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Research paper

Design, synthesis and antibacterial evaluation of novel pleuromutilin derivatives possessing piperazine linker

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ABSTRACT

A series of pleuromutilin derivatives bearing piperazine ring have been reported. The *in vitro* antibacterial activities of the synthetic derivatives against MRSA (ATCC 43300), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Enterococcus faecium* (ATCC35667) and *Escherichia coli* (ATCC25922) were evaluated by the broth dilution method. Most of the synthesized derivatives displayed potent activities. Compounds **11c**, **12a** and **12c** were found to be the most active antibacterial derivatives against MRSA (minimum inhibitory concentration = 0.015 μg/mL). The binding of compounds **11c**, **12a** and **12c** to the 50s ribosome were investigated by molecular modeling. Compound **11c** possessed lower binding free energy compared with compounds **12a** and **12c**. Compound **11c** was further evaluated in MRSA systemic infection model and displayed superior *in vivo* efficacy to that of tiamulin.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) are those strains of *S. aureus* that display intrinsic resistance to methicillin and other beta-lactam antibiotics, such as oxacillin, nafcillin and carbapenems. The emergence of MRSA infection has led to higher costs, longer hospitalization courses, as well as higher morbidity and mortality [1]. MRSA infection is now commonly acquired not only in hospital but also in community setting and its prevalence continues to increase in both hospital and community setting [2]. Although, there are several antibiotics have been proven effective for management of MRSA infection, concerns regarding the emergence of widespread resistance in MRSA to these antibiotics, including vancomycin [3], linezolid [4] and daptomycin [5], have also been raised. It is therefore urgent to discover and develop new antibiotics agents with novel mode of action against MRSA.

Pleuromutilin (**1**, Fig. 1), a tricyclic diterpenoid natural product, was first isolated from cultures of two basiomycetes species, *Pleurotus mutilis* and *Pleurotus passeckerianus*, in 1951 [6]. Pleuromutilin has good antibacterial activity *in vitro* against Gram-positive bacteria and mycoplasmas but insufficient potency *in vivo* [6,7]. Further studies demonstrated that the pleuromutilin class of antibiotics inhibits bacterial protein synthesis by interacting with the 23S rRNA of the 50S subunit of prokaryotic ribosomes [8]. Thus, the pleuromutilin class possesses rarely resistance with other classes of clinically used antibacterial drugs [9]. Additionally, this distinct mechanism of action of pleuromutilin also made it an attractive lead compound in the discovery and development of novel antibiotics to combat drug-resistant bacterial infections [10–12].

Thousands of semisynthetic pleuromutilin analogues have been made and evaluated to improve the antibacterial activities and *in vivo* efficacy after the clarification of the structure of pleuromutilin [13–15]. This has led to the discovery of tiamulin [16,17] (**2**, Fig. 1) and valnemulin [18] (**3**, Fig. 1) which were approved as therapeutic agents for veterinary clinical use. Further chemical modifications of pleuromutilin have been made for an attempt to discover an agent for human use after the discovery of tiamulin and valnemulin. These efforts resulted in the discovery of azamulin (**4**, Fig. 1), which entered phase I clinical trials in volunteers in 1980s [19,20]. Unfortunately, azamulin did not undergo further clinical trials because of its limited bioavailability as well as its CYP450 inhibition [21]. In 2007, retapamulin (**5**, Fig. 1) was approved as a topical antibiotic for human use to treat impetigo [22]. Although pleuromutilin analogues containing the sulfide linkage display potent *in vitro* antibacterial activity, their oral bioavailabilities were usually limited for their strong hydrophobic nature [23]. To

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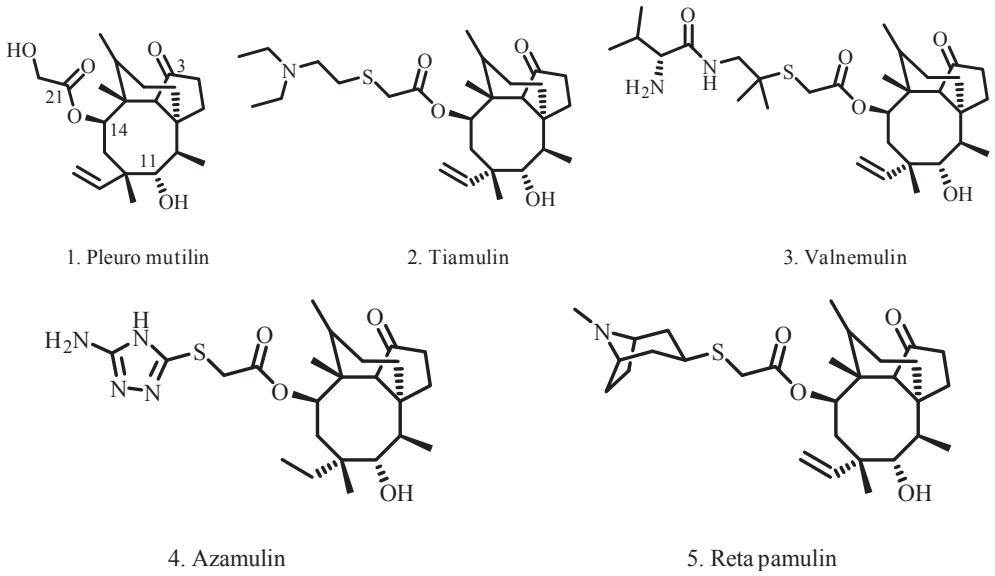


Fig. 1. Structure of pleuromutilin (1), tiamulin (2), valnemulin (3), azamulin (4) and retapamulin (5).

overcome this problem, a series of pleuromutilin derivatives having a piperazine ring linker had been prepared and evaluated by Hirokawa and coworkers [23,24]. Additionally, analogues having a triazole linker had been made and evaluated by Dreier and coworkers [25,26]. Considering those above pleuromutilin analogues with C14 side chains, in which without sulfide linkage, also possess good *in vitro* antibacterial activity, we have focused our efforts on pleuromutilin derivatives including nitrogen in the C14 side chains [27,28].

A general observation from our first series was that pleuromutilin derivatives bearing butyl amine group in the C14 side chain possessed moderate *in vitro* antibacterial activity [27]. In the second series, we identified the structurally novel pleuromutilin derivatives having a piperazine as linker and basic group. These pleuromutilin analogues displayed potent *in vitro* antibacterial activity against *S. aureus* (ATCC 29213) in our previous work [28]. As an extension to that study, we directed our efforts to explore the influence of the piperazine moiety as a linker in pleuromutilin analogues on the *in vitro* antibacterial activities. In the present study, we describe the design, synthesis, *in vitro* and *in vivo* antibacterial activities of novel pleuromutilin analogues bearing an additional piperazine ring.

2. Results and discussion

2.1. Chemistry

The general synthetic route of all pleuromutilin analogues is illustrated in Scheme 1. N-*tert*-butoxycarbonyl (Boc) -piperazine was used to react with compound **6** at first, the Boc group of the product was removed using TFA/DCM to yield compound **7**. Compound **8** was directly prepared by condensation reactions between the amino group of compound **7** and the acyl chloride group of chloracetyl chloride. Pleuromutilin derivatives **9**, **10**, **11a**-**11c**, **12a**-**12c**, **13a**-**13c**, **14a**-**14c** and **15a**-**15c** were prepared from the compound **8** which was synthesized in turn from compound **7** according to previous literature with modification [29].

2.2. Antibacterial activity

The MIC values of **7**, **8**, **9**, **10**, **11a**-**11c**, **12**a-**12c**, **13a**-1**3c**, **14a**-**14c** and **15a**-**15c** were tested against four Gram-positive bacteria, including ATCC 43300, ATCC 29213, ATCC 29212 and ATCC 35667 by the broth dilution methods. Pleuromutilin, tiamulin and valnemulin were used as positive controls. The results of these studies are summarized in Table 1.

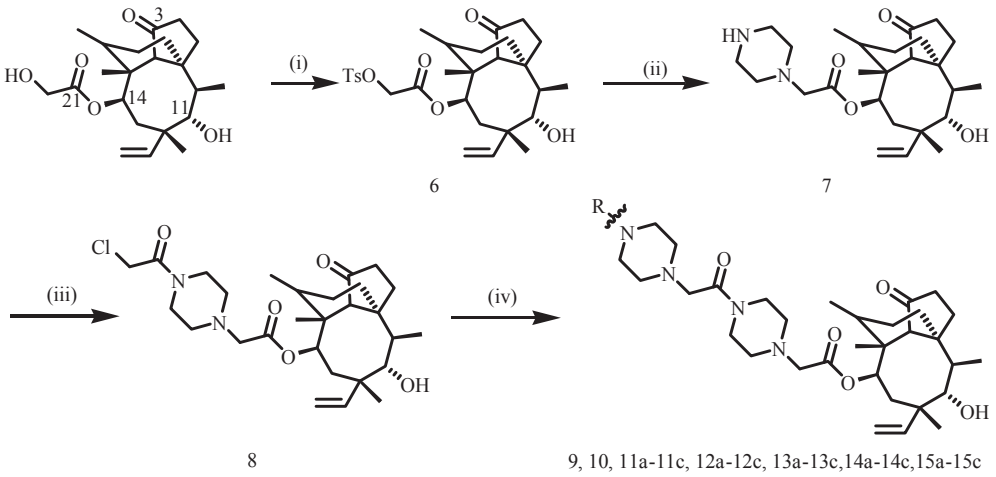
All target compounds showed potent activity against all four Gram-positive bacteria except *E. faecium* (ATCC 35667). All compounds possessed less potent against *E. faecalis* (ATCC 29212) than *S. aureus* (ATCC 43300 and ATCC 29213). It could be obtained that compounds **7** and **8** showed lower activities against *S. aureus* (ATCC 43300 and ATCC 29213) in comparison to that of tiamulin. Compound **9** possessed the same MIC value against MRSA and ATCC 29213 compared with tiamulin. The replacement of the methyl (R group in Table 1) of **9** with benzene ring resulted in compound **10**, which displayed nearly the same antibacterial activities compared with **9**. Considering that the substitution position in the aromatic ring have influence on the antibacterial activity of pleuromutilin analogues which bearing aromatic ring [10,28,30], further research on the types and position of benzene ring of **10** was carried on.

