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Design, synthesis, in vitro and in vivo evaluation against MRSA and molecular docking studies of novel pleuromutilin derivatives bearing 1, 3, 4-oxadiazole linker

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ABSTRACT

A class of pleuromutilin derivatives containing 1, 3, 4-oxadiazole were designed and synthesized as potential antibacterial agents against *Methicillin-resistant staphylococcus aureus* (MRSA). The ultrasound-assisted reaction was proposed as a green chemistry method to synthesize 1, 3, 4-oxadiazole derivatives (intermediates **85**–**110**). Among these pleuromutilin derivatives, compound **133** was found to be the strongest antibacterial derivative against MRSA (MIC = 0.125 μg/mL). Furthermore, the result of the time-kill curves displayed that compound **133** could inhibit the growth of MRSA *in vitro* quickly (- 4.36 log10 CFU/mL reduction). Then, compound **133** (- 1.82 $\log_{10}$ CFU/mL) displayed superior *in vivo* antibacterial efficacy than tiamulin (- 0.82 $\log_{10}$ CFU/mL) in reducing MRSA load in mice thigh model. Besides, compound **133** exhibited low cytotoxicity to RAW 264.7 cells. Molecular docking studies revealed that compound **133** was successfully localized in the binding pocket of 50S ribosomal subunit (ΔG_b = -10.50 kcal/mol). The results indicated that these pleuromutilin derivatives containing 1, 3, 4-oxadiazole might be further developed into novel antibiotics against MRSA.

1. Introduction

Methicillin-resistant staphylococcus aureus (MRSA) is one of the most important community-acquired (CA) and hospital-acquired (HA) pathogens [1]. MRSA caused severe skin and soft-tissue infections but on occasion causing life-threatening infections such as necrotizing pneumonia [2]. The annual cost of treating hospital acquired-MRSA was estimated to be $ 9.7 billion in the United States [3]. Vancomycin is one of the main drugs in the treatment for MRSA infection. However, it was reported that MRSA might acquire the vanA gene cluster during antibiotic therapy and became resistant to vancomycin [2]. The prevalence of MRSA has created a critical demand for the development of novel antimicrobial agents [4].

Pleuromutilin, a natural product, was isolated from *Pleurotus mutilus* and *Pleurotus passeckerianus* in 1951 [5]. It is composed of the tricyclic core (five-, six- and eight-membered rings), a C14 extension and a C21 keto group [6]. The pleuromutilin exhibited especially antibacterial potent towards Gram-positive bacteria and *Mycoplasma*. Pleuromutilin exhibited antibacterial activity through binding to the V domain of the peptidyl transferase center (PTC) of bacterial 50S ribosomal subunit [7,8]. For the unique mechanism, pleuromutilin rarely exhibit cross-resistance with other antibiotics. Thus, pleuromutilin has aroused considerable research. Most modifications focus on the C14 side chain of pleuromutilin [9]. These modifications have led to the approvement of tiamulin (2, Fig. 1) and valnemulin (3, Fig. 1) which were authorized as veterinary medicines in 1979 and 1999, respectively [10]. As the first pleuromutilin drug used in human medicine, retapamulin (4, Fig. 1) has been approved by the U.S. Food and Drug Administration (FDA) [11]. Lefamulin (5, Fig. 1) was approved by the FDA for treatment of community-acquired bacterial pneumonia (CABP) in humans in 2019 [12]. Success of lefamulin attracted researchers to further focus the development of pleuromutilin drugs.

Previous work in our laboratory has led to several semisynthetic pleuromutilin derivatives with potent antimicrobial activity against

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MRSA [13]. One of these pleuromutilin derivatives, with five-membered heterocyclic such as 1,2,3-triazole group in the C14 side chain [13]. The series of compounds possessed superior *in vivo* efficacy to that of tiamulin in MRSA thigh infection model. 1, 3, 4-Oxadiazole is a heterocyclic compound containing an oxygen atom and two nitrogen atoms in the five-membered ring. Owing to the π-conjugated system, hydrogen-bonding capacity, and rigidity, 1, 3, 4-oxadiazole possesses high affinity and acts as important pharmacophores by interacting with the biological receptors [14,15]. The antibacterial activity of norfloxacin against MRSA could be increased through the introduction of 1, 3, 4-oxadiazole and the target compound (6, Fig. 1) also displayed very low cytotoxicity [16]. Thus, we speculated that the introduction of 1, 3, 4-oxadiazole to C14 side chain of pleuromutilin might produce new derivatives with enhanced antibacterial activity compared to the prototype pleuromutilin.

In this study, a novel process for the synthesis of 5-substituted 1, 3, 4-oxadiazol-2-thiol derivatives was proposed via ultrasound-assisted reaction in the absence of basic or acidic. It is aligned with the principles of green chemistry. Then, 26 pleuromutilin derivatives were designed, synthesized and evaluated for their *in vitro* and *in vivo* antibacterial activities against four strains including MRSA.

2. Results and discussion

2.1. Chemistry

The synthesis methods of all 1, 3, 4-oxadiazole derivatives and pleuromutilin derivatives were displayed in Scheme 1. All the syntheses of pleuromutilin derivatives begun with 22-O-tosylpleuromutilin (compound **111**), which was obtained by the reaction of pleuromutilin and p-toluenesulfonyl chloride [17].

As illustrated in Scheme 1, the intermediates **85**–**110** were prepared in 3 steps starting from carboxylic acid derivatives (compounds **33**–**58**). First, different carboxylic acid reacted with EtOH to get the ethyl carboxylate (compounds **33**–**58**), respectively. These esters were hydrazinolysised in EtOH to obtain carboxyhydrazide (compounds **59**–**84**) under reflux. The compounds **85**–**110** were synthesized in the absence of basic or acidic via ultrasound-assisted reaction. Compounds **85**–**110** were produced in good to excellent yields under easy workup and purification conditions [18]. The target compounds **112**–**137** were prepared through reaction of the compounds **85**–**110** with compound **111** by a nucleophilic substitution reaction, respectively. Structures of starting materials (compounds 7–32) and corresponding intermediates (compounds 33–58, 59–84, 85–110) were disclosed in **Table SI 1**.

All pleuromutilin derivatives were purified by silica column chromatography. The structures of synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and high-resolution mass spectral (HRMS) analysis. All the spectra of synthesized pleuromutilin derivatives are supplied in the Supporting Information.

2.2. In vitro antibacterial activity

The *in vitro* antibacterial activity of these pleuromutilin derivatives containing 1, 3, 4-oxadiazole was evaluated against MRSA (ATCC 43300) and three *S. aureus* strains in accordance with the Clinical and Laboratory Standards Institute (CLSI)[19]. The results were displayed as the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) in Table 1. The pleuromutilin and tiamulin were used as the positive control drugs.

As illustrated in Table 1, most target compounds displayed stronger antibacterial activity than pleuromutilin or tiamulin against MRSA and *S. aureus*. The MIC values of 11 compounds are 0.125 μg/mL against MRSA. The MBC/MIC ratios of most target compounds against MRSA were ≤4. According to previous study, the antimicrobial drug could be considered bacteriostatic when the MBC/MIC is ≥4, while being bactericidal when the MBC/MIC is ≤4 [20]. Thus, the pleuromutilin derivatives containing 1, 3, 4-oxadiazole displayed potent and stable bactericidal effect.

Meanwhile, the preliminary structure–activity relationships (SARs) study was also explored. Among compounds **113**–**137**, all the compounds showed better antibacterial activity than compound **112**. Consistent with previous research [21], the results suggested that derivatives containing aromatic rings showed stronger antibacterial activity. The reason might be related to the exaltation of the binding ability between the C14 side and the hydrophobic portion of ribosomal subunits by aromatic rings. Then different electron-withdrawing and electron-donating groups were introduced on the benzene ring. When the hydrogen atom on the benzene ring was substituted by hydroxyl or nitro group, the compounds **120**–**122** were obtained. Their MIC values against MRSA were 0.125 μg/mL. Overall, the stronger electron-withdrawing or electron-donating ability of introduced groups, the better antibacterial activity of compounds. Increasing electron cloud density might change the physical and chemical properties of the compound molecules, which influenced pharmacological properties of target compound [22]. When the benzene ring on the 1, 3, 4-oxadiazole was substituted by thiophene group, the corresponding compound **133**

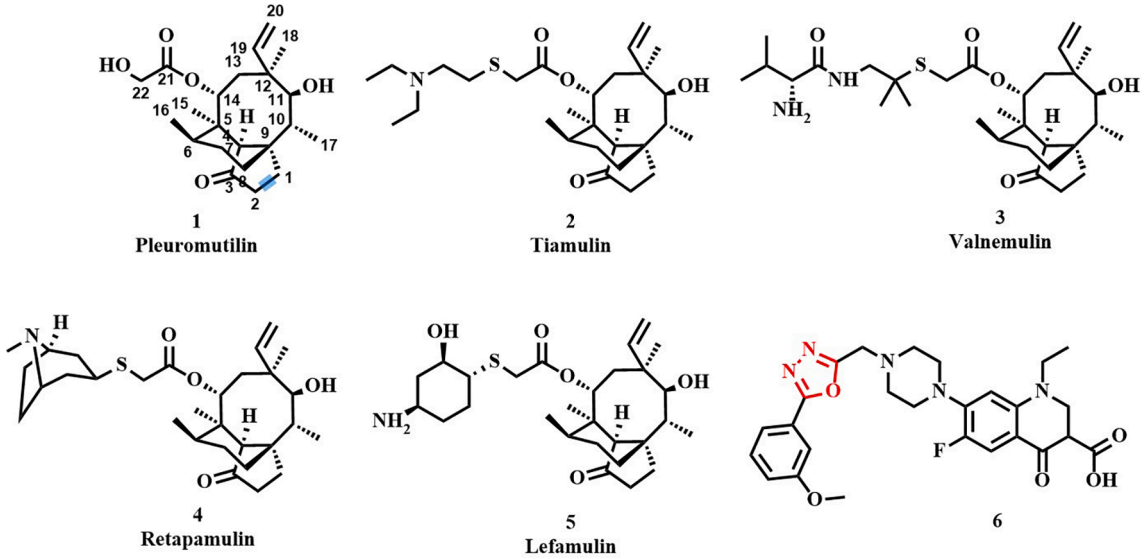
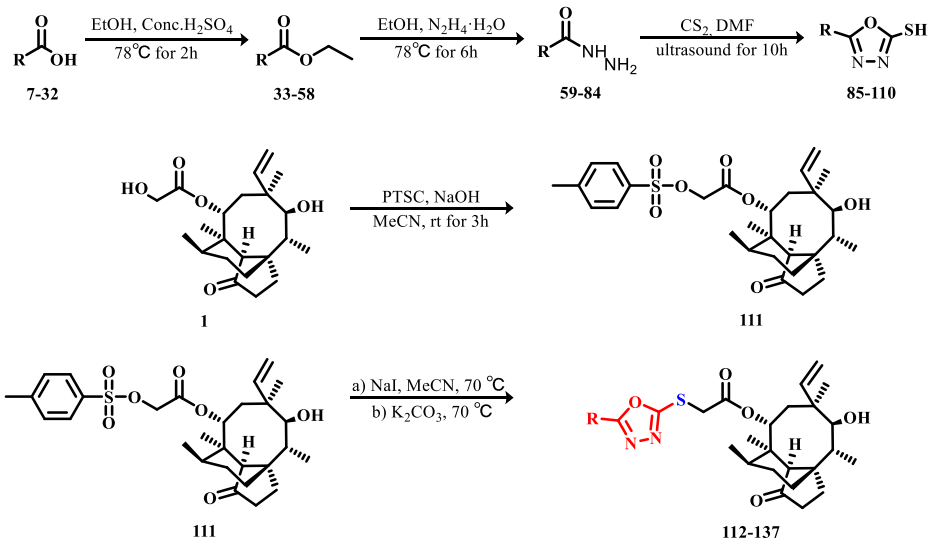


Fig. 1. Structure of pleuromutilin (***1***), tiamulin (***2***), valnemulin (***3***), retapamulin (***4***), lefamulin (***5***) and norfloxacin (***6***).



Scheme 1. Reagent and conditions: (i) ethanol, Concentrated sulfuric acid, 78 ℃, 2 h; (ii) ethanol, hydrazine,hydrate (1:1), 78 ℃, 6 h; (iii) DMF, CS_2, ultrasound, 10 h; (iv) acetonitrile, *p*-toluenesulfonyl chloride, NaOH, rt, 3 h; (v) a). acetonitrile, NaI, 70 ℃, 1.5 h; b). acetonitrile, K_2CO_3, 70 ℃, 3 h.

showed the most potent anti-MRSA activity among compounds **112**–**133**. The results might be related to the higher electron cloud density of the thiophene ring than that of the benzene ring.

The time-kill kinetics assay was used to analyze the antibacterial activity of compound **118** and compound **133** against MRSA. The results are displayed in Fig. 2**.**

In a concentration of 2 × MIC, compounds **118** and **133** had a significant inhibitory effect on MRSA. Compound **133** at 2 × MIC had induced killing of MRSA (- 1.28 $\log_{10}$ CFU/mL reduction) after 3 h of incubation, and conspicuous bacterial growth inhibition was observed at 24 h (- 4.36 $\log_{10}$ CFU/mL reduction). 99.9% of MRSA was killed by 2 × MIC compound **133** at 24 h. In contrast, some colonies were also detected after incubation for 24 h in the 4 × MIC tiamulin (the total bacterial − 1.90 $\log_{10}$ CFU/mL) [22]. The further increases in the concentration of the compound **133** to 4 × MIC did not further increase the antibacterial effect. According to time/concentration-killing bacteria relationships, compounds **118** and **133** can be divided into time-dependent groups, with a bactericidal effect at concentrations higher than 2 × MIC.

