Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5036–5040

A distal methyl substituent attenuates mitochondrial protein synthesis inhibition in oxazolidinone antibacterials

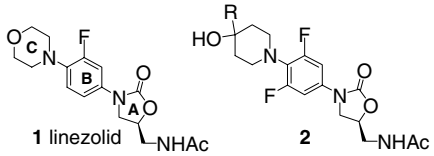
Adam R. Renslo,[a,*] Andy Atuegbu,[a] Prudencio Herradura,[a] Priyadarshini Jaishankar,[a] Mingzhe Ji,[a] Karen L. Leach,[b] Michael D. Huband,[b] Michael R. Dermyer,[b] Luping Wu,[b] J. V. N. Vara Prasad[b] and Mikhail F. Gordeev[a]

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

Received 6 June 2007; revised 3 July 2007; accepted 6 July 2007
Available online 13 July 2007

Abstract—Oxazolidinone analogs bearing substituted piperidine or azetidine C-rings are described. Analogs with a methyl group at the 3-position of the azetidine ring or the 4-position of the piperidine ring exhibited reduced mitochondrial protein synthesis inhibition while retaining good antibacterial potency.

Linezolid (**1**) is the first member of the oxazolidinone class of antibacterial protein synthesis inhibitors to reach the market.[1] The oxazolidinones bind to 23S rRNA in the 50S subunit of the bacterial ribosome, near the peptidyl transferase center.[2] Showing no significant cross-resistance with the existing antibacterial classes, linezolid has become an important addition to the clinician's armamentarium. Although linezolid is generally well tolerated, prolonged courses of therapy are sometimes complicated by reversible myelosuppression that can require cessation of treatment. It has been suggested[3] that mitochondrial protein synthesis (MPS) inhibition is responsible for this effect and in a recent report[4] the oxazolidinone eperezolid was shown to slow the proliferation of various mammalian cell lines via inhibition of MPS.

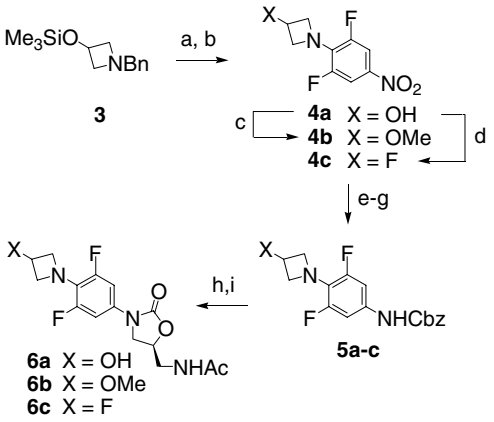


The identification of oxazolidinones with reduced MPS inhibitory activity could expand the utility of the class to include the treatment of deep-seated infections that require extended courses of therapy. In connection with this objective, we explored a series of oxazolidinone analogs bearing hydroxy-azetidine or hydroxy-piperidine C-rings (e.g., **2**). These heterocycles bear an obvious resemblance to the morpholine C-ring of linezolid but offer the option to introduce an additional substituent in proximity to the oxygen atom (i.e., R in **2**). In the course of our investigations, we made the unexpected and surprising observation that MPS inhibition is significantly affected by the nature of the alkyl substituent in these analogs. In particular, MPS inhibition was attenuated in the case where, R = methyl and furthermore, this modification only marginally affected antibacterial potency. Herein, we describe the synthesis and biological properties of a series of substituted azetidine and piperidine-containing oxazolidinone analogs.

