopyridinyl analogs with C-5 alkoxyheterocycle substituents were poorly active with the exception of the thiadiazoles **26** and **39**. This result suggests that the SAR of the C-5 substituent of oxazolidinone antibacterial agents is not only narrow in terms of acceptable replacements but also highly dependent on the nature of other substituents in the molecule.

References and notes

1. Mutnick, A. H.; Biedenbach, D. J.; Turnidge, J. D.; Jones, R. N. *Diagn. Microbiol. Infect. Dis.* **2002**, *43*, 65.
2. Linden, P. K. *Drugs* **2002**, *62*, 425.
3. Miller, D.; Urdaneta, V.; Weltman, A.; Park, S. *Morb. Mortal. Wkly. Rep.* **2002**, *51*, 902.
4. Jacoby, G.; Bush, K. In *Frontiers in Antimicrobial Resistance: a Tribute to Stuart B. Levy*; White, D. G., Alekshun, M. N., McDermott, P. F., Eds.; ASM Press: Washington, DC, 2005; Chapter 5, pp 53–65.
5. Paget, S. D.; Foleno, B. D.; Boggs, C. M.; Goldschmidt, R. M.; Hlasta, D. J.; Weidner-Wells, M. A.; Werblood, H. M.; Wira, E.; Bush, K.; Macielag, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4173.
6. Paget, S.; Boggs, C.; Foleno, B.; Goldschmidt, R.; Hilliard, J.; Melton, J.; Stryker, S.; Wira, E.; Bush, K.; Macielag, M. 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, 2002; F-1318.
7. Paget, S.; Hlasta, D. WO 01/42242 A, 2001.
8. Paget, S.; Boggs, C.; Foleno, B.; Goldschmidt, R.; Grant, E.; Hilliard, J.; Hlasta, D.; Weidner-Wells, M.; Werblood, H.; Wira, E.; Bush, K.; Macielag, M. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2001; F-1048.
9. Foleno, B. D.; Goldschmidt, R.; Licata, L.; Montenegro, D.; Paget, S.; Weidner-Wells, M.; Wira, E.; Macielag, M.; Bush, K. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2001; F-1049.
10. Hilliard, J.; Goldschmidt, R.; Fernandez, J.; Melton, J.; Paget, S.; Weidner-Wells, M.; Macielag, M.; Bush, K. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2001; F-1050.
11. Gregory, W. A.; Brittelli, D. R.; Wang, C.-L. J.; Wuonola, M. A.; McRipley, R. J.; Eustice, D. C.; Eberly, V. S.; Bartholomew, P. T.; Slee, A. M.; Forbes, M. *J. Med. Chem.* **1989**, *32*, 1673.
12. Singh, U.; Raju, B.; Lam, S.; Zhou, J.; Gadwood, R. C.; Ford, C. W.; Zurenko, G. E.; Schaadt, R. D.; Morin, S. E.; Adams, W. J.; Friis, J. M.; Courtney, M.; Palandra, J.; Hackbarth, C. J.; Lopez, S.; Wu, C.; Mortell, K. H.; Trias, J.; Yuan, Z.; Patel, D. V.; Gordeev, M. F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4209.
13. Gravestock, M. B. WO 99/64417 A2, 1999.
14. Gravestock, M. B.; Acton, D. G.; Betts, M. J.; Dennis, M.; Hatter, G.; McGregor, A.; Swain, M. L.; Wilson, G. R.; Woods, L.; Wookey, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4179.
15. Reck, F.; Zhou, F.; Girardot, M.; Kern, G.; Eyermann, C. J.; Hales, N. J.; Ramsay, R. R.; Gravestock, M. B. *J. Med. Chem.* **2005**, *48*, 499.
16. Hlasta, D. J. WO 01/42229 A1, 2001.
17. Petersen, U.; Krebs, A.; Schenke, T.; Grohe, K.; Bremm, K.-D.; Endermann, R.; Metzger, K.-G.; Zeiler, H.-J. US 94/5371090, 1994, CAN 119:49377.
18. 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.
19. Lwowski, W. *Synthesis* **1971**, *5*, 263.
20. Wittenbrook, L. S. *J. Heterocycl. Chem.* **1975**, *12*, 37.
21. Ohta, A.; Aoyagi, Y.; Kurihara, Y.; Yuasa, K.; Shimazaki, M.; Kurihara, T.; Miyamae, H. *Heterocycles* **1987**, *26*, 3181.
22. National Committee for Clinical Laboratory Standards: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 5th ed. Approved standard: NCCLS Document M7-A5, 2000.
23. All numbered compounds were characterized by high resolution ^{1}H NMR and MS. Purity of tested compounds was $\geqslant$95%.
24. Wookey, A.; Turner, P. J.; Greenhalgh, J. M.; Eastwood, M.; Clarke, J.; Sefton, C. *Clin. Microbiol. Infect.* **2004**, *10*, 247.
25. Jang, S.-Y.; Ha, Y. H.; Ko, S. W.; Lee, W.; Lee, J.; Kim, S.; Kim, Y. W.; Lee, W. K.; Ha, H.-J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3881.
26. Ogawa, H.; Aoyama, T.; Shioiri, T. *Heterocycles* **1996**, *42*, 75.