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Synthesis and Biological Evaluation of Novel Thioether Pleuromutilin Derivatives

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To develop new pleuromutilin derivatives as veterinary antibiotic medicines, we designed and synthesized a series of new thioether pleuromutilin derivatives possessing acylthiazolyl moiety based on previously designed derivatives. The antibacterial properties of the prepared pleuromutilin derivatives were assessed *in vitro* by the broth dilution method against five kinds of bacteria and the mycoplasma *Mycoplasma gallisepticum* (MG). All of the tested compounds displayed moderate to good antibacterial activity to methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-sensitive *Staphylococcus epidermidis* (MSSE), methicillin-resistant *S. aureus* (MRSA), *Streptococcus agalactiae* (*S. aga*) and MG. However, the activity to *Pyogenicoccus* (*Pyogens*) was generally poor. Compounds 13i and 1 showed potent antibacterial activity against MSSE and MRSA which are better than that of valnemulin. The structural modification for pleuromutilin affected the antibacterial activity. Amino substituents in the benzene ring can effectively improve activity. Compared with the analogue 13a that possesses unsubstituted benzoyl group, the nitro, methoxy, hydroxy and dichloro substituent contributed little to antibacterial activity. Increasing a methylene between benzene moiety and carbonyl group decreased the bioactivity of derivative. The analogues that obtained by the reaction of amino acids and intermediate 9 showed moderate activity.

Key words pleuromutilin derivative; antibacterial activity; acylation reaction; 2-aminothiazol

In 1951, Kavanagh first isolated the fused 5-6-8 tricyclic diterpenoid from *Clitopilus scyphoides* and *Pleurotus passckerianus* and named it as pleuromutilin (**1**)^{1,2)} (Fig. 1). Compound **1** was characterized as antibiotic with modest *in vitro* activity against Gram-positive bacteria and mycoplasmas.^{3,4)} Further structural studies of complexes of ribosomal subunits with pleuromutilin derivatives suggested that this class of antibiotics selectively inhibited the synthesis of bacterial protein by interaction with the 23S ribosomal RNA (rRNA) of the 50S bacterial ribosome subunit. Thus, this class of antibiotics prevents the correct positioning of the tRNA for peptide transfer and inhibits peptide bond formation.^{5,6)} The specific interactions of pleuromutilin drugs with the ribosome lead to the difficulty of pleuromutilin to produce cross resistance with other antimicrobial agents. Subsequently, Davidovich *et al.* found that these compounds bind to the peptidyl transferase center (PTC) with their core at A-site and their C14 extensions pointing towards the P-site. The C11 hydroxyl group was located in a position suitable for hydrogen bonding to G2505 phosphate, and the C21 keto group of the C14 extension was involved in two to three H-bonds with G2061.⁷⁾

Since pleuromutilin was determined to have good antibacterial activity, much synthetic effort had been done by using it as the lead compound. In 1976, Egger and Reinshagen synthesized 66 derivatives of pleuromutilin and tried to observe the relationship between the structure and antibacterial activity. Their group concluded that the carbonyl group of the five-membered ring and the C11-OH were necessary activity functional groups, but there was no effect on the antibiotic activity whether the exocyclic vinyl group was hydrogenated or not. At the same time, the glycolate ester at 14 position

was also necessary because the activity disappeared if pleuromutilin was transformed into mutilin with deacylation. Their group also suggested that the modifications of C14 side chain with a thioether group and a basic group offered the best bioactivities.^{8,9)} Subsequently, tiamulin (**2**) and valnemulin (**3**) were successfully developed as the veterinary antibiotic medicine.^{3,10)} As the first pleuromutilin class developed for human use, azamulin (**4**) is safe and has good antibacterial activity. Unfortunately, poor water solubility limits the bioavailability of azamulin and it has a strong irreversible inhibition effect on CYP P450, which leads to discontinued clinical research.¹¹⁾ Retapamulin (**5**), which was developed by GlaxoSmithKline in 2007, is the first pleuromutilin drug for human use as a topical antimicrobial agent to treat impetigo and secondarily infected traumatic lesions of skin infections.¹²⁾

More recently, BC-3205 (**6**), BC-7013 (**7**) and BC-3781 (**8**) are the new derivatives of pleuromutilin reported by Nabriva Therapeutics in the clinical research stage.¹³⁾ In 2011, **8** has successfully completed Phase II clinical trials and may be developed as the first systemic pleuromutilin antibiotic to treat acute bacterial skin and skin structure infections (ABSSSI).¹⁴⁾ In summary, all of these compounds either licensed or in clinical trials were attached with the different basic group through the thioether bond in C14 side chain. In 2012, Wang *et al.* reported a series of derivatives that were synthesized using 14-*O*-(2-aminothiazol)-4-methyl thioether mutilin (**9**) as the key intermediate.¹⁵⁾ These compounds had shown good antibacterial activity to methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-sensitive *Staphylococcus epidermidis* (MSSE), which were isolated from clinical bacteria. In 2014, Shang *et al.* reported a series of derivatives possess-

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Fig. 1. Structure of Pleuromutilin Derivatives and NTZ

ing substituted benzeneacyl group on the thiadiazole moiety of the 14-position side chain. Compound with *meta* amino substituted in the benzene ring showed the best antibacterial derivative among substituted benzeneamide derivatives.¹⁶ Derivatives with an amino group on the terminal C14 side chain presented improved activity.¹⁷ In addition, some efforts were focused on structural modification of valnemulin, one kinds of derivatives *via* modification the amino of dimethyl cysteine moiety of C14 side chain by general heterocyclic acid or chloromate or substituted benzene carboxylic acid.^{18,19} Shang *et al.* found that the stronger resonance effect of substituents, like methoxy or amino, the more excellent antibacterial activity.¹⁸ Xu *et al.* reported this kind of derivatives with furamide or pyrazolecarboxamide with excellent bioactivity.¹⁹ Ling *et al.* systematically described the activity of pleuromutilin derivatives possessing different heterocyclic group on C14 side chain and the bioactivity data showed that derivatives containing pyridine ring exhibited the best activity. And a heterocyclic ring at the C14 position of pleuromutilin derivatives may increase hydrogen bonding and π - π stacking interactions and thereby derivatives have strong antibacterial properties.^{15,20}

Nitazoxanide (NTZ) is a broad-spectrum anti-infective and antiviral agent,²¹ has a 2-acetyloxybenzamide-thiazole structure. We concerned whether the anti-infective activity could be improved when NTZ was used as a substituent at the C14 position to modify pleuromutilin. Therefore, combined with the above mentioned ideas, we designed some novel analogues by using compound 9 as a lead compound. We expected that derivatives containing aromatic amide group and heterocyclic ring will improve their activity through their resonance effect. Derivative that possesses 2-hydroxybenzamide group attached on thiazole is similar to that of NTZ with extended antimicro-

bial spectrum and better activity. In addition, we designed and synthesized three derivatives, which were obtained by the reaction of amino acid and intermediate 9. They have an amino group on C-14 side chain may improve bioactivity through the similar effects as tiamulin and valnemulin. A total of 15 new derivatives were synthesized by the amino of thiazol acylation reaction¹⁸ with a series of aromatic carboxylic acids, heterocyclic carboxylic acids and amino acids. The *in vitro* antibacterial activities of the prepared compounds were assessed by the broth dilution method against five bacteria, with erythromycin, tiamulin and valnemulin as positive controls. The control drug for *Mycoplasma gallisepticum* (MG) was tylosin instead of erythromycin.

MATERIALS AND METHODS

General Melting point was measured on a WRR-Y drug melting point instrument (Shenguang, China); the thermometer reading was not corrected. IR spectra were obtained by a Nicolet IS10 spectrometer (Thermo Fisher Scientific, U.S.A.) with KBr thin films. ¹H-NMR spectra were obtained on an Avance 600/400MHz spectrometer (Bruker, Switzerland). ¹³C-NMR data were collected on an Avance 150/100MHz instrument (Bruker) with complete proton decoupling. The chemical shift values (δ) were expressed in parts per million (ppm). The internal standard was tetramethylsilane (TMS). High-resolution (HR)-MS data were obtained on Q-TOF (Waters & Bruker, U.S.A. and Switzerland). All reagents and solvents (AR) were purchased from the Sinopharm Chemical Reagent and Aladdin Reagent companies without further purification.