PAE tests were conducted for compounds **118** and **133** to explore the potential of the measured compounds for continuous inhibition of bacteria and provide a more rational dosing regimen. The bacterial growth kinetics curve was displayed in Fig. 3 and the results of the PAEs are tabulated in Table 2. Following exposure to 2 × MIC and 4 × MIC for 2 h, the PAE of compound **118** was respectively 3.66 and 4.01 h, and the PAE at the same concentrations of compound **133** was 3.42 and 3.74 h. Correlatively, the PAE at the same concentrations of tiamulin was 1.65 and 2.04 h. The results indicated that the administration interval of compounds **118** and **133** might be longer than that of tiamulin. The results are displayed in Fig. 3.

2.3. *In vivo* antibacterial activity

Due to excellent *in vitro* antibacterial activity, the *in vivo* antibacterial activity of compound **133** was further assessed by neutropenic murine thigh infection model. The infection model was established by injecting MRSA solution about 10^7 CFU/mL into the thigh muscle of neutropenic mice. The mice were randomly divided into three groups and intravenously injected sterile normal saline (as a negative control), compound **133** (20 mg/kg) and tiamulin (20 mg/kg), respectively. The results are indicated in Fig. 4.

Compared to the negative control group, the positive control of tiamulin at 20 mg/kg could reduce MRSA load (- 0.85 $\log_{10}$ CFU/mL) against MRSA in thigh muscle ($P < 0.01$, n = 6/group), and the experimental group with compound **133** at 20 mg/kg performed a treatment effect (−1.82 $\log_{10}$ CFU/mL) in thigh ($P < 0.01$, n = 6/group). Comparing the clearance of bacteria in thigh muscle, compound **133** possessed more efficient antibacterial activity than tiamulin in reduce the MRSA load of thigh infected mice. It suggested compound **133** could do therapy MRSA infection *in vivo* initially.

2.4. *Cytotoxicity assay*

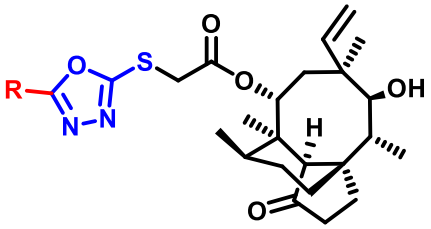
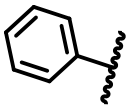
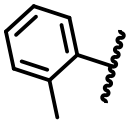
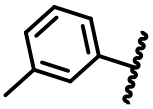
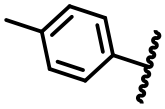
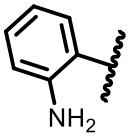
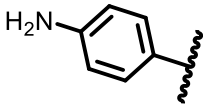
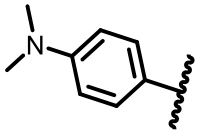
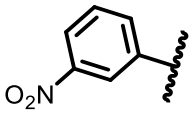
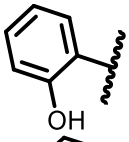
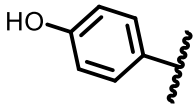
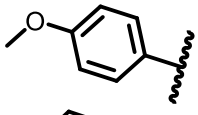
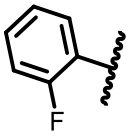
The presence of compounds can affect cellular basic physiological processes, inhibited proliferation, even reduce cell survival, etc. Macrophage generally exhibited microbicidal activity and was an important line of defense against invading microorganism [23]. Therefore, cytotoxicity was evaluated in order to explore the effect of compounds **112**–**133** on the macrophage viability [24]. The cytotoxicity of compounds **112**–**133** to RAW 264.7 cells was evaluated by MTT assay [25,26]. Most compounds displayed slight inhibition of the viability of RAW 264.7 cells at the concentration of 8.0 μg/mL and the result was shown in Figure SI 27. The cytotoxicity of compounds **118**, **133** and tiamulin was measured by MTT assay within different concentrations (Fig. 5). The cytotoxicity of compound was usually expressed as the IC_{50} value [27] (the concentration at which 50% of the cells are viable). The IC_{50} values for compounds **118**, **133** were calculated to be 16.41 μg/mL and 17.88 μg/mL, which were higher than that of tiamulin with 9.684 μg/mL, respectively. The present study indicated that the compounds **118**, **133** were less cytotoxic than tiamulin towards RAW 264.7 cells in our system. The safety profiles of compounds **118**, **133** were more reliable than that of tiamulin.

2.5. *Molecular docking study*

Based on the above theoretical and experimental findings, molecular docking experiments were carried out to predict the possible binding mode of compound **133** to 50S ribosomes (PDB: 1XBP) [7]. Through Autodock software, the redocking of tiamulin into 1XBP placed the ligand in the same conformation as that in the X-ray structure (RMSD = 0.781). The results for compound **133** present a similar binding mode to tiamulin and are displayed in Fig. 6a, which presented a superimposition of 2 kinds of docked compounds.

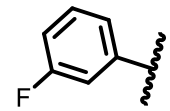
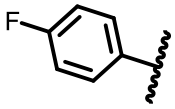
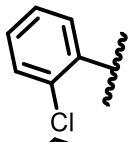
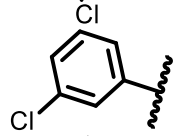
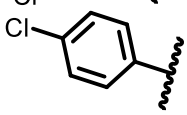
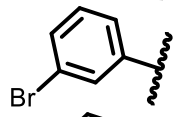
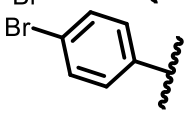
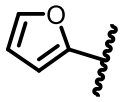
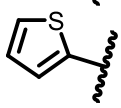
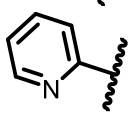
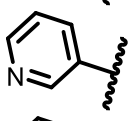
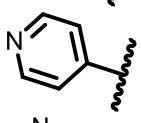
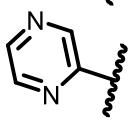
The binding free energy of compound **133** with the 50S ribosome was calculated to be −10.5 kcal/mol. As shown in Fig. 6b, four hydrogen bonds played an important part in the binding of compound **133** to 50S

Table 1
MIC and MBC (μg/mL) values of compounds 112–137, pleuromulin and tiamulin against *S. aureus* (ATCC 43300), *S. aureus* (ATCC 29213), *S. aureus* (AD3) and *S. aureus* (144).

Compound No	R	MIC/MBC(μg/mL)			
		MRSA ATCC 43300	*S. aureus* ATCC 29213	*S. aureus* AD3	*S. aureus* 144
					
112	**-H**	16/16	8/16	16/>16	16/>16
113		0.25/1	1/1	2/8	16/>16
114		1/1	16/16	16/16	16/>16
115		0.125/0.25	0.5/2	1/8	4/16
116		0.5/1	8/16	16/16	16/>16
117		0.25/0.25	1/2	1/4	8/16
118		0.125/0.125	0.25/1	0.125/0.5	0.25/2
119		0.125/0.5	0.5/2	0.5/8	2/4
120		0.125/0.125	0.5/1	0.5/4	8/16
121		0.125/0.5	0.5/2	0.5/4	8/16
122		0.125/0.125	0.25/1	0.125/1	0.5/2
123		0.25/0.5	0.5/2	0.5/4	8/16
124		0.125/0.25	0.5/2	0.5/8	2/4

(*continued on next page*)

Table 1 (*continued*)

Compound No	R	MIC/MBC(μg/mL)			
		MRSA ATCC 43300	*S. aureus* ATCC 29213	*S. aureus* AD3	*S. aureus* 144
125		0.125/0.25	0.25/2	0.25/2	1/2
126		1/4	8/16	8/16	8/16
127		16/>16	16/16	16/>16	16/>16
128		0.25/1	16/16	16/16	16/16
129		16/>16	>16/>16	>16/>16	>16/>16
130		0.25/0.5	16/>16	8/16	16/>16
131		>16/>16	8/16	4/8	16/>16
132		0.125/0.25	0.25/1	0.25/2	0.25/0.5
133		0.125/0.125	0.5/2	0.5/1	1/2
134		0.25/0.5	0.5/1	0.5/4	1/4
135		2/4	8/16	8/16	16/>16
136		0.125/0.25	0.5/2	0.5/2	0.5/2
137		0.25/0.25	0.5/2	1/1	1/4
Pleuromutilin	/	2/4	2/4	2/4	2/4
Tiamulin	/	0.5/1	1/1	1/2	1/2

ribosome. A hydrogen bond (distance: 3.3 Å) was formed between the ketone on the C3 atom of compound **133** (pleuromutilin core) and the residue of U2485. A hydrogen bond (distance: 2.6 Å) was constituted by the hydroxyl group on the C11 atom of compound **133** (pleuromutilin core) and the residue of G2484. A hydrogen bond (distance: 3.4 Å) was formed between the ketone on the C21 core and the residue of G2044. The other hydrogen bond was constituted by 1, 3, 4-oxadiazole of compound **133** and the residues of G2484 (distance: 3.4 Å). The results suggested that substituted oxadiazole rings might be acted as hydrogen bond acceptors forming hydrogen bonds with 50S ribosome residue to improve antibacterial activity [28]. The interaction of compound **133** with 50S ribosome should be further studied in our future work.

3. Conclusions

In summary, a series of novel pleuromutilin derivatives containing 1, 3, 4-oxadiazole have been prepared and evaluated for their antimicrobial effects against MRSA and *S. aureus*. The results indicated that

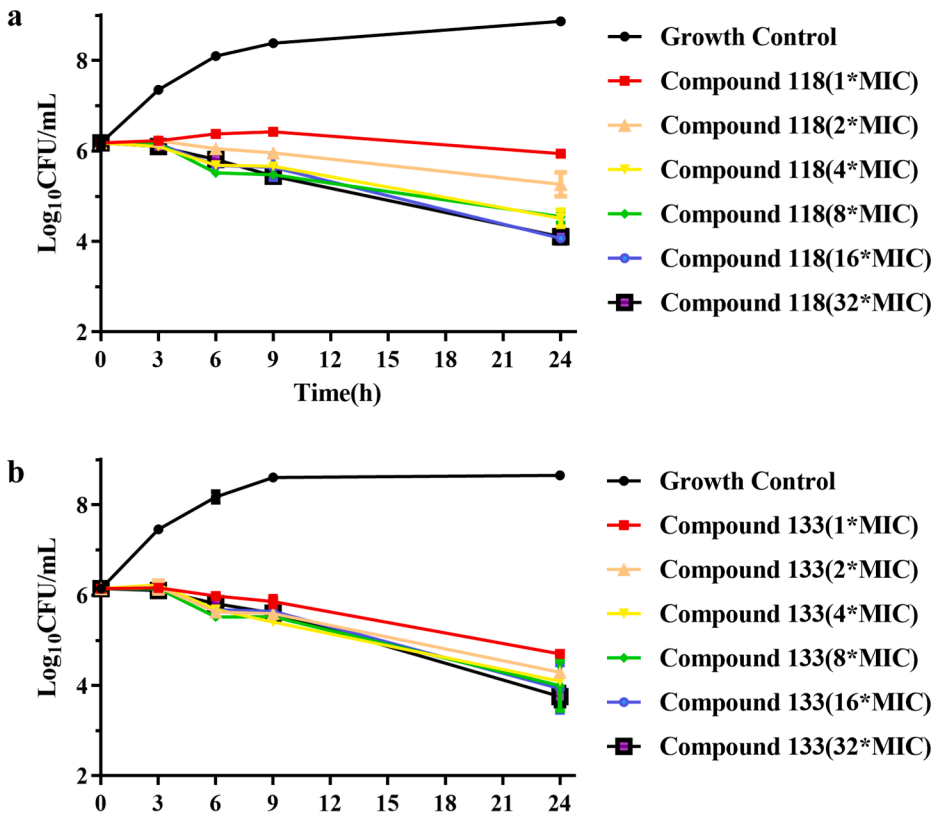


Fig. 2. Time-kill curves for MRSA ATCC 43300 with different concentrations of tiamulin and compounds ***118*** (a) and ***133*** (b).

