

## Novel pleuromutilin derivatives as antibacterial agents: Synthesis, biological evaluation and molecular docking studies

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### ABSTRACT

Owing to the increasingly serious problems caused by multidrug resistance in community-acquired infection pathogens, it has become an urgent need to develop new classes of antibiotics for overcoming the resistance. In this paper, we describe the design and synthesis of novel pleuromutilin derivatives containing the (2-aminothiazol-4-yl)-4-methyl group, as well as their in vitro antibacterial activities against Gram-positive clinical bacteria. Most of the tested compounds displayed strong antibacterial activities against these methicillin-susceptible and methicillin-resistant bacteria. Particularly noteworthy compound **15** and its derivative **16e**, both showed potent antibacterial properties (0.0625–0.5 µg/mL) that are superior to amoxicillin and tiamulin. Molecular docking studies suggested that the amino thiazole ring on the side chains of the pleuromutilin derivatives can in general be accommodated near the mutilin core in the binding pocket, and thus play an important role in the activity of the whole molecule. The findings reported herein may provide a new insight into the design of novel pleuromutilin derivatives for human clinical use.

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As a result of the increasing use or abuse of antibacterial agents for all kinds of infectious diseases on mankind, many drug-resistant pathogens have appeared in recent years. Especially, the methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *Streptococcus pneumoniae* (PRSP), have become serious problems in community settings and intensive care units (ICU) in the hospitals.<sup>1</sup> Therefore, developing novel classes of antibiotics against these resistant bacterial strains has become an urgent issue for scientists. The naturally occurring diterpene pleuromutilin can inhibit the growth of a variety of Gram-positive bacteria and mycoplasma, via a new action mechanism in that it selectively inhibits bacterial protein synthesis through interaction with 50s subunits of prokaryotic ribosomes, but has no effect on eukaryotic protein synthesis and does not bind to a mammalian ribosome,<sup>2</sup> and consequently, has no cross-resistance to marketed antibacterial agents. With the aim to develop new antibacterial agents, we decided to use pleuromutilin as the lead compound to design and synthesize a new class of pleuromutilins and evaluate their antibacterial bioactivities.

Since pleuromutilin **1** was first isolated in 1952 from two basidiomycete species (e.g., *Pleurotus mutilus* and *Pleurotus*

*passeckerianus*), two pleuromutilin derivatives,<sup>3</sup> tiamulin **2** and valnemulin **3**, have been successfully developed as therapeutic agents for veterinary use in markets until now. Tiamulin, developed by Sandoz in 1974, is a prophylactic and therapeutic agent for swine dysentery, and shows activity (MIC < 1 µg/mL) against anaerobic bacteria, intestinal spirochetes and *Mycoplasma* spp.<sup>4</sup> 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*.<sup>5</sup> The MIC value (0.03–2 µg/mL) of valnemulin on *B. pilosicoli* is about two times lower than that of tiamulin, and about 128 times lower than that of lincomycin (see Fig. 1).<sup>6,7</sup>

From then on, several other semisynthetic compounds **4–8** have also been designed for human use. Azamulin **4**, initially designed for this purpose, has entered into Phase I clinical studies on volunteers,<sup>8,9</sup> but due to its low solubility in water and a short half-life in vivo, no further progress was made for this compound.<sup>7</sup> Retapamulin **5** is the first antibacterial agent of the pleuromutilin class, which shows excellent in vitro antibacterial activity and was approved in April 2007 by FDA for the topical treatment of the impetigo and secondarily infected traumatic lesions of skin infection, mainly caused by the Gram-positive bacterial *S. aureus* (MIC = 0.12 µg/mL) and *Streptococcus pyogenes* (MIC = 0.016

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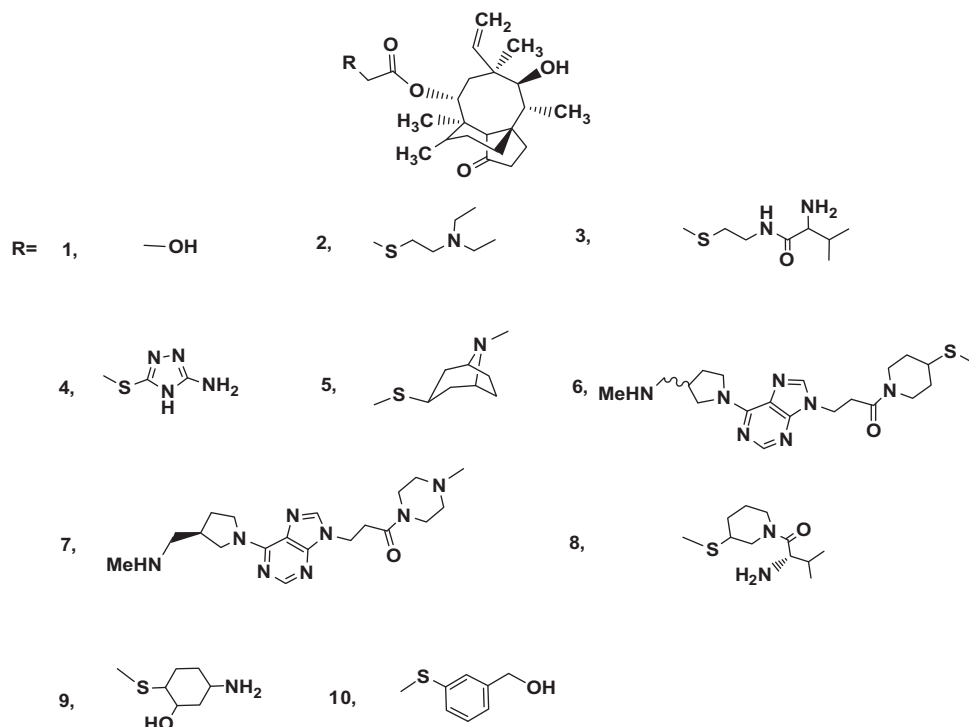


