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# Accepted Article

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**Authors:** li yun ge, Ju-Xian Wang, Guo-Ning Zhang, Mei Zhu, Xue-Fu You, Xin-Xin Hu, Fan Zhang, and Yu-Cheng Wang



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Chem. Biodiversity

# Antibacterial Activity and Structure-Activity Relationship of a Series of Newly Synthesized Pleuromutilin Derivatives

**Yun-Ge Li[a], Ju-Xian Wang[b], Guo-Ning Zhang[b], Mei Zhu[b], Xue-Fu You[b], Xin-Xin Hu[b], Fan Zhang*[a] and Yu-Cheng Wang*[b]**

[a] School of Pharmacy, Jinzhou Medical University, Jinzhou 121001, P. R. China;

[b]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, P. R. China.

*Corresponding authors: zhangfan830720@163.com (F. Zhang); wangyucheng@imb.pumc.edu.cn (Y. C. Wang)

**Abstract:** A series of novel thioether or sulfoxide-type pleuromutilin derivatives containing heteroaromatic substituents at the end of C14 side chain were designed and synthesized. All of the derivatives were evaluated for their anti gram-positive bacteria activity in vitro. And some of them show good to excellent anti-bacterial activity comparable to retapamulin and azamulin in most of the tested gram-positive pathogens. In this work, a five-membered heterocyclic moiety, a pyrimidine-membered heterocyclic moiety or a benzoheterocyclic moiety was introducted in the C14 side chain to increase the structural diversity of the pleuromutilin derivatives. The anti-bacterial result reveal that the thioether containing pleuromutilin derivatives exert a more potency activity than the sulfoxide-type derivatives against gram-positive pathogens. The structure-activity relationship summarized in this work may provide some interesting clues as to which functionalities are beneficial for high antimicrobial activity of the pleuromutilin derivatives.

**Keywords:** pleuromutilin derivatives; synthesis; antibacterial gram-positive activity; structure-activity relationship

## Introduction

The increasing prevalence of multidrug-resistant bacteria caused by the abuse of antibiotics has become a serious medical problem worldwide. More than 2 million people die each year due to infections caused by Staphylococcus aureus, Streptococcus pneumoniae, and Mycobacterium tuberculosis, and human health is seriously endangered[1 – 2]. Therefore, the discovery and development of new classes of antibacterial agents are very important to the medical community.

Pleuromutilin (Figure 1) is a tricyclic diterpenoid natural product with modest antibacterial activity and was first discovered and isolated by Kavanagh and his colleagues from Pleurotus mutilus and Pleurotus passeckeranius in 1951[3 – 5]. To date, many pleuromutilin derivatives have been prepared and evaluated, and derivatives in which the hydroxyl group in the side chain of the C14 glycolate is replaced with a sulfur-containing compound have achieved great success[6]. This has led to the discovery of tiamulin (Figure 1) and valnemulin (Figure 1) as veterinary medicines for poultry and pigs[7 – 8]. In the 1980s, azamulin (Figure 1), an extremely broad antibacterial drug for human use, was designed, but due to its strong inhibition of human cytochrome P450 and its low oral bioavailability and short half-life, its development was terminated in the first phase of clinical trials[9]. In 2007, retapamulin was developed by GlaxoSmithKline, which was the first approved topical antimicrobial agent to treat skin infections for human use[10]. Recently, the human drugs BC-7013 (Figure 1) and BC-3205 (Figure 1), developed by Nabriva Therapeutics, have been used for the treatment of bacterial infections[11]. As a topical drug, BC-7013 has a good therapeutic effect on skin infections caused by gram-positive bacteria. BC-3205 can be used either as a topical drug or as an oral drug[12]. Currently, these two drugs are in phase I clinical trials. In May 2018, Nabriva Therapeutics announced that the company's innovative antibiotic lefamulin (Figure 1) met all major US FDA and European Medicine Agency (EMA) endpoints in critical phase III clinical trials for treating community-acquired bacterial pneumonia (CABP) patients and showed good tolerance[13].

Biochemical and genetic studies have shown that pleuromutilin derivatives can inhibit bacterial protein synthesis by binding to the peptidyl transferase center (PTC) of the bacterial ribosome, thereby inhibiting bacterial growth[14 – 15]. Subsequent studies revealed that the nucleus of the pleuromutilin derivatives bound to the A site of the PTC, and the C14 side chains of these derivatives pointed toward the P-site, further demonstrating that the mechanism of action of this class of compounds is to prevent synthesis of bacterial proteins[16]. The mode of action is to use the flexible trait of the ribosome to bind the pleuromutilin derivatives tightly to the subunit pocket based on the induced fit motion[16].

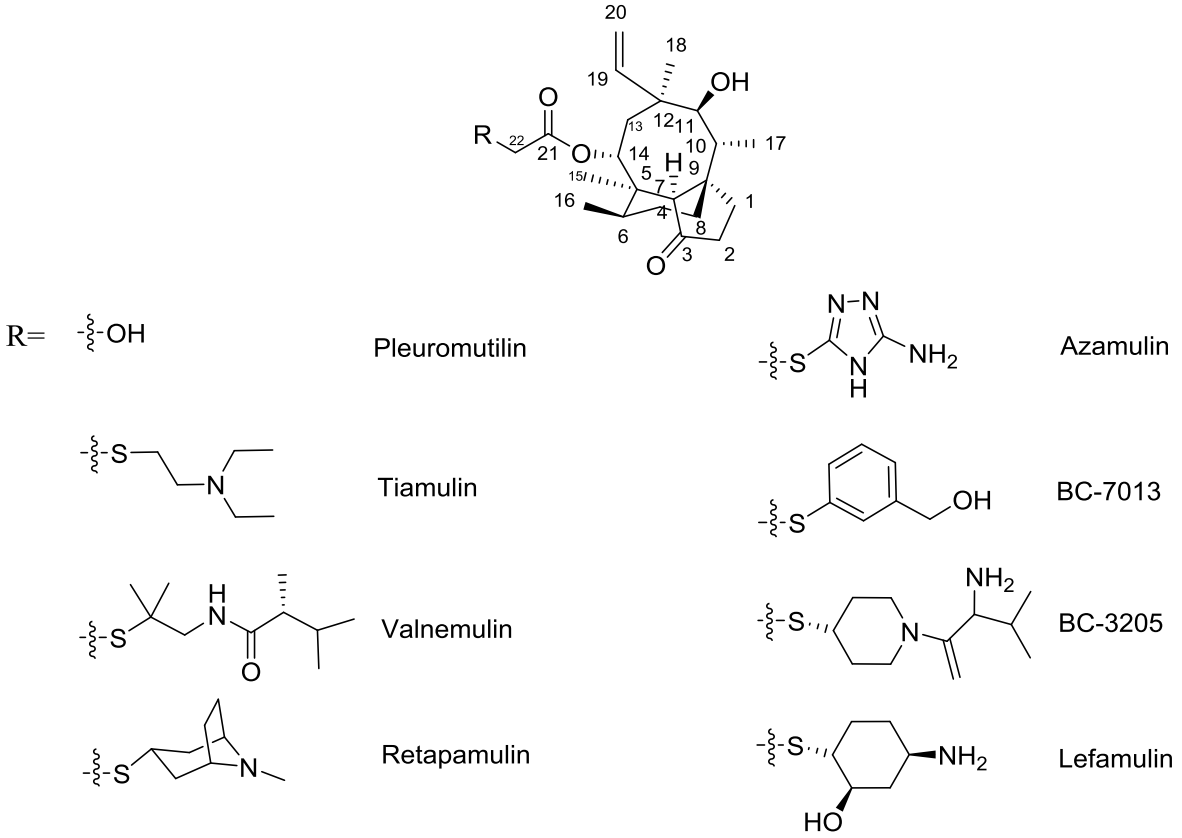



**Figure 1.** Structures of pleuromutilin and its derivatives.

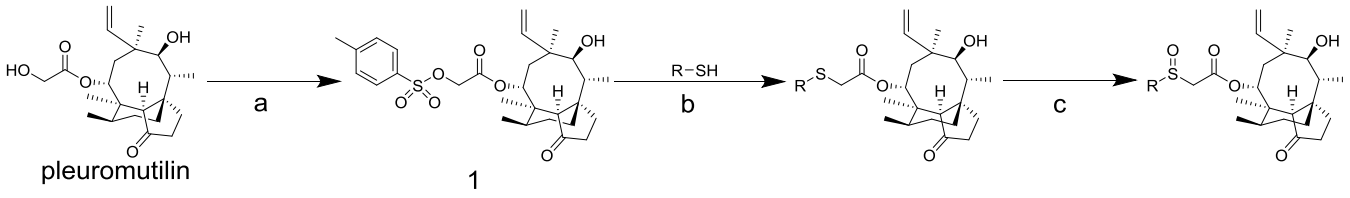
Chenyu Ling's group reported a series of pleuromutilin derivatives with heteroaromatic substituents incorporated into the C14 side chain with increased water solubility, hydrogen bonding and π-π stacking interactions, which showed excellent in vitro antibacterial activity and good in vivo efficacy[17]. In view of previous studies on pleuromutilin derivatives, we synthesized a series of thioether-type pleuromutilin derivatives and oxidized their thioether bonds to sulfoxides to obtain a series of novel sulfoxide pleuromutilin derivatives. In addition, three types of new thioether pleuromutilin derivatives were synthesized. Moreover, the antibacterial activity and structure-activity relationship of all compounds were described in detail.

## Results and Discussion

*Chemistry*

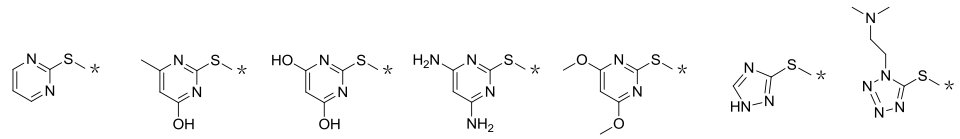
The general synthetic route of pleuromutilin derivatives is shown in Scheme 1. All of the pleuromutilin derivatives were synthesized from compound 1, using an easy and efficient method[18]. It is worth mentioning that we used 1, 8-Diazabicyclo[5.4.0]undec-7-ene (DBU) instead of sodium hydroxide in step b, which made the workup easier and produced a higher yield. Totally, 32 new and 5 known (2A, 2B, 3A, 4A, and 4D)[17][19] pleuromutilin derivatives were synthesized using this method.

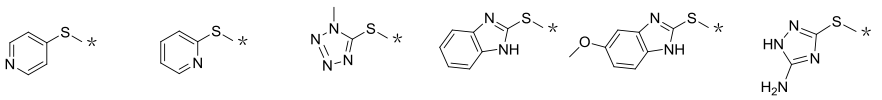
**Scheme 1** Synthetic route for all of the pleuromutilin derivatives



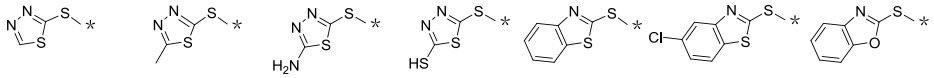

. **Reagents and conditions**: (a) TsCl, NaOH, t-butyl methyl ether/$H_2O$, reflux, 1 h; (b) Method 1: DBU, MeOH/DCM, rt, 12 h; Method 2: DBU, THF, Ar, 0℃, 2 h; (iv) *m*-CPBA, DCM, -5℃, 1 h.

**Method 1:R=**





**Method 2:R=**

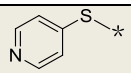
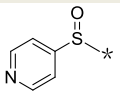
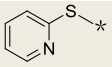
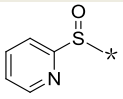
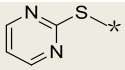
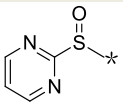
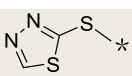
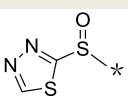
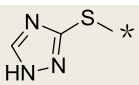
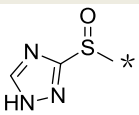


*Antibacterial Activity*

All derivatives were evaluated for resistant gram-positive pathogens by the agar dilution method, and retapamulin and azamulin were used as positive controls. The MIC (minimum inhibitory concentration) was determined as the lowest concentration of the antibiotic that inhibited the growth of the bacteria on the plate. All research results are shown in Tables 1-4.

The antibacterial data of Table 1 show that the thioether-type pleuromutilin derivatives have good antibacterial activity, with a minimum MIC ≤ 0.03 μg/mL and a maximum MIC = 0.25 μg/mL. The corresponding sulfoxide-type pleuromutilin derivative has an antibacterial activity that was 10-20 times lower. Our results revealed that even if the thioether structure is oxidized to a sulfoxide structure while retaining the sulfur atom at the C14 position, the antibacterial activity of the derivative decreased dramatically. Therefore, the structure-activity relationship further proved that the thioether structure was crucial for the antibacterial activity of pleuromutilin derivatives.