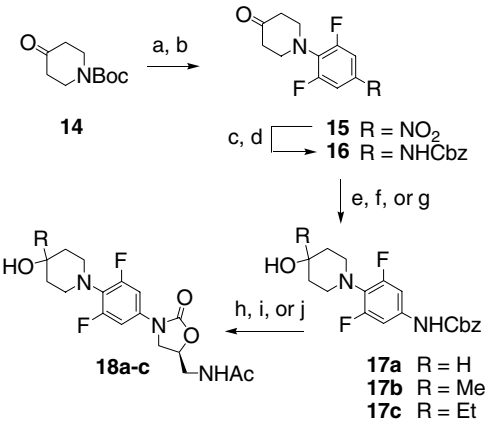
The preparation of the azetidine and piperidine analogs followed standard synthetic protocols for oxazolidinone synthesis[5] and is summarized in Schemes 1–5. Scheme 1 illustrates the synthesis of azetidine analogs **6a**–**c** bearing a single substituent (OH, OMe, or F, respectively) in the 3-position of the azetidine ring. Hydrogenolysis of the known azetidine **3**[6] was followed by a nucleophilic aromatic substitution reaction with 3,4,5-trifluoronitrobenzene to yield **4a**. Alcohol **4a** could then be converted to the methyl ether **4b** or the fluoro azetidine **4c**. Reduction

Keywords: Antibacterials; Oxazolidinones; Selectivity; Mitochondrial protein synthesis inhibition; Azetidines; Piperidines.
* Corresponding author at present address: Department of Pharmaceutical Chemistry, University of California, San Francisco, USA. E-mail: adam.renslo@ucsf.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.07.022



Scheme 1. Reagents and conditions: (a) H_2, $Pd(OH)_2/C$, MeOH; (b) 3,4,5-trifluoronitrobenzene, DIEA, DMF, 45 °C, 95% for two steps; (c) NaH, MeI, DMF, 94%; (d) DAST, CH_2Cl_2, −78 °C to rt; 72%; (e) Fe, NH_4Cl, EtOH, H_2O, 80 °C; (f) CbzCl, pyridine, CH_2Cl_2, 50–90% for two steps; (g) TBS–Cl, Et_3N, DMF (for **5a** only), 98%; (h) *t*-BuOLi, (*S*)-$ClCH_2CH(OAc)CH_2NHAc$, DMF, 50–70%; (i) Et_3N–HF, THF, 76% (for **6a** only).



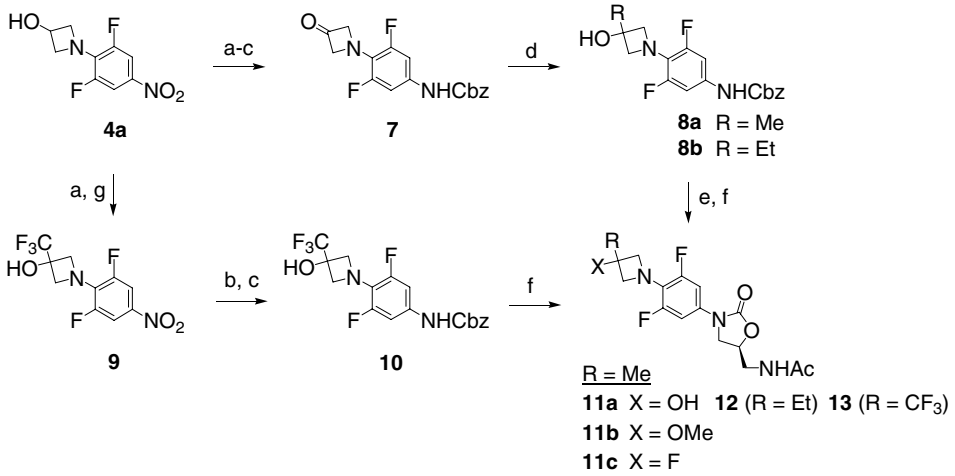
Scheme 3. Reagents and conditions: (a) TFA, $ClCH_2CH_2Cl$; (b) 3,4,5-trifluoronitrobenzene, DIEA, DMF, 50 °C, 12 h, 90% for two steps; (c) H_2 10% Pd/C, EtOAc, 18 h; (d) CbzCl, pyridine, CH_2Cl_2, 23 °C, 1 h, 80% for two steps; (e) DIBAL, CH_2Cl_2, −78 °C, 80% then TBSCl, Et_3N, DMF, 23 °C, 15 h, 52%; (for **17a**); (f) MeMgBr, THF, −78 °C, 79% (for **17b**); (g) EtMgBr, THF, −78 °C, 61% (for **17c**); (h) 3.0 equiv LiO*t*-Bu, MeOH, THF, DMF, 2.0 equiv (*S*)-$ClCH_2CH(OAc)CH_2NHAc$, then HF–Et_3N, THF, 23 °C, 27% for two steps (for **18a**); (i) 3.0 equiv LiO*t*-Bu, MeOH, THF, DMF, 2.0 equiv (*S*)-$ClCH_2CH(OAc)CH_2NHAc$, 44% (for **18b**); (j) 2.5 equiv LiO*t*-Bu, 1.3 equiv (*S*)-$ClCH_2CH(OH)CH_2NHBoc$, DMF, 20 h then 4 M HCl/dioxane; then Ac_2O, Et_3N, CH_2Cl_2, 17 h, 34% over three steps.

