Bioorganic & Medicinal Chemistry Letters 19 (2009) 170–174

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pleuromutilin derivatives having a purine ring. Part 2: Influence of the central spacer on the antibacterial activity against Gram-positive pathogens

Yoshimi Hirokawa [a,†], Hironori Kinoshita [a,*], Tomoyuki Tanaka [a], Takanori Nakamura [a], Koichi Fujimoto [b], Shigeki Kashimoto [b], Tsuyoshi Kojima [b], Shiro Kato [a]

[a] Chemistry Research Laboratories, Dainippon Sumitomo Pharma Co., Ltd, Enoki 33-94, Suita 564-0053, Japan
[b] Pharmacology Research Laboratories, Dainippon Sumitomo Pharma Co., Ltd, 3-1-98 Kasugade Naka, Konohana-ku, Osaka 554-0022, Japan

ARTICLE INFO

Article history:
Received 26 August 2008
Revised 23 October 2008
Accepted 28 October 2008
Available online 5 November 2008

Keywords:
Pleuromutilin
Gram-positive bacteria
MRSA
PRSP
VRE
Purine ring
Spacer
MIC
Sulfide
Piperazine ring

ABSTRACT

Structural modification of the 4-piperidinethio moiety, as a spacer of the first pleuromutilin analogues **2A** and **2B** having a purine ring, led to discovery of the novel pleuromutilin derivatives **14B** and **17B**. These compounds with good solubility in water showed promising in vitro antibacterial activity against various Gram-positive bacteria including MRSA, PRSP, and VRE and have potent in vivo efficacy.

The search for new antibacterial agents is important due to increasing multi-drug resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *enterococci* (VRE).[1–3] We have focused our attention on the natural product pleuromutilin[4–7] (**1**) with unique mechanism of action and consequently no cross-resistance to the marketed antibacterial agents. In part 1 of this series,[8] we identified the structurally novel pleuromutilin analogues **2A**[9] and **2B** (Table 1) having a purine ring as a polar and water solubilizing group. These compounds, with good solubility in water, displayed potent in vitro antibacterial activity against various Gram-positive bacteria including MRSA, PRSP, and VRE. Furthermore, their in vivo efficacy against *S. aureus* Smith systemic infection model in mice was essentially equipotent to that of the marketed antibiotic vancomycin. As an extension to that study, we have directed our efforts to understanding the influence of the 4-piperidinethio moiety as a central spacer in **2A** and **2B** on the in vitro and in vivo antibacterial activities. The present communication deals with the structure-activity relationships (SARs) of a novel series of pleuromutilin analogues **3A–17B** with several spacer moieties as shown in Table 1.

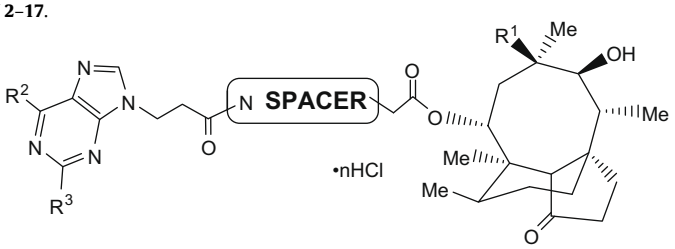
The synthesis of several nitrogen-containing heteroalicycles and the alkylene chains **26a–e**, **26g,h**, **27c**, and **28c** (the optically active pyrrolidine; *R* and *S* forms, respectively), and **29f** having a sulfide linkage is illustrated in Scheme 1. The thiol derivatives were prepared via the thioesters **21a–h** according to a method described in the patent literature[9,10] but using appropriate nitrogen-containing heteroalicycles (**20a–f**) and alkylene chains (**20g,h**), all of which are either commercially available or prepared according to a published procedure.[11] Reaction of the resultant thiol derivatives, without isolation, with the mutilin 14-tosyloxyacetate **18**[12] or the 19,20-dihydromutilin 14-tosyloxyacetate **19**, which was prepared from the 19,20-dihydromutilin[13] using a method described in the literature[12] for **18**, followed by deprotection of the corresponding *N*-Boc derivatives **22a–e**, **22g,h**, **23c**, **24c**, and **25f** gave the desired sulfur-linked derivatives **26a–e**, **26g,h**, **27c**, **28c**, and **29f** as a hydrochloride (see Fig. 1).

