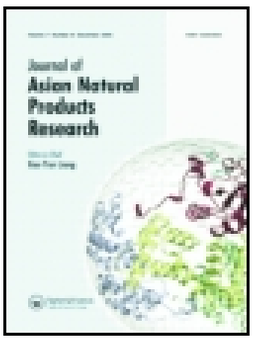

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Design, synthesis and antibacterial activity evaluation of pleuromutilin derivatives according to twin drug theory

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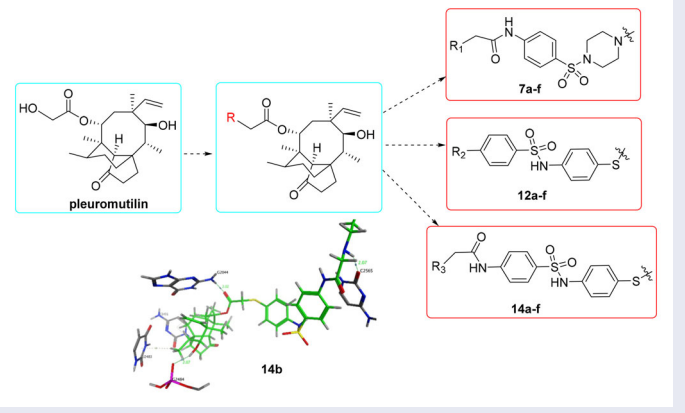
ABSTRACT

A series of novel pleuromutilin derivatives were designed and synthesized based on the twin drugs theory. Piperazinyl and thioether were used as the linkage to connect the pleuromutilin nuclear and sulfonamide to improve the biological activity and water solubility of derivatives. The *in vitro* antibacterial activities against drug-sensitive and drug-resistance bacteria were evaluated by agar dilution method. Most of the 25 compounds displayed excellent antibacterial activities against drug-sensitive bacteria, particularly, five compounds (**9**, **10**, **11**, **14a** and **14b**) exerted the excellent antibacterial activities against drug-resistance bacteria.



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Pleuromutilin derivatives; antibacterial activity; design; synthesis; twin drugs theory

1. Introduction

More and more drug-resistant pathogenic bacteria yielded in the past years because of the abuse of antibiotics, which resulted in many available drugs ineffective on those bacteria. More than two million people die from that each year and human

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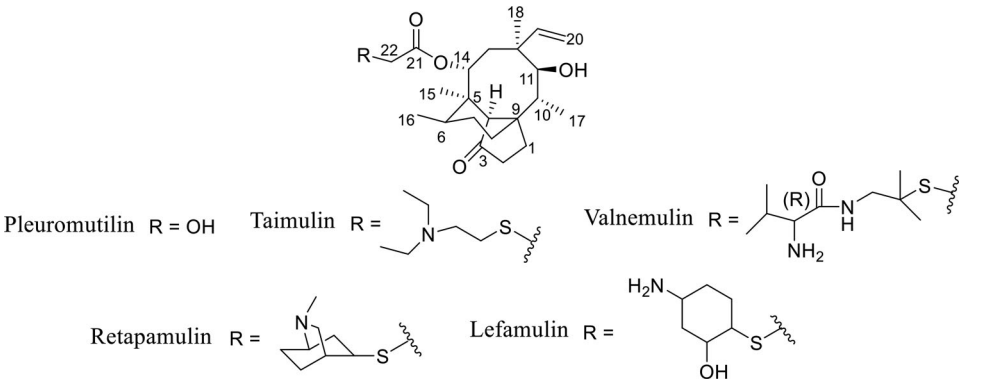

Figure 1. The structures of pleuromutilin and related drugs.

health was under dangerous severely [1]. Pleuromutilin and its derivatives showed great activities against the drug-resistant Gram-positive bacteria *in vitro* and had no target-specific cross-resistance to other antibiotics [2, 3].

Pleuromutilin was first isolated from *Basidiomycetes Pleurotusmutilus* in 1951 [4]. The unusual tricyclic diterpenoid structure of pleuromutilin can disturb the protein synthesis via interacting with the 50 s bacterial ribosome to exert its antibacterial activity against Gram-positive bacteria and mycoplasmas [5], therefore, it has become a lead compound of antibiotics. But pleuromutilin has many disadvantages, such as poor water solubility and pharmacokinetic properties, low oral bioavailability and mild toxicity, etc., so it is difficult to be developed directly as an antibacterial drug. In recent years, some new pleuromutilin derivatives were developed with higher antibacterial activities. According to the studies, the best design idea was to effectively modify the side chain at C-14 and the breakthroughs in antibacterial activity were made when both of the thioether and basic group were arranged in the same side chain. Further modifications within these groups led to the development of four drugs tiamulin, valnemulin, retapamulin and lefamulin (Figure 1). Tiamulin and valnemulin were approved as therapeutic agents for veterinary in 1974 and 1999, respectively. They display good antibacterial activities against mycoplasma and some gram-positive bacteria [6, 7]. In April 2007, retapamulin ointment (Altabax) was developed by Glaxo Smith Kline and approved by the Food and Drug Administration (FDA) for the treatment of skin and skin-structure infections (SSSIs) caused by *Staphylococcus aureus* and *Streptococcus pyogenes* infection. It became the first pleuromutilin approved for human use [8]. Lefamulin was approved by FDA on 19 August 2019 for the treatment of community-acquired bacterial pneumonia (CABP) [9]. The successful marketing of the above drugs further demonstrated the modification of C14 site was a great idea to develop drugs.

Piperazine was widely used in many antimicrobial drugs because of its good water solubility and electron-rich property, such as temafloxacin, piperaquine, delavirdine, norfloxacin and ketoconazole, etc. (Figure 2). We were inspired by those structures and eager to improve water solubility of pleuromutilin, and piperazine was also introduced to pleuromutilin as the linkage of C14 side chain in our previous work and some modified compounds showed good biological activity [10, 11].

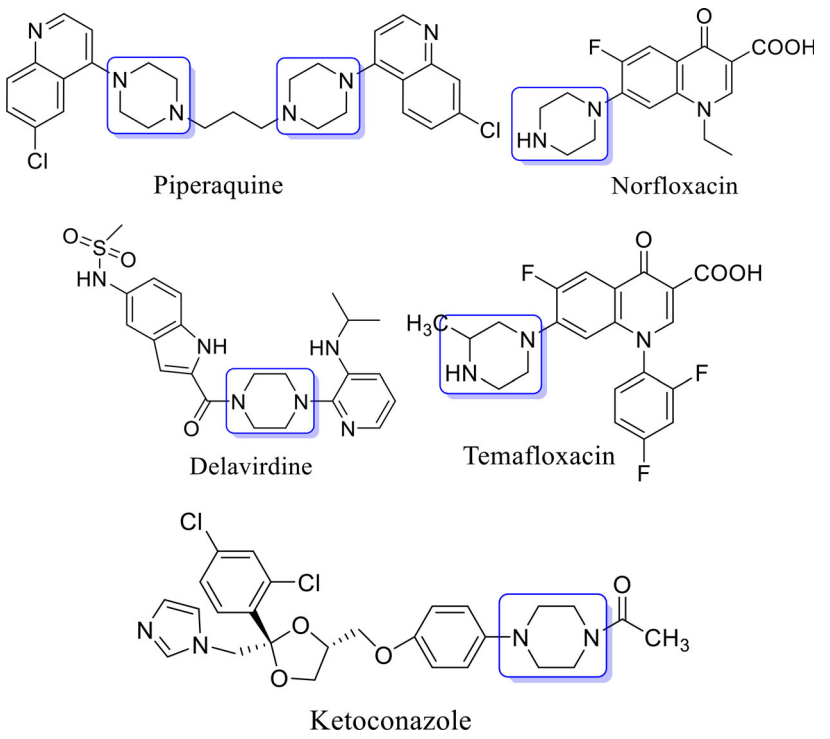

Figure 2. Antimicrobial drugs with piperazine rings.

