Letters

Water-Soluble Pleuromutilin Derivative with Excellent in Vitro and in Vivo Antibacterial Activity against Gram-Positive Pathogens

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Abstract: Although earlier pleuromutilin analogues showed potent in vitro antibacterial activity against some Gram-positive pathogens, their in vivo efficacy was low because of insufficient pharmacokinetic properties. We designed novel thioether pleuromutilin derivatives having a purine ring as a polar and water solubilizing group and identified a promising pleuromutilin analogue **6** with good solubility in water (~50 mg/mL). Compound **6** exhibited excellent in vitro and in vivo antibacterial activity against some Gram-positive strains, including drug-resistant pathogens.

Antibacterial resistance by hospital-acquired Gram-positive bacterial pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *enterococci* (VRE), has become a serious medical problem.[1,2] Although antibacterial agents including linezolid, quinupristin/dalfopristin, and teicoplanin are now available for the treatment of infections caused by resistant bacteria, these agents produce undesirable side effects and their efficacy is restricted because of development of resistant mutants. It is therefore necessary to identify and develop new antibacterial agents with novel mechanisms of action. For one approach in this search for novel antibiotics agents, we reassessed an earlier antibiotic pleuromutilin (**1**) analogue, which was not successfully developed for human therapy at that time.[3]

The antibiotic **1**, having an unusual tricyclic diterpenoid structure, was first isolated in 1951 from two basidiomycetes species and was characterized as a crystalline antibiotic with modest in vitro activity against Gram-positive pathogens and mycoplasmas and weak in vivo activity.[4,5] Further studies have shown that this antibiotic selectively inhibits bacterial protein synthesis through interaction with prokaryotic ribosomes but has no effect on eukaryotic protein synthesis and did not bind to mammalian ribosomes.[6,7] During the 1970s and early 1980s, the Sandoz group prepared a number of semisynthetic pleuromutilin analogues and reported initial structure–activity relationships (SARs) that focused on variations in the C14 glycolic acid side chain.[8–10] As a result, tiamulin (**2**) was successfully developed as a therapeutic agent for veterinary use.[7] A second veterinary agent, valunemulin (**3**), with more potent antibacterial activity than **2**, was launched in 1999.[11] Further chemical modifications of **1** aimed at producing an agent for human use that has sufficient efficacy and is less prone to metabolic degradation than **1**. These efforts resulted in the 1980s in the development of azamulin (**4**), which entered phase I clinical studies in volunteers.[12] Although **4** had good antibacterial activity, its bioavailability was severely limited by atrocious solubility in water. Thus, **4** showed a short half-life due to rapid metabolism and subsequent excretion, and the program for human therapy was subsequently abandoned.[13] Recently, researchers at GlaxoSmithKline have described their efforts in the development of a pleuromutilin derivative for human use. Their work led to identification of the novel retapamulin (**5**)[14] (Figure 1), which has excellent in vitro activity and was approved in 2007 as a topical antimicrobial agent for treatment of skin infections. Although semisynthetic pleuromutilin analogues generally exhibit potent antibacterial activity, they suffer from being rapidly and extensively metabolized in vivo because of their strong hydrophobic nature.[3]

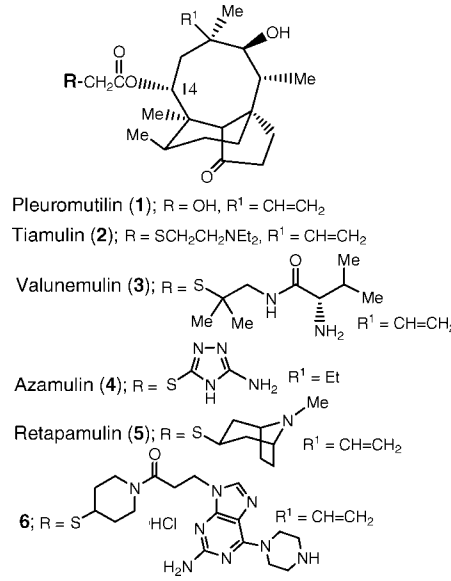

Figure 1. Pleuromutilin derivatives.