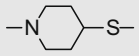
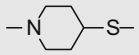
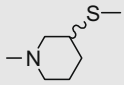
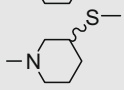
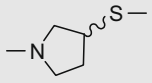
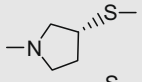
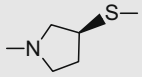
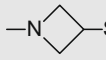
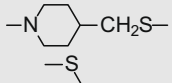
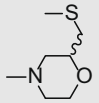
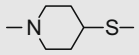
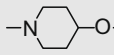
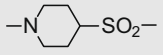
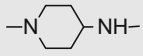
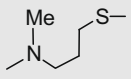
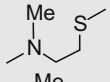
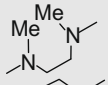
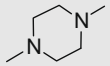
The corresponding ether-linked analogue **32** of **29f** having a sulfide linkage was prepared as follows. O-alkylation of 1-benzyloxycarbonyl-4-piperidinol, which was obtained by reaction of 4-piperidinol with benzyl chloroformate, with ethyl bromoacetate

* Corresponding author. Tel.: +81 6 6337 5906; fax: +81 6 6337 6010.
E-mail address: hironori-kinoshita@ds-pharma.co.jp (H. Kinoshita).
† Present address: Faculty of Pharmacy, Osaka Ohtani University, 3-11-1 Nishikiori-kita, Tondabayashi, Osaka 584-8540, Japan.

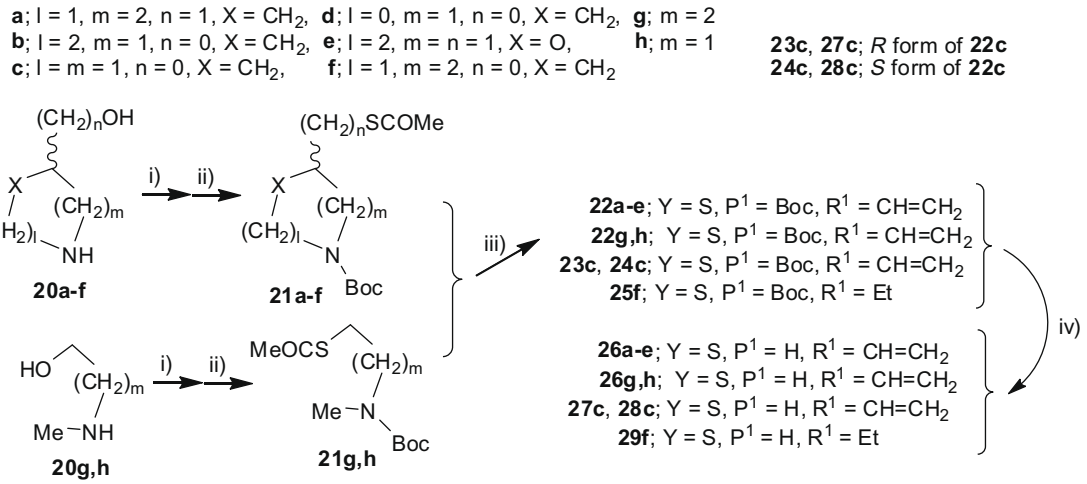
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.123

Table 1
In vitro and in vivo antibacterial activities of **2**–**17**.



