Bioorganic & Medicinal Chemistry Letters 19 (2009) 175–179

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Pleuromutilin derivatives having a purine ring. Part 3: Synthesis and antibacterial activity of novel compounds possessing a piperazine ring spacer

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
Piperazine ring

ABSTRACT

SAR studies on the water-soluble thioether pleuromutilin analogue **6**, which has excellent in vitro and in vivo antibacterial activities, led to discovery of the novel pleuromutilin derivatives having a piperazine ring spacer. These derivatives displayed potent and well-balanced in vitro antibacterial activity against various drug-susceptible and -resistant Gram-positive bacteria. In particular, the promising pleuromutilin analogues **37** and **40** were found to exhibit strong in vivo efficacy against *Staphylococcus aureus* Smith.

The antibiotic pleuromutilin[1–4] (**1**), having an unusual fused 5-6-8 tricyclic diterpenoid structure, was first isolated in 1951 from two basidiomycete species and was characterized as a crystalline antibiotic with modest in vitro antibacterial activity against Gram-positive pathogens and mycoplasmas and weak in vivo efficacy.[5] Pleuromutilin (**1**) selectively inhibits bacterial protein synthesis through interaction with prokaryotic ribosomes, but has no effect on eukaryotic protein synthesis and does not bind to mammalian ribosomes.[6] To find a novel class of antibiotics with a new mechanism of action, a Sandoz group reported initial structure–activity relationship (SAR) studies on a number of semisynthetic pleuromutilin derivatives.[7–9] Further studies led to the development of tiamulin (**2**) and valnemulin (**3**) as therapeutic agents for veterinary use.[10] Chemical modifications of **1**, with the aim of producing an agent for use in human, resulted in the 1980s in the development of azamulin (**4**).[11] Although **4** showed good in vitro antibacterial activity, its oral bioavailability was severely limited by atrocious solubility in water.[12] Recently, researchers at GlaxoSmithKline identified the new pleuromutilin analogue retapamulin (**5**)[13] with excellent in vitro antibacterial activity, and **5** was approved in 2007 as a topical antimicrobial agent for treatment of human skin infections. From previous SAR studies on **1**, analogues in which the hydroxyl of the C14 glycolic ester group is replaced with a substituent containing the sulfide linkage show potent in vitro antibacterial activity, but suffer from being rapidly and extensively metabolized in vivo because of their strong hydrophobic nature.[14] To overcome this problem, we designed structurally novel pleuromutilin derivatives having a purine ring as a polar and water solubilizing group and identified the novel pleuromutilin analogue **6** with potent in vitro and in vivo antibacterial activities, good solubility in water, and appreciable metabolic stability. Further structural modification of the 4-piperidinethio moiety as a central spacer of the first generation **6** led to discovery of the promising pleuromutilin analogue **7** having a piperazine ring spacer (see Fig. 1) refer to part 2[15] in this series. Like **6**, the originally identified **7** displayed excellent in vitro antibacterial activity against a number of Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *enterococci* (VRE). Furthermore, its in vivo efficacy against *S. aureus* Smith systemic infection model in mice was essentially equivalent to that of the marketed antibiotic vancomycin (VCM) (Table 1). The microbiological profile observed with the prototype

* 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 Nishikiorikita, Tondabayashi, Osaka 584-8540, Japan.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.127