Compounds **13a**-**13c**, **14a**-**14c**, and **15a**-**15c**, in which the electron donating group was introduced on the benzene ring of **10**, were much less active against MRSA. All those mention compounds except **15a** possessed superior antibacterial activity against MRSA in comparison to that of tiamulin and compound **10**. To our surprise, all of compounds **11a**-**11c** and **12a**-**12c** presented improved activity against MRSA compared with the compound **10**. The results suggested that the aromatic substituents with electron withdrawing groups could be introduced to design new pleuromutilin derivatives with higher antibacterial activities in the future.

Compounds **11c**, **12a** and **12c** possessed the most antibacterial activities which were superior or the same antibacterial activities against MRSA and ATCC 29213 to that of tiamulin. The MIC values have demonstrated that **11c**, **12a** and **12c** might act as potent antibacterial agent against MRSA.

2.3. Molecular docking study

For they displayed excellent antibacterial activities, **11c, 12a** and **12c** were chosen for molecular docking investigations. Compound **11c** was found to exhibit a hydrogen bond with 50s ribosome in the docking studies. As shown in Fig. 2, the oxygen



Scheme 1. Reagent and conditions: (i) *p*-toluenesulfonyl chloride, ethyl acetate, NaOH, 0 °C, 3 h. (ii) a. N-Boc-piperazine, K_2CO_3, acetonitrile, 70 °C; b. TFA/DCM, room temperature. (iii) Chloroacetyl chloride, K_2CO_3, DCM. (iv) piperazine derivatives, K_2CO_3, acetonitrile, reflux.

atom of **11c** (on the nitro group in the 4-position of the benzene ring) formed one H-bound with the amino group of A2041 (O/NH distance: 1.84 Å). The binding free energy of **11c** with 50s ribosome was calculated to be −8.67 kcal/mol. Compound **12a** exhibited a hydrogen bond between its OH group (eight-membered ring) and C2565 at a distance of 2.13 Å (Fig. 3). Although, the antibacterial activity of **12a** was the same as **11c**, the binding energy of **12a** (−6.95 kcal/mol) was higher than that of **11c**. One possible reason was that the distance of the hydrogen bond formed between the OH group of **12a** and C2565 was longer than the distance of the hydrogen bond formed between **11c**'s nitro group and A2041.

The molecular docking results of **12c** were shown in Fig. 4. Compound **12c** and **12a** displayed the similar docking mode. One hydrogen bond was formed between the OH group (eight-membered ring) of **12c** with C2565 with distance of 2.09 Å. Compound **12c** (−8.11 kcal/mol) exhibited a lower binding free energy than that of **12a** (−6.95 kcal/mol) while a higher binding free energy than that of **11c** (−8.67 kcal/mol), possibly because of the distance of the hydrogen bond formed by **12c** (OH/O distance: 2.09 Å) was longer than that of **11c** (O/NH distance: 1.84 Å) and shorter than that of **12a** (OH/O distance: 2.13 Å). The docking results of compounds **11c** suggested that the substituents with oxygen atom, which might form hydrogen bond with residues, could be introduced to produce pleuromutilin derivatives with excellent antibacterial activities as a potential drug design strategy.

2.4. In vivo efficacy of compound 11c

Considering compound **11c** showed potent *in vitro* activity against MRSA (MIC = 0.015 μg/ml) and possessed lower binding free energy compared with compounds **12a** and **12c**, the *in vivo* efficacy of **11c** and the reference agent tiamulin was examined in a systemic infection model in which mice were inoculated with MRSA. As shown in Fig. 5, intravenously administered with 40 mg/kg of test compounds, 11c provided significant protection (50%) while tiamulin provided weak protective effect with 10% survival against MRSA in the mouse systemic model. Thus, the *in vivo* efficacy of **11c** was higher than that of tiamulin. The result of *in vivo* efficacy demonstrated that **11c** was able to protect MRSA infection mice and might be a potent antibacterial drug candidate.

3. Conclusions

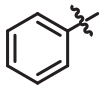
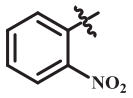
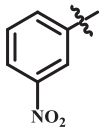
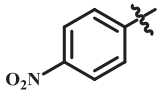
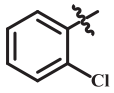
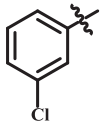
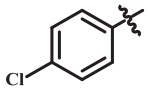
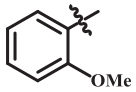
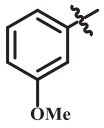
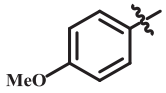
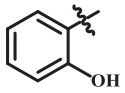
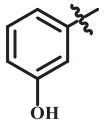
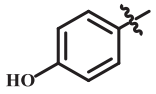
A series of novel pleuromutilin derivatives bearing piperazine ring have been reported. These new derivatives were investigated for their antibacterial activity *in vitro* against MRSA, *S. aureus*, *E. faecalis*, *E. faecium* and *E. coli*. The MIC values demonstrated that all the synthesized derivatives possessed potent antibacterial activity properties. Compounds **11c**, **12a** and **12c** possessed the most antibacterial activity against MRSA. Compounds **11c**, **12a** and **12c** were chosen for a molecular docking study. The results showed that the binding free energies were −8.67, −6.95 and −8.11 kcal/mol, respectively. Compounds **11c** was evaluated for *in vivo* efficacy using MRSA infected mouse model. It is interesting to note that compound **11c** exhibited superior *in vivo* efficacy to that of tiamulin against MRSA at the dose of 40 mg/kg in the mouse systemic infection model. The results indicated that the promising pleuromutilin derivative **11c** might serve as useful lead compound for further optimization and discovery of new antibiotics.

4. Experimental

4.1. Materials

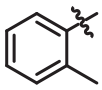
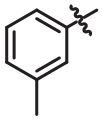
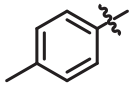
Pleuromutilin (>90% pure) was purchased from Great Enjoyhood Biochemical Co Ltd (Daying, China). Piperazine derivatives and chloracetyl chloride were obtained from J&K Scientific Ltd. The other reagents were all of analytical grade and purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). All reactions were monitored using thin-layer chromatography(TLC) on silica gel pre-coated plates. Purification of all compounds by column chromatography was carried out using silica gel (200–300 mesh, Branch of Qingdao Haiyang Chemical Co., Ltd., Shandong, China). Melting points were determined with a Shenguang X-4 apparatus (China) without corrected. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker AV-600 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) reported in hertz. Fourier transform infrared (FT-IR) spectra were obtained on a Nicolet 6700 Research FTIR spectrometer (Thermo Fisher) using KBr pellets, and the absorptions are reported in cm^{-1}. High-resolution mass spectra were obtained using a LTQ-Orbitrap

Table 1
MIC (μg/ml) for MRSA (ATCC 43300), *Staphylococcus aureus* (*S. aureus*) ATCC 29213, HN15 and SA1 (*S. aureus*, Guangdong isolated).

Compound	R	MRSA ATCC 43300	*S.aureus* ATCC 29213	*E.facalis* ATCC 29212	*E.facium* ATCC 35667
7		1	2	8	>32
8		>32	>32	>32	>32
9		0.125	0.5	2	>32
10		0.125	0.25	4	>32
11a		0.0625	0.0625	2	>32
11b		0.0625	0.25	2	>32
11c		0.015	0.125	1	>32
12a		0.015	0.5	4	>32
12b		0.03	0.25	2	>32
12c		0.015	0.125	2	>32
13a		0.5	0.5	4	>32
13b		0.25	0.25	2	>32
13c		0.25	0.5	2	>32
14a		0.5	1	2	>32
14b		0.25	0.5	2	>32
14c		1	2	8	>32

(*continued on next page*)

Table 1 (*continued*)

Compound	R	MRSA ATCC 43300	*S.aureus* ATCC 29213	*E.facalis* ATCC 29212	*E.facium* ATCC 35667
15a		0.0625	0.25	2	>32
15b		0.25	0.5	4	>32
15c		0.125	0.25	2	>32
pleuromutilin		2	8	16	>32
tiamulin		0.125	0.5	2	>32
valnemulin		0.03	0.125	1	>32
retapamulin		0.0625	0.0625		>32

mass spectrometer (Thermo Fisher) with an electro spray ionization(ESI) source. HPLC was carried out on an UltiMate 3000 separation system (Dionex, Germany).