Compound[a]	R^1	NSPACER	*n*	MIC[b] (μg/mL)								*MSSA*[c]
				MSSA[c]	*MRSA*[d]	*PSSP*[e]	*PRSP*[f]	*S. p.*[g]	*VRE*[h]	*M. c.*[i]	*H. j.*[j]	ED_{50}[k] (mg/kg, iv)
2A	Vinyl		1	0.25	0.5	0.063	0.063	0.032	0.125	0.25	1	1.86
2B	Vinyl		1	0.063	0.125	0.016	0.032	0.016	0.032	0.032	1	1.47
3A	Vinyl		1	0.5	1	0.032	0.032	0.016	0.25	0.125	2	3.13
3B	Vinyl		1	0.25	0.5	0.016	0.016	0.008	0.063	0.063	2	2.02
4A	Vinyl		1	0.25	1	0.063	0.125	≦0.004	0.25	0.125	1	2.61
5A	Vinyl		1	0.05	2	0.063	0.125	0.032	0.5	0.25	2	1.28
6A	Vinyl		1	0.5	1	0.063	0.125	0.016	0.25	0.25	2	3.16
7A	Vinyl		1	0.5	2	0.125	0.25	≦0.004	0.5	0.125	2	4.07
8A	Vinyl		1	0.25	0.5	0.032	0.032	0.016	0.063	0.125	4	2.77
9A	Vinyl		1	1	2	0.063	0.125	0.032	0.5	0.25	8	4.81
10A	Ethyl		1	0.25	0.25	0.063	0.063	0.032	0.125	0.125	1	1.66
11B	Ethyl		1	0.25	0.5	0.032	0.063	0.032	0.125	0.125	4	2.04
12B	Ethyl		1	0.5	2	0.063	0.125	0.032	1	0.125	4	1.79
13B	Vinyl		1	0.5	2	0.125	0.125	0.063	0.5	0.25	2	1.75
14B	Vinyl		1	0.125	0.25	0.016	0.016	0.008	0.063	0.063	1	0.86
15B	Vinyl		1	0.25	0.5	0.016	0.016	≦0.004	0.063	0.125	2	1.76
16B	Vinyl		2	1	4	0.063	0.125	0.063	1	0.25	8	>3.13
17B	Vinyl		2	0.25	1	0.063	0.063	0.032	0.5	0.25	4	0.80

[a] A; R^2 = 1-piperazinyl, $R^3 = NH_2$, B; R^2 = (±)-3-amino-1-pyrrolidinyl, R^3 = H.
[b] Minimum inhibitory concentration (MIC): lowest concentration of compound that inhibits visible growth of the organism.
[c] MSSA, methicillin-susceptible *S. aureus* Smith.
[d] MRSA, *S. aureus* KMP9.
[e] Penicillin-susceptible *S. pneumoniae* ATCC49619.
[f] *S. pneumoniae* KT2524.
[g] *S. pyogenes* ATCC12344.
[h] VRE, *E. faecium* KU1778.
[i] *M. catarrhalis* K1209.
[j] *H. influenzae* TH13.
[k] The efficacy criterion, ED_{50}, was calculated as the dose at which mice survival rate was 50%. Mice were inoculated with each organism intraperitoneally. Medication was given intravenously once, 1 h after inoculation.



Scheme 1. Reagents and conditions: (i) Boc_2O, $CHCl_3$, rt; (ii) ($^iPrCO_2N{=}$)$_2$, Ph_3P, THF, −20 °C, 0.5 h then MeCOSH, −10 °C to rt, 0.5 h; (iii) ^{t}BuOK, MeOH, reflux, 2 h then **18** or **19**, 0 °C to rt, 18 h; (iv) 30% HCl/EtOH, rt, 2 h.

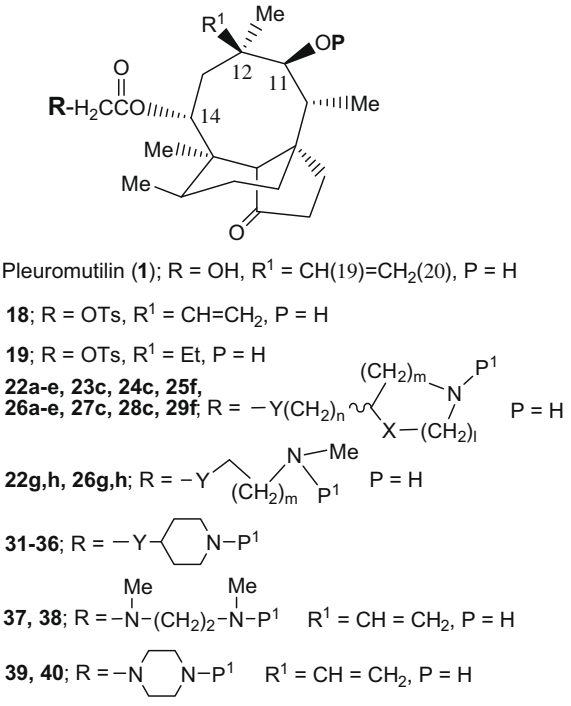


Figure 1. Structure of pleuromutilin derivatives.