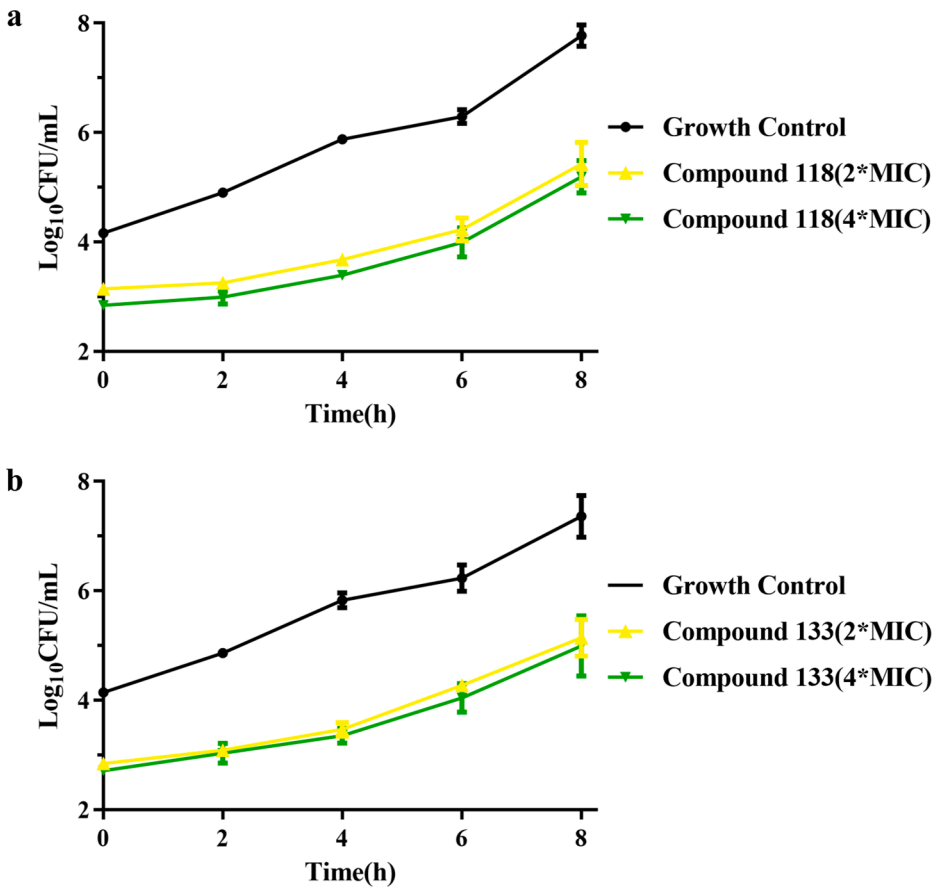


Fig. 3. The bacterial growth kinetic curves for MRSA ATCC 43300 exposed to compound ***118*** (a) and ***133*** (b) for 2 h.

Table 2
The PAEs values of compounds 118, 133 and tiamulin against MRSA ATCC 43300.

Compounds	Concentrations	PAE (h) Exposure for 2 h
Compound **118**	2 × MIC	3.66
	4 × MIC	4.01
Compound **133**	2 × MIC	3.42
	4 × MIC	3.74
Tiamulin	2 × MIC	1.65
	4 × MIC	2.04

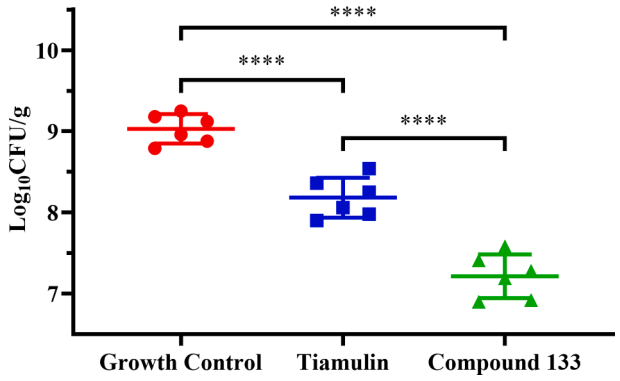


Fig. 4. Efficacy of tiamulin and compound ***133*** against MRSA ATCC 43300 in murine neutropenic thigh models: circular: growth control; square: tiamulin (20 mg/kg); triangle: compound ***133*** (20 mg/kg).

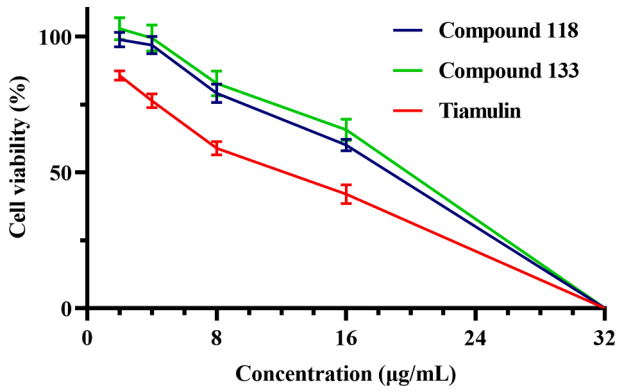


Fig. 5. The cytotoxicity of compounds ***118***, ***133*** and tiamulin against RAW 264.7 cells was determined using different concentrations of compound113 and tiamulin.

compounds **118** and **133** displayed an excellent antimicrobial effect against MRSA. The results of the time-killing curve indicated that compounds **118** and **133** manifested a more rapid bactericidal kinetic effect on MRSA than tiamulin. Compound **133** displayed a longer PAE time than tiamulin against MRSA. Compound **133** also exhibited a potent *in vivo* antibacterial effect than tiamulin in MRSA infection mice thigh models. Four strong hydrogen bonds were formed through the interaction of compound **133** with residues. In addition, compound **133** displayed lower cytotoxicity than tiamulin. Compound **133** could be served as a possible lead compound for the development of antibacterial drugs and is worthy of further clinical trials for the systematic treatment of infection.

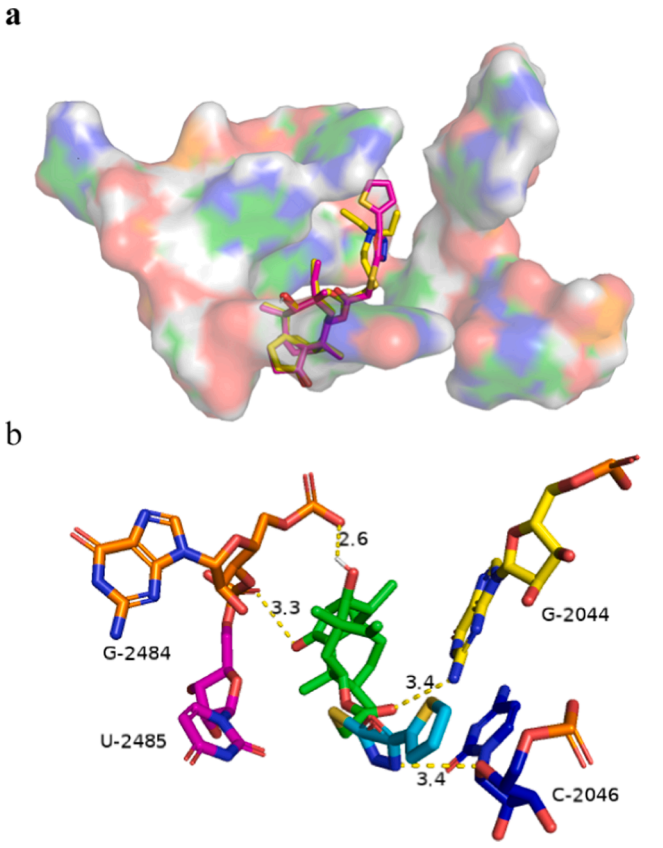


Fig. 6. (**a**) Docking mode of tiamulin (purple) and compound **133** (yellow) to 1XBP. (**b**) 3D representation of docking poses for the compound **133** in the 50S ribosome residues. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Experimental

4.1. Materials

Pleuromutilin (>90% pure) was purchased from Great Enjoyhood Biochemical Co. Ltd., (Daying, China). The carboxylic acids were purchased from TiTan Technology Co. Ltd., (Shanghai, China). The other analytical grade solvents were purchased from Guangzhou General Reagent Factory (Guangzhou, China). The target compounds were purified by silica gel column chromatography (200–300 mesh, Branch of Qingdao Haiyang Chemical Co. Ltd., Shandong, China). ^{1}H NMR and ^{13}C NMR spectra were processed with Bruker AV-600 spectrometer in chloroform-*d* using trimethylsilane as an internal standard. Chemical shift values (δ) were reported in ppm. High-resolution mass spectra were recorded by Thermo Scientific Q Exactive Focus Orbitrap LC-MS/MS with an electrospray ionization (ESI) source. Ultrasonic irradiation was supplied by Backer vCLEAN1-L03 (35 kHz frequency and 50 W output power).

4.2. Synthesis

A general synthetic route based on the compound 22-O-tosylpleuromutilin (compound **111**) and a variety of 2-mercapto-1, 3, 4-oxadiazole derivatives (compound **85**–**110**) were used to prepare pleuromutilin derivatives (Scheme 1).

The starting materials ethyl carboxylates (compound **33**–**58**), carboxyhydrazides (compound **59**–**84**), 22-O-tosylpleuromutilin (compound **111**) were prepared according to the literature.

4.2.1. General procedure for the synthesis of 2-mercapto-1, 3, 4-oxadiazole derivatives (compounds 85–110)

Carbon disulfide (50 mmol 4.7 mL) was added dropwise to the suspension including carboxyhydrazides (compound **59**–**84**, 50 mmol) in DMF (5 mL) processed to an ultrasonic bath for 10 h. Hydrogen sulfide emission was treated with a saturated solution of sodium bicarbonate. The reaction mixture was added to 20 g crushed ice containing excess of salt. The solid phase was filtered with Buchner funnel, washed with water, and dried to obtain compound **85**–**110**, respectively.

4.2.2. 22-[(1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 112)

Compound **6** (1 g, 2.16 mmol) was dissolved in acetonitrile (10 mL) and sodium iodide (0.35 g, 2.37 mmol) was added and stirred at 70 ℃ for 0.5 h. 2-mercapto-1, 3, 4-oxadiazole (0.24 g, 2.37 mmol) and potassium carbonate (0.60 g, 4.32 mmol) were added to the mixture and stirred again for 2 h at 70 ℃. Chloroform (30 mL) was added, and the mixture was then washed with water (30 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give the crude product. The crude product was concentrated in vacuo to remove the trifluoroacetic acid and yield the product, which was purified by column chromatography (dichloromethane: methanol = 70: 1) using silica gel to give the desired compound.

White powder; yield: 58%; ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 6.41 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.77 (1H, d, *J* = 8.5 Hz), 5.31 – 5.30 (1H, m, H14), 5.20 – 5.17 (1H, m, H20), 4.08 – 3.96 (2H, m, H20, H22), 3.36 (1H, dd, *J* = 10.4, 6.6 Hz, *H*11), 2.32 (1H, q, *J* = 6.9 Hz, H2), 2.26 (1H, dd, *J* = 19.5, 11.0 Hz, H4), 2.20 (1H, q, *J* = 9.7 Hz), 2.10 (1H, s, *H*10), 2.06 (1H, dd, *J* = 16.0, 8.6 Hz, H13), 1.77 (1H, dd, *J* = 14.4, 2.5 Hz, H6), 1.68 (1H, s, H8), 1.67 – 1.62 (2H, m, 11-OH), 1.58 – 1.52 (1H, m), 1.49 (1H, q, *J* = 4.0 Hz), 1.46 (1H, d, *J* = 6.6 Hz, H1, H7), 1.44 (3H, s, H13), 1.39 (1H, s), 1.36 (1H, s, H15), 1.18 (3H, s, H8), 1.13 (1H, td, *J* = 14.1, 4.3 Hz, H18), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.73 (3H, d, *J* = 7.1 Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) *δ* 216.76(C3), 166.38 (C21), 163.63, 138.809(C19), 117.24(C20), 74.62(C11), 70.65(C14), 58.08(C4), 53.41(C22), 45.42(C9), 44.59(C13), 43.98(C5), 41.86, 36.69, 36.26(C6), 36.02(C10), 34.42(*C*2), 30.39(C8), 26.84(C7), 26.44 (C18), 24.82(C1), 16.75(C16), 14.82(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{24}H_{34}N_2O_5S$ ($M+K^+$): 501.1820; Found: 501.1842.

4.2.3. 22-[(phenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 113)

White powder; yield: 72%; ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 7.53 – 7.49 (3H, m), 6.43 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.79 (1H, d, *J* = 8.5 Hz, H14), 5.33 – 5.30 (1H, m, H20), 5.19 (1H, dd, *J* = 17.4, 1.5 Hz, H20), 4.05 (2H, d, *J* = 3.0 Hz), 3.36 (1H, d, *J* = 6.2 Hz, H22), 2.34 – 2.29 (1H, m, H2), 2.26 – 2.23 (1H, m, H4), 2.20 (1H, q, *J* = 9.7 Hz, *H*10), 2.10 (1H, s, H13), 1.79 – 1.76 (1H, m, H6), 1.68 (2H, ddd, *J* = 9.6, 7.1, 3.5, H8), 1.66 – 1.62 (1H, m, 11-OH), 1.56 – 1.50 (2H, m, H1, H7), 1.49 – 1.46 (1H, m, H13), 1.45 (3H, s), 1.39 (1H, d, *J* = 3.1 Hz, H15), 1.37 (1H, d, *J* = 5.2 Hz, H8), 1.16 (3H, s), 1.13 (1H, dd, *J* = 14.2, 9.8 Hz, H18), 0.88 (3H, d, *J* = 7.1 Hz, H17), 0.76 (3H, d, *J* = 7.1 Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) *δ* 216.69(C3), 166.09(C21), 166.05, 162.72, 138.70(C19), 131.77, 129.06, 126.71, 123.47, 117.35(C20), 109.11, 74.59(C11), 70.91(C14), 58.06(C4), 45.42(C9), 44.64(C13), 44.00 (C12), 41.90(C5), 36.66(C6), 36.03(C10), 35.08, 34.41(*C*2), 30.39(C8), 26.83(C7), 26.40(C18), 24.82(C1), 23.38, 16.76(C16), 14.77(C15), 11.44.(C17); HR-MS (ESI): Calcd for $C_{30}H_{38}N_2O_5S$ ($M+Na^+$): 561.2393; Found: 561.2381.