4.2. Synthesis

A general synthesis strategy based on the usual 22-*O*-tosylpheuromutilin and piperazine derivatives were used (Scheme 1).

4.2.1. 22-*O*-tosylpleuromutilin (6)

A solution of pleuromutilin 1 (10.0 g, 26.42 mmol) in ethyl acetate (25.0 mL) was stirred at 0 °C in a three-necked round bottom flask, and *p*-toluenesulfonyl chloride (5.5 g, 29.06 mmol) was added. Sodium hydroxide (2.1 g, 52.84 mmol) was dissolved in 10 mL of water and dropped into the aforementioned solution. The mixture was then stirred at room temperature until complete consumption of pleuromutilin was obtained. $CHCl_3$(50 mL) and Ice-cold water (50 mL) were added to the solution. The organic phase was washed with brine and water. Then the organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a residue. The residue was precipitated from isopropanol to give a white solid (12.0 g, 85.71%).

4.2.2. 22-(Piperazine-1-yl)-22-deoxypleuromutilin (7)

Compound **6** (1 g, 1.87 mmol) was dissolved in acetonitrile (10 mL) and sodium iodide (0.31 g, 2.07 mmol) was added and stirred at 70 °C for 1 h. N-Boc-piperazine (0.38 g, 2.07 mmol) and potassium carbonate (0.52 g, 3.76 mmol) were added to the mixture and stirred again for 2 h at 70 °C. 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 oil. The crude oil was dissolved in 15 mL dichloromethane and 5 mL of trifluoroacetic acid was added and the mixture was stirred at room temperature for 8 h. The mixture was concentrated in vacuo to remove the trifluoroacetic acid and yield the crude product, which was purified by column chromatography (dichloromethane: methanol = 5:1) using silica gel to give the desired compound.

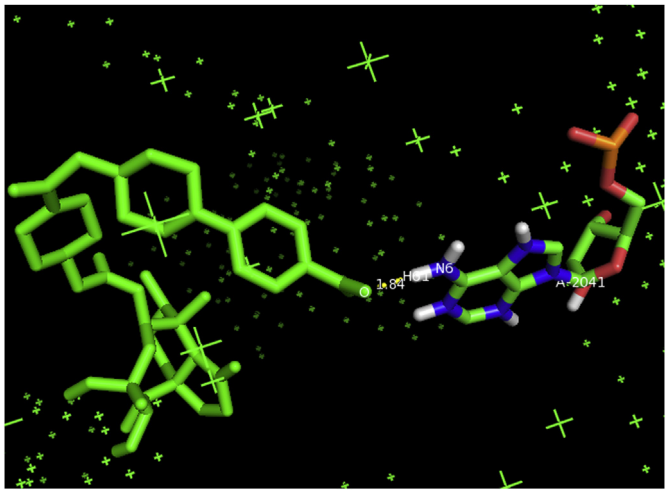


Fig. 2. Result of the docking of 11c (green) into the PTC model binding site. Residue numbers shown are according to *E. coli* 23s RNA numbering. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

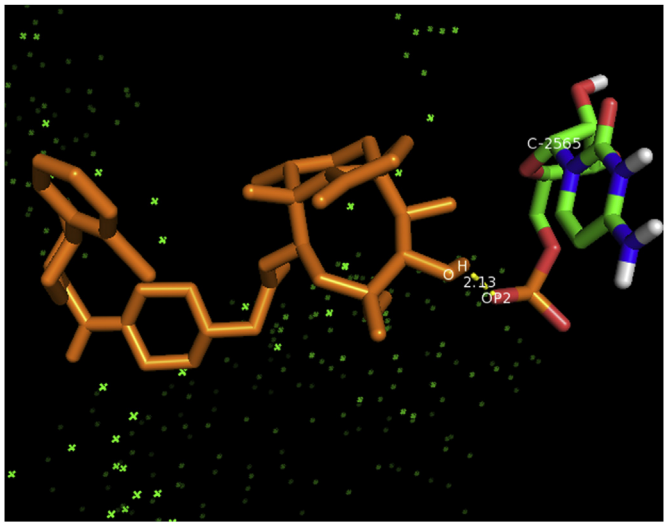


Fig. 3. Result of the docking of 12a (orange) into the PTC model binding site. Residue numbers shown are according to *E. coli* 23s RNA numbering. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

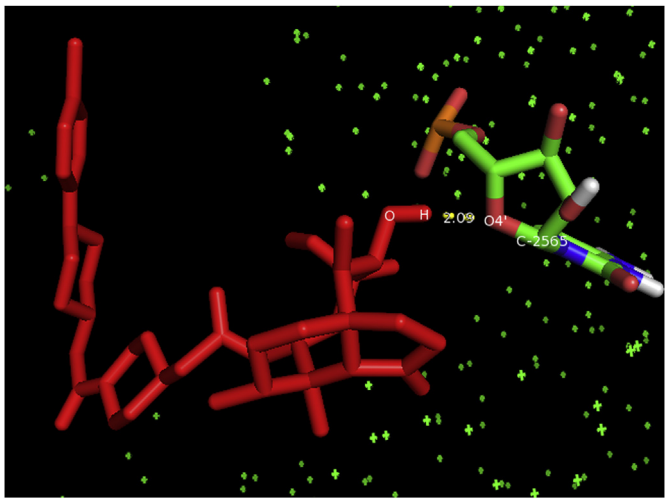


Fig. 4. Result of the docking of 12c (red) into the PTC model binding site. Residue numbers shown are according to *E. coli* 23s RNA numbering. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

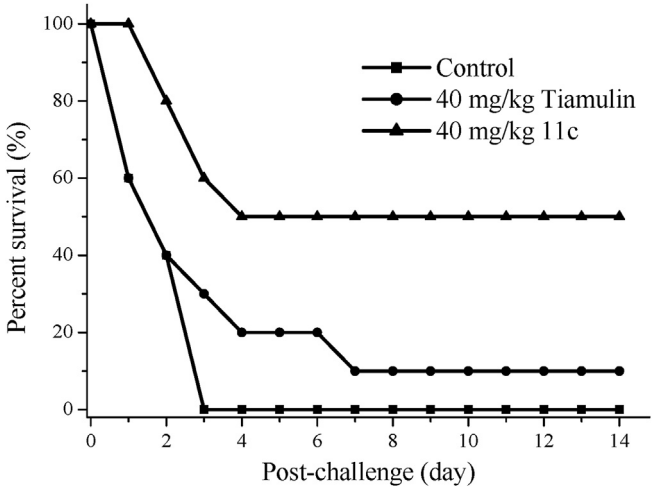


Fig. 5. Efficacy of compound **11c** and tiamulin in mouse systemic infection model.

White powder; Yield: 61.9%; m.p.: 56.7–57.2 °C; Purity of compound was 96.8% (determined by RP HPLC at 210 nm, t_R = 3.51 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.75 (2H, m), 7.22 (2H, d, *J* = 7.9 Hz), 6.46 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.77 (1H, d, *J* = 8.4 Hz, H14), 5.33 (1H, dd, *J* = 11.0, 1.6 Hz, H20), 5.19 (1H, dd, *J* = 17.3, 1.6 Hz, H20), 3.35 (1H, dd, *J* = 10.0, 6.6 Hz, H11), 3.15 (1H, d, *J* = 17.2 Hz, H22), 3.05 (1H, d, *J* = 17.1 Hz, H22), 2.38 (3H, s), 2.33 (1H, t, *J* = 7.0 Hz, H2), 2.16 (5H, m, H2, H4, H10, H14), 1.77 (1H, dd, *J* = 14.5, 3.2 Hz, H6), 1.56 (5H, m, H1, H7), 1.42 (3H, s, H15), 1.31 (3H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, dd, *J* = 14.1, 4.4 Hz, H8), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.68 (3H, d, *J* = 7.1 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 216.7 (C3), 168.4 (C21), 141.51, 139.1 (C19), 129.0, 117.2 (C20), 74.6 (C11), 68.8 (C14), 59.3, 58.2 (C4), 49.5 (C22), 45.4 (C9), 45.1 (C13), 44.0 (C12), 43.7, 41.8 (C5), 36.7 (C6), 36.1 (C10), 34.4 (C2), 30.4 (C8), 26.8 (C7), 26.4 (C18), 24.9 (C1), 16.7 (C16), 14.8 (C15), 11.4 (C17). IR (KBr, cm^{-1}) 3438, 2985, 2862, 1731, 1460, 1285, 1177, 1122, 1010, 910, 817. HR-MS (ESI): Calcd for $C_{26}H_{43}N_2O_4$ ($[M+H]^+$): 447.3217; Found: 447.3201.