Chemistry The synthetic route of the target compounds

(a) TsCl, Et₃N, CH₂Cl₂, r.t., 22 h; (b) KI, acetone, reflux, 1.5 h; (c) *S*-(2-amino-4-thiazolylmethyl)-isothioureia dihydrochloride, 10% NaOH, THF, -5°C, 2 h; (d) carboxylic acid, EDCI and TEA or DCC, HOBT, CH₂Cl₂, 32–48 h; (e) TFA, CH₂Cl₂, r.t., 0.5 h.

Chart 1. Reaction Conditions and Reagents

13a–o is shown in Chart 1. First, pleuromutilin (**1**) reacts with 4-toluene sulphonyl chloride in the presence of triethylamine (TEA) to afford 14-*O*-(*p*-toluene sulphonyloxyacetyl) mutilin (**11**). Iodide **12** is obtained with good yield by the nucleophilic attack of potassium iodide on **11**. Compound **12** reacts with *S*-(2-amino-4-thiazolylmethyl)-isothioureia dihydrochloride in the presence of 10% NaOH aqueous solution in tetrahydrofuran (THF) to afford **9**.^{22,23} Compound **9** is further treated with carboxylic acid or *N*-*tert*-butoxy-carbonyl (Boc) protected amino acid in presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) to give the compounds **13a–g**, **13j–l** and **14h–i**, **14m–o** at room temperature in CH₂Cl₂. Compound **14h–i**, **14m–o** is further treated with trifluoroacetic acid (TFA) in CH₂Cl₂ and turns into the final compound **13h–i**, **13m–o**. Compound **13g** was got from acetosalicylic acid instead of salicylic acid by using the general method without extra possessing. All of the target products were purified by column chromatography; their structures were characterized by IR, ¹H-NMR, ¹³C-NMR and HR-MS.

Synthesis

14-*O*-(*p*-Toluene Sulphonyloxyacetyl) Mutilin (**11**)

Pleuromutilin (10.00 g) and TEA (2.95 g, 1.1 eq) were dissolved in 80 mL CH₂Cl₂. 4-Toluene sulphonyl chloride (5.53 g, 1.1 eq) was added to the above mentioned solution by batch.

The mixture was stirred at room temperature for 22 h. The reaction mixture was washed with brine, dried with anhydrous sodium sulphate, filtered and evaporated in a vacuum. After recrystallization with ethyl acetate (EA):petroleum ether (PE)=1:1 (v/v), compound **11** was produced (8.44 g, 60%). mp 144.6–146.3°C. ¹H-NMR (CDCl₃) δ: 7.74 (d, *J*=7.7 Hz, 2H), 7.28 (d, *J*=7.9 Hz, 2H), 6.34 (dd, *J*=17.4, 11.0 Hz, 1H), 5.70 (d, *J*=8.6 Hz, 1H), 5.27 (d, *J*=11.7 Hz, 1H), 5.12 (d, *J*=17.4 Hz, 1H), 4.48–4.34 (m, 2H), 3.27 (dd, *J*=10.5, 6.6 Hz, 1H), 2.38 (s, 3H), 2.24–2.08 (m, 3H), 2.02–1.94 (m, 2H), 1.69 (dd, *J*=14.5, 2.0 Hz, 1H), 1.61–1.54 (m, 2H), 1.52 (s, 3H), 1.46–1.35 (m, 3H), 1.34 (s, 3H), 1.28 (dd, *J*=14.4, 3.0 Hz, 1H), 1.16 (dd, *J*=22.2, 11.1 Hz, 1H), 1.10–1.00 (m, 4H), 0.80 (d, *J*=6.9 Hz, 3H), 0.55 (d, *J*=7.0 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.74, 164.86, 145.28, 138.64, 132.56, 129.89, 128.07, 117.40, 74.50, 70.24, 65.00, 57.99, 45.36, 44.46, 43.93, 41.80, 36.51, 35.98, 34.37, 30.30, 26.73, 26.33, 24.78, 21.67, 16.51, 14.73, 11.46. IR (KBr) cm⁻¹: 3447, 2941, 1732, 1453, 1361, 1307, 1117, 1035, 832, 664, 560. MS *m/z*: 577.2 [M–H][–].

14-*O*-I-Iodide Mutilin (**12**)

Compound **11** (3.00 g) was reacted with potassium iodide (1.07 g, 1.18 eq) in 100 mL acetone with refluxing for 1.5 h. After the reaction was completed, the reaction solution was concentrated. The residue was dissolved with CH₂Cl₂ and

washed three times with a small amount of water. The organic phase was separated, dried, filtered and evaporated in a vacuum to obtain the white solid **12** (1.10 g) without purification, with a yield of 87%. mp 112.2–113.3°C. IR (KBr) cm^{-1} : 3585, 3301, 2989, 2933, 1735, 1711, 1454, 1278, 1084, 1023, 985, 976. MS m/z : 533.1 $[\text{M}-\text{H}]^-$ (Calcd for $\text{C}_{29}\text{H}_{40}\text{NO}_7\text{S}$: 532.6981).

14-*O*-(2-Aminothiazol)-4-methyl Thioether Mutilin (**9**)

2-Amino-4-methyl thiazolyl isothiurea dihydrochloride (1.00 g) and 10 mL water were added to the flask with three necks. The mixture was cooled in an ice-water bath. A solution of **12** (2.00 g/50 mL THF) was dropped into the mixture, followed by adding 6 mL of a 10% NaOH aqueous solution. The reaction mixture was stirred for 2 h after the dropwise addition. TLC monitored the end of the reaction. The aqueous layer was identified, whereas the organic phase was removed under decompression. A white foam of crude **9** was obtained (yield of 93%), which used in the next step without further purification. mp 79.9–83.1°C. $^1\text{H-NMR}$ (CDCl_3) δ : 6.50 (dd, $J=17.4$, 11.0 Hz, 1H), 6.33 (s, 1H), 5.78 (d, $J=8.4$ Hz, 1H), 5.37 (dd, $J=11.0$, 1.3 Hz, 1H), 5.23 (dd, $J=17.4$, 1.4 Hz, 1H), 3.68 (s, 2H), 3.46–3.32 (m, 1H), 3.09 (s, 2H), 2.43–2.32 (m, 1H), 2.29–2.16 (m, 2H), 2.14–2.05 (m, 2H), 1.79 (dd, $J=14.4$, 2.7 Hz, 1H), 1.72–1.61 (m, 2H), 1.58 (dd, $J=18.3$, 7.4 Hz, 2H), 1.51–1.42 (m, 4H), 1.42–1.32 (m, 2H), 1.24–1.05 (m, 4H), 0.90 (d, $J=7.0$ Hz, 3H), 0.75 (d, $J=6.9$ Hz, 3H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 217.07, 168.83, 168.20, 147.27, 139.21, 117.19, 105.78, 74.67, 69.26, 58.23, 45.48, 44.85, 43.96, 41.78, 36.81, 36.05, 34.48, 32.96, 31.84, 30.46, 26.87, 26.46, 24.87, 16.87, 14.97, 11.52. IR (KBr) cm^{-1} : 3431, 3336, 3199, 3119, 2933, 1724, 1617, 1526, 1281, 1115, 1017, 802, 691. FAB-MS m/z : 507.2342 $[\text{M}-\text{H}]^-$ (Calcd for $\text{C}_{26}\text{H}_{39}\text{N}_2\text{O}_4\text{S}_2$: 507.2351).