4.2.4. 22-[(2-Methylphenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 114)

White powder; yield: 74%; ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 7.42 (2H, td, *J* = 7.6, 1.2 Hz), 7.32 (2H, dd, *J* = 13.8, 6.4 Hz), 6.43 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.80 (1H, d, *J* = 8.5 Hz, H14), 5.32 – 5.30 (1H, m, H20), 5.18 (1H, dd, *J* = 17.4, 1.3 Hz, H20), 4.11 – 4.01 (2H, m), 2.68 (3H, s, H22, *H*11), 2.32 (2H, td, *J* = 12.8, 12.0, 5.9 Hz, H2, H4), 2.25 (1H, d, *J* = 9.6 Hz, *H*10), 2.19 (1H, dd, *J* = 19.4, 9.4 Hz), 2.12 – 2.07 (2H, m, H13), 1.77 (2H, dd, *J* = 14.5, 3.0 Hz, H6, H8), 1.67 (1H, d, *J* = 3.2 Hz, H1), 1.65 – 1.64 (1H, m, H7), 1.54 (1H, s), 1.48 (1H, s), 1.45 (3H, s, H13), 1.39 (1H, d, *J* = 7.0 Hz, H15), 1.17 (3H, s, H8), 1.14 – 1.11 (1H, m, H18), 0.89 (3H, d, *J* = 7.0, H17), 0.76 (3H, d, *J* = 7.1, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) *δ* 216.69(C3), 166.26(C21), 166.14, 162.32, 138.72(C19), 138.33, 131.73, 131.27, 128.75, 126.15, 122.54, 117.33 (C20), 74.60(C11), 70.89(C14), 58.07(C4), 45.42(C9), 44.63(C13), 44.00(C12), 41.90(C5), 36.67(C6), 36.03(C10), 35.02, 34.41(*C*2), 30.39 (C8), 26.84(C7), 26.39(C18), 24.82(C1), 22.01, 16.76(C16), 14.78 (C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{31}H_{40}N_2O_5S$ ($M+H^+$): 553.2736; Found: 553.2721.

4.2.5. 22-[(3-Methylphenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 115)

White powder; yield: 72%; ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 7.83 – 7.78 (2H, m), 7.39 – 7.34 (2H, m), 6.43 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.79 (1H, d, *J* = 8.5 Hz, H14), 5.34 – 5.30 (1H, m, H20), 5.18 (1H, dd, *J* = 17.4, 1.5 Hz, H20), 4.05 (2H, d, *J* = 2.5 Hz, H22), 3.38 – 3.34 (1H, m, *H*11), 2.43 (3H, s), 2.32 (1H, t, *J* = 6.9 Hz, H2), 2.26 – 2.23 (1H, m, H4), 2.18 (1H, dd, *J* = 19.5, 9.4 Hz, *H*10), 2.09 (2H, d, *J* = 10.8 Hz, H13, 11-OH), 1.79 – 1.76 (1H, m, H6), 1.73 (1H, s, H8), 1.71 – 1.66 (2H, m), 1.66 – 1.63 (1H, m, H1), 1.54 (1H, dd, *J* = 13.4, 2.9 Hz, H7), 1.50 (1H, d, *J* = 10.5 Hz, H13), 1.45 (3H, s), 1.39 (1H, d, *J* = 3.1 Hz, H15), 1.37 (1H, d, *J* = 5.1 Hz, H18), 1.16 (3H, s, H18), 0.88 (3H, d, *J* = 7.1, H17), 0.76 (3H, d, *J* = 7.1, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) *δ* 216.2(C3), 166.21(C21), 166.11, 162.57, 138.96(C19), 138.71, 132.60, 128.95, 127.20, 123.87, 123.32, 117.34(C20), 74.60(C14), 70.90(C14), 58.07(C4), 45.42(C9), 44.64(C13), 44.00(C12), 41.90(C5), 36.66(C6), 36.02(C10), 35.07, 34.41(*C*2), 30.39(C8), 26.83(C7), 26.41(C18), 24.82 (C1), 21.30, 16.75(C16), 14.77(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{31}H_{40}N_2O_5S$ ($M+H^+$): 553.2736; Found: 553.2721.

4.2.6. 22-[(4-Methylphenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 116)

White powder; yield: 71%; ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 7.87 (1H, d, *J* = 1.6 Hz), 7.30 (1H, d, *J* = 0.6 Hz), 7.29 (1H, s), 6.42 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.78 (1H, d, *J* = 8.5 Hz, H14), 5.32 – 5.30 (1H, m, H20), 5.18 (1H, dd, *J* = 17.4, 1.5 Hz, H20), 4.10 – 3.97 (2H, m), 3.37 – 3.34 (1H, m, H22), 2.42 (1H, s, H2), 2.34 – 2.28 (1H, m, H4), 2.24 (1H, dd, *J* = 11.1, 1.4 Hz, *H*10), 2.18 (1H, dd, *J* = 19.5, 9.4, H13), 2.10 (1H, s), 2.09 – 2.04 (1H, m,11-OH), 1.79 – 1.76 (1H, m, H6), 1.76 (1H, d, *J* = 2.6 Hz, H8), 1.69 – 1.61 (3H, m, H1, H7), 1.57 – 1.52 (1H, m), 1.51 (1H, d, *J* = 10.5 Hz, H13), 1.46 (1H, dd, *J* = 9.9, 3.1 Hz), 1.44 (3H, s), 1.39 (1H, d, *J* = 3.5 Hz, H15), 1.36 (1H, d, *J* = 5.5 Hz, H8), 1.16 (3H, s), 1.15 – 1.09 (1H, m, H18), 0.88 (3H, d, *J* = 7.1 Hz, H17), 0.75 (3H, d, *J* = 7.1 Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) *δ* 216.72(C3), 166.21 (C21), 166.13, 162.30, 142.36, 138.71(C19), 129.75, 126.67, 120.68, 117.33(C20), 74.59(C11), 70.88(C14), 58.07(C4), 45.42(C9), 44.64 (C13), 44.00(C12), 41.90(C5), 38.02, 36.66(C6), 36.02(C9), 35.09 (C10), 34.41(*C*2), 30.39(C8), 29.17, 26.83(C7), 26.41(C18), 24.81(C1), 21.61, 16.75(C16), 14.77(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{31}H_{40}N_2O_5S$ ($M+H^+$): 553.2736; Found: 553.2720.

4.2.7. 22-[(2-aminophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 117)

White powder; yield: 74%; ^{1}H NMR (600 MHz, Chloroform-*d*) *δ* 7.27 – 7.25 (1H, m), 6.81 – 6.73 (3H, m), 6.43 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.80 (1H, d, *J* = 8.5 Hz, H14), 5.34 – 5.31 (1H, m, H20), 5.20 – 5.17 (1H, m, H20), 4.08 – 3.99 (2H, m), 3.36 (1H, d, *J* = 6.3 Hz, H22), 2.33 (1H, q, *J* = 6.9 Hz, H2), 2.19 (1H, dd, *J* = 19.5, 9.4 Hz, H4), 2.09 (2H, d, *J* = 11.9 Hz, *H*10, H13), 1.79 – 1.76 (1H, m, H6), 1.69 – 1.67 (1H, m, H8), 1.67 – 1.66 (1H, m), 1.65 – 1.61 (1H, m, H1), 1.57 (1H, dd, *J* = 14.1, 3.5 Hz), 1.54 – 1.51 (1H, m, H7), 1.47 (1H, d, *J* = 6.9 Hz), 1.45 (3H, s, H13), 1.39 (2H, d, *J* = 3.4 Hz, H15), 1.37 (1H, d, *J* = 5.5 Hz, H8),

1.16 (3H, s), 1.12 (1H, dd, $J = 14.1$, 4.4 Hz, H18), 0.88 (3H, d, $J = 7.1$ Hz, H17), 0.76 (3H, d, $J = 7.1$ Hz, H16) . ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.70(C3), 166.12(C21), 166.01, 161.03, 146.73, 138.68 (C19), 132.57, 127.72, 117.36(C20), 116.85, 116.14, 105.30, 74.60 (C11), 70.88(C14), 58.07(C4), 45.42(C9), 44.64(C13), 43.99(C12), 41.90(C5), 36.67(C6), 36.03(C10), 35.03, 34.41(*C*2), 30.39(C8), 26.84 (C7), 26.39(C18), 24.82(C1), 16.75(C16), 14.78(C15), 11.45(C17); HR-MS (ESI): Calcd for $C_{30}H_{39}N_3O_5S$ ($M+H^+$): 554.2688; Found: 554.2674.

4.2.8. 22-[(4-aminophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 118)

White powder; yield: 74%; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.79 (2H, d, $J = 8.7$ Hz), 6.74 (2H, d, $J = 8.3$ Hz), 6.44 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.79 (1H, d, $J = 8.5$ Hz, H14), 5.39 – 5.15 (3H, m, H20), 4.03 (2H, d, $J = 2.5$ Hz), 3.36 (1H, d, $J = 6.5$ Hz, H22), 2.34 – 2.31 (1H, m, *H*11), 2.31 – 2.23 (1H, m, H2), 2.19 (1H, dd, $J = 19.5$, 9.3 Hz, H4), 2.12 – 2.07 (1H, m, *H*10), 1.78 (1H, dd, $J = 14.4$, 3.1 Hz, H6), 1.72 – 1.66 (2H, m, H8), 1.66 – 1.62 (1H, m, H1), 1.58 – 1.52 (1H, m, H7), 1.48 (1H, dd, $J = 9.6$, 2.8 Hz, H13), 1.45 (3H, s), 1.44 (1H, d, $J = 4.0$ Hz), 1.41 – 1.38 (1H, m,H15), 1.38 – 1.36 (1H, m, H8), 1.27 (1H, s), 1.17 (3H, s), 1.13 (1H, dd, $J = 14.0$, 4.5 Hz, H18), 0.89 (3H, d, $J = 7.1$ Hz, H17), 0.76 (3H, d, $J = 7.1$ Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.79 (C3), 166.48, 166.25, 161.18, 149.83, 138.72(C19), 128.44, 117.35 (C20), 114.62, 113.04, 74.60(C11), 70.81(C14), 65.93, 58.08(C4), 53.39(C22), 45.42, 44.63(C13), 44.00(C12), 41.89(C5), 36.67(C6), 36.02(C10), 35.12, 34.42(*C*2), 30.39, 26.83(C7), 26.41(C8), 24.81(C1), 16.75(C16), 14.77(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{30}H_{39}N_3O_5S$ ($M+H^+$): 554.2688; Found: 554.2674.

4.2.9. 22-[((4-dimethylamino)phenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 119)

White powder; yield: 74%; 1H NMR (600 MHz, Chloroform-*d*) δ 7.84 – 7.82 (2H, m), 6.73 (1H, s), 6.71 (1H, d, $J = 2.1$ Hz), 6.43 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.78 (1H, d, $J = 8.5$ Hz, H14), 5.32 (1H, dd, $J = 11.0$, 1.4 Hz, H20), 5.19 (1H, dd, $J = 17.4$, 1.5 Hz, H20), 4.01 (2H, d, $J = 2.9$ Hz), 3.35 (1H, t, $J = 7.6$ Hz, H22), 3.06 (6H, s, *H*11), 2.32 (1H, q, $J = 6.9$ Hz, H2), 2.20 (1H, q, $J = 9.7$ Hz, H4), 2.10 (1H, s, *H*10), 1.79 – 1.75 (1H, m, H6, H8), 1.74 (1H, s), 1.69 – 1.67 (1H, m, H1), 1.67 – 1.65 (1H, m, H7), 1.65 – 1.62 (1H, m), 1.54 – 1.52 (1H, m), 1.51 – 1.49 (1H, m), 1.46 (1H, dd, $J = 9.7$, 2.8 Hz), 1.44 (3H, s, H13), 1.39 (1H, d, $J = 3.8$ Hz, H15), 1.36 (1H, d, $J = 6.2$ Hz, H8), 1.16 (3H, s), 1.15 – 1.10 (1H, m, H18), 0.88 (3H, d, $J = 7.1$ Hz, H17), 0.75 (3H, d, $J = 7.1$ Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.77(C3), 166.79, 166.30, 160.81, 152.39, 138.71(C19), 128.12, 117.36(C20), 111.56, 110.46, 74.61 (C11), 70.75(C14), 58.08(C4), 45.42(C9), 44.63(C12), 44.40, 44.00, 42.66, 41.89(C5), 40.05, 36.68(C6), 36.02(C10), 35.16, 34.42(*C*2), 31.69, 30.40(C8), 26.83(C7), 26.40(C18), 24.82(C1), 16.75(C16), 14.78 (C15), 11.45(C17); HR-MS (ESI): Calcd for $C_{32}H_{43}N_3O_5S$ ($M+H^+$): 582.3001; Found: 582.2986.

4.2.10. 22-[(3-nitrophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 120)

White powder; yield: 74%; 1H NMR (600 MHz, Chloroform-*d*) δ 8.84 (1H, td, $J = 1.9$, 0.4 Hz), 8.52 – 8.19 (2H, m), 7.82 – 7.65 (1H, m), 6.44 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.81 (1H, d, $J = 8.5$ Hz, H14), 5.32 (1H, dd, $J = 11.0$, 1.4 Hz, H20), 5.20 (1H, dd, $J = 17.4$, 1.5 Hz, H20), 4.17 – 3.91 (2H, m), 2.35 – 2.16 (3H, m, H2, H4, *H*10), 2.11 (3H, q, $J = 8.6$ Hz, H13, 11-OH), 1.83 – 1.75 (1H, m, H6), 1.69 (3H, dddd, $J = 24.4$, 13.2, 9.1, 6.5 Hz, H1), 1.56 (2H, td, $J = 13.2$, 12.5, 2.9 Hz, H7), 1.51 – 1.47 (1H, m), 1.46 (3H, s, H14), 1.43 – 1.37 (2H, m, H15), 1.19 (3H, s, H8), 0.89 (3H, d, $J = 7.1$, H17), 0.78 (3H, d, $J = 7.1$ Hz) . ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.68(C3), 165.88, 164.12, 148.68, 138.73 (C19), 132.12, 130.44, 126.15, 125.10, 121.58, 117.33(C20), 74.58 (C11), 71.11(C14), 58.04(C4), 55.04, 45.42(C9), 44.66(C13), 44.02 (C12), 41.91(C5), 36.64(C6), 36.03(C10), 35.08, 34.41(*C*2), 30.37(C8), 26.83(C7), 26.44(C18), 24.81(C1), 16.76(C16), 14.77(C15), 11.43 (C17); HR-MS (ESI): Calcd for $C_{30}H_{37}N_3O_7S$ ($M-H^+$): 582.2274; Found: 582.2275.