Figure 1. Important pleuromutilin derivatives.

$\mu\text{g/mL}$ ).<sup>10</sup> Furthermore, to overcome strong hydrophobic problem, Yoshimi H. et al designed and synthesized the water-soluble new pleuromutilin derivatives **6** and **7** with amino purine group, which not only showed excellent in vitro antibacterial activity against MRSA, PRSP, VRE, *S. pyogenes*, and *M. catarrhalis* but also potent in vivo efficacy (MIC = 0.016  $\mu\text{g/mL}$ ).<sup>11,12</sup>

In recent years, several other pleuromutilins derivatives BC-3205 (**8**), BC-3781 (**9**) and BC-7013 (**10**) have already entered Phase I clinical studies in volunteers, respectively. Especially BC-3781 **9** has become the second pleuromutilin derivative that entered Phase II clinical studies, demonstrating excellent antimicrobial activity against relevant bacteria, including MRSA (see Fig. 1).<sup>13–15</sup> It is very important for BC-3781 to be well tolerated in patients and to keep a proper plasma concentration level, indicating a therapeutic potential for the treatment of skin infections and bacterial lung, including community-acquired *bacterial pneumonia* (CABP). Nevertheless, there have been no pleuromutilin derivatives for human oral use on the market so far to the best of our knowledge.

To develop new pleuromutilin derivatives for human use, we were inspired by the fact that many natural products isolated from marine sources bearing a thiazole ring, for example, Leucomide A **11**<sup>16</sup> and myxothiazol **12**,<sup>17</sup> can exhibit various types of bioactivities, including antibacterial, anticancer, and antiviral activities. Meanwhile, some of the new cephalosporins with a thiazole ring moiety (e.g., cefpiromes **13** and claforan **14**) have exhibited higher bioactivities than the prototype compounds. Thus, we speculated that the pleuromutilin derivatives containing a thiazole group may demonstrate somewhat enhanced antibacterial activity as compared to pleuromutilin (see Fig. 2). Also, we noted the interaction detail demonstrated by the crystal structure of the *Deinococcus radio-durans* 50S ribosomal subunit in complex with tiamulin and valnemulin.<sup>18,19</sup> In this crystal structure, besides that the ribosomal peptidyl transferase center (PTC) with their tricyclic mutilin core located inside a tight pocket confined by nucleotide residues at the A-site of 50S ribosomal subunit, it is very important that their

side chains, C-14 extensions, pointing toward the P-site, which enhances the interactions with 23S rRNA. This observation led us to speculate that modifying the side chain with the 2-aminothiazole ring moiety may improve their antibacterial activities. With these ideas in mind, we designed and synthesized a new pleuromutilin derivative with 2-aminothiazole side arm (**15**) as our lead compound, along with a novel class of target compounds with different 2-(substituted amino) acetyl amide group (Fig. 3, **16a–g**).

The synthesis of the lead compound **15** and the target compounds **16a–g** is shown in Scheme 1. Pleuromutilin (**1**) was reacted with 4-toluene sulfonyl chloride (TsCl) in ether using 30% aqueous NaOH as the catalyst, to yield 14-deoxy-14-*P*-tosyloxyacetoxymutilin **17**, which was further treated with potassium iodide in acetone to give the iodide **18** in good yield. The key intermediate **15** was obtained by the nucleophilic attack of 2-aminothiazol-4-yl)methanethiol on **18** in the presence of 30% NaOH in tetrahydrofuran (THF).<sup>20</sup> Further treatment of the lead compound **15** with 2-chloroacetyl chloride in the presence of potassium carbonate gave the intermediate **19**, which was directly treated with various amines in the presence of KI and potassium carbonate, to afford the corresponding target compounds **16a–g** in yields of 46–58%, respectively. The target products were purified by column chromatography, and their structures were characterized by IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, MS, and elemental analysis.<sup>21</sup>

The antibacterial properties of the novel pleuromutilin derivatives **16a–g** were assessed in vitro by the conventional agar dilution method, using test agar against a panel of selected Gram-positive clinical bacteria (Table 1). The minimal inhibitory concentrations (MIC,  $\mu\text{g/mL}$ ) were determined in comparison with amoxicillin and tiamulin as positive controls. An overnight culture of bacteria in tryptose broth was diluted to about  $10^5$  cells/mL with the same broth, and inoculated with an inoculating device onto agar containing serial two-fold dilutions of the tested compounds. Organisms were incubated at 37 °C for 18–20 h.