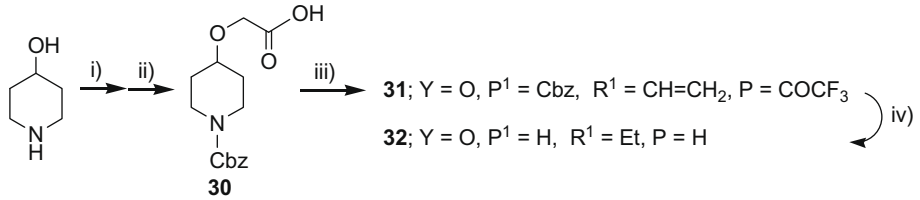
in the presence of NaH, followed by successive alkaline hydrolysis produced the glycolic acid **30**. Reaction of **30** with thionyl chloride gave the corresponding acid chloride, which was treated with the trifluroacetic acid mutilin 11-ester[14] to afford the mutilin 11,14-diesters **31**. Deprotection of trifluoroacetyl group at the 11-position in **31** by aqueous ammonia and successive hydrogenation with palladium on carbon produced the 19,20-dihydromutilin derivative **32** (Scheme 2).

The SO_2-linked derivative **34** was prepared as illustrated in Scheme 3. Oxidation of **25f** by *m*-chloroperbenzoic acid (MCPBA), followed by Boc-deprotection of the sulfone analogue **33** led to the desired compound **34** as hydrochloride in an excellent yield.

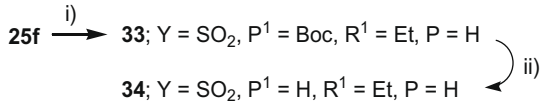
N-alkylation of 4-amino-1-(*tert*-butoxycarbonyl)piperidine, [*N*-(*tert*-butoxycarbonyl)-*N*,*N*′-dimethyl]ethylenediamine, and *tert*-butyl 1-piperazinecarboxylate with **18** and successive *N*-Boc-deprotection of the resultants **35**, **37**, and **39** gave the corresponding N-linked derivatives **36**, **38**, and **40**, respectively, as di-hydrochlorides (Schemes 4 and 5).

The pleuromutilin analogues **3A–17B** shown in Table 1 were prepared as illustrated in Scheme 6. Condensation of the 3-(purin-9-yl)propionic acids[8] **41** and **42** with several amines **26a–e, 26g,h, 27c, 28c, 29f, 32, 34, 36, 38**, and **40** in the presence of benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate as a coupling agent and Et_3N, followed by successive Boc-deprotection led to the target compounds **3A**–**17B** in moderate to good yields. The chemical structures of **3A**–**17B** were confirmed by ^{1}H NMR and mass spectra and the purity was demonstrated by HPLC analysis. Compounds **3A**–**17B**, which were obtained as mono- or di-hydrochlorides, showed good solubility in water (∼50 mg/mL).

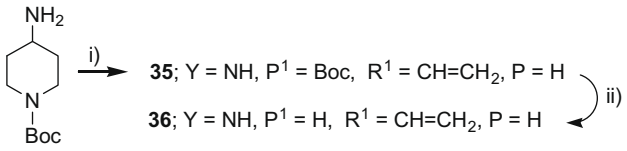
The pleuromutilin analogues **3A**–**17B** shown in Table 1 were tested for in vitro antibacterial activity[15] against well characterized drug-susceptible and -resistant Gram-positive and -negative bacteria including methicillin-susceptible *S. aureus* (MSSA), MRSA, penicillin-susceptible *S. pneumoniae* (PSSP), PRSP, VRE, *Streptococcus*



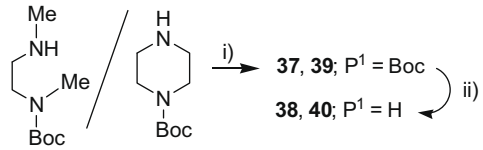
Scheme 2. Reagents and conditions: (i) $ClCO_2CH_2Ph$, Et_3N, CH_2Cl_2, rt, 4 h; (ii) $BrCH_2CO_2Et$, DMF, NaH, rt to 50 °C, 3 h then 2 N NaOH/MeOH, reflux, 3 h, 23%; (iii) $SOCl_2$, $CHCl_3$, reflux, 2 h then trifluoroacetic acid mutilin 11-ester, DMAP, pyridine, reflux, 18 h, 20%; (iv) 28% NH_4OH, MeOH, rt, 3 h, 98% then 10% Pd/C, H_2, EtOH, rt, quant.



