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Synthesis and Biological Activities of Novel Pleuromutilin Derivatives with a Substituted Thiadiazole Moiety as Potent Drug-Resistant Bacteria Inhibitors

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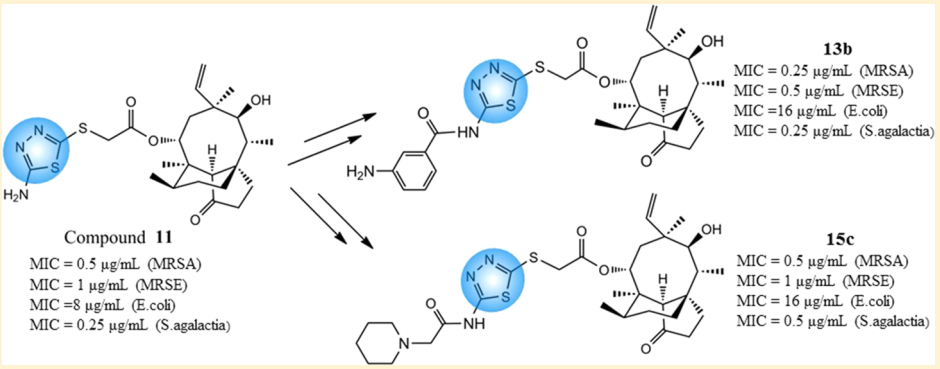
Supporting Information



ABSTRACT: A series of novel pleuromutilin derivatives possessing thiadiazole moieties were synthesized via acylation reactions under mild conditions. The in vitro antibacterial activities of the derivatives against methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis*, *Escherichia coli*, and *Streptococcus agalactiae* were tested by the agar dilution method and Oxford cup assay. The majority of the tested compounds displayed moderate antibacterial activities. Importantly, the three compounds with amino or tertiary amine groups in their side chains, **11**, **13b**, and **15c**, were the most active antibacterial agents. Docking experiments carried out on the peptidyl transferase center (PTC) of 23S rRNA proved that there is a reasonable direct correlation between the binding free energy (ΔG_b, kcal/mol) and the antibacterial activity. Moreover, the pharmacokinetic profiles of **11** and **15c** in rat were characterized by moderate clearance and oral bioavailability.

INTRODUCTION

The abuse of antibiotics has resulted in the emergence and spread of antibiotic resistance, leading to reductions or losses in therapeutic effects. Drug-resistant bacteria, especially *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Mycobacterium tuberculosis*, kill more than two million people each year and seriously endanger human health.[1,2]

Pleuromutilin (**1**) (Figure 1), a natural product composed of a 5−6−8 tricyclic carbon skeleton,[3,4] was first discovered and isolated from *Pleurotus mutilus* or *P. passeckerianus* in 1951.[5] The derivatives of pleuromutilin have received much investigation due to their high activities against mycoplasmas and drug-resistant Gram-positive bacteria,[2,6] their pharmacodynamic properties,[7] and their lack of target-specific cross-resistance to other antibiotics.[8]

Pleuromutilin derivatives interact with the 23S rRNA of the 50S bacterial ribosome subunit and interfere with bacterial protein synthesis.[9,10] The pleuromutilin derivative binding site is the domain V of 23S rRNA at the peptidyl transferase center (PTC). Their tricyclic core is positioned in a pocket next to the A-tRNA binding site, whereas the C-14 side chain extends to the P-tRNA binding site to prevent the peptide transfer from correct positioning of tRNAs and thus inhibit peptide bond formation.[11,12]

The introduction of a thioether at the C-22 position of pleuromutilin analogues and the presence of a basic group enhance antibacterial activity.[13−16] Thus, the structural modifications have focused basically on their C-14 side chain.

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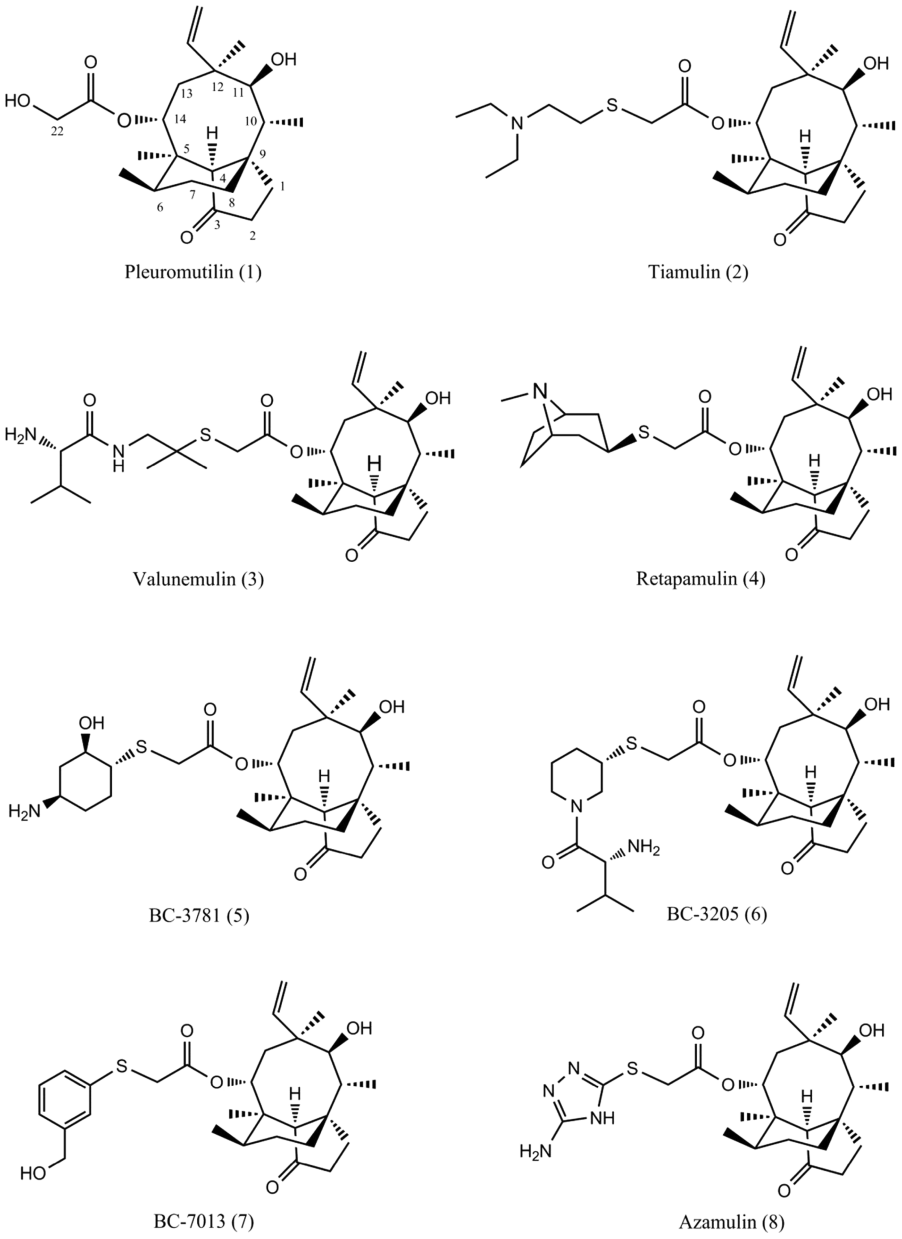

Figure 1. Structural formulas of pleuromutilin and derivatives thereof.

Further alterations on the C-14 side chain of pleuromutilin have led to three drugs: tiamulin (**2**), valnemulin (**3**), and retapamulin (**4**) (Figure 1). Tiamulin and valnemulin are used in veterinary medicine as a drug for poultry and pigs.[17−19] Retapamulin became the first pleuromutilin antibiotic to be approved for human use in 2007 by the Food and Drug Administration (FDA).[20,21] In addition to retapamulin, 14-*O*-{[(1*R*,2*R*,4*R*)-4-amino-2-hydroxy-cyclohexylsulfanyl]-acetyl}-mutilin (BC-3781, **5**),[22] 14-*O*-[(*N*-(3-methyl-2(*S*)-amino-buturyl-piperidin-3(*R*)-yl)-sulfanyl)-acetyl]-mutilin (BC-3205, **6**)[23] and 14-*O*-[(3-hydroxymethyl-phenylsulfanyl)-acetyl]-mutilin (BC-7013, **7**)[24] are being tested in clinical trial for human use. Azamulin (**8**) (Figure 1) was the next pleuromutilin designated for human use, and the drug was designed during the early 1980s. It did not undergo further clinical trials because of its poor solubility in water and its strong inhibition of human cytochrome P450.[25]

Thiadiazole-based structural scaffolds form an essential constituent of many synthetic drugs. Compounds containing a 1,3,4-thiadiazole moiety exhibit anticancer, anti-inflammatory, antibacterial, antifungal, antiviral, anticonvulsant, and antiparasitic activities (for some 1,3,4-thiadiazole-containing drugs available on the market, see Figure S1 in the Supporting Information).[26] Furthermore, thiadiazoles are bioisosteres of oxadiazole, oxazole, and benzene, which leads to analogues with improved activities because the sulfur atom imparts improved liposolubility.[27,28]

We have synthesized three types of pleuromutilin derivatives bearing a thiadiazole moiety: compounds **12a−f**, compounds **13a−c**, and compounds **15a−f**. All these compounds were synthesized from compound **11**, an important intermediate synthesized from 14-*O*-(iodoacetyl) mutilin (**10**) and 2-amino-5-mercapto-1,3,4-thiadiazole. In particular, compound **9** was synthesized using an efficient method characterized by a higher yield and shorter time compared with other published