The in vitro antibacterial activities of the new pleuromutilin derivatives **15** and **16a–g** prepared above against both Gram-positive

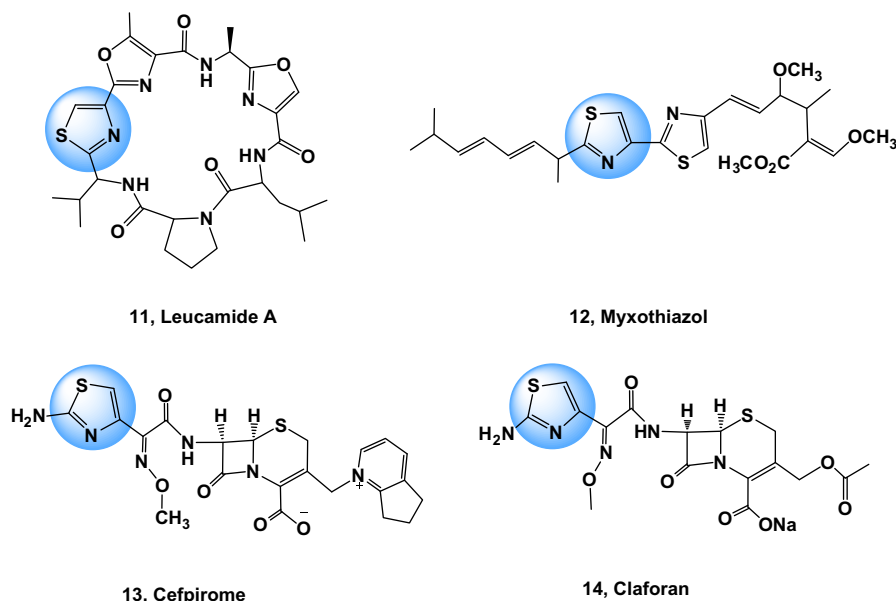


Figure 2. Some bioactive compounds containing thiazolyl group.

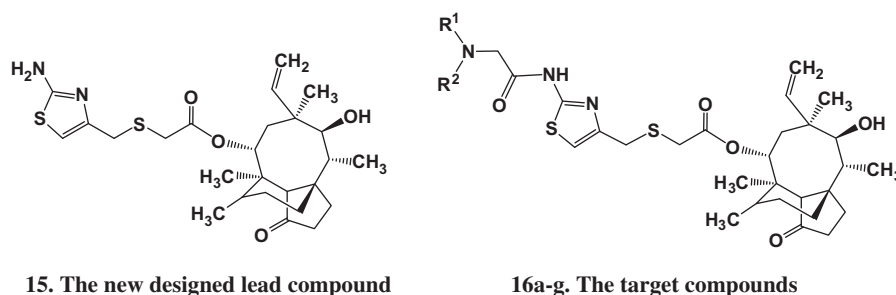


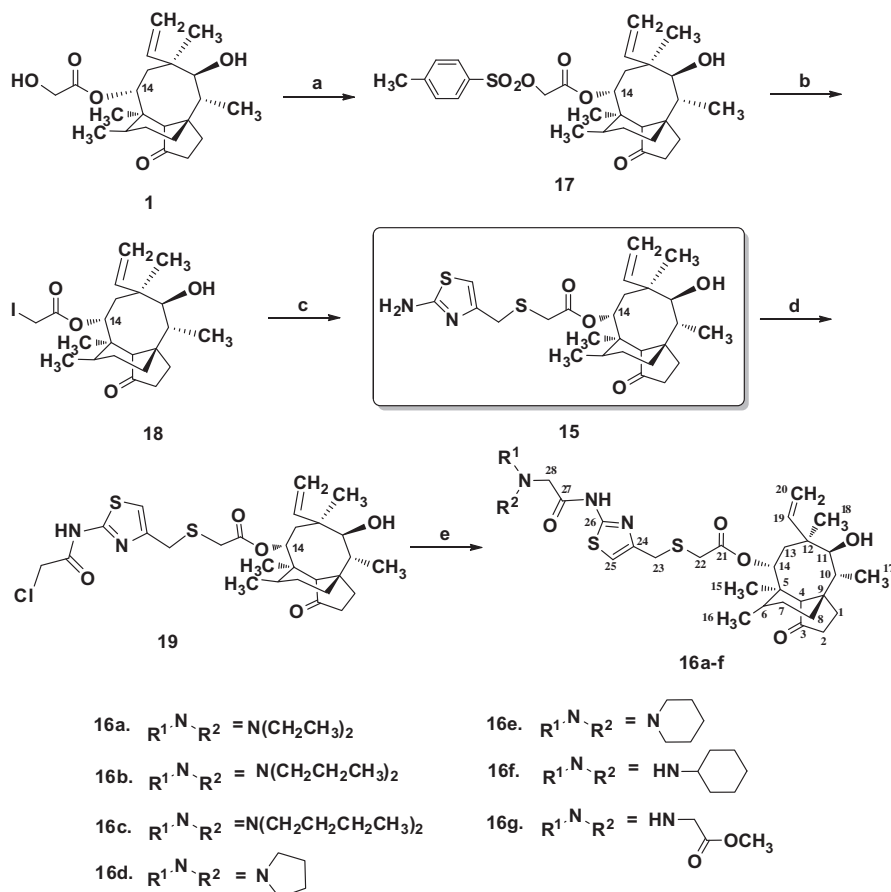
Figure 3. Novel pleuromutilin derivatives synthesized in the present work.

organisms, including methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus epidermidis* (MSSE), and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are listed in Table 1. For comparison, the MIC values of amoxicillin and tiamulin are also listed.