4.2.3. 22-(4-(2-chloro-acetyl)-piperazine-1-yl)-22-deoxypleuromutilin (8)

Compound **7** (1.0 g, 2.24 mmol) and potassium carbonate (0.62 g, 4.48 mmol) were added into 10 mL of dichloromethane and stirred at −15 °C (ice-sodium chloride bath). Chloroacetyl chloride (0.19 mL, 2.46 mmol) was added dropwise and the resulting mixture were stirred for 2.5 h at room temperature until complete consumption of compound 7 was obtained. The reaction mixture was extracted with 30 mL of chloroform and washed with water. The organic layer was dried using anhydrous Na_2SO_4, filtered and evaporated in vacuum to yield the curde product, which was chromatographed on silica gel (petroleum ether: ethyl acetate = 1:1) to obtain the pure product. White power; Yield: 60.7%; m.p.: 57.5–58.0 °C; Purity of compound was 96.4% (determined by RP HPLC at 254 nm, t_R = 6.40 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 6.51 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.78 (1H, d, *J* = 8.5 Hz, H14), 5.33 (1H, dd, *J* = 11.0, 1.6 Hz, H20), 5.19 (1H, dd, *J* = 17.3, 1.7 Hz, H20), 3.35 (1H, dd, *J* = 9.9, 6.3 Hz, H11), 3.16 (1H, d, *J* = 17.1 Hz, H22), 3.03 (1H, d, *J* = 17.0 Hz, H22), 2.6 (4H, m), 2.34 (1H, t, *J* = 7.0 Hz, H2), 2.16 (4H, m, H2, H4, H10, H14), 1.77 (1H, dd, *J* = 14.6, 3.3 Hz, H6), 1.57 (4H, m, H1, H7), 1.44 (3H, s, H15), 1.31 (3H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, dd, *J* = 14.1, 4.2 Hz, H8), 0.87 (3H, d, *J* = 7.0 Hz, H17), 0.72 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.1 (C3), 169.1 (C21), 139.1 (C19), 117.2 (C20), 74.6 (C11), 68.3 (C14), 60.0, 58.2 (C4), 52.8 (C22), 45.5 (C9), 45.0 (C13), 44.0 (C12), 41.8 (C5), 36.8 (C6), 36.1 (C10), 34.5 (C2), 30.5 (C8), 26.8 (C7), 26.4 (C18), 24.9 (C1), 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3353, 2930, 2863, 1736, 1457, 1210, 1172, 1118, 1017, 917, 838, 647. HR-MS (ESI): Calcd for $C_{28}H_{44}ClN_2O_5$ ($[M+H]^+$): 523.2933; Found: 523.2938.

4.2.4. General procedure for the synthesis of compounds 9, 10, 11a-11c, 12a-12c, 13a-13c, 14a-14c and 15a-15c

Compound **8** (1 g, 1.91 mmol), piperazine derivatives (2.00 mmol) and potassium carbonate (0.53 g, 3.82 mmol) were added into acetonitrile (10 mL) and stirred at 70 °C until complete consumption of compound **8** was obtained. The reaction mixture was extracted with chloroform (30 mL) and then washed with water (30 mL) twice. The organic layer was dried with anhydrous Na_2SO_4. After evaporation the organic solvent in vacuum, the residue was purified by silica gel column chromatography to give a pure product.

4.2.5. 22-(4-(2-(4-methyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (9)

White powder; Yield: 40.8%; m.p.: 38.9–40.5 °C; Purity of compound was 96.4% (determined by RP HPLC at 219 nm, t_R = 7.20 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 6.50 (1H, m, H19), 5.79 (1H, t, *J* = 8.9 Hz, H14), 5.34 (1H, m, H20), 5.20 (1H, m, H20), 3.48 (4H, m), 3.34 (1H, m, H11), 3.19 (1H, d, *J* = 17.1 Hz, H22), 3.05 (1H, d, *J* = 17.1 Hz, H22), 2.54 (4H, m), 2.35 (1H, m, H4), 2.22 (2H, m, H2, H10), 2.08 (2H, m, H13), 1.77 (1H, dd, *J* = 14.5, 2.9 Hz, H6), 1.66 (2H, m, H1), 1.59 (7H, s, H7), 1.55 (2H, m), 1.45 (3H, s, H15), 1.37 (2H, m, H8, H13), 1.27 (2H, dd, *J* = 16.7, 9.7 Hz), 1.19 (1H, m), 1.16 (3H, d, *J* = 6.3 Hz, H18), 1.12 (1H, dd, *J* = 14.1, 4.3 Hz, H8), 0.89 (3H, d, *J* = 9.6 Hz, H17), 0.73 (3H, d, *J* = 9.4 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 216.8 (C3), 169.0 (C21), 154.6, 139.2 (C19), 117.1 (C20), 79.6, 74.6 (C11), 68.5 (C14), 65.8, 60.0, 58.2 (C4), 52.7 (C22), 45.5 (C9), 45.2 (C13), 44.0 (C12), 41.8 (C5), 36.8 (C6), 36.1 (C10), 34.4 (C2), 30.5 (C8), 28.4, 26.9 (C7), 26.4 (C18), 24.9 (C1), 16.7 (C16), 15.2, 14.9 (C15), 11.4 (C17). IR (KBr, cm^{-1}) 3479, 2930, 2865, 1732, 1673, 1456, 1434, 1366, 1280, 1250, 1193, 1170, 982, 862, 763. HR-MS (ESI): Calcd for $C_{33}H_{55}N_4O_5$ ($[M+H]^+$): 587.4167; Found: 587.4180.

4.2.6. 22-(4-(2-(4-phenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (10)

White powder; Yield: 77.3%; m.p.: 73.8–74.9 °C; Purity of compound was 97.4% (determined by RP HPLC at 254 nm, t_R = 3.82 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.28 (1H, t, *J* = 6.0 Hz), 6.92 (2H, d, *J* = 17.4 Hz), 6.49 (1H, m, H19), 5.79 (1H, dd, *J* = 8.7, 4.2 Hz, H14), 5.34 (1H, m, H20), 5.20 (1H, dd, *J* = 17.4, 2.4 Hz, H20), 3.64 (4H, m), 3.37 (4H, m), 3.23 (1H, m, H22), 3.10 (1H, m, H22), 2.57 (4H, m), 2.34 (1H, t, *J* = 6.9 Hz, H2), 2.16 (4H, m, H2, H4, H10, H14), 1.77 (1H, dd, *J* = 14.3, 3.2 Hz, H6), 1.58 (5H, m, H1, H7), 1.44 (3H, d, *J* = 3.0 Hz, H15), 1.30 (4H, m, H8, H13, 11-OH), 1.17 (3H, d, *J* = 3.1 Hz, H18), 1.12 (1H, dd, *J* = 14.1, 4.3 Hz, H8), 0.88 (3H, d, *J* = 6.9 Hz, H17), 0.72 (3H, d, *J* = 7.0, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (C21), 167.8, 151.2, 139.1 (C19), 129.1, 119.8, 117.3 (C20), 116.1, 74.6 (C11), 68.5 (C14), 65.8, 64.4, 61.3, 59.7, 58.2 (C4), 53.2, 52.7, 49.1 (C22), 46.1 (C9), 45.0 (C13), 44.0 (C12), 41.7 (C5), 41.7, 36.7 (C6), 36.1 (C10), 34.5 (C2), 31.6, 30.4 (C8), 26.8 (C7), 26.4 (C18), 24.9 (C1), 22.6, 16.7 (C16), 15.3, 14.9 (C15), 14.1, 11.5 (C17). IR (KBr, cm^{-1}) 3450, 2935, 2820, 1732, 1634, 1599, 1454, 1192, 1151, 1116, 1009, 917, 759, 693. HR-MS (ESI): Calcd for $C_{38}H_{57}N_4O_5$ ($[M+H]^+$): 649.4323; Found:649.4329.

4.2.7. 22-(4-(2-(2-Nitrophenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (11a)

Yellow powder; Yield: 51.8%; m.p.: 79.3–80.5 °C; Purity of compound was 97.1% (determined by RP HPLC at 215 nm, t_R = 7.53 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.76 (1H, d, *J* = 8.0 Hz), 7.48 (1H, d, *J* = 7.8 Hz), 7.13 (1H, d, *J* = 8.3 Hz), 7.05 (1H, t, *J* = 7.7 Hz), 6.50 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.79 (1H, d, *J* = 8.5 Hz, H14), 5.33 (1H, *J* = 11.0 Hz, H20), 5.20 (1H, d, *J* = 17.3 Hz, H20), 3.65 (4H, m), 3.38 (1H, m, H11), 3.25 (2H, s, H22), 3.09 (4H, m), 2.60 (8H, m), 2.35 (1H, t, *J* = 6.9 Hz, H2), 2.15 (5H, m, H2, H4, H10, H14), 1.78 (1H, m, H6), 1.58 (5H, m, H1, H7), 1.44 (3H, s, H15), 1.31 (3H, m, H8, H13, 11-OH), 1.17 (3H, s, H18), 1.12 (1H, dd, *J* = 14.1, 4.4 Hz, H8), 0.89 (3H, d, *J* = 7.0 Hz, H17), 0.72 (3H, d, *J* = 7.1 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.1 (C3), 168.9 (C21), 167.6, 145.8, 143.4, 139.1 (C19), 133.5, 125.9, 121.9, 121.1, 117.2 (C20), 74.6 (C11), 68.5 (C14), 64.3, 60.7, 59.7, 58.2 (C4), 53.1, 52.6, 51.5 (C22), 45.5 (C9), 45.4, 45.0 (C13), 44.03 (C12), 41.8 (C5), 41.6, 36.7 (C6), 36.1 (C10), 34.4 (C2), 30.4 (C8), 29.7, 26.8 (C7), 26.5 (C18), 25.3, 24.8 (C1), 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3450, 2934, 2882, 1732, 1642, 1604, 1521, 1455, 1381, 1292, 1192, 1154, 1117, 1009, 915, 668. HR-MS (ESI): Calcd for $C_{38}H_{56}N_5O_7$ ($[M+H]^+$): 694.4174; Found: 694.4174.