of the nitro group in **4a**–**c** was followed by protection of the resulting amine as a benzyl carbamate (Cbz) to yield **5a**–**c** (in the case of **5a**, the hydroxyl was protected as a *tert*-butyldimethylsilyl ether). Finally, the oxazolidinone ring was installed by reaction with lithium *tert*-butoxide and (1*S*)-2-(acetylamino)-1-(chloromethyl)ethyl acetate according to the established procedure[5] to provide **6b** and **6c**. For **6a**, a final deprotection step was required (HF–Et_3N).

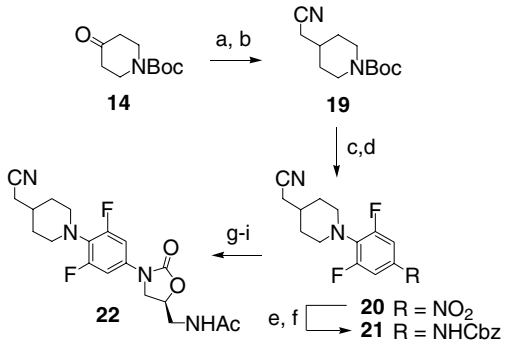
Scheme 2 illustrates the synthesis of analogs **11a**–**c**, **12**, and **13** in which a 3-alkyl substituent has been introduced adjacent to the hydroxy, methoxy, or fluoro substituents of the azetidine ring. The 3-methyl and 3-ethyl intermediates **8a** and **8b** were prepared by reaction of the appropriate Grignard reagent with the azetidinone **7** which was prepared in three steps from **4a**. Intermediate **8a** was converted to **11a**–**c** in a manner analogous to that described above for the synthesis of **6a**–**c** from **4a**.

Trifluoromethyl azetidine intermediate **9** was prepared in two steps from **4a** via oxidation to the azetidinone followed by reaction with trimethylsilyl trifluoromethane.[7] Compounds **8b** and **9** were converted to the oxazolidinones **12** and **13** as described above for the preparation of **6a**–**c**.

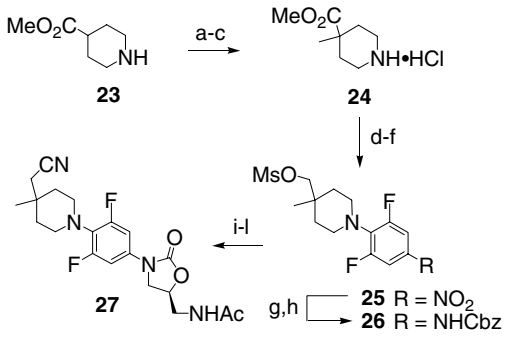
The synthesis of *piperidine*-containing oxazolidinones is described in Schemes 3–5. The preparation of analogs **18a**–**c** originated with the commercially available piperidone **14**, which was converted in two steps to the nitroaniline **15**. Reduction of the nitro function and conversion of the resulting amine to a benzyl carbamate provided **16**. At this stage, the ketone was reduced with



Scheme 2. Reagents and conditions: (a) Swern, 65–80%; (b) H_2, Pd/C, EtOAc; (c) CbzCl, pyridine, CH_2Cl_2, 75% for two steps; (d) MeMgBr or EtMgBr, THF, 0 °C, 75%; (e) DAST, CH_2Cl_2, −78 °C to rt, 30% (for **11c** only); (f) LiO*t*-Bu, MeOH, THF, DMF, (*S*)-$ClCH_2CH(OAc)CH_2NHAc$, 30–60%; (g) CF_3–$SiMe_3$, TBAF, THF, 16%.