Table 1. In vitro antibacterial activity of **2A, 2a, 2B, 2b, 3A, 3a, 4A, 4a** against gram-positive strains

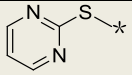
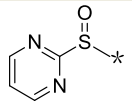
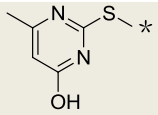
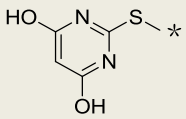
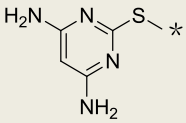
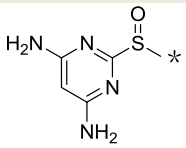
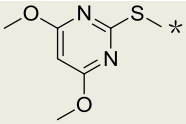
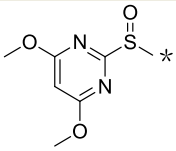
| Compound | Structure | MIC (μg/mL) | | | | |
|---|---|---|---|---|---|---|
| | | MSSE | MRSE | MSSA | MRSA | VISA |
| **2A** |  | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| **2a** |  | 1 | 1 | 1 | 1 | 1 |
| **2B** |  | ≤0.03 | ≤0.03 | 0.06 | 0.06 | ≤0.03 |
| **2b** |  | 0.12 | 0.25 | 0.25 | 0.5 | 0.12 |
| **3A** |  | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| **3a** |  | 1 | 1 | 1 | 1 | 0.5 |
| **4A** |  | ≤0.06 | ≤0.06 | 0.25 | 0.25 | 0.25 |
| **4a** |  | 1 | 1 | 1 | 2 | 1 |
| **4E** |  | 0.5 | 0.5 | 1 | 0.5 | 0.25 |
| **4e** |  | 1 | 2 | 2 | 1 | 0.5 |
| **Retapamulin** | - | ≤0.03 | ≤0.03 | 0.06 | 0.06 | ≤0.03 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Azamulin** | - | 0.12 | 0.12 | 1 | 0.5 | 0.25 |

Abbreviations are as follows: MIC, minimum inhibitory concentration; MSSE, methicillin-sensitive Staphylococcus epidermidis ATCC 12228; MRSE, methicillin-resistant Staphylococcus epidermidis 16-5; MSSA, methicillin-sensitive Staphylococcus aureus ATCC 29213; MRSA, methicillin-resistant Staphylococcus aureus ATCC 33591; VISA, vancomycin-intermediate Staphylococcus aureus ATCC 700699.

The in vitro antibacterial data in Table 2 suggest that increasing in the number of hydroxyl groups introduced into the pyrimidine ring leads to a significant decrease in the antibacterial activity of the derivatives (3B and 3C *vs.* 3A). The antibacterial activity was unchanged when the hydrophilic group amino group was introduced (3D *vs.* 3A) and was more effective than azamulin and is almost equivalent to retapamulin. This result indicates that the introduction of an amino group is a good choice for increasing water solubility.

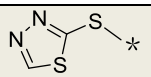
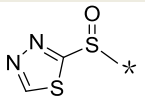
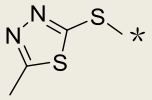
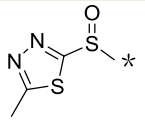
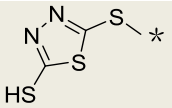
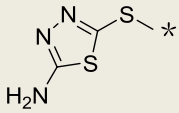
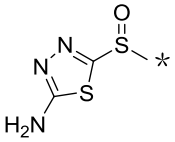
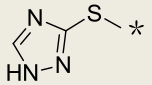
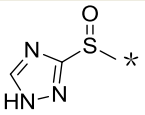
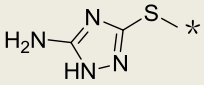
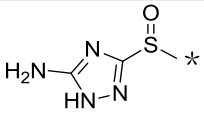
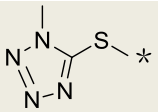
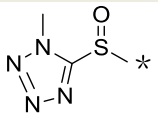
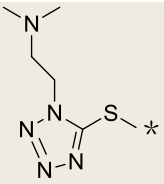
Table 2. In vitro antibacterial activity of 3A-E against gram-positive strains

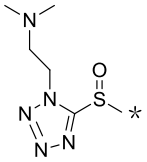
| **Compound** | **Structure** | **MIC (μg/mL)** | | | | |
|---|---|---|---|---|---|---|
| | | **MSSE** | **MRSE** | **MSSA** | **MRSA** | **VISA** |
| **3A** |  | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| **3a** |  | 1 | 1 | 1 | 1 | 0.5 |
| **3B** |  | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 |
| **3C** |  | 4 | 4 | 32 | 16 | 16 |
| **3D** |  | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| **3d** |  | 0.25 | 0.25 | 1 | 1 | 0.25 |
| **3E** |  | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| **3e** |  | 1 | 1 | 1 | 1 | 0.5 |
| **Retapamulin** | - | ≤0.03 | ≤0.03 | 0.06 | 0.06 | ≤0.03 |
| **Azamulin** | - | 0.12 | 0.12 | 1 | 0.5 | 0.25 |

Abbreviations: see footnote of Table 1.

Table 3 shows the following: 1) when the N atom on the five-membered heterocyclic ring increases, the antibacterial activity of the derivative decreases (4E *vs.* 4A); 2) the introduction of the amino side chain on the five-membered heterocyclic ring increases the antibacterial activity of the derivative (4D *vs.* 4A, and 4F *vs.* 4E). The structures of 4G is similar to that of azamulin, and the antibacterial activity is equivalent, which deserves further study.

Table 3. In vitro antibacterial activity of 4A-I against gram-positive strains

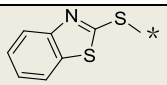
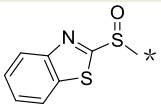
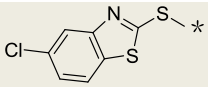
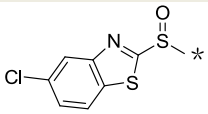
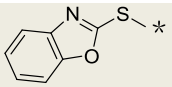
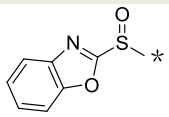
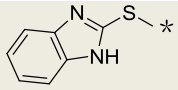
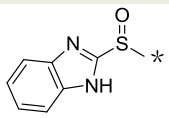
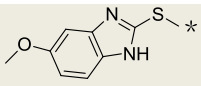
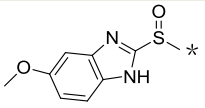
| Compound | Structure | MIC (μg/mL) | | | | |
|---|---|---|---|---|---|---|
| | | MSSE | MRSE | MSSA | MRSA | VISA |
| 4A |  | ≤0.06 | ≤0.06 | 0.25 | 0.25 | 0.25 |
| 4a |  | 1 | 1 | 1 | 2 | 1 |
| 4B |  | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| 4b |  | 1 | 1 | 1 | 0.5 | 0.5 |
| 4C |  | 0.25 | 0.25 | 0.5 | 0.25 | 0.12 |
| 4D |  | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06 |
| 4d |  | 0.25 | 0.25 | 1 | 1 | 0.25 |
| 4E |  | 0.5 | 0.5 | 1 | 0.5 | 0.25 |
| 4e |  | 64 | 64 | 128 | 128 | 64 |
| 4F |  | 0.125 | 0.125 | 1 | 0.5 | 0.25 |
| 4f |  | 16 | 16 | 128 | 128 | 32 |
| 4G |  | 1 | 1 | 1 | 1 | 0.5 |
| 4g |  | 1 | 2 | 2 | 2 | 1 |
| 4H |  | 1 | 1 | 2 | 2 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **4h** |  | 4 | 8 | 8 | 4 | 4 |
| **Retapamulin** | - | ≤0.03 | ≤0.03 | 0.06 | 0.06 | ≤0.03 |
| **Azamulin** | - | 0.12 | 0.12 | 1 | 0.5 | 0.25 |

Abbreviations: see footnote of Table 1. .

The derivatives 5A-E in Table 4 show higher antibacterial activity compared to the positive control. The introduction of the chlorine atom on the 5B benzene ring was detrimental to the activity. The introduction of the methoxy group on the 5D benzene ring made 5E more effective than 5D.

Table 4. In vitro antibacterial activity of 5A-E against gram-positive strains

| **Compound** | **Structure** | **MIC (μg/mL)** | | | | |
|---|---|---|---|---|---|---|
| | | **MSSE** | **MRSE** | **MSSA** | **MRSA** | **VISA** |
| **5A** |  | 0.125 | 0.125 | ≤0.06 | ≤0.06 | ≤0.06 |
| **5a** |  | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| **5B** |  | 0.5 | 0.5 | 0.5 | 0.5 | 0.25 |
| **5b** |  | 1 | 1 | 1 | 1 | 1 |
| **5C** |  | 0.12 | 0.12 | 0.5 | 0.12 | 0.12 |
| **5c** |  | 1 | 1 | 1 | 2 | 0.5 |
| **5D** |  | 0.12 | 0.25 | 0.25 | 0.25 | 0.06 |
| **5d** |  | 1 | 1 | 1 | 1 | 0.5 |
| **5E** |  | 0.06 | 0.06 | 0.125 | ≤0.06 | ≤0.06 |
| **5e** |  | 2 | 2 | 1 | 2 | 1 |
| **Retapamulin** | - | ≤0.03 | ≤0.03 | 0.06 | 0.06 | ≤0.03 |
| **Azamulin** | - | 0.12 | 0.12 | 1 | 0.5 | 0.25 |

Abbreviations: see footnote of Table 1.

## Conclusions

In summary, a series of thioether or sulfoxide-type pleuromutilin derivatives containing heteroaromatic substituents such as a five-membered heterocyclic moiety, a pyrimidine-membered heterocyclic moiety and a benzoheterocyclic moiety at the end of C14 side chain were designed and synthesized. All of the derivatives were evaluated for their anti gram-positive bacterial activity in vitro. Ten compounds (3B, 3D, 3E, 4B, 4C, 4F, 5A, 5C, 5D, and 5E) out of them

show good to excellent anti-bacterial activity comparable to retapamulin and azamulin in most of the tested gram-positive pathogens. The anti-bacterial result reveal that the thioether containing pleuromutilin derivatives exert a more potency activity than the sulfoxide-type derivatives against gram-positive pathogens. Conjugating and exploring these heterocyclic moieties at the end of C14 side chain contributed to further understanding of the design of this class of antibacterial agents. The structure-activity relationship summarized in this work can significantly provide some interesting clues as to which functionalities are beneficial for high antimicrobial activity.

## Experimental Section

Unless otherwise stated, the reagents involved are commercially available, analytically pure and chemically pure, and were used without special treatment. The reaction was monitored by thin layer analytical chromatography (TLC, Merck's silica gel 60 GF 254 aluminum foil plate) under UV light at 254 nm. The column chromatography silica gel is 100-200 mesh and 200-300 mesh silica gel (Qingdao Ocean Chemical Factory). The melting pointwas measured using a METTLER TOLEDO MP-70 melting point apparatus, and the temperature was not corrected. he Agilent-100 quadrupole LC/MS were used for Low-resolution mass spectrometry was measured on a Agilent-100 quadrupole LC/MS apparatus. High-resolution mass spectrometry was performed using a Bruker micrOTOF-QII mass spectrometer. The nuclear magnetic resonance spectrum and the carbon spectrum were measured on a Bruker 400 MHz, Bruker 500 MHz and Bruker AVANCE III HD 600 MHz nuclear magnetic resonance apparatus, and tetramethylsilane (TMS) was used as an internal standard.

Details of the route in Scheme 1 are described below.

(a) To a solution of Pleuromutilin (15.15 g, 40 mmol) and p-toluenesulfonyl chloride (8.4 g, 44 mmol) in the mixture of of water (10 mL) and tert-butyl methyl ether (4 mL), NaOH (10 N, 10 mL) was added dropwise. The reaction mixture was stirred under reflux for 1 hour and cooled to room temperature. Then the solvent was diluted with water (100 mL) and stirred for another 30 min in an ice bath. The suspension was suction filtered and washed 3 times with water (100 mLX3) and cold t-butyl methyl ether (40 mL) to afforded a white powdery solid.