In the course of our program aimed at the discovery of a pleuromutilin derivative for human use with potent antibacterial activity, good solubility in water, and metabolic stability over previous analogues, we designed the structurally novel thioether pleuromutilin analogues having a a purine ring as the polar and water solubilizing group. On the basis of our results of SARs,[15] we identified a promising compound **6** with good solubility in water. Herein, we describe the excellent in vitro and in vivo antibacterial activity of **6**.

The purine−propionic acid moiety **10** of **6** was prepared as shown in Scheme 1. Regioselective reaction of 7-amino-6-

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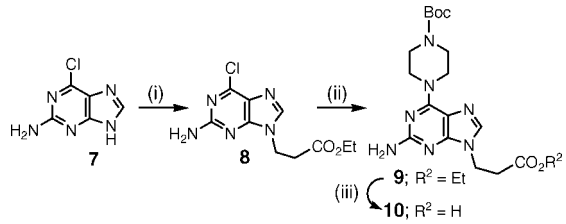
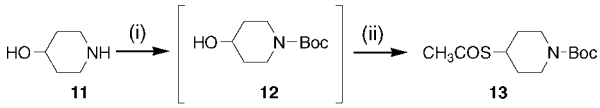
Scheme 1[a]



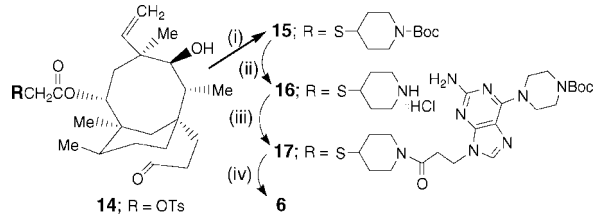
[a] Reagent and conditions: (i) ethyl acrylate or ethyl 3-bromopropionate, K_2CO_3, DMF, 80 °C, 64%; (ii) *tert*-butyl 1-piperazinecarboxylate, *N*,*N*-diisopropylethylamine, 2-propanol, reflux, 20 h, 92%; (iii) 2 M NaOH/MeOH, reflux, 2 h, then citric acid/H_2O, 94%.

Scheme 2[a]



[a] Reagent and conditions: (i) di-*tert*-butyl dicarbonate, $CHCl_3$, room temp, 2 h; (ii) diisopropyl azodicarboxylate, Ph_3P, THF, −20 °C, 0.5 h then thioacetic acid, −10 °C to room temp, 0.5 h, 98%.

Scheme 3[a]



[a] Reagent and conditions: (i) **13**, *t*-BuOK, MeOH, reflux, 2 h, then **14**, 0 °C to room temp, overnight, 98%; (ii) 30% HCl/EtOH, room temp, 2 h, 98%; (iii) **10**, benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate, Et_3N, DMF, room temp, 2 h, 72%; (iv) 4 M HCl/AcOEt, room temp, 2 h, 97%.

chloropurine (**7**) with ethyl acrylate or ethyl 3-bromopropionate gave the 9-purine propionic ester **8** in moderate yield. Treatment of **8** with 1-(*tert*-butoxycarbonyl)piperazine in refluxing 2-propanol followed by alkaline hydrolysis of the resulting ester **9** produced **10** in good yield.

The intermediate piperidine thioester **13** was prepared by Mitsunobu reaction of 1-(*tert*-butoxycarbonyl)-4-hydroxypiperidine (**12**), which was obtained in excellent yield from reaction of 4-hydroxypiperidine (**11**) and di-*tert*-butyl dicarbonate, with thioacetic acid (Scheme 2).

Compound **6** was prepared as shown in Scheme 3. Reaction of mutilin 14-tosyloxyacetate[8,9] **14** with the 4-piperidinethiol, which was generated by treatment of the thioester **13** with *t*-BuOK/MeOH, followed by deprotection of Boc group in the resultant **15** afforded **16** in excellent yield. Condensation of **16** with **10** in the presence of benzotriazole-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate as a coupling agent and successive acid hydrolysis of the resultant **17** gave the desired **6** as a hydrochloride with good solubility in water (~50 mg/mL).