The data in Table 1 showed that most of the tested compounds displayed superior or similar antibacterial activities to those of amoxicillin and tiamulin. In particular, the key intermediate compound **15** with the (2-aminothiazol-4-yl)methyl thioether group at C-14 position was found 4–8-fold more potent than amoxicillin and tiamulin against most of the selected Gram-positive organisms. For examples of the activity data to the four MRSAs, amoxicillin MIC = 16, 0.125, 16, and 32  $\mu\text{g/mL}$ , tiamulin MIC = 2, 0.5, 2, and 2  $\mu\text{g/mL}$ , compound **15** MIC = 0.25, 0.0625, 0.5, and 0.5  $\mu\text{g/mL}$ , respectively. These data indicate that pleuromutilin derivatives with thiazole group on the side chain at C-14 position can indeed demonstrate higher antibacterial activity against some organisms than their prototype compounds. Especially, compound **16e** having piperidinyl groups not only showed a similar activity as compared with compound **15** against MRSA, but also demonstrated the best activity (MIC = 0.0625–2  $\mu\text{g/mL}$ ) against MRSE, MSSE and MSSA, which is almost 2-fold more potent than compound **15**, 8–16-fold more potent than amoxicillin (MIC = 0.125–8  $\mu\text{g/mL}$ ), and tiamulin (MIC = 0.0625–8  $\mu\text{g/mL}$ ) against the same infections pathogens, respectively.

An examination of the structure–activity relationship (SAR) for compounds with different 2-(substituted amino) acetyl amide group on the thiazole ring **16a–g** revealed significant differences in their antibacterial activities. Firstly, the activities of compounds **16a–c** were found to decrease with the increasing steric bulkiness in the substituent amino group (bioactivities: **16a** > **16b** > **16c**). Especially, in case of **16c**, bulky *n*-butyl group led to a significant loss in antibacterial activity. This observation suggests that the C-14 extensions of the compound with a bulky substituent group pointing toward the P-site may not be favorable for its bioactivities. Secondly, the bioactivity of the compound with a secondary amino substituent group **16f–g** was a little lower than that of their counterparts with a tertiary amino group **16a–e**. It is very interesting to note that compound **16e** having a piperidine group showed the best activity against all the Gram-positive organisms. These results revealed that the pleuromutilin derivatives with the thiazole group in the side chain at C-14 position can demonstrate very promising bioactivities against Gram-positive organisms, and therefore, will provide a new insight into the design of novel antibacterial drugs.

To explore the antibacterial mechanism of these novel pleuromutilin derivatives, two tested compounds with excellent bioactivities, **15** and **16e**, and two representative compounds tiamulin and **6**, were chosen for molecular docking studies. The X-ray crystal structure of ribosome–Tiamulin complex (PDB code: 1XBP) with a resolution of 3.50 Å was applied to build the starting model of



**Scheme 1.** Reaction conditions and reagents: (a) TsCl, 30% NaOH, ether, reflux, 2hr, 60%; (b) KI, acetone, reflux, 3 h, 87%; (c) (2-aminothiazol-4-yl)methanethiol, 10% NaOH, THF, 0 °C to r.t., 4 h, 72%; (d) 2-chloroacetyl chloride,  $K_2CO_3$ ,  $CH_2Cl_2$ , 0 °C to r.t., 1 h, 92%; (e)  $R^1R^2NH$ ,  $K_2CO_3$ , THF, 40 °C, 4–8 h, 53%–65%.