(b) Method 1: To a solution of heteroaromatic thiol (2.25 mmol) in MeOH (10 mL), DBU (9 mmol) was added dropwise, and the mixture was stirred for 30 min at room temperature. Then a solvent of of Compound 1 (1.88 mmol) in dichloromethane (20 mL) was added to the solvent and the mixture was stirred until complete consumption of heteroaromatic thiol. The reaction mixture was concentrated in vacuo. EtOAc (25 mL) was added, and the mixture was washed with water and brine. The organic layer was separated and dried over anhydrous $Na_2SO_4$ overnight. Anhydrous $Na_2SO_4$ was filtered off and the solvent was evaporated in vacuo. The residue was purified on a silica gel column to afford intermediate as a white solid.

Method 2: To a mixed solution of heteroaromatic thiol (2.25 mmol) in 10% NaOH (12.5 mmol), a solution of compound 1 (1.88 mmol) in tetrahydrofuran (20 mL) was added dropwise. Then the mixture was stirred for 2 hours at 0 ℃ before the solvent was evaporated in vacuo., The residue was dissolved in EtOAc (25 mL), quenched with saturated aqueous $NH_4Cl$ and washed with water. The organic layer was separated and dried over anhydrous $Na_2SO_4$ overnight. The $Na_2SO_4$ was filtered off and the solvent was evaporated in vacuo. The residue was purified on a silica gel column to afford intermediate as a white solid.

(c) To a solution of thioether pleuromutilin derivative (300 mg) in $CH_2Cl_2$ (5 mL), 3-chloroperoxybenzoic acid (*m*-CPBA, 1 equivalent) was added at -5 ℃ and the mixture was stirred for 1 hour before it was washed with saturated $NaHCO_3$, water and brine, successively. The organic layer was dried over anhydrous sodium sulfate overnight. The solvent was evaporated in vacuo and the residue was purified on a silica gel column to afford the target compound as a white solid.

14-O-(p-Toluene-sulfonyloxyacetyl)-mutilin(1)

Yield: 92.6%. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 6.40 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.76 (d, *J* = 8.5 Hz, 1H), 5.32 (d, *J* = 12.2 Hz, 1H), 5.19 (d, *J* = 18.8 Hz, 1H), 4.48 (s, 2H), 3.35 (d, *J* = 6.5 Hz, 1H), 2.45 (s, 3H), 2.28 (p, *J* = 6.9 Hz, 1H), 2.24 (d, *J* = 9.7 Hz, 1H), 2.18 (dt, *J* = 19.4, 9.4 Hz, 1H), 2.10 – 2.02 (m, 2H), 1.75 (dd, *J* = 14.5, 2.8 Hz, 1H), 1.64 (td, *J* = 12.1, 11.1, 7.1 Hz, 2H), 1.56 (s, 1H), 1.53 – 1.42 (m, 2H), 1.40 (s, 3H), 1.34 (dd, *J* = 14.3, 3.0 Hz, 1H), 1.25 (d, *J* = 16.1 Hz, 1H), 1.15 (s, 3H), 1.11 (td, *J* = 14.2, 4.4 Hz, 1H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.62 (d, *J* = 7.1 Hz, 3H). 13C NMR (126 MHz, $CDCl_3$) δ 216.85 164.91, 145.35, 138.68 , 132.55 , 129.95 , 128.12 , 117.46, 74.53, 70.27, 65.06, 58.03, 45.40, 44.48, 43.96, 41.83 36.55, 36.02, 34.43, 30.34, 26.78, 26.38, 24.82, 21.74, 16.55, 14.78, 11.53. HRMS (ESI): calcd for C29H40O7SNa $[M + Na]^+$: 555.2392; found, 555.2395.

14-O-(((Pyridin-4-yl)sulfanyl)acetyl)mutilin(2A)

Yield: 81%. $^1$H NMR (500 MHz, $CDCl_3$) δ 8.40 (s, 2H), 7.17 (d, *J* = 4.0 Hz, 2H), 6.37 (dd, *J* = 20.0, 15.0 Hz, 1H), 5.76 (d, *J* = 10.0 Hz, 1H), 5.28 – 5.11 (m, 2H), 4.12 (d, *J* = 6.8 Hz, 1H), 3.67 (s, 2H), 3.34 (d, *J* = 5.0 Hz, 1H), 3.22 (s, 1H), 2.83 – 2.71 (m, 1H), 2.32 – 2.28 (m, 1H), 2.23 (s, 1H), 2.04 (s, 2H), 1.79 –

1.75 (m, 1H), 1.68 – 1.63 (m, 3H), 1.45 (s, 1H), 1.43 (s, 3H), 1.36 (d, *J* = 13.7 Hz, 1H), 1.26 (t, *J* = 6.9 Hz, 1H), 1.12 (s, 3H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.70 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.85, 167.11, 148.97, 138.77, 120.89, 117.27, 74.55, 70.39, 58.11, 45.42, 44.93, 43.87, 41.81, 36.66, 36.00, 34.41, 34.01, 30.36, 26.79, 26.48, 24.82, 16.75, 14.82, 14.19, 11.52 . HRMS (ESI): calcd for $C_{27}H_{37}NO_4SH$ $[M + H]^+$: 472.2522; found, 472.2536.

14-O-(((Pyridin-4-yl)sulfinyl)acetyl)mutilin(2a)

Yield: 31%. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.85 – 8.76 (m, 4H), 7.62 (dd, *J* = 12.2, 5.8 Hz, 4H), 6.43 (ddd, *J* = 17.5, 11.0, 7.9 Hz, 2H), 5.81 – 5.75 (m, 2H), 5.38 (d, *J* = 11.0 Hz, 2H), 5.25 (dd, *J* = 18.1, 10.2 Hz, 2H), 3.80 – 3.64 (m, 4H), 3.36 (d, *J* = 6.3 Hz, 2H), 2.32 – 2.25 (m, 4H), 2.25 – 2.16 (m, 4H), 2.10 – 2.04 (m, 5H), 1.77 (d, *J* = 14.6 Hz, 2H), 1.71 – 1.62 (m, 5H), 1.48 (s, 1H), 1.45 (s, 4H), 1.39 (s, 3H), 1.28 – 1.21 (m, 4H), 1.18 (d, *J* = 7.0 Hz, 6H), 1.13 (dd, *J* = 8.6, 4.9 Hz, 1H), 0.89 (dd, *J* = 7.0, 2.6 Hz, 6H), 0.75 (d, *J* = 7.1 Hz, 3H), 0.60 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.65, 216.61, 163.52, 163.49, 150.36, 150.29, 138.91, 138.66, 118.61, 118.59, 117.57, 117.42, 74.53, 71.13, 71.04, 61.31, 61.01, 58.00, 57.97, 45.41, 44.92, 44.82, 44.07, 44.02, 41.80, 41.78, 36.65, 36.55, 36.04, 34.40, 30.35, 30.32, 26.82, 26.80, 26.49, 26.34, 24.81, 16.87, 16.71, 14.85, 14.77, 11.49 . HRMS (ESI): calcd for $C_{27}H_{37}NO_5SNa$ $[M + Na]^+$: 510.2290; found, 510.2291.

14-O-(((Pyridin-2-yl)sulfanyl)acetyl)mutilin (2B)

Yield: 80%. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.33 (d, *J* = 4.8 Hz, 1H), 7.49 (td, *J* = 7.9, 1.7 Hz, 1H), 7.30 – 7.18 (m, 2H), 7.04 – 6.93 (m, 1H), 6.44 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.74 (d, *J* = 8.5 Hz, 1H), 5.27 (d, *J* = 11.0 Hz, 1H), 5.16 (d, *J* = 18.7 Hz, 1H), 4.00 – 3.81 (m, 2H), 3.33 (d, *J* = 6.3 Hz, 1H), 2.31 (p, *J* = 7.0 Hz, 1H), 2.27 – 2.14 (m, 2H), 2.08 (s, 1H), 1.99 (dd, *J* = 16.0, 8.6 Hz, 1H), 1.75 (dd, *J* = 14.5, 2.9 Hz, 1H), 1.67 – 1.60 (m, 2H), 1.54 (qd, *J* = 13.8, 3.6 Hz, 1H), 1.44 (td, *J* = 10.2, 9.8, 4.7 Hz, 1H), 1.41 (s, 3H), 1.37 – 1.28 (m, 2H), 1.14 (s, 3H), 1.11 (dd, *J* = 14.1, 4.4 Hz, 1H), 0.85 (d, *J* = 7.1 Hz, 3H), 0.76 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 217.09, 168.23, 156.85, 149.13, 139.03, 136.14, 122.13, 119.76, 117.06, 74.61, 69.53, 58.20, 45.46, 44.46, 43.90, 41.85, 36.82, 36.01, 34.48, 33.15, 30.45, 26.87, 26.43, 24.84, 16.68, 14.86, 11.45 . HRMS (ESI): calcd for $C_{27}H_{37}NO_4SNa$ $[M + Na]^+$: 494.2341; found, 494.2372.

14-O-(((Pyridin-2-yl) sulfinyl)acetyl)mutilin (2b)

Yield: 34%. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.68 – 8.55 (m, 1H), 8.06 – 7.89 (m, 2H), 7.41 (q, *J* = 7.5, 7.0 Hz, 1H), 6.44 (ddd, *J* = 17.4, 13.1, 11.1 Hz, 1H), 5.81 (dd, *J* = 19.0, 8.5 Hz, 1H), 5.41 – 5.28 (m, 1H), 5.24 (d, *J* = 17.4 Hz, 1H), 4.16 – 3.94 (m, 1H), 3.76 (dd, *J* = 93.1, 14.3 Hz, 1H), 3.36 (dd, *J* = 11.8, 6.5 Hz, 1H), 2.31 (dt, *J* = 9.7, 6.9 Hz, 1H), 2.26 – 2.22 (m, 1H), 2.18 (ddd, *J* = 19.4, 9.4, 4.6 Hz, 1H), 2.11 (q, *J* = 8.6 Hz, 1H), 2.07 (s, 1H), 2.05 (d, *J* = 4.1 Hz, 0H), 1.99 (dd, *J* = 16.0, 8.6 Hz, 1H), 1.76 (dd, *J* = 14.5, 2.9 Hz, 1H), 1.69 – 1.61 (m, 2H), 1.57 – 1.51 (m, 1H), 1.49 (d, *J* = 5.4 Hz, 3H), 1.46 – 1.42 (m, 1H), 1.37 (dt, *J* = 14.3, 3.4 Hz, 1H), 1.31 (s, 2H), 1.19 (d, *J* = 10.2 Hz, 3H), 1.12 (ddt, *J* = 14.0, 9.8, 4.8 Hz, 1H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.75 (dd, *J* = 15.1, 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.87, 216.85, 164.26, 163.88, 163.77, 163.54, 149.59, 149.53, 139.01, 138.90, 138.22, 138.17, 124.97, 120.57, 120.45, 117.35, 117.27, 74.61, 74.60, 70.62, 70.60, 58.83, 58.78, 58.12, 58.03, 45.44, 45.43, 44.84, 44.55, 44.06, 44.05, 41.83, 41.81, 36.76, 36.71, 36.07, 36.03, 34.45, 30.40, 30.37, 26.85, 26.83, 26.56, 26.50, 24.85, 24.82, 16.84, 14.95, 14.80, 11.47 . HRMS (ESI): calcd for $C_{27}H_{37}NO_5SNa$ $[M + Na]^+$: 510.2290; found, 510.2306.

14-O-(((Pyrimidin-2-yl)sulfanyl)acetyl)mutilin(3A)

Yield: 78.5%. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.48 (d, *J* = 4.6 Hz, 2H), 6.98 (t, *J* = 4.5 Hz, 1H), 6.46 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.78 (d, *J* = 8.5 Hz, 1H), 5.29 (dd, *J* = 11.0, 1.4 Hz, 1H), 5.18 (dd, *J* = 17.4, 1.5 Hz, 1H), 3.89 – 3.81 (m, 2H), 3.34 (d, *J* = 6.5 Hz, 1H), 2.35 – 2.29 (m, 1H), 2.26 – 2.17 (m, 2H), 2.10 – 2.07 (m, 1H), 2.05 – 2.01 (m, 1H), 1.76 (dd, *J* = 14.5, 3.0 Hz, 1H), 1.72 (s, 1H), 1.68 – 1.62 (m, 2H), 1.48 – 1.44 (m, 1H), 1.43 (s, 3H), 1.35 – 1.31 (m, 1H), 1.26 (t, *J* = 7.1 Hz, 1H), 1.15 (s, 3H), 1.12 (dd, *J* = 14.1, 4.4 Hz, 1H), 0.86 (d, *J* = 7.1 Hz, 3H), 0.77 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 217.03, 170.79, 167.76, 157.20, 139.09, 117.09, 116.76, 74.58, 69.71, 58.19, 45.46, 44.60, 43.91, 41.87, 36.79, 36.03, 34.47, 34.21, 30.45, 26.87, 26.45, 24.85, 16.67, 14.89, 11.46 . HRMS (ESI): calcd for $C_{26}H_{36}N_2O_4SNa$ $[M + Na]^+$: 495.2293; found, 495.2299.