Scheme 4. Reagents and conditions: (a) diethyl(cyanomethyl) phosphonate, LiBr, Et_3N, THF, 23 °C, 8 h, 99%; (b) H_2, 10% Pd/C, MeOH, 23 °C, 20 h, 98%; (c) 4.0 M HCl/dioxane, 23 °C, 25 h, 100%; (d) 3,4,5-trifluoronitrobenzene, DIEA, DMF, 70 °C, 82%; (e) Fe, NH_4Cl, EtOH, H_2O, 95 °C, 4 h, 87%; (f) CbzCl, pyridine, CH_2Cl_2, 0–23 °C, 65%; (g) 2.5 equiv Li*O*t-Bu, 1.3 equiv (*S*)-$ClCH_2CH(OH)CH_2NHBoc$, DMF, 20 h; (h) 20% TFA in CH_2Cl_2, 0–23 °C, 3 h; (i) Ac_2O, pyridine, CH_2Cl_2, 23 °C, 17 h, 38% over three steps.



Scheme 5. Reagents and conditions: (a) Boc_2O, THF, DIEA, 65 °C, 17 h, quant.; (b) LDA, MeI, THF, −78 to 23 °C, 25 h, 70%; (c) 4.0 M HCl/dioxane, 23 °C, 25 h, 93%; (d) 3,4,5-trifluoronitrobenzene, DIEA, DMF, 70 °C, 85%; (e) $CaCl_2$, $NaBH_4$, EtOH, 0–50 °C, 6 h, 94%; (f) MsCl, Et_3N, CH_2Cl_2, 0–23 °C, 20 h, 71%; (g) H_2, 10% Pd/C, EtOAc, 23 °C, 20 h; (h) CbzCl, pyridine, CH_2Cl_2, 23 °C, 90% over two steps; (i) 2.5 equiv Li*O*t-Bu, 1.3 equiv (*S*)-$ClCH_2CH(OH)CH_2NHBoc$, 0–23 °C, 17 h, 51%; (j) 20% TFA in CH_2Cl_2, 0–23 °C, 6 h; (k) Ac_2O, pyridine, CH_2Cl_2, 23 °C, 17 h; (l) KCN, DMSO, 80 °C, 20 h, 50% over three steps.

DIBAL to provide **17a**, or, alternatively, reacted with Grignard reagents to provide the 4-methyl and 4-ethyl intermediates **17b** and **17c**, respectively. The oxazolidinone pharmacophore was then installed as before[5] to provide analogs **18a**–**c**.

Recently, it was disclosed that piperidine-containing oxazolidinone analogs bearing a 4-cyanomethyl substituent exhibit excellent in vitro and in vivo properties.[8] We therefore targeted the cyanomethyl analog **22** along with the corresponding 4-methyl analog **27**, in order to evaluate the effect of a 4-alkyl substituent in this series. The synthesis of **22** began with a Horner–Wadsworth–Emmons reaction between piperidone **14** and diethyl(cyanomethyl)phosphonate (Scheme 4). Hydrogenation of the resulting cyanoacrylate then provided intermediate **19**, which was carried on to **22** using a sequence of reactions analogous to those used for the synthesis of **18** from **14**.

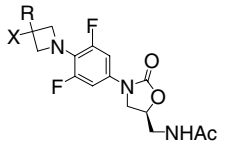
The 4-alkyl analog **27** was prepared using a different synthetic route starting with the commercially available amino ester **23** (Scheme 5). Following Boc-protection of the amine function in **23**, the 4-methyl substituent was introduced by reaction with LDA and iodomethane. Removal of the Boc group (affording **24**) was followed by coupling to 3,4,5-trifluoronitrobenzene as before. The methyl ester was then reduced and the resulting alcohol converted to the mesylate **25**. The oxazolidinone ring was installed, and in a final step, the mesylate group was displaced with cyanide to furnish the desired 4-methyl analog **27**.