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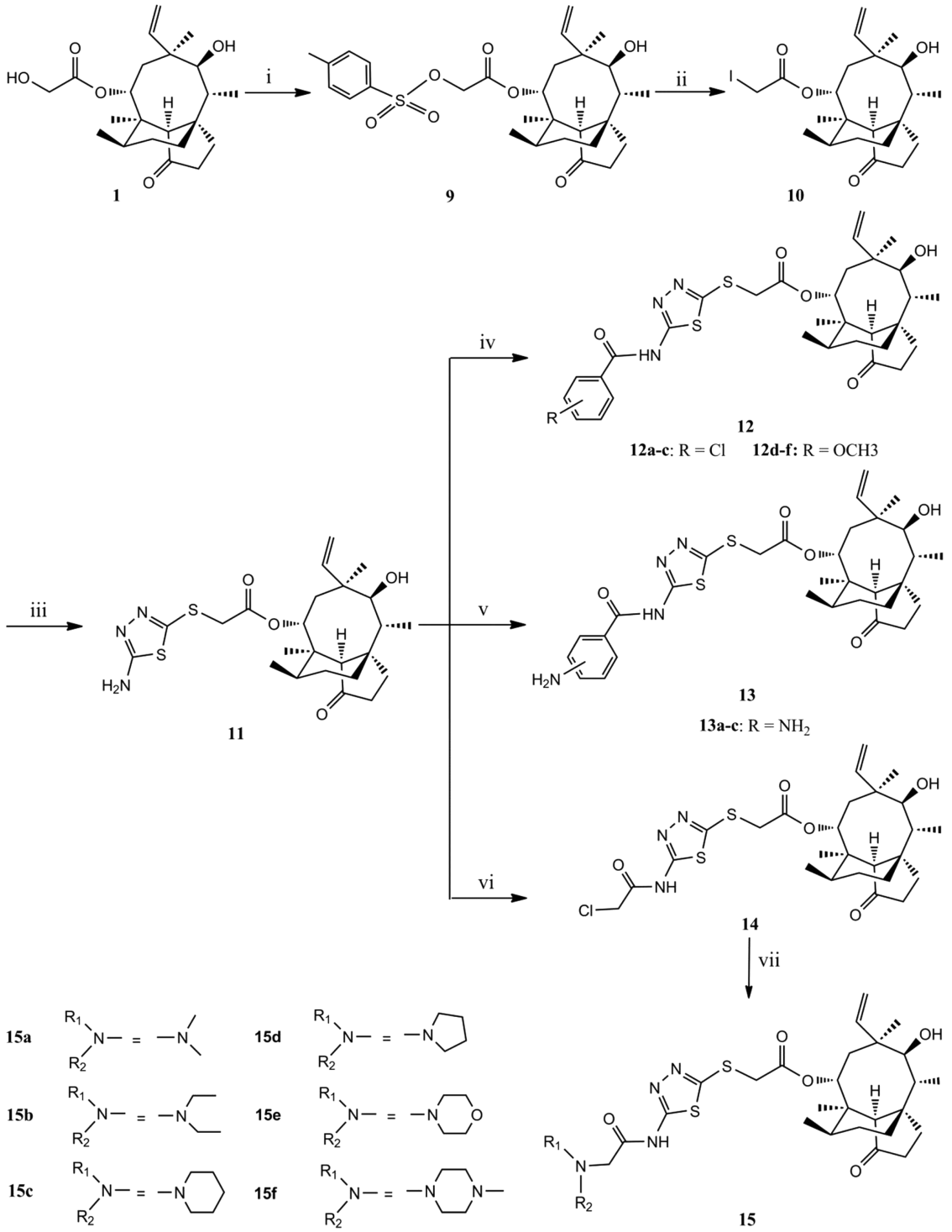
Scheme 1. Synthesis of Pleuromutilin Derivatives



Reagents and conditions: (i) TsCl, NaOH, *t*-butyl methyl ether/H_2O, reflux, 1 h, 93%; (ii) KI, acetone, reflux, 1 h, 81%; (iii) 2-amino-5-mercapto-1,3,4-thiadiazole, NaOH, THF, 0 °C, 2 h, 82%; (iv) benzoic acid, EDCI, HOBt, DCM, room temperature, 36−48 h, 62−75%; (v) *N*-Boc protected amino acids, EDCI, HOBt, DCM, room temperature, 36−48 h, then TFA, DCM, room temperature, 0.5 h, 58−61%; (vi) 2-chloroacetyl chloride, NMM, DCM, 0 °C, 2 h, 86%; (vii) secondary amines, TEA, THF, 45 °C, 2 h, 62−79%.

methods.[14,29] In this study, the antibacterial properties of all the above-mentioned synthesized compounds were evaluated using the agar dilution method and Oxford cup assay against three Gram-positive bacteria: methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), and *Streptococcus agalactiae* (*S. agalactiae*) and one Gram-negative bacteria: *Escherichia coli* (*E. coli*). Pleuromutilin derivatives are known to bind to the 23S rRNA of the 50S bacterial ribosome subunit. To explore the antibacterial mechanisms of compounds displaying different antibacterial activities but with similar substituents, compounds **12d−f**, **13a−c**, and **15a−d** were chosen for molecular docking studies.

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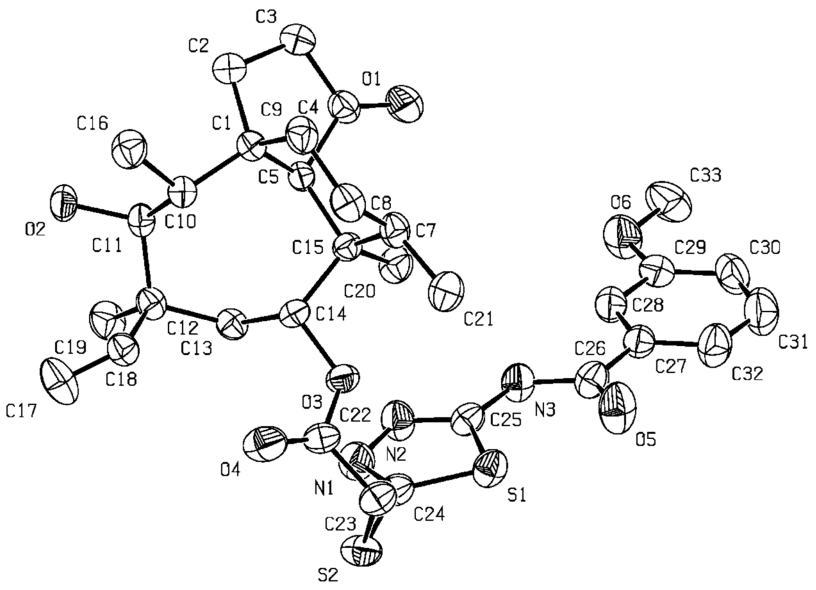

Figure 2. ORTEP diagram for compound **12e** with ellipsoids set at 50% probability (hydrogen atoms were omitted for clarity).

The antibacterial and docking results may provide new insights into the design of novel pleuromutilin derivatives and lay the basis for further studies of promising pleuromutilin antibiotics.

RESULTS AND DISCUSSION

Chemistry. The general synthetic routes for all the pleuromutilin derivatives are shown in Scheme 1. Almost all the syntheses of pleuromutilin derivatives begin from 22-*O*-tosylpleuromutilin (**9**), which was obtained by the reaction of pleuromutilin and *p*-toluenesulfonyl chloride to activate the 22-hydroxyl of pleuromutilin. In this paper, we describe a different synthesis method using NaOH as a base, in which pleuromutilin is rapidly converted to **9** in excellent yield. Intermediate **10** was prepared in good yield under reflux for 3 h in acetone, as previously described.[30,31] Compound **11** was then obtained in good yield by the nucleophilic attack of 2-amino-5-mercapto-1, 3, 4-thiadiazole on intermediate **10** under alkaline conditions and without further separation by column chromatography. In addition to the above synthetic method, compound **11** can be synthesized directly from compound **9** in a strongly alkaline environment. However, this method is undesirable because of the many byproducts produced in the residue and its lower yield.

Compounds **12a**−**f** were directly obtained by condensation reactions between the amino-group of compound **11** and the carboxyl group of substituted aryl carboxylic acids with 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt). Compounds **12a**−**f** could also be prepared by the reaction of compound **11** with correlative acyl chlorides, which were obtained from the acyl chlorination of substituted aryl carboxylic acids by $SOCl_2$ in refluxing conditions. Compounds **13a**−**c** were obtained in three steps: first, the amino groups of the amino acid derivative were protected by *tert*-butoxycarbonyl (BOC). Then, an amide was formed via a condensation reaction between compound **11** and the amino acid derivative using EDCI and HOBt. Finally, the protected amino groups were hydrolyzed with trifluoroacetic acid (TFA) and compounds **13a**−**c** were obtained. Compound **11** was treated with 2-chloroacetyl chloride using *N*-methylmorpholine to give the intermediate **14**, which was directly treated with various amines under alkaline conditions to give the target compounds **15a**−**f**.

All derivatives were obtained by column chromatography, followed by IR, ^{1}H NMR, and ^{13}C NMR spectral characterization as well as by high-resolution mass spectral (HRMS) analysis (for the spectra of all the synthesized compounds, see Supporting Information). A crystal of compound **12e** was formed as a colorless, block-like crystal in an acetone/ethanol solution, and X-ray diffraction unambiguously confirmed the structure (Figure 2; for discussion, see Supporting Information).

Biological Assays. The synthesized pleuromutilin derivatives (i.e., **12a**−**f**, **13a**−**c**, and **15a**−**f**) were screened for their in vitro antibacterial properties against MRSA, MRSE, *S. agalactiae*, and E. coli by the agar dilution method. The minimum inhibitory concentration (MIC) of the synthesized compounds, **11**, **12a**−**f**, **13a**−**c**, and **15a**−**f**, along with tiamulin and azamulin used as reference drugs, were determined in triplicate at pH 7.40 (Table 1). The MICs of the 16 new pleuromutilin derivatives in vitro against MRSA, MRSE, *S.agalactiae* and *E. coli* ranged from 8 to 0.25 μg/mL, 8 to 0.5 μg/mL, 8 to 0.25 μg/mL, and 64 to 8 μg/mL, respectively. The Oxford cup assays were carried out to evaluate the antibacterial activities against the above-mentioned four bacterial strains. The zones of inhibition for two concentrations (320 and 160 μg/mL) of the synthetic compounds and reference drugs were measured (Table 2). From Table 2, it can be observed that most compounds exhibited relatively moderate inhibitory activity against MRSA, MRSE, and *S. agalactiae* and weak activity against *E. coli*. Good inhibitory characteristics, exceeding those of tiamulin and azamulin, were observed for compounds **13b** and **15c**

Among the examined pleuromutilin derivatives, three compounds, **11**, **13b**, and **15c**, showed excellent antibacterial activity against all strains except *E. coli*. The others, especially compounds with chlorbenzene and methoxybenzene, showed moderate antibacterial activity. Compounds **11**, **13b**, and **15c** showed superior or comparable antibacterial activities to that of tiamulin. Compounds **11** and **15c** displayed the same antibacterial activities as azamulin against MRSA but lower

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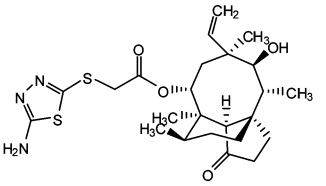
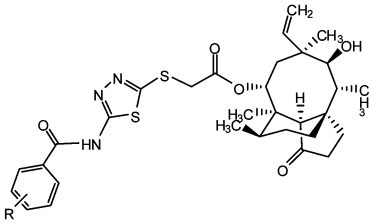
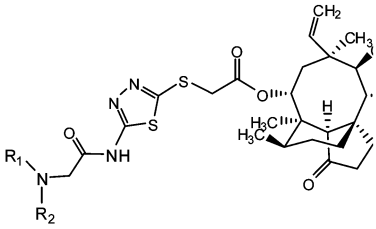
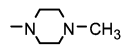
Table 1. In Vitro Antibacterial Activity (MIC) of the Novel Pleuromutilin Derivatives

Structure	Compound no.	R	MIC (μg/mL)			
			MRSA	MRSE	*E. coli*	*S. agalactiae*
	11	-	0.5	1	8	0.25
	12a	o-Cl	2	8	≥64	2
	12b	m-Cl	2	2	32	2
	12c	p-Cl	4	4	≥64	4
	12d	o-OCH_3	1	2	16	4
	12e	m-OCH_3	8	4	≥64	8
	12f	p-OCH_3	2	2	32	2
	13a	o-NH_2	4	2	16	4
	13b	m-NH_2	0.25	0.5	16	0.25
	13c	p-NH_2	2	1	16	2
	15a		1	2	32	2
	15b		8	4	64	8
	15c		0.5	1	16	0.5
	15d		4	4	32	2
	15e		1	8	≥64	4
	15f		8	4	16	8
Tiamulin			0.5	2	16	0.5
Azamulin			0.5	0.5	4	0.125

antibacterial activities than azamulin against MRSE, *E. coli*, and *S. agalactiae*. The antibacterial activities of compound **13b**, being the best antibacterial derivative, were superior or similar to those of azamulin against MRSA and MRSE but lower than those of azamulin against *E. coli* and *S. agalactiae*.

