Mutilins Derivatives: From Veterinary to Human-used Antibiotics

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Abstract: Mutilins derivatives, which were successfully developed in veterinary medicines such as tiamulin and valnemulin, have regained interest as promising antibacterial agents with potential for human use in the past few years. In 2007, Retapamulin, as the first in a new class of topical antibacterial in the nearly two decades, was approved for use in human skin infections. This review provides a developed process of mutilins derivatives from veterinary to human-used antibiotics and emphasizes the structure activity relationship (SAR) and antibacterial mechanism of mutilins derivatives. Moreover, the semi-synthetic strategy of water-soluble mutilins derivatives and related novel derivatives during 2006-2008 will also be reviewed.

Key Words: Antibacterial, pleuromutilin, tiamulin, valnemulin, retapamulin, structure-activity relationship (SAR), purine ring, piperazine ring spacer, water-soluble, metabolic stability.

INTRODUCTION

The emergence of multi-drug-resistant microorganisms leads to a concerted search for new antibacterial drugs with novel modes of action and activity, and results in the discovery of most of the antibacterial drugs derived from natural product leads [1-3]. Unfortunately, the last 50 years have seen the discovery of only three novel classes of antibacterials, namely oxazolidinones (linezolid), cyclic lipopeptides (daptomycin) and the recently identified platensimycin[4, 5]. Therefore the strategy of drug discovery and development has turned to the reassessment of previously discovered classes of antibacterial agents that have not been used in humans such as pleuromutilin [6].

Pleuromutilin (**1**, Fig. (**1**)), having an unusual fused 5-6-8 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 bacteria and mycoplasmas [7-9]. Initial structure–activity relationship (SAR) studies and chemical modifications focused on variations in the C_{14} glycolic acid side chain of pleuromutilin, as a result, a number of semisynthetic pleuromutilin analogues such as tiamulin (**2**, Fig. (**1**)) and valnemulin (**3**, Fig. (**1**)) were successfully developed as therapeutic agents for veterinary use [10-13]. In the past few years, efforts on pleuromutilin have been renewed at human therapeutics. Although the first human-used mutilins derivative azamulin (**4**, Fig. (**1**)) failed in Phase I clinical trials due to the fact that it showed a short half-life and rapid metabolism *in vivo,* retapamulin (**5**, Fig. (**1**)), a novel mutilins derivative, was approved in 2007 by FDA as the first in a new class of topical antimicrobial agent in the nearly two decades for treatment of human skin infections [6, 9, 14]. In the past two years, a series of novel water-soluble mutilins derivatives bearing a purine ring have been disclosed by Dainippon Sumitomo Pharma *Co., Ltd* [15-18].

The patent disclosures of mutilins antibacterial agents during 2001-2006 have been comprehensively reviewed [6]. This article provides the developed progress of mutilins derivatives from veterinary to human-used antibiotics and highlights on the SAR and antibacterial mechanism of mutilins derivatives. Moreover, the semi-synthetic strategy of water-soluble mutilins derivatives and related novel derivatives during 2006-2008 will also be described.

ANTIBACTERIAL MECHANISM AND RESISTANCE MECHANISM

The mutilins derivatives selectively inhibit bacterial protein synthesis through interaction with prokaryotic ribosomes but have no effect on eukaryotic protein synthesis and do not bind to mammalian ribosomes [19]. The antibacterial mechanism of mutilins derivatives was revealed from the crystal structure of the *Deinococcus radiodurans* 50S ribosomal subunit in complex with tiamulin and valnemulin [20,21]. The mutilins derivatives bind to the ribosomal peptidyl transferase center (PTC) with their tricyclic mutilin core located inside a tight pocket confined by residues G2061, A2451, C2452, A2503, U2504, G2505, U2506 at the A-site, and their C14 extensions pointing toward the P-site, at which enhances the interactions with 23S rRNA. Moreover, mutilins derivatives binding triggers an induced-fit mechanism that exploits the flexibility of nucleotides residing in and around the PTC, particularly U2585 and U2506, for tightening the binding pocket [22]. Chemical footprinting studies suggest that the mutilins dervatives with enhanced antimicrobial activity may be obtained by maximizing the number of interactions between the side chain moiety and the peptidyl transferase cavity [21]. These studies point out that mutilins dervatives may encounter less target-specific cross-resistance with other antibacterial agents, which make them as an important class of compounds to study for human clinical development.