General Procedure for the Synthesis of Compounds

13a–o A mixture of carboxylic acid (2 eq), DCC (0.41 g, 2 eq), HOBT (0.27 g, 2 eq) and CH_2Cl_2 (15 mL) was stirred at room temperature for 1 h. Compound **9** (0.5 g, 1 eq) was added into the mixture, which was continuously stirred for 48 h. The mixture was filtered to remove insoluble solids and the filtrate was concentrated under vacuum. The crude residue was purified by silica gel column chromatography to afford the pure desired compounds **13a–g**, **13j–l** and **14h–i**, **14m–o**. Compounds **14h–i** and **14m–o** (0.50 g) were treated with a mixture of 4 mL TFA and 10 mL dichloromethane at room temperature for half an hour. The reaction was quenched with saturated aqueous NaHCO_3 . The organic phase was separated, washed with water, dried with anhydrous Na_2SO_4 and removed under vacuum. The crude product was purified by silica gel column chromatography to obtain **13h–i**, **13m–o**.

14-*O*-(Benzamide-thiazolyl)-4-methyl Thioether Mutilin (**13a**)

White foam solid; yield: 44%; mp 104.2–105.8°C. $^1\text{H-NMR}$ (CDCl_3) δ : 10.24 (s, 1H), 7.92 (d, $J=7.4$ Hz, 2H), 7.58 (t, $J=7.4$ Hz, 1H), 7.49 (dd, $J=7.8$, 7.0 Hz, 2H), 6.79 (s, 1H), 6.47 (dd, $J=17.4$, 11.0 Hz, 1H), 5.74 (d, $J=8.4$ Hz, 1H), 5.33 (d, $J=11.0$ Hz, 1H), 5.20 (d, $J=17.4$ Hz, 1H), 3.70 (s, 2H), 3.43–3.31 (m, 1H), 3.00 (s, 2H), 2.39–2.29 (m, 1H), 2.26–2.13 (m, 2H), 2.13–2.02 (m, 2H), 1.75 (d, $J=14.4$ Hz, 1H), 1.62 (dd, $J=21.1$, 10.6 Hz, 4H), 1.57–1.47 (m, 1H), 1.47–1.38 (m, 4H), 1.38–1.28 (m, 2H), 1.21–1.04 (m, 4H), 0.86 (d, $J=6.8$ Hz, 3H), 0.70 (d, $J=6.7$ Hz, 3H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 216.99, 168.59, 164.59, 158.83, 146.38, 139.15, 132.94, 131.90, 128.99, 127.43, 117.17, 111.39, 74.67, 69.29, 58.18, 45.44, 44.83, 43.91, 41.73,

36.75, 36.01, 34.43, 32.92, 31.58, 30.40, 26.83, 26.45, 24.84, 16.84, 14.92, 11.51. IR (KBr) cm^{-1} : 3262, 2933, 1732 1668, 1540, 1455, 1285, 1115, 705. FAB-MS m/z : 609.2443 $[\text{M}-\text{H}]^-$ (Calcd for $\text{C}_{33}\text{H}_{41}\text{N}_2\text{O}_5\text{S}_2$: 609.2457).

14-*O*-(Benzacetamide-thiazolyl)-4-methyl Thioether Mutilin (**13b**)

White solid; yield: 65%; mp 191.9–193.7°C. $^1\text{H-NMR}$ (CDCl_3) δ : 9.00 (s, 1H), 7.38–7.29 (m, 3H), 7.26 (t, $J=6.3$ Hz, 2H), 6.72 (s, 1H), 6.44 (dd, $J=17.4$, 11.0 Hz, 1H), 5.72 (d, $J=8.4$ Hz, 1H), 5.31 (d, $J=11.0$ Hz, 1H), 5.17 (d, $J=17.4$ Hz, 1H), 3.78 (s, 2H), 3.72 (s, 2H), 3.35 (s, 1H), 3.03–2.92 (m, 2H), 2.37–2.25 (m, 1H), 2.25–2.10 (m, 2H), 2.05 (dd, $J=15.8$, 8.1 Hz, 2H), 1.74 (d, $J=14.3$ Hz, 1H), 1.68–1.48 (m, 4H), 1.49–1.37 (m, 4H), 1.36–1.20 (m, 2H), 1.16–1.04 (m, 4H), 0.86 (d, $J=6.9$ Hz, 3H), 0.68 (d, $J=6.8$ Hz, 3H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 217.09, 168.74, 168.61, 158.01, 146.23, 139.21, 132.78, 129.61, 129.41, 128.10, 117.18, 111.40, 74.65, 69.34, 58.22, 45.48, 44.86, 43.95, 43.46, 41.77, 36.79, 36.04, 34.47, 32.88, 31.64, 30.44, 26.86, 26.48, 24.88, 16.85, 14.95, 11.53. IR (KBr) cm^{-1} : 3500, 3193, 2931, 1730, 1549, 1455, 1279, 1114, 1015, 730. FAB-MS m/z : 623.2599 $[\text{M}-\text{H}]^-$ (Calcd for $\text{C}_{34}\text{H}_{43}\text{N}_2\text{O}_5\text{S}_2$: 623.2613).

14-*O*-(3-Methoxybenzamide-thiazolyl)-4-methyl Thioether Mutilin (**13c**)

White foam solid; yield: 32%; mp 87.7–89.4°C. $^1\text{H-NMR}$ (CDCl_3) δ : 9.81 (s, 1H), 7.89 (d, $J=8.6$ Hz, 2H), 6.98 (d, $J=8.6$ Hz, 2H), 6.78 (s, 1H), 6.48 (dd, $J=17.4$, 11.0 Hz, 1H), 5.76 (d, $J=8.4$ Hz, 1H), 5.35 (d, $J=11.0$ Hz, 1H), 5.21 (d, $J=17.4$ Hz, 1H), 3.87 (s, 3H), 3.78 (s, 2H), 3.36 (t, $J=7.4$ Hz, 1H), 3.03 (s, 2H), 2.39–2.28 (m, 1H), 2.27–2.13 (m, 2H), 2.12–2.03 (m, 2H), 1.76 (d, $J=14.3$ Hz, 1H), 1.69–1.59 (m, 2H), 1.59–1.48 (m, 2H), 1.48–1.40 (m, 4H), 1.40–1.29 (m, 2H), 1.20–1.07 (m, 4H), 0.87 (d, $J=6.9$ Hz, 3H), 0.72 (d, $J=6.8$ Hz, 3H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 216.95, 168.62, 163.94, 163.37, 159.03, 146.24, 139.16, 129.40, 123.95, 117.16, 114.26, 111.18, 74.66, 69.31, 58.19, 55.51, 45.44, 44.85, 43.92, 41.75, 36.76, 36.02, 34.42, 32.94, 31.66, 30.41, 26.84, 26.43, 24.84, 16.83, 14.92, 11.48. IR (KBr) cm^{-1} : 3437, 3265, 2932, 1731, 1667, 1607, 1541, 1513, 1456, 1285, 1260, 1114, 1019, 980, 843. FAB-MS m/z : 641.2714 $[\text{M}-\text{H}]^-$ (Calcd for $\text{C}_{34}\text{H}_{45}\text{N}_2\text{O}_6\text{S}_2$: 641.2719).

14-*O*-(2-Nitrobenzamide-thiazolyl)-4-methyl Thioether Mutilin (**13d**)

Light yellow foam solid; yield: 50%; mp 109.1–111.4°C. $^1\text{H-NMR}$ (CDCl_3) δ : 8.10 (d, $J=7.9$ Hz, 1H), 7.68 (ddd, $J=19.9$, 13.6, 7.4 Hz, 3H), 6.82 (s, 1H), 6.46 (dd, $J=17.4$, 11.1 Hz, 1H), 5.73 (d, $J=8.4$ Hz, 1H), 5.33 (d, $J=10.9$ Hz, 1H), 5.19 (d, $J=17.4$ Hz, 1H), 3.61 (s, 2H), 3.36 (dd, $J=9.9$, 6.6 Hz, 1H), 3.02 (s, 2H), 2.37–2.27 (m, 1H), 2.27–2.14 (m, 2H), 2.13–2.03 (m, 2H), 1.75 (d, $J=14.4$ Hz, 1H), 1.68–1.58 (m, 2H), 1.57–1.48 (m, 2H), 1.48–1.38 (m, 4H), 1.38–1.28 (m, 2H), 1.19–1.06 (m, 4H), 0.86 (d, $J=6.9$ Hz, 3H), 0.70 (d, $J=6.8$ Hz, 3H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 217.06, 168.60, 163.56, 158.15, 146.73, 146.48, 139.18, 133.87, 131.66, 130.46, 128.70, 124.87, 117.15, 111.71, 74.62, 69.35, 58.16, 45.43, 44.79, 43.91, 41.73, 36.74, 35.99, 34.43, 32.99, 31.34, 30.40, 26.83, 26.39, 24.83, 16.85, 14.91, 11.47. IR (KBr) cm^{-1} : 3448, 3256, 3193, 2933, 1725, 1685, 1533, 1458, 1347, 1286, 1116, 1017, 980, 721. FAB-MS m/z : 654.2299 $[\text{M}-\text{H}]^-$ (Calcd for $\text{C}_{33}\text{H}_{43}\text{N}_3\text{O}_7\text{S}_2$: 654.2308).