**Table 1**  
Antibacterial activity (MIC,  $\mu g/mL$ ) of pleuromutilin derivatives (**16a–g**) in vitro

Strains <sup>b</sup>	MIC <sup>a</sup> ( $\mu g/mL$ )									
	Amoxicillin	Tiamulin	15	16a	16b	16c	16d	16e	16f	16g
MSSA-cpu0211	4	2	<b>0.5</b>	<b>1</b>	<b>0.5</b>	4	0.125	<b>0.125</b>	<b>0.25</b>	<b>0.25</b>
MSSA-cpu0587	2	8	<b>2</b>	8	<b>0.5</b>	4	0.125	<b>2</b>	0.25	0.25
MSSA-cpu1044	1	8	2	1	<b>0.5</b>	4	0.25	<b>0.125</b>	0.5	0.5
MSSA-cpu1344	1	2	<b>0.125</b>	0.25	1	16	0.25	<b>0.0625</b>	0.5	0.5
MRSA-cpu0446	4	2	<b>0.25</b>	<b>0.5</b>	<b>0.5</b>	8	0.25	<b>0.5</b>	0.5	1
MRSA-cpu0887	0.125	0.5	<b>0.0625</b>	0.25	0.5	32	0.5	<b>0.0625</b>	0.5	1
MRSA-cpu1098	4	2	<b>0.5</b>	<b>0.5</b>	1	8	0.25	<b>0.25</b>	0.25	0.5
MRSA-cpu3256	8	2	<b>0.5</b>	2	1	8	0.25	<b>0.25</b>	0.5	0.5
MSSE-cpu0315	0.125	0.0625	<b>0.125</b>	0.25	8	8	2	<b>0.0625</b>	1	4
MSSE-cpu0347	8	0.5	<b>0.125</b>	<b>0.25</b>	0.5	8	0.25	<b>0.0625</b>	0.5	0.5
MSSE-cpu2089	0.125	0.5	<b>0.125</b>	0.25	0.5	4	0.125	<b>0.125</b>	0.25	0.25
MSSE-cpu1561	0.125	0.0625	<b>0.125</b>	0.25	4	16	0.5	<b>0.0625</b>	8	1
MRSE-cpu2623	8	1	<b>0.125</b>	<b>0.25</b>	<b>0.5</b>	4	0.5	<b>0.0625</b>	0.25	0.5
MRSE-cpu3889	8	1	<b>0.125</b>	2	2	16	0.25	<b>0.0625</b>	1	1
MRSE-cpu3569	8	0.25	<b>0.125</b>	0.25	4	8	0.5	<b>0.0625</b>	0.5	0.5
MRSE-cpu2736	0.125	0.0625	<b>0.125</b>	0.25	2	16	0.5	<b>0.0625</b>	1	0.5

<sup>a</sup> Minimal inhibitory concentrations (MIC): the lowest concentration of compound that inhibits visible growth of the organism.

<sup>b</sup> All of strains were isolated from the clinical bacteria in the Nanjing Gulou hospital and reserved in Department of Microbiology, College of Life Science and Technology, China Pharmaceutical University. Clinical strains were identified by the API bacteria analysis system.

50S ribosomal subunit from *Deinococcus radiodurans*. The active sites were calculated with the 'site finder' function of MOE.

Compound **15** was found to form two strong hydrogen bonds with ribosome in the docking studies, as shown in Figure 4. Specifically, the hydroxyl group of **15** (on C11) formed one H-bond with the oxygen atom of G2484 (OH/O distance: 1.42 Å), and the NH<sub>2</sub>

group on the thiazole ring of **15** formed the second H-bond with the hydroxyl group of residue C2565 (NH/O distance: 1.57 Å). Moreover, there was also a  $\pi$ - $\pi$  stacking interaction between thiazole ring of **15** and the purine ring of residue A2045, and ring to ring distance was 4.27 Å. The binding free energy of **15** with ribosome was calculated to be -12.51 kcal/mol.

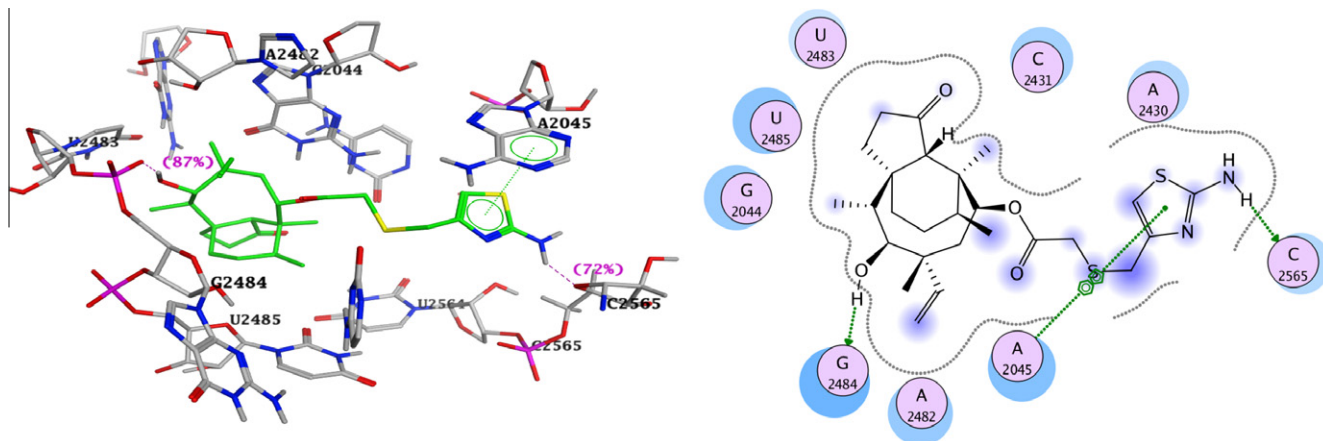


Figure 4. Docking models of compound **15** in the active site of the 50S ribosomal subunit.