14-O-(((Pyrimidin-2-yl) sulfinyl))acetyl)mutilin(3a)

Yield: 48%. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.88 (t, *J* = 4.6 Hz, 4H), 7.44 (td, *J* = 4.8, 1.9 Hz, 2H), 6.47 – 6.33 (m, 2H), 5.79 (dd, *J* = 8.3, 6.7 Hz, 2H), 5.38 – 5.28 (m, 2H), 5.23 (ddd, *J* = 17.4, 10.9, 1.4 Hz, 2H), 4.16 – 4.02 (m, 4H), 3.89 (d, *J* = 14.6 Hz, 1H), 3.35 (dd, *J* = 12.4, 6.5 Hz, 2H), 2.31 – 2.15 (m, 6H), 2.07 (d, *J* = 33.9 Hz, 5H), 1.98 (dd, *J* = 16.0, 8.6 Hz, 1H), 1.77 (s, 2H), 1.65 (ddt, *J* = 19.7, 9.3, 5.4 Hz, 5H), 1.58 (s, 4H), 1.49 (s, 1H), 1.46 (s, 4H), 1.45 – 1.42 (m, 1H), 1.37 (dt, *J* = 14.4, 3.0 Hz, 2H), 1.27 (d, *J* = 2.9 Hz, 4H), 1.25 (d, *J* = 7.1 Hz, 2H), 1.18 (d, *J* = 7.7 Hz, 6H), 1.15 – 1.08 (m, 2H), 0.87 (d, *J* = 7.0 Hz, 6H), 0.75 (d, *J* = 7.1 Hz, 3H), 0.72 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.85, 216.81, 172.66, 172.47, 164.12, 163.57, 158.56, 158.54, 138.92, 138.83, 122.01, 121.99, 117.35, 117.34, 74.60, 70.87, 70.82, 58.08, 57.99, 45.42, 44.69, 44.32, 44.09, 44.04, 41.83, 41.79, 36.71, 36.66, 36.06, 36.04, 34.44,

30.38, 30.35, 26.82, 26.56, 24.83, 24.81, 16.85, 16.79, 14.91, 14.79, 11.46 . HRMS (ESI): calcd for $C_{26}H_{36}N_2O_5SNa$ $[M + Na]^+$: 511.2243; found, 511.2256.

14-O-(((1, 3, 4-thiadiazole-5-yl)sulfanyl)acetyl)mutilin(4A)

Yield: 82%. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.02 (s, 1H), 6.41 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.77 (d, *J* = 8.5 Hz, 1H), 5.29 (d, *J* = 12.1 Hz, 1H), 5.18 (d, *J* = 18.7 Hz, 1H), 4.19 – 4.06 (m, 2H), 3.36 (d, *J* = 6.5 Hz, 1H), 2.31 (p, *J* = 7.9, 7.4 Hz, 1H), 2.21 (ddt, *J* = 28.8, 19.4, 9.5 Hz, 2H), 2.12 – 2.02 (m, 3H), 1.79 – 1.73 (m, 1H), 1.71 – 1.61 (m, 4H), 1.53 (qd, *J* = 14.0, 3.5 Hz, 1H), 1.46 (dd, *J* = 12.8, 2.7 Hz, 1H), 1.43 (s, 3H), 1.41 – 1.34 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 1H), 1.17 (s, 3H), 1.15 – 1.08 (m, 1H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.74 (d, *J* = 7.1 Hz, 3H).$^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.87, 166.42, 163.85, 151.73, 138.79, 117.25, 74.61, 70.65, 58.09, 45.43, 44.56, 43.99, 41.87, 36.69, 36.23, 36.01, 34.44, 30.39, 26.84, 26.47, 24.82, 16.74, 14.81, 11.46 . HRMS (ESI): calcd for $C_{24}H_{34}N_2O_4S_2Na$ $[M + Na]^+$: 501.1858; found, 501.1873.

14-O-(((1, 3, 4-thiadiazole-5-yl)sulfinyl)acetyl)mutilin(4a)

Yield: 44%. $^1$H NMR (600 MHz, $CDCl_3$) δ 9.37 (d, *J* = 3.0 Hz, 1H), 6.41 (ddd, *J* = 17.4, 14.3, 11.0 Hz, 1H), 5.84 (dd, *J* = 12.9, 8.6 Hz, 1H), 5.35 (d, *J* = 11.1 Hz, 1H), 5.23 (dd, *J* = 17.9, 7.7 Hz, 1H), 4.30 – 3.98 (m, 3H), 3.39 – 3.34 (m, 1H), 2.34 – 2.17 (m, 4H), 2.16 – 2.06 (m, 2H), 2.05 (d, *J* = 4.2 Hz, 1H), 1.78 (d, *J* = 15.8 Hz, 1H), 1.73 – 1.62 (m, 3H), 1.53 (d, *J* = 13.6 Hz, 1H), 1.48 (s, 2H), 1.45 (d, *J* = 2.8 Hz, 1H), 1.43 – 1.40 (m, 1H), 1.39 (s, 2H), 1.26 (t, *J* = 7.1 Hz, 1H), 1.19 (d, *J* = 6.8 Hz, 3H), 1.10 –1.14 (m, 1H), 0.89 (d, *J* = 7.0 Hz, 3H), 0.75 (t, *J* = 7.7 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.69, 216.66, 162.94, 162.70, 155.49, 155.43, 138.72, 138.54, 117.59, 117.51, 74.60, 71.65, 71.59, 61.06, 58.02, 57.96, 41.86, 36.65, 36.62, 36.07, 36.03, 34.41, 30.35, 30.34, 26.83, 26.81, 26.54, 26.50, 24.83, 24.81, 16.86, 16.85, 14.86, 14.72, 11.49, 11.48 . HRMS (ESI): calcd for $C_{24}H_{34}N_2O_5S_2Na$ $[M + Na]^+$: 517.1803; found, 517.1800.

14-O-(((1H-1, 2, 4-triazole-3-yl)sulfanyl)acetyl)mutilin(4E)

Yield: 85%. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.21 (s, 0H), 6.15 (dd, *J* = 17.8, 11.1 Hz, 1H), 5.36 (d, *J* = 17.8 Hz, 1H), 5.29 (d, *J* = 11.2 Hz, 1H), 4.35 (d, *J* = 7.6 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 0H), 3.86 (s, 1H), 3.45 – 3.38 (m, 1H), 2.28 – 2.20 (m, 2H), 2.17 (dd, *J* = 19.5, 9.1 Hz, 2H), 2.05 (d, *J* = 5.4 Hz, 2H), 1.91 (dd, *J* = 15.9, 7.7 Hz, 1H), 1.75 (dt, *J* = 14.5, 3.1 Hz, 1H), 1.65 – 1.67 (m, 1H), 1.63 – 1.56 (m, 2H), 1.48 (ddd, *J* = 19.9, 9.9, 5.2 Hz, 2H), 1.43 – 1.38 (m, 1H), 1.36 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 1H), 1.16 (s, 3H), 1.11 (dd, *J* = 14.0, 4.5 Hz, 1H), 0.96 (d, *J* = 7.1 Hz, 3H), 0.92 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 222.14, 171.63, 143.45, 119.13, 77.96, 74.34, 61.80, 57.33, 49.30, 48.15, 47.84, 45.69, 40.62, 40.25, 38.06, 37.81, 34.00, 30.93, 30.52, 28.35, 19.41, 17.84, 14.30 . HRMS (ESI): calcd for $C_{24}H_{35}N_3O_4SNa$ $[M + Na]^+$: 484.2246; found, 484.2258.

14-O-(((1H-1, 2, 4-triazole-3-yl)sulfinyl)acetyl)mutilin(4e)

Yield: 55%. $^1$H NMR (600 MHz, $CD_3OD$) δ 8.68 (s, 1H), 6.17 (ddd, *J* = 17.8, 11.2, 6.7 Hz, 1H), 5.72 (dd, *J* = 33.9, 8.4 Hz, 1H), 5.17 – 5.02 (m, 2H), 4.41 – 4.28 (m, 2H), 3.49 – 3.42 (m, 1H), 2.33 (d, *J* = 21.4 Hz, 1H), 2.25 (dt, *J* = 16.1, 6.9 Hz, 2H), 2.13 (dq, *J* = 17.9, 9.1 Hz, 2H), 2.00 (dd, *J* = 16.1, 8.5 Hz, 1H), 1.83 – 1.75 (m, 1H), 1.71 – 1.57 (m, 2H), 1.51 (dd, *J* = 25.5, 12.6 Hz, 1H), 1.40 (d, *J* = 16.6 Hz, 3H), 1.33 (dt, *J* = 28.3, 16.5 Hz, 3H), 1.12 (d, *J* = 16.5 Hz, 3H), 1.09 (s, 1H), 0.91 (t, *J* = 6.9 Hz, 3H), 0.70 (t, *J* = 6.4 Hz, 3H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 217.98, 163.73, 163.36, 145.51, 139.45, 115.37, 73.96, 71.12, 57.77, 56.27, 45.35, 44.30, 43.95, 41.74, 36.63, 36.35, 33.86, 30.04, 27.00, 26.57, 24.42, 15.61, 13.92, 10.41 . HRMS (ESI): calcd for $C_{24}H_{35}N_3O_5SNa$ $[M + Na]^+$: 500.2195; found, 500.2182.

14-O-(((4-Hydroxy-6-methylpyrimidine-2-yl)sulfanyl)acetyl)mutilin(3B)

Yield: 79%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.45 (dd, *J* = 17.4, 11.0 Hz, 1H), 6.06 (s, 1H), 5.75 (d, *J* = 8.5 Hz, 1H), 5.34 – 5.29 (m, 2H), 5.18 (d, *J* = 17.4 Hz, 1H), 3.93 – 3.84 (m, 2H), 3.36 (d, *J* = 6.4 Hz, 1H), 2.32 – 2.27 (m, 1H), 2.23 (d, *J* = 10.7 Hz, 1H), 2.20 (s, 3H), 2.18 – 2.15 (m, 1H), 2.09 (s, 1H), 2.04 (dd, *J* = 16.0, 8.6 Hz, 1H), 1.75 (d, *J* = 16.5 Hz, 1H), 1.64 (q, *J* = 11.0 Hz, 2H), 1.43 (s, 3H), 1.36 (d, *J* = 16.9 Hz, 1H), 1.30 – 1.23 (m, 2H), 1.14 (s, 3H), 1.12 – 1.08 (m, 1H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.73 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.97, 166.82, 165.54, 165.32, 159.18, 138.91, 117.25, 108.44, 74.56, 70.20, 58.09, 45.44, 44.50, 43.94, 41.88, 36.70, 36.01, 34.45, 33.30, 30.39, 26.85, 26.40, 24.82, 23.94, 16.83, 14.85, 11.47 . HRMS (ESI): calcd for $C_{27}H_{38}N_2O_5SNa$ $[M + Na]^+$: 525.2399; found, 525.2374.

14-O-(((4, 6-Dihydroxypyrimidine-2-yl)sulfanyl)acetyl)mutilin(3C)

Yield: 81.6%. $^1$H NMR (600 MHz, $CD_3OD$) δ 6.23 (dd, *J* = 17.4, 11.4 Hz, 1H), 5.71 (d, *J* = 8.5 Hz, 1H), 5.15 – 5.09 (m, 2H), 3.95 (q, *J* = 16.5 Hz, 2H), 3.47 (d, *J* = 6.1 Hz, 1H), 2.36 (d, *J* = 9.5 Hz, 1H), 2.31 – 2.22 (m, 2H), 2.14 (dt, *J* = 19.1, 9.4 Hz, 1H), 2.06 (dd, *J* = 16.0, 8.5 Hz, 1H), 2.01 (d, *J* = 5.4 Hz, 0H), 1.82 – 1.76 (m, 1H), 1.71 – 1.64 (m, 1H), 1.61 (ddd, *J* = 10.2, 7.0, 3.4 Hz, 1H), 1.47 – 1.45 (m, 1H), 1.43 (s, 3H), 1.41 – 1.37 (m, 1H), 1.36 – 1.32 (m, 1H), 1.17

(dd, *J* = 15.0, 6.1 Hz, 1H), 1.13 (s, 3H), 1.12 – 1.09 (m, 1H), 0.92 (d, *J* = 7.1 Hz, 3H), 0.70 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 219.60, 168.71, 162.66, 140.96, 116.69, 75.52, 72.33, 61.57, 59.24, 46.82, 45.66, 45.41, 43.28, 38.11, 37.73, 35.33, 34.15, 31.51, 28.24, 28.08, 25.84, 20.89, 17.02, 15.35, 11.77 . HRMS (ESI): calcd for $C_{26}H_{36}N_2O_6SNa$ $[M + Na]^+$: 527.2192; found, 527.2210.