We evaluated the antibacterial activities of the new oxazolidinone analogs by determining MIC_{90} values against 11 strains each of *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* using standard broth microdilution assay methods.[9] An in vitro *Escherichia coli* transcription and translation (TnT) assay[10] was used to provide a measure of intrinsic binding to bacterial ribosomes. Inhibition of MPS was assessed using an assay that measures [^{35}S]methionine incorporation into mitochondrial proteins.[4]

Table 1 summarizes the data for azetidine analogs **6a**–**c** and the corresponding 3-alkyl substituted derivatives **11a**–**c**, **12**, and **13**. These analogs displayed antibacterial potencies similar and in some cases superior to those of linezolid. The hydroxy-, methoxy-, and fluoro-azetidine analogs **6a**–**c** had identical MIC_{90} values against the three bacterial organisms examined, and were twofold more potent than linezolid against *S. aureus* and *E. faecalis* strains. The eight azetidine analogs in Table 1 exhibited very similar activities in the TnT assay, suggesting comparable intrinsic binding affinities to the ribosome target. Antibacterial activities were minimally impacted by the introduction of a 3-alkyl substituent in the azetidine ring; the alkylated analogs were equipotent or at most one dilution less potent than the corresponding unsubstituted analogs (cf. **6a**–**c** vs. **11a**–**c**).

The MPS inhibitory activity of analogs **6a** (hydroxy) and **6c** (fluoro) was within the range of values obtained for linezolid while the methoxy analog **6b** was a somewhat more potent inhibitor of MPS. Interestingly, the introduction of a 3-methyl substituent had a significant effect on MPS inhibition. Hence, the 3-methyl analogs **11a** and **11c** had more than threefold higher IC_{50} values than the *des*-methyl comparators **6a** and **6c**. A more dramatic effect was observed in the case of methoxy analogs **6b** and **11b**, with 3-methyl-3-methoxy analog **11b** exhibiting a 15- to 30-fold reduction in MPS inhibition. The 3-ethyl and 3-trifluoromethyl analogs (**12** and **13**) were prepared to probe steric and electronic effects of the 3-alkyl substituent. Surprisingly, neither of these analogs differed significantly from the parent *des*-alkyl analog **6a** in the MPS assay. Hence, from this limited survey of 3-alkyl substituents, it appears that a simple methyl group has the most favorable attenuating effect on MPS inhibition.

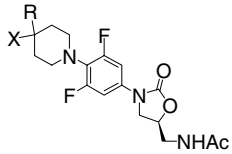
Table 1. Mitochondrial protein synthesis inhibition (IC_{50}, μM), *E. coli* in vitro transcription and translation assay (IC_{50}, μM), and antimicrobial activity (MIC_{90}, μg/mL) of azetidine-containing oxazolidinones



Compound	R	X	MPS IC_{50} (μM)	EC TnT IC_{50} (μM)	MIC_{90} S.a.	MIC_{90} S.p.	MIC_{90} E.f.
1	—	—	11–26		4	1	4
6a	H	OH	18	1.9	2	1	2
11a	Me	OH	68	3.4	4	1	4
12	Et	OH	15–34	2.7	4	2	2
13	CF_3	OH	19	2.4	4	2	2
6b	H	OMe	4	2.6	2	1	2
11b	Me	OMe	65–112	3.7	4	1	4
6c	H	F	13	1.4	2	1	2
11c	Me	F	42	2.3	4	1	2

S.a., *Staphylococcus aureus*; S.p., *Streptococcus pneumoniae*; E.f. *Enterococcus faecalis*.