The in vitro antibacterial activity of **6**, the earlier pleuromutilin analogue **4**, and other marketed antibacterial agents, i.e., a cephalosporin ceftriaxone (CTRX), a macrolide erythromycin (EM), a tetracycline minocycline (MINO), a glycopeptide vancomycin (VCM), and a quinolone levofloxacin (LVFX), was determined by broth microdilution according to standard procedures.[16] Their minimal inhibitory concentrations (MICs) against drug-susceptible and/or -resistant *Staphylococci*, *Streptococci*, and *Enterococci* strains are presented in Table 1. In addition to these strains, their antibacterial activity against *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Moraxella (Branhamella) catarrhalis*, and *Haemophilus influenzae* was also evaluated (Table 1).

The pleuromutilin analogues **4** and **6** exhibited remarkable antibacterial activity against all Gram-positive pathogens including MRSA, PRSP, and VRE, except for *E. faecalis*. In fact, **4** and **6** were not effective against the *E. faecalis* strain. Although **4** and **6** revealed almost the same profile of in vitro activity against susceptible and resistant Gram-positive microorganisms (MSSA[a] vs MRSA, PSSP vs PRSP, and VSE vs VRE), **6** showed a particularly potent antibacterial activity against *S. pyogenes* (MIC = 0.032 μg/mL). Compound **6** was also found to be more active against MRSA than all the marketed antibacterial agents tested here except for VCM. More importantly, **6** exhibited excellent antibacterial activity against susceptible and resistant *S. pneumoniae* with an MIC of 0.063 μg/mL and appeared as a possible alternative to the weak EM, MINO, and LVFX. Furthermore, **6** was found to have excellent antibacterial activity against *S. epidermidis* and relatively good potency against Gram-negative organisms such as *M. catarrhalis* and *H. influenzae*, both of which are common serious respiratory tract pathogens. In particular, its antibacterial activity against *S. epidermidis* was comparable to that of EM and MINO but significantly superior to that of VCM or CTRX. VCM, the traditionally last resort for serious infections caused by Gram-positive pathogens, is not active against VCM-resistant Gram-positive organisms including *E. faecium*. On the other hand, **6** exhibited superior antibacterial activity against VCM-resistant *E. faecium* strain (MIC = 0.063 μg/mL) compared to CTRX, EM, or LVFX. In general, **6** displayed potent antibacterial activity against MRSA, PRSP, and VRE pathogens, while marketed antibacterial agents were less active than **6** or inactive against these pathogens.

As shown in Table 2, **6** and five reference agents, i.e., ampicillin (ABPC), clarithromycin (CAM), MINO, VCM, and LVFX, were further evaluated for their in vitro activity against nine anaerobe and two mycoplasma strains. While **6** showed excellent activity against all anaerobe and mycoplasma strains, the reference agents appeared to have moderate to weak activity against some anaerobe and/or mycoplasma strains.

Next, **6**, **4**, CTRX, EM, MINO, VCM, and LVFX were examined for their in vivo efficacy (ED_{50}) against lethal *S. aureus* (MSSA and MRSA) and *S. pneumoniae* (PSSP and PRSP) systemic infection model in mice (Table 3). Compounds **4** and **6** and VCM showed moderate to potent protective effects against MRSA and PRSP strains compared with other marketed antibacterial agents. However, as in vitro evaluation, the in vivo antibacterial activity of **6** was more potent than that of **4**. Interestingly, **6** and VCM exhibited superior ED_{50} values (about 1−2 mg/kg) when given intravenously regardless of the frequency of dosing (once or twice), indicating the potential of **6** as an antibacterial agent with efficacy equal to that of VCM. The other reference agents showed relatively poor in vivo antibacterial efficacy against drug-resistant strains.