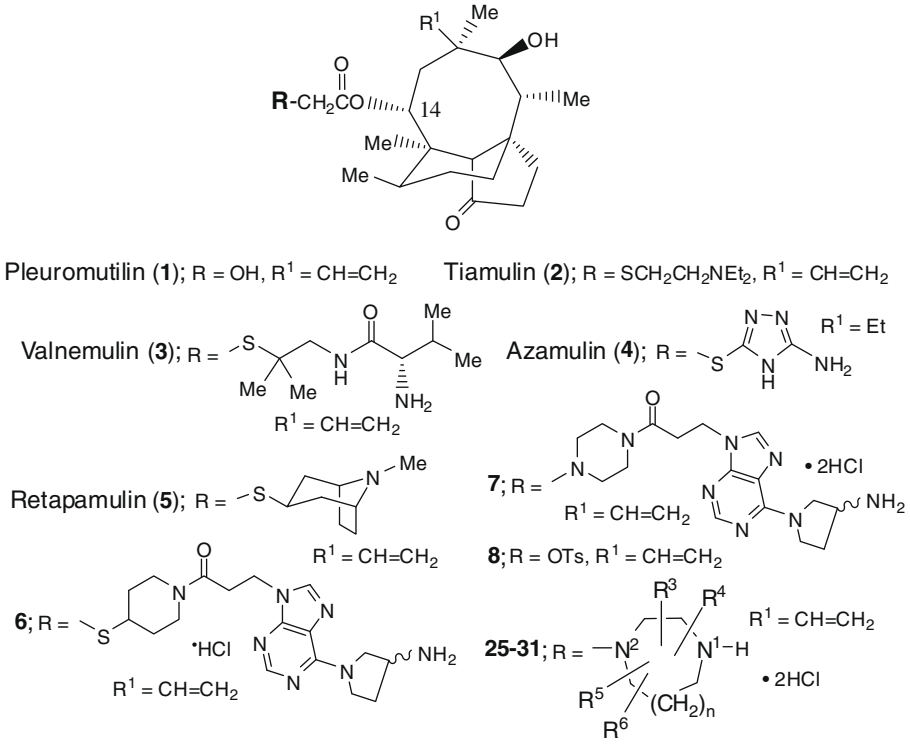


Figure 1. Structure of pleuromutilin derivatives.

7 is of clear interest, showing potent antibacterial activity against relevant bacterial strains in particular *Staphylococci* and *Streptococci*, with the exception of a borderline activity against *Haemophilus influenzae*. In this letter, we describe the synthesis and in vitro and in vivo antibacterial activities of novel pleuromutilin derivatives having a piperazine ring spacer.

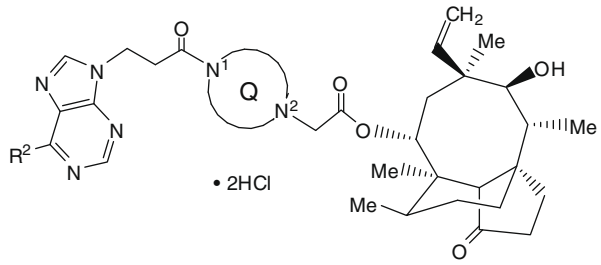
The new purine-propionic acids **14–17** with (±)-3-[*N*-(*tert*-butoxycarbonyl)-*N*-ethyl]amino, (*R*)- and (*S*)-3-[*N*-(*tert*-butoxycarbonyl)-*N*-methyl]aminomethyl, and (±)-3-dimethylaminopyrrolidines and 4-(*tert*-butoxycarbonyl)aminomethylpiperidine at the 6-position of the purine ring, except the previously described 3-(6-substituted purin-9-yl)propionic acids,[16] were prepared by the method described in a previous issue of this letter[16] (Scheme 1). In brief, reaction of 6-chloropurine **9** with the corresponding pyrrolidine and piperidine derivatives, followed by alkylation of the resultants **10–13** with ethyl 3-bromopropionate or ethyl acrylate gave the desired esters. Alkaline hydrolysis of the ethyl esters produced **14–17**.