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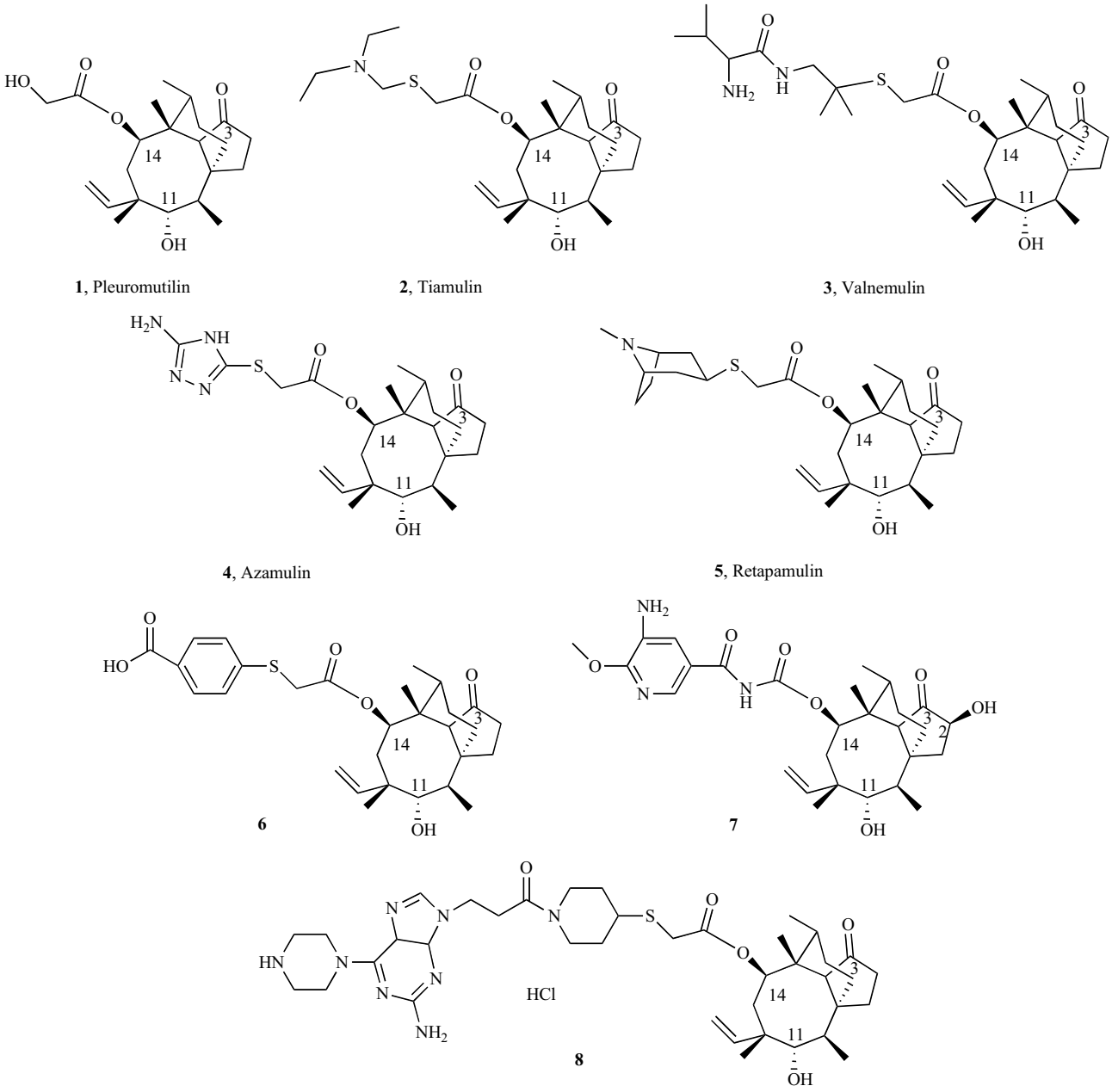


Fig. (1). Mutilins derivatives.

Nowadays, although some bacteria resistant to mutilins derivatives have been identified, they are not very widespread so far and apparently only slowly developing [23-25]. The mutations in amino acid positions 148 and 149 of the ribosomal protein L3 and nucleotide positions G2032, C2055, G2447, C2499, U2504 and A2572 of the 23S rRNA lead to reduced binding of tiamulin to the bacteria ribosomal subunits [24]. Cross-resistance to phenicols, lincosamides, oxazolidinones, streptogramin A antibiotics and mutilins derivatives may have something to do with the Cfr rRNA methyltransferase, which confers the addition of a single methyl group at nucleotide A2503 of 23S rRNA[26]. Moreover, structural basis for cross-resistance between mutilins derivatives and other PTC antibiotics indicates that almost all of the nucleotides mediating resistance are clustered in a distinct region [27]. Alterations of the identity of these nucleotides may not lethally affect ribosome function, but can hamper antibiotic binding through changes in the conformation and flexibility of specific PTC nucleotides [27]. It is interesting that some mutants of *Staphylococcus aureus* exhabit unidirectional cross-resistance between tiamulin and linezolid. Miller *et al.* recently illustrated that the copy number of 23S rRNA and the mutation in single-copy *rplC* gene are the main reasons for this phenomenon [28].