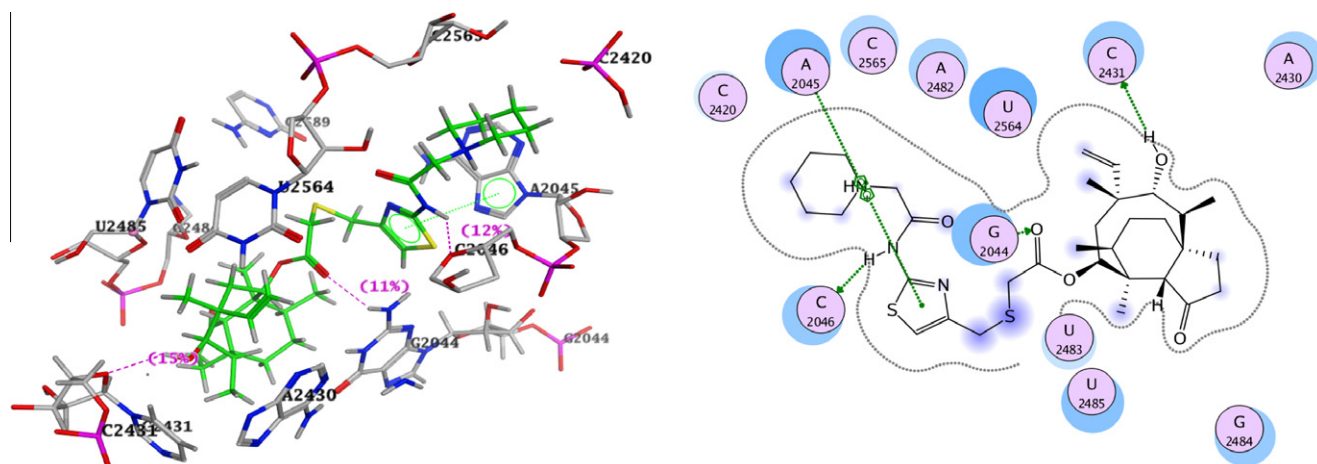


Figure 5. Docking models of compound **16e** in the active site of the 50S ribosomal subunit.

Compound **16e** with the best antibacterial activity also demonstrated a binding mode similar to that of **15**, as shown in Figure 5. In this case, there were three hydrogen bonds observed, through the interaction of **16e** with C-2431 (OH/O distance: 2.37 Å), G2044 (NH<sub>2</sub>/O distance: 2.89 Å), and C2046 (NH/O distance: 2.33 Å) respectively. A prominent  $\pi$ - $\pi$  stacking interaction between the thiazole ring of **16e** and the purine ring of A2045 with ring to ring distance of 4.61 Å was also found in docking mode. Compared to **15**, compound **16e** had an advantage on the number of hydrogen bonds formed with ribosome. Moreover, the higher binding free energy of **16e** with ribosome (−14.82 kcal/mol) was lower than that of **15**. This may explain why the antibacterial activity of **16e** was better than that of **15**.

We also performed the docking studies of the benchmark compound tiamulin and **6** with ribosome. From the docking result, both tiamulin and **6** exhibited a docking mode similar to that of **15** or **16e** in general (Figs. 6 and 7). An important difference was that no  $\pi$ - $\pi$  stacking interaction between tiamulin and ribosome was found, and only two hydrogen bonds with G2484 (OH/H distance: 1.32 Å) and U2564 (NH/O distance: 2.00 Å) were formed respectively. This may be the reason that tiamulin had the weaker antibacterial activity than that of the other three compounds **6**, **15**, **16e**, although its binding free energy was up to −13.49 kcal/mol.

On the contrary, there were three  $\pi$ - $\pi$  stacking interactions between the purine rings of **6** and A2045, and their ring to ring distances were 4.07 Å, 4.40 Å and 4.13 Å respectively, while

compound **15** and **16e** had only an  $\pi$ - $\pi$  stacking interaction with residue A2045 respectively besides the hydrogen bonds interactions with residues (as seen in Fig. 4~5). The binding free energy of **6** with ribosome was equal to −18.97 kcal/mol and seemed to testify the conclusion that the  $\pi$ - $\pi$  interactions between the heterocyclic moiety in the side chain of the compounds and nucleotide residues of ribosome may improve further its anti-bioactivity.

In a word, all these results also indicated that the side chains of the pleuromutilin derivatives played an important role in fine-tuning the physical-chemical properties of the molecule, and importantly, amino thiazole ring in general can be adopted near the mutilin core in the binding pocket and used to further improve the binding affinity.

In summary, via the introduction of (2-aminothiazol-4-yl)-methyl thioether group to the C-14 position of pleuromutilin, we have designed and synthesized new classes of pleuromutilin derivatives with different 2-(substituted amino) acetyl amide group on the thiazole ring in the side chain, and evaluated their antibacterial activities in vitro against Gram-positive clinical bacteria. Most of these compounds were found to display strong antibacterial activities against the methicillin-susceptible and methicillin-resistant bacteria. Particularly noteworthy are compound **15** and its derivative **16e**, both showed potent antibacterial properties (0.0625–0.5 µg/mL) that are superior to those of amoxicillin or tiamulin. The related activity data have been primarily rationalized, to some extent, by docking studies using MOE. These results may provide