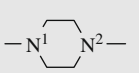
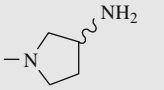
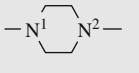
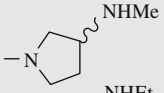
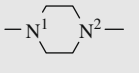
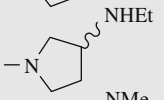
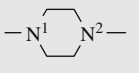
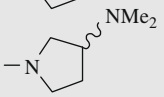
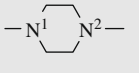
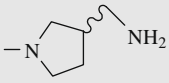
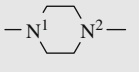
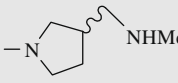
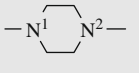
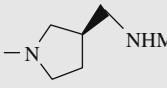
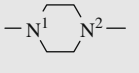
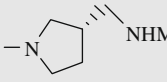
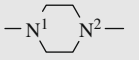
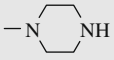
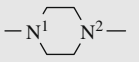
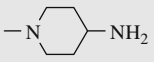
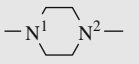
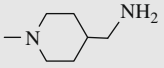
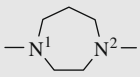
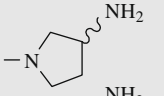
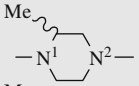
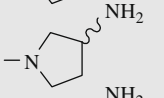
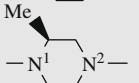
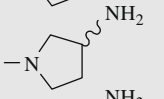
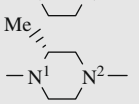
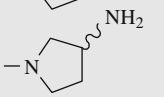
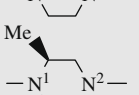
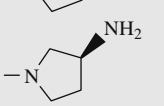
The piperazine and hexahydro-1,4-diazepine derivatives **25–31** (see Fig. 1) were prepared as illustrated in Scheme 2. Reaction of the commercially available piperazines **18–22**, $\mathbf{18}_R$, and $\mathbf{18}_S$, the *N*-Boc-protected hexahydro-1,4-diazepine **23**, and the *N*-Boc-protected piperazines **24**, $\mathbf{24}_R$, and $\mathbf{24}_S$ with the mutilin 14-tosyloxyacetate **8**[7,8] (see Fig. 1) followed by successive treatment with HCl afforded the desired **25–31** as di-hydrochlorides.

The pleuromutilin analogues **32–57** shown in Table 1 were prepared as follows. Condensation of the 3-(6-substituted purin-9-yl)propionic acids including **14–17** with the known pleuromutilin analogues[16] having a piperazine ring and **25–31** in the presence of benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate as a coupling agent and Et_3N, and successive acid treatment gave the desired compounds **32–57** as di-hydrochlorides in moderate to good yields. The chemical structures of all pleuromutilin analogues obtained were confirmed by ^1H NMR and mass spectra and the purity was demonstrated by HPLC analysis. The pleuromutilin derivatives **32–57** formed as di-hydrochlorides showed good solubility in water (~50 mg/mL). Using standard broth microdilution assay method,[17] the novel pleuromutilin derivatives **32–57** were tested 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 pyogenes*, *Moraxella catarrhalis*, and *H. influenzae*, all of which are common serious respiratory tract pathogen. In addition, compounds minimum inhibitory concentration (MIC) values were compared with those of the previously reported pleuromutilin analogue **7**[15] and VCM. The pleuromutilin derivatives **32–57**, except **53** and the reference compounds, displayed good to excellent in vivo efficacy against *S. aureus* Smith (MSSA) systemic infection model in mice. The lead compound **7** exhibited potent in vitro antibacterial activity comparable to that of VCM and had an interesting profile with good balanced in vitro activity against Gram-positive and -negative pathogens, except for a borderline activity against *H. influenzae*. Additionally, **7** revealed almost the same in vivo efficacy as VCM. In general, all the newly prepared pleuromutilin derivatives having a piperazine ring spacer retained potent in vitro antibacterial activity with only slight differences. Interestingly, PSSP, PRSP, *S. pyogenes*, and *M. catarrhalis* strains were highly susceptible, while *H. influenzae* strain exhibited somewhat higher MIC value. Lead optimization, aimed at improving the potency of **7**, proceeded stepwise.