The compounds with chlorine or amino substituents in the benzene ring at the position *meta* to the side chain showed

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Table 2. Zone of Inhibition for MRSA, MRSE, *E. coli*, and *S. agalactiae* in mm

	MRSA (μg/mL)		MRSE (μg/mL)		*E. coli* (μg/mL)		*S. agalactiae* (μg/mL)	
compound	320	160	320	160	320	160	320	160
11	15.64	13.01	15.15	12.75	14.33	12.36	17.30	14.98
12a	14.98	12.25	12.61	11.89	10.26	0.00	15.49	12.74
12b	14.66	13.36	15.02	12.94	12.60	11.68	13.54	12.69
12c	13.70	11.87	14.10	11.34	10.02	0.00	13.41	11.42
12d	14.77	12.16	14.25	11.92	11.54	9.96	13.37	11.29
12e	13.09	11.42	13.78	12.26	10.89	9.02	12.75	11.66
12f	15.54	12.18	14.44	9.52	11.18	10.72	14.32	11.53
13a	15.50	12.88	15.16	12.18	11.27	10.09	13.75	12.42
13b	19.23	17.48	17.82	16.15	13.71	12.65	18.38	17.42
13c	13.22	11.39	14.76	12.07	12.21	10.65	14.02	12.30
15a	17.62	15.44	16.02	14.35	11.97	10.10	14.51	11.54
15b	12.35	10.98	12.85	11.52	10.23	9.13	12.72	10.44
15c	18.82	13.75	17.37	14.54	13.31	11.70	17.69	13.54
15d	13.25	13.07	12.77	10.54	11.14	9.86	13.82	12.75
15e	15.44	13.39	10.83	0.00	11.90	0.00	13.25	10.91
15f	11.97	11.02	13.12	10.73	11.25	10.61	12.60	11.19
tiamulin	17.23	16.94	15.58	13.95	15.85	13.58	16.08	14.60
azamulin	17.98	16.52	17.02	15.36	17.20	15.96	18.86	17.16

Table 3. Binding Free Energy, Number of Noncovalent Molecular Interactions, and RMSD

		noncovalent molecular interaction				
compound	ΔG_b (kcal/mol)	hydro I interaction	atom of compound	residue	distance (Å)	RMSD (Å)
11	−10.88	H-bonding	OH (eight-membered ring)	G-2484	2.0	0.99
		H-bonding	NH_2 (triazole ring)	C-2565	2.3	
		H-bonding	C═O (ester)	G-2044	2.9	
		H-bonding	C═O (ester)	G-2044	3.3	
12d	−9.46	H-bonding	OH (eight-membered ring)	G-2484	2.2	0.99
		H-bonding	C═O (ester)	G-2044	2.9	
		$\pi-\pi$ interaction	benzene ring	C-2565	4.0	
12e	−8.40	H-bonding	OH (eight-membered ring)	G-2484	2.2	0.95
		H-bonding	OCH_3 (benzene ring)	C-2565	3.2	
		$\pi-\pi$ interaction	benzene ring	A-2045	3.9	
12f	−9.52	H-bonding	OH (eight-membered ring)	G-2484	2.2	0.86
		H-bonding	C═O (ester)	G-2044	2.8	
		H-bonding	OCH_3 (benzene ring)	C-2565	3.0	
		$\pi-\pi$interaction	benzene ring	A-2045	3.9	
13a	−8.16	H-bonding	OH (eight-membered ring)	G-2484	2.2	0.92
13b	−11.46	H-bonding	OH (eight-membered ring)	G-2484	2.2	0.81
		H-bonding	NH_2 (benzene ring)	C-2420	1.6	
		$\pi-\pi$ interaction	benzene ring	A-2045	3.9	
13c	−9.83	H-bonding	OH (eight-membered ring)	G-2484	2.1	0.75
		H-bonding	NH_2 (benzene ring)	C-2420	2.1	
		$\pi-\pi$ interaction	benzene ring	A-2045	4.0	
15a	−9.76	H-bonding	OH (eight-membered ring)	G-2484	2.2	0.87
		H-bonding	C═O (ester)	G-2044	3.4	
		H-bonding	C═O (amido bond)	C-2565	2.8	
15b	−9.69	H-bonding	OH (eight-membered ring)	G-2484	2.1	0.93
		H-bonding	C═O (amido bond)	U-2564	2.8	
15c	−11.13	H-bonding	OH (eight-membered ring)	G-2484	2.2	1.12
		H-bonding	C═O (five-membered ring)	C-2431	2.7	
		H-bonding	C═O (five-membered ring)	U-2485	3.2	
15d	−9.85	H-bonding	OH (eight-membered ring)	G-2484	2.1	0.93
		H-bonding	C═O (ester)	G-2044	3.5	
		H-bonding	C═O (amido bond)	C-2565	3.0	
		cation−π interaction	N (pyrrolidine)	G-2045	4.1	

stronger antibacterial activities than did those with *ortho* or *para* substituents in the benzene ring. For example, compound **13b** showed stronger antibacterial activity than did compounds **13a** or **13c**. In contrast, the compounds with a methoxyl group in

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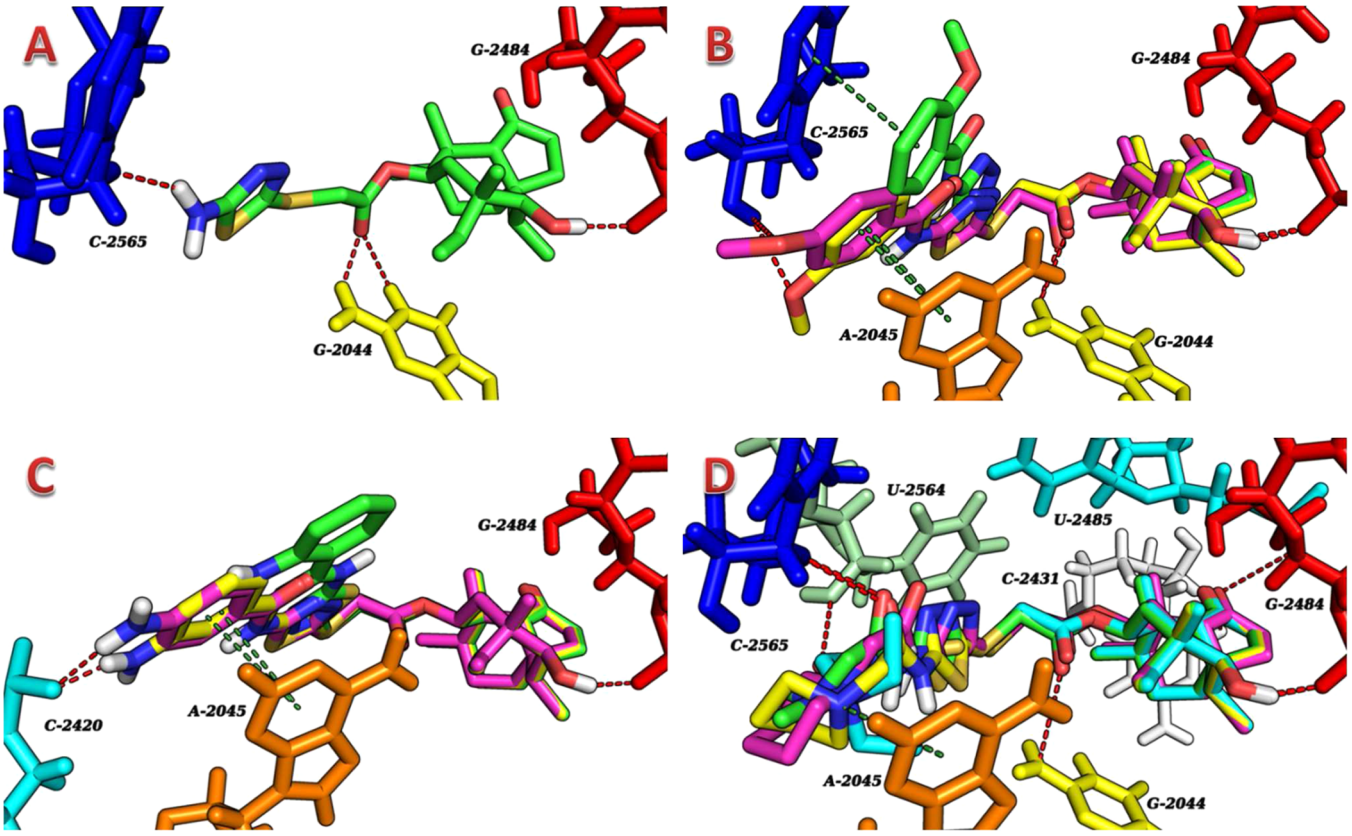

Figure 3. (A) Docking mode of compound **11** (green) to 1XBP. (B) Docking modes of compounds **12d** (green), **12e** (yellow), and **12f** (magenta) to 1XBP. (C) Docking modes of compounds **13a** (green), **13b** (yellow), and **13c** (magenta) to 1XBP. (D) Docking modes of compounds **15a** (green), **15b** (cyan), **15c** (magenta), and **15d** (yellow) to 1XBP. Important residues are drawn as sticks with different colors. Hydrogen bonds are shown as dashed red lines; $\pi-\pi$ and cation$-\pi$ interactions are shown as dashed green lines.

the benzene ring at the *meta* position showed lower antibacterial activities than did those with *ortho* or *para* substituents in the benzene ring. In addition, the compounds with an amino group in the benzene ring preferentially inhibited the growth of MRSE and *E. coli*. The antibacterial activity presented obvious variations among the 2-(substituted amino) acetyl amide analogues (**15a**–**f**) with similar substituents. The diethyl amino substitution (**15b**) and the piperidine substitution (**15c**) noticeably increased the anti-MRSA, MRSE and *S. agalactiae* activities compared with those of the dimethyl amino (**15a**) and pyrrolidine (**15d**) substitutions.

Compound **11** was structurally similar to azamulin but showed lower antibacterial activities. One possible reason was that 1,3,4-thiadiazole and 1,3,4-triazole were all mesoionic compounds, which contain distinct regions of positive and negative charges associated with a poly heteroatomic system, enabling them to cross cellular membranes and interact with biological targets with distinct affinities.[32,33] The replacement of the nitrogen in the triazole ring of azamulin with sulfur reduced the degree of polarity, which resulted in compound **11** displaying lower affinities compared with azamulin to biomolecules such as DNA and/or proteins.

To better understand the biological activities of the compounds bearing a substituent in the benzene ring, **12a**–**f** and **13a**–**c**, we examined their Hansch–Fujita hydrophobic parameters (π) and Hammett parameters (σ), two physical properties used to derive quantitative structure–activity relationships (QSAR) for a host of interactions of organic compounds with living systems. In our experience, there is a correlation between the two parameters and antibacterial activity. The antibacterial activity improved as the π and σ value of the substituted benzenes decreased below zero. For example, compound **13b** exhibited excellent antibacterial activity, and the π and σ values of the amine substitution in the benzene ring at the *meta* position were −1.23 and −0.16, respectively. This research may help us to design more potent antibacterial activities for pleuromutilin derivatives bearing substituted benzenes.