14-O-(((4, 6-Diaminopyrimidine-2-yl)sulfanyl)acetyl)mutilin(3D)

Yield: 74%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.55 – 6.46 (m, 1H), 5.72 (d, *J* = 8.5 Hz, 1H), 5.34 – 5.27 (m, 2H), 5.18 (d, *J* = 17.1 Hz, 1H), 4.73 (s, 2H), 3.82 (d, *J* = 16.2 Hz, 1H), 3.68 (d, *J* = 16.2 Hz, 1H), 3.36 (s, 1H), 2.91 (d, *J* = 7.3 Hz, 3H), 2.34 – 2.29 (m, 1H), 2.21 (dtd, *J* = 28.8, 19.3, 10.2 Hz, 2H), 2.10 (s, 1H), 2.05 – 1.96 (m, 1H), 1.75 (d, *J* = 14.4 Hz, 1H), 1.70 – 1.61 (m, 3H), 1.44 (s, 3H), 1.33 (t, *J* = 17.1 Hz, 2H), 1.27 (s, 3H), 1.14 (s, 2H), 0.87 (d, *J* = 7.0 Hz, 3H), 0.75 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 218.31, 168.91, 167.80, 163.63, 139.67, 115.29, 79.25, 74.06, 70.10, 57.90, 53.46, 45.38, 44.17, 43.91, 41.79, 36.73, 36.24, 33.96, 33.28, 30.10, 26.86, 26.69, 24.43, 15.71, 14.09, 10.42, 8.30. HRMS (ESI): calcd for $C_{26}H_{38}N_4O_4SNa$ $[M + Na]^+$: 525.2511; found, 525.2518.

14-O-(((4, 6-Diaminopyrimidine-2-yl) sulfinyl)acetyl)mutilin(3d)

Yield: 32%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.45 (td, *J* = 10.9, 5.5 Hz, 1H), 5.78 (dd, *J* = 30.4, 8.5 Hz, 1H), 5.51 (dd, *J* = 25.9, 9.2 Hz, 4H), 5.32 (d, *J* = 18.5 Hz, 2H), 5.19 (d, *J* = 17.4 Hz, 1H), 4.03 – 3.77 (m, 2H), 3.36 (dd, *J* = 12.3, 6.3 Hz, 1H), 2.33 – 2.21 (m, 2H), 2.21 – 2.14 (m, 1H), 2.13 – 2.05 (m, 1H), 1.98 (dd, *J* = 16.1, 8.5 Hz, 1H), 1.79 – 1.72 (m, 1H), 1.64 (p, *J* = 11.8, 10.3 Hz, 2H), 1.46 (d, *J* = 8.3 Hz, 3H), 1.41 (d, *J* = 16.0 Hz, 1H), 1.35 (s, 2H), 1.29 – 1.21 (m, 1H), 1.14 (d, *J* = 15.7 Hz, 3H), 1.08 (d, *J* = 4.2 Hz, 1H), 0.88 (t, *J* = 7.1 Hz, 3H), 0.74 (dd, *J* = 14.3, 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 217.05, 170.51, 163.73, 117.36, 74.55, 70.60, 57.95, 45.41, 44.56, 44.03, 41.79, 36.66, 35.97, 34.45, 30.33, 26.84, 26.41, 24.76, 16.90, 14.92, 14.71, 11.46 . HRMS (ESI): calcd for $C_{26}H_{38}N_4O_5SNa$ $[M + Na]^+$: 541.2461; found, 541.2459.

14-O-(((4, 6-Dimethoxypyrimidine-2-yl)sulfanyl)acetyl)mutilin(3E)

Yield: 90%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.48 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.79 – 5.69 (m, 2H), 5.31 (dd, *J* = 11.0, 1.4 Hz, 1H), 5.17 (dd, *J* = 17.4, 1.5 Hz, 1H), 3.89 (s, 6H), 3.88 (s, 1H), 3.82 (d, *J* = 16.2 Hz, 1H), 3.34 (d, *J* = 6.5 Hz, 1H), 2.30 (h, *J* = 7.2 Hz, 1H), 2.26 – 2.14 (m, 2H), 2.08 (t, *J* = 1.8 Hz, 1H), 2.05 – 1.97 (m, 1H), 1.70 – 1.76 (m, 1H), 1.63 (dd, *J* = 11.2, 8.7 Hz, 2H), 1.57 (d, *J* = 3.6 Hz, 1H), 1.44 (s, 3H), 1.38 – 1.33 (m, 1H), 1.28 – 1.19 (m, 2H), 1.12 (s, 3H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.71 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.91, 170.81, 167.87, 138.93, 117.08, 85.97, 74.47, 69.58, 58.06, 54.26, 44.49, 36.65, 35.90, 34.40, 34.17, 30.35, 26.81, 26.24, 24.74, 16.69, 14.81, 11.41 . HRMS (ESI): calcd for $C_{28}H_{40}N_2O_6SNa$ $[M + Na]^+$: 555.2505; found, 555.2517.

14-O-(((4, 6-Dimethoxypyrimidine-2-yl)sulfinyl)acetyl)mutilin(3e)

Yield: 52%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.39 (ddd, *J* = 16.1, 11.0, 4.8 Hz, 1H), 6.06 (s, 1H), 5.77 (dd, *J* = 21.3, 8.4 Hz, 1H), 5.32 (dd, *J* = 21.4, 11.0 Hz, 1H), 5.20 (dd, *J* = 17.4, 9.6 Hz, 1H), 4.14 (q, *J* = 14.1 Hz, 2H), 4.01 (s, 6H), 3.90 (d, *J* = 14.8 Hz, 1H), 3.35 (dd, *J* = 18.9, 6.2 Hz, 1H), 2.18 – 2.24 (m, 3H), 2.11 (s, 1H), 2.05 (d, *J* = 7.8 Hz, 2H), 1.92 (dd, *J* = 15.9, 8.4 Hz, 1H), 1.76 (d, *J* = 14.5 Hz, 2H), 1.69 – 1.60 (m, 2H), 1.56 – 1.49 (m, 1H), 1.46 (s, 3H), 1.36 (d, *J* = 11.1 Hz, 1H), 1.31 (d, *J* = 11.0 Hz, 1H), 1.26 (d, *J* = 7.0 Hz, 2H), 1.15 (d, *J* = 14.5 Hz, 3H), 1.10 (d, *J* = 10.3 Hz, 1H), 0.88 (d, *J* = 6.8 Hz, 3H), 0.74 (dd, *J* = 24.1, 6.9 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.79, 171.81, 170.37, 163.86, 138.60, 117.41, 91.18, 74.49, 70.61, 58.02, 57.56, 56.91, 54.96, 45.38, 44.65, 43.97, 41.73, 36.65, 35.99, 34.40, 30.30, 26.80, 24.77, 16.89, 14.87, 11.44 . HRMS (ESI): calcd for $C_{28}H_{40}N_2O_7SNa$ $[M + Na]^+$: 571.2454; found, 571.2469.

14-O-(((5-methyl-1, 3, 4-thiadiazole-2-yl)sulfanyl)acetyl)mutilin(4B)

Yield: 71%. $^1$H NMR (500 MHz, $CDCl_3$) δ 6.42 (dd, *J* = 20.0, 20.0 Hz, 1H), 5.77(d, *J* = 18.0 Hz, 1H), 5.33 – 5.29 (m, 1H), 5.21 – 5.16 (m, 1H), 4.07 (s, 2H), 3.38 – 3.33 (m, 1H), 2.73 (s, 3H), 2.33 – 2.29 (m, 1H), 2.27 – 2.19 (m, 2H), 2.10 (s, 1H), 2.07 – 2.03 (m, 1H), 1.79 – 1.74 (m, 1H), 1.69 – 1.64 (m, 2H), 1.62 (s, 1H), 1.44 (s, 3H), 1.38 (s, 1H), 1.35 (s, 1H), 1.28 (d, *J* = 21.3 Hz, 1H), 1.18 (s, 3H), 1.12 (d, *J* = 11.3 Hz, 1H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.75 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.89, 166.64, 165.37, 163.36, 138.83, 117.25, 74.62, 70.51, 58.11, 45.44, 44.57, 43.98, 41.88, 36.72, 36.05, 36.01, 34.45, 30.41, 26.85, 26.40, 24.83, 16.77, 15.63, 14.82, 11.47 . HRMS (ESI): calcd for $C_{25}H_{36}N_2O_4S_2Na$ $[M + Na]^+$: 515.2014; found, 515.2023.

14-O-(((5-methyl-1, 3, 4-thiadiazole-2-yl)sulfinyl)acetyl)mutilin(4b)

Yield: 40%. $^1$H NMR (500 MHz, $CDCl_3$) δ 6.42 (dd, *J* = 17.0, 11.0 Hz, 1H), 5.76 (d, *J* = 9.1 Hz, 1H), 5.31 (d, *J* = 10.3 Hz, 1H), 5.18 (d, *J* = 17.1 Hz, 1H), 4.03

(s, 2H), 3.37 – 3.32 (m, 1H), 2.72 (s, 3H), 2.34 – 2.29 (m, 1H), 2.26 – 2.18 (m, 2H), 2.10 (s, 1H), 2.07 – 2.02 (m, 1H), 1.76 (d, *J* = 14.2 Hz, 1H), 1.65 (q, *J* = 10.9 Hz, 4H), 1.53 (d, *J* = 13.4 Hz, 1H), 1.43 (s, 3H), 1.38 (s, 1H), 1.34 (s, 1H), 1.28 (d, *J* = 20.1 Hz, 2H), 1.17 (s, 3H), 1.12 (d, *J* = 12.0 Hz, 1H), 0.87 (d, *J* = 7.0Hz, 3H), 0.74 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.89, 166.63, 163.06, 138.83, 117.25, 76.82, 74.63, 70.52, 60.91, 58.12, 45.44, 44.58, 43.99, 41.88, 36.72, 36.06, 34.46, 30.41, 26.85, 26.41, 24.83, 16.77, 16.18, 15.62, 14.82, 11.47 . HRMS (ESI): calcd for $C_{25}H_{36}N_2O_5S_2Na$ $[M + Na]^+$: 531.1963; found, 531.1963.

14-O-(((5-mercapto-1, 3, 4-thiadiazole-2-yl)sulfanyl)acetyl)mutilin(4C)

Yield: 43%. $^1$H NMR (600 MHz, $CDCl_3$) δ 11.21 (s, 1H), 6.43 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.78 (t, *J* = 9.9 Hz, 1H), 5.42 – 5.30 (m, 1H), 5.22 (d, *J* = 16.7 Hz, 1H), 3.91 – 3.76 (m, 2H), 3.39 (d, *J* = 6.5 Hz, 1H), 2.25 – 2.30 (m, 2H), 2.24 – 2.17 (m, 1H), 2.10 (q, *J* = 8.3 Hz, 2H), 1.78 (d, *J* = 14.5 Hz, 1H), 1.68 – 1.66 (m, 2H), 1.53 (d, *J* = 11.2 Hz, 1H), 1.45 (s, 3H), 1.42 – 1.35 (m, 2H), 1.26 (dd, *J* = 7.5, 4.7 Hz, 2H), 1.18 (s, 3H), 1.16 – 1.12 (m, 1H), 0.88 (t, *J* = 6.5 Hz, 3H), 0.72 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.96, 189.15, 165.99, 157.11, 138.83, 117.48, 74.61, 70.89, 58.11, 45.47, 44.77, 43.96, 41.89, 36.66, 36.03, 35.46, 34.46, 30.40, 26.84, 26.46, 24.86, 16.77, 14.83, 11.52 . HRMS (ESI): calcd for $C_{24}H_{34}N_2O_4S_3Na$ $[M + Na]^+$: 533.1578; found, 533.1592.