14-*O*-(4-Nitrobenzamide-thiazolyl)-4-methyl Thioether Mutilin (**13e**)

Saffron foam solid; yield: 54%; mp 118.6–120.1°C.

¹H-NMR (CDCl₃) δ: 10.16 (s, 1H), 8.44–8.26 (m, 2H), 8.12 (d, *J*=6.9 Hz, 2H), 6.86 (s, 1H), 6.48 (dd, *J*=17.4, 11.1 Hz, 1H), 5.76 (d, *J*=8.0 Hz, 1H), 5.35 (d, *J*=11.0 Hz, 1H), 5.21 (d, *J*=17.5 Hz, 1H), 3.79 (s, 2H), 3.42–3.30 (m, 1H), 3.05 (s, 2H), 2.39–2.29 (m, 1H), 2.28–2.15 (m, 2H), 2.15–2.03 (m, 2H), 1.77 (d, *J*=13.1 Hz, 3H), 1.64 (dd, *J*=21.2, 11.7 Hz, 2H), 1.58–1.49 (m, 2H), 1.49–1.41 (m, 4H), 1.41–1.21 (m, 4H), 1.21–1.05 (m, 4H), 0.88 (d, *J*=6.4 Hz, 3H), 0.72 (d, *J*=6.6 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.98, 168.59, 162.57, 158.14, 150.33, 146.66, 139.18, 137.32, 128.64, 124.20, 117.18, 111.88, 74.65, 69.41, 58.17, 45.44, 44.84, 43.93, 41.75, 36.74, 36.02, 34.43, 32.96, 31.55, 30.40, 26.85, 26.42, 24.84, 16.86, 14.92, 11.50. IR (KBr) cm⁻¹: 3450, 3253, 2932, 1727, 1676, 1548, 1528, 1346, 1288, 1115, 1015, 980, 850, 713. FAB-MS *m/z*: 654.2286 [M–H][–] (Calcd for C₃₃H₄₃N₃O₇S₂: 654.2308).

14-*O*-(2,4-Dichlorobenzamide-thiazolyl)-4-methyl Thioether Mutilin (**13f**)

White foam solid; yield: 46%; mp 102.5–104.1°C. ¹H-NMR (CDCl₃) δ: 7.82 (d, *J*=8.4 Hz, 1H), 7.50 (d, *J*=2.0 Hz, 1H), 7.39 (dd, *J*=8.4, 2.0 Hz, 1H), 6.85 (s, 1H), 6.48 (dd, *J*=17.4, 11.0 Hz, 1H), 5.76 (d, *J*=8.5 Hz, 1H), 5.35 (d, *J*=11.0 Hz, 1H), 5.21 (dd, *J*=17.4, 1.4 Hz, 1H), 3.78 (s, 2H), 3.36 (s, 1H), 3.06 (s, 2H), 2.40–2.30 (m, 1H), 2.30–2.15 (m, 2H), 2.14–2.03 (m, 2H), 1.77 (dd, *J*=14.3, 2.8 Hz, 1H), 1.69–1.61 (m, 2H), 1.61–1.48 (m, 2H), 1.48–1.41 (m, 4H), 1.40–1.29 (m, 2H), 1.20–1.06 (m, 4H), 0.88 (d, *J*=7.0 Hz, 3H), 0.73 (d, *J*=6.9 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.87, 168.58, 162.32, 157.65, 146.88, 139.17, 138.65, 132.13, 132.04, 130.65, 130.25, 127.94, 117.15, 111.70, 74.64, 69.34, 58.18, 45.44, 44.86, 43.94, 41.76, 36.76, 36.03, 34.42, 33.03, 31.54, 30.42, 26.85, 26.41, 24.84, 16.83, 14.92, 11.44. IR (KBr) cm⁻¹: 3449, 3256, 3193, 3080, 2960, 2927, 1728, 1676, 1541, 1454, 1284, 1262, 1103, 1018, 802, 697. FAB-MS *m/z*: 677.1660 [M–H][–] (Calcd for C₃₃H₃₉N₃O₅S₂Cl₂: 677.1677).

14-*O*-(2-Hydroxybenzamide-thiazolyl)-4-methyl Thioether Mutilin (**13g**)

White solid; yield: 37.7%; mp 218.5–220.1°C. ¹H-NMR (CDCl₃) δ: 7.64 (d, *J*=8.0 Hz, 1H), 7.46 (t, *J*=7.7 Hz, 1H), 7.03 (d, *J*=8.4 Hz, 1H), 6.94 (t, *J*=7.6 Hz, 1H), 6.85 (s, 1H), 6.48 (dd, *J*=17.4, 11.0 Hz, 1H), 5.76 (d, *J*=8.4 Hz, 1H), 5.35 (d, *J*=11.0 Hz, 1H), 5.21 (d, *J*=17.3 Hz, 1H), 3.82 (s, 2H), 3.41–3.32 (m, 1H), 3.05 (s, 2H), 2.40–2.30 (m, 1H), 2.28–2.17 (m, 2H), 2.17–2.04 (m, 2H), 1.76 (d, *J*=14.2 Hz, 2H), 1.70–1.59 (m, 4H), 1.58–1.49 (m, 2H), 1.49–1.41 (m, 4H), 1.39–1.29 (m, 2H), 1.20–1.07 (m, 4H), 0.88 (d, *J*=7.0 Hz, 3H), 0.73 (d, *J*=6.9 Hz, 3H). δ: 11.9 (s, 1H), 11.65 (s, 1H), 7.96 (d, *J*=7.8 Hz, 1H), 7.44 (s, 1H), 6.97 (d, *J*=10.7 Hz, 3H), 6.13 (d, *J*=11.2 Hz, 1H), 5.54 (d, *J*=8.1 Hz, 1H), 5.05 (d, *J*=8.2 Hz, 2H), 4.46 (d, *J*=5.5 Hz, 1H), 3.79 (s, 2H), 3.4 (s, 1H), 2.37 (s, 1H), 2.33–1.94 (m, 4H), 1.7–1.53 (m, 2H), 1.52–1.32 (m, 5H), 1.31–1.18 (m, 5H), 1.07–0.92 (m, 4H), 0.8 (d, *J*=8 Hz, 3H), 0.61 (d, *J*=6.5 Hz, 3H). ¹H-NMR (DMSO-*d*₆) δ: 11.9 (s, 1H), 11.65 (s, 1H), 7.96 (d, *J*=7.8 Hz, 1H), 7.44 (s, 1H), 6.97 (d, *J*=10.7 Hz, 3H), 6.13 (d, *J*=11.2 Hz, 1H), 5.54 (d, *J*=8.1 Hz, 1H), 5.05 (d, *J*=8.2 Hz, 2H), 4.46 (d, *J*=5.5 Hz, 1H), 3.79 (s, 2H), 3.4 (s, 1H), 2.37 (s, 1H), 2.33–1.94 (m, 4H), 1.7–1.53 (m, 2H), 1.52–1.32 (m, 5H), 1.31–1.18 (m, 5H), 1.07–0.92 (m, 4H), 0.8 (d, *J*=8 Hz, 3H), 0.61 (d, *J*=6.5 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.98, 168.60, 139.14, 135.54, 119.67, 118.72, 117.21, 111.86, 74.65, 69.38, 58.18, 45.44, 44.84, 43.92, 41.74, 36.75, 36.01, 34.43, 32.96, 31.53, 30.41, 26.84, 26.39, 24.84, 16.87, 14.93, 11.50. ¹³C-NMR (DMSO-*d*₆) δ: 217.64, 168.66, 164.60, 157.82, 147.62, 141.39,

134.92, 130.71, 120.22, 117.64, 115.63, 111.47, 73.10, 69.99, 57.76, 45.46, 44.52, 44.22, 41.96, 36.87, 34.48, 33.81, 31.62, 30.61, 30.31, 29.02, 27.05, 24.96, 16.62, 15.07, 12.01. IR (KBr) cm⁻¹: 3446, 3247, 2952, 1731, 1646, 1540, 1463, 1262, 1114, 1017, 802, 756. δ: 217.64, 168.66, 164.60, 157.82, 147.62, 141.39, 134.92, 130.71, 120.22, 117.64, 115.63, 111.47, 73.10, 69.99, 57.76, 45.46, 44.52, 44.22, 41.96, 36.87, 34.48, 33.81, 31.62, 30.61, 30.31, 29.02, 27.05, 24.96, 16.62, 15.07. FAB-MS *m/z*: 625.2387 [M–H][–] (Calcd for C₃₃H₄₁N₂O₆S₂: 625.2406).