4.2.8. 22-(4-(2-(3-Nitrophenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (11b)

Yellow powder; Yield: 53.5%; m.p.: 71.6–72.8 °C; Purity of compound was 96.8% (determined by RP HPLC at 225 nm, t_R = 4.57 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.72 (1H, m), 7.65 (1H, dd, *J* = 7.9, 2.0 Hz), 7.38 (1H, t, *J* = 8.2 Hz), 7.21 (1H, m), 6.49 (1H, dd, *J* = 17.4, 10.9 Hz, H19), 5.79 (1H, t, *J* = 6.8 Hz, H14), 5.33 (1H, t, *J* = 10.6 Hz, H20), 5.20 (1H, dd, *J* = 17.4, 6.3 Hz, H20), 4.81 (1H, s), 4.03 (1H, m), 3.67 (3H, dd, *J* = 11.3, 6.2 Hz), 3.35 (1H, dd, *J* = 10.0, 5.9 Hz, H11), 3.30 (3H, t, *J* = 4.9 Hz), 3.26 (1H, d, *J* = 2.4 Hz), 3.21 (1H, m, H22), 3.09 (1H, m, H22), 2.83 (1H, d, *J* = 5.1 Hz), 2.69 (3H, t, *J* = 4.9 Hz), 2.57 (3H, m), 2.35 (1H, t, *J* = 6.6 Hz, H2), 2.17 (4H, m, H2, H4, H10, H14), 1.91 (1H, s), 1.77 (1H, m, H6), 1.58 (5H, m, H1, H7), 1.43 (3H, d, *J* = 3.6 Hz, H15) 1.32 (3H, m, H8, H13, 11-OH), 1.17 (3H, d, *J* = 5.4 Hz, H18), 1.12 (1H, d, *J* = 4.4 Hz, H8), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.71 (3H, d, *J* = 7.1 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.1 (C3), 168.9 (C21), 167.6, 151.7, 149.2, 139.1 (C19), 129.7, 121.2, 117.2 (C20), 113.8, 109.6, 74.6 (C11), 68.5 (C14), 64.3, 60.9, 59.7, 58.2 (C4), 53.1, 52.5, 48.5, 48.3 (C22), 45.4 (C9), 45.0 (C13), 44.0 (C12), 41.7 (C5), 39.7, 36.7 (C6), 36.1 (C10), 34.4 (C2), 30.4 (C8), 28.8, 26.8 (C7), 26.5 (C18), 25.3, 24.8 (C1), 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3445, 2935, 2862, 1732, 1635, 1526, 1455, 1346, 1290, 1242, 1193, 1153, 1116, 1009, 914, 866, 761, 738, 669. HR-MS (ESI): Calcd for $C_{38}H_{56}N_5O_7$ ($M + H^+$): 694.4174; Found: 694.4143.

4.2.9. 22-(4-(2-(4-Nitrophenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (11c)

Yellow powder; Yield: 49.3%; m.p.: 90.0–91.0 °C; Purity of compound was 97.7% (determined by RP HPLC at 215 nm, t_R = 9.25 min); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.28 (1H, d, *J* = 2.6 Hz), 8.15 (1H, m), 6.84 (1H, m), 6.48 (1H, m, H19), 5.80 (1H, m, H14), 5.34 (1H, m, H20), 5.21 (1H, dd, *J* = 17.3, 1.6 Hz, H20), 4.15 (1H, s), 3.64 (1H, m), 3.45 (1H, m), 3.36 (1H, dd, *J* = 10.4, 6.5 Hz, H11), 3.27 (1H, m, H22), 3.17 (1H, dd, *J* = 18.1, 10.4 Hz, H22), 2.66 (3H, s), 2.44 (1H, d, *J* = 3.4 Hz), 2.34 (1H, t, *J* = 6.7 Hz, H2), 2.16 (5H, m, H2, H4, H10, H14), 1.78 (1H, m, H6), 1.57 (5H, m, H1, H7), 1.43 (3H, d, *J* = 2.0 Hz, H15), 1.30 (3H, m, H8, H13, 11-OH), 1.17 (3H, s, H18), 1.13 (1H, m, H8), 0.89 (3H, dd, *J* = 7.0, 1.4 Hz, H17), 0.71 (3H, dd, *J* = 7.0, 2.9 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (C21), 167.4, 154.8, 139.1 (C19), 138.7, 125.9, 117.3 (C20), 112.8, 74.6 (C11), 68.5 (C14), 60.7, 59.7, 58.2 (C4), 53.0, 52.8, 52.5, 47.0 (C22), 46.1, 45.4 (C9), 45.0 (C13), 44.0 (C12), 41.7 (C5), 41.2, 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 29.7, 28.4, 26.8 (C7), 26.4 (C18), 25.4, 24.9 (C1), 21.3, 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3445, 2929, 2862, 1732, 1636, 1597, 1455, 1373, 1325, 1243, 1194, 1154, 1115, 1006, 916, 829, 754, 693, 666. HR-MS (ESI): Calcd for $C_{38}H_{56}N_5O_7$ ($[M+H]^+$): 694.4174; Found: 694.4182.

4.2.10. 22-(4-(2-(2-chlorophenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (12a)

White powder; Yield: 55.8%; m.p.: 96.8–97.0 °C; Purity of compound was 96.0% (determined by RP HPLC at 254 nm, t_R = 5.80 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.35 (1H, d, *J* = 7.9, 1.5 Hz), 7.04 (1H, m), 6.97 (1H, t, *J* = 7.6 Hz), 6.50 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.79 (1H, d, *J* = 8.4 Hz, H14), 5.34 (1H, m, H20), 5.20 (1H, dd, *J* = 17.4, 1.5 Hz, H20), 3.68 (4H, t, *J* = 5.2 Hz), 3.36 (1H, d, *J* = 6.0 Hz, H11), 3.26 (2H, s, H22), 3.21 (1H, d, *J* = 17.1 Hz), 3.09 (5H, m), 2.71 (4H, s), 2.56 (4H, m), 2.34 (1H, t, *J* = 7.0 Hz, H2), 2.16 (5H, m, H2, H4, H10, H14), 1.77 (1H, dd, *J* = 14.4, 3.1 Hz, H6), 1.59 (5H, m, H1, H7), 1.44 (3H, s, H15), 1.29 (3H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, dd, *J* = 14.1, 4.4 Hz, H8), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.72 (3H, d, *J* = 7.1 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (C21), 167.9, 149.1, 139.1 (C19), 130.7, 128.8, 127.5, 123.7, 120.4, 117.2 (C20), 74.6 (C11), 68.5 (C14), 65.8, 61.1, 59.7, 58.2 (C4), 53.3, 53.2, 52.7, 51.1 (C22), 45.4 (C9), 45.0 (C13), 44.0 (C12), 41.7 (C5), 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.5 (C18), 24.8 (C1), 16.7 (C16), 15.3, 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3450, 2935, 2819, 1734, 1639, 1453, 1230, 1192, 1153, 1121, 1010, 933, 751. HR-MS (ESI): Calcd for $C_{38}H_{56}ClN_4O_5$ ($[M+H]^+$): 683.3934; Found: 683.3933.

4.2.11. 22-(4-(2-(3-chlorophenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (12b)

White powder; Yield: 52.9%; m.p.: 56.1–57.5 °C; Purity of compound was 95.8% (determined by RP HPLC at 254 nm, t_R = 4.77 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.27 (1H, d, *J* = 1.4 Hz), 6.50 (1H, dd, *J* = 17.3, 10.9 Hz, H19), 5.78 (1H, d, *J* = 8.4 Hz, H14), 5.33 (1H, d, *J* = 10.9 Hz, H20), 5.19 (1H, d, *J* = 17.4 Hz, H20), 3.67 (4H, m), 3.36 (1H, m, H11), 3.23 (1H, d, *J* = 4.0 Hz, H22), 3.20 (6H, m), 3.07 (1H, m, H22), 2.65 (4H, t, *J* = 4.9 Hz), 2.55 (3H, m), 2.34 (1H, t, *J* = 7.0 Hz, H2), 2.16 (5H, m, H2, H4, H10, H14), 1.78 (3H, m, H6), 1.58 (5H, m, H1, H7), 1.43 (3H, d, *J* = 1.4 Hz, H15), 1.29 (3H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, dd, *J* = 14.2, 4.4 Hz, H8), 0.88 (3H, d, *J* = 6.9 Hz, H17), 0.71 (3H, d, *J* = 6.9 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (21), 167.7, 152.2, 139.1 (C19),

135.0, 130.0, 119.4, 117.3 (C20), 115.8, 114.0, 74.6 (C11), 68.5 (C14), 64.4, 61.2, 59.7, 58.2 (C4), 53.1, 52.9, 52.7, 48.7 (C22), 45.5 (C9), 45.1 (C13), 44.0 (C12), 41.7 (C5), 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 29.7, 26.8 (C7), 26.4 (C18), 25.4, 24.9 (C1), 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3451, 2934, 2824, 1732, 1640, 1594, 1453, 1383, 1192, 1152, 1116, 914, 770. HR-MS (ESI): Calcd for $C_{38}H_{56}ClN_4O_5$ ($[M+H]^+$): 683.3934; Found: 683.3940.