THE DEVELOPED COURSE OF HUMAN-USED MUTILINS DERIVATIVES

Although there is a concise synthesis of the tricyclic skeleton of pleuromutilin [29], the major method for ana-

logues discovery is semi-synthesis from the readily available and inexpensive natural product itself [30]. Structural modification of pleuromutilin has provided several derivatives with enhanced activity against bacteria and mycoplasmas, and has permitted conclusions to be reached about SAR [11, 12]. The carbonyl group in the five-membered ring and the hydroxyl group at C_{11} are essential for activity. The vinyl group can be hydrogenated without loss of activity. Mutilin and other compounds with a free OH at C_{14} are inactive. Chemical modification at C_{14} offers the most possibilities for achieving the best activity and solubility properties. Experiments demonstrate that the antibacterial activity of pleuromutilin can be greatly enhanced if the glycolic side chain in position C_{14} of the molecule is replaced by acyl residues other than aromatic ring. Moreover, it is shown that mutilin esters of substituted thioglycolic acids had distinctly superior minimum inhibitory concentration (MIC) values than O or N- nucleophile.

Tiamulin, the first mutilins derivative in market, was developed by Sandoz in 1974. It is a prophylactic and therapeutic agent for swine dysentery, and shows activity (MIC < 1 μg/mL) against anaerobic bacteria, intestinal spirochetes and *Mycoplasma* spp [31]. However, other gram-negative organisms like *Pseudomonas aeruginosa*, *Proteus* species, and *Alcaligenes faecalis* are proved to be naturally resistant to tiamulin [31]. Although tiamulin exhibits potent *in vitro* activity against staphylococcal isolated from humans (MIC_{50} ≤0.5 μg/mL), it is rapidly metabolized *in vivo* by cytochrome P450-mediated hydroxylation of the tricyclic nucleus at the C_2 and C_8 positions, and then eliminated [32]. Baggetto *et al*. reported that low concentrations of tiamulin (0.1 to 10 μM) sensitized three highly resistant P-glycoprotein (Pgp)-overexpressing tumor cell lines to several multi-drug resistance (MDR)-related anticancer drugs [33]. It is suggested that tiamulin is a potent chemosensitizer that antagonizes the Pgp-mediated chemoresistance in many tumor cell lines.

Valnemulin was originally approved in 1999 in the European Union (EU) for the prevention and treatment of swine dysentery caused by *Brachyspira hyodysenteriae* and enzootic pneumonia caused by *Mycoplasma hyopneumoniae*. It is the first veterinary medicinal premix to be centrally approved across the EU and categorized as a prescription only medicine (POM). In January 2004 it was approved by the European Commission for the new indications for the prevention of porcine colonic spirochaetosis (colitis) caused by *Brachyspira pilosicoli* and the treatment of porcine proliferative enteropathy (ileitis) caused by *Lawsonia intracellularis*. The MIC value (0.03-2 μg/mL) of valnemulin on *B. pilosicoli* is about 2 times lower than tiamulin, and about 128 times lower than lincomycin [13]**.** *In vitro* study of tiamulin resistance shows that *B. hyodysenteriae* and *B. pilosicoli* strains became resistant to tiamulin are also susceptible to valnemulin [34]. To some extent, valnemulin is the most active with MIC values (0.5-4 μg/mL) against *Mycobacterium tuberculosis* resistant strains which showed MIC values of 8 μg/mL to isoniazid, rifampicin and streptomycin [6]. Among the compounds evaluated against five *Helicobacter pylori* ATCC strains, valnemulin exhibits potent antibacterial activity with MIC values in the range of < 0.0125-0.05 μg/mL compared with metronidazole and tetracycline with the MIC value ranges of 2-128 and 0.2-0.4 μg/mL, respectively [6]. Moreover, Heilmann *et al.* reported the successful treatment of resistant human mycoplasma infection in immunocompromised patients with primary antibody deficiency (PAD) using orally administered valnemulin (MIC = 0.05-2.5 μg/mL) [35]. For some mycoplasma strains, valnemulin was shown to be more potent than presently available antimicrobials such as doxycycline, quinolones and macrolides [35].

Further chemical modifications of pleuromutilin aimed at producing an agent with sufficient efficacy and less metabolic degradation for human use. These efforts resulted in the development of azamulin in 1980s, a azole derivative of pleuromutilin, which entered Phase I clinical studies in volunteers [36, 37]. Unfortunately, azamulin did not progress further due to its low solubility in water and a short half-life *in vivo* [6]. However, a recent study indicates that azamulin is a highly selective inhibitor of human CYP3A *in vitro*. Preincubation of 4.8 mM azamulin in the presence of NADPH for 10 min inhibited approximately 95% of testosterone β-hydroxylase activity [38].