4.2.11. 22-[(2-hydroxyphenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 121)

White powder; yield: 64%; 1H NMR (600 MHz, Chloroform-*d*) δ 6.42 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.72 (1H, d, $J = 8.5$ Hz, H14), 5.36 (1H, dd, $J = 11.0$, 1.3 Hz, H20), 5.22 (1H, dd, $J = 17.4$, 1.4 Hz, H20), 3.67 (1H, d, $J = 10.5$ Hz), 3.59 (1H, d, $J = 10.5$ Hz), 3.38 (1H, dd, $J = 10.4$, 6.6 Hz, *H*11), 2.37 – 2.16 (3H, m, H2, H4, *H*10), 2.13 – 2.05 (2H, m, H13, 11-OH), 1.78 (1H, dq, $J = 14.4$, 2.8 Hz, H6), 1.71 – 1.62 (3H, m, H8, H1, H17), 1.56 (1H, qd, $J = 13.9$, 3.6 Hz, H13), 1.51 – 1.43 (5H, m), 1.47 (4H, s), 1.42 – 1.31 (2H, m, H15, H8), 1.21 – 1.10 (4H, m, H18), 0.90 (3H, d, $J = 7.1$, H17), 0.75 (3H, d, $J = 7.1$, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.68(C3), 165.87, 165.47, 162.17, 157.24, 138.66(C19), 133.76, 126.43, 119.98, 117.57, 117.36(C20), 107.73, 74.58(C11), 71.06(C14), 58.05(C4), 45.42(C9), 44.63(C13), 43.98 (C12), 41.89(C5), 36.64(C6), 36.03(C10), 35.04(*C*2), 34.40, 30.38(C8), 26.83(C7), 26.40(C18), 24.81(C1), 16.74(C16), 14.77(C15), 11.44 (C17); HR-MS (ESI): Calcd for $C_{30}H_{38}N_2O_6S$ ($M+H^+$): 555.2529; Found: 555.2517.

4.2.12. 22-[(4-hydroxyphenyl −1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 122)

White powder; yield: 67%; 1H NMR (600 MHz, Chloroform-*d*) δ 7.85 (3H, dd, $J = 8.5$, 6.6 Hz), 7.03 – 6.91 (2H, m), 6.42 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.78 (1H, d, $J = 8.5$ Hz, H14), 5.39 – 5.07 (2H, m, H20), 4.10 – 3.97 (2H, m), 3.36 (1H, dd, $J = 10.3$, 6.5 Hz, H22), 2.34 – 2.27 (1H, m), 2.27 – 2.22 (1H, m), 2.19 (1H, dd, $J = 19.5$, 9.4 Hz, H2), 2.11 – 2.04 (2H, m, H4, *H*10), 1.93 (1H, s, H13), 1.77 (1H, dq, $J = 14.6$, 3.2 Hz, H6), 1.68 – 1.63 (1H, m, H1), 1.61 (1H, d, $J = 10.6$, H7), 1.54 (1H, td, $J = 13.3$, 12.6, 3.2 Hz, H13), 1.50 – 1.45 (1H, m), 1.43 (3H, s), 1.40 – 1.34 (2H, m, H15), 1.15 (3H, s, H8), 1.11 (1H, dd, $J = 14.1$, 4.4 Hz, H18), 0.88 (3H, d, $J = 7.0$ Hz, H17), 0.75 (3H, d, $J = 7.1$, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 217.28(C3), 166.28, 162.00, 159.92, 138.59 (C19), 128.78, 117.49(C20), 116.29, 115.05, 74.66(C11), 70.99(C14), 58.13(C4), 45.45(C9), 44.62(C13), 43.98(C12), 41.91(C5), 36.68(C6), 36.00(C10), 35.74, 35.02, 35.02, 34.46(*C*2), 30.39(C8), 26.80(C7), 26.37(C18), 24.80(C1), 23.87, 16.75(C16), 14.77(C15), 11.48(C17); HR-MS (ESI): Calcd for $C_{30}H_{38}N_2O_6S$ ($M+H^+$): 555.2529; Found: 555.2513.

4.2.13. 22-[(4-methoxyphenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 123)

White powder; yield: 64%; 1H NMR (600 MHz, Chloroform-*d*) δ 7.93 – 7.92 (2H, m), 7.00 – 6.98 (2H, m), 6.42 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.78 (1H, d, $J = 8.5$ Hz, H14), 5.18 (1H, dd, $J = 17.4$, 1.4 Hz, H20), 4.03 (2H, d, $J = 3.1$ Hz), 3.88 (3H, s, H22), 3.36 (1H, s, *H*11), 2.32 (1H, q, $J = 6.9$ Hz, H2), 2.27 – 2.24 (1H, m, H4), 2.19 (1H, dt, $J = 19.4$, 9.4 Hz, *H*10), 2.10 (1H, s, H13), 1.77 (2H, dd, $J = 14.5$, 2.9 Hz, H6, H8), 1.67 (2H, dd, $J = 8.1$, 4.2 Hz, H1), 1.58 – 1.50 (3H, m, H7), 1.47 – 1.45 (1H, m, H13), 1.44 (3H, s), 1.39 (1H, d, $J = 4.0$, H15), 1.36 (1H, d, $J = 6.1$, H8), 1.16 (3H, s), 1.15 – 1.11 (1H, m, H18), 0.88 (3H, d, $J = 7.1$ Hz, H17), 0.75 (3H, d, $J = 7.1$, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.72(C3), 166.16, 166.01, 162.39, 161.92, 138.72(C19), 128.49, 117.33(C20), 115.97, 114.51, 74.60(C11), 70.86(C14), 58.07(C4), 55.46, 45.42(C9), 44.64(C13), 44.00(*C*2), 43.03, 41.90(C5), 36.66(C6), 36.33, 36.03(C10), 35.11, 34.41(*C*2), 30.39(C8), 26.83(C7), 26.41 (C18), 24.82(C1), 16.75(C16), 14.77(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{31}H_{40}N_2O_6S$ ($M+H^+$): 569.2685; Found: 569.2671.

4.2.14. 22-[(2-fluorophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 124)

White powder; yield: 81%; 1H NMR (600 MHz, Chloroform-*d*) δ 8.01 – 7.99 (1H, m), 7.55 – 7.52 (1H, m), 7.31 – 7.28 (1H, m), 7.26 – 7.23 (1H, m), 6.42 (1H, dd, $J = 17.4$, 11.0, H19), 5.79 (1H, d, $J = 8.5$ Hz,

H14), 5.32 – 5.30 (1H, m, H20), 5.19 – 5.16 (1H, m, H20), 4.09 – 4.02 (2H, m), 3.36 (1H, d, J = 5.8 Hz, H22), 2.32 (1H, q, J = 7.0 Hz, *H*11), 2.26 – 2.23 (1H, m, H2), 2.22 – 2.17 (1H, m, H4), 2.09 (2H, d, J = 9.1 Hz, *H*10, H13), 1.79 – 1.75 (1H, m, H6), 1.67 – 1.65 (1H, m, H1), 1.65 – 1.62 (1H, m, H7), 1.56 (1H, dd, J = 14.1, 3.7 Hz, H13), 1.54 – 1.51 (1H, m), 1.46 – 1.45 (1H, m), 1.44 (3H, s), 1.39 (1H, d, J = 4.1 Hz, H15), 1.37 (1H, d, J = 5.8H8), 1.16 (3H, s), 1.13 (1H, dd, J = 14.2, 9.8 Hz, H18), 0.88 (3H, d, J = 7.1 Hz, H17), 0.75 (3H, d, J = 7.1, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.69(C3), 166.04, 163.32, 162.73, 160.68, 158.96, 138.71(C19), 133.58, 133.53, 129.53, 124.67, 117.31(C20), 112.03, 74.59(C11), 70.93(C14), 58.06(C4), 45.42(C9), 44.63(C13), 44.00(C12), 41.90(C5), 36.66(C6), 36.03(C10), 35.03, 34.41(*C*2), 30.39 (C8), 26.83,(C7) 24.82(C18), 16.73(C16), 14.75(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{30}H_{37}FN_2O_5S$ (M+Na$^+$): 579.2299; Found: 579.2288.

4.2.15. 22-[(3-fluorophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 125)

White powder; yield: 82%; 1H NMR (600 MHz, Chloroform-*d*) δ 7.80 (1H, ddd, J = 7.8, 1.6, 1.0 Hz), 7.70 (1H, ddd, J = 9.1, 2.6, 1.5 Hz), 7.49 (1H, td, J = 8.0, 5.5 Hz), 7.24 (1H, tdd, J = 8.4, 2.6, 1.0 Hz), 6.43 (1H, dd, J = 17.4, 11.0 Hz, H19), 5.80 (1H, d, J = 8.5 Hz, H14), 5.35 – 5.10 (2H, m, H20), 4.14 – 3.99 (2H, m), 3.36 (1H, dd, J = 10.4, 6.5 Hz, H22), 2.33 – 2.23 (2H, m, H2, H4), 2.19 (1H, dd, J = 19.5, 9.4 Hz, *H*10), 2.12 – 2.05 (2H, m, H13, 11-OH), 1.77 (1H, dq, J = 14.5, 3.2 Hz, H6), 1.67 (2H, s H8), 1.67 – 1.62 (1H, m, H1), 1.59 – 1.51 (1H, m, H7), 1.50 – 1.46 (1H, m, H13), 1.45 (3H, s), 1.42 – 1.34 (2H, m, H15, H8), 1.17 (3H, s), 1.13 (1H, dd, J = 14.2, 4.4 Hz, H18), 0.88 (3H, d, J = 7.1 Hz, H17), 0.76 (3H, d, J = 7.1 Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.69(C3), 166.00, 165.05, 163.26, 161.99, 138.70(C19), 130.96, 130.91, 125.32, 122.45, 118.93, 118.79, 117.35(C20), 113.84, 113.68, 74.59(C11), 70.99(C14), 58.06(C4), 45.42(C9), 44.01(C12), 41.90(C5), 36.65(C6), 36.03(C10), 34.41(*C*2), 30.38(C8), 26.83(C7), 24.82, 16.76(C16), 14.76 (C15), 11.43(C17); HR-MS (ESI): Calcd for $C_{30}H_{37}FN_2O_5S$ (M+Na$^+$): 579.2299; Found: 579.2288.

4.2.16. 22-[(4-fluorophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 126)

White powder; yield: 78%; 1H NMR (600 MHz, Chloroform-*d*) δ 8.00 (2H, td, J = 5.2, 2.8 Hz), 7.21 – 7.18 (2H, m), 6.43 (1H, dd, J = 17.4, 11.0 Hz, H19), 5.79 (1H, d, J = 8.5 Hz), 5.32 – 5.30 (1H, m, H20), 5.19 (1H, d, J = 17.4 Hz, H20), 4.05 (2H, d, J = 3.3 Hz), 3.36 (1H, dd, J = 10.4, 6.5 Hz, H22), 2.32 (1H, q, J = 6.9 Hz), 2.29 – 2.22 (1H, m, H2), 2.19 (1H, dd, J = 19.5, 9.4 Hz, H4), 2.09 (2H, d, J = 16.5 Hz, *H*10, H13), 1.77 (1H, dd, J = 14.5, 2.9 Hz, H6), 1.71 (1H, s, H8), 1.67 – 1.64 (1H, m, H1), 1.57 – 1.49 (2H, m, H7, H13), 1.47 (1H, d, J = 11.6 Hz), 1.45 (3H, s), 1.38 (1H, d, J = 5.2 Hz, H15), 1.38 – 1.35 (1H, m, H8), 1.17 (3H, s), 1.16 – 1.10 (1H, m, H18), 0.88 (3H, d, J = 7.0 Hz, H17), 0.75 (3H, d, J = 7.1 Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.71(C3), 166.05, 165.63, 165.23, 163.95, 162.79, 138.72(C19), 129.04, 128.98, 117.33 (C20), 116.51, 116.36, 74.59(C11), 70.95(C14), 58.06(C4), 45.42(C9), 44.64(C13), 44.00(C12), 41.90(C5), 36.65(C6), 36.02(C10), 35.08, 34.41(*C*2), 30.38(C8), 26.83(C7), 26.41(C18), 24.81(C1), 16.75(C16), 14.77(C15), 11.43(C17); HR-MS (ESI): Calcd for $C_{30}H_{37}FN_2O_5S$ (M+Na$^+$): 579.2299; Found: 579.2289.