4.2.12. 22-(4-(2-(4-chlorophenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (12c)

White powder; Yield: 63.7%; m.p.: 79.2–80.0 °C; Purity of compound was 96.4% (determined by RP HPLC at 254 nm, $t_R = 4.56$ min); ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.29 (1H, s), 7.20 (1H, d, $J = 8.5$ Hz), 6.83 (1H, d, $J = 8.6$ Hz), 6.50 (1H, m, H19), 5.78 (1H, d, $J = 8.1$ Hz, H14), 5.33 (1H, t, $J = 9.7$ Hz, H20), 5.20 (1H, dd, $J = 17.4$, 5.8 Hz, H20), 4.79 (1H, m), 4.02 (1H, m)3.67 (3H, m), 3.36 (1H, s, H11), 3.24 (1H, d, $J = 3.9$ Hz, H22), 3.18 (4H, m), 3.07 (1H, m, H22), 2.79 (1H, t, $J = 4.9$ Hz), 2.66 (3H, t, $J = 4.8$ Hz), 2.55 (5H, m), 2.34 (1H, t, $J = 6.7$ Hz, H2), 2.16 (5H, m, H2, H4, H10, H14), 1.78 (1H, m, H6), 1.58 (5H, m, H1, H7), 1.43 (3H, s, H15), 1.32 (3H, m, H8, H13, 11-OH), 1.16 (3H, d, $J = 4.7$ Hz, H18), 1.11 (1H, dd, $J = 14.0$, 4.2 Hz, H8), 0.88 (3H, d, $J = 7.0$ Hz, H17), 0.71 (3H, d, $J = 6.2$ Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.1 (C3), 168.9 (C21), 167.7, 149.8, 139.1 (C19), 128.9, 124.6, 117.2 (C20), 117.2, 74.6 (C11), 68.5 (C14), 68.3, 64.3, 61.1, 59.9, 59.7, 58.2 (C4), 53.1, 53.3, 52.8, 52.6, 49.1 (C22), 45.4 (C9), 45.0 (C13), 44.0 (C12), 41.7 (C5), 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.4 (C18), 25.3, 24.8 (C1), 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3451, 2935, 2882, 1732, 1596, 1496, 1454, 1383, 1304, 1237, 1192, 1151, 1116, 1009, 916, 819. HR-MS (ESI): Calcd for $C_{38}H_{56}ClN_4O_5$ ($[M+H]^+$): 683.3934; Found: 683.3939.

4.2.13. 22-(4-(2-(2-methoxyphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (13a)

White powder; Yield: 43.8%; m.p.: 64.7–65.8 °C; Purity of compound was 95.7% (determined by RP HPLC at 254 nm, $t_R = 4.67$ min); ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.01 (1H, m), 6.94 (2H, m), 6.87 (1H, dd, $J = 8.0$, 1.2 Hz), 6.51 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.80 (1H, d, $J = 8.5$ Hz, H14), 5.34 (1H, dd, $J = 11.0$, 1.6 Hz, H20), 5.21 (1H, dd, $J = 17.4$, 1.6 Hz, H20), 3.69 (4H, m), 3.37 (1H, dd, $J = 10.4$, 6.5 Hz, H11), 3.29 (2H, m), 3.21 (1H, d, $J = 17.2$ Hz, H22), 3.12 (4H, s), 3.08 (1H, d, $J = 17.1$ Hz, H22) 2.76 (4H, s), 2.57 (4H, m), 2.35 (1H, t, $J = 6.9$ Hz, H2), 2.16 (5H, m, H2, H4, H10, H14), 1.78 (1H, dd, $J = 14.4$, 3.1 Hz, H6), 1.59 (5H, m, H1, H7), 1.45 (3H, s, H15), 1.30 (3H, m, H8, H13, 11-OH), 1.17 (3H, s, H18), 1.13 (1H, dd, $J = 14.0$, 4.3 Hz, H8), 0.89 (3H, d, $J = 7.0$ Hz, H17), 0.73 (3H, dd, $J = 108.5$, 7.0 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (C21), 167.8, 152.3, 141.1, 139.1 (C19), 123.0, 121.0, 118.2, 117.3 (C20), 111.3, 74.6 (C11), 68.5 (C14), 64.4, 61.2, 59.8, 58.2 (C4), 55.4, 53.3, 52.7, 50.5 (C22), 45.5 (C9), 45.0 (C13), 44.0 (C12), 41.7 (C5), 41.7, 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 29.7, 26.8 (C7), 26.4 (C18), 25.4, 24.9 (C1), 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3452, 2936, 2817, 1728, 1500, 1453, 1302, 1242, 1191, 1152, 1117, 915, 749. HR-MS (ESI): Calcd for $C_{39}H_{59}N_4O_6$ ($[M+H]^+$): 679.4429; Found: 679.4409.

4.2.14. 22-(4-(2-(3-methoxyphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (13b)

White powder; Yield: 46.1%; m.p.: 90.0–91.2 °C; Purity of compound was 97.1% (determined by RP HPLC at 254 nm, $t_R = 3.63$ min); ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.17 (1H, t, $J = 8.2$ Hz), 6.49 (1H, m), 6.46 (1H, t, $J = 2.4$ Hz, H19), 6.42 (1H, dd, $J = 7.9$, 2.3 Hz), 5.78 (1H, d, $J = 8.5$ Hz, H14), 5.33 (1H, dd, $J = 10.9$, 1.6 Hz, H20), 5.19 (1H, dd, $J = 17.4$, 1.6 Hz, H20), 3.79 (3H, s), 3.66 (4H, m), 3.35 (1H, dd, $J = 10.5$, 6.5 Hz, H11), 3.23 (1H, d, $J = 4.2$ Hz, H22), 3.19 (5H, m), 3.07 (1H, d, $J = 17.1$ Hz, H22), 2.66 (4H, t, $J = 5.0$ Hz), 2.54 (2H, m), 2.34 (1H, t, $J = 6.9$ Hz, H2), 2.18 (5H, m, H2, H4, H10, H14), 1.77 (1H, dd, $J = 14.5$, 3.1 Hz, H6), 1.54 (5H, m, H1, H7), 1.43 (3H, s, H15), 1.30 (3H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, dd, $J = 14.0$, 4.4 Hz, H8), 0.88 (3H, d, $J = 7.0$ Hz, H17), 0.71 (3H, d, $J = 7.0$ Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (C21), 167.8, 160.6, 152.6, 139.1 (C19), 129.8, 117.3 (C20), 108.9, 104.6, 102.6, 74.6 (C11), 68.5 (C14), 64.4, 61.3, 59.7, 58.2 (C4), 55.2, 53.1, 52.7, 49.0 (C22), 45.5 (C9), 45.0 (C13), 44.0 (C12), 41.7 (C5), 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 28.4, 26.8 (C7), 26.4 (C18), 25.4, 24.9 (C1), 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3440, 2936, 2830, 1732, 1642, 1496, 1454, 1374, 1291, 1259, 1203, 1168, 970, 839, 757, 690.HR-MS (ESI): Calcd for $C_{39}H_{59}N_4O_6$ ($[M+H]^+$): 679.4429; Found:679.4416.

4.2.15. 22-(4-(2-(4-methoxyphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (13c)

White powder; Yield: 55.7%; m.p.: 81.6–82.0 °C; Purity of compound was 96.2% (determined by RP HPLC at 215 nm, $t_R = 9.07$ min); ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.02 (1H, d, $J = 8.8$ Hz), 6.95 (1H, d, $J = 2.9$ Hz), 6.85 (2H, m), 6.78 (1H, dd, $J = 8.8$, 2.9 Hz), 6.50 (1H, dd, $J = 17.4$, 10.9 Hz, H19), 5.79 (1H, dd, $J = 8.6$, 3.0 Hz, H14), 5.34 (1H, m, H20), 5.20 (1H, m, H20), 3.89 (1H, d, $J = 4.8$ Hz), 4.03 (1H, m), 3.77 (3H, d, $J = 4.3$ Hz), 3.68 (2H, s), 3.60 (2H, s), 3.36 (1H, dd, $J = 10.6$, 6.5 Hz, H11), 3.27 (2H, s), 3.21 (1H, d, $J = 7.9$ Hz, H22), 3.10 (1H, dd, $J = 17.2$, 7.7 Hz, H22), 2.58 (3H, m), 2.34 (1H, t, $J = 7.0$ Hz, H2), 2.17 (5H, m, H2, H4, H10, H14), 1.78 (1H, dd, $J = 14.6$, 3.1 Hz, H6), 1.57 (5H, m, H1, H7), 1.44 (3H, d, $J = 2.4$ Hz, H15), 1.30 (3H, m, H8, H13, 11-OH), 1.17 (3H, d, $J = 2.4$ Hz, H18), 1.12 (1H, m, H8), 0.88 (3H, d, $J = 6.9$ Hz, H17), 0.72 (3H, d, $J = 7.1$ Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (C21), 167.8, 153.9, 145.6, 139.1 (C19), 118.2, 117.3 (C20), 114.5, 74.6 (C11), 68.5 (C14), 61.3, 59.8, 58.2 (C4), 55.6, 53.2, 52.7, 50.6 (C22), 45.5 (C9), 45.0 (C13), 44.0 (C12), 41.8 (C5), 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.4 (C18), 25.4, 24.9 (C1), 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3472, 2934, 2862, 1733, 1511, 1455, 1382, 1292, 1244, 1191, 1151, 1117, 1011, 915, 824, 668. HR-MS (ESI): Calcd for $C_{39}H_{59}N_4O_6$ ($[M+H]^+$): 679.4429; Found: 679.4434.