In the 1970s, a number of pleuromutilin derivatives were synthesized and the structure-activity relationships (SAR) studies implied that introduction of thioether and alkaline group in the C14 side chain would improve antibacterial activity [6]. Beyond that, lots of studies have also confirmed this opinion. Such as Cheng-Hong Li's team reported the novel semisynthetic pleuromutilin analogues embracing 7H-pyrrolo[2,3-d]pyrimidine backbone in the C14 side chain [12]. A series of novel acetamide phenyl pleuromutilin derivatives incorporating 2-aminothiophenol moieties into the C14 side chain were synthesized by You-zhi Tang's team and one of them has potential drug-like properties for the treatment of MRSA infection [13].

The (SAR) studies showed that the ester group at C14, the carbonyl group at C3 and the hydroxyl group at C11 are all required for antibacterial activity, and the only valid modification site is C22 side chain on C14 group [14–16]. This can be confirmed by the four marketed drugs (tiamulin, valnemulin, retapamulin, lefamulin). So, in our study, the modification of pleuromutilin derivatives was focused on the hydroxy group of C22 that links in C14 side chain. A new class of pleuromutilin derivatives was designed and synthesized based on the previous SAR studies and twin drug theory. We spared no effort to link the benzene-sulfonamide group to piperazine and thioether group, and then the combined two groups were linked together with C14 side chain of pleuromutilin, which aims to improve water solubility and antibacterial activity of pleuromutilin. The design, synthesis as well as detailed antibacterial activity of these novel pleuromutilin derivatives will be reported and discussed in this paper.

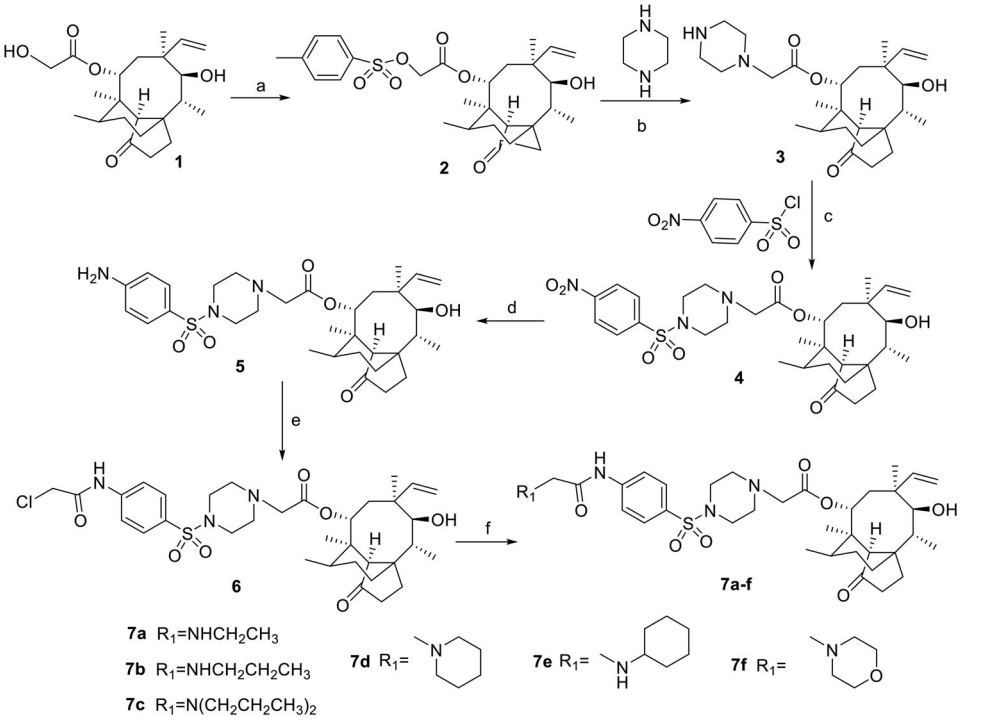

Figure 3. The synthetic rout of derivatives containing piperazinyl and benzenesulfonyl: (a) TsCl, triethylamine, DCM, r.t., (b) acetonitrile, r.f., (c) K_2CO_3, acetonitrile, r.f., (d) Fe powder, NH_4Cl, CH_3COOH, ethyl alcohol, 80 °C, (e) 2-chloroacetyl chloride, DCM, triethylamine, r.t., (f) alkylamine or arylamine, triethylamine, DMF, 100 °C.

2. Results and discussion

2.1. Chemistry

As shown in Figure 3, pleuromutilin (**1**) reacts with p-toluene sulfochloride in the presence of triethylamine in CH_2Cl_2 to provide 22-O-(p-toluenesulfonyl) pleuromutilin (**2**) in 52% yield. Compound **2** reacts with piperazine in acetonitrile to obtain compound **3**. Compound **4** was prepared by electrophilic addition of p-nitrobenzene sulfonyl chloride with **3** under a basic condition in 90% yield. Compound **4** was reduced to **5** by iron powder. Compound **5** reacts with chloroacetyl chloride to afford **6**, and then the novel derivatives were obtained after electrophilic substitution reactions of **6**.

The SAR studies indicated that thioether group has great value in improving the activity of pleuromutilin, so in the following compounds we retained the thioether group and it was built by p-aminothiophenol. p-Nitrobenzene sulfonyl chloride was connected to the end of the side chain as twin drug component. As shown in Figure 4, compound **2** was prepared in the same method as Figure 3. Compound **8** was obtained in the present of base by substitution reaction. The obtained crude product was treated with trifluoroacetic acid at room temperature for 8 hours to give compound **9**. Next, K_2CO_3 was employed to promote the condensation of p-nitrobenzene sulfonyl chloride with compound **9** in acetonitrile to yield **10**, and the nitryl of

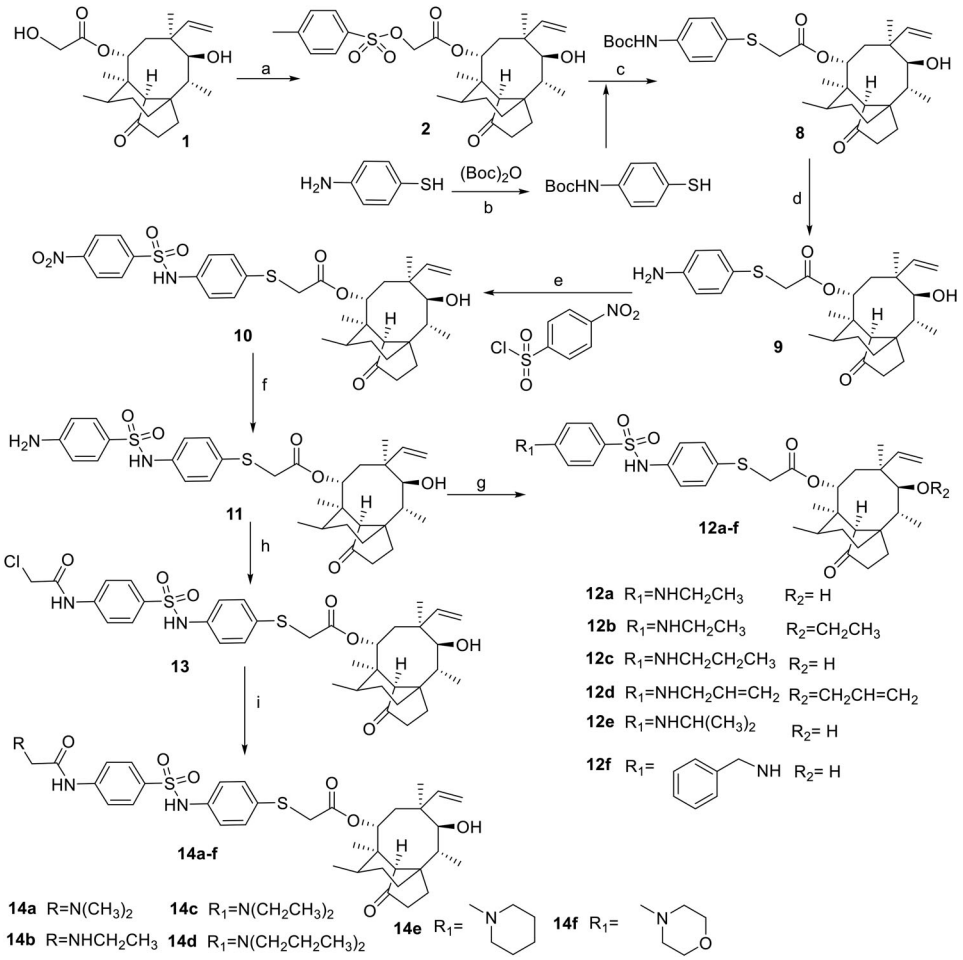

Figure 4. The synthetic rout of derivatives containing thioether and benzenesulfonyl: (a) TsCl, triethylamine, DCM, r.t., (b) $(Boc)_2O$, triethylamine, acetonitrile, 0 °C-r.t., (c) anhydrous K_2CO_3, acetonitrile, r.f., (d) CF_3COOH, DCM, r.t., (e) anhydrous K_2CO_3, acetonitrile, r.t., (f) Fe powder, NH_4Cl, CH_3COOH, ethyl alcohol, 80 °C, (g) halohydrocarbon, anhydrous K_2CO_3, DMF, 100 °C, (h) 2-chloroacetyl chloride, DCM, triethylamine, r.t., (i) alkylamine, anhydrous K_2CO_3, DMF, 100 °C.