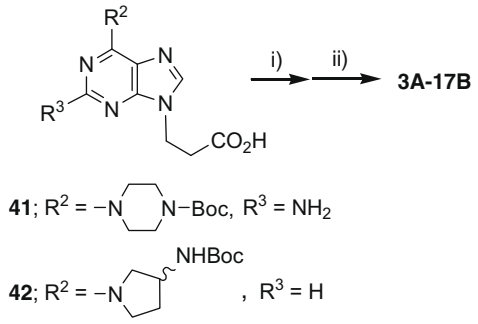
Scheme 3. Reagents and conditions: (i) MCPBA, CH_2Cl_2, rt, 24 h, 90%; (ii) 30% HCl/EtOH, rt, 2 h, quant.



Scheme 4. Reagents and conditions: (i) **18**, K_2CO_3, NaI, MeCN, reflux, 16 h, 76%; (ii) 30% HCl/EtOH, rt, 2 h, quant.



Scheme 5. Reagents and conditions: (i) **18**, K_2CO_3, NaI, MeCN, reflux, 16 h, (**37**; 97%, **39**; 95%); (ii) 30% HCl/EtOH, rt, 2 h, quant.



Scheme 6. Reagents and conditions: (i) **26a**–**e**, **26g**,**h**, **27c**, **28c**, **29f**, **32**, **34**, **36**, **38**, and **40**, benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate, Et_3N, DMF, rt, 2 h; (ii) 30% HCl/EtOH, rt, 2 h.

pyogenes, *Moraxella catarrhalis*, and *Haemophilus influenzae*, all of which are common serious respiratory tract pathogens. Furthermore, **3A**–**17B** were evaluated for in vivo efficacy against *S. aureus* Smith (MSSA) systemic infection model in mice. For comparison, the previously reported pleuromutilin analogues[8,9] **2A** and **2B** were used. All compounds were found to possess potent in vitro antibacterial activity against all strains (Table 1). In general, PSSP, PRSP, and *S. pyogenes* strains were highly susceptible, while MRSA and *H. influenzae* strains had somewhat higher MIC values. For these derivatives, no significant differences in minimum inhibitory concentration (MIC) for drug-susceptible versus -resistant strains was observed.

Influence of the sulfide linkage of **2A** and **2B** on the antibacterial activity was first examined. Compounds **3A** and **3B**, bearing the 3-piperidinylthio ring as a central spacer, while keeping the mutilin framework with its 2-amino-6-(piperazin-1-yl)purine (Type A) or 6-[(±)-3-aminopyrrolidin-1-yl]purine (Type B) ring intact, showed in vitro antibacterial activity practically comparable to that of **2A** and **2B** against all strains, but no improvement in in vivo efficacy. It was subsequently found that the direction of the connecting vector on the piperidine ring is important, since in vivo efficacy was reduced by using the (±)-3-piperidinythio spacer (**3A** and **3B**). The in vivo efficacy of Type B as in **2B** and **3B** was more potent than that of Type A as in **2A** and **3A**. Compound **4A** bearing the pyrrolidinyl spacer exhibited in vitro antibacterial activity comparable to that of **2A**, while its in vivo efficacy was lower than that of **2A**. The in vitro antibacterial activity of the optically active derivatives **5A** and **6A** of **4A** was very similar to that of **4A**. On the other hand, *R* configuration at the 3-position on the pyrrolidine ring, such as in **5A**, increased the in vivo efficacy, while the *S* configuration (**6A**) showed slightly worse efficacy. Although replacement of the piperidinyl spacer of **2A** with an azetidinyl or a 2-morpholinyl spacer (yielding **7A** or **9A**, respectively) caused a significant or slight decrease in in vitro and in vivo activities, the in vitro antibacterial activity of **8A**, containing the 4-piperidinylmethyl spacer, was essentially the same as that of **2A**. Compound **8A** in vivo efficacy, however, was weaker than that of **2A**.