Table 2. Mitochondrial protein synthesis inhibition (IC_{50}, μM), *E. coli in vitro* transcription and translation assay (IC_{50}, μM), and antimicrobial activity (MIC_{90}, μg/mL) of piperidine-containing oxazolidinones



Compound	R	X	MPS IC_{50} (μM)	EC TnT IC_{50} (μM)	MIC_{90} S.a.	MIC_{90} S.p.	MIC_{90} E.f.
1	—	—	11–26		4	1	4
18a	H	OH	11	1.7	2	1	2
18b	Me	OH	34–56	2.1	4	1	2
18c	Et	OH	13–23	2.1	4	1	2
22	H	CH_2CN	6	1.6	2	1	1
27	Me	CH_2CN	26	nt	2	1	1

S.a., *Staphylococcus aureus*; S.p., *Streptococcus pneumoniae*; E.f., *Enterococcus faecalis*; nt, not tested.

The antibacterial, TnT, and MPS inhibitory activities of *piperidine*-containing oxazolidinones are presented in Table 2. The larger piperidine ring places the distal substituents (i.e., R and X) further from the aromatic B-ring than is the case in the azetidine series. For this reason, it was unclear whether the effects observed for the 3-methyl azetidine analogs would hold also for 4-methyl piperidine analogs. In fact, a similar trend was observed. All of the piperidine analogs exhibited comparable IC_{50} values in the TnT assay. Likewise, antibacterial activities (MIC_{90}) were minimally affected by the introduction of 4-methyl or 4-ethyl substituents (cf. **18a** vs. **18b**–**c** and **22** vs. **27**). In the MPS assay, however, 4-methyl analogs **18b** and **27** exhibited between three and fivefold lower levels of inhibition. The effect was again most significant with a methyl substituent; the 4-*ethyl* analog **18c** was only marginally less active than *des*-alkyl analog **18a**. To further establish the significance of these effects, we evaluated analogs such as **11a** and **18b** in two different cell proliferation assays (data not shown). The trends observed in the MPS assay were also apparent in the cell-based assays, with methyl substituents conferring a favorable effect.

The data presented here suggest that subtle structural modifications far removed from the oxazolidinone pharmacophore can significantly impact MPS inhibition in oxazolidinone antibacterials. Importantly, attenuation of MPS inhibition can be achieved with little or no impact on the desired (antibacterial) bioactivity. At least four compounds presented in the current work exhibit significantly reduced MPS inhibition as compared to linezolid. These findings are suggestive of structural differences between the bacterial and mitochondrial ribosomes that might be exploited to design safer and more selective oxazolidinones.

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; (e) Renslo, A. R.; Luehr, G. W.; Gordeev, M. F. *Bioorg. Med. Chem.* **2006**, *14*, 4227.

2. (a) Shinabarger, D. *Exp. 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*, 2197.
3. Sciotti, R. J.; Pliushev, M.; Wiedeman, P. E.; Balli, D.; Flamm, R.; Nilius, A. M.; Marsh, K.; Stolarik, D.; Jolly, R.; Ulrich, R.; Djuric, S. W. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2121.
4. (a) Nagiec, E. E.; Wu, L.; Swaney, S. M.; Chosay, J. G.; Ross, D. E.; Brieland, J. K.; Leach, K. L. *Antimicrob. Agents Chemother.* **2005**, *49*, 3896; (b) McKee, E. E.; Ferguson, M.; Bentley, A. T.; Marks, T. A. *Antimicrob. Agents Chemother.* **2006**, *50*, 2042.
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. Higgins, R. H. *J. Heterocycl. Chem.* **1987**, 1489.
7. Qiu, X.; Qing, F. *J. Org. Chem.* **2002**, *67*, 7162.
8. Chugh, Y.; Shetty, N.; Deshpande, P. K.; Sindkhedkar, M. D.; Jafri, M. A.; Yeole, R. D.; Shukla, M. C.; Gupte, S. V.; Patel, M.; Desouza, N. J. International PCT Publication WO 2004/007488, 2004.
9. NCCLS (National Committee for Clinical Laboratory Standards). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard. NCCLS Document M7-A5, 5th ed.; Vol. 20, No 2.2000.
10. (a) Murray, R. W.; Melchior, E. P.; Hagadorn, J. C.; Marotti, K. R. *Antimicrob. Agents Chemother.* **2001**, *45*, 1900; (b) Zubay, G. *Annu. Rev. Genet.* **1973**, *7*, 267.