4.2.17. 22-[(2-chlorophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 127)

White powder; yield: 80%; 1H NMR (600 MHz, Chloroform-*d*) δ 7.93 (1H, dd, J = 7.8, 1.7 Hz), 7.55 (1H, dd, J = 8.1, 1.3 Hz), 7.48 (1H, ddd, J = 8.0, 7.4, 1.7 Hz), 7.41 (1H, td, J = 7.6, 7.6, 1.3 Hz), 6.43 (1H, dd, J = 17.4, 11.0 Hz, H19), 5.80 (1H, d, J = 8.5H14), 5.36 – 5.14 (2H, m, H20), 4.14 – 4.00 (2H, m), 3.36 (1H, dd, J = 10.6, 6.5 Hz), 2.32 (1H, p, J = 7.5, 7.5, 7.3, 7.3 Hz, H2), 2.27 – 2.22 (1H, m, H4), 2.19 (1H, dd, J = 19.5, 9.4 Hz, *H*10), 2.13 – 2.04 (2H, m, H13, 11-OH), 1.77 (1H, dq, J = 14.6, 3.2, 3.2, 3.2 Hz, H6), 1.67 (2H, s, H1, H7), 1.59 – 1.51 (1H, m), 1.50 – 1.46 (2H, m, H13), 1.45 (3H, s), 1.38 (2H, dd, J = 15.4, 4.2 Hz, H15, H8), 1.16 (3H, s), 1.12 (1H, dd, J = 14.1, 4.4 Hz, H18), 0.88 (3H, d, J = 7.0 Hz, H17), 0.75 (3H, d, J = 7.1 Hz, H16).^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.71(C3), 166.04, 164.34, 163.46, 138.70(C19), 133.08, 132.46, 131.24, 130.98, 127.07, 122.79, 117.35(C20), 74.60 (C11), 70.95(C14), 58.07(C4), 45.42(C9), 44.64(C13), 44.00(C12), 41.90(C5), 36.66(C6), 36.03(C10), 35.03, 34.41(*C*2), 30.39(C8), 26.83 (C7), 26.39(C18), 24.82(C1), 16.76(C16), 14.77(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{30}H_{37}ClN_2O_5S$ (M+Na$^+$): 595.2004; Found: 595.1993.

4.2.18. 22-[(3-chlorophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 128)

White powder; yield: 84%; 1H NMR (600 MHz, Chloroform-*d*) δ 7.99 (1H, td, J = 1.8, 0.5 Hz), 7.89 (1H, ddd, J = 7.8, 1.6, 1.1 Hz), 7.51 (1H, ddd, J = 8.0, 2.1, 1.1 Hz), 7.48 – 7.39 (1H, m), 6.43 (1H, dd, J = 17.4, 11.0 Hz, H19), 5.79 (1H, d, J = 8.5, H14), 5.33 – 5.30 (1H, m, H20), 5.19 (1H, dd, J = 17.4, 1.5 Hz, H20), 4.12 – 4.00 (2H, m), 3.36 (1H, dd, J = 10.5, 6.5 Hz, H22), 2.35 – 2.29 (1H, m, H2), 2.28 – 2.22 (1H, m, H4), 2.19 (1H, dd, J = 19.5, 9.4 Hz, *H*10), 2.12 – 2.06 (2H, m, H13. 11-OH), 1.77 (1H, dq, J = 14.5, 3.1 Hz, H6, H8), 1.68 (1H, s, H1), 1.67 – 1.62 (1H, m, H7), 1.58 – 1.52 (1H, m), 1.51 – 1.46 (2H, m), 1.45 (3H, s, H13), 1.42 – 1.34 (2H, m, H15, H8), 1.17 (3H, s), 1.16 – 1.10 (1H, m, H18), 0.88 (3H, d, J = 7.1 Hz, H17), 0.76 (3H, d, J 7.1, H17).^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.69(C3), 165.99, 164.88, 163.32, 138.70 (C19), 135.25, 131.82, 130.44, 126.66, 125.05, 124.77, 117.36(C20), 74.59(C11), 71.00(C14), 58.06(C4), 45.42(C9), 44.65(C13), 44.01 (C12), 41.90(C5), 36.65(C6), 36.03(C10), 35.06, 34.41(*C*2), 30.38(C8), 26.83(C7), 26.41(C18), 24.82(C1), 16.76(C16), 14.77(C15), 11.44 (C17); HR-MS (ESI): Calcd for $C_{30}H_{37}ClN_2O_5S$ (M+Na$^+$): 595.2004; Found: 595.1991.

4.2.19. 22-[(4-chlorophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 129)

White powder; yield: 82%; 1H NMR (600 MHz, Chloroform-*d*) δ 7.96 – 7.92 (2H, m), 7.51 – 7.47 (2H, m), 6.44 (1H, dd, J = 17.4, 11.0 Hz, H19), 5.80 (1H, d, J = 8.5 Hz, H14), 5.34 – 5.31 (1H, m), 5.20 (1H, dd, J = 17.4, 1.4 Hz, H20), 4.06 (2H, d, J = 3.0, H20, H22), 2.34 – 2.25 (2H, m, H2, H4), 2.20 (1H, dd, J = 19.5, 9.4 Hz), 2.10 (2H, d, J = 8.7 Hz, *H*10, H13), 1.78 (1H, dd, J = 14.5, 3.0 Hz, H6), 1.69 (2H, ddd, J = 12.9, 4.8, 2.8 Hz, H8), 1.60 – 1.54 (3H, m, 11-OH), 1.46 (3H, s, H1, H7), 1.41 – 1.35 (3H, m, H13, H15), 1.18 (3H, s, H8), 1.13 (1H, dd, J = 14.1, 4.4 Hz, H18), 0.89 (3H, d, J = 7.1, H17), 0.77 (3H, d, J = 7.1, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.70(C3), 166.02, 165.24, 163.04, 138.71 (C19), 138.09, 129.48, 127.96, 121.91, 117.34(C20), 99.99, 74.59 (C11), 70.98(C14), 58.05(C4), 48.14, 45.42(C9), 44.64(C13), 44.01 (C12), 41.90(C5), 36.65(C6), 36.02(C10), 35.08, 34.41(*C*2), 30.38(C8), 26.83(C7), 26.42(C18), 24.81(C1), 16.76(C16), 14.77(C15), 11.44 (C17); HR-MS (ESI): Calcd for $C_{30}H_{37}ClN_2O_5S$ (M+Na$^+$): 595.2004; Found: 595.1990.

4.2.20. 22-[(3-bromophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 130)

White powder; yield: 81%; 1H NMR (600 MHz, Chloroform-*d*) δ 8.15 – 8.14 (1H, m), 7.94 – 7.93 (1H, m), 7.67 – 7.66 (1H, m), 7.38 (1H, t, J = 7.9 Hz), 6.42 (1H, dd, J = 17.4, 11.0 Hz, H19), 5.79 (1H, d, J = 8.5 Hz), 5.31 (1H, dd, J = 9.6, 1.4 Hz, H14), 5.21 – 5.17 (1H, m, H20), 4.06 (2H, d, J = 3.2, H20, H22), 3.36 (1H, d, J = 6.4, *H*11), 2.34 – 2.30 (1H, m, H2), 2.26 – 2.23 (1H, m, H4), 2.20 (1H, q, J = 9.7, *H*10), 2.10 (2H, d, J = 8.6 Hz, H13), 1.79 – 1.75 (1H, m, H6), 1.67 (1H, dd, J = 5.2, 3.0 Hz, H8), 1.56 (1H, dd, J = 13.9, 3.7 Hz), 1.54 – 1.49 (2H, m), 1.48 – 1.46 (1H, m, H1), 1.45 (3H, s, H7, H13), 1.39 (1H, d, J = 5.7, H15), 1.37 (1H, d, J = 7.9 Hz), 1.17 (3H, s), 1.12 (1H, dd, J = 14.1, 4.4 Hz, H8), 0.88 (3H, d, J = 7.1, H17), 0.76 (3H, d, J = 7.1 Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.66(C3), 165.97, 164.72, 163.32, 138.71(C19), 134.73, 130.63, 129.52, 125.27, 125.20, 123.11, 117.35(C20), 74.59(C11),

71.00(C14), 58.05(C4), 45.42(C9), 44.65(C13), 44.01(C12), 41.90(C5), 36.65(C6), 36.03(C10), 35.06, 34.41(*C*2), 30.38(C8), 26.83(C7), 26.43 (C18), 24.82(C1), 16.76(C16), 14.77(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{30}H_{37}BrN_2O_5S$ ($M+H^+$): 619.1664; Found: 619.1647.

4.2.21. 22-[(4-bromophenyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 131)

White powder; yield: 81%; 1H NMR (600 MHz, Chloroform-*d*) δ 7.96 – 7.92 (1H, m), 7.67 – 7.63 (1H, m), 7.38 (1H, t, $J = 7.9$ Hz), 6.43 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.80 (1H, d, $J = 8.5$ Hz), 5.33 – 5.30 (1H, m, H14), 5.19 (1H, dd, $J = 17.4$, 1.4 Hz, H20), 4.09 – 4.03 (2H, m, H20, H22), 3.36 (1H, s, *H*11), 2.32 (1H, q, $J = 6.9$ Hz, H2), 2.29 – 2.25 (1H, m, H4), 2.21 (1H, q, $J = 9.7$ Hz, *H*10), 2.09 (2H, d, $J = 16.0$ Hz, H13), 1.77 (1H, dd, $J = 14.5$, 2.9 Hz, H6), 1.69 – 1.67 (1H, m, H8), 1.66 – 1.62 (1H, m, 11-OH), 1.52 (1H, dd, $J = 13.3$, 2.9 Hz), 1.47 (2H, dd, $J = 9.7$, 2.8 Hz, H1, H7), 1.45 (3H, s, H13), 1.40 (1H, d, $J = 5.9$ Hz, H15), 1.37 (1H, d, $J = 7.9$ Hz), 1.17 (3H, s, H8), 1.13 (1H, dd, $J = 14.1$, 4.4 Hz, H18), 0.89 (3H, d, $J = 7.1$ Hz, H17), 0.76 (3H, d, $J = 7.1$, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.66(C3), 165.97, 164.72, 163.32, 138.71(C19), 134.73, 130.63, 129.52, 125.27, 125.20, 123.11, 117.35(C20), 74.59 (C11), 71.00(C14), 58.05(C4), 45.42(C9), 44.65(C13), 44.01(C12), 41.90(C5), 36.65(C6), 36.03(C10), 35.06, 34.41(*C*2), 30.38(C8), 26.83 (C7), 26.43(C18), 24.82(C1), 16.76(C16), 14.77(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{30}H_{37}BrN_2O_5S$ ($M+Na^+$): 641.1478; Found: 641.1466.

4.2.22. 22-[(2-furanyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 132)

White powder; yield: 67%; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.64 (1H, dd, $J = 1.8$, 0.8 Hz), 7.13 (1H, dd, $J = 3.6$, 0.8 Hz), 6.60 (1H, dd, $J = 3.5$, 1.8 Hz), 6.44 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.80 (1H, d, $J = 8.5$ Hz), 5.33 (1H, dd, $J = 11.0$, 1.5 Hz, H14), 5.21 (1H, dd, $J = 17.5$, 1.5 Hz, H20), 4.05 (2H, d, $J = 1.9$ Hz, H20, H22), 3.36 (1H, s, *H*11), 2.33 (1H, p, $J = 7.1$ Hz, H2), 2.28 – 2.23 (1H, m, H4), 2.20 (1H, dd, $J = 19.4$, 9.4 Hz), 2.11 (1H, s, *H*10), 2.10 – 2.06 (1H, m, H13), 1.78 (1H, dd, $J = 14.5$, 3.2 Hz, H6), 1.70 – 1.64 (1H, m, H8), 1.55 (2H, d, $J = 13.1$ Hz, 11-OH, H1), 1.52 – 1.46 (1H, m, H7), 1.45 (3H, s, H13), 1.44 – 1.39 (1H, m), 1.37 (1H, d, $J = 5.5$, H15), 1.28 (1H, d, $J = 8.6$ Hz), 1.18 (3H, s, H8), 1.17 – 1.11 (1H, m, H18), 0.89 (3H, d, $J = 7.0$ Hz, H17), 0.77 (3H, d, $J = 7.1$ Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.71(C3), 165.96 (C21), 162.27, 158.77, 145.75, 138.90(C19), 138.69, 117.35(C20), 114.10, 112.15, 74.60(C11), 70.97(C14), 58.06(C4), 45.42(C9), 44.64 (C13), 44.00(C12), 41.90(C5), 36.65(C6), 36.02(C10), 35.12, 34.41 (*C*2), 30.38(C8), 26.82(C7), 26.42(C18), 24.81(C1), 16.74(C16), 14.75 (C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{28}H_{36}N_2O_6S$ ($M+Na^+$): 551.2186; Found: 551.2177.