14-O-(((5-amino-1, 3, 4-thiadiazole-2-yl)sulfanyl)acetyl)mutilin(4D)

Yield: 74%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.41 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.74 (d, *J* = 8.5 Hz, 1H), 5.34 – 5.28 (m, 2H), 5.19 (d, *J* = 18.7 Hz, 1H), 3.86 (s, 2H), 3.37 (d, *J* = 6.4 Hz, 1H), 2.31 (p, *J* = 7.0 Hz, 1H), 2.15 – 2.21 (m, 2H), 2.11 (s, 1H), 2.09 – 2.02 (m, 2H), 1.79 – 1.74 (m, 1H), 1.65 (td, *J* = 12.1, 11.0, 7.1 Hz, 2H), 1.48 – 1.44 (m, 1H), 1.43 (s, 3H), 1.39 – 1.30 (m, 2H), 1.16 (s, 3H), 1.12 (td, *J* = 14.2, 4.4 Hz, 1H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.71 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 217.19, 169.59, 166.95, 151.77, 138.94, 117.22, 74.60, 70.45, 58.14, 53.48, 45.45, 44.62, 44.00, 41.86, 36.72, 36.02, 34.48, 30.39, 26.84, 26.57, 24.83, 16.75, 14.86, 11.50 . HRMS (ESI): calcd for $C_{24}H_{35}N_3O_4S_2Na$ $[M + Na]^+$: 516.1967; found, 516.1992.

14-O-(((5-amino-1, 3, 4-thiadiazole-2-yl)sulfinyl)acetyl)mutilin(4d)

Yield: 64%. $^1$H NMR (600 MHz, $CD_3OD$) δ 6.19 – 6.21 (m, 1H), 5.75 (dd, *J* = 16.8, 8.4 Hz, 1H), 5.48 (s, 1H), 5.18 – 5.08 (m, 2H), 3.48 (dd, *J* = 9.7, 6.2 Hz, 1H), 2.35 (d, *J* = 11.6 Hz, 1H), 2.23 – 2.27 (m, 2H), 2.19 – 2.05 (m, 2H), 1.83 – 1.77 (m, 1H), 1.71 – 1.65 (m, 1H), 1.65 – 1.59 (m, 1H), 1.51 (d, *J* = 15.7 Hz, 1H), 1.46 (s, 3H), 1.42 (s, 2H), 1.37 (t, *J* = 15.4 Hz, 2H), 1.29 (d, *J* = 16.0 Hz, 1H), 1.14 (d, *J* = 9.3 Hz, 3H), 1.11 (d, *J* = 5.2 Hz, 1H), 0.95 – 0.91 (m, 3H), 0.75 – 0.71 (m, 3H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 219.45, 219.41, 174.55, 164.57, 164.31, 162.10, 140.77, 140.73, 116.92, 116.89, 75.35, 75.34, 72.83, 72.76, 61.51, 59.12, 59.07, 54.81, 46.72, 46.70, 45.78, 45.74, 45.38, 45.36, 43.13, 43.12, 38.01, 37.96, 37.70, 37.64, 35.28, 31.42, 31.40, 28.35, 28.32, 27.97, 25.80, 25.76, 17.11, 17.10, 15.42, 15.39, 11.84, 11.81 . HRMS (ESI): calcd for $C_{24}H_{35}N_3O_5S_2Na$ $[M + Na]^+$: 532.1916; found, 532.1935.

14-O-((( 3-amino-1, 2, 4-triazole-5-yl)sulfanyl)acetyl)mutilin(4F)

Yield: 69%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.35 (dd, *J* = 17.6, 11.2 Hz, 1H), 5.70 (d, *J* = 8.6 Hz, 1H), 5.30 (s, 1H), 5.19 (d, *J* = 11.2 Hz, 1H), 5.13 (d, *J* = 18.6 Hz, 1H), 3.80 – 3.68 (m, 3H), 3.37 (d, *J* = 6.3 Hz, 1H), 2.27 (dd, *J* = 12.8, 6.2 Hz, 2H), 2.19 (td, *J* = 20.0, 19.3, 10.3 Hz, 2H), 2.10 (s, 1H), 2.03 (dd, *J* = 15.9, 8.5 Hz, 1H), 1.75 (d, *J* = 14.3 Hz, 1H), 1.63 (t, *J* = 10.1 Hz, 2H), 1.52 – 1.44 (m, 2H), 1.43 (s, 3H), 1.35 – 1.29 (m, 2H), 1.28 – 1.22 (m, 1H), 1.12 (s, 3H), 1.11 – 1.08 (m, 1H), 0.87 (d, *J* = 7.0 Hz, 3H), 0.68 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 217.18, 168.64, 157.35, 155.31, 139.03, 117.10, 74.61, 70.50, 58.14, 45.45, 44.53, 44.03, 41.89, 36.68, 36.02, 34.91, 34.48, 30.38, 26.87, 26.67, 24.83, 16.63, 14.85, 11.51 . HRMS (ESI): calcd for $C_{24}H_{36}N_4O_4SNa$ $[M + Na]^+$: 499.2355; found, 499.2311.

14-O-((( 3-amino-1, 2, 4-triazole-5-yl)sulfinyl)acetyl)mutilin(4f)

Yield: 30%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.39 – 6.43 (m, 1H), 5.83 (dd, *J* = 29.2, 8.5 Hz, 2H), 5.32 (t, *J* = 11.0 Hz, 1H), 5.17 (dd, *J* = 17.4, 12.3 Hz, 1H), 4.16 – 4.01 (m, 2H), 3.92 (dd, *J* = 43.9, 14.9 Hz, 1H), 3.40 – 3.30 (m, 1H), 2.33 – 2.22 (m, 2H), 2.19 (dd, *J* = 19.5, 9.8 Hz, 1H), 2.11 (q, *J* = 10.5, 8.5 Hz, 2H), 2.05 (s, 1H), 1.80 – 1.73 (m, 1H), 1.66 (dq, *J* = 13.6, 7.3 Hz, 2H), 1.56 – 1.50 (m, 1H), 1.47 (s, 3H), 1.37 (s, 2H), 1.26 (t, *J* = 7.1 Hz, 2H), 1.17 – 1.10 (m, 3H), 1.08 (s, 1H), 0.88 (t, *J* = 6.6 Hz, 3H), 0.75 (t, *J* = 7.3 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 215.76, 162.20, 159.99157.10, 138.10, 116.18, 73.53, 70.11, 59.38, 56.92, 44.42, 43.00, 40.83, 35.63, 35.07, 33.40, 29.35, 25.86, 25.31, 23.80, 20.03, 15.86, 13.60, 10.47 . HRMS (ESI): calcd for $C_{24}H_{36}N_4O_5SNa$ $[M + Na]^+$: 515.2304; found, 515.2293.

4-O-((( 1-methyltetrazole-5-yl)sulfanyl)acetyl)mutilin(4G)

Yield: 57%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.38 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.75 (d, *J* = 8.5 Hz, 1H), 5.29 (dd, *J* = 11.0, 1.1 Hz, 1H), 5.19 (dd, *J* = 17.4, 1.3

Hz, 1H), 4.13 – 4.05 (m, 2H), 3.97 (s, 3H), 3.36 (d, *J* = 6.5 Hz, 1H), 2.29 (p, *J* = 6.9 Hz, 1H), 2.25 (d, *J* = 11.0 Hz, 1H), 2.19 (dt, *J* = 19.4, 9.4 Hz, 1H), 2.10 (s, 1H), 2.06 (dd, *J* = 16.1, 8.6 Hz, 1H), 1.76 (dd, *J* = 14.5, 2.9 Hz, 1H), 1.69 – 1.62 (m, 2H), 1.60 (s, 1H), 1.46 (ddd, *J* = 13.0, 9.7, 2.7 Hz, 1H), 1.42 (s, 3H), 1.39 – 1.36 (m, 1H), 1.34 (s, 1H), 1.17 (s, 3H), 1.13 (td, *J* = 14.2, 4.4 Hz, 1H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.71 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.77, 166.09, 152.84, 138.66, 117.35, 74.58, 71.03, 58.03, 45.41, 44.53, 44.00, 41.85, 36.61, 36.01, 35.88, 34.42, 33.58, 30.36, 26.82, 26.47, 24.81, 16.71, 14.75, 11.46 . HRMS (ESI): calcd for $C_{24}H_{36}N_4O_4SNa$ $[M + Na]^+$: 499.2305; found, 499.2363.

4-O-((( 1-methyltetrazole-5-yl)sulfinyl)acetyl)mutilin(4g)

Yield: 11%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.41 – 6.29 (m, 1H), 5.79 (dd, *J* = 8.5, 5.3 Hz, 1H), 5.34 – 5.25 (m, 1H), 5.20 (dd, *J* = 16.8, 5.2 Hz, 1H), 4.49 (d, *J* = 17.3 Hz, 1H), 4.34 (d, *J* = 15.5 Hz, 3H), 4.23 (dd, *J* = 44.6, 15.5 Hz, 1H), 3.40 – 3.32 (m, 1H), 2.30 – 2.22 (m, 2H), 2.22 – 2.17 (m, 1H), 2.14 – 2.03 (m, 2H), 1.80 – 1.74 (m, 1H), 1.71 – 1.61 (m, 3H), 1.47 (d, *J* = 5.6 Hz, 3H), 1.41 – 1.36 (m, 3H), 1.28 (dd, *J* = 16.5, 9.9 Hz, 1H), 1.17 (d, *J* = 9.7 Hz, 3H), 1.12 (dd, *J* = 13.8, 4.1 Hz, 1H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.74 – 0.71 (m, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.54, 216.51, 163.07, 162.05, 154.89, 154.77, 138.67, 138.40, 117.61, 117.39, 74.53, 71.80, 71.75, 58.45, 58.38, 57.92, 57.90, 45.38, 44.64, 44.10, 44.05, 41.82, 41.80, 36.55, 36.54, 36.01, 35.48, 35.45, 34.38, 30.30, 26.81, 26.49, 26.42, 24.79, 16.81, 14.79, 14.68, 11.47, 11.46 . HRMS (ESI): calcd for $C_{24}H_{36}N_4O_5SNa$ $[M + Na]^+$: 515.2304; found, 515.2296.

4-O-(((1-[2-(Dimethylamino)ethyl]-1H-tetrazole -5-yl)sulfanyl)acetyl)mutilin(4H)

Yield: 53 %. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.37 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.73 (d, *J* = 8.5 Hz, 1H), 5.29 (dd, *J* = 11.0, 1.2 Hz, 1H), 5.18 (dd, *J* = 17.4, 1.3 Hz, 1H), 4.42 (t, *J* = 5.8 Hz, 2H), 4.14 – 3.99 (m, 2H), 3.35 (s, 1H), 2.87 (s, 2H), 2.35 (s, 6H), 2.30 – 2.27 (m, 1H), 2.25 (d, *J* = 11.1 Hz, 1H), 2.15 – 2.19 (m, 1H), 2.09 (s, 1H), 2.05 (dd, *J* = 16.1, 8.6 Hz, 1H), 1.76 (dd, *J* = 14.5, 2.9 Hz, 1H), 1.69 – 1.61 (m, 2H), 1.45 (ddd, *J* = 13.0, 9.6, 2.5 Hz, 2H), 1.42 (s, 3H), 1.37 (dd, *J* = 14.3, 3.2 Hz, 1H), 1.31 (d, *J* = 16.1 Hz, 1H), 1.17 (s, 3H), 1.12 (td, *J* = 14.2, 4.4 Hz, 1H), 0.87 (d, *J* = 7.1 Hz, 3H), 0.70 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.78, 166.33, 153.28, 138.68, 117.36, 74.60, 70.86, 58.05, 57.51, 45.42, 45.21, 44.56, 44.00, 41.86, 36.63, 36.22, 36.01, 34.43, 30.37, 29.70, 26.83, 26.44, 24.82, 16.73, 14.77, 11.47 . HRMS (ESI): calcd for $C_{27}H_{43}N_5O_4SNa$ $[M + Na]^+$: 556.2933; found, 556.2920.

4-O-(((1-[2-(Dimethylamino)ethyl]-1H-tetrazole-5-yl)sulfinyl)acetyl)mutilin(4h)

Yield: 16%. $^1$H NMR (600 MHz, $CDCl_3$) δ 6.42 – 6.32 (m, 1H), 5.77 – 5.68 (m, 1H), 5.29 (dq, *J* = 16.0, 9.9, 8.1 Hz, 1H), 5.18 (dt, *J* = 16.2, 7.9 Hz, 1H), 4.40 (t, *J* = 6.2 Hz, 1H), 4.17 – 3.97 (m, 2H), 3.49 (s, 1H), 3.35 (d, *J* = 6.2 Hz, 1H), 3.12 (q, *J* = 7.3 Hz, 1H), 2.84 (t, *J* = 6.1 Hz, 1H), 2.81 – 2.61 (m, 2H), 2.38 – 2.14 (m, 6H), 2.06 (dd, *J* = 26.6, 14.7 Hz, 2H), 1.76 (d, *J* = 14.4 Hz, 1H), 1.65 (q, *J* = 11.8, 10.7 Hz, 2H), 1.46 (d, *J* = 11.8 Hz, 1H), 1.42 (s, 3H), 1.39 – 1.24 (m, 4H), 1.17 (s, 3H), 1.11 (d, *J* = 14.7 Hz, 1H), 0.99 – 0.91 (m, 1H), 0.87 (d, *J* = 6.8 Hz, 3H), 0.75 – 0.66 (m, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.85, 166.34, 138.71, 117.32, 74.59, 70.87, 58.06, 57.53, 45.54, 45.42, 45.24, 44.56, 44.00, 41.86, 36.63, 36.22, 36.01, 35.75, 34.43, 30.37, 26.82, 26.48, 24.82, 16.72, 14.77, 11.47 . HRMS (ESI): calcd for $C_{27}H_{43}N_5O_5SNa$ $[M + Na]^+$: 572.2883; found, 572.2883.