14-*O*-(3-Aminobenzamide-thiazolyl)-4-methyl Thioether Mutilin (**13h**)

White foam solid; yield: 40%; mp 108.5–110.4°C. ¹H-NMR (CDCl₃) δ: 7.26 (t, *J*=5.3 Hz, 2H), 7.23–7.19 (m, 1H), 6.87 (dd, *J*=7.1, 1.7 Hz, 1H), 6.80 (s, 1H), 6.49 (dd, *J*=17.4, 11.0 Hz, 1H), 5.76 (d, *J*=8.4 Hz, 1H), 5.35 (d, *J*=11.0 Hz, 1H), 5.21 (dd, *J*=17.4, 1.4 Hz, 1H), 3.80 (s, 2H), 3.36 (s, 1H), 3.05 (s, 2H), 2.41–2.30 (m, 1H), 2.28–2.14 (m, 2H), 2.14–2.04 (m, 2H), 1.76 (d, *J*=14.3 Hz, 1H), 1.69–1.59 (m, 3H), 1.59–1.50 (m, 1H), 1.48–1.40 (m, 4H), 1.39–1.30 (m, 2H), 1.19–1.06 (m, 4H), 0.88 (d, *J*=7.0 Hz, 3H), 0.73 (d, *J*=6.9 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.99, 168.63, 164.60, 158.67, 147.15, 146.39, 139.14, 132.87, 129.89, 119.28, 117.21, 116.59, 113.82, 111.33, 74.64, 69.29, 58.18, 45.44, 44.83, 43.92, 41.75, 36.77, 36.01, 34.44, 32.93, 31.66, 30.41, 26.84, 26.40, 24.84, 16.85, 14.94, 11.49. IR (KBr) cm⁻¹: 3455, 3370, 3235, 2929, 1727, 1667, 1541, 1456, 1280, 1115, 1017, 941, 844, 744. FAB-MS *m/z*: 626.2730 [M–H][–] (Calcd for C₃₃H₄₄N₃O₅S₂: 626.2722).

14-*O*-(4-Aminobenzamide-thiazolyl)-4-methyl Thioether Mutilin (**13i**)

White solid; yield: 16.4%; mp 134.8–137.4°C. ¹H-NMR (CDCl₃) δ: 7.78 (d, *J*=7.2 Hz, 2H), 6.76 (s, 1H), 6.69 (d, *J*=7.2 Hz, 2H), 6.48 (dd, *J*=17.0, 11.1 Hz, 1H), 5.76 (d, *J*=8.3 Hz, 1H), 5.36 (d, *J*=11.0 Hz, 1H), 5.21 (d, *J*=17.4 Hz, 1H), 3.80 (s, 2H), 3.36 (s, 1H), 3.04 (s, 2H), 2.39–2.29 (m, 1H), 2.29–2.15 (m, 2H), 2.14–2.04 (m, 2H), 1.76 (d, *J*=14.2 Hz, 1H), 1.64 (dd, *J*=21.4, 10.6 Hz, 2H), 1.56 (d, *J*=12.9 Hz, 1H), 1.52–1.40 (m, 5H), 1.40–1.29 (m, 2H), 1.19–1.06 (m, 4H), 0.87 (d, *J*=6.4 Hz, 3H), 0.73 (d, *J*=6.2 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.97, 168.61, 164.02, 159.63, 151.00, 145.47, 139.14, 129.55, 120.65, 117.22, 114.25, 111.00, 74.64, 69.29, 58.19, 45.44, 44.83, 43.92, 41.74, 36.77, 36.01, 34.44, 32.88, 31.43, 30.42, 26.84, 26.39, 24.84, 16.86, 14.93, 11.50. IR (KBr) cm⁻¹: 3466, 3362, 3231, 2926, 1724, 1624, 1540, 1282, 1182, 1016, 734. FAB-MS *m/z*: 624.2585 [M–H][–] (Calcd for C₃₃H₄₂N₃O₅S₂: 624.2566).

14-*O*-(2-Furoyl-thiazolyl)-4-methyl Thioether Mutilin (**13j**)

White foam solid; yield: 50%; mp 105.8–107.2°C. ¹H-NMR (CDCl₃) δ: 9.84 (s, 1H), 7.59–7.45 (m, 1H), 7.31 (d, *J*=3.6 Hz, 1H), 6.78 (s, 1H), 6.56 (dd, *J*=3.5, 1.7 Hz, 1H), 6.46 (dd, *J*=17.4, 11.0 Hz, 1H), 5.74 (d, *J*=8.4 Hz, 1H), 5.32 (d, *J*=11.0 Hz, 1H), 5.19 (dd, *J*=17.4, 1.4 Hz, 1H), 3.79 (s, 2H), 3.39–3.29 (m, 1H), 3.03 (s, 2H), 2.37–2.28 (m, 1H), 2.28–2.12 (m, 2H), 2.11–2.01 (m, 2H), 1.74 (dd, *J*=14.5, 2.4 Hz, 1H), 1.68–1.57 (m, 3H), 1.57–1.47 (m, 1H), 1.47–1.37 (m, 4H), 1.38–1.26 (m, 2H), 1.18–1.04 (m, 4H), 0.86 (d, *J*=7.0 Hz, 3H), 0.70 (d, *J*=6.8 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.99, 168.58, 157.58, 155.05, 146.64, 145.82, 145.52, 139.20, 117.10, 117.07, 112.88, 111.42, 74.60, 69.30, 58.18, 45.43, 44.83, 43.92, 41.73, 36.75, 36.02, 34.42, 32.91, 31.65, 30.40, 26.82, 26.49, 24.82, 16.81, 14.91, 11.47. IR (KBr) cm⁻¹: 3447, 3410, 2933, 1728, 1671, 1588, 1541, 1328, 1296, 1115, 1015, 856, 759. FAB-MS *m/z*: 599.2243 [M–H][–] (Calcd for C₃₁H₃₉N₂O₆S₂: 599.2250).

14-*O*-(Thiophenecarbonyl-thiazolyl)-4-methyl Thioether Mutilin (**13k**)

White solid; yield: 47%; mp 127.3–129.7°C. ¹H-NMR (CDCl₃) δ: 7.73 (d, *J*=3.7 Hz, 1H), 7.63 (d, *J*=5.0 Hz, 1H), 7.15 (t, *J*=4.4 Hz, 1H), 6.80 (s, 1H), 6.48 (dd, *J*=17.4, 11.1 Hz, 1H), 5.76 (d, *J*=8.4 Hz, 1H), 5.36 (d, *J*=11.0 Hz, 1H), 5.21 (d, *J*=17.4 Hz, 1H), 3.80 (s, 2H), 3.43–3.30 (m, 1H), 3.04 (s, 2H), 2.40–2.29 (m, 1H), 2.29–2.15 (m, 2H), 2.14–2.04 (m, 2H), 1.76 (d, *J*=14.5 Hz, 1H), 1.71–1.59 (m, 3H), 1.59–1.51 (m, 1H), 1.51–1.39 (m, 5H), 1.39–1.29 (m, 2H), 1.19–1.06 (m, 4H), 0.88 (d, *J*=7.0 Hz, 3H), 0.73 (d, *J*=6.8 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.96, 168.60, 158.95, 158.38, 146.28, 139.15, 136.31, 132.61, 129.95, 128.28, 117.20, 111.53, 74.64, 69.32, 58.18, 45.44, 44.84, 43.92, 41.75, 36.76, 36.02, 34.43, 32.89, 31.60, 30.41, 26.84, 26.39, 24.84, 16.85, 14.93, 11.49. IR (KBr) cm⁻¹: 3391, 3092, 2966, 1732, 1652, 1557, 1455, 1412, 1287, 1115, 1048, 849, 723. FAB-MS *m/z*: 615.2046 [M–H][–] (Calcd for C₃₁H₃₉N₂O₅S₂: 615.2021).