10 was catalyzed by iron powder to obtain amino of compound **11**. Then compound **11** was reacted with some different halohydrocarbon in dimethylformamide (DMF) to give compounds **12a–f**. Compound **11** was also reacted with chloroacetyl chloride in the presence of triethylamine and CH_2Cl_2, and the obtained crude product was treated with K_2CO_3 and some different alkylamine in DMF to give compounds **14a–f**.

2.2. In vitro antibacterial activity

The antibacterial properties of 25 novel pleuromutilin derivatives **4**, **5**, **6**, **7a–f**, **9–11**, **12a–f**, **13** and **14a–f** were assessed *in vitro* by the broth microdilution method and

Table 1. The MIC value (μg/ml) of novel pleuromutilin derivatives.

Cpd. No.	*S. aureus* ATCC 25923	MRSA NY3	*S. epidermidis* ATCC 12228	*S. suis* ATCC 43765	*E. coli* CVCC 231
4	0.5	>64	0.06	>64	>64
5	0.06	>64	0.06	>64	>64
6	0.06	>64	0.06	>64	>64
7a	0.06	32	0.06	8	32
7b	0.25	32	0.25	32	64
7c	1	>64	1	>64	>64
7d	0.06	>64	0.06	>64	>64
7e	0.06	>64	0.25	>64	>64
7f	0.06	>64	0.25	>64	>64
9	0.03	4	0.03	>64	>64
10	0.125	2	0.06	>64	>64
11	0.06	4	0.06	>64	>64
12a	0.25	>64	0.25	>64	>64
12b	0.5	>64	1	>64	>64
12c	0.5	>64	0.5	>64	>64
12d	4	>64	4	>64	>64
12e	0.25	>64	0.25	>64	>64
12f	2	>64	1	>64	>64
13	0.25	32	0.25	64	64
14a	0.03	4	0.03	64	64
14b	0.03	2	0.03	64	8
14c	0.25	64	0.06	64	64
14d	0.25	64	0.25	64	64
14e	0.25	>64	0.25	64	64
14f	0.03	>64	0.06	64	64
Tiamulin fumaric	0.125	16	0.125	32	16
Erythromycin thiocyanate	0.125	8	0.125	0.5	16

determined in comparison with tiamulin fumaric and erythromycin thiocyanate. The MIC values are provided in Table 1. The data show that most of the tested compounds display good to excellent *in vitro* antibacterial activities against Gram-positive bacteria except *S. suis* to those of the positive controls. The MICs of 25 new pleuromutilin derivatives *in vitro* against MSSA and *S. epidermidis* are from $\geq 8\,\mu$g/ml to $0.03\,\mu$g/ml. Compound **5** displays better MIC value against MSSA compared with the control drugs, while compound **4** shows worse antibacterial activity against MSSA in comparison to that of compound **5**. This data demonstrate that derivatives containing amino group exhibit better activity compared to those with nitro group. The alkyl amino groups were connected on the benzene ring of compound **6** and resulted in compounds **7a–f**, and most of these derivatives possess no significant increase in activity against drug-resistant bacteria compared with both tiamulin and compound **8**. The MIC value of the most active compounds in this series is $0.06\,\mu$g/ml.

The activity of compound **9** was evaluated (bioactivities: **9** > **5**), and this observation suggests that the replacement of the piperazine on the starting position of the C14 side chain with diphenyl sulfide can increase the electron densities of C14 side chain of pleuromutilin derivatives, which resulted in compound **9** possessing higher antibacterial activities compared with compound **5**. The activity decreases when the amino group of diphenyl sulfide was replaced by p-toluensulfonyl chloride. The nitro group on benzene of compound **10** was reduced to amino group to obtain compound **11**, leading to increased activity, and it is proved that the alkaline center is the key factor to improve antibacterial activity. Subsequent derivatives **12a–f** exhibiting poor activity may be due to the steric hindrance. Unexpectedly, compounds **14a** and **14b**

show excellent antibacterial activity, their MIC against MSSA and MSSE are 0.03 μg/ml, compound **14f** (0.03 μg/ml) displayed 4-fold enhanced antibacterial activity against MSSA in comparison with that of tiamulin fumaric and erythromycin thiocyanate. It is worth mentioning that compounds **9**, **10**, **11**, **14a** and **14b** show the excellent activity (2 μg/ml to 4 μg/ml) to drug-resistance bacteria, 2–8 fold enhance than the control drugs. This result indicates the increase in the electron cloud density affects the binding strength of receptor pocket with pleuromutilin derivatives. We can see from **14a–f** that the antibacterial activity decreases with the increase of steric hindrance and increases with the increase of electron cloud density.

2.3. Molecular docking study

Crystal structure studies have proposed that pleuromutilin derivatives block peptide-bond formation directly by interfering with the substrate binding at the ribosome's peptidyl-transferase center (PTC) domain. Since their excellent anti-bacterial activity, we conducted the molecular docking research of **9**, **10**, **11**, **14a** and **14b**, and tiamulin was selected as the control. As shown in Figure 4, the molecular docking of **9** bearing p-amino thiophenol displays four interaction forces (Figure 5A), three of them are hydrogen bonds and formed between pleuromutilin nucleus and residues G2484 (1.65 Å) and G2044 (3.1 Å). The other one is H-π interaction, and it is formed between C14 side chain and residue A2045.

There are seven interaction forces between compound **11** (Figure 5C) and the active pocket, including four hydrogen bonds and three H-π interactions. It was found that OH on C11 of pleuromutilin had a strong hydrogen bond (1.91 Å) with residue C2565. It should be notable that two hydrogen bonds are formed between C14 side chain and active pocket, NH with U2485 (2.37 Å) and S = O with G2426 (2.22 Å), respectively. The calculation showed that the binding energy of **11** with ribosome is −9.78 kcal/mol, which is equivalent to **9** (−9.65 kcal/mol). In terms of **14a** (Figure 5D), there are three hydrogen bonds between compound and active pocket. One of the three hydrogen bond is formed between NH of acylamino and residues C2565 (2.24 Å), another one is formed between C22-S- and G2484 (3.83 Å) and the last one is formed between C22-H- and G2484 (2.35 Å). It is worth mentioning that thioether plays a role in the formation of hydrogen bonding. The CH between thioether and ester plays a crucial role, and it forms a hydrogen bond with G2484 at a distance of 2.35 Å. There are also three H-π interactions that are attached by the nuclear to the residue. Furthermore, the total score of the optimal conformation of **14a** is calculated to be −11.82 kcal/mol, which indicates that the affinity activity of **14a** with 23S rRNA is significantly higher than those of tiamulin (−8.62 kcal/mol) and compound **11** (−9.78 kcal/mol). Though compound **14a** owns fewer bonds, the lower binding energy may explain why it shows better *in vitro* antibacterial activity than others. It can be explained that why **14b** (Figure 5E) owns the equal antibacterial activity.