In the past few years, efforts on pleuromutilins to develop human-used mutilins derivatives have been renewed. Retapamulin is the first in a new class of antibacterial drugs known as pleuromutilins to be approved for use in humans [14]. It was approved in April 2007 by FDA for the topical treatment of impetigo and secondarily infected traumatic lesions (SITL) of skin infection, which are most commonly caused by the gram-positive bacteria *S.aureus* (MIC = 0.12 μg/mL) and *Streptococcus pyogenes* (MIC = 0.016 μg/mL) [14]. The antibacterial mechanism, clinical trials, medical applications and pharmacokinetics of retapamulin have been recently concluded [6, 14, 39-44]. Like other mutilins derivatives, retapamulin selectively inhibits bacterial protein synthesis by binding to a site on the 50S subunit of the bacterial ribosome through an interaction that is different from that for other ribosomally targeted antibiotics, such as macrolides [22, 45-47]. In addition, retapamulin has a low potential to generate resistance in *S.aureus* compared with other topical antibiotics [48]. To date, it is demonstrated that retapamulin do not exhibit any clinically relevant, target-specific cross-resistance with other antibiotic classes [42]. Furthermore, retapamulin maintains activity against organisms resistant to methicillin, erythromycin, fusidic acid, mupirocin, azithromycin and levofloxacin [44]. Recent report showed that 99.9% of 664 *S.aureus* isolate from the UK, including many resistant to fusidic acid (MIC > 1 μg/mL) and/or highly resistant to mupirocin (MIC > 256 μg/mL) are inhibited by retapamulin at <= 0.25 μg/mL [49]. It is also active against bacterial pathogens commonly associated with respiratory tract infections such as *Streptococcus pneumoniae* (MIC = 0.12 μg/mL), *Haemophilus influenzae* (MIC = 2 μg/mL), *Moraxella catarrhalis* (MIC = 0.03 μg/mL) and vancomycin(VCM)-susceptible strains of *Enterococcus faecium*, but inactive against *Enterococcus faecalis* [6]. Moreover, retapamulin exhibits the most active *in vitro* (MIC ≤ 1 μg/mL) against 141 clinical isolates of *Propionibacterium* species responsible for acne vulgaris, including seven multi-resistant strains [50]. The 5 days twice daily course of 1% retapamulin ointment (success rates = 89.5%) showed com-

parable efficacy to a 10-day course of oral cephalexin 500 mg twice-daily (success rates = 91.9%) for the empiric treatment with SITL in 1904 patients [51].

Other mutilins derivatives from Nabriva Therapeutics such as BC-3205, BC3781 and BC-7013 combine excellent oral bioavailability with substantial activity against gram-positive pathogens and atypicals as well as some gram-negative pathogens such as MDR pathogens including methicillin resistant *S.aureus* (MRSA), MDR *S.pneumonia* (i.e. macrolide and quinolone resistance), and vancomycin resistant *E.faecium* (VRE). BC-3205 and BC-3781 are intended for the treatment of serious MDR skin & complicated skin structure infections (CSSI) and moderate to severe pneumonia and are scheduled to enter Phase II and Phase I clinical trials in 2009 respectively,. Meanwhile, BC-7013 is currently undergoing Phase I clinical trials and is intended for the treatment of gastrointestinal complications caused by the over-proliferation of *Clostridium difficile*.

A lot of novel mutilins derivatives were summarized in review reported by Phillips and Sharaf, for example, derivative **6** (Fig. (**1**)), which shows MIC values of 0.125 and 0.5 μg/mL against *S. aureus* Oxford and *S. pneumoniae* 1629, respectively [6]. Another 2-hydroxy derivative **7** (Fig. (**1**)) for the treatment and prophylaxis of drug-resistant bacterial infections claimed oral efficacy. The compound dosed at 300 and 600 mg/kg was highly effective in reducing MRSA WCUH29 counts by 3.6 and 4.3 $\log_{10}$ CFU/abscess, respectively, compared with untreated controls, in a murine groin abscess infection model [6]. Hanson *et al.* reported that the hydroxyl-mutilins derivatives (Fig. (**2**)), for example, (8S)-, (7R)-, (2S)-hydroxymutilin and (2R)-hydroxypleuromutilin were harvested by biotransformation of mutilin or pleuromutilin using the strains *Streptomyces griseus* SC 1754 and SC 13971 (ATCC 13273) and/or *Cunninghamella echinulata* SC 16162 (NRRL 3655) [52]. The detailed spectroscopic properties of stereochemistry in (2*R*)-and (2*S*)-hydroxymutilin were measured and calculated recently by combination of computational and spectroscopic study using Gas-phase structural models, which is shown to be a useful tool in the analysis of unknown mutilin derivatives or other compounds, such as those present as minor impurities or metabolites in pharmaceutical samples [53].