4.2.16. 22-(4-(2-(2-Hydroxyphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (14a)

White powder; Yield: 64.2%; m.p.: 104.0–106.1 °C; Purity of compound was 95.4% (determined by RP HPLC at 215 nm, $t_R = 8.67$ min); ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.15 (1H, dd, $J = 7.9$, 1.5 Hz), 7.08 (1H, m), 6.94 (1H, dd, $J = 8.2$, 1.5 Hz), 6.86 (1H, m), 6.51 (1H, dd, $J = 17.4$, 11.0 Hz, H19), 5.79 (1H, d, $J = 8.4$ Hz, H14), 5.34 (1H, dd, $J = 10.9$, 1.5 Hz, H20), 5.20 (1H, dd, $J = 17.3$, 1.6 Hz, H20), 4.03 (1H, m), 3.67 (4H, m), 3.36 (1H, dd, $J = 10.4$, 6.5 Hz, H11), 3.27 (2H, s), 3.22 (1H, d, $J = 17.2$ Hz, H22), 3.09 (1H, d, $J = 17.0$ Hz, H22), 2.91 (3H, t, $J = 4.8$ Hz), 2.70 (3H, s), 2.58 (3H, m), 2.35 (1H, t, $J = 7.0$ Hz, H2), 2.17 (5H, m, H2, H4, H10, H14), 1.78 (1H, dd, $J = 14.8$, 3.3 Hz, H6), 1.60 (5H, m, H1, H7), 1.45 (3H, s, H15), 1.35 (3H, m, H8, H13, 11-OH), 1.17 (3H, s, H18), 1.13 (1H, dd, $J = 14.1$, 4.4 Hz, H8), 0.88 (3H, d, $J = 7.1$ Hz, H17), 0.73 (3H, d, $J = 7.0$ Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (C21), 151.4, 139.1 (C19), 138.7, 126.6, 121.4, 120.1, 117.3 (C20), 114.2, 74.6 (C11), 68.5 (C14), 60.7, 59.7, 58.2 (C4), 53.6, 53.1, 52.6, 52.3 (C22), 45.4 (C9), 45.4, 45.0 (C13), 44.0 (C12), 41.8 (C5), 41.6, 36.7 (C6), 36.1 (C10), 34.4 (C2), 30.4 (C8), 29.7, 26.8 (C7), 26.4 (C18), 24.8 (C1), 16.7 (C16), 14.9 (C15), 14.2, 11.5 (C17). IR (KBr, cm^{-1}) 3450, 2934, 2882, 1732, 1604, 1521, 1455, 1381, 1343, 1292, 1192, 1154, 1117, 1009, 915, 668. HR-MS (ESI): Calcd for $C_{38}H_{57}N_4O_6$ ($[M+H]^+$): 665.4273; Found: 665.4271.

4.2.17. 22-(4-(2-(3-Hydroxyphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (14b)

White powder; Yield: 68.3%; m.p.: 35.6–35.8 °C; Purity of

compound was 96.1% (determined by RP HPLC at 215 nm, t_R = 6.32 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.07 (1H, t, *J* = 8.0 Hz), 6.46 (3H, m), 6.36 (1H, d, *J* = 7.9 Hz, H19), 5.78 (1H, d, *J* = 8.3 Hz, H14), 5.32 (1H, d, *J* = 10.8 Hz, H20), 5.19 (1H, d, *J* = 17.4 Hz, H20), 4.79 (1H, s), 4.12 (1H, t, *J* = 7.1 Hz), 3.66 (4H, d, *J* = 5.1 Hz), 3.49 (1H, t, *J* = 7.1 Hz), 3.37 (1H, d, *J* = 7.1 Hz, H11), 3.22 (2H, d, *J* = 11.2 Hz, H22), 3.16 (4H, m), 3.09 (1H, d, *J* = 17.2 Hz, H22), 2.64 (4H, t, *J* = 4.7 Hz), 2.54 (2H, m), 2.34 (1H, t, *J* = 7.0 Hz, H2), 2.15 (4H, m), 1.77 (1H, d, *J* = 14.5 Hz, H6), 1.60 (4H, m, H1, H7), 1.44 (3H, s, H15), 1.30 (3H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, d, *J* = 4.5 Hz, H8), 0.88 (3H, d, *J* = 6.9 Hz, H17), 0.71 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.4 (C3), 169.0 (C21), 168.0, 157.3, 152.6, 139.0 (C19), 129.9, 117.3 (C20), 108.1, 107.1, 103.4, 74.6 (C11), 68.6 (C14), 61.0, 59.7, 58.2 (C4), 53.0, 52.6 (C22), 49.0, 45.5 (C9), 45.1 (C13), 44.0 (C12), 41.8 (C5), 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.4 (C18), 24.8 (C1), 16.8 (C16), 15.0 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3437, 2936, 2823, 1731, 1629, 1453, 1194, 1153, 1117, 1009, 914, 866, 765, 690. HR-MS (ESI): Calcd for $C_{38}H_{57}N_4O_6$ ($[M+H]^+$): 665.4273; Found: 665.4276.

4.2.18. 22-(4-(2-(4-Hydroxyphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (14c)

White powder; Yield: 66.8%; m.p.: 103.0–103.5 °C; Purity of compound was 96.8% (determined by RP HPLC at 215 nm, t_R = 5.75 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 6.79 (4H, m), 6.49 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.78 (1H, d, *J* = 8.5 Hz, H14), 5.32 (1H, d, *J* = 10.9 Hz, H20), 5.19 (1H, d, *J* = 17.4 Hz, H20), 3.66 (4H, m), 3.49 (1H, q, *J* = 7.0 Hz), 3.37 (1H, d, *J* = 7.4 Hz, H11), 3.24 (1H, s, H22), 3.19 (1H, d, *J* = 17.2 Hz, H22), 3.06 (5H, m), 2.66 (4H, t, *J* = 4.8 Hz), 2.55 (3H, m), 2.34 (1H, t, *J* = 6.9 Hz, H4), 2.16 (5H, m, H2, H4, H10, H14), 1.77 (1H, m, H6), 1.57 (5H, m, H1, H7), 1.43 (3H, s, H15), 1.27 (3H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, m, H8), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.71 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.3 (C3), 168.9 (C21), 168.1, 150.6, 145.0, 139.0 (C19), 118.5, 117.3 (C20), 116.0, 74.6 (C11), 68.6 (C14), 65.9, 61.1, 59.7, 58.2 (C4), 53.2, 52.6, 50.8 (C22), 45.3 (C9), 45.0 (C13), 44.9, 44.0 (C12), 41.7 (C5), 36.7 (C6), 36.0 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.4 (C18), 24.8 (C1), 16.7 (C16), 15.2, 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3427, 2936, 2819, 1732, 1632, 1514, 1455, 1374, 1302, 1227, 1153, 1117, 1009, 916, 824, 723. HR-MS (ESI): Calcd for $C_{38}H_{57}N_4O_6$ ($[M+H]^+$): 665.4273; Found: 665.4277.

4.2.19. 22-(4-(2-(2-Methylphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (15a)

White powder; Yield: 46.7%; m.p.: 68.2–70.3 °C; Purity of compound was 96.7% (determined by RP HPLC at 225 nm, t_R = 6.15 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.00 (1H, m), 6.92 (2H, dd, *J* = 8.0, 2.0 Hz), 6.86 (1H, d, *J* = 8.0 Hz), 6.50 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.79 (1H, dd, *J* = 8.8, 5.2 Hz, H14), 5.34 (1H, m, H20), 5.20 (1H, m, H20), 4.72 (1H, d, *J* = 37.0 Hz), 4.01 (1H, m), 3.86 (2H, s), 3.68 (3H, m), 3.50 (1H, t, *J* = 5.1 Hz), 3.36 (1H, dd, *J* = 9.9, 6.2 Hz, H11), 3.24 (1H, d, *J* = 2.0 Hz, H22), 3.20 (1H, m, H22), 3.08 (4H, m), 2.70 (3H, s), 2.55 (3H, m), 2.35 (1H, t, *J* = 6.9 Hz, H2), 2.17 (5H, m, H2, H4, H10, H14), 1.91 (1H, s), 1.77 (1H, dd, *J* = 14.5, 3.2 Hz, H6), 1.57 (5H, m, H1, H7), 1.44 (3H, s, H15), 1.26 (3H, m, H8, H13, 11-OH), 1.17 (3H, d, *J* = 3.3 Hz, H18), 1.12 (1H, dd, *J* = 13.9, 4.2 Hz, H8), 0.88 (3H, d, *J* = 6.8 Hz, H17), 0.72 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.1 (C3), 168.9 (C21), 168.0, 141.2, 139.1 (C19), 123.0, 121.0, 117.2 (C20), 111.3, 74.6 (C11), 68.5 (C14), 64.3, 61.3, 58.2 (C4), 55.4, 53.4, 53.2, 52.8, 52.5, 50.6 (C22), 46.1, 45.5 (C9), 45.0 (C13), 44.0 (C12), 41.8 (C5), 41.6, 41.2, 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.4 (C18), 25.3, 24.8 (C1), 21.3, 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3445, 2935, 2862, 1733, 1500, 1455, 1382, 1373, 1302, 1270, 1241, 1192, 1153, 1118, 1011, 915, 750. HR-MS (ESI): Calcd for $C_{39}H_{59}N_4O_5$ ($[M+H]^+$): 663.4480; Found: 663.4485.