Molecular Docking Study. Compounds with similar substituents can exhibit large differences in antibacterial activity. To understand this phenomenon, a series of docking studies were conducted to compare the binding modes of **12d**–**f**, **13a**–**c**, **15a**–**d**, and **11**. On applying Homdock software,[34] the redocking of tiamulin **2** into 1XBP[37] placed the drug in the same conformation as that in the X-ray structure. The results for the 11 compounds present a similar binding mode, with a RMSD range of 0.75 to 1.12 Å, as documented for tiamulin and presented in Figure S2 in the Supporting Information, which displys a superimposition of the 11 docked compounds.

The lowest binding free energies (ΔG_b, kcal/mol) and the lowest root-mean-square difference (RMSD) values in comparison to the native cocrystallized ligand, were considered as the best parameters to evaluate the dock binding affinities.[36] Upon flexible docking into the 50S ribosomal subunit, the above-mentioned compounds revealed binding free energies (ΔG_b) in the range of −8.40 to −11.46 kcal/mol. Moreover, three types of noncovalent molecular interactions, namely,

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hydrogen bonding and $\pi-\pi$ and cation$-\pi$ interactions, played an important role in the binding of the compounds to 1XBP. For all the docking models, at least one hydrogen bond was formed between the hydroxyl group of the eight-membered ring and the residue of G-2484 (Table 3 and Figure 3). In the docking results for compounds **12d**, **12e**, **12f**, **13b**, and **13c**, a $\pi-\pi$ interaction was formed between the benzene ring and the purine of A-2045. Cation$-\pi$ interactions between pyrrolidine and the purine of A-2045 were only present in the docking model for compound **15d**.

The molecular docking results revealed why compounds **11**, **13b**, and **15c** were the most active agents. When compound **11** was docked, it exhibited four hydrogen bonds between its OH (eight-membered ring), NH_2 (triazole ring) and C═O (ester) groups with G-2484, G-2565, and G-2044, with an RMSD of 0.99 Å (see Figure 3A and Table 3). When compound **13b** was docked, it exhibited two hydrogen bonds between its OH (eight-membered ring) and NH_2 (benzene ring) groups with the phosphates of G-2484 and C-2420 with distances of 2.2 and 1.6 Å, respectively. In addition, a $\pi-\pi$ interaction was formed between the benzene ring and the purine of A-2045 with a distance of 3.9 Å. Although **13c** showed the same hydrogen bonds and $\pi-\pi$ interactions as did **13b**, it exhibited a higher binding free energy than that of **13b**, possibly because of the greater distances of the hydrogen bond between the NH_2 (benzene ring) and C-2420 and of the $\pi-\pi$ interaction between the benzene ring and the purine of A-2045 (see Figure 3C and Table 3). Compounds **15a**$-$**d** adopted very similar conformations, and their entire molecules were superimposed well. Compound **15c** formed three hydrogen bonds between its OH (eight-membered ring) and C═O (five-membered ring) groups with G-2484, C-2431 and U-2485, with distances of 2.2, 2.7, and 3.2 Å, respectively. To our surprise, compound **15d** showed a better docking model than did **15c**, with three hydrogen bonds and a cation-π interaction formed, but displayed a lower binding affinity, perhaps because of the longer distance of the hydrogen bond between its C═O (ester and amido bond) group and C-2420 and C-2565 (see Figure 3D and Table 3). Compounds **12d** and **12f** were not the most active agents, but they showed higher antibacterial activities than did their analogue, **12e**. Molecular docking studies revealed that **12f** formed three hydrogen bonds (OH, C═O, and OCH3) and a $\pi-\pi$ interaction. Compound **12e** displayed a docking model similar to that of **12f**, except with the absence of one hydrogen bond formed between C═O (ester) and G-2044 (see Figure 3B and Table 3). The superior docking modes of compounds **12d**, **12f**, **13b**, and **13c** suggested that the substituents with hydrogen donators or with groups with lower Hammett constants, which enhance $\pi-\pi$ stacking interactions,[37] should be introduced to produce new analogues with higher antibacterial activities as a potential design strategy.

Based on the docking results, the predicted binding affinities (binding free energies) were in agreement with the biological results, revealing that there is a direct and rational correlation between the binding free energy and the antibacterial activity. Because X-ray structures of the 50S ribosomal subunits are available only for *E. coli* (PDB ID: 2AW4), we investigated the linear relationships between the binding free energy (ΔG_b, kcal/mol) and the zone of inhibition (mm) for 320 and 160 μg/mL concentrations of *E. coli* on the basis of the high similarity in domain V of 23S rRNA at the PTC between 1XBP and 2AW4 (the superimposed results of the key residues of 1XBP and 2AW4 are shown in Figure S3 in the Supporting Information). The results revealed a direct reasonable correlation between the binding free energy and the zone of inhibition, with correlation coefficients (R^2) of 0.6626 and 0.7658 for compounds **11**, **12d**$-$**f**, **13a**$-$**c**, and **15a**$-$**d**, as illustrated in Figure S4 in the Supporting Information.

Pharmacokinetics (PK) Studies. The intravenous and oral pharmacokinetics of **11** and **15c** were investigated in the rat. The compounds were administered in either single intravenous (iv) injections of 5 mg/kg or in an oral dose of 15 mg/kg. The main pharmacokinetic profiles of **11** and **15c** are displayed in Table 4, and the kinetic profiles of distribution in plasma of **11** and **15c** after intravenous (iv) and oral (po) administration are shown in Figure S5 in the Supporting Information.

Table 4. Pharmacokinetic Properties of 11 and 15c in Rats

	11		15c	
parameter	iv	po	iv	po
C_{max}[a] (μg/mL)	13.57	3.07	11.26	4.65
T_{max}[b] (h)	0.08	0.75	0.08	0.75
$t_{1/2}$[c] (h)	3.40	6.07	3.89	5.98
Cl[d] (mL/h·kg)	324.57	611.01	353.81	621.41
V_{ss}[e] (L/kg)	0.92	5.14	1.02	4.91
MRT[f] (d)	2.84	8.42	2.88	7.91
$AUC_{0\rightarrow\infty}$[g] (μg·h/mL)	15.41	24.55	14.13	24.14
F[h] (%)		35.81		39.73

[a]Maximum concentration. [b]Time to reach C_{max}. [c]Half-life. [d]Clearance. [e]Volume of distribution at steady state. [f]Mean resident time. [g]Area under the curve. [h]Oral bioavailability.

After a single iv administration of **11** and **15c** (5 mg/kg), the mean values of $t_{1/2}$ were 3.40 and 3.89 h, reflecting a high rate of elimination. The similar basic nature and lipophilicity of **11** and **15c** produced similar distribution volumes (0.92 and 1.02 L/kg) and $AUC_{0\rightarrow\infty}$ values (15.41 and 14.13 μg·h/mL) after iv administration. Following po administration, compounds **11** and **15c** were absorbed rapidly, with C_{max} values of 3.07 and 4.65 μg/mL, respectively, at 0.75 h (T_{max}). Then, the concentrations of the two compounds dropped rapidly, with elimination half times ($t_{1/2}$) of 6.07 and 5.89 h, respectively, and moderate plasma clearance (CL) rates of 611.01 and 621.41 mL/h·kg, respectively.

Compounds **11** and **15c** had higher C_{max} values following oral dosing than did valnemulin in ducks (0.12 μg/mL) and chicken (0.66 μg/mL). Furthermore, the plasma $t_{1/2}$ values for **11** and **15c** after po dosing were longer than that of valnemulin in ducks (4.83 h) but shorter than that of valnemulin in chickens (11.10 h).[38,39] Based on the MICs of compounds **11** and **15c** against MRSA and *S. agalactiae*, the drug plasma concentrations were all above the MIC for a minimum of 6 h following iv and po administration. In clinical practice, a dose of 15 mg/kg body weight is suggested every 6 h following oral administration. The systemic bioavailabilities of **11** and **15c** were 35.81% and 39.73% following oral administration in rats, respectively. Our results are similar to those for valnemulin in ducks (36.68%) but far lower than those for valnemulin in chickens (74%) and in Sprague$-$Dawley (SD) rats (100%).[38,39] One of the reasons was that the two compounds were administered as the basic form, whereas valnemulin was administered as the hydrochloride with a higher solubility in water. These relatively low bioavailabilities indicate that it is necessary to develop proper preparations of compounds **11**and

15c to increase their bioavailability if they are to serve as new drugs.

CONCLUSION

New series of novel pleuromutilin derivatives containing thiadiazole moieties were synthesized by acylation under very mild conditions. These new derivatives were investigated for their antibacterial activity in vitro against MRSA, MRSE, *S. agalactiae*, and *E. coli*. The obtained MIC values and antibacterial activities revealed that all the synthesized compounds showed potent activity properties. Compounds **11**, **13b**, and **15c** were the most active antibacterial agents against MRSA, MRSE and *S. agalactiae*. Docking studies were performed on some compounds (**11**, **12d−f**, **13a−c**, and **15a−d**) with similar substituents but differences in antibacterial activity. The results showed that the binding free energies (ΔG_b) were in the range of −8.40 to −11.46 kcal/mol, with an RMSD range of 0.75 to 1.12 Å. Furthermore, three interactions were found to occur: hydrogen bonding, $\pi-\pi$ interactions, and cation$-\pi$ interactions. These results reveal that there is a direct and rational correlation between the binding free energy and the antibacterial activity. Compounds **11** and **15c** were further profiled in a rat model for pharmacokinetic studies. The results indicated that **11** and **15c** may serve as possible lead compounds for the development of antibacterial drugs.

EXPERIMENTAL SECTION

Synthesis. The reagents and solvents were obtained commerically and used without further purification. The melting points were recorded on a Tianda Tianfa YRT-3 apparatus (China) with open capillary tubes and were uncorrected. Infrared (IR) spectra were obtained on a Thermo Nicolet NEXUS-670 spectrometer. NMR spectra were recorded using Bruker 400 MHz spectrometers in $CDCl_3$or DMSO-d_6. The chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane. ^{13}C NMR spectra were recorded on 100 MHz spectrometers. All reactions were monitored by TLC (silica gel GF254) using elution (petroleum ether-ethyl acetate) followed by visualization after spraying with a 0.05% $KMnO_4$ aqueous solution or under UV illumination directly. The final compounds was determined to be ≥95% pure by HPLC analysis using a Waters 2695 series instrument equipped with a Waters 2998 DAD detector and a Hypersil ODS column (4.6 mm × 250 mm, 5 μm) at a mobile phase flow rate of 1 mL/min. Peaks were detected at 254 nm and the system was operated at 25 °C. The mobile phase was a mixture of acetonitrile (90%) and water (10%) containing 0.1% disodium hydrogen phosphate.