The efficacy of compounds **4** and **6**, given orally twice a day, against *S. aureus* Smith (MSSA) was next evaluated. While **4** had an ED_{50} of 13.3 mg/kg, po, the ED_{50} of **6** was more than 50 mg/kg, po (data not shown).

Finally, the efficacy of **6** and the reference agents EM and VCM was examined in a pulmonary infection model in which

[a] Abbreviations: MSSA, methicillin-susceptible *S. aureus*; PSSP, penicillin-susceptible *S. pneumoniae*; VSE, vancomycin-susceptible enterococci.

Table 1. In Vitro Antibacterial Activity of **6**, **4**, and Reference Agents (CTRX, EM, MINO, VCM, and LVFX)

	MIC (μg/mL)						
organism	**6**	**4**	CTRX	EM	MINO	VCM	LVFX
S. aureus FDA 209P (MSSA)	0.063	0.25	2	0.063	0.063	0.5	0.125
S. aureus Smith (MSSA)	0.25	0.5	2	0.125	0.063	1	0.125
S. aureus KT0116 (MRSA)[a]	0.5	1	>128	>128	16	1	>128
S. aureus KMP9 (MRSA)[a]	0.5	2	>128	32	4	0.5	>128
S. epidermidis ATCC12228	0.063	0.25	2	0.063	0.125	1	0.25
S. pneumoniae ATCC49619 (PSSP)	0.063	0.5	0.063	0.016	0.032	0.25	0.5
S. pneumoniae KT2524 (PRSP)[b]	0.063	0.5	0.5	>128	8	0.5	8
S. pyogenes ATCC12344	0.032	0.25	0.016	0.008	0.063	0.5	0.5
E. faecalis ATCC29212	64	128	4	2	1	4	0.5
E. faecium ATCC19434 (VSE)	0.063	0.5	4	1	0.063	0.5	4
E. faecium KU1778 (VRE)[c]	0.063	0.25	>128	>128	0.063	>128	64
M. catarrhalis K1209	0.032	0.032	0.25	0.032	0.25	64	0.063
H. influenzae TH13	0.125	0.5	0.008	2	0.5	>128	0.032

[a] A clinical isolate of MRSA. [b] A clinical isolate of PRSP. [c] A clinical isolate of VRE.

Table 2. In Vitro Antibacterial Activity of **6** and Reference Agents (ABPC, CAM, MINO, VCM, and LVFX) against Anaerobe and Mycoplasma Strains[a]

	MIC (μg/mL)					
organism	**6**	ABPC	CAM	MINO	VCM	LVFX
anaerobes						
Staphylococcus saccharolyticus GAI 5520	0.06	0.008	0.063	0.125	2	0.125
Streptococcus intermedius GAI 7416	0.25	0.125	<0.004	0.25	1	0.5
Atopobium parvulum GAI 5542	0.25	0.125	<0.004	0.25	2	0.25
Peptostreptococcus asaccharolyticus GAI 5534	0.032	0.25	>128	16	0.125	2
Peptostreptococcus anaerobius GAI 5506	0.032	0.125	<0.004	0.032	0.25	0.125
Peptostreptococcus magnus GAI 5528	0.063	0.25	2	0.125	0.25	0.125
Clostridium perfringens GAI 5526	0.063	0.063	1	0.063	0.5	0.25
Propionibacterium acnes GAI 5568	0.008	0.125	<0.004	0.125	0.5	0.25
Propionibacterium granulosum GAI 7414	0.016	0.063	<0.004	0.25	0.5	0.25
mycoplasma						
Mycoplasma pneumoniae FH Liu	0.008	NT	0.016	0.25	>128	0.125
Mycoplasma pneumoniae Mac	0.008	NT	0.016	0.25	>128	0.063

[a] NT: Not tested.