The docking research of the positive control compound tiamulin was also made to compare its binding mode with that of above docking compounds. The result showed

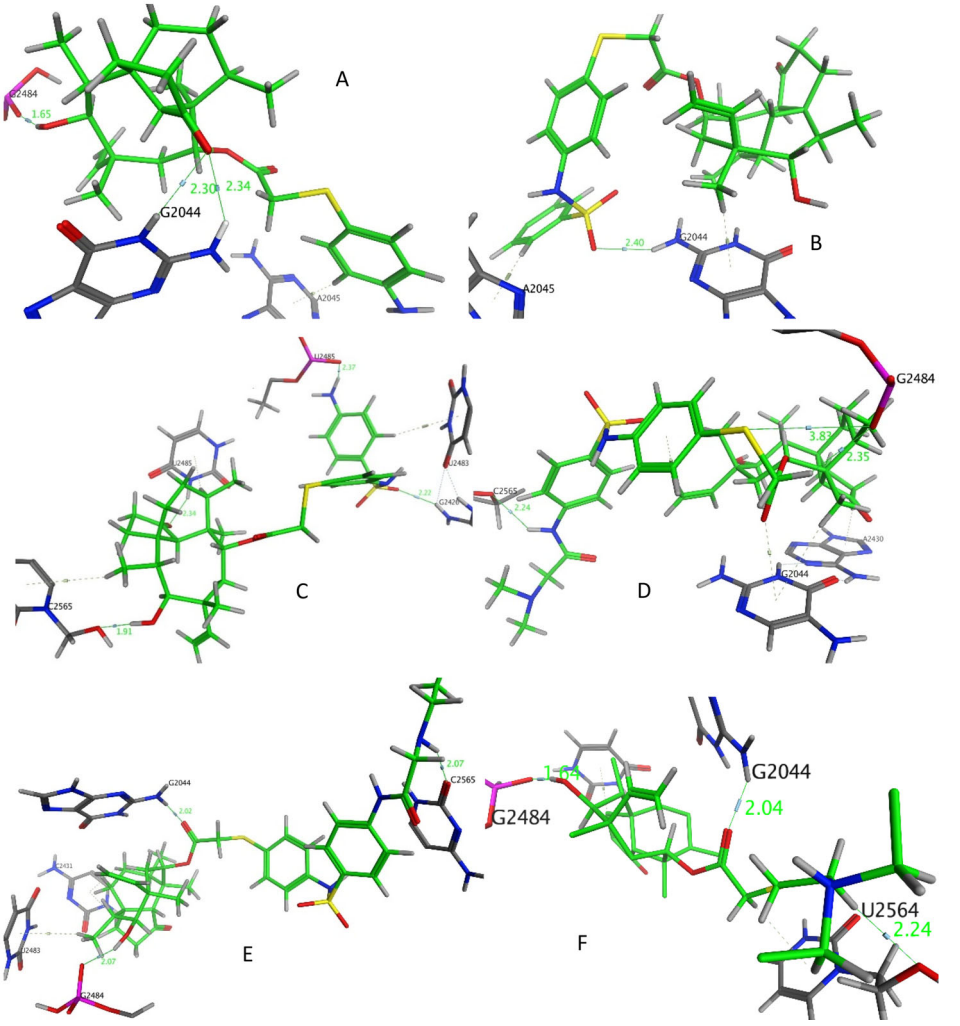

Figure 5. The molecular docking results of compounds **9** (A), **10** (B), **11** (C), **14a** (D), **14b** (E), and tiamulin (F).

that tiamulin formed three hydrogen bonds with the 23S rRNA and rendered a similar docking mode to that of **9**, **10**, **11**, **14a** and **14b** in general. The calculated binding free energy of tiamulin was −8.62 kcal/mol, being higher than that of **9**, **10**, **11**, **14a** and **14b**. It is consistent with the *in vitro* assay that the antibacterial activity of these five compounds were better than that of tiamulin. It can be observed that the conformation of all the above docking compounds are consistent with that of tiamulin in the pocket, and formed some hydrogen bonds with G2044, G2484, U2483, C2565, A2430, U2485, G2426 and A2045, which are rendered a similar docking mode to that of tiamulin (Figure 5F) in general. The docking result may explain why **9**, **10**, **11**, **14a** and **14b** exhibit better *in vitro* antibacterial activity and suggest that the introduction of sulfonyl and NH_2 to the C14 side chain would be effective tactics for the design of pleuromutilin derivatives.

2.4. Conclusion

In summary, a series of piperazine type and thioether type new pleuromutilin derivatives containing sulfanilamide substituents were designed and synthesized. In comparable with tiamulin and erythromycin, 10 compounds (**5**, **6**, **7a**, **7d**, **9**, **10**, **11**, **14a**, **14b** and **14f**) showed good to excellent anti-bacterial activities to most of the tested Gram-positive pathogens and of them, 5 compounds (**9**, **10**, **11**, **14a** and **14b**) exhibited good antibacterial activities against the drug-resistant Gram-positive bacteria. Therefore, the result of anti-bacterial test revealed that thioether group in pleuromutilin played more important role in antibacterial activity than piperazinyl in pleuromutilin.

3. Experimental

3.1. General experimental procedures

All reagents were purchased from commercial sources and were used as received. Pleuromutilin (>90% pure) was obtained from Beijing Ouhe Technology Co., Ltd. (Beijing, China). p-toluene sulfochloride, chloroacetyl chloride, p-aminothiophenol and alkylamine were obtained from Aladdin Reagent Co. (Shanghai, China). p-Nitrobenzene sulfonyl chloride and piperazine were purchased from Adamas Reagent Co. (Shanghai, China).

Routine monitoring of reaction was performed by TLC using pre-coated GF254 TLC plate, which were purchased from Qingdao Ocean Chemical Co., Ltd. (Qingdao, China). NMR spectra were recorded on a Bruker AVANCE 600 spectrometer at 600 MHz instrument (Bruker Daltonik, Bremen, Germany) in $CDCl_3$ using tetramethylsilane (TMS) as the internal reference. MS was performed with electron spray ionization (ESI) mode and recorded on a high performance liquid chromatography tandem mass spectrometer by Waters (Waters, Milford, Massachusetts, America). Melting points of all compounds were defined using Kerui X-5 apparatus (Gongyi Kerui, Gongyi, Henan, China) without correction. Elemental analysis was performed on an Elementar vario EL cube elemental analyzer (Elementar, Germany). IR was performed by Nicolet IS10 Fourier Infrared transform spectrometer (Thermo Fisher Scientific, Waltham, America).

3.2. Synthesis

Compounds **2** and **3** were synthesized from pleuromutilin according to the pathway in our previous work [10], and the yield and melting point were consistent with the reference.

3.2.1. Synthesis of 22-(4-(p-nitrobenzene sulfonyl) piperazinyl)-22-deoxypleuromutilin (4)

Compound **3** (6 g, 13.4 mmol) and anhydrous K_2CO_3 (1.86 g, 13.4 mmol) were added into 30 ml acetonitrile, followed by stirring and heating under reflux. p-Nitrobenzenesulfonyl chloride (2.98 g, 13.4 mmol) was dissolved in acetonitrile and dropwise added into the above reaction mixture. Most acetonitrile was evaporated *in*