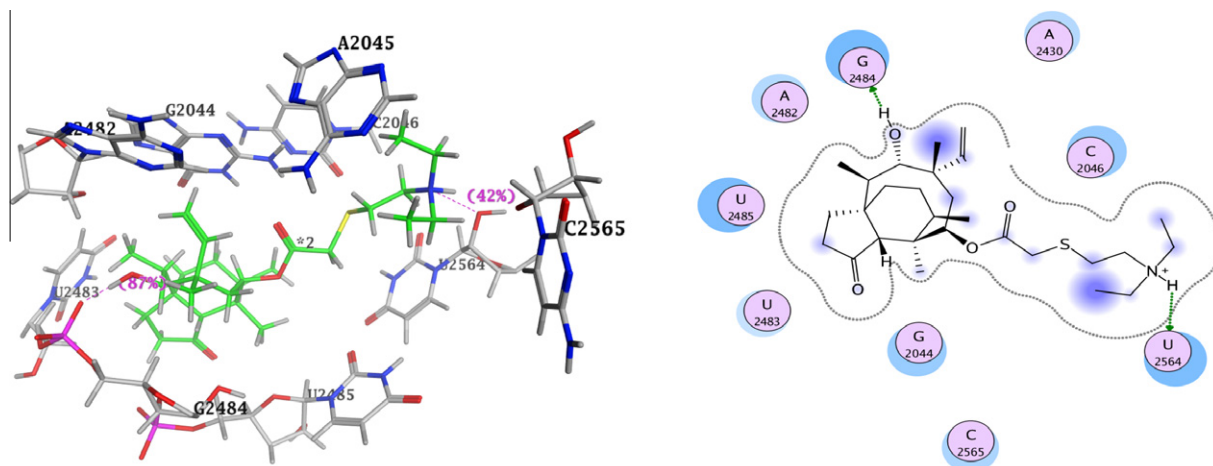


Figure 6. Docking models of tiamulin in the active site of the 50S ribosomal subunit.

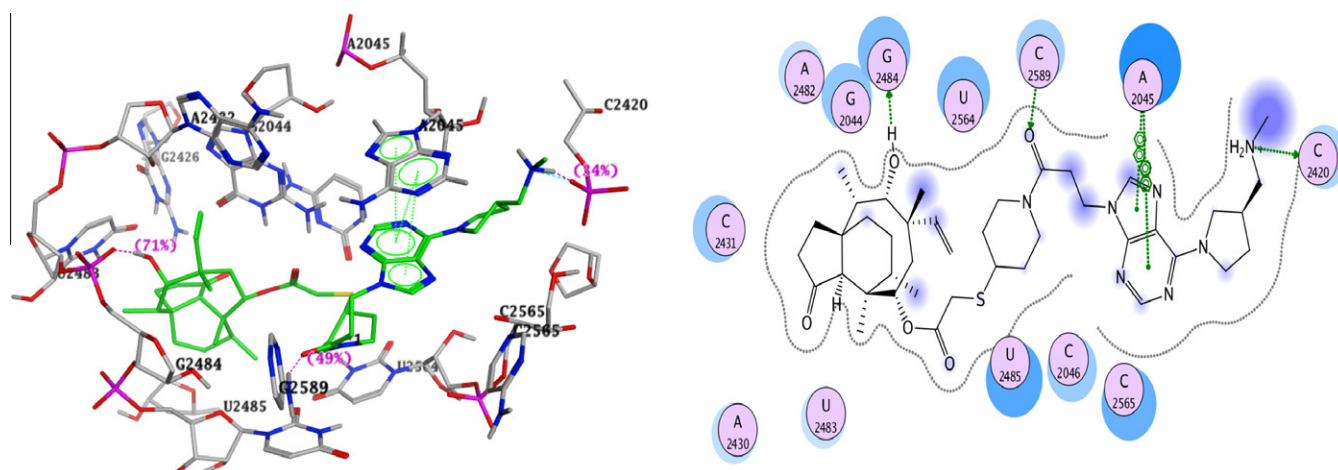


Figure 7. Docking models of compound 6 in the active site of the 50S ribosomal subunit.

new insights into the design of novel pleuromutilin derivatives, and lay the basis for further studying on the promising antibiotics for human clinical use.

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- The synthesis of the lead compound 15: To a solution of 2-aminothiazol-4-yl)methanethiol (0.33 g, 2.24 mmol) in 2 mL 10% aqueous NaOH solution, compound 18 (1.00 g, 2.04 mmol) in 25 mL THF was added dropwise. The reaction mixture was stirred in the ice bath for 1 h. After the reaction was completed, the solvent was removed under reduced pressure. The crude residue was dissolved in ethyl acetate, and the resulting solution was washed sequentially with water and brine, dried over  $\text{MgSO}_4$ , filtered and concentrated, before being purified by column chromatography on silica gel (PE/EtOAc = 5:1–1:1) to yield 15 as a white solid (0.75 g, 72%). M.p.: 67–70 °C. Analytical data for 15: IR (KBr,  $\text{cm}^{-1}$ ): 3436.30, 3353.02, 2984.70, 2856.58,