4-O-(((Benzothiazole-2-yl)sulfanyl)acetyl)mutilin(5A)

Yield: 80%. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 1H), 7.74 (d, *J* = 12.0, 1H), 7.40 (t, *J* = 7.4 Hz, 1H), 7.29 (t, *J* = 7.6 Hz, 1H), 6.42 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.77 (d, *J* = 8.6 Hz, 1H), 5.26 (d, *J* = 11.7 Hz, 1H), 5.12 (d, *J* = 16.6 Hz, 1H), 4.15 – 4.10 (m, 1H), 4.03 (d, *J* = 16.3 Hz, 1H), 3.32 (d, *J* = 6.4 Hz, 1H), 2.32 – 2.28 (m, 1H), 2.24 – 2.16 (m, 2H), 2.05 (d, *J* = 15.3 Hz, 2H), 1.99 (dd, *J* = 16.0, 8.6 Hz, 1H), 1.74 (d, *J* = 14.5 Hz, 1H), 1.63 (dd, *J* = 22.2, 11.2 Hz, 3H), 1.43 (s, 3H), 1.35 (d, *J* = 14.0 Hz, 1H), 1.32 – 1.24 (m, 2H), 1.12 (dd, *J* = 14.2, 4.4 Hz, 1H), 1.07 (s, 3H), 0.85 (d, *J* = 7.0 Hz, 3H), 0.77 (d, *J* = 7.2 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.93, 166.82, 164.61, 152.67, 138.77, 135.45, 126.04, 124.49, 121.67, 121.06, 117.20, 74.58, 70.23, 58.11, 45.42, 44.43, 43.91, 41.87, 36.75, 35.99, 35.71, 34.44, 30.41, 26.85, 26.32, 24.82, 16.80, 14.83, 11.45 . HRMS (ESI): calcd for $C_{29}H_{37}NO_4S_2Na$ $[M + Na]^+$: 550.2062; found, 550.2075.

4-O-((( Benzothiazole-2-yl)sulfinyl)acetyl)mutilin(5a)

Yield: 77%. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 8.2, 3.6 Hz, 2H), 8.01 (dd, *J* = 8.0, 2.2 Hz, 2H), 7.60 – 7.56 (m, 2H), 7.53 – 7.49 (m, 2H), 6.45 – 6.38 (m, 2H), 5.83 (dd, *J* = 19.0, 8.6 Hz, 2H), 5.35 – 5.29 (m, 3H), 5.21 (dd, *J* = 17.4, 1.3 Hz, 2H), 4.20 – 4.17 (m, 1H), 4.16 (s, 1H), 4.13 – 4.08 (m, 1H), 3.99 (d, *J* = 14.9 Hz, 1H), 3.35 (dd, *J* = 12.3, 6.5 Hz, 2H), 2.92 (d, *J* = 45.1 Hz, 0H), 2.31 – 2.16 (m, 6H), 1.95 – 2.05 (m, 5H), 1.79 – 1.74 (m, 2H), 1.68 – 1.61 (m, 5H), 1.48 (s, 3H), 1.45 (dd, *J* = 8.0, 4.6 Hz, 2H), 1.43 (s, 1H), 1.38 (td, *J* = 9.9, 9.4, 5.5 Hz, 2H), 1.31 (s, 3H), 1.27 – 1.24 (m, 1H), 1.15 (d, *J* = 5.0 Hz, 6H), 1.11 (ddd, *J* = 14.4, 10.4, 4.2 Hz, 2H), 0.88 (d, *J* = 7.0 Hz, 6H), 0.78 (d, *J* = 7.2 Hz, 3H), 0.73 (d, *J* = 7.2 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.72, 216.70, 175.87, 175.87, 163.30, 163.00, 153.64, 153.62, 138.72, 138.67, 127.13, 127.11, 126.50, 126.47, 124.11, 124.10, 122.32, 122.27, 117.43, 117.39, 74.56, 71.23, 71.20, 60.85, 60.64, 58.05, 57.95, 45.41, 45.39, 44.70, 44.53, 44.05, 41.85, 41.82, 36.68, 36.60, 36.06, 36.03, 34.41, 30.36, 30.33, 26.82, 26.81,

26.56, 26.50, 24.83, 24.79, 16.87, 16.84, 14.88, 14.62, 11.48, 11.46 . HRMS (ESI): calcd for $C_{29}H_{37}NO_5S_2Na$ $[M + Na]^+$: 566.2011; found, 566.2018.

4-O-((( 5-Chloro-benzothiazole-2-yl)sulfanyl)acetyl)mutilin(5B)

Yield: 93%. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 1.8 Hz, 1H), 7.65 (d, *J* = 8.5 Hz, 1H), 7.28 (d, *J* = 1.9 Hz, 1H), 6.41 (dd, *J* = 17.4, 11.0 Hz, 1H), 6.15 (dd, *J* = 17.8, 11.2 Hz, 1H), 5.78 (d, *J* = 8.5 Hz, 1H), 5.37 (d, *J* = 17.8 Hz, 1H), 5.27 (dd, *J* = 16.8, 11.8 Hz, 2H), 5.14 (d, *J* = 17.4 Hz, 1H), 4.35 (d, *J* = 7.7 Hz, 1H), 4.11 (d, *J* = 16.3 Hz, 1H), 4.02 (d, *J* = 16.3 Hz, 1H), 3.47 – 3.37 (m, 1H), 3.32 (d, *J* = 6.5 Hz, 1H), 2.32 – 2.28 (m, 1H), 2.26 – 2.14 (m, 5H), 2.08 – 2.03 (m, 2H), 2.00 (dd, *J* = 16.0, 8.6 Hz, 1H), 1.91 (dd, *J* = 15.8, 7.7 Hz, 1H), 1.79 – 1.72 (m, 2H), 1.69 – 1.64 (m, 3H), 1.63 – 1.57 (m, 3H), 1.55 (d, *J* = 13.0 Hz, 5H), 1.46 (dd, *J* = 15.3, 6.8 Hz, 2H), 1.43 (s, 3H), 1.36 (s, 3H), 1.31 – 1.24 (m, 2H), 1.15 (s, 3H), 1.12 (d, *J* = 4.3 Hz, 1H), 1.08 (s, 3H), 0.96 (d, *J* = 7.1 Hz, 3H), 0.92 (d, *J* = 7.1 Hz, 3H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.76 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.93, 166.86, 166.61, 153.42, 138.69, 133.69, 132.08, 124.79, 121.67, 121.59, 117.23, 74.51, 70.23, 58.06, 45.40, 44.34, 43.85, 41.84, 36.70, 35.97, 35.66, 34.42, 30.38, 26.81, 26.19, 24.79, 16.79, 14.80, 11.47 .HRMS (ESI): calcd for $C_{29}H_{36}ClNO_4S_2Na$ $[M + Na]^+$: 584.1672; found, 584.1678.

4-O-((( 5-Chloro-benzothiazole-2-yl)sulfinyl)acetyl)mutilin(5b)

Yield: 49%. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.93 (dd, *J* = 8.6, 1.4 Hz, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 6.41 (ddd, *J* = 17.0, 11.0, 5.3 Hz, 1H), 5.90 – 5.74 (m, 1H), 5.33 (dd, *J* = 10.9, 7.3 Hz, 1H), 5.22 (dd, *J* = 18.0, 2.2 Hz, 1H), 4.25 – 4.04 (m, 2H), 3.98 (d, *J* = 14.8 Hz, 0H), 3.41 – 3.28 (m, 1H), 2.24 – 2.29 (m, 2H), 2.15 – 2.20 (m, 2H), 2.13 – 2.06 (m, 2H), 2.06 – 2.00 (m, 1H), 1.77 (d, *J* = 13.7 Hz, 1H), 1.72 – 1.60 (m, 3H), 1.53 (d, *J* = 13.3 Hz, 1H), 1.48 (d, *J* = 3.8 Hz, 2H), 1.47 – 1.42 (m, 1H), 1.43 – 1.35 (m, 2H), 1.32 (s, 2H), 1.26 (t, *J* = 7.1 Hz, 1H), 1.17 (s, 3H), 1.14 – 1.08 (m, 1H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.75 (dd, *J* = 22.4, 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.69, 216.66, 178.33, 178.19, 163.19, 162.87, 154.40, 154.40, 138.75, 138.66, 134.56, 134.52, 133.32 , 133.31, 127.15, 127.11, 123.88, 123.87, 123.08, 123.04, 117.51, 117.42, 74.57, 71.35, 71.31, 60.71, 60.63, 58.04, 57.95, 45.42, 45.41, 44.75, 44.53, 44.06, 41.86, 41.84, 36.68, 36.61, 36.08, 36.05, 34.41, 30.36, 30.34, 26.83, 26.82, 26.51, 26.46, 24.84, 24.80, 16.88, 16.87, 14.89, 14.63, 11.48 . HRMS (ESI): calcd for $C_{29}H_{36}ClNO_5S_2Na$ $[M + Na]^+$: 600.2621; found, 600.2637.

4-O-(((Benzoxazole-2-yl)sulfanyl)acetyl)mutilin(5C)

Yield: 85 %. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.2 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.43 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 6.42 (ddd, *J* = 18.8, 11.0, 8.0 Hz, 2H), 5.78 (dd, *J* = 13.8, 8.6 Hz, 2H), 5.30 (dd, *J* = 32.3, 11.0 Hz, 2H), 5.17 (dd, *J* = 22.3, 17.1 Hz, 2H), 4.48 (s, 1H), 4.08 – 3.95 (m, 2H), 3.34 (t, *J* = 6.5 Hz, 2H), 2.45 (s, 2H), 2.25 – 2.30 (m, 2H), 2.10 – 2.20 (m, 3H), 2.08 (s, 2H), 2.06 – 2.00 (m, 2H), 1.76 (dd, *J* = 8.6, 5.9 Hz, 2H), 1.69 – 1.62 (m, 3H), 1.60 – 1.52 (m, 4H), 1.49 – 1.44 (m, 2H), 1.43 (s, 4H), 1.41 (s, 2H), 1.37 (d, *J* = 10.4 Hz, 3H), 1.28 – 1.23 (m, 2H), 1.16 (s, 2H), 1.11 (s, 4H), 0.88 (d, *J* = 6.0 Hz, 3H), 0.86 (s, 1H), 0.77 (d, *J* = 7.1 Hz, 3H), 0.63 (d, *J* = 7.1 Hz, 1H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.88, 166.52, 152.04, 138.75, 129.92, 128.10, 124.41, 124.13, 118.61, 117.28, 109.99, 74.59, 70.52, 65.05, 58.12, 45.44, 44.52, 43.95, 41.90, 36.72, 36.03, 34.94, 34.45, 30.41, 26.85, 26.40, 24.83, 16.76, 14.80, 11.47 . HRMS (ESI): calcd for $C_{29}H_{37}NO_5SNa$ $[M + Na]^+$: 534.2290; found, 534.2303.