Influence of the 6-substituent on the purine ring was first examined. The 6-(3-methylamino, 3-ethylamino, and 3-dimethylaminopyrrolin-1-yl)purine analogues **32–34** had essentially the same in vitro antibacterial activity as **7**, which bears a 3-aminopyrrolidine ring, although their in vivo efficacy was lower than that of **7**. Replacement of the amino group of **7** with an aminomethyl or a methylaminomethyl substituent (yielding **35** or **36**, respectively) resulted in a slight decrease in in vitro and in vivo antibacterial activities. These results indicate that the in vivo efficacy is highly sensitive to small structural changes at the 3-position of the pyrrolidino ring. Compounds in vivo efficacy following substitution at the 3-pyrrolidine ring decreased generally in this order NH_2

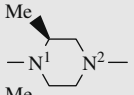
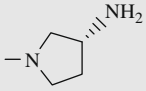
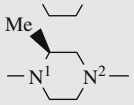
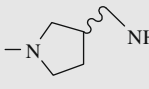
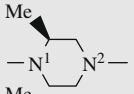
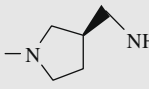
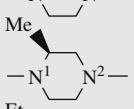
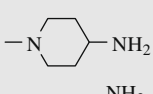
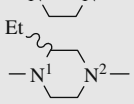
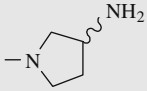
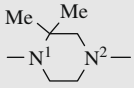
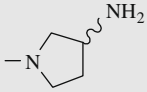
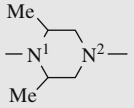
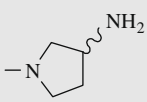
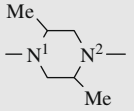
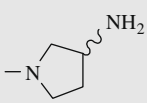
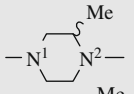
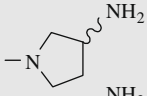
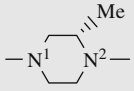
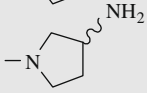
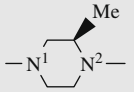
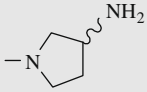
Table 1
In vitro and in vivo antibacterial activities of **7** and **32**–**57**.



Compound	Q	R^2	MIC[a] (μg/mL)								MSSA[b] ED_{50}[j] (mg/kg, iv)
			MSSA[b]	*MRSA*[c]	*PSSP*[d]	*PRSP*[e]	*S. p.*[f]	*VRE*[g]	*M. c.*[h]	*H. i.*[i]	
7			0.25	1	0.063	0.063	0.032	0.5	0.25	4	0.80
32			0.25	1	0.063	0.125	0.032	0.25	0.25	4	1.30
33			0.25	0.5	0.125	0.125	0.063	0.25	0.125	4	2.21
34			0.25	0.25	0.125	0.063	0.063	0.125	0.125	4	2.94
35			0.25	2	0.063	0.125	0.032	1	0.5	4	1.21
36			0.25	2	0.125	0.125	0.032	1	0.5	4	1.10
37			0.25	2	0.125	0.063	0.032	0.5	0.125	0.5	0.83
38			0.25	2	0.125	0.125	0.063	0.5	0.125	2	1.18
39			0.125	0.25	0.063	0.063	0.032	0.063	0.063	2	1.65
40			0.25	1	0.125	0.125	0.032	0.25	0.125	2	0.97
41			0.25	1	0.125	0.125	0.032	0.25	0.125	2	1.51
42			0.5	1	0.063	0.063	0.032	0.25	0.25	4	2.51
43			0.25	0.25	0.032	0.032	0.032	0.125	0.125	2	1.39
44			0.125	0.25	0.032	0.063	0.032	0.125	0.125	2	1.47
45			0.25	0.5	0.125	0.125	0.063	0.25	0.25	8	2.21
46			0.25	0.5	0.032	0.032	0.016	0.125	0.125	4	1.10