14-*O*-(4-Pyridinecarbonyl-thiazolyl)-4-methyl Thioether Mutilin (**13l**)

Light yellow foam solid; yield: 78%; mp 125.5–127.8°C. ¹H-NMR (MeOD) δ: 8.67 (d, *J*=4.7 Hz, 2H), 7.98 (d, *J*=4.7 Hz, 2H), 6.87 (s, 1H), 6.30 (dd, *J*=17.4, 11.3 Hz, 1H), 5.72 (d, *J*=8.3 Hz, 1H), 5.16 (dd, *J*=14.4, 7.7 Hz, 2H), 3.83 (s, 2H), 3.48 (d, *J*=6.0 Hz, 1H), 3.16 (s, 2H), 2.38–2.20 (m, 3H), 2.19–2.04 (m, 2H), 1.79 (d, *J*=14.0 Hz, 1H), 1.73–1.49 (m, 4H), 1.47–1.36 (m, 4H), 1.36–1.25 (m, 2H), 1.19–1.07 (m, 4H), 0.91 (d, *J*=6.9 Hz, 3H), 0.71 (d, *J*=6.0 Hz, 3H). ¹³C-NMR (MeOD) δ: 218.30, 169.14, 166.17, 149.30, 146.32, 143.62, 139.88, 122.16, 115.11, 110.37, 74.00, 69.83, 57.92, 45.39, 44.63, 43.87, 41.70, 36.76, 36.33, 33.90, 32.92, 31.47, 30.13, 26.88, 26.61, 24.43, 15.66, 14.04, 10.36. IR (KBr) cm⁻¹: 3446, 2932, 1730, 1670, 1550, 1456, 1284, 1115, 1018, 908, 801, 718. FAB-MS *m/z*: 612.2564 [M+H]⁺ (Calcd for C₃₂H₄₂N₃O₅S₂: 612.2566).

14-*O*-(2-Pyrrolidinecarbonyl-thiazolyl)-4-methyl Thioether Mutilin (**13m**)

White foam solid; yield: 70%; mp 137.8–140.2°C. ¹H-NMR (Pyr) δ: 7.06 (s, 1H), 6.91 (s, 1H), 6.75 (ddd, *J*=17.6, 14.0, 11.3 Hz, 2H), 6.17 (dd, *J*=15.8, 7.7 Hz, 1H), 6.04 (t, *J*=7.5 Hz, 2H), 5.85 (s, 1H), 5.57–5.48 (m, 2H), 5.43 (t, *J*=9.7 Hz, 2H), 4.97 (s, 3H), 4.30 (td, *J*=8.4, 4.3 Hz, 1H), 4.17–4.10 (m, 2H), 4.04 (dd, *J*=13.7, 7.8 Hz, 2H), 3.94 (d, *J*=13.5 Hz, 1H), 3.81 (d, *J*=5.9 Hz, 2H), 3.53 (s, 2H), 3.37 (d, *J*=14.5 Hz, 1H), 3.30 (d, *J*=14.5 Hz, 1H), 3.05 (dd, *J*=10.6, 4.0 Hz, 1H), 2.88 (dt, *J*=10.0, 6.8 Hz, 1H), 2.83 (dt, *J*=10.3, 6.5 Hz, 1H), 2.69 (td, *J*=11.9, 7.5 Hz, 1H), 2.57–2.42 (m, 5H), 2.34–2.25 (m, 3H), 2.20–2.14 (m, 4H), 2.06 (dt, *J*=20.4, 7.9 Hz, 1H), 1.98 (td, *J*=12.8, 6.4 Hz, 1H), 1.81–1.65 (m, 17H), 1.60–1.48 (m, 2H), 1.43 (s, 5H), 1.38–1.33 (m, 2H), 1.29 (t, *J*=13.3 Hz, 2H), 1.15 (t, *J*=6.6 Hz, 6H), 1.07 (dd, *J*=20.6, 8.1 Hz, 2H), 0.88 (d, *J*=6.6 Hz, 3H), 0.83 (d, *J*=6.4 Hz, 3H). ¹³C-NMR (Pyr) δ: 216.94, 177.19, 174.09, 169.98, 168.79, 168.77, 158.43, 147.80, 145.27, 141.37, 141.34, 115.84, 115.75, 110.52, 109.48, 73.93, 73.90, 70.18, 61.11, 60.71, 58.19, 54.84, 47.28, 47.17, 45.68, 45.00, 44.95, 44.82, 44.79, 42.24, 42.20, 37.16, 37.13, 37.03, 34.52, 33.81, 33.16, 32.72, 32.13, 30.82, 30.46, 29.80, 28.70, 27.07, 27.03, 26.29, 26.08, 25.10, 16.64, 16.62, 15.08, 15.06, 11.99. IR (KBr) cm⁻¹: 3445, 3257, 2935, 1728, 1575, 1457, 1280, 1115, 1017, 715. FAB-MS *m/z*: 602.2723 [M–H][–] (Calcd for C₃₁H₄₄N₃O₅S₂: 602.2722).

14-*O*-(2-Amino-3-methyl)butanoyl-thiazolyl)-4-methyl

Thioether Mutilin (**13n**)

White foam solid; yield: 64%; mp 110.8–116.0°C. ¹H-NMR (Pyr) δ: 7.05 (s, 1H), 6.76 (dd, *J*=17.7, 11.1 Hz, 1H), 6.04 (d, *J*=8.2 Hz, 1H), 5.84 (s, 1H), 5.52 (d, *J*=17.7 Hz, 1H), 5.43 (d, *J*=11.2 Hz, 1H), 4.98 (s, 4H), 4.18–4.09 (m, 2H), 3.80 (d, *J*=4.4 Hz, 1H), 3.65 (d, *J*=5.0 Hz, 1H), 3.53 (s, 2H), 3.40 (s, 1H), 2.57–2.45 (m, 2H), 2.34–2.23 (m, 2H), 2.20–2.12 (m, 2H), 1.81–1.65 (m, 9H), 1.43 (s, 3H), 1.40–1.31 (m, 1H), 1.27 (d, *J*=11.9 Hz, 1H), 1.19–1.11 (m, 4H), 1.10–1.03 (m, 1H), 1.02–0.93 (m, 6H), 0.87 (d, *J*=6.6 Hz, 4H). ¹³C-NMR (Pyr) δ: 216.94, 174.41, 168.79, 158.83, 147.84, 141.34, 115.82, 110.42, 73.92, 70.18, 60.92, 58.19, 45.67, 45.01, 44.81, 42.23, 37.13, 37.03, 34.51, 33.86, 32.26, 32.19, 30.45, 28.69, 27.06, 25.09, 19.59, 16.94, 16.64, 15.08, 11.99. ¹H-NMR (CDCl₃) δ: 6.79 (s, 1H), 6.49 (dd, *J*=17.4, 11.0 Hz, 1H), 5.77 (d, *J*=8.4 Hz, 1H), 5.37 (dd, *J*=11.0, 1.1 Hz, 1H), 5.22 (dd, *J*=17.4, 1.3 Hz, 1H), 3.80 (s, 1H), 3.49 (s, 1H), 3.37 (s, 1H), 3.12–3.00 (m, 2H), 2.45 (s, 1H), 2.41–2.29 (m, 1H), 2.29–2.15 (m, 2H), 2.15–2.05 (m, 2H), 1.78 (dd, *J*=14.4, 2.6 Hz, 1H), 1.72–1.60 (m, 2H), 1.54–1.42 (m, 5H), 1.41–1.30 (m, 2H), 1.21–1.08 (m, 4H), 1.04 (d, *J*=7.0 Hz, 3H), 0.88 (dd, *J*=11.7, 7.0 Hz, 6H), 0.74 (d, *J*=6.8 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 217.04, 168.70, 139.18, 117.23, 110.98, 74.67, 69.30, 59.80, 58.23, 45.48, 44.86, 43.96, 41.78, 36.81, 36.05, 34.47, 32.93, 31.77, 30.77, 30.45, 26.87, 26.44, 24.88, 19.60, 16.88, 16.11, 14.97, 11.52. IR (KBr) cm⁻¹: 3437, 2957, 1731, 1538, 1456, 1280, 1114, 1017, 730. FAB-MS *m/z*: 604.2875 [M–H][–] (Calcd for C₃₁H₄₇N₃O₅S₂: 604.2879).