4.2.20. 22-(4-(2-(3-Methylphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (15b)

White powder; Yield: 60.6%; m.p.: 53.0–54.0 °C; Purity of compound was 95.9% (determined by RP HPLC at 254 nm, t_R = 4.31 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.15 (1H, t, *J* = 7.8 Hz), 6.73 (2H, dd, *J* = 11.2, 2.7 Hz), 6.69 (1H, d, *J* = 7.4 Hz), 6.50 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.78 (1H, d, *J* = 8.4 Hz, H14), 5.33 (1H, dd, *J* = 10.9, 1.6 Hz, H20), 5.19 (1H, dd, *J* = 17.4, 1.6 Hz, H20), 3.68 (4H, m), 3.35 (1H, dd, *J* = 10.7, 6.5 Hz, H11), 3.23 (2H, d, *J* = 4.0 Hz, H22), 3.19 (5H, m), 3.06 (1H, d, *J* = 17.1 Hz, H22), 2.66 (4H, t, *J* = 4.9 Hz), 2.53 (3H, m), 2.34 (1H, t, *J* = 6.9 Hz, H2), 2.18 (7H, m, H2, H4, H10, H14), 1.77 (1H, dd, *J* = 14.5, 3.1 Hz, H6), 1.54 (14H, m, H1, H7), 1.43 (3H, s, H15), 1.27 (4H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, dd, *J* = 14.1, 4.4 Hz, H8), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.71 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.0 (C3), 168.9 (C21), 167.8, 151.3, 139.1 (C19), 138.8, 129.0, 120.8, 117.3 (C20), 113.2, 74.6 (C11), 68.5 (C14), 64.4, 61.3, 59.8, 58.2 (C4), 52.9, 49.2 (C22), 45.3 (C13), 44.0 (C12), 41.8 (C5), 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.4 (C18), 25.4, 24.9 (C1), 21.8, 16.7 (C16), 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3484, 2932, 2863, 1733, 1602, 1494, 1455, 1366, 1307, 1192, 1170, 1117, 1013, 941, 846, 772, 693. HR-MS (ESI): Calcd for $C_{39}H_{59}N_4O_5$ ($[M+H]^+$): 663.4480; Found: 663.4470.

4.2.21. 22-(4-(2-(4-Methylphenyl-piperazin-1-yl)-acetyl)-piperazin-1-yl)-22-deoxypleuromutilin (15c)

White powder; Yield: 48.9%; m.p.: 87.1–88.4 °C; Purity of compound was 96.3% (determined by RP HPLC at 254 nm, t_R = 6.26 min); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.16 (2H, m), 6.99 (2H, m), 6.50 (1H, dd, *J* = 17.4, 11.0 Hz, H19), 5.79 (1H, d, *J* = 8.4 Hz, H14), 5.33 (1H, d, *J* = 10.8 Hz, H20), 5.19 (1H, d, *J* = 17.3 Hz, H20), 3.69 (4H, m), 3.36 (1H, s, H11), 3.25 (2H, s), 3.21 (1H, d, *J* = 17.2 Hz, H22), 3.08 (1H, d, *J* = 17.1 Hz, H22), 2.93 (4H, d, *J* = 4.9 Hz), 2.64 (4H, d, *J* = 26.4 Hz), 2.53 (4H, m), 2.34 (1H, t, *J* = 6.9 Hz, H2), 2.20 (7H, m, H2, H4, H10, H14), 1.77 (1H, d, *J* = 14.6 Hz, H6), 1.59 (4H, m, H1, H7), 1.44 (3H, s, H15), 1.27 (3H, m, H8, H13, 11-OH), 1.16 (3H, s, H18), 1.12 (1H, m, H8), 0.88 (3H, d, *J* = 7.0 Hz, H17), 0.72 (3H, d, *J* = 7.0 Hz, H16). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) 217.1 (C3), 168.9 (C21), 168.0, 151.3, 139.1 (C19), 132.6, 131.1, 126.5, 123.2, 118.9, 117.2 (C20), 74.6 (C11), 68.5 (C14), 65.8, 61.4, 59.8, 58.2 (C4), 53.6, 53.2, 52.7, 51.7 (C22), 45.5 (C9), 45.1 (C13), 44.0 (C12), 41.9, 41.9 (C5), 41.7, 36.7 (C6), 36.1 (C10), 34.5 (C2), 30.4 (C8), 26.8 (C7), 26.5 (C18), 24.9 (C1), 17.9, 16.7 (C16), 15.3, 14.9 (C15), 11.5 (C17). IR (KBr, cm^{-1}) 3456, 2937, 2862, 1734, 1642, 1599, 1493, 1456, 1375, 1302, 1268, 1192, 1152, 1116, 1011, 932, 822. HR-MS (ESI): Calcd for $C_{39}H_{59}N_4O_5$ ($[M+H]^+$): 663.4480; Found: 663.4488.

4.3. Minimum inhibitory concentration(MIC) testing

The MIC values of the target compounds against MRSA (ATCC 43300), *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Enterococcus faecium* (ATCC35667) and *Escherichia coli* (ATCC25922) were determined using pleuromutilin, tiamulin and valnemulin as positive controls. MIC values were determined by broth dilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) [31].

Stock solutions of these compounds were dissolved in methanol to make a stock solution with a concentration of 1280 μg/mL. The working solutions (320 μg/mL) of these compounds were made by diluting stock solutions in sterile Mueller-Hinton (MH) broth or Brain Heart Infusion (BHI) broth (for ATCC 29212 and ATCC 35667). The control test with the same medium as used in the MIC test was carried out to eliminate the effect of methanol on the growth of

bacteria.

The tested organisms were recovered and then incubated in broth medium for 18–24 h at 37 °C in 5% CO_2 atmosphere. Inocula was then prepared by transferring several colonies of the tested organisms to saline. The bacterial suspensions were mixed and corrected to 0.5 McF arland standard using saline. Further dilutions in saline were made to get the required working suspensions (10^6 CFU/mL).

The compounds (with a final concentration of 0.016–32 mg/mL) and bacteria solutions were dispensed into 96-well plate with total volume at 200 μL, the mixture were then cultured for 24 h at 37 °C. The minimum inhibitory concentration (MIC, μg/mL) of compounds is the lowest concentration of the compound that inhibit the visible growth of bacteria after overnight incubation. All dates were tested in duplicate in each plate. Two columns served as drug-free controls (no cultures were added in one column and drugs replaced by blank solvent in the other column). Pleuromutilin, tiamulin and valnemulin were used as positive controls against tested organisms. The final concentration of methanol in the first well column was 1.25%. Initially, preliminary analyses were conducted with 1.25% (v/v) methanol/broth and this did not affect neither the growth of the tested bacteria nor the determination of MIC.

4.4. Molecular modeling

AutoDock 4.2 was used for docking study. The crystal structure of 50S ribosomal subunit from *Deinococcus radiodurans* in complex with tiamulin (PDB ID: 1XBP) [32] was used to construct the starting model of the 50S ribosomal subunit. The ribosomal subunit used for docking was extracted from the crystal structure of 1XBP in which the tiamulin was removed. The starting model consists of all residues within 30 Å around the tiamulin in 1XBP. The docking position was set to the position of tiamulin in 1XBP. All compounds were prepared with Avogadro 1.1.1 [33], with a 5000 steps Steepest Descent as well as 1000 steps Conjugate Gradients geometry optimization using MMFF94 force field.

The binding affinity between pleuromutilin analogues and receptor were estimated using AutoDockTools. All the figures were performed using PyMol 1.8.0.4.

4.5. MRSA infection model

Institute of Cancer Research mice (female, the Medical Experimental Animal Center of Guangdong Province, Guangzhou, China), weighing between 23 and 25 g, were rendered neutropenic upon treatment with 150 mg/kg cyclophosphamide intraperitoneally 4 days prior to infection and with 100 mg/kg 1 day prior to infection. The neutropenic animals (10 per group) were inoculated intraperitoneally with 0.5 mL of an inoculum containing ~10^7 CFU/mL MRSA. Mice were then administered with the test compounds intravenously 1 h after infection at dose of 40 mg/kg. Tiamulin was used as a control in the same manner at the same doses as **11c**. Formulation of the test compounds was a filtered solution in 10% DMSO, 10% Tween-80, and 80% normal saline.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.01.004.

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