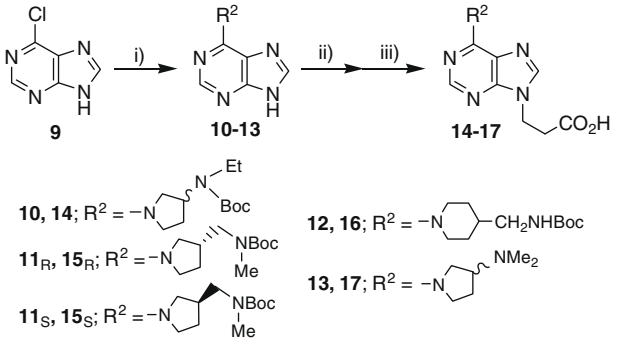
(*continued on next page*)

Table 1 (*continued*)

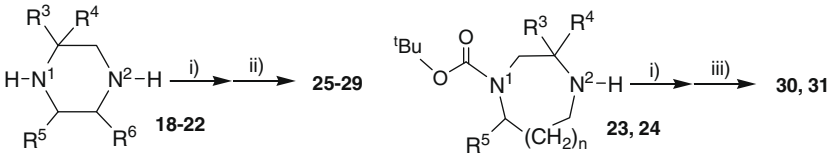
Compound	Q	R^2	MIC[a] (μg/mL)								MSSA[b] ED_{50}[j] (mg/kg, iv)
			MSSA[b]	*MRSA*[c]	*PSSP*[d]	*PRSP*[e]	*S. p.*[f]	*VRE*[g]	*M. c.*[h]	*H. i.*[i]	
47			0.125	0.25	0.032	0.032	0.016	0.125	0.125	2	1.56
48			0.125	1	0.063	0.032	0.063	0.25	0.125	2	1.20
49			0.25	0.5	0.063	0.063	0.032	0.25	0.125	2	1.10
50			0.25	0.5	0.063	0.063	0.032	0.125	0.125	4	1.66
51			0.25	0.25	0.063	0.063	0.032	0.125	0.125	4	2.95
52			0.125	0.125	0.032	0.032	0.016	0.063	0.032	4	2.32
53			0.125	0.125	0.016	0.016	0.016	0.063	0.063	4	>3.13
54			0.25	0.25	0.063	0.063	0.032	0.125	0.125	4	3.13
55			0.25	0.5	0.063	0.063	0.032	0.125	0.125	2	1.42
56			0.25	0.5	0.125	0.063	0.032	0.125	0.125	4	1.28
57			0.25	0.5	0.032	0.063	0.016	0.063	0.063	2	1.42
VCM			1	0.5	0.25	0.5	0.5	>128	64	>128	0.88

[a] Minimum inhibitory concentration (MIC): lowest concentration of compound that inhibits visible growth of the organism.
[b] MSSA, methicillin-susceptible *S. aureus* Smith.
[c] MRSA, *S. aureus* KMP9.
[d] *S. pneumoniae* ATCC49619.
[e] *S. pneumoniae* KT2524.
[f] *S. pyogenes* ATCC12344.
[g] VRE, *E. faecium* KU1778.
[h] *M. catarrhalis* K1209.
[i] *H. influenzae* TH13.
[j] 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.

(**7**) > CH_2NHMe (**36**) > CH_2NH_2 (**35**) > NHMe (**32**) >> NHEt (**33**) >> NMe_2 (**34**). As for **36**, both compounds **37** and **38** with the optically active 3-methylaminomethyl substituent showed approximately similar in vitro antibacterial activity to **36** having the racemic pyrrolidine ring. However, the in vivo efficacy of **37** was slightly better than that of the racemic compound **36** or **38**. The ED_{50} value (0.83 mg/kg) of **37** was essentially equivalent to that of **7** or VCM. On the other hand, replacement of the pyrrolidine ring in **7** with a piperazine (giving **39**), a 4-aminopiperidine (giving **40**), or a 4-aminomethylpiperidine (giving **41**) ring uniformly retained in vitro antibacterial activity against all strains, but the in vivo efficacy of **39** and **41** was not favorable. Compound **40** exhibited potent in vivo efficacy with ED_{50} value of 0.97 mg/kg, which is slightly less than that of **7**.