NOVEL WATER-SOLUBLE MUTILINS DERIVATIVES HAVING A PURINE RING

Although mutilins derivatives in which the hydroxyl of the C_{14} glycolic ester group is replaced with a substituent containing the sulfide linkage show potent *in vitro* antibacterial activity, but these compounds suffer from being rapidly and extensively metabolized *in vivo* because of their strong hydrophobic nature [9]. Therefore the following research aims at the discovery of a novel derivative for human use with potent antibacterial activity, good solubility in water, good pharmacokinetics and absorption, distribution, metabolism, and excretion (ADME) properties, and metabolic

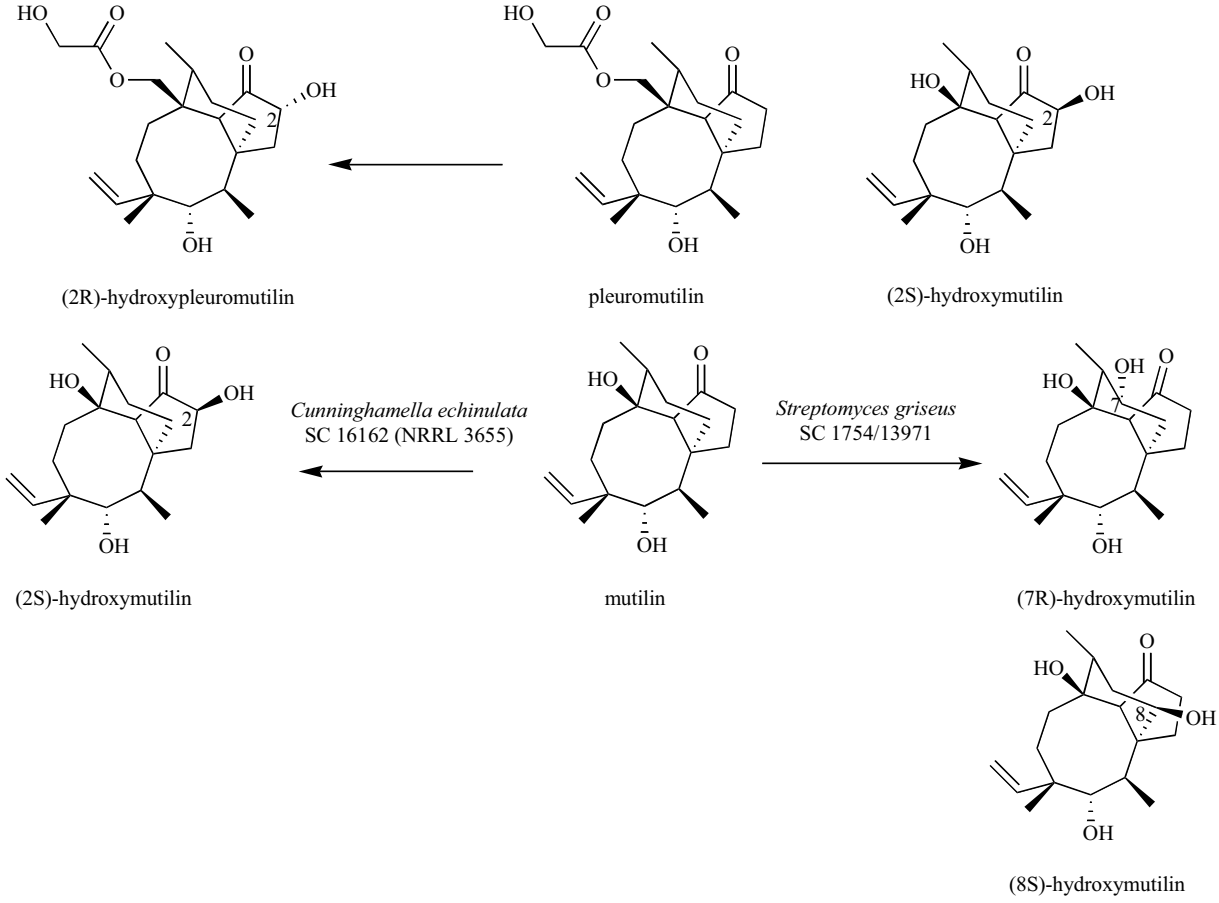
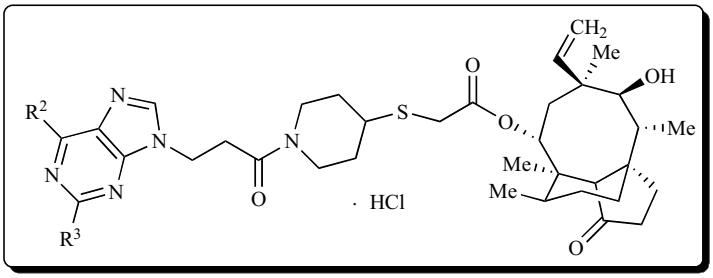


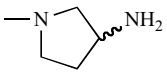
Fig. (2). Hydroxymutilin derivatives.

stability over previous analogues. As a result, Hirokawa *et al.* reported a structurally novel thioether pleuromutilin analogue **8** (Fig. (**1**)) having a purine ring as the polar and water solubilizing group [15]. The SAR analysis shows that compound **8** has a good solubility in water (~50 mg/mL). Compound **8** was prepared by 3 steps and was shown in good yield [15].