As described in a previous paper,[16] hydrogenation of the vinyl group in the 12-position on the mutilin ring of **2A** (giving an ethyl group as in **10A**) did not negatively affect the in vitro or in vivo activity when compared to that of **2A**. Replacement of the sulfur atom in **2B** by an oxygen atom, sulfone linkage, or nitrogen atom (yielding **11B**, **12B**, or **13B**, respectively) did not, in general, increase the in vitro or in vivo activity. Furthermore, removal of the methylene or ethylene moiety from the piperidine ring of **2B** provided the 3-methylaminopropylsufide **14B** and 2-methylaminoethylsulfide **15B**, both of which exhibited very good in vitro antibacterial activity against all strains with MIC values between 2 and $\leqslant$0.004 μg/mL. In particular, compound **14B** showed the most favorable in vitro and in vivo activities in this series, while the in vivo efficacy of **15B** was less than that of **2B**. Replacement of the sulfur atom of **15B** with a methylamino group (giving **16B**) considerably reduced both the vitro and in vivo activities. A similar finding has been reported with the addition of a basic nitrogen in the spacer.[16] Quite surprisingly, formation of the piperazine ring from **16B** as in **17B** led to a considerable increase in both the in vitro and in vivo activities. Compound **17B** as well as the 3-methylaminopropylsulfide **14B** conferred the highest in vivo efficacy. The excellent in vivo efficacy of **14B** and **17B**, which also have good solubility in water, may reflect improved pharmacokinetics and ADME properties compared to derivatives **2A** and **2B**. Further SARs of a novel series of pleuromutilin derivatives containing the piperazine ring as a central spacer will be reported in due course.

With the purpose of finding novel antibacterial agents for use in human, the new pleuromutilin analogues **2A** and **2B** with good solubility in water and excellent in vitro and in vivo activities were identified. Modification of the sulfur-linked piperidinyl spacer of **2A** and **2B** resulted in the discovery of the novel pleuromutilin derivatives **14B** and **17B** having the 3-methylaminopropylsulfide and the piperazine ring, respectively, as a central spacer. Both compounds showed much higher in vivo efficacy than the parent compound **2B** and appeared to have a well-balanced in vitro antibacterial activity profile against MRSA, PRSP, VRE, *S. pyogenes, M. catarrhalis*, and *H. influenzae*, all of which are known to cause respiratory tract infections. The promising pleuromutilin derivatives **14B** and **17B** may serve as useful lead compounds for the discovery of new antibiotics.

References and notes

1. Projan, S. J.; Youngman, P. J. *Curr. Opin. Microbiol.* **2002**, *5*, 463.
2. Livermore, D. M. *Clin. Infect. Dis.* **2003**, *36*, 1058.
3. Ohno, A. *Infect. Control* **2004**, *13*, 1012.
4. Hunt, E. *Drug Future* **2000**, *25*, 1163.
5. Anchel, M. *J. Biol. Chem.* **1952**, *199*, 133.
6. Arigoni, D. *Gazz. Chim. Ital.* **1962**, *92*, 884.
7. Birch, A. J.; Cameron, D. W.; Holzapfel, C. W.; Rihards, R. W. *Chem. Ind.* **1963**, 374.

8. Hirokawa, Y.; Kinoshita, H.; Tanaka, T.; Nakamura, T.; Fujimoto, K.; Kashimoto, S.; Kojima, T.; Kato, S. *BioMed. Chem. Lett.* **2008**, *18*, 3556.
9. Hirokawa, Y.; Kinoshita, H.; Tanaka, T.; Nakata, K.; Kitadai, N.; Fujimoto, K.; Kashimoto, S.; Kojima, T.; Kato, S. *J. Med. Chem.* **2008**, *51*, 1991.
10. WO 99/21855.
11. (a) Sum, F.-W.; Wong, V.; Han, S.; Largis, E.; Mulvey, R.; Tillett, J. *BioMed. Chem. Lett.* **2003**, *13*, 2191; (b) Kojima, T.; Niigata, K.; Fujikura, T.; Tachikawa, S.; Nozaki, Y.; Kagami, S. *Chem. Pharm. Bull.* **1985**, *33*, 3766.
12. (a) Egger, H.; Reinshagen, H. *J. Antibiotics* **1976**, *29*, 915; (b) Riedl, K. *J. Antibiotics* **1976**, *29*, 132.
13. (a) Birch, A. J.; Holzapfel, C. W.; Richards, R. W. *Tetrahedron* **1966**, (Suppl. 8), part II, 359.; (b) Berner, H.; Schulz, G.; Fischer, G. *Monat. Chem.* **1981**, *112*, 1441.
14. (a) WO 97/25309.; (b) WO-2000/73287.
15. *National Committee for Clinical Laboratory Standards 2000. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 5th ed.; Approved Standard M7 A5; National Committee for Clinical Laboratory Standards: Wayne, PA, 2000.
16. Egger, H.; Reinshagen, H. *J. Antibiotics* **1976**, *29*, 923.