Scheme 1. Reagents and conditions: (i) R^2H, $^iPr_2NEt$, ^iPrOH, reflux, 15 h; (ii) $BrCH_2CH_2$-CO_2Et (CH_2=$CHCO_2Et$), K_2CO_3, DMF, 80 °C, 24 h; (iii) 2 N NaOH/MeOH, reflux, 3 h.



18, 25; R^3 = Me, R^4 = R^5 = R^6 = H
18_R, 25_R; R^3 = Me, R^4 = R^5 = R^6 = H
18_S, 25_S; R^3 = Me, R^4 = R^5 = R^6 = H
19, 26; R^3 = Et, R^4 = R^5 = R^6 = H
20, 27; R^3 = R^4 = Me, R^5 = R^6 = H
21, 28; R^3 = R^5 = Me, R^4 = R^6 = H
22, 29; R^3 = R^6 = Me, R^4 = R^5 = H

23, 30; R^3 = R^4 = R^5 = H, n = 1
24, 31; R^3 = Me, R^4 = R^5 = H, n = 0
24_R, 31_R; R^3 = Me, R^4 = R^5 = H, n = 0
24_S, 31_S; R^3 = Me, R^4 = R^5 = H, n = 0

Scheme 2. Reagents and conditions: (i) 8, K_2CO_3, NaI, MeCN, reflux, 16 h; (ii) 4 M HCl/AcOEt, rt, quant; (iii) 30% HCl/EtOH, rt, 2 h, quant.

Compound **42**, bearing the hexahydro-1,4-diazepine ring as a central spacer, showed in vitro antibacterial activity practically comparable to that of **7** against all strains, but its in vivo efficacy was not improved. Next, we studied the influence of a substituent in the piperazine ring on the antibacterial activity. Introduction of a methyl group into the piperazine-carbon atom (N^1) on the side of the purine ring of **7** (yielding the racemic compound **43**) caused a slight increase in in vitro antibacterial activity. Compound **43** appeared to have good in vitro profile against *Staphylococci* and *Streptococci*. However, the in vivo efficacy was less than that of **7**. Comparison of the antibacterial activity of **44**, having an (*S*)-methylpiperazine spacer, to that of **45**, having an (*R*)-methylpiperazine spacer, indicated that the former compound was more potent. However, **44** showed no improvement in in vivo efficacy when compared to **7**. As for **44** bearing the racemic 3-aminopyrrolidine ring, compounds **46** and **47** with the optically active 3-aminopyrrolidine ring were prepared to probe the steric effect of the amino group in **44**. Neither of these analogues differed significantly from the parent analogue **44** in vitro antibacterial activity, but the in vivo efficacy of **46** with (*S*)-3-aminopyrrolidine was more potent than that of **44**, and the ED_{50} value was 1.10 mg/kg. The in vivo efficacy of **47** having the 6-[(*R*)-3-aminopyrrolidin-1-yl]purine moiety was substantially weaker than that of **44**. On the other hand, the racemic and the (*R*)-(3-methylaminomethylpyrrolidin-1-yl)purine derivatives **48** and **49** showed potent in vivo efficacy, and the ED_{50} value of **49** was 1.10 mg/kg. Compound **50** bearing the 4-aminopiperidine ring showed an in vitro antibacterial activity practically comparable to that of **7** against all strains, but its in vivo efficacy was not improved.

Introduction of an ethyl group or two methyl groups into the piperazine ring spacer of **7** (yielding racemic compound **51** or **52–54**, respectively) significantly decreased the in vivo efficacy despite high in vitro antibacterial activity.

Finally, introduction of a methyl group into the piperazine-carbon atom on the side (N^2) of the mutilin ring of **7** gave the racemic compound **55**, whose in vitro and in vivo antibacterial activities was comparable to that of the corresponding counterpart **43**. Next, the stereochemistry at the methyl group on the pyrrolidine ring of **55** was examined. Compound **56** with (*S*)-methylpiperazine and the (*R*)-methylpiperazine analogue **57** showed no improvement in the in vitro and in vivo antibacterial activities compared with the racemic compound **55**. For methylpiperazine analogues, the position and stereochemistry of the methyl group in the piperazine ring spacer did not significantly influence the antibacterial activity.