vacuum after TLC test showed the reaction was completed. The slurry was cooled to room temperature and treated with water (100 ml) and extracted with ethyl acetate (50 ml × 3). Yellow solid (7.54 g) was obtained after concentrating the organic phase. The yield of the crude product was 90%. The next step of reaction could be carried out directly without purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.37 (d, $J = 8.4$ Hz, 2H, phenyl H-3, 5), 7.93 (d, $J = 8.4$ Hz, 2H, phenyl H-2, 6), 6.43 (dd, $J = 17.4$, 10.8 Hz, 1H, H-19), 5.74 (d, $J = 8.4$ Hz, 1H, H-14), 5.29 (d, $J = 10.8$ Hz, 1H, 1H from H-20), 5.16 (d, $J = 17.4$ Hz, 1H, 1H from H-20), 3.33 (dd, $J = 10.8$, 6.6 Hz, 1H, H-11), 3.14 (d, $J = 16.8$ Hz, 1H, 1H from H-22), 3.11–3.13 (m, 4H, piperazinyl H-3, 5), 3.04 (d, $J = 16.8$ Hz, 1H, 1H from H-22), 2.58 − 2.70 (m, 4H, piperazinyl H-2, 6), 2.27–2.32 (m, 1H), 2.14 − 2.25 (m, 2H), 2.02 − 2.06 (m, 2H), 1.75 (dd, $J = 14.4$, 3.0 Hz, 1H), 1.64 − 1.66 (m, 1H), 1.60 − 1.62 (m, 1H), 1.48 − 1.53 (m, 1H), 1.42 − 1.47 (m, 2H), 1.40 (s, 3H), 1.32 − 1.35 (m, 1H), 1.23 (d, $J = 15.6$ Hz, 1H), 1.14 (s, 3H), 1.08 − 1.11 (m, 1H), 0.86 (d, $J = 7.2$ Hz, 3H), 0.66 (d, $J = 7.2$ Hz, 3H).^{13}C NMR (151 MHz, $CDCl_3$) δ 217.1, 168.8, 150.4, 141.7, 139.1, 129.1 (phenyl C-3, 5), 124.4 (phenyl C-2, 6), 117.4, 74.7, 68.7, 59.3, 58.2, 51.9 (piperazinyl C-2, 6), 45.9 (piperazinyl C-3, 5), 45.5, 45.1, 44.0, 41.8, 36.7, 36.1, 34.5, 30.5, 26.9, 26.5, 24.9, 16.8, 14.9, 11.6. ESIMS: *m/z* 654.7 $[M + Na]^+$. Elemental analysis: Found: C, 60.78%, H, 7.24%, N, 6.71%, S, 5.16%; calcd for $C_{32}H_{45}N_3O_8S$, C, 60.84%, H, 7.18%, N, 6.65%, S, 5.07%.

3.2.2. Synthesis of 22-(4-(p-aminobenzene sulfonyl) piperazinyl)-22-deoxypleuromutilin (5)

To a solution of ammonium chloride (0.95 g, 17.4 mmol) and reduced iron powder (1.46 g, 26.1 mmol) in acetic acid (10 ml), compound **4** (5.5 g, 8.7 mmol) was dissolved in absolute ethyl alcohol (20 ml) and then added slowly to the above mixed solution. The mixture was stirred and heated to 80 °C. After TLC test showed the reaction was completed, saturated sodium bicarbonate was employed to adjust the pH of the mixture to 7. The ethyl acetate extract was washed with water (30 ml × 3), and the organic phase was concentrated under reduced pressure, equivalent petroleum ether was added, followed by recrystallizing to give the pure product (3.96 g) in 75.6% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (d, $J = 8.4$ Hz, 2H, phenyl H-2, 6), 6.62 (d, $J = 8.4$ Hz, 2H, phenyl H-3, 5), 6.43 (dd, $J = 17.4$, 10.8 Hz, 1H, H-19), 5.74 (d, $J = 8.4$ Hz, 1H, H-14), 5.29 (dd, $J = 10.8$, 1.4 Hz, 1H, 1H from H-20), 5.17 (d, $J = 17.4$ Hz, 1H, 1H from H-20), 4.18 (s, 2H, phenyl-NH_2), 3.33 (dd, $J = 10.8$, 6.6 Hz, 1H, CHOH), 3.12 (d, $J = 17.4$ Hz, 1H, 1H from H-22), 3.02 − 3.04 (m, 4H, piperazinyl H-3, 5), 3.01 (d, $J = 17.4$ Hz, 1H, 1H from H-22), 2.56 − 2.61 (m, 4H, piperazinyl H-2, 6), 2.28 − 2.31 (m, 1H), 2.16 − 2.23 (m, 2H), 2.02 − 2.08 (m, 2H), 1.73 − 1.75 (m, 2H), 1.59 − 1.65 (m, 1H), 1.47 − 1.51 (m, 1H), 1.42 − 1.45 (m, 2H), 1.39 (s, 3H), 1.32 − 1.34 (m, 1H), 1.23 (d, $J = 16.2$ Hz, 1H), 1.13 (s, 3H), 1.08 − 1.11 (m, 1H), 0.85 (d, $J = 6.6$ Hz, 3H), 0.66 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.2, 169.0, 150.9, 139.1, 130.1 (phenyl C-3, 5), 123.2 (phenyl C-2, 6), 117.4, 114.0, 74.7, 68.6, 59.5, 58.3, 52.2 (piperazinyl C-2, 6), 45.5 (piperazinyl C-3, 5), 45.9, 45.1, 44.0, 41.8, 36.8, 36.1, 34.6, 30.5, 26.7, 26.5, 24.9, 16.8, 14.9, 11.6. ESIMS:

m/z 624.5 $[M+Na]^+$. Elemental analysis: Found: C, 63.96%, H, 7.75%, N, 6.95%, S, 5.29%; calcd for $C_{32}H_{47}N_3O_6S$, C, 63.87%, H, 7.87%, N, 6.98%, S, 5.33%.

3.2.3. Synthesis of 22-((4-(4-chloroacetyl amino) benzenesulfonyl) piperazinyl)-22-deoxypleuromutilin (6)

To a stirred solution of compound **5** (7.1 g, 11.8 mmol) in CH_2Cl_2 (100 ml) were added chloroacetyl chloride (1.33 g, 11.8 mmol) and triethylamine (0.6 g, 5.9 mmol) at room temperature. After the materials were consumed completely, water (100 ml) was added to the reaction system. The resulting solution was extracted with CH_2Cl_2 (50 ml × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated *in vacuum*, to obtain the crude yellowish-brown solid (6.98 g) in 87.2% yield, which was directly used in the next step without purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.46 (s, 1H, NHCO), 7.71 − 7.75 (m, 4H, phenyl), 6.44 (dd, J = 17.4, 10.8 Hz, 1H, H-19), 5.74 (d, J = 8.4 Hz, 1H, H-14), 5.28 (d, J = 10.8 Hz, 1H, 1H from H-20), 5.17 (d, J = 17.4 Hz, 1H, 1H from H-20), 4.22 (s, 2H, NHCOC$\underline{H}_2$Cl), 3.33 (dd, J = 10.8, 6.6 Hz, 1H, C$\underline{H}$OH), 3.13 (d, J = 17.4 Hz, 1H, 1H from $\underline{H}$-22), 3.04 − 3.06 (m, 4H, piperazinyl $\underline{H}$-3, 5), 3.03 (d, J = 17.4 Hz, 1H, 1H from $\underline{H}$-22), 2.57 − 2.66 (m, 4H, piperazinyl H-2, 6), 2.29 − 2.31 (m, 1H), 2.16 − 2.23 (m, 2H), 2.02 − 2.07 (m, 2H), 1.75 (dd, J = 14.4, 3.0 Hz, 1H), 1.60 − 1.66 (m, 2H), 1.50 (dd, J = 14.4, 3.0 Hz, 1H), 1.41 − 1.47 (m, 2H), 1.39 (s, 3H), 1.33 (dd, J = 14.4, 3.0 Hz, 1H), 1.24 (d, J = 15.6 Hz, 1H), 1.14 (s, 3H), 1.08 − 1.12 (m, 1H), 0.85 (d, J = 7.2 Hz, 3H), 0.67 (d, J = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.2, 168.9, 164.3, 141.0, 139.1, 131.3, 129.3 (phenyl C-3, 5), 119.7 (phenyl C-2, 6), 117.4, 74.7, 68.7, 59.5, 58.3, 52.1 (piperazinyl C-2, 6), 45.9 (piperazinyl C-3, 5), 45.5, 45.0, 44.0, 42.9, 41.8, 36.8, 36.1, 34.6, 30.5, 26.9, 26.5, 24.9, 16.8, 14.9, 11.6. MS (ESI, *m/z*): 700.8 $[M+Na]^+$. Elemental analysis: Found: C, 60.23%, H, 7.18%, N, 6.28%, S, 4.71%; calcd for $C_{34}H_{48}ClN_3O_7S$, C, 60.21%, H, 7.13%, N, 6.20%, S, 4.73%.