Compound **8** exhibits remarkable antibacterial activity against gpathogens including *S.aureus* KMP9 (MRSA)(MIC = 0.5 μg/mL), *S.pneumoniae* KT2524 penicillin resistant (PRSP)(MIC = 0.063μg/mL), and *E.faecium* KU1778 (VRE)(MIC = 0.063 μg/mL) as well as a particularly potent antibacterial activity against *S.pyogenes* ATCC12344 (MIC = 0.032 μg/mL). Furthermore, compound **8** is found to have excellent antibacterial activity against *Staphylococcus epidermidis* (MIC = 0.063 μg/mL) and relatively good potency against gram-negative organisms such as *M. catarrhalis* K1209 (MIC = 0.032 μg/mL) and *H. influenzae* TH13 (MIC = 0.125 μg/mL). Compound **8** also exhibits superior antibacterial activity against VRE strain (MIC = 0.063 μg/mL) compared to ceftriaxone (CTRX), erythromycin (EM) or levofloxacin (LVFX). In addition, compound **8** shows excellent activity against all anaerobe and mycoplasma strains, which the agents ampicillin (ABPC), clarithromycin (CAM), minocycline (MINO), VCM and LVFX appear to have moderate to weak activity against. *In vivo* efficacy median effective dose (ED_{50}) against lethal *S.aureus* (methicillin susceptible *Staphylococcus aureus*, MSSA and MRSA) and *S. pneumoniae* (penicillin susceptible *Streptococcus pneumoniae*, PSSP and PRSP) has been studied between **8** and VCM in systemic infection model of mice. Both of them exhibit 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 **8** as an antibacterial agent with efficacy equal to that of VCM. The efficacy of **8** and the

Table 1. ***In Vitro*** **and** ***In Vivo*** **Antibacterial Activities of 20, 21 and 22 Compared with Azamulin and VCM**



Compound	R^2	R^3	MIC (μg/mL)								MSSA[b]
			MSSA[b]	MRSA[c]	PSSP[d]	PRSP[e]	*S. p.*[f]	VRE[g]	*M. c.*[h]	*H. i.*[i]	ED_{50}[j] (mg/kg, iv)
22		H	0.063	0.125	0. 016	0.032	0.016	0.032	0.032	1	1.47
20		H	0.125	0.5	0.016	0.032	0.008	0.125	0.063	1	0.59
21		H	0.25	0.5	0.063	0.016	0.125	0.125	0.125	2	0.76
azamulin			0.5	2	0.5	0.5	0.25	0.25	0.032	0.5	6.88[k]
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, methicillin resistant *S. aureus* KMP9.

d PSSP, penicillin susceptible *S. pneumoniae* ATCC49619.

e PRSP, penicillin resistant *S. pneumoniae* KT2524.

f *S. pyogenes* ATCC12344.

g VRE, vancomycin-resistant enterococci *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.

k Mice were inoculated with each organism and medication was given intravenously twice, 1 and 4 h after inoculation.

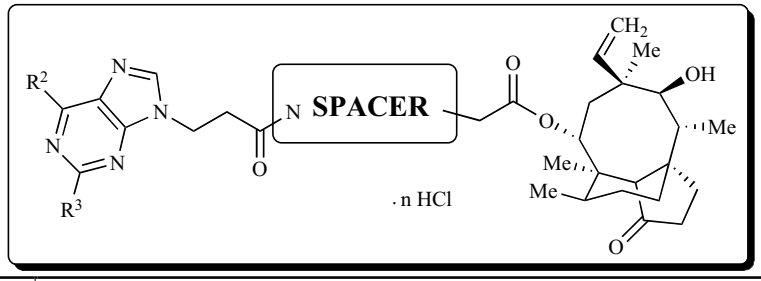
agents EM and VCM are examined in a pulmonary infection model in which mice are inoculated with penicillin-susceptible *S. pneumoniae* ATCC6303, and it is shown that twice daily intraperitoneal treatment with 25 mg/kg of **8** (MIC = 0.032 μg/mL), VCM (MIC = 0.5 μg/mL), or EM (MIC = 0.032 μg/mL) for 3 consecutive days provided significant protection (83-67% survival rate) against lethal pulmonary challenge with *S. pneumoniae* ATCC6303.