In summary, further studies aimed at the development of pleuromutilin derivatives for use in human led to identification of a novel class of pleuromutilin analogues having a piperazine ring spacer. As a result of SAR, compounds **37** and **40** showing not only excellent in vitro antibacterial activity against MRSA, PRSP, VRE, *S. pyogenes*, and *M. catarrhalis*, but also potent in vivo efficacy were identified. The excellent in vivo efficacy of both compounds, which have good solubility in water, may reflect good pharmacokinetics and ADME properties. Therefore, this new class of pleuromutilin appears to have a very promising profile for the treatment of infections caused by respiratory pathogens.

References and notes

1. Hunt, E. *Drugs Future* **2000**, *25*, 1163.
2. Anchel, M. *J. Biol. Chem.* **1952**, *199*, 133.
3. Arigoni, D. *Gazz. Chim. Ital.* **1962**, *92*, 884.
4. Birch, A. J.; Cameron, D. W.; Holzapfel, C. W.; Richards, R. W. *Chem. Ind.* **1963**, 374.
5. Drews, J.; Georgopoulos, G.; Laber, G.; Schutze, E.; Unger, J. *Antimicrob. Agents Chemother.* **1975**, *7*, 507.
6. Hogenauer, G. *Eur. J. Biochem.* **1975**, *52*, 93.
7. Riedl, K. *J. Antibiot.* **1976**, *29*, 132.
8. Egger, H.; Reinshagen, H. *J. Antibiot.* **1976**, *29*, 915.
9. Egger, H.; Reinshagen, H. *J. Antibiot.* **1976**, *29*, 923.
10. Hannan, P. C. T.; Windsor, H. M.; Ripley, P. H. *Res. Vet. Sci.* **1997**, *63*, 157.
11. (a) Berner, H.; Fischer, G.; Hildebrandt, J. F.; Laber, G.; Vyplel, H. *Curr. Chemother. Immunother.* **1982**, *1*, 345; (b) Hildebrandt, J. F.; Berner, H.; Laber, G.; Turnowsky, F.; Schütze, E. *Curr. Chemother. Immunother.* **1982**, *1*, 346; (c) Wenzel, A. F.; Baumgartner, R.; Georgopoulos, A.; Hildebrandt, J. F.; Laber, G.; Schütze, E.; Walzl, H. *Curr. Chemother. Immunother.* **1982**, *1*, 347.
12. Ganzinger, U.; Stephen, A.; Obenaus, H.; Baumgartner, R.; Walzl, H.; Brüggemann, S.; Schmid, B.; Racine, R.; Schatz, F.; Haberl, H. *Abstract PS 4.6/2-14*, Presented at the 13th International Congress on Chemotherapy, Vienna, August 28–September 2, 1983.
13. Draghi, D.; Scangarella, N.; Shawar, R.; Sahm, D. *Abstract 1900*, Presented at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, December 16–19, 2005.
14. Nefzger, M.; Batting, F.; Czok, R. *Abstract PS 4.6/2-12*, Presented at the 13th International Congress on Chemotherapy, Vienna, August 28–September 2, 1983.
15. Hirokawa, Y.; Kinoshita, H.; Tanaka, T.; Nakamura, T.; Fujimoto, K.; Kashimoto, S.; Kojima, T.; Kato, S. BioMed. Chem. Lett., preceding communication in this issue.
16. Hirokawa, Y.; Kinoshita, H.; Tanaka, T.; Nakamura, T.; Fujimoto, K.; Kashimoto, S.; Kojima, T.; Kato, S. *BioMed. Chem. Lett.* **2008**, *18*, 3556.
17. *National Committee for Clinical Laboratory Standards. 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.*