*14-O-(p-Toluene-sulfonyloxyacetyl)-mutilin (**9**).* A 10 M NaOH (5 mL, 50 mmol) was added dropwise to a mixture of pleuromutilin (7.57 g, 20 mmol) and *p*-toluenesulfonyl chloride (4.2 g, 22 mmol) in water (5 mL) and *t*-butyl methyl ether (20 mL). The result mixture was stirred under reflux for 1 h followed by dilution with water (50 mL) and stir under an ice bath for 15 min. The result suspension was washed with water (50 mL) and cold *t*-butyl methyl ether (20 mL) 3 times. The crude production was filtered to give 93% (9.8 g) compound **9** as a white powder; mp: 147−148 °C. IR (KBr): 3446, 2924, 2863, 1732, 1633, 1597, 1456, 1371, 1297, 1233, 1117, 1035, 832, 664, 560 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.63 (d, 3H, J = 6.8 Hz), 0.87 (d, 3H, J = 6.8 Hz), 1.11−1.15 (m, 1H), 1.22−1.26 (s, 5H), 1.33−1.36 (m, 1H), 1.41−1.44 (m, 1H), 1.46−1.50 (m, 5H), 1.63−1.65 (dd, 2H,J_1 = 10 Hz,J_2 = 7.2 Hz), 2.01−2.08(m, 3H), 2.21−2.29 (m, 3H), 2.45(s, 3H), 3.34 (d, 1H, J = 6.4 Hz), 4.48 (s, 2H), 5.17−5.21 (d, 1H, J = 8.8 Hz), 5.31−5.34 (d, 1H, J = 6.4 Hz), 5.75−5.78 (d, 1H, J = 4.2 Hz), 6.43(q, 1H, J = 17.2 Hz, 10.8 Hz); 7.35−7.37 (d, 2H, J = 4.0 Hz), 7.80−7.82 (d, 2H, J = 4.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.7, 164.8, 145.2, 138.6, 132.5, 129.9, 127.9, 117.2, 74.4, 70.2, 64.9, 57.9, 45.3, 44.4, 43.9, 41.7, 36.4, 35.9, 34.3, 30.2, 26.7, 26.3, 24.7, 21.6, 16.4, 14.6, 11.4. HRMS (ESI): $[M + Na]^+$ calcd for $C_{29}H_{40}O_7S$, 533.2501; found, 533.2507.

*14-O-(Iodoacetyl)-mutilin (**10**).* Compound **10** was prepared on the basis of a published method from a solution of compound **9** and KI, which was refluxed for 1 h.[30,31] The crude production was purified by recrystallization with acetone to give 81% compound **10** as a pale yellow powder; mp: 117−119 °C. IR (KBr): 3307, 2979, 2933, 1736, 1712, 1456, 1417, 1279, 1084, 1023, 985, 967 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.26 (s, 1H), 6.41 (dd, J = 17.4, 11.0 Hz, 1H), 5.70 (d, J = 8.5 Hz, 1H), 5.34 (d, J = 11.0 Hz, 1H), 5.20 (dd, J = 17.4, 1.1 Hz, 1H), 3.65 (d, J = 10.4 Hz, 1H), 3.57 (d, J = 10.5 Hz, 1H), 3.35 (s, 1H), 2.36−2.28 (m, 1H), 2.26−2.14 (m, 2H), 2.07 (dd, J = 16.0, 8.5 Hz, 2H), 1.79−1.60 (m, 4H), 1.57−1.47 (m, 2H), 1.47−1.42 (m, 4H), 1.35 (dd, J = 26.4, 9.7 Hz, 2H), 1.17 (s, 3H), 1.12 (d, J = 4.3 Hz, 1H), 0.87 (d, J = 7.0 Hz, 3H), 0.73 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.76, 166.88, 138.57, 117.28, 74.71, 70.49, 58.20, 45.48, 44.20, 43.93, 41.98, 36.71, 35.86, 34.31, 30.10, 26.78, 26.42, 24.80, 16.75, 14.78, 11.46. HRMS (ESI): $[M + NH_4]^+$ calcd for $C_{22}H_{33}IO_4$, 506.1762; found, 506.1758.

*14-O-[(2-Amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**).* A solution of compound **10** (2.45 g, 5 mmol) in 60 mL of tetrahydrofuran was added dropwise to a mixture of 2-amino-5-mercapto-1,3,4-thiadiazole (0.73 g, 5.5 mmol) in 5 mL of 10% NaOH. It was stirred at 0 °C for 2 h, then evaporated in vacuum, and ethyl acetate (100 mL) was added to the residue. The obtained mixture was quenched with saturated aqueous NH_4Cl and washed with water, followed by separation of the organic layer and drying under anhydrous Na_2SO_4 overnight. After evaporating most of the ethyl acetate under vacuum, the residue was purified by recrystallization or silica gel column chromatography (petroleum ether/ethyl acetate 1:2 v/v) to afford compound **11** as a white solid in 82% yield (2.3 g); mp: 74−75 °C. IR (KBr): 3419, 3330, 2931, 1731, 1616, 1507, 1456, 1417, 1373, 1282, 1190, 1152, 1117, 1019, 980, 953, 938,916 cm^{-1}. ^1H NMR (400 MHz, DMSO) δ: 7.28 (s, 2H), 6.08 (dd, J = 17.8, 11.2 Hz, 1H), 5.52 (d, J = 8.1 Hz, 1H), 5.03 (dd, J = 21.0, 14.7 Hz, 2H), 4.51 (d, J = 5.9 Hz, 1H), 4.02 (q, J = 7.1 Hz, 1H), 3.90 (q, J = 16.0 Hz, 2H), 3.45−3.32 (m, 2H), 2.39 (s, 1H), 2.19 (dd, J = 18.8, 10.8 Hz, 1H), 2.11−1.97 (m, 4H), 1.64 (dd, J = 18.4, 9.5 Hz, 2H), 1.54−1.42 (m, 1H), 1.36−1.21 (m, 6H), 1.16 (d, J = 7.1 Hz, 1H), 1.08−0.94 (m, 4H), 0.82 (d, J = 6.7 Hz, 3H), 0.59 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ: 217.08, 169.73, 166.70, 148.89, 140.68, 115.39, 72.63, 70.27, 59.75, 57.23, 44.95, 44.14, 41.51, 36.33, 34.00, 30.10, 28.70, 26.60, 24.47, 20.75, 16.09, 14.51, 14.08, 11.54. HRMS (ESI): $[M + H]^+$ calcd for $C_{24}H_{35}N_3O_4S_2$, 494.2142; found, 494.2139.

*General Procedure for the Synthesis of Compounds **12a−f**.* A mixture of benzoic acid (3.6 mmol), compound **11** (1.48 g, 3 mmol), EDCI (0.69 g, 3.6 mmol), HOBt (0.51 g, 3.6 mmol), and 60 mL of dichloromethane was stirred at room temperature for 36−48 h. The mixture was washed with saturated aqueous $NaHCO_3$ and water, dried with anhydrous Na_2SO_4 overnight

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and rotary evaporated to dryness. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 1:1 v/v) to afford the desired compounds **12a**−**12f**.

*14-O-[(2-Chlorobenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**12a**).* Compound **12a** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *o*-chlorobenzoic acid. The crude product was purified by silica gel column chromatography in 75% yield (1.42 g) as a white solid; mp: 74−76 °C. IR (KBr): 3446, 2932, 1731, 1683, 1539, 1456, 1374, 1305, 1248, 1189, 1152, 1117, 1046, 1017, 980, 938, 916, 895, 746 cm^{-1}. ^1H NMR (400 MHz, CDCl3) δ: = 7.79 (s, 1H), 7.57−7.34 (m, 3H), 6.38 (dd, J = 17.4, 11.0, 1H), 5.73 (d, J = 8.4, 1H), 5.27 (d, J = 11.0, 1H), 5.13 (d, J = 17.4, 1H), 3.94−3.77 (m, 2H), 3.32 (d, J = 6.1, 1H), 2.32−2.09 (m, 3H), 2.09−1.96 (m, 3H), 1.78−1.68 (m, 1H), 1.65−1.47 (m, 3H), 1.45−1.29 (m, 5H), 1.23 (dd, J = 12.2, 5.1, 2H), 1.10 (d, J = 8.5, 4H), 0.84 (d, J = 7.0, 3H), 0.67 (d, J = 6.9, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.79, 166.40, 164.40, 160.13, 158.44, 138.71, 130.84, 130.47, 127.08, 117.20, 74.48, 70.36, 60.28, 57.98, 45.33, 44.62, 43.84, 41.77, 36.57, 35.85, 34.33, 30.28, 26.73, 26.35, 24.73, 16.64, 14.75, 14.10, 11.37. HRMS (ESI): $[M + Na]^+$ calcd for $C_{31}H_{38}ClN_3O_5S_2$, 654.1834; found, 654.1828.

*14-O-[(3-Chlorobenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**12b**).* Compound **12b** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *m*-chlorobenzoic acid. The crude product was purified by silica gel column chromatography in 62% yield (1.18 g) as a white solid; mp: 115−118 °C. IR (KBr): 3427, 2928, 1731, 1673, 1538, 1454, 1411, 1309, 1250, 1188, 1152, 1116, 1019, 980, 914, 805, 733 cm^{-1}. ^1H NMR (400 MHz, CDCl3) δ: 8.13 (d, J = 6.5 Hz, 2H), 7.60 (d, J = 6.6 Hz, 1H), 7.51 (dd, J = 9.9, 5.3 Hz, 1H), 6.40−6.29 (m, 1H), 5.72 (d, J = 5.3 Hz, 1H), 5.27−5.19 (m, 1H), 5.09 (dd, J = 17.4, 1.8 Hz, 1H), 4.05−3.92 (m, 2H), 3.31 (s, 1H), 2.30−2.11 (m, 3H), 2.04 (s, 2H), 1.97 (dd, J = 14.6, 6.9 Hz, 1H), 1.73 (d, J = 14.3 Hz, 1H), 1.61 (d, J = 10.1 Hz, 2H), 1.46 (dd, J = 16.4, 13.0 Hz, 2H), 1.34 (t, J = 13.3 Hz, 4H), 1.28−1.16 (m, 3H), 1.09 (t, J = 10.4 Hz, 4H), 0.84 (d, J = 4.8 Hz, 3H), 0.65 (d, J = 3.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.20, 163.91, 161.20, 158.54, 138.37, 133.00, 132.35, 129.84, 128.48, 126.62, 116.93, 74.21, 70.06, 60.04, 57.71, 45.06, 44.25, 43.53, 41.47, 36.29, 35.58, 34.07, 30.00, 26.43, 25.99, 24.46, 20.71, 16.35, 14.44, 13.85, 11.12. HRMS (ESI): $[M + Na]^+$ calcd for $C_{31}H_{38}ClN_3O_5S_2$, 654.1834; found, 654.1827.

*14-O-[(4-Chlorobenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**12c**).* Compound **12c** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *p*-chlorobenzoic acid. The crude product was purified by silica gel column chromatography in 64% yield (1.21 g) as a white solid; mp: 101−103 °C. IR (KBr): 3420, 2927, 1731, 1670, 1594, 1539, 1493, 1456, 1409, 1310, 1152, 1116, 1014, 980, 891, 846, 749 cm^{-1}. ^1H NMR (400 MHz, CDCl3) δ: 8.19 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.3 Hz, 2H), 6.38 (dd, J = 17.4, 11.0 Hz, 1H), 5.74 (d, J = 8.4 Hz, 1H), 5.23 (d, J = 10.9 Hz, 1H), 5.10 (d, J = 17.3 Hz, 1H), 3.98 (s, 2H), 3.31 (d, J = 6.4 Hz, 1H), 2.29−2.15 (m, 3H), 2.05−1.94 (m, 2H), 1.73 (d, J = 14.3 Hz, 1H), 1.61 (d, J = 10.4 Hz, 2H), 1.50−1.37 (m, 5H), 1.33−1.20 (m, 3H), 1.08 (s, 4H), 0.84 (d, J = 6.9 Hz, 4H), 0.68 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.88, 166.48, 164.44, 161.65, 158.79, 139.91, 138.71, 130.18, 129.18, 128.85, 117.26, 74.53, 70.44, 58.03, 45.38, 44.59, 43.85, 41.80, 36.62, 35.87, 34.39, 30.33, 26.75, 26.28, 24.77, 16.69, 14.78, 11.44. HRMS (ESI): $[M + Na]^+$ calcd for $C_{31}H_{38}ClN_3O_5S_2$, 654.1834; found, 654.1829.