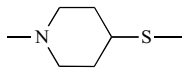
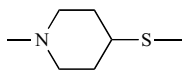
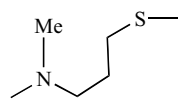
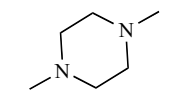
In the publication reported by Hirokawa *et al*, a series of compounds bearing a 4-piperidinethio moiety are prepared and evaluated [16]. From SAR studies, two compounds **20** ((±)-6-(3-aminomethylpyrrolidine)) purine and **21** (introduction of a methyl group at the amino group of **20**) show not only excellent *in vitro* antibacterial activity against MRSA, PRSP, VRE, *S .pyogenes*, *M. catarrhalis* and *H. influenzae* but also potent *in vivo* efficacy. The *in vitro* antibacterial activity and *in vivo* efficacy of **20** and **21**compared to azamulin and VCM are summarized in Table **1**. Moreover, these compounds, which also have good solubility in water, reflect good pharmacokinetics and ADME properties. In addition, compound **22** ((±)-6-(3-aminopyrrolidin-1-yl)purine) also displays excellent *in vitro* and in *vivo* activities, and the different stereochemistry at the 3-position of the pyrrolidine ring do not affect its any activities.

Next, the influence of the central spacer of compounds **8** and **22** on the antibacterial activity has been investigated. Structural modification of the sulfur-linked piperidinyl spacer of **8** and 2**2** resulted in the discovery of two novel derivatives **23** and **24** having the 3-methylaminopropylsulfide and the piperazine ring, respectively, as a central spacer [17]. The *in vitro* antibacterial activity and *in vivo* efficacy of **23** and **24** compared to **8** and **22** are summarized in Table **2**. Both compounds show much higher *in vivo* efficacy than the parent compound **22** and appear to have a well-balanced *in vitro* antibacterial activity profile against MRSA, PRSP, VRE, *S pyogenes*, *M.catarrhalis*, and *H.influenzae*. Furthermore, removal of the methylene or ethylene moiety from the piperidine ring of **22** provide the 3-methylaminopropylsufide **23**, which exhibits very good *in vitro* antibacterial activity against all strains with MIC values between 2 and ≤ 0.004 μg/mL. More importantly, compounds **23** and **24** reflect improved pharmacokinetics and ADME properties compared to **8** and **22**.

Recently, a series of novel derivatives possessing a piperazine ring spacer have been synthesized [18]. The *in vitro* antibacterial activity and *in vivo* efficacy of compounds **25**, **26** compared to **23** and VCM are summarized in Table **3**. In general, the MIC values of all compounds are compared with those of **22** and VCM, and the compounds **25** and 2**6** are found to exhibit strong *in vivo* efficacy against *S.aureus* Smith. Replacement of the amino group of **24** with an aminomethyl or a methylaminomethyl substituent results in a slight decrease in *in vitro* and *in vivo* antibacterial activities. These results indicate that the *in vivo* efficacy is highly sen-

Table 2. ***In Vitro*** **and** ***In Vivo*** **Antibacterial Activities of 22, 8, 23 and 24**

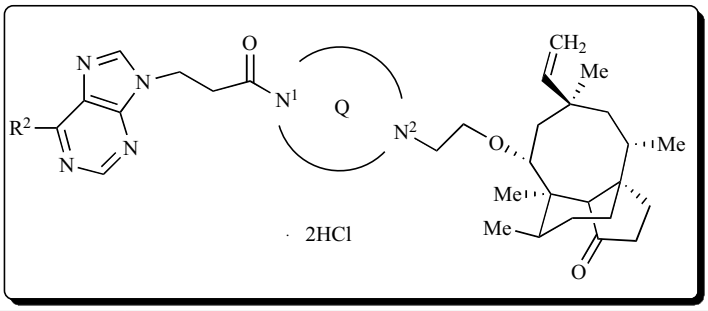


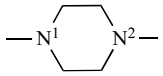
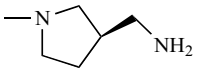
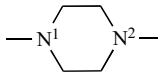
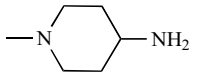
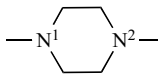
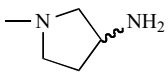
Compound※	N SPACER	n	MIC (μg/mL)[a]								MSSA[b]
			MSSA[b]	MRSA[c]	PSSP[d]	PRSP[e]	*S. p.*[f]	VRE[g]	*M. c.*[h]	*H. i.*[i]	ED_{50}[j] (mg/kg, iv)
22		1	0.063	0.125	0.016	0.032	0.016	0.032	0.032	1	1.47
8		1	0.25	0.5	0.063	0.063	0.032	0.125	0.25	1	1.86
23		1	0.125	0.25	0.016	0.016	0.008	0.063	0.063	1	0.86
24		2	0.25	1	0.063	0.063	0.032	0.5	0.25	4	0.80

※ **22**: R^2 = 1-piperazinyl, R^3 = NH_2; **8, 23** and **24**: R^2 = (±)-3-amino-1-pyrrolidinyl, R^3 = H.

a~j are described as the same as table 1.