3.2.4. General procedure for the synthesis of 7a–g

A three-neck round bottom flask equipped with a magnetic stirrer was added compound **6** (1.2 g, 1.8 mmol) and 20 ml of DMF. The stirred solution was then added anhydrous K_2CO_3 (0.19 g, 1.4 mmol) and equal molar quantities of alkylamine/arylamine at 100 °C for 5 h. After completion, about 15 ml DMF was evaporated *in vacuum*. The residue was treated with 20 ml H_2O and stirred, the solid was filtered, rinsed with water and dried at 80 °C. The residue was purified by silica gel chromatography with ethyl acetate/petroleum ether (V/V 20/1 to 5/1) as eluent to give **7a–g** as a white solid.

3.2.4.1. 22-(4-(4-(2-Ethylamino acetamino) benzenesulfonyl) piperazinyl)-22-deoxypleuromutilin (7a). White powdery solid, yield 73.5%. m.p.: 180 − 182 °C. IR (KBr, cm^{-1}): 3372, 2958, 2935, 2861, 1733, 1694, 1593, 1520, 1456, 1403, 1348, 1306, 1204, 1163, 1118, 1016, 940, 738. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.79 (s, 1H, NHCO), 7.76 (d, J = 8.4 Hz, 2H, phenyl H-3, 5), 7.68 (d, J = 8.4 Hz, 2H, phenyl H-2, 6), 6.44 (dd, J = 17.4, 10.8 Hz, 1H, H-19), 5.73 (d, J = 8.4 Hz, 1H, H-14), 5.28 (d, J = 10.8 Hz, 1H, H-20), 5.16 (d, J = 17.4 Hz, 1H, H-20), 3.47 (s, 2H, H-22), 3.33 (d, J = 6.0 Hz, 1H, H-

11), 3.13 (d, $J = 17.4$ Hz, 1H, H-22), 3.04 – 3.06 (m, 4H, piperazinyl H-3, 5), 3.02 (d, $J = 17.4$ Hz, 1H, H-22), 2.78 (q, $J = 7.2$ Hz, 2H, NH-CH_2-CH_3), 2.57 – 2.64 (m, 4H, piperazinyl H-2, 6), 2.28 – 2.31 (m, 1H), 2.14 – 2.24 (m, 3H), 2.02 – 2.07 (m, 2H), 1.74 (d, $J = 12.6$ Hz, 1H), 1.60 – 1.66 (m, 2H), 1.49 – 1.53 (m, 1H), 1.43 – 1.45 (m, 1H), 1.39 (s, 3H), 1.32 – 1.34 (m, 1H), 1.23 (d, $J = 15.6$ Hz, 1H), 1.19 (t, $J = 7.2$ Hz, 3H, NH-CH_2-CH_3), 1.13 (s, 3H), 1.08 – 1.11 (m, 1H), 0.85 (d, $J = 7.2$ Hz, 3H), 0.66 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.2 (C-3), 170.1 (C-21), 168.9 (CONH), 141.9 (C-19), 139.1 (phenyl C-1), 129.9 (phenyl C-4), 129.3 (phenyl C-3, 5), 119.1 (phenyl C-2, 6), 117.4 (C-20), 74.7, 68.6, 59.5, 58.3, 52.6, 52.1 (piperazinyl C-2, 6), 45.9 (piperazinyl C-3, 5), 45.5, 45.1, 44.7, 44.0, 41.8, 36.7, 36.1, 34.6, 30.5, 26.9, 26.4, 24.9, 16.8, 15.1, 14.9, 11.6. ESIMS: *m/z* 687.3 $[M + H]^+$. Elemental analysis: Found: C, 62.82%, H, 7.96%, N, 8.10%, S, 4.61%; calcd for $C_{36}H_{54}N_4O_7S$, C, 62.95%, H, 7.92%, N, 8.16%, S, 4.67%.

The spectral data of **7b–7f** are listed in the supplementary information.

3.2.5. Synthesis of 22-(4-(N-Boc) thiophenyl)-22-deoxypleuromutilin (8)

To a stirred solution of K_2CO_3 (2.01 g, 14.6 mmol) and compound **2** (9.7 g, 18.2 mmol) in acetonitrile (30 ml), 4-N-Boc thiophenol (4.1 g, 18.2 mmol) was dissolved in acetonitrile (5 ml) and added at reflux temperature. After the materials were consumed completely, about 15 ml acetonitrile was evaporated *in vacuum*, and then water (150 ml) was added to the residue. The resulting solution was extracted with ethyl acetate (50 ml × 3). The combined organic layers were evaporated *in vacuum* to obtain the crude yellowish-brown solid (9.94 g) in 93.2% yield.

3.2.6. Synthesis of 22-(4-amino thiophenyl)-22-deoxypleuromutilin (9)

Compound **8** (13.3 g, 22.7 mmol) was dissolved into CH_2Cl_2 (50 ml) and trifluoroacetic acid (10 ml) was dropwise added into the mixture at room temperature. After the materials were consumed completely, the formed slurry was treated with water (150 ml) and saturated sodium bicarbonate was employed to adjust the pH of the mixture to 7. The resulting solution was extracted with CH_2Cl_2 (50 ml × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated *in vacuum*, to obtain the crude yellowish-brown solid (9.52 g) in 86.3% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.18 (d, $J = 8.4$ Hz, 2H, phenyl H-2, 6), 6.50 (d, $J = 8.4$ Hz, 2H, phenyl H-3, 5), 6.39 (dd, $J = 17.4$, 10.8 Hz, 1H, H-19), 5.64 (d, $J = 8.4$ Hz, 1H, H-14), 5.27 (d, $J = 10.8$ Hz, 1H, 1H from H-20), 5.12 (d, $J = 17.4$ Hz, 1H, 1H from H-20), 3.67 (s, 2H, phenyl NH_2), 3.32 (dd, $J = 15.0$, 10.8 Hz, 2H), 3.26 (dd, $J = 10.2$, 6.6 Hz, 1H, CHOH), 2.23 – 2.27 (m, 1H), 2.08 – 2.20 (m, 2H), 2.00 (s, 1H), 1.91 (dd, $J = 15.6$, 8.4 Hz, 1H), 1.68 (dd, $J = 14.4$, 2.4 Hz, 1H), 1.53 – 1.59 (m, 2H), 1.42 – 1.49 (m, 1H), 1.35 – 1.40 (m, 2H), 1.32 (s, 3H), 1.26 (dd, $J = 14.4$, 3.0 Hz, 1H), 1.11 (d, $J = 16.2$ Hz, 1H), 1.07 (s, 3H), 1.01 – 1.05 (m, 1H), 0.79 (d, $J = 7.2$ Hz, 3H), 0.59 (d, $J = 7.2$ Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.3, 168.8, 146.8, 139.2, 134.7 (phenyl C-3, 5), 121.8, 117.2 (phenyl C-2, 6), 115.6, 74.7, 69.2, 58.3, 45.5, 44.7, 43.9, 41.8, 39.7, 36.9, 36.1, 34.6, 30.5, 26.9, 26.4, 24.9, 16.8, 15.0, 11.6. ESIMS: *m/z* 508.5 $[M + Na]^+$. Elemental analysis: Found: C, 69.32%, H, 8.02%, N, 2.91%, S, 6.67%; calcd for $C_{28}H_{39}NO_4S$, C, 69.24%, H, 8.09%, N, 2.88%, S, 6.60%.