14-*O*-(2-Amino-3-phenyl)propanoyl-thiazolyl)-4-methyl Thioether Mutilin (**13o**)

White foam solid; yield: 67%; mp 98.5–100.3°C. ¹H-NMR (Pyr) δ: 7.26 (dd, *J*=14.2, 6.9 Hz, 4H), 7.22–7.15 (m, 2H), 7.03 (s, 1H), 6.77 (dd, *J*=17.6, 11.1 Hz, 1H), 6.05 (d, *J*=8.3 Hz, 1H), 5.83 (s, 1H), 5.53 (d, *J*=17.8 Hz, 1H), 5.44 (d, *J*=11.4 Hz, 1H), 5.01 (s, 3H), 4.21–4.07 (m, 1H), 3.81 (d, *J*=5.4 Hz, 1H), 3.50 (s, 2H), 2.99 (dd, *J*=12.9, 9.3 Hz, 1H), 2.60–2.47 (m, 2H), 2.29 (dd, *J*=15.7, 8.3 Hz, 1H), 2.17 (dd, *J*=9.7, 6.3 Hz, 2H), 1.82–1.66 (m, 8H), 1.44 (s, 3H), 1.39–1.32 (m, 1H), 1.32–1.25 (m, 1H), 1.15 (d, *J*=7.0 Hz, 3H), 1.11–1.02 (m, 1H), 0.88 (t, *J*=10.9 Hz, 3H). ¹³C-NMR (Pyr) δ: 216.97, 168.81, 141.36, 138.39, 129.57, 128.71, 126.77, 115.84, 73.92, 70.18, 58.19, 57.07, 54.84, 45.68, 45.04, 44.81, 42.24, 37.13, 37.04, 34.53, 30.46, 28.70, 27.07, 25.10, 16.66, 15.10, 12.00. IR (KBr) cm⁻¹: 3458, 3294, 2929, 2860, 1730, 1538, 1455, 1279, 1114, 1017, 701. FAB-MS *m/z*: 652.2873 [M–H][–] (Calcd for C₃₅H₄₇N₃O₅S₂: 652.2879).

14-*O*-(Boc-2-pyrrolidinecarbonyl-thiazolyl)-4-methyl Thioether Mutilin (**14m**)

White foam solid; yield: 58%; mp 79.8–81.3°C. ¹H-NMR (CDCl₃) δ: 10.31 (s, 1H), 9.47 (s, 1H), 6.70 (s, 1H), 6.42 (dd, *J*=17.4, 11.0 Hz, 1H), 5.70 (d, *J*=8.4 Hz, 1H), 5.29 (dd, *J*=11.0, 1.4 Hz, 1H), 5.15 (dd, *J*=17.4, 1.4 Hz, 1H), 4.46 (s, 1H), 4.32 (s, 1H), 3.73 (s, 2H), 3.40 (s, 1H), 3.30 (s, 1H), 2.99 (s, 2H), 2.36–2.08 (m, 4H), 2.08–1.96 (m, 3H), 1.91–1.80 (m, 2H), 1.71 (dd, *J*=14.4, 2.6 Hz, 1H), 1.65–1.48 (m, 3H), 1.48–1.34 (m, 12H), 1.33–1.14 (m, 5H), 1.14–0.97 (m, 4H), 0.82 (d, *J*=7.0 Hz, 3H), 0.66 (d, *J*=6.8 Hz, 3H). ¹³C-NMR (CDCl₃) δ: 216.05, 167.69, 156.87, 145.55, 138.17, 116.21, 110.11, 80.29, 73.65, 68.26, 59.15, 57.21, 52.44, 46.35, 44.46, 43.85, 42.93, 40.76, 35.79, 35.04, 33.46, 31.94, 30.62, 29.44, 27.34, 25.85, 25.44, 23.86, 15.85, 13.95, 10.51. IR (KBr) cm⁻¹: 3491, 3214, 2978, 1698, 1549, 1409, 1279, 1160, 1116, 1017, 912, 731. FAB-MS

m/z : 702.3234 $[M-H]^-$ (Calcd for $C_{36}H_{52}N_3O_7S_2$: 702.3247).

14-*O*-(Boc-2-amino-3-methyl)butanoyl-thiazolyl)-4-methyl Thioether Mutilin (**14n**)

White foam solid; yield: 52%; mp 103.8–106.9°C. 1H -NMR ($CDCl_3$) δ : 6.70 (s, 1H), 6.42 (dd, $J=17.4$, 11.0 Hz, 1H), 5.70 (d, $J=8.3$ Hz, 1H), 5.29 (dd, $J=11.0$, 1.2 Hz, 1H), 5.15 (dt, $J=10.7$, 5.4 Hz, 2H), 4.20 (s, 1H), 3.78–3.66 (m, 2H), 3.34 (dd, $J=10.2$, 6.5 Hz, 1H), 2.97 (s, 2H), 2.34–2.24 (m, 1H), 2.24–2.08 (m, 3H), 2.08–1.96 (m, 2H), 1.71 (dd, $J=14.4$, 2.4 Hz, 1H), 1.65–1.44 (m, 4H), 1.43–1.34 (m, 13H), 1.26 (ddd, $J=30.1$, 17.1, 6.2 Hz, 3H), 1.13–0.99 (m, 4H), 0.96–0.73 (m, 9H), 0.66 (d, $J=6.9$ Hz, 3H). ^{13}C -NMR ($CDCl_3$) δ : 216.12, 169.40, 167.72, 154.93, 145.32, 138.16, 116.23, 110.31, 79.60, 73.66, 68.34, 58.97, 57.19, 52.44, 44.48, 43.84, 42.94, 40.76, 35.78, 35.03, 33.47, 31.85, 30.66, 29.65, 29.44, 27.29, 25.87, 25.44, 23.88, 18.29, 16.59, 15.88, 13.95, 10.53. IR (KBr) cm^{-1} : 3443, 3268, 2931, 1716, 1557, 1328, 1161, 1017, 732. FAB-MS m/z : 704.3421 $[M-H]^-$ (Calcd for $C_{36}H_{54}N_3O_7S_2$: 704.3403).