4-O-(((Btobenzoxazole-2-yl)sulfinyl)acetyl)mutilin(5c)

Yield: 20%. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 7.8, 4.3 Hz, 1H), 7.65 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.48 (dt, *J* = 28.7, 7.4 Hz, 2H), 6.25 – 6.32 (m, 1H), 5.79 (dd, *J* = 23.0, 8.5 Hz, 1H), 5.32 – 5.24 (m, 1H), 5.23 – 5.05 (m, 2H), 4.41 (dd, *J* = 21.3, 15.1 Hz, 1H), 4.24 (dd, *J* = 15.0, 13.6 Hz, 1H), 3.32 (d, *J* = 19.2 Hz, 1H), 2.29 – 2.22 (m, 2H), 2.18 (dd, *J* = 19.4, 9.7 Hz, 1H), 2.08 – 2.02 (m, 2H), 1.94 (d, *J* = 8.7 Hz, 1H), 1.78 – 1.72 (m, 1H), 1.62 (dd, *J* = 23.6, 10.6 Hz, 2H), 1.48 – 1.45 (m, 1H), 1.42 (d, *J* = 5.6 Hz, 3H), 1.35 (d, *J* = 10.6 Hz, 1H), 1.30 (s, 1H), 1.26 (d, *J* = 7.2 Hz, 1H), 1.24 – 1.19 (m, 1H), 1.12 (s, 3H), 1.06 (s, 1H), 0.86 (dd, *J* = 7.0, 4.7 Hz, 3H), 0.73 (dd, *J* = 17.6, 7.1 Hz, 3H). $^{13}$CNMR (151MHz, $CDCl_3$)δ216.70, 216.66, 163.25, 162.85, 151.76, 151.73, 140.25, 140.20, 138.42, 138.40, 127.45, 125.80, 125.77, 121.43, 117.46, 117.35, 111.60, 111.57, 74.50, 74.48, 71.23, 71.22, 57.98, 57.87, 57.57, 45.36, 45.34, 44.59, 44.30, 43.94, 43.90, 41.76, 36.59, 36.00, 35.98, 34.36, 30.31, 30.26, 26.74, 26.39, 24.79, 24.75, 16.79, 16.72, 14.73, 14.58, 11.47, 11.45 . HRMS (ESI): calcd for $C_{29}H_{37}NO_6SNa$ $[M + Na]^+$: 550.2239; found, 550.2226.

4-O-(((Benzimidazole-2-yl)sulfanyl)acetyl)mutilin(5D)

Yield: 90%. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.52 (dd, *J* = 5.8, 3.1 Hz, 2H), 7.24 – 7.18 (m, 2H), 6.41 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.79 (d, *J* = 8.5 Hz, 1H), 5.24 (d, *J* = 11.1 Hz, 1H), 5.13 (d, *J* = 18.5 Hz, 1H), 3.93 (d, *J* = 2.9 Hz, 2H), 3.36 (d, *J* = 6.5 Hz, 1H), 2.37 – 2.28 (m, 1H), 2.27 – 2.15 (m, 2H), 2.09 (s, 1H), 2.04 (q, *J* = 8.6 Hz, 1H), 1.77 (dd, *J* = 14.5, 2.8 Hz, 1H), 1.70 – 1.60 (m, 2H), 1.58 – 1.46 (m, 2H), 1.44 (s, 3H), 1.40 – 1.31 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 1H), 1.14 (dd, *J* = 14.3, 4.4 Hz, 1H), 1.11 (s, 3H), 0.89 (d, *J* = 7.1 Hz, 3H), 0.73 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.87, 168.81, 148.20, 138.77, 122.74, 117.29, 74.63, 70.87, 58.10, 45.44, 44.55, 44.00, 41.86, 36.70, 36.07, 35.24, 34.44, 30.40, 26.86, 26.47, 24.85, 16.83, 14.83, 11.51 . HRMS (ESI):

calcd for C29H38N2O4SNa [M + Na]$^+$: 533.2450; found, 533.2474.

4-O-(((Benzimidazole-2-yl)sulfinyl)acetyl)mutilin(5d)

Yield: 33%. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.68 (t, *J* = 9.4 Hz, 2H), 7.35 (td, *J* = 6.5, 3.1 Hz, 2H), 6.37 (ddd, *J* = 61.5, 17.4, 11.0 Hz, 1H), 5.80 (dd, *J* = 38.5, 8.5 Hz, 1H), 5.29 (d, *J* = 12.4 Hz, 1H), 5.19 – 5.05 (m, 2H), 4.31 (dd, *J* = 15.1, 3.4 Hz, 1H), 4.23 – 4.10 (m, 1H), 3.33 (dd, *J* = 30.0, 6.4 Hz, 1H), 2.32 – 2.13 (m, 3H), 2.05 (dd, *J* = 22.0, 15.6 Hz, 2H), 1.91 (dd, *J* = 16.0, 8.6 Hz, 1H), 1.74 (d, *J* = 14.6 Hz, 1H), 1.68 – 1.58 (m, 2H), 1.49 (d, *J* = 11.1 Hz, 1H), 1.46 (s, 2H), 1.42 (d, *J* = 10.4 Hz, 1H), 1.36 (d, *J* = 16.0 Hz, 1H), 1.30 – 1.25 (m, 3H), 1.10 (s, 3H), 1.06 (s, 1H), 0.87 (dd, *J* = 18.0, 7.0 Hz, 3H), 0.72 (dd, *J* = 24.6, 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.70, 216.67, 163.58, 163.14, 138.75, 138.51, 151.41, 124.19, 117.35, 117.23, 74.60, 74.53, 71.26, 59.53, 58.90, 58.02, 57.91, 45.41, 45.38, 44.71, 44.34, 44.03, 43.98, 41.85, 41.81, 36.61, 36.59, 36.05, 35.99, 34.40, 30.34, 30.32, 26.83, 26.75, 26.42, 26.38, 24.84, 24.78, 16.96, 16.82, 14.85, 14.61, 11.51, 11.47 . HRMS (ESI): calcd for C29H38N2O5SNa [M + Na]$^+$: 549.2399; found, 549.2404.

4-O-(((5-Methoxy-benzimidazole-2-yl)sulfanyl)acetyl)mutilin(5E)

Yield: 90%. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.8 Hz, 1H), 7.02 – 6.97 (m, 1H), 6.85 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.42 (dd, *J* = 17.4, 11.0 Hz, 1H), 5.78 (d, *J* = 8.5 Hz, 1H), 5.32 – 5.23 (m, 1H), 5.14 (dd, *J* = 17.5, 1.2 Hz, 1H), 3.90 (d, *J* = 4.1 Hz, 2H), 3.82 (s, 3H), 3.36 (d, *J* = 6.5 Hz, 1H), 2.31 (h, *J* = 7.9, 7.4 Hz, 1H), 2.27 – 2.15 (m, 2H), 2.09 (s, 1H), 2.06 – 2.01 (m, 1H), 1.78 – 1.74 (m, 1H), 1.68 – 1.61 (m, 2H), 1.47 – 1.44 (m, 1H), 1.43 (s, 3H), 1.38 – 1.31 (m, 2H), 1.16 – 1.12 (m, 1H), 1.12 (s, 3H), 0.89 (d, *J* = 7.0 Hz, 3H), 0.71 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.92, 168.81, 156.55, 147.10, 138.83, 117.25, 112.15, 74.62, 70.82, 58.10, 55.83, 45.44, 44.53, 44.00, 41.85, 36.69, 36.07, 35.46, 34.44, 30.39, 26.85, 26.51, 24.85, 16.80, 14.82, 11.52 . HRMS (ESI): calcd for C30H40N2O5SNa [M + Na]$^+$: 563.2556; found, 563.2571.

4-O-(((5-Methoxy-benzimidazole-2-yl)sulfinyl)acetyl)mutilin(5e)

Yield: 41%. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.56 (t, *J* = 9.6 Hz, 2H), 7.09 (dd, *J* = 10.7, 2.4 Hz, 2H), 6.90 – 6.98 (m, 2H), 6.38 – 6.40 (m, 1H), 6.30 (ddd, *J* = 17.4, 11.0, 1.2 Hz, 1H), 5.75 (d, *J* = 8.5 Hz, 1H), 5.15 (dt, *J* = 17.4, 1.3 Hz, 1H), 5.10 – 5.04 (m, 2H), 4.29 (dd, *J* = 15.0, 8.7 Hz, 2H), 4.22 (d, *J* = 2.8 Hz, 1H), 4.19 (d, *J* = 3.3 Hz, 1H), 3.86 (dd, *J* = 2.8, 1.0 Hz, 6H), 3.36 (d, *J* = 6.4 Hz, 1H), 3.31 (d, *J* = 6.3 Hz, 1H), 2.30 – 2.13 (m, 7H), 2.08 – 2.02 (m, 4H), 1.90 (dd, *J* = 16.1, 8.6 Hz, 1H), 1.74 (dd, *J* = 14.4, 3.2 Hz, 2H), 1.66 – 1.59 (m, 4H), 1.47 (d, *J* = 3.1 Hz, 1H), 1.45 – 1.44 (m, 4H), 1.35 (d, *J* = 16.0 Hz, 2H), 1.31 (s, 4H), 1.27 – 1.24 (m, 3H), 1.12 (d, *J* = 4.2 Hz, 1H), 1.10 (s, 3H), 1.08 (d, *J* = 4.4 Hz, 1H), 1.05 (s, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 7.0 Hz, 3H), 0.74 (d, *J* = 7.3 Hz, 3H), 0.67 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 216.74, 216.72, 163.66, 163.13, 157.59, 149.85, 138.68, 138.49, 117.29, 117.13, 114.62, 74.53, 74.48, 71.11, 60.39, 59.39, 58.74, 57.97, 57.86, 55.76, 45.36, 45.33, 44.58, 44.21, 43.97, 43.90, 41.78, 36.53, 36.03, 35.95, 34.36, 30.28, 30.26, 26.79, 26.68, 26.47, 26.39, 24.79, 24.73, 16.92, 16.73, 14.79, 14.60, 11.48, 11.45 .HRMS (ESI): calcd for C30H40N2O6SNa [M + Na]$^+$: 579.2505; found, 579.2489.

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## Author Contribution Statement

Yun-Ge Li, Fan Zhang and Yu-Cheng Wang designed and planned the experiments. Yun-Ge Li and Mei Zhu synthesized the compounds. Xue-Fu You tested their anti gram-positive bacteria activity. Yun-Ge Li wrote the manuscript with support from Guoning Zhang, Ju-Xian Wang and Fan Zhang. Yucheng Wang supervised the project.

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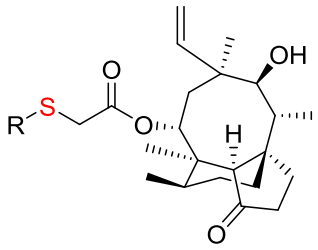
Graphical Illustration

# Antibacterial Activity and Structure-Activity Relationship of a Series of Newly Synthesized Pleuromutilin Derivatives

**Yun-Ge Li[a], Ju-Xian Wang[b], Guo-Ning Zhang[b], Mei Zhu[b], Xue-Fu You[b], Xin-Xin Hu[b], Fan Zhang*[a] and Yu-Cheng Wang*[b]**

[a] School of Pharmacy, Jinzhou Medical University, Jinzhou 121001, P. R. China;

[b]Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, P. R. China.

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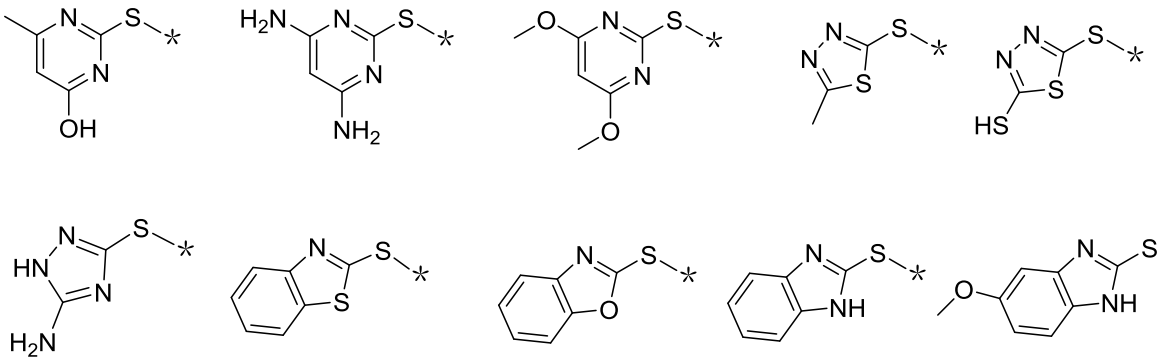


**Abstract:** A series of novel thioether or sulfoxide-type pleuromutilin derivatives containing heteroaromatic substituents at the end of C14 side chain were designed and synthesized. All of the derivatives were evaluated for their anti gram-positive bacteria activity in vitro. And some of them show good to excellent anti-bacterial activity comparable to retapamulin and azamulin in most of the tested gram-positive pathogens. In this work, a five-membered heterocyclic moiety, a pyrimidine-membered heterocyclic moiety or a benzoheterocyclic moiety was introducted in the C14 side chain to increase the structural diversity of the pleuromutilin derivatives. The anti-bacterial result reveal that the thioether containing pleuromutilin derivatives exert a more potency activity than the sulfoxide-type derivatives against gram-positive pathogens. The structure-activity relationship summarized in this work may provide some interesting clues as to which functionalities are beneficial for high antimicrobial activity of the pleuromutilin derivatives.