*14-O-[(2-Methoxybenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**12d**).* Compound **12d** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *o*-methoxybenzoic acid. The crude product was purified by silica gel column chromatography in 68% yield (1.28 g) as a white solid; mp: 89−92 °C. IR (KBr): 3304, 2934, 1732, 1662, 1601, 1527, 1487, 1466, 1398, 1373, 1288, 1242, 1184, 1163, 1118, 1044, 1017, 980, 938, 916, 895, 756, 666 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: = 11.18 (s, 1H), 8.20 (dd, J = 7.9, 1.7, 1H), 7.58−7.53 (m, 1H), 7.11 (t, J = 7.6, 1H), 7.05 (d, J = 8.4, 1H), 6.38 (dd, J = 17.4, 11.0, 1H), 5.72 (d, J = 8.4, 1H), 5.29−5.23 (m, 1H), 5.14 (dd, J = 17.4, 1.2, 1H), 4.12−4.00 (m, 6H), 3.97 (t, J = 10.2, 2H), 3.32 (d, J = 6.0, 1H), 2.29−2.24 (m, 1H), 2.16 (dd, J = 11.4, 6.3, 2H), 2.07−1.97 (m, 5H), 1.71 (dd, J = 14.4, 2.4, 2H), 1.64−1.53 (m, 4H), 1.52−1.45 (m, 1H), 1.43−1.27 (m, 9H), 1.21 (t, J = 7.1, 2H), 1.14−1.06 (m, 5H), 0.82 (s, 3H), 0.70 (d, J = 6.9, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.91, 166.77, 162.45, 159.06, 158.51, 157.98, 138.83, 135.12, 132.74, 121.81, 118.16, 117.20, 111.76, 74.55, 70.38, 60.34, 58.08, 56.44, 45.40, 44.59, 43.96, 41.83, 36.69, 35.99, 34.42, 30.37, 26.82, 26.45, 24.80, 16.74, 14.79, 11.46. HRMS (ESI): $[M + Na]^+$ calcd for $C_{32}H_{41}N_3O_6S_2$, 650.2329; found, 650.2322.

*14-O-[(3-Methoxybenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**12e**).* Compound **12e** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *m*-methoxybenzoic acid. The crude product was purified by silica gel column chromatography in 62% yield (1.17 g) as a white solid; mp: 142−145 °C. IR (KBr): 32894, 2934, 1728, 1678, 1585, 1557, 1489, 1456, 1407, 1310, 1273, 1226, 1186, 1153, 1114, 1042, 1009, 979, 922, 736, 675 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.81 (d, J = 8.1 Hz, 1H), 7.65−7.62 (m, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.15 (dd, J = 8.1, 2.2 Hz, 1H), 6.35 (dd, J = 17.4, 11.0 Hz, 1H), 5.72 (d, J = 8.4 Hz, 1H), 5.22 (dd, J = 11.0, 1.1 Hz, 1H), 5.08 (dd, J = 17.4, 1.2 Hz, 1H), 4.00 (dd, J = 36.4, 16.1 Hz, 2H), 3.84 (s, 3H), 3.35−3.27 (m, 1H), 2.29−2.10 (m, 3H), 2.03 (d, J = 5.7 Hz, 1H), 1.96 (dd, J = 16.0, 8.5 Hz, 1H), 1.72 (dd, J = 14.4, 2.2 Hz, 1H), 1.63−1.47 (m, 3H), 1.41−1.30 (m, 4H), 1.25−1.19 (m, 1H), 1.06 (s, 4H), 0.83 (d, J = 7.0 Hz, 3H), 0.65 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.39, 166.05, 164.73, 160.88, 159.22, 157.88, 138.12, 131.64, 129.29, 120.30, 119.08, 116.68, 112.92, 73.98, 69.78, 57.47, 54.89, 44.82, 43.99, 43.28, 41.22, 36.06, 35.40, 35.11, 33.84, 29.77, 26.19, 25.75, 24.22, 16.11, 14.20, 10.90. HRMS (ESI): $[M + K]^+$ calcd for $C_{32}H_{41}N_3O_6S_2$, 666.2068; found, 666.2061.

*14-O-[(4-Methoxybenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**12f**).* Compound **12f** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *p*-methoxybenzoic acid. The crude product was purified by silica gel column chromatography in 66% yield (1.24 g) as a white solid; mp: 131−133 °C. IR (KBr): 3426, 2933, 1731, 1666, 1606, 1537, 1513, 1456, 1416, 1374, 1299, 1256, 1176, 1117, 1027, 980, 938, 892, 845, 759, 691, 649, 609 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 8.20 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H), 6.35 (dd, J = 17.3, 11.0 Hz, 1H), 5.72 (d, J = 8.4 Hz, 1H), 5.21

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(d, J = 11.0 Hz, 1H), 5.08 (d, J = 17.4 Hz, 1H), 4.11 (q, J = 7.1 Hz, 1H), 3.98 (t, J = 9.3 Hz, 2H), 3.87 (d, J = 18.7 Hz, 3H), 3.30 (dd, J = 10.1, 6.6 Hz, 1H), 2.29−2.11 (m, 3H), 2.03 (s, 2H), 1.95 (dd, J = 16.0, 8.5 Hz, 1H), 1.72 (d, J = 14.2 Hz, 1H), 1.60 (dd, J = 21.1, 11.3 Hz, 2H), 1.54−1.40 (m, 3H), 1.37−1.29 (m, 4H), 1.24 (t, J = 7.1 Hz, 2H), 1.15−1.02 (m, 4H), 0.84 (d, J = 6.9 Hz, 3H), 0.66 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.99, 166.64, 164.82, 163.89, 158.25, 138.77, 130.95, 123.07, 117.34, 114.19, 74.62, 70.47, 60.46, 58.12, 55.64, 45.47, 44.62, 43.93, 41.89, 36.72, 36.05, 34.49, 30.43, 26.85, 26.37, 24.87, 21.12, 16.75, 14.82, 14.27, 11.54. HRMS (ESI): $[M + K]^+$ calcd for $C_{32}H_{41}N_3O_6S_2$, 666.2068; found, 666.2059.

*General Procedure for the Synthesis of Compounds **13a**−**c**.* A 1 N NaOH solution (6 mL) was added to a suspension of amino acid derivative (5 mmol) in tetrahydrofuran (50 mL) and water (20 mL), followed by the addition of 1.1 g di-*tert*-butyl dicarbonate (5 mmol) dropwise at room temperature. The tetrahydrofuran was evaporated under reduced pressure after stirring for 4 h, then ethyl acetate (50 mL) and 5% citric acid (30 mL) were added to the result residue. The organic layer was separated, washed with water (20 mL), dried over Na_2SO_4, and rotary evaporated to dryness. The crude residue was used in the next reaction without purification.

A mixture of the above *N*-Boc protected amino acids (3.6 mmol), 1.48 g compound **11** (3 mmol), 0.69 g EDCI (3.6 mmol), 0.51 g HOBt (3.6 mmol), and 60 mL of dichloromethane was stirred at room temperature for 36−48 h. The mixture was washed with saturated aqueous $NaHCO_3$ and water and then evaporated under reduced pressure. The residue was treated with a mixture of TFA and dichloromethane (1:1; 20 mL) at room temperature for 30 min. The reaction mixture was quenched with 25% aqueous $NaHCO_3$ (50 mL), washed with water, dried with anhydrous Na_2SO_4 overnight, and rotary evaporated to dryness. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate 1:2 v/v) to give the desired compounds.

*14-O-[(2-Aminobenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**13a**).* Compound **13a** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *o*-aminobenzoic acid. The crude product was purified by silica gel column chromatography in 61% yield (1.12 g) as a white solid; mp: 146−149 °C. IR (KBr): 3473, 3367, 2931, 1731, 1651, 1617, 1557, 1525, 1455, 1385, 1297, 1241, 1161, 1117, 1017, 980, 937, 896, 751, 696, 670 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, J = 8.2 Hz, 1H), 7.29 (dd, J = 15.0, 7.4 Hz, 1H), 6.72 (t, J = 6.9 Hz, 2H), 6.38 (dd, J = 17.4, 11.0 Hz, 1H), 5.74 (d, J = 8.4 Hz, 1H), 5.26 (d, J = 11.0 Hz, 1H), 5.12 (d, J = 17.4 Hz, 1H), 3.99 (q, J = 16.1 Hz, 2H), 3.33 (d, J = 5.9 Hz, 1H), 2.35−2.10 (m, 4H), 2.09−1.91 (m, 3H), 1.73 (d, J = 15.4 Hz, 1H), 1.57 (ddd, J = 27.7, 23.3, 12.8 Hz, 3H), 1.48−1.28 (m, 6H), 1.25 (t, J = 7.1 Hz, 1H), 1.19−0.99 (m, 4H), 0.95 (t, J = 7.4 Hz, 1H), 0.85 (d, J = 6.9 Hz, 3H), 0.69 (d, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 217.14, 166.84, 166.65, 161.15, 158.06, 150.56, 138.82, 134.51, 129.17, 117.50, 116.83, 111.52, 74.71, 70.48, 58.21, 45.53, 44.69, 44.01, 41.95, 36.81, 36.09, 34.56, 30.49, 26.91, 26.45, 24.93, 16.84, 14.94, 11.60. HRMS (ESI): $[M + H]^+$ calcd for $C_{31}H_{40}N_4O_5S_2$, 613.2513; found, 613.2509.

*14-O-[(3-Aminobenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**13b**).* Compound **13b** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *m*-aminobenzoic acid. The crude product was purified by silica gel column chromatography in 61% yield (1.12 g) as a white solid; mp: 135−138 °C. IR (KBr): 3432, 3370, 2927, 1729, 1667, 1626, 1587, 1531, 1456, 1410, 1374, 1305, 1189, 1152, 1117, 1049, 1017, 980, 939, 847, 743, 677 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, J = 1.4 Hz, 1H), 7.49 (d, J = 7.4 Hz, 1H), 7.31−7.25 (m, 1H), 6.89 (d, J = 7.9 Hz, 1H), 6.41−6.31 (m, 1H), 5.73 (d, J = 8.0 Hz, 1H), 5.25 (d, J = 10.9 Hz, 1H), 5.10 (d, J = 17.4 Hz, 1H), 4.11 (q, J = 7.1 Hz, 1H), 3.93 (dd, J = 20.6, 4.6 Hz, 2H), 3.31 (s, 1H), 2.32−2.10 (m, 3H), 2.01 (dt, J = 26.7, 8.0 Hz, 3H), 1.72 (d, J = 14.4 Hz, 1H), 1.60 (dd, J = 20.2, 10.1 Hz, 2H), 1.52−1.29 (m, 6H), 1.28−1.18 (m, 3H), 1.10 (d, J = 14.2 Hz, 4H), 0.84 (d, J = 6.7 Hz, 3H), 0.66 (d, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.90, 166.70, 165.57, 162.01, 158.11, 147.14, 138.68, 131.50, 129.71, 119.66, 118.47, 117.28, 114.54, 74.51, 70.41, 60.34, 58.01, 45.35, 44.58, 43.84, 41.76, 36.56, 35.94, 34.37, 30.31, 26.74 26.26, 24.75, 21.00, 16.68, 14.75, 14.15, 11.44. HRMS (ESI): $[M + H]^+$ calcd for $C_{31}H_{40}N_4O_5S_2$, 613.2513; found, 613.2510.