4.2.23. 22-[(2-thienyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 133)

White powder; yield: 67%; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.72 (1H, dd, $J = 3.7$, 1.2 Hz), 7.56 (1H, dd, $J = 5.0$, 1.2 Hz), 7.17 (1H, dd, $J = 5.0$, 3.7 Hz), 6.44 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.80 (1H, d, $J = 8.5$ Hz, H14), 5.33 (1H, dd, $J = 11.0$, 1.4 Hz, H20), 5.20 (1H, dd, $J = 17.4$, 1.4 Hz, H20), 4.04 (2H, d, $J = 2.4$ Hz), 3.36 (2H, s, H22, *H*11), 2.35 – 2.31 (1H, m, H2), 2.21 (1H, q, $J = 9.8$ Hz, H4), 2.10 (2H, d, $J = 8.7$ Hz, *H*10, H13), 1.78 (1H, dd, $J = 14.5$, 3.1 Hz, H6), 1.68 – 1.63 (1H, m, H8), 1.57 (2H, d, $J = 10.4$, 11-OH), 1.54 – 1.49 (1H, m, H1), 1.46 (3H, s, H7, H13), 1.40 (1H, d, $J = 5.4$ Hz), 1.38 – 1.35 (1H, m, H15), 1.28 (1H, d, $J = 7.9$ Hz, H8), 1.18 (3H, s), 1.13 (1H, dd, $J = 14.1$, 4.4 Hz, H18), 0.89 (3H, d, $J = 7.1$ Hz, H17), 0.77 (3H, d, $J = 7.1$ Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.72(C3), 166.03(C21), 162.22, 162.12, 138.69(C19), 130.21, 129.78, 128.13, 124.60, 117.36(C20), 74.60 (C11), 70.94(C14), 58.06(C22), 45.42(C9), 44.64(C13), 44.01(C12), 41.90(C5), 36.66(C6), 36.02(C10), 35.11, 34.42(*C*2), 30.39(C8), 26.83 (C7), 26.40(C18), 24.82(C1), 16.76(C16), 14.77(C15), 11.44(C17); HR-MS (ESI): Calcd for $C_{28}H_{36}N_2O_5S_2$ ($M+Na^+$): 567.1958; Found: 567.1946.

4.2.24. 22-[(2-pyridy-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 134)

White powder; yield: 69%; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.78 – 8.77 (1H, m), 8.20 – 8.19 (1H, m), 7.89 (1H, td, $J = 7.8$, 1.7 Hz), 7.47 (1H, ddd, $J = 7.6$, 4.8, 1.1 Hz), 6.43 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.80 (1H, d, $J = 8.5$ Hz, H14), 5.32 (1H, dd, $J = 11.0$, 1.4 Hz, H20), 5.20 (1H, dd, $J = 17.4$, 1.5 Hz, H20), 4.13 – 4.04 (2H, m), 3.36 (1H, d, $J = 6.4$ Hz, H22), 2.33 – 2.18 (3H, m, H2, H4, *H*10), 2.12 – 2.00 (3H, m, H13, H6), 1.79 – 1.76 (1H, m, H8), 1.71 – 1.68 (1H, m, 11-OH), 1.67 – 1.65 (1H, m, H1), 1.65 – 1.62 (1H, m, H7), 1.54 (2H, qd, $J = 14.0$, 3.7, H13), 1.44 (3H, s), 1.42 – 1.38 (2H, m, H5, H8), 1.17 (3H, s, H18), 0.88 (3H, d, $J = 7.1$ Hz, H17), 0.76 (3H, d, $J = 7.1$ Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.72(C3), 166.00(C21), 165.14, 164.38, 150.26, 143.12, 138.71(C19), 137.24, 125.84, 122.88, 117.34(C20), 74.60, 70.94, 58.06, 45.42(C9), 44.61(C13), 44.00(C12), 41.90(C5), 36.66 (C6), 36.02(C10), 35.02, 34.41(*C*2), 30.38(C8), 26.82(C7), 26.42(C18), 24.81(C1), 16.75(C16), 14.75(C15), 11.43(C17); HR-MS (ESI): Calcd for $C_{29}H_{37}N_3O_5S$ ($M+H^+$): 540.2532; Found: 540.2516.

4.2.25. 22-[(3-pyridy-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 135)

White powder; yield: 68%; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.27 – 9.18 (1H, m), 8.78 (1H, dd, $J = 4.9$, 1.7 Hz), 8.30 (1H, dt, $J = 8.0$, 1.9 Hz), 7.47 (1H, ddd, $J = 8.0$, 4.9, 0.9 Hz), 6.43 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.80 (1H, d, $J = 8.5$ Hz, H14), 5.33 – 5.17 (2H, m, H20), 4.16 – 4.01 (2H, m), 3.37 (1H, s, H22), 2.35 – 2.24 (2H, m, H2), 2.20 (1H, dt, $J = 19.4$, 9.3 Hz, H4), 2.13 – 2.06 (2H, m, *H*10, H13), 1.78 (1H, dq, $J = 14.5$, 3.1 Hz, H6), 1.71 – 1.64 (1H, m, H8), 1.57 – 1.48 (2H, m, H1, H7), 1.46 (3H, s, H13), 1.39 (2H, dd, $J = 15.4$, 3.7 Hz, H15), 1.31 – 1.25 (1H, m, H8), 1.18 (4H, s, H18), 1.17 – 1.11 (1H, m), 0.89 (3H, d, $J = 7.0$ Hz, H17), 0.77 (3H, d, $J = 7.1$, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.67(C3), 165.94(C21), 163.94, 163.71, 152.47, 147.68, 138.70 (C19), 133.87, 123.77, 120.02, 117.36(C20), 74.60(C11), 71.05(C14), 58.06(C4), 45.42(C9), 44.66(C13), 44.01(C12), 41.91(C5), 36.65(C6), 36.03(C10), 35.09, 34.41(*C*2), 30.38(C8), 26.83(C7), 26.41(C18), 24.82 (C1), 16.76(C16), 14.77(C15), 11.43(C17); HR-MS (ESI): Calcd for $C_{29}H_{37}N_3O_5S$ ($M-H^+$): 538.2376; Found: 538.2376.

4.2.26. 22-[(4-pyridy-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 136)

White powder; yield: 73%; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.81 (2H, d, $J = 5.5$ Hz), 7.85 – 7.84 (2H, m), 6.42 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.80 (1H, d, $J = 8.5$ Hz, H14), 5.33 – 5.30 (1H, m, H20), 5.21 – 5.17 (1H, m, H20), 4.08 (2H, d, $J = 3.7$ Hz), 3.38 – 3.33 (2H, m, H22, *H*11), 2.32 – 2.29 (1H, m,H2), 2.20 (1H, q, $J = 9.7$ Hz, H4), 2.11 (1H, s), 1.79 – 1.77 (1H, m), 1.76 (1H, d, $J = 3.0$ Hz,*H*10), 1.66 (1H, dd, $J = 4.4$, 2.8 Hz, H1), 1.56 (1H, dd, $J = 14.0$, 3.6, H7), 1.52 – 1.48 (2H, m, H13), 1.48 – 1.46 (1H, m), 1.45 (3H, s), 1.39 (1H, s), 1.37 (1H, d, $J = 4.7$ Hz, H15), 1.17 (3H, s, H8), 1.12 (1H, dd, $J = 14.1$, 4.4 Hz, H18), 0.88 (3H, d, $J = 7.1$ Hz, H17), 0.76 (3H, d, $J = 7.1$ Hz, H16). ^{13}C NMR (151 MHz, Chloroform-d) δ 216.63(C3), 165.84(C21), 164.44, 164.20, 150.89, 138.72(C19), 130.48, 120.02, 117.33(C20), 74.58(C11), 71.11(C14), 58.04(C4), 45.42(C9), 44.66(C13), 44.02(C12), 42.79, 41.90(C5), 36.63 (C6), 36.03, 35.99(C10), 35.06, 34.40(*C*2), 30.37(C8), 26.83(C7), 26.44 (C18), 24.81(C1), 16.75(C16), 14.76(C15), 11.43(C17); HR-MS (ESI): Calcd for $C_{29}H_{37}N_3O_5S$ ($M+H^+$): 540.2532; Found: 540.2518.

4.2.27. 22-[(pyrazinyl-1, 3, 4-oxadiazol-5-yl) thio] deoxy pleuromutilin (compound 137)

White powder; yield: 73%; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 9.43 (1H, d, $J = 1.5$ Hz), 8.85 – 8.66 (2H, m), 6.44 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.81 (1H, d, $J = 8.6$ Hz, H14), 5.40 – 5.16 (2H, m, H20), 4.23 – 4.00 (2H, m), 3.37 (1H, t, $J = 8.0$ Hz, H22), 2.32 (1H, p, $J = 7.0$ Hz, H2), 2.28 – 2.23 (1H, m, H4), 2.20 (1H, dd, $J = 19.4$, 9.4, *H*10), 2.13 – 2.07

(2H, m, H13), 1.78 (1H, dd, $J = 14.5$, 3.2 Hz, H6), 1.73 – 1.63 (2H, m, H8, 11-OH), 1.55 (2H, d, $J = 14.9$ Hz, H1, H7), 1.53 – 1.46 (1H, m, H13), 1.46 (3H, s), 1.44 – 1.37 (2H, m, H15, H8), 1.19 (3H, s), 1.15 (1H, td, $J = 14.2$, 4.5 Hz, H18), 0.89 (3H, d, $J = 7.0$ Hz, H17), 0.78 (3H, d, $J = 7.1$ Hz, H16). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 216.68(C3), 165.83 (C21), 165.25, 163.33, 146.49, 144.56, 143.92, 139.24(C19), 138.72, 117.33(C20), 74.59(C11), 71.09(C14), 58.04(C4), 45.41(C9), 44.63 (C13), 44.01(C12), 41.90(C5), 36.64(C6), 36.02(C10), 35.04, 34.40 (C2), 30.37(C8), 26.82(C7), 26.44(C18), 24.81(C1), 16.75(C16), 14.75 (C15), 11.43(C17); HR-MS (ESI): Calcd for $C_{29}H_{37}N_3O_5S$ ($M+Na^+$): 563.2298; Found: 563.2289.

4.3. In vitro efficacy of pleuromutilin derivatives

4.3.1. Minimal inhibitory concentration (MIC) testing and minimum bactericidal concentration (MBC) testing

The minimal inhibitory concentrations MIC of the target compounds against methicillin-resistant *S. aureus* (ATCC 43300), *S. aureus* (ATCC 29213), *S. aureus* (AD3) and *S. aureus* (1 4 4) were determined using tiamulin as positive controls. MIC values were determined by broth dilution in accordance with the Clinical and Laboratory Standards Institute (CLSI). Stock solutions of these compounds were made a stock solution with a concentration of 1280 mg/mL by ultrapure-water and Tween 80. The working solutions (320 mg/mL) of these compounds were made by diluting stock solutions in sterile Mueller-Hinton (MH) broth. The amount of inoculation in each well was 5×10^5 CFU/mL, and a series of diluents of 2 times were prepared in Mueller-Hinton broth medium (MHB). There were three parallel experiments for each compound concentration. The plate was incubated at 37 ℃ for 24 h, and the MIC value was recorded as the lowest inhibitory concentration of the sample on the visible growth of the tested bacteria.

After obtaining the MIC value, the 96-well plate was incubated at 37 ℃ in 5% CO_2 atmosphere for 24 h, and the MBC was determined by inoculating the borehole bacterial solution from no obvious bacterial growth on Mueller-Hinton Agar plate (MHA). Next, the MH agar plate was further cultured at 37 ℃ for 24 h according to previous reference, and the lowest concentration of the compound required to reduce the bacterial count by more than 99.9% on the plate was MBC.

4.3.2. Constant concentration time-kill curves

The bactericidal activities of compounds **118** and **133** were determined by the constant concentration time-kill curve as reported in our previous work. MRSA (ATCC 43300) grown in Muller-Hinton (MH) was diluted to about 1×10^6 CFU/mL and treated with compound **118**, compound **133** and tiamulin of 2 × MIC, 4 × MIC, 8 × MIC, 16 × MIC, 32 × MIC. Samples were taken 100 μL respectively from the subculturing inoculums at 0, 3, 6, 9, 12 and 24 h and serially diluted 10-flod with sterile normal saline. Then 25 μL of the dilutions were plated on sterile MH agar plates. The total bacterial CFU/mL on the plates were counted to calculate the bacterial colonies after incubated at 37 ℃ for 20 h. The time-kill curve was constructed by plotting $\log_{10}$ CFU/mL of bacteria counts versus time.

4.3.3. Determination of the postantibiotic effect (PAE)

The PAE of compound **118**, compound **133** and tiamulin against MRSA (ATCC 43300) were determined using time-kill methods according previous work.

At first, the MRSA suspension was diluted to 1×10^6 CFU/mL in MH broth as the culture medium. Then each test compound at final concentrations of 2 × MIC and 4 × MIC was added into test tubes that contained the culture solution. The test tubes were incubated at a 37 ℃ constant temperature vibration incubator for 2 h. After incubating, the test compounds were removed by centrifuging the tubes for 10 min at 3600 revolutions per minute, decanting and suspending in pre-warmed MH broth. The bacterial pellet was washed using sterile normal saline and then suspended in 5 mL MH broth in new tubes, diluted 1000-fold. The tubes were placed in 37 ℃ and the growth of bacteria colonies was recorded from each tube at 0, 1, 2, 4, 6 and 8 h. The sample solutions (100 μL) were diluted 10-fold by sterile normal saline and plated on MH agar plates. After spreading plates, the MH agar plates were incubated at 37 ℃ for 20 h. Finally, the colonies on the plates were counted. The PAE value was calculated by the equation $PAE = T_A - T_C$ (T_A is the time required for the bacteria in the test and control groups to increase by 1 $\log_{10}$ CFU/mL) and expressed in an hour.

4.4. In vivo efficacy of pleuromutilin derivatives

4.4.1. Neutropenic murine thigh infection model

Five-week-old, specific-pathogen-free (SPF), female ICR/Swiss mice weighing 24–26 g were used for the experiment. As described previously, Neutropenic was rendered ($<0.1 \times 10^9$/L) by injecting intraperitoneally with 150 mg/kg and 100 mg/kg cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, IN) 3–5 days and 1 day, before MASR infection. Each mouse was injected with 100 μL MRSA solution (approximately 10^7 CFU/mL) into the thigh to establish the thigh infection model 2 h before dosing. Then these mice were randomly divided into three groups (5 per group) and intravenously injected sterile normal saline, compound **133** (20 mg/kg), and tiamulin (20 mg/kg) as a control group and two experimental groups respectively. Mice were euthanized 24 h after intravenous injection. Thigh tissue of each mouse was removed, collected, weighed, and homogenized in 5 mL of iced saline. In the end, aliquots of five to six serial dilutions (10-fold) were plated in duplicate on MH agar.