Table 3. In Vivo Antibacterial Activity of **6**, **4**, and Reference Agents (CTRX, EM, MINO, VCM, and LVFX)

	ED_{50} (mg/kg/dose)[a]						
organism	**6**	**4**	CTRX	EM	MINO	VCM	LVFX
*S. aureus*Smith (MSSA)	1.86 (1.51)	(6.88)	3.72	2.55	0.515	0.88	0.78
*S. aureus*KT0116 (MRSA)	2.15 (0.91)	(5.20)	>100	>100	50	1.01	>100
S. pneumoniae ATCC49619 (PSSP)	1.54 (1.34)	(12.9)	0.22	1.48	0.968	1.06	24.7
S. pneumoniae KT2524 (PRSP)	1.55 (1.88)	(17.7)	27.5	>100	50	2.37	>100

[a] The efficacy criterion, ED_{50}, was calculated as the dose at which mice survival rate was 50%. Mice were inoculated intraperitoneally. Medication was given intravenously once, 1 h after infection. Values in parentheses represent ED_{50} given as intravenous twice, 1 and 4 h after infection.

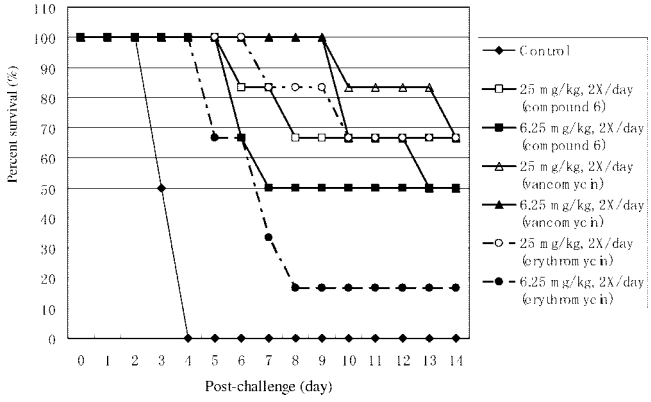


Figure 2. Dose–response study showing prevention by **6**, EM, and VCM of mortality in mice exposed to a lethal challenge of *S. pneumoniae* ATCC6303 by intratracheal instillation.

mice were inoculated with penicillin-susceptible *S. pneumoniae* ATCC6303. As shown in Figure 2, twice daily intraperitoneal treatment with 25 mg/kg of **6** (MIC = 0.032 μg/mL), VCM (MIC = 0.5 μg/mL), or EM (MIC = 0.032 μg/mL) for 3 consecutive days provided significant protection (83−67% survival rate) against lethal pulmonary challenge with *S. pneumoniae* ATCC6303. Although the treatment dose was reduced to 6.25 mg/kg, **6** and VCM provided potent protective effect with 50% survival in both cases. In general, the in vivo efficacy of **6** was comparable to that of VCM and somewhat higher than that of EM. Clearly, in vitro antibacterial activity is not the only parameter that influences in vivo efficacy in this study, as evidenced by identical MIC values of **6** and EM against *S. pneumoniae* ATCC6303. Pharmacokinetics and ADME properties also have considerable impact on efficacy.

In summary, **6** with good solubility in water not only conferred in vitro antibacterial activity against a number of Gram-positive and several Gram-negative pathogens, including MRSA, PRSP, VRE, anaerobes, and mycoplasmas, but also showed potent in vivo efficacy in mice. Furthermore, **6** was very effective at preventing mortality induced by penicillin-susceptible *S. pneumoniae* ATCC6303 and exhibited approximately the same antibacterial activity as VCM in this animal model. *S. aureus*, *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. pyogenes*, and mycoplasma are known to cause

respiratory tract infections. In particular, *S. pneumoniae* is the main causative pathogen of community-acquired *pneumoniae*. Because **6** showed potent in vitro and in vivo antibacterial activity against almost all of the main causative pathogens of community-acquired *pneumoniae* tested, it is suggested that **6** has potential as an antibacterial agent for use in humans. In future publications, we will give extensive details on the in vitro SARs and in vivo antibacterial activity of this compound. In addition, a more comprehensive study directed at optimization for an even more potent in vivo efficacy will be reported in due course.

Supporting Information Available: Experimental details and characterization data for **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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