3.2.7. Synthesis of 22-(4-(p-nitrobenzene sulfamine) thiophenyl)-22-deoxypleuromutilin (10)

Compound **9** (7.05 g, 14.5 mmol) and anhydrous K_2CO_3 (2.0 g, 14.5 mmol) were added in acetonitrile (30 ml) and stirred at room temperature. p-Nitrobenzene sulfonyl chloride (3.22 g, 14.5 mmol) was dissolved in acetonitrile (10 ml) and dropwise added into the mixture. After the materials were consumed completely, about 25 ml acetonitrile was evaporated *in vacuum*, and then the formed slurry was treated with water (150 ml) and extracted by ethyl acetate (50 ml × 3). The combined organic layers were evaporated *in vacuum* to obtain the crude yellowish-brown solid (8.74 g) in 89.7% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.11 (d, $J = 6.6$ Hz, 2H, phenyl H-3′, 5′), 7.90 (d, $J = 7.8$ Hz, 2H, phenyl H-2′, 6′), 7.19 (d, $J = 7.8$ Hz, 2H, phenyl H-2, 6), 6.93 (d, $J = 7.8$ Hz, 2H, phenyl H-3, 5), 6.35 (dd, $J = 16.8$, 11.4 Hz, 1H, H-19), 5.65 (d, $J = 7.2$ Hz, 1H, H-14), 5.21 (d, $J = 11.4$ Hz, 1H, 1H from H-20), 5.10 (d, $J = 16.8$ Hz, 1H, 1H from H-20), 3.47 (t, $J = 16.8$ Hz, 2H), 3.32 (s, 1H), 2.15 − 2.25 (m, 3H), 2.03 (s, 1H), 1.94 (q, $J = 7.2$ Hz, 1H), 1.73 (d, $J = 13.2$ Hz, 1H), 1.59 − 1.63 (m, 2H), 1.42 − 1.53 (m, 3H), 1.28 (s, 5H), 1.15 (d, $J = 15.6$ Hz, 1H), 1.09 (s, 4H), 0.85 (d, $J = 5.4$ Hz, 3H), 0.56 (d, $J = 5.4$ Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.8, 168.4, 149.9, 145.9, 139.1, 131.6 (phenyl C-2, 6), 128.5 (phenyl C-2′, 6′), 124.3, 122.6 (phenyl C-3′, 5′), 117.2 (phenyl C-3, 5), 74.7, 69.8, 60.6, 58.3, 53.8, 45.6, 44.9, 44.0, 41.8, 37.6, 36.9, 36.1, 34.7, 30.5, 26.9, 26.6, 24.9, 16.8, 14.9, 11.6. ESIMS: *m/z* 693.8 $[M + Na]^+$. Elemental analysis: Found: C, 60.93%, H, 6.39%, N, 4.24%, S, 9.49%; calcd for $C_{34}H_{42}N_2O_8S_2$, C, 60.88%, H, 6.31%, N, 4.18%, S, 9.56%.

3.2.8. Synthesis of 22-(4-(p-aminobenzene sulfamine) thiophenyl)-22-deoxypleuromutilin (11)

Reduced iron powder (2.4 g, 42.9 mmol) and ammonium chloride (1.54 g, 28.6 mmol) were added into acetic acid (30 ml) and water (20 ml). Acetonitrile solution of compound **10** (9.6 g, 14.3 mmol) was dropwise added into the above mixture and stirred at 80 °C for 3 h. After completion, saturated sodium bicarbonate was employed to adjust the pH of the mixture to 7 and the mixture was extracted with ethyl acetate (30 ml × 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuum*. The residue was purified by recrystallization with ethyl acetate/petroleum ether (V/V: 2/3) to give **11** as a white solid (6.32 g, 68.9% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (d, $J = 8.4$ Hz, 2H, phenyl H-2, 6), 7.21 (d, $J = 8.4$ Hz, 2H, phenyl H-2′, 6′), 6.97 (d, $J = 8.4$ Hz, 2H, phenyl C-3, 5), 6.55 (d, $J = 8.4$ Hz, 2H, phenyl C-3′, 5′), 6.36 (dd, $J = 17.4$, 10.8 Hz, 1H, H-19), 5.65 (d, $J = 8.4$ Hz, 1H, H-14), 5.23 (d, $J = 10.8$ Hz, 2H, 1H from H-20), 5.11 (d, $J = 17.4$ Hz, 1H, 1H from H-20), 4.23 (s, 2H, phenyl NH_2), 3.42 − 3.48 (m, 2H), 3.32 (s, 1H), 2.13 − 2.28 (m, 3H), 2.03 (s, 1H), 1.89 (dd, $J = 15.6$, 8.4 Hz, 1H), 1.72 (d, $J = 13.8$ Hz, 1H), 1.55 − 1.65 (m, 3H), 1.40 − 1.50 (m, 2H), 1.29 (s, 3H), 1.24 − 1.28 (m, 1H), 1.08 (s, 4H), 1.05 (s, 1H), 0.84 (d, $J = 6.6$ Hz, 3H), 0.55 (d, $J = 6.6$ Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.7, 168.5, 151.1, 138.9, 137.0, 131.8 (phenyl C-2, 6), 130.0, 129.5 (phenyl C-2′, 6′), 126.9, 121.2 (phenyl C-3, 5), 117.4, 114.1 (phenyl C-3′, 5′), 74.6, 69.6, 58.2, 45.5, 44.7, 43.9, 41.8, 37.8, 36.8, 36.0, 34.6, 30.5, 26.9, 26.5, 24.9, 16.7, 14.9, 11.6. ESIMS: *m/z* 663.8 $[M + Na]^+$. Elemental analysis: Found: C, 63.79%, H,

6.86%, N, 4.32%, S, 10.06%; calcd for $C_{34}H_{44}N_2O_6S_2$, C, 63.72%, H, 6.92%, N, 4.37%, S, 10.01%.

3.2.9. General procedure for the synthesis of 12a–f

Compound **11** (1.0 g, 1.56 mmol), anhydrase K_2CO_3 (0.17 g, 1.25 mmol) and equal molar quantities of halohydrocarbon were added into 15 ml DMF and stirred at 100 °C for 5 h. After completion, about 10 ml DMF was evaporated *in vacuum*. The residue was treated with 30 ml H_2O and stirred, the solid was filtered, rinsed with water and dried at 80 °C. The residue was purified by silica gel chromatography with ethyl acetate/petroleum ether (V/V 20/1 to 3/1) as eluent to give **12a–f** as a white solid.

3.2.9.1. 22-(4-(p-ethylamine benzene sulfamine) thiophenyl)-22-deoxypleuromutilin (12a). White powdery solid, yield 67.2%. m.p.: 198 − 199.5 °C. IR (KBr, cm^{-1}): 3470, 3376, 2982, 2934, 2880, 1725, 1629, 1596, 1491, 1457, 1340, 1321, 1281, 1147, 1116, 1087, 1015, 938, 739, 679, 548. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.33 (d, J = 8.4 Hz, 2H, phenyl H-2′, 6′), 7.27 (d, J = 8.4 Hz, 2H, phenyl H-2, 6), 6.97 (d, J = 8.4 Hz, 2H, phenyl H-3′, 5′), 6.61 (d, J = 8.4 Hz, 2H, phenyl H-3, 5), 6.42 (dd, J = 17.4, 10.8 Hz, 1H, H-19), 5.73 (d, J = 8.4 Hz, 1H, H-14), 5.29 (d, J = 10.8 Hz, 1H, H-20), 5.15 (d, J = 17.4 Hz, 1H, H-20), 4.16 (s, 2H), 3.56 (s, 2H, H-22), 3.52 (q, J = 7.2 Hz, 2H, $NHCH_2CH_3$), 3.33 (dd, J = 10.2, 6.6 Hz, 1H, H-11), 2.28 − 2.33 (m, 1H), 2.21 − 2.27 (m, 1H), 2.14 − 2.20 (m, 1H), 2.00 − 2.07 (m, 2H), 1.75 (dd, J = 14.4, 2.4 Hz, 1H), 1.60 − 1.66 (m, 3H), 1.47 (m, 1H), 1.41 − 1.44 (m, 1H), 1.39 (s, 3H), 1.34 (dd, J = 14.4, 2.4 Hz, 1H), 1.23 (d, J = 15.6 Hz, 1H), 1.13 (s, 3H), 1.08 − 1.11 (m, 1H), 1.02 (t, J = 7.2 Hz, 3H, $NHCH_2CH_3$), 0.86 (d, J = 6.6 Hz, 3H), 0.65 (d, J = 6.6 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.2 (C-3), 168.2 (C-21), 150.7 (phenyl C-4′), 139.0 (C-19), 137.9 (phenyl C-4), 134.8, 129.8 (phenyl C-2, 6), 129.6 (phenyl C-2′, 6′), 129.5 (phenyl C-3, 5), 126.4, 117.3, 113.9 (phenyl C-3′, 5′), 74.7, 69.9, 58.3, 45.5, 45.2, 44.9, 44.0, 41.9, 37.0, 36.8, 36.1, 34.6, 30.5, 26.9, 26.6, 24.9, 16.9, 14.9, 14.0, 11.6. ESIMS: m/z 691.8 $[M + Na]^+$. Elemental analysis: Found: C, 64.69%, H, 7.16%, N, 4.17%, S, 9.66%; calcd for $C_{36}H_{48}N_2O_6S_2$, C, 64.64%, H, 7.23%, N, 4.19%, S, 9.59%.