14-*O*-(Boc-2-amino-3-phenyl)propanoyl-thiazolyl)-4-methyl Thioether Mutilin (**14o**)

White foam solid; yield: 54%; mp 92.2–94.0°C. 1H -NMR ($CDCl_3$) δ : 7.24–7.12 (m, 3H), 7.08 (d, $J=6.7$ Hz, 2H), 6.69 (s, 1H), 6.41 (dd, $J=17.4$, 11.0 Hz, 1H), 5.69 (d, $J=8.4$ Hz, 1H), 5.28 (dd, $J=11.0$, 1.3 Hz, 1H), 5.15 (dd, $J=17.4$, 1.4 Hz, 1H), 4.60 (s, 1H), 3.77–3.62 (m, 2H), 3.33 (dd, $J=10.2$, 6.5 Hz, 1H), 3.17 (d, $J=11.5$ Hz, 1H), 3.07–2.88 (m, 3H), 2.35–2.21 (m, 1H), 2.21–2.08 (m, 2H), 2.08–1.95 (m, 2H), 1.70 (dd, $J=14.4$, 2.4 Hz, 1H), 1.65–1.42 (m, 4H), 1.41–1.34 (m, 4H), 1.34–1.16 (m, 11H), 1.14–1.00 (m, 4H), 0.82 (d, $J=7.0$ Hz, 3H), 0.65 (d, $J=6.9$ Hz, 3H). ^{13}C -NMR ($CDCl_3$) δ : 216.09, 169.18, 167.66, 154.51, 145.29, 138.16, 135.00, 128.19, 127.73, 126.11, 116.21, 110.35, 79.81, 73.63, 68.31, 57.17, 54.73, 52.43, 44.45, 43.83, 42.91, 40.74, 36.90, 35.76, 35.01, 33.45, 31.79, 30.63, 29.42, 27.18, 25.85, 25.43, 23.86, 15.85, 13.93, 10.51. IR (KBr) cm^{-1} : 3348, 2932, 1717, 1541, 1279, 1164, 1017, 731. FAB-MS m/z :

752.3403 $[M-H]^-$ (Calcd for $C_{40}H_{54}N_3O_7S_2$: 752.3403).

RESULTS AND DISCUSSION

Tested Strains Methicillin-sensitive *Staphylococcus aureus* ATCC25923 (MSSA), methicillin-sensitive *Staphylococcus epidermidis* ATCC12228 (MSSE), *Streptococcus agalactiae* ATCC13813 (*S. aga*), *Pyogenicococcus* (*Pyogens*) ATCC19615 and methicillin-resistant *S. aureus* (MRSA) were purchased from Weilu Co., Ltd. (Shanghai, China). MG was obtained from the Department of Avian Infectious Disease of the Shanghai Veterinary Research Institute.

Bioassay The antibacterial properties of the pleuromutilin derivatives **13a–o** were evaluated *in vitro* against MSSA, MSSE, MRSA, *S. aga*, *Pyogens* and MG via the broth dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS). The minimum inhibitory concentration (MIC, $\mu g/mL$) results are listed in Table 1. Erythromycin, tiamulin and valnemulin were used as reference drugs; tylosin was used instead of erythromycin for MG. All of the tested compounds exhibited moderate to good antibacterial activity against all strains. The MICs of derivatives against MSSA, MSSE, MRSA, *S. aga*, *Pyogens* and MG *in vitro* were in range of >1.78 –0.00085, >4 –0.0017, >1.78 –0.0039, >16 –0.25, >1.78 –0.056 and >0.25 –0.0039 $\mu g/mL$, respectively. The antibacterial effects for most aromatic acyl derivatives and the intermediate **9** were better than those of tiamulin and erythromycin against MSSA, MSSE, MRSA and MG. Compounds **13j** or **13a**, **c** and **e** moderately inhibited the growth of *S. aga*, which were equal to those of tiamulin or valnemulin respectively. Compounds **13i** and **l** showed potent activity against MSSE and MRSA, which were better than that of valnemulin. However, all the tested compounds exhibited weak activity against *Pyogens* compared with the reference drugs.

Table 1. Antibacterial Activity (MIC, $\mu g/mL$) *in Vitro*

Compound	(MIC, $\mu\text{g/mL}$)					
13a–g	MSSA	MSSE	MRSA	<i>S. aga</i>	<i>Pyogens</i>	MG
13a	0.014	0.028	0.063	0.5	0.11	0.063
13b	0.056	0.22	0.25	8	0.89	0.031
13c	0.056	0.056	0.125	0.5	0.44	0.031
13d	0.056	0.11	0.25	4	>1.78	0.016
13e	0.056	0.056	0.063	0.5	>1.78	0.0078
13f	0.11	0.11	0.5	>16	>1.78	0.25
13g	0.11	0.44	0.5	>16	>1.78	0.125
13h	0.0017	0.0069	0.0078	0.25	0.056	0.016
13i	0.00085	0.0035	0.0078	0.25	0.056	0.0078
13j	0.028	0.056	0.063	1	1.78	0.13
13k	>1.78	>1.78	>4	>16	>1.78	>0.25
13l	0.00085	0.0017	0.0039	2	0.22	0.0078
13m	0.11	0.22	0.5	8	0.22	0.031
13n	0.44	0.44	1	8	0.44	0.13
13o	0.22	0.44	0.5	2	0.44	0.031
9	0.028	0.056	0.125	2	0.22	0.0078
Erythromycin	0.11	0.22	>4	>16	0.0017	0.031*
Tiamulin (2)	0.11	0.11	0.5	1	0.028	0.031
Valnemulin (3)	0.0035	0.028	0.063	0.5	0.0035	0.0039

*The MIC of Tylosin.

From the MIC data, we can conclude that the structural modification of pleuromutilin can markedly affect the activity and the results further confirm that modification on the side chain at C-14 position of pleuromutilin derivatives with thiazole group can indeed improve antibacterial activity of the prototype compound against organisms.¹⁵⁾ Amino substitution in the benzene ring can improve activity in comparison with the reference drugs except against *Pyogens* and MG, and *para* substitution (**13i**) is better for antibacterial activity than *meta* substitution (**13h**) with being found 3–10-fold more potent than erythromycin and tiamulin against MSSA and MSSE. The result is consistent with the previous research results^{16,17)} which amino group could increase the antibacterial effect whether linking with benzene ring or not. Nitro, methoxy, hydroxy and dichloro substituents contribute little to the increased activity compared with the analogue (**13a**) that possesses unsubstituted benzoyl group. The *para* nitro-substitution derivative (**13d**) shows a little better antibacterial activity against MSSA and MSSE than the *ortho* substitution derivative (**13e**). Increasing a methylene between benzene moiety and carbonyl group decreases the bioactivity of derivative (**13b**). Introducing pyridine ring to C14 side chain can significantly improve activity (**13l**) and the effect is better than that of all the controls against MSSA, MSSE and MRSA, but in contrast with the previous study,¹⁵⁾ modification by pyridine group not shows satisfactory bioactivity, even not as good as modified by piperidinyl group. We think it may be caused by the methylene group between piperidinyl and carbonyl and needs more detail research in future. Compound modified by furancarboxyl group (**13j**) presents antibacterial activity, whereas derivative possessing thiophenecarboxyl (**13k**) group almost does not have bioactivity. The amino acid analogues (**13m–o**) showed moderate activity. The derivative (**13g**) possessing structure similar to NTZ doesn't show outstanding activity as expected and displays even worse than **13a** which bears phenyl group, it illustrates that simple association of antibacterial agent with pleuromutilin cannot increase bioactivity, and *ortho*-hydroxyl group substituent contributed little to the increased antibacterial activity, furthermore, more detail work about the position of hydroxyl group substituent maybe need to reveal.

In a word, some of the designed pleuromutilin derivatives showed the improvement antibacterial activity against Gram-positive, which indicates that the side chain of pleuromutilin derivatives indeed has great effect on the properties of the pleuromutilin derivatives and further on the bioactivity. The results will give new ideas for modification of novel pleuromutilin derivatives on the position C14, and be served as the foundation for screening the promising antibiotics for clinical use.

CONCLUSION

We designed and synthesized a series of new pleuromutilin derivatives that incorporated the acylthiazolyl moiety. The antibacterial activity of these new pleuromutilin derivatives were evaluated *in vitro* against five kinds of bacteria and the mycoplasma MG. All the derivatives exhibited moderate to good inhibitory characteristics against sensitive and resistant Gram-positive bacteria. Compounds **13i** and **l** showed better activities among the synthesized analogues.

The MICs of **13i**, bearing a 4-aminobenzamide-thiazolyl group on the C-14 glycolic acid side chain, and **13l**, bearing a 4-pyridinecarbonyl-thiazolyl group on the C-14 glycolic acid side chain, had better activities against MSSA (MIC=0.0017 µg/mL), MSSE (MIC=0.0017, 0.0035 µg/mL) and MRSA (MIC=0.0078 µg/mL). The MICs of synthesized new pleuromutilin derivatives against *S. aga*, *Pyogens* and MG were weak.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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