*14-O-[(4-Aminobenzamide-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**13c**).* Compound **13c** was prepared according to the general procedure from 14-*O*-[(2-amino-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**11**) and *p*-aminobenzoic acid. The crude product was purified by silica gel column chromatography in 58% yield (1.07 g) as a white solid; mp: 151−153 °C. IR (KBr): 3441, 3371, 2930, 1728, 1627, 1603, 1568, 1518, 1455, 1374, 1296, 1261, 1183, 1117, 1049, 980, 938, 890, 842, 760, 693, 609 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06−7.96 (m, 2H), 6.69 (d, J = 8.6 Hz, 2H), 6.35 (dd, J = 17.4, 11.0 Hz, 1H), 5.71 (d, J = 8.1 Hz, 1H), 5.15 (dd, J = 49.9, 14.1 Hz, 2H), 4.35−4.23 (m, 2H), 4.01 (t, J = 9.9 Hz, 2H), 3.35−3.26 (m, 1H), 2.20 (ddd, J = 23.9, 11.0, 4.8 Hz, 3H), 2.07−1.81 (m, 3H), 1.72 (d, J = 14.2 Hz, 1H), 1.67−1.53 (m, 3H), 1.49−1.30 (m, 5H), 1.28−1.18 (m, 2H), 1.06 (s, 4H), 0.83 (d, J = 6.7 Hz, 3H), 0.67 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.92, 166.790, 164.770, 161.78, 157.81, 151.60, 138.73, 130.82, 119.69, 117.28, 114.06, 74.55, 70.48, 60.41, 58.04, 45.40, 44.52, 43.89, 41.83, 36.65, 35.91, 34.43, 30.34, 26.56, 24.79, 16.73, 14.78, 11.47. HRMS (ESI): $[M + H]^+$ calcd for $C_{31}H_{40}N_4O_5S_2$, 613.2513; found, 613.2507.

*14-O-[(2-Chloroacetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**14**).* To a solution of 1.48 g of compound **11** (3 mmol) and 0.61 g of *N*-methylmorpholine (6 mmol) in dry dichloromethane (20 mL), 0.51 g of $ClCH_2COCl$ (4.5 mmol) in dry dichloromethane (5 mL) was slowly dropped at 0 °C. After stirring for 1 h, the solution was washed with water three times, and then the organic layer was dried with $MgSO_4$, filtered, concentrated, and purified by column chromatography (petroleum ether/ethyl acetate = 1:1) to yield **14** as a white solid in 86% yield (1.47 g); mp: 82−84 °C. IR (KBr): 3446, 2936, 1731, 1652, 1558, 1540, 1456, 1405, 1286, 1189, 1153, 1117, 1056, 1017, 980, 668 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.47−6.32 (m, 1H), 5.72 (s, 1H), 5.33−5.20 (m, 1H), 5.13 (d, J = 13.6 Hz, 1H), 4.38 (s, 2H), 4.14−4.03 (m, 1H), 3.98 (s, 2H), 3.33 (d, J = 5.6 Hz, 1H), 2.23 (d, J = 33.3 Hz, 3H), 2.03 (t, J = 11.2 Hz, 3H), 1.73 (d, J = 13.6 Hz, 1H), 1.62 (d, J = 7.5 Hz, 2H), 1.42 (t, J = 17.2 Hz, 6H), 1.10 (d, J = 4.0 Hz, 4H), 0.84 (d, J = 3.4 Hz, 3H), 0.69 (d, J = 6.8 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.94, 171.07, 166.43, 164.90, 159.41, 138.80, 117.24, 74.47, 70.46, 60.43, 57.97, 45.46, 44.55, 43.87, 41.94, 41.79, 36.48, 35.89, 34.31, 30.28, 26.75, 26.34, 24.60,

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16.71, 14.74, 11.41. HRMS (ESI): $[M + Na]^+$ calcd for $C_{26}H_{36}ClN_3O_5S_2$, 592.1678; found, 592.1669.

*General Procedure for the Synthesis of Compounds **15a–f**.* The appropriate secondary amine (4.5 mmol) was added to a solution of 1.76 g of compound **14** (3 mmol) and 0.61 g of triethylamine (6 mmol) in tetrahydrofuran (60 mL) and stirred at 45 °C for 2 h. Then, the solvent was removed under reduced pressure, and ethyl acetate (50 mL) was added. The mixture was quenched with saturated aqueous NH_4Cl (30 mL). The organic layer was separated, washed with water (20 mL) three times, dried with anhydrous Na_2SO_4, and rotary evaporated to dryness. The crude residue was purified by silica gel column chromatography (ethyl acetate/ethanol 20:1 v/v) to afford the desired compounds.

*14-O-[(2-(bis(Methyl)-amino)-acetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**15a**).* Compound **15a** was prepared according to the general procedure from 14-*O*-[(2-chloroacetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**14**) and dimethylamine. The crude product was purified by silica gel column chromatography in 69% yield (1.20 g) as a white solid; mp: 74–76 °C. IR (KBr): 3444, 2937, 1731, 1557, 1519, 1403, 1284, 1152, 1117, 1047, 1017, 980, 953, 916, 864, 665 cm^{-1}. ^1H NMR (400 MHz, acetone) δ: 6.36 (dd, J = 17.8, 11.2 Hz, 1H), 5.83 (d, J = 8.6 Hz, 1H), 5.22 (dd, J = 35.5, 14.5 Hz, 2H), 4.17 (dt, J = 14.2, 5.8 Hz, 4H), 3.70 (d, J = 5.4 Hz, 1H), 3.40 (s, 2H), 2.48 (s, 7H), 2.35 (dd, J = 18.0, 11.0 Hz, 2H), 2.25–2.10 (m, 14H), 2.06 (d, J = 1.4 Hz, 9H), 1.92–1.75 (m, 2H), 1.68 (dd, J = 34.3, 21.7 Hz, 2H), 1.56–1.34 (m, 6H), 1.30 (t, J = 7.1 Hz, 3H), 1.21 (d, J = 16.0 Hz, 4H), 1.04 (d, J = 7.0 Hz, 3H), 0.81 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.79, 175.22, 168.65, 166.36, 158.38, 138.44, 116.94, 74.23, 70.08, 61.50, 60.10, 57.74, 45.48, 45.09, 44.21, 43.60, 41.50, 36.34, 35.67, 34.12, 30.03, 26.47, 26.10, 24.46, 20.70, 16.43, 14.46, 13.87, 11.18. HRMS (ESI): $[M + H]^+$ calcd for $C_{28}H_{42}N_4O_5S_2$, 579.2669; found, 579.2661.

*14-O-[(2-(bis(Ethyl)-amino)-acetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**15b**).* Compound **15b** was prepared according to the general procedure from 14-*O*-[(2-chloroacetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**14**) and diethylamine. The crude product was purified by silica gel column chromatography in 64% yield (1.17 g) as a white solid; mp: 70–73 °C. IR (KBr): 3444, 2971, 2932, 1732, 1698, 1519, 1455, 1403, 1285, 1202, 1152, 1117, 1065, 1018, 980, 953, 938, 916, 724, 665 cm^{-1}. ^1H NMR (400 MHz, acetone) δ: 6.25 (t, J = 14.0 Hz, 1H), 5.72 (s, 1H), 5.11 (dd, J = 32.6, 14.1 Hz, 2H), 4.18–4.02 (m, 2H), 3.59 (s, 1H), 3.38 (s, 2H), 3.11 (s, 1H), 2.69 (d, J = 5.3 Hz, 3H), 2.37 (s, 1H), 2.28–2.03 (m, 4H), 1.73 (dd, J = 27.5, 12.4 Hz, 2H), 1.63–1.49 (m, 2H), 1.39 (d, J = 20.0 Hz, 5H), 1.30 (d, J = 13.1 Hz, 1H), 1.11 (dd, J = 25.5, 14.0 Hz, 12H), 0.93 (s, 3H), 0.70 (s, 3H). ^{13}C NMR (100 MHz, acetone) δ: 217.09, 171.44, 167.44, 159.08, 141.33, 116.42, 74.70, 71.63, 58.52, 57.46, 49.35, 46.34, 45.18, 42.88, 37.65, 36.86, 34.94, 31.24, 30.56, 30.07, 28.74, 27.82, 27.38, 25.65, 16.88, 15.12, 12.51, 11.81. HRMS (ESI): $[M + H]^+$ calcd for $C_{30}H_{46}N_4O_5S_2$, 607.2982; found, 607.2972.

*14-O-[(2-(Piperidine-1-yl)-acetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**15c**).* Compound **15c** was prepared according to the general procedure from 14-*O*-[(2-chloroacetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**14**) and piperidine. The crude product was purified by silica gel column chromatography in 62% yield (1.15 g) as a white solid; mp: 49–51 °C. IR (KBr): 3434, 2932, 1731, 1557, 1519, 1455, 1403, 1286, 1188, 1152, 1117, 1019, 980, 954, 939, 916, 667 cm^{-1}. ^1H NMR (400 MHz, acetone) δ: 6.26 (dd, J = 17.7, 11.2 Hz, 1H), 5.72 (d, J = 8.4 Hz, 1H), 5.12 (dd, J = 32.7, 14.4 Hz, 2H), 4.16–4.03 (m, 2H), 3.60 (d, J = 5.9 Hz, 1H), 3.30 (s, 2H), 2.57 (s, 3H), 2.38 (s, 1H), 2.31–2.23 (m, 1H), 2.19–2.08 (m, 2H), 1.96 (s, 6H), 1.77 (d, J = 16.7 Hz, 1H), 1.69–1.51 (m, 6H), 1.50–1.35 (m, 7H), 1.31 (d, J = 15.8 Hz, 1H), 1.21–1.09 (m, 4H), 0.94 (d, J = 7.0 Hz, 3H), 0.71 (d, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, acetone) δ: 217.24, 172.50, 170.26, 167.42, 159.22, 141.30, 116.40, 74.68, 71.60, 62.29, 58.48, 55.44, 46.31, 45.14, 42.85, 37.62, 36.81, 34.90, 28.67, 27.45, 27.83, 26.69, 25.61, 24.46, 20.62, 16.96, 15.25, 11.88. HRMS (ESI): $[M + H]^+$ calcd for $C_{31}H_{46}N_4O_5S_2$, 619.2982; found, 619.2974.