This study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the South China Agricultural University.

4.5. Cytotoxicity assay

As previously described, cell viability was assessed using the MTT assay. The cell line used in the experiment was RAW 264.7 cells. The cells were seeded into 96-well plates at a density of 10^5 cells per well and incubated at 37 ℃ overnight. The cells were then treated with compounds **112**–**137** (8 mg/mL) and cultured at 37 ℃. After incubation for 16 h, the cells were incubated with 100 μL per well of MTT (0.5 mg/mL in PBS) for another 4 h at 37 ℃ under 5% CO_2. The medium was removed, and the remaining cells were dissolved in 150 μL DMSO for 30 min at 37 ℃. Finally, the absorbance of the samples at 540 nm was recorded by the microplate spectrophotometer (BIO-TEK Instrument Inc., USA)[4].

The RAW 264.7 cells were incubated by the same method. The compound **118,** compound **133** and tiamulin was chosen as the compound to be tested. The cells were then treated with the resulting solution with varying concentrations (2, 4, 6, 8, 16, 32 μg/mL) of these coumponds and cultured for 16 h incubation at 37 ℃. Then the cells were incubated with 100 μL per well of MTT (0.5 mg/mL in PBS) for another 4 h at 37 ℃ under 5% CO_2. The medium was removed, and the remaining cells were dissolved in 150 μL DMSO for 30 min at 37 ℃. Finally, the absorbance of the samples at 540 nm was recorded by the microplate spectrophotometer (BIO-TEK Instrument Inc., USA).

4.6. Molecular modeling

In order to reveal the binding modes of synthesized pleuromutilin analogs, docking was performed based on the crystal structure of *S. aureus* 50S ribosomal in complex with tiamulin (PDB ID code: 1XBP). The peptidyl transferase center (PTC) model was built that consists of all residues within 40 Å around the tiamulin in 5HL7. The binding site of tiamulin in 5HL7 was set to the docking position. All compounds were prepared with Avogadro 1.1.1, with a 5000 steps Steepest Descent as well as 1000 steps Conjugate Gradients geometry optimization using MMFF94 force field. Docking experiments were performed using

AutoDock[29] Vina and Pymol.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.104956.

References

[1] F.C. Leonard, B.K. Markey, Meticillin-resistant Staphylococcus aureus in animals: A review, Vet. J. 175 (1) (2008) 27–36, https://doi.org/10.1016/j.tvjl.2006.11.008.
[2] F. Rossi, L. Diaz, A. Wollam, D. Panesso, Y. Zhou, S. Rincon, A. Narechania, G. Xing, T.S.R. Di Gioia, A. Doi, T.T. Tran, J. Reyes, J.M. Munita, L.P. Carvajal, A. Hernandez-Roldan, D. Brandão, I.M. van der Heijden, B.E. Murray, P.J. Planet, G.M. Weinstock, C.A. Arias, Transferable Vancomycin Resistance in a Community-Associated MRSA Lineage, New Engl. J. Med. 370 (16) (2014) 1524–1531, https://doi.org/10.1056/NEJMoa1303359.
[3] J. Feng, A. Paparella, G. Booker, S. Polyak, A. Abell, Biotin Protein Ligase Is a Target for New Antibacterials, Antibiotics 5 (2016) 26, https://doi.org/10.3390/antibiotics5030026.
[4] Z. Zhang, K. Li, G.-Y. Zhang, Y.-Z. Tang, Z. Jin, Design, synthesis and biological activities of novel pleuromutilin derivatives with a substituted triazole moiety as potent antibacterial agents, Eur. J. Med. Chem. 204 (2020) 112604, https://doi.org/10.1016/j.ejmech.2020.112604.
[5] F. Kavanagh, A. Hervey, W.J. Robbins, Antibiotic Substances From Basidiomycetes: VIII. Pleurotus Multilus (Fr.) Sacc. and Pleurotus Passeckerianus Pilat, Proc Natl Acad Sci U S A 37 (9) (1951) 570–574, https://doi.org/10.1073/pnas.37.9.570.
[6] E. Llabani, R.W. Hicklin, H.Y. Lee, S.E. Motika, L.A. Crawford, E. Weerapana, P. J. Hergenrother, Diverse compounds from pleuromutilin lead to a thioredoxin inhibitor and inducer of ferroptosis, Nat. Chem. 11 (6) (2019) 521–532, https://doi.org/10.1038/s41557-019-0261-6.
[7] F. Schlünzen, E. Pyetan, P. Fucini, A. Yonath, J.M. Harms, Inhibition of peptide bond formation by pleuromutilins: the structure of the 50S ribosomal subunit from Deinococcus radiodurans in complex with tiamulin, Mol. Microbiol. 54 (2004) 1287–1294, https://doi.org/10.1111/j.1365-2958.2004.04346.x.
[8] K.S. Long, J. Poehlsgaard, L.H. Hansen, S.N. Hobbie, E.C. Böttger, B. Vester, Single 23S rRNA mutations at the ribosomal peptidyl transferase centre confer resistance to valnemulin and other antibiotics in Mycobacterium smegmatis by perturbation of the drug binding pocket, Mol. Microbiol. 71 (2009) 1218–1227, https://doi.org/10.1111/j.1365-2958.2009.06596.x.
[9] Y.Z. Tang, Y.H. Liu, J.X. Chen, Pleuromutilin and its derivatives-the lead compounds for novel antibiotics, Mini Rev. Med. Chem. 12 (2012) 53–61, https://doi.org/10.2174/138955712798868968.
[10] E. van Duijkeren, C. Greko, M. Pringle, K.E. Baptiste, B. Catry, H. Jukes, M. A. Moreno, M.C.M.F. Pomba, S. Pyorala, M. Rantala, M. Ru auskas, P. Sanders, C. Teale, E.J. Threlfall, J. Torren-Edo, K. Torneke, Pleuromutilins: use in food-producing animals in the European Union, development of resistance and impact on human and animal health, J. Antimicrob. Chemoth. 69 (8) (2014) 2022–2031.
[11] S. Rittenhouse, C. Singley, J. Hoover, R. Page, D. Payne, Use of the Surgical Wound Infection Model To Determine the Efficacious Dosing Regimen of Retapamulin, a Novel Topical Antibiotic, Antimicrob. Agents Chemother. 50 (11) (2006) 3886–3888, https://doi.org/10.1128/AAC.00183-06.
[12] Y.R. Lee, K.L. Jacobs, Leave it to Lefamulin: A Pleuromutilin Treatment Option in Community-Acquired Bacterial Pneumonia, Drugs 79 (17) (2019) 1867–1876, https://doi.org/10.1007/s40265-019-01219-5.
[13] G.-Y. Zhang, Z. Zhang, K. Li, J. Liu, B.o. Li, Z. Jin, Y.-H. Liu, Y.-Z. Tang, Design, synthesis and biological evaluation of novel pleuromutilin derivatives containing piperazine and 1,2,3-triazole linker, Bioorg. Chem. 105 (2020) 104398, https://doi.org/10.1016/j.bioorg.2020.104398.
[14] S.H. Derawey, M.N. Mosa, E.Q. Jasim, R.M.O. Hraishawi, Synthesis, characterization and antibacterial evaluation of 1,3,4-oxadiazole derivatives, Int. J. Res. Pharmaceut. Sci. 10 (2019) 2342–2350, https://doi.org/10.26452/ijrps.v10i3.1477.
[15] B.B. Kashid, P.H. Salunkhe, B.B. Dongare, K.R. More, V.M. Khedkar, A.A. Ghanwat, Synthesis of novel of 2, 5-disubstituted 1, 3, 4- oxadiazole derivatives and their in vitro anti-inflammatory, anti-oxidant evaluation, and molecular docking study, Bioorg. Med. Chem. Lett. 30 (12) (2020) 127136, https://doi.org/10.1016/j.bmcl.2020.127136.
[16] Y. Guo, T. Xu, C. Bao, Z. Liu, J. Fan, R. Yang, S. Qin, Design and synthesis of new norfloxacin-1,3,4-oxadiazole hybrids as antibacterial agents against methicillin-resistant Staphylococcus aureus (MRSA), Eur. J. Pharm. Sci. 136 (2019) 104966, https://doi.org/10.1016/j.ejps.2019.104966.
[17] M. Gao, J. Zeng, X. Fang, J. Luo, Z. Jin, Y. Liu, Y. Tang, Design, synthesis and antibacterial evaluation of novel pleuromutilin derivatives possessing piperazine linker, Eur. J. Med. Chem. 127 (2017) 286–295, https://doi.org/10.1038/s41557-019-0261-6.
[18] E. Yarmohammadi, H. Beyzaei, R. Aryan, A. Moradi, Ultrasound-assisted, low-solvent and acid/base-free synthesis of 5-substituted 1,3,4-oxadiazole-2-thiols as potent antimicrobial and antioxidant agents, Mol. Divers (2020), https://doi.org/10.1007/s11030-020-10125-y.
[19] K. Povilas, G. Birute, M. Vytautas, P. Ruta, G. Ramune, P. Rita, G. Philip, P. Alius, P. Vidmantas, Synthesis, ADMET Properties, and In Vitro Antimicrobial and Antibiofilm Activity of 5-Nitro-2-thiophenecarbaldehyde N-((E)-(5-Nitrothienyl) methylidene)hydrazone (KTU-286) against *Staphylococcus aureus* with Defined Resistance Mechanisms, Antibiotics 9 (2020), https://doi.org/10.3390/antibiotics9090612.
[20] M.H. Hannoun, M. Hagras, A. Kotb, A.M.M. El-Attar, H.S. Abulkhair, Synthesis and antibacterial evaluation of a novel library of 2-(thiazol-5-yl)-1,3,4-oxadiazole derivatives against methicillin-resistant Staphylococcus aureus (MRSA), Bioorg. Chem. 94 (2020), 103364, https://doi.org/10.1038/s41557-019-0261-6.
[21] X. Zuo, X. Fang, Z. Zhang, Z. Jin, G. Xi, Y. Liu, Y. Tang, Antibacterial Activity and Pharmacokinetic Profile of a Promising Antibacterial Agent: 22-(2-Amino-phenylsulfanyl)-22-Deoxypleuromutilin, Molecules 25 (2020) 878, https://doi.org/10.3390/molecules25040878.
[22] Z. Jin, L.e. Wang, H. Gao, Y.-H. Zhou, Y.-H. Liu, Y.-Z. Tang, Design, synthesis and biological evaluation of novel pleuromutilin derivatives possessing acetamine phenyl linker, Eur. J. Med. Chem. 181 (2019) 111594, https://doi.org/10.1016/j.ejmech.2019.111594.
[23] S. Dreschers, C. Platen, A. Ludwig, C. Gille, N. Köstlin, T. Orlikowsky, Metalloproteinases TACE and MMP-9 Differentially Regulate Death Factors on Adult and Neonatal Monocytes After Infection with Escherichia coli, Int. J. Mol. Sci. 20 (2019) 1399, https://doi.org/10.3390/ijms20061399.
[24] N.A. Scott, A. Andrusaite, P. Andersen, M. Lawson, C. Alcon-Giner, C. Leclaire, S. Caim, G. Le Gall, T. Shaw, J.P.R. Connolly, A.J. Roe, H. Wessel, A. Bravo-Blas, C. A. Thomson, V. Kästele, P. Wang, D.A. Peterson, A. Bancroft, X. Li, R. Grencis, A. M. Mowat, L.J. Hall, M.A. Travis, S.W.F. Milling, E.R. Mann, Antibiotics induce sustained dysregulation of intestinal T cell immunity by perturbing macrophage homeostasis, Sci. Transl. Med. 10 (464) (2018) eaao4755, https://doi.org/10.1126/scitranslmed.aao4755.
[25] K. Morikawa, H. Watabe, M. Araake, S. Morikawa, Modulatory effect of antibiotics on cytokine production by human monocytes in vitro, Antimicrob. Agents Ch. 40 (1996) 1366–1370, https://doi.org/10.1016/j.ejps.2020.105596.
[26] E.T. Da Silva, G.F. de Andrade, A.D.S. Araújo, M.C.S. Lourenço, M.V.N. de Souza, Antibacterial activity of new substituted 4-N-alkylated-2-trifluoromethyl-quinoline analogues against sensitive and resistant Mycobacterium tuberculosis strains, Eur. J. Pharm. Sci. 157 (2021), 105596, https://doi.org/10.1038/s41557-019-0261-6.
[27] P. Cos, A.J. Vlietinck, D.V. Berghe, L. Maes, Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept', J. Ethnopharmacol. 106 (3) (2006) 290–302, https://doi.org/10.1016/j.jep.2006.04.003.
[28] F.A. Khan, A.U. Rehman, M.A. Abbasi, S.G. Afridi, A. Khan, M.A. Lodhi, A. Khan, Structural basis of binding and justification for the urease inhibitory activity of acetamide hybrids of N-substituted 1,3,4-oxadiazoles and piperidines, J. Mol. Struct. 1223 (2021) 129141, https://doi.org/10.1016/j.molstruc.2020.129141.
[29] O. Trott, A.J. Olson, AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, J. Comput. Chem. (2009) NA–NA. https://doi.org/10.1002/jcc.21334.