1716.37, 1617.08, 1524.02, 1280.78, 685.05;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  6.50 (1H, dd,  $J_1 = 11.2$ ,  $J_2 = 17.6$ , H19, 6.33 (1H, s, H25), 5.77 (1H, d,  $J = 8$ , H14), 5.37 (1H, d,  $J = 11.2$ , H20), 5.23 (1H, d,  $J = 17.2$ , H20), 3.67 (2H, s, H23), 3.08 (2H, s, H22), 3.38 (1H, m, H11), 2.38 ~ 2.05 (5H, m, H2, H4, H10, H13), 1.83 ~ 1.31 (11H, m, H1, H6, H7, H8, H15, H13, 11-OH), 1.19 (3H, m, H, H18), 0.89 (3H, d,  $J = 6.8$ , H17), 0.74 (3H, d,  $J = 6.8$ , H16);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  217.12 (C3), 168.82 (C21), 168.18 (C26), 147.13 (C24), 139.10 (C19), 117.27 (C20), 105.89 (C25), 74.82 (C11), 69.16 (C14), 58.20 (C4), 45.46 (C22), 44.76 (C9), 43.9 (C13), 41.73 (C12), 36.78 (C5), 36.00 (C6), 34.48 (C10), 32.80 (C2), 31.77 (C23), 30.42 (C8), 26.84 (C7), 26.39 (C18), 24.85 (C1), 16.90 (C16), 14.99 (C15), 11.58 (C17); ESI-MS ( $m/z$ ): 507  $[\text{M}+\text{H}]^+$ ; Anal. Calcd for  $\text{C}_{26}\text{H}_{38}\text{N}_2\text{O}_4\text{S}_2$ : C, 61.63; H, 7.56; N, 5.53; Found: C, 61.41; H, 7.81; N, 5.46.

21. **General procedure for the synthesis of the target compounds 16a–g:** To a mixture of compound **15** (1.00 g, 1.98 mmol) and  $\text{K}_2\text{CO}_3$  (0.41 g, 2.96 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (20 mL), a solution of  $\text{ClCH}_2\text{COCl}$  (0.33 g, 2.96 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (5 mL) was added drop wise at 0 °C. The reaction mixture was stirred for 2 h. After completion of the reaction, the solution was washed with water for three times, and the organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered, concentrated, and purified by column chromatography (PE/EtOAc = 3:1) to yield **19**. A solution of compound **19** (1.72 mmol),  $\text{R}^1\text{R}^2\text{NH}$  (8.60 mmol), and  $\text{K}_2\text{CO}_3$  (5.16 mmol) in 20 mL THF was stirred at 40 °C for 4–8 h. The solvent was removed under

reduced pressure. The crude residue was dissolved in chloroform and washed with saturated NaCl solution. The organic layer was dried with  $\text{MgSO}_4$ , filtered, concentrated, and purified by column chromatography (PE/EtOAc = 2:1–1:4) to yield **16a–g**.

**Analytical data for selected compounds 16e:** yield 65%, white foam solid, IR (KBr,  $\text{cm}^{-1}$ ): 2936.22, 2861.18, 1732.36, 1529.90, 1453.83, 1420.38, 1374.36, 1327.54, 1279.07, 1154.30, 1115.30, 1017.60;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  10.42 (1H, br, NH), 6.79 (1H, s, H25), 6.51 (1H, dd,  $J_1 = 10.8$  Hz,  $J_2 = 17.2$  Hz, H19), 5.79 (1H, d,  $J = 8.4$  Hz, C14), 5.39 (1H, d,  $J = 10.8$  Hz, C20), 5.24 (1H, d,  $J = 17.6$  Hz, C20), 3.83 (2H, s, H23), 3.38 (1H, m, H11), 3.18 (2H, s, H22), 3.06 (2H, s, H28), 2.52 (4H, br,  $2 \times \text{NCH}_2$ ), 2.39 ~ 2.05 (5H, m, H2, H4, H10, H13), 1.80 ~ 1.19 (20H, m, H1, H6, H7, H8, H15, H13, 11-OH, H18,  $3 \times \text{CH}_2$ ), 0.89 (3H, d,  $J = 6.8$  Hz, H17), 0.75 (3H, d,  $J = 6.8$  Hz, H16);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  217.10 (C3), 168.64 (C21), 157.83 (C27), 146.17 (C26), 139.08 (C24), 117.30 (C19), 111.32 (C20), 107.03 (C25), 74.61 (C11), 69.19 (C14), 61.89 ( $\text{CH}_2\text{NCH}_2$ ), 58.17 (C4), 56.68 (C28), 45.44 (C22), 45.38 (C9), 43.89 (C13), 41.72 (C12), 36.78 (C5), 35.99 (C6), 34.46 (C10), 32.74 (C2), 31.65 (C23), 30.41 (C8), 26.84 (C7), 26.36 (C18), 25.85 ( $2 \times \text{NCH}_2\text{CH}_2$ ), 24.84 (C1), 23.42 ( $\text{NCH}_2\text{CH}_2\text{CH}_2$ ), 16.91 (C16), 14.94 (C15), 11.56 (C17); ESI-MS ( $m/z$ ): 632  $[\text{M}+\text{H}]^+$ ; Anal. Calcd for  $\text{C}_{33}\text{H}_{49}\text{N}_3\text{O}_5\text{S}_2$ : C, 62.73; H, 7.82; N, 6.65; Found: C, 62.67; H, 7.80; N, 6.58.