Table 3. ***In Vitro*** **and** ***In Vivo*** **Antibacterial Activities of 25, 26 Compared with 23 and VCM**



Compound	Q	R^2	MIC (μg/mL)[a]								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]	
25			0.25	2	0.125	0.03063	0.032	0.5	0.125	0.5	0.83
26			0.25	1	0.0125	0.125	0.032	0.25	0.125	2	0.97
23			0.25	1	0.063	0.063	0.032	0.5	0.25	4	0.80
VCM			1	0.5	0.25	0.5	0.5	>128	64	>128	0.88

a~j are described as the same as Table **1**.

sitive to small structural changes at the 3-pyrrolidine ring. Compounds *in vivo* efficacy following substitution at the 3-pyrrolidine ring decreased generally in this order NH_2 > CH_2NHMe > CH_2NH_2 > NHMe >> NHEt >>NMe_2.

In summary, further studies aim at the development of pleuromutilin derivatives for use in human turn to identification of a novel class of pleuromutilin analogues having a piperazine ring spacer. This new class of pleuromutilin appears to have a very promising profile for the treatment of infections caused by respiratory pathogens.

CONCLUSIONS

Nowadays, bacterial infectious diseases remain the second-leading cause of death worldwide, which cause 17 million deaths globally, particularly in children and the elderly [2]. The pace of drug resistance, particularly as gram-positive pathogens, has outstripped the discovery of new antimicrobial agents and there is an urgent need for new antibiotic drugs with novel mechanisms of action. In the last 25 years, out of 155 small molecules used as chemotherapeutics, 47% are directly taken from the natural products and an additional 26% are derivatives or synthetic natural product mimics [54]. In contrast to this apparent dominance of natural products in pharmaceuticals, over the last two decades synthetic combinatorial chemistry and associated high throughput drug discovery screening yielded only one *de novo* chemical which was approved for drug use. Hence, it is not surprising that the current chemical-based drug discovery is now focusing on introducing structural and chemical diversity to natural product scaffolds to identify novel therapeutic molecules [55].

The fungal kingdom as an important scaffold for novel antibiotics includes many species with unique and unusual biochemical pathways, and also displays a broad range of useful activities for pharmaceutical and agricultural purposes [56, 57]. The total number of bioactive fungal product (at the end of 2002) is approximately 8600, including 57% antibiotics (approximately 4900) [58]. Although the ascomycetes and several other filamentous and entophytic fungal species are the main producing stains, basidiomycetes have been reinvestigated recently mainly due to it holds an excellent promise for new compounds with interesting biologic activities [59-61]. Pleuromutilin, as the previously discovered classes of antibacterial agents that has not been used in humans, belongs to a tricyclic diterpenoid. It was first isolated in 1951 from two basidiomycetes species, and was characterized as a crystalline antibiotic with modest *in vitro* activity against gram-positive bacteria and mycoplasmas. SAR studies focused on variations in the C_{14} glycolic acid side chain and a number of semi-synthetic pleuromutilin derivatives have been successfully developed. The compounds inhibit bacterial protein synthesis by specifically targeting the large subunit (50S) of the bacterial ribosome and scarcely exhibit target-specific cross-resistance to other nosocomia antibacterials., In addition, a series of novel derivatives bearing a purine ring with potent antibacterial activity, good solubility

in water, good pharmacokinetics and ADME properties, and metabolic stability over previous analogues have been developed recently. More importantly, a click chemistry approach to pleuromutilin conjugates with nucleosides or acyclic nucleoside derivatives is benefited to promote rational design of pleuromutilin based drugs[62]. The outcomes will be very vital in demonstrating the potential uses of mutilins derivatives for human clinical development.

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ABBREVIATIONS

ABPC	=	Ampicillin
ADME	=	Absorption, distribution, metabolism, and excretion
CAM	=	Clarithromycin
CFU	=	Colony-Forming Units
CSSI	=	Complicated skin structure infections
CTRX	=	Ceftriaxone
CYP	=	Cytochrome P450
ED_{50}	=	Median effective dose
EM	=	Erythromycin
EU	=	European Union
FDA	=	Food and Drug Administration
LVFX	=	Levofloxacin
MDR	=	Multi-drug resistance
MIC	=	Minimum inhibitory concentration
MINO	=	Minocycline
MRSA	=	Methicillin resistant *Staphylococcus aureus*
MSSA	=	Methicillin susceptible *Staphylococcus aureus*
NADPH	=	Reduced form of nicotinamide adenine dinucleotide phosphate
PAD	=	Primary antibody deficiency
Pgp	=	P-glycoprotein
POM	=	Prescription only medicine
PPC	=	Clinical per protocol (PPC)
PRSP	=	Penicillin resistant *Streptococcus pneumoniae*
PSSP	=	Penicillin susceptible *Streptococcus pneumoniae*
PTC	=	Peptidyl transferase center
SAR	=	Structure–activity relationship
SITL	=	Secondarily infected traumatic lesions
VCM	=	Vancomycin
VRE	=	Vancomycin-resistant enterococci

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