The spectral data of **12b–12f** are listed in the supplementary information.

3.2.10. Synthesis of 22-(4-(4-(2-chloroacetamide) benzene sulfonamido) thiophenyl)-22-deoxypleuromutilin (13)

Compound **11** (5.2 g, 8.1 mmol) and triethylamine (1.23 g, 12.2 mmol) were dissolved into 50 ml CH_2Cl_2. Chloroacetyl chloride (1.38 g, 12.2 mmol) was then dropwise added into the mixture and stirred for about 5 h. After completion, the formed slurry was treated with water (50 ml) and extracted with CH_2Cl_2 (50 ml × 3). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuum* to obtain the crude yellowish-brown solid (4.73 g) in 81.3% yield. The next step of reaction could be carried out directly without purification.

3.2.11. General procedure for the synthesis of 14a–f

Compound **13** (1.0 g, 1.4 mmol), anhydrous K_2CO_3 (0.15 g, 1.1 mmol) and equimolar alkylamine/arylamine were added in 20 ml DMF and stirred at 100 °C for 5 h. After the materials were consumed completely, about 15 ml DMF was evaporated *in vacuum*. The residue was treated with 30 ml H_2O and stirred, the solid was filtered, rinsed with water and dried at 80 °C. The residue was purified by silica gel column chromatography with ethyl acetate/petroleum ether (V/V 20/1 to 5/1) as eluent to give **14a–f** as a white solid.

3.2.11.1. 22-(4-(4-(2-Dimethylamino acetamino) benzene sulfonamido) thiophenyl)-22-deoxypleuromutilin (14a). White powdery solid, yield 62.1%. m.p.: 208 − 209.5 °C. IR (KBr, cm^{-1}): ν_{max} 3262, 2982, 2934, 2860, 1717, 1593, 1517, 1493, 1457, 1402, 1336, 1278, 1159, 1116, 1093, 1015, 917, 828, 638, 574. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.37 (s, 1H, NHCO), 7.71 (d, J = 8.4 Hz, 2H, phenyl H-2′, 6′), 7.60 (d, J = 8.4 Hz, 2H, phenyl H-3′, 5′), 7.19 (d, J = 7.8 Hz, 2H, phenyl H-2, 6), 6.97 (d, J = 7.8 Hz, 2H, phenyl H-3, 5), 6.35 (dd, J = 17.4, 10.8 Hz, 1H, H-19), 5.66 (d, J = 7.8 Hz, 1H, H-14), 5.22 (d, J = 10.8 Hz, 1H, H-20), 5.09 (d, J = 17.4 Hz, 1H, H-20), 3.44 − 3.49 (m, 2H), 3.32 (s, 1H), 3.07 (s, 2H, CH_2CONH), 2.35 (s, 6H, $N(CH_3)_2$), 2.22 − 2.27 (m, 2H), 2.15 − 2.19 (m, 1H), 2.05 (s, 1H), 1.93 (dd, J = 15.6, 8.4 Hz, 1H), 1.73 (dd, J = 14.4, 2.4 Hz, 1H), 1.57 − 1.65 (m, 3H), 1.41 − 1.49 (m, 2H), 1.31 (s, 3H), 1.25 − 1.29 (m, 2H), 1.09 − 1.12 (m, 2H), 1.08 (s, 3H), 0.85 (d, J = 6.6 Hz, 3H), 0.59 (d, J = 6.6 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 217.5 (C-3), 169.9 (NHCO), 168.4 (C-21), 141.6 (phenyl C-4′), 139.1 (C-19), 134.7, 131.8 (phenyl C-2, 6), 128.6 (phenyl C-2′, 6′), 121.9 (phenyl C-1), 119.3 (phenyl C-3′, 5′), 117.3 (phenyl C-3, 5), 74.7, 69.7, 63.6 ($COCH_2N(CH_3)_2$), 58.3, 53.6, 46.2 ($CH_2N(CH_3)_2$), 45.6, 44.8, 43.9, 41.8, 37.9, 36.9, 36.1, 34.6, 30.5, 29.81, 26.9, 26.6, 24.9, 16.8, 14.9, 11.6. ESIMS: *m/z* 748.8 $[M + Na]^+$. Elemental analysis: Found: C, 62.81%, H, 7.02%, N, 5.73%, S, 8.78%; calcd for $C_{38}H_{51}N_3O_7S_2$, C, 62.87%, H, 7.08%, N, 5.79%, S, 8.83%.

The spectral data of **14b–14f** are listed in the supplementary information.

3.3. Minimum inhibitory concentrations (MIC) testing

The evaluation of the *in vitro* anti-bacterial activity of all prepared derivatives was performed against a panel of well characterized clinical susceptible (S.aureus, S.epidermidis and S.suis) and resistant Gram-positive (MRSA-NY3) bacteria isolated from clinic as well as against one Gram-negative (E.coli) bacteria strain. The minimum inhibitory concentrations (MICs) of target compounds against above bacteria were determined by broth dilution method, tiamulin fumaric and erythromycin thiocyanate were employed as the reference agents based on the Clinical and Laboratory Standards Institute (CLSI) [17]. The MIC values are showed in Table 1.

3.4. Molecular modeling

Molecular docking study was performed by Molecular Operating Environment (MOE) 2018.0101 release of Chemical Computing Group Inc., Montreal, Quebec,

Table 2. Docking scores as "S" (kcal mol^{-1}) and bond interactions of **9, 10, 11, 14a, 14b** and tiamulin with active pocket of 23s rRNA.

Cpd. No.	S (kcal mol^{-1})	No. of bonds	Distance (Å)	Amino acids involved	Interacting groups
9	−9.6458	4	2.34	G2044	H-bond with C = O of pleuromutilin C3
			2.30	G2044	H-bond with C = O of pleuromutilin C3
			1.65	G2484	H-bond with OH of pleuromutilin C11
				A2045	H-π interaction
10	−10.4873	3	2.40	G2044	H-bond with S = O of pleuromutilin C14
				G2044	H-π interaction
				A2045	H-π interaction
11	−9.7800	7	1.91	C2565	H-bond with OH of pleuromutilin C11
			2.22	G2426	H-bond with S = O of pleuromutilin C14
			2.34	U2485	H-bond with C = O of pleuromutilin C3
			2.37	U2485	H-bond with CH_3 of pleuromutilin C14
				U2483	H-π interaction
				U2485	H-π interaction
				C2565	H-π interaction
14a	−11.8241	6	2.24	C2565	H-bond with NH of pleuromutilin C14
			2.35	G2484	H-bond with H of pleuromutilin C14
			3.83	G2484	H-bond with S of pleuromutilin C14
				G2044	H-π interaction
				G2044	H-π interaction
				A2430	H-π interaction
14b	−10.3751	6	2.02	G2044	H-bond with C = O of ester group
			2.07	U2483	H-bond with OH of pleuromutilin C11
			2.07	C2565	H-bond with NH of pleuromutilin C14
				C2431	H-π interaction
				C2431	H-π interaction
				U2483	H-π interaction
Tiamulin	−8.6283	5	1.64	G2484	H-bond with OH of pleuromutilin C11
			2.04	G2044	H-bond with C = O of ester group
			2.24	U2564	H-bond with H of C14 side chain
				U2483	H-π interaction
				U2564	H-π interaction

Canada to fully understand the interaction between those new derivatives with receptor targets and further study the structure-activity relationship of this series of compounds [18]. The X-ray crystal structure of Deinococcus radiodurans with tiamulin (PDB 1 D: 1XBP) was obtained from the Protein Data Bank and was used to construct the initial model [19]. Binding score, number and lengths of the hydrogen bonds and amino acid interactions are determined and showed in Table 2. The docking method was validated by the redock of tiamulin, with the root mean square deviations (RMSD) of tiamulin in crystal structure relative to tiamulin in the redock model being 0.35 Å, which confirmed the approach was appropriate.

Disclosure statement

The authors declare that they have no conflict of interest.

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