*14-O-[(2-(Pyrrolidine-1-yl)-acetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**15d**).* Compound **15d** was prepared according to the general procedure from 14-*O*-[(2-chloroacetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**14**) and piperidine. The crude product was purified by silica gel column chromatography in 71% yield (1.29 g) as a white solid; mp: 82–84 °C. IR (KBr): 3418, 2937, 1732, 1519, 1456, 1402, 1374, 1289, 1167, 1149, 1117, 1013, 981, 954, 940, 918, 835, 671 cm^{-1}. 1H NMR (400 MHz, acetone) δ: 6.27 (dd, J = 17.7, 11.2 Hz, 1H), 5.74 (d, J = 8.4 Hz, 1H), 5.22–5.05 (m, 2H), 4.11 (s, 1H), 4.06 (d, J = 7.1 Hz, 1H), 3.50 (d, J = 1.8 Hz, 2H), 2.74 (d, J = 6.5 Hz, 3H), 2.26 (dd, J = 12.7, 5.8 Hz, 1H), 2.11–2.04 (m, 8H), 1.98 (s, 4H), 1.83 (d, J = 14.1 Hz, 4H), 1.74 (dd, J = 19.5, 6.0 Hz, 1H), 1.63–1.53 (m, 1H), 1.44 (s, 4H), 1.32 (d, J = 14.0 Hz, 1H), 1.21 (t, J = 7.1 Hz, 3H), 1.14 (s, 3H), 0.95 (d, J = 7.1 Hz, 3H), 0.72 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.89, 171.07, 169.17, 166.57, 158.51, 138.65, 117.12, 74.42, 70.26, 60.27, 58.00, 54.49, 45.28, 44.42, 43.80, 41.69, 36.54, 35.88, 34.31, 30.23, 26.67, 26.31, 24.66, 23.89, 20.94, 16.62, 14.65, 14.06, 11.37. HRMS (ESI): $[M + H]^+$ calcd for $C_{30}H_{44}N_4O_5S_2$, 605.2826; found, 605.2818.

*14-O-[(2-(Morpholine-4-yl)-acetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**15e**).* Compound **15e** was prepared according to the general procedure from 14-*O*-[(2-chloroacetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**14**) and morpholine. The crude product was purified by silica gel column chromatography in 73% yield (1.36 g) as a white solid; mp: 69–71 °C. IR (KBr): 3438, 2927, 2892, 1731, 1519, 1454, 1403, 1295, 1190, 1152, 1116, 1015, 981, 954, 912, 897, 668 cm^{-1}. ^1H NMR (400 MHz, acetone) δ: 6.26 (dd, J = 17.7, 11.2 Hz, 1H), 5.72 (d, J = 8.4 Hz, 1H), 5.12 (ddd, J = 14.5, 12.5, 1.4 Hz, 2H), 4.12–4.03 (m, 2H), 3.74–3.67 (m, 4H), 3.59 (d, J = 6.0 Hz, 1H), 3.38 (s, 2H), 2.67–2.59 (m, 4H), 2.38 (s, 1H), 2.29–2.21 (m, 1H), 2.18–2.03 (m, 5H), 1.97 (s, 2H), 1.81–1.64 (m, 2H), 1.56 (ddd, J = 16.2, 10.3, 4.1 Hz, 2H), 1.47–1.35 (m, 5H), 1.36–1.27 (m, 1H), 1.20 (t, J = 7.1 Hz, 1H), 1.17–1.06 (m, 4H), 0.94 (d, J = 7.1 Hz, 3H), 0.71 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, acetone) δ: 216.16, 168.81, 166.46, 158.32, 158.10, 140.33, 115.46, 73.65, 70.65, 66.36, 60.98, 57.52, 53.54, 45.34, 44.18, 41.89, 36.66, 35.84, 33.95, 30.22, 27.75, 26.86, 24.65, 19.97, 19.66, 16.01, 14.31, 10.93. HRMS (ESI): $[M + H]^+$ calcd for $C_{30}H_{44}N_4O_6S_2$, 621.2775; found, 619.2770.

*14-O-[(2-(4-Methylpiperazine-1-yl)-acetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**15f**).* Compound **15f** was prepared according to the general procedure from 14-*O*-[(2-chloroacetamido-1,3,4-thiadiazole-5-yl)-thioacetyl]-mutilin (**14**) and 4-methylpiperazine. The crude product was purified by silica gel column chromatography in 79% yield (1.50 g) as a white solid; mp: 70–71 °C. IR (KBr): IR (KBr): 3435, 2935,

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1731, 1556, 1519, 1455, 1403, 1284, 1189, 1153, 1117, 1018, 981, 955, 916, 863, 665 cm^{-1}. 1H NMR (400 MHz, acetone) δ: 6.27 (dd, J = 17.7, 11.2 Hz, 1H), 5.74 (d, J = 8.4 Hz, 1H), 5.22−5.05 (m, 2H), 4.11 (d, J = 3.5 Hz, 1H), 4.06 (d, J = 7.1 Hz, 2H), 3.61 (s, 1H), 3.36 (s, 2H), 2.65 (s, 3H), 2.43 (d, J = 24.2 Hz, 4H), 2.30−2.16 (m, 5H), 2.14−2.05 (m, 6H), 1.98 (s, 4H), 1.83−1.66 (m, 2H), 1.56 (dt, J = 26.2, 10.6 Hz, 2H), 1.47−1.37 (m, 5H), 1.32 (d, J = 14.3 Hz, 1H), 1.21 (t, J = 7.1 Hz, 4H), 1.16−1.01 (m, 5H), 0.95 (d, J = 7.1 Hz, 3H), 0.72 (d, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 216.86, 168.43, 166.50, 158.58, 158.17, 138.66, 117.02, 74.33, 70.24, 60.48, 60.21, 57.89, 54.55, 53.30, 45.71, 45.23, 44.38, 43.77, 41.64, 36.49, 35.84, 34.27, 30.18, 26.63, 26.34, 24.63, 20.90, 16.59, 14.62, 14.03, 11.34. HRMS (ESI): $[M + H]^+$ calcd for $C_{31}H_{47}N_5O_5S_2$, 634.3091; found, 634.3087.

Pharmacology. *MIC Determination.* The MIC values of the synthesized compounds were determined using the agar dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS). A 12 800 μg sample of each synthesized compound was dissolved in approximately 5 mL of dimethyl sulfoxide (DMSO). Then, distilled water was added to the solution to bring the volume to 10 mL. Tiamulin fumarate and azamulin as reference drugs were dissolved in 10 mL of distilled water directly. All the obtained solutions were then diluted 2-fold with distilled water. A 2 mL volume of the 2-fold serial dilution of each test compound/drug was incorporated into 18 mL of hot Mueller−Hinton agar medium. Inoculums of MRSA, MRSE, *S. agalactiae*, and *E. coli*, which were all obtained from the clinic, were prepared from blood slants and adjusted to approximately 10^5−10^6 cfu/mL with saline solution (0.90% NaCl). A 10 μL amount of bacterial suspension was spotted onto Mueller−Hinton agar plates containing 2-fold serial dilutions of the compounds/drugs. The plates were incubated at 36.5 °C for 48 h. The MIC was defined as the minimum concentration of the compound needed to completely inhibit bacterial growth. The same procedure was repeated in triplicate.

Oxford Cup Assays. Inoculums were prepared in 0.9% saline using McFarland standard and spread uniformly on nutrient agar plates. All the compounds were prepared in the same manner used for the MIC test, and the resulting solutions (320 and 160 μg/mL) were added individually to the Oxford cups, which were placed at equal distances above the agar surfaces. The zone of inhibition for each concentration was measured after a 24 h incubation at 37 °C. The same procedure was repeated in triplicate.

Molecular Modeling. Because all the compounds share a common pleuromutilin scaffold, similarity-based molecular docking was introduced to analyze the different activities of compounds **11**, **12d**, **12e**, **12f**, **13a**, **13b**, **13c**, **15a**, **15b**, **15c**, and **15d**. The conformation of tiamulin in the X-ray crystal structure (PDB ID: 1XBP)[35] was used as a control to evaluate the accuracy of the docking performance. Homdock software in the Chil^2 package[34] was used for docking study. Briefly, the starting conformations of all compounds were obtained from alignment to tiamulin in the X-ray crystal stucture. Molecular superposition was carried out by a graph-based molecular alignment (GMA) method. Then the interaction between the receptor and compounds was optimized by a 200 steps gradient optimization to remove partial overlap. Optimization was performed by Glamdock, and the binding affinity was evaluated by Chil^2 Score. The receptor used for docking was extracted from the X-ray structure of a 50S ribosomal subunit (1XBP). The compounds were built with with Avogadro software,[40] with 5000 steps Steepest Descent and 1000 steps Conjugate Gradients optimization using MMFF94 force field.

Pharmacokinetic Studies. Intravenous and oral PK studies (n = 3) were performed with male SD rats (Lanzhou Veterinary Research Institute, CAAS, Lanzhou, China) weighing 220−250 g and approximately 6−8 weeks old. The rats were fasted overnight before use and for 6 h after dosing. Compounds **8** and **15c** were formulated as a solution of 5% Tween in water for iv administration at a dose of 5 mg/kg and for po at 15 mg/kg, respectively. Serial blood samples (0.5 mL) were collected via the retrobulbar vein at various time points after the compounds were administered and placed into EDTA−K_2-coated tubes. Then, the samples were centrifuged to yield plasma samples, and the concentrations of the compounds in plasma were determined by HPLC (for methods and equipment, see Supporting Information). The pharmacokinetic parameters were calculated from the mean plasma concentration by noncompartmental analysis.

X-ray Diffraction. The crystals of compound **12e** with dimensions of 0.31 mm × 0.28 mm × 0.26 mm was mounted on an Agilent SuperNova-CCD diffractometer equipped with a mirror-monochromatic Mo Kα (λ = 0.7107 Å) radiation at 296.35(10) K. The total of reflections including 4476 independent scans (R_{int} = 0.0250) were collected in the range of $3.07 \le \theta \le 26.37°$ by using an ω scan mode, of which 3825 with $I > 2\sigma(I)$ were observed and used in the succeeding refinements. The structure was refined by full-matrix least-squares techniques on F^2 with the SHELXL program.[41] The final refinement gave R = 0.0399, wR = 0.0841 ($w = 1/[\sigma^2 (F_0^2) + (0.0405P)^2 + 0.0182P]$, where $P = (F_0^2 + 2Fc^2)/3$), S = 1.033, $(\Delta\rho)_{max}$ = 0.179, and $(\Delta\rho)_{min}$ = −0.225 e/Å^3 (for more details and for hydrogen bond information, see Tables S1 and S2 in the Supporting Information). PLATON 1.17[42] was used for the molecular representations, and SHELXL[41] was used for the packing diagrams (for the molecular packing, see Figure S6 in the Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

The biological activities of various compounds containing a thiadiazole group, the correlations between the binding free energy and the antibacterial activity, the kinetic profiles in rat plasma, the data for hydrogen bonding and X-ray crystal structure information, and the characterizations of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

R.S. and Z.X. contributed to chemistry. J.L., X.P., C.Z., and E.G. carried out biological evaluation and pharmacokinetics studies. X.X. and Y.L. performed the molecular docking experiments.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

BOC, *tert*-butoxycarbonyl; DMSO, dimethyl sulfoxide; EDCI, 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride; FDA, Food and Drug Administration; GMA, graph-based molecular alignment; HOBt, 1-hydroxybenzotriazole; HRMS, high-resolution mass spectral; MC/SA, Monte Carlo/Simulated Annealing; MRSA, *Staphylococcus aureus*; MRSE, methicillin-resistant *Staphylococcus epidermidis*; MIC, minimum inhibitory concentration; NCCLS, National Committee for Clinical Laboratory Standards; QSAR, quantitative structure−activity relationships; PTC, peptidyl transferase center; RMSD, root-mean-square difference; SD, Sprague−Dawley; TFA, trifluoroacetic acid

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